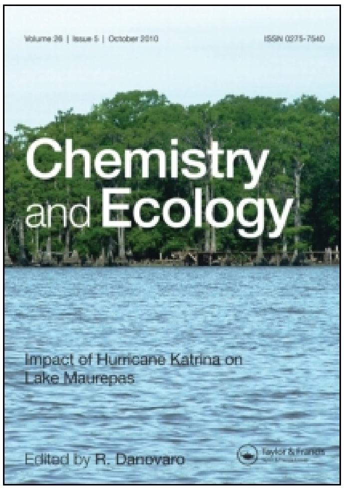

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Ferric Oxide Incrustations on Cell Walls of the Green Alga *Cladophora rupestris* Growing near an Iron Ore Unloading Terminal

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Cladophora rupestris plants growing near an iron ore unloading terminal bore red-brown cell wall incrustations. These were shown to be of ferric oxide by histochemical tests. Iron-free *Cladophora* plants transplanted to this same location developed Fe_2O_3 incrustations after 3–4 weeks. Similar crustose deposits of smaller sizes were observed on *Cladophora* plants growing near rusty chains in a harbour at some distance from the ore terminal. *Cladophora* would seem to be a useful factor monitoring organism for iron ore dust spillage in the sea.

INTRODUCTION

The iron-ore and coal unloading terminal forms part of the British Steel complex on the Hunterston peninsula in the Firth of Clyde. The terminal became operational in November 1979. In an earlier paper (Boney, 1978) the rôle of marine algae as iron ore dust collectors was described. It was shown that intertidal algae with different modes of thallus construction could accumulate and retain appreciable quantities of iron ore dust after shaking in sea water suspensions. It was further shown (Boney, 1980a), that *Fucus* eggs could be centres of dust accumulation in sea water suspensions, and would show enhanced rates of sinking as a result. Particles of coal dust are retained by tufted growths of the red alga *Audouinella purpurea* in localities with exposed coal seams in the region of high water mark (Boney, 1980b). These data are relevant to particulate matter, which in the case of iron-ore may well be spilled at the times of unloading or may be broadcast over the sea by wind-winnowing from stackyards. It is well known that iron in solution is present in very small quantities in the sea. According to Sillen (1961), the major inorganic ionic species is $Fe(OH)_2^+$ in concentrations of about $10^{-4}\mu g$ ions l^{-1}, and the remainder is present almost entirely as colloidal sized particles of hydrous ferric oxide. Iron ore dust particles in sea water form aggregates which sink rapidly. The rates at which any part of these aggregates pass into solution is clearly very slow.

In the construction of the unloading jetty and associated industrial plant an appreciable area of the sandy foreshore was reclaimed (Figure 1), (Clokie and Boney, 1980). As part of this construction work, the new intertidal structures were lined on the seaward side with an "armouring" of large stones—mainly from broken up waste blast furnace linings. The 'armouring' has been colonized by algae with varying densities of plant cover—possibly due to the slow localized leaching of toxic substances. In time a typical intertidal succession was observed with a canopy of fucoid algae eventually dominating the shoreline and a subflora of green and red algae. *Cladophora rupestris* is a common member of this subflora.

OBSERVATIONS

The observations to be described were made on algae collected from sites 1, 2 and 3 (Figure 1) in close proximity to the jetty. "Spot" tests and histochemical examinations for the presence of Fe^{3+} iron compounds were made after immersing the plant material in 1–2 ml of dil-HCl and adding either 2–3 drops of 2.5% potassium ferrocyanide solution or 2–3 drops of 2% ammonium thiocyanate solution. In the former instance the presence of Fe^{3+} iron is indicated by the formation of a deep blue colour or precipitate (Turnbull's blue), and in the latter by the formation of a deep crimson colour.

The results of "spot" tests with various regions of the thalli of five species are summarized in Table I. One only (*Ulva lactuca*) failed to show the presence of iron, and in two species (*Gigantine stellata* and *Fucus serratus*), iron was not detected in the apical regions of fronds, but was present near the bases of the thalli. In both *Cladophora rupestris* and *Polysiphonia macrocarpa* iron was detected in all three regions of the thalli. Especially dense colourations were obtained with the mid- and basal regions of *Cladophora*.

Cladophora plants from sites 1, 2 and 3 were examined microscopically. All showed red-brown incrustations of irregular shape on the cell walls, with both the sizes and numbers increasing from the apical cells down to cells in the basal regions (Figure 2). These incrustations were further

TABLE I

Results of "spot" tests for the presence of Fe^{3+} iron. Marine algae collected from site 1 (Figure 1)

		Potassium ferrocyanide	Ammonium thiocyanate
Chlorophyceae			
Cladophora rupestris	B	+	+
	C	+ +	+ +
	D	+ +	+ +
Ulva lactuca		−	−
Rhodophyceae			
Polysiphonia macrocarpa	B	+	+
	C	+	+
	D	+	+
Gigartina stellata	B	−	−
	D	+	+
Phaeophyceae			
Fucus serratus L.	B	−	−
	D	+ +	+ +

+ + deep colour + pale colour − no colour change
B apical region C mid-region D basal region.

examined by acidifying the filaments on slides with dilute HCl and then adding a drop of ammonium thiocyanate solution. The dissolution of the red-brown incrustations was then observed under the microscope, with the soluble ferric thiocyanate so formed producing a red "halo" around each of the cells. In further tests for the presence of Fe^{3+}, *Cladophora* plants from the neighbourhood of the Hunterston jetty were compared with those from other localities in the Fairlie Channel (Table II). Whilst *Cladophora* plants from Great Cumbrae Island gave no reactions for iron after "spot" tests, those from the rocks near the jetty at Fairlie (some 200 m N.E. of the Hunterston jetty) gave positive reactions. On further examination of the Fairlie site it was found that the plants had been growing nearby some rusty anchor chains. These plants also bore red-brown incrustations on their cell walls, but in smaller amounts than the plants from Hunterston. *Polysiphonia* plants from Fairlie showed some evidence of iron oxide incrustations on basal regions of their thalli. No such depositions were observed on *Fucus serratus* and *Gigartina stellata*, nor was there evidence of any particulate ore dust attached to the plant surfaces.

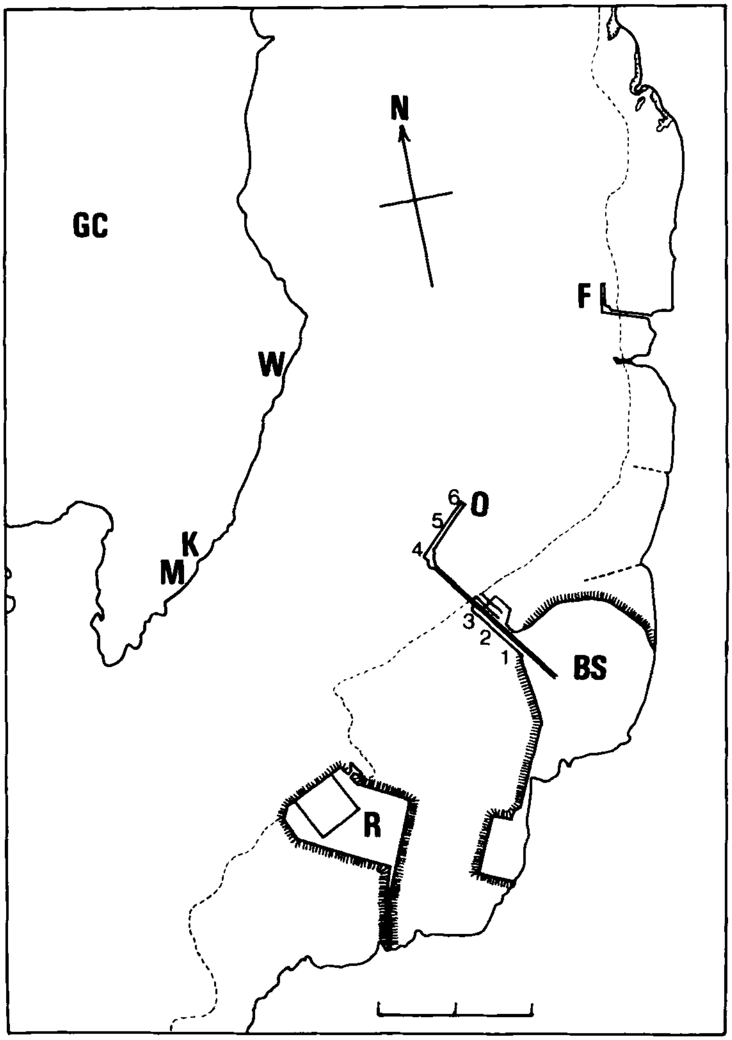

FIGURE 1 Outline map of the Fairlie Channel, Firth of Clyde. F = Fairlie Jetty; O = Ore-unloading terminal; BS = British Steel plant site; R = Oil Rig construction yard; GC = Isle of Great Cumbrae; 1–3 = Sites from which iron-encrusted *Cladophora* was first collected, and to which transplants were made; 4–6 = Sites on outer face of unloading jetty to which transplants were made; M = Marine station slipway; K = North of Keppel pier; W = "Wishing Well". The broken line shows the outer limit of sandy foreshore. Scale line = 100 m.

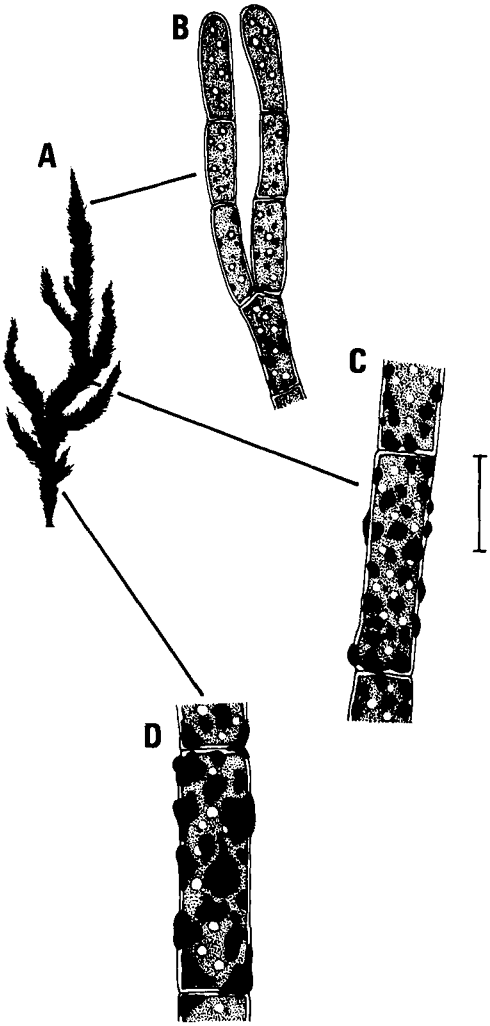

FIGURE 2 Ferric oxide incrustation on *Cladophora rupestris* cells. A—plant (about half natural size); B—terminal cells; C—cells from mid-region; D—cells from basal region. (Ferric oxide incrustations shown in black.) Scale line by C = 150 μm.

TABLE II

Results of tests for Fe^{3+} iron on *Cladophora* plants from localities at Hunterston and in the Fairlie Channel, Firth of Clyde

Locality	Potassium ferrocyanide solution	Ammonium thiocyanate solution
Hunterston terminal		
Sites 1	+ +	+ +
2	+ +	+ +
3	+ +	+ +
Fairlie jetty	+	+
Gt. Cumbrae Island		
Marine Station Slipway	–	–
North of Keppel Pier	–	–
Rocks at "Wishing Well"	–	–

(Reactions as described in Table I)

This seeming ability of *Cladophora* plants to accumulate superficial ferric oxide deposits was further tested by transplanting iron-free *Cladophora* plants on stones from the Isle of Great Cumbrae to sites 1–3 near the ore terminal and to sites 4, 5 and 6 on the outer face of the unloading jetty. After 3–4 weeks the red-brown incrustations were observed on the cell walls of all the transplanted *Cladophora* plants.

DISCUSSION

The incorporation of iron compounds in and on cell wall structures of certain algae is well known. Some genera of placoderm desmids show iron incorporation in the outer pectic layers (Brook, 1981), and euglenoid flagellates of the Phacotaceae may become encapsulated in iron-containing structures (Leedale, 1967). Colloidal sized particles of hydrous ferric oxide adhere to the cell walls of microbial marine algae (Harvey, 1937; Davies, 1970), and there is evidence that such externally adherent compounds can be utilised as iron sources in cell metabolism by the algae (Harvey, 1937; Goldberg 1952; Hayward, 1968). Of the five species of marine algae examined, *Cladophora rupestris,* and to a lesser extent *Polysiphonia macrocarpa,* appear to be able to concentrate the ferric oxide in crustose form on their cell walls. When iron ore dust accumulations by marine algae were measured it was observed that *Cladophora* plants accumulated less particulate matter on their terminal cells than on older cells in the mid- and basal regions (Boney, 1978). Accumulation of particulate iron ore dust, however, is not the same process as the formation of ferric oxide incrustations on the cell walls, and in no instance where ore dust was used was there evidence of these rust-like incrustations. The smaller quantities of both ore-dust particles and ferric oxide incrustations on the terminal cells of *Cladophora* filament is probably an indication of their increased metabolic activity, with the accompanying enhanced release of extracellular products and loss of pectinaceous material from the outermost layers of the cell wall. Cells in the mid- and basal regions are presumably less active in this respect. It is likely that the absence of ferric oxide incrustation on *Ulva* fronds is due to a mobile pectic outer layer. In both *Gigartina stellata* and *Fucus serratus* it is possible that the iron compound is incorporated in the outer "cuticle" region of the thallus, without formation of the crustose deposit. The absence of the iron compound on the terminal frond regions of both plants is probably linked with enhanced mucilage production in what are fertile areas of the frondage.

Since the solubility of iron compounds in the sea is low, it seems likely

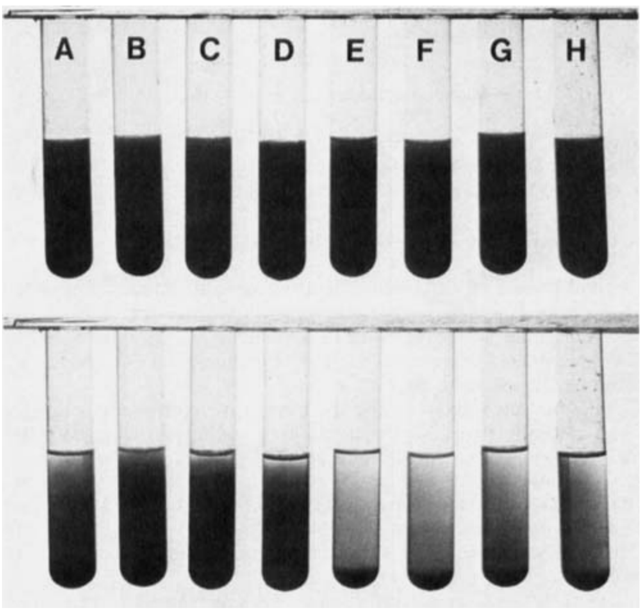

FIGURE 3 Iron ore dust sedimentation in distilled water and sea water. The upper row of test tubes shows ore dust samples immediately after suspension in distilled water (A–D) and in sea water (E–H). Similar weights of ore dust were used in each tube. The lower row of test tubes is the same series 3 h after suspension of the ore dust. A, E—Lac Jeannine; B, F—Tazadit; C, G—Bolivar; D, H—Mang ore.

that iron is present in the sea both at Hunterston and Fairlie as colloidal hydrous ferric oxide, and that the *Cladophora* plants take up the element in this form. Site 1 at Hunterston lies close to a drainage outlet from the ore stackyard. Ore dust particles in sea water forms aggregates and sink rapidly (Figure 3). Whilst we have no data on the quantities of iron present in the sea off Hunterston, the evidence from both the ore-terminal and Fairlie indicates that *Cladophora* is a sensitive and rapid indicator organism of the element in marine habitats. All plants examined appeared healthy with green chloroplasts and starch-filled pyrenoids in cells in all regions, including those with a dense "loading" of ferric oxide. Whether the plants can mobilize this iron source for their metabolism is at present unknown. Whilst corroded iron debris can produce similar incrustations, *Cladophora* could nevertheless serve as a useful factor monitoring organism for the seaborne spread of iron-ore dust from ore unloading terminals.

Acknowledgment

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