

## Chapter 12

GROWTH OF THE FOULING ALGA *CLADOPHORA GLOMERATA* (L.) Kütz. AT VARIOUS CONCENTRATIONS OF COPPER.

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## 12.1 INTRODUCTION

Trace amounts of copper are essential for metabolic processes of algae (Sorrentino, 1978) whereas higher concentrations inhibit growth or kill the algae (Francke and Hillebrand, 1980). Copper sulphate has widely been used as an algicide to control or prevent considerable algal growth (Whittaker et al., 1978). For instance, copper-based paints are used as antifouling coatings on ships. The effectivity of such paints is restricted, after a few months algae will start to settle on the treated surface. This may be due to the loss of algicide properties of the paint and/or settlement of highly tolerant algae. Hall et al. (1979) observed that strains of the marine alga *Ectocarpus siliculosus* (Dillw.) Lyngb. collected from panels coated with antifouling paint were tolerant to high concentrations of copper. In this study preliminary results are presented from a survey for copper tolerant strains of the freshwater alga *Cladophora glomerata* (L.) Kütz..

## 12.2 MATERIALS AND METHODS

The algae were collected from two antifouling coated shipwalls at the yacht harbor at the lake Nieuwe Meer, Amsterdam. A strain isolated from a sheet piling in a ditch at the University campus served as a reference. Unialgal filaments of *Cladophora glomerata* were isolated and transferred into a synthetic medium, Wood's Hole slightly modified (Table 12.1).

The strains were identified according to the classification of Van den Hoek (1963). Aggregations of algae were fragmented and 5-cell filaments were inoculated in Petri dishes, containing 20 ml of the synthetic medium in 0.5% agar. Copper ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) was added in a series containing 0.002, 0.16, 0.33, 0.48, and 0.58  $\text{mg} \cdot \text{l}^{-1}$ , including the copper in the standard medium. The experiments were run in a climate room at  $20 \pm 2$  °C. Light was provided with TL 32 fluorescent tubes in a 16 - 8 hr light-dark regime at  $90 \mu\text{Einsteins} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , measured with the LI-COR LI-185A Quantum meter. The cell numbers of the filaments were counted under the microscope, at 400 x. Specific growth rate was calculated as doublings per day with the formula :

$$\mu = \ln(x_2/x_1)/(t_2-t_1) \quad (1)$$

where  $x_2$  and  $x_1$  represent the number of cells per filament at day  $t_2$  and  $t_1$ , respectively.

Table 12.1. Components of the synthetic growth medium

Component concentration mg.l <sup>-1</sup>	Concentration of the elements/ions mg.l <sup>-1</sup>			
CaCl <sub>2</sub> .2H <sub>2</sub> O	36.8	Ca 10.0	Cl 17.7	
MgSO <sub>4</sub> .7H <sub>2</sub> O	37.0	Mg 3.6	SO <sub>4</sub> 14.4	
NaHCO <sub>3</sub> .7H <sub>2</sub> O	100.0	Na 27.4	HCO <sub>3</sub> 72.6	
NaNO <sub>3</sub>	85.0	Na 23.0	N 14.0	NO <sub>3</sub> 62.0
K <sub>2</sub> HPO <sub>4</sub>	8.7	K 4.2	P 1.75	HPO <sub>4</sub> 4.8
Na <sub>2</sub> EDTA.2H <sub>2</sub> O	4.4	Na 0.53		
FeCl <sub>3</sub> .6H <sub>2</sub> O	1.5	Fe 0.30	Cl 0.6	
MnCl <sub>2</sub> .4H <sub>2</sub> O	0.2	Mn 0.055	Cl 0.07	
ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.02	Zn 0.0046	SO <sub>4</sub> 0.007	
NaMoO <sub>4</sub> .2H <sub>2</sub> O	0.006	Mo 0.0026		
CoCl <sub>2</sub> .6H <sub>2</sub> O	0.01	Co 0.0024		
CuSO <sub>4</sub> .5H <sub>2</sub> O	0.01	Cu 0.0025		
H <sub>3</sub> BO <sub>3</sub>	0.5	B 0.09		
HEPES	500			
Thiamine	0.1			
Biotine	0.0005			
Cyanobalamine (vit. B12)	0.0005			

### 12.3 RESULTS AND DISCUSSION

The growth responses to the various concentrations of copper are presented in Fig. 12.1. The amounts of cells are more or less the same for the three tested strains at low concentrations of 0.002 mg.l<sup>-1</sup> Cu, but after addition of 0.16 mg.l<sup>-1</sup>, the amount of cells was less for strain 3 (reference) than for strains 1 and 2. Filaments of a relatively larger number of cells were observed with strain 2 in comparison with strains 1 and 3 at 0.48 and 0.58 mg.l<sup>-1</sup> Cu.

Specific growth rates ( $\mu$ ) of the filaments at different concentrations of copper, expressed as doublings per day, are presented in Table 12.2.

The growth of the strains is identical at 0.002 mg.l<sup>-1</sup> Cu, but at increasing concentrations the responses deviate. Growth slightly diminished for strain 2, whereas specific growth rates dropped drastically for the filaments of strains 1 and 3 (reference). So strains 1 and 3 are less tolerant to copper than strain 2. Although strains 1 and 2 were both collected from surfaces treated with an antifouling substance, only strain 2 showed a higher tolerance for copper. This indicates that Cu-intolerant as well as Cu-tolerant strains of *Cladophora glomerata* are found on such substrates.

Several examples have been reported of marked differences in metal resistance within a single species. These include planktonic species, e.g. Ni-tolerant *Scenedesmus* species (Stokes et al., 1973) and Cu-tolerant *Scenedesmus* species (Mierle and Stokes, 1976) and *Chlorella vulgaris* Beyerinck (Foster, 1977)

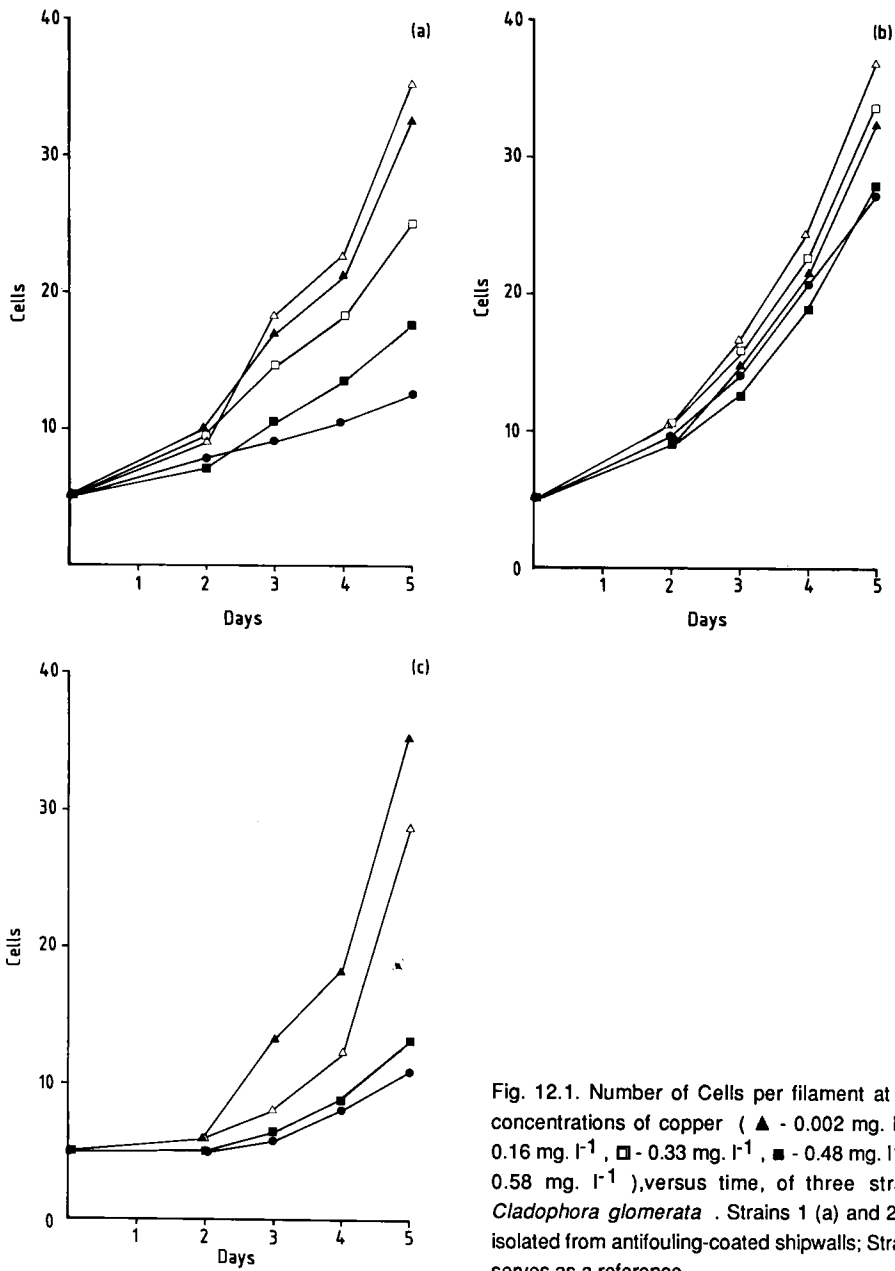


Fig. 12.1. Number of Cells per filament at various concentrations of copper ( ▲ - 0.002 mg. l<sup>-1</sup> , △ - 0.16 mg. l<sup>-1</sup> , ◻ - 0.33 mg. l<sup>-1</sup> , ■ - 0.48 mg. l<sup>-1</sup> , ● - 0.58 mg. l<sup>-1</sup> ), versus time, of three strains of *Cladophora glomerata* . Strains 1 (a) and 2 (b) are isolated from antifouling-coated shipwalls; Strain 3 (c) serves as a reference.

Table 12.2. Growth rate ( $\mu$ ) for filaments of *Cladophora glomerata* at different concentrations of copper;  $\mu$  in doublings per day.

Concentration mg.l <sup>-1</sup> Cu	Strain 1	Strain 2	Strain 3
0.002	0.37	0.37	0.39
0.16	0.39	0.40	0.34
0.33	0.32	0.38	
0.48	0.25	0.34	0.19
0.58	0.18	0.34	0.15

as well as Zn-tolerant strains of filamentous algae, e.g. *Horridium* species (Say et al., 1977) and *Stigeoclonium tenue* Kütz. (Harding and Whitton, 1976).

The presence of Cu-tolerant strains from antifouling coated surfaces as described in this study agrees with the observations of Hall et al. (1979). They isolated a Cu-tolerant strain of the marine brown alga *Ectocarpus siliculosus*. Although *Cladophora glomerata* is regarded as sensitive to heavy metals (Whitton, 1970) it was, besides the dominant alga *Oedogonium*, present on the antifouling-coated shipwall. As the coating will steadily lose its algicide properties it can be expected that more tolerant strains settle in an early stage; with diminishing toxicity more tolerant strains may survive. This phenomenon can be responsible for the presence of Cu-tolerant and Cu-intolerant strains on the coatings.

#### 12.4 ACKNOWLEDGEMENTS

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