COPPER-ALGAE INTERACTIONS: INHERITANCE OR ADAPTATION?

JUAN A. CORREA,* PALOMA GONZÁLEZ and PABLO SÁNCHEZ
Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago, Chile

and

JORGE MUÑOZ and MARÍA C. ORELLANA
Departamento de Biología, Facultad de Ciencias del Mar, Universidad Católica de la Santísima Concepción

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Abstract. This study evaluated the responses of wild, adult plants of *Enteromorpha compressa*, and their progeny, to various copper concentrations. Experiments were designed to test the hypotheses that: 1) individuals of *E. compressa* from Caleta Palito, a copper-enriched coastal locality, tolerate higher copper concentrations than those from a place with no history of copper pollution and 2) such copper tolerance is under genetic control and therefore, was an inherited character. Our results indicate that algae which inhabit a copper-enriched environment tolerate higher concentrations of copper than those from waters with low copper concentrations. On the other hand, our results suggest that generalizations regarding heritability of the tolerance to copper do not apply to the Chilean *E. compressa*, as no differences in growth or rhizoid production were found between the progeny from Caleta Palito and Caleta Zenteno. These findings are an indication that heritability and adaptation may represent alternative strategies used by different populations of the same algal species to tolerate copper.

1. Introduction

Copper is an essential micro-nutrient for marine macro- and micro algae. It is involved in the electron transport during photosynthesis and acts as cofactor in a number of crucial enzymatic reactions (McLachlan, 1982; Lobban *et al.*, 1985; Sandman, 1985). The concentration of total copper in sea water varies, with reported values ranging from 0.002 to 0.6 μg L^{-1}(Lobban *et al.*, 1985; Maeda and Sakaguchi, 1990; but see Phillips, 1977). These values can increase several times in coastal waters affected by wastes of industrialized human settlements (Seeliger and Edwards, 1977; Chan *et al.*, 1974; Stenner and Nickless, 1975; Gupta and Arora, 1978; Shioi *et al.*, 1978; Wallner *et al.*, 1985; Chung and Brinkhuis, 1986; Ho, 1987). Concentration of this metal in tissues of some algae may reach, on the other hand, 15 μg g^{-1} (0.6–80 μg g^{-1}), which indicates that these organisms have the capacity to concentrate copper (Seeliger and Edwards, 1977; Rai *et al.*, 1981; Talbot and Chegwidden, 1982; Seeliger and Cordazo, 1982; Reed and Moffat, 1983; Lobban *et al.*, 1985; Ho, 1987).

At high concentrations, copper (as Cu^{+2}) is toxic for most photosynthetic marine and brackish-water organisms (Rai *et al.*, 1981; Lobban *et al.*, 1985; Maeda and

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* To whom correspondence should be addressed.

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Sakaguchi, 1990). However, available information indicates that seaweeds vary in their capability to tolerate the presence of copper at concentrations higher than normal (Seeliger and Edwards, 1977; Shubert, 1984; Evans and Hoagland, 1986; Maeda and Sakaguchi, 1990). This variation is determined by the species under consideration and also by intra-specific variability, which allows different individuals of the same species to diminish or overcome the toxic effects of copper with different degree of success (Russell and Morris, 1970; Goodman *et al.*, 1976; Hall *et al.*, 1979; Seeliger and Braga, 1982; Reed and Moffat, 1983; Wallner *et al.*, 1986; Cullinane *et al.*, 1987).

In this context, green algae belonging to *Ulva*, *Enteromorpha* and *Chaetomorpha* and to the phaeophycean *Ectocarpus siliculosus* (Dillw.) Lyngb. are the main organisms, on a world-wide basis, which consistently appear in copper-enriched marine environments (Phillips, 1977; Talbot and Chegwidden, 1982; Seeliger and Cordazzo, 1982; Ho, 1987, 1990; Maeda and Sakaguchi, 1990; Vermeer and Castilla, 1991; Castilla, 1995). The same taxa are the dominant fouling flora developing on the hull of ships (Evans and Hoagland, 1986), in spite of the widespread use of copper-based anti-fouling paints. For these reasons and also because they are cosmopolitan, the above species are commonly used as bio-indicators of waters contaminated with copper.

It has been hypothesized that copper-enriched environments could exert selective pressure on the algae and lead to the development of copper-tolerant ecotypes (Russell and Morris, 1970; Reed and Moffat, 1983). Furthermore, experimental studies strongly suggest that copper-tolerance in both *Enteromorpha* and *Ectocarpus* is transmitted to the progeny (Russell and Morris, 1970; Reed and Moffat, 1983). The suggestion of genetic involvement is based on experiments where individuals of these species, from 'copper-polluted' areas, are taken to the laboratory and their progeny displays levels of copper tolerance similar to that of the parental generation (Reed and Moffat, 1983).

There are areas in the world, like northern Chile, that have rich copper deposits, a number of which are currently exploited as copper mines. Although in some cases all the mining operation occurs far away from coastal waters, in some others there is a long history of dumping mining wastes into the sea (Vermeer and Castilla, 1991; Castilla, 1995). In northern Chañaral Bay, the original dumping site of wastes from El Salvador mine, dramatic changes occurred in the topography of the coast. Large sandy beaches developed as the result of accumulation of particulate matter from the mine, brought to the sea through the Río Salado bed until 1975 (Castilla, 1995). Caleta Palito, a locality near Chañaral and since 1975 the disposal site for El Salvador mine, receives now (since 1990) the so called 'clear water' or water without particulate matter. The intertidal algal community of Caleta Palito has become dominated mainly by a single species, *Enteromorpha compressa* (L.) Grev., which consistently forms a carpet on the rocks and in the intertidal pools. This dominance suggests that, regardless the partial treatment of the tailing, the disposal site remains a copper-enriched environment.

In spite of the magnitude of the changes in the algal community at Caleta Palito, very little has been done in order to understand the causes of those changes, particularly regarding the potential role of copper as a key factor determining the dominance by *E. compressa*. Due to the duration of the exposure to the mining wastes (treated and untreated), algae at Caleta Palito constitute a unique possibility to understand long term copper-algae interactions in marine systems. In this context, the main objectives of this study were designed to experimentally test the hypotheses that: 1) individuals of *E. compressa* from Caleta Palito tolerate higher copper concentrations than those from a place with no history of copper pollution and 2) such copper tolerance was under genetic control and therefore, was an inherited character.

2. Materials and Methods

2.1. STUDY SITES AND COPPER LEVELS

Caleta Palito, a rocky beach 8 Km north from Chañaral (26° 15′ S, 69° 34′ W), was used as the copper-enriched environment. To assess the level of copper in the system, duplicate water samples were taken at the dumping canal, 20 m before it reaches the sea, and 50 m southward, at the same place where adult individuals of *E. compressa* were collected for the experiments. Water samples were also taken at Caleta Zenteno, a rocky beach 90 km south from Caleta Palito. This site was selected as control because there is no history of copper-pollution affecting the area. The intertidal community at Caleta Zenteno is characterized by a high variety of benthic algae colonizing most of the primary intertidal space (Castilla, 1995), with *E. compressa* restricted mainly to the pools. It is important to consider that the main water current in this part of the Chilean coast moves from south to north.

Water samples were filtered through 0.45 μm Sartorious membrane filters and fixed with nitric acid (Merck, supra pur). Fixation was done by adding 0.5 mL of concentrated nitric acid to each of the filtered, 500 mL water samples. Dissolved copper was quantified by potentiometric striping analysis in stationary solution, using a computerized Radiometer ISS 820 analyzer. All glassware and plastic material used to collect and store water samples was acid-clean.

To assess the presence of copper in the tissues of *E. compressa*, algae from Caleta Palito and Caleta Zenteno were collected at the same spots used to collect experimental individuals. Algae were treated with nitric acid and copper was quantified in a GBC 909 PBT atomic absorption spectrophotometer. An atom trap mode was used to improve sensitivity when concentrations of the metal were low.

Copper certified standards were run simultaneously to the water and tissue samples. Standards were CASS-2 for water and DOLT-1 for algal tissue. All the standards were provided by the National Research Council of Canada, Division of Chemistry, Marine Analitical Chemistry Standards Program.

2.2. ALGAL MATERIAL

Adult individuals of *E. compressa* were collected during low tide at Caleta Palito, the copper-enriched environment, 50 m southward from the site where the water from the dumping canal mixes with the sea. Individuals of the same species were simultaneously collected at Caleta Zenteno.

Algae were kept in plastic bags and transported in a cooler with abundant ice packs. Algae reached the laboratory within 12 h of collection. These plants were maintained in 1 L beakers with 0.45 μm filtered sea water, at 10 °C and 40–50 μmol photons m^{-2} s^{-1}. Water was changed twice a week, and algae were used within 10 days of arrival to the laboratory.

Adult plants were used to obtain a new generation of *E. compressa*. The progeny was obtained from spores released by the adult plants, following an inductive treatment consisting of incubating fragments of healthy thalli with ASP-12 culture medium (Provasoli, 1963) without copper, in 15×60 mm glass Petri dishes. This medium was sterile-filtered with 0.2 μm Sartorius membrane filters. Fragments were incubated at 15 °C, 55 μmol photons m^{-2} s^{-1} and 12:12 photoperiod. Culture medium was changed every two days. Once spores were released, tissue fragments were discarded and germlings were maintained in the Petri dishes until a size of 2 mm was reached. Subsequently, germlings were transferred to 250 mL beakers and cultured at the same conditions outlined above.

2.3. ELONGATION

To assess the effect of copper on elongation of adult, wild *E. compressa*, 3 mm long fragments were excised from the apical region of healthy thalli collected at Caleta Palito and Caleta Zenteno. These fragments were cultured during 2 weeks in ASP-12 medium with various copper concentrations. Cupric chloride ($Cu\ Cl_2 \cdot 2\ H_2O$ Merck p.a.) was used throughout the study and concentrations in the medium were 0 (control), 1, 10 and 100 μmol L^{-1}. Each treatment was replicated, using 8 fragments of tissue per treatment. Growth conditions were 13 °C, 12:12 photoperiod and, to avoid spontaneous sporulation of the thallus, light was reduced to 6.5 μmol photons $m^{-2}s^{-1}$. Culture medium was changed every two days.

An identical experimental design was applied to assess the effect of copper on the progeny of *E. compressa* from both Caleta Palito and Caleta Zenteno.

At the end of the experimental period, fragments were measured and daily growth rates (DGR) were calculated as

$$\mathrm{DGR} = \frac{[(L_t - L_0)/L_0]\ 100}{t}$$

where L_0 is the initial length and L_t the length of the fragments at t days.

TABLE I

Mean values of copper in water, in *Enteromorpha compressa*, and concentration factors. Values in parenthesis represent standard deviation of duplicates

	Water (μg L^{-1})	E. compressa (μg g^{-1})[a]	Concentration factor[b]
Caleta Palito	2416.2	–	–
(Canal)	(34.6)		
Caleta Palito	29.3	63.3	21.6×10^2
(50 m from discharge)	(3.6)	(11.9)	
Caleta Zenteno	3.1	2.0	6.5×10^2
	(0.4)	(0.2)	
Standards	0.692[c]	20.2[d]	

[a] Values are based on dry weight of algal tissue.
[b] Calculated as CF: μg Cu g^{-1} dry biomass divided by μg Cu mL^{-1} water.
[c] Certified concentration: 0.675.
[d] Certified concentration: 20.8.

2.4. RHIZOIDS

The effect of copper on the production of rhizoids by *E. compressa* was assessed using an experimental design similar to that for testing elongation. As above, adults and the progeny of *E. compressa* from Caleta Palito and Caleta Zenteno were tested. At the end of the experiment, rhizoid production was estimated as the number of fragments with rhizoids over the total number of fragments.

2.5. CELL VIABILITY

The same experimental design outlined for the experiments described above was followed to test cell viability of adults and progeny from Caleta Palito and Caleta Zenteno. Cell viability was assessed by using 0.25% Evan's blue in ASP-12, for 5 min. This vital stain penetrates and accumulates in the cytoplasm of non viable cells, which appear homogeneously blue (positive reaction) and are clearly different from viable cells, which remain green and with the parietal plastid and central vacuole easily recognizable (Reed and Moffat, 1983).

A total of 5 areas, 0.2 mm^2, were randomly selected in each of the experimental fragments and the cells with positive reaction were recorded. Using the same staining procedure and fragments from the same population used for the experiments, cell viability of 98–100% was recorded at the beginning of the experiment. This ensured the healthy status of the cells before the fragments were cultured under the various copper concentrations.

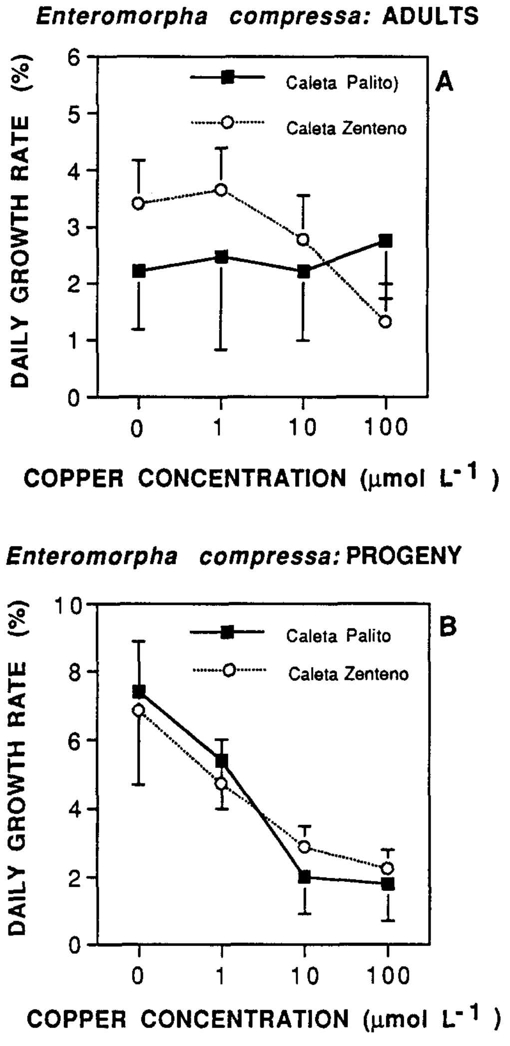

Fig. 1. Effect of copper on daily growth rate of *Enteromorpha compressa* from Caleta Palito and Caleta Zenteno. Standard deviation shown for each mean value. A. Wild, adult individuals. B. Progeny.

Data analysis (ANOVA) were done using the SAS software (version 5) and original values were arcsin-transformed when necessary. Using the same software, an *a posteriori* LSM test was used to assess the significance of the differences between treatments.

3. Results

In Caleta Palito, values of dissolved copper are much higher in water from the canal than in the sea water collected at 50 m from the discharge (Table I). In Caleta

TABLE II

Significance of the differences between daily growth rate means in *Enteromorpha compressa* from Caleta Palito and Caleta Zenteno. Values represent the probability that $LSM_{(i)} = LSM_{(j)}$

		ADULTS								PROGENY							
		Caleta Palito				*Caleta Zenteno*				*Caleta Palito*				*Caleta Zenteno*			
		0	1	10	100	0	1	10	100	0	1	10	100	0	1	10	100
Caleta Palito	0	–	ns	ns	ns	*	**	ns	*	–	**	***	***	ns	***	***	***
	1		–	ns	ns	***	**	ns	ns		–	***	***	*	ns	***	***
	10			–	ns	**	**	ns	ns			–	ns	***	***	**	ns
	100				–	ns	ns	ns	**				–	***	***	**	ns
Caleta Zenteno	0	–	–	–	–	–	ns	ns	***	–	–	–	–	–	***	***	***
	1		–	–	–		–	ns	***		–	–	–		–	***	***
	10			–	–			–	**			–	–			–	ns
	100				–				–				–				–

$* p < 0.05$.
$** p < 0.01$.
$*** p < 0.001$.

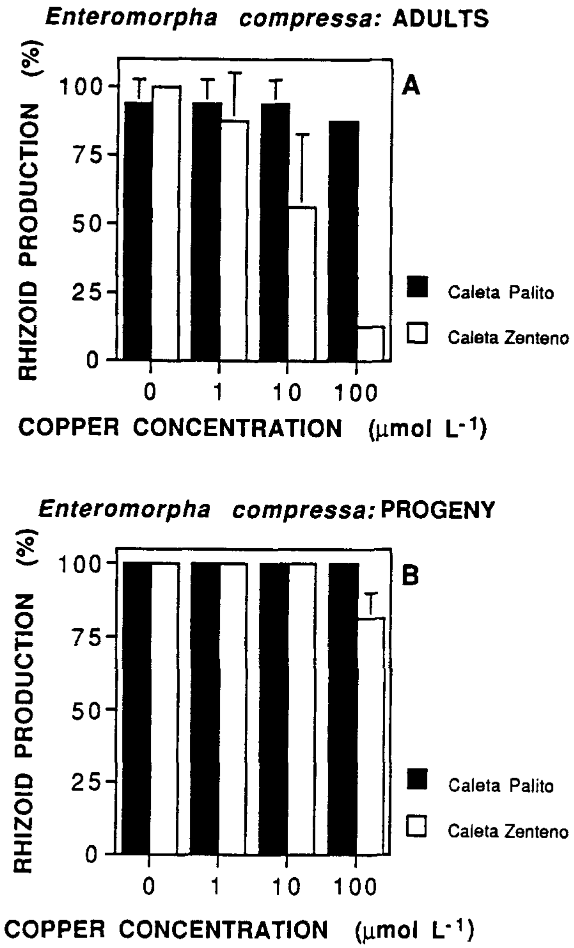

Fig. 2. Effect of copper on rhizoid production in *Enteromorpha compressa* from Caleta Palito and Caleta Zenteno. Standard deviation shown for each mean value. A. Wild, adult individuals. B. Progeny.

Zenteno, on the other hand, copper values in sea water were lower than those from Caleta Palito (Table I).

Tissues of *E. compressa* from Caleta Palito showed a copper content significantly higher than the levels detected in plants from Caleta Zenteno (Table I). Furthermore, concentration factors in algae from Caleta Palito were three times higher than those of plants from Caleta Zenteno (Table I).

A negative effect of copper on DGR was found in adult *E. compressa* from Caleta Zenteno (Figure 1a). Mean DGR ranged from 3.5% at 0 μmol L^{-1} copper to 2.7% at 10 μmol L^{-1} copper, and differences between these treatments were not significant (Table II). However, mean DGR decreased significantly (Table II) when tips were grown at 100 μmol L^{-1} (Figure 1a). Increased copper concentration, on

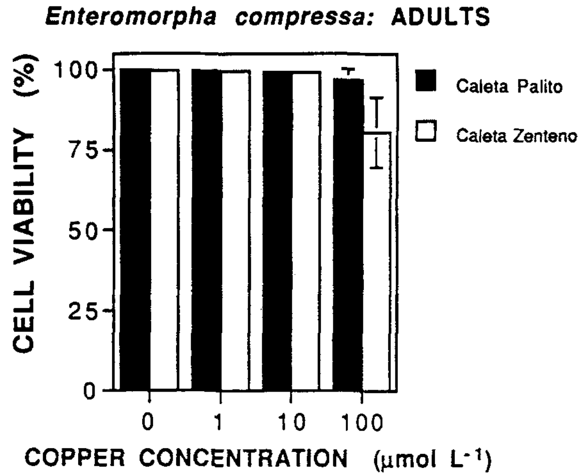

Fig. 3. Effect of copper on cell viability of *Enteromorpha compressa* from Caleta Palito and Caleta Zenteno. Standard deviation shown for each mean value.

the other hand, did not affect DGR of adult plants from Caleta Palito (Figure 1a). Differences in DGR among treatments were not significant (Table II).

When localities are compared, adult plants from Caleta Zenteno grew significantly faster than those from Caleta Palito at 0 and 1 μmol L^{-1} copper, but significantly slower at 100 μmol L^{-1} copper (Fig 1A, Table II).

Progeny of *E. compressa* from both Caleta Zenteno and Caleta Palito was negatively affected by increased copper concentration (Figure 1b). The highest DGR was obtained in culture medium without copper (Figure 1b). DGRs decreased significantly at 1 μmol L^{-1} copper, reaching a minimum at 10 μmol L^{-1} copper (Figure 2b, Table II); DGRs obtained at 100 μmol L^{-1} copper did not differ significantly from those at 10 μmol L^{-1} (Figure 1b, Table II). Differences in DGRs between the progenies from Caleta Palito and Caleta Zenteno were not significant, with the exception of 10 μ mol L^{-1} copper (Table II).

The production of rhizoids in adult plants from Caleta Palito occurred, as average, in over 85% of the fragments under any of the experimental copper concentrations (Figure 2a), and the differences were not significant (LSM test, $p > 0.05$). Adult plants from Caleta Zenteno, on the other hand, were affected negatively as copper concentration increased, falling from 100% of the fragments with visible rhizoids at 0 μmol L^{-1} copper to less than 15% at 100 μmol L^{-1} copper (Figure 2a). Although the differences between 0–1 μmol L^{-1} copper were not significant (LSM test, $p > 0.05$), the drop of the mean values for rhizoid production at 10 and 100 μmol L^{-1} copper was significant (LSM test, $p < 0.001$). The progeny of *E. compressa* from Caleta Palito did not show significant differences with that from Caleta Zenteno when grown at 0–10 μmol L^{-1} copper; in all cases 100% of the fragments produced rhizoids (Figure 2b). At 100 μmol L^{-1} copper, however, a significantly lower (LSM test, $p < 0.001$) number of fragments from Caleta Zenteno produced rhizoids (Figure 2b).

Cell viability in adult plants from Caleta Palito did not differ significantly (LSM test, $p > 0.05$) from that detected in plants collected at Caleta Zenteno, when cultured at 0–10 μmol L^{-1} copper (Figure 3). However, cell viability in plants from Caleta Zenteno showed a significant decline at 100 μmol L^{-1} copper (LSM test, $p < 0.001$); such effect was not observed in plants from Caleta Palito. Progeny of *E. compressa* from both Caleta Palito and Caleta Zenteno showed 100% viability (not shown).

4. Discussion

Our study demonstrates that wild adult individuals of *E. compressa* from a copper-enriched environment tolerate copper concentrations higher than algae from a place with no history of copper pollution. This response was consistent in all three aspects assessed under laboratory conditions, where plants from Caleta Palito performed in the same fashion throughout the experimental range (0–100 μmol L^{-1} copper). By comparison, *E. compressa* from the non-polluted site at Caleta Zenteno grew slower, displayed a reduced capacity to produce rhizoids and showed a decrease in viable cells. Similar patterns of responses to copper-enrichment have been reported for *E. compressa* from St. Andrews Bay, Scotland (Reed and Moffat, 1983) and for *Ectocarpus siliculosus* from England (Russell and Morris, 1970). In the case of *E. compressa*, ship-fouling wild individuals tolerated concentrations up to 9.6 μmol L^{-1} copper, whereas non-fouling individuals were negatively affected, and showed symptoms of toxicity, at 1.8 μmol L^{-1} copper and higher (Reed and Moffat, 1983). In *E. siliculosus* (non-tolerant strain) concentrations of 0.01 mg L^{-1} copper reduced growth in half (Russell and Morris, 1970). Growth ceased at 0.05 mg L^{-1}, approximately a third of our 1 μmol L^{-1} copper concentration, (Russell and Morris, 1970). However, growth of the tolerant strains from the copper-treated hull of vessels ceased only at 0.5 mg L^{-1} copper, almost a third of our 10 μmol L^{-1} copper concentration, (Russel and Morris, 1970). Also working with *E. siliculosus*, Hall *et al.* (1979) found that their tolerant strain grew at less than a fourth of the growth rate recorded at 0 mg L^{-1} copper if it was cultured at 0.25 mg L^{-1} copper, and at less than 1/13 if the culture medium contained 0.5 mg L^{-1} copper. The non-tolerant strain, on the other hand, did not grow at all at 0.25 and at 0.5 mg L^{-1} copper in the medium (Hall *et al.*, 1979). It becomes apparent from the above analysis, however, that in spite of trend similarities, there are major differences between the levels of copper-enrichment at which tolerance or non-tolerance operate with the algae used in previous studies, and those affecting the Chilean *E. compressa*. In this context, the non-tolerant Chilean *E. compressa*, obtained from a place with no history of copper-pollution, responded without significant differences at concentrations ranging from 0 to 10 μmol L^{-1} copper (the whole range used by Reed and Moffat, 1983) in terms of DGR and cell viability (this study). Similar arguments apply for comparisons between *E. compressa* and *E. siliculosus* (Russell and Morris, 1970; Hall *et al.*, 1979). This evidence stresses the notion that 'tolerant' and 'non-tolerant' should not be used as absolute terms. Populations of *E. compressa*, like the one at Caleta Zenteno for example (non-tolerant in this study), might well be considered copper-tolerant under the standards provided by Redd and Moffat (1983). Furthermore, comparisons of the present study with that of Reed and Moffat strongly suggest the inconvenience of making broad generalizations regarding the levels of copper tolerance in a given species based upon responses of algal populations from a restricted geographic range.

Copper concentration at which adults of *E. compressa* from Caleta Zenteno are affected depends of the response being measured (i.e. growth, rhizoids, cell viability, etc). It is clear from our results that DGR is not affected until the copper in the medium is 100 μmol L^{-1}. A similar pattern was observed in the cell viability trials, where a detectable negative impact was significant only at 100 μmol L^{-1} copper. The production of rhizoids, however, appeared to be more sensitive to increases in copper concentrations, as a deleterious effect appeared at 10 μmol L^{-1} and it was followed by a second significant impact at 100 μmol L^{-1}. These responses differ from the patterns found in other populations of *E. compressa*, which displayed a gradual, step-by-step decrease in growth, rhizoid production and cell viability (Reed and Moffat, 1983).

Suggestions that the progeny of copper-tolerant individuals could also be copper-tolerant are not fully supported by this study. This becomes apparent when the progenies from Caleta Zenteno and Caleta Palito are compared in terms of their DGRs and no differences appear at the various copper concentrations. Furthermore, the progeny of *E. compressa* from the two sites showed a quite different overall pattern of response to copper, in comparison to that of the parental generation. Thus, whereas adults modified their DGRs only at 100 μmol L^{-1} copper (plants from Caleta Zenteno only), their progeny appeared more sensitive to the metal and showed significant negative effects even at 1 μmol L^{-1}. Finally, whereas an increase from 10 to 100 μmol L^{-1} copper resulted in lower DGRs of adults, the same change in copper concentration did not trigger an equivalent response in the progeny. Neither heritability of copper tolerance is supported by our results on cell viability, where the differences observed between adults from Caleta Zenteno and Caleta Palito at 100 μmol L^{-1} copper were not detected in their progeny. Some support to the heritability hypothesis comes from the experiments on rhizoid production, where the responses by adults at 100 μmol L^{-1} copper were also found in their progeny. However, a more detailed analysis indicates that the magnitude of the effect, and the concentration at which copper exerts a noticeable impact, is different in adults and their progeny. Thus, our results differ from those provided by the previous work on *E. compressa* from Scotland (Reed and Moffat, 1983), where the progeny of copper-tolerant adults was also tolerant to the metal. At present, we do not have a satisfactory explanation to the lack of concordance between the European and Chilean *E. compressa* regarding heritability of copper tolerance. However, physiological adaptation to copper, rather than heritability, was recently reported in cyanobacteria (Takamura *et al.*, 1990). Accordingly, we should be aware that alternative strategies to tolerate copper (i.e. heritability or physiological adaptation) might have evolved in different populations of the same species.

Adults of *E. compressa* from both Caleta Palito and Caleta Zenteno are able to concentrate copper. Our data also indicate that *E. compressa* accumulated more copper in environments with higher copper-enrichment, like Caleta Palito. Similar trends were reported for *Enteromorpha* spp (Seeliger and Edwards, 1977), *Fucus* spp (Preston *et al.*, 1972; Bryan and Hummerstone, 1973; Foster, 1976) and *Ascophyllum* (Foster, 1976). However, the fact that the Chilean *E. compressa* concentrate copper provides no insight regarding the spatial location of the metal (i.e. intra- or extracellular) nor clarify the mechanisms involved in tolerance to the metal. It is known that some algal species can incorporate copper into the cells, where it forms complexes and looses toxicity (Silverberg *et al.*, 1976; Daniel and Chamberlain, 1981). Other algae sequester copper and detoxify it by synthesis of phytochelating peptides (Gekeler *et al.*, 1988). Polysaccharides present in the cell wall of algae also have the properties to bind, and render non-toxic, ionic heavy metals (Veroy *et al.*, 1980). Our preliminary attempts to detect copper bound to the cell walls of *E. compressa* using X-Ray microanalysis have not succeeded.

5. Conclusions

It is concluded that high copper concentrations decreased the DGR of adult *E. compressa* from Caleta Zenteno but not from Caleta Palito. However, the progeny of the same species was equally (i.e. negatively) affected, regardless the locality where their parents were collected. Thus, copper tolerance does not seem to be a heritable character in the chlorophycean alga *E. compressa* from Chile. Instead, the better performance in the assays displayed by adults from Caleta Palito suggests that physiological adaptation rather than heritability might be involved in the survival and dominance of *E. compressa* in that locality.

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