Atti Soc. Tosc. Sci. Nat., Mem., Serie B, **101** (1994)
pagg. 35-45 , figg. 5

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A PRELIMINARY STUDY OF THE EFFECTS OF Hg, Pb AND Cu ON THE EARLY LIFE-HISTORY STAGES OF *CALLITHAMNION GRANULATUM* (DUCLUZEAU) C. AGARDH (CERAMIALES, RHODOPHYTA)

Abstract — The toxicity of some heavy metals (Hg, Pb and Cu) on the early life-history stages of *Callithamnion granulatum* was investigated by means of laboratory essays. The biological parameters examined were mortality, germination of the tetraspores, and growth rate of the germlings developing from them. The results show that the parameters chosen are affected by the presence of pollutants in the culture medium, with the exception of spores germination when exposed to copper. Generally, mortality levels increased while germination and growth rates decreased as a function of metals concentrations. The importance of field studies together with physiological and biochemical research to correctly interpret the laboratory results is also discussed.

Riassunto — *Studio preliminare sugli effetti di Hg, Pb e Cu sulle prime fasi di sviluppo di* Callithamnion granulatum *C. Agardh (Ceramiales, Rhodophyta).* Saggi di laboratorio sono stati utilizzati per valutare la tossicità di Hg, Pb e Cu sulle prime fasi di sviluppo di *Callithamnion granulatum.* L'effetto dei metalli è stato valutato sui seguenti parametri: mortalità e germinazione delle tetraspore e tasso di accrescimento dei germogli. In generale la mortalità è incrementata con la concentrazione dei metalli mentre i tassi di germinazione e di accrescimento hanno manifestato un andamento opposto. L'unica eccezione è rappresentata dalla germinazione delle tetraspore esposte al rame, per cui non sono stati rilevati effetti significativi. L'importanza di affiancare gli esperimenti di laboratorio con indagini di campo e con studi a carattere fisiologico e biochimico viene enfatizzata nella discussione.

Key words — Heavy metals, Macroalgae.

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Introduction

Contaminants in the marine environment can have toxic effects on many different organisms and may affect biological processes at a cellular, population, community and ecosystem level (Boyle, 1984). In recent years much attention was devoted to finding organisms suitable as biological indicators in monitoring the marine environment (Levine, 1984; Ronnberg *et al.*, 1990). Various toxicological tests were developed using plant and animal species with very different biological and ecological characteristics (Gould *et al.*, 1988; MacDonald *et al.*, 1988; Vranken *et al.*, 1988; Wong, 1989).

Recent studies have shown that benthic macroalgae accumulate heavy metals (Haritonidis *et al.*, 1983; Kangas and Autio, 1986; Khristoforova, 1985; Munda, 1984) and that these pollutants may inhibit the growth of adult thalli (Munda and Hudnik, 1986; Munda and Hudnik, 1988). Anderson *et al.* (1990) stressed the importance of working with early life stages of a given species because they usually are more sensitive than adult organisms and may better indicate the toxicity of the pollutants. Little information, however, is available in the literature about the effects of heavy metals on the early life stages of macroalgae (but see Anderson and Hunt, 1988; Boney, 1980; Levine, 1984).

In this work, the tetraspores of *Callithamnion granulatum* and the germlings originated from them were used to test the toxicity of three heavy metals: Pb, Cu and Hg. *C. granulatum*, a benthic interdital alga, was chosen because it was easely recognized in the field, amenable to laboratory culture and fertile for most of the year, thus ensuring a conspicuous supply of spores for the essays.

Results indicated that the biological parameters considered were affected by the presence of the heavy metals in the culture medium.

Material and Methods

Callithamnion granulatum (Ducluzeau) C. Agardh (Ceramiales, Rhodophyta) was collected along the coast south of Livorno (Central Italy) between November 1988 and June 1990. Fertile tetrasporophytes were selected in the laboratory and fronds placed on slides in Petri dishes. These were then randomly assigned to treatments and controls. The culture medium for both treatment and control dishes was prepared with sterile artificial seawater (Instant Ocean), enriched with 0.15 g 1^{-1} $NaN0_3$ and 0.014 g 1^{-1} Na_2HP0_4. The salinity level was maintained at 38‰.

Petri dishes with different metal concentrations were prepared by serial diluition of nitrate salts in the culture medium. A stock solution of 1 mg 1^{-1} normally used for atomic absorption spectroscopy (A.A.S) was employed. The metals tested were: Cu, Pb and Hg. The concentration used were of 20, 50, 250, 500 and 1000 ug 1^{-1} for the mortality/germination tests, and of 10 and 50 ug 1^{-1} for the growth essays. Four Petri dishes were replicated for each metal treatment and for the relative control (no metals were added to the culture medium in control dishes). The mortality/germination, tests were run for two days. This exposure period was chosen because in culture conditions approximatively all the tetraspores of *C. granulatum* germinated within 48 hours from their release (CINELLI *et al.*, umpubl. data). Thus, because our aim was to test the effects of the metals on the spores and not on the germlings of *C. granulatum*, we choose to stop the essays after two days of exposure.

A pilot study indicated that no germlings survived more than five or six days when exposed to concentrations higher than 50 ug 1^{-1} of each metal. As a conseguence, a narrower range of concentrations of those used in the mortality/germination tests was employed in the growth essays which ran for ten days. Once treatments were prepared, the Petri dishes were placed in a growth chamber at 15°C with 12 LD photo period at 35 uE m 2 s 1 and left undisturbed overnight to ensure spore release and attachment. The following day the fronds were removed from the dishes and then the slides were ready for data collection (BONEY and CORNER, 1959; BONEY and COERNER, 1962; BONEY, 1963; STEELE, 1977).

The number of spores present on each slide, for both treatment and control dishes, were counted after one day (initial count) and two days (final count) from Petri dish preparation. Mortality was calculated according to the following formula:

$$G = \frac{Ni - Nf}{Ni} \cdot 100$$

where Ni and Nf were respectively the numbers of spores present at the initial and final count (after 2 days contact with pollutants). The difference between Ni and Nf was the number of spores disappearing (because of total depigmentation) during the time elapsing between counts. This value was taken as indicative of the number of spores dead in this time interval.

Germination percentage was calculated as follows:

$$G = \frac{Ng}{Ni} \cdot 100$$

where Ng was the number of germinated spores after two days of exposure to metals and Ni was the number of spores initially present.

Plant growth was measured every two days up to the 10th day of exposure. At each sampling date the length of ten germlings selected at random was measured for each dish; rhizoids were not included in the measurement. The solution in the dishes was renewed every two days during the experimental period to maintain conditions as standardised as possible (ANDERSON and HUNT, 1988; DENTON and BURDON-JONES, 1981; MUNDA, 1984; MUNDA, 1986; MUNDA and HUDNIK, 1986).

Single-factor analysis of variance was used to test for differences between slides exposed to different concentrations of heavy metals (including the control treatment), for both the mortality and the germination experiments. All percentages were arcsine transformed before the statistical comparisons (SOKAL and ROHLF, 1981). The effects of the metals on *C. granulatum* growth were tested using ANOVA for randomized bloch designs. Separate tests were performed for each sampling date. The Bonferroni correction was also applied to maintain the total probability of type one error to the 0.05 level (MORRISON, 1976).

RESULTS

Mortality and germination rates for *C. granulatum* tetraspores after treatment with heavy metals were reported in Figs. 1 and 2.

Spores treated with mercury (Figs. 1, 2) showed marked reactions for both the biological parameters considered, with a sharp increase in mortality and a strong inhibition in germination, the latter being 100% at concentrations higher than 500 ug l^{-1} (Fig. 2). The toxicity of mercury was confirmed by the high significant levels obtained for both treatments ($P < 0.001$, ANOVA).

Copper did not affect tetraspore mortality so drastically (Fig. 1). Constant levels of mortality (never exceed 30%) were observed even at high concentrations (1000 ug l^{-1}). The all-round effect was however statistically significant ($P < 0.05$, ANOVA). Such effect probably was due to the differences between the control and the treatments. The restrained toxic effect of copper on *C. granulatum* was further confir-

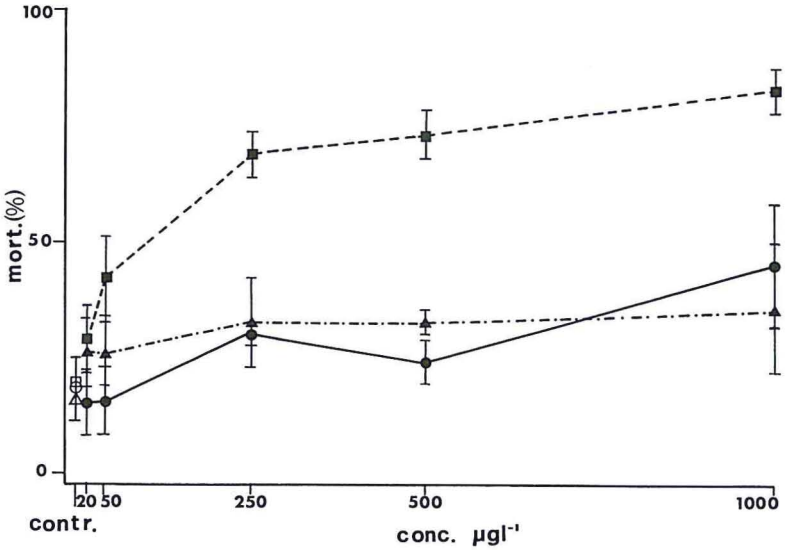

Fig. 1 - Effects of Pb (•), Cu (▲) and Hg (■) on mortality rate of *C. granulatum*. Open symbols refer to the control treatments: o = control for the Pb treatment; Δ = control for the Cu treatment; □ control for the Hg treatment. Value are means (± 1 SD) on four replicates.

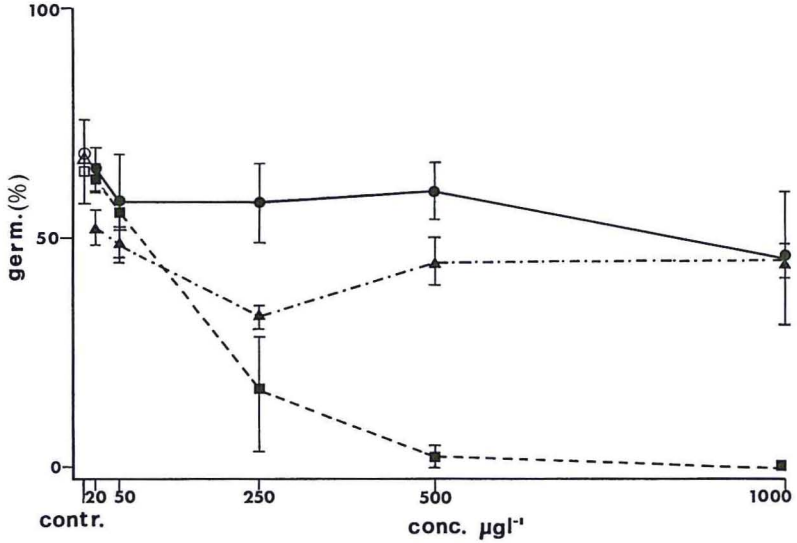

Fig. 2 - Effect of Pb, Cu and Hg on germination rate of *C. granulatum*. Symbols as in figure 1. Values are means (± 1 SD) on fours replicates.

med in the germination tests (Fig. 2). In this instance the treatment effect was not significant ($P > 0.05$, ANOVA).

Analougously, lead exerted only a slight effect on mortality and germination of tetraspores (Figs. 1, 2), although significant differences among concentrations (including the control treatment) appeared significant ($P < 0.01$) in both tests.

Growth of juvenile sporophytes exposed to mercury was reported in Fig. 3. Differences among treatments (including the control dishes) were evident after two days from the start of the experiment ($P < 0.001$, ANOVA).

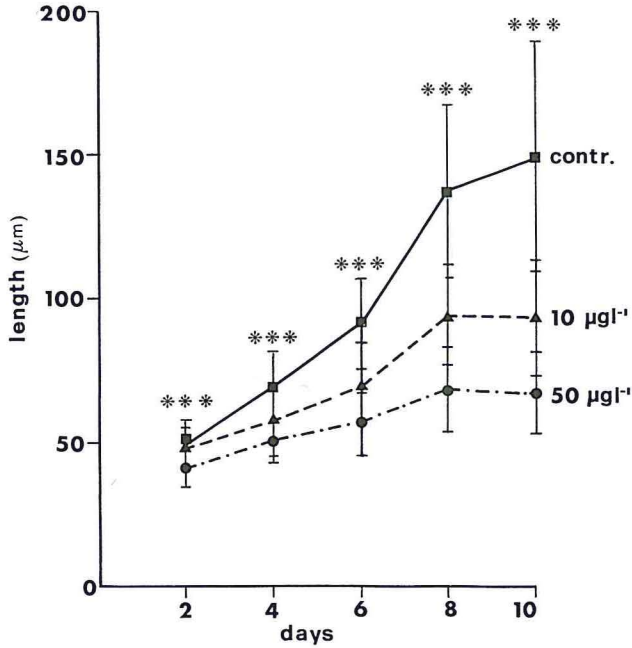

Fig. 3 - Growth response of C. granulatum germlings exposed to Hg. Values are means (± 1 SD) on four replicates: (*** = $P < 0.001$, ANOVA).

Fig. 4 shows the effect of copper on the growth rate of *C. granulatum* tetraspores. A graphical comparison of the growth curves indicated that the main differences occured among the control and the other treatments, while the growth rate of germlings treated with 10 and 50 ug 1^{-1} of copper was very similar. The statistical analyses, however, detected significant differences among treatments (including the control dishes) from the 4th day of exposure.

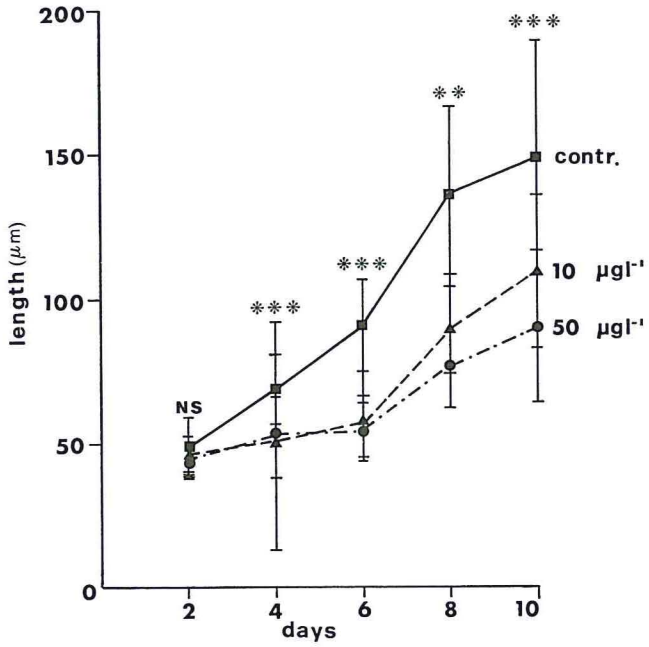

Fig. 4 - Growth response of *C. granulatum* germlings exposed to Hg. Values are means (± 1 SD) on four replicates. (** = $P < 0.01$, *** = $P < 0.001$. ANOVA.

A similar result was obtained with lead; again, significant differences among treatments were evident from the fourth day of exposure (Fig. 5).

Discussion

Our results showed that tetraspores of *C. granulatum* were affected by the presence of heavy metals in the culture medium.

Generally, mortality levels increased and germination rates decreased as a function of metals concentrations. This was particularly striking with mercury (Figs. 1, 2), while copper did not show the same pattern.

Our results indicated that the germination of *C. granulatum* was not significantly affected by copper. This contrasts with the results reported by other authors (Anderson *et al.*, 1990). The toxicity of copper is well documented for a wide range of marine organisms (Ahsanullah *et al.*, 1981; Blust *et al.*, 1988; Gould *et al.*, 1988; Munda and Hudnik,

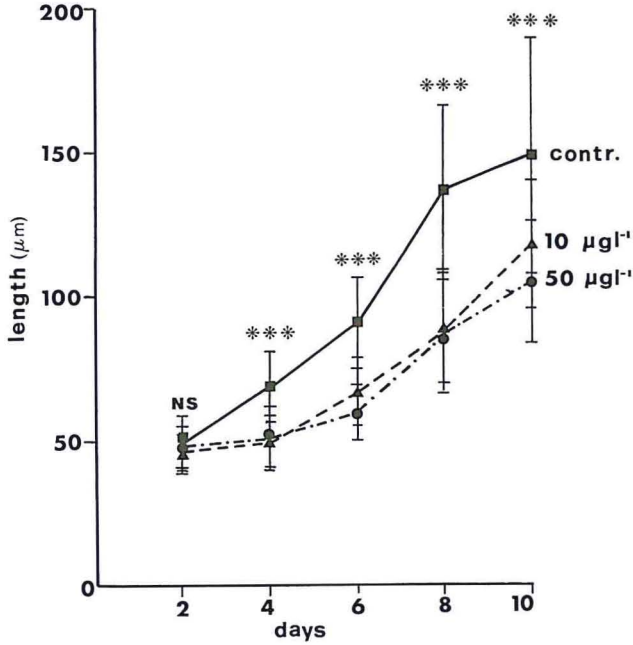

Fig. 5 - Growth response of *C. granulatum* germlings exposed to Pb. Values are means (± 1 SD) on four replicates. (*** = $P < 0.001$, ANOVA).

1986; STROMGREN, 1982). In marine macroalgae this metal might alter germination inhibiting spore photosynthesis or the use of stored photosynthate (ANDERSON *et al.*, 1990). In *Laminaria hyperborea*, for example, gametophyte's germination was inhibited at 100 ug 1^{-1} (HOPKIN and KAIN, 1978). A lack of a significant response in our germination tests suggests that for *C. granulatum* inhibition could be triggered off at higher concentrations than those used in our experiments. Such interspecific variability could be related to the ion selectivity of the polysaccarides that constitute the cell walls of the algae (KANGAS and AUTIO, 1986). For example the existence of ion-binding substances with a high affinity for zinc was demonstrated for brown algae (SKIPNES *et al.*, 1975). No analogous information, however, is available for the red algae.

The growth of germlings was also affected by the metals tested. Growth inhibition was reported as one of the most common algal response to increasing levels of heavy metals in water (ELNABARAWY and WELTER, 1984) and it seems more sensitive than other parameters to detect the toxic effects of these pollutants. For example in *L. hyper-*

borea, as reported earlier, gametophyte germination was inhibited at 100 ug 1^{-1}, while sporophyte growth was inhibited at 10 ug 1^{-1} (HOPKIN and KAIN, 1978). Our results also corroborated this view, in fact spores germination was not significantly affected by copper, while growth rates were strongly inhibited from the fourth day of exposure (Fig. 4).

In synthesis our experiments indicate that the early life history stages of *C. granulatum* are sensitive to the presence of heavy metals in laboratory conditions, and that several biological parameters may be affected by these pollutants. With the exception of mercury, however, these parameters appeared only little affected by an increase of metal concentrations in the culture medium; in contrast the main differences were evident among the controls and the other treatments.

Finally, the knowledge concerning the effects of heavy metals at cellular and sub-cellular levels in macroalgae is still scarce. This is a limiting factor on the interpretation of results from the present laboratory experiments, particularly in the assumption that they represent the real situation. For this reason laboratory tests should be considered as complementary to field studies and to physiological and biochemical research to better understand the effects of pollutants on natural populations.

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(*ms. pres. il 14 giugno 1994; ult. bozze il 10 febbraio 1995*)