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THE TOXICITY OF COPPER, ZINC, AND MERCURY TO THE BROWN MACROALGA LAMINARIA SACCHARINA

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ABSTRACT

The effects of copper, zinc, mercury, and methyl mercury on the growth in culture of stages in the life history of Laminaria saccharina were investigated. The developmental stages between microscopic gametophyte and young sporophyte (sporeling) were the most sensitive, with significant reductions in the size of the sporelings at nominal concentrations of 10 $\mu g\ Cu.l^{-1}$, 100 $\mu g\ Zn.l^{-1}$, 5 $\mu g\ Hg.l^{-1}$, and 0.5 μg Hg(methyl).l^{-1}. These results suggest that the technique using L. saccharina is among the more sensitive of those reported for these metals.

Preliminary investigations of the accumulation of copper by sporophytes cultured in sublethal concentrations of the metal are described, and the potential of L. saccharina for field monitoring, as well as laboratory toxicity testing, is discussed.

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KEYWORDS

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INTRODUCTION

Toxicity tests with algae are recognised as important in the assessment of the potential impact of chemicals on the marine environment. Macroalgae (seaweeds) have a number of advantages over the more commonly used planktonic microalgae (Burrows, 1971), and the use of Laminaria saccharina has been reported as a bioassay organism for seawater quality (Burrows and Pybus, 1971), and as a toxicity test organism for detergents (Pybus, 1973), and cadmium (Markham et al., 1980).

The purpose of the present study was to investigate the sensitivity of Laminaria saccharina to heavy metals, and to assess the potential of this alga for both laboratory and field pollution studies.

MATERIALS AND METHODS

ESTABLISHMENT OF CULTURES

Fertile sporophytes of Laminaria saccharina were obtained from shores in Anglesey, North Wales. Zoospores were released within a few hours when reproductive portions of the frond were placed in filtered seawater. Volumes of this zoospore suspension were used to inoculate culture medium in vessels containing glass microscope slides, onto which the zoospores settled and attached within 24 h.

Under the conditions employed, the zoospores germinated within 3 days and developed to form the microscopic male and female gametophytes. After fertilisation the sporelings developed by cell division and enlargement, the majority comprising eight or more cells within 20 to 30 days of zoospore release. When macroscopic, after approximately 45 days, the young sporophytes were removed from the slides and cultured in a free-floating condition.

CULTURE CONDITIONS

The stock and experimental cultures were maintained under semistatic conditions with renewal of the medium twice per week. The culture medium was prepared from filtered (1 µm) seawater, collected from a position 16 km offshore in the Irish sea, and is described in Table I.

Table I. Composition of the culture medium

Compound	Quantity (mg)
$NaNO_3$	42.0
$Na_2HPO_4.12H_2O$	11.0
$FeCl_3.6H_2O$	0.27
$MnSO_4.4H_2O$	0.024
KI	1.7
Vitamin B_{12}	0.001
Filtered seawater	to 1 000 ml

Glass culture vessels containing 5 l of medium were employed, with gentle aeration of the cultures except during zoospore settlement. The cultures were maintained at a temperature of 8 ± 1 °C with a photoperiod of 18 h at a light intensity of approximately 2 500 lux.

TOXICITY EXPERIMENTS

The separate effects of copper, zinc, mercury (inorganic) and methyl mercury were investigated on :

1. the growth of macroscopic sporophytes ;
2. the development of zoospores, through the gametophyte stages, to microscopic sporelings.

The growth of the macroscopic sporophytes was expressed as the percentage increase in surface area of the fronds. Groups of 20 or 30 sporophytes, cultured from the zoospores of a single "parent" were cultured in a range of concentrations of each metal for 14 days, and the growth compared with that of a control group.

The development of zoospores inoculated into control and metal treatments was determined by microscopic examination of randomly selected slides removed from the vessels at intervals, the final assessment being based on the size (cell number) and survival (density of plants on the slide) of the sporelings.

The concentrations of the metals were achieved by the addition of concentrated stock solutions of the metal chloride or sulphate (zinc) in deionised water, the maximum addition being 4 $ml.l^{-1}$. Concentrations were expressed as weight of the metal ion per litre.

ACCUMULATION

The accumulation of copper by sporophytes exposed to a range of concentrations of copper for 14 days was investigated, by removing plants at intervals for analysis of the frond tissues by atomic absorption spectrophotometry following acid digestion. The distal portion of the frond, not growing during the exposure period, was analysed separately from the growing portion (close to the junction of frond and stipe).

Three plants were sampled from a control and each metal treatment on each occasion. Analyses of 35 additional plants, sampled from the population used to provide the experimental groups, were carried out to provide an additional "control" baseline for comparison.

RESULTS

TOXICITY

The effects of copper, zinc and inorganic and methyl mercury on the growth of the macroscopic sporophytes are shown in Fig. 1. The results for each metal suggest a concentration related response above a certain threshold, with significant ($P = 0.05$) inhibition of growth at 50 $\mu g\ Cu.l^{-1}$, 1 000 $\mu g\ Zn.l^{-1}$, 50 $\mu g\ Hg.l^{-1}$, and 5 μg Hg(methyl)$.l^{-1}$.

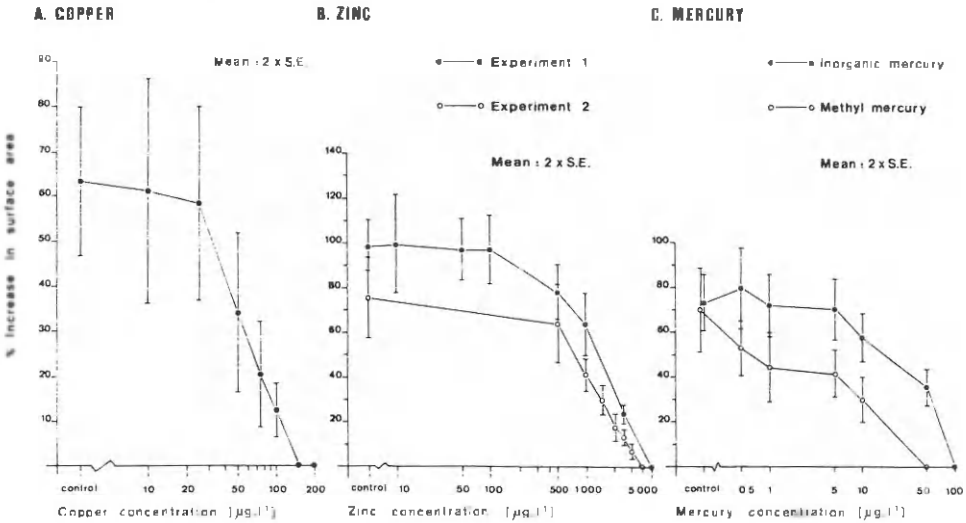

Fig. 1. The effects of copper (a), zinc (b), and inorganic and methyl mercury (c) on growth (14 days) of the sporophyte of Laminaria saccharina.

The effects on the size of sporelings cultures from zoospores in the metal treatments are shown in Fig. 2. The concentrations found to cause a significant inhibition of growth in these experiments were lower, in each case, than for the sporophyte, and were 10 µg $Cu.l^{-1}$, 100 µg $Zn.l^{-1}$, 5 µg $Hg.l^{-1}$, and 0.5 µg Hg(methyl).l^{-1}. Survival at these "effect concentrations" was reduced only for inorganic mercury. For copper, zinc, and methyl mercury, the concentrations causing a significant decrease in survival were 25 $\mu g.l^{-1}$, 1 000 $\mu g.l^{-1}$ and 1 $\mu g.l^{-1}$ respectively.

ACCUMULATION

The accumulation of copper by the distal portion of the frond (not growing during exposure), when cultured in sublethal concentrations of the metal, is shown in Fig. 3. Significant accumulation had occurred, at all concentrations of copper examined, within the 14 day period, and the tissue copper concentrations were reasonably well related to concentration in the medium.

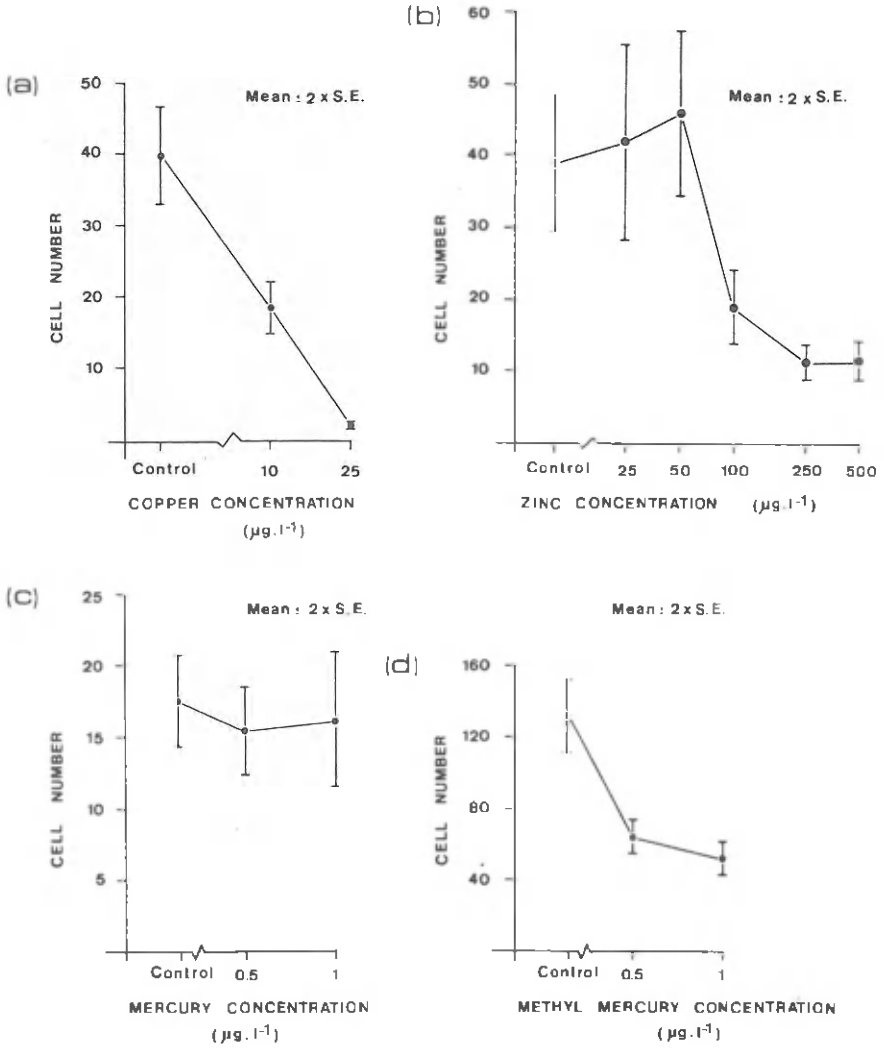

Fig. 2. The effects of copper (a), zinc (b), inorganic mercury (c), and methyl mercury (d) on the cell number of sporelings of L. saccharina, cultured from zoospores in the metal treatments.

The accumulation of copper by the growing portion of the frond was lower, and more erratic, than for the distal parts and therefore the results are not described here in detail. It is probable that growth occurring in this part of the frond tended to "dilute" the tissue concentrations resulting from uptake of copper.

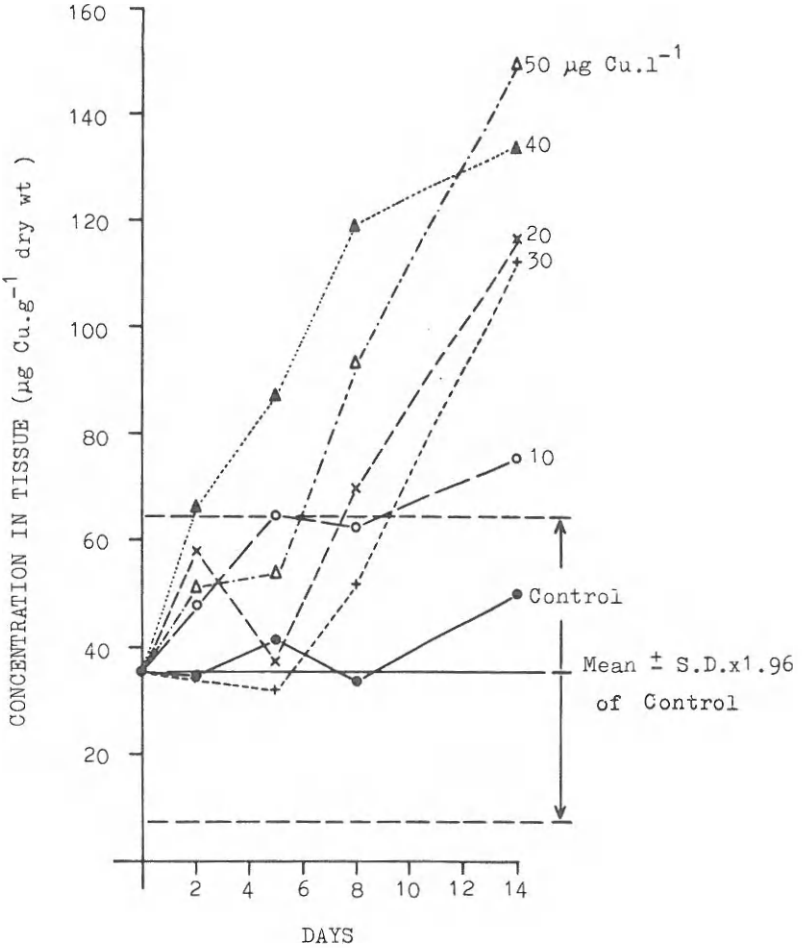

Fig. 3. The accumulation of copper by the distal frond tissues of the sporophyte of Laminaria saccharina.

DISCUSSION AND CONCLUSIONS

Until more is known about the form, or speciation, of heavy metals in seawater, and the factors affecting their biological availability, the relevance of the results of laboratory toxicity tests to the field situation is uncertain. However, consideration of the sensitivity of an organism to heavy metals, and the techniques available for studying that organism's

response, is worthwhile. Firstly, an understanding of the biological significance of elevated concentrations of metals must be gained, ultimately, from biological systems that are sensitive to small differences in biological availability. Secondly, because of the wealth of information available in the literature, the relative sensitivity of a technique to heavy metals can be evaluated, which may help in considering its value for studying the effects of other types of contaminant of the marine environment.

It is beyond the scope of this paper to review fully the literature on the toxicity of these metals. However, the results of this study are compared in Table II with those for several of the more sensitive organisms that have been reported. Comprehensive comparisons of the sensitivity of organisms to copper and mercury are made by Taylor (1978) and Taylor (1977) respectively. It was concluded that the sensitivity of Laminaria saccharina was similar to that of the more sensitive of the marine organisms for which data are available, for the metals employed.

Table II. Sensitivity "threshold" reported for selected marine organisms

Organism	Threshold ($\mu g.l^{-1}$)			Reference
	Cu	Zn	Hg	
Laminaria saccharina (alga)	10	100	5	This study
Campanularia flexuosa (hydroid)	10	-	2	1
Cristigera sp. (protozoan)	-	125	3	2
Skeletonema costatum (microalga)	10	50	5	3, 4, 5

Key to references : 1 = Stebbing (1976)
2 = Gray and Ventilla (1973)
3 = Burrows and Sharples (1972)
4 = Jensen et al. (1974)
5 = Rice et al. (1973)

Jensen (1981) reviewed the various approaches that have been used to investigate the effects of heavy metals on algae in both the laboratory and the field. Many of the difficulties associated with use of unicellular algae for such purposes might be overcome by the use of macroalgae such as Laminaria. Although this study employed static cultures, regular replacement of the medium probably helped to reduce the interactions between the metal and the extracellular products of the alga and the changes in algal physiology associated with cultures in a limited volume of medium. In addition, both the microscopic and macroscopic stages of Laminaria saccharina are amenable to culture under continuous-flow conditions which could enable toxicant concentration, nutrient status and algal growth rate to be more fully controlled.

The results of the preliminary accumulation study with copper reported here, and the work of Bryan (1969) with Laminaria digitata and zinc, suggest that the frond tissue of Laminaria may exhibit relationships between internal and external concentrations of metals which could be established in the laboratory to help the interpretation of field monitoring programmes. Jensen (1981) described some of the difficulties associated with the species of seaweed that are currently used in such monitoring work. Many of these would be overcome using Laminaria saccharina because the localised growth zone enables tissue to the reliably aged using holes punched in the frond (Parke, 1948). Furthermore the ability to transplant laboratory cultured sporophytes successfully to an artificial substrate in the field has been described by Thompson (1977), and the growth of natural populations has been studied in detail (Parke, 1948).

Thus Laminaria saccharina may be potentially valuable not only as a laboratory test organism, but also as a means of bridging the gap between laboratory and field observations.

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