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Cadmium effects on growth and physiology of *Ulva lactuca*

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ABSTRACT: The chlorophycean *Ulva lactuca* L. was grown in the laboratory in unialgal culture to sufficient size so that up to 70 discs, 24 mm in diameter, could be punched out of a single plant. Using such discs, *U. lactuca* was then tested with various concentrations of Cd under continuous-flow conditions. A concentration of 4.5 ppm Cd was lethal to *U. lactuca* within 6 days. Control discs in unpolluted water increased in diameter at a rate of 8 to 13 % day^{-1} over a 6-day period. At sublethal concentrations of Cd a sharp reduction in growth rate was observed at increasing concentrations up to approximately 0.3 ppm Cd, whereas from 0.3 ppm Cd to the lethal concentration the reduction of the growth rate was significantly less. Reduction in photosynthetic performance corresponded closely to the reduction in growth rate. At ambient concentrations of 0.8 ppm Cd, the plants concentrated Cd by a factor of approximately 50 in 6 days. Much higher concentration factors were attained in lower ambient concentrations. After removal from Cd-polluted water into flow-through culture in unpolluted water, a subsequent loss of Cd was indicated and the plants recovered rapidly. Plants exposed up to 3 d to 0.7 ppm Cd recovered sufficiently to produce viable gametes 7 days after removal from Cd. Because it has a relatively short life span and apparently loses Cd subsequent to exposure to Cd-polluted water, *Ulva lactuca* is not recommended as an alga for monitoring in-situ environmental pollution.

INTRODUCTION

Cadmium has attracted increasing attention in recent years as an environmental pollutant. Although it is found in only minute traces in unpolluted marine environments, certain coastal and inshore waters can contain several μg Cd l^{-1}. Numerous studies of Cd accumulation have been carried out, although relatively few of these studies have involved marine algae. It is known that marine algae, especially brown algae, can concentrate heavy metals to a very great extent (Gutknecht, 1965; Bryan & Hummerstone, 1973; Morris & Bale, 1975; Pak et al., 1977; Seeliger & Edwards, 1977).

Burrows (1971) reported on pollution effects on two common algae, the annual green alga *Ulva lactuca* and the perennial brown alga *Laminaria saccharina*. She noted that *U. lactuca* grew better in the presence of organic pollution. Zavodnik (1977) reported that Pb in concentrations up to 1 ppm had no measurably toxic effect on *U. rigida* over 6 days,

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as determined by measurements of oxygen production. On the other hand, Hägerhäll (1973) reported that *U. lactuca* was absent from areas where it had previously been recorded, which had subsequently become polluted with heavy metals including Cd. Burrows (1971) suggested that *U. lactuca* and *L. saccharina* might be good indicator organisms for environmental pollution because they are sessile and thus unable to move away from the pollutant and they have rapid growth rates which are easy to measure. For these reasons and because it is ubiquitous and successful culture methods have been devised for it, we have previously tested the effects of Cd on *Laminaria saccharina* (Markham et al., 1980). For the same reasons and to provide a comparison with *Laminaria* we have tested the effects of Cd on *U. lactuca.*

MATERIAL AND METHODS

Plant material

Small pieces were cut out of unialgal stock-culture plants of *Ulva lactuca* L. and grown in an automatic flow-through culture system as described previously (Markham et al., 1979). After approximately 40 days growth (pretreatment) in enriched sea water (Provasoli, 1968) at 12 °C under white fluorescent light (Osram 65 W/19; 2000 lux) each piece had grown to sufficient size so that up to 70 discs, 24 mm in diameter, could be punched out of a single plant. In any one experimental series only discs cut out of the same plant were used. Growth of the discs under test conditions was measured as increase in diameter, then calculated as specific growth rate (SGR; % increase/day) using the formula:

$$SGR = \frac{100\ [\log_e\ (D_2/D_1)]}{t}$$

where D_1 = initial diameter
D_2 = diameter on day t.

Cadmium treatment

In the treatment the *Ulva* discs were tested in a continuous-flow test system (Markham et al., 1979). In this system filtered sea water containing the desired concentration of Cd (added as $CdCl_2$) was pumped continuously from 20-l glass bottles into test culture vessels (2-l capacity; 22-cm diameter, 9 cm high) at a rate of 2 ml/min. This flow rate had previously been demonstrated as sufficient to maintain the Cd concentration in the test vessels despite uptake by the plants (Markham et al., 1979). All test cultures were maintained at 12 °C, 16:8 light:dark, white fluorescent light at 5000 lux. The solution in the test vessels was stirred continuously by magnetic stirrer. Most experiments were run for a period of 6 days. One control and 5 test concentrations of Cd were used in most experiments, with 10–12 discs per test concentration. In initial experiments to determine the lethal concentration, concentrations of 0.08 ppm Cd to 4.6 ppm Cd were employed. Most subsequent experiments employed concentrations of 0.21, 0.29, 0.92, 1.73, and 2.75 ppm Cd. For further details of experimental conditions, see Markham et al. (in press).

Cadmium determination

Water samples from each test vessel were analyzed for Cd content by atomic absorption spectrophotometry (AAS) as described by Sperling (1977). Plant discs to be analyzed for Cd content were removed from the test vessels at the end of the experiment with glass forceps, passed rapidly (20 sec) through a dehydration series from distilled water to 100 % methanol, weighed for wet weight, dried at 85 °C for 20–21 h, weighed for dry weight, and then analyzed by flameless AAS as described by Sperling et al. (1977). Cd-uptake rates were determined by removing discs after 1, 2, 4 and 6 days in 0.88 ppm Cd for AAS analysis.

In order to test recovery from exposure to Cd, plants were grown in 0.72 ppm Cd. After 1, 3, and 6 days 10 discs were transferred into uncontaminated sea water in new vessels, where they were allowed to grow further in flow-through conditions until 15 days after the beginning of the experiment. At 6 days one disc from each exposure time was measured for growth and then analyzed for Cd content. At 15 days all the remaining plants were measured and samples from each exposure time were analyzed for Cd content.

Photosynthetic ^{14}C assimilation

To examine possible effects of Cd on primary metabolism, *Ulva* discs which had been cultured in Cd in continuous-flow conditions as described above were then allowed to assimilate ^{14}C from a $H^{14}CO_3$–seawater medium for 30 min in the light (20,000 lux). The incubation medium contained the same amount of Cd as the culture medium plus 10 μCi $NaH^{14}CO_3$/10 ml. After the appropriate incubation time, the samples were harvested, briefly rinsed in tap water, rapidly deep frozen, and subsequently lyophilized. For further details on the incubation and analytical procedures, see Kremer (1978).

RESULTS

Growth

Ulva discs in concentrations of approximately 4.5 ppm were killed in the standard 6-day test. Control discs increased at a rate of approximately 13 %/day over 6 days. The decrease in growth rate with increasing Cd concentration within the sublethal range was very steep to approximately 0.3 ppm Cd (Fig. 1). Above this concentration the decrease was markedly less.

In the experiment to test recovery after exposure to Cd, linear regression analysis of the growth measurements made after 6 days (Fig. 2) indicates a significant effect ($r = 0.95$; significant to 0.05 level) of increased exposure time in reducing growth. When measured after 15 days, however, i. e. after 14, 12, or 9 days in uncontaminated water, the evident reduction in growth was much less (Fig. 2).

In the recovery experiment almost all the discs exposed 1 day to Cd became fertile 7 days after being removed to uncontaminated water. Almost all the discs exposed 3 days to Cd also became fertile 7 days after removal to clean water. Of the discs exposed 6 days to Cd, one became fertile 8 days after removal to clean water. The control discs all

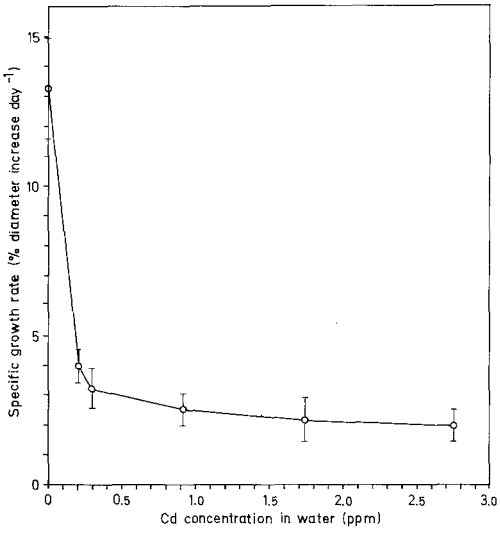

Fig. 1. *Ulva lactuca*. Percentage diameter increase per day of discs measured after 6 days in various Cd concentrations (ppm.) Vertical bars indicate standard deviations

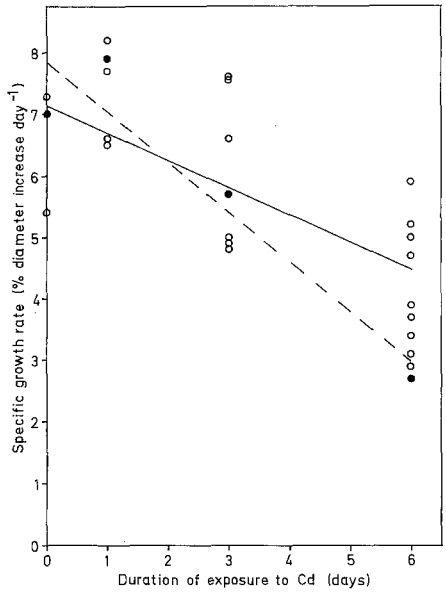

Fig. 2. *Ulva lactuca*. Percentage diameter increase per day of discs after various durations (days) of exposure to 0.7 ppm Cd, measured after 6 days (broken line) and 15 days (solid line). Lines: linear regressions, significant at the 0.05 level. Only discs which had not released swarmers were measured. (N = 4 for 6 days, N = 21 for 15 days)

became fertile 14 days after the start of the experiment. All fertile discs released viable male gametes. In many discs 100 % of the cells produced gametes and thus after gamete swarming nothing remained for growth measurements. This accounts for the fact that N is greater for plants exposed longer to Cd for the 15-day measurements in Figure 2.

Uptake of cadmium

Cd uptake as plotted against time was very rapid at first, with over 30 ng Cd/mg tissue accumulated after 1 day in 0.9 ppm Cd, but slowed considerably after the first day (Fig. 3). After 6 days in the same concentration only about 59 ng Cd/mg tissue had been accumulated. Measurements made after 6 days in various Cd concentrations show that Cd accumulation is proportional to the concentration in the water (corr. coeff. = r = 0.973, significant at 0.01 level for N = 6), although relatively more is taken up from lower ambient concentrations (Fig. 4). Calculation of the concentration factor (= Cd concentration in plant/Cd concentration in water) illustrates this point better, as this factor is much larger in lower ambient concentrations (Fig. 4). It also reveals that above ambient concentrations of about 1.0 ppm Cd the concentration factor does not rise above 50 × and tends to remain constant with increasing ambient concentration.

When plants were exposed to Cd and then returned to clean water, there was an apparent loss of Cd in plants which had taken up more than 200 μg Cd/plant (= ca. 5 ng

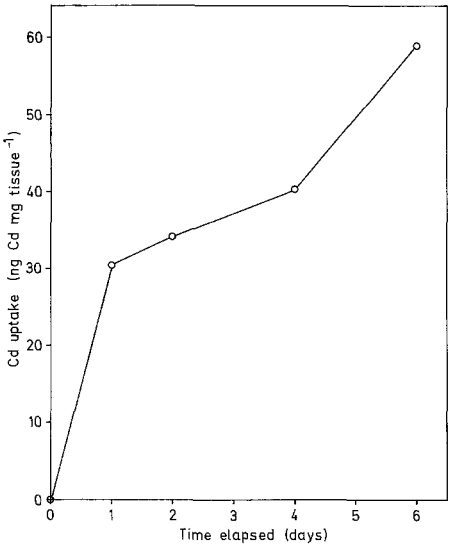

Fig. 3. *Ulva lactuca*. Rate of Cd uptake by discs exposed to 0.88 ppm Cd. One disc sampled at each time interval

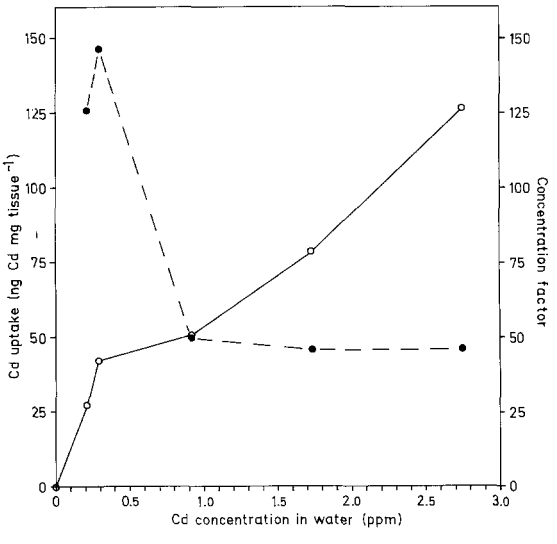

Fig. 4. *Ulva lactuca*. Solid line: uptake of Cd from various solution concentrations, measured after 6 days; broken line: concentration factors (Cd concentration in tissue / Cd concentration in solution). One disc measured per concentration

Cd/mg tissue) (Fig. 5). The relative loss was greater in plants which had accumulated more Cd.

Carbon assimilation

The photosynthetic assimilation of ^{14}C as measured after 6 days in various concentrations of Cd was very sensitive to Cd (Fig. 6). The curve for the reduction of light-dependent carbon assimilation with increasing Cd concentration is very similar to that for growth reduction (cf Fig. 1). There is the same sharp drop at low concentrations and relatively less assimilation-rate reduction with increasing Cd concentration. Similar experiments (data not included in Fig. 6). indicate that this effect of Cd is time-dependent as well. No attempts have been made to determine to what extent a recovery of the potential for photosynthetic carbon fixation might be achieved.

DISCUSSION AND CONCLUSIONS

These experiments have shown that *Ulva lactuca* is sensitive to Cd, as is shown by reduction in growth, rate of photosynthetic carbon assimilation and considerable delay in sporulation. This sensitivity is not a straight-line relationship with ambient Cd concentration. A comparison of Figures 1, 4 and 6 indicates a correlation between the photosynthetic and growth responses and the uptake and concentration-factor curves. Growth, photosynthesis and concentration factor fall steeply with increasing Cd concentration at lower ambient concentrations, then much less rapidly as concentration

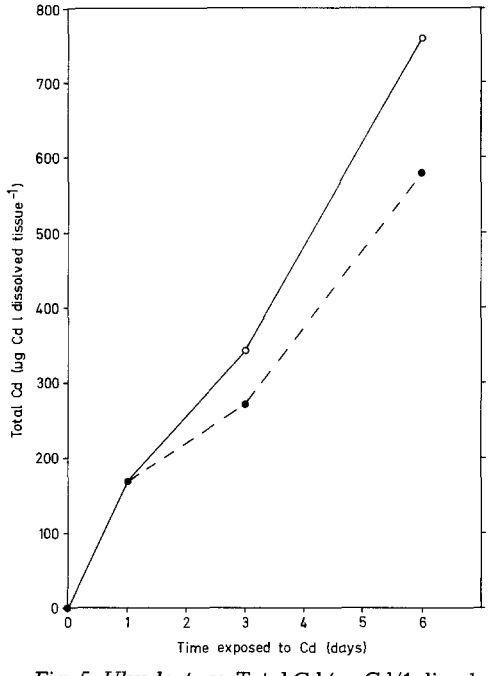

Fig. 5. *Ulva lactuca*. Total Cd (μg Cd/1 dissolved tissue) measured in discs after various durations (days) of exposure to Cd. Solid line: discs analyzed 6 days from start of Cd exposure; broken line: discs analyzed 15 days from start of Cd exposure

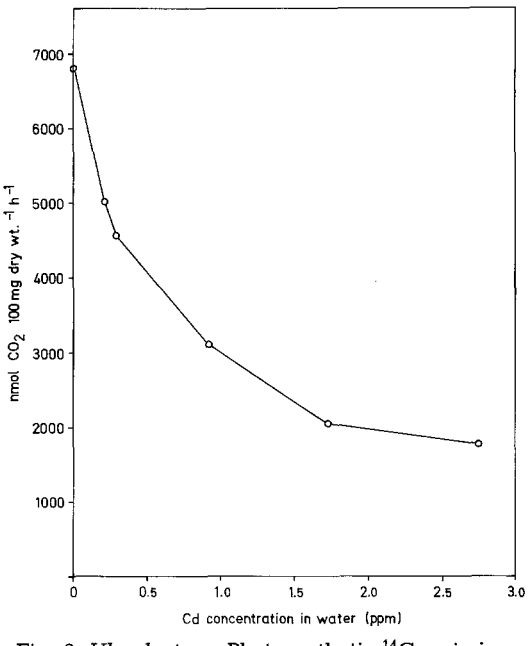

Fig. 6. *Ulva lactuca*. Photosynthetic ^{14}C assimilation by discs after 6 days exposure to various Cd concentrations

increases. Cd uptake rises sharply to ambient concentrations of about 0.3 ppm Cd, then rises more gradually with increasing concentration. This is about the same point at which there is a break in the growth curve. Earlier work with the brown alga *Laminaria saccharina* (Markham et al., 1980) showed that in that alga the response of growth, photosynthesis, uptake and concentration factor to increasing concentration was essentially a straight-line relationship and it was concluded that uptake was an uncontrolled process. Further, in that alga, there was no evidence of a loss of Cd after removal from Cd-polluted media, nor was there any subsequent recovery from the Cd-inhibited growth rate. For *Ulva*, the lack of a straight-line relationship with increasing Cd concentrations and especially the constant concentration factors above approximately 1.0 ppm Cd indicate that there may be active control over the amount of Cd taken up and retained. This is further indicated by the relatively greater loss of Cd by plants which had taken up more Cd (Fig. 5). Although comparisons are difficult because of the different concentrations employed, it is of interest that Sivalingam (1978) subjected *U. reticulata* to extremely high concentrations of Cd (up to 500 ppm) and reported a Cd loss due to an apparent "regulatory discharge mechanism".

The improved growth rate after removal from Cd indicates recovery from the toxic effects of Cd and provides indirect evidence of a loss of Cd. The fact that many of the plants became fertile complicated the measurements of growth rate. The factors inducing and controlling sporulation in *Ulva* are complex and not fully elucidated (see

Nordby, 1977) and are beyond the scope of this paper. Important with regard to Cd pollution is that the sporulation of the plants a week after removal from Cd indicates nearly complete recovery from the Cd effects.

Although *Ulva* is very sensitive to Cd, at least over a short time, its uptake of Cd is very low, when compared with that by other green algae (Pak et al., 1977) or *Laminaria* (Markham et al., 1980). It also takes up less Cu and Pb than do other green algae (Pak et al, 1977) or *Laminaria* (Markham et al., 1980). It also takes up less Cu and Pb than do the similar green algae *Enteromorpha* and *Blidingia* (Seeliger & Edwards, 1977) and much less ^{65}Zn than does *Fucus* (Gutknecht, 1965). Seeliger & Edwards (1977) also found a poorer correlation between tissue concentrations of Cu and Pb in *Ulva* and dissolved Cu and Pb than was the case for *Blidingia, Enteromorpha* or *Fucus.*

Because of its low uptake and subsequent loss of Cd and because it shows little or no long-term aftereffects from exposure to Cd, and also because it has a life span of less than a year, *Ulva* will not integrate environmental Cd concentrations over any period of time. Since it is apparently able to avoid uptake of Cd above a certain concentration, it will give a distorted picture of the in-situ Cd concentration. For these reasons, *Ulva*, in contrast to an alga such as *Laminaria*, is probably not a good alga to use as an in-situ indicator of environmental pollution by Cd or other heavy metals.

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