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Effects of Cadmium on *Laminaria saccharina* in Culture

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ABSTRACT: Cadmium effects were tested on 50-d-old laboratory-grown sporophytes of the kelp *Laminaria saccharina* under continuous-flow laboratory conditions. In standard 6-d experiments the rapid rate of growth of the plants (30 % length increase d^{-1} in controls) provided a suitable parameter for the measurement of cadmium effects. Over a 6-d period a concentration of 4.5 ppm Cd was lethal. There is evidence that a lower concentration would be lethal over a longer period of exposure. In the range of 0.2 to 4.5 ppm Cd the reduction in growth rate with increasing Cd concentration was nearly linear. The growth rate diminished to 50 % of the controls at approximately 2.15 ppm Cd. When plants exposed to cadmium for 6 d were measured after a further 8 d in unpolluted seawater, the concentration causing growth-rate reduction to 50 % of the controls was 0.86 ppm Cd. Long-term after effects are more serious than is immediately evident and exposure time is more important than concentration. Cadmium uptake begins almost immediately and is apparently unregulated, indicating potentially very high tissue concentrations. Over 100 ng Cd mg dry wt $^{-1}$ was accumulated by plants in 6 d from a 0.78 ppm Cd solution. Saturation was not reached. Relatively more cadmium is taken up from lower ambient concentrations. Slower-growing plants and slower-growing regions of the thallus (stipe/holdfast; distal blade region) take up relatively more cadmium. At concentrations over 2.3 ppm Cd the blades show a sharply delimited distal loss of pigment. Cadmium inhibits photosynthesis, as well as dark carbon assimilation. The degree of inhibition depends on time and concentration. In distal plant regions the photosynthetic potential is more sensitive to cadmium. The reduction in photosynthesis in the most actively growing region with increasing cadmium concentration follows a curve very similar to that for the reduction in growth. The decrease in growth and photosynthetic potential caused by cadmium is not correlated with pigment loss or cadmium accumulation.

INTRODUCTION

Since cadmium was found to be the cause of the endemic 'itai itai' disease in the Toyama District of Japan (Kobayashi, 1970) this metal has attracted increasing attention as an environmental pollutant. It is found only in minute traces in unpolluted environments. Its normal concentration in ocean waters seems to lie below 0.1 $\mu g\ l^{-1}$ (Goldberg, 1965; Riley and Taylor, 1968; Bryan, 1971; Preston, 1973), whereas polluted coastal and inshore waters can, in certain cases, contain several $\mu g\ l^{-1}$. In general, Cd concentrations of the German Bight (North Sea) range between 0.02 and 0.2 $\mu g\ l^{-1}$ (Schmidt, 1976; Sperling, unpublished).

It is well known that low concentrations of Cd can cause serious chronic effects (Friberg et al., 1974). Although several studies have been conducted recently regarding the influence of Cd on marine organisms, relatively few have dealt with marine plants and the majority of these were conducted on unicellular algae. Species of the kelp *Laminaria* have been tested more frequently with a variety of other pollutants. Pieces cut from fronds of *L. digitata* were examined for the effects of zinc (Bryan, 1969). *L. saccharina* was tested in seawater containing undefined pollutants (Burrows, 1971; Burrows and Pybus, 1971) and detergents (Pybus, 1973); *L. hyperborea* was tested with a variety of detergents, herbicides, insecticides, Zn, Cu, Hg (Hopkin and Kain, 1971, 1978) and Cd (Hopkin and Kain, 1978). In these studies, growth measurements were conducted using zoospores, gametophytes, and early sporophyte stages, whereas respiration, when tested, was measured in discs cut from adult, field-collected plants. These *Laminaria* studies, and analyses of the metal content of brown algae growing in polluted areas, especially species of *Fucus* (Bryan and Hummerstone, 1973; Fuge and James, 1973, 1974; Morris and Bale, 1975; Seeliger and Edwards, 1977) have established that uptake of heavy metals by brown algae is not regulated, reflects the concentration in the ambient seawater, and results in

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very high concentration factors, although the concentration factors are greater where the environmental concentration is lower.

Laminaria saccharina is ubiquitous in temperate northern hemisphere coastal waters and has a rapid growth rate which is very sensitive to culture conditions and can be measured by increase in length. Most previous tests of heavy metals on multicellular marine algae, including the above-noted tests on *Laminaria*, have been conducted in standing culture, where the growth rate of the algae may be less than optimal, and the concentration of the metal being tested declines over the course of the experiment due to uptake by the algae. The development of automatic flow-through culture systems for producing and testing uniform, unialgal cultures of *L. saccharina* has made it possible to examine the effects of Cd under controlled conditions of rapid growth and constant pollutant concentration.

MATERIAL AND METHODS

Plant Material

Sporophytes of *Laminaria saccharina* (L.) Lamour. were initiated from stock cultures of gametophytes and grown in the laboratory in unialgal culture on polyethylene rods in an automatic flow-through culture system as described previously (Markham et al., 1979). After 50 d growth (pretreatment) in enriched seawater (Provasoli, 1968) at 12 °C under white fluorescent light (Osram 65 W/19; 2000 lux), the sporophytes were cut back to 20 mm blade length and two 1.5 mm holes were punched in each blade at 10 and 15 mm from the transition zone, respectively. The first hole was for measurements of growth, as previous experiments had shown that the first 10 mm in small plants like these increased in length by a factor of 2.5 to 3.0 in 5 d, whereas the next 5 mm only increased by a factor of 1.2 to 1.3. The second hole was punched to provide an indication of possible distal loss of tissue. After measurement of the plants, the specific growth rate (% increase d^{-1}) was calculated using the formula:

$$SGR = \frac{100\ [log_e\ (L_2/L_1)]}{t}$$

where L_1 = initial length (= stipe/blade transition to first punched hole), L_2 = length on Day t (= stipe/blade transition to first punched hole on Day t)

Cadmium Treatment

In the treatment, the prepared plants, each growing attached to its individual polyethylene rod, were tested in a continuous-flow system (Markham et al., 1979). In this system, filtered seawater containing the desired concentration of Cd (added as $CdCl_2$) was pumped continuously by a multichannel peristaltic pump from 20-l glass bottles through polyethylene tubing to test culture vessels (2 l capacity; 22 cm diameter, 9 cm high). All test cultures were maintained at 12 °C, 16 : 8 light : dark, fluorescent light at 5000 lux (previously determined to be saturating for growth of plants of this age). Before running tests with new concentrations, the desired concentrations of Cd were pumped once through the system to 'condition' the glassware and minimize possible later adsorption of Cd. Prior to addition of the plants, the test culture vessels were filled again with test solutions from the reservoir bottles by rapid pumping and then allowed to stand overnight. After addition of the plants, the solution was pumped continuously into the vessels at a rate of 2 ml min^{-1} for a period of 6 d. The solution in the test vessels was stirred continuously by magnetic stirrer. The stirrer was separated from the culture vessel by a 1-mm air space and a 5-mm thick sheet of Plexiglas, which prevented overheating of the culture solution by the stirrer. The concentration of Cd in the water showed essentially no change over 6 d at any concentration, indicating that the flow rate was sufficient to maintain the concentration despite uptake by the plants.

Due to the rapid rate of growth of control plants, tests longer than 6 d resulted in overcrowding of the control vessel. One control and 5 test concentrations of Cd were used in most experiments, with 10–15 plants per test concentration. Cd was added in concentrations of 0.07, 0.46, 0.97, 4.37 and 8.4 ppm Cd^{++} in initial experiments to determine the lethal concentration. Later experiments were restricted to a lower range (mean values = 0.2, 0.39, 0.82, 1.87, 2.64 ppm Cd^{++}).

Cadmium Determination

Water samples were taken from each culture vessel after 2 and again after 6 d of each experiment and analyzed for Cd content by atomic absorption spectrometry (AAS) as described by Sperling (1977). At the end of the experiment the plants were removed from the culture vessels with glass forceps and the distance from the transition zone to the first punched hole was measured on each plant.

In order to determine the amount of Cd taken up by the plants, one or two entire plants from each concentration were passed rapidly (20 s) through a dehydration series from distilled water to 100% methanol, dried at 85 °C for 20–21 h, weighed for dry weight and then analyzed by flameless AAS for Cd content as described by Sperling et al. (1977).

In one experiment, samples were taken after 6 d from 5 regions of the plant (holdfast/stipe, transition zone, 10 mm above transition zone, mid-blade, distal end) and analyzed separately for Cd content to determine relative uptake rates by different parts of the plant. Uptake rates were determined for whole plants by removing entire plants for Cd analysis after 1 h and 1, 3, 5 and 6 d in 0.86 ppm Cd.

To test recovery from exposure to Cd, plants were grown in sublethal concentrations of 0.38 and 0.86 ppm Cd. After 1, 2, 3, 5 and 6 d, 2 plants were removed from each concentration to uncontaminated seawater in new vessels, where they were allowed to grow further in flow-through conditions until 14 d after the beginning of the experiment. At this time all plants were measured.

^{14}C Assimilation

To examine possible effects of Cd on primary metabolism (cf. Overnell, 1976), *Laminaria* sporophytes (50 d old) were treated for 24 and 96 h in Cd-polluted media ranging from 0.16 to 1.5 ppm Cd and then allowed to assimilate ^{14}C from $H^{14}CO_3^-$ for 30 min both in the light and in the dark. The incubation medium contained the same amount of Cd as the culture medium plus 10 μCi $NaH^{14}CO_3$ $(10\ ml)^{-1}$. All experiments were conducted with 3 replicates. Further details on the incubation procedure have already been described earlier (Kremer, 1978). After the incubation the plants were briefly rinsed in tap water, cut into stipe, growing zone and distal part of the blade, and separately deep frozen in liquid N_2. Assimilation rate calculations were based on (a) the total alkalinity of the seawater medium and (b) the amounts of radiocarbon incorporated in the appropriate conditions. Radioactivity was determined by counting aliquots of ethanolic extracts of the freeze-dried algal material in a scintillation spectrometer. For further analytical details see Kremer (1978).

RESULTS

Growth of Plants

Preliminary experiments over the concentration range of 0.07 to 8.40 ppm Cd established that all plants in concentrations of 4.5 ppm or greater were killed in the standard 6-d test. Subsequent experiments concentrated on the sublethal range of 0.2 to 2.6 ppm.

In the pretreatment culture system, the plants attained lengths of 50 to 70 mm in 50 d (approx. 13 % to 16 % increase d^{-1}). In the treatment system, where there were fewer plants per dish and a higher light intensity, the control plants grew 35 to 40 mm (as measured to the first hole) in 6 d (28 % to 30 % increase d^{-1}; Fig. 1, conc. 'O'; Fig. 2, '1') after having been cut back to 20 mm at the start of the experiment. While the proximal 10 mm of the blade increased by a factor of 4.5 to 5.0, the distal 10 mm only increased by a factor of 1.5 to 2.0. Thus measurement from the transition zone to the first hole gave an easy measure of growth in the most rapidly growing region and accounted for most of the growth. This rapid growth rate provided a sensitive parameter for measuring the effects of Cd. With plants of this age, no distal loss of tissue occurred.

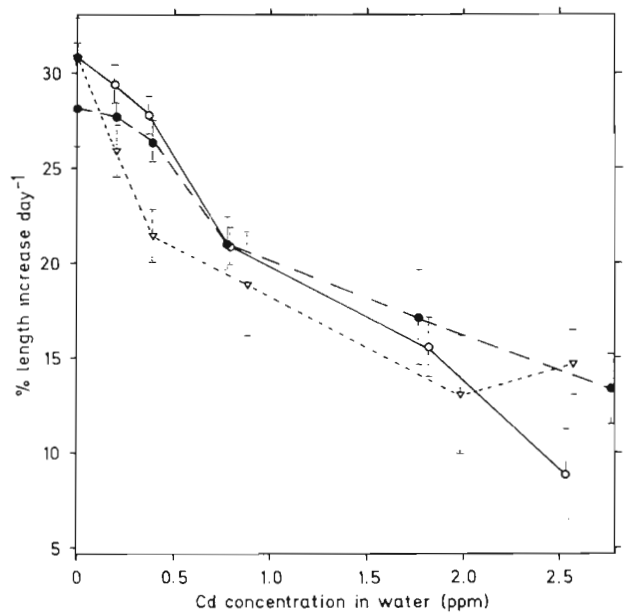

Fig. 1. *Laminaria saccharina*. Percentage length increase d^{-1} of the original lowermost 10 mm of plants, measured after 6 d in various Cd concentrations (ppm). Vertical bars indicate standard deviations (3 experiments)

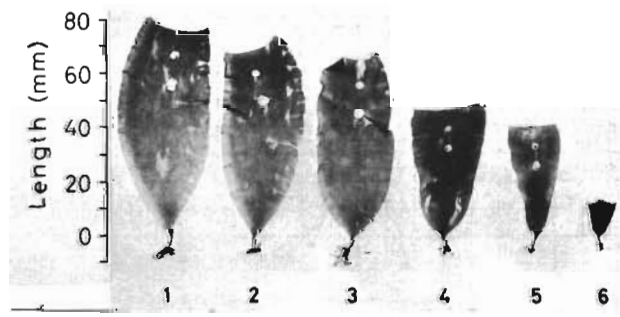

Fig. 2. *Laminaria saccharina*. Young plants after 6 d in various Cd concentrations (ppm). All plants (50 d old) were cut back to 20 mm blade length and had holes punched at 10 and 15 mm from the transition zone at the start of the experiment. Cd concentrations employed: 1 = 0.0 ppm (control); 2 = 0.19 ppm; 3 = 0.38 ppm; 4 = 0.8 ppm; 5 = 1.83 ppm; 6 = 2.78 ppm

Linear regression analysis of the data for the 3 experiments presented in Figure 1 (N = 18, correlation coefficient = –0.93, significant at the 0.01 level) yields a growth rate 50 % of the control rate at a calculated concentration of 2.15 ppm Cd. At concentrations of approximately 2.3 ppm Cd or more, the blades showed a sharply delimited distal loss of pigment (Fig. 2).

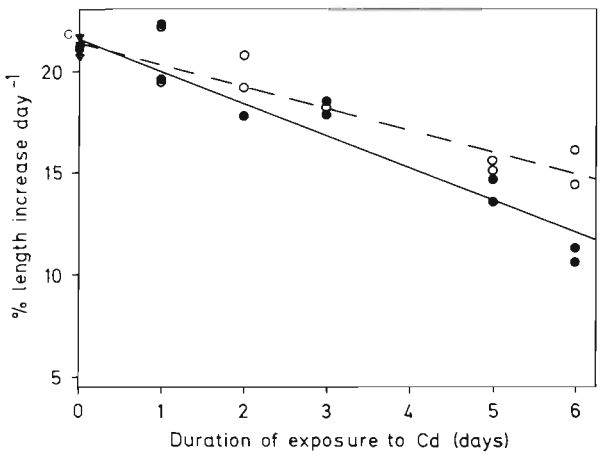

Fig. 3. *Laminaria saccharina*. Percentage length increase d^{-1} of the original lowermost 10 mm of plants after various durations (d) of exposure to Cd, followed by culture in Cd-free water, for 2 Cd concentrations. All plants were measured after 14 d from the beginning of the experiment. Lines: linear regressions, significant at the 0.01 level (N = 16, including control plants). C (▽) = control; ○ = 0.38 ppm Cd (broken line); • = 0.06 ppm Cd (solid line)

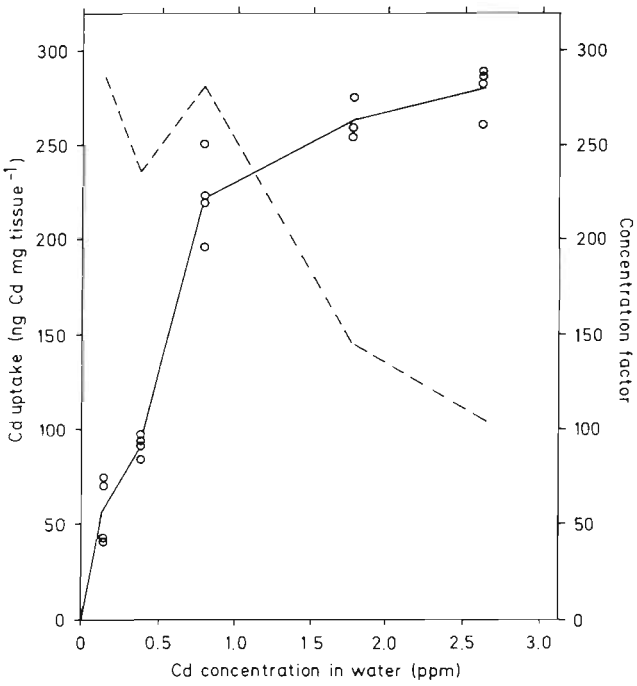

Fig. 4. *Laminaria saccharina*. Solid line: uptake of Cd in 6 d from various concentrations, expressed as ng Cd (mg dry weight tissue)$^{-1}$; broken line: concentration factors (concentration in tissue/concentration in solution). Four plants measured per concentration

In experiments testing recovery of plants after Cd exposure, all plants grew after being returned to uncontaminated seawater, but there was no recovery from the reduced growth rates observed over the 6-d exposure period (Fig. 3). The growth per day over the whole 14 d (exposure plus subsequent culture in uncontaminated seawater) was poorer for all concentrations employed than that observed over 6 d of exposure to Cd (cf. Fig. 1). For plants which were 6 d in Cd and then 8 d in clean seawater, linear regression analysis (N = 12, including control plants) indicates a growth rate 50 % of the control rate for plants exposed 6 d to a calculated concentration of 0.86 ppm Cd. This is less than half the concentration producing this rate when growth is measured immediately after 6 d exposure. An increased time of exposure to a given concentration of Cd depresses growth more than does an increased concentration over a given time (Fig. 3). The control plants in the recovery experiments showed a mean increase d^{-1} of 21.16 %, when measured after 14 d. Plants exposed 1 d to 0.86 ppm Cd showed essentially no effect: 20.97 % increase over the 14-d period. However, plants exposed to 0.38 ppm Cd for 6 d increased over the 14-d period only 15.27 % d^{-1} and those exposed 6 d to 0.86 ppm Cd increased only 10.97 % d^{-1} over the 14 d, or approximately 50 % of the control rate.

Uptake of Cadmium

Uptake of Cd corresponded roughly to the concentration available in the water, although the concentration factor (concentration in plants/concentration in water) was greater at lower ambient concentrations (Fig. 4). Measurements of the rate of uptake indicated that the plant is not at all saturated after 6 d in 0.75 ppm Cd, as the curve shows no signs of leveling out (Fig. 5). Uptake begins almost immediately and 9.5 ng Cd mg dry weight^{-1} was detectable in the plant tissue after 1 h exposure to 0.75 ppm Cd (Fig. 5).

Cd is taken up most rapidly by the slowest-growing regions of the plant, i. e., the stipe and most distal portions of the blade, and least of all by the meristematic transition zone between the stipe and the blade (Fig. 6). Initially it seemed that the amount of uptake might be the same everywhere, with expansion of the growing region leading to a decline of Cd concentration mg^{-1} in the rapidly growing region by 'dilution' as suggested by Fuge and James (1973). This may play a small role in determining the relative concentrations. However, measurements of relative uptake after 1 d already showed essentially the same pattern as those

after 6 d, with only the total concentration in each region increasing with time (Fig. 6).

Carbon Assimilation by Cd-Polluted Sporophytes

Despite the rapid uptake of Cd from the culture medium, preliminary experiments showed that rates of carbon assimilation were little affected when 50-d-old plants were transferred from the Cd-free pretreatment medium into a radioactive incubation medium containing defined amounts of Cd. However, when young sporophytes were grown for 24 h in a Cd-polluted medium and subsequently tested for potentials of carbon dioxide fixation, marked differences between Cd-treated plants and unpolluted control plants were observed. The results are compiled in Table 1. Since experiments on Cd uptake showed that different parts of the sporophytes accumulate different amounts of Cd, various regions of the young *Laminaria* sporophytes were measured separately. The values of Table 1 demonstrate several facets of photosynthetic carbon assimilation in a *Laminaria* thallus. On a dry weight basis, rates of photosynthesis are highest in the distal part of the blade and somewhat lower in the region of the holdfast and stipe. The relatively lowest rates of photosynthesis are encountered in the intercalary growing zone of the young sporophyte. In all regions of the thallus, rates of photosynthesis show concentration-dependent depressions. This Cd-dependent effect is relatively lower in the basal parts, whereas the distal region of the plant is more sensitive: in this region of the plant only 35 % of the control rate is achieved after a 24-h treatment in a medium containing 1.5 ppm Cd. However, Cd toxicity is not only a concentration effect, but also implies time-dependent actions, as may be seen from a comparison of 24-h treated and 96-h treated specimens (Table 1).

Since various representatives of the Laminariales have been found to achieve appreciable rates of light-independent carbon assimilation (cf. Kremer and Küppers, 1977), dark fixation in Cd-treated sporophytes

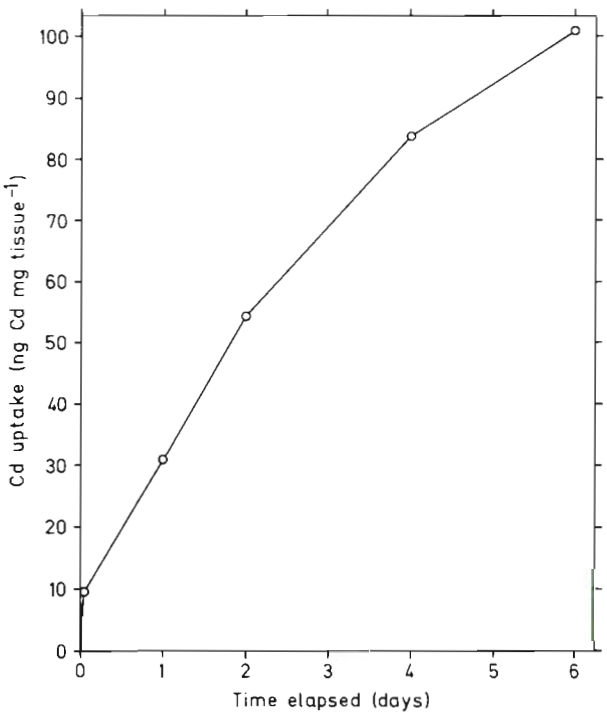

Fig. 5. *Laminaria saccharina*. Rate of uptake by whole plants exposed to 0.75 ppm Cd. Concentration in tissue = ng Cd (mg dry weight tissue)$^{-1}$. One plant sampled at each time interval

Table 1. *Laminaria saccharina*. Photosynthetic carbon assimilation – in nmol CO_2 (100 mg dry weight)$^{-1}$ h^{-1} – by different parts of the plant, and effects of 24-h and 96-h treatment with various Cd concentrations

Condition	Base holdfast/stipe	Blade growing region	Blade distal part
24-h series			
Control	1748	1654	2150
0.16 ppm Cd	1559	1556	1978
0.28 ppm Cd	1453	1428	1601
0.70 ppm Cd	1385	1289	1445
1.07 ppm Cd	1175	1188	1175
1.51 ppm Cd	1258	1144	759
96-h series			
Control	1598	1407	2288
0.16 ppm Cd	1733	1368	1929
0.28 ppm Cd	1450	1215	1446
0.70 ppm Cd	1319	1112	998
1.07 ppm Cd	1155	927	776
1.51 ppm Cd	1082	828	523

Table 2. *Laminaria saccharina*. Light-independent (dark) carbon fixation – in nmol CO_2 (100 mg dry weight)$^{-1}$ h^{-1} – by different parts of the plant, and effects of 24-h and 96-h treatment with various Cd concentrations

Condition	Base holdfast/stipe	Blade growing region	Blade distal part
24-h series			
Control	165	346	258
0.16 ppm Cd	161	222	211
0.28 ppm Cd	126	167	231
0.70 ppm Cd	97	76	176
1.07 ppm Cd	95	114	141
1.51 ppm Cd	69	93	148
96-h series			
Control	186	313	202
0.16 ppm Cd	157	217	189
0.28 ppm Cd	134	103	184
0.70 ppm Cd	110	98	166
1.07 ppm Cd	76	90	129
1.51 ppm Cd	59	76	72

was also tested. The results are shown in Table 2. Control specimens showed the highest rates of dark carbon fixation in the growing zone (proximal part of the blade). In contrast to the results of photosynthetic carbon assimilation, this zone in particular is rather sensitive to Cd. The inhibition of dark fixation by Cd, seen also to a lesser extent in the distal part of the blade and in the holdfast region, proved to be time and concentration dependent as well (Table 2).

Pigment Content

Young sporophytes of *Laminaria* showed notable loss of pigment, particularly in the distal part of the blade, after some days incubation in sublethal Cd concentrations. Table 3 gives a quantification of this effect for various regions of young plants, which were also tested for their carbon assimilation potentials. It is obvious that differential sensitivity to Cd is exhibited by different thallus regions. Holdfasts and stipes seem to be rather unaffected even by Cd concentrations over 1.5 ppm and like the growing region (proximal half of the blade) are bleached only by relatively high Cd concentrations. The distal part of the young blade, on the other hand, is the most sensitive region of the sporophyte, losing over 90 % of its total pigment (chlorophyll *a* and fucoxanthin) at concentrations over 1.5 ppm Cd after 6 d.

DISCUSSION AND CONCLUSIONS

The concentrations of Cd tested in these experiments are all considerably higher than those found in the North Sea or in open-ocean waters. However, all these

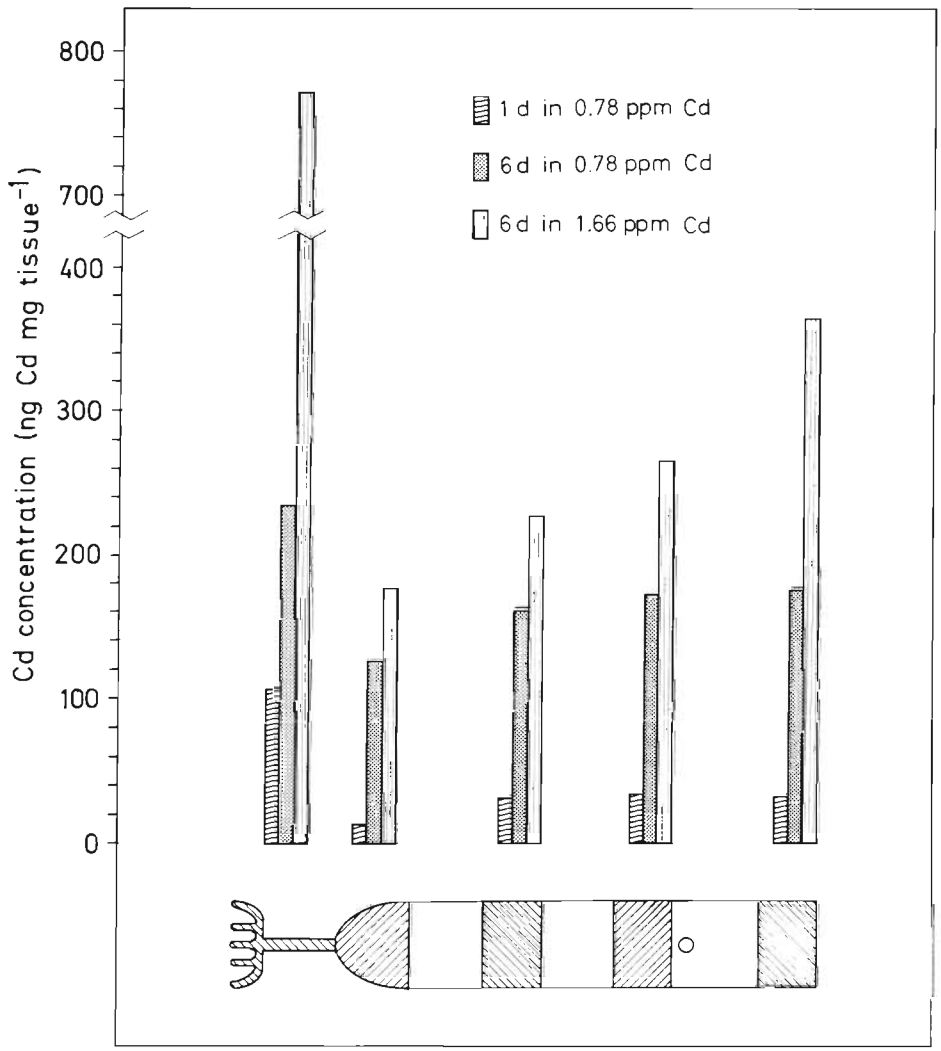

Fig. 6. *Laminaria saccharina*. Concentration of Cd, expressed as ng Cd (mg dry weight tissue)$^{-1}$ in different regions of a plant after 1 d and 6 d

Table 3. *Laminaria saccharina*. Pigment (chlorophyll *a* and fucoxanthin) content of sporophytes treated for 6 d with various Cd concentrations. Values expressed as μg pigment / 100 mg dry weight. Average values of three replicates

Conditions	Base holdfast/stipe		Blade growing zone		Blade distal part	
	chl *a*	fucox.	chl *a*	fucox.	chl *a*	fucox.
Control	80.1	26.9	139.6	32.6	203.8	65.4
0.23 ppm Cd	81.9	27.6	142.2	38.9	182.6	59.1
0.46 ppm Cd	79.0	26.2	144.2	39.5	196.2	63.6
0.80 ppm Cd	85.7	31.2	177.8	48.7	152.3	49.4
1.05 ppm Cd	82.4	27.1	128.3	35.0	103.1	33.4
1.66 ppm Cd	87.4	29.5	80.6	21.9	17.7	5.5

experiments were short-term experiments and designed to give an indication of the sublethal effects of Cd within a workable time span. As the plants were growing rapidly during the experiments, the results give a good indication of the effects of Cd. The rate of growth also necessarily limited the time which the experiments could be run under these conditions. Results of thinning in cultures where plants were grown to test size for the experiments reported here show that crowding of culture vessels inhibits growth greatly. These experiments indicate that this is true also when the plants are subjected to Cd pollution. Thus where plants were removed over the course of the experiment, growth of the remaining plants was greater than in the same Cd concentration when more plants were present. Previous studies and these experiments have shown that slower-growing plant parts take up more Cd. These experiments indicate that slower-growing plants as a whole also take up more Cd.

Experiments in which plants were exposed for various periods to Cd and then removed to grow further in clean water indicate two important points with regard to time. First, long-term after effects are far more serious than is apparent from measurements taken immediately after Cd exposure. Thus, after 14 d, the concentration producing a reduction in growth to 50 % of the control was less than half that required for the same effect in 6 d, although in both cases exposure to Cd lasted only 6 d. Secondly, time of exposure to Cd was more important in reducing growth than was the concentration to which the plants were exposed. Thus, a 1-d exposure to Cd at any concentration had not produced a significant reduction in growth when the plants were measured 2 weeks later, whereas plants exposed 6 d showed significant growth reductions at all concentrations tested. These results indicate that chronic low-level Cd pollution may be as serious in the long run as the higher concentrations employed here. It is probable that the lethal dose is far below 4.5 ppm, if it is applied over a longer time.

One visible symptom of Cd toxicity below 2.0 ppm was a decrease in growth, which corresponds to results reported for a variety of terrestrial plants (Haghiri, 1973; Turner, 1973; Lamoreaux and Chaney, 1977). Another symptom of Cd effects is a significant dose and time-dependent reduction of both photosynthetic as well as light-independent carbon assimilation rates with different sensitivity in different regions of the test plants (Tables 1 and 2). Inhibitory effects on algal photosynthesis by several heavy metals including Cd were earlier reported by Overnell (1976) for a variety of unicellular marine algae.

A third symptom, the distal loss of pigment, which occurs particularly at higher concentrations, is a curious feature. Pybus (1973) reported a similar effect of detergent on *Laminaria*, but did not mention a sharp boundary to the unpigmented zone, as observed here. It was reported that soybeans show chlorosis of the younger leaves at higher Cd concentrations (Haghiri, 1973), but in contrast, the distal portions of the *Laminaria* blades which show chlorosis are older rather than younger. In lethal concentrations, the pigment loss extended down into the meristematic zone, leaving only the stipe still pigmented. We have shown, however, that the stipe accumulates the greatest amount of Cd. The pigment loss is thus not correlated with accumulated Cd content. A comparison of Tables 1 and 3 shows that assimilation-rate depressions are also not well correlated with pigment decay. This may indicate that a decrease in the photosynthetic potential (Table 1) of Cd-polluted plants is not caused primarily by pigment loss, but is probably due to other effects. The same is true for dark carbon assimilation, since the enzyme responsible for light-independent carboxylation, phosphoenolpyruvate carboxykinase, is located outside the chloroplasts.

The results here agree with previous reports that the uptake of Cd is not regulated and leads to high concentration factors. These short-term experiments did not give an indication of the time to reach equilibrium.

Bryan (1969), working with pieces of *Laminaria* exposed to Zn, reported that after 30 d plant pieces were still not in equilibrium with their environment. As Cd is chemically related to Zn in many ways, the time required for uptake may be similar. As equilibrium was not reached, no figures can be given at this time for the potential concentration of Cd which could be accumulated by *Laminaria*, but it is obviously high.

These experiments suggest that the use of field-collected plants of *Laminaria saccharina* as indicators of environmental Cd pollution could be a promising method. The concentration of Cd in the *Laminaria* tissues is higher where the environmental concentrations are higher and the plants integrate Cd over an entire period of exposure because uptake begins almost immediately. As the concentration factors are high, even small amounts of environmental Cd can be detected. On the basis of available data, there is no evidence that Cd was lost from plants after they were returned to clean water, but this should be tested further. If there is indeed no loss, this would agree with other authors and is another point in favor of *Laminaria* plants as possible pollution indicators. Caution should be exercised, however, in interpreting the results quantitatively, as there are several potential sources of error. Although no Cd may be lost from living plants, above a certain size, *Laminaria* plants slough off distal tissue as they grow. As the distal tissues were shown to contain the second highest concentrations of Cd after the stipe, this loss of tissue could seriously affect estimates of total Cd taken up. As shown in this study and pointed out earlier by Bryan (1969), uptake to equilibrium is slow and thus rapid environmental fluctuations would not be indicated. Fuge and James (1974) as well as Morris and Bale (1975) have reviewed several sources of error in using brown algae as indicators. There are marked seasonal changes in concentration within the same species, as would be expected, since this study has shown that the rate and amount of uptake depends on growth rate. Fuge and James (1974) further noted that older plant tissues contain higher concentrations, as has been shown in this study as well. Considering all these factors, and in agreement with Bryan and Hummerstone (1973), *Laminaria* plants should be useful as indicators of changes, if entire plants of the same age, or the same portions of such plants, are collected for analyses on the same site in the same season in subsequent years. A test of this method, taking into account the data in this study, remains to be carried out.

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