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Inhibition of Marine Algal Photosynthesis by Heavy Metals

J. Overnell

Institute of Marine Biochemistry; Aberdeen, Scotland

Abstract

The maximum rate of light-induced evolution of oxygen by suspensions of cells of the unicellular marine algae *Attheya decora, Brachiomonas submarina, Dunaliella tertiolecta, Isochrysis galbana, Monochrysis lutheri, Phaeodactylum tricornutum* and *Skeletonema costatum* was measured. With this bioassay the relative sensitivities of these species of algae towards Cu, Hg, Cd and Zn were compared with that towards the herbicide 3(3,4-dichlorophenyl)-1,1-dimethyl urea. The algae were uniformly sensitive to low concentrations of DCMU; *S. costatum* and *A. decora* were especially sensitive to Cu and Hg ions. In contrast, *D. tertiolecta* was remarkably insensitive to Hg. These results suggest that *A. decora* could be used as a sensitive indicator of the quality of estuarine water, and a bioassay system based on this is proposed.

Introduction

The possible deleterious effects of heavy metals, released by man's activities, on the primary producers in the food chain must be a cause for concern. Indeed much useful work investigating these effects in the marine environment has already been reported and this has recently been reviewed by Rice *et al.* (1973).

The choice of conditions and methods for assaying the toxicity of heavy metals to marine unicellular algae is difficult, however, because each suffers from some serious drawback. While the most obvious parameter to measure is growth, natural seawater is found to have a variable ability to support growth and to complex heavy metals (Johnston, 1964). If artificial media are used, the added chelators (usually EDTA) which are needed to complex trace metal nutrients, especially iron, swamp the toxic effects of small quantities of added heavy metals. If chelators are omitted, growth is only possible during the first week after making up the medium during which time the concentration of iron (and perhaps other metals) falls to levels insufficient to support growth (Davey *et al.*, 1973). Moreover, in growth experiments marine algae would be expected to liberate products capable of forming complexes with heavy metals. Thus, unicellular algae have a tendency to release a considerable proportion of the carbon they fix into the medium in the form of organic compounds (Hellebust, 1974). In addition, hydrogen sulphide, thioethers and mercaptans are all known to be evolved by unicellular algae (Ishida and Kadota, 1967). Of the strains used here, *Attheya decora* produces a strong smell of dimethyl sulphide and *Dunaliella tertiolecta* a smell of hydrogen sulphide.

As well as measurements of growth, measurements of photosynthesis have been made, either by ^{14}C bicarbonate incorporation or by evolution of oxygen. Here, the tacit assumption is made that reduction of photosynthesis is the primary effect of the toxin or rapidly reflects the toxic effect.

In this case the problem of chelating agents can be eliminated by resuspending the cells in a fresh medium that is not required to support growth, i.e., free of chelating agents and metals. Of necessity these experiments have to be short-term, which indeed is one of their attractions, and so any long-term effects of the metals at the concentration tested could be missed. Thus, assays based on photosynthesis and on growth are essentially complementary.

The purpose of this paper is first to compare the sensitivities of photosynthesis of several species of algae to the herbicide 3(3,4-dichlorophenyl)-1, 1-dimethyl urea and heavy metals under standard conditions of short-term exposure and to compare the results obtained with previously published results on the inhibition of growth by these substances. The reason for doing this is that photosynthesis measurements are more easily made, and so if there is good correlation between these measurements and growth measurements then photosynthesis experiments could be used exclusively as a rapid screening technique. The second purpose is to use the results obtained to propose a quick and simple bioassay based on photosynthesis measurements and to identify possible limitations.

Materials and Methods

Abbreviations

The abbreviations used through this paper are as follows: DCMU: 3(3,4-dichlorophenyl)-1, 1-dimethyl urea; HEPPS: N-2-hydroxyethylpiperazine-N'-3-propane sulphonic acid; MOPS: morpholino-3-propane sulphonic acid.

Experimental Organisms

Attheya decora, Isochrysis galbana, Monochrysis lutheri and *Phaeodactylum tricornutum* were kindly donated by Dr. M.R. Droop, Dunstaffnage Marine Research Laboratory, P.O. Box 3, Oban, Argyll, Scotland, and *Skeletonema costatum* Skel-5 and *S. costatum* Skel-O by Dr. S. Myklestad, Institute of Marine Biochemistry, University of Trondheim, N-7034 Trondheim NTH, Norway. *Brachiomonas submarina* Strain 7/1a was obtained from the Culture Collection of Algae and Protozoa, 36, Storey's Way, Cambridge, England. *Dunaliella tertiolecta* Strain no. 999 was obtained from the Culture Collection of Algae at Indiana University, Department of Botany, Bloomington, Indiana 47401, USA. The *D. tertiolecta* was freed of bacteria by treatment with benzylpenicillin and streptomycin, and a bacteria-free culture has been deposited with the Culture Collection of Algae and Protozoa, Cambridge.

Culture Conditions

The organisms were grown in the S50 medium of Droop (1958) as described previously (Overnell, 1975c). For cultures of diatoms, SiO_2 was added, 3 mg/l dissolved in NaOH. Air without added CO_2 was used exclusively.

Cell Suspensions

The harvested cells were washed twice before use with a medium containing the major ions of the growth medium in the concentrations present in the growth medium, i.e., 0.26 M NaCl, 0.0123 M $MgCl_2$, 2.9 mM $CaSO_4$, 5.4 mM KCl. These were buffered at either pH 8.0 ± 0.1 or 7.0 ± 0.1 with HEPPS and MOPS respectively, 0.01 M.

Chlorophyll *a* was determined spectrophotometrically using the parameters of Ziegler and Egle (1965) in an 80% (v/v) acetone extract. It was found, however, that 80% acetone did not extract all the chlorophyll from *Phaeodactylum tricornutum* and *Isochrysis galbana*. For chlorophyll *a* determinations of these species the cell suspensions were disrupted in a French press prior to extraction (*P. tricornutum* and *I. galbana*) or alternatively the pellet from the 80% acetone extraction was re-extracted with 100% acetone and the supernatant diluted to 80% with water (*I. galbana*). The optical density of the second extraction was then added to that of the first. To facilitate routine assays, a calibration curve was constructed of absorbance at the red maximum (674 or 678 nm) for whole cells against weight of chlorophyll *a* per ml determined from the acetone extracts. Because of light-scattering due to the whole cells, the cuvette holder near the photomultiplier was used (Unicam SP 1700) and a straight-line graph of absorbance versus concentration was obtained. The slopes varied considerably from species to species.

Preparation of Suspensions of Attheya decora in Metal Free Medium

A column of Chelex 100, sodium form, was prepared as described by Davey *et al.* (1970). The pH 8.0 (HEPPS) medium was passed through this column until the effluent was pH 8.0. The next 500 ml to be eluted was collected and stored in a covered washed 600 ml polypropylene beaker. Cells of *Attheya decora* grown in the usual way were washed twice with the heavy metal free medium and then resuspended (at 5 µg of chlorophyll *a* per ml) in the metal free medium and also in the untreated original medium. Cell suspensions (5 ml) were pipetted into washed Bio-Cult clear plastic test tubes, 16 x 125 mm and incubated at 1000 lux for 24 h.

Measurement of Light-Induced Evolution of Oxygen

This was carried out as described earlier (Overnell, 1975c).

Measurement of ^{14}C Bicarbonate Uptake

To the suspension of cells was added carrier bicarbonate plus ^{14}C bicarbonate to give 10 µ moles, 1 µCi in each 5 ml aliquot. These aliquots, in stoppered clear plastic tubes, were illuminated at 2900 lux, 10 cm from the front of a 20 W warm-white fluorescent tube for 30 min. The contents were then filtered through Whatman GF/C glass-fibre papers, the papers washed with non-radioactive (bicarbonate-free) medium, and counted in a scintillation counter using Instagel scintillation fluid.

Heavy Metal Pre-incubation

The photosynthesis measurements were preceded by a period of incubation with the heavy metals (or herbicide) under a variety of conditions, but all at room temperature (18° to 20°C). These conditions were darkness, 1000 or 2900 lux for 15 min, 4.5 or 24 h. The conditions used for each experiment are given in Table 1 and in the legends to the figures. The medium used was the same as that used for photosynthesis measurements, with the exception that the bicarbonate was added 5 min before the start of the photosynthesis measurements. At this time the cell suspension was also transferred to the Clark electrode.

Chemicals

DCMU was obtained from Pfaltz and Bauer and recrystallized before use. The buffers HEPPS and MOPS appeared suitable for this study because of their chemical similarity to the buffers studied by Good *et al.* (1966) which showed very small or negligible stability constants with Ca, Mg, Mn and Cu. HEPPS and MOPS were obtained from British Drug Houses. Bicarbonate ^{14}C was obtained from the Radiochemical Centre, Amersham, England. $HgCl_2$, $CuSO_4$, $Cd(CH_3COO)_2$ and $ZnSO_4$ were the forms of the heavy metals used. These and the common reagents were AnalaR grade. Heavy metals were used from stock solutions made up in 0.1 N HCl.

Results

Inhibition of Photosynthesis after 15-min Incubation

For all species used, the effect of heavy metals on light-induced evolution of oxygen was first studied using the standard conditions of 15 min dark pre-incubation with pollutants. DCMU, Cd^{2+} and Hg^{2+} were incubated with the cells at pH 8.0 to represent the pH of sea water. Initial experiments indicated that high concentrations of Cu and Zn produced precipitates of their hydrated oxides at pH 8.0. For these metals the inhibition experiments were conducted at pH 7.0. The results were plotted on semi-log paper as in Overnell (1975a, b). The curves were examined, and the concentration of the metal required to reduce the net rate of O_2 evolution to 50% of the control is recorded in Table 1 as the I_{50} value. Table 1 also records the absolute value for the control rates of O_2 evolution and the concentration of chlorophyll *a* per unit absorbance of whole cells. For metals showing less than 50% inhibition over the concentration range studied, the concentration giving rise to 80% of the control activity is quoted as I_{80}. Typical sigmoidal inhibition curves were obtained for most algae and most metals, and the curves for *Attheya decora* are given in Fig. 1A as representative of other algae. The results obtained for treatment of all the algae with copper were somewhat anomalous even at pH 7.0, in that at the highest concentrations the inhibition is reduced. Results for *Isochrysis galbana* do not lend themselves to tabular presentation of I_{50} values since after the 15-min exposure the curves for the rates of O_2 evolution level off at about the 50% value. For this reason the complete plotted results are given in Fig. 1B.

The I_{50} for *Dunaliella tertiolecta* with mercuric chloride is 1×10^{-4}M. This is somewhat higher than for *Brachimonas submarina* at 4×10^{-5}M, and much higher than for all other algae which range from 1×10^{-6} to 3×10^{-6}M. For copper, *D. tertiolecta* showed a smaller I_{50} of 7×10^{-5}M which, in this case, is similar to the I_{50} values for all the other algae except *Phaeodactylum tricornutum* for which little or no effect could be found. For DCMU, however, there is a remarkable consistency of results, with I_{50} for all the species ranging from 3×10^{-8} to 1×10^{-7}M.

Inhibition of Photosynthesis after Increased Times of Incubation

Since previous work (Overnell, 1975b) on the action of copper on *Phaeodactylum tricornutum* had indicated O_2 evolution to be more sensitive to copper than was observed in the present 15 min experiments, the time of pre-incubation was increased. The results of incubations for 15 min

Table 1. Rates of photosynthesis, concentrations of chlorophyll *a* per unit absorbance and inhibition (*I*) of the photosynthesis of 7 strains of algae by DCMU and heavy metals. The photosynthesis was measured by light-induced evolution of oxygen unless otherwise stated, after a 15 min dark pre-incubation with the inhibitor. Inhibition results for *Isochrysis galbana* were not applicable to tabular presentation, na; and some positions in the table were not measured, nm. When activity was >80% of the control at 10^{-3}M the inhibitor was judged to be non-toxic, nt. SD: standard deviation

Species	Rate of photosynthesis in absence of pollutants [μ mole O_2 min^{-1} (mg chlorophyll a)$^{-1}$, mean ± SD]	Concentration of chlorophyll *a* per unit absorbance (A) at λ_{max} μg chlorophyll a ml^{-1} A^{-1} (λ_{max} in nm in parenthesis)	I_{50} values in molarity (number of measurements in parentheses)				
			DCMU	Hg^{2+}	Cu^{2+}	Cd^{2+} I_{80}	Zn^{2+} I_{80}
Attheya decora	3.61±0.54	19.9 (674)	$3x10^{-8}$ (14) $3x10^{-8}$ (10)[a]	$2.5x10^{-6}$ (12) $1x10^{-6}$ (19)[a]	$7x10^{-5}$ (8)	nt (2)	$6x10^{-4}$ (4)
Brachiomonas submarina	4.93±0.42	18.3 (678)	$4x10^{-8}$ (9)	$4x10^{-5}$ (7)	$2\text{-}5x10^{-5}$ (10)	nt (2)	nm
Dunaliella tertiolecta	2.39±0.21	15.8 (678)	$1x10^{-7}$ (13)	$1x10^{-4}$ (12)	$7x10^{-5}$ (11)	nm	$1x10^{-3}$ (3)
Isochrysis galbana	2.79±0.58	11.3 (678)	na	na	na	na	na
Monochrysis lutheri	1.97±0.30	14.6 (678)	$7x10^{-8}$ (9)	$1x10^{-6}$ (8)	(80% value) ~$2x10^{-4}$ (4)	nt (2)	$2\text{-}3x10^{-5}$ (4)
Phaeodactylum tricornutum	1.86±0.34	12.5 (674)	$1x10^{-7}$ (7)	$2\text{-}3x10^{-6}$ (12)	nt (9)	nm	nt (3)
Skeletonema costatum Clone Skel-5	3.26±0.85	12.0 (674)	$7x10^{-8}$ (9)	$2.5x10^{-6}$ (10)	$5x10^{-5}$ (14)	nt (2)	nt (2)

[a]Measured by ^{14}C bicarbonate uptake, 15 min dark pre-incubation.

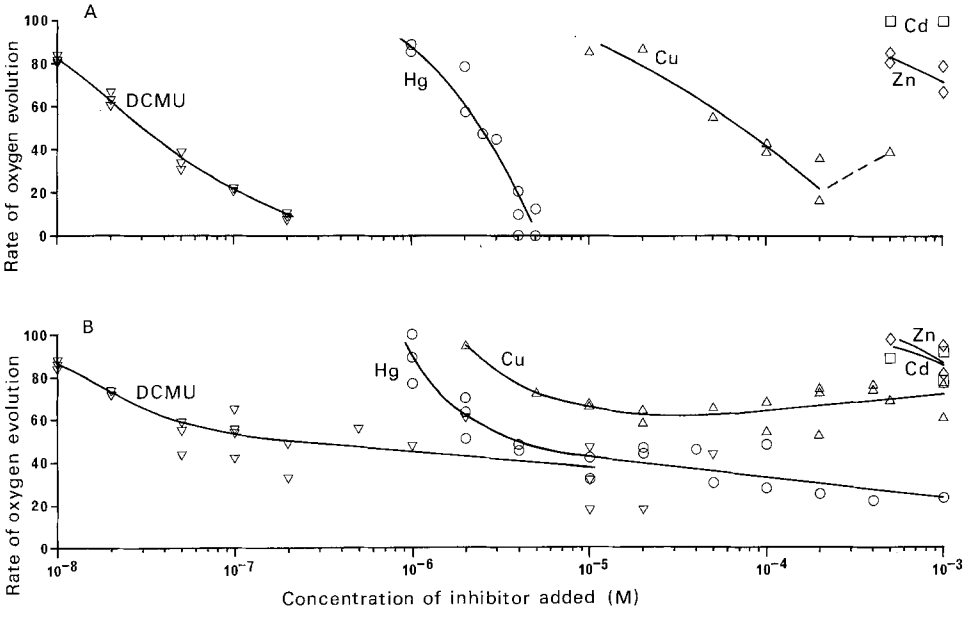

Fig. 1. Light-induced evolution of oxygen expressed as percentage of control value, as function of inhibitor concentration; in (A) *Attheya decora*, and (B) *Isochrysis galbana*. 5 μg chlorophyll *a*/ml; 15-min incubation in the dark with inhibitors as shown

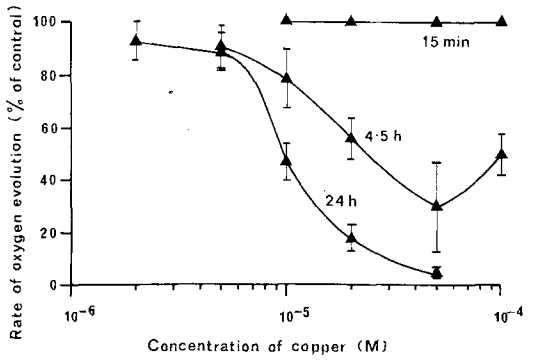

Fig. 2. *Phaeodactylum tricornutum*. Light-induced evolution of oxygen expressed as percentage of control value, as function of copper concentration. 5 µg chlorophyll *a*/ml; 15-min incubation in the dark, 4.5 h dark and 4.5 h light at 2900 lux (values for the two conditions indistinguishable and therefore combined), and 24 h incubation at 2900 lux. Bars indicate standard deviations

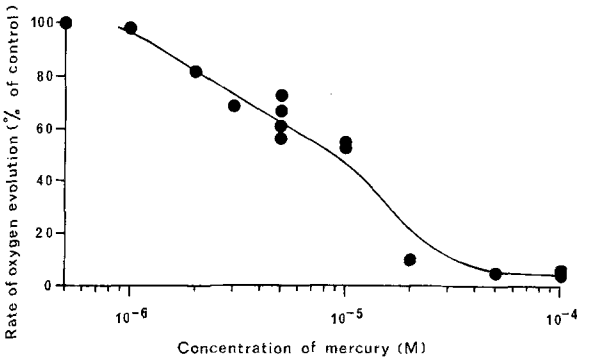

Fig. 3. *Isochrysis galbana*. Light-induced evolution of oxygen expressed as percentage of control value, as function of mercuric concentration. 5 µg chlorophyll *a*/ml; 24 h incubation at 1000 lux

(dark), 4.5 h (dark), 4.5 h (light) and 24 h (light) are compared in Fig. 2. The increased time of incubation gave rise to a more pronounced inhibition of photosynthesis. There was no apparent difference between the curves for 4.5 h light and 4.5 h dark.

In Fig. 1B we see a curious apparent lower limit to the effect of all the pollutants added to *Isochrysis galbana* of about 50% of the control activity. This effect was investigated in the case of mercury. The time of incubation was increased to 24 h in a light regime of 1000 lux. The result of this is illustrated in Fig. 3. Although the lower limit of activity has been reduced to nearly zero, the first part of the inhibition curve remains the same.

Effect of Cell Concentration on I_{50}

The effect of varying the cell concentration on the sensitivity of the algae is important for determining the best concentration of cells needed for an assay of polluted water. For these experiments, the algae *Brachiomonas submarina* and *Attheya decora* were chosen because they provided a high output of oxygen per unit of chlorophyll. The I_{50} values were determined, with 15 min dark pre-incubation, for inhibition by mercury:

Brachiomonas submarina, chlorophyll *a* concentration = 0.4 µg/ml, I_{50} = 1.1 x 10^{-5}M; *Attheya decora*, chlorophyll *a* concentration = 0.5 µg/ml, I_{50} = 1.3 x 10^{-6}M.

Extrapolation to zero chlorophyll concentration was carried out in the following way: Let C = concentration of metal added, Let c = concentration of metal in solution at end of incubation, Let ^{c}algae = concentration of metal absorbed by algae, let (algae) = concentration of algae. Thus, $C = c + {}^{c}\text{algae}$. Now for 50% inhibition $\frac{^{c}\text{algae}}{(\text{algae})}$ = constant. Therefore C = (algae) constant + c; i.e., in a plot of C versus (algae), c is determined by the intercept on the C axis.

Using the I_{50} values above and those in Table 1 (5 µg of chlorophyll *a* per ml) gave the Hg concentrations at zero chlorophyll concentration of 1 x 10^{-6}M for *Attheya decora* and 9 x 10^{-6}M for *Brachiomonas submarina* for 50% inhibition.

Exposure of Attheya decora Cells to Metals and DCMU at 1000 lux for 24 h, 0.5 µg Chlorophyll a/ml

Using the conditions of the proposed assay (see "Discussion"), cells of *Attheya decora* were incubated at room temperature (ca. 20°C) for 24 h in light of 1000 lux (warm-white fluorescent tube) at a concentration of 0.5 µg chlorophyll *a*/ml. This was concentrated by centrifugation to 2 µg chlorophyll *a*/ml, and light-induced evolution of oxygen was measured. The results obtained are summarized below:

Herbicide/metal	DCMU	Hg^{2+}	Cu^{2+}
I_{50}	3 x 10^{-8}M	5 x 10^{-7}M	3 x 10^{-6}M

Comparison of O_2 Evolution with ^{14}C Bicarbonate Uptake

In order to test the hypothesis that the inhibitory effects on light-induced evolution of oxygen and on ^{14}C bicarbonate uptake are the same, experiments were conducted with *Attheya decora* and DCMU and $HgCl_2$. The results of the inhibition of ^{14}C uptake were plotted in the same way as for the oxygen evolution experiments, and the results are included in Table 1. For DCMU the agreement was good, but for $HgCl_2$ the ^{14}C uptake method gave an apparently greater sensitivity than the O_2 evolution.

Zinc Tolerance of Skeletonema costatum

Two strains of *Skeletonema costatum* (Skel-O and Skel-5), which had been isolated by Dr. S. Myklestad, were shown to be zinc-tolerant and zinc-sensitive by Jensen and Rystad (1974). In order to determine whether or not this difference in the effect of zinc on the growth of the two strains was paralleled by differences in sensitivities of light-induced evolution of oxygen, I treated the cultures of these at pH 7.0 for 4.5 h in the dark with various concentrations of zinc before measuring the light-induced evolution of oxygen. Both strains were equally insensitive to zinc, giving rates of oxygen evolution of 72 and 61%, respectively of the controls at a zinc concentration of 10^{-3}M.

Effect of Prior Removal of Heavy Metals from Assay Medium

Davey *et al.* (1970) found a concentration of 2×10^{-7}M Cu^{2+} in a sample of freshly prepared artificial sea water. In order to determine whether heavy metals presumably present in my suspension medium were having a deleterious effect on the control rates of photosynthesis, cells of *Attheya decora* were washed twice in Chelex 100-treated medium. Five measurements were made of the light-induced evolution of oxygen by cells incubated in the Chelex 100-treated medium and also by cells incubated in the untreated medium for 24 h at 1000 lux. No real difference between the two could be detected: the control and the sample in the Chelex 100-treated medium gave values of 1.2 ± 0.06 and 1.08 ± 0.04 (mean $\pm$ standard deviation) mV/5 min output on the recorder attached to the oxygen electrode.

Discussion

Table 1 gives the observed rates of photosynthesis per unit chlorophyll *a* in saturating red light. Maximum rates of photosynthesis may be slightly higher, since the oxygen electrode cell used was not completely and uniformly illuminated. The chlorophyll *a* concentration per unit absorbance at the red maximum is also given. For diatoms this maximum appears at 674 nm and for the other algae at 678 nm. The differences in the measured chlorophyll *a* contents may be partially due to shading within the cell in the case of the larger cells. The differences in the rates of photosynthesis probably reflect differences in the rates of the dark reactions to CO_2 fixation.

This study indicates that *Phaeodactylum tricornutum* is insensitive to copper after 15 min dark pre-incubation and has an I_{50} for Hg^{2+} of 2 to 3 x 10^{-6}M. In buffered sodium chloride only, the I_{50} values are 7 x 10^{-6} and 5 x 10^{-6}M for Cu^{2+} and Hg^{2+}, respectively (Overnell, 1975b). In contrast, *Dunaliella tertiolecta* has I_{50} values of 7 x 10^{-5} and 1 x 10^{-4}M for Cu^{2+} and Hg^{2+} in the half-strength sea water medium and 1 x 10^{-5} and 2.5 x 10^{-5}M in the sodium chloride medium (Overnell, 1975b). The anomalous result is the protection against copper poisoning afforded to the diatom *P. tricornutum* by the Ca^{2+}, Mg^{2+} and K^+ ions in half-strength sea water.

In many of the inhibition curves, copper effects are variable with anomalously high rates of O_2 evolution for copper concentrations in the range 1 x 10^{-4} to 1 x 10^{-3}M. This is presumably due to the formation of precipitates of hydrated copper oxide. It implies that some of the points on the curves were obtained with supersaturated solutions of copper.

Mandelli (1969) measured the effect of copper on growth of axenic cultures of 6 species of unicellular marine algae in continuous light. *Dunaliella tertiolecta* grown at 30°C at a concentration of 5 x 10^4 cells/ml was the only species still capable of growth at a copper concentration of 7.7 x 10^{-6}M, whereas growth of *Skeletonema costatum* at 20° to 30°C at a concentration of 3 x 10^5 cells/ml was inhibited in the range 3.8 to 2.5 x 10^{-6}M; *S. costatum* grown at 18°C in a light-dark cycle was found to be more sensitive. Davies (1974) found that growth of *D. tertiolecta* was little affected by 5 x 10^{-6}M Hg^{2+}, whereas *S. costatum, Phaeodactylum tricornutum* and *Isochrysis galbana* were increasingly sensitive to mercury. 1.5 x 10^{-7}M Hg^{2+} was

lethal to *I. galbana*. However, it may be that the tolerance of *D. tertiolecta* is an artefact due to release of hydrogen sulphide into the medium giving rise to a concentration of hydrogen sulphide greatly in excess of that found in nature. This could give rise to precipitation of insoluble sulphides of mercury and copper in the medium. The present work eliminated this possibility by using freshly washed cells, and we see from Table 1 that *D. tertiolecta* is in fact still much more tolerant than most of the other algae towards Hg, in fact more so by a factor of about 20. With copper this is not the case, nor is it the case with DCMU. There would appear to be a particular resistance to mercury in this species. The lack of a differential effect with copper found here and in Mandelli's experiments could be explained by detoxification of the copper in the growth medium in the case of Mandelli's experiments or too short a time of incubation with my experiments. Clearly, the relative rates of uptake and distributions of the metals need to be examined.

Increasing the time of exposure of *Phaeodactylum tricornutum* to copper from 15 min to 24 h resulted in a typical sigmoidal curve with an I_{50} of 10^{-5}M. This is similar to the value of 7×10^{-6}M for *P. tricornutum* in sodium chloride medium (Overnell, 1975b). Thus the result of the Ca^{2+}, Mg^{2+} and K^{+} ions is apparently to slow the rate of inactivation. It would be interesting to study the time course of uptake of copper for the purpose of comparison, and such experiments are in progress. In the case of *Isochrysis galbana* and mercury poisoning, a plateau at about 50% of the control value is seen for a 15 min incubation. This is largely abolished after a 24 h incubation and a full sigmoidal curve is obtained. However, for the portion 100% of control to 50% of control there is no displacement to lower copper concentrations, i.e., in this range increasing the time of incubation from 15 min to 24 h does not increase the sensitivity. The plateau implies more than one site of action and also a concentration-independent effect. One possibility for this would be the slow transport of bound copper from an insensitive (external) site to a sensitive (internal) one.

It appears that the effect of zinc on the photosynthetic apparatus of the two strains of *Skeletonema costatum*, Skel-5 and Skel-0, is virtually the same and also is small. This should be compared with the extreme sensitivity of growth of Skel-5 to zinc (Jensen and Rystad, 1974). It would appear that zinc exerts its toxic effect on some part of cell metabolism remote from photosynthesis, for example cell division.

If photosynthesis is to be measured for the purpose of monitoring waters for heavy metal contamination, a decision must be made between using ^{14}C bicarbonate uptake and oxygen evolution. This may be determined by the availability of apparatus. The two methods give essentially the same results for DCMU inhibition in *Attheya decora*, but slightly different I_{50} values for Hg^{2+} inhibition (Table 1).

Of the species tested here, the two diatoms *Skeletonema costatum* and *Attheya decora* would appear to be the most generally sensitive. *A. decora* is easier to culture and gives somewhat more consistent and higher rates of photosynthesis per unit chlorophyll. *A. decora* may be pelagic or littoral, although it is only rarely observed. It is found only in inshore waters whose salinities may vary from 5 to 30% (Edler, 1975). It should thus be an ideal organism for studying polluted marine waters, since it is likely that these would be restricted to estuaries and enclosed bays. For a bioassay of possible polluted waters I suggest that a culture of *A. decora* grown in a medium having the same salinity as the suspected water is suspended in a filtered sample of this water and incubated for 24 h at a light intensity of 1000 lux. Occasional agitation would be necessary, since the cells tend to settle. A concentration of 0.5 µg chlorophyll *a*/ml would be suitable since, at least for mercury, there is very little potential increase in sensitivity to be gained by reducing the concentration further. For measurement of oxygen evolution the cells could be concentrated by centrifugation before measurement. A trial using these conditions was carried out and the method was found to be satisfactory, although centrifugation of the copper-treated cells proved difficult and so oxygen evolution of the unconcentrated suspension had to be measured. This trial showed that compared with the 15 min incubation at 5 µg chlorophyll *a*/ml, the I_{50} for DCMU inhibition under the above conditions remained unchanged, suggesting that the uptake of DCMU is small and rapid, but that I_{50} for mercury and copper inhibition was reduced as expected.

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Dr. Julian Overnell
Institute of Marine Biochemistry
St. Fittick's Road
Torry, Aberdeen AB1 3RA
Scotland

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