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EFFECTS OF SALINITY AND TOXIC HEAVY METALS ON OXYGEN RELEASE BY *FUCUS VESICULOSUS* L.

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Continuous measurements with an oxygen electrode applied to thalloid algal material were used to determine O_2 production/consumption at light and dark phases in an open system. The balance of photosynthesis-related O_2 release by *Fucus vesiculosus* L. in seawater shows a decrease within only 20 min. after addition of $Cu(II)SO_4 \cdot 5\,H_2O$ at concentrations known to be toxic. Short response time and continuous recording allowed to show that in the case of $ZnCl_2$ addition, the increase in O_2 release is followed by a decrease. Effects of interactions between zinc and copper compounds and seawater salinities can be recorded in the same way.

INTRODUCTION

Toxicity of heavy metals is an intensively explored field in algological and ecophysiological research [for a survey see Round (1985) and Schlee (1986)]. When selecting algae as test organisms in marine or brackish water ecosystems, much more work seems to have already been done on microalgae because of the use of the existing microbiological methods which can be applied to those organisms, particularly in cultures during long-term and growth experiments (cf. Kosakowska et al. 1987). On the other hand, macroalgae are considered difficult to handle and are therefore often disregarded. In their survey of bioindicators, Arndt et al. (1987) mentioned that macroalgae were occasionally viewed as unsuitable indicators, since tests showed responses indicating both sensitivity and lack of it, which is probably connected with the accumulation activities of the algal cells (Arndt et al. 1987).

An attempt is made to demonstrate that *Fucus vesiculosus,* an alga common in the Baltic Sea and growing within a wide salinity range is a suitable laboratory test organism for toxicity assays, one that may exhibit synergistic effects of salinity and toxic

heavy metals. A continuous measurement technique of recording relative O_2 concentration levels was applied to show also the time dependence and sequence of toxic effects. Thus an attempt is made to support recommendations for including *F. vesiculosus* and other macroalgae in the bioindicators check list, these photosynthetically active organisms offering valuable experimental and biological advantages.

MATERIALS AND METHODS

Fucus vesiculosus specimens were collected in Kiel Fjord, in the vicinity of Kiel-Friedrichsort where also the seawater was collected from the surface. Salinity of the water was determined twice a month within July 1988 – June 1989; each time salinity proved to be different, the values ranging within 11.8–20.30‰.

Within about 3 h from collection, the algae were transported wet (in plastic bags) and cooled to the laboratory where they were kept at 6°C in darkness, submersed in well-aerated seawater for not longer than 4 days.

Before assays, small (1 cm diameter) discs were cut off from the tips of the thalli at places where the algal surface was relatively sparsely overgrown by epiphytic organisms.

The dark/light bottle experiments were performed similarly to the A–Z test of Knöpp (Knöpp 1961). Oxygen measurements were carried out with WTW electrodes (Wissenschaftl.–technischen Werkstatten, Weilheim, BRD); reference values were measured in O_2 – saturated water of the sample. Salinity was determined conductometrically, using an electrosalinometer (Electronic Instruments, Chertsey, Kent, Great Britain).

Relatively small portions of the algae (discs), i.e., about 0.6 g wet weight/250 ml were tested, i.e. about 0.15 g wet weight/1000 ml reaction medium, in the continuous recording experiments.

The experimental procedure of the continuous oxygen measurement in the open system is as follows:

A single 1 cm diameter *Fucus* disc is fastened to the tip of the electrode in an open plexiglass chamber; the electrode is placed in a 1000 ml open glass flask, incubated in 14°C water bath. A 60 W Osram bulb served as the light source. The O_2 release was continuously recorded by an electric compensation recorder (Servogor S). The constant water movement inside the flask was provided by using a magnetic stirrer (Plötz 1975).

The detectable O_2 release activity of each *Fucus* disc had to be calibrated prior to each experiment because oxygen concentration levels at dark and light periods would often differ initially, due to biological properties of the algal material and the physical and chemical properties of the measurement system.

The experiment can be started once a constant balance between oxygen consumption and production of at least 3 mg O_2/dm^3 (relative oxygen concentration) has been reached. To test the biological activity, short dark/light phases of about 5–10 min. each are applied, whereby constant relative oxygent contents are being obtained for half an hour or more. As the oxygen data obtained reflect only a balance, it is possible to plot dark/light oxygen lines for e.g. 4 days or with permanent light phases of e.g. 12 days. In this way the "reference line" based on photosynthesis and respiration of the algal material is found in a short time (about half an hour) and intoxication or salinity effects can be subsequently recorded.

Long-term measurements are possible with *F. vesiculosus* for 4 days. Similar experiments were carried out on *Ulva lactuca* L. (Plõtz 1975); the method seems to be applicable both to algae from the open Baltic Sea and from rivers.

There was no water flow through the open system used. A 5% exchange of the incubation volume (1000 ml) is allowed; if it is higher, a new calibration is necessary.

Some of the samples tested were incubated in the Baltic seawater diluted with tap water. Other samples were only slightly altered by adding seawater from the Baltic or the North Sea (Wilhelmshaven, 31.5‰) to the water samples obtained on a day of algal collection.

The pH values of the reaction media, measured with a glass electrode, were between 7.3 and 7.8; the pure tap water had pH 8.1. Effects of pH on O_2 release are integrated into the calibrated balance between oxygen production and consumption, for which reason pH control in the experiments was not permanent.

When the balance shows a constant line, every loss in O_2 release detected after addition of heavy metals indicates toxicity. Toxic effects can always be reproduced. The continuous measurement method presented does not provide quantitative data; the results are possibly affected by copper complexing with organic ligands and by pH changes. The method presented facilitates demonstration whether toxicity at low concentrations of heavy metals and synergistic effects of the metals, salinity, and time take place. The toxicity indicated does not mean termination of a lowered O_2 production, neither does it deal with adaptation problems.

For the bottle test, an acutely toxic, high copper sulphate concentration was chosen to keep effects of pH and complexing ligands low.

RESULTS

The O_2 release of *F. vesiculosus* was determined in the dark/light bottle test (0.6 g algal wet weight/250 ml tap water, brackish, or seawater; 12°C; 13 h at permanent light). The results are given in Table 1. There was a reduction in O_2 release when the seawater was diluted, the release being stimulated by salinity exceeding about 18 ‰.

Table 1

O_2-release (mg O_2/dm^3) of *Fucus vesiculosus* at different seawater salinities, without heavy metal, and with 10 mg/dm^3 $Cu(II)SO_4 \cdot 5\,H_2O$ added

	Tapwater	Seawater (Baltic Sea)							Seawater (North Sea)
‰ :	1	3	5	10	15	18.4	20	25	31.2
mg O_2/dm^3:	1.5	1.2	1.5	1.6	2.2	2.4	2.7	2.7	2.9
mg O_2/dm^3: [$Cu(II)SO_4 \cdot 5\,H_2O$] [10 mg/dm^3]	0.6	0.7	0.5	0.8	1.2	1.3	1.9	2.7	2.2

Table 2

O_2-release (mg O_2/dm^3) of *Fucus vesiculosus* at different seawater salinities. Intoxication = bottom line, 1 mg/dm^3 $Cu(II) \cdot SO_4 \cdot 5\,H_2O$ added to seawater

‰ : Seawater (Baltic)	15	18*	25
mg O_2/dm^3:	1.5	2.0	2.0
mg O_2/dm^3: [$CuSO_4 \cdot 5\,H_2O$] [1mg/dm^3]	1.3	1.5	2.0

* salinity when sample was taken

Table 3

O_2-release (mg O_2/dm^3) of *Fucus vesiculosus* (without heavy metal). Bottom line: Intoxication is due to exposure of algae to 1 mg/dm^3 $Cu(II)SO_4 \cdot 5\,H_2O$ in the former experiment (the same algae used here; see Table 2 and text)

‰ : Seawater (Baltic)	18 (15)	18 (18)	18 (25)
mg O_2/dm^3	1.6	1.5	1.5
mg O_2/dm^3 [without $CuSO_4 \cdot 5\,H_2O$]	0.9	1.0	0.9

The oxygen concentration values (mg O_2/dm^3) are based on oxygen saturation of about 8.5 mg O_2/dm^3 seawater.

The bottom line of Table 1 shows detrimental effect of Cu(II)$SO_4 \cdot 5 H_2O$ at a highly toxic concentration (10 mg/dm^3) seen as a reduction in O_2 release in the corresponding salinities. This allows to infer that the intoxication is irreversible, perhaps also at lower copper levels. At higher salinities, there is still more oxygen in the sample, certainly because of the stimulation effect mentioned above.

To test these inferences, another bottle test was run, the results being shown in Tables 2 and 3. Initially, a normal activity of *Fucus* was measured under slightly altered salinities. The copper sulphate concentration was only 1 ppm here and the impairment of O_2 release was low or even undetectable (last column of Table 2: 2.0 mg O_2/dm^3 in both cases) after 11 h exposure. Subsequently, the same algal material was subject to another experiment (Table 3), with salinity of 18‰ only; the salinity values under which the algae were incubated formerly, are given in brackets. No toxic heavy metal was added now. The two tests were separated by a 10 h dark period at 12°C, without any Cu(II)$SO_4 \cdot 5 H_2O$ added; salinities applied were those given in Table 2.

The lowered O_2 release from 2 (Table 2) to 1.5 mg O_2/dm^3 (Table 3) is probably due to the alga storage conditions and the short dark period. However, it seems interesting that the formerly intoxicated algae (cf. Table 2) still showed a strong decrease in their oxygen release values, and the so far undetected toxic effect (cf. 25‰ column in Table 2) appears now in Table 3 as well, although the algae were "washed" durig the dark period and no new copper sulphate was added.

In the following, a few experiments on copper and zinc intoxication of *F. vesiculosus*, carried out with the use of the continuous measurement method described, are discussed.

Fig. 1. illustrates the pattern of dark/light intervals and calibrated O_2 balance. Constant levels have to be obtained before toxicity test measurements can be started.

Fig. 2 shows time courses of O_2 release after additions of different concentrations of Cu(II)$SO_4 \cdot 5 H_2O$ (1, 2 and 3). The additional toxic effect (2) is considered significant because there is no other deviation of this nature and it was reproducible. Slight decreases like that were irreversible, i.e. the reference line was never again reached after a decrease.

When zinc ($ZnCl_2$) was applied as the toxic metal, a timedependent increase in O_2 release could be seen (Fig. 3), followed by a significant decrease. If copper was added at this phase as well, no additional effects were observed in the recorded O_2 release; no additional effects could also be observed when the copper sulphate concentration was much higher than that of $ZnCl_2$ (10 mg/dm^3).

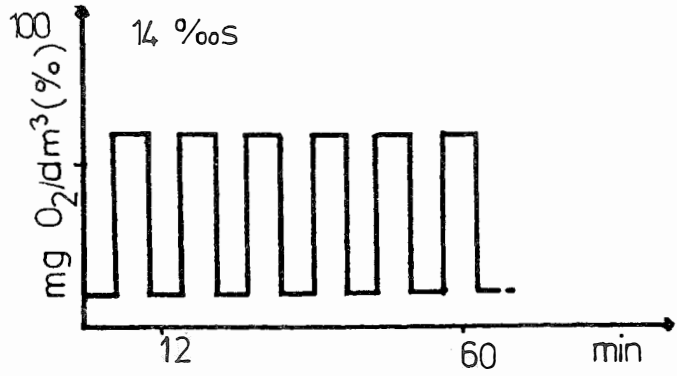

Fig. 1. Relative O_2-release of *Fucus vesiculosus* in short dark/light periods. Calibration of balance. (10 mg O_2/dm^3= 100%)

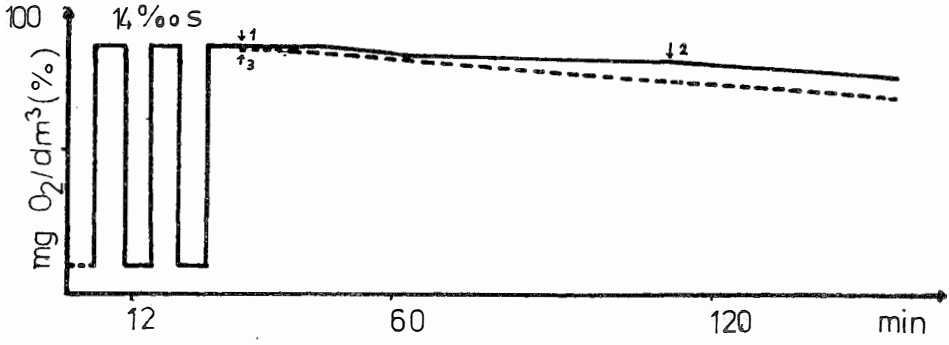

Fig. 2. Toxic effects and time-dependence on O_2-release of *Fucus vesiculosus*, ↓1) 0.5 mg, 2) 0.5 mg, 3) 10 mg $Cu(II)SO_4 \cdot 5\,H_2O$ is added per 1 dm^3 Baltic Seawater (10 mg O_2/dm^3 = 100%)

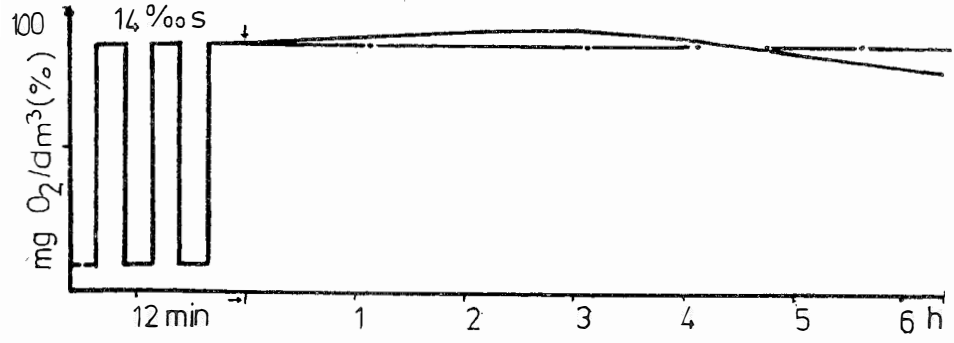

Fig. 3. Time-dependence and stimulated O_2-release of *Fucus vesiculosus*, when 10 mg $ZnCl_2$ (↓ upper line) is added per 1 dm^3 Baltic Seawater (10 mg O_2/dm^3 = 100%)

DISCUSSION

Salinity is in nature a very important factor ("master factor") for growth of widespread algae such as *F. vesiculosus* in the brackish Baltic Sea having many areas, e.g. the Kiel Fjord, of salinities constantly changing at unpredictable time intervals (Gessner 1955, 1959; Gessner and Schramm 1871). Stimulation and depression effects on photosynthesis and respiration rates are known (cf. Gessner and Schramm 1971). Photosynthesis-related O_2 release, basically important for algal growth, can be considered significant for growth and/or survival, also when it is necessarily recorded only as a balance value.

Copper is known to be essential for plant growth, but concentrations of about 0.3 ppm are considered algicidal. A zinc-copper antagonism has been reported as well (see Round 1985).

The present data from toxicity tests demonstrate the sensitivity of *F. vesiculosus* and applicability of the method. The sensitivity is time-dependent, but growth is not considered. A new emphasis is placed on the synergism with salinity; the synergism can now be determined using the continuous O_2 recording method. A large number of samples can easily be tested for the time dependence. After certain toxic events, non-toxic seawater (of different salinities) can result in a postponement of a total breakdown of the O_2 producing system because the algae no longer exhibit the normal salinity stress reaction patterns (cf. Tables 2 and 3).

At the first sight, the Baltic Sea water will probably have no such copper or zinc concentrations which could lead to a total breakdown of the *Fucus* O_2 production. Nevertheless, although the limit values for heavy metal pollution in aquatic ecosystems cannot be derived from the data presented, it is advisable to prevent any further pollution with heavy metals.

The experiments described were performed to show that *F. vesiculosus* and other macroalgae are useful test organisms for a rapid determination of the quality, time course and/or synergistic effects of acute toxicity events and for their prediction if the method is adjusted to the ecologically relevant algae.

Thus the experiments described may be of a practical interest to all those who are concerned with photosynthesis, the photosystem II and copper, or salinity– and cation-controlled processes. Additionally, perhaps one can speculate, based on the above, on experimental conditions for "wash-and-reload-models" in terms of salinity for sewage treatment in marine and brackish waters or salted rivers.

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