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Fluorometry - a method for ecotoxicological purposes

Introduction

Algae are often used for testing the toxicity of herbicides and for assessing water quality. The toxic action of a substance or water sample is generally measured by means of a growth test or by determining the rate of photosynthesis (maesurement of the effective concentration - EC_{50}).
Both tests involve considerable effort in terms of time and equipment. Fluorometry, on the other hand, seems to be a promising way of measuring toxic properties quickly and cheaply. This would be particularly true for in vivo fluorescence measurements, if it can be proved that they yield results are consistant with those yielded by growth and photsenthesis test.
Fluoremetric methods are based on the fact that the fluorescence of chlorophyll is effected by substances which suppress, partly or completely, the transport of electrons during photosynthesis.
Fluorometry has been in use for some time as a routine method for measuring biomass in laboratory cultures, and despite certain drawbacks it has also found application for in vivo biomass measurements on natural populations (OWENS et al. 1980, PARKER and TRANTER 1981). Although its value as a tool for ecotoxicological studies seams abvious, fluorometry has very seldom been used hitherto for such purposes as far as we know (LIVINGSTON 1977, LITTLE 1978, SASTRY and MILLER 1981, BENECKE et al. 1982). We have therefore studied fluorometry with the aim of developing a standard fluorometric screening method which can be used to assess both the acute (short duration) and chronic (long term) toxicities of substances.
The normal fluorescence of chlorophyll is equivalent to the biomass. Herbicides interrupt the photosynthetic transport of electrons, and the degree of fluorescence enhancement is approximately proportional to the inhibitory action of the active ingredient of the herbicide. The fluorescence enhancement induced by DCMU is equivalent to the potential photosynthesis. We have used this findings for the development of a fast screening method for ecotoxicological purposes described below.

Material and Methods

1 Algal species and cultures

Batch cultures of *Phaeodactylum tricornutum, Skeletonema costatum, Selenastrum capricornutum, Chlorella pyrenoidosa* and *Scenedesmus subspicatus* and chemostat cultures of *Chlorella pyrenoidosa* were used for the investigations. The principal invesitgations were performed on algae in the exponential growth phase because pilot studies had shown that responses to the test substances were greatly reduced when the experiments were performed on algae during the stationary growth phase (cf. also SAMUELSSON et al. 1978).

The algae were grown in continuous light in a medium of synthetic freshwater or nutrient enriched natural seawater at 20 °/oo salinity. The growth temperature was 20°C.

The biomass of the cultured algae samples were standardised (except for the toxicity tests with sewage samples, for which the algal biomasses were to low) by extinction measurements at 750, 680 and 530 nm in 5 cm cuvettes before the fluorescence measurements. The subsequent cell counts were done with a Coulter Counter model FN, and LORENZEN'S (1967) method was used for determination of the chlorophyll a concentration.

The algal material used for the fluorometric investigations was used in addition for measurements of growth and photosynthetic rates. The rate od photosynthesis was measured by the 14-C-method in a light incubator (GARGAS et al. 1976).

Phytoplankton samples collected from a small eutrophic pond were used for comparative studies. The dominant algal groups in these samples were the chlorococcal green algae, blue-greens and the euglenoid *Phacus.*

2 Fluorescence equipment and investigated substances

The fluorescence was measured with an Aminco solid state blank substract fluorocolorimeter 4-7440. The samples were excited by light at 436 nm after it had passed the Corning 4/7110 primary filter and aperture 4. All data were recorded on a W + W 1100 recorder (Struers).

The substances tested were 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU), the auxine herbicides 2,4-dichlorphenoxyacetic acid (2,4-D) and 4-chlor-3-methylphenoxyacetic acid (MCPA), and 4-nitrophenol (4-NP). The MCPA was on a water soluble from containing 16,8 % filler, and the 2,4-D was on a water soluble from (Herbatox DP 667) containing 33 % filler. The DCMU and 4 -NP were applied as pure substances. The concentrations tested ranged from 10^{-8} to 10^{-4} for DCMU, 0,1 - 500 μg ml^{-1} for MCPA, 0,02 - 5 μml^{-1} for 2,4-D and 0,05 - 51,0 μg for 4-NP.

The effects of waste water containing copper, organically loaded sewage and untreated waste water from a herbicide factory producing hormonal herbicides were also investigated.

3 Standardisation of the test procedure

The in vivo effects of herbicides and other substances depend not only on the pysiological statūs of the algae, but also on the species concerned, the used concentrations and the time of contact with the substances. This is shown in Fig. 1 for three of our test algae in the case of DCMU. *Skeletonema* responded most sensitively, as seen by the quick inhibition of the fluorescence enhancement at 10^{-4} M DCMU; but even 10^{-4} DCMU did not inhibit the fluorescence of *Chlorella* during the 10 minutes. Supplementary studies on *Scenedesmus subspicatus* and *Seleneastrum capricornutum* showed that the algae in our study has a more or less narrow range of DCMU concentrations at which fluorescence enhancement is at its optimum. Within this range, fluorescence reaches a quasi-steady state, under the weak light excitation conditions of our experiments, after about 5 minutes and then remains at a constant plateau level for the next 5 - 10 min. This observation serves as the basis for the herbicide screening test we have developed. The test procedure, which is described in the following, involves four fluorescence measurements.

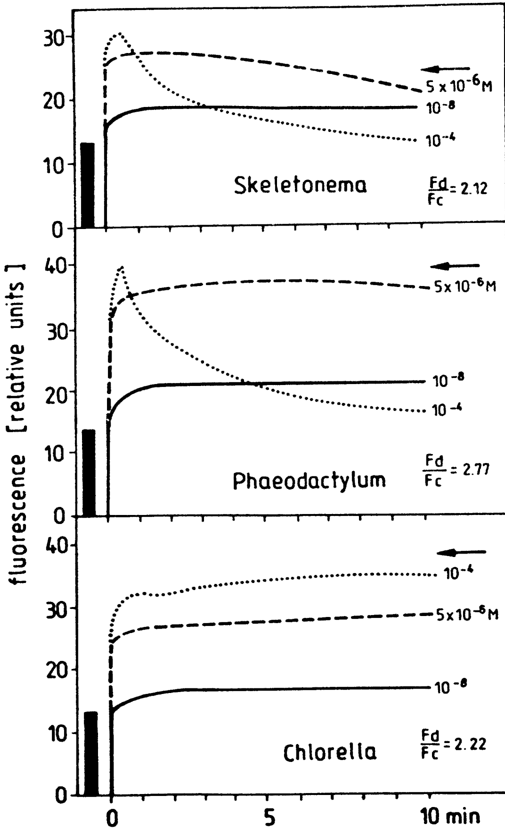


Fig. 1
Increase in fluorescence induced by 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) in various algae. Columns = chlorophyll fluorescence (Fc), curves = variation of fluorescence vs. time after addition of DCMU (Fd). Dotted arroes = steady state fluorescence after addition of 10^{-8} M DCMU: Fd/Fc ratio applies for 10^{-8}M DCMU. Representative example from five experiments

4 Used and recommended test procedure

a) Test algae are cultured under standardised conditions. The algae used for fluorescence measurements must be in the exponential growth phase. Natural algal populations must be tested as soon as possible after collection from the biotope, and great care must be taken to avoid harmful effects of transport.

b) The algal biomass used for the test must be standardised if cultured algae are used. This can be done by cell counts (e.g. with Coulter Counter) or, more simply, by measuring the extinction at 750, 680, 650 and 490 nm for green algae (instead of 650 nm, for diatom 580 nm (fucoxanthin) and 625 nm (phycocyanin) for blue-greens).

c) The background fluorescence of the media or, for natural phytoplankton samples, of the particle-free sample water must be measured. This also applies to the fluorescence of the test substances in alga-free medium or particle-free sample water. The values obtained during this step are needed to correct the subsequent fluorescence measurements.

d) The fluorescence of the untreated sample can now be measured (1st measurement: Fc valus = equivalent of biomass).

e) DCMU must be added to the sample to give the optimal concentration, and the DCMU induced fluorescence enhancement can be measured after 5 min contact with the DCMU (2nd measurement: Fd value = potential photosynthesis).

f) The test substance is now added to untreated samples to abtain the different test concentrations, and the corresponding fluorescence values can be measured after a contact time of 5 min (3rd measurement: Fh value = decoupling effect of test substance; short type measuring), or after longer periods of incubation (long type measuring).

g) The reference value relative to the action of DCMU alone must be measured after adding DCMU to give the optimum DCMU concentration in all samples loaded with the test substance. The DCMU induced fluorescence enhancement can again be measured after 5 min. of contact (4th measurement: Fdh). h) After the measured fluorescences have been corrected for background fluorescence of the medium and/or the intrinsic fluorescence of the test substance, the following ratios must be calculated:

- Fd/Fc = maximum potential photosynthetic activity per unit biomass;
- Fh/Fc = decoupling action of the test substance per unit biomass;
- Fdh/Fc = interaction between test substance and optimal DCMU concentration per unit biomass; and
- Fdh/Fh = relative influence of test substance on the maximum potential photosynthetic performance.

The value F/dFc = 1,0 is equivalent to a zero fluorescence enhancement.

The differences between the values

$$\frac{Fdh - Fh}{Fh} \quad \text{and} \quad \frac{Fd - Fc}{Fc}$$

are expressed as percentages in order to obtain the percentage inhibitation and enhancement respectively induced by the test substance relative to that induced by DCMU. If the values of the quotient Fdh/Fh are about 1,0, no algal growth can be expected. if they are less than 1,0 it can be assumed that the algae are in a damaged state.

We used a DCMU concentration of 10^{-6} M in all experiments. Since a concentration of ca. 10^{-4} M DCMU is necessary to obtain optimal fluorescence yields in the case of the green algae, the values of the quotient Fd/Fc given here are too low for this group of algae.

Results

1 Acute effects of herbicides

The immediate effects of the three substances we tested on the fluorescence of chlorophyll differed distinctly. At the high concentrations used for the experiments, 2,4-D has basically the same effect as DCMU (Fig. 2). Fluorescence is enhanced at low concentrations, but at higer concentrations this initial enhancement is followed by inhibition. The later addition of DCMU leeds to a lower enhancement than the control with 10^{-6} M DCMU when the 2,4-D concentration is high. The relative sensitivities of the algae to 2,4-D were about the same as their sensitivities of DCMU.

The MCPA preparation we used had almost no effect during the short test. None of the 5 algae reacted to the concentrations that were applied. Only the most sensitive of them, *Skeletonema*, showed signs of fluorescence enhancement upon the simultaneous

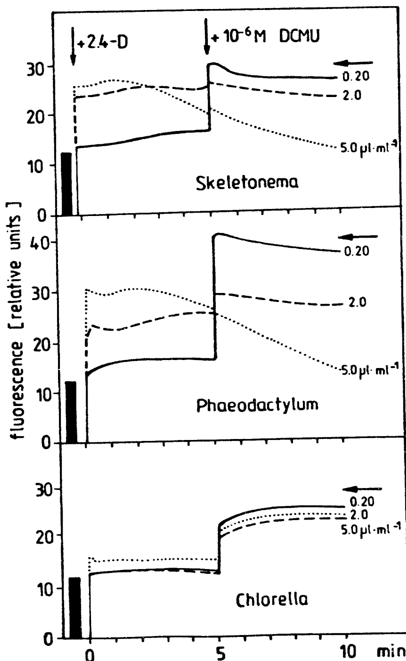


Fig. 2
Effect of 2,4-diclorophenoxyacetic acid (2,4-D). Columns = chlorophyll fluorescence (Fc), curves = influence of 2,4-D alone after 5 min (Fh) and interaction with 10^{-6}M DCMU during the next 5 min (Fdh). Cf. Fig. 1 for further particulars. Representative example from four experiments.

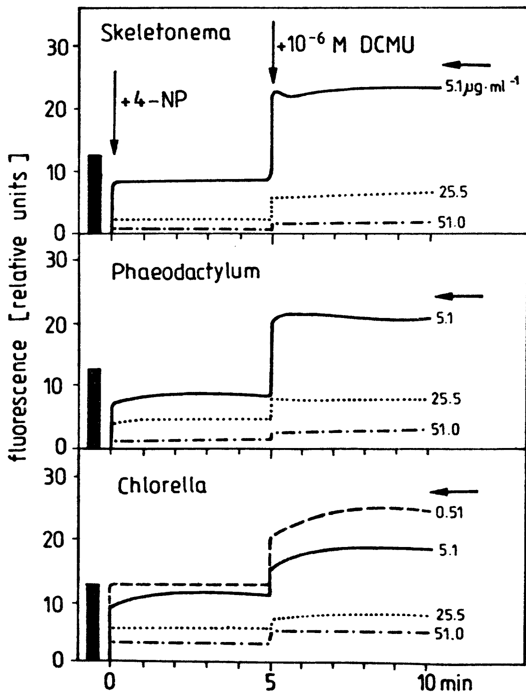
application of 100 μg ml^{-1} MCPA and 10^{-6} M DCMU and of fluorescence quenching at 500 $μml^{-1}$ MCPA and 10^{-6} M DCMU. Extension of the incubation time with MCPA from 10 to 45 min led to distinct fluorescence quenching even after exposure to 500 $μml^{-1}$ alone. This absence of acute effects may be caused by slow uptake and/or the slow enzymatic conversion of the MCPA into the actual active substance. This surmise is supported by the fact that in a five day growth test with *Phaeodactylum* a concentration of 500 μg ml^{-1} MCPA proved to have a significant inhibitory action. Partial inhibition was observed at a MCPA concentration of 100 μg ml^{-1} (Table 1).

Table 1 MCPA induced increase in chlorophyll (Fc value) of *Phaeodactylum tricornutum* during a long term test. Batch culture.
Fc = chlorophyll fluorescence;
Fdh/Fh ratios as steady state fluorescence after 5 min contact with additional DCMU (10^{-6} M). MCPA concentrations in μg ml^{-1}.

Days		0		1		3		4		6
	Fc	FdH/Fh	Fc	FdH/Fh	Fc	FdH/Fh	Fc	FdH/Fh	Fc	FdH/Fh
Contro MCPA	6,7	1,19	7,2	1,39	12,3	2,21	39,7	2,68	191,6	1,4
0.001	6,0	1,25	7,3	1,42	12,6	2,17	34,1	2,68	190,7	1,43
0,01	6,2	1,26	8,5	1,25	12,0	2,08	34,7	2,60	191,6	1,38
0,1	6,4	1,20	7,1	1,27	12,4	2,07	38,1	2,65	198,4	1,38
1,0	6,3	1,19	7,0	1,39	12,3	2,08	34,7	2,63	202,7	1,38
10,0	5,0	1,20	6,7	1,42	12,4	2,14	40,0	2,60	196,2	1,40
100,0	5,5	1,26	6,3	1,25	10,6	1,84	26,7	2,52	123,4	1,52
500,0	3,9	1,15	4,7	1,00	5,1	1,15	6,4	1,19	12,1	1,08

4-nitrophenol represents another group of substances. In contrast to 2,4-D and MCPA, 4-NP always induces fluorescence quenching at the concentrations used for our experiments (Fig. 3). It is caused by absorption of the excitation light at 436 nm by 4-NP. In this case the measured values must be appropriately corrected. Comparative studies involving growth tests showed that for *Phaeodactylum* the EC_{50} concentration of 4-NP is 25 - 30 μg ml^{-1}. The EC_{50} concentration determined by means of the quotient Fdh/Fh is about 25 μg ml^{-1} 4-NP, which is in good agreement with the above mentioned EC_{50}.

We performed tests with phytoplankton samples taken from an eutrophic pond in addition to the studies with standardised algal cultures. In order to measure the fluorescence during these tests it was necessary to compensate for the intrinsic fluorescence of the particle-free sample water. The results (Fig. 4) show that the response of the phytoplankton community to the herbicides being investigated was similar to that of the green algae we used except in the case of MCPA,



which enhanced fluorescence at concentrations as low as 10 $\mu g\ ml^{-1}$. This may be due to the relatively large portion of blue-greens or to the presence of the euglenoid *Phacus*.

Fig. 3
Effect of 4-nitrophenol (4-NP) on fluorescence. Cf. Fig. 2 for further details. Representative example from five experiments.

2 Long term effects of herbicides and sewage samples

In order to check the suitability of fluorometry as screening method for ecotoxicological purposes, growth tests and photosynthetic rate measurements were performed parallel to the fluorometric investigations. The pollutants used in this case were 4-NP and sewage samples.

2.1 4-Nitrophenol

The short term responses to different 4-NP concentrations shown in Fig. 3 and results obtained earlier from growth and photosynthesis experiments yielded calculated EC_{50} values of 25 - 30 $\mu g\ ml^{-1}$ at 20 °C for *Phaeodactylum*. The two curves in Fig. 5. shows the inhibition of 14-C-fixation and fluorescence ratio of *Skeletonema* relative to the control. The two curves are almost parallel. The stronger inhibition percentages induced by 4-NP when fluorescence technique is used is striking.

The results of a long term test with 20 μg^{-1} 4-NP *Phaeocdactylum* cultured at temperatures of 20 °C and 25 °C respectively, are given in Table 2. The algae was preadapted to the temperatures.

The immediate reactions detected by means of the fluorescence method on the first day indicated that the inhibitory action of 4-NP at this concentrations will be much stronger at 20 °C than at 25 °C (The inhibition of the fluorescence ratio is 85 % at 20 °C, and only 35 % at 25 °C.

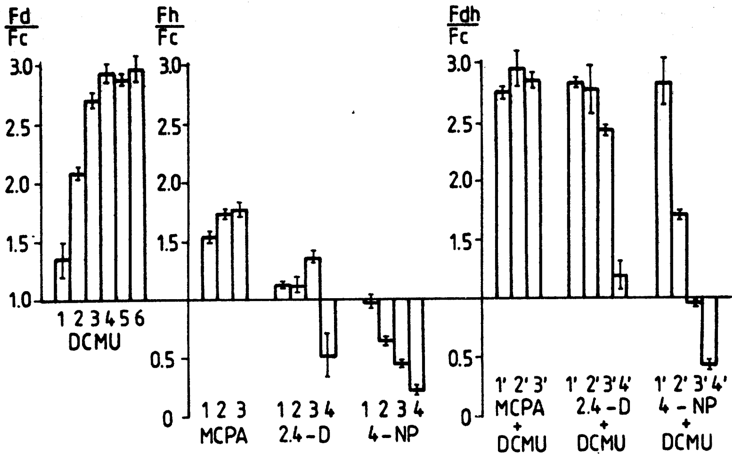


Fig. 4 Fluorescence variations in pond samples. Fd/Fc ratios after 5 min exposure to DCMU 10^{-8}, 10^{-7}, 10^{-6}, 5 x 10^{-6}, 10^{-5} and 10^{-4}M) = 1 ... 6. Fh/Fc ratios after 5 min contact with MCPA wi thout DCMU (10, 100 and 500 μg ml^{-1}) = 1 ... 3 and Fdh/Fc ratios after 5 min contact with 10^{-6}M DCMU in addition = 1' ... 3' and; Fh/Fc ratios after5min contact with 2,4-D without DCMU (0,02, 0,2, 2,0 and 5,0 μl ml^{-1}) = 1 ... 4 and Fdh/Fh ratiosaf'-ter 5 min contact with 10^{-6} DCMU in addition = 1' ... 4'; Fh/Fc ratios after 5 min con tact with 4-NP without DCMU (0,51, 5,1, 25,5 and 51,0 μg ml^{-1}) = 1 ... 4 and Fdh/Fh ratios after 5 min contact with 10^{-6}M DCMU in addition = 1' ... 4'. Means from three experiments.

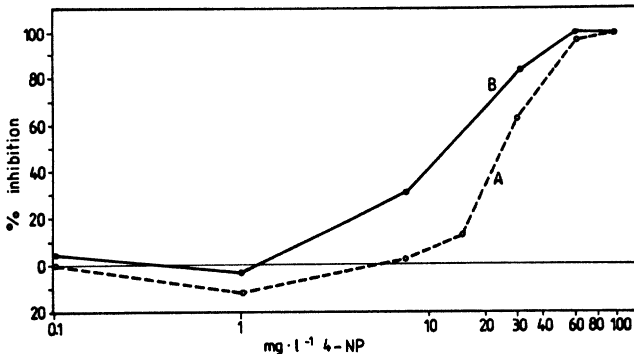


Fig.5 Influence of 4-nitrophenole on 14-C-fixation rate (A) and the Fdh/Fh ratio (B) after 4 hours preincu bation with 4-nitrophenol in light. 14-C-fixation rate was measured after 2 hours incubation. Fluorescence measurement: means of measurements at beginning and end of 14-C-incubation time with and without 10^{-6}M DCMU, 5 min contact.

Table 2 Comparsion of effects of 4-nitrophenol (4-NP) on *Phaeodactylum tricornutum* at culturing temperatures of 20°C and 25 °C. Test conditions: 20 $\mu g \cdot ml^{-1}$ 4-NP, batch culture. Cell count by Coulter Counter. Biomasse equivalents given as Fc values. Fdh/Fh ratios as steady state fluorescence after 5 min contact with additional DCMU (10^{-6} M). Differences calculated as percentages relative to controls.

20°C									
	Control			20 $\mu g \cdot ml^{-1}$ 4-NP			% of Control		
Days	Cells x 10^7	Fc	$\frac{Fd}{Fc}$	Cells x 10^7	Fc	$\frac{Fdh}{Fh}$	Cells	Fc	$\frac{Fdh-Fh}{Fh}$
0	1,0	4,5	1,53	1,0	4,5	1,08	100,0	100,0	15,0
1	5,4	14,0	2,04	3,7	7,5	1,39	68,5	53,6	37,5
2	28,5	120,0	2,72	9,6	24,5	1,48	33,7	20,4	27,9
3	113,0	410,0	2,48	16,2	34,5	1,45	13,4	8,4	30,4
4	325,0	700,0	1,71	30,5	76,5	1,52	9,4	10,9	73,2
25°C									
	Control			20 $\mu g \cdot ml^{-1}$ 4-NP			% of Control		
Days	Cells x 10^7	FC	$\frac{Fd}{Fc}$	Cells x 10^7	Fc	$\frac{Fdh}{Fh}$	Cells	Fc	$\frac{Fdh-Fh}{Fh}$
0	1,0	4,5	1,26	1,0	4,5	1,17	100,0	100,0	65,4
1	3,9	8,0	1,79	2,8	6,5	1,28	72,7	81,3	35,4
2	16,5	61,0	2,43	10,8	20,5	1,61	65,5	33,6	42,7
3	75,0	200,0	2,49	45,0	55,8	1,72	60,0	27,9	48,3
4	200,0	460,0	1,99	107,6	142,8	1,84	53,8	31,0	84,8

2.2 Organically loaded sewage

The organic load of the sewage consisted of oils and acids. In viev of this composition, it was impossible to use the Coulter Counter for analysis. The small initial biomass of the algas (10^7 cells 1^{-1}) at the beginning ot the test did not permit exact measurement of the optical density until after incubation for 48 h. The fluorescence technique passes abvious advantages under these conditions and can be used to follow the whole of growth.

Comparision of the fluorescence ratio with measurements of 14-C-fixation rate showed that the inhibition of the 14-C-fixation rate and the fluorescence ratio were also in good agreement in the case of this organically loaded sewage (Fig. 6). Moreover, the Fdh/Fh ration can be used to determine quickly and exactly the changes in the potential photosynthesis of the algae while they are being cultured (Fig. 7). The measurements reveal that the control cultures were influenced by the limitation imposed by the nutrient situation as early as the third day. This means that an accurate picture regarding the inhibitory action of the test substance can be obtained only from the results yielded up to including the third day. Hence, application of fluorometry permits not

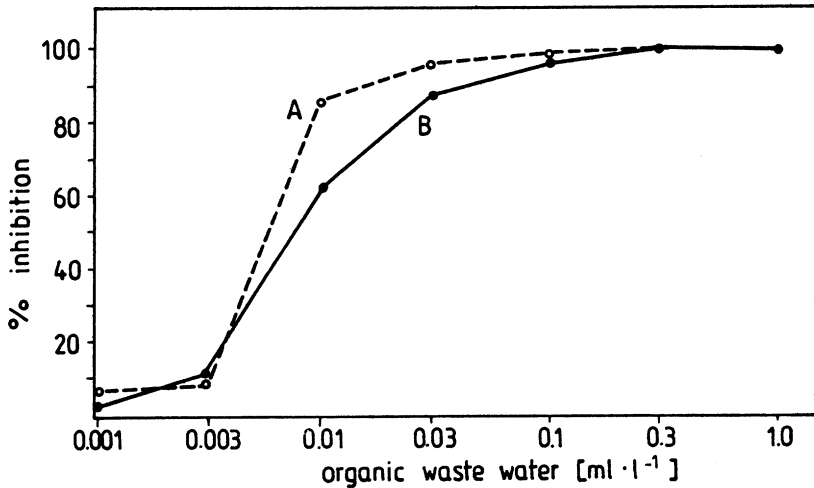


Fig. 6 Influence of organically loaded sewage on 14-C-fixation rate (A) and the (Fdh - Fh)/Fh ratio (B) after 4 hours preincubation in light with different sewage concentrations. The measu rements was as in Fig. 5.

only an estimation of the inhibitory effect produced by the sewage or substances being tested but also an assessment of the culture conditions, so that interactions that can cause errors may be excluded.

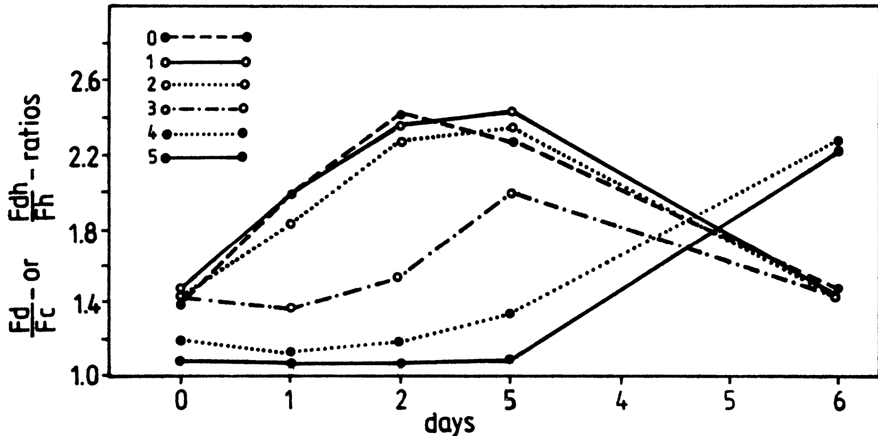


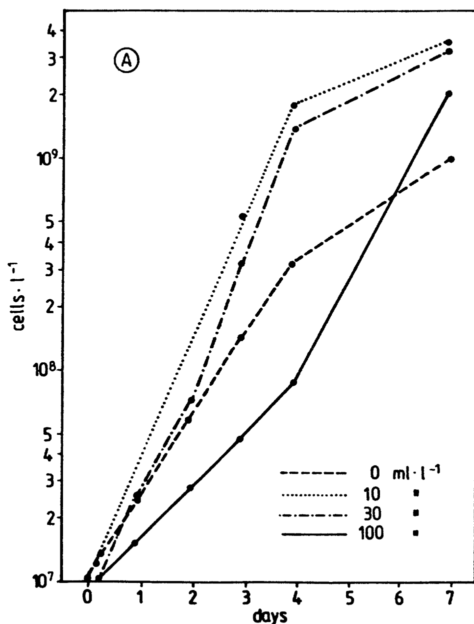
Fig. 7 Changes in the Fdh/Fh ratio upon contact with organically loaded sewage. Batch cultures with 0; 0,001; 0,003; 0,01 and 0,1 ml 1^{-1} sewage (0; 1 - 5); Fdh/Fh ratios as steady state values after 5 min contact with 10^{-6}M DCMU

2.3 Waste water samples loaded with herbicide and copper

The fluorometric immediate response (Fdh/Fc ration) shows that the samples containing Cu differ distinctly from the sewage samples: the substantial decrease in the Fdh/Fh ratio observed in all sewage samples is markedly in contrast to the initial rise in the Fdh/Fc ratio at low Cu concentrations followed by a reduction in the ratio as the Cu concentration in the waste water increases (Table 3). Toxic effects could be predicted only for the waste water sample with the highest Cu concentration tested.

Table 3 Short time influence of sewage containing copper (B)and herbicide (phenoxyacetic acid: D) on the Fdh/Fcratios. Fdh/Fc ratio used as control. Means from tree experiments.

	waste water B			
Control Fd / Fc	1 Fdh / Fc	10	100	330 ml · 1^{-1}
1,91	2,10	1,90	1,79	1,07
	waste water D			
Control Fd / Fc	3 Fdh / Fc	10	100 ml · 1^{-1}	
2,11	1,68	1,67	1,57	



The growth test failed to confirm this prediction regarding the influence of copper. In the culture experiments the effect of copper proved to be much stronger: the EC_{50} concentration was found to be 1 - 3 ml 1^{-1}. As Fig. 8a and 8b shows, the difference between the results yielded by fluorometry and growth measurement for the waste water containing herbicide differed considerably at first. This is due mainly to a reduc-

Fig. 8 a
Influence of sewage containing herbicide (530 ppm phenoxyacetic acid). A = cell divition after addition of 0, 10, 30 and 100 ml sewage 1^{-1} to culture medium

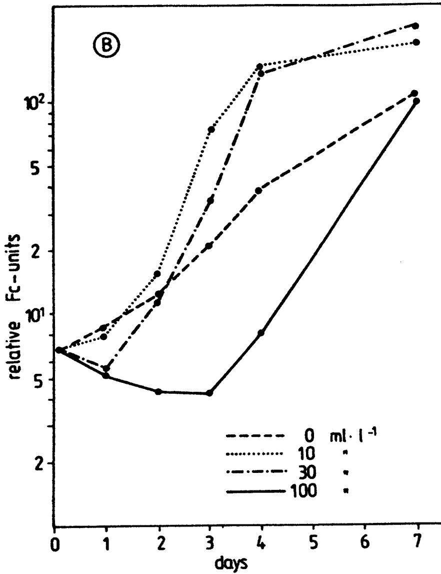


Abb. 8b B = increase in chlorophyll concentration (Fc values) in relation units

tion in the chlorophyll content of the severely loaded samples. The later results, and also the final situation, yielded by the two methods are almost identical.

Analysis of the potential photosynthesis (Fdh/Fh ratio, Fig. 8c) shows that in the water containing herbicide the relative magnitude of the Fdh/Fh decrease remains the same until the fifth hour of incubation, whereupon the photosynthetic potential of the algae with mean concentration load increases greatly. This is caused by the high organig nitrogen and phosphate concentrations in the waste water, which have a stimulatory action on the growth rate and final yield. The highest waste water concentration led to a clearly delayed development of photosynthetic performance. The Fdh/Fh

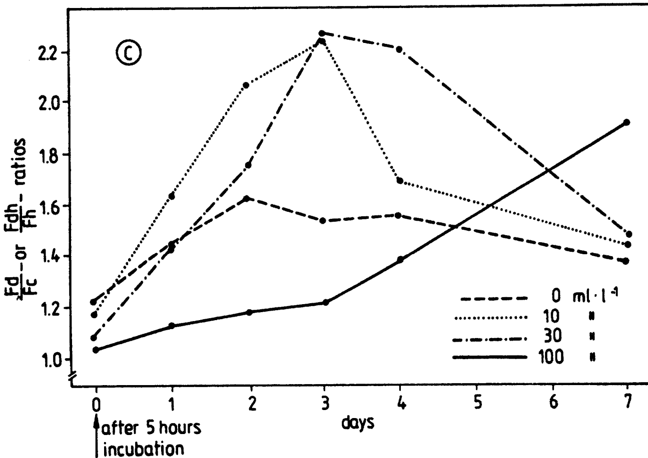


Fig. 8c C = changes in Fdh/Fh ratios under influence of sewage measured as steady state values after 5 min contact with $1o^{-6}$M DCMU. Control as Fd/Fc ratio.

ratio showed no signs of a definite increase until after the third day, when the gradual decline of the inhibitory action or the adaptation of the algae had progressed sufficiently. It seems likely that if the test at this concentration had been prolonged, far greater increases in biomass would have been the result.

Discussion

The results we have presented show that fluorometry can be applied for ecotoxicological purposes and posses major advantages due to the simplicity of in vivo fluorescence measurement and partly to its economy in term of equipment and time. Additional benefits are that it can also be used when the media consist of emulsions or corrosive substances and when the algal biomasses are very low. The later property also permits the use of algal populations taken from the field and thus supplement the applications suggested by YENTSCH and YENTSCH (1979).

The fourstage method we have described permits the toxicity to be estimated reslistically. This method yields results that are in very close agreement with the EC_{50} values for the rate of photosynthesis. Measurement of the background fluorescence (Fc), the Fd/Fc ratio and the Fdh/Fh ratio and the application of DCMU as a reference substance permit simultaneous determination of the biomass, growth in terms of biomass and potential photosynthesis capacity of algal populations. The comprehensive information these values give regarding the course of the test can help to avoid false conclusions.

The Fc values can be used directly to determine the EC_{50} values for growth inhibition. The results they yield do not differ appreciably from those yielded by cell counts. Short term changes in the Fdh/Fh ratio permit conclusions to be drawn regarding the toxicity of the substance being tested or regarding the presence of toxic substances, although this obviously does not apply to the effects of heavy metals, which impair the transport of electrons only slightly.

The good agreement between the 14-C-fixation rate at light saturation and the Fdh/Fh ratio at the low light intensity we used to excite fluorescence (4 $W \cdot m^{-2}$) is remarkable. The quasi-steady state reached by the fluorescence ensures high standards of reproductability.

Analysis of the short time test with 2,4-D, MCPA and 4-NP shows that these substances differ substantially in effectiveness. MCPA can be considered harmless, whereas 2,4-D and 4-NP are relatively toxic. The tested marine diatoms and freshwater green algae shows a different sensitivity. Tests on dinoflaggellates and blue-greens would complete the picture and would give indications regarding possible shifts in the population under natural conditions following herbicidal loading of waters.

When studying the long term effects of herbicides or sewage samples, the ratio Fd/Fc and Fdh/Fh give a quick and reliable impression of the state of the culture and the changes in the toxicity or adaptation of the algae that are taking place. Measurement of the Fd/Fc ratio when culturing starts also permits the exact, checkable assessment of the physiological status of the

algae being used for the test and thus helps reduce the risk of obtaining false results due to differences in the initial conditions.
For long term tests it is advisable to use the plateau range of the Fd/Fc ratio for the controls in order to obtain comparable calculations of the percentage inhibitions induced by different substances. The experiments should be terminated after this range has been left, whereupon the cell counts and the biomass measurements must be performed.

Acknowledgement
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Zusammenfassung
Beschrieben wird eine Screening-Methode für ökotoxikologische Zwecke auf der Grundlage der Fluoreszenzmessung bei Algen. Vergleichsuntersuchungen zur Ermittlung der Wachstums- und Photosyntheseraten umfaßten verschiedene Algenarten *(Phaeodactylum tricornutum, Skeletonema costatum, Selenastrum capricornutum, Chlorella pyrenoidosa und Scenedesmus subspicatus).* Übereinstimmende Ergebnisse zwischen der fluorimetrischen und den konventionellen Methoden wurden sowohl beim Einsatz von 3-(3,4-Dichlorphenyl)-1.1-Dimethylharnstoff (DCMU), 2,4-Dichlorphenoxyessigsäure (2,4-D), 4-Chlor-2-Methylphenoxessigsäure (MCPA), 4-Nitrophenol (4-NP) als auch beim Einsatz verschiedener Abwässer erzielt. Der Vergleich mit den konventionellen Methoden zeigte, das die Fluoreszenzmethode schnell, einfach und billig ist und gleichzeitig zusätzliche Informationen liefert.

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