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Use of Algal Fluorescence for an Automated Biological Monitoring System

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In the field of stream-quality monitoring some bioassays have been developed to a high standard: the Haematococcus pluvialis-test (MÄCKLE 1977), the Gnathonemus-test (GELLER & MÄCKLE 1977) and other fish-tests (in the FRG especially the Goldorfen-test (FISCHER & GODE 1978). A survey of the application of bioassays monitoring the toxicity of water pollutants is given by OTT & IRRGANG (1977), VON OERTZEN (1977), BESCH (1977), LITTLE (1976) and BENECKE (1980).

A standard bioassay must concentrate on organisms, which have a representative sensitivity to a wide spectrum of pollutants and a low sensitivity to matrix-effects. We wish to present a new bioassay procedure, which uses algae (Scenedesmus, Chlorella) as test-organisms. The test is based on the registration of the spontaneous, variable fluorescence as one parameter of the algal physiological state. The change of the fluorescence curve due to substances, which affect or block photosynthesis, is described by KAUTSKY (1943) and FRANCK et al. (1969). ARNDT (1972, 1974) was the first to employ this measurement to look at the effects of air-pollution. Todays research mainly concentrates on the problem of chlorophyll recording in vivo (HEANEY 1978; NUSCH 1982).

This paper describes the apparatus for the continous recording of the algae suspension fluorescence curve and demonstrates the possible use of the bioassay for stream-quality monitoring.

The fluorescence phenomenon

All influences, which retard the primary processes of photosynthesis, cause variations in the extent of the fluorescence emission of a plant. These changes refer to the spontaneous, variable fluorescence as well as to the delayed. During the excitation with short-waved light the electrons of the chlorophyll-molecule are

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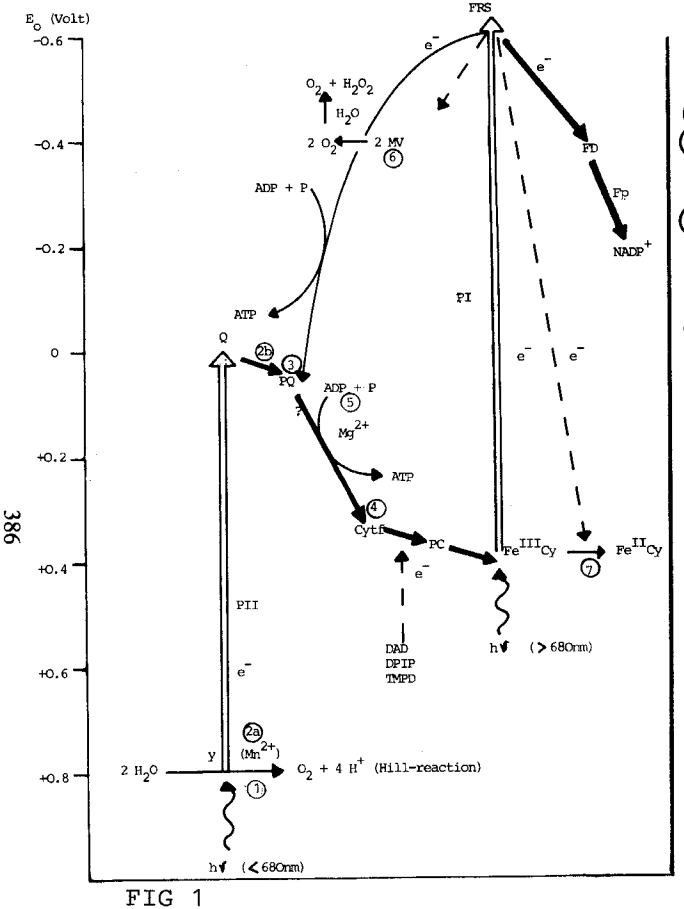


FIG 1

Photosynthetic reactions
(after MORELAND & HILTON 1976; BÖGER & VETTER 1977)

Influence of several substances on photosynthesis

(1) m-Cl-CCP: Carbonylcyanide-3-Chloro-Phenylhydrazone
(2a a./o. 2b: Diuron

Inhibition of electron-transport between PI and PII

(2b Carbamate, Thiocarbamate, Pyridazone, Triazine, Uracile, Diphenyl ether, 1,2,4-Triazine

PQ-Antagonists

(3) DBMIB: Dibromothymoquinone, Ioxynil

Decoupling substances

(4) NH_4^+, Methylamine, Perfluidone, Atebrin, Chlorocholine chloride, Propandinitrile

Inhibition of energy-transfer

(5) DIO 9, Phlorozine

Electron-acceptors

(6) Dipyridin, several Naphtoquinones, PMS: Phenazine methosulfate, several Flavines

(7) K-Hexacyanoferrate (III)

y=electron acceptors PI=Photosystem I PII=Photosystem II
Q=electron acceptors of PII PQ=Plastoquinone
Cytf=Cytochrome f PC=Plastocyanin FRS=Ferredoxine red. subst. Fp=Ferredoxine-NADP-reductase FD=Ferredoxine

Artificial e^--acceptors: MV=Paraquat FeIICy=K-Hexacyanoferrate (III)

Artificial e^--donators: DAD=Diaminodurol ascorbate DPIP=red. 2,6-Dichlorophenol indophenol TMPD=N'N'N'N'-Tetramethyl-p-phenylenediamine

Place of detection	Active substance	Concentration ppb	Author
Sewage			
Herbicide-product.	MCPB	660.000	SCHÖBEL et al. (1972)
	2,4-D	3.900.000	
	S-Triazine	250-1000	TORTIR (1977)
	2,4-DP	1	STRUIF et al. (1978)
Drainage	Alachlor	98.8	KADOUM & MOCK (1978)
	Atrazine	1.074.8	
	Cyanazine	54.0	
	Propazine	269.4	
	Atrazine	1.49	MUIR et al. (1976)
	Cyanazine	0.68	
	Cyprazine	0.57	
	Metribuzine	1.65	
	Atrazine	3.5	von STRYK & BOLTON (1977)
	Simazine	32.800	DJUMJA et al. (1977)
	Atrazine		
Surface discharge			
5-10 m distance (highest recommended amount)	Diuron	1.800	EVANS & DUSEJA (1973)
	Sumitol	500 (0 m)	
	2,4-D	4.200	
	2,4,5-T	1.400	
	Picloram	2.700	
	Atrazine	900	
Artificial rain	Atrazine	11.000	BAILEY et al. (1974)
	Dichlorobenil	5.300	
Rain	Diuron	10	WILLIS et al. (1975)
	Linuron	124	
	TCPA	310	
	Trifluraline	1.9	
	2,4-D	8.1	WHITE et al. (1976)
Percolation water	Buturon + Metabol.	6	HAQUE et al. (1977)
Percolation and spring water	Picloram	1	BOVEY et al. (1975)
	2,4,5-T		
Ground water (Lysimeter)	Picloram	4	"
	Prometryne	48.2	LA FLEUR et al. (1975)
	MCPA	?	SYNEK et al. (1973)
Ground water and drinking water	Atrazine	?	WOLFE et al. (1976)
	Atrazine	5	JUNK et al. (1976)
River and drainage	Benthiocarb	10	SUZUKI et al. (1977)
River	2,4,5-T (ester)	3	THARALDSEN (1973)
	2,4-D	1.5	WATSON (1977)
River (300 m downstream a sprayer-cleaning)	Dinoseb	4.800	ZITKO et al. (1976)

TABLE 1

Content of Herbicides in Waters of different Origin and Quality
(References in BENECKE 1980)

Place of detection	Active substance	Concentration ppb	Author
Surface water			
River	2,4-D + 2,4,5-T (acid + ester)	o.249	JUNK et al. (1976)
	Atrazine	3.7	
	Atrazine	6.3	
Brook (measured 2,5 mth. aft. an accident)	Pentachlorophenol	82	PIERCE et al. (1977)
River	"	1o.ooo	FOUNTAINE et al. (1976)
River	2,4-D-Acid	2	CHOI et al. (1976)
River	2,4-D	2.ooo	CROLL (1975)
	2,4-D (Butylester)	500	ANAN EV & BEL GIBAEV (1977)
	2,4-D (Amine)	20	
River	CNP	16.6	SUZUKI et al. (1978)
River	2,4-D	21.000	STRUIF et al. (1978)
	MCPA	0.3	
	MCPP	1.3	
	MCPB	0.15	
	2,4,5-T	40	
Pond	Simazine (+Metab.)	100 (after 14 d)	MAIER-BODE (1972)
	Atrazine (+Metab.)	20 (after 56 d)	
	Terbutryne	25 (after 14 d)	
	Ametryne	8 (after 7 d)	
Rhine (Düsseldorf)	4-Chloranil (Buturon + Monolinuron dgrdn. pr.)		HERZEL & SCHMIDT (1977)
Rhine (km 865)	Buturon	0.076	STÖBER & REUPERT (1978)
Rhine (km 865)	Chlorotoluron	0.20	
Rhine (km 698)	Linuron	0.09	
Wupper (mouth)	Diuron	0.28	
Rhine (km 698)	Metoxuron	0.21	
Rhine (km 640)	Monolinuron	0.21	
Rhine (km 640)	Phenmedipham	0.09	

TABLE 1 (continued)

lifted to a higher energy-level. Normally, the excitation energy is transmitted from the chlorophyll to acceptor-molecules (see Fig. 1). The quantity of acceptors is limited, so that 2-20% of the energy is lossed as fluorescence. Excitated ions emit quanta in the red range of the spectrum during their return to the normal level. The fluorescence reaches high values after a dark period: acceptor-molecules have to accomodate during sudden radiation to the new situation.

Herbicides may inhibit photosynthesis; Fig.1 combines a model of photosynthesis with possible inhibition positions. Table 1 shows the concentration of several herbicides in the water. A decision about the influence of herbicides on the plant based on the knowledge of the concentration in the water is impossible.

Fluorescence measurement

In the fluorometry we use the optical characteristics of the photosynthetic pigments to measure certain parameters. These pigments emit fluorescent light at 660 nm. Maximum values are reached after a dark period when the pigments are excitated with red light (650 nm) or blue light (450 nm). Fluorescence intensity is dependent on temperature, pH, O_2 and other factors.

Fig. 2 shows a normal fluorescence curve. After a first increase, we notice a typical depression followed by a second increase. The phases 1-5 are typical for that curve.

Fig. 3 illustrates the measuring device we have chosen. The algal culture in field A is a normal chemostate. Field B includes light source and a photomultiplier, a flow-through cell and an oscillograph. The instruments are fitted on an optical bench.

The algae are pumped from the culture into the cell. The flow-through cell guarantees stable temperature and aeration during the dark phase. After a defined time in the dark the aeration is stopped and the algae are exposed to light. A shutter controls the exposure time. The light passes a blue-filter, the cell and a red filter with a transmission maximum at 660 nm. The photomultiplier amplifies the signal and the curve is recorded on the oscillograph. With a Polaroid-camera the curve can be photographed.

We can dose certain substances or raw-water to the algae in the cell. If a water sample is analyzed, the water first passes a filter to remove suspended matter and thus prevent light scattering.

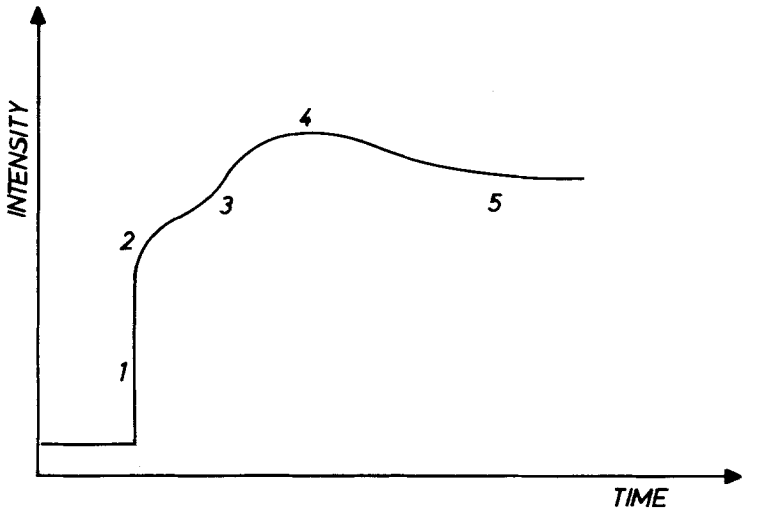


FIG. 2

Fluorescence curve

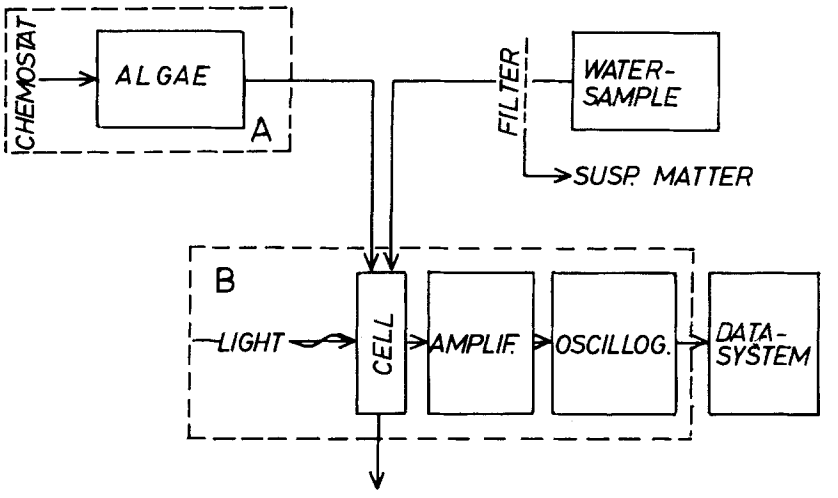


FIG. 3

Key plan of measurement

RESULTS

The results presented here show the principle function of the bioassay and its sensitivity. Till now, we concentrated on the reproduction of the normal fluorescence curve and the dosage of single substances.

Fig. 4 and 5 show several fluorescence curves photographed from the oscillograph. The normal fluorescence shows the above mentioned characteristics (phases 1-5). The height and the curve's slope depend on very defined conditions. Chlorophyll concentration and physiological state of the algae have the main effect on the curve. We can reproduce a normal curve at a cell concentration of $4\text{-}7 \times 10^6$ cells/mL. The age of the culture must be controlled and the culture punctually renewed.

The fluorescence intensity is proportional to the exposure time and the light intensity in certain limits. Thus, exposure time can be chosen variable, we used 1/2 sec., 150 Watt and a dark-phase of 10 min.

Nearly all curves show two effects: changes in the fluorescence intensity and levelling of phases 1-3. Both effects can be demonstrated by temperature change, aeration with N_2, several herbicides and other chemicals. Both effects can be demonstrated together and separately, they are not coupled.

If the pollutants can affect the algae for 15 min or longer, a full depression of fluorescence can be noticed in some cases.

TABLE 2

Sensitivity of the Fluorescence-Test in Comparison (mg/L)

	Fluorescence	Growth (BRINGMANN & KÜHN)
Atrazine	0.0325	0.003
$HgCl_2$	0.005	0.005
$CuSO_4$	0.003	0.03
$Cd(NO_3)_2$	0.007	0.07
KCN	0.007	0.07
Monolinuron	0.014	0.14
1,3-Dinitrobenzene	0.034	0.17
2-Chlorotoluene	3.1	31.0

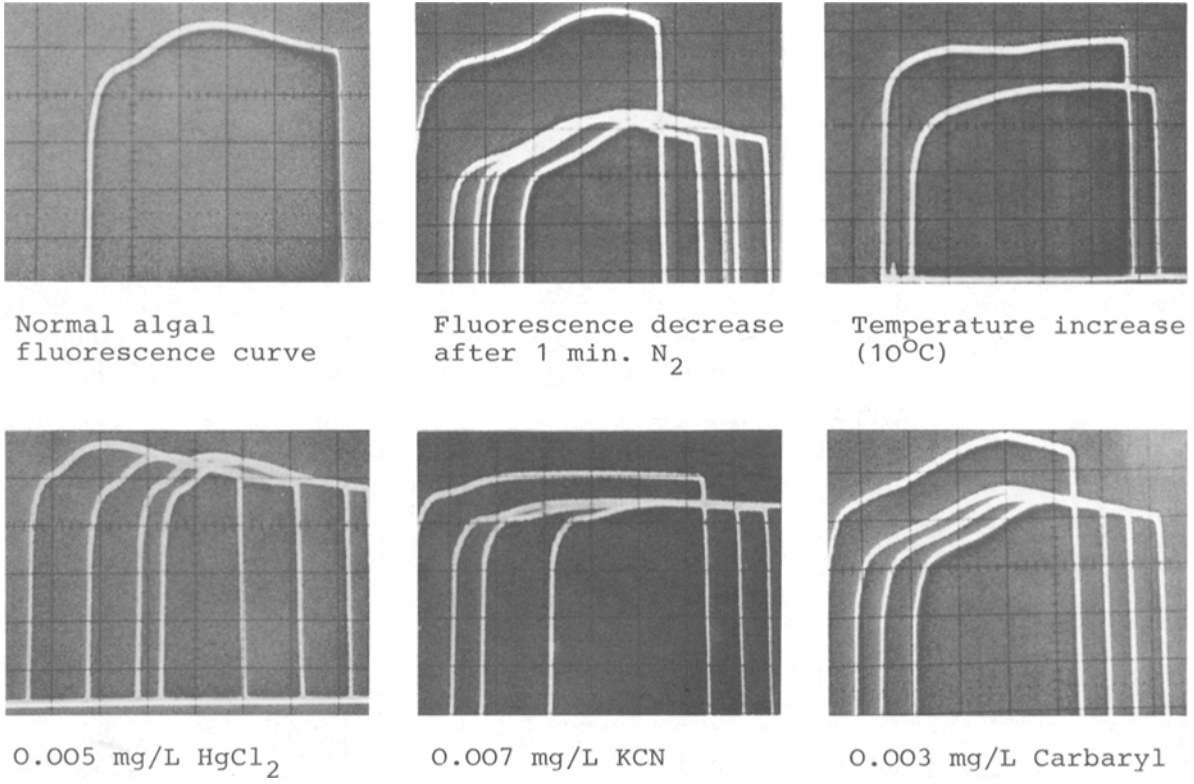
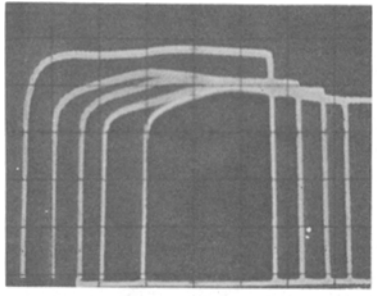
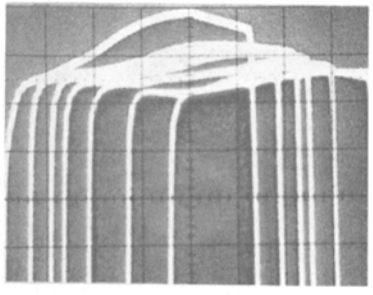


FIG. 4

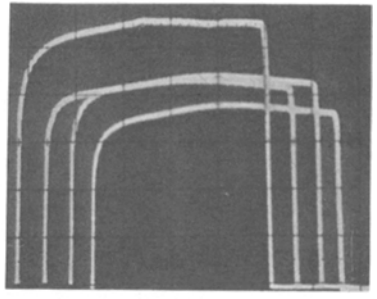
Fluorescence curves after dosage of a toxicant; several curves on the photographs: normal curve on the left, the following 5, 10, 15, 20, 25 min after dosage. Exception: temperature increase, normal curve on the right)



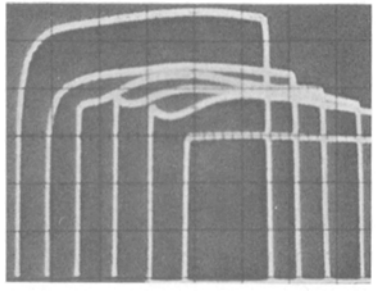
0.033 mg/L
Atrazine



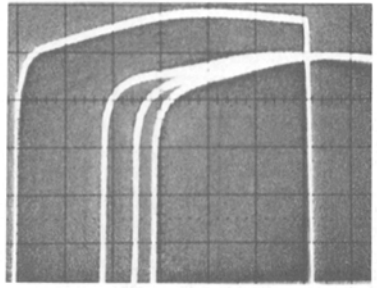
0.024 mg/L
Prometryne



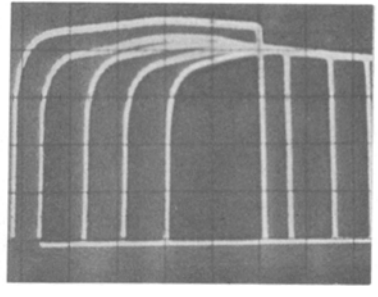
0.0014 mg/L
Linuron



3.1 mg/L
2-Chlorotoluene



0.034 mg/L
1,3-Dinitrobenzene



Brook-water (Dortmund), contaminated with a green substance (Uranin?)

FIG. 5

Fluorescence curves after dosage of a toxicant; legend see Fig 4.

With the demonstrated changes of the fluorescence curve we can detect an immediate effect on the photosynthetic process, which mainly results in a change of the first fluorescence phases. A curve levelling in this phase can be explained as a deactivation of the acceptor regeneration. In the phase 1-2, the number of acceptors, which have been regenerated in the dark-phase, decreases. The following slight depression may be caused by a regeneration of these molecules or an adaptation.

Curve-levelling is a characteristic for all dosed herbicides and other chemicals. A change in the curve's height may be the result of changing algal concentration and not of a damage. A decrease in fluorescence intensity could also be interpretated as a direct damage of the chlorophyll-molecule. The energy-yield of the fluorescence would then be diminished. The connection of both effects as a result of a beginning damage must still be cleared up.

Till now, a prediction about the changes of the fluorescence is not possible. When pollutants influence the algae very long we can sometimes measure an adaptation of the algae to the new conditions. We never noticed a full regeneration of the normal fluorescence curve, but the initial intensity sometimes returns.

Generally, a pollutant causes a greater damage when it can affect the algae for a long time. The beginning of a damage depends on the concentration and the chemical nature of a pollutant. We used concentrations, which exceeded the concentration of typical pollutants in the raw water. A comparison with the concentrations used by other authors (BRINGMANN & KÜHN 1975) and a former research (ZULLEI & BENECKE 1978) shows, that we can demonstrate effects on the algae with concentrations far below those. The time from the dosage of a toxicant to a clear reaction of the algae is very short (30 sec. to 5 min.; see Table 2).

An advantage of the system is the use of algae, which are simple to culture. A stable algal concentration and species-composition is one of the problems, we did not solve sufficiently. An algae-screening could be successful to find more sensitve species. Algae counting and determination of the actual chlorophyll-concentration should be replaced by an automatic registration.

A further problem arises with the influences of a heterogenous matrix. A direct influence of the matrix could not be measured; on the other hand, we could not measure a clear reaction of the fluorescence to raw water (River Ruhr).

The sensitivity to several toxicants must be tested. This is now at work using the substances of other authors, especially the list of reference chemicals proposed by SCHEELE (1980). If the sensitivity to a representative number of chemicals is clear, the interactions between toxicants, physical parameters and the algae can be analyzed. A connection to a data system, automatic registration and alarming would then be useful.

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