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Waste-Water Assay with Continuous Algal Cultures: The Effect of Mercuric Acetate on the Growth of Some Marine Dinoflagellates

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Abstract

The effect of mercuric acetate was studied in culture experiments with the dinoflagellates *Scrippsiella faeroense* (Paulsen) Balech et Soares, *Prorocentrum micans* Ehrenberg and *Gymnodinium splendens* Lebour. Impairment of growth rates, *in vivo* chlorophyll fluorescence, maximum cell densities and morphological changes served as criteria for assessing sublethal influences. Tests were made using the batch- and continuous-culture techniques. Addition of Hg at concentrations of 0.001 $mg.1^{-1}$ and higher resulted in reduction of relative growth rates. In a few cases populations recovered from the initial decline and showed new growth. Cell counts corresponded very closely to *in vivo* chlorophyll fluorescence measurements. Morphological variations were observed in *S. faeroense,* which responded (even in sublethal concentrations) by bursting it's thecae, releasing naked motile cells and forming vegetative resting stages. The problems of optimal algal-bioassay methods are discussed also, in the light of results obtained by other authors.

Introduction

Rearing experiments using marine plankton algae are useful for the investigation of toxic wastes and waste products. Even in sublethal concentrations, impairment of growth rates, cell densities and morphological or physiological peculiarities of the cells can become evident. In the present investigation an attempt was made to develop an algal bioassay by means of culturing sensitive test forms of the North Sea in non-axenic monocultures. Two cultivation methods were used for the test: (1) batch cultures, (2) continuous cultures utilizing the turbidostat principle. Batch cultures consist of closed systems with limited experimental time and volume. The cell density, the nutrient content, and possibly the concentrations of dissolved toxicants change within one experiment. Toxic substances can be added only once at the beginning of the experiments. Therefore, the batch cultures serve only for first estimations of sublethal and lethal-limit concentrations. In continuous cultures the cell density of the culture system can be held at a constant and relatively low level by means of the flow rate of the culture medium. The addition of the toxic agents can take place continuously together with the medium inflow. Thus, as opposed to the batch-culture method, the algae are exposed permanently to the influence of fresh, running, waste water. The experimental time is theoretically unlimited, as long as the control culture maintains it's exponential growth. Therefore, the continuous-culture method can be used for the examination of long-term effects of pollutants.

The dinoflagellates *Scrippsiella faeroense, Prorocentrum micans* and *Gymnodinium splendens* served as test forms. These algae are common members of the North Sea plankton. They were chosen because they could be cultivated during the experiments in pure sea water enriched only with nitrate and phosphate. The addition of chelating agents and trace metals could thereby be avoided; this is a very important precondition of a test on heavy metals, which otherwise would be combined in a complex form even at the start of the experiments.

The test was used to investigate the effect of mercuric acetate. Mercury was chosen as an example of a toxic industrial waste product. The experiments were restricted to culture effects. Multipli-

cation rates, maximum cell densities, *in vivo* chlorophyll fluorescence and morphological alterations served as criteria of toxic impairment. Chemical analyses on the location of the mercuric acetate and it's accumulation by the cells during the experimental time will be considered in later experiments. Nearly the same test method has been used in earlier investigations of industrial waste products of a titanium dioxide factory (Kayser, 1969, 1970) and of "red mud" – a waste material from bauxite processing (Kayser, 1973).

Materials and Methods

The dinoflagellates *Scrippsiella faeroense* (Paulsen) Balech et Soares[1], *Prorocentrum micans* Ehrenberg and *Gymnodinium splendens* Lebour were isolated near Helgoland and cultivated in non-axenic monocultures. The culture medium consisted of seawater from Helgoland (32 ± 1.5‰S, sterile-filtered through Millipore filter of 0.22 µm pore diameter), enriched with 0.1 g $NaNO_3$ and 0.02 g $NaH_2PO_4.l^{-1}$. The algae were kept in series of 2-l glass bottles (Jenaer Glas, Duran 50) and in an 8-l fermenter. In the turbidostat experiments the continuous flow of the culture medium was secured by two 13-canal-peristaltic-micropumps with Tygon tubes. The pumps raised the culture medium continuously from 5-l stock bottles into the 2-l culture vessels in an amount corresponding to the multiplication rate of the control cultures. Simultaneously, the pumps withdrew exactly the same quantity of the algal culture from the culture vessels. Mercury was added as $Hg(CH_3COO)_2$ from a distilled-water stock-solution [0.159 g $Hg(CH_3COO)_2$ in 100 ml H_2O, 20°C]. In the batch-culture series, the mercury was added once at the beginning of the experiments, whereas in the 2-l turbidostat series mercury was added together with the inflow of the culture medium. Only in the 8-l fermenter experiments was the mercury added directly from the stock solution in amounts corresponding to the medium inflow.

All cultures were set up in constant-temperature rooms at 18°C ± 1C°. The bottles were illuminated by laterally-positioned daylight fluorescent lamps (Osram - L 40 W/15) with a light intensity of about 6,000 lux. The distance from the light source to the bottles was 10 cm. A 14h:10h light:dark period was maintained. Cell counts and microphotos were made with an inverted microscope. In addition, a Coulter Counter was used. The *in vivo* fluorescence of the algal cultures was determined by a Turner Fluorometer. Samples were taken daily and at the same time (10.00 hrs). To achieve a homogeneous distribution of the algae in the continuous-culture experiments, a slight turbulence of the culture medium was obtained by aeration (40 ml air.min^{-1}).

[1] In a prior publication by the author (Kayser, 1973), this species was named erroneously *Peridinium trochoideum* (Stein) Lemmermann (Balech and Soares, 1966)

Results

Scrippsiella faeroense

Batch Cultures

The growth curves of 6 algal cultures are presented in Fig. 1 as cell number versus time. Comparison with the control culture shows that the addition of 0.001 mg $Hg.l^{-1}$ resulted in a slightly decreased maximum cell density. After addition of 0.01 mg $Hg.l^{-1}$, the culture stagnated on the first day, showed a reduced multiplication rate, and reached a diminished maximum cell density of only 45% that of the control. Addition of 0.05 mg $Hg.l^{-1}$ caused an immediate decrease in cell density to 17% of the initial value within the first few days, followed by a stagnation phase. Then recovery took place, so that at the end of the experiment nearly the same density had been reached as the control. In 0.1 mg $Hg.l^{-1}$ no recovery stage was visible. In Table 1, the limiting concentrations are summarized for comparison with the results of the following experiments. The *in vivo* chlorophyll fluorescence measurements of the cultures are shown in Fig. 2 as fluorometric units versus time. The results agree very well with the cell counts in Fig. 1. There was no evidence that *in vivo* chlorophyll fluorescence was more affected than was cell number.

In addition to the reduction of growth capacity a further, very conspicuous, reaction was observed. With the addition of mercury concentrations of 0.01 mg $Hg.l^{-1}$ and higher, the majority of the cells began within as little as 1 h to sink and settle on the bottom of the culture vessels. One hour later microscopic examination revealed that in 0.01 mg $Hg.l^{-1}$ a few cells, and in higher concentrations nearly all cells, burst their thecae, displaying a split between the epitheca and the cingulum. Epitheca and hypotheca were gaping. A few minutes before bursting the protoplast withdrew somewhat from the apex of the epitheca. Subsequently, either (1) the protoplast

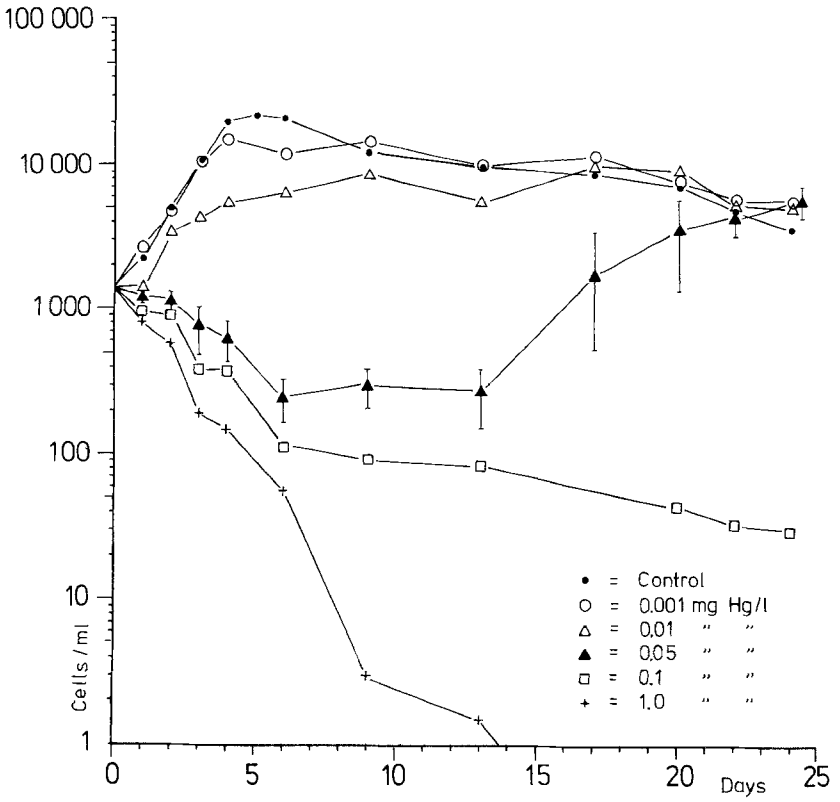

Fig. 1. *Scrippsiella faeroense*. Batch culture. Cell density after addition of mercuric acetate in amounts of 0.001 to 1 mg $Hg.l^{-1}$ at beginning of experiments in 2-l bottles. Mean values and standard deviations of 5 replicates are given for 0.05 mg $Hg.l^{-1}$

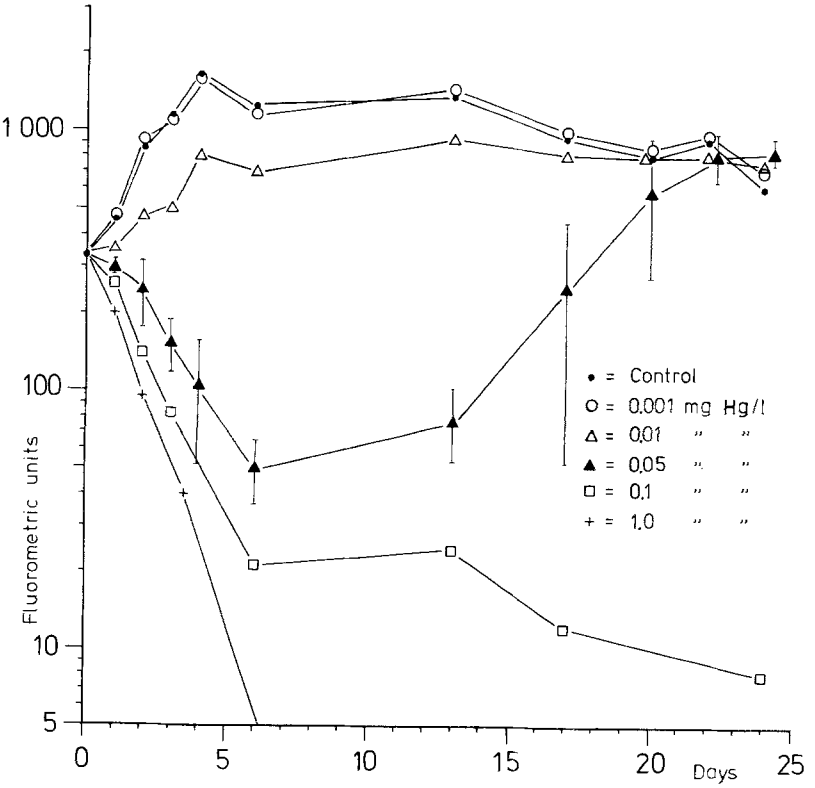

Fig. 2. *Scrippsiella faeroense*. Batch culture. *In vivo* chlorophyll fluorescence after addition of mercuric acetate in amounts of 0.001 to 1 mg $Hg.l^{-1}$ at beginning of experiments in 2-l bottles. Mean values and standard deviations of 5 replicates are given for 0.05 mg $Hg.l^{-1}$

Table 1. *Scippsiella faeroense, Prorocentrum micans* and *Gymnodinium splendens*. Limiting concentrations (mg $Hg.l^{-1}$) of $Hg(CH_3COO)_2$ at which toxic impairment occurs in batch and continuous cultures

Sequence of decreasing toxic effects	*G. splendens*	*S. faeroense*		*P. micans*	
	Continuous	Batch	Continuous	Batch	Continuous
(a) Break-down of culture within experimental period	0.5 -0.05	1	1	1	1
(b) Decreasing cell density without recovery effect	0.01	0.1		0.1	0.5
(c) Decreasing cell density with recovery effect		0.05	0.1 -0.05		0.1 -0.05
(d) Reduced multiplication rate, diminished maximum cell density		0.01 -0.001	0.01 -0.001	0.01	

left the theca via the split and swam away, or (2) it remained in the theca and, in the course of a few weeks, assumed a spherical thick-walled shape.

Case 1. Immediately after bursting the theca, the protoplast, with it's apical end foremost, squeezed through the lateral split by means of a lateral flexure. For a short time a longitudinal flagellum was visible at the antapical end of the emerging cell; then the cell swam away by longitudinal rotation. It was oblong, with slower movements than that of a normal thecated cell. This entire process lasted approximately 1 min. By the following day most of the oblong cells had dropped to the bottom and lay motionless. Fig. 3A shows such cells, after the addition of 0.05 mg $Hg.l^{-1}$, beside empty thecae. Divisions (?) could be observed occasionally (Fig. 3 B). At times the oblong cells could not come off from the thecae entirely. In such cases they formed posteriorly one or a chain of empty, smooth, oblong pelliculae (Fig. 3 C). Such pelliculae were also formed when oblong cells, already resting on the bottom, resumed swimming (Fig. 3 D). Fairly common at this stage of the experiment were empty thecae and pelliculae or chains of empty pelliculae, attached to a motionless oblong cell. In 0.05 mg $Hg.l^{-1}$ all the oblong cells had settled after 1 day. In the succeeding days they began to die. Only very few survived, apparently by forming solid membranes. The increase in cell numbers that occurred after 13 days in 0.05 mg $Hg.l^{-1}$ (Figs. 1 and 2) was caused by normally thecated cells, which survived the initial toxic decrease of the culture.

Case 2. The protoplasts remained in the burst thecae, enlarged somewhat in the course of the next few days, and formed a solid, smooth membrane (Fig. 3 E). The contents of the cells were stained strongly by Lugol solution. Some examples of this type remained unaltered for more than 3 weeks in 0.05 mg $Hg.l^{-1}$ (Fig. 3 F). In the culture to which 1 mg $Hg.l^{-1}$ had been added, all cells burst laterally, but the protoplasts did not leave the thecae, presumably because of the immediate acute toxic effect of the mercury. All cells died in this stage within 2 weeks.

In the control cultures comparable processes were not observed in the exponential phase of the culture. Only when the culture entered the stationary phase at densities of 10,000 to 20,000 $cells.ml^{-1}$ were both laterally-burst thecae and oblong naked motile cells observed. In addition weakly stained microcells (ca. 20 µm length), strongly stained macrocells (ca. 50 µm length) and cysts well armoured with calcite spiculae occurred (Fig. 3 G,H,I, respectively). These cysts had an orange-coloured spot at the core. It is possible that these stages constitute parts of a sexual life cycle of this species (see "Discussion").

Continuous Cultures

The growth curves from six 2-l turbidostats are shown in Fig. 4. A flow rate of 400 $ml.l^{-1}.day^{-1}$ maintained the con-

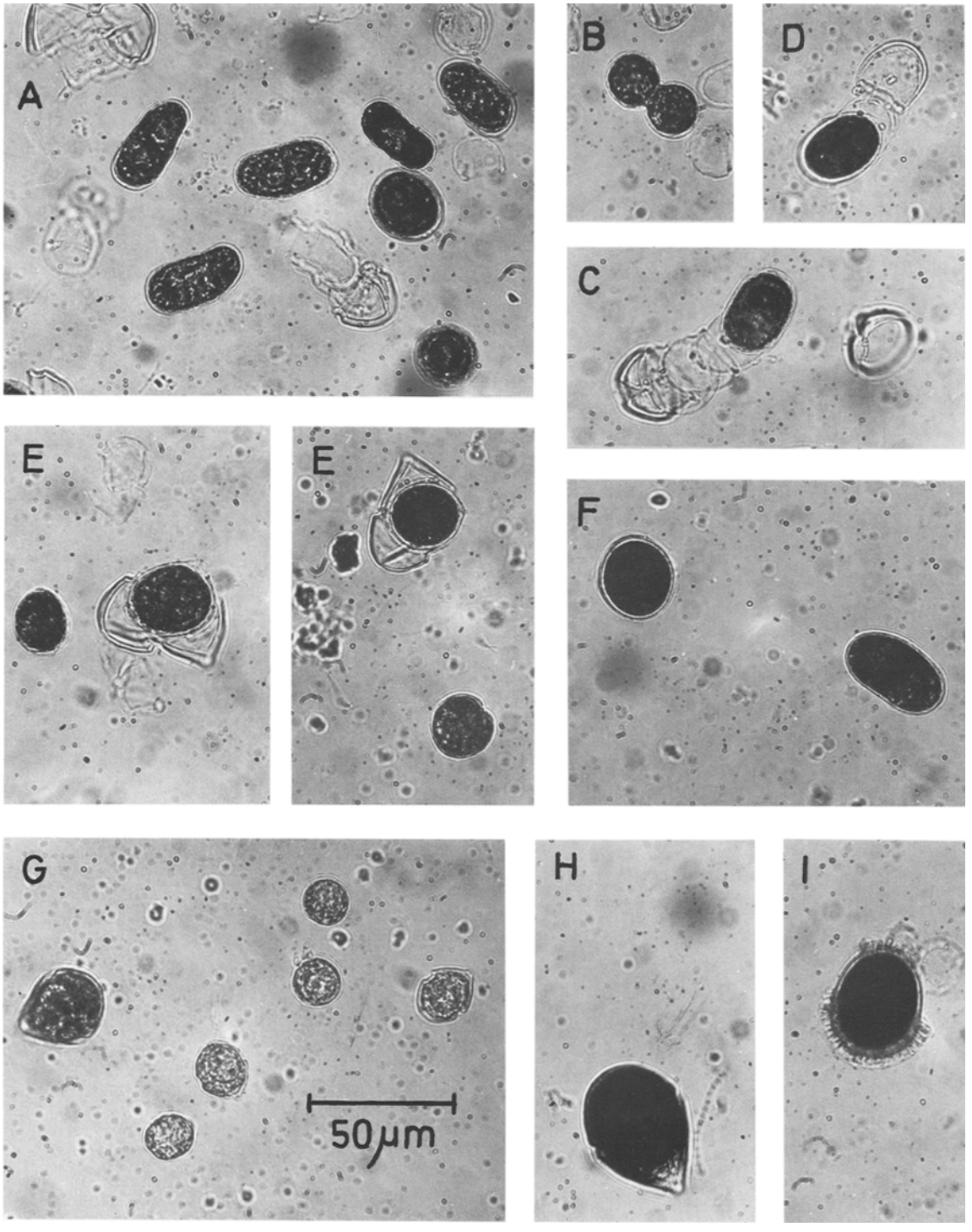

Fig. 3. *Scrippsiella faeroense*. Morphological variations after addition of mercuric acetate. (A) Naked oblong cells and empty thecae at bottom of the culture vessel, 1 day after addition of 0.05 mg $Hg.l^{-1}$; (B) division stage of an oblong cell; (C) oblong cell which has not come off from the hypotheca and has formed posteriorly a chain of empty, smooth, oblong pelliculae; (D) oblong cell with empty, hyaline pelliculae; (E) oval protoplasts, remaining in the burst thecae; (F) oval and oblong resting stages with a solid, smooth membrane, 3 weeks after addition of 0.05 mg $Hg.l^{-1}$; (G) control culture — microcells in culture with a density of 11,000 cells.ml^{-1}; (H) macrocell; (I) cyst, partly armoured with calcite spiculae from 24th experimental day. (All cells coloured by Lugol-fixation)

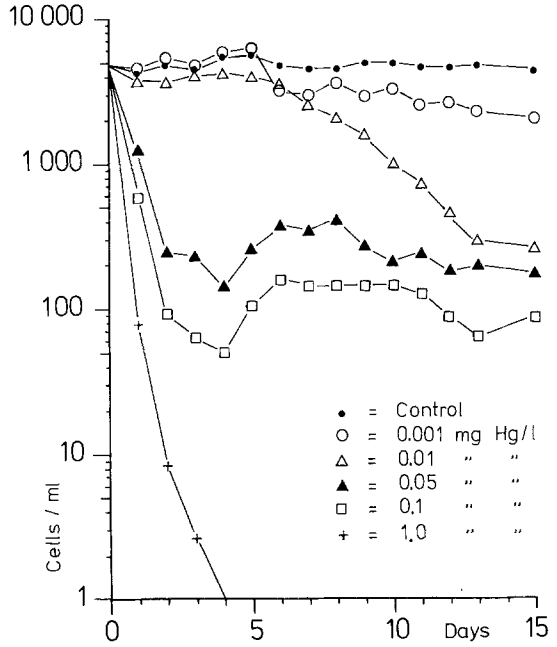

Fig. 4. *Scrippsiella faeroense*. Continuous culture. Cell density during addition of mercuric acetate in amounts of 0.001 to 1 mg $Hg.l^{-1}$ in 2-l turbidostats

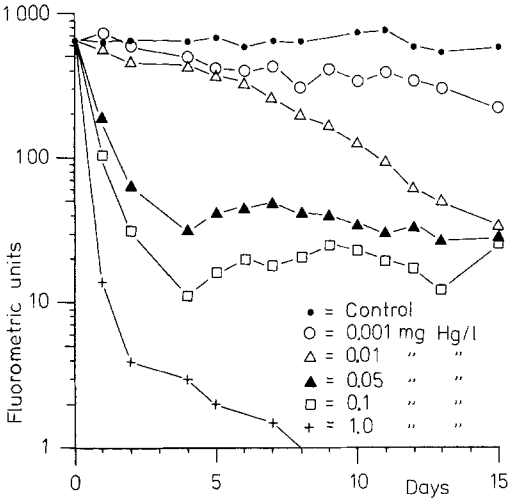

Fig. 5. *Scrippsiella faeroense*. Continuous culture. *In vivo* chlorophyll fluorescence during addition of mercuric acetate in amounts of 0.001 to 1 mg $Hg.l^{-1}$ in 2-l turbidostats

trol culture at a nearly constant density. As in batch cultures the first effect on multiplication rate was observed after additions of 0.001 and 0.01 mg Hg. l^{-1} (Table 1). After 5 days the cultures began to decrease in the course of the following 10 days to 43 and 5.6%, respectively, of the initial density. Addition of 0.05 and 0.1 mg $Hg.l^{-1}$ caused immediate rapid decrease in cell number. After 4 days recovery occurred, and subsequently the cell density remained nearly constant at 4.3 and 2.1%, respectively, of the initial density. After 4 days in 1 mg $Hg.l^{-1}$, the culture decreased without recovery to 1 $cell.ml^{-1}$. The *in vivo* chlorophyll measurements of the cultures are presented in Fig. 5. The courses of the curves follow very closely those of the cell counts.

Microscopic observation showed that the cells in Hg concentrations of 0.01 $mg.l^{-1}$ and higher settled within the first day despite aeration turbulence. As in batch cultures, empty, laterally split thecae and motile naked cells appeared. Oval, thick-walled resting stages which had remained in the burst thecae were also observed. The non-thecated motile cells died in the course of 1 week. Only normally thecated motile cells survived during the initial decrease in cell number of the cultures. In 1 mg $Hg.l^{-1}$ no motile cells were found after the first day of the experiment.

Prorocentrum micans

Batch Cultures

The first visible effect on multiplication rate occurred after addition of 0.01 mg $Hg.l^{-1}$, which caused stagnation of the culture density for the first 3 days (Fig. 6, Table 1). After this initial lag phase, culture density paralleled control density very closely. Addition of 0.1 mg $Hg.l^{-1}$ decreased culture density rapidly from the first day. No recovery occurred. In 1 mg $Hg.l^{-1}$, the culture density decreased to zero at the end of the first day. Fig. 7 shows the corresponding *in vivo* chlorophyll fluorescence measurements, which correspond closely with the cell counts. After 5 days, *in vivo* fluorescence in the 0.1 mg $Hg.l^{-1}$-cultures varied between 1 and 10 fluorometric units. These measurements lie near the lower limits of precision of the Turner Fluorometer. The results of 5 experiments are therefore presented separately for this concentration (Fig. 7).

Continuous Cultures

Figs. 8 and 9 show the results of a series of experiments run in six 2-l-

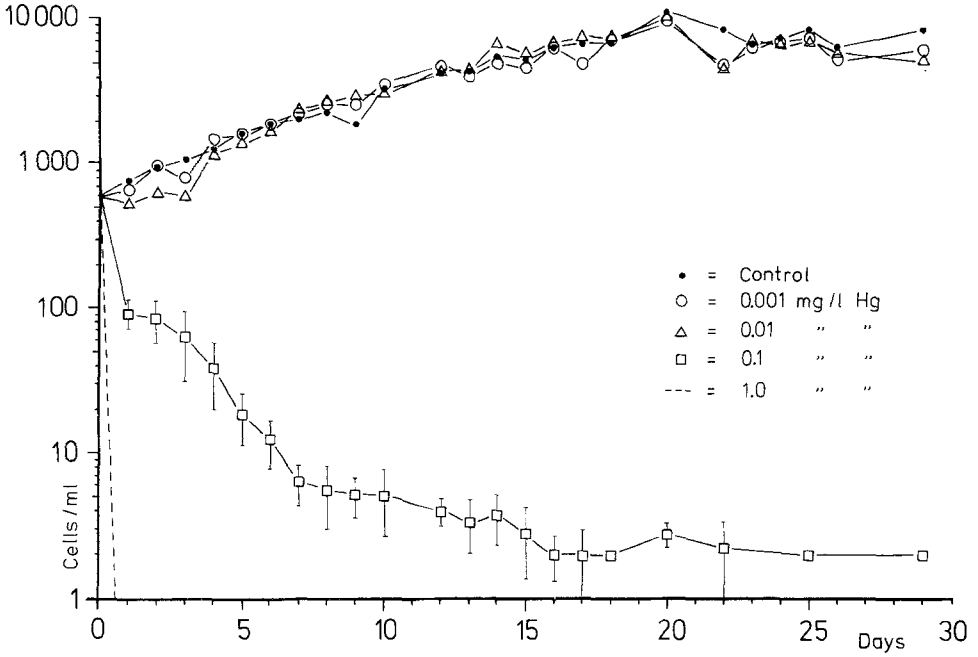

Fig. 6. *Prorocentrum micans*. Batch culture. Cell density after addition of mercuric acetate in amounts of 0.001 to 1 mg $Hg.l^{-1}$ at beginning of experiments in 2-l bottles. Mean values and standard deviations of 5 replicates are given for 0.1 mg $Hg.l^{-1}$

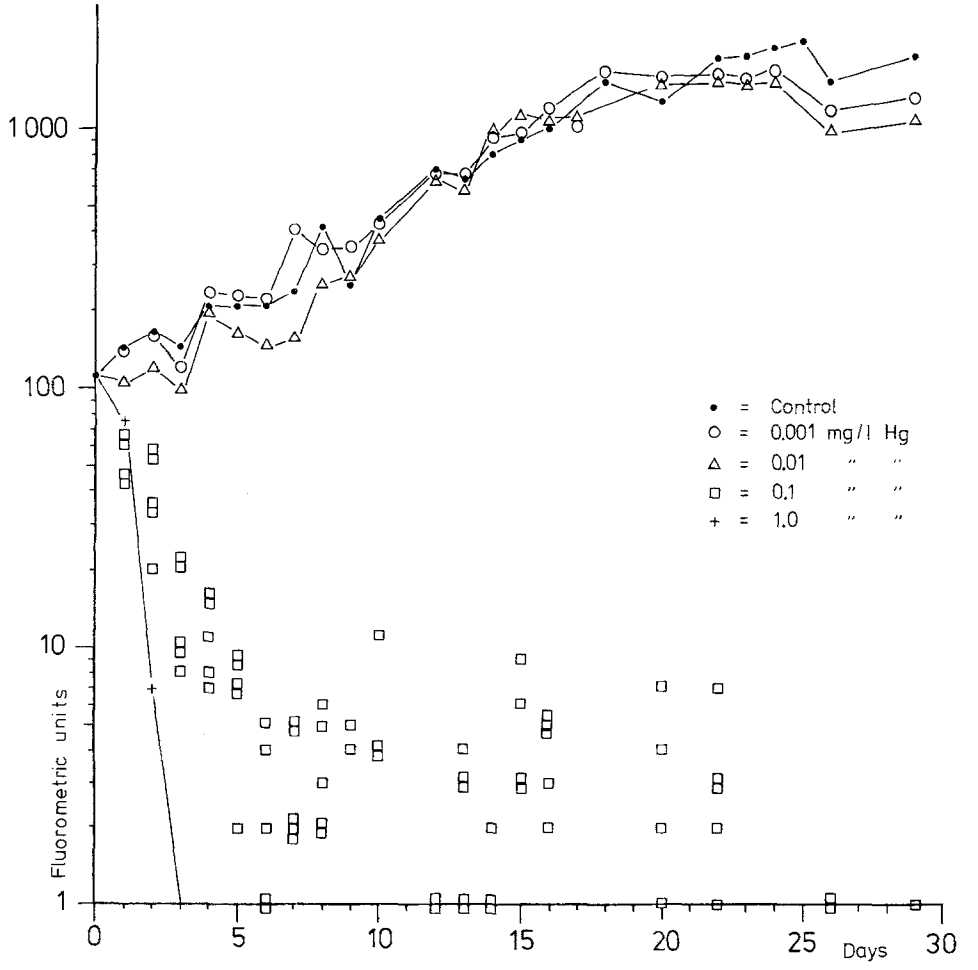

Fig. 7. *Prorocentrum micans*. Batch culture. *In vivo* chlorophyll fluorescence after addition of mercuric acetate in amounts of 0.001 to 1 mg $Hg.l^{-1}$ at beginning of experiments in 2-l bottles. Separate measurements of 5 parallel replicates are illustrated for 0.1 mg $Hg.l^{-1}$

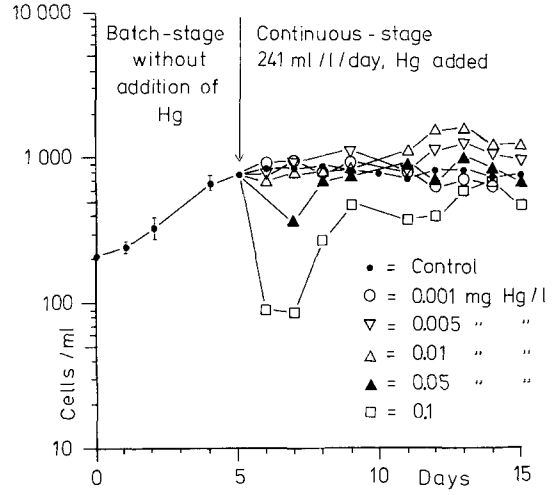

Fig. 8. *Prorocentrum micans*. Continuous culture. Cell density during addition of mercuric acetate in amounts of 0.001 to 0.1 mg $Hg.l^{-1}$ on 5th day of experiment in 2-l turbidostats

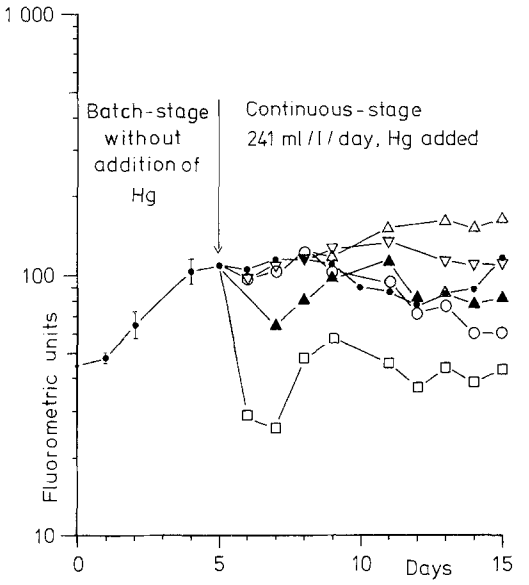

Fig. 9. *Prorocentrum micans*. Continuous culture. *In vivo* chlorophyll fluorescence during addition of mercuric acetate in amounts of 0.001 to 0.1 mg $Hg.l^{-1}$ on 5th day of experiment in 2-l turbidostats. Symbols as in Fig. 8

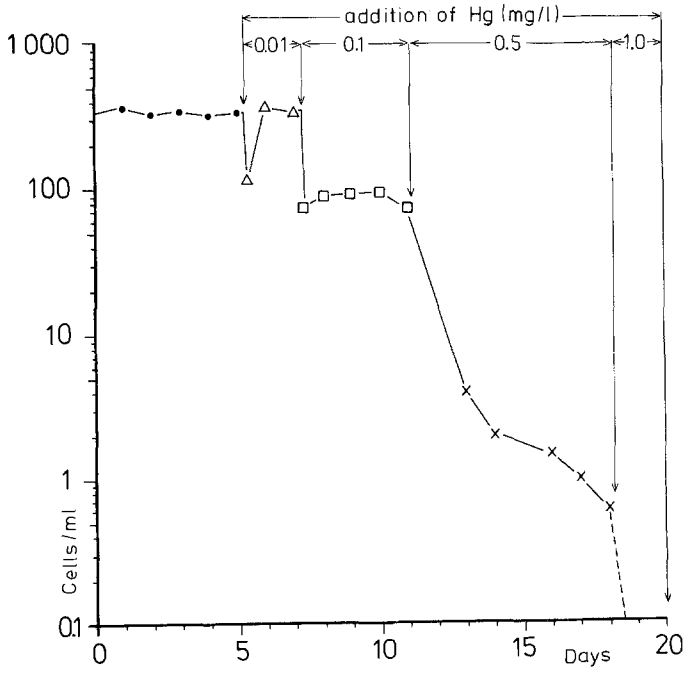

Fig. 10. *Prorocentrum micans*. Continuous culture. Cell density in 8-l turbidostat (fermenter). Mercuric acetate was added once daily directly to culture vessel in amounts of 0.01 to 1 mg $Hg.l^{-1}$ in succession from 5th day of experiment on

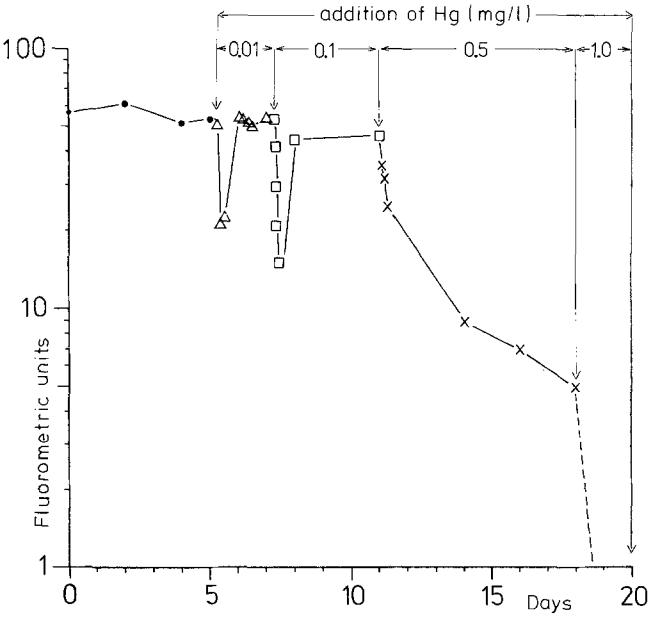

Fig. 11. *Prorocentrum micans*. Continuous culture. *In vivo* chlorophyll fluorescence in 8-l turbidostat (fermenter). Mercuric acetate was added once daily directly to culture vessel in amounts of 0.01 to 1 mg $Hg.l^{-1}$ in succession from 5th day of experiment on

turbidostats. During the first 5 days the algae were cultivated as batch cultures. Then the continuous medium flow was switched-on at a flow rate of 241 ml. $l^{-1}.day^{-1}$. Simultaneously, mercury acetate was added continuously together with the medium inflow. The 0.001 to 0.01 mg $Hg.l^{-1}$-cultures varied little from the constant cell numbers of the control culture. In 0.05 mg $Hg.l^{-1}$, however, a distinct decrease in cell number occurred during the first 2 days. After this initial decrease the culture recovered rapidly. The same results were obtained with the 0.1 mg $Hg.l^{-1}$-culture. The *in vivo* chlorophyll fluorescence measurements displayed analogous results (Fig. 9).

A second continuous-flow experiment, to determine the lethal Hg-concentrations under turbidostatic conditions, was conducted in an 8-l fermenter (Figs. 10,11). The experiment varied from the first in two details: (1) the fermenter had only 1 culture vessel – therefore different Hg concentrations could be investigated only in succession; (2) the mercury was added directly once daily to the culture vessel – this semicontinuous addition was designed to prevent possible inactivation of the mercury in the medium stock bottles before it reached the culture vessel by the flow-through system. A flow rate of 325 $ml.l^{-1}.day^{-1}$ maintained the initial density at a constant level (Fig. 10). Addition of 0.01 mg $Hg.l^{-1}$ on the fifth day caused, within 3 h, a short-term decrease in cell numbers, presumably by settlement since on the following day the culture had recovered to pre-mercury values. The same occurred in the *in vivo* fluorescence measurements (Fig. 11). Subsequent increase of Hg concentration to 0.1 mg $Hg.l^{-1}$ caused a renewed decrease in cell numbers within 2 h. In this case, however, cell numbers remained around this level for the following 4 days. Upon addition of 0.5 mg $Hg.l^{-1}$ the cell numbers and corresponding *in vivo* fluorescence decreased rapidly within the next week and with no signs of recovery. Addition of 1 mg $Hg.l^{-1}$ caused total destruction of the culture within 1 day.

Gymnodinium splendens

Continuous Culture

The experiment was carried out in a series of five 2-l turbidostats (Fig.12). After an initial batch stage of 3 days, a continuous flow-through of 350 $ml.l^{-1}.day^{-1}$ maintained the control culture at nearly constant cell density. Continuous

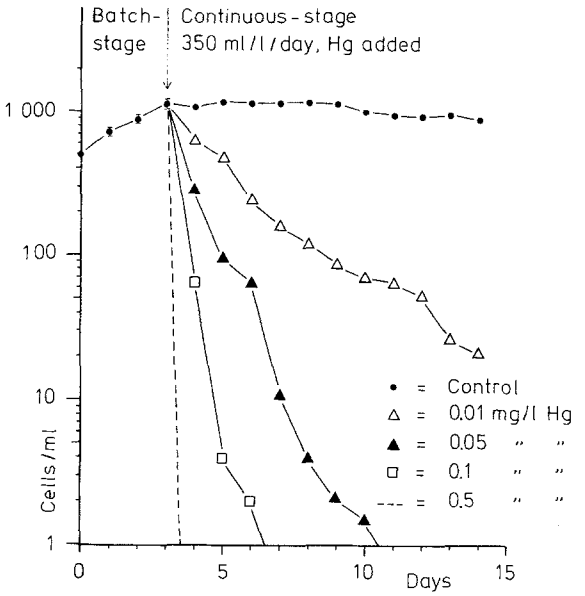

Fig. 12. *Gymnodinium splendens*. Continuous culture. Cell density during addition of mercuric acetate in amounts of 0.01 to 0.5 mg $Hg.l^{-1}$, beginning on 3rd day of experiment in 2-l turbidostats

addition of 0.01 mg $Hg.l^{-1}$ decreased the test culture density to nearly 2% of control density within 2 weeks. In 0.05 and 0.1 mg $Hg.l^{-1}$ cell density dropped to less than 1 $cell.ml^{-1}$ even after 11 and 7 days, respectively. In 0.5 mg $Hg.l^{-1}$, all cells died within the first day of Hg addition. No recovery was observed at any of the concentrations tested.

Discussion

Comparison of the three algal species shows that *Gymnodinium splendens* is most sensitive to mercuric acetate (Table 1), followed by *Scrippsiella faeroense* and *Prorocentrum micans*. Effects of toxicity were most obvious immediately after the addition of mercury in both batch and continuous-culture experiments. The expected intensified toxicity under continuous-flow conditions did not occur. The Hg-addition via the continuous medium inflow, as carried out in the 2-l turbidostat series, had the same effect on the algae as had the semicontinuous direct input of Hg into the culture vessel daily by hand, as carried out in the 8-l fermenter experiment. The recovery observed in *S. faeroense* and *P. micans* may have resulted from volatilization or fixation of ionic Hg to walls or to particulate or dissolved organic compounds, in which form it is less toxic to the algae. Perhaps these compounds

are produced by the algae themselves. Chemical analyses of the location of the Hg during the experiments may provide precise information.

Davies (1974) reported a comparable recovery effect with cultures of *Isochrysis galbana:* addition of 10.5 $\mu g.l^{-1}$ mercuric chloride at the beginning of the experiments prevented growth after 5 days. On the 15th day recovery occurred, followed by renewed exponential growth of the culture. With the difference that in the present study growth was hindered even from the first day on (Figs. 1 and 2), Davies' experiment corresponded to my 0.05 mg $Hg.l^{-1}$ experiment on *Scrippsiella faeroense.* According to Ben-Bassat *et al.* (1972), *Chlamydomonas rheinhardii* is extremely tolerant to inorganic mercury and continues to grow in concentrations of 1 mg $Hg.l^{-1}$ although with a lengthened lag phase. At 2 mg $Hg.l^{-1}$, however, growth ceases. In their experiments, the total mercury levels of the aerated cultures initially containing 0.2 mg $Hg.l^{-1}$ decreased by about 40% over a period of 8 days. The authors suggested that the mercury had become volatile through some biological process, thus allowing it to leave the cultures.

During culture experiments inorganic mercury compounds can be changed into organic forms by microbial activity (Magos *et al.*, 1964). This is important in view of the fact that Harris *et al.* (1970) and Nuzzi (1972) reported that monoalkyl- and aryl-mercury salts are considerably more toxic than their inorganic forms. Matida *et al.* (1971) showed also that *Scenedesmus dimorphus* was not inhibited by 50 ppb mercuric chloride, but that growth was retarded by 10 ppb methyl mercuric chloride. According to Knauer and Martin (1972), assimilation of mixed phytoplankton communities was slowly inhibited by concentrations of over 0.5 ppb mercuric chloride, whereas as little as 0.1 ppb methyl mercury inhibited production to 30%. On the other hand, Hannan and Patouillet (1972) and Hannan *et al.* (1973) indicated that mercuric chloride is more toxic than dimethyl mercury. Similar effects of diphenyl mercury on fresh-water phytoplankton was noted by Harris *et al.* (1970).

Results of toxicity tests depend on the composition of the test medium used. Toxicity varies inversely with the concentration of nutrients present (Hannan and Patouillet, 1972). In a practical waste-water tolerance test it is best, therefore, to use pure local sea water from that area which is being considered for waste-water discharge purposes (Kayser, 1971; Jensen and Rystad, 1974). This makes the choice of the test species very difficult, since only a few forms can grow without addition of nutrients and other substances to their culture medium. Particularly stenoplastic and sensitive forms may require special media for normal growth. Sensitivity of the test forms is nevertheless a very important criterion for evaluating results from a waste-water test. Test organisms should be characteristic members of the plankton communities in the area. The test should not be restricted to one species but should include as many forms as can be cultivated within the assay conditions.

Morphological changes in test algae provide very reliable and distinct criteria for a bioassay. In *Scrippsiella faeroense,* Hg-addition causes the emergence of naked motile cells, and the observation of non-motile resting stages even at low cell densities in the exponential phase of the cultures. In the control cultures, comparable processes occurred only after maximum cell densities had been reached. This phenomenon was also observed in aged cultures by Braarud (1958) and Sousa e Silva (1962). Boltovskoy (1973) pointed out that the emergence of naked cells in dinoflagellates occurs under unfavourable environmental conditions, and Professor H.A. von Stosch (personal communication) reports this even from damaged plankton samples. This phenomenon is, therefore, not a specific effect of mercury. Comparison with the unaffected control cultures indicates, however, that the addition of Hg induces the process. Cu-ions produce the same effect (Dr. S.M. Saifullah, personal communication).

The additional occurrence of macro- and microcells and of calcitic resting spores at maximum cell densities in the control cultures suggests that some stages of a sexual life cycle may be involved. According to Professor von Stosch (personal communication), the macrocells could be planozygotes, that later change into hypnozygotes (Fig. 3 H). The microcells could be gametes (Fig. 3 G). However, only an exact analysis of the nuclei and long-term observations of the living cells can provide valid data (von Stosch, 1973).

Nuzzi (1972) described phathological changes in the size and form of *Phaeodactylum tricornutum* and *Chlorella* sp. after the addition of organomercurial compounds. Davies (1974) observed a considerable effect of mercuric chloride upon the mean cell volumes of *Isochrysis galbana;* cell volumes almost doubled at the highest sublethal concentrations he examined (10.5 $\mu g.l^{-1}$).

The measurements of the *in vivo* chlorophyll fluorescence corresponded closely to the cell counts in the experiments described here. This is mainly because the samples were taken simultaneously. Blasco (1973) showed that in the dark the fluorescence of algae is significantly enhanced. In his experiments the ratio of *in vivo* fluorescence:chlorophyll *a* was not constant for different species and, for the same species, was negatively correlated with the concentration of chlorophyll per cell. *In vivo* fluorescence depends also on the physiological characteristics of the cells. Measurements of photosynthesis, however, seem to constitute a very much more sensitive method for the examination of toxic substances. Harris *et al.* (1970), for example, showed a reduced assimilation rate in the diatom *Nitzschia delicatissima* in as little as 0.1 ppb of organomercurial fungicides. The same was reported by Knauer and Martin (1972) for mixed populations of phytoplankton with methyl mercury.

So far, algal bioassays in the laboratory have been made almost exclusively on monocultures in the exponential growth phase and under optimal culture conditions. These correspond only to a small extent to conditions in the natural environment. Interspecific competition, nutrient limitation, non-optimal light and temperature conditions, and grazing rates of plankton predators limit algal populations *in situ* and, therefore, may increase their sensitivity to additional stresses by toxicants. The objective imitation of natural stress factors in a laboratory test is very difficult. Interspecific competition could be imitated by multispecies cultures. Fisher *et al.* (1974) showed in two-species cultures of *Dunaliella tertiolecta* and *Thalassiosira pseudonana* and in natural plankton communities that 0.01 ppb of polychlorinated biphenyl (PCB) did not effect algal growth in pure cultures but caused substantial disruption in continuous-culture communities. Nutrient-limited conditions in continuous-test cultures could be achieved by using chemostats. This method, however, involves relatively high and unnatural cell densities. In the present work the turbidostat technique was preferred because it allows observation of low cell densities, by means of the culture flow-through system, and avoids nutrient limitation. In future work it is intended to supplement the present experiments by continuous multispecies cultures using the chemostat technique, and by chemical analyses of the locations of the added toxicants.

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