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The effect of pesticides on the growth of green and blue-green algae cultures*

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A b s t r a c t — The sensibility of 9 green algae: *Chlorella pyrenoidosa, C. mucosa, Ankistrodesmus minutissimus, Chlorococcum* sp., *Dictyospaerium pulchellum, Scenedesmus acutus, S. quadricauda, Hormidium flaccidum*, and *Stichococcus* sp., and 3 blue-green algae: *Anabaena variabilis, Spirulina platensis*, and *Oscillatoria* sp. to 9 pesticides: TCA, 2,4-D acid, monuron, diuron, simazine, atrazine, p. naphtoquinone, DDT, and methoxychlor was determined on the basis of growth kinetics and the criterion of LC_{50} (50% growth inhibition). The LC_{50} values of 0.15—24 $\mu g \cdot dm^{-3}$ denote the species sensitive to 2,4-D and urea- and triazine-derivative herbicides; the values of 40—50 $\mu g \cdot dm^{-3}$ characterize the species sensitive to p-naphtoquinone while those within 0.3—0.9 $mg \cdot dm^{-3}$ determine the species sensitive to TCA, DDT and methoxychlor.

K e y w o r d s: green algae, blue-green algae, sensibility to pesticides.

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1. Introduction

The constantly increasing amounts of pesticides used in agriculture and forestry are drained to water bodies, bringing about drastic changes in their biocenosis. Until the seventies most investigations were limited to the determination of toxicity of herbicides to aquatic animals (A l a b a s t e r 1969, M u l l i s o n 1970, K a m l e r et al. 1974). Algal tests

* Praca wykonana w problemie MR. II. 15.

were less frequently used, being chiefly applied in the investigations on algicides (Braginskij et al. 1968, 1969, Fitzgerald 1971). In recent years algae have been more and more often used the determination of toxic substances in the environment (Zweig et al. 1968, Pillay, Than 1972, PN-74, Wright 1975) and even for the identification of groups of chemical compounds (Böhm 1973, Noll, Bauer 1973). Usually the investigations were carried out either with one species and different preparations or with larger number of species, one concentration of the given substance, and a short period of its action.

The aim of the work was to investigate the effect of pesticides on several species of algae in cultures grown to the stationary phase of growth. The investigations were motivated by the results of experiments with 3 algae species grown on sewage water from agricultural areas treated with monuron (Bednarz, Żarnowski 1979). It was found that the toxic agent in the drainage water severely affected the growth of algae cultures and that the reaction of individual species was different. The conclusions drawn from these findings were checked upon in laboratory investigations which included 12 algae species and 9 pesticides. Attempts were made to determine the sensitivity of the algae to the preparations, and also the usefulness of this reaction in the quantitative and qualitative detection of these compounds in aquatic environments.

2. Material and method

The investigation was carried out in the years 1974—1977 in the Institute of Zootechnics at Zator, and in the years 1978—1979 in the laboratory of Water Biology of the Polish Academy of Sciences in Cracow.

The biological material was composed of algalogically pure cultures of 12 algae species from the collection of the Institute of Zootechnics at Zator (Bednarz, Nowak 1971). The cultures were treated with 9 chemically pure pesticides obtained from the Pedagogical Institute at Këthen (GDR).

Nine algae species belonged to *Chlorophyceae* and three to *Cyanophyceae*. Unicellular *Chlorophyceae* were represented by *Chlorella pyrenoidoea* Chick., strain No 366; *C. mucosa* Korschik., No 594; *Ankistrodesmus minutissimus* Korschik., No 1193; and *Chlorococcum* sp., No 564; caenobial *Chlorophyceae* were represented by *Scenedesmus acutus* Meyen, No 1608; *S. quadricauda* (Turp.) Brèb., No 1097; and *Dictyosphaerium pulchellum* Wood, No 1616. Filamentous

Chlorophyceae were represented by *Hormidium flaccidum* Braun, No 494 and *Stichococcus* sp., No 1619. Filamentous algae *Anabaena variabilis* Kütz, No 1618, *Spirulina platensis* (Gom.) Geitl., No 1620, and *Oscillatoria* sp., No 1621 represented the *Cyanophyceae* group. Apart from the last two species, all investigated algae were freshwater organisms, isolated from fish ponds, rivers, municipal sewage, heated waters, and also from mineral springs (Bednarz, Nowak 1971);; halophilous blue-green algae *Spirulina platensis* and *Oscillatoria* sp. were isolated from flood-waters of Lake Czad in Africa (Compère 1968). The selected pesticides were in current use in agriculture and belonged to the most important structural types of compounds. Particular reference was made to herbicides, 6 preparations having been included in the experiment: 1 — TCA, sodium salt of trichloracetic acid, 2 — 2,4-D acid, sodium salt of 2,4-dichlorophenoxyacetic acid (derivates of carboxylic acids), 3 — monuron, N-(4-chlorophenyl)-NN-dimethylurea, 4 — diuron, N-(3,4-dichlorophenyl)-NN-dimethylurea (urea derivates), 5 — simazine, 2-chloro-4,6-(bisethylamino)-1,3,5-triazine, and 6 — atrazine, 2-ethylamino-6-isopylamino-4-chloro-1,3,5,-triazine (triazine derivatives).

Fungicides were represented by: 1 — p. naphtoquinone, (2,3-dichloro--1,4-naphtoquinone) (quinone derivates), and the insecticides were: 1 — DDT, (1,1,1-trichloro-2,2-di-(4-chlorophenyl)-ethane), and 2 — methoxychlor, (1,1,1-trichloro-2,2-bis(4-methoxyphenyl)-ethane) (chlorinated hydrocarbons).

The algae were grown on liquid media at 23—25°C; the volume of the medium was 150 ml in 300-milliliter flasks. During 14-day cultures up (to the stationary phase of growth), the flasks were shaken in a laboratory shaker. Fluorescence lamps of the day-light type at light intensity 2000 lx were applied. Freshwater species were grown in L_8m medium (Jankowski 1964) while halophilous algae were inoculated in Zarrouck's medium (1966).

Pesticides in the form of water suspensions were added to the media directly before the inoculation with algae. The applied tenfold gradation of concentrations ranged from 0.01 to 100 $mg \cdot dm^{-3}$. In preparing the decreasing and increasing range of concentrations, 1 $mg \cdot dm^{-3}$ was accepted as the starting value, because this magnitude is generally regarded as a dangerous concentration and is sometimes actually noted in aquatic environments (FAO 1970, Braginskij 1972, Taylor et al. 1972, Khan 1977, Melnikov et al. 1977). The concentration of pesticides was decreased to 0.0001 for some sensitive species and increased to 1 $g \cdot dm^{-3}$ for tolerant ones.

The initial density of the cultures was 0.01—0.02 $g \cdot dm^{-3}$ of dry weight. The growth of cultures was checked every two days by counting cells in Bürker's chamber. Except for *Hormidium flaccidum* and *Stichococcus* sp. which appeared in the form of filaments composed of several cells, the growth of filamentous algae was checked by counting the number of filaments per 1cm^3 of the culture, using the drop method (J a n u s z k o et al. 1977). The final effect of the culture was evaluated by the dry weight content, determined by filtering (*Spirulina platensis* and *Oscillatoria* sp.) or centrifuging (other species), and then drying to constant weight at 105°C. The culture was carried out in 3 replications while the control cultures, without pesticides, were conducted separately for each experimental series.

The concentrations which brought about a 50% decrease in the growth of algae cultures were calculated for all chemical compounds, the decrease being expressed as the dry weight content (LC_{50}) in relation to the yield of control cultures taken as 100%.

3. Results

3.1. Sensibility of algae determined by the LC_{50} criterion

It was found that some of the applied substances caused similar reactions in closely related species while other compounds brought about different reactions even in species which belonged to the same genus (figs 1—5). The least variable reaction to all treatments was found in the cultures of *Hormidium flaccidum* and *Stichococcus* sp. The greatest similarity of reaction within the systematic units was observed with regard to methoxychlor and diuron.

The range of concentrations which caused a 50% inhibition of growth was very wide. Even for the same compound it varied from 0.0001 to 100 mg · dm^{-3} (Table I, fig. 6). The species at the top of Table I were marked as sensitive; those which were listed at the lowest positions of the Table were marked (*) as resistant.

The investigated species of algae reacted very slightly to DDT while higher concentrations of this compound stimulated the growth of *Oscillatoria* sp. (fig. 1A).

The reaction to TCA was also poor, *Chlorella pyrenoidosa* being the only sensible species (Table I). In spite of similar LC_{50} values, *Chlorella mucosa* responded with good growth at all applied concentrations. This was also found with the remaining species (fig. 2B).

Oscillatoria sp. and *Spirulina platensis* were distinctly stimulated by low concentrations of p. naphtoquinone. At concentrations higher than 0.1 mg · dm^{-3} their growth was completely stopped. The reaction of other species this to compound was less pronounced (fig. 2A).

Methoxychlor at the concentration of 100 mg · dm^{-3} was toxic to all investigated species (fig. 1B) while the reaction of algae to a 10-fold

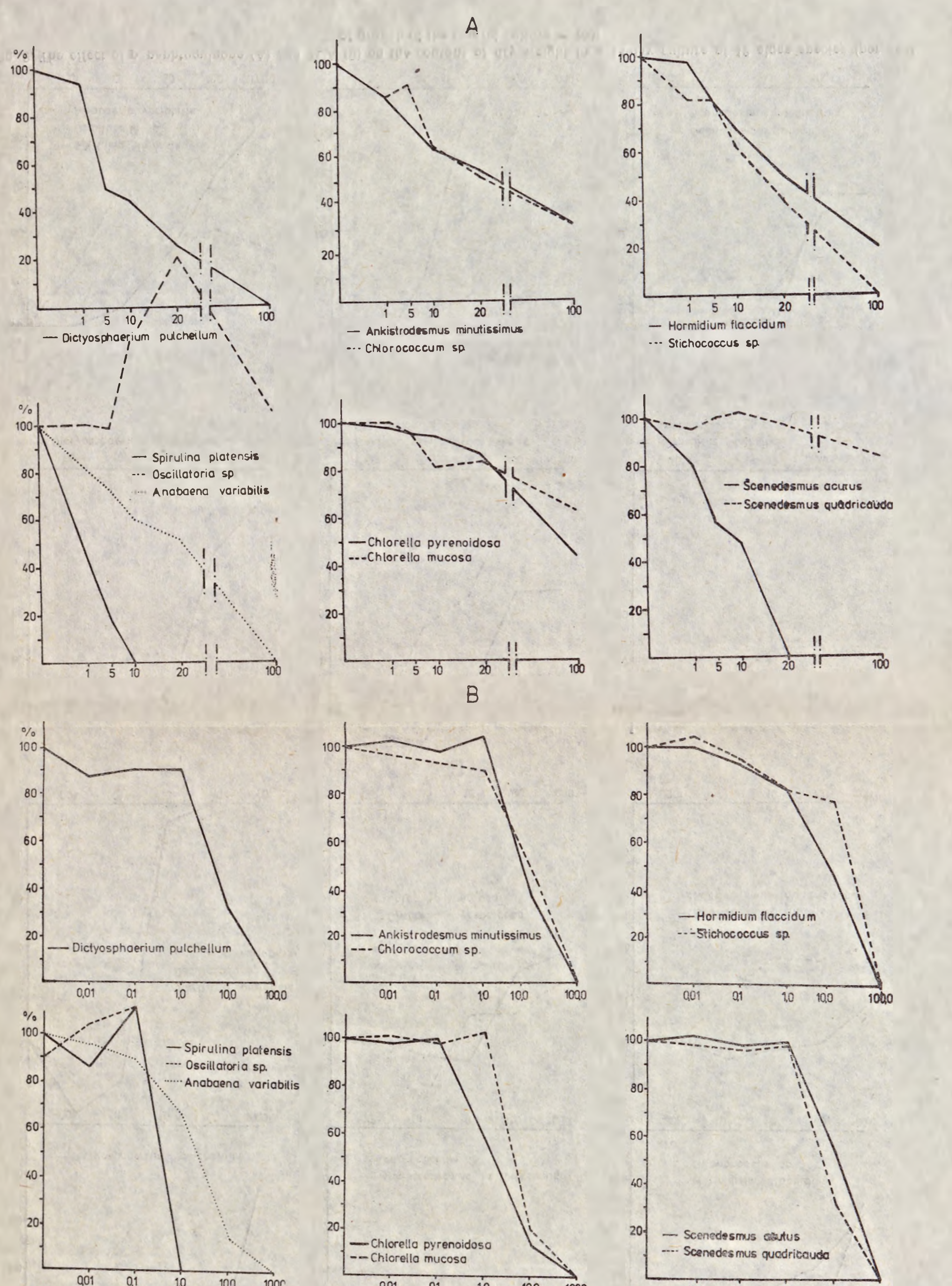

Fig. 1. The effect of DDT (A) and methoxychlor (B) on the content of dry weight in a 14-day culture of 12 algae species (per cent of growth of the control culture — 100)

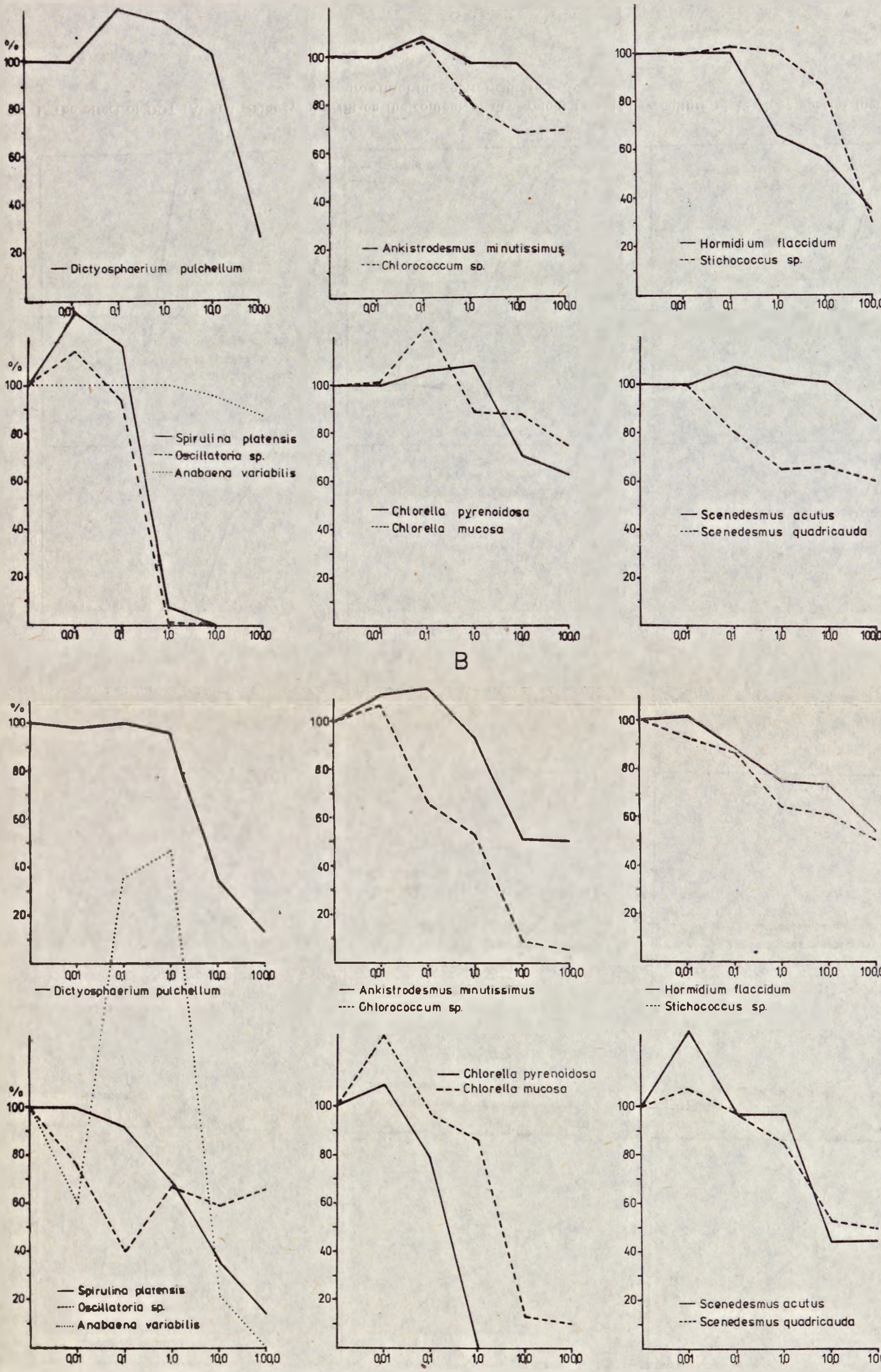

Fig. 2. The effect of p. naphtoquinone (A) and TCA (B) on the content of dry weight in a 14-day culture of 12 algae species (per cent of growth of the control culture = 100)

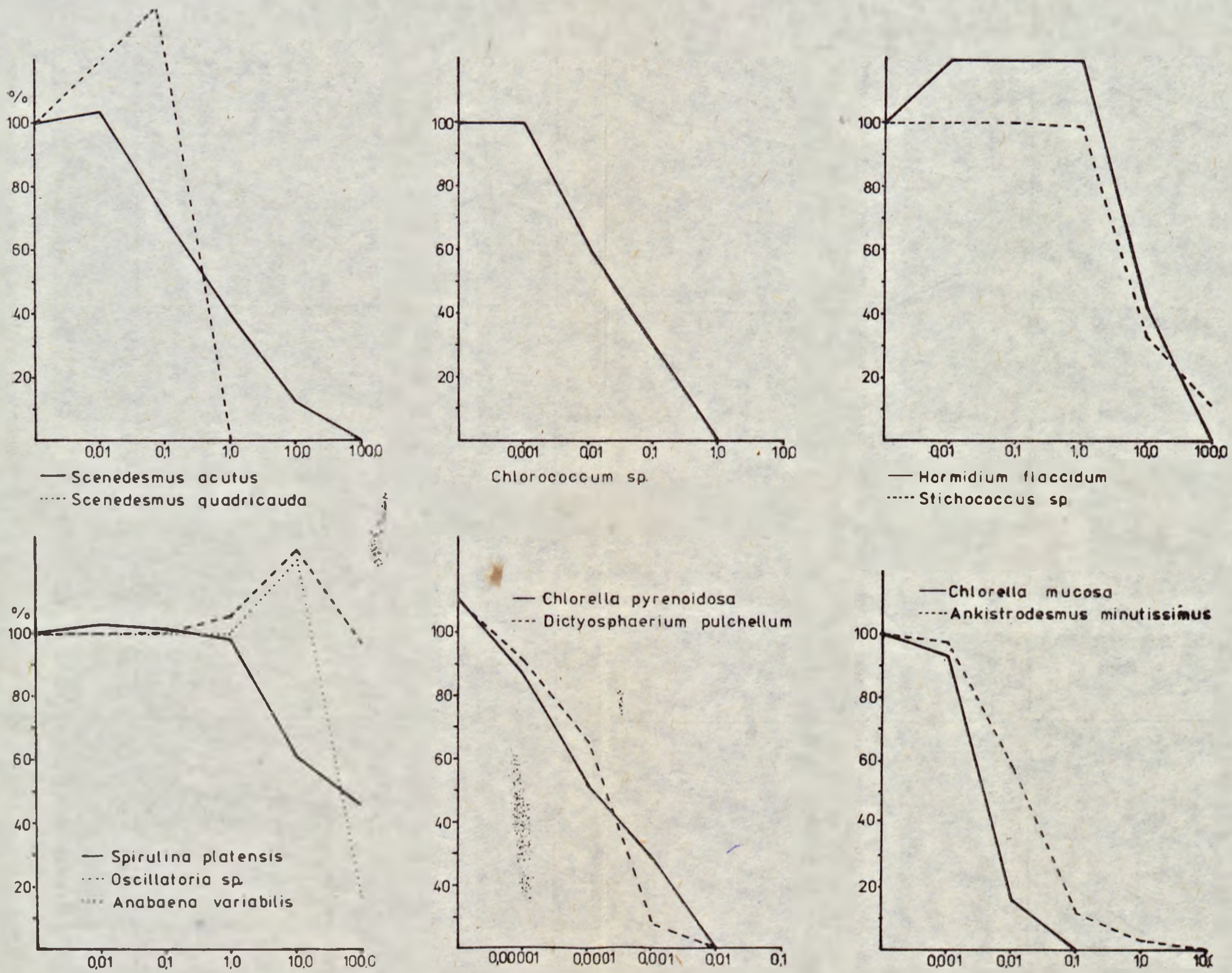

Fig. 3. The effect of 2.4-D on the content of dry weight in a 14-day
ture = 100)

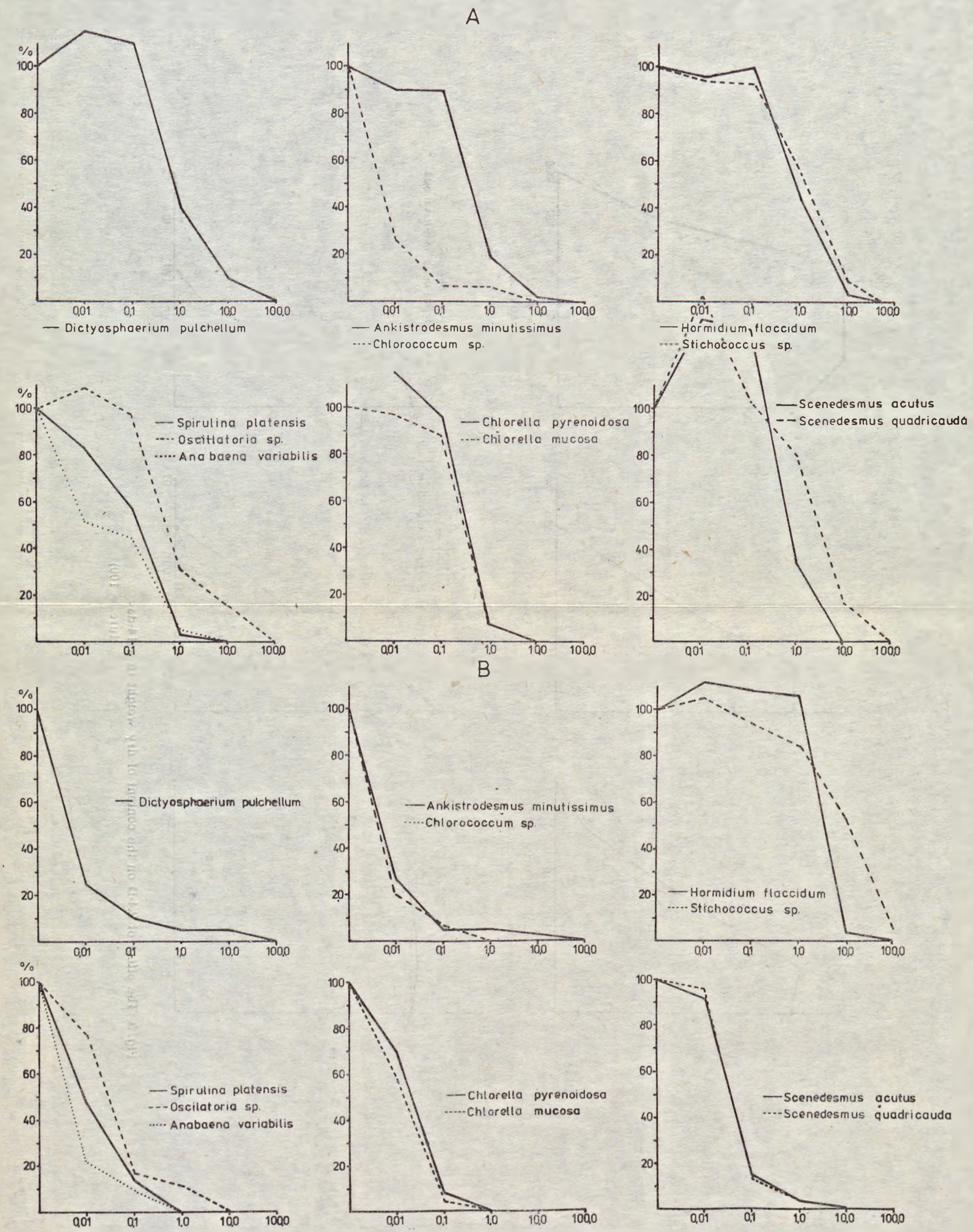

Fig. 4. The effect of monuron (A) and diuron (B) on the content of dry weight in a 14-day culture of 12 algae species (per cent of growth of the control culture = 100)

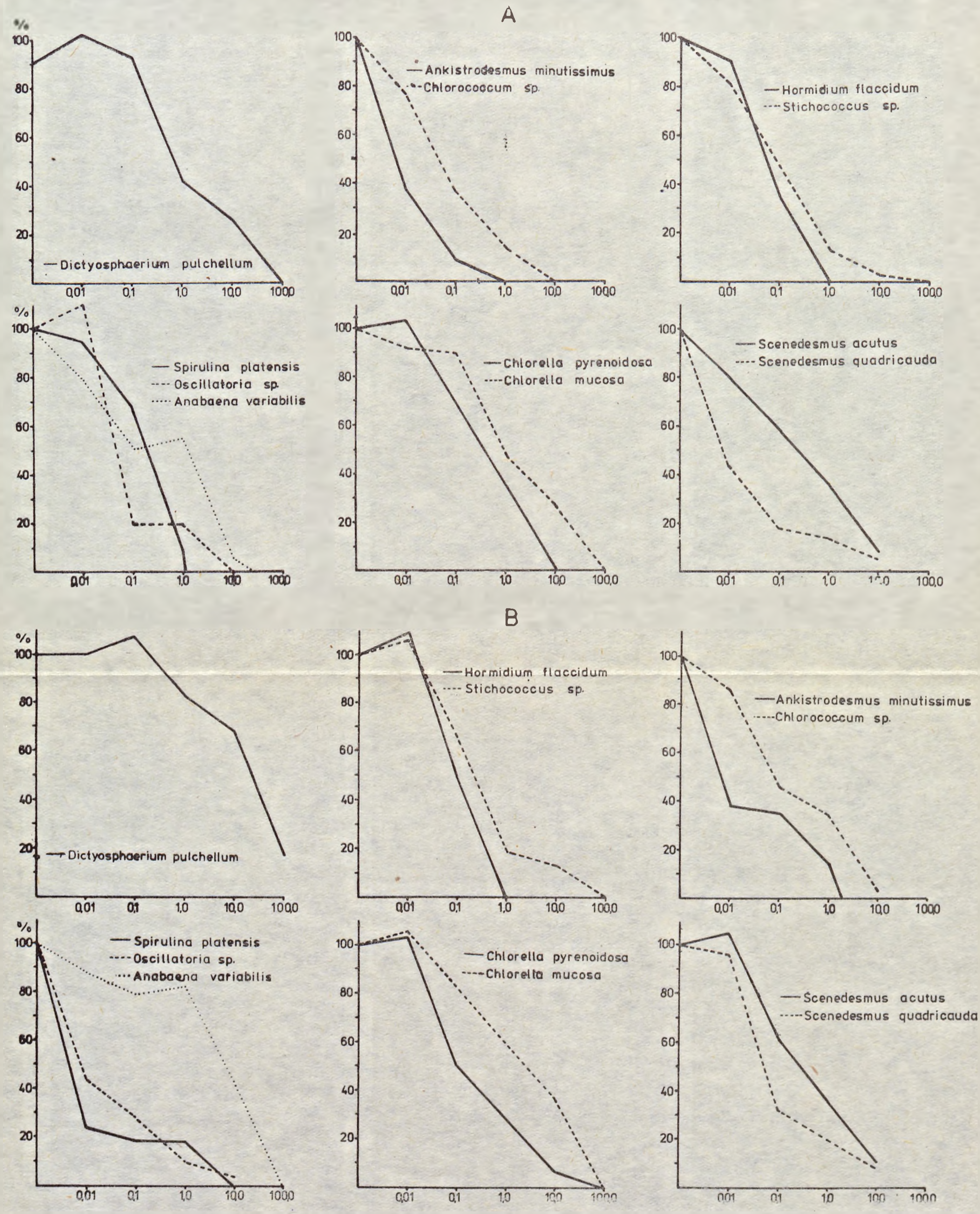

Fig. 5. The effect of atrazine (A) and simazine (B) on the content of dry weight in a 14-day culture of 12 algae species (per cent of growth of the control culture = 100)

Table I. Sensibility of the investigated species of algae to different pesticides expressed in LC_{50} in $\mu g \cdot dm^{-3}$. The continuous line divides sensible species from the remaining ones. • - resistant species.

Legend to symbols:

Chl p - Chlorella pyrenoidosa
Chl m - Chlorella mucosa
Sc ac - Scenedesmus acutus
Sc q - Scenedesmus quadricauda
Hor - Hormidium flaccidum
Stich - Stichococcus sp.
Chloc - Chlorococcum sp.
Ank - Ankistrodesmus minutissimus
Dic - Dictyosphaerium pulchellum
Anab - Anabaena variabilis
Spir - Spirulina platensis
Oscil - Oscillatoria sp.

Diuron		Monuron		Atrazine	
species	LC_{50}	species	LC_{50}	species	LC_{50}
Chloc	5	Chloc	6.5	Ank	7.5
Anab	9.8	Anab	24	Sc q	8
Dic	6	Oscil	100	Chloc	60
Ank	6	Chl p	400	Oscil	60
Spir	8.5	Ank	500	Hor	70
Chl p	30	Chl m	530	Stich	90
Oscil	40	Spir	600	Spir	300
Chl m	40	Sc ac	710	Sc ac	300
Sc ac	50	Dic	800	Chl p	500
Sc q	50	Hor	900	Dic	810
Hor	500	•Stich	2500	Chl m	880
•Stich	1500	•Sc q	4100	•Anab	1500

Simazine		2,4-D acid		Methoxychlor	
species	LC_{50}	species	LC_{50}	species	LC_{50}
Spir	6	Chl p	0.15	Spir	480
Chloc	8	Dic	0.24	Oscil	480
Oscil	8.2	Chl m	5	•Chl p	1800
Sc q	65	Ank	18	•Anab	2200
Ank	87	Chloc	28	•Dic	6000
Hor	90	Sc q	500	•Chl m	6000
Chl p	100	•Sc ac	600	•Sc q	7000
Stich	200	•Stich	7000	•Ank	7500
Sc ac	400	•Hor	9000	•Hor	8000
•Chl m	3300	•Spir	60000	•Chloc	10000
•Anab	7400	•Oscil	80000	•Sc ac	13000
•Dic	28000	•Anab >	100000	•Stich	30000

1.4 p-naphtoquinone		TCA		DDT	
species	LC_{50}	species	LC_{50}	species	LC_{50}
Oscil	40	Chl p	300	Spir	900
Spir	90	•Chl m	460	•Dic	5000
•Hor	20000	•Chloc	1200	•Sc ac	8500
•Stich	60000	•Spir	5000	•Stich	15500
•Dic	65000	•Dic	7000	•Hor	20000
•other >	100000	•Anab	8000	•Anab	22000
		•Sc ac	8800	•Chloc	50000
		•Ank	98000	•Ank	55000
		other >	100000	•Chl p	[illegible]
				•other >	100000

concentration of this pesticide was similar to their reaction to higher concentrations of DDT (fig. 1A, B).

The greatest variation in the sensitivity of algae was noted with 2.4-D and atrazine (fig. 6). In spite of low LC_{50} values, *Scenedesmus acutus* was also determined as tolerant to 2.4-D because its growth was stopped at concentrations exceeding 100 mg $\cdot$ dm^{-3} (fig. 3).

Atrazine at the concentration of 100 mg $\cdot$ dm^{-3} was toxic to the investigated species while a 10-fold lower concentration inhibited the growth of all culture (fig. 5A). The growth of *Oscillatoria* sp. decreased under the influence of atrazine at the concentration of 0.1 mg $\cdot$ dm^{-3} and

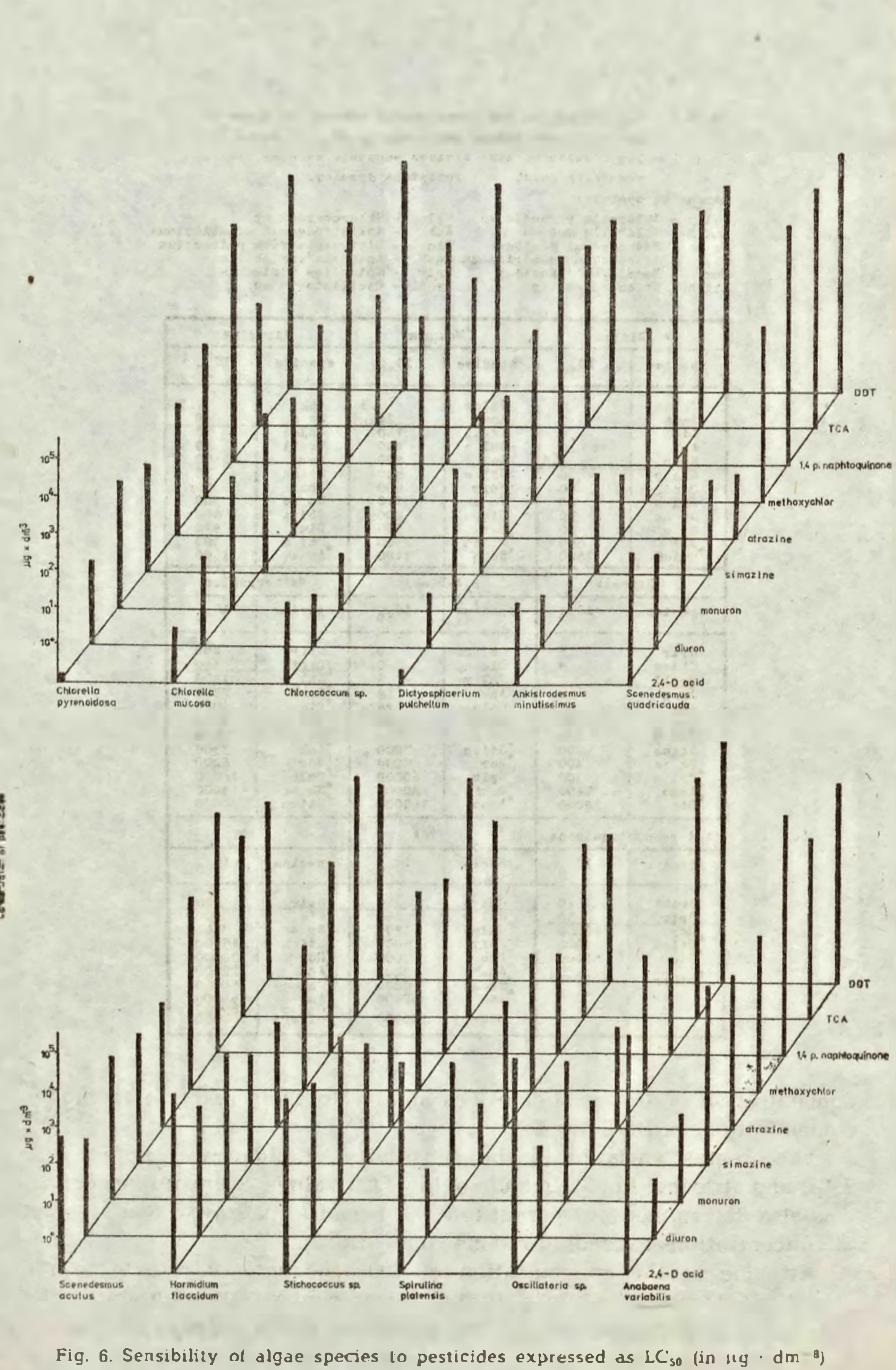

Fig. 6. Sensibility of algae species to pesticides expressed as LC_{50} (in µg · dm^{-3})

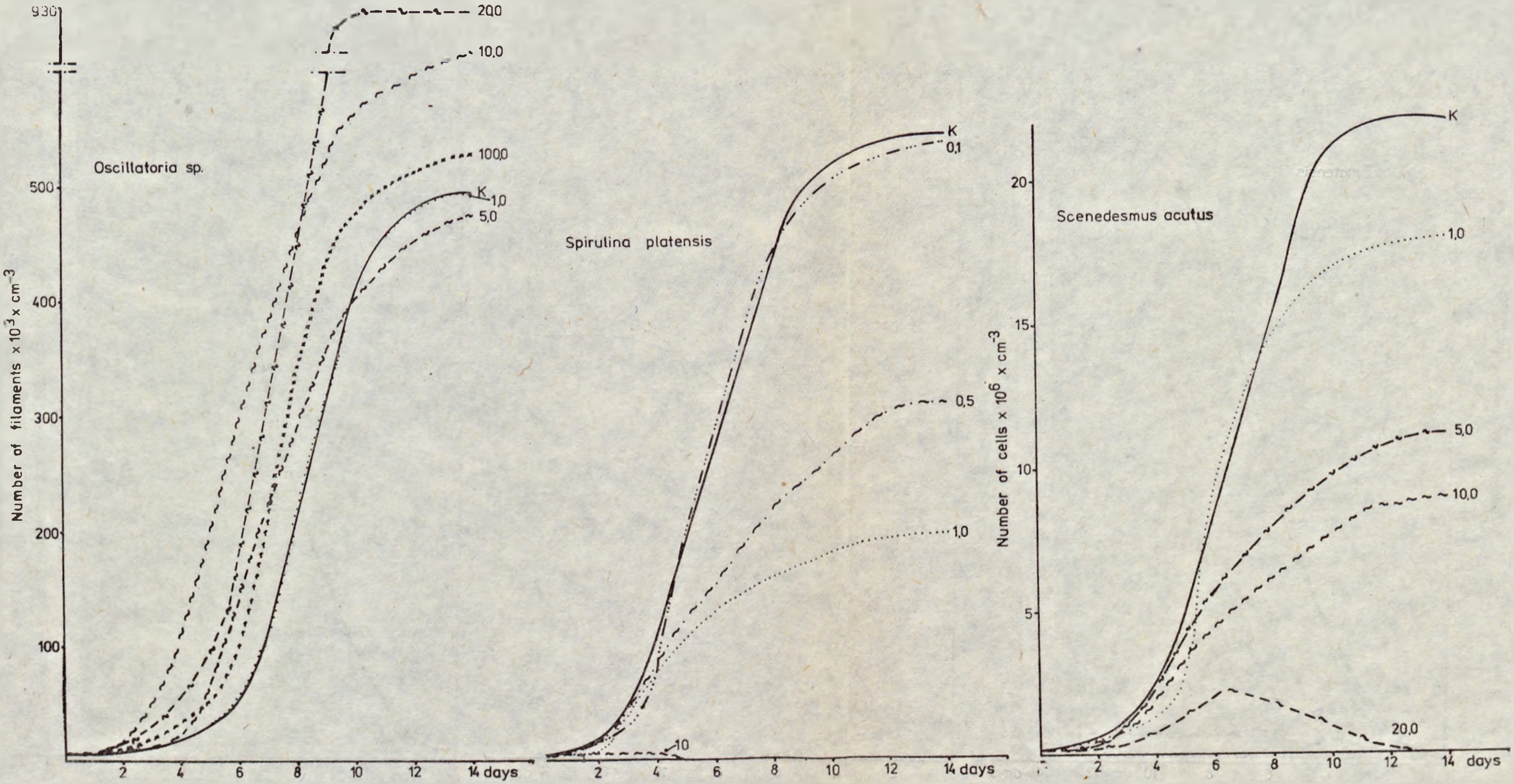

Fig. 7. The effect of DDT on the kinetics of growth of algae cultures

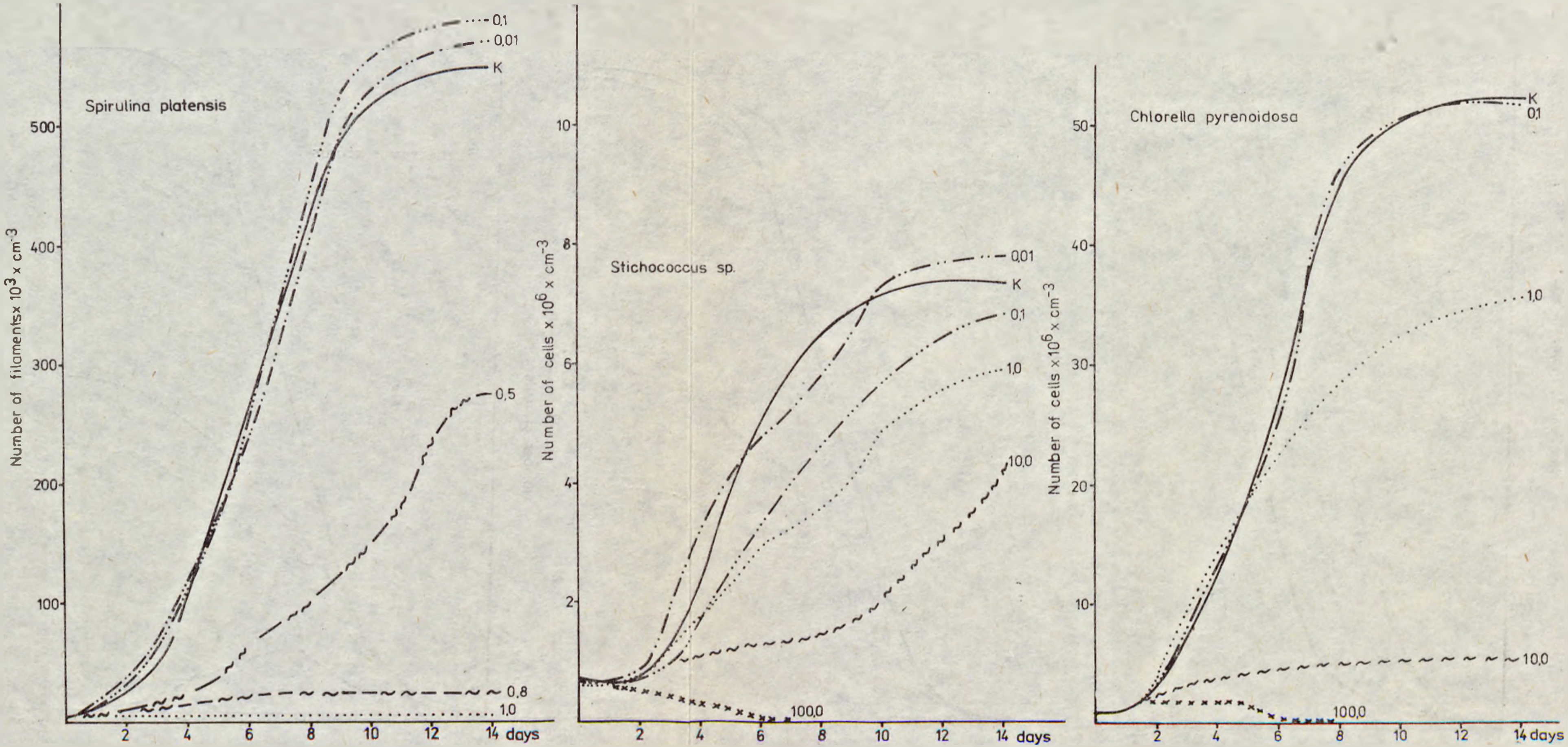

Fig. 8. The effect of methoxychlor on the kinetics of growth of algae cultures

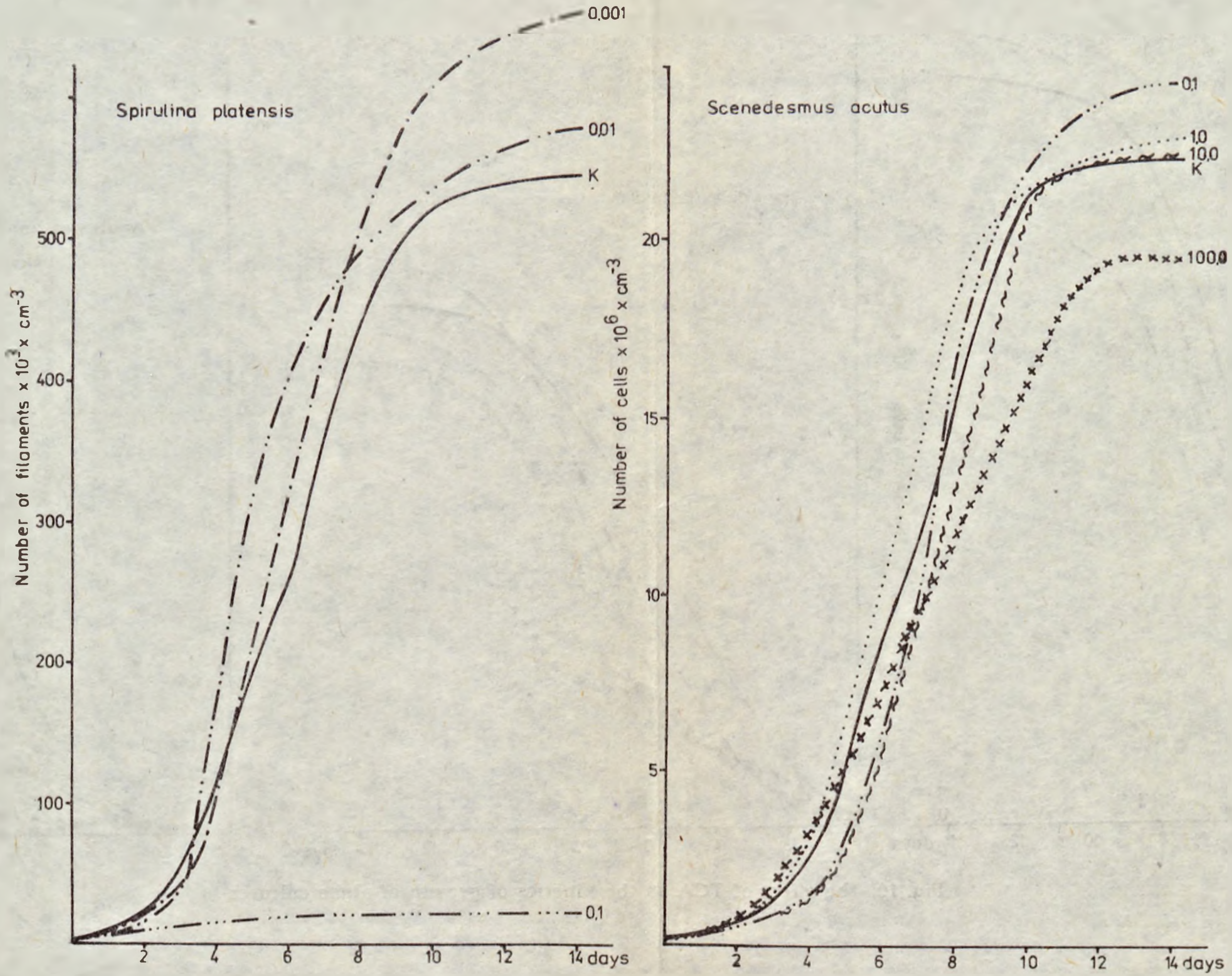

Fig. 9. The effect of 1,4-p. naphtoquinone on the kinetics of growth of algae cultures

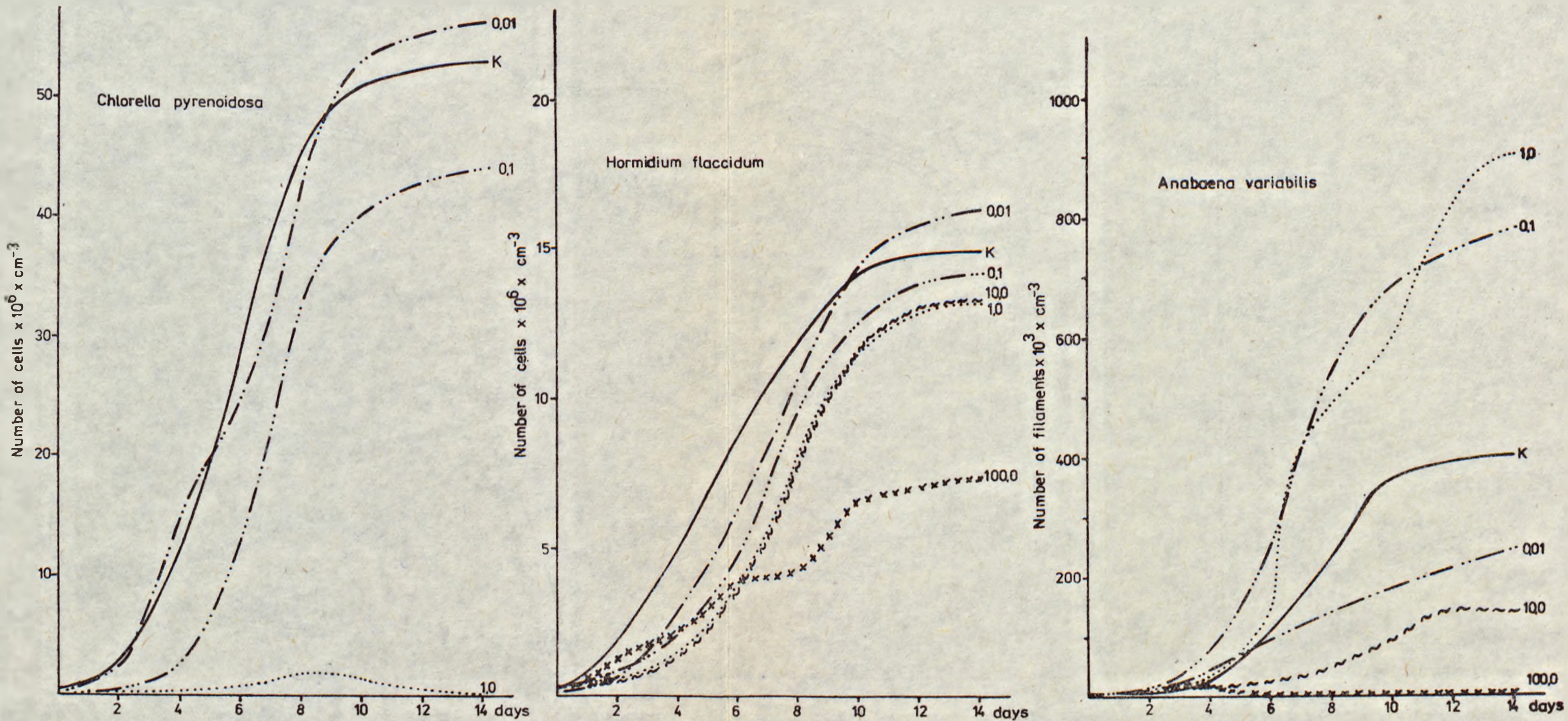

Fig. 10. The effect of TCA on the kinetics of growth of algae cultures

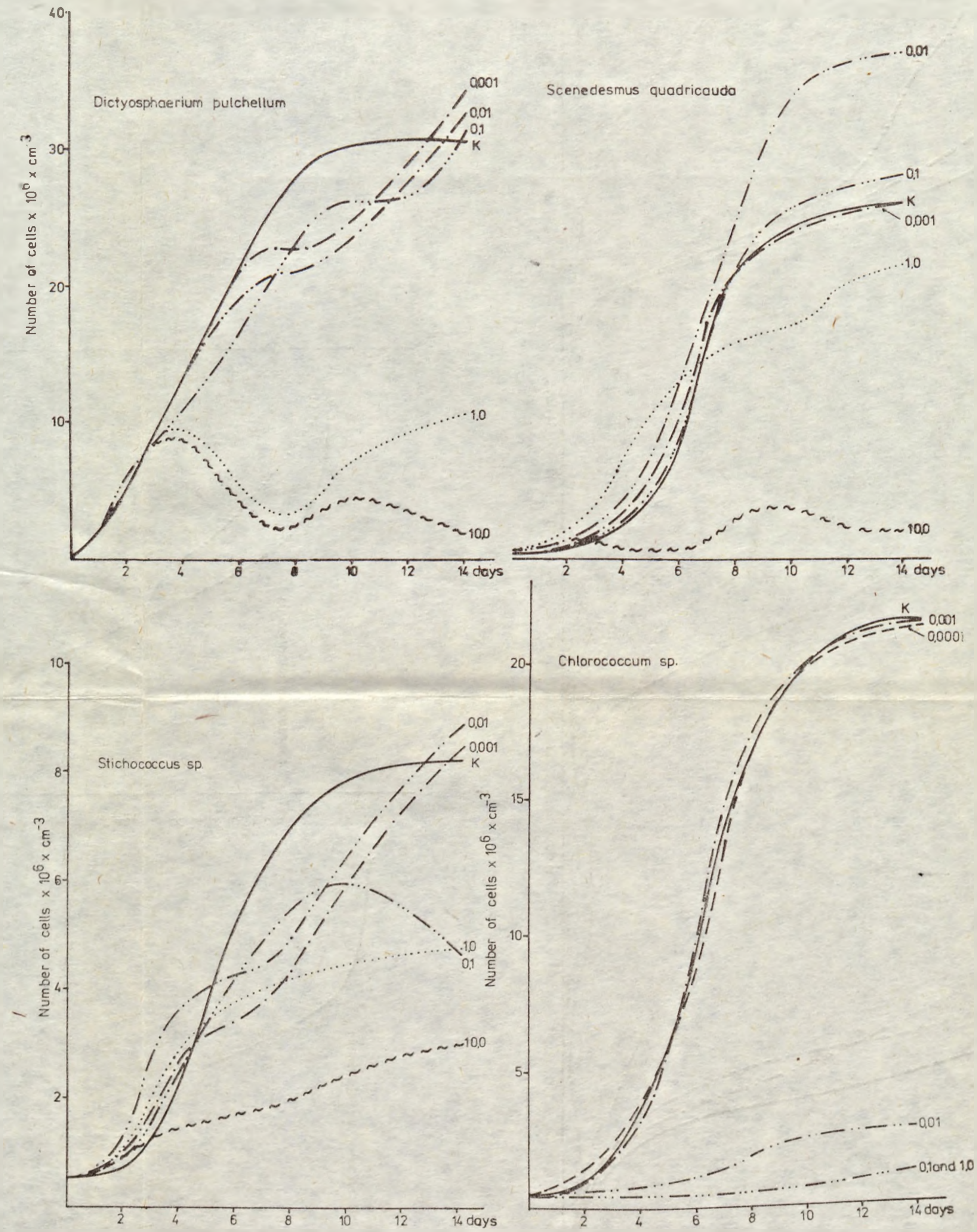

Fig. 11. The effect of monuron on the kinetics of growth of algae cultures

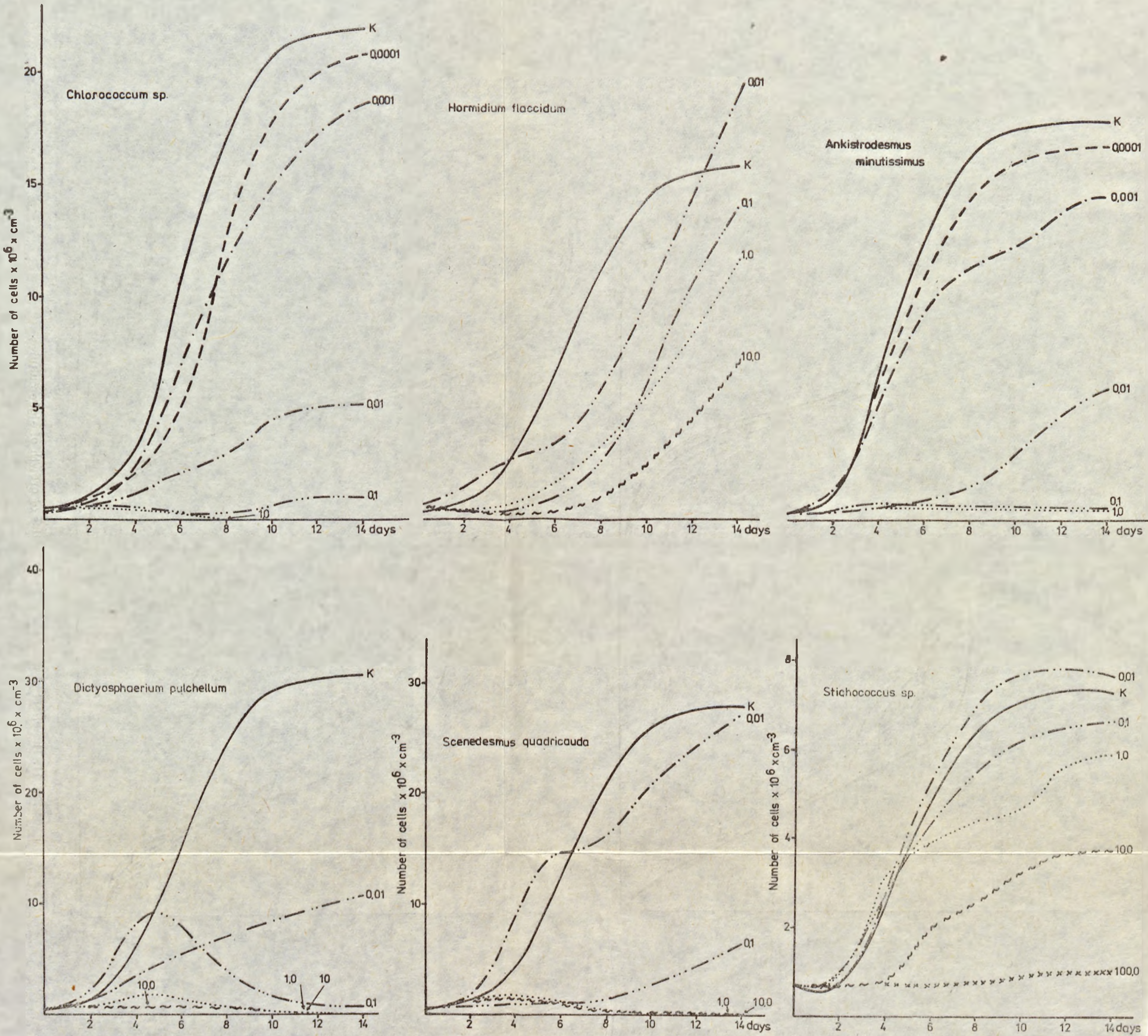

Fig. 12. The effect of diuron on the kinetics of growth of algae cultures

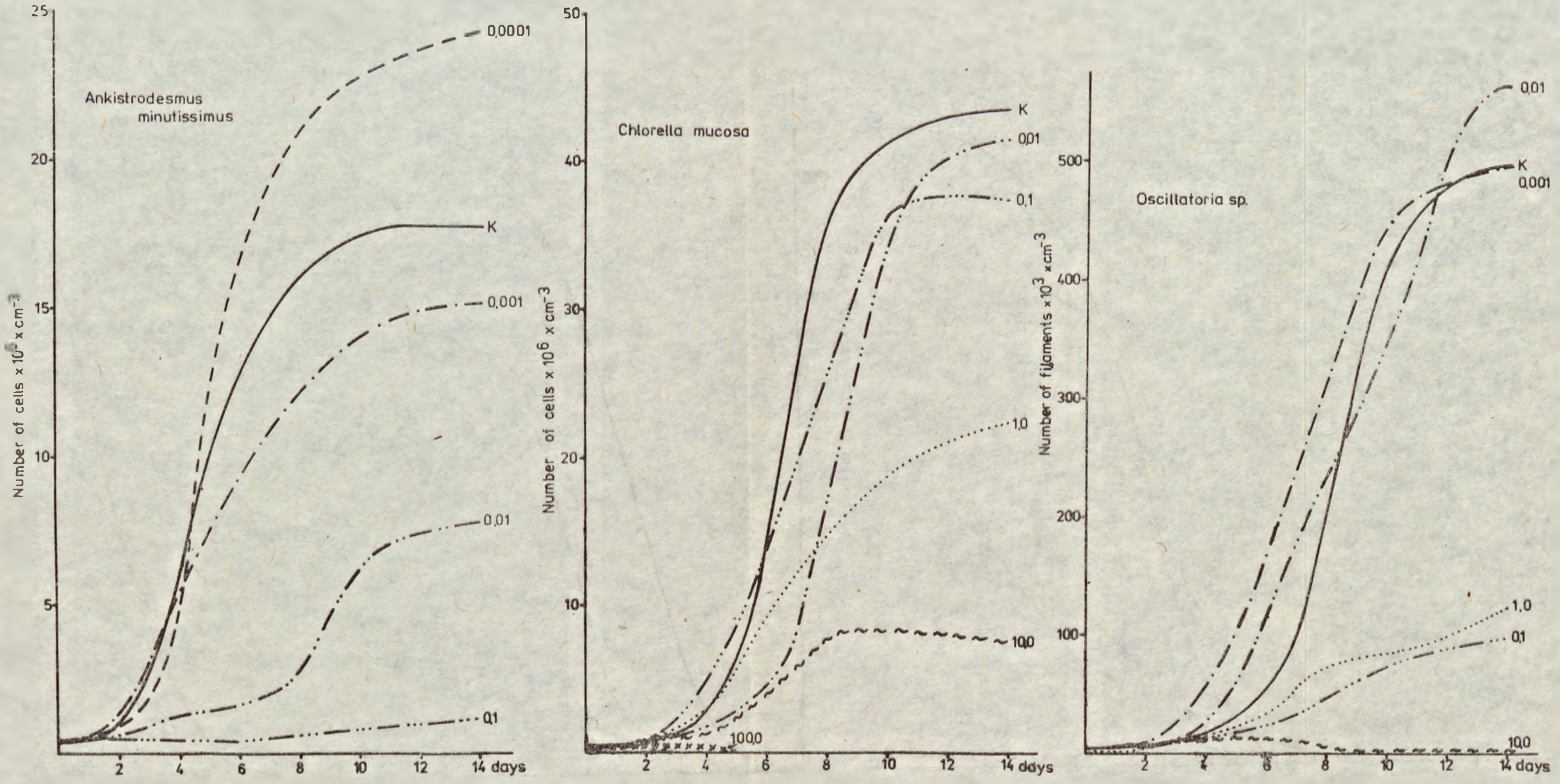

Fig. 13. The effect of atrazine on the kinetics of growth of algae cultures

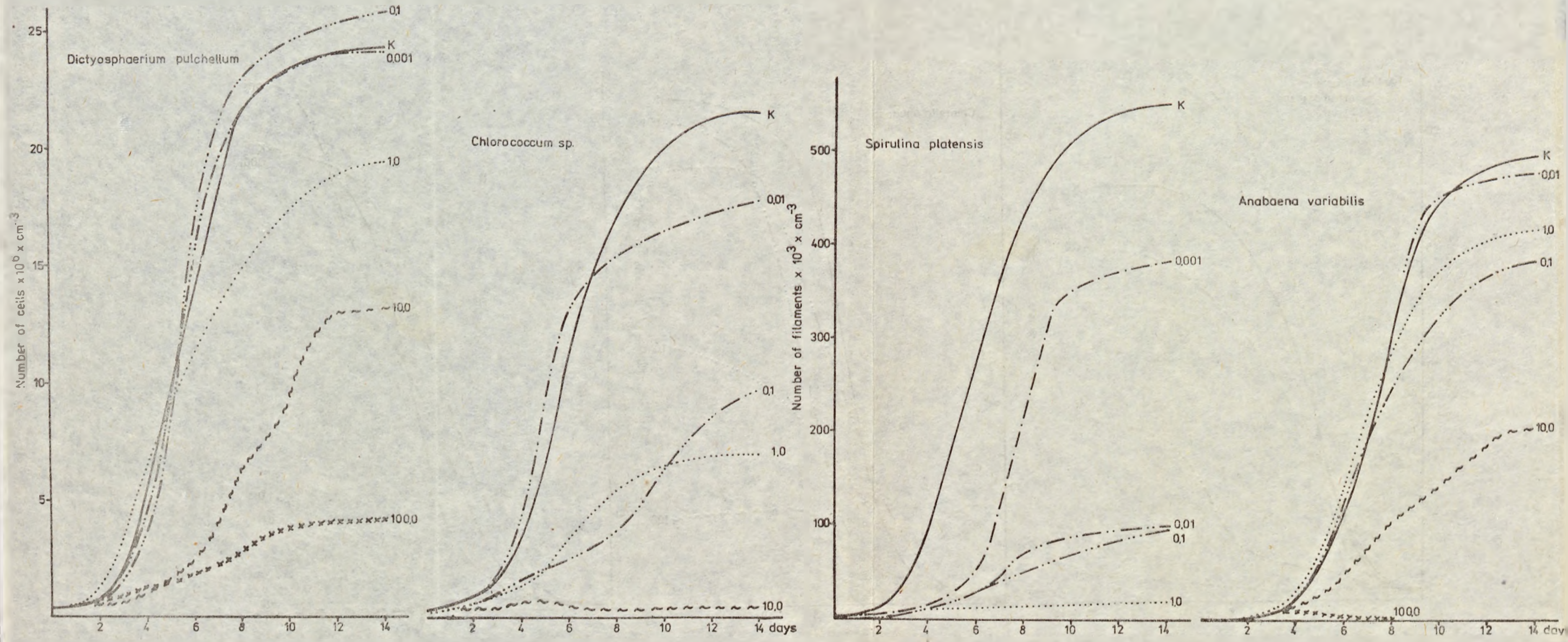

Fig. 14. The effect of simazine on the kinetics of growth of algae cultures

was maintained at the same level at a 10-fold increased concentration. With *Anabaena variabilis* an inversion of toxicity was noted at atrazine concentrations of 0.1 and 1.0 mg $\cdot$ dm^{-3} (fig. 5A).

Simazine was less toxic than atrazine by one order of magnitude (fig. 6). The concentration of 100 mg of this compound was toxic to all species (fig. 5B), except for *Dictyosphaerium pulchellum*. Similarly as atrazine, the concentrations for 0.01 and 1.0 mg $\cdot$ dm^{-3} were similar toxic to *Oscillatoria* sp. and *Spirulina platensis*, while an inversion of toxicity of these concentrations was noted with *Anabaena variabilis* (fig. 5).

The urea derivative herbicides monuron and diuron differentiated the sensibility of algae to the smallest degree. In general, the species were more sensible to diuron by one order of order of magnitude (fig. 6). At the concentration of 100 mg $\cdot$ dm^{-3} both substances were toxic to the investigated species (fig. 4), except for *Stichococcus* sp. which showed slight growth at this dose of monuron (fig. 4A).

3.2. The effect of pesticides on the kinetics of growth of algae cultures

The effect of pesticides on the kinetics of growth of the algae was greatly differentiated and was manifested in all stages of culture development. The algicidal effect of high concentrations of such compounds as monuron, diuron, simazine, atrazine, 2.4-D acid, and also of methoxychlor at the concentration of 100 mg $\cdot$ dm^{-3}, was already noted on the second day of the culture. The growth was completely stopped and the algae died off during a few days.

DDT, TCA, p. naphtoquinone and lower concentration of the remaining pesticides modified the development of algae by changing the different phases of growth (figs 7—14).

The most pronounced effects were observed in species treated with 2.4-D. This problem will be discussed in a separate work.

The following effects of the remaining pesticides on the kinetics of growth of the algae were observed in the experiment:

— The lag phase was shortened under the influence of DDT (in *Oscillatoria* sp., fig. 7), TCA (in *Anabaena variabilis*, fig. 10, and in most other species), and low concentration of monuron, diuron and atrazine.

— The lag phase was extended under the influence of DDT (in *Spirulina platensis* and *Scenedesmus acutus*, fig. 7), methoxychlor (in *Spirulina platensis* and *Stichococcus* sp., fig. 8), p. naphtoquinone (in *Scenedesmus acutus*, fig. 9), TCA (in *Chlorella pyrenoidosa* and *Hormidium flaccidum*, fig. 10), monuron (in *Chlorococcum* sp. and *Scenedesmus quadricauda*, fig. 11), diuron (in *Hormidium flaccidum*, *Ankistrodesmus minutissimus* and *Stichococcus* sp., fig. 12), simazine (in *Spirulina platensis*, *Dictyosphaerium pulchellum* and *Chlorococcum* sp., fig. 14) and atrazine (in *Ankistrodesmus minutissimus* and *Chlorella mucosa*, fig. 13).

— In the stage of linear growth an increased rate of cell division was observed in cultures treated with DDT (in *Oscillatoria* sp. and *Scenedesmus acutus*, fig. 7), low concentrations of methoxychlor (in most species, e.g., fig. 8), p. naphtoquinone (in *Spirulina platensis* and *Scenedesmus acutus*, fig. 9), TCA (in *Chlorella pyrenoidosa* and *Anabaena variabilis*, fig. 10), monuron and diuron (in *Scenedesmus quadricauda*, fig. 11, 12), simazine (in *Dictyosphaerium pulchellum* and *Chlorococcum* sp., fig. 14), and under the influence of atrazine (in *Ankistrodesmus minutissimus* and *Chlorella mucosa*, fig. 13).

— A decrease or inhibition of the growth rate was found in cultures treated with DDT (in *Scenedesmus acutus* and *Spirulina platensis*, fig. 7), p. naphtoquinone (in *Spirulina platensis*, fig. 9), TCA (in *Chlorella pyrenoidosa* and *Anabaena variabilis*, fig. 10), monuron, diuron, simazine and atrazine. In most species this modification was particularly pronounced at higher concentrations of pesticides (0.1—1.0 mg $\cdot$ dm^{-3}).

In *Dictyospaerium pulchellum* (fig. 11) changes in the growth rate within the linear phase appeared at all concentrations of monuron, while in *Hormidium flaccidum* and *Scenedesmus quadricauda* (fig. 12) the growth rate changed under the influence of diuron at the concentration of 0.01 mg $\cdot$ dm^{-3}.

The cultures treated with various concentrations of pesticides entered the stationary phase of growth in periods similar to those of control cultures (most observations), but also earlier or later at different densities of cultures. Increased densities of cultures in the stationary phase of growth appeared in *Oscillatoria* sp. treated with DDT at nearly every concentration (fig. 7). A increase in the density of cultures in the stationary phase also appeared under the influence of low concentrations of methoxychlor (in *Spirulina platensis*, fig. 9), p. naphtoquinone (in *Spirulina platensis* and *Scenedesmus acutus*, (fig. 9), TCA (in *Chlorella pyrenoidosa, Hormidium flaccidum* and *Anabaena variabilis*, fig. 10), monuron (in *Scenedesmus quadricauda*, fig. 11), diuron (in *Stichococcus* sp., fig. 12), simazine (in *Dictyosphaerium pulchellum*, fig. 14), and atrazine (in *Ankistrodesmus minutissimus* and *Oscillatoria* ss., fig. 13).

In general, at nearly all higher concentrations of pesticides the stationary phase of growth was reached with the number of cultures lower than in the controls.

Earlier appearance of the stationary phase in cultures was rare. It was only found in *Scenedesmus acutus* treated with 20 mg $\cdot$ dm^{-3} of DDT (fig. 7), in *Chlorella pyrenoidosa* treated with 10 mg $\cdot$ dm^{-3} methoxychlor (fig. 8), and in *Dictyosphaerium pulchellum* treated with 0.1 mg $\cdot$ dm^{-3} of diuron (fig. 12).

Sometimes the extension of the linear phase of growth, even up to 14 days, was observed in cultures treated with pesticides while the stationary phase of growth did not appear at all. This was observed in *Anabaena* *variabilis* under the influence of TCA (fig. 10), in *Stichococcus* sp. and *Dictyosphaerium pulchellum* under the influence of monuron (fig. 11), in *Hormidium flaccidum, Scenedesmus quadricauda, Dictyosphaerium pulchellum, Chlorococcum* sp., and *Ankistrodesmus minutissimus* treated with diuron (fig. 12), in *Spirulina platensis, Dictyosphaerium pulchellum* and *Chlorococcum* sp. treated with simazine (fig. 14), and in *Oscillatoria* sp. under the influence of atrazine (fig. 13).

Apart from the already discussed response of algae to pesticides, an unusual action of diuron on the kinetics of growth of *Hormidium flaccidum* was observed. In the initial period not only the inhibition of growth but also a decrease in the number of cells, followed by a slow increase in the density of the culture, were noted (fig. 12).

Also an inversion of toxicity was found in some species of algae under the influence of atrazine (fig. 13), simazine (fig. 14), TCA (fig. 10), and DDT (fig. 7).

Macroscopic observations of cultures treated with lethal doses of various pesticides led to the differentiation of three types of response as shown by the algae.

1. Cultures treated with 2.4-D, monuron and diuron. Lethal doses of these compounds brought about the discoloration of the cultures while the medium remained clear. In the microscopic picture swollen cells of algae with destructed chloroplasts devoid of green colour, prevailed. Later on the cells became „optically empty".

2. Cultures treated with p. naphtoquinone, methoxychlor, atrazine, and simazine turned yellow (simazine and atrazine) of brown (p. naphtoquinone and methoxychlor). The microscopic picture showed shrunken protoplasts and the granulation of the browned cell contents.

3. Cultures treated with toxic concentrations of DDT and TCA. The bleaching of cultures was accompanied by the turbidity of the medium. The microscopic picture showed shrunken protoplasts and the granulation of the bleached cell contents. „Optically empty" swollen cells were also encountered.

4. Discussion

Among other objectives, chemistry should detect and record the content of pesticides in aquatic environments. However, apart from the fact that chemical methods call for complicated and expensive laboratories, they are not sufficiently selective and record the biologically active substances together with other structurally similar but biologically inactive products. Therefore, biological tests are used in detecting pesticides and in evaluating their toxicity (Addison, Bardsley 1968, Mullison 1970, FAO 1970, Braginskij 1972, Böhm 1973, PN-74, 1975, Khan 1979). The criteria used in the evaluation of the toxicity of pesticides were physiological factors such as the photosynthetical oxygen release, intensity of respiration, content of photosynthetical pigments, changes in the redox potential, amount of nucleic acids, and the activity of certain enzymes measured during 24, 48 and 72 hours or during a few days of observations (Gramlich, Franz 1964, Schröder et al. 1967, Sikka, Pramer 1969, Paromenskaja 1967, Sumida Seizo, Veda Minoru 1973, and Petrov et al. 1974). The physiological effects, though usually correlated with the growth of cultures (Ashton et al. 1966, Zweig et al. 1964 Paromenskaja, Ljalin 1968), most often indicated the acute toxicity of preparations, but did not allow for the identification of the chronic sensibility of species. Moreover, the observed phenomena could have been only transitory, causing no lethal changes in the organisms of the algae. The above reasons and the different methods used by various authors resulted in considerable discrepancies in the evaluation of the toxicity of pesticides to different species of algae.

According to Maloney and Palmer (1956) the range of concentrations which inhibited the growth of algae was 0.9—150 mg $\cdot$ dm^{-3} of the pesticide; the species which responded by growth inhibition to 0.9—50 mg $\cdot$ dm^{-3} were classified as sensible, those reacting to higher concentrations were classified as tolerant.

The obtained results suggest the range of concentrations within 0.15 μg — 100 mg $\cdot$ dm^{-3}. The LC_{50} values of 0.15—24 μg $\cdot$ dm^{-3} denote species sensible to 2.4-D and the urea and triazine herbicides. The values of 40—50 μg $\cdot$ dm^{-3} are characteristic of species sensible to the fungicide p-naphtoquinone. The values of 0.3—0.9 mg $\cdot$ dm^{-3} suggest sensibility to the insecticides and TCA. The resistant species were charaterized by LC_{50} values equal or greater than 1 mg $\cdot$ dm^{-3} of pesticides concentration.

Arvik et al. (1971) claimed that 2.4-D at concentration below 50 μg $\cdot$ dm^{-3} did not affect the growth of *Chlorella vulgaris* and *Chlorococcus* sp. He, therefore determined these species as tolerant of 2.4-D. Fletcher et al. (1970) reported that *Chlorella* was sensible to this substance because the dose of 50 mg $\cdot$ dm^{-3} inhibited its growth. Dushkova and Dencheva (1973) observed that the growth of *Scenedesmus acutus* was retarded with the dose of 50 mg $\cdot$ dm^{-3} and, therefore, this species was classified as sensitive to 2,4-D. However, in the present work *Scenedesmus acutus* was found to be tolerant of this pesticide (LC_{50} amounting to 0.6 mg $\cdot$ dm^{-3}). Bertagnolli and Nadakavukaren (1974) found that *Chlorella pyrenoidosa* was tolerant of 2,4-D, no toxic effect appearing at the concentration of 600 mg $\cdot$ dm^{-3}. Valentine and Bingham (1974) did not observe the toxicity of 2,4-D at a concentration of 200 mg $\cdot$ dm^{-3} to *Scenedesmus quadricauda, Chlorella pyrenoidosa* and *Euglena gracilis.* According to these authors, the preparation was decomposed under the influence of *Scenedesmus quadricauda* while the development of the two other species was not affected by the absorption of the pesticide from the medium. In the author's experiments *Chlorella pyrenoidosa* proved very sensitive and *Scenedesmus quadricauda* less sensitive to 2,4-D (LC_{50} of 0.15 µg and 0.5 mg · dm^{-3}), being killed by the dose of 1 mg · dm^{-3}.

In the author's experiments the toxicity of TCA was observed only in case of *Chlorella pyrenoidosa* (LC_{50} of 0.3 mg · dm^{-3}). Other authors (Balezina 1967) claimed that at the concentration of 0.1—10 mg · dm^{-3} this compound unfavourably affected the development of all soil algae. Boev and Minibaev (1975) found the toxic effect of TCA at concentration of 0.1—19 mg · dm^{-3} on soil blue-green algae and at higher concentrations on all other systematic groups of algae. The results obtained by the present author suggested that TCA was slightly more toxic to chlorococcous green algae.

Maloney (1958) reported that the concentration of 0.5 mg · dm^{-3} of urea herbicides, and particularly of monuron, inhibited the growth of 33 algae species. Taking this finding into consideration, Maloney concluded that all urea herbicides were toxic to algae. Similarly, Kruglov (1975) found that the range of toxic concentrations of these compounds was 0.5—70 mg · dm^{-3}. Sumida Seizo and Veda Minoru (1973) observed the growth inhibition of *Chlorella* sp. at a concentration of 5.0 mg · dm^{-3}, while Geoghegan (1957) used *Chlorella vulgaris* and *Scenedesmus quadricauda* for detecting the content of 0.5 and 12 mg · dm^{-3} of monuron in the environment. In the present study *Chlorococcus* sp. and *Anabaena variabilis* were classified as sensible to monuron and diuron, while *Dictyosphaerium pulchellum, Ankistrodesmus minutissimus* and *Spirulina platensis* (LC_{50} within 5—24 µg · dm^{-3}) also responded to diuron.

Some authors compared the toxicity of triazine herbicides to that of urea derivatives (Ashton et al. 1966, Paromenskaja 1967, Sumida Seizo, Veda Minoru 1973, Kruglov 1975) while Gramlich and Frans (1964) determined the dose of 0.2 mg · dm^{-3} of these compounds as toxic to *Chlorella pyrenoidosa*, and Maloney and Palmer (1956) reported that the toxic concentration ranged from 0.9 to 20 mg · dm^{-3}. The author's results suggest that the species classified as sensitive (*Spirulina platensis, Chlorococcum* sp. and *Oscillatoria* sp. to simazine, and *Ankistrodesmus minutissimus* and *Scenedesmus quadricauda* to atrazine) responded with growth inhibition already at concentrations of 6—10 µg · dm^{-3} of triazine compounds.

In the opinion of many authors (e.g., Braginskij 1972, Byrdy et al. 1976) p. naphtoquinone is an algicide. Yet, its effect on the growth of the investigated algae was poor. Only *Oscillatoria* sp. and *Spirulina platensis* with LC_{50} of 40 and 50 µg · dm^{-3}, were sensitive to this pesticide.

The same was found by Dushkova and Dencheva (1973) and by Pristavu (1975). According to these authors, the growth of *Scenedesmus acutus* and *Chlorella pyrenoidosa* was inhibited by the doses of 200 and 243 mg · dm^{-3}. On the other hand, Zweig et al. (1968) compared the action of 5.73 mg · dm^{-3} of p. naphtoquinone to that of the same concentration of diuron, which after 48 hours caused a drastic decrease in the photosynthesis of algae. Gramlich and Franz (1964). also claimed that the low concentrations of p. naphtoquinone (0.22 mg · dm^{-3}) inhibited the growth of *Chlorella pyrenoidosa*.

According to Czeczuga and Gerasimov (1973), DDT affected the growth of algae. This opinion was supported by the observations of *Anabaena cylindrica* and *Chlorella pyrenoidosa*. Kirchner et al. (1975) also reported the sensibility of *Chlorella pyrenoidosa* to DDT. Mosser et al. (1972) arrived at similar conclusions on the basis of observations on the growth of halophilous *Dunaliella bioculata* and *Thalasiosira pseudonana* treated with DDT. On the other hand, Koning and Mortimer (1971) and Batterton et al. (1972) did not observe any toxic action of DDT on the growth of algae. In the present investigation the toxicity of DDT was found only in *Spirulina platensis* (the sensitive species, and *Scenedesmus acutus* (the tolerant species).

Methoxychlor at a concentration of 100 mg · dm^{-3} was toxic to all investigated species, while at the 10-time lower concentration this insecticide was toxic only to halophilous blue-green algae *Spirulina platensis* and *Oscillatoria* sp. (LC_{50} of 480 μg · dm^{-3}). According to other authors (e.g., Kircher et al. 1975), methoxychlor was toxic to *Chlorella pyrenoidosa*. This species was classified as sensitive to methoxychlor with the inhibitory dose of 0.1 mg · dm^{-3}.

On the basis of the general sensibility of soil algae, Pillay and Tchan (1972) classified the investigated herbicides in the following order: diuron > monuron > atrazine > simazine > atraton. The present results made it possible to complement this series with the following compounds: simazine > 2,4-D acid > methoxychlor > p. naphtoquinone > TCA > DDT. The series shows the lowest toxicity of DDT and TCA to the investigated species.

Numerous authors (among other, Addison, Bardsley 1968, Zweig et al. 1968, Arvik et al. 1971, Sullivan et al. 1972 and Noll, Bauer 1973) described the use of alga tests in the determination of water pollution. In some papers (e.g. Bohm 1973) the growth curves of synchronistic cultures were used in the identification of different chemical groups of pesticides. Addison and Bardsley (1968) drew the curves of *Chlorella vulgaris* sensitivity to urea herbicides, and calculated the value of coefficients based on an increase in the density of cultures during 6 days after the herbicide treatment. They found that the value of the coefficient depended upon the type of the herbicide and that it could be applied in the identification of pollution. Similar conclusions can be drawn from the present author's results.

The variability of effect of pesticides on the kinetics of growth of the investigated algae made it impossible to connect the reaction of the individual species to the chemical structure of the compounds, and, therefore, attention was rather paid to the dependence of the reaction of algae upon the concentration of the pesticides. A similar dependence was found by Arvik et al. (1971) and also by Żurek (1980). These authors observed the inversion of toxicity within a certain range of concentrations, and also the irregularity of the growth curves in algae cultures treated with pesticides.

The growth of the untreated control cultures of different species was typical and during 14 days all phases of development described in the literature, appeared in them (Myers 1953, Tamiya et al. 1953, Bednarz, Nowak 1971, Eloranta 1978).

On the basis of other authors data and the obtained results it can be postulated that the variable response of algae to different pesticides and different concentrations of a given compound depend upon many factors not easily definitable. Among other reasons, a significant role is played by the chemical structure of compounds, the pathways of their action, the variability of species and clones of algae, and the adaptation of organisms to environments polluted with pesticides.

5. Conclusions

It seems that the algae characterized by different sensibility to chemical compounds can be used as test species in detecting the occurrence of pesticides (particularly herbicides) in aquatic environments. For the identification and quantitative determination of polluting substances the following group of particularly sensitive species, complemented with resistant or selective ones, can be suggested:

for 2,4-D acid — *Chlorella pyrenoidosa* and *Dictyosphaerium pulchellum*, with *Ankistrodesmus minutissimus* and *Scenedesmus acutus* as complementary species,

for diuron — *Chlorococcum* sp., *Anabaena variabilis*, *Dictyosphaerium pulchellum* and *Ankistrodesmus minutissimus*, with *Spirulina platensis* as a complementary species,

for monuron — *Chlorococcum* sp. and *Anabaena variabilis*, with *Ankistrodesmus minutissimus* and *Dictyosphaerium pulchellum* as complementary species,

for simazine — *Spirulina platensis*, *Chlorococcum* sp., and *Oscillatoria* sp., with *Chlorella pyrenoidosa* and *Dictyosphaerium pulchellum* as complementary species, for atrazine — *Ankistrodesmus minutissimus* and *Scenedesmus quadricauda* with *Chlorella pyrenoidosa* and *Anabaena variabilis* as complementary species,

for TCA — *Chlorella pyrenoidosa* and *C. mucosa*, with some randomly selected species,

for p. naphtoquinone — *Spirulina platensis* and *Oscillatoria* sp., with *Scenedesmus quadricauda* as a complementary species,

for methoxychlor — *Spirulina platensis* and *Oscillatoria* sp., with *Scenedesmus quadricauda* and *Chlorella pyrenoidosa* as complementary species,

for DDT — *Spirulina platensis* and *Scenedesmus acutus*, with *Oscillatoria* sp. and *Chlorella pyrenoidosa* as complementary species.

The concentration of pesticides in water can be also determined with biological tests by using diluted of condensed samples of the investigated water and comparing the obtained results with the standard growth curves. However, the methods in this type of analyses should be separately discussed.

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7. Polish summary

Wpływ niektórych pestycydów na wzrost kultur wybranych gatunków zielenic i sinic

Przeprowadzone badania pozwoliły na określenie wrażliwości 12 gatunków glonów, w tym 9 zielenic: *Chlorella pyrenoidosa, C. mucosa, Ankistrodesmus minutissimus, Chloorcoccum* sp., *Dictyosphaerium pulchellum, Scenedesmus acutus, S. quadricauda, Hormidium flaccidum, Stichococcus* sp., oraz 3 sinice: *Anabaena variabilis, Spirulina platensis* i *Oscillatoria* sp., na 9 pestycydów, w tym 6 herbicydów: TCA, kwas 2,4-D, monuron, diuron, symazyna, atrazyna, 1 fungicyd, p. naftochinon, oraz dwa insektycydy: DDT i metoksychlor. Czternastodniowe hodowle glonów poddane działaniu pestycydów w stężeniach od 0,0001 do 100 mg $\cdot$ dm^{-3} wykazywały najczęściej różny od kontroli plon suchej masy (ryc. 1—5). Niskie stężenia pestycydów nie wywierały wpływu hamującego, przeważnie miały działanie stymulujące na wzrost glonów, natomiast największe ich stężenia działały toksycznie (ryc. 1—5).

Oznaczono stężenia pestycydów, wywołujące 50% zahamowanie wzrostu kultur (LC_{50}), wyrażonego suchą masą, uzyskaną na końcu hodowli. Pozwoliło to na porównanie toksyczności preparatów i wrażliwości badanych gatunków (ryc. 6). Za wrażliwe na herbicydy mocznikowe, triazynowe i kwas 2,4-D uznano gatunki o LC_{50} od 0,15 do 24 $\mu g \cdot dm^{-3}$, na p. naftochinon o LC_{50} od 40 do 50 $\mu g \cdot dm^{-3}$ oraz na TCA i chlorowane węglowodory alifatyczne o LC_{50} od 0,3 do 0,9 $mg \cdot dm^{-3}$ (tabela I).

Oddziaływanie pestycydów na kinetykę wzrostu kultur glonów było różnorodne i bardzo zmienne, nawet w przypadku jednej i tej samej substancji, uzależnione raczej od stężenia preparatu niż jego budowy chemicznej. Niskie stężenia pestycydów przeważnie działały stymulująco na wzrost kultur w fazie eksponencjalnej wzrostu (ryc. 7—14). Hamujące działanie preparatów najsilniej zaznaczało się w obrębie fazy liniowego wzrostu kultur, powodując przejście kultur w fazę stacjonarną przy mniejszej niż w kontroli liczebności komórek (ryc. 7—14), a także powodując niekiedy dwufazowy typ wzrostu (ryc. 11, 12). Toksyczne stężenia pestycydów wywoływały całkowite zahamowanie wzrostu glonów, występujące od pierwszego dnia hodowli, lub powodowały obumieranie kultur po zaledwie parodniowym okresie słabego wzrostu (ryc. 7—14).

Gatunki odznaczające się szczególną wrażliwością uznano za przydatne do opracowania zestawu gatunków testowych, umożliwiających wykrywanie i określenie stężenia pestycydów w wodach. W przypadku symazyny są to: *Spirulina platensis*, *Chlorococcum* sp. i *Oscillatoria* sp., dla atrazyny: *Ankistrodesmus minutissimus*, *Scenedesmus quadricauda*, dla monuronu: *Chlorococcum* sp., *Anabaena variabilis*, dla diuronu: *Chlorococcum* sp., *Anabaena variabilis*, *Dictyosphaerium pulchellum*, *Ankistrodesmus minutissimus* i *Spirulina platensis*, dla TCA: *Chlorella pyrenoidosa*, dla kwasu 2,4-D: *Chlorella pyrenoidosa*, *Dictyosphaerium pulchellum* i *Chlorella mucosa*, dla DDT: *Spirulina platensis*, oraz dla p. naftochinonu: *Oscillatoria* sp. i *Spirulina platensis*. Natomiast za odporne uznano te gatunki, których LC_{50} wynosiło 1 mg i więcej, a więc dla symazyny: *Chlorella mucosa*, *Anabaena variabilis* i *Dictyosphaerium pulchellum*, dla atrazyny: *Anabaena variabilis*, dla monuronu: *Stichococcus* sp. i *Scenedesmus quadricauda*, dla diuronu: *Stichococcus* sp., dla kwasu 2,4-D: *Stichococcus* sp., *Hormidium flaccidum*, *Spirulina platensis*, *Oscillatoria* sp., *Anabaena variabilis* i *Scenedesmus acutus*, dla TCA: wszystkie badane gatunki, oprócz *Chlorella pyrenoidosa*, dla metoksychloru: wszystkie badane glony poza *Oscillatoria* sp. i *Spirulina platensis*, dla DDT: pozostałe gatunki poza *Spirulina platensis* i dla p. naftochinonu: pozostałe gatunki poza *Spirulina platensis* i *Oscillatoria* sp. (tabela I).

Dane doświadczalne uzyskane dla badanych gatunków planktonowych potwierdziły obserwacje Pillay i Tchan (1972), dotyczące wrażliwości glonów glebowych na pestycydy. Pozwoliły uzupełnić proponowany przez tych autorów szereg, ułożony według toksyczności pestycydów, o następujące preparaty: symazyna $>$ kwas 2,4-D $>$ metoksychlor $>$ p. naftochinon $>$ TCA $>$ DDT.

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