

The evaluation of the adaptation ability of some green algae to 2,4-D acid, monuron, and diuron admixtures, under laboratory conditions*

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Abstract — The toxic action of monuron, diuron, simazine, atrazine and 2,4-D upon *Ankistrodesmus minutissimus*, *Chlorella pyrenoidosa*, *Dictyosphaerium pulchellum*, *Scenedesmus acutus*, *S. quadricauda*, and *Hormidium flaccidum* was irreversible even if the algae were transferred to media free of these substances. Increased tolerance of monuron and 2,4-D was obtained in *Dictyosphaerium pulchellum* while in case of *Chlorella pyrenoidosa* the tolerance of these two compounds was not increased. Both species of algae did not develop an increased tolerance of diuron.

Key words: Green-algae, adaptation to pesticides: monuron, diuron, 2,4-D acid.

1. Introduction

In earlier laboratory experiments (Bednarz 1981a, b) some green and blue-green algae species treated with various pesticides showed different sensitivity to these compounds. The data were obtained with the biological material which was for the first time exposed to the action of pesticides.

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The two-phase type of growth found in some species treated with herbicides (Bednarz 1981 a, b) suggests that the adaptability of algae to these compounds developed in the polluted cultures.

The aim of the work is to elucidate the question whether the successive contacts with pesticides can increase the tolerance of algae in relation to these compounds in the environment, and whether the observed symptoms of the toxic action are permanent or could be removed by providing an unpolluted medium.

2. Material and method

The investigation was carried out in the Laboratory of Water Biology of the Polish Academy of Sciences in Cracow, in the years 1978—1979. The following algae cultures were included in the experiments; *Ankistrodesmus minutissimus* Korschik, strain No 1193, *Scenedesmus quadricauda* (Turp.) Bréb., No 1097, *S. acutus* Meyen, No 1608, *Dictyosphaerium pulchellum* Wood, No 1616, *Chlorella pyrenoidosa* Chick, No 366, and *Hormidium flaccidum* Braun, No 494, obtained from the Institute of Zootechnics at Zator (Bednarz, Nowak, 1971). The applied herbicides were chemically pure form of 2,4-D acid, monuron, diuron, simazine, and atrazine obtained from the Pedagogical Institute in Käthen (GDR).

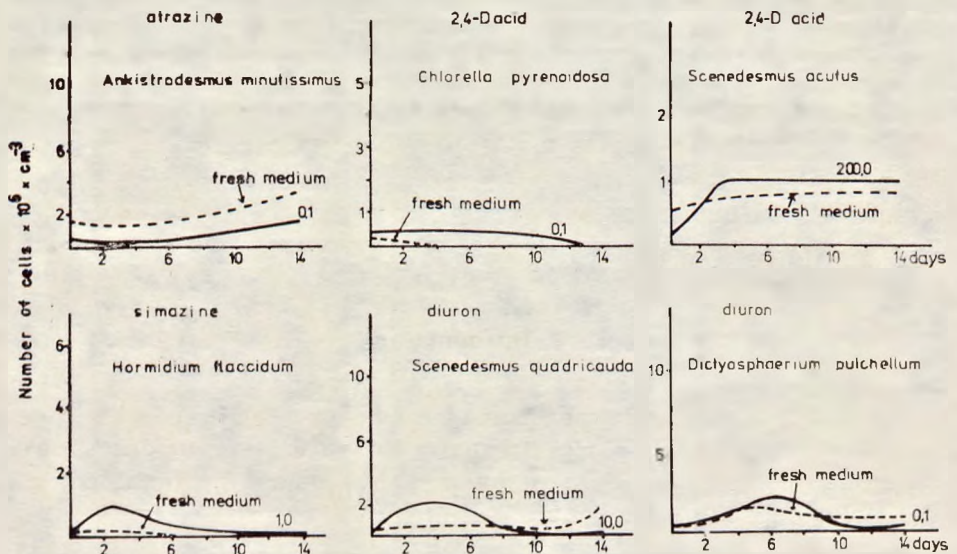


Fig. 1. Growth of algae in a pesticide-free medium after a pre-culture with toxic concentrations of herbicides ($\text{mg} \cdot \text{dm}^{-3}$)

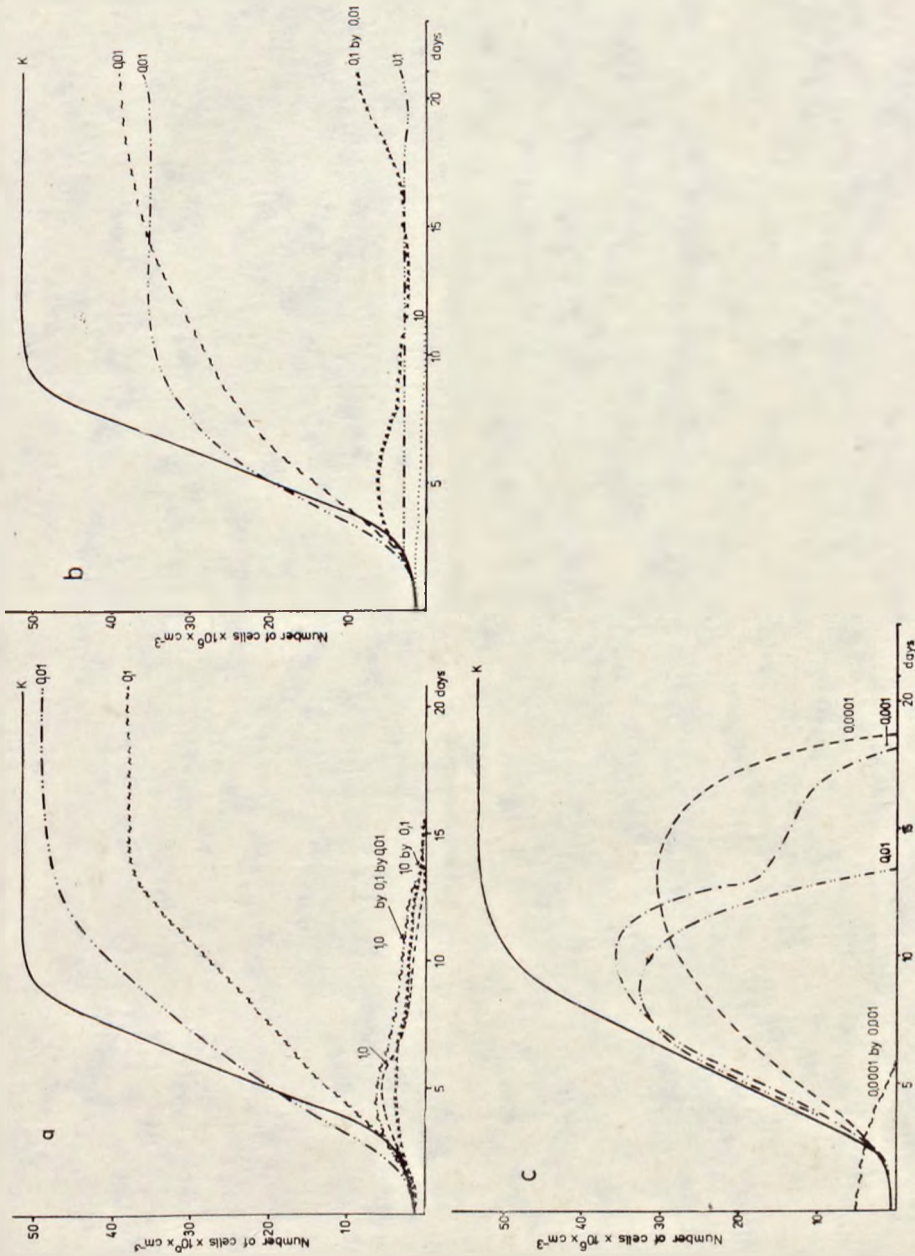
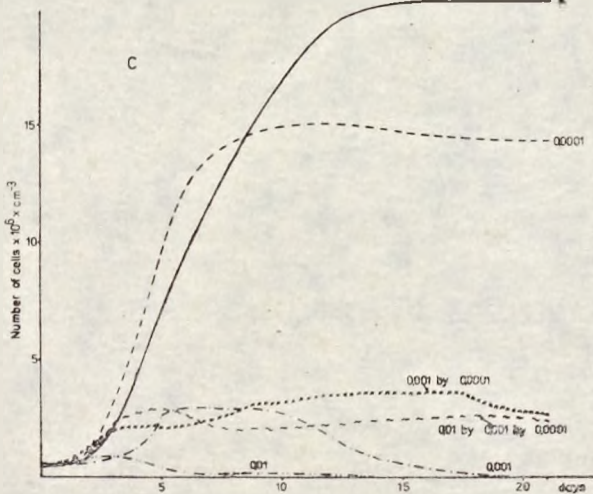
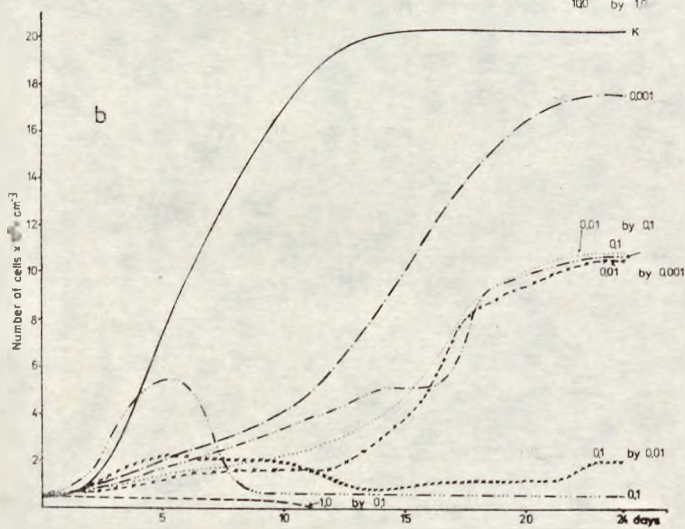
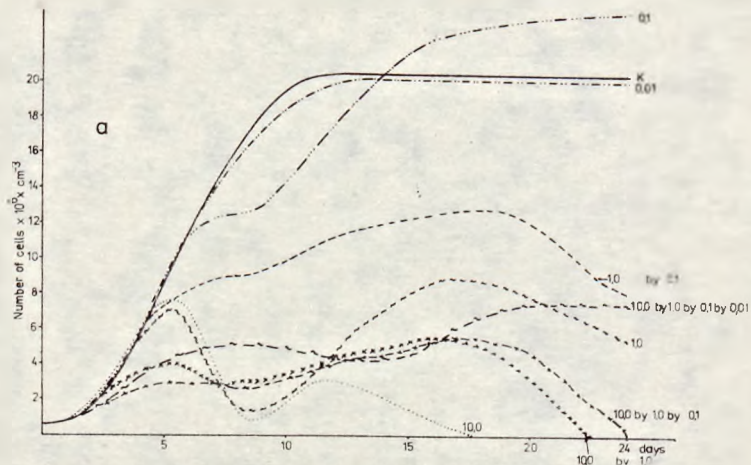


Fig. 2. Growth of *Chlorella pyrenoidosa* in: a — monuron, b — diuron, c — 2,4-D acid ($\text{mg} \cdot \text{dm}^{-3}$), K — control



The algae cultures were conducted on the L₅m medium (Jankowski 1964). The cultures were checked every other day under conditions described by Bednarz (1981a, b).

Two different tasks were set in the study:

1) The determination of the possible reversibility of the toxic action of herbicides. Algae cultures were treated with toxic concentrations of herbicides and the specimens which showed injury symptoms were centrifuged, washed in a fresh medium, and transferred to a medium free of herbicides. The cultures were conducted for 14 days, the control cultures being grown in media with toxic admixtures of herbicides.

2) It was attempted to increase the tolerance of algae in relation to herbicides. The method of successive passages to media containing increasing concentrations of 2,4-D acid, monuron, and diuron was used, with an initial harmless dose and a tenfold gradation of concentrations. Two species: *Chlorella pyrenoidosa* and *Dictyosphaerium pulchellum* were included in the experiment. The following range of concentrations was applied: a) 2,4-D acid, 0.00001—0.01 for the two species, b) monuron, 0.01—1.0 for *Chlorella pyrenoidosa*, and 0.01—10.0 for *Dictyosphaerium pulchellum*, c) diuron, 0.01—1.0 for *Chlorella pyrenoidosa*, and 0.001—1.0 mg·dm⁻³ for *Dictyosphaerium pulchellum*. The cultures were cultivated for 24 days; the cultures of algae which were not subjected to passages, either not treated with herbicides or treated with various concentrations of these compounds, were used as the control. All cultures were carried out in three replications.

3. Results

In spite of the fact that the algae were passaged to herbicide-free media, no regeneration of cultures was observed. During a 14-day culture the investigated algae species showed the symptoms of sensibility which were characterized by very poor or completely stopped growth (fig. 1).

In *Chlorella pyrenoidosa* in spite of its numerous passages, no increase in tolerance of 2,4-D (fig. 2a) was noted while its sensitivity to monuron and diuron was slightly increased (fig. 2b, c).

In *Dictyosphaerium pulchellum* the tolerance of monuron distinctly increased after four passages and that of 2,4-D acid after three passages (fig. 3a, b) as compared with the tolerance showed by impassaged specimens. However, in case of diuron the tolerance of this species was only insignificantly increased (fig. 3c).

Fig. 3. Growth of *Dictyosphaerium pulchellum* in: a — monuron, b — diuron, c — 2,4-D acid (mg·dm⁻³), K — control

4. Discussion and conclusions

The results of experiments with herbicide treated algae showed their poor adaptability to environments polluted with toxic compounds. In case of heavy pollution of the environment the toxic symptoms were irreversible. In the present experiments the increased adaptability was only found in *Dictyosphaerium pulchellum* in relation to two herbicides. With *Chlorella pyrenoidosa* this effect was not observed. However, using a two-stage mutation of *Chlorella pyrenoidosa*, Cho et al. (1972) obtained an increased tolerance of monuron, reaching $20 \text{ mg} \cdot \text{dm}^{-3}$. Increased tolerance of monuron was also manifested by a greater tolerance of diuron, simazine, and atrazine, i.e. the inhibitors of photosynthesis.

In the investigation on the effects of herbicides, the two-phase growth of some algae cultures was observed. This was particularly pronounced with *Dictyosphaerium pulchellum* treated with monuron (Bednarz 1981a). The obtained results suggest that this type of growth was caused by some kind of selection occurring in the population of algae cells treated with herbicides. Probably, as a result of the herbicide action the cells characterized by increased tolerance began to predominate in the culture in the second phase of growth. These observations were supported by the present results. According to Cho et al. (1972) increased tolerance of pesticides, obtained by the method of successive passaging, is caused by the mutation tending towards the development of a physiological race of the algae for whom the presence of pesticides in the environment would be an indispensable growth factor. Similar observations were made in the investigations on the effect of antibiotics on algae cultures. The obtained physiological races were antibiotics-dependent, growing only in the presence of these substances in the medium (Bednarova et al. 1976, Tugarinov 1965).

5. Polish summary

Oceńa zdolności adaptacyjnych niektórych zielenic do zanieczyszczeń kwasem 2,4-D, monuronem i diuronem, w warunkach laboratoryjnych

Toksyczne działanie monuronu, diuronu, symazyny, atrazyny i kwasu 2,4-D na *Ankistrodesmus minutissimus*, *Chlorella pyrenoidosa*, *Dictyosphaerium pulchellum*, *Scenedesmus acutus*, *S. quadricauda* i *Hormidium flaccidum* było zjawiskiem nieodwracalnym, mimo przeniesienia glonów do pożywki wolnej od tych związków (ryc. 1).

Drogą kolejnych pasażów *Dictyosphaerium pulchellum* i *Chlorella pyrenoidosa* w pożywce o wzrastającej koncentracji monuronu, diuronu i kwasu 2,4-D próbowano uzyskać zwiększenie tolerancji glonów na te związki. Osiągnięto wyraźne zwiększenie odporności *Dictyosphaerium pulchellum* na monuron i kwas 2,4-D (ryc. 3a, b), lecz

nie otrzymano pozytywnych rezultatów w przypadku *Chlorella pyrenoidosa* (ryc. 2a, b). U obydwóch badanych gatunków uzyskano negatywne wyniki adaptacyjne odnośnie do diuronu (ryc. 2c, 3c).

6. References

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