ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLIII, nr 4
1974

Influence of sodium humate on the growth of *Scenedesmus quadricauda* (Turp. Brèb.) and *Gonium pectorale* in the case of different calcium and iron doses

KRYSTYNA JURAJDA

Department of Plant Physiology, Institute of Botany, University of Wrocław

(Received: April 19, 1974)

Abstract

The influence of sodium humate on calcium and iron uptake by two species of algae was studied. It was found that sodium humate influence differentially the growth of these algae in various culture conditions by regulating the iron uptake. The action of sodium humate is especially favourable in non optimal pH of the medium and its effectiveness increases with time.

INTRODUCTION

A number of papers from the field of ecology indicate that the presence of various species in a given water body is largely dependent on the iron content, water pH and the presence of humic substances (Uspeński, 1927; Lund, 1965; Lepnieva, 1950).

The analyses performed by Uspeński showed that in natural water bodies only iron concentration may change suddenly, therefore it decides of the spread of algae.

It is mentioned in the literature that, among algae, calciphils and calciphobes may be distinguished (Lepnieva, 1950; Lund, 1965). Lund claims that the amount of calcium influences the specific composition in natural water bodies. Golovin (1964) investigated ecological groupments of algae and stressed the existence of forms which choose consistently an alkaline or acidic medium. The reaction of this medium is not without meaning for the occurrence of definite species and indirectly influences the spread of algae (Uspeński, 1927). The pH limits within which algae are able to grow are very wide; as reported by Lund the population density in natural conditions may be high between pH 4.5 and 9.5. Other authors claim that the pH of the medium is a factor which influences not the quantity, but the quality of the phytoplankton.

At the same time, the buffer role of humic (organic) compounds at unfavourable pH in natural conditions is stressed (Uspeński, 1927; Lepnieva, 1950). It has also been found in experimental work that humic compounds have a favourable effect at nonoptimal pH. In such cases Mainx (1927) explains the influence of humus by its buffering effect in the medium, whereas Badura (1965) attributes the protective role of humus to its ion-exchanger properties. This action, as affirmed by the author, consists in regulation of mineral components uptake at certain pH values.

The presence of humic compounds and their fractions in mineral medium has mostly a stimulating action on the development of algae, the increment of their mass and their morphology. It has, however, been found that the result of the physiological influence of humic compounds is dependent on external physical and chemical factors (Uspeński, Uspeńskaya, 1925; Uspeński, 1927; Fogg, 1942; Gumiński 1947; Lhotaký 1954, 1955, 1960; Květ, 1957; Prát, 1960, 1964, 1967; Stoklasa, 1962; Tichý, 1962; Rypáček, 1962; Minář, Mannsbartová, Tichý, 1964; Flanderková, Tichý, Minář (1969); Kábelová, Minář, Tichý, 1969; Kyć, 1970, Nechutová, Tichý, 1970 and others).

The aim of the present study was the investigation of the effect of humic compounds on two species of algae against three variable parameters Ca^{2+}, Fe^{3+} and pH.

METHODS

The investigations were carried out on cultures of *Scenedesmus quadricauda* (Turp. Brèb.) and *Gonium pectorale* originating from the collection of autotrophic cultures of the Czech Academy of Sciences.

After numerous preliminary experiments Pringsheim's medium (1954) was chosen for culture as the most suitable for both kinds of algae. The medium was prepared from water bidistilled in a glass distiller and salts pure p. a. The composition of the medium was as follows: KNO_3 — 0.2 g, $(NH_4)_2\,HPO_4$ — 0.02 g, $MgSO_4 \cdot H_2O$ — 0.01 g, $CaCl_2 \cdot 6\,H_2O$ — 0.0005 g, $FeCl_3$ — 0.0005 g and distilled water — 1000 ml. Pringsheim's medium was modified by increasing iron or calcium concentration and used in two combinations: without sodium humate and with it. The sodium humate solution was prepared from compost by the method described by Gumiński (1950, 1965).

Medium pH was adjusted for several days with the use of a L. B. S. lamp pH-meter to 6.8. Then the medium was sterilized in an autoclave and pH was checked once more.

The algae were cultured in 25 ml of medium in 100-ml Erlenmeyer flasks. The experimental cultures were inoculated unter sterile conditions by introducing 1 ml of well stirred 15-day basic culture into each flask.

The experiments were performed in a photothermostat room at light intensity 5000 lux and temperature 21.2° C over a period of two weeks.

The influence of sodium humate was expressed in terms of the number of cells and dry mass of the algae.

In order to obtain univocal results the algae were killed with ethaform (3 parts of ethanol and 1 part of formalin) by adding 5 drops of this substance to each flask. The algae were counted in a Fuchs-Rosenthal chamber. Samples were taken four times from a well stirred mixture. The number of cells is given as arithmetic mean from five replications for 1/4 of the chamber volume.

Then the contents of each flask were transferred on to weighed hard filter paper and washed with acidified water in order to wash out the salt, and with slightly alkalized water for washing out humic compounds. The whole was dried to constant weight, first at 105°C and later at 80°C.

RESULTS

1. Influence of sodium humate in the case of different iron doses

The results of the experiments shown in diagrams 1 and 2 prove that *Scenedesmus quadricauda* is an alga requiring much larger iron amounts (ca. 1.4 mg/l.) as compared with *Gonium pectorale* (ca. 0.5 mg/l.).

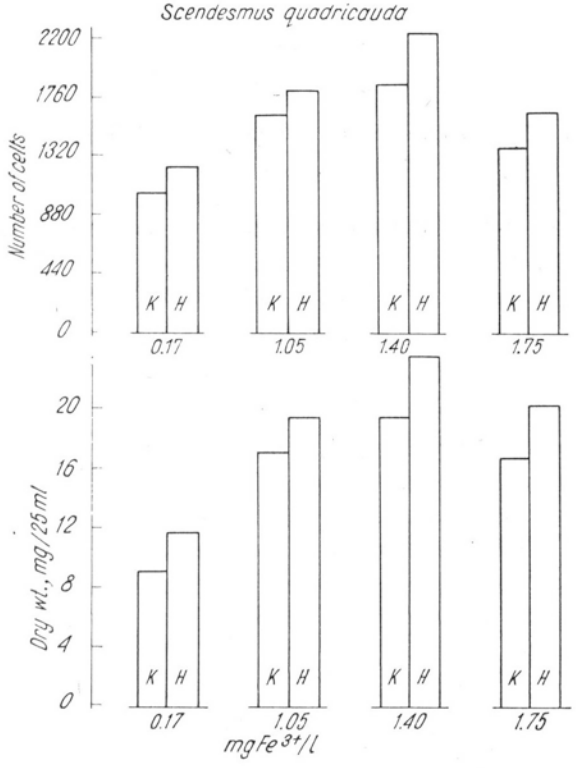


Fig. 1. Influence of sodium humate with different iron doses
K—control; H—sodium humate

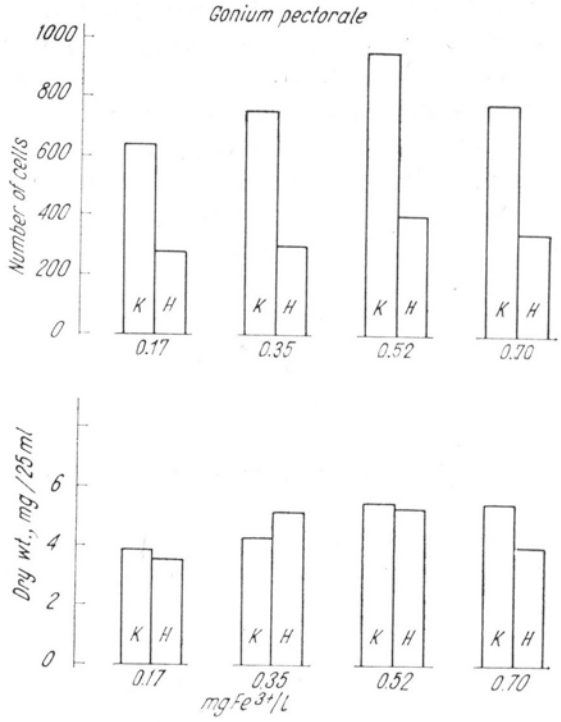


Fig. 2

Fig. 2. The influence of sodium humate with different iron doses
K — control; *H* — sodium humate

The influence of sodium humate was studied in media containing various iron levels as follows:

for *Scenedesmus* 0.17 mg Fe^{3+}/l. (iron level in Pringsheim's medium), 1.05 mg Fe^{3+} /1., 1.40 mg Fe^{3+}/l., 1.75 mg Fe^{3+}/l;

for *Gonium pectorale* 0.17 mg Fe^{3+} /l., 0.35 mg Fe^{3+}/l., 0.52 mg Fe^{3+} /l., 0.70 mg Fe^{3+}/1.

In all combinations a stimulating effect of sodium humate on cell number and dry mass increment of the algae was noted in all *Scenedesmus quaericauda* cultures.

The presence of sodium humate in *Gonium pectorale* cultures greatly inhibited the increase in the number of cells, it was, however, observed that their size markedly increased. No negative influence of humate on dry mass increment of the algae was observed in media with a low iron concentration.

2. Influence of sodium humate in the case of various calcium doses

The experiments illustrated in diagrams 3 and 4 prove differences in the calcium requirement of the two algae. Maximum growth of *Scenedesmus quadricauda* was

obtained at concentrations of 4.5 mg Ca^{2+}/l. and of *Gonium pectorale* at 9.0 mg Ca^{2+}/l.

The role of sodium humate was studied in the case of the following calcium doses:

for *Scenedesmus quadricauda* 0.09 mg Ca^{2+} /l. (calcium level in Pringsheim's medium), 0.9 mg Ca^{2+}/l., 4.5 mg Ca^{2+} /l., 9.0 mg Ca^{2+}/l.

for *Gonium pectorale* 9.0 mg Ca^{2+}/l., 13.5 mg Ca^{2+}/l.

It was found that the addition of sodium humate to the medium causes an increase in dry mass and in the number of cells in *Scenedesmus quadricauda*, notwithstanding the calcium concentration in the medium.

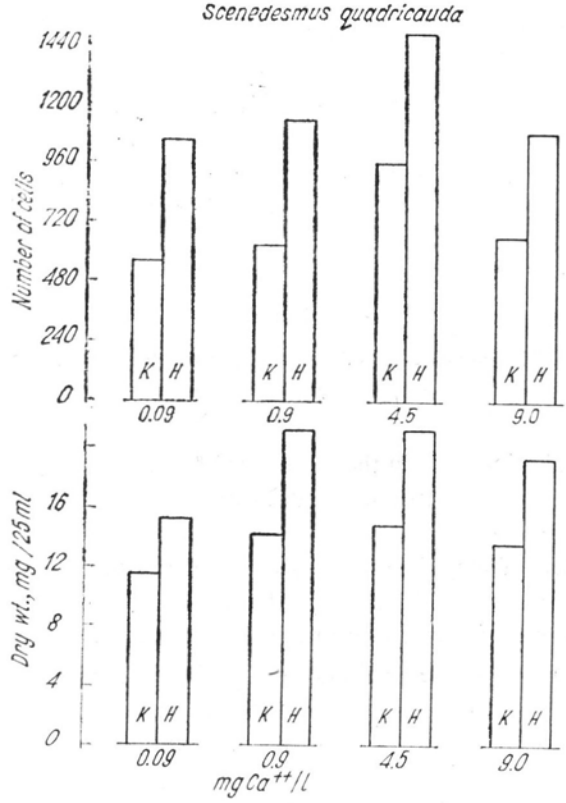
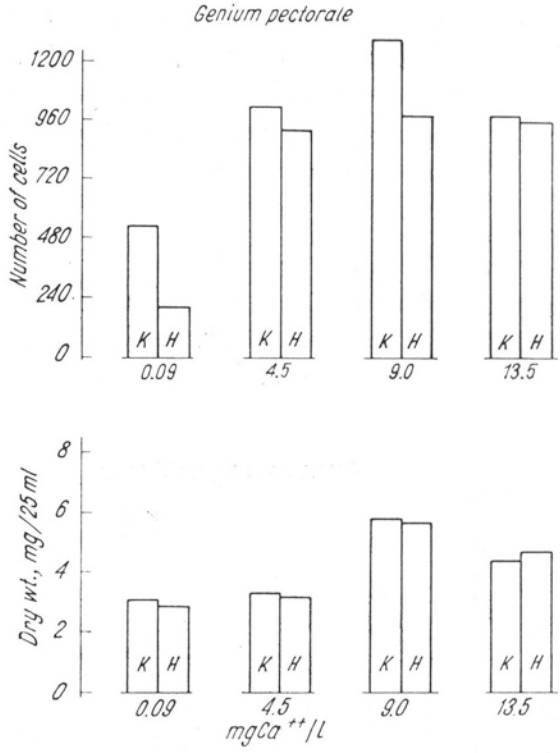


Fig. 3

The presence of humate evoked quite a different reaction in the *Gonium pectorale* culture. This alga responded by an inhibition of the increase in cell number and dry mass. Only when an excess of calcium was added to the medium was no detrimental effect of sodium humate observed.



Figs 4

Fig. 4. The influence of sodium humate with different calcium doses
K — control; *H* — sodium humate

3. Influence of sodium humate in the case of optimal calcium and iron doses after 5, 10 and 15 days of culture

In the next step of investigations the development of *Scenedesmus quadricauda* and *Gonium pectorale* was studied in optimal media established in experiments 1 and 2.

Experiment 3 was performed according to the following scheme:

1. optimal calcium concentration — no sodium humate
   „ „ „ — sodium humate added
2. „ iron „ — no sodium humate
   „ „ „ sodium humate added
3. „ calcium and iron concentration — no sodium humate added
   „ „ „ „ „ — sodium humate added

After 5, 10 and 15 days of culture 5 lots from each combination were taken for analysis which demonstrated an unequal cell growth and dry mass increment of the algae in the different combinations.

After 10 and 15 days the largest increment in cell number and dry mass of *Scenedesmus quadricauda* was obtained in medium with optimal calcium and iron content,

somewhat lower in the medium enriched only in iron, whereas a considerably lower increment in medium with only calcium added (diagram 5). The stimulating effect of sodium humate increased with time and proved most pronounced in medium defective in iron.

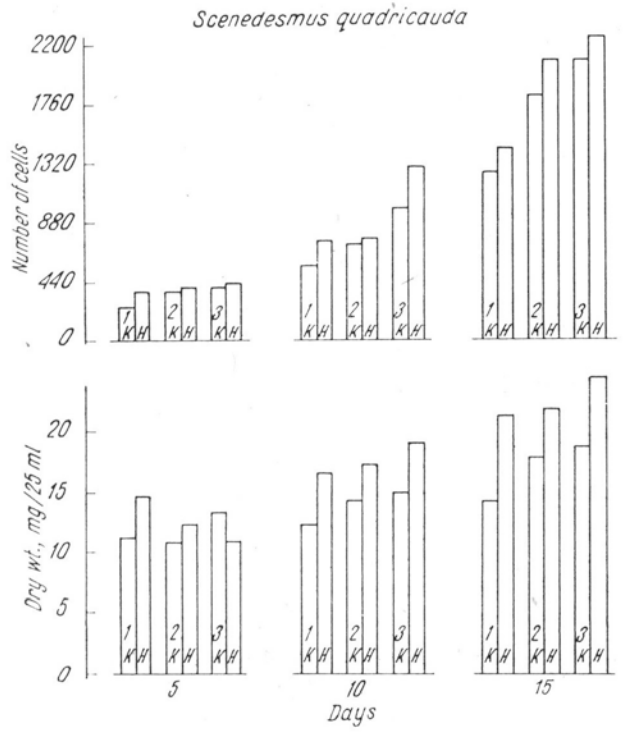
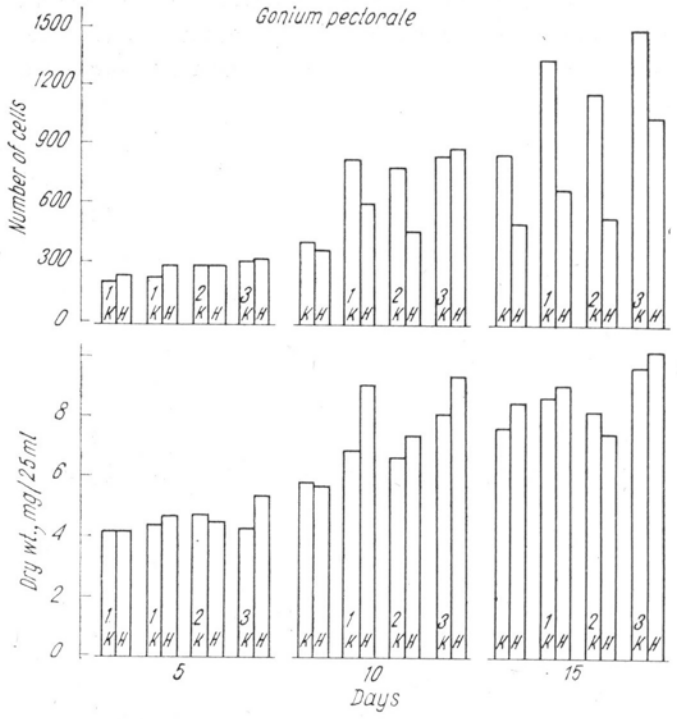


Fig. 5. The influence of sodium humate with optimal calcium and iron doses — after 5, 10 and 15 days

As could be expected a different response was noted in *Gonium pectorale* cultures. As the culture aged, differences in growth became apparent. Highest increments were also obtained in medium with optimal concentrations of both elements. On the other hand, this alga grew better in medium containing an optimal calcium dose than in that with optimal iron concentration. Observations of growth dynamics, in media lacking sodium humate and those with this substance added, for a two-week period showed that the presence of humate pregressively limits the increase in the number of cells and amount of dry mass, particularly when calcium is deficient (diagram 6).

4. Influence of initial pH of medium on the effect of sodium humate in the case of optimal calcium and iron doses

As demonstrated by the foregoing experiments (1, 2, 3) *Scenedesmus quadricauda* and *Gonium pectorale* have different requirements as regards calcium and iron concentration in the medium. Since the effectiveness of iron and calcium ions is



Figs 6

Fig. 6. The influence of sodium humate with optimal calcium and iron doses — after 5, 10 and 15 days

K — control; *H* — sodium humate

dependent on the pH of the solution, it was necessary to test the reaction of both algae in media with optimal calcium and iron doses with the application of three variants of the initial pH of the medium: 5.8, 6.8 and 7.8. This scheme was applied to media with and without sodium humate.

The most intensive growth of *Scenedesmus quadricauda* was obtained in media with initial pH 7.8 (diagram 7). Acid pH caused a distinct growth inhibition (small rounded cells, in many cenobia spines lacking). Medium with optimal iron concentration proved more advantageous than that with optimal calcium content. The presence of sodium humate stimulated growth in all the variants of the medium.

The same experiment performed with *Gonium pectorale* demonstrated that medium with initial pH 6.8 gives the highest algae mass increment (diagram 8). Both acidic and alkaline medium considerably inhibited growth. In all combinations a greater cell number and dry mass increment was found in media with optimal pH 7.8, particularly in the variants with calcium deficit. At pH 5.8 humate stimulates growth of cells and dry mass increase in *Gonium pectorale*.

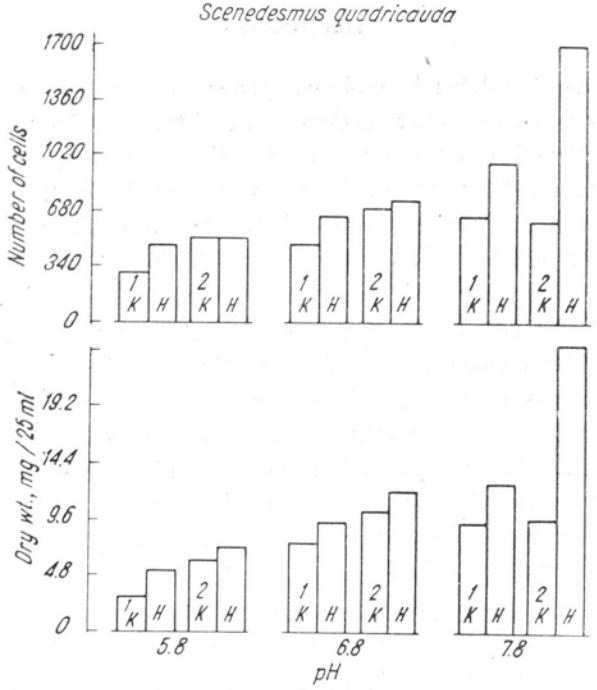


Fig. 7

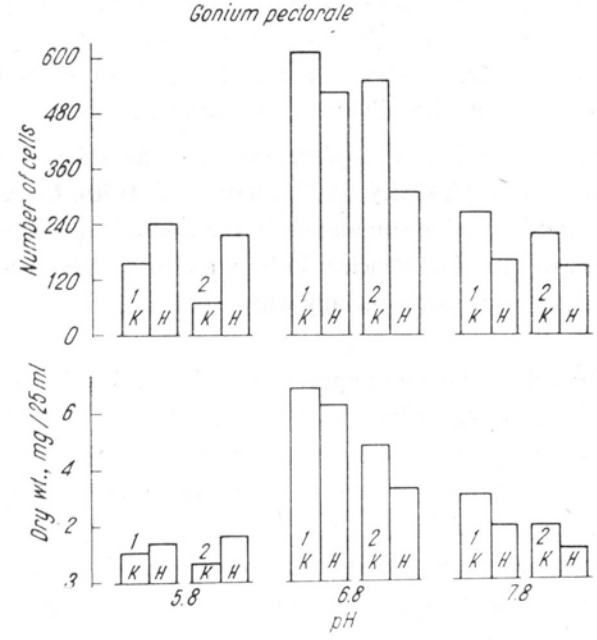


Fig. 8

Figs 7 and 8. The influence of initial pH of the medium on the action of sodium humate with optimal iron and calcium doses

K — control; *H* — sodium humate

DISCUSSION

Sodium humate stimulated *Seenedesmus quadricauda* cultures to an increase in cell number and dry mass. Control tests demonstrated the sensitivity of this alga to F^3 deficit. It may, therefore, be assumed that sodium humate compensates the iron deficit in the medium (Horner, Burk, Hoover, 1934; Ihotoký, 1955; Erdtman, 1955; Dyakonova, 1962; Aso and Sekai, 1963; Gumiński et al. 1965; Gumiński, 1965; Czerwiński, 1967; Gumiński and Sulej, 1967; Tatkowska, 1970; Kyć, 1970).

Numerous investigators are of the opinion that humic compounds regulate too low or too high concentrations of the medium, making possible to the plants the uptake of cations occurring in insufficient amounts, or inhibit the adsorption of those which are present in excess (a buffering action) (Söchtig, 1964; Gumiński, 1967; Tatkowska, 1970; Kyć, 1970; Badura, 1965). Pringsheim's medium is characterized by a relatively low salt concentration, and this may explain the stimulating action on the population of *Scenedesmus quadricauda* even when iron is present in excess in the nutrient solution.

Analysis of the results of all the experiments with *Gonium pectorale* shows unequivocally that sodium humate inhibits the development of this alga. This negative effect decreased with growing calcium concentration in the medium. Such an action is probably associated with the complex-forming properties of the humate. It is known from other studies that humic compounds limit the uptake of certain ions, for instance Cu^{2+} or Ca^{2+}, forming difficultly decomposable chelates (Kappen, 1943, Čatsky, 1958; Jurkowska, 1957; Zajcev, 1959; B. Niklewski, 1949; Gumiński et al. 1965; Bleim, 1966; N. Niklewski, 1968).

The results of investigations up to date prove that the effect of humate becomes more intensive with time (Ihotaký, 1955, 1960; Kyć, 1970). In the light of these communications, the increasing stimulation in the case of *Scenedesmus quadricauda*, particularly in nutrient medium deficient in iron, and the inhibitory effect of humate in cultures of *Gonium pectorale*, especially when calcium is deficient, become understandable.

The protective role of humate appeared also in media with pH different from optimal, as noted by Sladký, 1959, 1965; Sladký, Tichý, 1959; Prát, 1960; Kyć, 1970. These results may perhaps be interpreted as follows: in an acidic medium F^{3+}, Ca^{2+} and Al^{3+} are precipitated in the form of phosphates (Ellenberg, 1958), whereas when pH changes in alkaline direction Ca^{2+}, Mg^{2+} and Fe^{3+} precipitate (Granick, 1958). Since humic compounds inhibit phosphate precipitation, they at the same time make cations available to plants, and the range of favourable pH increases.

It would thus seem, in the light of these results that the stimulating influence of humate on *Scenedesmus quadricauda* (increase of cell number and of dry mass), and the inhibitory effect on the same parameters in *Gonium pectorale* are connected with the different requirements of these organisms as regards iron and calcium.

CONCLUSIONS

1. *Scenedesmus quadricauda* requires relatively large amounts of iron and is very sensitive to its deficit.
2. *Gonium pectorale* is highly sensitive to calcium deficit.
3. Sodium humate causes an increase in the number of cells and dry mass in *Scenedesmus quadricauda*. This phenomenon cannot be explained solely by the availability of iron in this case.
4. Sodium humate in general inhibits growth of the *Gonium pectorale* population.
5. Sodium humate has a favourable effect when pH is not optimal. One cannot, however attribute this solely to its buffer role. In these conditions it should rather be ascribed to the regulation of ion uptake by this substance.
6. The effectiveness of humate increases with time.

Acknowledgement.

The author wishes to express her gratitude to professor S. Gumiński her promoter for indicating the theme and for valuable guidance in the course of this study.

REFERENCES

Aso S., Sakai I., 1963. Soil Sci. and Plant Nutr. 9: 85—91.
Badura L., 1965. Acta Soc. Bot. Pol., 34: 287—328.
Blaim K., 1966. Post. Nauk. Roln., 4/100: 100—109.
Čatsky J., 1958. Folia biol., 4: 247—259.
Czerwiński W., 1967. Acta Soc. Bot. Pol., 36: 549—554.
Dyakonova K. W., 1962. Poczviedienie, 7: 19—25.
Ellenberg H., 1958. Hb. Pflanzenphysiol., 4/: 638—708.
Erdtman H., 1955. Z. Pflanzenern, Düng. Bodeng., 69: 38—43.
Fogg G. E., 1942. Journ. Exp. Biol., 19:78—87.
Flanderková J., Tichý V., Minář J., 1969. Spisy prirodov. Fak. Univ. J. E. Purkyne w Brne., 502: 123—138.
Granick G., 1958. Trace Elem. Lamb. C. A. O. G. Bently and J. M. Beattie, Akad. Press., New York, 365—382.
Gołowin S., 1964. Frag. Flor. et Geob., 10/1/: 121—169.
Gumiński S., 1947. Acta Soc. Bot. Pol., 18/1: 91—115.
Gumiński S., Gumińska Z., Sulej J., 1965. J. Exp. Bot. 16: 151—162.
Gumiński S., Sulej J., 1967. Acta Soc. Bot. Pol. 36: 109—122.
Gumiński S., Badurowa M., 1965. Acta Soc. Bot. Pol. 24: 83—96.
Gumiński S., 1967. Studies about Humus, Prague, 1967.
Jurkowska H., 1957, Zeszyty nauk. W. S. R. w Krakowie., Roln. 2,2: 93—140.
Kábelová J., Minář J., Tichý V., 1969. Spisy Prirodov. fak. Univer. J. E. Purkyne w Brne. 502: 139—148.
Kappen H., Dhaal J., Kreir A., 1943. Bk. u. Pfl. Ern. 31: 357—371.
Kvét J., 1958, Preslia. 30: 126—137.
Kyć S., 1970. Acta Soc. Bot. Pol., 39/4: 681—700.
Lepnieva S. G., 1950. Żhyźń priesnych vod ZSRR. T. III. Moskva-Leningrad.
Lhotský S., 1954. Cs. Biol. 3/1: 45—48.
Lhotský S., 1955. Biologika, 1: 155—213.

Lhotský S., 1960. Acta Agrobot. 9: 113—116.
Lund J. W. G., 1965. Biol. Rev. 40: 231—293.
Mainx F., 1929. Biolobie der Algen, Tab. Biol. 5: 1—24.
Minář J., Mannsbartová E., Tichý V., 1964. Biol. Plant. 6/4: 265—272.
Nechutová H., Tichý V., 1970, Arch. Hydrobiol. 39, Algological Studies 2/3: 26—37.
Niklewski B., 1949. Nawożenie roślin na ziemiach polskich. Poznań, Inst. Wyd. Ruchu Lud.
Niklewski M., 1968. Wiad. Bot. XII, 3: 211—228.
Prát S., 1960. Acta Agrobot. 9/1: 117—122.
Prát S., 1964. Humus a jeho wyznam, Praha, Cesk. Akad. VED.
Prát S., 1967. Rev. roumaine biol. ser. bot. 12/2-3: 207—210.
Pringsheim E. G., 1954. Algenreinkulturen ihre Herstellung und Erhaltung, Jena.
Rypáček V., 1962. Studies about Humus, Prague, 235—243.
Sladký Z., 1959. Biol. Plant. 1: 199—204.
Sladký Z., 1959. Biol. Plant. 1: 142—159.
Sladký Z., Tichy V., 1959. Biol. Plant. 1: 9—15.
Stoklasa J., 1962. Biol. Plant. 4/2: 98—100.
Tatkowska E., 1970. Acta Agrobot. 23/1: 73—85.
Tichý V., 1962. Studies, about Humus, Prague.
Uspeński E. E., Uspeńskaja W. J., 1925. Zeitsch. f. Bot. 17: 273—308.
Uspeński E. E., 1927. Pflanzenforschung 9: 1—104.
Zajcev B. D., 1959. Poczviedienje. 3: 72—76.

Author's address:
Dr. Krystyna Jurajda
Department of Plant Physiology
Institute of Botany, University of Wrocław
ul. Kanonia 6/8; 50-328 Wrocław, Poland

Wpływ humianu sodowego na wzrost Scenedesmus quadricauda (Turp. Brèb.) oraz Gonium pectorale na tle zróżnicowanych dawek wapnia i żelaza

Streszczenie

Celem pracy było zbadanie działania związków próchniczych na dwa gatunki glonów na tle trzech zmiennych parametrów: Ca^{+++}, Fe^{+++} i pH.

Badania przeprowadzono na kulturach *Scenedesmus quadricauda* (Turp. Brèb.) oraz *Gonium pectorale* pochodzących ze zbioru Kultur Autotroficznych przy Czeskiej Akademii Nauk.

Uzyskane wyniki pozwalają na wyciągnięcie następujących wniosków:

1. *Scenedesmus quadricauda* wymaga stosunkowo dużych ilości żelaza i jest bardzo czuły na jego niedostatek.
2. *Gonium pectorale* wykazuje dużą wrażliwość na niedobór wapnia.
3. Humian sodowy działa stymulująco na ilość komórek oraz przyrost suchej masy *Scenedesmus quadricauda*, czego nie można tłumaczyć jedynie udostępnieniem żelaza.
4. Humian sodowy powoduje na ogół hamowanie wzrostu populacji *Gonium pectorale*.
5. Humian sodowy działa korzystnie w pozaoptymalnym pH. Nie można jednak mówić jedynie o buforującej jego roli, a jego wpływ w takich warunkach należy tłumaczyć raczej regulacją pobierania jonów.
6. Efektywność humianu wzrasta z upływem czasu.

Mojemu Promotorowi Wielce Szanownemu
Panu Profesorowi dr St. Gumińskiemu
składam serdeczne podziękowanie za
wskazanie tematu i okazaną w pomoc postaci
cennych rad i wskazówek w trakcie wykonywania
niniejszej pracy.