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**Występowanie wolnych aminokwasów
w wodzie stawowej**

**Occurrence of free amino acids
in pond water**

Wpłynęło 17 grudnia 1970 r.

Abstract — The content of free amino acids in pond water was examined and found to vary from some scores to some hundred $\mu\text{g/l}$. In this water the following amino acids appeared most frequently: cystine, aspartic acid, serine with glycine, glutamic acid, alanine, valine, leucine. The concentration of free amino acids dissolved in pond water depends, to a certain extent, on the fertility of the pond, the time of day, and the layer in vertical section.

In natural waters amino nitrogen occurs in colloidal and molecular form in peptides and amino acids. As a rule, it constitutes only a small part of the dissolved organic matter in lake waters, forming only 5.5 to 10 per cent (Gocke 1970).

The content of free amino acids in natural waters varies from a few to some hundred μg per litre (Domagalla et al. 1925, Enaki 1967, Meistrenko 1965, Vlasova 1965). Free amino acids play an important role in the vital functions of aquatic organisms. They serve as nutrient, or as a source of energy for fungi, bacteria, and some algae (Brehm 1967, Fogg 1962, Gaertner 1970, Gocke 1970, Hobbie et al. 1968, Kuznenko 1970). By means of phytoplankton cultures it was demonstrated that some amino acids added to the nutrient solution do not influence the growth of different species of algae in the same way. Moreover, it was observed that the concentration of a given amino acid in the environment is not without effect for the development of these organisms (Clémenton, Erisman 1964, Kuznenko 1969, Litvinenko, Volovski 1962). Besides, amino acids, as constituents

of organic matter, play a significant part in the nourishment of zooplankton and other invertebrate aquatic animals (G ro v e r et. al. 1961).

Aquatic organisms, such as phytoplankton, zooplankton, and other invertebrates, contribute to an increase in the amount of organic matter dissolved in the water; not only do they take up amino acids from the water but also excrete them into it (A n d e r s o n, Z e u t s c h e l 1970, F o g g 1962 a, K e n n e t h, W e b b 1967, P i n e n o v a et al. 1969).

The main source of amino acids is organic matter dissolved in natural water, which undergoes autolysis and decomposition both of autochthonic and allochthonic character. It is well known that allochthonic organic matter penetrates into the inland waters from outside with rain, thawing snow, atmospheric contaminations, and municipal waste waters.

Purpose of the investigation and material

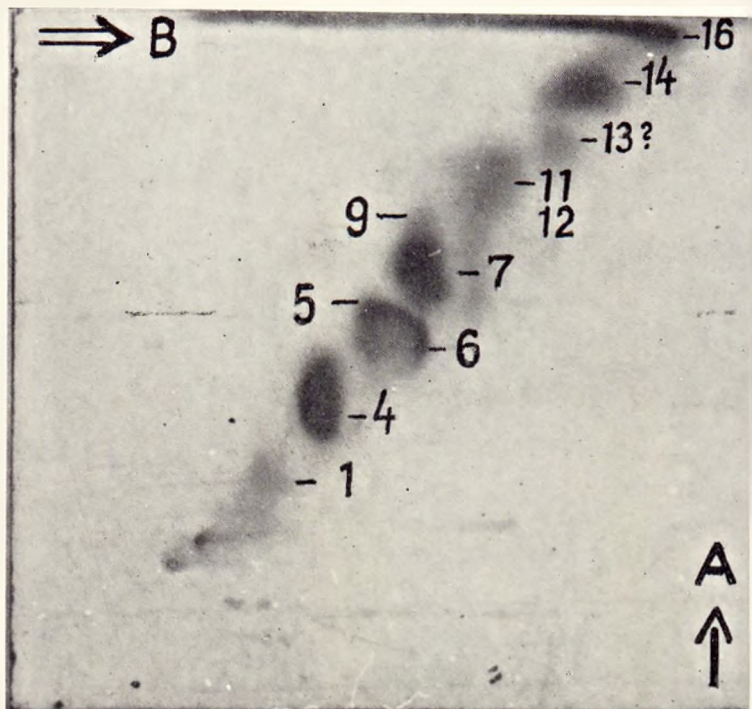
In 1969 a number of preliminary investigations on the content of free amino acids in pond water were carried out in the Laboratory of Water Biology PAN (Polish Academy of Sciences). These investigations aimed at checking the qualitative and quantitative variations of the mentioned substances in the vertical section of the water in a pond, time of day of sampling being considered. Water samples were collected from breeding ponds with a population of carp K 2/3.

The ponds were of equal area, amounting to 1 500 m², and situated close to each other. The two ponds were equally fertilized. The difference lay only in the amount of feeding stuff supplied for the fish: pond No 21 received 1 325 kg granulate and 195 kg wheat and pond No 23 received 1 337 kg wheat.

Methods

Temperature, oxygen content, oxydability, and pH were determined each time according to the method given by J u s t and H e r m a n o w i c z 1964. The obtained data are presented in Table I. Besides the chemical analysis, the qualitative composition of phytoplankton was determined in the examined water samples.

For the determination of the total amounts of free amino acids and their quality, the pond water was treated with chloroform for preservation purposes and subsequently filtered through hard filter paper in order to remove seston and possible mineral suspension. After this the water was passed through a column 0.9 cm in diameter and 10 cm high filled with cationite in hydrogenic form (Zerolit 225-SRC-13). The resin was prepared according to the method given by K a r p o v (1962). Adsorbed amino acids



Ryc. 1. Rozdział wolnych aminokwasów przy pomocy chromatografii cienkowarstwowej dwukierunkowej (woda ze stawu nr 23, 26. IX, powierzchnia, po południu): A — pierwszy kierunek rozwijania, B — drugi kierunek rozwijania; 1. cystyna, 4. kwas asparaginowy, 5. seryna, 6. glikokol, 7. kwas glutaminowy, 9. alanina, 11. tyrozyna, 12. tryptofan, 13. metionina?, 14. walina, 16. leucyna (fot. A. Siemińska)

Fig. 1. Separation of free amino acids followed by thin-layer two-dimensional chromatography (pond water No 23, from 26th September, surface p.m.): A — first direction of development, B — second direction of development: 1. cystine, 2. aspartic acid, 5. serine, 6. glycine, 7. glutamic acid, 9. alanine, 11. tyrosine, 12. tryptophan, 13. methionine?, 14. valine, 16. leucine (phot. by A. Siemińska)

were eluted with 2 N ammonia to the amount 80 ml per litre of the examined water. Ammonia was evaporated in a rotary vacuum flask at 40—50°C. The dry residue from 1 litre of water after elution was divided into two halves and the determination carried out in two repetitions. Amino nitrogen was determined quantitatively by means of ninhydrin. The reaction was carried out in a water-butanol solution, producing a cadmic complex with hydridantin resulting from the ninhydrin reaction. The results were read on a colorimeter at the wave-length 508 m μ and calculated in terms of glycine (Semenov 1962).

For chromatographic purposes the dry residue from 2 l of water after evaporation of ammonia was extracted by means of 96 per cent ethanol acidified with 10 N HCl (Baliga, Krishwamur 1955). The residue was diluted in 1 ml redistilled water, after evaporation of ethanol. Amino acids were determined qualitatively by means of thin-layer-chromatography, MN 300 cellulose being used as adsorbent. The latter was prepared as follows: 90 ml water and 15 g cellulose were homogenized for 90 sec. This suspension was subsequently spread over 17×17 cm glass plates. The cellulose layer was 0.4 cm thick (Opieńska-Blaut et al. 1967). For one-dimensional-ascending-chromatography a composition of butanol, acetic acid, and water in the proportion 4:1:1 was used, whereas in two-dimensional-chromatography the above given composition was applied for the first direction, and for the second propanol, formic acid, and water in the proportion 40:2:10 or phenol and water in the proportion 3:1 were used with the addition of 8-oxychinoline and ammonia in order to saturate the chamber with its vapour (Kański 1957). Amino acids were detected by means of a ninhydrin reagent in methanol with the addition of 2, 4, 6 colidine. Identification was made by comparing the R_f of standard and investigated amino acids. Besides, colour tests were made for tryptophan and tyrosine (Opieńska-Blaut et al. 1965, Stahl 1967). Separation of aspartic acid, serine, and glycine as well as methionine with valine was a difficult problem. Hence, two directional separation was also applied (fig. 1).

Results

In the first period (August) considerable amounts of free amino nitrogen were recorded in the water sample from pond No 21; the greatest amounts were found close to the bottom (Table I). The water taken at the same time from pond No 23 also contained fairly large amounts of amino acids but at the surface their content was much poorer. At 2 p.m. a fall in amino acid content was noted in both ponds at almost all sampling points.

In the water from pond No 21 taken at the same time from both these depths and times of day, the following amino acids were found: cystine,

Tabela I. Niektóre cechy fizyko-chemiczne wody stawów
 Table I. Some physico-chemical features of pond waters
 a - powierzchnia - surface, b - dno - bottom

Staw nr Pond No 21								
Data poboru Date of sampling	Czas poboru Time of sampling	T°C	pH	Tlen rozpuszczony Oxygen dissolved O ₂ /mg/l	Utlenialność Oxydability O ₂ mg/l		Azot aminowy Amino nitrogen ug/l	
					a	b	a	b
29.VIII.	rano a. m.	14	7.2	2.88	13.94	15.52	135	180
	popołudniu p. m.	16	7.2	7.28	18.06	17.11	48	36
26.IX.	rano a. m.	10.9	7.2	6.16	21.64	21.32	62	38
	popołudniu p. m.	10.9	7.2	8.08	21.02	22.26	44	33
Staw nr Pond No 23								
29.VIII.	rano a. m.	14	7.0	3.25	12.92	12.67	34	124
	popołudniu p. m.	16	7.0	6.66	13.94	15.52	37	33
26.IX.	rano a. m.	10.9	7.0	6.08	15.08	13.80	55	56
	popołudniu p. m.	10.9	7.0	7.04	13.17	13.17	51	63

aspartic acid, serine probably with glycine, glutamic acid, alanine, valine, and leucine. Moreover, in the afternoon hours lysine was found in traces in the surface water of this pond. It should be noted that the water from the bottom of this pond was characterized by a greater variety in the qualitative composition of amino acids than the surface water. Thus, besides the above mentioned amino acids, proline was found at this depth. Identification of this amino acid was made by comparing the Rf of the examined sample with the standard one. The isatin test (Noworytko 1955) proved not to be satisfactory in this case. In the water close to the bottom tyrosine was recorded at both sampling times, whereas in the afternoon only lysine and arginine were found.

In pond No 23 the pattern of free amino acids in the surface water taken in the morning and in the afternoon was similar to analogous samples taken from pond No 21, while at the bottom this dependence did not appear. In the surface water from pond No 21 certain qualitative variations between the samples were noted depending on the time of day, alanine occurring only in the water taken in the morning hours and tyrosine being present in traces in the afternoon. As has already been mentioned, the water close to the bottom demonstrated a considerable fall in the amount of free amino acids during the day (from 124 µg in the morning to 33 µg

in the afternoon). In this water layer variations in the quantitative composition of free amino acids were observed. Besides the most frequently occurring amino acids, the following were found in the morning: lysine, threonine, unidentified amino acids, and phenylalanine recorded only here. In the afternoon the composition of free amino acids proved to be much poorer than that in the morning sample. Threonine, valine, unidentified amino acids, phenylalanine, and leucine were absent. Tyrosine and tryptophan, on the other hand, were found in large dark spots (fig. 2).

Samples collected in the second period (September) in very windy weather, which was evidenced by an equal temperature in the whole water column (Table I), demonstrated smaller variations in the amount of free amino acids than in the previous samples between individual depths of the pond and times of the year. However, this time too, the amounts of free amino nitrogen in the water taken in the morning both from the surface and from the bottom were slightly greater than in that taken in the afternoon. Water taken from the bottom of pond No 23 was an exception in this respect, as in the afternoon a slight increase in the amino nitrogen content was recorded.

In pond No 21 the following free amino acids occurred both in the surface water and in that taken from the bottom of the pond: cystine, aspartic acid, probably serine with glycine, glutamic acid, alanine, valine, and leucine. In water samples taken in the morning both from the surface and from close to the bottom tyrosine and tryptophan were found. These were absent in the water taken in the afternoon. Lysine was identified in the water close to the bottom both in the morning and in the afternoon.

The pattern of free amino acids from pond No 23 in the second period of samplings, was on the whole similar to that from pond No 21 (fig. 3). Moreover, in the morning samples arginine, occurred in the surface water and threonine in the samples from the bottom; the latter occurred also in an analogous sample taken during the previous sampling period (fig. 2). Lysine was found in traces in all samples, with the exception of the surface water collected in the afternoon.

Discussion

In spite of numerous drawbacks the thin-layer-chromatographic method proved to be successful because it permits the identification of the substances sought in a relatively small amount of the basic sample, which in the case of free amino acid identification in natural waters is an essential problem. Hence, on the basis of the literature (identification was carried out by means of various methods) and of the present investigations it was established that aspartic acid and alanine are amino

acids which were identified in a free state in natural waters by the majority of authors investigating amino acids in water, whereas glutamic acid was detected only by a few of them (B r e h m 1967, G o c k e 1970, F o g g 1962, J u r š i k 1961, L i c h t f i e l d, P r e s c o t t 1970, P a l u c h, S t a n g r e t 1969, P o v o l e d o 1960). Besides the mentioned amino acids, various authors indicated the occurrence of only one or a few of the amino acids mentioned in the present paper, i.e. cystine, lysine, arginine, glycine, serine, tyrosine, tryptophan, threonine, valine, leucine (G o c k e 1970, F o g g 1962, J u r š i k 1961, L i c h t f i e l d, P r e s c o t t 1970, P a l u c h, S t a n g r e t 1969, P e t e r s e n et al. 1925, P o v o l e d o 1960). Histidine and ornithine were detected only by P e t e r s e n et al. (1925) and by H o b b i e et al. (1969).

In the first period the greatest number of amino acids was recorded in morning samples from the layer close to the bottom. In the afternoon exhaustion of these substances was observed, so that their amounts at this time were lower than in the surface water. In the second period (end of September) when sampling took place in windy weather, which made the water turbulent, only a slightly larger amount of free amino acids was observed in almost all samples in the morning, not only at the bottom but also on the surface.

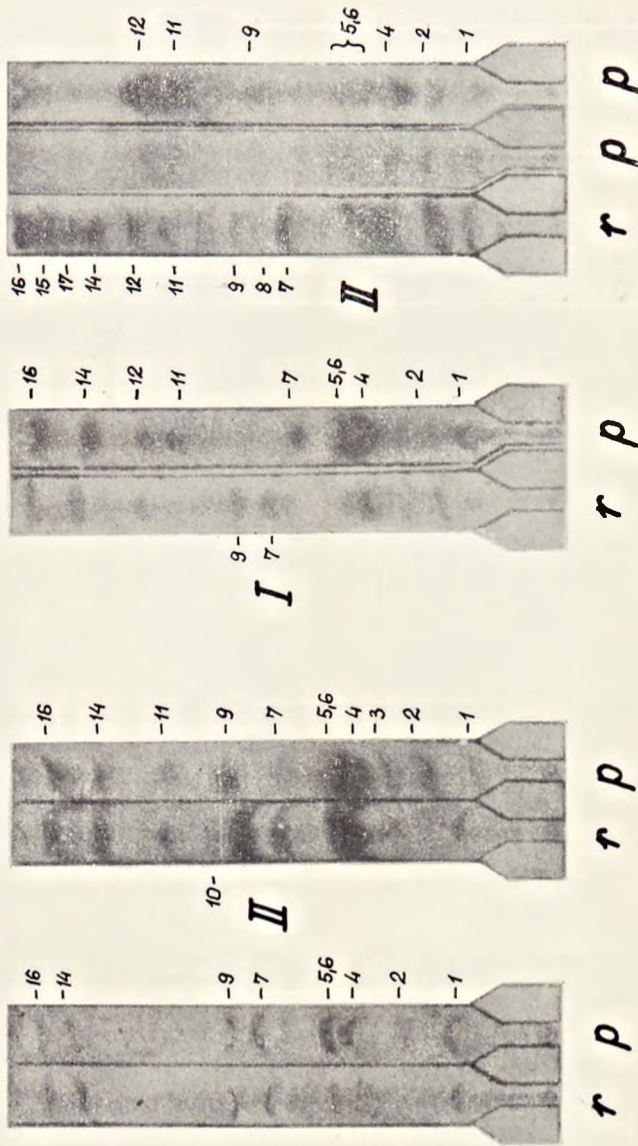
Accumulation of free amino acids at the bottom in the morning indicates the sinking of organic matter to the bottom and that a decomposition process takes place, connected with excretion of the mentioned substances. According to F o g g (1962), accumulation of extracellular products increases with an increase in the depth of the reservoir. G o c k e (1970) maintains that the maximum of free amino substances occurring in the morning is conditioned by an intensive division of algae during the night. Due to the increase in the number of algae cells in this period of time, the amount of extracellular substances released into the environment increased several times. Conformity of the results reported in the present paper with those quoted by the two above-mentioned authors, is probably connected with vertical migration of the plankton during the day.

When comparing the two examined ponds it should be noted that pond No 21 was more fertile than No 23 as it was richer in free amino acids, this could also be confirmed by other coefficients of water fertility, such as oxydability (Table I), higher in the first pond, and the predominance of chlorococcous algae which usually appear in fertile ponds. In pond No 23, of lower oxydability *Conjugales* algae predominated, this algae being characteristic of less fertile waters (B u c k a, K y s e l o w a 1967, B u c k a 1968, K r z e c z k o w s k a - W o ł o s z y n 1966, 1967).

Staw 21
Pond

Sierpień
August

Staw 23
Pond



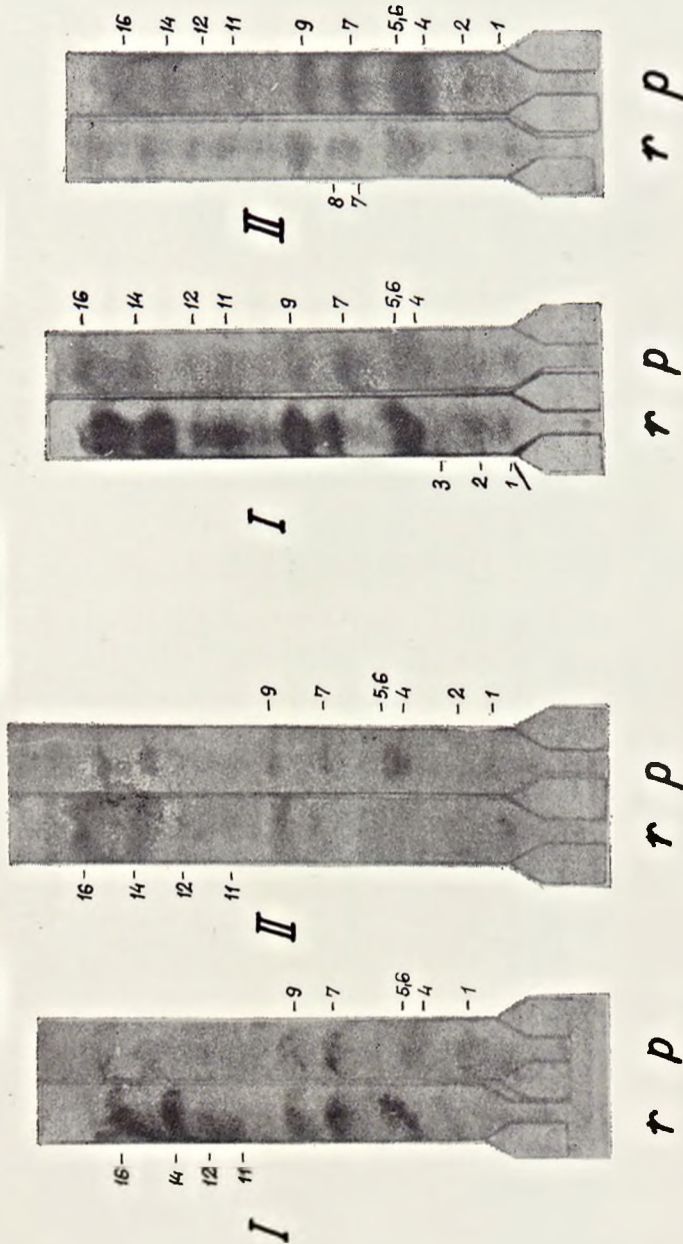
Ryc. 2. Rozdział wolnych aminokwasów przy pomocy chromatografii cienkowsłupkowej (woda ze stawów nr 21 i 23, 29. VIII): 1. cystyna, 2. lizyna, 3. arginina, 4. kwas asparaginowy, 5. seryna, 6. glikokol, 7. kwas glutaminowy, 8. treonina, 9. alanina, 10. prolina, 11. tyrozyna, 12. tryptofan, 14. walina, 15. fenylalanina, 16. leucyna, 17. niezidentyfikowany. I — powierzchnia, II — dno, r — rano, p — po południu (fot. A. Siemińska)

Fig. 2. Separation of free amino acids followed by thin-layer one-dimensional chromatography (water of ponds Nos 21 and 23, from 29 th August): 1. cystine, 2. lysine, 3. arginine, 4. aspartic acid, 5. serine, 6. glycine, 7. glutamic acid, 8. threonine, 9. alanine, 10. proline, 11. tyrosine, 12. tryptophan, 14. valine, 15. phenylalanine, 16. leucine, 17. unidentified, I — surface, II — bottom, r — a. m., p — p. m (phot. by A. Siemińska)

Staw 23
Pond

Wrzesień
September

Staw 21
Pond



Ryc. 3. Rozdział wolnych aminokwasów przy pomocy chromatografii cienkowarstwowej jednokierunkowej (woda ze stawów nr 21 i 23, 26. IX): 1. cystyna, 2. lizyna, 3. arginina, 4. kwas asparaginowy, 5. seryna, 6. glikokol, 7. kwas glutaminowy, 8. treonina, 9. alana-
nina, 11. tyrozyna, 12. tryptofan, 14. walina, 16. leucyna I — powierzchnia, II — dno, r — rano, p — po południu
(fot. A. Siemińska)

Fig. 3. Separation of free amino acids followed by thin-layer one-dimensional chromatography (water of ponds Nos 21 and 23, from 26 th September): 1. cystine, 2. lysine, 3. arginine, 4. aspartic acid, 5. serine, 6. glycine, 7. glutamic acid, 8. threonine, 9. alanine, 11. tyrosine, 12. tryptophan, 14. valine, 16. leucine. I — surface, II — bottom, r — a. m. p — a. p. (phot. by A. Siemińska)

Conclusions

1. Lack of conformity in the determination of the composition of free amino acids present in natural waters is caused by using dissimilar methods.

2. Increased excretion of free amino acids into the water and their concomitant lower uptake by aquatic organisms takes place in the morning.

3. Variations in the content of free amino acids at various times of the day on the surface and at the bottom of the pond seem correspond with the vertical migration of the plankton in the water.

4. The content of free amino acids in pure waters of a type similar to those investigated may reflect their fertility: the greater the concentration of amino acids the higher fertility of water.

Acknowledgements

I am greatly indebted to Dr Henryk Kraczkowski, Head of the Institute of Biochemistry of the Agricultural College, Lublin, for enabling me to become acquainted with the thin-layer-chromatographic method. My thanks are also due to Mrs Krystyna Kyselowa for identifying the phytoplankton in the examined material.

STRESZCZENIE

W stawach hodowlanych Zespołu Gospodarstw Doświadczalnych PAN w Gołyszach wykonano wstępne badania nad zawartością wolnych aminokwasów w wodzie. Próby wody pobierano dwukrotnie: 29.VIII i 26.IX.1969, o godz. 6 rano i o 14 po południu, z dwu poziomów: 30 cm od powierzchni i 30 cm nad dnem. W wodzie stawów każdorazowo mierzono temperaturę i pH, jak również oznaczono utlenialność i skład fitoplanktonu. Ogólną zawartość azotu aminowego oznaczano metodą kolorymetryczną ninhydrynową. Poszczególne aminokwasy określano, stosując technikę chromatografii cienkowarstwowej.

Stwierdzono, że w wodzie stawów prawie zawsze występowały takie wolne aminokwasy, jak: cystyna, kwas asparaginowy, seryna z glikokolem, kwas glutaminowy, alanina, walina i leucyna. Znacznie rzadziej w próbach wody notowano lizynę, argininę, treoninę, tyrozynę i fenylalaninę.

Wymienione wolne aminokwasy, zidentyfikowane przez autora w wodzie stawów, nie zawsze były podawane przez badaczy zajmujących się tym problemem. Brak zgodności wyników uzyskanych w niniejszej pracy, z danymi z literatury, dotyczącymi składu wolnych aminokwasów, jest spowodowany stosowaniem niejednakowych metod. Stwierdzono, że wystąpiły różnice w zawartości wolnych aminokwasów w zależności od pory dnia i od warstwy w pionowym przekroju.

W pierwszym terminie (koniec sierpnia) notowano większe nagromadzenie wolnych aminokwasów przy dnie, w porze rannej. Natomiast w drugim terminie (koniec września), gdzie próby pobierano przy wietrznej pogodzie, obserwowano tę

prawidłowość w porze rannej, nie tylko jednak nad dnem, ale także przy powierzchni.

Porównując badane stawy, należy zauważyć, że staw nr 21 był żyzniejszy niż staw nr 23, ponieważ miał większą zawartość wolnych aminokwasów. Potwierdzały to także inne wskaźniki żyzności wody, jak utlenialność (Tab. 1) większa w pierwszym stawie i przewaga glonów chlorokokkowych, występujących zwykle w żyznych stawach. W stawie nr 23, z mniejszą utlenialnością przeważały glony *Conjugales*, charakterystyczne dla wód o mniejszej żyzności.

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