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MICROBIOLOGICAL EXAMINATION OF THE CARSKA BARA SWAMP WATER

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Abstract

Microbiological examinations of the Carska bara swamp water performed from the middle of the 1981 to middle of the 1984. They included the total count of bacterioplankton by means of direct method, and the count of heterotrophic bacteria by means of cultivation methods. Electron microscopic examinations of plankton and attached forms of bacteria were done in order to get more complete pattern of microbe populations of the investigated ecosystem.

The results of examination of bacterioplankton occurence in Carska bara swamp water show the significant fluctuations but not strictly sesonaly dependent. Between the count of bacteria and observed physico-chemical parameters in swamp water relatively low coefficients of correlation were found.

Introduction

A large number of smaller and bigger water pools which were the part of flooded region originated between the Danube, the Tisza and the Begej were located in the area of the west part of Banat once, thanks to geomorphological and pedological factors, but to the contrary of relatively arid climate. The Theiss flow was regulated in the last century and in that way the water regime of the whole region was changed, so came to the significant reduction of flooded terrains. The constructions of canals of the hydrosystem Duna—Tisza—Duna (Danube—Theis—Danube), led to the configuration changes of this terrain, which made the number of natural, especialy statical water pools in this area more decraesed (HAM 1975). The largest part of the area, within 10 km of the former Begej, was surrounded with embankments and separated of the flows of the Theiss and the Begej.

On the right side of the Begej, between its bank and defensive embankment, the well known swamp Carska bara is situated. It is the special type of the natural reservation, in the part of which, Vojtina mlaka is protected since 1955. Carska bara swamp is autochthonous pool over 300 ha (BUJNOVIĆ 1973). That is swampy area in the broadest sence, the water level beeing regulated mostly by the waters of the Old Begej flow. The water level changes during the year lead to the various residences changes. During the steady water level of the Begej, Carska bara is covered by water macrophytes, and it is surrounded by valley flooded forests (DJERFI 1962; ŠOTI. and DIMITRIJEVIĆ 1974). Such conditions make possible for the great number of swamp birds to find exceptional conditions for permanent stay, for bilding nests, as well as for the longer or shorter stop during the migration. That is the very reason for

numerous ornithologic observations and the examination of this pool. We did not find however any details on former microbiological examinations of Carska bara swamp water. Considering the importance of microorganisms in the processes of matter cycling in nature, in the nutrition chaines, we have examined bacterial component in the Carska bara swamp water, which was not studied so far.

Materials and Methods

Microbiological examinations of Carska bara water were performed since the summer of 1981 (the month of July) to the summer of 1984 (the month of August). The total count of bacteria was examined by ultrafiltration method (RAZUMOV 1932), on membrane filters „Sartorius No 2". The count of heterotrophic bacteria (on nutrient agar) was determinated by standard cultivation method (RODINA 1965). Agarised examined water was used for more complete ecological examinations, as the medium the most similar to the nature of examined environment. Electron-microscopic examinations were also done in order to achiece the complete pattern of micropopulations of the ecosystem in question, to recognise that part of bacterial population which can not be seen by classical methods of cultivation.

The variety of planktons and attached forms of bacteria was followed during the microflora examinations by means of electron-microscopic. The examined water was centrifugated in the amount of 10 cm^3, and the fixatif (4% glutar-aldehyde) was poured over the sediment, in order to recognize various planktonic bacteriol forms. The fixations were performed at the room temperature during 2 hours. The cells were centrifugated and rinsed by destillated water afterwards. Such bacterial suspension was poured on the mash and dried on the air. The mashes were contrasted by phosphotungstenic acid pH 1,7 immediately before electronmicroscope (Jeol TEM — 100 C) screening.

Covering glases were dived in the tested water and exposed 2—7 days in the thermostat on 27 °C in order to recognize the attached bacterial forms. After that, without previous fixation covered glasses were put into fluid nitrogen, they were frozen and dried in vacuum. The glasses dried in that way overgrow with bacteria, were glued for carriers, were covered with gold and observed using scanning electron microscope (Jeol SEM — 35).

Paraell to theses analyses some standard physicochemical factors (the amount of dissolved oxigen, pH and water temperature) were followed.

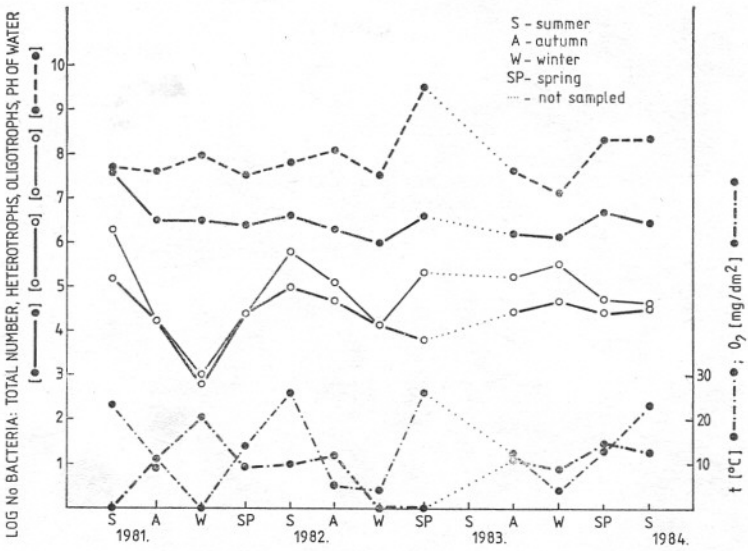
Results

The results of microbiological examinations of Carska bara swamp water showed the variations of amounts of the total count of bacteria as well as their separate groups. These variations depend on seasons although with deviations.

The total count of bacteria went from one to nearly five million cells/cm^3 of water, with exception of summer period of 1971, when even 42 390 000/cm^3 was registrated. Contrary to the total bacterial count (determined by direct method) the number of heterotrophic bacteria (determined on nutrient agar) have shown more significant fluctuations. The number of heterotrophs went from 700—155 000 colonies/cm^3 of water, while the number of colonies obtained on agarised tested water was usually higher and was 1000—1 915 000/cm^3 of water. None of the bacterial groups in question have shown the regular seasonal changes in amount which is partly understandible considering the characteristic of the tested water object.

In the Carska bara swamp water some physico-chemical parameters (pH, water temperature and the amount of the dissolved oxygen) were determined parallel with the following of the count of bacteria. These parameters also changed significantly in tested water.

It should be noted that the obtained values of tested water pH which were shown graphically and compared with logarithm of total count of bacteria (Graf. 1) had similar tendencies. However, correlation coefficient of these two parameters is very low and was only $r = 0{,}23$. The total number of bacteria in the tested water had the



Graf. 1. Number of bacteria and values of physico-chemical parameters in water of Carska bara swamp

higher coefficient of correlation with the temperature (r = 0,40) and with the amount of dissolved oxygen in water (r = −0,42).

Very low coefficient of correlation was found between the number of bacterial colonies and followed physico-chemical factors of the water in question. Heterotrophs had the lowest coefficient of correlation with concentration O_2 in water (r = −0,29), with pH of water (r = −31), and the highest one with water temperature (r = 0,55), while the number of bacteria counted on agarised tested water was the least coased by pH values of water (r = 0,015), a little bit more by dissolved oxygen (r = −0,44), and it correlated the best with water temperature also (r = 0,59).

Relatively low correlation coefficients of bacterial count and aberved physico-chemical parameters in Carska bara water are certainly the result of the complex ecological conditions in this relatively shallow water ecosystem in which we have included only a minor number of the most important factors in our investigations. In such natural residences which are under the very high influence of changes of a borad spectrum of interrelated abiotic and biotic factors, which can hardly be all included in investigations, it is almost impossible to find direct correlation among certain factors. Other autors came to the similar conclusion. Brković—Popović and Popović (1977) found the abundance of the bacterioplankton inwater current to be naturally variable and dependable on large number of physico-chemical and biological factors which, acting in the same time, very often could influence opositly the development of autochthonous microflora. Brasfield (1972), using multiple linear regression analyses in the determination of the dependance between the size of bacterial population in the river water and some physico-chemical factors of the environment, for the largest number of tested parameters also did find statisticaly reliable correlations.

The tested Carska bara water, according to the number of saprophytic bacteria/ cm^3 (Sladeček 1973; Tümpling 1969) belonged to the second class of quality mostly,

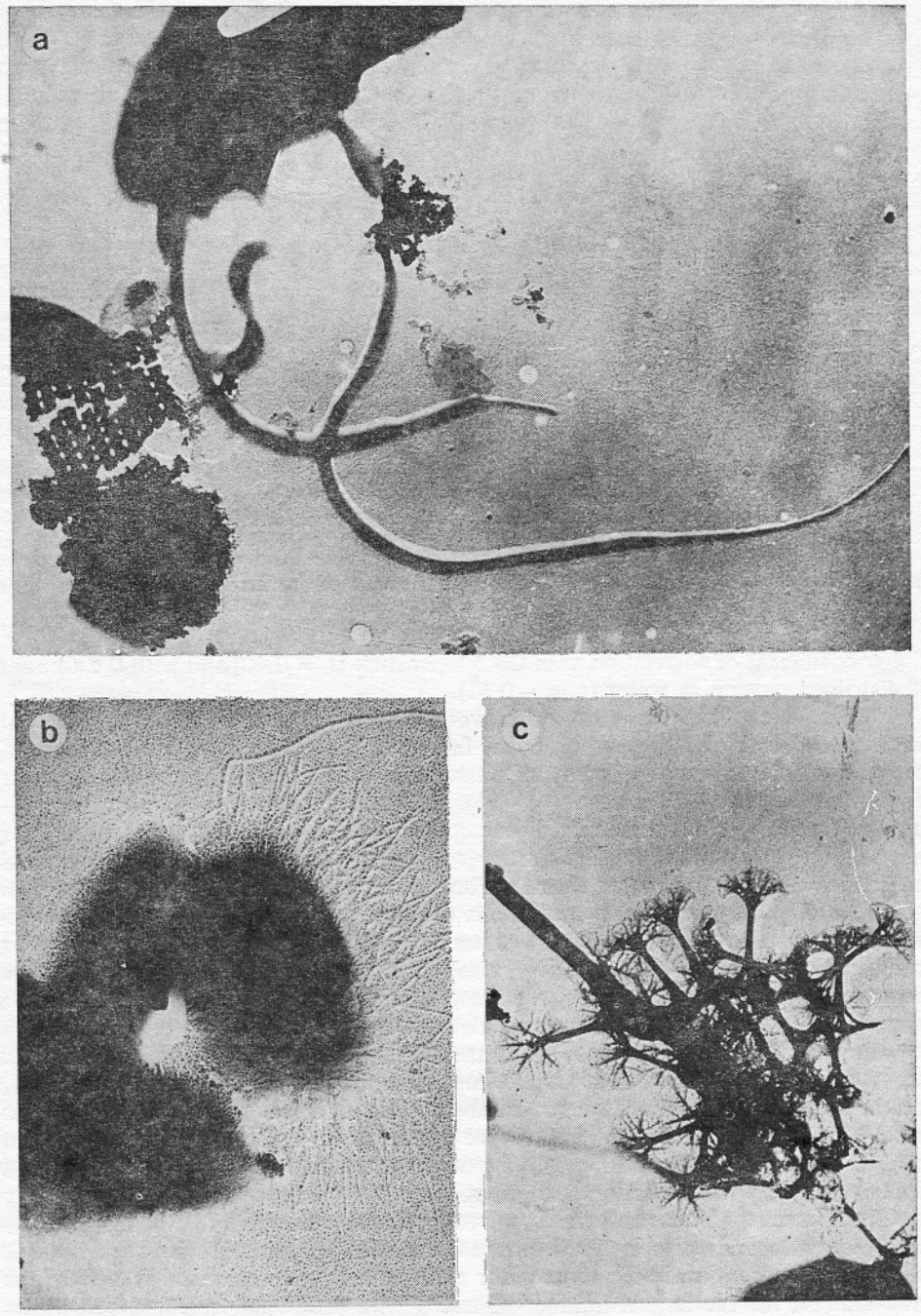


Fig. 1 Various forms of bacterioplankton from the examined water. Caulobacter-like organisms (a), fimbria bearing bacteria (b), unidentified forms (c). Bar indicates 1 μm

with exception in the summer period 1981 and 1982 when it slowly decreased to the third class quality, and the winter period 1982 when it belonged to the first class. The second microbiological indicator of water quality — Korsh's index (the relation between the total count of bacteria and the number of heterotrophic bacteria) have shown that the Carska bara swamp water during the period of investigation was mostly in the category of polluted waters. Moderately polluted was only in 1981 as well as in summer periods during next years of investigations (1982—1984).

A great differences between the total bacterial count and number of heterotrophic bacteria obtained on nutrient agar, were visible regarding to the obtained results. The participation of heterotrophs in the total count of bacterioplankton was only 0,02—3,4%. That was the very reason for us too, wishing to find also those bacteria which could not be grown by standard microbiological methods in laboratory conditions, to use also the electronmicroscopy. Betwen the plankton of the tested water dominated filamentous bacteria which resemble *Caulobacter* (Fig. 1a) by a narrowed and expanded end. Among rod-shaped bacteria, which were numerous, we have noticed also such cells with thick tuft of fimbria on their surface (Fig. 1b). Except the microbial forms which, according to its morphology and dimensions could be easely identified as bacteria or algae, such forms whose origin and nature could not be identified have been noticed (Fig. 1c).

The attempt to observe microflora in its natural sample by method of overgrown slides and observing by scanning electron microscope, have even more shown the wealth and variety of bacterial forms of Carska bara water. Filamentous bacteria *Sphaerotilus — Leptothrix* type, which covered the largest part of overgrown glasses surfaces (Fig. 2a) occured the most frequently as the attached bacterioflora. Among this forms there were such filamentous forms in which individual cells were clearly visible (Fig. 2b) or they were not visible due to the thick slimy sheath (Fig. 2c). Slimy sheath had characteristic irregular form probably as the result of drying. These filamentous bacteria, beside the developed slimy sheath had also slurry filaments which they use to be attached to the substrat (Fig. 2d). In certain cases branching of these bacteria were noted (Fig. 2b, c). Beside the bacteria of *Sphaerotilus — Leptothrix* type, also rod-shaped bacteria of various forms were observed. They occured the most frequently in microcolonies and rarely separatly. The cells themselves had pointy (Fig. 3a) or rounded (Fig. 3b) tips. The characteristic of such microcolonies is the presence of extracellular polysaccharide resembling net matrix (Fig. 3c, d) Such form of slimy matrix leads to the conclusion that racextellular polysaccharides form interrelation, ie. certain communication between cells, besides their role as fixator to substrate. Even when the slime was not very distinct the connections between cells in form of thinner filaments was noticed.

Scanning electron microscopy of original natural sample has advantage over microscopy of cultivated material because it excludes the complex factor of change of environment. This is the way how the forms which usually cannot be obtained in culture have been observed. Apart of that, applied method made the observations of interrelationship among microorganisms in situ possible.

Conclusion

The results of investigation of bacterioplankton quantity in Carska bara swamp water shaw significant fluctuations which are not seasonal always. Relatively low coefficient of correlation between the count of bacteria and observed physico-chemical parameters in water were found.

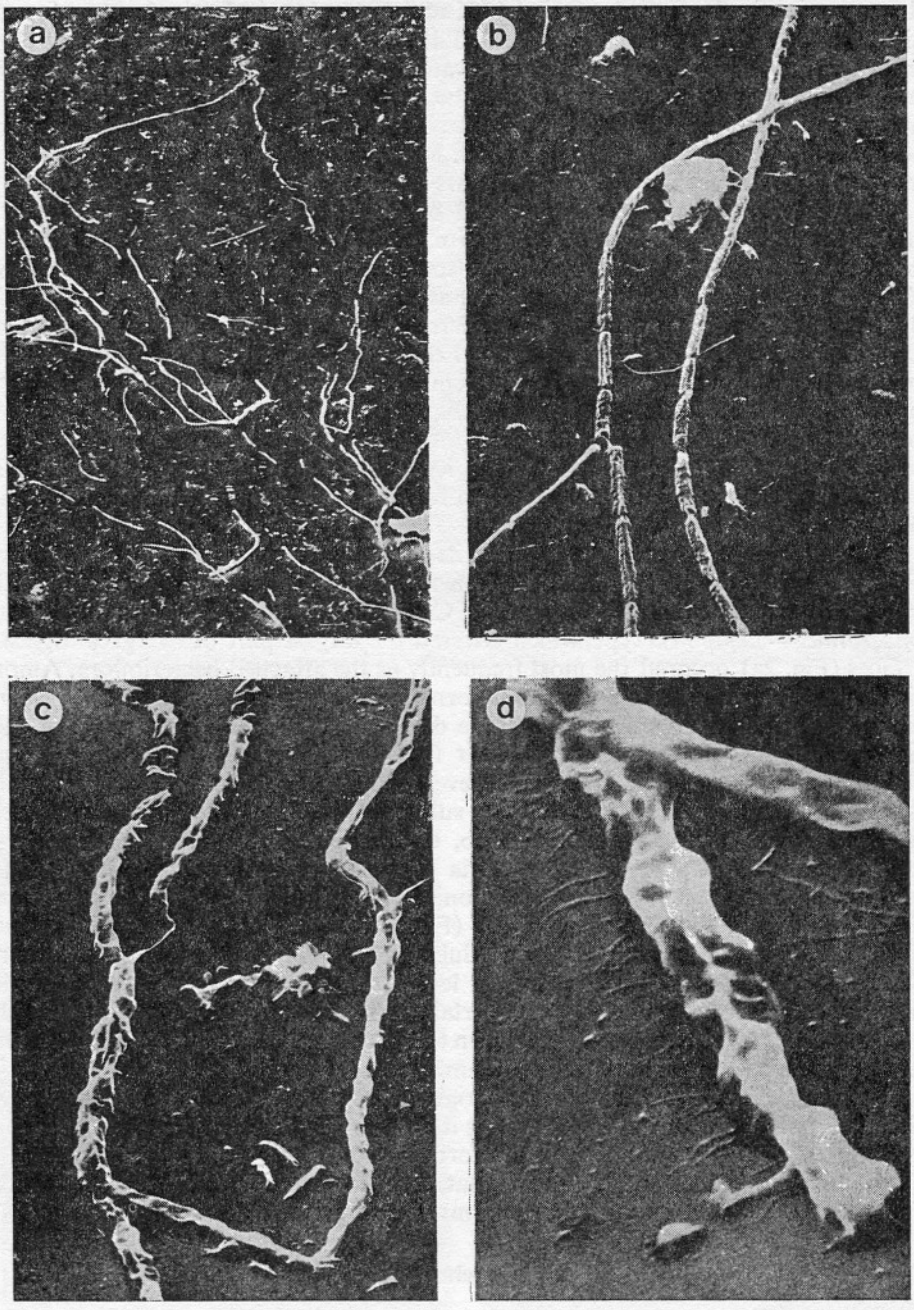


Fig. 2 Attached filamentous microorganisms. Abundance od Sphaerotilus-Lepthotrix-like microorganisms (a), filaments formed of rod-shaped cells (b), filaments embeded in a thick mucilagenous sheath (c), tiny mucilagenous threads protruding from the sheath (d). Bar indicates 1 μm

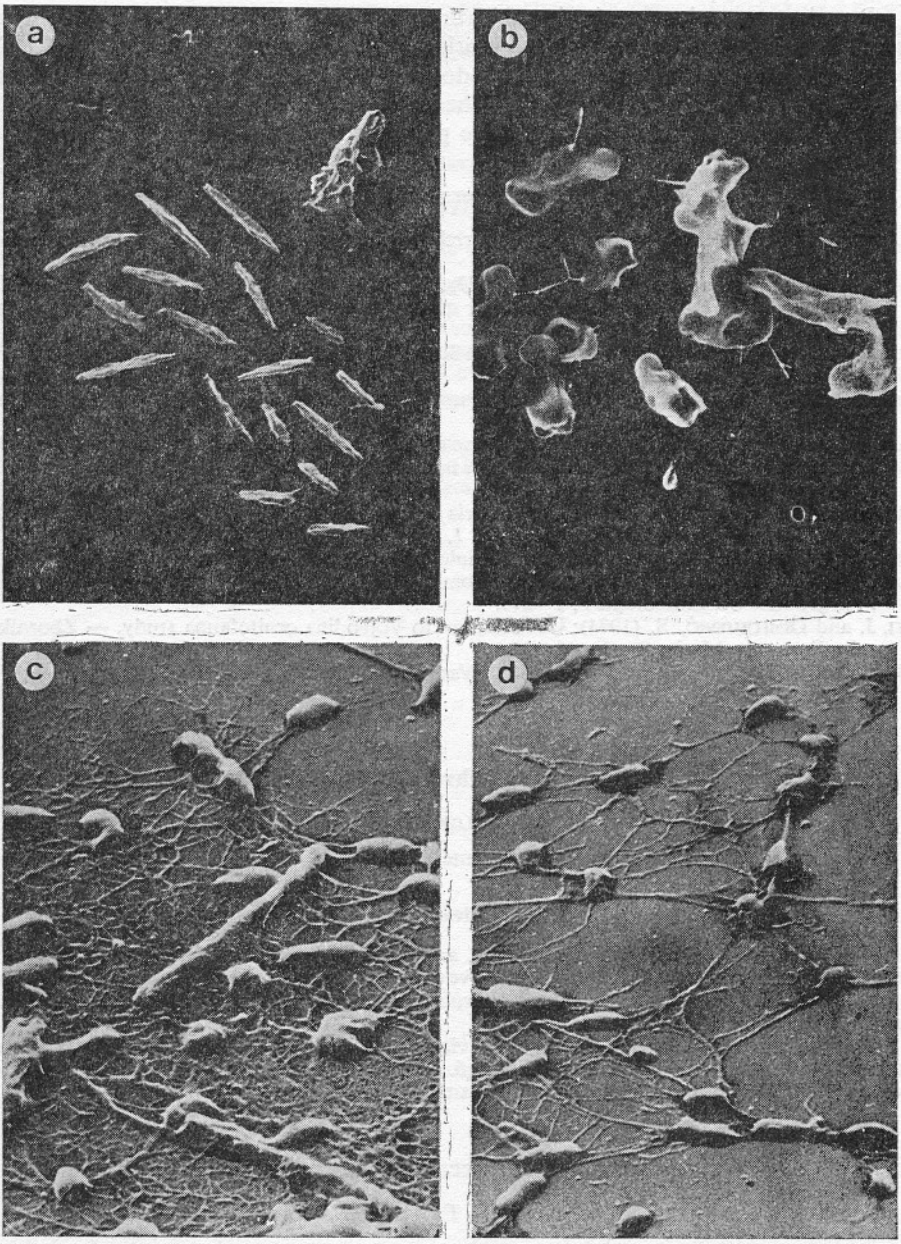


Fig. 3 Attached rod-shaped bacteria. Microcolony of cells with pointed (a) and with rounded (b) ends, and abundant (c) and less abundant (d) presence of mucilagenous threads interconnecting cells in a microcolony. Bar indicates mμ.

By electron microscopic investigation of bacterioplankton microorganisms resembling *Caulobacter,* rods with fimbriae, but also forms of unidentified origin were found. Among the attached forms dominated filamentous bacteria of *Sphaerotilus — Leptothrix* type, coated by slimy sheath and connected by slurry filaments and the rods with visible extracellular netlike polysaccharide matrix.

Literature

BRASFEILD, H. (1972): Environmental factors correlated with size of bacterial populations in a pollu ted stream. — Appl. Microbiol. *24*,349.

BRKOVIĆ — POPOVIĆ, I. and POPOVIČ, M. (1977): Dependance of the quantity of bacteria on the amount of organic matter in surface water quality examination. — Mikrobiologija (Belgrade) *14*, 117.

BUJNOVIĆ, D. (1973): Protected natural objects in Socialistic Autonomous Province of Vojvodina. — Priroda Vojvodine *1*, 23,

DJERFI, B. (1969): Structure of biocenosis of „Carska bara" swamp. — Proc. Symp. Ecol., Belgrade, 12—14 Febr. 1969, p. 41.

HAM, I. (1975): Qualitative composition of Heron colony *(Ardeidae)* and the influence of individual factors of environment on its formation in the region of the Lower Begej in Vojvodina. — Larus *26—28*, 143

RAZUMOV, A. S. (1932): Direct method for bacteria counting in water. A comparison with the method after Koch., — Mikrobiologiya (Moscow), *1*, 131.

RODINA, A. G. (1965): Methods of water microbiology, — Moscow — Leningrad, Nauka.

SLADEČEK, V. (1973): System of water quality from the biological point of view. — Arch. Hydrobiol. *7*, Ergebnisse der Limnologie 7, 1—218.

ŠOTI, J. and DIMITRIJEVIĆ, S. (1974): Contribution to Vojvodina ornitofauna study. — Zbornik za prirodne nauke Matice Srpske *46*, 125.

TÜMPLING, W. (1969): Zur Klassifizierung der Wasserbeschaffenheit aus biologischer Sicht. — Wiss. Z. Univ. Rostock *18*, 793—798.

A Carska bara mikrobiológiai vizsgálata

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Kivonat

A Carska bara mikrobiológiai vizsgálata az 1981—1984-es időszakban a bacterioplankton összmeghatározására - közvetlen módszerrel, illetve tenyészetben a bactériumok számbeli meghatározása mellett az adott ökoszisztéma plankton mikróbái, valamint rögzített formái elektron-mikroszkópos vizsgálatával történt.

Megállapítást nyert, hogy a Carska bara vizében a bactérioplankton összetételének jelentős ingadozása nem szabályos évszakonkénti jellegű. A bactériumok számbeli változása viszonylag alacsony értékeket mutat a víz fizikai-kémiai paramétereihez viszonyítva.

Микробиологические исследования царской лужи

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Резюме

Микробиологические исследования воды Царской лужи произведены в 1981—1984 годах и они охватили совокупное поголовье бактерий прямым методов и поголовье бактерий методами выращивания-разведения, а в целях получения всеохватывающей катрины микробных популяций исследуемой экосистемы проведены также электрон-микроскопические исследования планктонных и прикрепченных форм бактерий.

Результаты исследования представительства (наличия, количества) бактериопланктонов в воде Царской лужи указывают на значительные колебания, не являющийся колебаниями правильного сезонного характера. Также найдены и сравнительно низкие коэфициенты корреляции поголовья бактерий и сопровождающих физико-химических параметров в воде.

Mikrobiološka ispitivanja vode Carske bare

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Abstrakt

Mikrobiološka ispitivanja vode Carske bare su vršena od sredine 1981 do sredine 1984 godine, obuhvatila su ukupnu brojnost bakterioplanktona — direktnom metodom i brojnost bakterija-odgajivačkim metodama, a u cilju dobijanja sveobuhvatne slike mikrobnih populacija ispitivanog ekosistema vršena su i elektron -mikroskopska ispitivanja planktonskih i pričvršćenih oblika bakterija.

Rezultati ispitivanja zastupljenosti bakterioplanktona u vodi Carske bare ukazuju na znatna kolebanja koja nisu pravilno sezonskog karaktera. Nadjeni su i relativno niski koeficijenti korelacije brojnosti bakterija i pra enih fizičko-hemijskih parametara u vodi.