MICROBIOLOGICAL INDICATORS OF THE WATER QUALITY OF BACKWATER TISZA WITH SPECIAL REFERENCE TO THE OLIGOTROFIC MICROFLORA

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Gajin, S., Gantar, M., Matavulj, M., Petrovic, O., Obreht, Z., Radnovic, D. and Budakov, Lj. (1992): Microbiological indicators of the water quality of Backwater Tisza with special reference to the oligotrofic microflora. - TISCIA 26,43-48.

Abstract. Samples for microbiological water analysis of the Mrtva Tisza (the backwater of the river Tisza) were taken every two months during the period from November 1987 to September 1990.
During these investigations we estimated the total number of bacterioplankton, number of heterotrophic and facultatively oligotrophic bacteria, and enzymatic (phosphatase) activity of water. The classification of waters was carried out on the basis of number of heterotrophs, T/H index and enzyme activity of water. In addition, the morphology of oligotrophic bacteria were investigated by electron microscopy.
The obtained results revealed that water quality of the Backwater Tisza ranges from moderately polluted to fully polluted waters (II-III class). The dominant part of bacterioflora was the population of oligotrophic bacteria. Electron microscopy showed that the morphology of bacteria was effected by the concentration of nutrients in the media.

Keywords: facultative oligotrophs, microbial indicators, organic load, water.

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Introduction

Microbiological investigations of surface waters are mostly confined to sanitary aspect in the water state estimation, which are carried out by institutes of Public Health. Also, the level of the microbiological parameters are prescribed by law and regulations. These investigations are, certainly, of significance, but recent results indicate that the dominant microflora of surface water is represented by oligotrophic microorganisms. This group of microorganisms (which represents 50-90% of the total bacterioflora in surface waters) is not included by standard microbiological investigations, although, thanks to their multienzymatic nature, they participate significantly in self-purification processes of surface waters.

The study of ecological conditions in water habitats has led to the conclusion of irregularity in access to quantitative studies of water bacterioplankton by cultivation methods. Even nowadays, nutrient agar is used in routine way for cultivation of heterotrophic bacteria from water, although the results obtained by many authors: Ishida and Katoda (1977, 1979, 1981), Kuznecov et al. (1979), Ishida et al. (1980-a, 1980-b, 1982), Olsen and Bakken (1987), Stilinovic and Futac (1990), Gajin et al. (1990), and others indicate that at such investigations it is necessary to apply media poorer in nutrients than the nutrient agar.

For these facts, we decided to draw our particular attention to oligotrophs in these investigations of autochthonous microflora of surface waters. The results of classical microbiological investigations of water have been used in this paper for standard estimation of water condition.

Methods

Samples for microbiological water analysis of the Backwater Tisza were taken every two months in the period from November 1987 to September 1990, and were analyzed in the Microbiological Laboratory of the Institute of Biology in Novi Sad.

The total number of bacterioplankton was estimated applying the direct method of bacteriological filtration (Razumov, 1932) using bacteriological filters "Sartorius No 2". The number of aerobic heterotrophic bacteria was determined by cultivation on nutrient agar, and the number of oligotrophic bacteria by the same method on diluted nutrient agar (1:10, 1:100, 1:1000), as well as on medium F-5 (Ishida and Katoda, 1977).

Besides, enzyme (phosphatase) activity of water was determined on the basis of p-Nitrophenylphosphate hydrolysis (Matavulj et al., 1984).

Estimation of the condition of the investigated waters and categorization into classes were carried out according to the number of aerobic heterotrophic bacteria (Kohl, 1975), according to the level of water phosphate activity (Matavulj et al., 1990) and T/H index (ratio of the total number of bacteria and heterotrophs) values (Matavulj et al., 1989).

Morphologically different bacterial colonies grown on nutrient media were isolated and tested with respect to the requirements concerning the nutrient quantity, by transferring each isolate on the nutrient agar, also on the same but diluted medium (1:10, 1:100, 1:1000), and on the medium F-5. The capability and intensity of growth were recorded on these substrata, while the changes of morphology of bacterial strains in dependence of quantity of nutrients in media were followed by transmission electron microscopy.

Results and Discussion

The results of our three-year investigation of the Backwater Tisza water point to the fluctuation of the water quality during the year. The average values of the microbiological parameters indicate that in the most cases it was a question of the polluted waters. Most of the samples belonged to the II-III class, with the tendency of mild improvement of water quality during the three years of investigation (that should be confirmed by further investigations). We assume that this mild improvement of the water quality could be the result of measures undertaken for the protection of the waters investigated.

It can be seen from Fig. 1. that number of heterotrophic bacteria, taken as an indicator of water quality, fluctuates to a great extent depending on a season but remaining within the limits of II-III and II classes of water according to Kohl (1975). However, the percentage of heterotrophs in the total bacterioplankton, and the ratio of total bacterioplankton to heterotrophs (T/H) certainly gives more real picture. These parameters vary in the summer-autumn period, but on the other hand we obtained clearly expressed winter minimums in the percentage of heterotrophs in the total bacterioplankton, that is maximums in the T/H ratio in winter period.

Phosphatase activity of water, representing the enzyme activity of all microorganisms present in the water, point to summer or spring-summer maximums, that is to the waters which are the most loaded in spring-summer period during a year, but all values are within the limits of III-A category of water (Matavulj et al., 1988).

Values of determination of heterotrophic and oligotrophic bacteria number indicate that bacterioplankton of the Backwater Tisza water had far better conditions for growth on the substrata poorer in quantity of nutrients than on the standard nutrient agar. So, viable bacteria count on nutrient agar diluted ten times was up to 5.1 times greater than on the standard media (yearly averages: 3.2 in 1988, 2.5 in 1989, 3.4 in 1990); (Gajin et al., 1990). It should also be emphasized that from all media applied, in all cases of investigation, the highest number of colonies has been found on the nutrient agar diluted ten times.

The dominance of (facultative) oligotrophic bacteria over the heterotrophic ones, found also in the Backwater Tisza water, point to the incompleteness of such investigation of water quality when only classical methods of determination of number of (heterotrophic) bacteria, corresponding only to one level of trophy are applied, that is, when only standard nutrient agar is being used.

At the investigation of bacterioplankton of waters by cultivation method, the usual period of incubation is 5-7 days. By prolongation of this period, we noticed that at the incubation temperature of 26°C period of 5 days was enough for full growth of heterotrophs, but bacterial colonies on the media poorer in nutrients grew slower. On the basis of these observations we concluded that one of the reasons of exclusion of autochthonous oligotrophic microflora of waters at routine investigations is certainly the insufficient routine period of incubation as well.

From the total number of 412 tested bacteria, isolated from the Backwater Tisza water during three-year investigation, 80.1% of isolates were capable to grow on diluted nutrient agar and 74.5% on the standard nutrient agar (Fig. 2.).

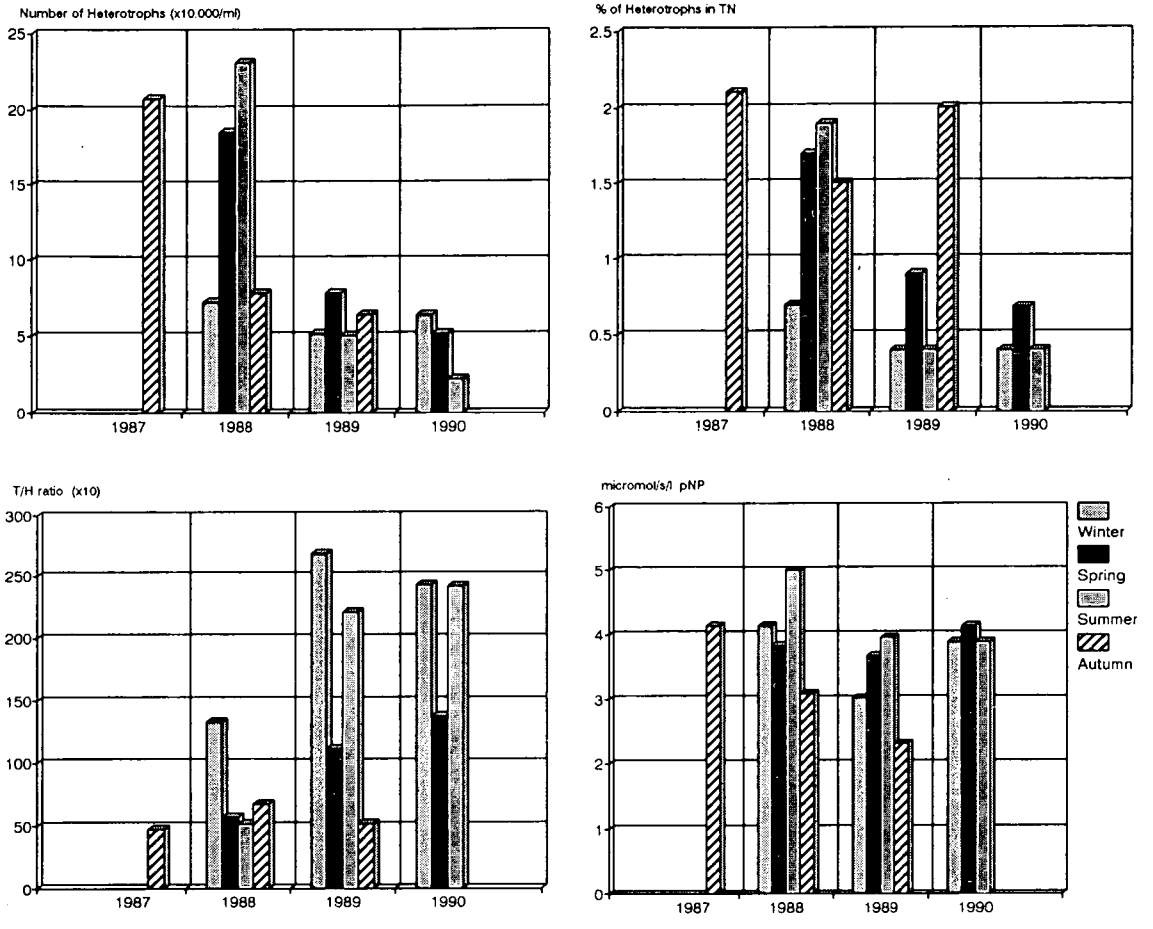

Fig. 1. The water quality of Backwater Tisza according to microbiological and enzymological indicators

These results also point to the abundance of autochthonous oligotrophic microflora in the investigated waters, as well as to a possible methodological error at routine investigation of microflora of surface waters carried out only on one level of trophy, and only on the standard nutrient agar respectively.

Examination of microflora of the investigated water by an electron microscopy revealed, besides the usual bacterioflora, the abundance of forms with noticeable numerous pili. However, by examination of isolates taken from the investigated water such forms could not be noticed. For this reason we assume that the forms observed in the intact sample were strict oligotrophs, or oligonitrophiles. Oligotrophic strains grown on nutrient agar were polymorphic, or with a great number of lysing cells, and typical morphology of cells was noticed only when grown on the medium with reduced quantity of nutrients (Fig. 3.). Morphological

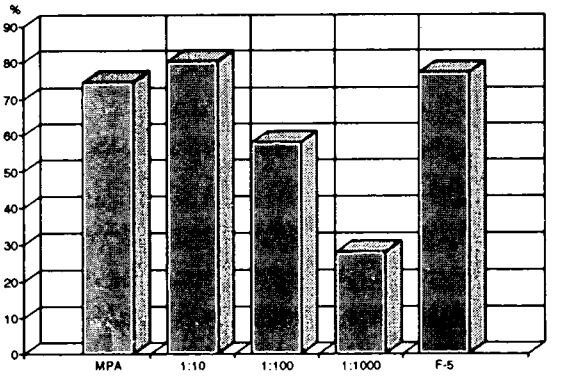

Fig. 2. Percentage of bacterial isolates growing on media with different nutrient concentrations

ambiguity was the most expressive in the isolates of stalked bacteria grown on the nutrient agar, whose typical morphology was noticed only when grown on the media with reduced quantity of nutrients (Fig. 4.).

Conclusion

The results obtained during the three years of investigation of microbiological indicators of water quality allow the categorization of the Backwater Tisza water into II-III and II classes (according to the number of heterotrophs). This is the water most loaded in spring-summer period during a year, but within the limits of III-A category (according to the enzyme activity of water).

The numbers of bacteria obtained by cultivation methods, as well as the capability of growth of bacteria isolated from the Backwater Tisza water on media with different concentrations of nutrients, point to the dominance of oligotrophic microflora in investigated waters. With this respect we point to the incomplete investigation when this important part of microflora is neglected and to the possible methodological error at routine investigation of microflora of surface waters carried out only on one level of trophy, that is on the standard nutrient agar only.

Morphological investigations of oligotrophic bacteria cultivated on standard nutrient agar also point to the fact that the media with reduced concentrations of nutrient are more suitable for investigation of microflora of surface waters .

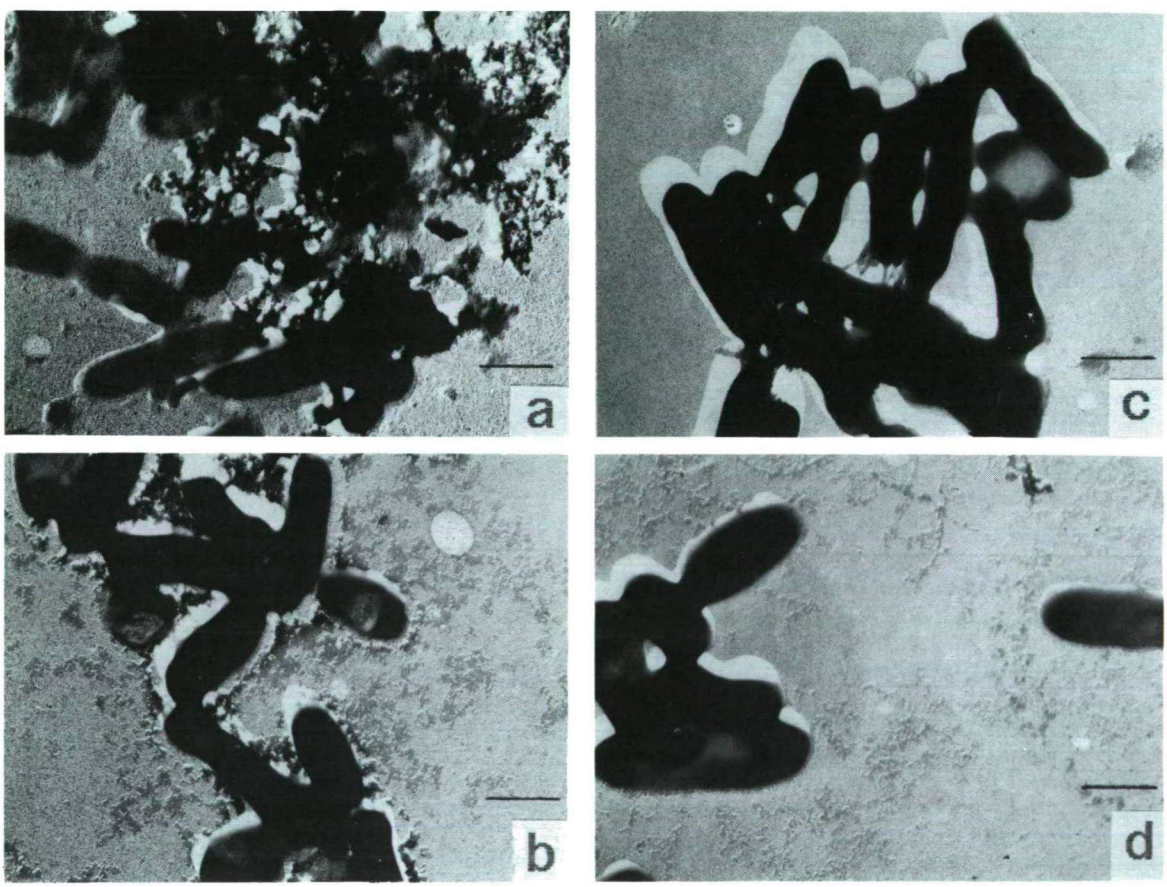

Fig. 3. Electron micrographs of bacterial cells showing morphological changes as affected by different nutrient concentrations. a - standard nutrient agar; b - nutrient agar diluted 1:10; c - nutrient agar diluted 1:100; d - nutrient agar diluted 1:1000.; (index = 1μm)

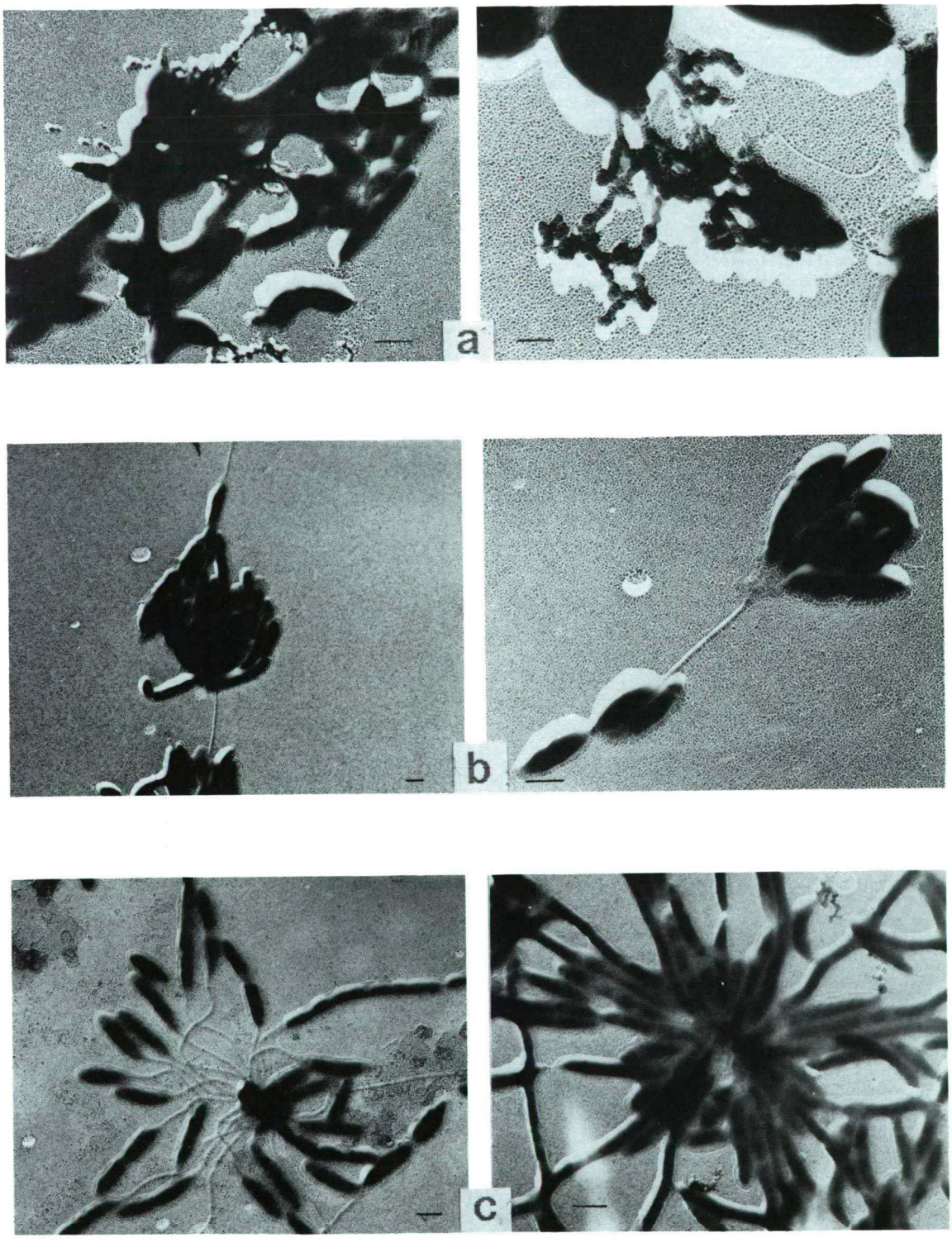

Fig. 4. Electron micrographs of stalked bacteria showing morphological changes as affected by different nutrient concentrations. a - standard nutrient agar; b - nutrient agar diluted 1:10; c - nutrient agar diluted 1:100; (index = 1μm)

References

Gajin, S., Gantar, M., Bokorov, M., Matavulj, M., Petrovic, O., Obreht, Z. and Radnovic, D. (1990): Oligotrofna microflora povrsinskih voda. Zbornik radova II Jugoslovenskog simpozija mikrobne ekologije, Zagreb, pp. 3-12.

Ishida, Y., Imai, I. and Katoda, H. (1980a): Growth and activity of an aquatic bacteria in low nutrient media. Abstract. 79th Ann. Meet. Amer. Soc. Microbiol., Los Angeles, California

Ishida, Y. and Katoda, H. (1977): Distribution of oligotrophic bacteria in Lake Mergozzo. Bull. Jpn. Soc. Sci. Fish., 43,1417-1424.

Ishida, Y. and Katoda, H. (1979): A new method for enumeration of oligotrophic bacteria in lake water. Arch. Hydrobiol. Beih. Ergbn. Limnol., 12,77-85.

Ishida, Y. and Katoda, H. (1981): Growth patterns and substrate requirements of the first isolated naturally occurring obligate oligotrophs. Microb. Ecol. 7,123-130.

Ishida, Y., Shibahara, K., Uchida, H. and Katoda, H. (1980b): Distribution of obligately oligotrophic bacteria in lake Biwa. Bull. Jpn. Soc. Sci. Fisc. 46,1151-1158.

Ishida, Y., Imati, I., Miyagaki, T. and Katoda, H. (1982): Growth and uptake kinetics of a facultatively oligotrophic bacterium at low nutrient concentrations. Microbial Ecology, 8,23-32.

Kohl, W. (1975): Über die Bedeutung bakteriologisher untersuchungen für die beurteilung von Fleissgewässern, Dargestellt an Beispiel der Osterreich. Donau. Arch. Hydrobiol. 44,392-461.

Kuznetsov, S.,I., Dubinina, G.,A. and Lapteva, N.A. (1979): Biology of oligotrophic bacteria. Ann. Rev. Microbiol. 33,377-387

Matavulj, M., Gajin, S., Gantar, M., Petrovic, O., Erbeznik, M., Bokorov, M. and Stojilkovic, S. (1984): Phosphatase activity as an additional parameter of water condition estimate in some lakes of Vojvodina province. Mikrobiologija (Beograd), 21 (1),53-61.

Matavulj, M., Gajin, S., Erbeznik, M., Bokorov, M. and Petrovic, O. (1989): Phosphatase activity of water as a parameter of the river Tisza water monitoring. Tiscia 23,29-36.

Matavulj, M., Bokorov, M., Gajin, S., Gantar, M., Stojilkovic, S. and Flint, K.P. (1990): Phosphatase activity of water as a monitoring parameter. Water Sci. and Technol. 22 (5),63-68.

Olsen, R.A. and Bakken, L.K. (1987): Viability of soil bacteria: optimization of plate-counting technique and comparison between total counts and plate counts within different size groups. Microbiol. Ecology 13,59-74.

Razumov, A. (1932): Prjamoj metod uceta bakterij v vode. Sravnenie ego s metodom Kocha. Mikrobiologija, 1,131.

Stilinovic, B. and Futac, N. (1990): Zastupljenost oligotrofnih i politrofnih bakterija u sedimentu akvatorija Plitvickih jezera. Zbornik radova II Jugoslovenskog simpozija mikrobne ekologije. pp. 13-19.