

BACTERIOLOGICAL INVESTIGATIONS OF THE WATERS IN YUGOSLAVIA

BAKTERIOLOŠKA ISPITIVANJA VODA U JUGOSLAVIJI

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Due to its geographic and hydrographic situation, Yugoslavia has waters for the development of extremely varied flora of microorganisms. The waters of Yugoslavia include: the Adriatic Sea, many natural and artificial lakes, rivers, canals, hot springs, mineral springs and fish ponds.

Before Yugoslavia developed its own staff of bacteriologists, the bacterial flora was, to a very limited extent, studied by foreign bacteriologists. The first studies were done of the flora of the *Adriatic Sea*. At the end of the 19th and beginning of the 20th century, several types of heterotrophic bacteria were described (Hansgirg, 1890, 1892) as well as luminescent, purple and colorless sulphur bacteria in the water and silt of the Bay of Trieste (Molisch, 1904, 1907, 1912). The study of Marine bacteria in Yugoslavia began to develop after the Second World War when the Laboratory of Marine Bacteriology was established in the Institute of Oceanography and Fisheries in Split.

The ecological problems of the bacterial populations of the Adriatic were studied with the aim of finding the significance of bacteria in organic production, especially in supplying the ecosystem with usable assimilatives. For this purpose the daily, seasonal, horizontal, and vertical distribution of the aerobic and anaerobic bacteria were analysed in the waters of the central and southern Adriatic, especially those active in the nitrogen and sulphur cycles (Cvijić, 1955, 1962, 1963). Other studies are concerned with the relationship of bacterioplankton to phyto and zooplankton from the point of view of organic production in the central Adriatic (Cvijić, 1953; Ristić and Pucher-Petković, 1969).

Research on the flora confirmed some already known types, as well as discovering some new types, of heterotrophic bacteria in the area of

the Bay of Kaštela (Vlajnić, 1955) and of sulphur bacteria from the coastal region near Split and Dubrovnik (Klas, 1936, 1936a, 1938). Sulphur bacteria were studied not only from the point of view of flora but also from the cytological stand-point (Devidić, 1954).

Experiments testing the influence of certain antibiotics and various pH environments on pure and mixed cultures of bacteria from the sea were done in the Laboratory of Marine Bacteriology (The Institute of Oceanography and Fisheries in Split), (Cvijić, 1953a, 1956a).

A new Bacteriological Laboratory has been organized in the Institute for Marine Biology in Kotor in order to investigate the marine bacteria and their activity in organic production of the southern Adriatic.

Salty lake water of some of the Adriatic islands was subjected to bacteriological researching. Of special interest was the explanation for the phenomenon of pink or reddish waters in the deeper layers of the Malo (Little) and Veliko (Big) Lakes on the island of Mljet as deriving from a special type of chromogenic bacteria (Cvijić, 1955a, 1960a).

The bacterial flora of *semi-salty* (brackish) water and silt of the brackish Adriatic area (Lakes: Modrić, Kutli, Desne, and the Bačine lakes) were studied in the Microbiological Laboratory of the Biological Institute of the University in Sarajevo. The flora in various degrees of saltiness was examined with respect to halotolerance and fermentative activity of aerobic and facultative anaerobic proteolytic bacteria and cellulose decomposing bacteria (Ristanović, 1963, 1969b, 1969c). The quantitative relationship of physiological groups of microorganisms in the carbon, nitrogen, and phosphorus cycles was established and the necessity of a more complete explanation of the organic production of these waters was indicated (Ristanović, 1970).

In the oligotrophic *fresh-water lakes* near the Adriatic (Deran, Jelim) the participation of bacteria in the transformation processes of sediment materials on the bottom was studied (Ristanović, 1967).

In the far south of Yugoslavia near the Greek and Albanian borders, The Ohrid, Prespa, and Dojran lakes have been under study by the Hydrobiological Institute in Ohrid for the past two decades. The vertical and horizontal distribution of the bacterial population of heterotrophs have been studied both qualitatively and quantitatively (Ocevski, 1953, 1958, 1958a, 1960, 1966). The aerobes and anaerobes of various physiological groups of microorganisms have been studied in the contact zone of water and various levels of the silt (Ocevski, 1969). Studies were also done of the periphytic bacteria of Prespa Lake as well as of the rhizosphera microflora in the littoral zone of Lake Ohrid (Ocevski, 1959, 1963).

The bacterial flora of the Plitvice Lakes, in the western part of Yugoslavia, have been studied mostly with regard to the sanitary aspect (Emili, 1958; Pavletić and Stilinović, 1965).

Among the earliest studies of microflora of the rivers of Yugoslavia is the study by Protić (1933). Researching the Miljacka river in Sarajevo the author showed the distribution of filamentous forms of bacteria in relation to the population of river. Cvijić (1955) analysed the heterotrophic bacteria in the Krka and Zrmanja rivers which flow into the Adriatic, to study the significance of river flora in the changes of composition of the bacterial population in the sea. A similar study of the water and silt of the Neretva and its tributary, the Krupa, which also flow into the Adriatic was done to gain insight into the participation of microorganisms in the transformation processes of nitrogen, carbon and phosphorus compounds (Ristanović, 1967, 1970).

Bacterial heterotrophic types from the Velgoška, Čerave and Studenčiška rivers which flow into Lake Ohrid were studied by Očevski (1958) to establish the horizontal distribution of these populations in relation to the autochthonous bacteria of the lake itself.

The sudden development of urban settlements and industrial centers near river streams presents important and immediate problems requiring study by bacteriologists. There are two aspects to research in this area: 1) sanitation and hygiene concerning the evaluation of the quality of water on the basis of certain bacterial types (with legally prescribed regulations) and 2) the significance of microorganisms in the process of natural self-purification and in organic production. Research with concern for the sanitary aspect has been done on the rivers: Danube (Bulajić, 1960), Dobra (Emili, 1956; Hryby, 1956), Kupa (Emili, 1965), Sava (Matoničkin et al., 1969), and others. Study of bacterial activity in the transformation process of certain toxic materials in polluted rivers and changes which take place in certain physiological groups of microorganisms was done in the Veseočica and Vrbanja rivers polluted by waste waters of the leather industry (Ristanović, 1969, 1969a, 1970a). Recently studies have been made of the participation of bacteria in the processes of degradation of complex toxic materials in equipment for purification of industrial waste and sewage waters from settlements.

The artificial watercourses — i. e. canals — have also been studied from the bacteriological stand-point. In the new biocenosis of the Danube — Tisa — Danube Hydrosystem the dependence relationship was established between the bacterio — and zooplankton (Ristić and Pujin, 1969). In the far southern Adriatic area, in the canal and bay of Milena near Ulcinj, a study was made of the flora of sulphur and iron bacteria of sulphur mud used for therapeutic purposes (Ristanović, 1966).

The southern Adriatic area has many sea bottom sulphur springs. Devidé (1957) found a number of a new types of sulphur bacteria in this area.

Yugoslavia also has many *thermal and mineral springs*, but they have not been thoroughly studied from the bacteriological stand-point, although study of the algae has been given special attention. Vouk (1919) even set up a classification of thermal waters on this basis. Some types of thermophilic bacteria were described in the thermal springs of Vranjska Banja (Georgevitch, 1910, 1910a) and of Ilidža near Sarajevo (Karliniski, 1895). Recent research on the qualitative composition of heterotrophic and sulphur bacteria at Ilidža showed that this flora consists of ecotypes of mesophiles (Tešić et al., 1963; Todorović and Ristanović, 1963).

The bacterial populations in *fish-ponds* have been studied both qualitatively and quantitatively. A study of the composition of flora of the bacterial population in the Crna Mlaka fish-pond (Ehrlich, 1953), showed only a small number of nitrogen fixators, nitrifying bacteria, denitrifying and cellulolytic bacteria, which made for conditions of poor productivity in the fish-pond. With the introduction of fertilization, however, an increase was found in the number of species and of cells both in the water and silt, e. g. in the »Jegrička« hatchery (Plančić and Ristić, 1961). In the »Čurug« experimental fish-pond tests were made of the stimulative effects of organic and mineral fertilizers upon the tempo of development of bacterial populations, especially upon the biomass and the size of the cells (Ristić, 1959, 1965, 1966, 1966a). The participation of sulphur bacteria on the size of the biomass of bacterioplankton was shown. The same author found one new species of the *Thiothrix* sp. (Ristić, 1966b). The significance of bacteria as the first link in the trophic food chain was analysed. Special attention was given to the correlational connection between bacteria and zooplankton (Ristić and Pujin, 1964).

The Yugoslav hydrobacteriologists apply standard microbiological *methods* in their work, but give special attention to the discovery and perfecting of new and better methods. Among the first significant contributions in this area was made by Cvijić (1955). He modified the J-Z ZoBell sampler for taking test waters from various depths by replacing the glass bottle with a large tube for capturing the water and by placing a rubber ball on the metal frame.

For taking samples of the silt and contact zone of water Očevski (1954) constructed a simple apparatus which consists of a part for taking the sample and another part which carries weights and a mechanism for closing the glass cylinder. Očevski (1966a) also developed a microcapillary glass cell as a replacement for the peloscopic capillary of Perfiljev and Gabe. Očevski's cell makes it possible to examine the undisturbed structure of the microbe population in the waters.

A constant problem for the bacteriologist-ecologist has been how to make the artificial alimentary media as similar as possible to the quality of the conditions in the natural environment. Ristić (1958, 1964)

contributed to the solution with the introduction of agarized extract of silt for laboratory usage. Ristanović (1965) proposed the use of 0,8% agar-agar in the composition of the media in order to bring to a minimum the heating effect of the melted agar at the time of inoculation.

In addition to various foreign codes, Yugoslav hydrobacteriologists make use of a simplified *key*, for the determination of bacteria to the genera, which was worked out by Tešić (1962). The same author, with a group of his colleagues, put together a *manual* (Coll. authors, 1966) which aids bacteriologists to apply the methods on the basis of the most reliable and recognized approaches in the world.

The *future program* of bacteriological studies of water in Yugoslavia emphasizes the need for a more extensive study of bacterial flora in a larger number of water environments and for the present concepts to be made more thorough and more specialized. One of the ways in which this can be achieved is by means of further and constant collaboration among Yugoslav and foreign hydrobacteriologists. Thus we can together make hydrobacteriology a still more relevant and comprehensive biological discipline.

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BAKTERIOLOŠKA ISPITIVANJA VODA U JUGOSLAVIJI

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Pre nego što se razvio kadar jugoslovenskih hidrobakteriologa bakterijsku floru su, istina, u malim okvirima proučavali inostrani bakteriolozi. Tek poslednjih dvadesetak godina hidrobakteriologija se kod nas razvija kao veoma važna biološka disciplina. Osnivaju se naučno-istraživački centri u: Splitu, Ohridu, Rijeci, Beogradu, Zagrebu, Novom Sadu, Sarajevu i Kotoru. U tom periodu dati su prilozi o učešću bakterija u organskoj produkciji, posebno u transformacionim procesima organskih materija i odnosima kako među članovima bakterijskih populacija tako i prema ostalim biotičkim faktorima. Saznanje o florističkom sastavu bakterijskih populacija dopunjeno je priložima o novoopisanim vrstama bakterija, naročito iz Jadranskog mora. Postoje brojni radovi i iz sanitarne bakteriologije.

U radu su dati podaci o bakteriološkim ispitivanjima Jadranskog mora (Cviić 1953, 1953a, 1955, 1956, 1956a, 1960, 1962, 1963; Devidić 1954; Klas 1936, 1936a, 1938; Ristić et Puchar-Petković 1969; Vlajnić 1955), zatim jezera sa slanom vodom na ostrvu Mljetu (Cviić 1955, 1960a), te brakičnih voda u jezerima Neretvine delte: Modrić, Kutli, Desne i Bačinska jezera (Ristanović 1963, 1969c, 1969d, 1970). Daljim osvrtom prikazani su radovi o bakterijskim populacijama jezera sa slatkom vodom u blizini Jadranskog mora — Deran i Jelim (Ristanović 1967), zatim u Makedoniji: Ohridsko, Prespansko i Dojransko (Oceviski 1953, 1958, 1958a, 1959, 1960, 1963, 1966, 1969), te Plitvičkih jezera (Emili 1958; Pavletić et Stilinović 1965). Bakterijska flora naših reka ispitivana je širom zemlje: Miljacka (Protić 1933), Krka, Zrmanja (Cviić 1955), Velgoška reka, Čerave, Studenčište (Oceviski 1958), Dunav (Bulajić 1960), Dobra (Emili 1956, 1965; Hrybu 1956), Kupa (Emili 1965), Sava (Matoničkin et al. 1969), Neretva, Krupa, Desanka, Veseočica, Vrbanja (Ristanović 1969, 1969a, 1969b, 1970a). I bakterijske zajednice u veštačkim vodotocima proučavane su u kanalu Milena kod Ulcinja (Ristanović 1966), kao i u kanalu Dunav-

-Tisa-Dunav (Ristić et Pujin 1969). Bakteriološki aspekt sagledavan je i u sastavu biocenoza ribnjaka: Crna Mlaka (Ehrlich 1953), Jegrička, Živača i Čurug (Plančić et Ristić 1961; Ristić 1959, 1965, 1966, 1966a, 1966b; Ristić et Pujin 1964). Sumporna vrela i termalne vode ispitivane su u ograničenim razmerama: sumporna vrela na području Južnog Jadrana (Devidé 1957), zatim u Vranjskoj Banji (Georgevitch 1910, 1910a) i u Ilidži (Tešić et al. 1963; Todorović et Ristanović 1963).

Jugoslovenski hidrobakteriolozi dali su svoj doprinos u iznalaženju i usavršavanju već postojećih i pogodnijih metoda kako u eksperimentalnom radu (Cvijić 1955; Očevski 1954, 1966a; Ristić 1958, 1964; Ristanović 1965), tako i u izradi uprošćenog ključa za determinaciju bakterija do rodova (Tešić 1962) i, najzad zajedničkog priručnika za bakteriološka ispitivanja prirodnih sredina (Coll. authors 1966).

