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INTRODUCTION

On the initiative of the Committee of Hydrobiology, Polish Academy of Sciences, a Scientific Session was held in Wrocław on 7—9 June 1972, concerning the microbiology of surface waters.

During the debates 29 lectures and reports were read touching the most essential questions concerning the methodology of research, the qualitative and quantitative relations of bacteria and phytoplankton, the influence of noxious and toxic substances on biocenoses which populate surface waters, and the possibilities of biodegradation of some compounds reaching surface waters with sewage.

An extensive discussion emphasized the urgent need of continuing and enlarging microbiological research mainly in consideration of the role it should play in problem connected with the preservation of waters.

Most lectures delivered during the Session have been printed in the present number of the *Polish Archives of Hydrobiology*.

Prof. dr. Hab. M. Pawlaczyk-Szpilowa

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METHODS IN AQUATIC MICROBIOLOGY. A STORY OF APPARENT PRECISION AND FRUSTRATED EXPECTATIONS

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ABSTRACT

Techniques of study of aquatic bacterial microflora are evaluated and their suitability for ecological research is discussed. A new method of a semi-continuous culture on membrane-filters, combined with radioisotope labelling, is described and discussed.

„Trace science then, with modesty thy guide"
Alexander Pope *An Essay on Man*

1. INTRODUCTION

This review will deal only with some methodological problems of microbial ecology. Its scope will be limited to a critical commentary on experimental approaches and methods of qualitative and quantitative study of bacterial populations, their growth, function and role in water bodies. The epiphytic microflora and that of bottom sediments will be mentioned only in connection with the chief topic of the discussion. These problems, as well as particulars of many earlier experimental techniques, have been described in recent reviews and monographs (Rodina 1965, Van Niel 1965, Wood 1967, Brock 1971) and will not be considered here.

Many research approaches to the study of water bacteria are possible. Some among them, e.g. the plate and dilution techniques are important not because of their doubtful cognitive values but because of their undubitable traditional popularity among many bacteriologists. Others, as for instance submerged-glass technique or immersed-bottle culture procedure, furnish different observations and data scarcely comparable even with each other, and difficult for interpretation.

The more recently proposed methods making use of radioisotope labelling and various application of continuous culture techniques tend to be overestimated by some and ignored by many microbiologists.

The role of aquatic microbiology for the protection and preservation of the natural human environment is steadily increasing. As a consequence, many new research and control laboratories are being established all over the world, attracting to aquatic microbiology a vast number of people with different scientific background and experience. This obviously helps in developing interdisciplinary contacts. On the other hand, it often leads to the application of traditionally used methods without sufficient insight into their limitations. Accordingly, it seems a proper time for a critical discussion of the problem.

2. PLATE AND DILUTION TECHNIQUES

The classical dilution-plate count has been applied for examination of water microflora since Koch's time. Although for a long time it has been shown that rich in nutrients agar- and gelatine-broth media, as well as high temperature of incubation, are unfavourable for water microbiota (Waksman et al. 1933 a, b, Waksman, Renn 1936, Zo Bell, Conn 1940, Zo Bell 1946, Vinogradski 1949, Kuznetsov 1959, Kuznetsov, Romanenko 1963, Wood 1967, and others), the use of such conditions is prevalent even now in numerous laboratories concerned with sanitary and pollution problems.

The different modifications of nutrient media (e.g. Ferrer et al. 1963, Strzelczyk et al. 1967) more suitable for water bacteria, and the lowering of the incubation temperature to 20—28°C, advised by many investigators, enable cultivation of more forms. In spite of this, the fraction of bacteria capable of growth on these media never exceeds 10% and often is as low as 0.01% of the direct count on filter membranes (Kuznetsov, Karzinkin 1931, Waksman 1934, Jannasch 1953, 1958, 1963, 1967 a, Kuznetsov 1959, Prokešová 1963, Bohdanowicz-Strucińska 1965, Wood 1967). That this discrepancy is due not to low viability of water bacteria, but to the intrinsic limitations of the plate method, has been shown by Kunicka-Goldfinger (1973). Using the technique of semi-continuous culture on membrane filters she observed that almost 100% of bacteria found on the filters are able to divide and form microcolonies.

The plate count remains a useful although of limited applicability method in sanitary control of water quality. For other purposes it should be applied only with highest care and all its inherent limitations and defects (Zo Bell 1946, Van Niel 1965, Wood 1967, Brock 1971) should be taken into account during interpretation of its results.

The cultivation of bacteria on membrane filters spread on the selective media is widely employed in sanitary microbiology. Its usefulness for ecological research is, however, very small due to the limitations common with the plate methods. The modifications of this technique are based on replacement of routine agar-media with filtered natural water solidified with agar (Melchiorri-Santolini, Cafarelli 1967) or with a pile of paper-discs soaked with diluted nutrient medium (Prokešová 1963) or natural water (Mucha, Daubner 1971). It is thus an endeavour to imitate to some extent natural nutritional conditions. It is undoubtedly a progress in relation to the plate method, not only in this respect but also owing to the possibility of examination of much greater volumes of water, what may be really important, especially when oligotrophic waters are concerned. Nevertheless, as has been shown by Bohdanowicz-Strucińska (1965), the counts

obtained by similar modifications are very low, only by a trifle higher than in the plate method. This seems to be due to the limited supply of nutrients and accumulation of growth-inhibiting metabolites in the surface layer of the medium.

The dilution technique, advised by Rasumov (1927, acc. to Kuznetsov 1959) and adopted by many bacteriologists has comparatively many shortcomings. Its use for obtaining a measure of the quantity of bacteria has no foundation. Laurent (1964) has shown, for instance, that the "total count" may be smaller than the count of a specific physiological group, e.g. ammonificators. What we really get with this technique is a rough approximation of the "potential" of a given physiological process (e.g. ammonification, denitrification, nitrification etc.) in the examined biocenosis. It gives us some information about the past of the examined biotope, which has influenced the present appearance of a given physiological form. It may, as well, give some hints about the future possibilities of the realization of found "potentials". But it does not measure the present qualitative and quantitative status of the water microflora. The technique may be thus a useful tool in the hands of an experienced research-worker, but may, as well, lead to bias and unfounded conclusions.

3. THE DIRECT MICROSCOPIC METHODS

The methods are based on counting of stained or unstained bacterial cells. Initially (Cholodny 1929, 1930, Henrici 1933) examined water was spread on the surface of a glass slide. Such procedure, useful for soil examination, was less suitable for counting of aquatic microbes because generally only few microbial cells could be found in a small volume of water smeared over the glass. Certain improvement has been achieved with condensing of the examined water by partial evaporation (Kuznetsov, Karzinkin 1931, Collins, Kipling 1957). A real advancement was the application by Cholodny (1929) and Rasumov (1933, acc. to Kuznetsov 1959) of cellulose membrane filters for this purpose. Depending on the expected density of bacterial population, samples of a chosen volume could be filtered through the membrane, and microbes settled on its surface could be stained and observed directly.

There are, nevertheless, certain deficiencies of this procedure. Firstly, there are difficulties in differentiation between living and dead cells. There is general agreement that a fraction of unviable cells among water bacteria is relatively small (less than 10%). Kunicka-Goldfinger (1973) found less than 1.0% of such cells. The fraction of unviable cells may be yet different in various experiments, and in

specific conditions (massive toxic pollution, sudden increase of water temperature) it may change abruptly. In those cases the differentiation between live and dead cells may be significant for ensuing interpretation of the results. Numerous procedures have been proposed to attain this end. Differential staining with acridine dyes or mixture of dyes gives hardly replicable and dubious results. Labelling of bacteria with radioisotope of phosphorus (MacLeod et al. 1966) requires incubation of the sample with ^{32}P substrate before filtration, thus introduces an additional unknown factor of selection.

The second source of a possible bias of the direct count is the difficulty of differentiation of bacterial cells, nannoplanktonic algae and particles of detritus (Ward, Whipple 1959, Van Niel 1965). This is one of the reasons for poor replicability of the results, stressed already by Zo Bell (1946). Dahlman-Łebkowska (1956) and Gołębiowska (1965) in our laboratory found that the counts made by two persons on the same samples were significantly different in about 30% of cases. The repeated counts made on the same filter differed often more than by 100%.

The further shortcoming of technique is an uncertainty as to the quantity and role of "dwarf" bacterial forms described by Jannasch (1958) and Nikitin, Kuznetsov (1967).

The last and until now unsurmountable difficulty is connected with the impossibility to tell anything about actual physiological activity of the observed cells. The resting cells and fully active forms are stained in the same manner.

In spite of these limitations, direct microscopic count remains the powerful research tool, on condition that the said limitations are well remembered.

Some improvements upon the technique, based on combining semi-continuous culture on membrane-filters and labelling with ^{3}H-thymidine will be described in the last chapter.

4. ASSESSMENT OF THE GROWTH RATE BY "IMMERSED BOTTLE TECHNIQUE"

In this technique, a sample of the examined water is incubated in a glass bottle, immersed in the water body at the site of sample collection. Subsamples of the water are filtered through the membrane filters before filling the bottle and after incubation of the sample in the immersed bottle. The number of bacteria is assayed by direct microscopic count. The difference between the counts before and after incubation time, gives the increase of the cell numbers and on this basis the growth rate may be calculated.

The technique has been introduced by Rasumov (1948) and Iva-

nov (1955) (according to Kuznetsov 1959) and is widely used by Russian workers (e.g. Kuznetsov 1959, Kriss 1959, Kuznetsov, Romanenko 1963, Rodina 1965). Due to the simplicity of the technique and its apparent precision it is attractive for many ecologists having no bacteriological experience. Yet this technique is based on a false assumption of simulation of the natural habitat. In reality, it gives no information even about aggregate growth rate of tested bacterial population. The water sample in the closed bottle is not a part of the examined water body. Numerous processes are going on, such as adhesion of bacteria to the walls, adsorption of nutrients and metabolites on the glass, changes in oxygen content, rH and pH of the water.

Adhesion of bacteria to the glass and the wall-growth are well documented (Zo Bell, Anderson 1936, Zo Bell 1937, 1943, 1946, Jannasch, Johnes 1959, Van Niel, Stanier 1959, Larsen, Dimmick 1964, Wuhrman 1964, Rodina 1965, Szymanowski 1965, Anderson, Meadows 1965 a, b, Stotzky, Rem 1966 a, b, c, Wood 1967, Zvyagintsev 1967, Zvyagintsev, Velikanov 1968). Further complication is caused by differences among bacteria in their capability for adhesion and wall-growth (e.g. Fig. 1). Adhesion may be also reversible and irreversible

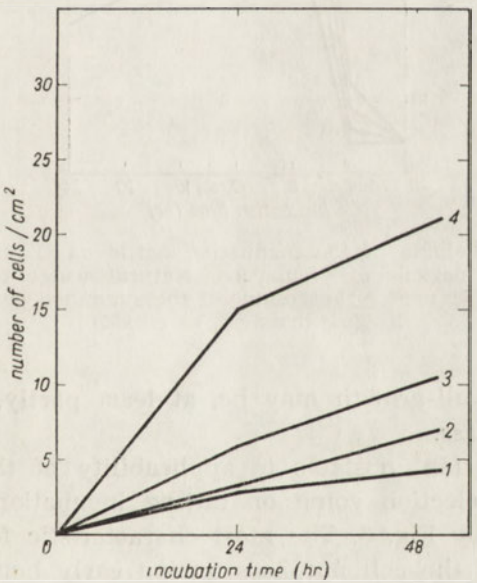

Fig. 1. Adhesion to glass surface and wall-growth of various bacteria. Mixture of equal numbers of cells of *Pseudomonas fluorescens* (1), *Escherichia coli* (2), *Bacillus subtilis* (3) and *Sarcina lutea* (4) was incubated in artificial fresh water in bottles (20 ml in volume). After the incubation, the cultures were poured out, the bottles gently washed with sterile water. Then, bacteria settled on the glass walls were transferred from the glass-surface into the water by use of a special blending device as described by Szymanowski (1965). The bacteria were then counted by the plate method (according to Szymanowski 1965)

as has been noted by Marschall et al. (1971 a, b). Owing to those facts and to changeable adsorption of various nutrients (Hotchkiss, Waksman 1936, Conn, Conn 1940, Heukelekian, Heller 1940, Zo Bell, Grant 1943, Renn 1964, Floodgate 1965) the role of all these factors is unknown and unpredictable. The result is the well known influence of the volume of the test-bottle on the increase of the cell number (Zo Bell 1943, 1946, Szymanowski 1965, see Fig. 2). Even this relation is, however, changeable and depends on the characteristic of water and microflora.

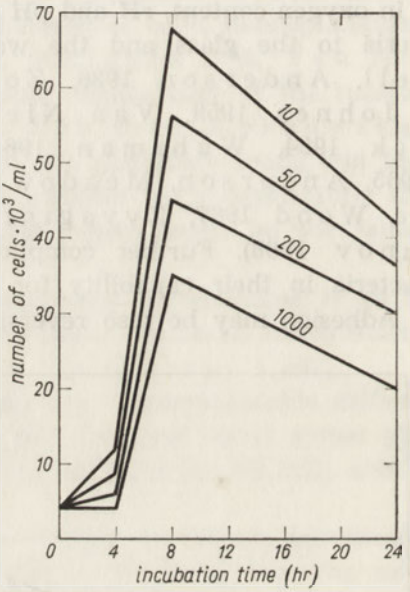

Fig. 2. Effect of the volume of the incubation bottle on the number of bacteria found after different periods of incubation. Natural water from an unpolluted river. 10, 50, 200 and 1000 denote the volume of the examined bottles (ml) (according to Szymanowski 1965)

Adhesion and wall-growth may be, at least partly, avoided by the use of siliconized glass.

The more important obstacle to applicability of the technique are the processes of selection going on during incubation of the sample. This is portrayed by Fig. 3. The most characteristic for the culture is high fluctuation of the cell numbers during early hours of incubation, probably caused by reversible and irreversible adhesion of bacteria to glass-wall. After 32 hours of incubation the culture is overgrown by the dominant form. No such selection and dominance could be observed during this time in the natural habitat.

The growth curve of the bottle population has nothing in common with processes typical for the natural environment. It is a result of

the interplay of various unknown and unmeasurable factors like adhesion, de-adhesion, adsorption and selection, taking place in the closed, artificial environment of the bottle volume.

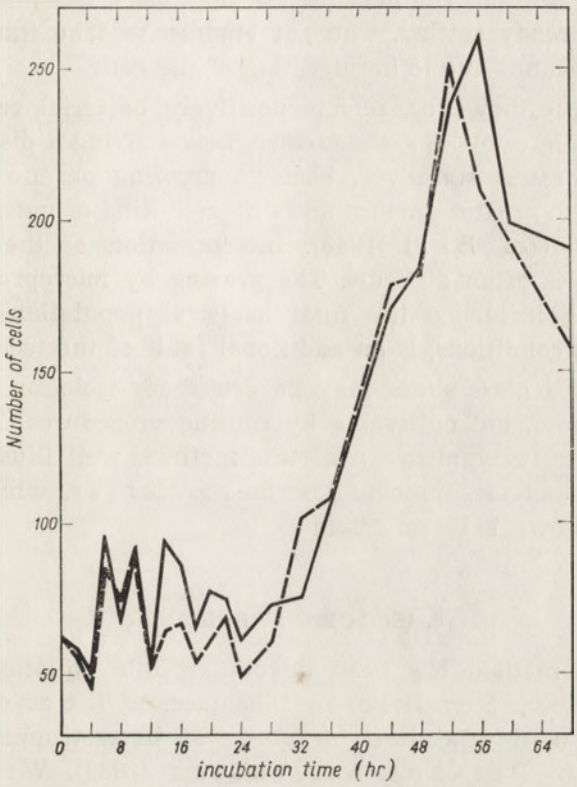

Fig. 3. Growth of the natural bacterial population in the bottle. The examined water from the Lake Mikołajskie had been previously filtered through a filter paper for elimination of a majority of algae and Protozoa. Bacterial cell numbers were assayed by the direct count on membrane filters (Coli 5). The two curves illustrate the growth in the two parallel bottles. The volume of the examined here bottles — 50 ml. The fluctuation of the cell numbers was similar independently of the volume of the bottle (according to Bohdanowicz-Strucińska 1965)

5. SUBMERGED GLASS SLIDES

The technique described for soil examination by Cholodny (1930) has been adopted to aquatic microbiology by Henrici (1933). It takes advantage of a tendency of bacteria to adhere to a glass surface and of the ability of the glass surface to adsorb and concentrate nutrients from the water. In its application for growth rate measurement it involves repeated microscopic counts of bacterial cells and their groupings on the glass slide immersed in the examined water (Zo Bell 1943, Kriss, Rukina 1952, Ierusalimskij 1954, Paluch 1958, Kriss

1959, Wood 1967). The source of errors due to the immigration of the cells settling on the glass during the incubation period may be overcome by irradiation of the glass slides with ultraviolet after various periods of incubation (Bott, Brock 1970). The UV-irradiation does not hinder further immigration on the glass of the new cells, but inhibits the division of cells already settled. Thus, it enables to take into account the increase of cell count due to immigration of the cells.

The technique, however, selects positively bacterial cells with high capability to adhere to the glass surface, hence giving a distorted picture of natural processes. Moreover, bacteria growing on the glass surface are supplied with greater amount and different kind of nutrients adsorbed to the surface (Zo Bell 1943). Interpretation of the results, that being the case, is often difficult. The grazing by micropredators, which may differently influence the final bacterial population on the glass slide in various conditions, is an additional fault of the technique.

The immersed glass slides may be useful for isolation of indigenous aquatic microbiota, not cultivable by routine procedures. There are yet pitfalls to successful application of this method, well illustrated by the story of the genus Krassilnikovia described by Kriss, which subsequentely has been shown to be an artefact.

6. ISOTOPIC LABELLING

The isotope method has been introduced into aquatic microbiology by Kuznetsov, Sorokin in 1955 (acc. to Kuznetsov 1959). The presently used procedure for study of heterotrophic growth has been described by Parsons, Strickland (1962), Wright, Hobbie (1965, 1966), Hamilton et al. (1966), Hamilton, Austin (1967), Allen (1971).

The sample of the examined water is incubated in the presence of ^{14}C labelled substrate (most often glucose or acetate) in the glass bottle, as described in Chapter 4. Since the incubation period is, in this case, short, the errors due to wall-growth, nutrient adsorption or selection are greatly diminished. At the end of the incubation period, the sample is filtered through the membrane filter and after additional treatment, the unsoluble products of assimilation of ^{14}C are assayed. The method gives fairly good approximation of the so-called "heterotrophic potential" of the examined water. There is also possibility to differentiate, at least partially, between bacterial and algal assimilation of ^{14}C labelled substrate. The chief limitation, apart from the effects of the sorption of nutrients and accumulation of intermediary metabolites (Wood 1967), is due to the possibility of a selective assimilation of various carbon sources present in the natural water and added ^{14}C labelled compound.

This technique, combined eventually with direct microscopic examination, seems to be the most promising among the discussed here procedures.

7. CONTINUOUS CULTURES

The chemostat type of continuous cultures, as described by Monod (1950), has been modified and adopted for study of aquatic bacteria by Jannasch (1963, 1965 a, b, 1967 a), Postgate (1973) and Veldekamp, Kuenen (1973). There are yet drawbacks in the application of the technique for study of microbial ecology. The hard to overcome wall-growth is one among them. The other, is a difficulty in obtaining balanced growth at very low dilution rate (i.e. low growth rate) in presence of trace amounts of nutrients. The most important obstacle is the fact that the continuous culture does not simulate natural conditions. The process going on in the natural environment is not a steady-state process. Hence, the steady-state established in the chemostat is a powerful selective force, the nature of the selection depending on the culture conditions and the initial type of microflora in the examined water.

The ingenious modifications as e.g. constructing a series of chemostats (Jannasch 1967 b) or measuring wash-out rate instead of growth rate (Jannasch 1969) do not evade these obstacles.

The technique is, however, useful in the study of bacterial physiology, of selective agents which may operate in natural environment, and for isolation of indigenous aquatic bacteria.

The highly unsatisfactory and critical state of experimental techniques in water bacteriology has been recently thoroughly discussed by Van Niel (1965), Wood (1967), Brock (1971). Apart from the isotope technique or direct microscopic observation, and to the lesser extent the method of submerged glass-slides and of continuous cultures — the other procedures now being commonly applied in research and control laboratories are of very small value. Their use by unexperienced workers and uncritical interpretation or generalizations of their results is a real hindrance in the development of aquatic microbial ecology.

8. NEW APPROACHES

The elegant and ingenious technique of Perfil'ev, Gabe (1961) is very helpful in the study of soil and bottom sediment microflora. Its future application to water microbiology may be very promising. The full and detailed description of the technique may be found in the cited book by Perfil'ev, Gabe (1961).

THE SEMI-CONTINUOUS CULTURE ON MEMBRANE FILTERS

The technique originally proposed by Kunicki-Goldfinger et al. (1967), has been used for the assay of the assimilable nutrients in natural water (Kunicka-Goldfinger, Kunicki-Goldfinger 1972) and for enumeration of the total number of viable bacteria in water (Kunicka-Goldfinger 1973).

In this technique the sample of the examined water is filtered through the membrane filter. The filter is then placed in the culture-device (Fig. 4). The natural water from which the sample for filtration was drawn, is used as a nutrient medium after preliminary sterilization by filtration.

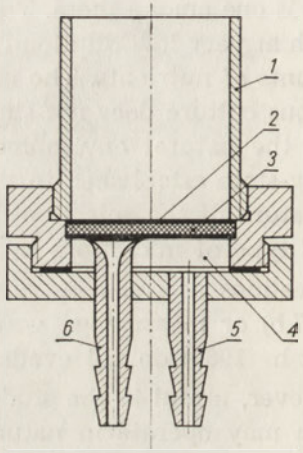

Fig. 4. The apparatus for the semi-continuous culture on membrane filters. Chamber (1) shelters the membrane filter (2) from contamination and dessication. A sintered-glass disc (3) supports the membrane filter. Flow chamber (4) with inlet (5) and outlet (6). The inlet is connected with a plastic tubing and a peristaltic pump (according to Kunicka-Goldfinger, Kunicki-Goldfinger 1972)

The medium is continuously flowing under the filter, thus steadily supplying new nutrients and eliminating intermediary metabolites. Bacteria are growing on the surface of the filter. At appriopriate time intervals the number of single cells, cell-groupings (microcolonies) and number of cells in microcolonies is assayed by microscopic count. On basis of these data growth rate and fraction of dividing bacteria may be calculated.

The natural water may be enriched with additional nutrients enabling in this manner the study of effects of such enrichment on water microflora.

The technique made possible measuring of exceedingly low growth rates of water bacteria at trace concentrations of nutrients (Fig. 5). It allowed also to assess the viability and physiological activity of the total microflora in natural water and after its enrichment with an additional carbon source (Table I).

The technique proved to be useful for isolation and enumeration of nitrifying bacteria (Łukaszkiewicz 1968).

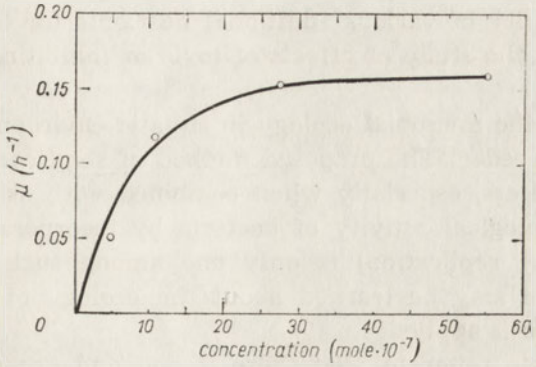

Fig. 5. Influence of the concentration of the limiting substrate (glucose) upon the growth rate of *Pseudomonas sp.* Measured by the technique of the semi-continuous culture on membrane filters (according to Kunicka-Goldfinger 1970)

Table I. Comparison of the direct count of membrane filters, plate count and viable count in the semi-continuous culture on membrane filters (according to Kunicka-Goldfinger 1973)

Method of counting	Number of cells and microcolonies per microscopic field and number of colonies per plate	Number of bacteria per 1 ml
Direct count on the membrane filter	0.227	1544,000
Plate count	165	165,000
Viable count: semicontinuous culture on membrane filter:		
medium — the examined water	0.282	1907,000
medium — the water enriched with phenol (1 mg per 1 ml)	0.364	2475,000

By application of a closed circuit for medium flow and an addition of H^3-thymidine to it, it might be possible to test the viability and physiological activity of the natural water microflora. The modification enables the measuring of not only the aggregate growth rate of total

microflora, but also, with the help of the autoradiographic technique, the quantitative differentiation among bacterial forms characterized by different viability and activity. Further, it makes possible the assessment of the assimilability of various additional nutrients for bacteria. It may be also useful in the study of effects of toxic or inhibiting substances on water bacteria.

For study of the microbial ecology in aquatic environment many new approaches are needed. The proposed method of semi-continuous culture on membrane filters, especially when combined with assessing the viability and physiological activity of bacteria by incorporation of H^3-thymidine (i.e. DNA replication) is only one among such approaches. It seems, that more may be learned about the ecology of water bacteria when this method is applied.

I would like to reiterate, that there is not, and probably never will be, a simple method for the study of microbial ecology. Only the combining of many techniques and critical evaluation of obtained results may help us to learn more in this important field of biology.

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METHODS OF MICROBIOLOGICAL INVESTIGATIONS OF WATER IN THE LIGHT OF THE REQUIREMENTS OF HYDROBIOLOGY

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ABSTRACT

The present paper is a discussion of the methods used in microbiological investigations of water ecosystems and their usefulness in investigations concerning the productiveness of inland waters. It has been emphasized that such methods should be used as would permit experimenting in conditions as much approximating natural conditions as possible, with a simultaneous observation of the transformation processes of organic matter due to microbes. The method of determining the total number of bacteria, of their generation time and of the heterotrophic activity of multi-species bacteria populations occurring in natural waters have been discussed in detail. Those parameters are no less characteristic of the trophic types of water ecosystems than the amount of the primary production of organic matter. The necessity of adapting new methods from related sciences such as biochemistry and biophysics was insisted upon.

Water ecosystems form a specific environment as regards their biological, physical and chemical features and they are characterized by a great variety and changeability. Consequently the methods used in their investigation should be so selected and worked out as to make it possible to study the dynamics of processes occurring in whole ecosystems as well as to determine certain laws and generalizations which control those processes.

In the recent years the main interest of biologists as well as of other specialists has been concentrating on problems connected with the productivity of water ecosystems, and on entrophication processes of inland waters. In numerous cases the methods used up to the present have proved insufficient and new ones are being worked out or the existing ones adapted, but there arise great difficulties.

The methods now used in hydromicrobiology are various, frequently quite groundless and including errors, the extent of which is often unknown. Methods used in diagnostics and epidemiology have been in-

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troduced in hydromicrobiology without any adaptation. That is why the interpretation of the results obtained is frequently wrong and they do not characterize the investigated environment.

One of the parameters determining the microbiology of water bodies is the total number of bacteria in the water and in the bottom deposits.

The commonly used method of inoculation on Petri's dishes containing meat-infusion agar with peptone as well as the methods of cultivating bacteria on selective media are thoroughly foundless in hydromicrobiology though the number of colonies growing on the dishes is assumed to be the total number of bacteria or the total number of heterotrophic bacteria. Both assumptions arouse reservations. On Petri's dishes, in due time, there grow small amounts of bacteria and it is unknown what percentage of all the bacteria in the investigated environment they make; besides, different results are frequently obtained depending on the way of inoculation, on the time and temperature of the exposure.

When the cultivated bacteria are isolated from natural water, particular care should be given to the chemical composition of the medium. The generally used meat-peptone media contain, on the average, 0.5—5.0 g/l of organic matter while in the most eutrophized water the organic matter content does not exceed 30—40 mg/l. Therefore bacteria growing on dishes are not characteristic of the environment since the bacterioplankton prevailing in water is adapted to the micro-amounts of nutritive substances. Of course, there occur, in water ecosystems, tribes adapted to rich trophic conditions, e.g. in bottom deposits, in detritus, but neither those tribes nor those microbiotopes prevail.

The results obtained on Petri's dishes do not concern the total number of heterotrophic bacteria either, as the medium used is not specific of water biotopes. Besides, the culture usually contains but a low percentage of heterotrophic bacteria.

The titration method must be criticized for the same reasons since its use is foundless in hydromicrobiology, though undoubtedly very useful in medical microbiology and diagnostics.

In cultures of bacteria isolated from water, if the use of the titration method is well-founded and necessary in the given experimental conditions, the range of incubation temperature should be appropriately chosen. Usually this temperature should be the same as in the natural environment 19—20°C as it prevails in summer in our climate, if the investigations are carried out in summer, and 6—10°C if processes occurring in the cold season are under examination. Higher temperatures may be used only during investigations of bacterial microflora isolated from warmed water or if it is necessary to eliminate autochtonous bacterial microflora from natural water, e.g. sea water, in order to state its pollution with fecal microflora, e.g. with *Escherichia coli;* in such

case, as those are diagnostic investigations, the temperature of 40 or 44°C is justified since it is to eliminate all bacteria except fecal bacteria which are of interest as a pollution index.

The proposed temperature of 19—20°C is not an optimum temperature for microbiological processes occurring in natural water biotopes, this optimum is slightly higher and ranges from 20 to 25°C (Godlewska-Lipowa 1972), nevertheless it is a temperature at which, in natural conditions, all the processes of organic metabolism in ecosystems occur, may be at a slower rate, but characteristic of our climatic conditions.

It seems that the most objective method, and the one used more and more commonly in determining the total number of bacteria in water, is that of direct counting on membrane filters under the microscope (Jannasch 1958, Kuznetsov, Romanenko 1963).

When the Gram method dyeing is used on membrane filters, Gram positive and Gram negative bacteria can be distinguished: Victoria B blue is used to detect living and dead bacteria. In the method of direct counting attention should be paid to the size of pores in the membrane filters used. In bacteria populations living in natural water coming from lakes of different trophicity, the sizes of bacteria cells differ greatly though usually a certain range of cell sizes prevails. As our investigations on the variously eutrophized Mazurian lakes have proved, coccoidal forms of bacteria prevail in oligotrophic and weakly eutrophized lakes. They constitute averagely 80—90% of the total number of bacteria and their sizes are 0.1—0.4 μ^3. As eutrophication and, at the same time, the degree of pollution grows, the percentage of coccoidal forms decreases, as those forms are eliminated by the increasingly numerous bacilli-forms and rod-forms where the cells are much larger. In the Szczytno lakes, Długie and Miejskie (Godlewska-Lipowa et al. 1973), which are strongly polluted with municipal and industrial sewage, over 90% of the whole bacteria population consist of bacilli and rod-forms with an average size of 1.0—3.0 μ^3; the percentage of coccoidal forms is less than 10%.

Thus, in the investigations of "clean" waters, not polluted with sewage, the use of small-pore filters 0.1—0.3 μ in diameter is proper, while in highly polluted waters it seems advisable to use filters of the coli-5 type, produced in the Warsaw Factory of Serums and Vaccines, with pores of 0.5—0.7 μ in diameter.

As investigations have proved, the morphological forms of bacteria prevailing in populations, in natural waters, are highly characteristic of the kind of pollution and the type of a given water medium.

The total number of bacteria and the types of biocenoses in bottom deposits can be estimated by means of the luminiscence method, in the presence of fluorochroms such as rhodamin, primulin, koriphosphin,

acridin orange (Rodina 1965, 1971). Those dyes react with nucleic acids of the DNA and RNA type; each of these acids, in ultraviolet light, produces a different type of luminescence, a different colour of luminescence and on this basis it is possible to distinguish living and dead forms of bacteria cells in the specimen. Owing to the luminescence method it is also possible to study spiral of bacteria which are invisible under the microscope in usual light (Babiuk, Paul 1970).

An index of bacteria reproduction is their generation time, the time during which the numerical force of the population doubles, i.e. the time between two cell fissions. At the present stage of studies on the productivity of water ecosystems the knowledge of the generation time of bacteria is one of the parameters indispensable for the estimation of the productivity of the bacterial biomass.

The method of investigating multi-species groups of bacteria, occurring in natural water bodies is far from being perfect and needs much further detailed work but it permits an approximate evaluation of the reproduction rate of bacteria. The estimation of the numerical increase of bacteria cells in an environment devoid of consumers is the basis for such evaluation. The increase is determined according to the final and initial number of bacteria, after some time of incubation while the population is reproducing. This method (Ivanov 1955), though not devoid of imperfections, permits experimenting in conditions maximally approximating natural conditions. Among all the known and used methods this one seems to be the best adapted to investigation on the reproduction rate of bacteria in water ecosystems (Vollenveider 1969, Sorokin, Kadota 1972).

Analyses of the number of bacteria in different exposure times (1—24 hours) have shown that changes of number in multi-species groups of bacteria occur in the same way as in mond's monocultures (quoted after Clifton 1957). The analysis of the curve of growth of a multi-species population has proved that, in all the instances examined and in water taken from lakes of different trophicity, numerical changes occur only during the first hours of exposure (Godlewska-Lipowa 1969, 1970). After 10—12 hours of exposure a gradual decrease of the population occurs, which is expressed in the drop of the number of bacteria cells (Fig. 1, 2). After a 24 hours exposure the death rate often reaches 80% of the increase obtained during that period (Fig. 3). The death rate of bacteria populations is different in lakes of different trophicity.

While measuring the generation time of bacterioplankton it seems right to consider the number of bacteria cells in the logarithmic phase of the population's increase, as it is in that phase that the death rate may be assumed to be equalling zero. In the stationary phase there exists an equilibrium between reproduction and dying, while after an

exposure of 24 hours dying prevails over reproduction. The generation time calculated after a 24 hours exposure is the resultant of the intensity of reproduction and of the autolysis of the bacterioplankton (Fig. 4). Investigations have shown that it is necessary to shorten the exposure periods to 4—8 hours.

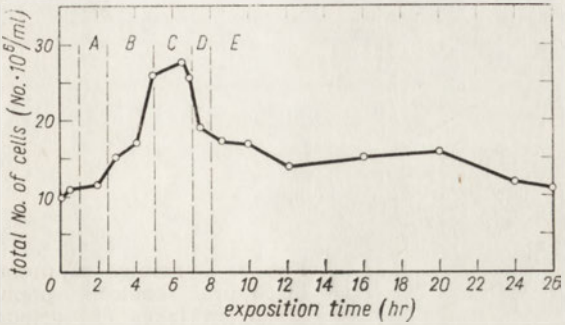

Fig. 1. Interdependence of number of bacteria cells in multi-species population and exposure time in successive phases of growth of bacteria population. A — initial stationary phase, B — logarithmic growth phase, C — stationary growth phase, D — logarithmic death phase, E — slowed-down death phase

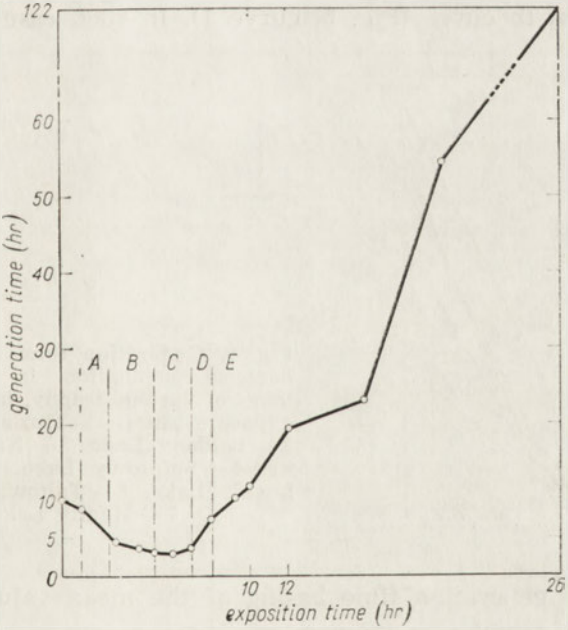

Fig. 2. Interdependence of generation time in multi-species bacteria population and exposure time in successive phases of growth of bacteria population. A — initial stationary phase, B — logarithmic growth phase, C — stationary growth phase, D — logarithmic death phase, E — slowed-down death phase

The reproduction of multi-species bacterial groups in lakes polluted with sewage proceeds in a slightly different way (Godlewska-Lipowa et al. 1973). In the Miejskie Lake, in Szczytno, after two hours of incubation of isolated water samples, the first population appears in the logarithmic phase, then it undergoes lysis and after 5 hours there

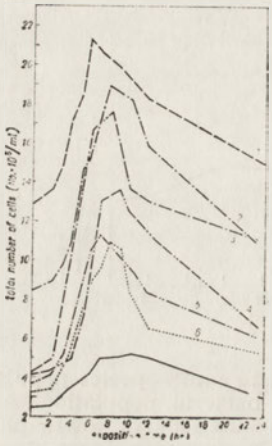

Fig. 3. Dynamics of numerousness of natural bacteria population in Mazurian lakes of various trophy, in different exposure times. 1 — Guzianka Lake, 2 — Bełdany Lake, 3 — Nidzkie Lake, 4 — Śniardwy Lake, 5 — Mikołajskie Lake, 6 — Tałtowisko Lake, 7 — Mamry Lake.

appears the second population (Fig. 5, curve 2). The population of the Długie Lake, Szczytno, which is also polluted with sewage, has a similar shape of its growth curve (Fig. 5, curve 1). In such case it is possible

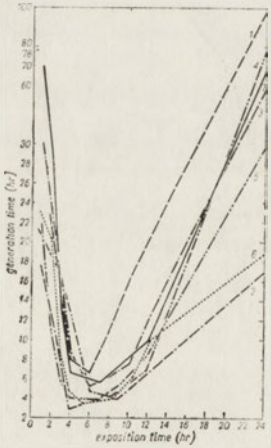

Fig. 4. Generation time of natural bacteria population in Mazurian lakes of various trophy, in different exposure times. 1 — Guzianka Lake, 2 — Bełdany Lake, 3 — Nidzkie Lake, 4 — Śniardwy Lake, 5 — Mikołajskie Lake, 6 — Tałtowisko Lake, 7 — Mamry Lake

to calculate the generation time basing of the mean values of the generation times of population one and two.

The prevailing bacterial group in water ecosystems are heterotrophic bacteria which use organic matter as a source of energy. The essential feature of ecosystems is not their numeric force but their activity.

The first attempts to determine the heterotrophic activity of bacterial microflora (Parsons, Strickland 1962, Wright, Hobbie 1965, Vaccaro, Jannash 1966, Allen 1967, Wetzel 1967) carried out with the use of media marked with ^{14}C have shown that populations of bacteria assimilate organic media with various intensity. This also concerns organic matter coming from algae previously marked with ^{14}C (Sorokin et al. 1971).

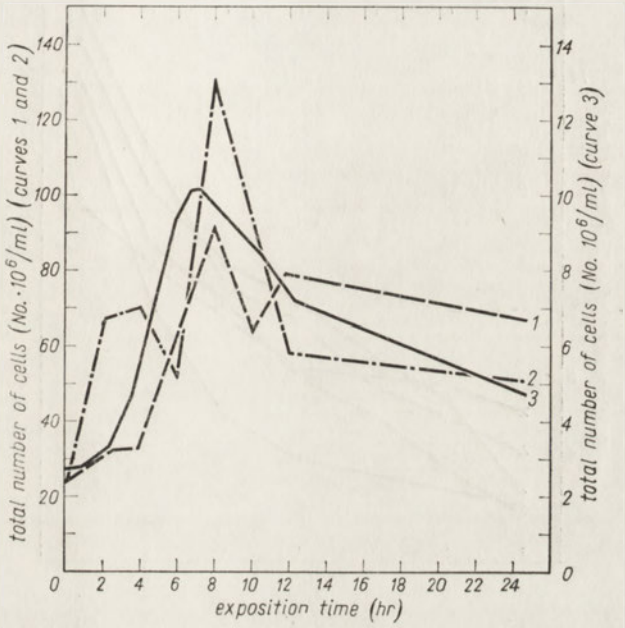

Fig. 5. Curves of growth of natural bacterial groups in polluted lakes, Szczytno Town, (1) Długie Lake and (2) Miejskie Lake and in the eutrophic (3) Mikołajskie Lake

Investigations on the heterotrophic activity of bacterial microflora in the natural water of the Mazurian lakes with their different trophic types, were carried out by means of a method based on the determination of the oxygen quantity used by bacteria (Godlewska-Lipowa 1973). Lake water, cleared of zoo- and phytoplankton was incubated at a temperature of 19—20°C, in darkness, with or without (in control samples) organic matter, glucose and bactopeptone, 10 mg per litre. After an exposure of 24 hours the particular samples were liquidated and their oxygen content was determined by Winkler's method. From the quantity of oxygen used in the water samples with and without added organic matter, the coefficient of heterotrophic activity was calculated and expressed by the ratio A/B, where A was the oxygen consumption (mg O_2 per litre per 24 hours) in the water sample containing

glucose or bactopeptone and B was the oxygen consumption (mg O_2 per litre per 24 hours) in the sample without any substratum.

The Mazurian lakes chosen for investigations form a kind of trophic gradient where the total number of bacteria and the amount of bacterial biomass increases with the increase of trophicity (Fig. 6). In the least

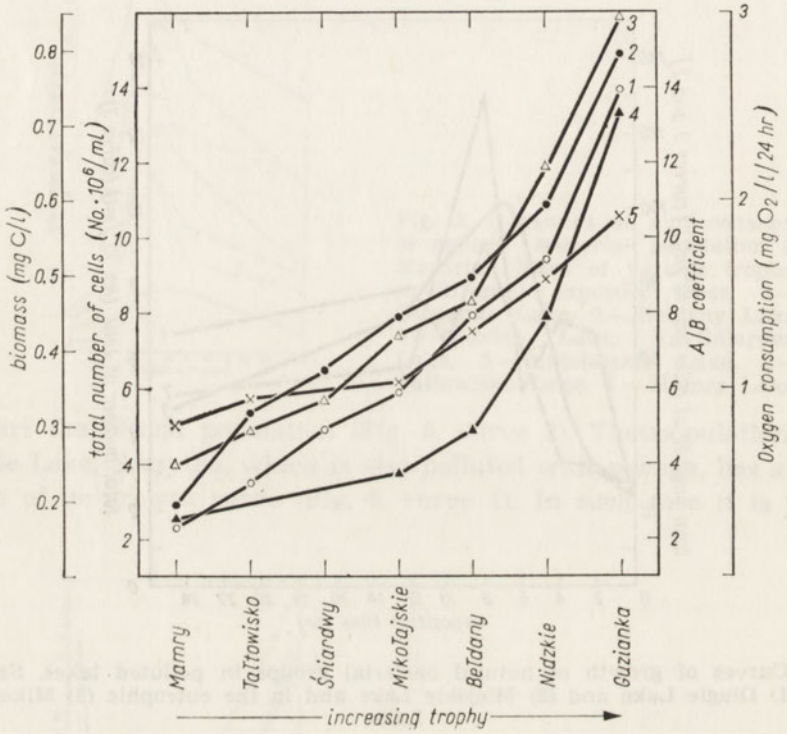

Fig. 6. Total number of bacteria (1), biomass (2), oxygen consumption by bacteria (3) and coefficients of heterotrophic activity A/B in relation to glucose (4) and bactopeptone (5) in Mazurian lakes of different trophy

eutrophized lakes where the number of bacteria is the lowest (the Mamry Lake) the heterotrophic activity, determined in relation to glucose and bactopeptone, was the smallest. In lakes which are more eutrophized and polluted with industrial sewage (the Nidzkie and Guzianka lakes) the heterotrophic activity proved to be the highest (Fig. 6).

The method of determining the heterotrophic activity of bacterial microflora occurring in natural lake water may be treated as a diagnostic method. It allows to determine not only the degree of eutrophy but also that of pollution of water ecosystems. Besides, this method makes it possible to study the processes of destruction of organic matter, occurring in whole ecosystems.

The methods discussed above do not comprise all the methods applied in hydromicrobiology. Only those have been considered which allow to carry out investigations in conditions possibly most resembling natural conditions. Among the methods used many bring information concerning only some fragment of the processes which occur in water bodies, but they do not give an analysis of the dynamics of those processes.

Hydromicrobiology is practically a new branch in natural science and its methods should be appropriate to the new requirements of modern science. Hydromicrobiology as well as hydrobiology are experimental sciences, their aim is to study the processes of circulation of organic matter in nature; that is why the principal methods of experimentation should be frequently adapted from methods used in physics, biophysics and biochemistry. In working out new methods of research it is undoubtedly advisable to refer to classical methods but they should be applied in a new and throughly different aspect.

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BIOECOLOGICAL CHARACTERISTICS OF BLUE-GREEN ALGAE AND CONDITIONS OF THEIR DEVELOPMENT. A REVIEW

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ABSTRACT

The characteristics of blue-green algae as to their cell structure, taxonomy and evolution has been given. The influence of environmental factors on development and distribution of species, on nourishment, photosynthesis, nitrogen and phosphorus assimilation, secretion of substances into the medium from living and dead blue-green algae has been discussed. Moreover, it is remarked that recently the blue-green algae have been more and more spreading in waters all over the world due to growing eutrophication of water-bodies under the influence of municipal sewages and fertilizers glowing down from the fields under cultivation.

1. INTRODUCTION

Blue-green algae have a number of favourable and even exceptional properties which make their life and development possible in conditions altogether not convenient to other algae. They can be found on dry rocks with strong insolation, on and in soils, in hot springs reaching a temperature of 85°C, and in all kinds of polluted environment. Especially in waters, which are now undergoing an ever higher eutrophication almost everywhere, blue-green algae compete successfully with other algae, frequently as the only representants of the vegetation, displaying luxuriant water blooms. Inland waters more and more polluted with sewage from towns and agglomerations, with leached fertilizers flowing from intensely fertilized fields and meadows, as well as with all kinds of pesticides, waters sometimes warmed by electric plants cooling their condensers in open circulation have become a very favourable environment for the development of blue-green algae. In eutrophized lakes and ponds, in dam reservoirs built on rivers, which always wash all floatable and soluble matters away from the whole drainage-basin, planktonic blue-green algae develop in the first place.

Interest in blue-green algae and particularly in their biology, growth conditions and development, has been considerably growing in the last twenty years. Those interesting algae, probably the oldest autotrophic plants on the Earth, have always been an object of numerous investigations, but lately the research work has been stimulated by the practical aspect, that is, on the one hand, the protection of surface waters against the excessive development of blue-green algae and, on the other hand, the exploitation, in mass cultures, of their high production affording valuable organic matter.

The protection of surface waters against the blooming of blue-green algae has become a necessity in all cases where the water is used to supply water-works. Such water must meet a number of requirements: it must be clean, colourless, odourless and without bacteria. Waters abounding in blue-green algae have always

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such defects as: a yellow-brown colour, a bad smell, etc. Water-supply services can remove those defects with difficulty and at high cost. The adaptation of water taken from surface reservoirs is the more difficult the more blue-green algae the water contains.

Mass cultures of blue-green algae have been lately undertaken in many countries mainly because of the fact that blue-green algae, particularly *Spirulina platensis*, have proved to be a more digestible food for people and animals than e.g. Chlorella or Scenedesmus which have strong cellulose cell membranes. Besides, the spreading of blue-green algae cultures over cultivated fields causes a visible crop increase.

That is why it seems right to present some recent data characterizing this interesting plant group.

2. CHARACTERISTICS OF BLUE-GREEN ALGAE

A general feature of blue-green algae which distinguishes them from other algae is a more primitive differentiation of the cell protoplast, consisting in a lack of a formed nucleus and of chromatofores. In the place of the latter there occurs, on the border of the protoplasm, a layer of the so-called chromatoplasm which has the same structure as that of chromatofores in vascular plants. It contains chlorophyll-a, carotenoides and phycobilins, especially c-phycocyanine and c-phycoerythrine as assimilatory pigments. The protoplasm contains various grainy and rod-shaped outgrowths, the function of which has not been fully explained, and gas vacuoles. There occur particularly characteristic grains giving a positive Feulgen reaction; because of their DNA content they are regarded as an equivalent of the nucleus and have been called nucleoids. It has been stated under the electronic microscope that it is a ball of thread, so it corresponds with a chromosome but, as it seems, a chromosome structurally and chemically different from that of vascular plants. Besides, there have been found forms resembling ribosomes and containing RNA, grains of glycogen (Cyanophyceae starch), cyanophycin, metachromatin granules and forms of the lipoid type. Instead, no mitochondria have been found though some scientists suppose that some corresponding forms occur in the protoplasm. Gas vacuoles were regarded, until lately, as specific for blue-green algae, but they have been found in other algae and bacteria (Bowen, Jensen 1965). The function of those interesting forms has not been fully explained yet.

Thus, in their cytological features, blue-green algae differ visibly from other algae and most resemble gram positive bacteria. Their connection with bacteria can be seen in the occurrence of nurein and diaminopimelin acid in their membranes, the lack of mitochondria (though this is not sure), a structural resemblance of nucleatides, the occurrence of glycogen granules and the amitotic cell fission. Also some biochemical features are similar, such as the absence of sterols in the cells of blue-green algae and the resemblance of the peptide complex. As compared with other algae, blue-green algae contain more amino acids, lisine and arginine.

All the features mentioned above might prove that blue-green algae and bacteria have a common origin. This was stressed upon in taxonomic works as early as in the middle of the nineteenth century and this opinion has still many followers, though there also exist other theories. Taxonomists still divide the vegetable kingdom into Acarionta (Procariota) and Carionta (Eucariota) though more recent research has stated the doubtless occurrence of nucleic elements and a specific chromosome in bacteria and blue-green algae. In the cells of blue-green algae we can distinguish chromatoplasm and centroplasm regarded by some authors, e.g. Gusev (1966), as a nucleus consisting of a basic substance, hyaloplasm, and of chromatin elements. Yet there is no nuclear membrane and the centroplasm of the cell performs the functions of the nucleus as well as those fulfilled by the cytoplasm in other algae. Bacteria cells possess no chromatoplasm and no differentiation into centroplasm and chromatoplasm even if they contain pigments active in photosynthesis (e.g. Chlorobacteriaceae). Up to now transition forms between bacteria and blue-green algae have been found. E.g. the Caryophanon which contain a differentiated protoplast cannot be regarded as such a form because they have flagella.

The opinion represented, among others, by Gollerbach, Kuck (1964), that, from the point of view of phylogenetic taxonomy, bacteria, blue-green algae and other plants should be treated as three separate and independent developmental stages seems to be justified. The biochemical and physiological features of their nuclear apparatus are resembling but this apparatus has a different structure in all the three groups. The first two (bacteria and blue-green algae) resemble each other more than the third group and that is why it may be assumed that they have some common progenitor, but as soon as a division of the protoplast of this hypothetical progenitor into two functionally different parts occurred, a new type of plants was born: the Cyanophyta.

If we accepted Oparin's well-known idea of the heterotrophic origin of organisms we could imagine that blue-green algae were separated from some hypothetical common stock at the time when some forms of this stock passed to autotrophic life. It is supposed that this occurred in the Pre-Cambrian. Anyway, as early as in the Ordovician formation, highly organized forms of blue-green algae have been found in fossils. More or less saprophytic organisms such as green and sulphur bacteria might have been, according to some authors, the transition link. But, contary to those bacteria, in their developmental process blue-green algae have formed their own assimilation apparatus adapted to the light reaction connected with the photolysis of water and the molecular emanation of oxygen. Thus, in the process of photosynthesis, they have approached vascular plants and are visibly separated from photosynthesi-

zing bacteria. This peculiarity of the blue-green algae group, appearing in the cell structure, the pigment content and the phytosynthesis process, has persisted in further development.

The characteristic structure of the chromatoplasm of blue-green algae comformable to the structure of the chromatofores of vascular plants allows to suppose that blue-green algae might have been the predecessors of the chromatofores of those plants. Mereschkowsky's (1905) hypothesis, that the chromatofores of plants are separate organisms living in symbiosis with the cell in which they occur, is well known. The number of arguments confirming this hypothesis has been growing lately. Among others, the so-called Cyanellae in the cells of the colourless alga *Gloeochaete Wittrockiana* undoubtedly play the part of chromatofores but, when isolated, they can also live outside the cell of their host. The whole group of algae containing in their cells, blue-green algae which perform the function of chromatofores has been called, by Skuja, Glaucophyta to emphasize its particularity which might be a very old developmental stage of nuclear plants (Eucarionta). The great resemblance of blue-green algae and chromatofores, the same microstructure and the absence of a well-formed nucleus are arguments for such a supposition. Evidently, the symbiosis of blue-green algae with colourless plants has led, in the developmental process, to some modifications, to a retardation of the independence of blue-green algae cells, to the atrophy of nuclear elements and particularly of such an important physiological function as respiration. Instead, it has led to specialization in CO_2 assimilation.

Mereschkowsky's (1905) hypothesis assumes a double nature of the vegetal kingdom. Thus, at a very distant epoch, primarily heterotrophic, colourless plants got into permanent symbiosis with blue-green algae and in this way they developed chromatofores. Blue-green algae would then be a group, phylogenetically independent, and not related to the rest of the organic world, not even to bacteria. An argument for this is the fact that they are the only organisms which combine the capacity of photosynthesis with that of molecular assimilation of nitrogen.

3. INFLUENCE OF ENVIRONMENTAL FACTORS

Among environmental factors influencing the life of blue-green algae, temperature should be named in the first place. Blue-green algae possess a high ability of adaptation to different temperatures so that there is no such place on the Earth where blue-green algae would not occur. They live in temperatures of 0—85°C, but their mass development occurs only at optimum temperatures for each species. Hence the temperature affects, in natural conditions, the changes in the domination of species in waters. E.g. *Anabaena flos aquae* begins to develop at 5°C but its

maximum development occurs only at 15—20°C; *Microcystis aeruginosa* develops at 0°C but it appears in masses only at 20°C and reaches its maximum at 23—27°C. The differences of optimum temperatures in the development of particular species are the reason of the unequal occurrence of blue-green algae in various climatic zones or in the same zone in years of different thermic conditions.

Limit temperatures affect the morphological features of the cells, the qualitative content of pigments and the intensity of the photosynthesis and of the growth of organic matter. Both at the upper and lower limits of the temperature proper for the development of a given species the fission of cells may be checked through their growth may proceed. That is why it is not unfrequent to observe giant forms among blue-green algae.

The great hardiness of blue-green algae to freezing and drying up is well known. Particular species can tolerate temperatures from −10 to −70°C. Rock and soil blue-green algae tolerate drying up and to not lose their growing capacity for a long time. Some species of the families Nostocaceae and Oscillatoriaceae revive after several years of dry state, but there also exist species which cannot endure more than ten days of dry state.

4. NOURISHMENT

As regards nourishment, blue-green algae belong, in principle, to the type of myxotrophic plants, but photosynthesis plays, in their nourishment, a very important and sometimes a dominating part owing to their content of assimilatory pigments. An essential element in the complex of pigments important in photosynthesis is chlorophyll. Beside it there occur supplementary pigments, carotenoides and phycobilins which, in some way, replace the absent chlorophyll-b in the cycle of photosynthetic reactions.

The functioning of the pigmental system of blue-green algae differs depending on the species, the physiological state and light conditions. But in general, the effectiveness of photosynthesis in blue-green algae is lower than in plants containing chlorophyll-b. It has been proved, for example, that the time of passage of excitation energy from phycocyanin into chlorophyll-a is $0.3 \cdot 10^{-9}$ sec in *Aphanotece nidulans* and the effectiveness of the migration amounts to 86%, while in Chlorella the migration from chlorophyll-b to chlorophyll-a lasts for $0.0 \cdot 10^{-9}$ sec with 100% effectiveness. The passage of energy between chlorophyll-a and phycocyanin is then slower and its effectiveness lower than between chlorophyll-a and -b. So, the formation of a system of chlorophylls a and b was a progress in the development of plants, which blue-green algae have not attained.

Blue-green algae can draw carbon dioxide from different sources which again depends on the properties of particular species. The representants of the genus Nostoc, Aphanotece, and some other can utilize CO, in light and in the presence of oxygen, oxygenating it into CO_2. It has been also stated that they draw CO_2 from carbonates more easily than from free CO_2 which is probably connected with the fact that blue-green algae have their developmental optimum in alkaline reaction (pH 8.0—9.5), i.e. in conditions where usually no free CO_2 occurs in water. Blue-green algae can also draw carbon from organic combinations: acetates, D-fructose, glucose, from amino acids: glutaminic acid, proline, arginine, leucine and from polysaccharides and other organic compounds. Amino acids added to the culture medium have caused an increase in growth and biomass of blue-green algae. They can also utilize amino acids dissolved in surface waters the amount of which is fairly high, according to Stangret (1970).

There are various ways and various conditions of assimilating organic matter by blue-green algae. *Tolypothrix temus*, for example, assimilates glucose only in the presence of oxygen which is also absorbed. *Anabaena variablis* assimilates glucose in darkness, in anaerobic conditions. *Nostoc punctiforme* does not assimilate glucose at all.

Blue-green algae are also capable of photoreduction of reconstruction of CO_2 in light, in the atmosphere of hydrogen or sulphuretted hydrogen, and of chemosynthesis in darkness by taking adventage of the energy of hydrogen oxydation.

The assimilation products of blue-green algae are hexoamines, which occur in much larger quantities than in vascular plants, and glutaminic acid. The quantities of liberated oxygen depend on the intensity of light which also influences the absorption of oxygen. It has been stated that less oxygen is absorbed in a low intensity of light as compared with the check-up in darkness. Respiration in darkness in directly proportional to the concentration of oxygen and it is different from respiration in light. That is why in investigations of primary productivity the value of the actual intensity of photosynthesis cannot be obtained by a simple comparison of the consumption and increase of oxygen in darkened and not darkened bottles.

The attitude of blue-green algae towards the oxygen content in water is interesting. It is principally negative which can be proved by the fact that their growth is always stronger in weakly oxidized and slowly flowing water containing much organic matter, and while the oxido-reducing potential increases. The cells of blue-green algae are resistant to periodic deficiencies of oxygen and, at the same time, they defend themselves in a particular way against an excess of oxygen, as it has been proved by Sirenko et al. (1969) in their work on

Microcystis. A protective role against an excess of oxygen is played, in *Microcystis aeruginosa*, by mucillaginous envelopes of the colonies, which are alwasy a seat of bacteria consuming oxygen, and contain compounds absorbing the oxygen liberated in the process of photosynthesis. Such are, in the first place, compounds of sulphur, particularly of glutanian and cysteine combined with ascorbic acid. This alga displays a particularly strong growth on the bottom of poorly oxidized waters and then it forms only small tremellose envelopes on the surface of the colonies. It does not develop in well oxidized bottom layers of the water. In summer, when the colonies come up from the bottom to the upper layers of the water they always form thick mucillaginous envelopes which protect the cells against an excess of oxygen.

Blue-green algae are then, so to say, primitive photosynthesizers sensitive to the excess of oxygen isolated in the photosynthesis, which they seem not to be able to utilize yet and are forced to defend themselves against its excess, wasting thus large amounts of energy. Though the problem of the negative attitude of blue-green algae towards oxygen has not been solved definitively yet, it may be supposed that it was the result of a violent transition they passed from anaerobic conditions of life of their presumable progenitors, which were, according to some scientists, photosynthesizing bacteria, to oxygen conditions characteristic of Chlorophyllic plants. Thus blue-green algae are not yet able to manage stronger oxidation caused by photosynthesis and are forced to defend themselves against oxygen, which is well illustrated by the ecological and physiological properties of species growing in masses and in soil, such as Microcystis, Anabaena, Aphanizomenon, etc. A stronger oxidation can practically check the development of blue-green algae and, on the contrary, this may be increased through the decrease of the oxygen quantity in water which occurs, as a rule, in dam reservoirs and in waters polluted with sewage.

5. ASSIMILATION OF NITROGEN AND PHOSPHORUS

Blue-green algae can assimilate all forms of nitrogen in waters: nitrites, nitrates, ammonia, organic nitrogen and free nitrogen. There occur, evidently, specific abilities of particular species in utilizing the particular forms of nitrogen. But their characteristic property is the assimilation of molecular nitrogen which is of great importance in the circulation of nitrogen and its increase in water and soil. The general outlines of the action of assimilating free nitrogen are known. The centers of assimilation are situated in the lamellae of the chromatofores and the energy is supplied by carbohydrates produced in photosynthesis. Thus the assimilation of free nitrogen is closely connected with photo-

synthesis and proceeds in the same organelles of the cell. The assimilation of nitrogen in darkness is accompanied by the isolation of CO_2 thus being connected with respiration. An intense assimilation of free nitrogen begins in water after nitrates and ammonia have been used up. Assimilation is affected by the biological features of individual species, the age and physiological state of the cells, the ecological conditions of development and growth and the environmental factors such as temperature, pH, alimentary salts, light, etc.

A particular interest was aroused by blooms in dam reservoirs and ponds caused by some species of blue-green algae capable to assimilate free nitrogen. Those species are: *Anabaena flos aquae, A. lemmermanni, A. scheremetievi, A. spiroides* and also *Microcystis aeruginosa* and *Aphanizomenon flos aquae.* Alive or dead they secrete large amounts of substances containing nitrogen and particularly amino acids such as: alamine, glycine, serine, leucine, treonine, valine, asparagin acid and glutaminic acid.

The ability to assimilate molecular nitrogen as well as the photosynthesis qualifies blue-green algae as a kind of hyperautotrophes.

Phosphorus is absorbed by blue-green algae in smaller quantities than nitrogen, yet the part of this element in the energetic processes of cells is not sufficiently known. There is evidence that phosphorus combinations are not utilized by blue-green algae in the same way as by vascular plants. No oxidative phosphorilation has been proved in them, either.

6. SECRETION OF SUBSTANCES INTO THE MEDIUM

During their life blue-green algae secrete large amounts of organic substances, such as: oxalid, tartaric, succinic, citric and malic acids; peptides, mucus and substances of the polysaccharides type; vitamines, e.g. B-12; aldehides and terpenes derivates. The physiological and biological role of these secreted substances is not fully known, but undoubtedly it plays an important part in the formation of biocenoses in waters. It is a well-known fact that during the mass development of blue-green algae other species of algae such as Scenedesmus and Pediastrum recede or pass into an inactive state. Oscillatoria checks the growth of Rhizoclonium and Chlorella; *Microcystis aeruginosa* checks the development of *Aphanizomenon flos aquae* and vice versa. The action of the substances secreted by blue-green algae (as well as by other algae) may occur, according to L e f è v r e (1964) in three directions: 1. the secreted metabolites check the cell fission but do not disturb the assimilatory apparatus. As a result the cells store large quantities of reserve materials and they burst, 2. an inverse situation may occur: assimilation is checked

but not cell fission. Then the dividing cells become dwarfed, 3. both processes may be checked and the cells die. Owing to metabolites there occur, too, various morphological changes which lead to teratological forms.

Terpenes and their derivatives have a checking influence on the growth of other algae. They accumulate abundantly in waters where *Microcystis aeruginosa, Aphanizomenon flos aquae* and *Phormidium uncinatum* live in masses. A derivative of terpenes — pheniloethyl alcohol, when concentrated to 8.4 mg/l, checks the growth of algae cells and the sytnhesis of DNA and of chlorophyll. In higher concentrations it checks all photosynthesis.

Secretions lead finally to an autointoxication of secreting organisms which is sometimes the cause of a sudden decrease of blooms. The intoxicating and irritating action of blue-green algae on animals and human organisms is well known. People bathing in waters containing large quantities of planktonic blue-green algae get mucosa and skin irritations. Workers of fish-ponds, where blue-green algae blooms are common, speak of "water nettles" which sting the skin.

7. CLOSING REMARKS

The above presented main data concerning the biological properties of blue-green algae show how particular and different from other algae this group is. Blue-green algae are characterized by a considerable resistance to outer factors and an adaptability to various environmental conditions. They become particularly active in extreme ecological conditions where other algae have no chance of development. As they are very old plants, probably the oldest autotrophic organisms on the Earth, they had time to spread all over world and take every possible ecological recess. The high cosmopolitism of blue-green algae, especially those belonging to the orders Chroococcales and Oscillatoriales, is well known. So the same species may be found in waters, pools, on soils and on rocks, in Greenland and on the Spitzbergen as well as in temperate zones, in the tropics and on the Antarctic. So far, only few species have been found that seem to be attached exclusively to the tropical zone. They constitute only ca. 2% of known freshwater species the total number of which is estimated at ca. 1300.

The ubiquity of blue-green algae suggests their great importance in Nature's economy. This problem has not been sufficiently studied yet. The recent decades have been mainly devoted to investigations of the role of blue-green algae in reservoirs supplying usable water and much less to their role in the soil. It has but recently been noticed that there are possibilities of mass cultivation of blue-green algae: *Spirulina platen-*

sis and, to a certain degree, *Spirulina massartii*, as it results from observations on the traditional use of these algae as food by the natives of the Tchad Lake neighbourhood in Africa.

Investigations on the development of blue-green algae in dam reservoirs tend towards a detailed study of their biological properties in order to find the most efficient measures to fight them and to exclude blooms which always cause serious disturbances in the utilization of water. On the contrary, in fish-ponds a mass (though not exaggerated) development of blue-green algae is accompanied by a favourable increase of fish and indicates a maximum fecundity of the water, obtained through fertilization.

A good aeration of water has proved to be the best protection against mass development of blue-green algae. A decrease of oxygen content in water causes, in summer and until late autumn, an intensive development of blue-green algae. The fight against them by means of copper sulphate or various pesticides is of relative and only emergency value. In dam reservoirs on the Dniepr good results were obtained in reducing the development of blue-green algae by infecting them with a special virus.

The study on the role of blue-green algae in the soil, though not yet completed, is an important achievement. Blue-green algae take part in the processes of soil formation, contribute to the accumulation of organic matter, enrich the soil in nitrogen, affect its mineral composition, improve its physical features, by storing calcium in their thalli; they contribute to the aeration of the soil and to retain moisture. In some districts of South Russia the fertilization of fields with mass cultivated blue-green algae has been introduced; its effect on the increase of crops is visible. The important part played by blue-green algae in the rice fields of sub-tropical and tropical conditions is well known. They supply those soils with so much nitrogen that no more additions of this element are necessary.

To finish, here is a general remark of a prospective character. It is known for certain now that the growing pollution of water, its saturation with organic compounds, as a result of the whole of economic activities, and — at the same time — a high deoxidization, are favourable to the development of blue-green algae and facilitates their rivalry with other algae. Thus there appears a prospect of the conquest of the world's lands and waters by blue-green algae.

In the Cambrian and Silurian, and partly in the Ordovician blue-green algae were nearly the only existing plants. Fossils dating of those periods show a great differentiation of their thalli and cells fully corresponding to the forms found in water nowadays. Should the present conditions create prospects of a revival of blue-green algae and their renewed domination on the Earth? What is the world tending to?

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BACTERIAL FLORA OF WATER BODIES OF THE PERIGLACIAL TUNDRA (WEST SPITSBERGEN)

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ABSTRACT

Materials were collected from three tundra shallow water bodies, situated in the Fuglebergetsletta lowland. The total number of bacteria was calculated by direct microscopic count, as well as the number of heterotrophic bacteria cultivated on an MPA medium. Moreover bacteria decomposing cellulose, starch, proteolytic bacteria and those assimilating atmospheric nitrogen were determined. The calorific value of sestone samples was also determined. The results revealed that in all water bodies occurs a rich bacterial flora, and in water bodies No. 1 and 2 sestone — both in amounts typical for eutrophic water bodies of a moderate climate. On the other hand, the low percentage of heterotrophic bacteria, developed on MPA medium, participating in the total number of bacteria indicates the oligotrophic character of investigated water bodies.

1. INTRODUCTION

The significance of water bodies bacterial flora for their biocenosis is basically connected with the relations of that flora to the trophicity of water body. Owing to the activity of the bacterial flora, mineral components, e.g. nitrogen, phosphorus and others, as well as carbon are introduced into alimentary chains. Thanks to microorganisms, the organic substance of the water body is recuperated and once more takes part in life processes, either directly by sustaining by its energy heterotrophic bacteria, which constitute the nourishment of numerous water bacteriophages, or by a more complex route, by mineralization of that substance and the liberation of non-organic compounds, indispensable in photo- and chemo-autrotrophic production. That is why we wanted to collect microbiological information, which notions would help us to evaluate the trophicity of investigated water bodies.

We wanted to confirm the presence of some characteristic groups of bacteria of which the presence should, according to us, exert an important effect on the trophization of arctic tundra water bodies. In the first place, we assigned to these groups the assimilators of free nitrogen, and further those groups of bacteria which desintegrate protein, starch, and cellulose into simple, easily assimilated by other organisms organic compounds. Cellulose, in regard of numerous Lichenes growing on the shores of investigated water bodies, may reach them in form of allochtonic substances. The total number of bacterial cells within surface and bottom water layers of the pools was also counted, as well as the amount of heterotrophic bacteria. From these the percentage of heterotrophic bacteria in

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total bacteria was counted. This is called the bacterial index of production of water bodies, considered by some authors (Santolini 1966) as a magnitude characterizing the trophicity of water masses.

As complementary investigations, studies on the caloricity of sestone in near bottom water layers were carried out in 1972. Moreover, on the basis of the culture materials systematic determination of some microorganisms was performed.

2. AREA OF INVESTIGATION AND METHODS

Materials for studies were collected in the course of two short stays in the Spitsbergen, in autumn of 1971 and in autumn 1972. In 1971 the water bodies were ice-covered. The ice was 5 cm thick, whereas in 1972 the surface of the water bodies was free.

The material was collected from three tundra pools, situated in the Fuglebergetsletta lowland, stretching in a broad band along the sea-shore (Fig. 1).

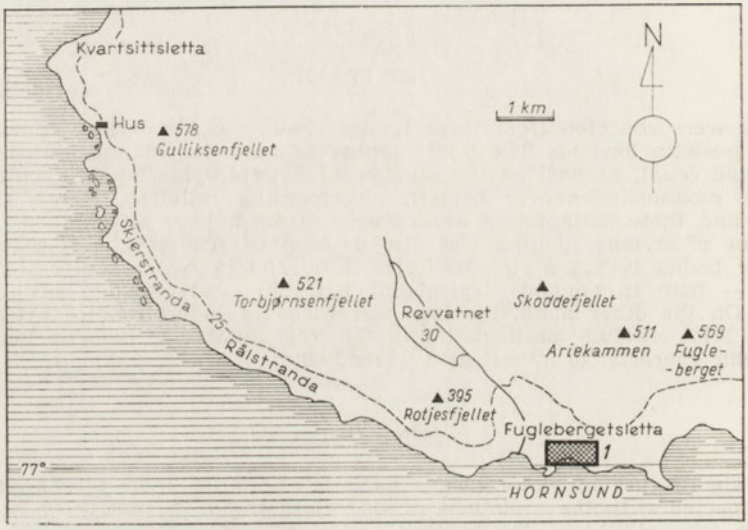

Fig. 1. North-west coast of Hornsund fiord. 1 — the area of investigations

The water bodies marked No. 1 and No. 2 are serially connected flow pools (Fig. 2) on the left branch of the Arie torrent which, flowing through a flat terrace (6 m altitude) forms in its cavities shallow pools. At a distance of about

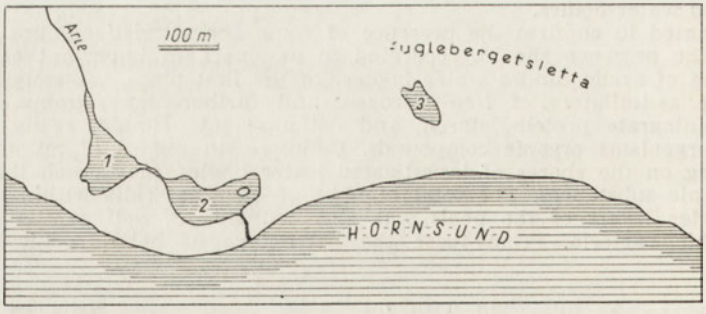

Fig. 2. The dimension sketch of the investigated pools. 1–3 — pool numbers

300 m from pools No. 1 and 2, lies pool No. 3 through which the water is not flowing. The distance of the water bodies from the sea amounts to about 50 m.

The depth of pool No. 2 amounts, in its central part to about 1.5 m, whereas pools No. 1 and 3 are 0.7 m deep. Because of their comparative shallowness, these pools are intensely pertorbed by winds. The bottom of these pools is not uniform: in each of them are parts covered with detritus layers of up to 30 cm thickness, and parts with sand and gravel. This differentiation is most obvious in pool No. 3 where the bottom in its western part is covered with stones and gravel and in its eastern part passes flatly into a muddy shore.

The water bodies are surrounded from N and W low rocky hills, partly covered with Lichenes. Towards the sea, the pools are surrounded by flat shores which, a score or so of meters further drop sharply and steeply to the sea. The soil surrounding the pools is very poor (Szerszeń 1968, Boratyński et al. 1968). No sounding of the pool shores could be carried out deeper than 5 cm, where a rocky substratum appeared.

Small pools of the Fuglebergetslette lowland have been investigated from a thermal point of view, by Kuziemski (1968) and Rakusa-Suszczewski (1968). According to Kuziemski (1968), the water temperature of these pools does not exceed 12°C in the course of the whole year. Chemical examination of the water, performed in summer 1968 (Rakusa-Suszczewski 1968a) classify these pools after Alekin (1956) to the hydrocarbonic class, calcium group.

Investigations of shallow tundra pools of W. Spitsbergen, carried out by Doroszewski (1968) and Rakusa-Suszczewski (1968b) on zooplankton revealed the existance of numerous species of Rhizopoda and Ciliata, beside the presence of Orthocladiinae, Tendipedinae, *Daphnia pulex mitdendorfiana* as well as an important quantity of *Euphyllopods nothostraca*.

The pool, marked by us with No. 2 is particularly eagerly visited by birds (Doroszewski 1958).

The Fulebergetsletta lowland was the site, between 1957 and 1972 of numerous Polish geophysical expeditions. Baranowski (1968) collected extensive data on the thermic properties of the tundra and the solar radiation in this area. In connection with the high significance of temperature course for bacterial metabolic processes, we give some characteristic mean diurnal temperatures, taken from the paper of Baranowski (1968) (Table I).

Table I. The maximum and minimum mean daily temperatures of air and soil in the Fuglebergetsletta lowland (after Baranowski 1968)

Layer	Mean daily temperatures (°C)	
	minimum	maximum
100 cm above the soil surface	−24 (Dec.)	+8 (June)
at the soil surface	−20 (Dec.)	+10 (June)
50 cm under the soil surface	−14 (Dec.)	+4 (Aug.)
160 cm under the soil surface	−8 (April)	0 (June-Oct.)

The results of water temperature measurements performed during the above mentioned investigations are listed in Table II.

Table II. The temperatures of surface and bottom water in the investigated tundra water bodies (°C)

Pool No.	1971 (ice cover)		1972 (open water)	
	surface	bottom	surface	bottom
1	0.5	2.0	—	2.5
2	0.5	2.3	—	2.5
3	0.5	2.2	—	3.0

In the same paper Baranowski (1968) states that the total radiation of the sun in a year amounts for the Fuglebergetsletta lowland 47 kcal/cm² which represents one of the lowest insolation values on the globe.

Water samples were taken from surface and bottom layers of the pools. Surface water in case of ice-covered pools was sampled at a depth of 5 to 10 cm under the ice, where there was no ice 5 cm below water mirror. Bottom water samples were collected 15 cm from the bottom. Water samples were collected by means of sub-pressure into aseptic vessels, previously brought. The scheme of sampling is presented in Figs 3, 4, 5.

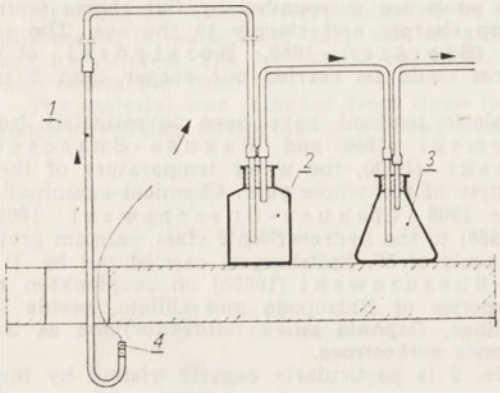

Fig. 3. The scheme of the sampling of water from beneath the ice. 1 — pipe sucking up a sample, 2 — vessel for a sample, 3 — sub-pressure flask, 4 — rubber stopper

Figure 3 presents a scheme of water sample collection from beneath the ice, whenever the access to any point of the pool is possible. A sterelized sound (1) of which one end is fitted with a stopper (4) is plunged at the requested depth under the ice. The stopper (4) is plunged at the requested depth under the ice.

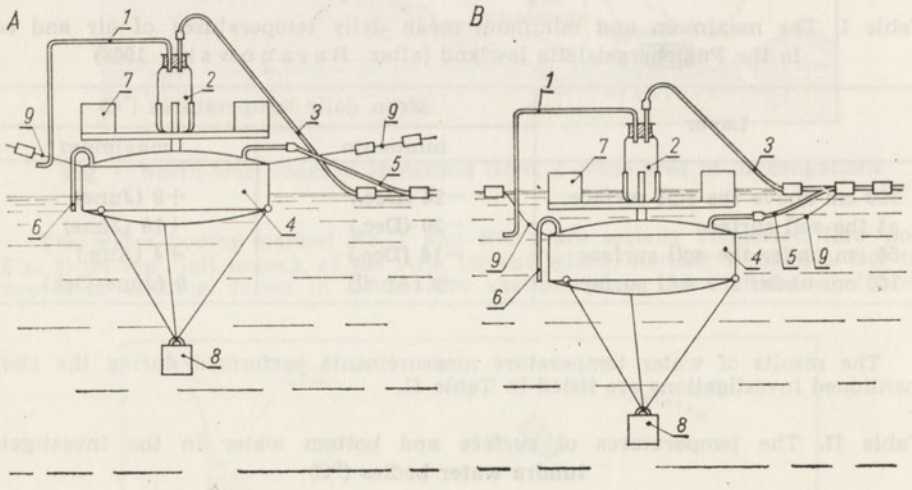

Fig. 4. The scheme of the sampling of surface water when water was free of ice A — towage of device through the water surface (the float (4) is filled with air), B — collection of a sample (the float (4) is ballasted with water). 1 — pipe sucking up a sample, 2 — vessel for a sample, 3 — vacuum tube creating a sub-pressure in the vessel, 4 — float ballasted with water, 5 — sub-pressure tube creating a sub-pressure in the float (4), 6 — pipe sucking up the ballasting water, 7 — stabilizating floats, 8 — sounding-lead, 9 — towing lines

The stopper is then carefully removed (4) thus creating a subpressure in the vessel (2), which draws in the material. Subpressure is produced by means of a vacuum hand-pump.

In the successive Figs 4 and 5, a scheme of surface and bottom water samples collection is presented at free water surface.

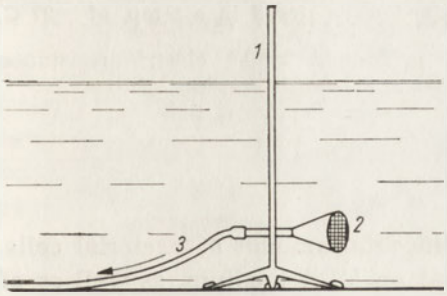

Fig. 5. The scheme of the sampling of bottom water when water was free of ice. 1 — stand placed in the sampling point with the cable-stays, 2 — conical end protected with net, 3 — sub-pressure tube sucking out a sample into the vessel placed on the shore

Surface water samples were taken by means of the original method of balast-loats, elaborated by the present authors. This method was prepared for pools, not exceeding in diameter 100–200 m. The basic aim of the authors was to facilitate the collection of surface water samples with the least disturbance of pool stratification. This method presented in Fig. 4, consists in collecting samples by subpressure into a vessel, fastened to a special float, anchored previously in a chosen place of the pool. Anchoring is done from the shore by means of three lines. The float may be transported to the chosen place in the pools, in regard of pool size and ground surface conditions either by towing over water surface or on strained lines, and fixing in the point foreseen. The float recipient and sample vessel are connected by light, empty tubes with the stand on the shore. The tubes are supported by cork floats.

Sampling runs as follows: after a space of time indispensable for return to natural stratification of water mass disturbed by the activities connected with anchoring of the float, there develops within the float vessel a subpressure, balasting it thus with water and producing immersion of the sound outlet (1) at a depth of about 5 cm under water. There follows sub-pressure within the sample vessel, drawing in the material. After uptake of material, water from the balasting collector is eliminated by pressed-in air, and the inlet of the sound emerges thus above the water surface. After that, the whole of the device, with the collected sample is pulled out onto the shore.

Bottom water is sampled by a special tripod, fixed in the bottom (Fig. 5). The material was taken up by sub-pressure method directly into vessels placed on the shore.

The total number of bacteria was determined by the method of direct microscopic counting. The water, supplied to the boat was filtered on the a Millipore filtering device with membrane filters Millipore, with filtering pores of 0.22 μ diameter. The water from each sample taken was filtered through 5 filters. The bacterial sediment was stained with erytrosine. The filters with sediments intended for counting were transported in containers preserving them from any damage.

The number of heterotrophic bacteria were denoted basing on cultures incubated at 8°C temp. in meat-peptone-agar medium (MPA), fixed by agar. From each sample taken, 0.5 ml were sown on a plate. From each sample taken 5 plates were incubated. After 25 days of incubation, the number of grown colonies was determined, and a series of strains were isolated, in order to investigate their morphologic and physiologic features. Thus were determined percentage of proteolitic bacteria and of those desintegrating starch, among the number of grown heterotrophic microorganisms, as well as the species of isolated strains.

The presence in the examined water of bacteria assimilating atmospheric nitrogen and decomposing cellulose were denoted by means of inoculation on selective media. For bacteria, assimilating atmospheric nitrogen. a medium with glucose was used recommended for Azotobacter. On the other hand, in order to

establish the presence in water of bacteria decomposing cellulose in oxygenated conditions, two media were applied: agar Riviera and silica gel, saturated with a medium after Charpentier (R o d i n a 1965).

The caloricity of sestone was quantitatively determined exclusively within bottom water of the pools, by the method of wet dichromate oxidation (Maciołek 1962). Samples of water filtered in amounts of 10.5 l through glass paper filters, of GF/C, Whatman type. The sediments obtained were stored in a temp. of −27°C, up to the time of determination.

Water temperature measurements were performed by an electric resistance telethermometer with platinum detectors (100Ω).

3. RESULTS

The results from determination of the total amount of bacterial cells, of number of heterotrophic bacteria bred on MPA medium, as well as of bacterial index of water productivity are collected in Table III.

Table III. Density of bacteria in tundra water bodies and bacterial index of productivity

Pool No.	Level	Direct microscopic count (N/ml)		No. of heterotrophic bacteria (MPA medium) (Nh/ml)		Index of productivity $\frac{N_h}{N} \cdot 100$ (%)	
		1971	1972	1971	1972	1971	1972
1	surface	24,650	1 386,000	133	272	0.54	0.02
	bottom	442,000	3 819,000	179	942	0.04	0.024
2	surface	53,650	1 631,000	70	472	0.13	0.028
	bottom	239,000	2 286,200	102	870	0.05	0.038
3	surface	10,700	605,500	57	152	0.53	0.024
	bottom	2 175,000	2 038,000	1913	190	0.08	0.018

Table IV. The percentage share of bacteria desintegrating protein and starch in the total incubated heterotrophic bacteria from water of tundra pools

Pool No.	Bacteria desintegrating			Other bacteria (%)
	Protein (%)	Starch (%)	Both protein and starch (%)	
1	41.5	53.0	3.5	2.0
2	74.4	9.3	3.7	12.6
3	33.4	36.6	25.4	4.6

In Table III attention is drawn to the distinct stratification on the total number of bacterial cells in 1971, when the water bodies were ice-covered, as well as the decrease of the stratification in 1972, when the water mirror of the pools was free. One should also stress the low

participation of heterotrophic bacteria bred on an MPA medium in the total number of bacteria.

Table IV presents percentage of cells desintegrating proteins and starch in the number of bred heterotrophic bacteria.

The results presented in that table stress the different character of the heterotrophic flora collected from pool 2, as compared with other water bodies. In samples taken from that pool, the amount of bacteria desintegrating protein exceed about 8 times the number of bacteria desintegrating starch. In the remaining two pools, the ratio of these numbers was approximately 1 : 1.

Cellulolytic bacteria were bred as well on the medium Riviera as Charpantier. The cellulolytic bacteria revealed morphological as well as physiological features approximating those of the species Cytophaga and Cellvibrio. The number of colonies on plates inoculated with bottom water in the amount of 0.5 ml to each plate was 2 to 5 colonies after 30 days of growth at a temp. of 8°C for all pools. The decay of cellulose at 8°C was very slow. The microorganisms inoculated and incubated at 26°C developed rapidly and already after 10 days a notorious decay of cellulose could be observed.

The inoculation performed on plates with nitrogenless medium gave also positive results. The cultures obtained enabled to isolate a few groups of organisms of which one revealed features approximating those of Azotobacter.

Systematic denotation enabled to isolate the following kinds of bacteria: Bacillus, Flavobacterium, Achromobacter, Sarcina, Chromobacterium and Thiocapsa. From determined species, strains with features approximating those of *Bacillus brevis* were denoted, *Flavobacterium Harrisoni, Flavobacterium rhenanum, Chromobacterium violaceum, Achromobacter xerosis, Achromobacter iophagus, Achromobacter cycloclastes, Sarcina ventriculi, Sarcina Henseni* and *Thiocapsa roseopersicina.*

The sestone caloricity is given in Table V, and results of temperature measurements in Table II.

Table V. Calorific values of sestone of bottom water of the investigated pools

Pool No.	Calorific value (cal/l)
1	7.9
2	12.3
3	2.85

4. DISCUSSION

The present study was meant to give merely information concerning the bacterial flora of investigated pools. The samples taken to this use (water) represent rather poor material, but the results obtained from them seem to build an interesting and characteristic pattern for the zone of arctic climate.

A high density of bacterial cells, as well as large amounts of sestone represent a characteristic feature of eutrophic waters in a moderate climate (Potajenko 1970, Kowalewskaja 1970a, b). This situation is also stated for pools No. 1 and 2. On the other hand, the established by us in these water bodies low percent of heterotrophic bacteria, bred on MPA medium, as well as the slight amount of carbon dioxide in those waters [1] (denoted by M. Pulina, unpublished information) are characteristic for oligotrophic water (Santolini 1966, Lityński 1952).

Thus, when studying the data obtained in categories of a moderate climate, we have a contradictory picture. In this case, however, the contradiction is merely apparent, as in various climatic zones the same criteria of water body trophicity cannot be applied. There occur particularly another correlation between the productivity of pools and the state of their standing crop, as well in regard to sestone as to plankton and bacterioplankton. In arctic climate, the low amounts of solar energy reaching the surface of pools in the course of the year are limiting the production of phytoplankton. However, low temperatures which in consequence limit metabolism, and thus the rate of flow of energy within biocenosis induce, in spite of low production, an accumulation within the pool of important amounts of sestone.

The analysis of results mentioned above bears on the two connected pools, No. 1 and No. 2. In pool No. 3, unconnected with the two first ones and of different bottom character, only insignificant amounts of seston were found. This would seem to testify to the possibility of high differentiation between tundra water bodies in regard to their biocenotic structure. This may be corroborated by denotations in Table IV concerning bacteria desintegrating starch and protein. Here may be noted the distincly different character of pools No. 2 from the remaining two. In the samples taken from that pool the amount of bacterial cells desintegrating protein is eight times that of cells desintegrating starch, whereas in the pools No. 1 and No. 3 the proportion is like 1 : 1.

The difference of character of pool No. 2 appears not only in the microbiological picture. Rakusa-Suszczewski (1968a) reve-

[1] On August 19, 1972, 3.3 mg CO_2/l was found in pool No. 1, and 4.4 mg CO_2/l in pool No. 3.

aled in that pool a higher content of calcium and magnesium than in pools No. 1 and No. 3, where these values are almost identical. Doroszewski (1968) discovered in that pool exceptionally important amounts of Ciliata, and noted that in the vicinity of that pool unusual numbers of birds are nestling (Doroszewski 1958). It would seem that the birds visiting that pool exert some influence on its chemical properties, as well as on biocenotic composition, what in turn is closely connected with the high caloricity of seston within that pool (Table V).

Thus various microbiological characteristics of the investigated pools occur parallelly to their chemical ones and to different zoological observations.

Finally, we should take position in relation to divergences between total number of bacterial cells in 1971 and 1972. It draws attention that a distinct stratification in bacterial density at the time of their freezing (1971) and a lack of such stratification in 1972, when the water surface was free of ice. In 1972 also, a higher amount of bacteria was observed in all water bodies, as well in surface layers as near the bottom. It seems that as well lack of stratification as higher amount of bacterial cells in the water of the pool, devoid of ice cover may be ascribed to the insignificant depth of the pools. Thus, at the time when there is no ice cover on the pools their waters are continually disturbed by frequent in that area and sometimes very violent winds. In such conditions, a large number of bacteria, mostly keeping to the bottom when the pool is frozen, carried by the motion of the water, are lifted up, thus increasing their number within the water and leveling the stratification between bottom and surface water.

Acknowledgement

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HETEROTROPHIC ACTIVITY OF BACTERIAL MICROFLORA IN MAZURIAN LAKES OF VARIOUS TROPHY

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ABSTRACT

Investigations carried out in six Mazurian lakes indicate a clear dependence between the total number of bacteria in water of various trophies and value of the coefficient of heterotrophic activity of bacterial microflora. Mean values of the activity coefficient were calculated from data obtained at different depth in the lakes and it was established that those values calculated for glucose and bactopeptone revealed a distinct connection with the degree of trophicity of the lakes. They are called coefficients of the eutrophication degree. Investigations of cellulolytic activity have shown that this activity also visibly tends to grow as the trophy of the lakes grows; besides, the coefficient of cellulolytic activity supplies information on the degree of pollution of the lakes with sewage from cellulose works.

1. INTRODUCTION

Heterotrophic bacteria using organic matter as a source of carbon are the prevailing bacterial group in water ecosystems. Their number is of no importance in characterizing various trophic types since it is a well known fact that they constitute 90% of all the bacteria in natural waters (Kuznetsov 1970); what matters is their activity — the ability of destroying organic matter.

Investigations tending towards a determination of the heterotrophic activity of natural groups of heterotrophic bacteria were carried out with the use of media labelled with carbon ^{14}C (Parsons, Strickland 1962, Wright, Hobbie 1965, Hobbie, Wright 1965, Vaccaro, Jannasch 1966, Allen 1967). Media most frequently used were glucose and sodium acetate, as well as hydrolyzates of algae cells previously labelled with carbon ^{14}C (Sorokin et al. 1971). The investigations have shown a varying intensity in utilizing the dissolved organic matter by uni- and multi-species bacteria groups. It has been also found that there occurred seasonal changes in the rate of assimilation of organic media (Wetzel 1967).

The aim of the present paper is to investigate the heterotrophic activity of bacterial microflora occurring in the Mazurian lakes of various trophic types.

2. MATERIAL AND METHODS

Investigations were carried out in August 1971, in the following lakes; Taltowisko — slowly eutrophized; Śniardwy — slowly eutrophized, pleomictic; Mikołaj-

This work was supported with the Polish Academy of Sciences within the project PAN 09.1.7.4.

skie — eutrophic, holomictic; Bełdany — eutrophized, Nidzkie — eutrophized, polluted with sewage from cellulose works, Guzianka — eutrophized.

Water samples were collected from the central part of the lakes, far from the shore and the influence of the littoral, by means of a plankton sampler of Bernatowicz type [1], from the surface down to the bottom zone, at 5 metre spaces.

The method used to investigate the heterotrophic activity of bacterial microflora was based on the measurement of oxygen utilized by the natural group of bacteria. After the elimination of zoo- and phytoplankton (filtering through cottonwool) more and less easily dissolvable organic media were added to the water, 10 mg per litre; they were: glucose, bactopeptone, cellobiose and cellulose. Water samples without any substratum were used as control samples.

The water was incubated in bottles, of 120 ml capacity with ground glass stoppers, in darkness, at a temperature of 19–20°C, during 24–48 hours depending on the kind of substratum. After appropriate duration of exposure in the successive variants of experiments the oxygen content was determined by the Winkler method and the loss of oxygen in relation to the control (starting) sample was measured. Each variant of the experiment was performed twice.

In order to obtain a characterization of the investigated lakes as regards their trophy and microbiology, the total number of bacteria was determined by the method of direct counting on membrane filters Coli-5 Sartorius (Kuznetsov, Romanenko 1963) and the biomass was measured (Rodina 1965).

3. RESULTS

On the basis of the investigations described above it was established that the mentioned lakes form a kind of trophic gradient characterized by the increase of the number of bacteria and of the bacteria biomass as trophy grows (Fig. 1). The lowest numbers occurred in the Tałtowisko Lake and they amounted averagely to 3.0—3.5 million per one ml of water, the highest — in Guzianka Lake where they reached 12.0—13.0 million of bacteria cells per ml of water. The increase of bacterial biomass and the amount of oxygen utilized by the bacterial microflora in natural lake water were also put under observation.

Basing on the amount of oxygen utilized in the presence of substratum, after an exposure of 24 hours, the coefficient of heterotrophic activity *A/B* of bacterial microflora in the mentioned lakes was counted (Fig. 2). This coefficient equals the ratio of the amount of oxygen (mg O_2 per litre) utilized by the bacteria, after 24 hours incubation on substratum (*A*) to the amount of oxygen (mg O_2 per litre) utilized in the control sample, without any substratum, after the same duration of exposure (*B*). Glucose and bactopeptone were used as media since it was assumed that they are organic substrata generally utilized by all heterotrophic bacteria as they are the most easily assimilated sources of organic carbon. The coefficient of heterotrophic activity calculated in relation to glucose was assumed as the coefficient of the general heterotrophic activity of multi-species bacteria populations in natural lake water (Fig. 2 A). High values of this coefficient were stated in strongly eutrophized and polluted

[1] It is a metal device of 5 l capacity automatically closed in water by means of a weight lowered on a cord directly upon a closing mechanism placed in the cover, commonly used in collecting plankton samples.

lakes, containing the highest number of bacteria, that is, among the investigated ones, in Nidzkie and Guzianka lakes. In less eutrophized lakes this coefficient attained much lower values.

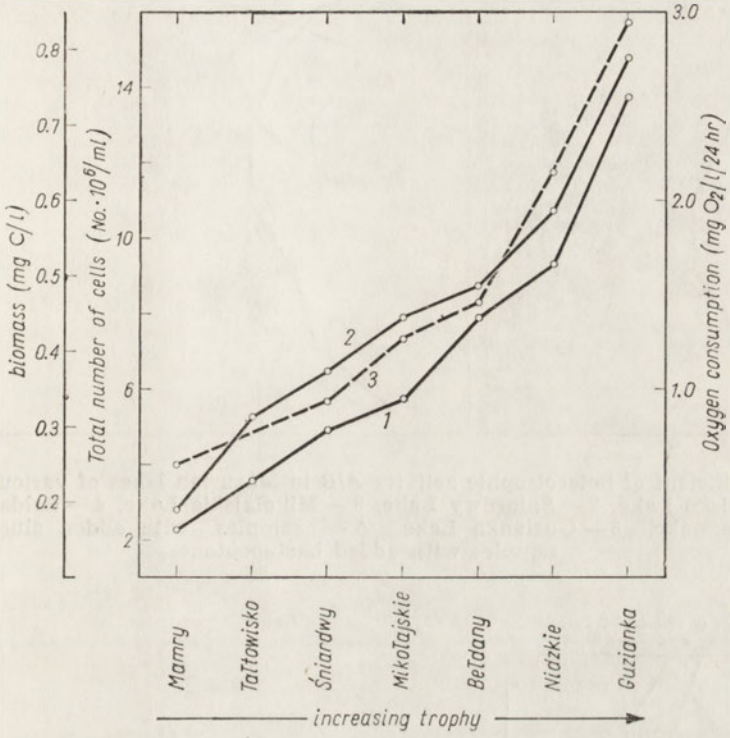

Fig. 1. Total number of bacteria (1), bacterial biomass (2) and oxygen consumption (3) in Mazurian lakes of various trophy

The results obtained when bactopeptone was used as substratum (Fig. 2 B) were similar to those acquired with the use of glucose. The only exception was a very high coefficient of activity in the Śniardwy Lake, many times higher than in other lakes. This might be due to the occurrence, in the water of the Śniardwy Lake, of large amounts of bacteria washed out of bottom deposits.

Cellobiose belongs to substances which are less easily dissolved than the former. As a product of the hydrolysis of cellulose it is utilized by some bacteria groups only. The value of the coefficient of cellobiotic activity in the investigated lakes is low (Fig. 3) and does not depend on the degree of eutrophy.

The coefficient of cellulolytic activity is worthy of notice. Cellulose is utilized by a group of bacteria producing cellulase — an enzyme hydrolyzating cellulose. It is a group of bacteria, heterogeneous from the

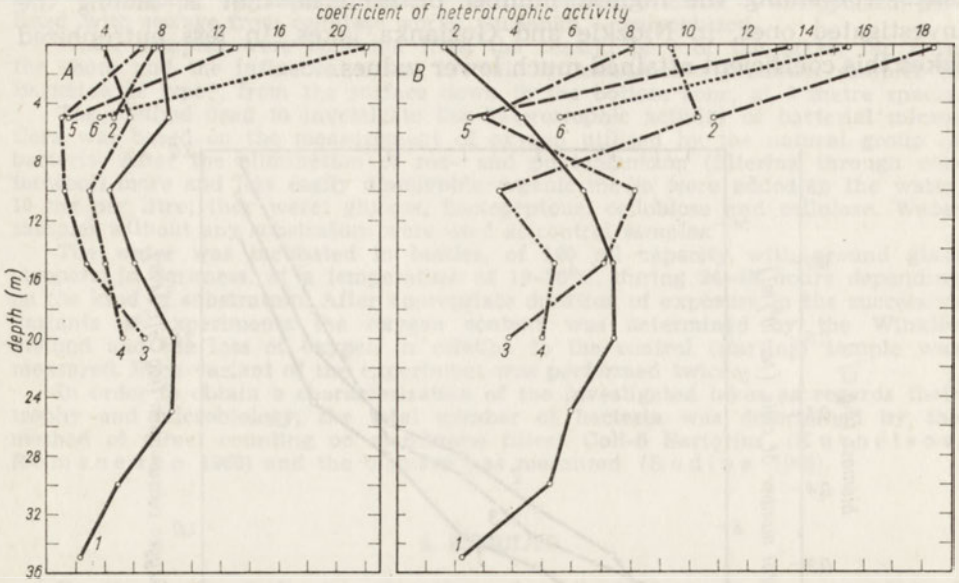

Fig. 2. Coefficient of heterotrophic activity *A/B* in Mazurian lakes of various trophy. 1 — Tałtowisko Lake, 2 — Śniardwy Lake, 3 — Mikołajskie Lake, 4 — Bełdany Lake, 5 — Nidzkie Lake, 6 — Guzianka Lake. A — samples with added glucose, B — samples with added bactopeptone

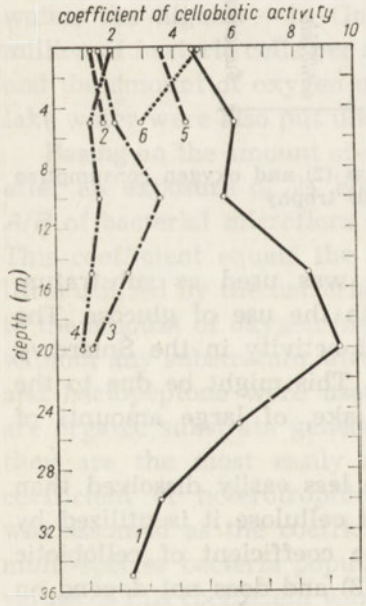

Fig. 3. Coefficient of cellobiotic activity (samples with added cellobiose) in Mazurian lakes of various trophy (lakes marked as in Fig. 2)

systematic point of view, and specific in its physiology on account of the occurrence of cellulase. In all the lakes here discussed the cellulolytic activity is low except the Nidzkie Lake where high values of the

coefficient of cellulolytic activity have been stated (Fig. 4). In the recent years this lake has been systematically polluted with sewage from the

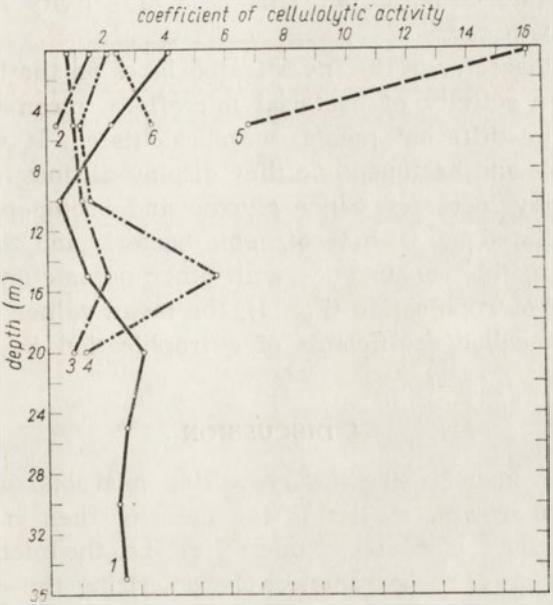

Fig. 4. Coefficient of cellulolytic activity (samples with added cellulose) in Mazurian lakes of various trophy (lakes marked as in Fig. 2)

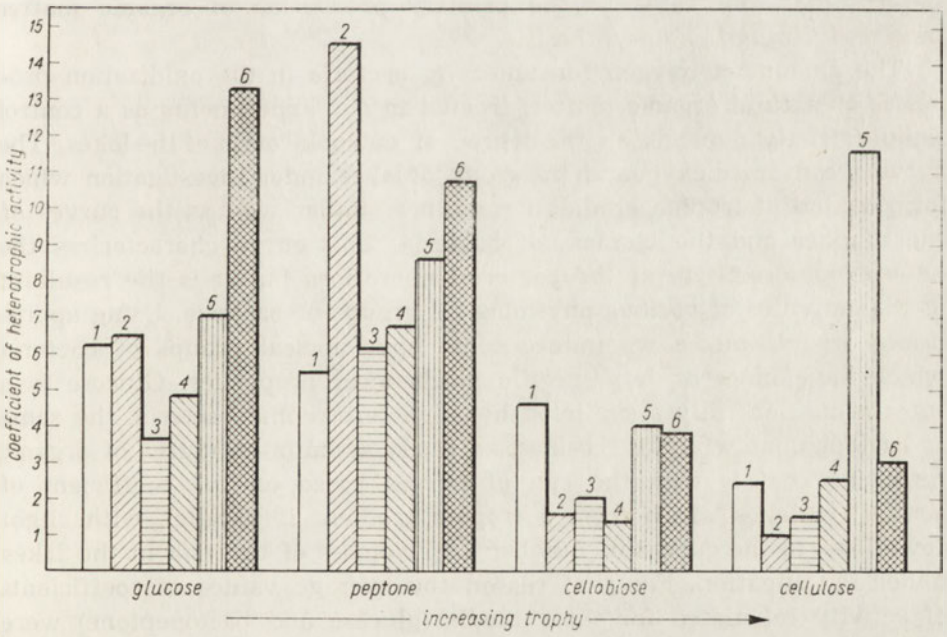

Fig. 5. Average values of coefficients of heterotrophic activity of bacterial microflora in Mazurian lakes of various trophy (lakes marked as in Fig. 2)

factory of cellulose and hard-board, situated in the vicinity. High values of cellulolytic activity considerably exceeding those of other lakes indicate a high state of pollution. This type of activity may be treated here as a diagnostic test.

In order to characterize the investigated lakes on the basis of a value summing up the activity of bacterial microflora, mean values of coefficients, with the different media, were calculated. It was established that with glucose and bactopeptone they display distinct increase tendencies as the trophy increases. Since glucose and bactopeptone are media generally assimilated by all heterotrophic bacteria and their mean coefficients show a visible concurrence with other parameters characterizing the processes of eutrophication (Fig. 1), the mean values of those coefficient have been called coefficients of eutrophication *Eu* (Fig. 5).

4. DISCUSSION

Heterotrophic bacteria are the prevailing microbial group in inland waters; dissolved organic matter is the basis of their metabolism. The heterotrophic activity of bacterial microflora, i.e. the intensity of utilizing organic media is one of the parameters characterizing the extent of trophication of water ecosystems, to the same degree as the total number of bacteria, their biomass and generation time (Godlewska-Lipowa 1970). Those parameters may constitute a criterion in the classification of lakes no less than the value of the primary production of organic matter produced through photosynthesis.

The amount of oxygen consumed by bacteria in the oxidization processes of natural organic matter, treated in our experiments as a control sample strictly depends on the degree of eutrophication of the lakes. The curve of consumed oxygen, in the group of lakes under investigation which form a kind of trophic gradient, runs in a similar way as the curves of the number and the biomass of bacteria. This curve characterizes the heterotrophic activity of the bacterial microflora but it is the resultant of the activities of various physiological groups of bacteria. Using appropriate organic media we induce some physiological groups of bacteria which have more or less specific biochemical properties. Glucose is a substratum generally assimilated by all heterotrophic bacteria, the same as bactopeptone which is, besides, an easily assimilated source of organic nitrogen. That is why the run of curves based on the coefficient of activity in lakes which form a trophic gradient is parallel, with slight deviations, to the curves of number and biomass of bacteria in the lakes under investigation. For that reason the average values of coefficients of activity calculated for those media (glucose and bactopeptone) were assumed to be the coefficients of the degree of eutrophication *Eu* of the lakes.

The coefficients of heterotrophic activity visibly tend to decrease with depth. It might be caused by the oxygen gradient occurring in lakes. High values were usually stated in surface water and in the trophogenic zones; as depth grew those values decreased.

The Tałtowisko Lake, untill now considered a mesotrophic lake (Hillbricht-Ilkowska, Spodniewska 1969), seems to be an eutrophic lake since it has a high coefficient of heterotrophic activity in relation to glucose and to bactopeptone and a great number of bacteria reaching 3.5—4.2 million per ml of water.

A high coefficient of heterotrophic activity in relation to bactopeptone occurs in the Śniardwy Lake. This is probably due to the turbulence of the water mass owing to which large numbers of bacteria are washed out of bottom deposits. The total number of bacteria in this lake ranges from 5.5 to 6.5 million per ml of water. These numbers are frequently higher than those of the eutrophic Mikołajskie Lake though the Śniardwy Lake displays a low degree of eutrophy.

The cellobiotic activity does not seem to depend on the degree of eutrophication while the cellulolytic activity visibly shows such a dependence. Cellulose is one of the basic products of photosynthesis of macrophytes and phytoplankton. This might account for the fact that the curve of cellulolytic activity rises as trophy grows, because it can be also considered, to a certain degree, an index of primary production of organic matter which is a fundamental criterion of classification of lakes.

An exception in the gradient system of the investigated lakes, owing to its very high coefficient of cellulolytic activity, is the Nidzkie Lake polluted with sewage from cellulose factory. It seems that, owing to the accumulation of large quantities of slowly dissolved cellulose, there occur large numbers of cellulolytic bacteria. In this case such high values of the coefficient of cellulolytic activity are an index of the degree of pollution of the lake.

Investigations on the heterotrophic activity of bacterial microflora in water ecosystems of various trophy are in their initial stage; much more thorough and detailed research is needed. The results obtained, however, seem to prove that they will be an additional and new criterion in the estimation of eutrophication and, perhaps, the degree of pollution of lakes, not only as statistical indices but as indices characterizing the dynamics of processes of the destruction of organic matter.

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UPTAKE OF ORGANIC MATTER BY NATURAL BACTERIA GROUPS IN THE MAZURIAN LAKES

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ABSTRACT

The investigations were carried out in seven Mazurian lakes of various degrees of eutrophication and concerned the intensity of the uptake of organic substrates: sucrose lactose, asparagin and salicyn, by natural bacteria groups occurring in natural lake water. The coefficient of heterotrophic activity of the bacterial microflora was calculated and it was established that in the case of asparagin it shows a visible tendency to grow as the eutrophy increases. Such a distinct dependence has not been observed with the other substrates.

1. INTRODUCTION

Heterotrophic planktonic bacteria are the most numerous group of organism participating in the decay processes of organic matter and, at the same time, the dominant group among microbes in water ecosystems (Zobell 1946, Kuznetsov 1959, 1970, Parsons, Strickland 1962). In the characteristics of bio-physico-chemical processes occurring in ecosystems their number is not essential but their activity, i.e. the ability of transforming organic matter.

Investigations of the assimilation of organic compounds by uni- and multi-species bacteria groups in water, carried out by means of substrates marked with radioactive carbon ^{14}C, (Hobbie, Wright 1965, Wright, Hobbie 1965, 1966, Vaccaro, Jannasch 1967, Hobbie, Crawford 1969) show a varying intensity of uptake of organic matter. The rate of growth of the number of bacteria cells in multi-species groups in lake waters of various trophy, in the presence of various media, differed visibly (Godlewska-Lipowa 1972a, b). This is a proof of the various activity of bacterial microflora.

The degree of trophy is closely connected not only with the amount of primary production, as it was commonly assumed up to now, but with the intensity of processes of destruction of organic matter (Caspers, Karbe 1966, 1967, Olszewski 1971).

The rate of decomposition of organic matter may be determined not only on the basis of the quantity of the uptake substrate (Hamilton, Austin 1967, Hamilton, Preslan 1970, Sorokin 1970, Takahashi, Ichimura 1971) but also of the consumption of oxygen from the environment (Godlewska-Lipowa 1973).

The aim of this paper was to investigate the assimilation of several organic compounds used by heterotrophic bacteria as a source of carbon, in lakes of various trophy. The investigated substrates were: lactose, sucrose, salicin and aspara-

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gin in quantities of 10 mg/l. The selected substrates are products of photosynthesis of water plants and for that reason they occur, in smaller or greater quantities, in the natural environment. Lactose is an exception it is foreign to natural waters but it was used as it is organic matter flowing into water with industrial and municipal sewage.

2. MATERIAL AND METHODS

Investigations were carried out in July and August 1972 in the mesotrophic Mamry Lake, the eutrophic Tałtowisko Lake, the eutrophic Śniardwy Lake, the eutrophic, holomictic Mikołajskie Lake, the eutrophic Bełdany Lake, the eutrophic Nidzkie Lake, polluted with industrial sewage from a factory of cellulose, and the eutrophic Guzianka Lake (Olszewski, Paschalski 1959).

Water samples were collected from the central part of the lakes by means of a plankton sampler of Bernatowicz type, from the surface to the bottom zone at 5 m spaces.

The water was devoid of zoo- and phytoplankton (filtering through cotton-wool) and organic substrates were added. Water samples without substrates were regarded as control samples. The water was incubated in bottles of 120 ml capacity with ground glass stoppers, in darkness, at the temperature of 19–20°C during 12–24 hours. After appropriate periods of exposure in the successive variants of the experiment the oxygen content was determined by Winkler's method and the loss of oxygen in relation to the initial sample was measured (mg/l). Each variant of the experiment was performed twice.

On the base of the quantity of oxygen uptaken by bacteria, the coefficient of heterotrophic activity of bacteria microflora in relation to the investigated substrates was calculated. It was assumed to be the relation A/B, where A was the amount of oxygen (mg O_2/l) uptaken by bacteria after an exposure of 24 hours, in samples with substrate added, while B was the amount of oxygen consumed by bacteria, in the same time, in samples not containing any substrate.

Basing on the values of coefficients of activity calculated for different depths in the investigated lakes, mean coefficients, characterizing the particular lakes, were calculated.

In order to characterize the lakes as regards their microbiology the total number of bacteria was determined by the method of direct counting on membrane filters Coli-5 Sartorius (Kuznetsov, Romanenko 1963) and the biomass was calculated (Rodina 1965).

3. RESULTS

The lakes selected for investigations form a trophic gradient characterized by the increase of the total number of bacteria and of the bacterial biomass simultaneous with the growth of trophy (Fig. 1). The gradient of number range within large limits — from 2.1 milion per ml in the mesotrophic Mamry Lake to 13.0 milion per ml of bacteria cells in the most eutrophized among the lakes, the Guzianka Lake. The biomass increases parallelly to the number. The amount of oxygen uptake by bacteria in the water samples devoid of phyto- and zooplankton after 24 hours of exposure, without any substrate, visibly depends on the degree of trophy of the lake. In the Mamry Lake the quantity of the uptake oxygen merely amounted to 0.3 mg/l · 24 hours while in the Guzianka Lake — containing the highest number of bacteria — it was 3.0 mg/l · 24 hours i.e. ten times as much (Fig. 1, curve No. 3).

The coefficient of activity calculated for lactose (Fig. 2 A) showed

visible differences depending on the depth; the lowest values were obtained from the bottom zone, the highest — in the trophogenic zone. The lowest mean values of the coefficient of activity (Fig. 2 B) were obtained for the Mamry Lake, slightly higher values — for the lakes: Tałtowisko, Śniardwy and Mikołajskie; in the Bełdany Lake a slight decrease of the coefficient was observed as compared with the values obtained for the lakes mentioned above, and in the Guzianka Lake there was a slight increase.

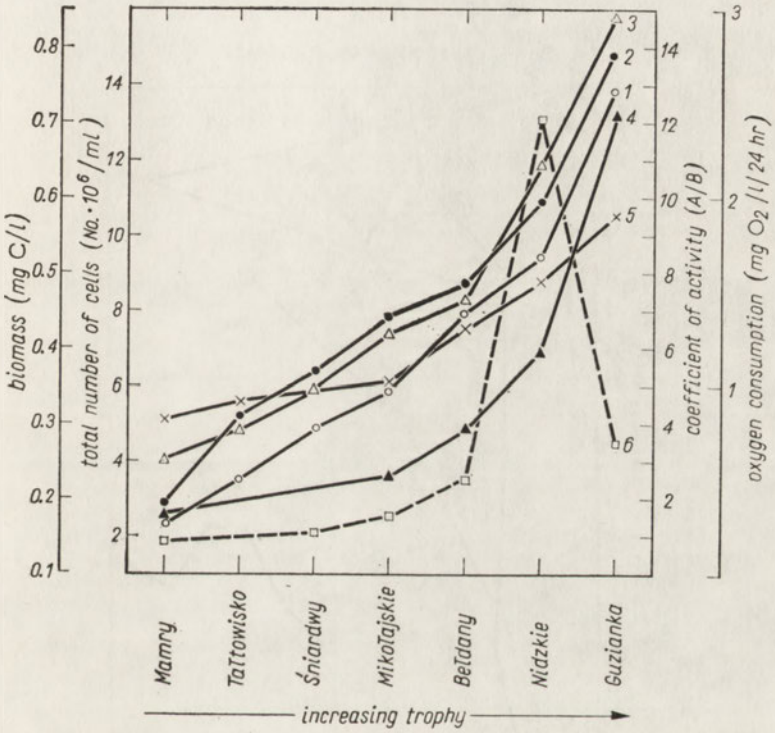

Fig. 1. Total number of bacteria (million per ml) (1), biomass (2), mg O_2/l/24 hours uptaken by bacteria (3) and coefficients of heterotrophic activity calculated for glucose (4) for bactopepton (5) and for cellulose (6), in Mazurian lakes of various trophy (Godlewska-Lipowa 1973)

A similar structure of curves was obtained for sucrose (Fig. 3) with the only difference that the coefficient did not so visibly depend on depth. The same as with lactose, an increase of the mean values of the coefficient could be observed progressing from the Mamry Lake to the Mikołajskie Lake parallelly to the growth of the number of bacteria, thus to the degree of trophy, then a visible decrease in the Bełdany and Guzianka lakes.

Asparagin belongs to the group of organic compounds easily assimilated by bacteria and is used by them as a source of carbon and organic nitrogen. In the system of curves of the activity coefficient calculated for various depths, in the investigated lakes, no distinct dependence of its uptake on the depth has been observed. In the lakes: Mamry, Tałtowisko, Śniardwy and Bełdany (Fig. 4 A) higher values of the coefficient occurred in the bottom zone, slightly lower values — in the trophogenic zone, while in the lakes Mikołajskie and Guzianka the value of the coefficient of activity decreased as depth grew.

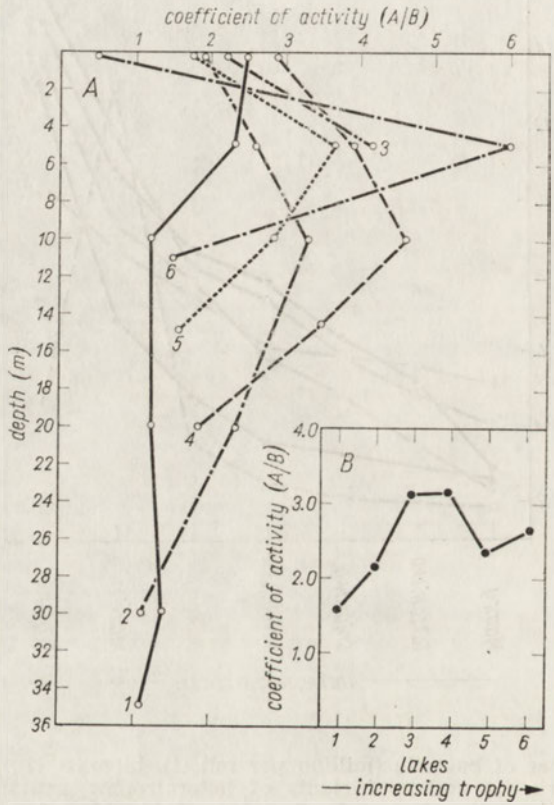

Fig. 2. Coefficient of heterotrophic activity (water samples with lactose) in Mazurian lakes of various trophy. 1 — Mamry, 2 — Tałtowisko, 3 — Śniardwy, 4 — Mikołajskie, 5 — Bełdany, 6 — Guzianka. A — dependence on depth, B — mean values

The mean values of the coefficient (Fig. 4 B) were the lowest for the Mamry Lake, while they were highest for the Guzianka Lake parallelly to the increase of the number of bacteria and, consequently, of the degree of trophy.

Salicin is a glycoside assimilated only by some physiologic groups of bacteria. The degree of its uptake in the investigated lakes, except the Mamry Lake, showed a tendency to grow with depth, the values of the coefficient were higher in the bottom zone than in the epilimnion (Fig. 5 A). The mean values of the coefficient (Fig. 5 B) did not indicate any great differences between the lakes, nevertheless the lowest values were obtained for the Mamry Lake and the highest for the Guzianka Lake.

The degree of uptake of the media discussed above was also investigated in the Nidzkie Lake which is polluted with sewage from the cellu-

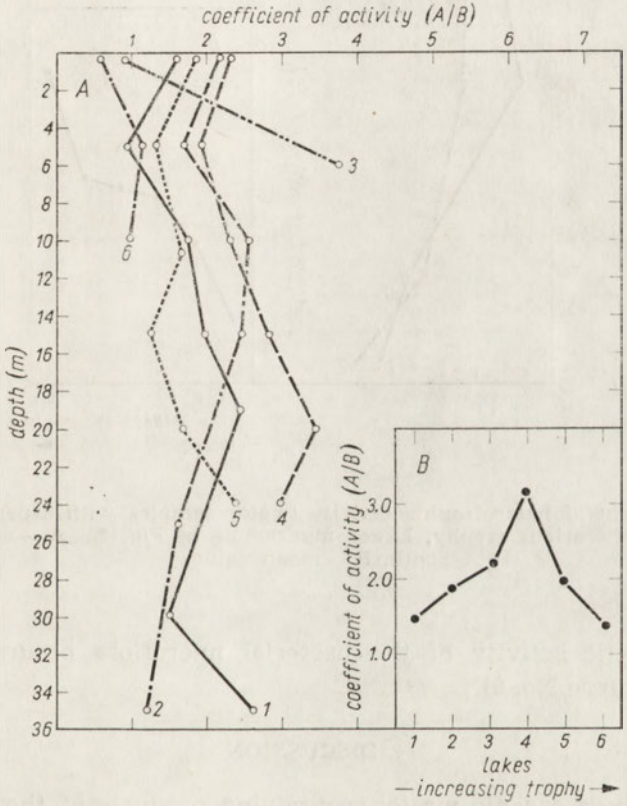

Fig. 3. Coefficient of heterotrophic activity (water samples with sucrose) in Mazurian lakes of various trophy. Lakes marked as in Fig. 2. A — dependence on depth, B — mean values

lose works. In all the cases the values of the coefficient of activity were very low, frequently they were lower than these values in the Mamry Lake. Though other parameters of the Nidzkie Lake indicate its high trophy (Fig. 1), it seems that the mentioned fact is due to the unusually

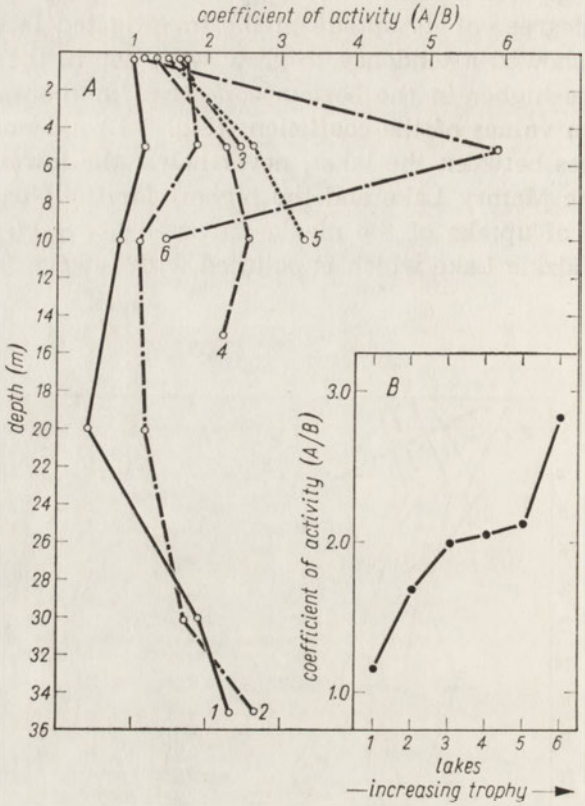

Fig. 4. Coefficient of heterotrophic activity (water samples with asparagin) in Mazurian lakes of various trophy. Lakes marked as in Fig. 2. A — dependence on depth, B — mean values

high cellulolytic activity of the bacterial microflora occurring in this lake (Fig. 1, curve No. 6).

4. DISCUSSION

The dissolved organic matter, containing products of the metabolism of plant and animal organisms, is used by heterotrophic microbes, in a complicated process of bio-physico-chemical transformations, for the biosynthesis of the bacterial mass. Glucose and bactopepton are substrates assimilated by all heterotrophic bacteria, apart a few exceptions. They are easily oxydized and the rate of their uptake, expressed by the coefficient of heterotrophic activity of bacterial microflora (Godlewska-Lipowa 1973) is proportional to the number of bacteria in water ecosystems and thus it characterizes the degree of eutrophication (Fig. 1, curves 4 and 5). Organic compounds of the disaccharide type, e.g. sucrose,

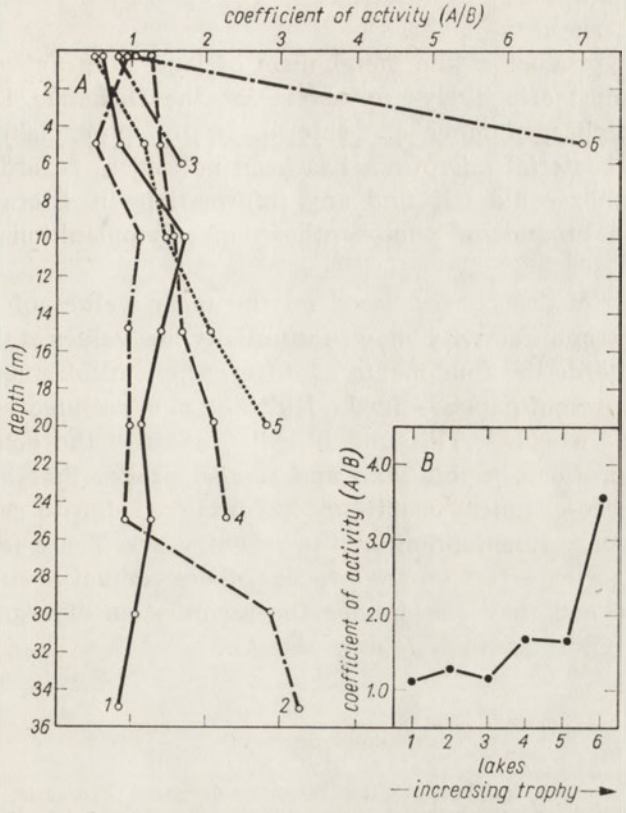

Fig. 5. Coefficient of heterotrophic activity (water samples with salicin) in Mazurian lakes of various trophy. Lakes marked as in Fig. 2. A — dependence on depth, B — mean values

are used by some physiological groups of bacteria. In less eutrophized lakes sucrose is more intensely uptaken than in more eutrophized lakes. It seems, then, that the effectiveness of its assimilation is not connected with number but with the physiological activity of bacterial groups occurring in lakes and possibly also with some species groups of bacteria.

Lactose, which is also a disaccharide though one of another type, is uptaken by bacteria from land environments which get into water ecosystems with allochtonic matter, municipal and industrial sewage, containing lactose. The effectiveness of its uptake is higher in less eutrophized lakes with smaller amounts of organic matter; may be this is due to the fact that highly eutrophized lakes are richer in organic matter of another type, which is used by heterotrophic bacteria as a source of energy.

Asparagin is a compound easily assimilated by various physiological groups of bacteria which can be observed in the visible dependence of

the degree of its uptake on the degree of eutrophication and thus on the number of bacteria in the lakes.

Salicin — a product of the metabolism of land flora *Salix sp.* in the first place is most effectively uptaken in the Guzianka Lake which contains the highest number of bacteria. In the other lakes no high activity of the bacterial microflora has been noticed as regards this substrate. The author could not find any information in literature as to whether it is a product of photosynthesis of phytoplankton and water flora.

The analysis of the curves based on the mean values of the coefficients of heterotrophic activity showed unusually low values of these coefficients — as regards the four media of different chemical structure investigated in the present paper — in the Nidzkie Lake, polluted with sewage from the cellulose works. This undoubtedly indicates the domination of cellulolytic microflora in this lake and it also proves that an essential change of physico-chemical conditions has occurred due to sewage from the cellulose works functionning in the recent years. These factors seem to have a hampering effect on the process of destruction of organic matter in this lake and they also hinder the assimilation of organic matter even if it is as highly assimilable as asparagin.

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The author is much indebted to the recently deceased Professor dr. Przemysław Olszewski for his valuable suggestions which have helped her in formulating the conception of the present paper.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	21	1	69-73	1974

H. PETRYCKA

THE EFFECT OF TEMPERATURE ON THE NUMBER OF BACTERIA IN WATER DETERMINED BY THE METHOD OF POURED PLATES

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ABSTRACT

River water bacteria were cultivated at the temperatures of 22, 27, 32, 37°C during periods of 24, 48, 72 and 96 hours and of 10 days, on agar medium. It was established that the most suitable conditions for the development of the greatest number of bacteria in river water inoculations were the temperatures of 22 and 27°C in the winter–spring period and 32°C in the other seasons, the best time was 96 hours. Instead, at the temperature of 37°C there grew several times less bacteria than at 22–32°C which proves that this temperature is useless in the investigations of saprophytic microflora in water environment.

1. INTRODUCTION

The determination of the number of saprophytic bacteria in water is most usually carried out by the method of poured plates on nutrient agar, on gelatin broth and recently also on "natural feeding". Inoculations are cultivated at the temperatures of 20, 22, 23°C or 25, 26 and 28°C during a period of 48–72 hours and up to 10 or even 14 days (Paluch 1963, Rodina 1965, Daubner 1967, Strzelczyk et al. 1969).

Though this method does not show all the microorganisms occurring in water and it bears numerous methodical errors (Paluch 1958), in systematic check-up investigations it is an index of the degree of pollution of the water body with organic substances. On the other hand, when connected with other microbiological, hydrobiological and physico-chemical investigations, it completes the results which characterize the processes of metabolism in water. In the present paper an attempt has been made to show which of the temperatures enumerated above and how long cultivation on nutrient agar are most favourable for bacteria to grow in greatest numbers.

2. MATERIAL AND METHODS

Water samples were collected from the Ruda River in Wielopole and in Stodoły and from the Nacyna River in Rybnik.

The Ruda River in Wielopole was slightly polluted. The amount of organic nitrogen ranged from 0.0 to 1.68 mg/l, and that of ammonium nitrogen — from 0.30 to 2.5 mg/l. The temperature of water was, in autumn, winter and spring from 1.5 to 8.4°C, and in summer from 11 to 16°C. Instead, in the village of Stodoły, below the estuary of the Nacyna, the pollution of the Ruda River increased. Organic nitrogen ranged from 0.0 to 8.0 mg/l and ammonium nitrogen — from 0.15 to 2.5 mg/l. The temperature of water, with slight differences, was the same as in Wielopole.

The Nacyna River in Rybnik was strongly polluted. It carried large quantities of mud and coal and of organic refuse. Organic nitrogen ranged from 3.08 to 7.87 mg/l, and ammonium nitrogen — from 0.15 to 6.00 mg/l. The temperature of water in the autumn–winter–spring period oscillated between 4 and 13°C while in summer it reached 16–18°C.

On the ground of hydrobiological, microbiological and physico-chemical investigations the Ruda River in Wielopole was qualified as belonging to the β-mesosaprobic zone and in Stodoły — to the α-mesosaprobic zone. The Nacyna River was recognized as highly polluted; it was characterized by a lack of typical indices of the polysaprobic zone (Kluczycki et al. 1967).

Water samples for microbiological investigations were collected once a month during one year. The water was inoculated, by the method of poured plates, on nutrient agar produced by the firm "DIFCO", in four parallel repetitions. Plates with inoculated material were cultivated at the temperatures of 22, 27, 32 and 37°C during 24, 48, 72 and 96 hours and up to 10 days. After those periods of cutlivation the colonies of bacteria appearing on the medium were counted and marked with Indian ink on the outside bottom part of the plate.

3. RESULTS AND DISCUSSION

The results presented in Fig. 1 ABC illustrate the amounts of bacteria colonies obtained on nutrient agar from 1 ml of water after ten days cultivation at the temperatures of 22, 27, 32 and 37°C, as well as the changes in the numbers of bacteria in water depending on the season.

The amounts of bacteria colonies obtained at 22, 27 and 32°C after 10 days of cultivation are similar, apart from slight differences in the winter-spring period.

From April to May for the Ruda River and from January to April for the Nacyna, at 1.5 to 10°C of temperature of water, the total average numbers of thousands of bacteria colonies cultivated at 22, 27 and 32°C were: for the water from the Ruda River at Wielopole 2.3, 2.0 and 2.0, at Stodoły — 46.0, 64.0 and 37.0; for the Nacyna River they were 303.0, 198.0 and 91.0.

It results that in the winter–spring period a higher average number of cultivated bacteria grew at the temperatures of 22 and 27°C than at 32°C. Instead, the results obtained at the temperature of 37°C were several times smaller than at 22, 27, and 32°C. The cleaner and colder the water the greater were these differences. It results that the temperature of 37°C checks the development of numerous bacteria occurring in water.

One of the more important factors affecting the life processes of microorganisms and their number in water particularly in polluted water carrying considerable amounts of allochthonous microflora, is the temperature. This is evidenced by the numerical results obtained in the inoculations of water in the autumn–winter–spring season (Fig. 1 ABC). In November when the temperature of water was 2.0–2.5°C in the Ruda and 4.0°C in the Nacyna, the number of bacteria in inoculations of water from those rivers was several times lower as compared with the results obtained in summer. The greatest differences between the summer months

and November were noted in cultures kept at 37°C. In December and January, in the inoculations on water from the α-mesosaprobic and highly polluted zone of the Nacyna, the number of bacteria increased

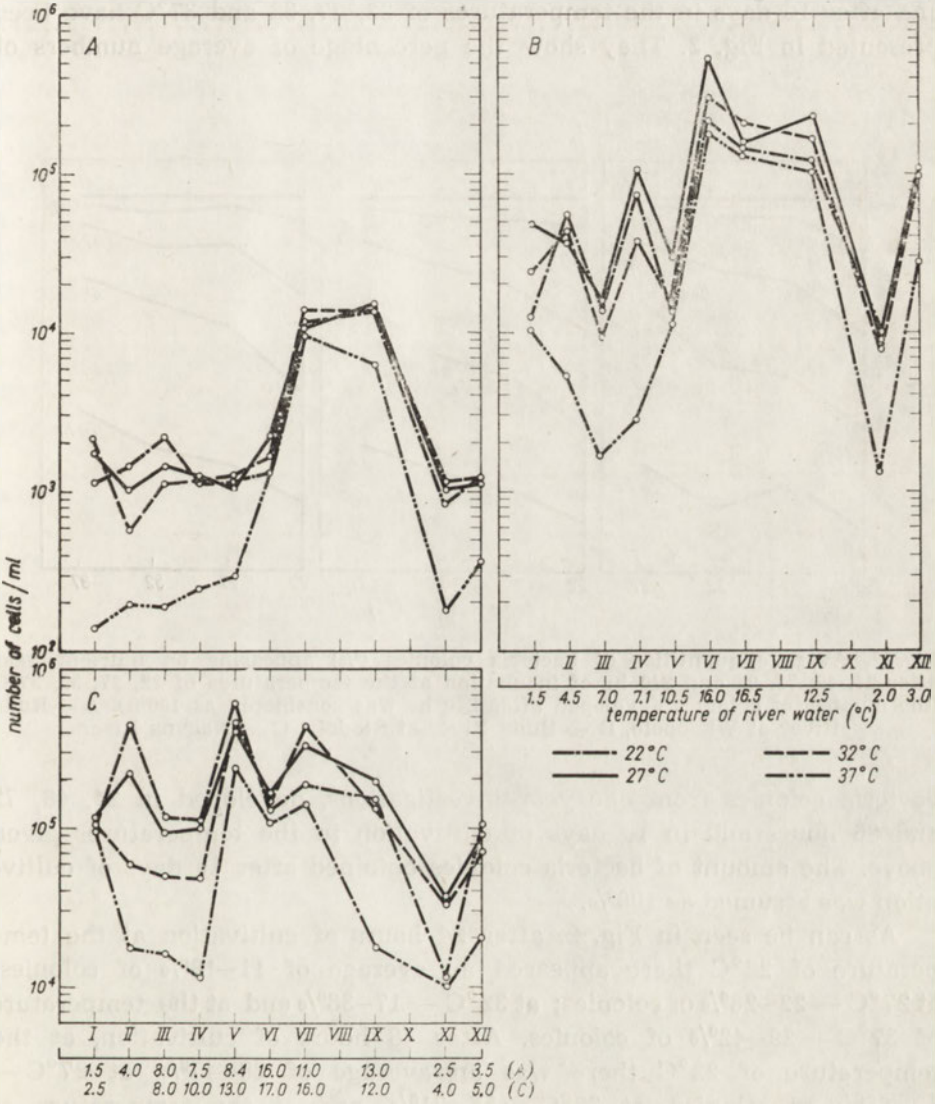

Fig. 1. Number of bacteria colonies obtained from 1 ml of river water on nutrient agar after 10 days cultivation at 22, 27, 32, 37°C (semi-log scale). A — Ruda River at Wielopole, B — Ruda River at Stodoły, C — Nacyna River

several times, but the number of bacteria from the β-mesosaprobic zone was only slightly higher than that obtained in November. Such numbers of bacteria persisted until April in the α-mesosaprobic and highly polluted zone of the Nacyna, and until June in its β-mesosaprobic zone.

The development of maximum numbers of bacteria on nutrient agar from inoculated water frequently depends not only on environmental factors but also on the duration of the cultivation.

The results of cultivation obtained after 24, 48, 72, and 96 hours and after 10 days in the temperatures of 22, 27, 32 and 37°C have been presented in Fig. 2. They show the percentage of average numbers of

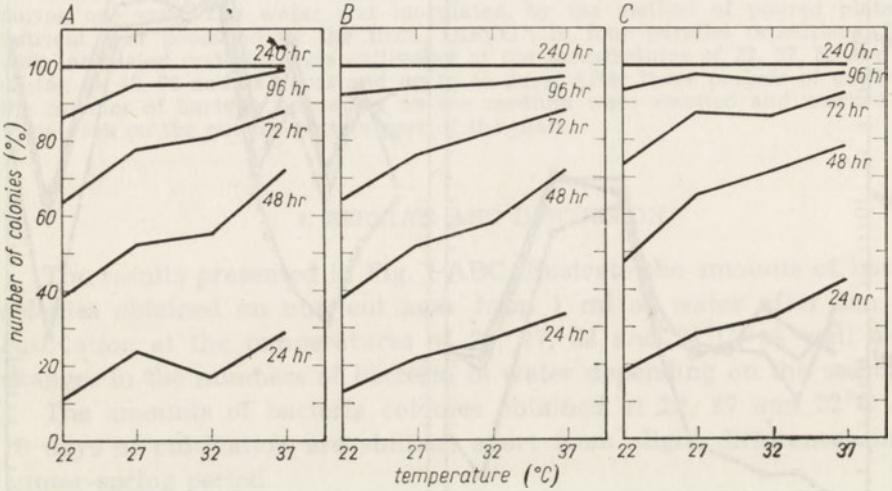

Fig. 2. Average quantities of bacteria colonies (%) appearing on nutrient agar after 24, 48, 72, 96 and 240 hr of incubation at the temperatures of 22, 27, 32, 37°C (the number of colonies developed after 240 hr was considered as 100%). A — Ruda River at Wielopole, B — Ruda River at Stodoły, C — Nacyna River

bacteria colonies from one year investigations, developed in 24, 48, 72 and 96 hours and in 10 days of cultivation in the temperatures given above. The amount of bacteria colonies obtained after 10 days of cultivation was assumed as 100%.

As can be seen in Fig. 2, after 24 hours of cultivation at the temperature of 22°C there appeared an average of 11–18% of colonies; at 27°C — 22–28% of colonies; at 32°C — 17–38% and at the temperature of 37°C — 29–42% of colonies. After 48 hours of cultivation, at the temperature of 22°C there was an average of 38–47%, at 27°C — 52–65% of colonies, at 32°C — 55–71%, and at the temperature of 37°C — 72–78% of colonies. After 72 hours of incubation, at 22°C there grew an average of 64–73% of colonies, at 27°C — 76–87% of colonies; at 32°C the average was 81–86% and at the temperature of 37°C — 88–92% of colonies. After 96 hours of cultivation the average development at 22°C was 86–93% of colonies, at 27°C — 95–97%; at 32°C there was 95% of colonies and at the temperature of 37°C — 95–99% of colonies.

Thus the growing rate of bacteria colonies on the medium increased in proportion to the height of temperature.

It results from the data given above that the best temperatures for a maximum development of saprophytic bacteria in inoculations of surface waters range between 22 and 32°C. However, in winter-spring months, when the temperatures of water are lower, the temperatures of 22 and 27°C may be more appropriate and 96 hours may be a sufficient time of cultivation.

The temperatures of 22, 27, and 32°C proposed for cultivation in order to show the greatest number of bacteria in surface water come within the range of temperatures used by Strzelczyk et al. (1969) and by Kuznetzov, Romanenko (1963). Instead, the duration of the culture, 10 and 14 days, practiced by Strzelczyk et al. (1969) and 7 days by Niewolak (1968) may be useful in investigations of water where autochthonous microflora prevails. But with inoculations on nutrient agar of water containing large quantities of allochthonous microflora the period of 96 hours proved to be sufficient.

In the present investigations it is also worthy of notice that the results were characterized by their frequent recurrence and the number of bacteria in the cultures grew in proportion to the degree of pollution of the river, which may have a practical meaning particularly in test investigations of river water polluted with e.g. municipal or industrial sewage.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	21	1	75-82	1974

A. SOLSKI

THE INFLUENCE OF DISCHARGED HEATED WATERS FROM THE POWER STATION AT SKAWINA ON MICROFLORA OF VISTULA RIVER

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ABSTRACT

The influence of discharges of heated water from the power station at Skawina on the occurrence of microorganisms in water and bottom sediments of Vistula River was studied. The investigations were made from May 16, 1968 to February 18, 1969, in the 13 km section of Vistula River and in the estuary of Skawinka River. The total number of bacteria, among which psychro-, meso- and thermophilic bacteria were distinguished, as well as some physiological groups of bacteria were investigated in the present study.

1. INTRODUCTION

Thermic pollution of surface waters has recently become a problem in Poland. It will be increasingly difficult with the development of new power stations, mostly lignite operated, but prospectively also nuclear.

That is why the Meteorology and Water Economics Research Institute, in cooperation with some scientific institutions, has undertaken the investigations of heated water from the power station at Skawina. The investigations comprised the physico-chemical analyses as well as examinations of the communities of aquatic organisms, thus also microorganisms, representing the main trophic levels of a water body.

Microbiological analyses of water and bottom sediments were made in the Department of Agricultural Microbiology of the Agricultural University at Cracow (Florczyk et al. 1970).

2. METHODS

The investigations were made between May 16, 1968 and February 18, 1969 in the section of Vistula River, 13 km long, (km 60th–73rd), as well as in the estuary of Skawinka River (Fig. 1).

Five sampling stations were established (No. 1, 3, 4, 5, 6) on Vistula River and one (No. 2) on Skawinka River. Water and bottom sediments were sampled near both river banks and in the middle part of a stream; water was sampled from all the stations and bottom sediments from stations No. 1, 2, 4, 6.

The following microbiological analyses were made:

1. Quantitative determinations of total number of bacteria, in which psychro-, meso- and thermophilic bacteria were distinguished, as well as proteolytic, nitrifying and denitrifying bacteria, *Escherichia coli* bacteria group and fungi;

2. Qualitative determinations of bacteria fixing free nitrogen, Sphaerotilus-Leptothrix bacteria group, sulphur bacteria, phosphate-positive bacteria, cellullose decomposing bacteria and photosynthetic bacteria.

These determinations were made according to the methods used in the Department of Agricultural Microbiology of the Agricultural University at Cracow (S m y k 1969).

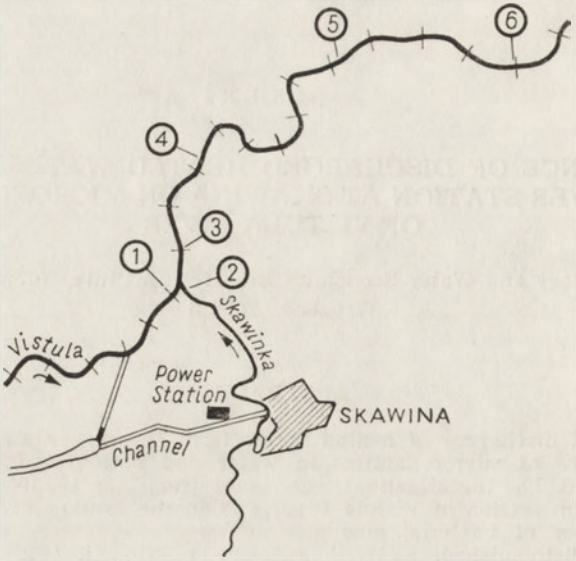

Fig. 1. Situation layout of the studied section of Vistula River. 1–6 — sampling stations

3. RESULTS

THE CHANGES OF TOTAL NUMBER OF BACTERIA IN WATER

The total number of bacteria in water sampled from the regions not affected by heated water (Fig. 2) varied from dozen thousands to about 400,000 cells/ml, with two extremes in November—December (about 280,000 cells/ml) and March—April (about 400,000 cells/ml) (Fig. 2).

The total number of bacteria in the zone affected by the heated water (stations No. 3, 4, 5 at the right bank) varied from dozen thousands to about 80,000 cells/ml without any visible changes during a year (Fig. 2). The similar pattern of the total number of bacteria was observed in water of Skawinka River (Fig. 3).

The psychrophilic bacteria were the most numerous in the unheated region: 10,000—370,000 cells/ml. The two mentioned extremes in autumn and early spring were depending on the intense development of this group. In the heated region, the psychrophilic bacteria were distinctly less numerous, at the estuary of Skawinka River being as scarce as 2000—20,000 cells/ml. In water of Skawinka River they were in numbers not higher than few thousands cells per 1 ml during the whole period of investigations (Fig. 3).

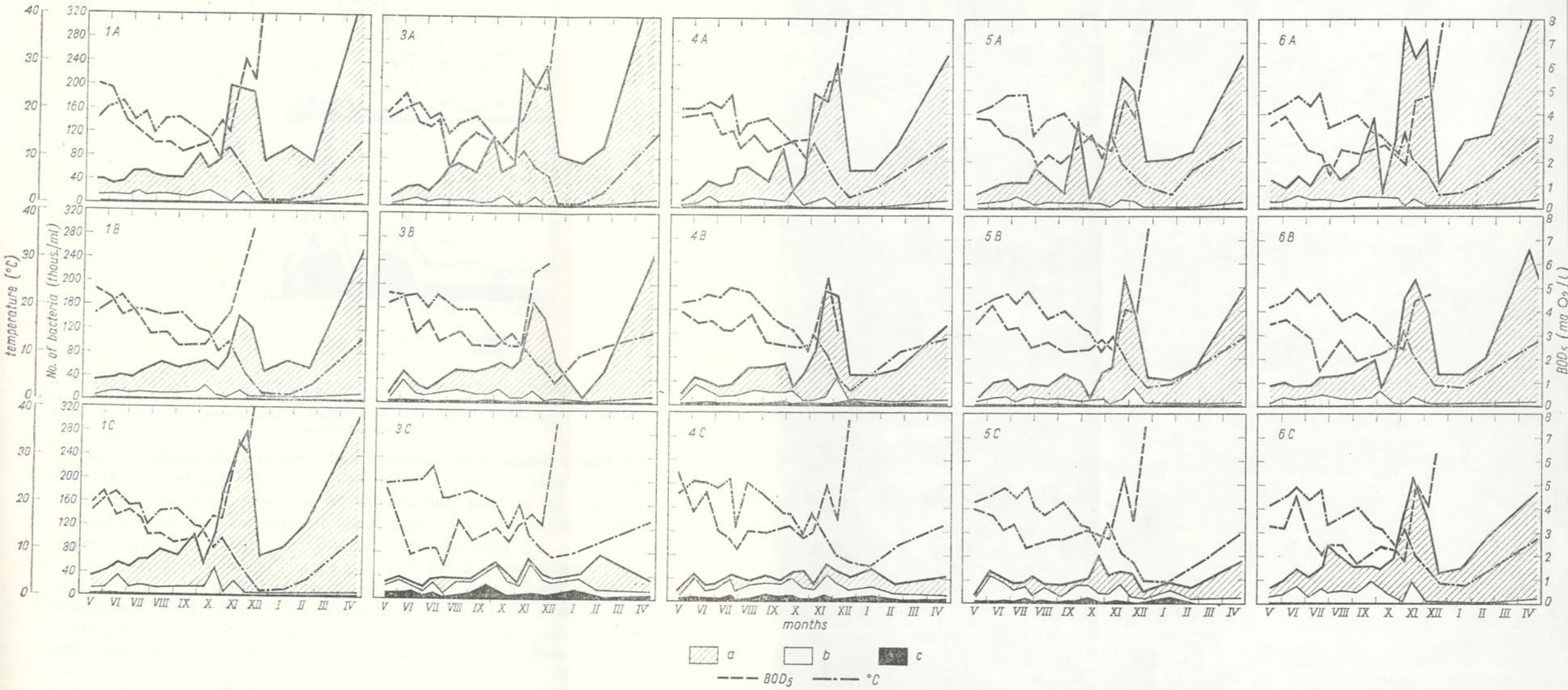

Fig. 2. The changes of total number of bacteria in water of Vistula River depending on temperature and BOD_5 (May 16, 1968 — April 24, 1969). 1–6 — sampling stations, A — left bank, B — midstream, C — right bank, *a* — psychrophilic bacteria, *b* — mesophilic bacteria, *c* — thermophilic bacteria

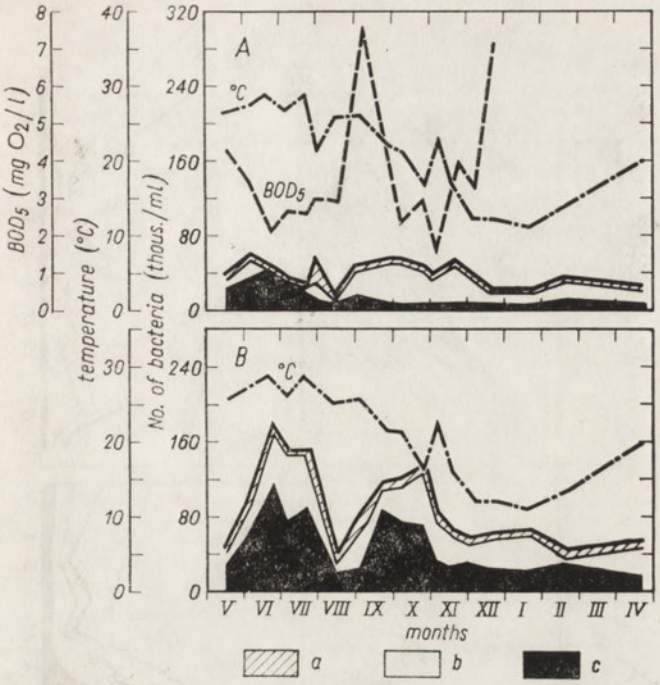

Fig. 3. The changes of total number of bacteria in water (A) and bottom sediment (B) of Skawinka River depending on temperature and BOD_5 (May 16, 1968 — April 24, 1969), *a* — psychrophilic bacteria, *b* — mesophilic bacteria, *c* — thermophilic bacteria

The mesophilic bacteria were less numerous in unheated waters (up to 20,000 cells/ml and only about 1000 cells/ml in winter) than in heated waters (varying from 10,000 to 50,000 cells/ml) (Fig. 2). In water of Skawinka River they amounted to about 1000 cells/ml in summer period (June–August) and about 45,000 cells/ml in autumn (Fig. 3).

The thermophilic bacteria were scarce in the unheated region. They were visibly more numerous in Vistula River just at the estuary of Skawinka River (10,000–50,000 cells/ml). Their numbers gradually decreased down the river and in station No. 6 (the right bank) the thermophilic bacteria were in numbers similar to those in the unheated region. The most numerous these bacteria were in Skawinka River from May to July (up to 40,000 cells/ml) (Fig. 3). In other periods there were 5500–13,000 cells/ml.

THE CHANGES OF TOTAL NUMBER OF BACTERIA IN BOTTOM SEDIMENTS

In the bottom sediments of the investigated section of Vistula River the total number of bacteria varied from 4000 to 550,000 cells/ml during the period of investigations (Fig. 4); the least numbers were recorded in May and June while at the beginning of July they increased up to about

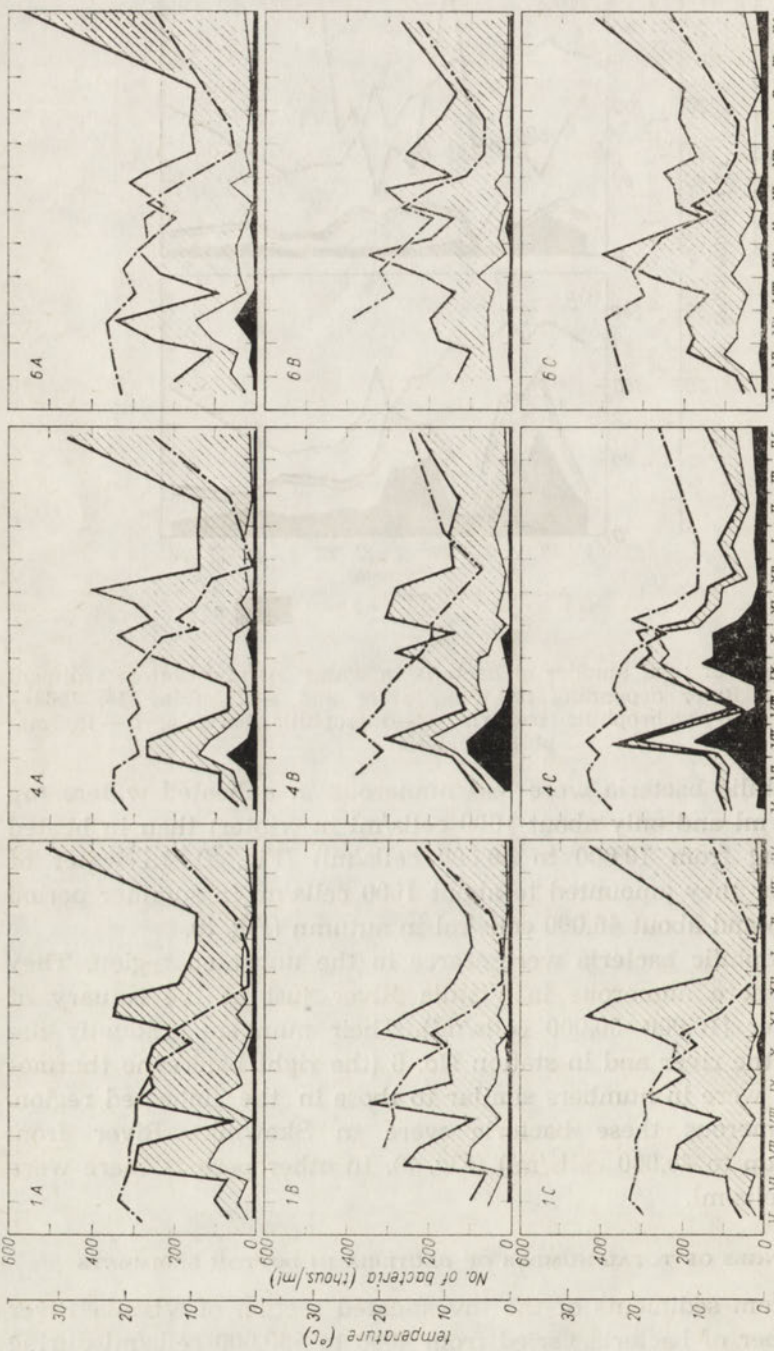

Fig. 4. The changes of total number of bacteria in bottom sediments of Vistula River depending on temperature and BOD_5 (May 16, 1968 — April 24, 1969). 1, 4, 6 — sampling stations, A — left bank, B — midstream, C — right bank; *a* — psychrophilic bacteria, *b* — mesophilic bacteria, *c* — thermophilic bacteria

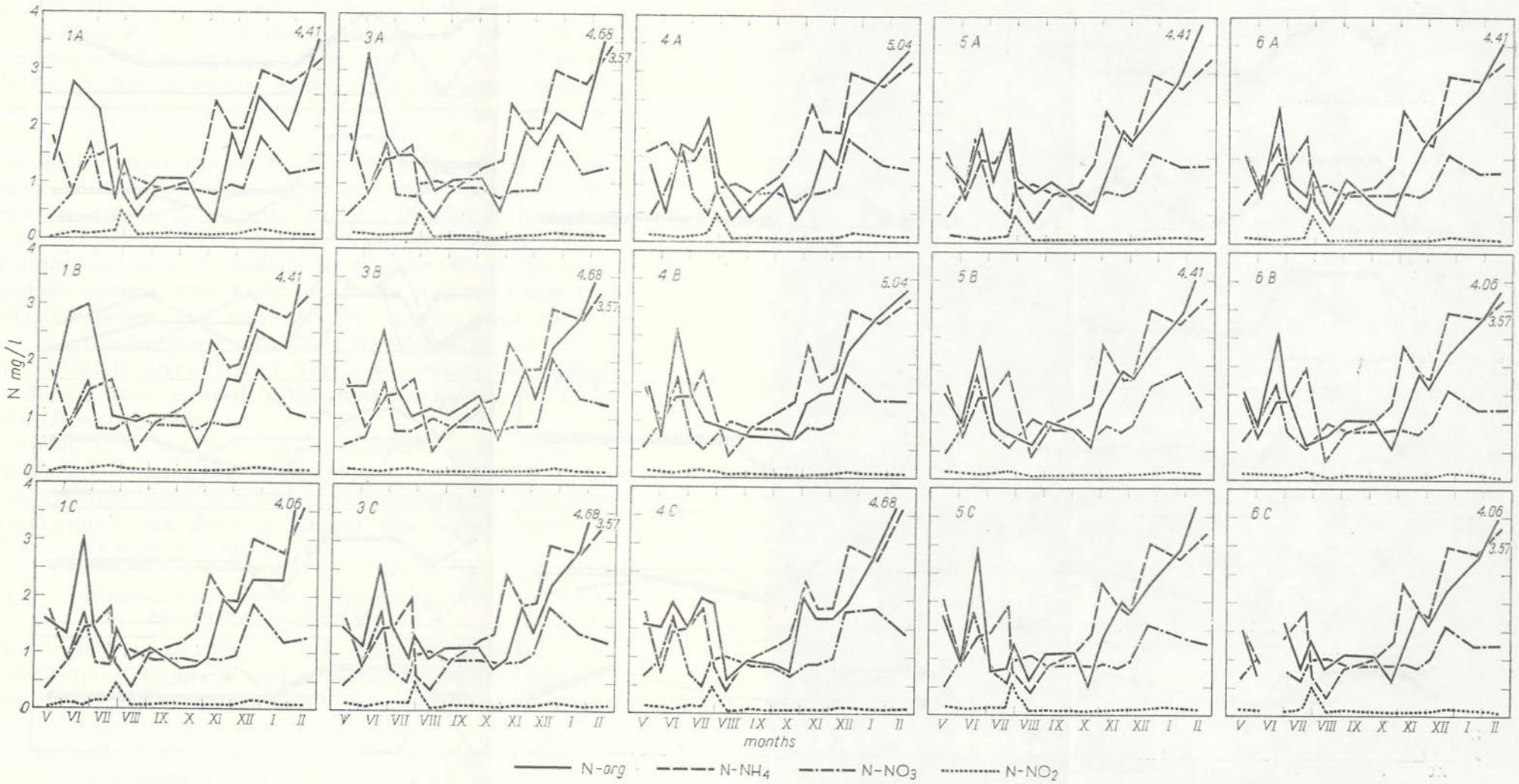

Fig. 5. The changes of mineral and organic nitrogen content in Vistula River (May 16, 1968 — February 18, 1969). 1–6 — sampling stations
A — left bank, B — midstream, C — right bank

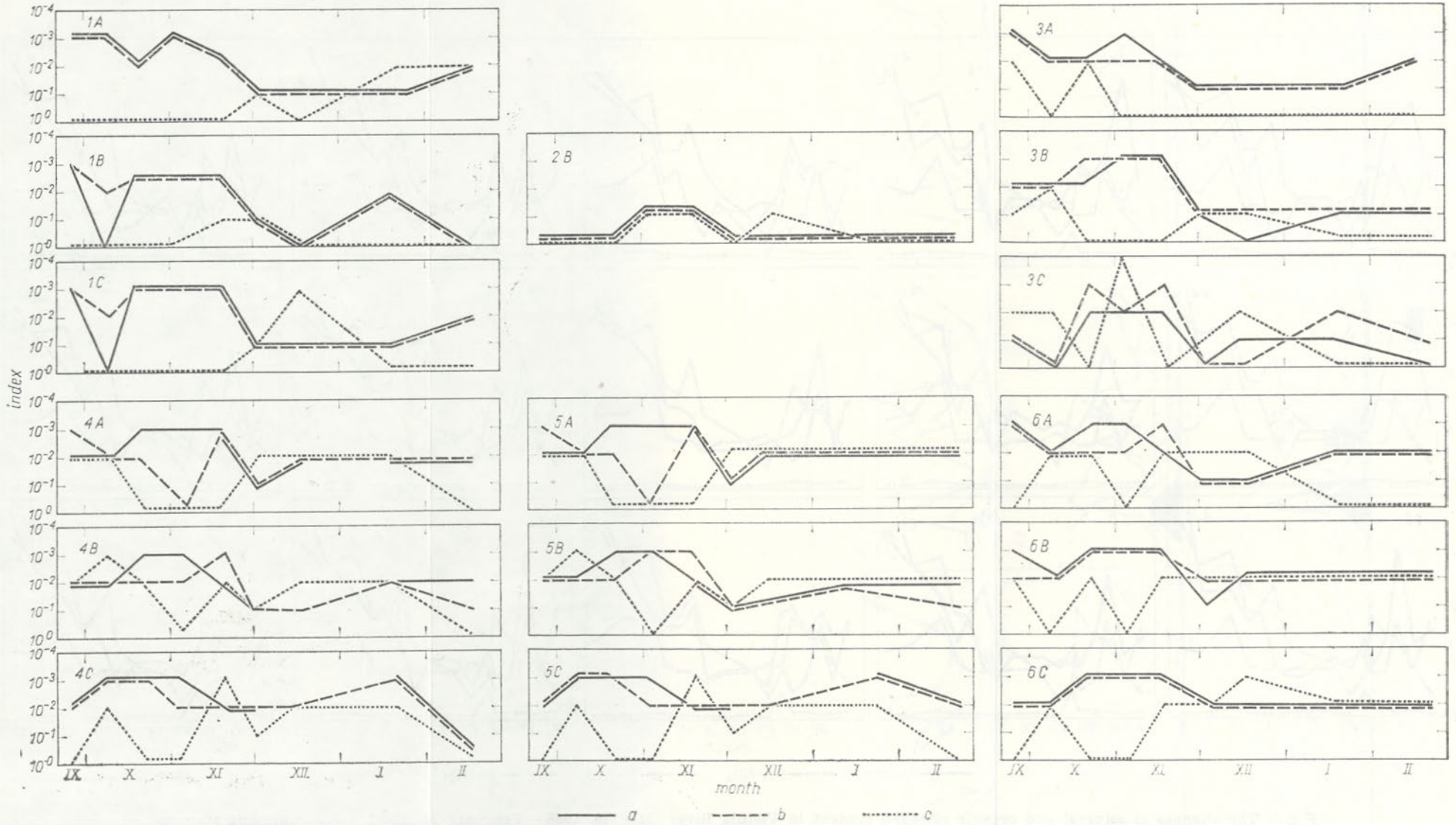

Fig. 6. The changes of numbers of nitrifying and denitrifying bacteria in Vistula (stations No. 1 and 3–6) and Skawinka (station No. 2) rivers (September 25, 1968 — January 18, 1969). A — left bank, B — midstream, C — right bank, *a* — nitrifying bacteria phase I, *b* — nitrifying bacteria phase II, *c* — denitrifying bacteria

320,000 cells/ml. Later, till December, the numbers varied markedly. The significant decrease of the total number of bacteria in winter (December—March) could be caused by the lower water temperature. The repeated increase in spring (April) was one of the highest in the investigation period.

The course of changes of the total number of bacteria in bottom sediments of Skawinka River was different from that in Vistula River. The maximum numbers of bacteria were in summer at the highest water temperatures (Fig. 3). The significant decrease of the number of bacteria in August was probably caused by the flow of water coming from heavy rainfalls.

The psychrophilic bacteria were the predominating group in the unheated region, particularly in the winter and early spring periods; they were more than 95% of the total number of bacteria. The least numbers of psychrophilic bacteria were recorded in Skawinka River (2—15%) and at the right bank of Vistula River in station No. 3 (Fig. 3, 4).

The mesophilic bacteria were found in all the stations during the whole investigation period. The most numbers (up to about 150,000 cells/ml) were found in summer in Skawinka River (Fig. 3) and in the heated region of Vistula River (Fig. 4). The least numbers (not higher than 30,000 cells/ml) were recorded in the unheated region of Vistula River in winter (December—March).

The thermophilic bacteria were found but minimally in the unheated region of Vistula River during the whole investigation period (Fig. 4). The more numbers were recorded in Skawinka River (Fig. 3), and their maximum development took place in summer (the highest record — 225,000 cells/ml — was made on June 19, 1968).

THE OCCURRENCE OF PHYSIOLOGICAL BACTERIA GROUPS IN WATER OF VISTULA AND SKAWINKA RIVERS

Mineral and organic nitrogen (Fig. 5). The numbers of proteolytic, nitrifying and denitrifying bacteria as well as bacteria fixing free nitrogen were observed in dependence on the contents of organic nitrogen, ammonia salts, nitrites and nitrates in water of Vistula River.

The nitrifying bacteria (Fig. 6) (phase I and II) were studied in Skawinka River from September 1968 to February 1969. In November they were found in scarce numbers and nearly not found at all in winter period. In general, the index of nitrifying bacteria in water of Vistula River was from 10^{-1} to 10^{-3}.

The relatively small numbers of these bacteria in water of Vistula River in the unheated region could be caused, probably, by another inhibiting factor, e.g. industrial pollutions. The local absence of nitrifying bacteria in the heated region (station No. 3) was resulted, first of all, in "diluting" effect of water from Skawinka River flowing into Vistula.

The course of the curves illustrating the numbers of nitrifying and denitrifying bacteria in the investigated stations (Fig. 6) allows to conclude that they were not the discharges of heated water which inhibited the processes of nitrogen conversions in water of Vistula River.

The bacteria fixing free nitrogen were found but sporadically both in seston and at the bottom of Vistula River during the whole half-yearly investigation period.

Sphaerotilus-Leptothrix bacteria group. They were found neither in water nor in bottom sediments of Skawinka River, however in the heated and unheated regions of Vistula River they were found quite often.

The photosynthetic bacteria of Thiorhodaceae, Athiorhodaceae and Chlorobacteriaceae families were investigated mainly in bottom sediments. They were not found in those of Skawinka River, whereas in Vistula River they were found sporadically.

The cellulose decomposing bacteria were studied in bottom sediments only. They were often found in Skawinka River. In Vistula River they were more frequent in the heated than in unheated region.

The phosphate-positive bacteria. They were investigated in the bottom zone. The occurrence of those bacteria in different seasons in the highly heated Skawinka River as well as in Vistula River can point to their eurythermic character.

The sulphur bacteria (bacteria deoxidizing sulphates, bacteria oxidizing H_2S and thiosulphates). No significant differences of occurrence of these bacteria in bottom sediments of both rivers were observed between the heated and unheated regions.

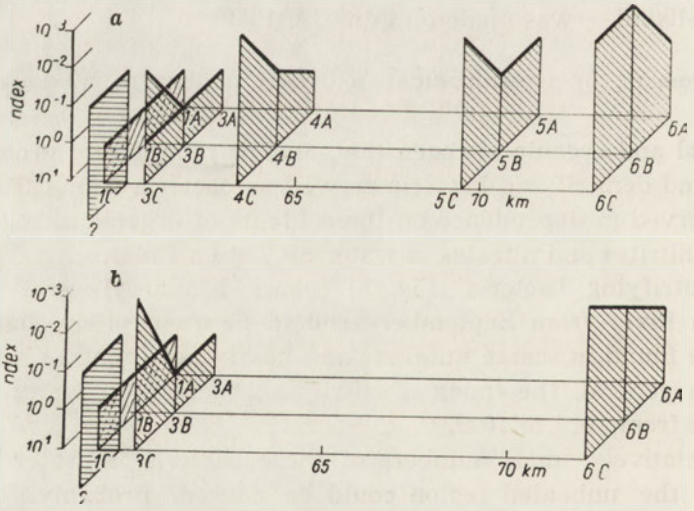

Fig. 7. The changes of numbers of *Escherichia coli* bacteria in water (a) and bottom sediment (b) of Vistula River (stations No. 1, 3–6) and Skawinka River (station No. 2) (November 19, 1968). A — left bank, B — midstream, C — right bank

The *Escherichia coli* bacteria group. These bacteria occurred commonly in water and bottom sediments of Vistula River. In general, the higher temperatures influenced the development of these bacteria in Vistula River (Fig. 7). This phenomenon is clear due to the thermic requirements of this bacteria group.

THE OCCURRENCE OF FUNGI IN SKAWINKA AND VISTULA RIVERS

About 16 genera and more than 20 species of fungi were the most frequently found in the investigation period. The predominating species were: *Rhizopus nigricans, Mucor strictus, Trichoderma lignorum, Aspergillus niger, Absidia glauca,* and others. *Mucor strictus* is worthy to mention since in spite of its common occurrence it disappeared in the heated region in summer.

4. DISCUSSION

The rapid decrease of bacteria numbers in bottom sediments of Skawinka and Vistula rivers in the middle of August 1968 could be caused by the abundance of rainfall water. This assumption was confirmed by the observations of Stangenberg, Pawlaczyk (1961) who stated that during the high water levels of Nysa Łużycka River the bottom sediments were intensely washed away and *Tubifex tubifex* — a typical sedimentic organism — was periodically found in seston.

According to Rheinheiner (1965) it can be assummed that the reason of the increase of bacteria number in water of Vistula River in late autumn could be the increment of organic matter contents (Fig. 2, 5).

The bacteria of Sphaerotilus-Leptothrix group were completely disappearing in water and bottom sediments of Skawinka River; such a recession was not to be observed in Vistula River. Stangenberg, Pawlaczyk (1961) found out that *Sphaerotilus natans* and *Lephothrix ochracea* disappeared in seston of Nysa Łużycka River only at the highest temperature, close to 36°C.

In the investigation period, the temperature of water of Skawinka River varied from 11 to 29.5°C. The highest water temperatures of Vistula River were observed at the right bank in station No. 3: 10—27.7°C.

According to Turoboyski (1969), the water temperature of Vistula River down the place of discharges of heated waters can reach 34°C, whereas in the present investigations no water temperatures higher than 30°C were recorded in Vistula River.

The results of the present paper confirmed the opinion of Stangenberg, Pawlaczyk (1961) that in our climatic conditions, the only temperatures which can harm the river biocenosis are those higher than 30°C.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	21	1	83-91	1974

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SANITARY CONDITIONS OF THE SURFACE WATERS IN THE WATER SUPPLY INTAKES

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ABSTRACT

The sanitary conditions of water in the 31 small water-supply intakes in the Wrocław province may be considered as satisfactory, and even good in some cases. The two largest water intakes in the Kaczawa River (Legnica) and the Oława River (Wrocław) showed periodically inadequate quality of water.

1. INTRODUCTION

The problem of sanitary measures for protection of the surface waters used for the pure water supply has been for many years one of the fundamental and subsistent issues in the research programme carried on in laboratories of the Communal Hygiene Department, at the Provincial Sanitary-Epidemiologic Station, in Wrocław. The Station operates a permanent sanitary control of all the surface waters, used for the public utility purposes, especially at the sites of the water intake for the municipal water supply.

The region of Lower Silesia is in a rather difficult situation from the viewpoint of water economics, due to its specific hydro-geological conditions, resulting from the fact that the greater part of its territory is in the mountain area. Underground and well water is scarce and the flow of rivers is inadequate and irregular. In dry years the annual rate of flow may decrease to 50% of the rate of flow in the normal year or increase to 160% in wet years. In result of the economic development of the mountain region great changes are occurring in its natural topographical conditions causing a decrease in the natural retention of water in that area.

Economic activization of the Lower Silesia region has brought about an increased demand for water and the imminence of pollution of the existing, meager, water resources. Already by now the rate of flow of the Silesian rivers does not assure sufficient supply of water for the technological needs of industry and the population, likewise, begins to be aware of the water shortage. There are times when deficiencies in water supply arise and now and then they reach up to 94% of the daily water requirements. Particularly acute water shortages occur frequently in the Dzierżoniów area.

The Statute passed on February 17, 1960, in respect of "provision of potable and household water to supply the needs of town and rural population" declares that the government is under obligation to supply water for public use and stipulates that the quality of water must meet the requirements specified in the Decree of the Ministry of Health and Social Welfare issued on November 16, 1961. Thus, the public service administration was bound to exploit the surface water resources. The level of those waters reserves is characterized by great fluctuations, throughout the course of a year.

There are 33 surface water reservoirs in the territory of the Wrocław Province; 31 of them are in permanent or periodical operation, while the remaining two (at

Kamienna Góra and Miedzianka) were recently excluded from the permanent service. Almost all those water intakes are grouped south from the line Zgorzelec–Lwówek–Bolków–Złoty Stok (Fig. 1). One cannot rely on the possibility of finding in those areas supplementary underground water resources. The above mentioned water intakes serve to provide water for small water supply networks.

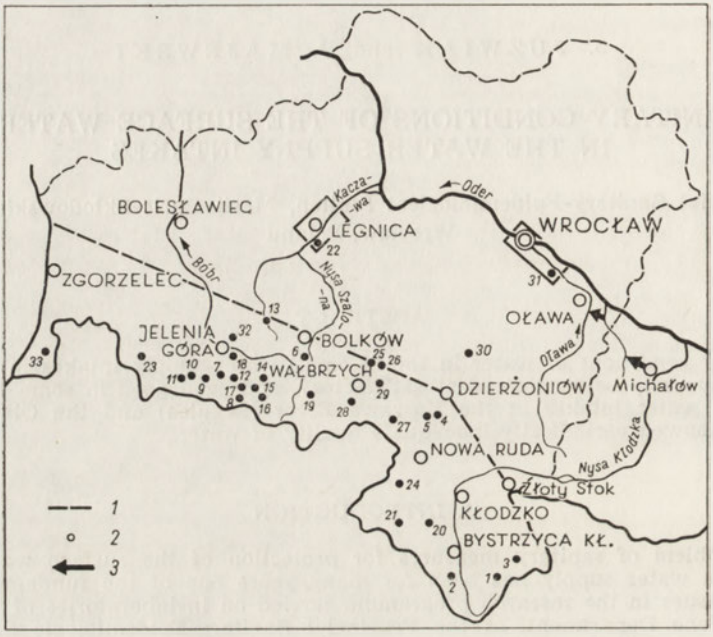

Fig. 1. The map of the observation area in the Wrocław province. 1 — the line limiting the area poor in the undergroung waters (south), 2 — surface water supply intakes, 3 — route of water transfer from Nysa Kłodzka River to Oława River

The largest, in this province, municipal waterworks in Wrocław and Legnica base their whole water supply system on the intake of the surface water from the Oława and Kaczawa rivers. The water intakes are located at the lower part of the rivers course, where the effects of pollution, occurring in the drainage area, are already distinctly visible.

The total input of the surface waters amounts to 2.0 mln m³/day which, counted together with 0.5 mln m³/day of the ground water input, gives about 2.5 million m³/day. The problem of water supply from the surface water intakes (there are 96 towns and housing estates supplied from municipal waterworks) are linked with sewage economics of that area (the Wrocław Presidium of the People's Province Council, 1970).

There are altogether 49 sewage treatment plants (14 mechanical and 35 bio-mechanical ones) in that region. 61 towns do not have communal sewage treatment plants. In that case sewage is treated in individual septic tanks, attached to a homestead.

The existing situation puts the sanitary engineering officers under the obligation of a particularly watchful supervision of the water supplied for use of the population. The sanitary control of the quality of the surface waters to be used as potable water is carried out in accordance with the following programme.

The water intakes situated in the higher regions of the mountains supplying water for use of small communities are examined, on the average, once a year. The quality of water from the reservoirs on the Kaczawa River (town of Legnica)

and the Oława River (city of Wrocław) as well as the sanitary conditions of the Nysa Kłodzka River are controlled 4 times a year. The quality of "natural" water (at the water-intake site) and of purified water (from the mains) is tested once a month in laboratories of the District Sanitary-Epidemiologic Stations in the scope of routine analyses consisting of 13 basic determinations of physico-chemical characteristics of water (turbidity, colour, odor, reaction, hardness, alkalinity, iron, manganese, chlorides, oxygenation, ammonia, nitrites, nitrates) and bacteriological tests.

In the laboratories at the Provincial S-E Station examination of water is carried out on a larger scale with additional analyses for determination of calcium, magnesium, copper, zinc, lead, fluor, contents in water and the degree of salinity, as well. The quality of "natural" water, i.e. at the water intake site, is classified by standards published in the Official Bulletin No. 17, § 144, July 18, 1970.

2. RESULTS OF EXAMINATION OF SURFACE WATERS

The basic indicators for sanitary evaluation and classification of the analysed water is presented in Table I. The values of various indicators of the quality of water are as follows:

BOD_5. The values of that indicator, in the water intakes located at the mountain brooks (Kowary Górne, Sosnówka, Kuźnice), ranged from 0.6 mg O_2/l to 3.6 mg O_2/l. In the Kaczawa River water intake (town of Legnica) — 3.8 mg O_2/l, in 1972; in the Oława River (Siechnice) — 1.8 mg O_2/l; in the Nysa River (near the border line of the Opole province) — 10 mg O_2/l.

Thus, if the permissible boundary value of the BOD_5 for waters of first class purity is 4.0 mg O_2/l, then that value was exceeded in 1972 only in the waters of the Nysa Kłodzka River. No doubt, this was caused by contamination of the water by industrial wastes from the Cellulose-Paper Factory at Bardo.

Oxygenation. The standard value for this indicator is 10.0 mg O_2/l, for waters of the first class purity.

Oxygenation values in water from small reservoirs ranged from 1.4 mg O_2/l (Długopole, Podgórzyn) to 7.3 mg O_2/l (Szklarska Poręba). Thus, the standard value was not exceeded, in that case. On the other hand, in the Nysa River below Kamieniec Ząbkowicki oxygenation value was 21.5 mg O_2/l, i.e. it was over twice as much as the standard value. In the Kaczawa River (waterworks of the town of Legnica) oxygenation value was 3.6 mg O_2/l, and in the Oława River (Siechnice) — 3.3 mg O_2/l.

Suspensions. The permissible value of suspensions content in water is up to 20 mg/l.

In the majority of the water supply intakes traces of suspensions were found in the water. In the stream of Międzygórze the content of suspensions was 11 mg/l; in the Kaczawa River, near the town of Legnica — 15 mg/l; in the Oława River at Siechnice, as much as 81 mg/l; and in the Nysa Kłodzka River on the border of the province — 25 mg/l. So, in the latter two a drastic transgression beyond the permissible limits of the suspension content in water has been observed.

Table I. Sanitary conditions in the water supply intakes in the Wrocław region

Water supply	Water intake	Indicators of water quality					
		BOD_5 (mgO_2/l)	Oxygenation (mgO_2/l)	Total suspension (mg/l)	Organic nitrogen (mgN_{org}/l)	Coli titre scato-type	Saprogenic zone
The permissible values for the water of first class purity		$\geqslant$4,0	$\geqslant$10.0	$\geqslant$20	$\geqslant$1.0	$\leqslant$1.0	oligo-β-meso
Międzygórze	stream Bogoryja	2.4	2.2	11	0.4	<1.0	oligo
Długopole	unnamed stream	1.4	1.4	6	0.4	0.1	o β m
Stronie Śląskie	Morawa River	3.4	3.1	10	0.4	0.1	β m
Dzierżoniów 1	unnamed stream	1.4	1.7	traces	0.7	<1.0	o β m
Dzierżoniów 2	unnamed stream	1.4	2.1	traces	1.0	<1.0	o β m
Wierzchosławice	unnamed stream	2.8	3.1	traces	1.0	<1.0	o β m
Podgórzyn	Podgórna River	2.2	1.4	traces	0.4	<1.0	oligo
Sosnówka *	Kacza River	3.6	2.8	traces	0.2	<1.0	o β m
Szklarska Poręba 1	Kamieńczyk River	1.0	3.8	traces	0.2	<1.0	oligo
Szklarska Poręba 2	unnamed stream	1.8	7.3	traces	0.2	<1.0	oligo
Szklarska Poręba 3	unnamed stream	1.8	3.9	traces	0.7	<1.0	oligo
Jagniątków *	Sopot River	1.6	2.2	traces	0.2	<1.0	oligo
Miedzianka	Bóbr River	—	—	—	—	—	—
Kowary (Wojków)	Kolnica River	0.8	3.0	traces	0.7	<1.0	oligo
Kowary Średnie	Malina River	3.0	2.1	traces	1.2	<1.0	oligo
Kowary Górne	unnamed stream	0.6	1.8	traces	0.7	<1.0	oligo
Karpacz	Bystrzyk River	2.8	2.1	traces	0.1	<1.0	o β m

Jelenia Góra *	Rakownica River	1.0	4.6	traces	4.7	<1.0	o β m
Kamienna Góra *	Pisarzowski Brook	3.2	4.5	traces	0.9	1.0	o β m
Duszniki Zdrój	Bystrzyca D. Brook	0.8	2.6	traces	0.8	<1.0	o β m
Polanica Zdrój	unnamed stream	1.2	3.6	traces	1.1	<1.0	oligo
Legnica	Kaczawa River	3.8	3.6	15	1.1	0.001	L β m
Świeradów Zdrój	unnamed stream	2.6	3.1	traces	0.8	<1.0	oligo
Radków	unnamed stream	2.6	1.9	traces	0.2	1.0	o β m
Świebodzice 1	Lubachowska W. stream	3.4	4.0	traces	1.1	0.001	β m
Świebodzice 2	unnamed stream	2.4	4.2	traces	1.1	<1.0	o β m
Głuszyca *	Złota Woda River	1.8	1.6	traces		<1.0	o β m
Kuźnice	Lesk River	3.6	2.6	traces	0.8	<1.0	oligo
Wałbrzych (Dziećmorowice)	unnamed stream	2.8	4.3	16	0.7	0.1	β m
Sobótka (Sulistrowiczki)	Sulistrowicki Brook	2.0	4.1	traces	1.3	<1.0	o β m
Wrocław (Siechnice)	Oława River	1.8	3.3	81	1.6	0.1	β m
Wojcieszów	Kaczawa River	3.2	3.9	traces	0.3	<1.0	β m
Zatonie	Plebanka River	2.4	4.8	9	1.2	1.0	o β m
Nysa Kłodzka River down Kamieniec Z.		10.0	21.5	25	1.4	0.01	β m

* In 1971.

Organic Nitrogen. In the waters of the first class purity the organic nitrogen concentration is permissible up to 1 mg N_{org}/l.

In the course of the present study the lowest concentration of organic nitrogen (0.1 mg N_{org}/l) was found in the Bystrzyk, a mountain brook supplying water to the waterworks at Karpacz. The highest concentration was observed in the Oława River at Siechnice — 1.6 mg N_{org}/l. Transgression of the permissible standard value was observed also at Kowary Średnie (1.2 mg/l). Polanica Zdrój, Legnica, Świebodzice, and Sobótka (1.1 mg N_{org}/l). In the Nysa Kłodzka River, on the border of the province, organic nitrogen concentration was 1.4 mg N_{org}/l.

Coli titre (scato-type). The standard value of the Coli titre in the waters supplying waterworks is not allowed to be lower than 1.0.

In the majority of the examined waters the scato-type Coli titre value was higher than 1.0. The standard value was not reached in the waters supplying waterworks at Długopole and Stroń (0.1), Legnica (0.001), Świebodzice (0.001), Wałbrzych (0.1), and Wrocław (the Oława River 0.1). The waters of the Nysa Kłodzka River, at Kamieniec Ząbkowicki, showed the Coli titre value 0.1.

Saprogenic zone. The waters of the first class purity should be of the oligo saprogenic zone.

The observations of plankton and periphyton in the waters at the water supply intakes have shown, in most cases, the oligo- and β-meso--saprogenic character of the examined colonies of organisms. The presence of biocenesis of a L-β-meso-saprogenic character was found only in the Kaczawa River at the water intake supplying Legnica. Observations carried out in previous years have shown, in some periods, similar conditions in the Oława and Nysa Kłodzka rivers.

Examination of the waters of mountain brooks revealed, in general, the presence of poor colonies of organisms characterized by small variety of species and inconsiderable total number of individuals. There were found, now and then, single filiform bacteria, single or scarce flagelliform phytoplanton, and diatomaceous species, such as: Asterionella, Fragilaria, Melosira, Pinnularia, Navicula, Synedra, Nitzschia, etc. Sometimes, the presence of single Chlorophyceae (Pediastrum and Scenedesmus) was detected. There were also found small numbers of Ciliata (Vorticella and Nassula), rare Rotatoria (e.g. *Colurella bicupidata),* and sometimes Tartigrada (e.g. Macrobiotus) observed in the Podgórna River. Rhizopoda (Arcella and Actinophorys) were seen sporadically. The most frequently differentiated in trypton were scarse mineral particles, organic detritus, and remnants of plant tissues.

In the waters of the Kaczawa, Nysa Kłodzka, and Oława rivers, near the water supply intakes, colonies of organisms were observed to be fairly rich and with strongly differentiated species. The phytoplankton species were prevalent in here, most frequently, however, the quantita-

tive ratio between particular groups of organisms was of great variety. For instance, at the waterworks at Legnica (mill-race of the Kaczawa River) filiform bacteria were sometimes predominant in number and this fact together with an abundant occurrence of some Ciliata species (*Paramaecium caudatum*) or Flagellata (*Anthophysa vegetans*) indicated water pollution. This was confirmed also by the results from physico-chemical and bacteriological analyses.

In the Oława and Nysa Kłodzka rivers, in most cases, there were found colonies of organisms composed of fairly numerous filiform bacteria, infrequent or fairly frequent Flagellata (Phacus and *Synura uvella*), rare Cyanophyta (*Oscillatoria limosa, Oscillatoria brevis,* and Lyngbya), abundant or very abundant Bacillariophyta (Asterionella, Cymbella, Melosira, Synedra, Nitzschia, and Navicula), single or rather numerous Chlorophyta (Scenedesmus, Pediastrum, and Tribonema). Among protozoans there were present fairly numerous Rhizopoda (Arcella), Ciliata (Paramaecium, Vorticella, and Chilidon), now and then, infrequent Nematoda, and sometimes Rotatoria (*Colurella bicuspidata*) were also detected. Small crustaceans (Copepoda — Nauplius) were found quite often. Considerable amount of organic detritus was encountered, likewise.

The Oława, Kaczawa, and Nysa Kłodzka rivers have been examined along their whole course through territories of the province of Wrocław. Water from those rivers is the main source of water supply for the population of the cities of Wrocław and Legnica and consequently it is under special official care and protection. (Instruction No. 10/71 of the Presidium of the People Province Council in Wrocław, July 19, 1971, "in respect of the establishment of categories of purity of the inland surface waters").

The Oława River is investigated by the Provincial Sanitary-Epidemiologic Station four times a year, along its course between Ziębice and Siechnice, at five basic Control Posts (above Ziębice, at Henryków, above the town of Strzelin, below Strzelin, and the water intake at Siechnice).

The level of water pollution in the river varies throughout the year and also in the course of consecutive years. As results from the observations carried on during the year 1971 at the Siechniwice Control Post, the composition of water in January did not arouse any doubts as to its quality, in the main. However, as regards bacteriological analyses water was strongly polluted (the scato-type Coli titre — 0.001). In April, also, physico-chemical characteristics of water met the standard requirements, in general, except the increased content of organic nitrogen (3.6 mg/l). As concerns hydrobiological and bacteriobiological indicators there were no reservations. In December, as well the physico-chemical composition of water as the hydrobiological and bacteriological indicators met the requirements of the standard for the first class purity of water.

Along the whole course of the examined segment of the river a distinct increase in water pollution was observed near Ziębice and Wiązowa, further on below the town of Oława a gradual decrease in the water pollution level was noted. The main source of the water pollution in the Oława River is due to a sugar factory at Ziębice and its industrial wastes which during the production season are increasing the level of water pollution many times, all the way down to the water intake at Siechnice.

Taking into consideration a low level of water in the Oława River and low rate of the flow (0.7 m^3/sec) in cross-section of the river (Wrocław), a transfer of water from the Nysa Kłodzka River into the Oława River has been operated since August 1971 during the critical periods. The rate of the transferred water may be in the range from 1.25 to 2.5 m^3/sec depending on the requirements. Examinations of the transferred water carried out in 1970–1971 showed a varying quality of water, nevertheless it was approximate to the required standard, in the main.

The Nysa Kłodzka River. The waters of the Nysa Kłodzka River are examined along a segment between Bystrzyca Kłodzka–Kamieniec Ząbkowicki, at 10 Control Posts, four times a year.

The quality of water above the town of Bystrzyca may be considered as satisfactory, in the light of the obtained results. Below that town there was observed, merely, an increase in the bacteriological contamination of water (the scato-type Coli titre decreases from 1.0 down to 0.1). Below the town of Kłodzko there was noted a further increase in the water pollution exceeding the limits of permissible standards.

Industrial wastes from the Cellulose-Paper Factory at Bardo are the principal source of water pollution in the Nysa River. Moreover, in the sugar production season the quality of the water deteriorates additionally due to the industrial wastes from the Sugar Factory at Ząbkowice brought into the Nysa Kłodzka River by the inflowing waters of the Budzówka River.

As results from the observations carried on during 1971 at the Control Post below Kamieniec Ząbkowicki, at the time of all the four examinations (March, May, September, November) of the water, an excessive oxygenation (36.5–52.0 mg O_2/l) was noted and also a high BOD_5 (20.4–32.0 mg O_2/l). In general, the physico-chemical composition of water is below the permissible value of the standards determined for the waters of the third class purity. As follows from the hydrobiological observations, colonies of plankton organisms detected in that spot of the river had usually a β-meso-saprogenic character. The scato-type Coli titre ranged from 0.001 (March) to over 1.0 (November).

The Kaczawa River. In 1971, the sanitary conditions of the waters in the Kaczawa River were controlled three times (April, October, December) at 7 Control Posts covering the segment of the river between the town of Złotoryja and the water intake supplying waterworks at

Legnica. The main source of that river pollution is due to the sewage from the town of Jawor and wastes, from the sugar Factory, inflowing with waters of the Nysa Szalona River. At the water intake at Legnica the BOD_5 values ranged from 5.4 mg O_2/l (April) to 20.8 mg O_2/l (December). Water oxygenation was from 5.3 mg/l (October) to 28.8 mg O_2/l (December). Suspensions content ranged from 6 mg/l (October) to 720 mg/l (December). The scato-type Coli titre was from 0.01 (December) to 0.1 (April and October). In December, there was also noticed the occurrence of colonies of plankton organisms of a L-β-meso-saprogenic character.

The results of general evaluation showed that the sanitary conditions did not meet the requirements of the first class purity water standards. The results of estimation from the previous years were similar.

Thus, the examinations of water from the small water supply intakes did not show any serious deviations from the recommended standards. However, though the quality of water (as a staple material) met, in general, the requirements of the proper standards, nevertheless the waters from the examined intakes required some improvement, as a rule. Water purification treatment is carried on in all waterworks with more or less complicated operations including, always, disinfection performed regularly and continuously. To that purpose chlorine is used in the majority of the water supply systems. Most of the water intakes are enclosed in special zones of safeguard and direct preservation.

The quality of water from the upper and middle course of the Oława and Kaczawa rivers does not meet, for the most part, the requirements of the proper standards and this is mainly due to inadequate physico-chemical composition and bacteriological contamination.

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K. CZYŻ and A. PRASZKIEWICZ

THE QUANTITATIVE DISTRIBUTION OF ACTINOMYCETES (ORDER ACTINOMYCETALES) IN THE BOTTOM SEDIMENTS OF THE VISTULA RIVER ON THE STRETCH FROM SANDOMIERZ TO WARSAW

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ABSTRACT

The bottom sediments of the Vistula River on the stretch from Sandomierz to Warsaw are richly populated by Actinomycetes. Their numbers oscilate in the range from 100,000 to 10,000,000 cells/g of dry sediment weight. The factors which decide about quantitative differentiation of Actinomycetes in the bottom of the examined stretch of the Vistula are the pollutions introduced into the Vistula by its tributaries Wyżnica and Jeziorka (15,000,000–20,000,000 cells/g of dry weight) and industrial wastes from the Nitrogen Plant of Puławy below which the maximum numbers of Actinomycetes have always been recorded. The Actinomycetes which occur in the Vistula above the water supply for Warsaw exceed as a rule 1000,000 cells/g of dry weight of sediment and therefore can be regarded as one of the reasons of the deterioration of the water taste and odor.

1. INTRODUCTION

Actinomycetes ecologically connected with soil in land environment and with bottom sediments in water reservoirs contribute with their mass growth of cells to the formation of characteristic soil odors, which can cause disadvantageous changes of organoleptic properties of water taken for drinking purposes (Silvey, Roach 1956).

From among many factors suspected of an unfavorable influence on the taste and odor of Vistula water (Dojlido et al. 1967, 1972) Actinomycetes, a group of microorganisms which actively produce smelling substances, were found to be one of the reasons which cause the deterioration of the water odor in the Vistula in the water supply for Warsaw.

The hitherto existing literature on the subject of how to detect the presence of Actinomycetes and their quantitative existance in the bottom sediments of running waters is relatively poor. Adams (1929) when examining the Nile River noticed that the taste and smell of soil in water are caused by the abundant presence of Actinomycetes which the author considered as a biological factor contaminating the Nile River.

In the comparative investigations as to the abundance of Actinomycetes in the bottom sediments of the River Don it was discovered that the number of these microorganisms on the bottom is 10 to 10,000 times greater than in the running water. Moreover, it was ascertained that they were responsible for the unpleasant smell and taste of the water from the Don because the same characteristic smells arose during the cultivation of the Actinomycetes cultures which were isolated from the bottom sediments. It appears from the data in literature that a certain interdependence exists between the number of Actinomycetes and the kind of

subsoil. Erikson (1941) noted that an extensive growth of Micromonospora cultures isolated from water and from bottom sediments of lakes took place on the organic medium containing chitin and cellulose. The author drew a conclusion that organic substances from lake can be used by Actinomycetes.

Morris (1962) noticed a twofold increase of Actinomycetes numbers in the spots situated below the sources of pollution on the River Cedar.

The allochtonic pollutions led into the river were a stimulator of Actinomycetes growth. The author has also proved that when the number of Actinomycetes equaled 60,000 cells per 1 g of dry weight of bottom sediment, characteristic, fusty-soily odors appeared, easily perceptible in water. As these smells were identical with those produced by Actinomycetes in plate cultures, this group of organisms has been recognized to be responsible for the deterioration of the quality of water in the River Cedar.

In his monograph Waksman (1959) gave other examples of the abundance of Actinomycetes discovered in river sediments. It appears from these examples

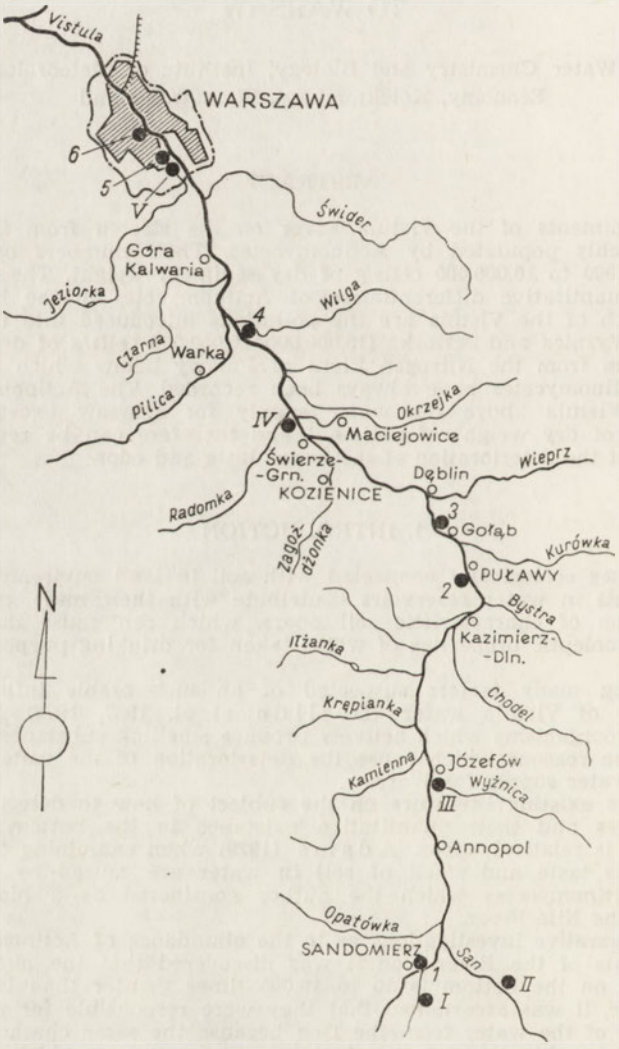

Fig. 1. Location of sampling stations. 1–6 — stations on Vistula River, I–V — stations on the tributaries

that the mass growth of Actinomycetes exceeding 1000,000 cells in 1 g dry sediment has always been the cause of unpleasant taste and smell of water. There were no similar publications in Polish literature.

The present work is the first report in Poland on the subject of the quantitative occurrence of Actinomycetes in the river bottom sediments and it concerns the middle part of the Vistula on the stretch from Sandomierz to Warsaw.

2. MATERIAL AND METHOD

The investigations were conducted in a yearly cycle from October 1970 to September 1971 and additionally in the winter period of 1972. The distribution of stations is represented on Fig. 1.

During the first stage of investigations, i.e. from October 1970 to March 1971, some indicatory data were collected concerning the numbers of Actinomycetes in the bottom of the Vistula in the following spots: above the San (st. 1), above Puławy (st. 2), below Puławy (st. 3), above the tributary of the River Wilga (st. 4), above the tributary of the River Jeziorka (st. 5) and in the region of the water intake for Warsaw (st. 6). From among the tributaries Trześniówka (st. I), Wyżnica (st. III), Radomska (st. 4) and Jeziorka (st. V) were included in the investigation.

Beginning with the vegetation period of 1971 (from May to September) and then during the winter season of 1972 (from January to March) the investigations of the microflora of the river bottom were concentrated on the above mentioned six stations in the Vistula River bed. The area near the tributary of the River San (st. 2) was also included into the investigations. The samples of the bottom sediment were taken on the average each month with the Szczepański (1953) bottom sampler. During the investigation period, 75 samples were taken, and from each sample the three Petri plates were prepared.

For the detection of the Actinomycetes the plate method was employed, and the four times diluted Ringer liquid was used.

For the Actinomycetes cultivation starch agar of the following composition was prepared: starch 10.0 g, KNO_3 1.0 g, K_2HPO_4 0.5 g, NaCl 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, agar 15.0 g, water 1000 ml.

The material was seeded on the Petri plates from 4 successive dilutions, repeated three times for each dilution and then incubated for 10 days at temperature of 30°C. An average of every three plates was taken into consideration. The final result was the number of Actinomycetes colonies grown on the medium in relation to 1 ml of bottom sediment suspension and then reduced to 1 g of dry weight of sediment determined by the gravimetric method. Thus the number of colonies corresponding to 1 g of dry weight of bottom sediment was accepted as a result.

For the detection of Actinomycetes colonies the Waksman (1959) monograph was used. An additional factor which made the identification of Actinomycetes easier was the very strong and characteristic smell which came from the produced metabolites and which was emited by the culture in the process of the growth of the colonies.

3. RESULTS

The preliminary investigations (1971—1972) conducted on the quantitative existance of Actinomycetes in the bottom sediments of the Vistula River have indicated the abundance of these microorganims in the bottom of the river bed. The numbers of Actinomycetes exceeding the value of 1000,000 cells/g of dry weight, above which the organoleptic changes of the water can be observed, are presented in Fig. 2. Besides, the numbers of phytoplankton in the sampling spots of the Vistula River where the density of green cells exceeded the value of $5 \cdot 10^5$/l of water are also presented. When the phytoplankton exceeds the mentioned value in number, the taste and odor of water are changing (Palmer 1959).

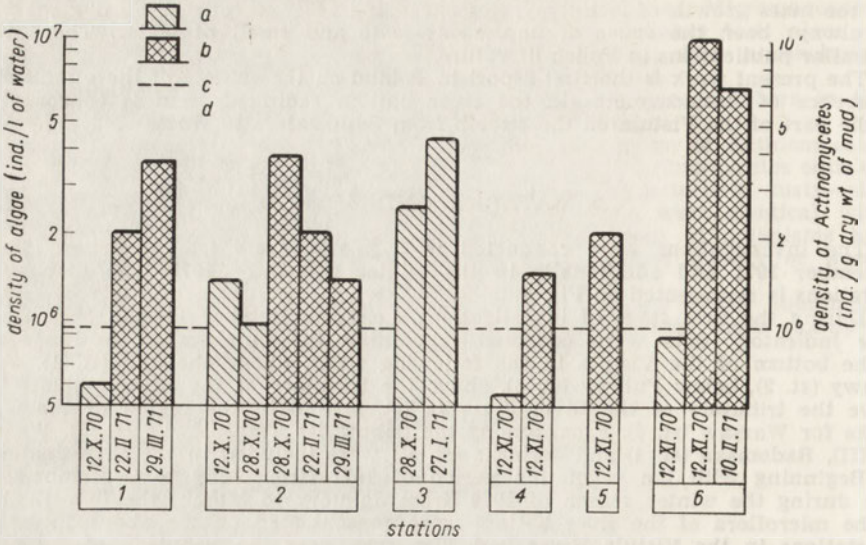

Fig. 2. Density of algae (a) and Actinomycetes (b) with reference to the limit values above which the changes of taste and odor of water can be observed. c — the border value for Actinomycetes, d — the border value for algae. 1–6 — stations on Vistula River

The further investigations conducted from January to September 1971 and continued from January to March 1972 confirmed the preliminary data that the excessive growth of Actinomycetes (considering their activity in the production of substances affecting taste and smell) constitutes a threat to the river. These microorganisms were many times discovered in numbers greater than 1000,000 cells/g of dry weight of sediment (Table I). The average numbers of Actinomycetes oscilated within the bounds 1000,000—10,000,000 cells/g of dry weight in the Vistula and were even greater in the tributaries. The highest number of Actinomycetes (20,000,000 cells/g of dry weight) were recorded in the San River in March 1971 and January 1972 (Table I). It was concluded that contamination of the Vistula River by Actinomycetes resulted from the examined tributaries mainly Wyżnica and Jeziorka. Therefore these tributaries can be suspected of the negative influence on the water quality of Vistula River.

It has also been proved that the intensity of the growth of Actinomycetes depends on the season as is shown in Fig. 2, 3 and 4. During two winter periods 1971/1972 and 1972 (Fig. 2 and 4) and in spring in May (Fig. 3) Actinomycetes were represented in very great numbers, especially on the section of the river below Puławy, at spots 3 and 4.

As the vegetation season set in, beginning from June of 1971 (Fig. 3) the numbers of Actinomycetes in the bottom sediments of the Vistula were greatly reduced in comparison to the data from the winter period and that of the early spring. The analysis of the samples taken in the

Table I. Quantitative distribution of Actionomycetes in the bottom sediments of the Vistula River and its some tributaries

Period		Actinomycetes density (thous./g dry weight of sediment)										
		Sampling station										
		1	I	II	III	2	3	IV	4	5	V	6
1971	Jan.	500	20		2 500	500		2 000	600			
	Febr.	2 000				1 500			500		15 000	2 000
	March	4 000	300	2 000	20 000	—			—			—
	April	—		—		1 000		7 000	300		10 000	500
	May	500		1 500		600	10 000		4 000	1 300		3 000
	June	300		700		900	1 500		100	300		500
	July	200		1 000		1 200	1 700		2 300	600		900
	Aug.	500		400		800	1 000		700	2 000		200
	Sept.	100		400		600	1 300		800	2 400		1 000
1972	Jan.	5 000		2 000		2 000	7 000		5 000	3 000		600
	Febr.	330		1 100		300	830		320	4 000		260
	March	130				670	470		560	3 000		800

periods from June to September revealed somewhat higher values of Actinomycetes numbers in the month of July. This increase of Actinomycetes numbers in July was probably caused by the flow of waters

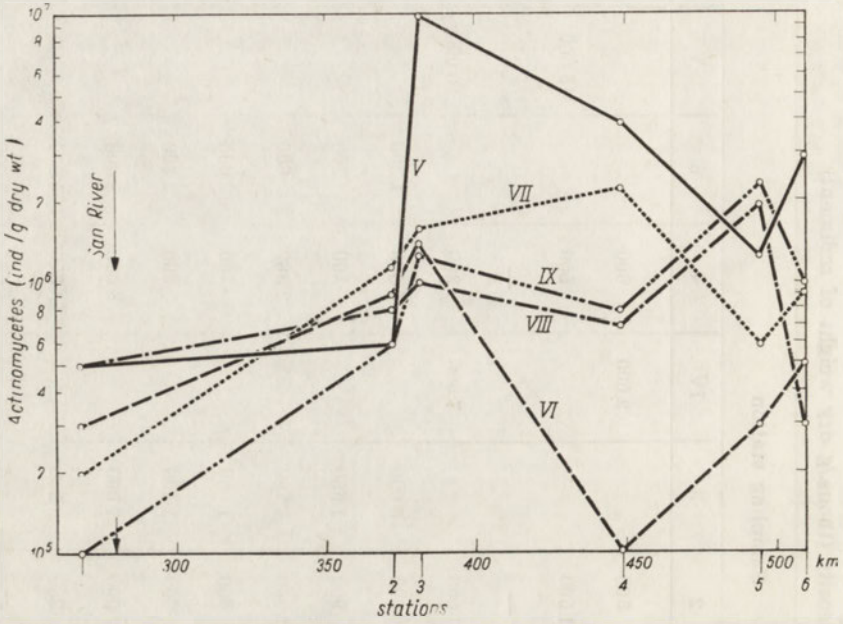

Fig. 3. Actinomycetes population in the Vistula River bottom sediments in the vegetation period in 1971. V–IX — months

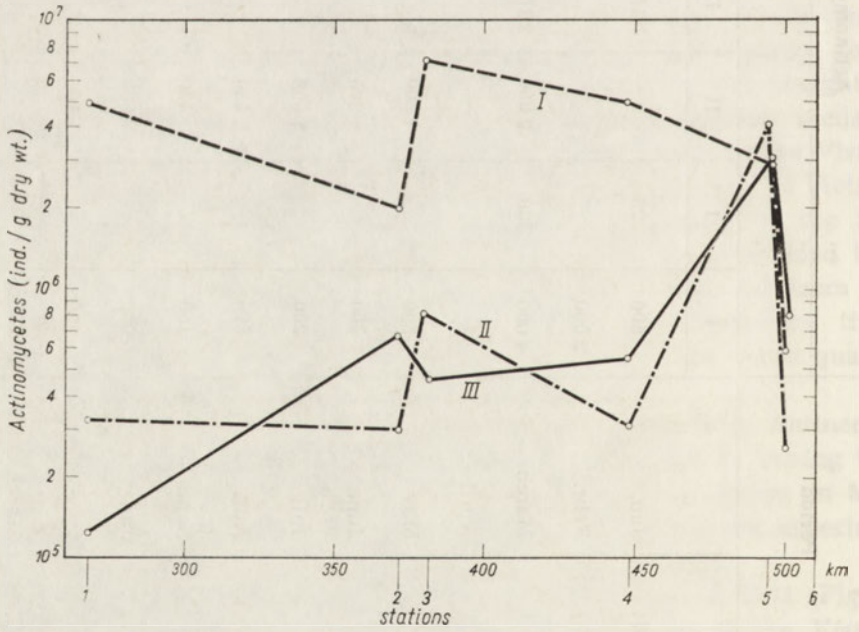

Fig. 4. Actinomycetes population in the Vistula River bottom sediments in the winter of 1972. I–III — months

from the catchment area and by washing out of the soil caused by the longlasting and heavy rains which were observed at that time. As it is known, soil is the proper habitat for Actinomycetes and for that reason they could be allochtonically introduced into the river.

After the hydrological conditions had become settled and after the coming of the sunny weather which lasted during the whole month of August of 1971, a distinct fall of Actinomycetes numbers on nearly the whole examined section of the Vistula was observed. It seems that these distinct seasonal differences in Actinomycetes numbers in the bottom sediments of the Vistula (the maximum growth falling on the winter--spring period and the minimum in the summer season) are in some degree dependent upon the abundance of bioseston, mainly phytoplankton in the Vistula water. The intensive growth of Actinomycetes in the Vistula usually followed with a delay in relation to the mass appearance of algae. For instance, the numbers of algae of about 15,000,000 ind./l which had been observed in the beginning of October 1970 at spot 2 caused at the end of this month the increase of the Actinomycetes numbers to 4000,000 cells/g of dry weight (Fig. 2). Likewise the high increase of Actinomycetes numbers on the section of the river from Puławy to Warsaw observed in November was probably caused by the algae bloom observed below Puławy (Fig. 2). Analogically the winter bloom of the algae Cyanophyceae appearing annually in the Vistula below Puławy (Dojlido et al. 1972) can be regarded as the cause of the occurrence of such great numbers of Actinomycetes in winter at a point above Warsaw (Fig. 2 and 4). The interpretation according to which the increase of the Actinomycetes numbers in the river bottom sediments is due to the blooming of the phytoplankton seems proper inasmuch that the whole mass of algae carried in the river current, after their cells die become part of the bottom sediment creating thus a suitable bedding for the growth and development of Actinomycetes. This dependence is consistent with Erikson's (1941) theory who has proved that the most extensive growth of Actinomycetes take place on organic bedding containing cellulose and chitin, namely the substances entering into the composition of plant and animal cells.

The investigations of the bottom sediments of the Vistula from the point of view of the Actinomycetes population have also permitted to ascertain the quantitative differentiation of this group of microorganisms along the examined section of the Vistula. Irrespective of the season of the year, a visible increase of the numbers of Actinomycetes have always been observed at spot 3 below the Fertilizer Plant of Puławy which discharged wastewaters to the Vistula. In May the Actinomycetes numbers at the spot below Puławy increased as many as 16 times in comparison with the numbers recorded above Puławy (Fig. 2); in the remaining months of the vegetation season and in winter (January, February

1972) this increase was on the average twofold. This corroborates Morris (1962) observations made on the River Cedar that sewage discharged into a river causes the intensive growth of Actinomycetes.

It seems necessary to stress the particular threat to the water quality of the Vistula in the Puławy region — first on account of the amount of industrial wastewaters from the fertilizer plant, secondly on account of the more intensive growth of Actinomycetes in this region. The combined effect of both these factors, namely wastewaters and Actinomycetes, as well can exert directly or indirectly unfavorable influence of the organoleptic properties of Vistula water.

From other regions so called "threatened" by the excessive growth of Actinomycetes special attention should be paid to the areas near the estuaries of considerably polluted tributaries such as Wyżnica and Jeziorka. Especially alarming is the great increase of Actinomycetes in the bottom sediments of Jeziorka where the numbers of Actinomycetes amounted to 10,000,000–15,000,000 cells/g of dry weight of sediment.

As the tributary of Jeziorka is situated near (15 km above) to the water inlet for Warsaw supply there are misgivings that Jeziorka on account of the great pollution by wastewaters and also on account of the great abundance of Actinomycetes in the bottom sediments can have negative effects on the taste and smell of Vistula water in Warsaw. Though the numbers of Actinomycetes in the region of the water inlet were as a rule lower than those recorded below Puławy (Fig. 3 and 4) yet these values were sufficiently high that Actinomycetes might be suspected of their participation in the deterioration of the water taste and odor.

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HYDRO-BACTERIOLOGICAL CORRELATIONS IN THE ODER RIVER

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ABSTRACT

The correlation between the number of bacteria in 1 ml of water and the intensity of flow, and between the load of bacteria and the intensity of flow has been established for the Oder River. It was thus shown that the correlation in respect to loading is more significant. This correlation has been established by means of regression equations: it takes the form of double logarithmic scale in straight lines. From the equations determining the load of bacteria has been established the adequate number of bacteria for determined volume of flow.

1. INTRODUCTION

The norms obligatory in Poland of tolerable inland surface waters pollution are referred to the reliable flow (Rozporządzenie...). By competent or adequate flow of current water we understand the mean low flow of many years, called MLF, or guarantee flow, occurring in the given cross-section. The norms of tolerable pollution of inland surface waters concern 49 indices and kinds of pollution: physico-chemical, radiological, and biological, for which numerical values are given. These values, called tolerable, cannot be exceeded at flows greater than the reliable flow. Three classes of water purity are distinguished: class I concerns waters meant to supply the population with drinking water, and water for alimentation plants, as well as fish-breeding of the highest kind. Class II — concerns fish-culture with the exception of salmon-like species. This water also serves to satisfy animal husbandry, bathing and water sports places. Class III supplies the needs of industry and agriculture.

The admissible values of Index Coli of fecal bacteria amount to for the waters of class I — 1 and above, for class II — 0.1 and above, and for class III — 0.01 and above. The corresponding maximal amounts of coli-bacteria in 1 ml are as follows: for class I — 1, for class II — 10 and for class III — 100.

In order to compare the number of bacteria checked in a given period with admissible values, one has to denote the reliable numbers of bacteria. By this notions is understood such a number of bacteria which has been statistically denoted from a larger number of momentary results, e.g. 48 or more (Krasnodębski 1971, personal information) and from the dependence of bacteria number from the flow (Mańczak 1963, 1971). The present paper contains a tentative list of these dependences for the Oder River, basing on the results of bacteriological and hydrological research in 1969, carried out in a cross-section of the Automatic Water Quality Monitory Station (AWQMS) at Wrocław.

In searching for these correlations one might have expected these correlations to follow the course of soluble compounds, i.e. that at increased flow the number of bacteria would diminish as a result of dilution. But in the majority of cases, simultaneously with flow intensification, an increase of bacteria number may be observed, as well in pure as in polluted waters.

The reasons for change in number of bacteria in polluted waters were in-

vestigated by Fair (1966), in 1963/1964 in the River Purde. At intensified flow, due to spring waters (rain), he observed an increase of bacteria number up to the moment of maximal flow intensification. With weakening of flow, the number of bacteria dropped.

The occurrence of correlation between flow intensity and number of bacteria (Fig. 1) was also established in the Ohio River (Mańczak 1963). Basing on

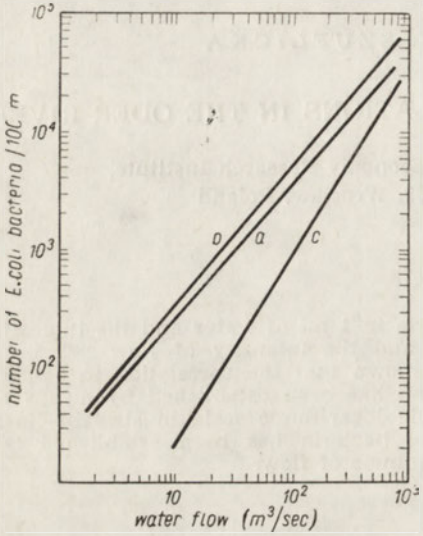

Fig. 1. The correlation between the mean probable number of *E. coli* bacteria and the water flow of Ohio River (acc. to Ohio River Commission 1953). a — at water supply plant (Louisville), b — at water supply plant (Evansville), c — at water supply plant (Cairo)

the monthly mean flows for that river and the corresponding number of bacteria Coli calculated to 100 ml of water from the river, a diagram was plotted in sheape of logarithmic straight lines for three different cross-sections of control measurements. It results from the diagram that the course of straight lines for three various cross-sections bears the same character and indicates a close correlation between intensity of flow and number of bacteria.

Rheinheimer (1971) basing on experiments carried out along a 200 km segment of the Łaba River suggests the possibility of increase in bacteria number during summer high waters, as a result of wash-out of bacteria from arable soil and meadows.

2. METHODS

The bacteriological investigations of the Oder River waters were carried out from January to the end of December 1969, at the rate of 1 every week, and comprised the determination of the number of psychro- and mesophylic bacteria, as well as bacteria from the Coli group. Samples were taken at a depth of 20–30 cm below water surface.

The total amount of psychro- and mesophylic bacteria were determined by culture method on a standard agar medium, prepared from a standard culture medium. For psychrophylic bacteria, a 72 hour incubation period was applied, at 20°C, for mesophylic ones — 24 hrs incubation at 37°C.

The number of Coli bacteria was determined by filter method (membranes). The bacteria retained on filters were incubated for 24 hrs at 37°C, applying the differentiating Endo agar medium. If not-typical colonies grew on the filters, corroborating analyses were carried out on lactose medium. The colonies fermenting lactose with gas production were ascribed to the Coli group.

All these determinations were carried out in two parrallel repetitions, reading them on the basis of plates, on which the number of colonies did not exceed 300. The results obtained were recalculated to 1 ml of river water.

3. RESULTS

The characteristic amounts of bacteria and magnitudes of intensity of flow, that is minimal, mean and maximal have been established during investigations in 1969, and are listed in Table I.

Table I. The characteristic amounts of bacteria and intensity of flows of the Oder River at Wrocław (km 249.4th) in 1969

Group of bacteria	Number of cells/1 ml		
	minimum	mean	maximum
Psychrophilic	800	47,050	350,000
Mesophilic	130	2240	15,000
Coli bacteria	10	160	800
Intensity of flow Q (m^3/sec)	40	90	157

Basing on analytical research as well as on results of flow intensity measurements, corresponding to the time of investigations, the correlations, between the number of bacteria determined in 1 ml of river water and flow intensity, as well as between bacterial load (No. of cells/sec) and flow intensity. By bacterial load we understand the product of bacteria number in 1 m^3 of water and corresponding intensity of flow. In order to simplify calculations the magnitude — loading $\cdot 10^{-6}$ was applied.

The comparison of these correlations for psychrophylic, mesophylic, as well as for the Coli group bacteria has shown, that the correlation of flow intensity and bacteria load may be considered as univocal, as the scattering of points is here lesser than when comparing the correlation of flow and number of bacteria.

The correlation between load of mentioned groups of bacteria and intensity of flow is expressed in the formula:

$$y = a \cdot x^b,$$

where y — load of bacteria $\cdot 10^{-6}$, x — intensity of flow, a, b — equation constants.

In the calculations, the logarithmic form of the above equation was used:

$$\log y = \log a + b \log x$$

The thus calculated curves of regression for psychrophylic (Fig. 2), mesophylic (Fig. 3) and for bacteria of the coli group (Fig. 4) denote the

mean load of these groups as the function of flow intensity. The reality of the discussed correlation was determined by means of the coefficient of correlation r_o. The calculation of regression equation was done on a computer.

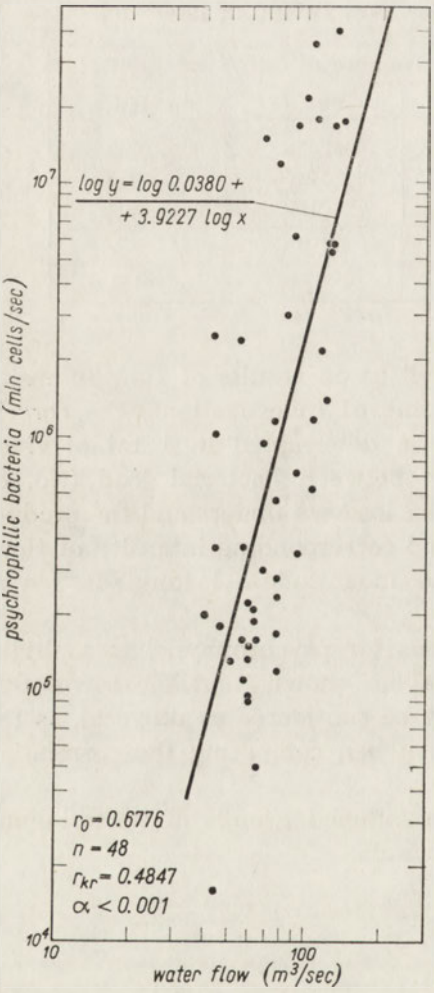

Fig. 2. The correlation between the psychrophilic bacteria and water flow

For a full characteristics of the degree of precision of the calculations, next to the curves were given also the values of correlation coefficients r_o, as well as the critical values of these coefficients r_{kr}, corresponding to the level of reality α, at which the coefficients obtained from calculations may be considered as essential.

For all three groups of bacteria discussed, the obtained correlation coefficients may be considered as highly essential, at a level of reality

of <0.001, which means that the accepted equation approximates the observed phenomena with sufficient precision.

Basing on the correlations calculated, between bacterial load and flow intensity one might trace the curve showing the correlation bet-

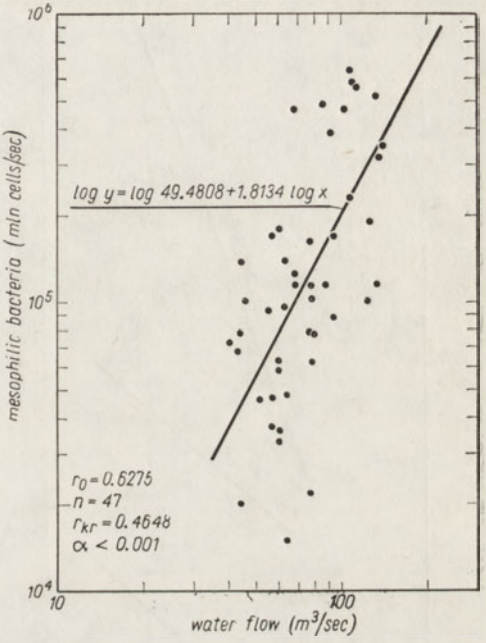

Fig. 3. The correlation between the mesophilic bacteria and water flow

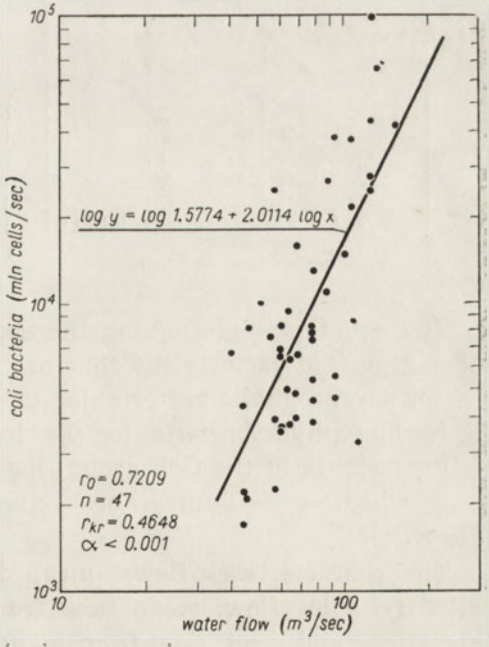

Fig. 4. The correlation between the Coli group bacteria and water flow

ween number of bacteria and flow intensity, dividing the load by the corresponding volume of flow. The thus traced curves for psychrophylic, mesophylic and Coli-group bacteria are presented in Fig. 5.

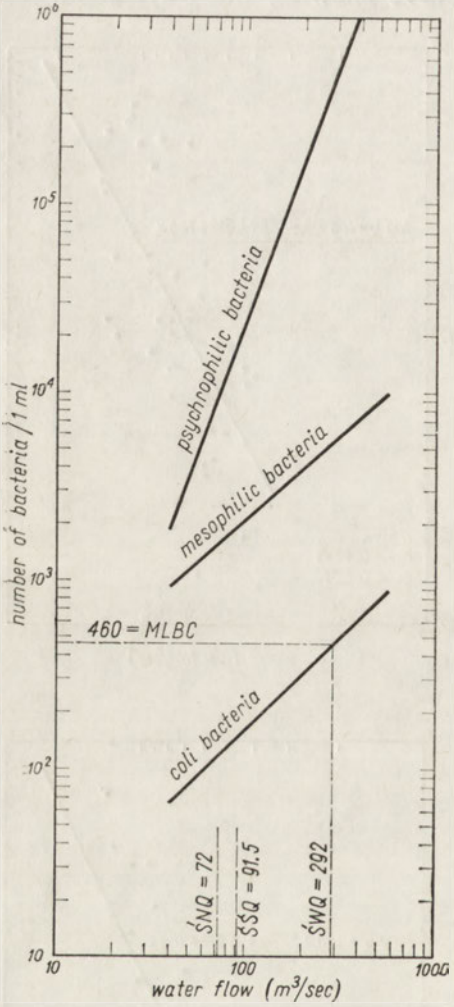

Fig. 5. The correlation between the number of bacteria and water flow. MLBC — the determinating number of bacteria of coli group, ŚNQ — the mean from the lowest flows of many years, ŚSQ — the mean flow from many years, ŚWQ — the mean from highest flows of many years

The equations determining the correlation between number of particular groups of bacteria and flow are the following ones:

for psychrophylic bacteria: $\log y = \log 0.0380 + 2.9227 \log x$

for mesophylic bacteria: $\log y = \log 49.4808 + 0.8134 \log x$

for bacteria of the Coli group: $\log y = \log 1.5775 + 1.0115 \log x$

in which: y — number of bacteria/1 ml, x — intensity of flow in m^3/sec.

The characteristics flows (mean from lowest flows of many years, called reliable flow, mean flow from many years, called also mean large flow, and mean flow from highest flows of many years, called also

large mean flow) (Mańczak 1963) of the Oder River at the cross-section ASPJW — Wrocław (km 249.4), from the years 1965–1970, and the corresponding number of bacteria are presented in Table II.

Table II. The numbers of bacteria corresponding to the characteristic flows of the Oder River at the cross-section ASPJW — Wrocław

Group of bacteria	Number of cells/1 ml		
Psychrophilic	9000	20,000	510,000
Mesophilic	1550	1950	5500
Coli bacteria	115	150	460
Characteristic flows (m^3/sec)	ŚNQ = 72	ŚSQ = 91.5	ŚWQ = 292

4. DISCUSSION

From the compared number of bacteria, occurring at various intensities of flow, results clearly that at larger flows the number of bacteria is higher decidedly than at lesser ones. This is also a result of the hydrotechnical installations on the Oder river, which is piled up on the distance from Koźle to Brzeg Dolny. During sailing time, particular mill-dams pile up the water, what favours the sedimentation process of suspensions and the development of bottom sediments. During non-sailing time and at the period of extensive water-flow, these sediments are floated down the river, and together with them the adsorbed in them bacteria (Mańczak 1972).

As reliable number of bacteria, one should consider those which occur at large mean flow from many years, and amounts to 292 m^3/sec. These values amount to for: psychrophylic bacteria — 510,000 cells/ml, mesophylic bacteria — 5500 cells/ml, Coli bacteria — 460 cells/ml.

From the comparison of reliable number of bacteria of the Coli group amounting to 460 cells/ml with admissible values amounting to 1 — for class I of waters, 10 — for the class II waters and 100 — for class III, it results that from the bacteriological point of view the Oder waters exceed admissible norms for class III.

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E. RZEWUSKA and A. WERNIKOWSKA-UKLEJA

RESEARCH ON THE INFLUENCE OF HEAVY METALS ON THE DEVELOPMENT OF *SCENEDESMUS QUADRICAUDA* (TURP) BRÈB. PART I. MERCURY

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ABSTRACT

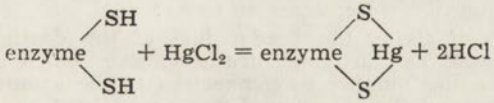
In the investigations of the effect of the mercury salt ($Hg(NO_3)_2$, $HgCl_2$) on the growth of *Scenedesmus quadricauda* (Turp) Brèb., the increase of the quantity of organisms and intensity of photosynthesis was used as a criterion of toxic effect. As the criterions adopted for evaluation did not tally with each other, an additional criterion was applied, namely the value of the coefficient obtained from the proportion of the size of assimilation processes to the respiration and decay processes in the tests with specified salt concentrations.

1. INTRODUCTION

The dynamic progress of civilization which is the condition of social development constitutes a potential threat to the natural environment of which an integral part are surface waters. Quantity and quality of water become often a decisive factor as regards the possibility of putting into practice new technologies. At the same time, we observe an increase of trade wastes which after having been drained off into the collector cause the destruction of its biocoenosis. Przewłocki (1970) quoting Tenn and Stanley mentions 6 groups of industrial works, the wastes of which contain heavy metals. These are the works of metal wares production, metal technology, steel industry, chemical industry, textile industry and of electroplating. Mercury compounds which are used in agriculture for grain treatment constitute an additional source of bringing mercury into the environment.

In the cation toxicity sequence presented by Mroziński (1965) ($Hg > Cu > Ni > Cd \geqslant Pb > Zn > Co > Mn > NH_4 \geqslant Cr > K > Ca > Mg \geqslant Na$) with the constant anion Cl^-, Hg^{++} is on the first place.

Ions of heavy metals constitute special danger for organisms that live in resevoirs. Their toxicity is due to their coming into reaction with proteins, among which enzymes constitute an essential group. The influence of the ions of heavy metals cause the inhibition of the enzyme as a result of the reaction of the metal ion with a functional group of the enzyme according to the following reaction



active enzyme passive enzyme

Polish water legislation (Rozporządzenie...) defines the following permissible concentration of mercury according to water class: class I — 0.001, class II — 0.005, class III — 0.010 mg/l.

The aim of this work was:

1. To examine the influence of cations Hg^{++} on the development of *Scenedesmus quadricauda* measured by the increase of the quantity of organisms and by the intensity of photosynthesis.
2. To examine whether the toxicity of mercury is dependent on the type of accompanying anion and on the water used for diluting processes.

2. MATERIAL AND METHODS

Investigations were carried out making use of alga *Scenedesmus quadricauda* (Turp) Brèb. — a representative of autotrophic organisms to be found in plankton of surface waters, both running and stagnant (Starmach 1969). *S. quadricauda* is, according to the saprobic system of Kolkwitz and Marson, included into organisms of the β-mesosaprobic sphere. This species grows well in laboratory conditions (Jankowski 1964) and at the same time is characterized by rapid increase of cell numbers — which enables the carrying out of experiments in a short period and with considerable differentiation of quantities of organisms according to the concentration of toxic substance. Bringmann, Kühn (1959a, b, 1960) first used *Scenedesmus quadricauda* for toxicological investigations.

The *Scenedesmus quadricauda* population used for experiments comes from the culture of the Institute of Zootechnics in Zator. The basic cultivation was carried on in laboratory conditions for 10 days in 200 ml flasks made from Silvit glass and with the use of Uspenski's medium (Kyć 1970). During the culture time the flasks with the algat were placed in a glow photostat with a light intensity of 3500 thousand lux and temperature 20–22°C. For the purpose of airing the culture the flasks were shaken twice every day. After 10 days the contents of the seperate flasks were blended with a magnetic mixer and the number of organisms in 1 ml was counted under a microscope. Afterwards, 4 ml of algat suspension were removed to a fresh medium for the purpose of carrying on the culture. The remaining algats were used for experiments.

Investigations were divided into two stages. The first stage comprised preliminary experiments, the aim of which was to define such concentration, which, due to the toxical effect of the mercury compound, resulted in considerable inhibition of increase of *Scenedesmus quadricauda*.

For this purpose, with the help of a medium and using titre 2, series of dilutions of $HgCl_2$ and $HgNO_3$ salts were prepared.

Moreover a second similar variant of experiment with the salt $HgNO_3$ was conducted, where instead of medium aquarium water was used for diluting purposes (total hardness $c = 280$ mg/l). Afterwards, to each particular flask to 100 ml of solution, 500 thousand of specimen of organisms *Scenedesmus quadricauda* were added and they were placed in a photostat in analogical conditions as when carrying on the cultivation. After 10 days a visual appraisal was carried out and the growth of organisms in respective concentrations was checked under a microscope. On the basis of such a preliminary analysis a concentration was fixed which in turn became the starting point for the proper experiment.

The second stage comprised the proper experiment. We tried to conduct it in such a way that in a sequence of dilutions after 10 days exposition it would be possible to single out spheres of effects of poisons, defined by Świętochowski et al. (1962) as follows: zone of death, of inhibition, of stimulation and of the absence of activity.

In the experiment proper investigations concerning the toxicity of mercury salts were conducted in the following ranges of concentration.

$Hg^{++}(NO_3)$ — medium from 1.5 to 0.10 mg/l,
$Hg^{++}(Cl^-)$ — medium from 1.0 to 0.005 mg/l,
$Hg^{++}(Cl^-)$ — aquarium water from 4.0 to 0.29 mg/l.

For the purpose of fixing the border line of the death zone the threshold concentration was used, i.e. the concentration above which a total absence of organisms or their smaller number in comparison to the amount introduced during this test at the beginnig of the experiment was ascertained.

The sequence of dilutions in the experiment proper starting with the threshold concentration, was prepared using titre 1.3 in all three repetitions. The con-

trol test — without the mercury salt — has always taken the last place in the sequence. The further procedure was as in the experiment proper.

After a period of 10 days exposition the quantity of *S. quadricauda* organisms was counted under a microscope in a Fuchs-Rosenthal chamber and their number in 1 ml of solution was established. Then the respective solutions of each concentration from the repetitions were poured into one common flask, where they were diluted in the proportion 1 part of the solution with accumulation of algats to 2 parts of medium. All this was thoroughly blended and then poured out into 6 bottles with ground glass stoppers made from clear glass.

In the first pair of bottles the content of dissolved oxygen was fixed at once as the initial quantity. The second pair of bottles was placed in a photostat while the third in a dimmed thermostat in a temperature similar to that of the photostat. After 24 hours the oxygen content in all the samples was fixed and recorded.

The results of calculation of the amount of *Scenedesmus quadricauda* organisms and of the increase of oxygen by photosynthesis in respective concentrations of mercury salt were presented as percentages in proportion to the values arrived at during the control tests.

3. RESULTS

The toxicity of mercury in the form of nitrate (Fig. 1) caused death and inhibition of *Scenedesmus quadricauda* growth in the range of concentrations from 1.5 to 0.52 mg Hg^{++}/l and stimulation in the range from 0.40 to 0.10 mg Hg^{++}/l.

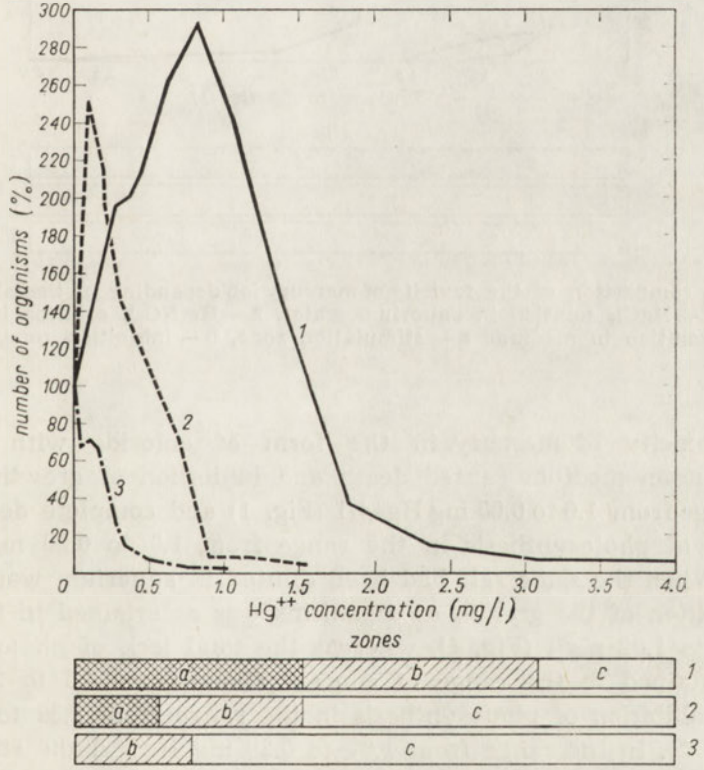


Fig. 1. The comparison of the toxicity of mercury ion depending on the salt in which it occurs. 1 — $HgCl_2$ solution in aquarium water, 2 — $Hg(NO_3)_2$ solution in medium, 3 — $HgCl_2$ solution in medium. a — stimulation zone, b — inhibition zone, c — death zone

The investigations of photosynthesis (Fig. 2) proved its complete decline in concentrations>1.5, inhibition in the range from 1.15 to 0.23 mg Hg^{++}/l and stimulation from 0.14 to 0.10 mg Hg^{++}/l. The comparison of these results shows that using both criterions of estimation, that is the growth of organisms and photosynthesis, in the range of concentrations from 0.14 to 0.10 mg Hg^{++}/l the stimulation of the development of algats was ascertained.

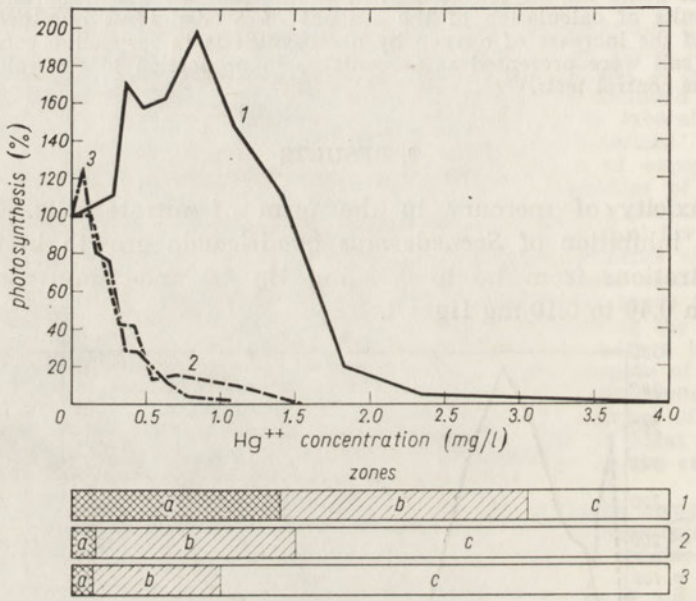


Fig. 2. The comparison of the toxicity of mercury ion depending on the salt in which it occurs. 1 — $HgCl_2$ solution in aquarium water, 2 — $Hg(NO_3)_2$ solution in medium, 3 — $HgCl_2$ solution in medium. a — stimulation zone, b — inhibition zone, c — death zone

The toxicity of mercury in the form of chloride with dilutions carried out on medium caused death and inhibition of growth of cells in the range from 1.0 to 0.05 mg Hg++/l (Fig. 1) and complete decline and inhibition of photosynthesis in the range from 1.0 to 0.20 mg Hg^{++}/l (Fig. 2). When the same salt had been diluted in aquarium water, death and inhibition of the growth of organisms was ascertained in the range from 4.0 to 1.82 mg/l (Fig. 1), whereas the total lack of photosynthesis was ascertained in the range of concentrations from 4.0 to 3.07 mg/l and the inhibition of photosynthesis in the range from 2.36 to 1.40 mg Hg/l (Fig. 2). In the range from 1.07 to 0.29 mg Hg^{++}/l the stimulation of *Scenedesmus quadricauda* growth was ascertained. This stimulation was shown both by an increased quantity of organisms in 1 ml and by the increase of oxygen as a result of photosynthesis.

For the purpose of a broader analysis of results and first of all in order to find the starting point for calculations of the admissible concentrations of toxic substances in water, a ratio between assimilation and dissimilation processes taking place in respective tests was calculated.

The values of ratios which were obtained for *Scenedesmus quadricauda* during the tests with $HgNO_3$ were as follows:

at a concentration $\geqslant 1.5$ mg $Hg(NO_2)_3$/l ratio $= 0$,

at a concentration $= 1.15$ mg $Hg(NO_3)_2$/l ratio $= 1.46$,

at a concentration < 1.15 mg $Hg(NO_3)_2$/l ratio always > 1.46.

On the other hand during the tests with $HgCl_2$ (medium) values of the ratios were as follows:

at a concentration $= 1$ mg $HgCl_2$/l value of the ratio $= 0$,

at a concentration $\geqslant 0.59$ mg $HgCl_2$/l value of the ratio < 1,

at a concentration $\leqslant 0.45$ mg $HgCl_2$/l value of the ratio always > 1.

During the tests with $HgCl_2$ (aquarium water) the values of the ratios were as follows:

at a concentration $\geqslant 1.82$ mg $HgCl_2$/l value of the ratio $= 0$,

at a concentration $\leqslant 1.07$ mg $HgCl_2$/l value of the ratio > 1.

4. DISCUSSION

The investigation of the toxicity of mercury ions proved that this cation, when in the nitrate compound, is characterized by a smaller toxicity than in the form of the chloride.

The confrontation of the results with ions Hg^{++} in the form of chlorides in medium and in aquarium water shows their greater toxicity in the medium. While, in the range of concentrations where during the test with aquarium water mercury stimulated growth and photosynthesis, in the test with the medium it caused death or inhibition or both processes. Besides, we think that test investigations on the subject of permissible concentrations of the toxic substances should be performed on unified standard cultures. An analysis of the results presented in Fig. 1 and 2 proves the necessity of carrying out the evaluation of the toxicity of the heavy metal ions towards *Scenedesmus quadricauda* alga by means of, at least, two criterions. The range of zones as regards the effect of poisons on *Scenedesmus quadricauda* delimited by the criterion of the increase of the quantity of organisms does not always correspond with the range delimited by the second criterion, namely the measurement of photosynthesis. Therefore there is the necessity of conducting further investigations to establish the criterions with the help of which it would be possible to define the permissible concentrations of toxic substances for plant organisms in the water habitat. In the literature which is within our reach we do not find proposals as to the means of

defining the permissible concentrations of substances in waters bases on experiments with plants.

The problem of fixing the admissible concentrations of toxic substances bases on the investigations carried out on animal organisms is broadly discussed in world literature. Hart et al. (1945) and Edwards, Brown (1966) who are quoted by Solski (1971) propose to adopt as the criterion of value LC-50/48 and to apply the safety coefficients ranging from 0.1 to 0.3. The latest recommendations of the U.S. National Advisory Commitee suggest the acceptance of the safety coefficient = 0.1 for the putrefactive poisons and 0.01 for the durable substances (Solski 1971). In our opinion the criterion LC-50 used in animal toxicology cannot be applied for calculation of the admissible concentrations for plants, because it serves to estimate other processes. Moreover, in this way one estimates only the final effect of the action of poisons manifested by death, whereas the deviations from a norm resulting from the disturbance of physiological processes are not analysed.

Taking into account the role which the autotrophical organisms play in the alimentary chain, special attention should be given to studies on their subject. Moreover, in comparison to the animal organisms they present a more suitable though not less sensitive object of research.

The presented estimation of the results of the investigations based on the employed criterions does not give an unequivocal answer to the question, which concentrations of the examined metals could be accepted as admissible for a given species. On account of this an additional attempt was made to interpret the obtained results by calculating in the form of a coefficient the proportion of the quantity of oxygen which increases during the tests owing to the process of photosynthesis to the quantity of oxygen used for dissimilation purposes (in the samples exposed during 24 hours). For the interpretation of data obtained in this manner it was assumed that the concentrations at which the calculated ratios are <1 influence of population of algats by causing it to die out; on the other hand the concentrations which correspond to the value of the coefficient $=1$ (which constitutes the compensation point) were treated as the border value defining the balance point. Moreover, it was assumed that the first concentration in the test at which the value of the given ratio $\geqslant 1$ will be treated as the initial value for the purpose of defining the admissible concentration of the examined substance in the habitat with a simultaenous use of the safety coefficient in accordance with the previously presented proposals in literature. In our opinion the safety coefficient = 0.01 should be accepted for the mercury salt. The proposals to fix the admissible for *Scenedesmus quadricauda* concentrations of mercury salt with a successive use of the safety coefficient 0.1 and 0.01 against the background of the effect of these concentrations expressed by the increase of the quantity of organisms and of the oxygen liberated

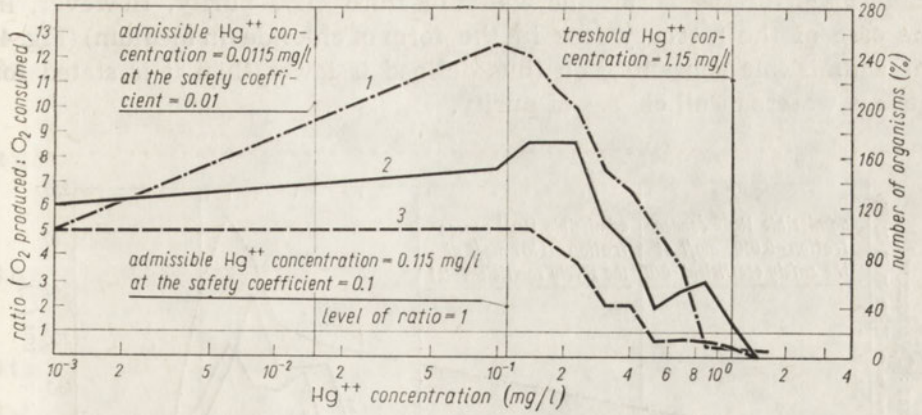


Fig. 3. Estimation of the admissible mercury concentration (diluted in medium) in the form of nitrates using the ratio of O_2 produced in assimilation : O_2 consumed in dissimilation. 1 — number of organisms in % from control, 2 — ratio of O_2 produced : O_2 consumed, 3 — photosynthesis in % from control

by photosynthesis were presented in Fig. 3, 4 and 5. The value of the concentration arrived at in our test with the use of the safety coefficient = 0.1 and when mercury is in the form of chloride (in medium) inhibits the growth of *Scenedesmus quadricauda* organisms (Fig. 4) which suggests the necessity of accepting the coefficient = 0.01.

The admissible concentration of mercury in the form of nitrates calculated in the present work with the use of the safety coefficient = 0.01 amounts to 0.0115 mg Hg^{++}/l (Fig. 3) and approximates the concentration

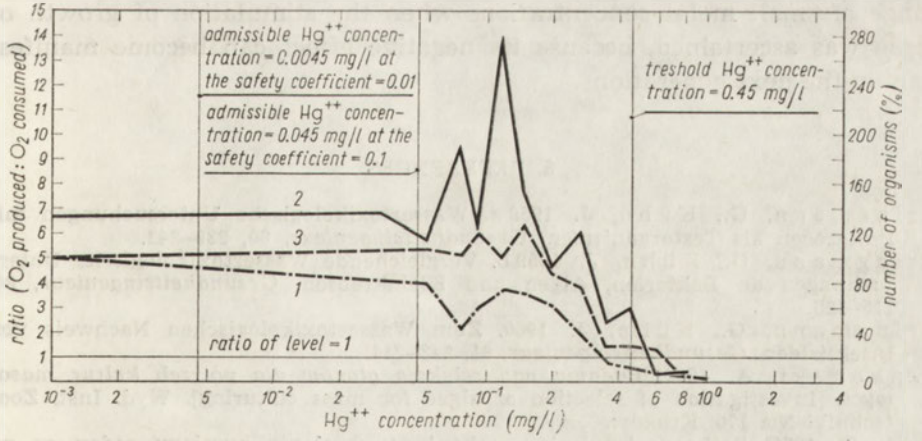


Fig. 4. Estimation of the admissible mercury concentration (diluted in medium) in the form of chlorides using the ratio of O_2 produced in assimilation : O_2 consumed in dissimilation. 1, 2, 3 as in Fig. 3

value given in the norms for water of third class purity. However, in the case of the mercury ions in the form of chloride (in medium) (Fig. 4) the addmissible concentration thus defined is lower than it is stated for surface waters of all classes of purity.

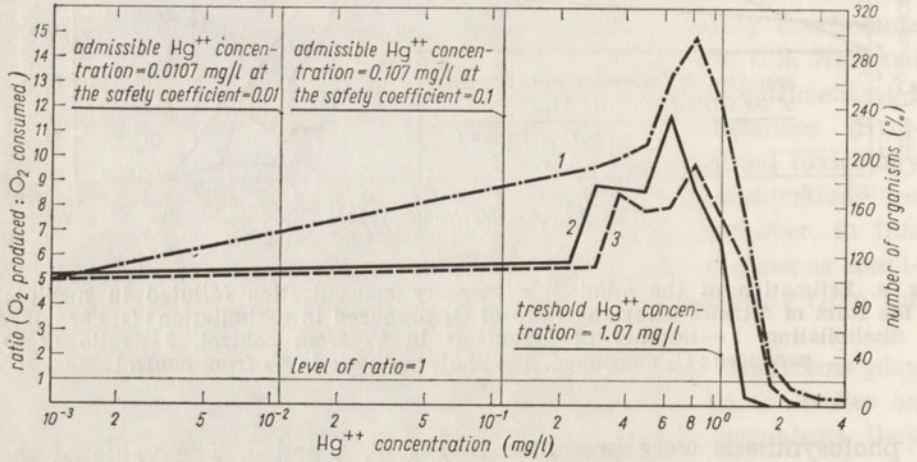


Fig. 5. Estimation of the admissible mercury concentration (diluted in aquarium water) in the form of chlorides using the ratio of O_2 produced in assimilation : O_2 consumed in dissimilation. 1, 2, 3 as in Fig. 3

The possibility of the cumulation of metal in the cells of the algae is an additional important argument for the necessity of applying more acute values of the safety coefficient. This problem was not examined in the tests under discussion. This cumulation can even take place in the range of small metal concentrations when the stimulation of growth of algae was ascertained, because its negative effect can become manifest only in the next generations.

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EFFECT OF AMMONIA AND UREA ON NITROBACTERIA IN WATER ENVIRONMENT

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ABSTRACT

The effect of urea and ammonia on the groups of nitrobacteria living in water environment has been studied in order to determine the strength limit of concentrations inhibiting some metabolic processes in bacteria. It has been found that nitrobacteria have a high degree of tolerance for ammonia and urea, since fairly high concentrations of the examined compounds seldom encountered in the surface water environment had to be used to produce an effect.

1. INTRODUCTION

A considerable development of some branches of chemical industry, the intensification of agriculture, and urbanization of the country have resulted in an increasing contamination of the surface waters with nitrogen compounds. Urea is found in municipal sewage, in industrial wastes from nitrogen production plants, and is also washed away in large amounts from the soil. Ammonia is found in the wastes of chemical industry e.g. in effluents from nitrate fertilizers production plants, in the wastes from coking, refining, and from pharmaceutical plants and municipal sewage, as well. Both urea and ammonia at low concentration have a stimulating effect on the plant growth, and therefore they are widely utilized in agriculture. Nitrogen, carbon and phosphorus, act as fertilizers by stimulating the development of bacteria and algae. At higher concentrations, however, both urea and ammonia show toxic effects.

The hydrolysis of urea is produced by urea bacteria (Maciaszek 1968, Kleczkowski 1971). Only after the hydrolysis urea can be the source of nitrogen for other bacteria, whereas, ammonia occurs in water in the form of NH_4^+ ions and can be absorbed directly by bacteria and be used for biosynthesis of proteins; it can be also utilized by nitrifying bacteria.

We have not found any mention on the effect of urea and ammonia on nitrobacteria in the papers of other authors. On the other hand, their effect on other water organisms, e.g. Cyanophyceae, Diatomae, Chlorophyceae, and Crustacea is well known. Concentrations of both those compounds fluctuate within a wide range of values. Low concentrations of urea and ammonia, ranging from 0.08 to 0.9 mg/l, have a favourable effect on the development of microorganisms. Slightly higher concentration have not caused any disorders in the physiology of water plants. *Asterionella formosa* (Bacillariophyceae) multiplies at 100 mg/l concentration of ammonium nitrate, while Pediastrum (Chlorophyta) multiplies at 2 mg/l concentration, exclusively (Liebmann 1960).

The toxic effect of ammonia in water environment depends on the pH value, the concentration of non-dissociated ammonium base, and the content of carbon dioxide (McKee, Wolf 1963). Liebmann (1960) has given data on the limits of tolerance of the harmful effects of ammonia. Thus, for fish the tolerance limits are within the range of 0.2–2 mg/l, and for other water organisms 0.2–9 mg/l.

McKee, Wolf (1963) give the following toxicity thresholds of ammonia for indicator organisms: 0.08–0.9 mg/l for organisms from oligo- and mesosaprobic zones, 0.3–4.9 mg/l for organisms from mesosaprobic zone, 3.2–220 mg/l for organisms from polysaprobic zone.

Noxious concentrations of urea are much higher (McKee, Wolf 1963), Liebmann (1960) indicates that for fish urea at 16 g/l concentration is harmful while the 30 g/l concentration is a lethal dose.

The purpose of the present study is determination of ammonia and urea concentrations which have an inhibiting or even toxic effect on the physiologic development of nitrobacteria groups.

2. MATERIAL AND METHODS

Studies on the action of urea and ammonia were carried out on a pure nitrobacteria strain isolated from the Oława River. In order to isolate pure ammonifying and denitrifying cultures agar was used as a base. Then, the pure ammonifying bacteria were cultured in peptone medium and denitrifiers in broth with KNO_3 (Rodina 1968). Proteolytic bacteria were isolated and cultured in gelatin medium. Pure cultures of nitrifying bacteria were obtained by the following procedure: samples of water from the Oława River were passed through the Sartorius type membrane filters with 0.2 μ pores. Then filtrates were placed on polyurethane foam pads submerged in liquid medium on the Petri plates.

Pure cultures of nitrobacteria used as experimental material were incubated in the above mentioned media. The pH value of the culture medium was not the same for all groups of bacteria. For nitrifying bacteria (phase I) pH was 7.7, for nitrifiers (phase II) — 8.0, and for all others — 7.2. In order to compare the effect of urea and ammonia on the examined bacteria the material was suspended in Tris buffer, at pH of identical value as mentioned above.

The initial density of bacterial suspension was determined by means of the Spekol type spectrophotometer and was constantly equal to 70% of the transmitted light. Measuring of the nitrifying bacteria density in the "Spekol" was connected with some difficulties due to the settling of those bacteria on the insoluble particles of the medium components (calcium carbonate). Thus, there would be a gross error in the measurements of the density of suspension. Therefore, in the case of the nitrifiers suspension the following procedure was used: bacteria together with calcium carbonate were collected always from a 14-day-old culture and centrifuged. Nitrites and nitrates in the supernatant layer were examined colorimetrically. The quantity of nitrites and nitrates was always constant while the centrifuged bacteria constituted the initial suspension to be used in further experiments. Next, urea and ammonia were added to those previously prepared samples in such quantities so as to obtain an adequate range of concentrations of the examined compounds. Thus, for the nitrifying bacteria they ranged from 10^{-7} to 20 g/l, and for the other nitrobacteria from 0.1 to 26 g/l. The determined time for the contact of bacteria with the compound was 24 hrs; the temperature during the course of the experiment was 25°C; the pH value was the same as during incubation, respectively.

The effect of the examined substances was estimated on the basis of changes in the activity of the investigated bacteria strains. The ability of the bacteria for proteolysis, ammonification, and nitrification served as a criterion of their activity. With this purpose in view, bacteria were centrifuged after 24 hour contact with urea and ammonia, washed in Tris buffer and centrifuged once more. The supernatant liquid was decanted and the bacteria were suspended in NaCl physiological solution. After dilution the bacteria were inoculated on the appropriate media. Each of the urea and ammonia concentrations had a distinct range of dilutions. The control sample was prepared in the same manner but it did not contain the examined compounds.

The estimation of results was based on the ability of bacteria to produce some specific metabolic processes, such as: proteolysis, ammonification, denitrification, and nitrification. Therefore, proteolytic bacteria from proper dilutions were inoculated by pricking method in gel medium and cultured at 25°C for 72 hours. Then, their proteolytic activity was estimated in relation to various concentrations of the examined compounds. The activity of ammonifying and denitrifying bacteria was estimated respectively by detecting the presence of ammonia after incubation period with the Nessler reagent. Nitrifiers were cultured

during 14 days at 25°C and their activity was estimated on the basis of the presence of NO_2^- detected by means of Griss reagent and of NO_3^- detected by phenolobisulphonic acid. Intensity of staining in both cases was read calorimetrically with Spekol. Then the quantity of products in mg/l was determined from the calibration curve by taking the extinction value as basis of calculation.

3. RESULTS

The effect of ammonia on nitrobacteria is shown in Fig. 1. Ammonia at 0.1 g/l concentration does not show any negative effects in proteolytic bacteria, whereas a 0.5 g/l dose reduces the titer of those bacteria to

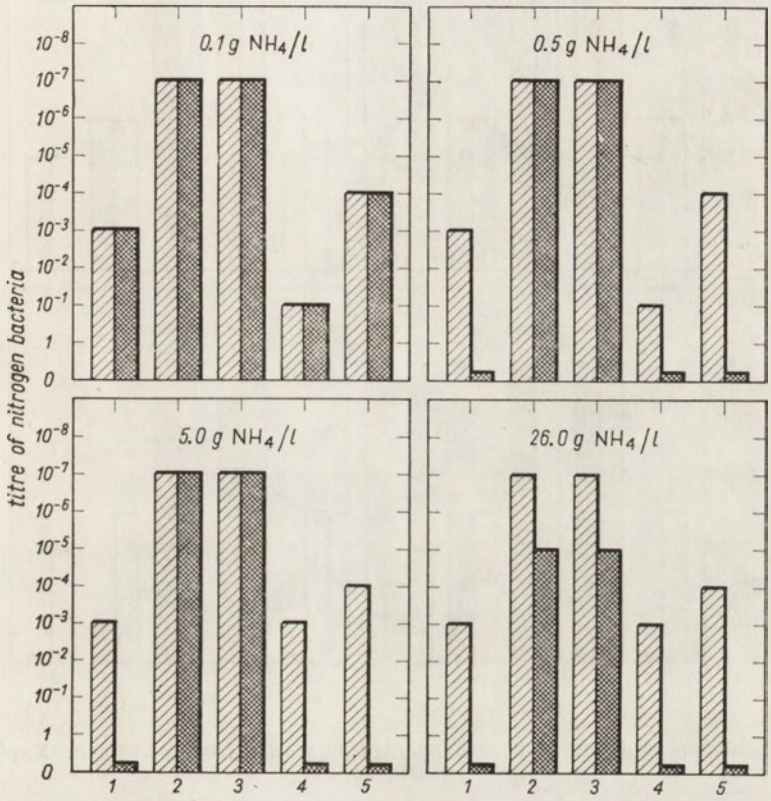

Fig. 1. Influence of different ammonia concentrations on nitrogen bacteria. 1 — proteolytic bacteria, 2 — ammonifying bacteria, 3 — denitrifying bacteria, 4 — nitrifying bacteria (phase I), 5 — nitrifying bacteria (phase II). Lighter bars — controls, darked bars — experimental data

naught. Ammonia in the range of 0.1—20 g/l does not show any negative effects in ammonifying and denitrifying bacteria, since their titre remains at the same level as in the control sample. Only at 26 g/l concentration their titer decreased from 10^{-7} to 10^{-5}, as compared with the control sample. The action of nitrifying bacteria (phase I and II) becomes completely inhibited at 0.5 g/l ammonia concentration.

The effect of urea on nitrobacteria is shown in Fig. 2. As can be seen, the titer of proteolytic bacteria is reduced from 10^{-3} to 10^{-2} along with the increase in urea concentrations from 0.5 to 1.0 g/l and their action becomes completely inhibited at 10 g/l urea concentration. Within the range of urea concentrations from 0.5 g/l to 20 g/l the

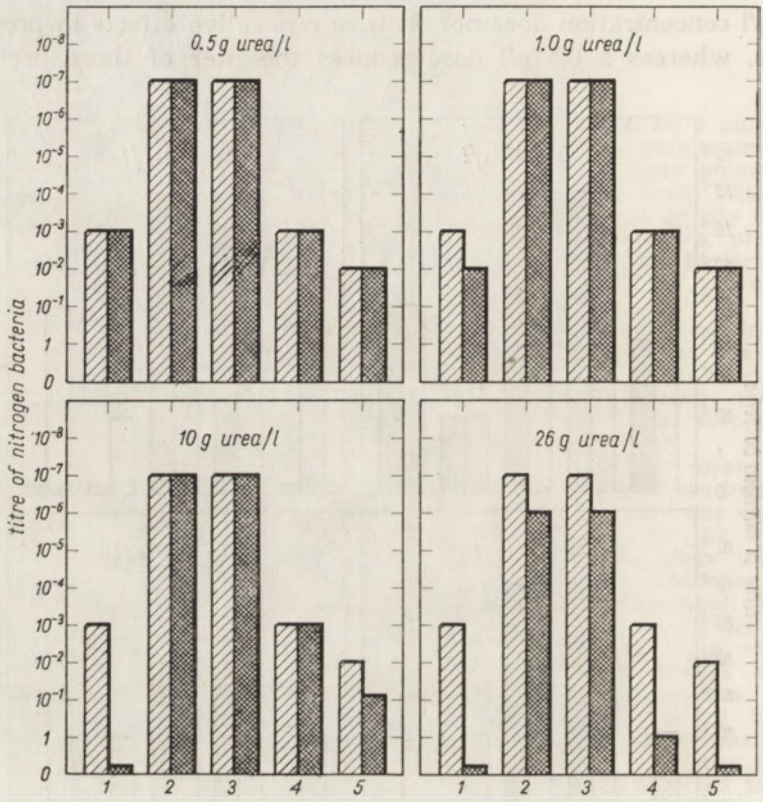

Fig. 2. Influence of different urea concentrations on nitrogen bacteria. Explanations as in Fig. 1

titre af ammonifying and denitrifying bacteria does not show any changes, only at 26 g/l concentration it gets reduced from 10^{-7} to 10^{-5}. The titre of the nitrifying bacteria (phase I) does not show any decrease at concentrations ranging from 0.5 to 20 g/l but it gets reduced from 10^{-3} to 1 at the 26 g/l concentration. The nitrifying bacteria (phase II) are less resistant to the effects of ammonia since at the 10 g/l concentration their titre is reduced from 10^{-3} to 10^{-2}. At 15 g/l the titre is reduced to 1, and at 26 g/l concentration this type of bacteria does not show any ability to produce specific metabolites.

4. DISCUSSION

The present study showed that ammonia is much more toxic than urea. Of all the strains of nitrobacteria the most resistant to the effects of both compounds were the strains of ammonifying and denitrifying bacteria while proteolytic and nitrifying bacteria strains appeared to be the most sensitive. The range of concentrations used in the experiment has produced only the reduction but no stimulation of the examined metabolic processes. Concentrations of ammonia ranging from 0.1 to 20 g/l have not shown negative effects in denitrifying and ammonifying bacteria, whereas, in proteolytic and nitrifying bacteria no changes were produced at 0.1 g/l concentration. In the range from 0.1 to 26 g/l the latter bacteria were reduced to naught while the former showed a decrease at 26 g/l concentration of ammonia. Proteolytic bacteria occurred at the 10 g/l urea concentration and the nitrifying ones (phase II) at a concentration below 20 g/l. The number of denitrifying and ammonifying bacteria was only slightly reduced.

The higher tolerance of ammonifying and denitrifying bacteria may be explained by the fact that in physiological processes produced by the action of those bacteria large quantities of ammonia are released. Since both compounds produce inhibitory effects on some groups of nitrobacteria at high concentrations only, their action cannot be considered as being toxic.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	21	1	125-131	1974

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EFFECT OF PHOSPHORUS ON THE ENZYMATIC ACTIVITY OF WATER BACTERIA

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ABSTRACT

The examined inorganic phosphorus compounds, even in small doses (>0.1 mg P/1) had inhibitory effects on the development of water bacteria whereas the presence of organic phosphoric compounds had a stimulating effect on them. The frequency of bacteria producing phosphatase in the total number of water bacteria depends on the degree of water pollution, expressed in BOD_5. In all kinds of the examined bacteria basic phosphatases were found showing the maximum of activity at 9.0–10.0 pH. Phosphatases are enzymes not capable of adaptation. Both, potassium phosphate and β-sodium glycerophosphate have a positive effect on respiration intensity, however, organic phosphates are more effective in increasing the activity of respiration processes.

1. INTRODUCTION

Phosphorus participates in many enzymatic reactions, such as, e.g. cyclic photophosphorylation, photochemical decomposition of water, assimilation of carbon dioxide, and some other processes essential in biological oxidation. The effect of phosphorus on oxidation processes in organic matters is of particular interest and importance from the viewpoint of conservation of natural waters.

It is a well known fact that an increase of phosphates in water increases the trophic conditions in water bodies by stimulating the bacteria and algae development (Liebmann 1960, Schmidt 1963, Phillips 1964, Stangenberg 1967). Ohle (1953) believes that the main role in the eutrophic processes can be ascribed to the content of orthophosphates in the waters, whereas, Vogler (1965) is of opinion that besides orthophosphates, also, the dissolved inorganic polyphosphates and dissolved organic phospho-compounds may supply phosphorus required by water organisms. The eutrophic processes occurring in the water bodies are a highly unfavourable phenomenon, since an increase of organic substances has a particularly negative effect on the oxygen conditions and, also, changes to the worse the physico-chemical properties of water.

Phosphorus is one of the factors conditioning the growth of the bacterial flora in the water. However, the knowledge concerning participation of bacteria in utilization and transformation of phosho-compounds and about the effect of phosphorus on the bacterial life and growth is still rather incomplete. To explain, at least, some points of that interesting problem, studies were undertaken with the view to learn to what degree the development of bacteria is determined by various kinds of phosphorus sources and concentrations. The effects of phospho--compounds on the changes in respiration intensity in the selected species of water bacteria were examined, as well, basing on the fact that the respiratory enzymes activity is strongly associated with the presence of inorganic phosphates.

2. MATERIALS AND METHODS

Bacteria cultures were grown in mineral agar with asparagine and glucose. The culture medium was complemented with a solution of microelements, as by

Fiodorow (1952). Monobasic potassium phosphate, sodium pyrophosphate, washing powder "IXI", and sodium β-glycerophosphate in doses ranging form 0.0 to 100.0 g/l were used as the source of phosphorus for bacteria.

Detection of bacterial phosphatases was performed with the phenolphthalein disphosphoric acid sodium salt method (Collins 1964, Rodina 1965). In order to determine whether the phosphatases of the examined species of bacteria are of the acid or basic enzymes type a modified Mienkin's culture medium was prepared (Rodina 1965), the pH ranging from 4.0 to 11.0. The medium was inoculated with pure strains of *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Serratia marcescens* and incubated for 120 hrs at 20 and 37°C.

Adaptation of *Escherichia coli* and *Alcaligenes faecalis* strains to the phosphatase production was carried on in a partly modified Mienkina's culture medium (without the supplement of chalk and lecithin) during 30 days, at 20°C. Sodium β-glycerophosphate or phenolphthalein diphosphoric acid sodium salt were used to induce the formation of phosphatases.

A Warburg respirometer was used for determining the effect of various doses of organic and inorganic phosphorus on the intensity of respiration. As an organic source of phosphorus sodium β-glycerophosphate was used and from mineral salts a K_2HPO_4 and KH_2PO_4 mixture. The experiment was carried out in a pure *Pseudomonas aeruginosa* culture suspended in a modified Mienkina's culture medium, without glucose, at 7.3 pH. The number of bacteria in suspension was always the same.

3. RESULTS

DEPENDENCE OF THE NUMBER OF BACTERIA ON THE SOURCE AND DOSAGE OF PHOSPHORUS

The colony of water bacteria cultured in the medium containing inorganic compounds, as the source of phosphorus, showed a weaker development than the others grown on common agar-agar medium (Fig. 1, curves 1—3). In the case of the tests with monobasic potassium phosphate (Fig. 1, curve 1), the greatest number of bacteria obtained at the optimal

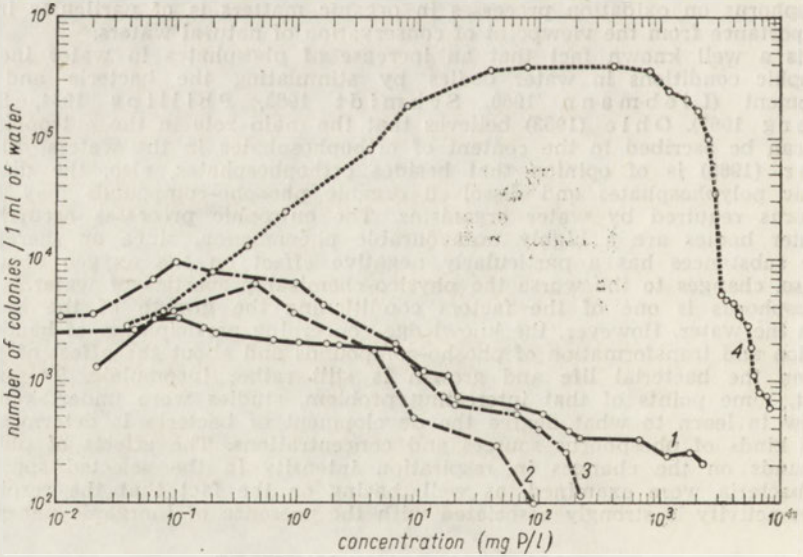

Fig. 1. Dependence of the number of bacteria on the doses of the chemical compounds containing phosphorus. 1 — KH_2PO_4, 2 — $Na_2H_2P_2O_7$, 3 — washing powder "IXI", 4 — sodium β-glycerophosphate (log-log scale)

dose of 0.1 mg P/l amounted to 3350 colonies, which was only 61% of the number of bacteria cultivated in common agar-agar medium (5450 colonies per 1 ml). Higher doses had an inhibitory effect on the bacterial flora development and the decrease in the number of bacteria was very distinct (Fig. 1).

Slightly better results were obtained with the use of sodium pyrophosphate (Fig. 1, curve 2). At the optimal dose of 0.46 mg P/l the number of bacteria amounted to 6100 and was 11% greater than the number of bacteria grown on common agar-agar (5450 colonies per 1 ml). At a higher dose the number of bacteria was decreasing very sharply which may lead to conclusion that larger amounts of sodium pyrophosphates are noxious to the development of bacteria.

Studies were also carried out on the effects of phosphorus, contained in detergents, upon the growth of bacteria. The washing powder "IXI" was used in the experiments. In this series of tests the number of bacteria grown in agar-agar medium amounted to 7950 colonies per 1 ml. The obtained results (Fig. 1, curve 3) suggest that in this case, also, there is an interrelation between the amount of phosphorus and the number of bacteria. Small doses of the washing powder are stimulating bacterial growth very markedly — up to 0.1 mg P/l dose but above that value the number of bacteria decreases rather strikingly. One cannot allege unequivocally that phosporus is the sole agent limiting the growth of bacteria in that case. The other components of that detergent may also have a negative effect on the development of microflora.

The experiments, having in view explication of the organic phosphocompounds effects on the development of bacteria, have shown that complementing a poor mineral medium with sodium β-glycerophosphate had a stimulating effect on the microorganisms growth (Fig. 1, curve 4). This effect was distinctly noticeable already at 0.4 mg P/l dose and reached its maximum at the concentration of 400 mg P/l. At that point, the number of bacteria was fiftyfold more numerous than the number of bacteria cultivated on common agar-agar medium (7950 colonies per 1 ml). At a higher dose than 400 mg P/l there was a striking decrease in the number of bacteria giving evidence to the harmful effects of those β-glycerophosphate concentrations on the development of bacteria.

PROPORTION OF THE BACTERIA PRODUCING PHOSPHATASE TO THE TOTAL NUMBER OF BACTERIA FOUND IN THE SURFACE WATERS

There is a great variety of bacteria species in the surface waters but not all of them are capable of splitting the organic phosphorus compounds. This is associated with the possession of enzymes causing hydrolysis of the phosphate esters, i. e. the presence of phosphatases.

With the purpose of determining what is the proportion of the phosphatase producing bacteria in the total number of the water microflora

and to find out whether their presence is conditioned by the degree of water pollution, studies were carried out in several lakes in the region of Konin and some stations allocated on the Warta and Bogdanka rivers. On the background of physico-chemical conditions characterizing the degree of water pollution, thorough examinations were carried on to study the changes in the number of bacteria participating directly in hydrolytic processes of the organic phospho-compounds. A complex of six lakes in the region of Konin town, viz., the Ślesińskie, Mikorzyńskie, Wąsowskie, Pątnowskie, Licheńskie and Gosławickie lakes showed only a slight degree of water contamination and can be characterized as mesotrophic water bodies with exception of the Gosławickie Lake showing a fairly high degree of water pollution (BOD_5: 5.6—8.0 mg O_2/l). The water of the Warta River within the municipal area of Poznań was strongly polluted (BOD_5: 13.8—15.4 mg O_2/l).

The proportion of the bacteria producing phosphatases to the total number of bacteria is dependent on the degree of water pollution expressed in BOD_5 (Fig. 2). This interrelation was very distinctly marked in the case of the samples collected from the Warta River and the Gosławickie Lake, i.e., the polluted waters. In the waters contaminated with organic substances the number of bacteria producing phosphatase was

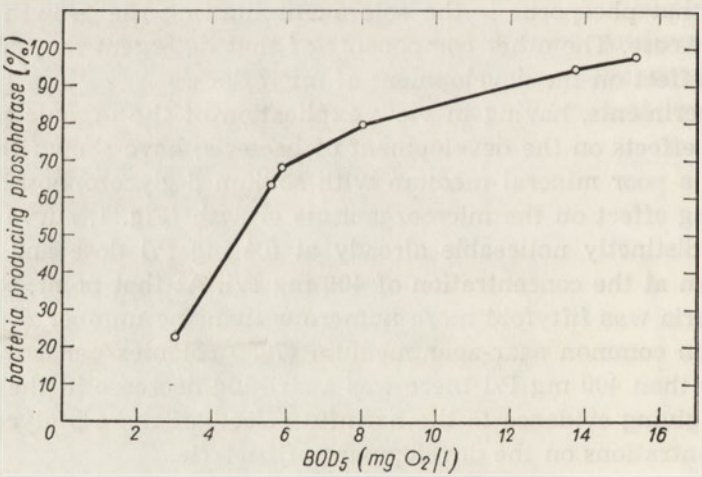

Fig. 2. Dependence of the percentage of bacteria producing phosphatase and the total number of bacteria on the changes in BOD_5 values

very often over 90% of the total number of bacteria (Fig. 3). In waters with a lower degree of contamination the number of bacteria producing phosphatase is, as a rule, smaller than the number of the phosphatase-negative bacteria and does not exceed 35% of the total number of bacteria (Fig. 3).

Phosphatases belong to the very common and widespread enzymes catalyzing the hydrolysis of organic phosphate esters. Experiments were carried out to find out whether the phosphatases produced by *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Serratia marcescens*, are of a basic or acid character.

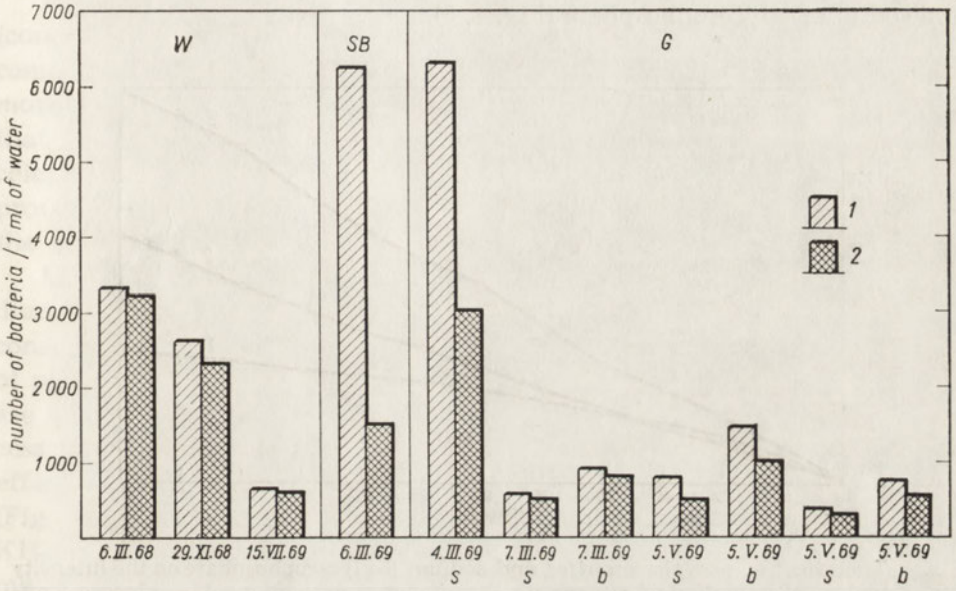

Fig. 3. Comparison of the total number of bacteria (1) with the number of the bacteria producing phosphatase (2) in the Warta River (W), the Struga Biskupia River (SB) and the Gosławickie Lake (G), s — surface samples, b — bottom samples

The obtained results indicated that the examined bacteria species produced basic phosphatase and the maximum of the enzyme activity in case of *Pseudomonas aeruginosa* was reached at 9.0 pH, while in the other two species at 10.0 pH, at the temperature of 20 and 37°C, alike.

The ascertainment of the presence of bacteria producing phosphatase along with those not-producing phosphatase made it necessary to determine whether phosphatases are the adaptive enzymes.

Observations were carried out on pure strains of two species of bacteria without phosphatases, namely *Escherichia coli* and *Alcaligenes faecalis*. Sodium β-glycerophosphate and phenolphthalein diphosphoric acid sodium salt, at 0.1—1000 mg/l doses, were used as inductive compounds to produce phosphatase. Despite a good development of bacteria, it was found after a 30 day adaptation period that the microorganisms have not produced enzymes capable of catalysing the hydrolysis of those compounds.

THE EFFECT OF PHOSPHORUS ON THE RESPIRATION INTENSITY

With the purpose of a thorough examination of the effects of inorganic and organically combined phosphorus on the intensity of the biological oxidation of glucose by *Pseudomonas aeruginosa*, several series of experiments were carried on using mono- and dibasic sodium phosphate and sodium β-glycerophosphate (Fig. 4, 5).

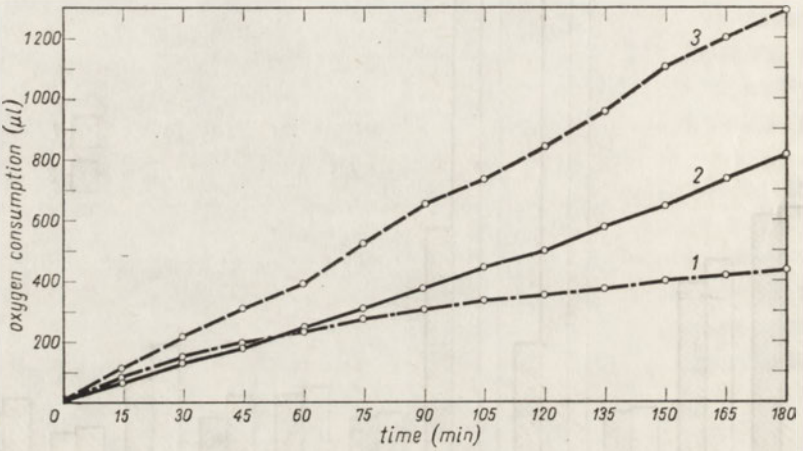

Fig. 4. The effect of phosphate buffer and sodium β-glycerophosphate on the intensity of the biological oxidation of glucose by *Pseudomonas aeruginosa*. 1 — glucose (control), 2 — glucose + sodium β-glycerophosphate (1000 mg P/l, 3 — glucose + phosphate buffer (KH_2O_4 + K_2HPO_4) (10 mg P/l)

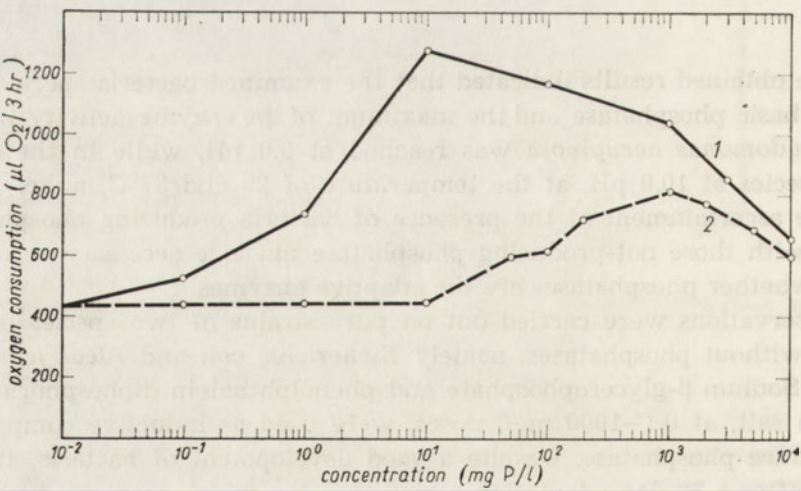

Fig. 5. Comparison of the effect of KH_2PO_4 + K_2HPO_4 (1) and sodium glycerophosphate (2) on the intensity of the biological oxidation of glucose by *Pseudomonas aeruginosa* (semi-log scale)

At an optimal dose of mono- and dibasic sodium phosphate (10 mg P/l) the oxygen consumption, after 180 min, is threefold greater (Fig. 4, curve 3) than in the test without the addition of phosphates (Fig. 4, curve 1). It serves to show that inorganic phosphates accelerate markedly the oxidation of the substratum and in that way they are increasing the activity of the respiratory enzymes.

Following the pattern of the test with sodium β-glycerophosphate (concentration 1000 mg P/l) the effects of phosphorus occurring in organic compounds on the speed of the process of glucose oxidation by *Pseudomonas aeruginosa* were also examined. The obtained results indicated that in this case the biochemical processes of glucose oxidation were accelerated likewise, though the oxygen consumption in the respiration process was only twofold greater, as compared with the tests without the addition of phospho-compounds (cf. curve 1 and 2 in Fig. 4).

Using various doses of mono- and dibasic sodium phosphate and sodium β-glycerophosphate, as substances affecting the intensity of oxygen consumption by bacteria, it has been observed that even small quantities of phosphorus derived from inorganic compounds give in effect an increase of the oxygen volume assimilated by bacteria in the respiration process (Fig. 5, curve 1). Organic phospho-compounds have, also, a positive effect on the intensity of respiration, though only at much greater doses (Fig. 5, curve 2). In both cases the optimal doses were determined, for KH_2PO_4 and K_2HPO_4 — 10 mg P/l, for sodium β-glycerophosphate — 1000 mg P/l; above those values a distinctly negative effect of those compounds on the biochemical process of oxidation has been observed.

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L. BOŻKO, Z. KAŃSKA and K. MICHAŁOWSKI

DEHYDROGENASE ACTIVITY OF ACTIVATED SLUDGE IN PURIFICATION OF INDUSTRIAL AND MUNICIPAL WASTES

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ABSTRACT

The dehydrogenase activity of activated sludge microorganisms according to the changing BOD load by the amount of pollutions in purification of municipal sewage and industrial wastes arising from the production of synthetic fibres was investigated. The obtained results proved that the dehydrogenase activity measured by the TTC test is a very sensitive indicator of the course of the sewage purification process by the activated sludge method. The TTC test should be used to supplement the control investigations of activated sludge.

1. INTRODUCTION

The continuous increase of municipal and industrial wastes creates the necessity to develop and perfect the methods of their purification. A subject of interest at the present stage of research is among others the putting into practice of enzymatic methods, serving to measure the oxidative capacity of activated sludge. The metabolism of organic substrates is catalyzed by a number of enzymatic systems and therefore the inhibition or activation of enzymes affects the course of the purification process.

Certain research workers (Vaicum et al. 1965) recommended using many enzymes as indicators of the activity of activated sludge however in practice dehydrogenase are used (Bucksteeg, Thiele 1959, Lenhard et al. 1964, Ford 1967). The method of determining the activity of these enzymes is based on the reduction of the colourless triphenyltetrazolium chloride (TTC) to triphenylformazan (TF) which is red. The intensity of the colour of the sample characterizes the dehydrogenase activity of the microorganisms.

The subject of the present investigations was to determine the dehydrogenase activity of microorganisms of the activated sludge collected from the municipal wastewater-treatment plant "ZASPA" in Gdańsk and of the activated sludge which purifies the industrial wastes from the production of synthetic fibres.

The main aim of the investigations was to characterize the dehydrogenase activity according to the effect of purification of sewage expressed in the reduction of BOD_5, COD-permanganate, COD, total Kjeldahl nitrogen and also dependent on the value of the sludge volume index and using BOD load. The process of sewage purification by the method of activated sludge was carried out at different technical parameters.

2. METHODS

The scope of the investigations included the determination of dehydrogenase activity of activated sludge using the TTC test (Ford 1967) and the control

of the course of the sewage purification process. During the course of the investigations the technical parameters of the process and the physical and chemical composition of the sewage flowing into the aeration tank and flowing out of the tank were determined. A biological analysis of the activated sludge was also carried out. The technological investigations were carried out according to Imhoff (1969), Gańczarczyk 1969) and physical and chemical investigations according to Hermanowicz et al. (1967). The biological analysis was carried out on the basis of the quantitative and qualitative investigations of the organisms to be found.

3. RESULTS

CHARACTERISTICS OF THE EXAMINED SEWAGE

The composition of the industrial wastes was characterized on the basis of the chosen properties which could influence the purification process. The examined industrial wastes had a specific odor. Their pH was about 7.2. The average values of the pollution indicators were as follows: BOD_5 270 mg O_2/l, COD-permanganate 90 mg O_2/l, COD 755 mg O_2/l. Specific substances: ethylene glycol, methanol and nonionic surfactans defined on the basis of average values occurred in concentrations: 170, 180 and 50 mg/l, respectively. The nitrogen compounds were mainly represented by nitrite nitrogen and organic nitrogen (average value 3.22 mg $N\text{-}NO_2$/l and 4.13 mg N_{org}/l). Ammonia nitrogen and nitrate nitrogen occurred in small amounts (0.15 mg $N\text{-}NH_4$/l and 0.05 mg $N\text{-}NO_3$/l). The amount of phosphates was 1.45 mg PO_4/l.

On account of the unsufficient contents of biogenic compounds of nitrogen and phosphorus the wastes were enriched with salts of these elements in the generally accepted proportion $BOD_5 : N : P$ as $100 : 6.0 : 1.5$.

The sewage flowing into the treatment plant "ZASPA" in Gdańsk contained municipal sewage with a small addition of industrial wastes with a pH of 6.7—7.4, BOD_5 109—354 mg O_2/l, COD-permanganate 71—141 mg O_2/l and the suspended solids (SS) of 107—354 mg/l.

INVESTIGATIONS ON THE PROCESS OF INDUSTRIAL WASTES PURIFICATION

The process of purification of the wastes was carried out in laboratory conditions in the unit presented in Fig. 1. The activated sludge adapted for the purification of these wastes was used in the investigations. The dehydrogenase activity of microorganisms of the activated sludge was determined according to the increase of BOD load. The range of the employed load amounted to 1029—1920 mg BOD_5/l · day (BOD_5 is given here per liter and per day). Figure 2 shows the results of the biological investigations and the effect of the purification of the wastes expressed in the reduction of BOD_5, COD, COD-permanganate and specific substances.

Throughout the whole period of investigations the presence of the filamentous bacteria of the Sphaerotilus genus was observed. During

the course of the purification process when the BOD load of the aeration tank amounted to about 1100 mg BOD_5/l · day, the number of Sphaerotilus fell from 60 to 20 thousand/ml. During the further stage of the

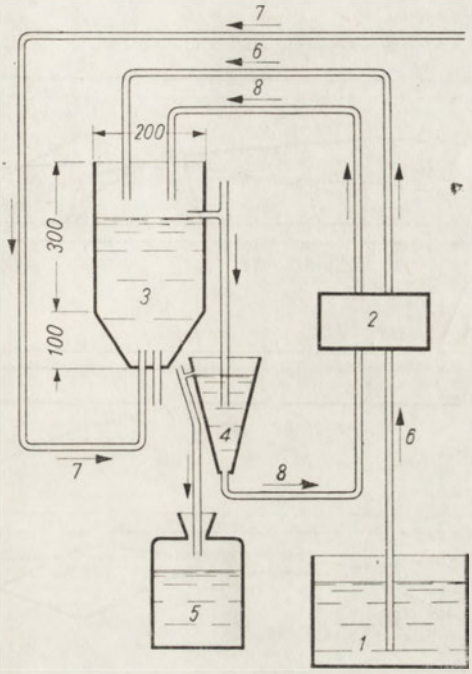

Fig. 1. The scheme of the laboratory installation for purification of wastes using the activated sludge method. 1 — tank of inflowing wastes, 2 — feeding pump, 3 — aeration tank, 4 — settling tank, 5 — tank of outflowing wastes, 6 — channels of inflowing wastes, 7 — channels of compressed air, 8 — channels of returned sludge

process when the load was gradually increased to 1400 and 1900 mg BOD_5/l · day, the numbers of Sphaerotilus increased respectively to 108 and 132 thousand/ml, reaching the maximum value. However, in the final stage with the greatest load employed the number of Sphaerotilus suddenly diminished to 2 thousand/ml. At the same time bacteria from the Zoogloea genus and fungi appeared.

Stalked Ciliata dominated among the Protozoa. Their number diminished with the increase of the load 1100—1400 mg BOD_5/l · day amounting on the average from 20 to 1.2 thousand/ml. At the highest examined load of 1900 mg BOD_5/l · day they disappeared at first, but then appeared again once in the number of 2.0 thousand/ml. Free swimming Ciliata appeared periodically in small numbers reaching maximum numbers of 19.2 thousand/ml at the highest load. This number was three times greater than the number present at lower loads of the aeration tank. Rhizopoda appeared in the final stage of applying of the load of

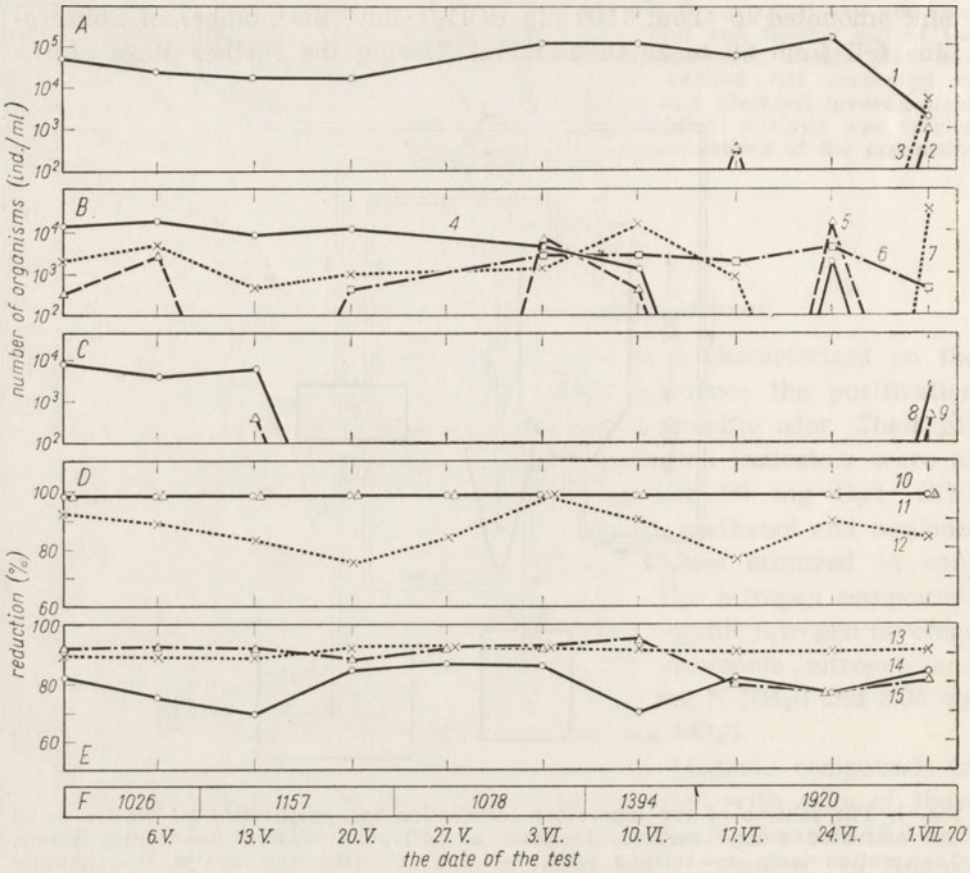

Fig. 2. The investigations on the process of purification of industrial wastes using the activated sludge method (April 29 — July 1). The effect of purification expressed in numbers of organisms. A — Bacteriophyta: 1 — *Sphaerotilus sp.*, 2 — *Zoogloea sp.*, 3 — Fungi; B — Protozoa: 4 — free swimming Ciliata, 5 — stalked Ciliata, 6 — Rhizopoda, 7 — Mastgophora; C — other animal organisms: 8 — Rotatoria, 9 — Nematodes; D — reduction of specific substances: 10 — methanol, 11 — ethylene glycol, 12 — nonionic surfactans; E — reduction of the basic pollution indicators: 13 — BOD_5, 14 — COD-permanganate, 15 — COD; F — the load of aeration tank (mg BOD_5/l · day)

about 1100 mg BOD_5/l · day and remained at a small level till the end of the investigations, independent of the load.

Other animal organisms, mainly at the lowest load, were represented by Rotatoria and twice by few Nematodes.

On the basis of the reduction of the pollution in the raw sewage and in the purified sewage the aeration tank, the effect of the sewage purification was as given below. In the whole period of investigations irrespective of the increase of the load the reduction of BOD_5 was high amounting to 92—94%. A similar trend showed the reduction of COD

reaching 98% at the load 1400 mg BOD_5/l · day. During the increase of the load to 1900 mg BOD_5/l · day the reduction of COD fell and till the end of the investigations remained at the level of reduction of COD-permanganate which in the period under discussion was the lowest in comparison with BOD_5 and COD and amounted in the final stage to 82%.

The dehydrogenase activity of the activated sludge in the examined range of loads of the aeration tank increased in general with the load (Fig. 3). In the initial stage of the investigations at the constant load of about 1100 mg BOD_5/l · day the activity of the sludge and the mixed

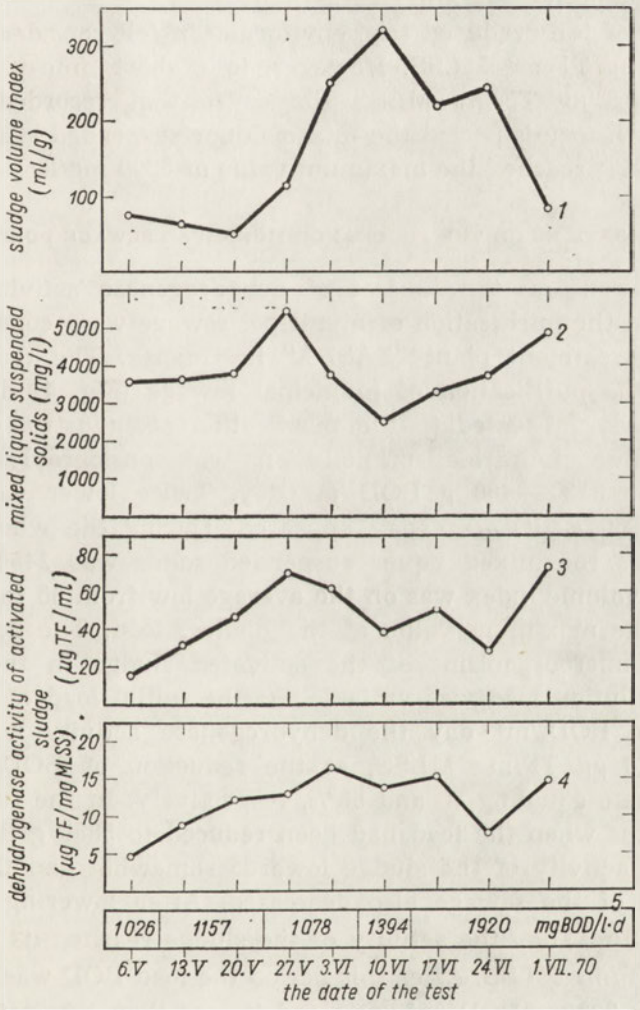

Fig. 3. The investigations on the process of purification of industrial wastes using the activated sludge method (April 29 — July 1). The technological characteristics and dehydrogenase activity of the activated sludge (aeration tank No. I). 1 — sludge volume index, 2 — mixed liquor suspended solids, 3 — dehydrogenase activity of the activated sludge (µg TF/mg MLSS), 5 — load of aeration tank (mg BOD_5/l · day)

liquor suspended solids (MLSS) gradually increased from 4.2 to 12.7 μg TF/mg MLSS and 3600 to 5500 mg/l, respectively. The sludge volume index was low in the range from 52 to 119 ml/g. In the latter part of this period the dehydrogenase activity was characterized by the greatest value 16.3 μg TF/mg MLSS but at the same time there appeared a considerable decrease of the mixed liquor suspended solids to 3800 mg/l, and the sludge index increased from 119 to 253 ml/g. It should be stated that a considerable growth of filamentous bacteria from the Sphaerotilus genus was observed in this period. This in turn influenced the increase of the sludge index. In the further course of the process the activity showed certain fluctuations. At first, at the highest load the amount of the produced triphenylformazan fell considerably amounting to 7.5 μg TF/mg MLSS. However, in a short time it reached the value of 14.8 μg TF/mg MLSS similar to that recorded before the described fall. In this period the mixed liquor suspended solids gradually increased till it reached the maximum value of 5000 mg/l.

INVESTIGATIONS ON THE PROCESS OF MUNICIPAL SEWAGE PURIFICATION

Investigations on the subject of dehydrogenase activity of microorganisms in the purification of municipal sewage were conducted in the wastewater-treatment plant "ZASPA" in Gdańsk. The technical parameters of the purification of municipal sewage (Fig. 4) differed from those formerly presented in the purification of industrial wastes. The range of value of the load of pollutions was considerably greater and amounted to 6130—9400 g $BOD_5/m^3 \cdot$ day. Twice lower values namely 4600 and 5850 g $BOD_5/m^3 \cdot$ day appeared. During the whole period of investigations the mixed liquor suspended solids was 2450—3150 mg/l. The sludge volume index was on the average low from 65 to 81 ml/g and it reached its maximum value at the highest load. The dehydrogenase activity of microorganisms of the activated sludge in the mentioned range of pollution load was variable. At the initial load of the aeration tank 6130 g $BOD_5/m^3 \cdot$ day the dehydrogenase activity was high and equaled 34.8 μg TF/mg MLSS, at the reduction of BOD_5 and COD--permanganate equaling 73 and 66%, respectively. In the further course of the process when the load had been reduced to 5850 g $BOD_5/m^3 \cdot$ day (Fig. 4) the activity of the sludge lowered somewhat and the degree of purification of the sewage also decreased. After lowering the load to 4600 g $BOD_5/m^3 \cdot$ day the activity of the sludge reached its lowest value of 21.5 μg TF/mg MLSS. After this period the load BOD was considerably increased to 7320 g $BOD_5/m^3 \cdot$ day and it was then ascertained that the sludge activity increased reaching the former maximum value of 34.8 μg TF/mg MLSS. With the further increase of the load to 9400 g $BOD_5/m^3 \cdot$ · day a small fall of activity to 27.5 μg TF/mg MLSS was observed. The

effect of wastes purification during the period April 8–15, 1970, improved, reduction of BOD_5 and COD reached maximum values amounting to 78 and 80%, respectively.

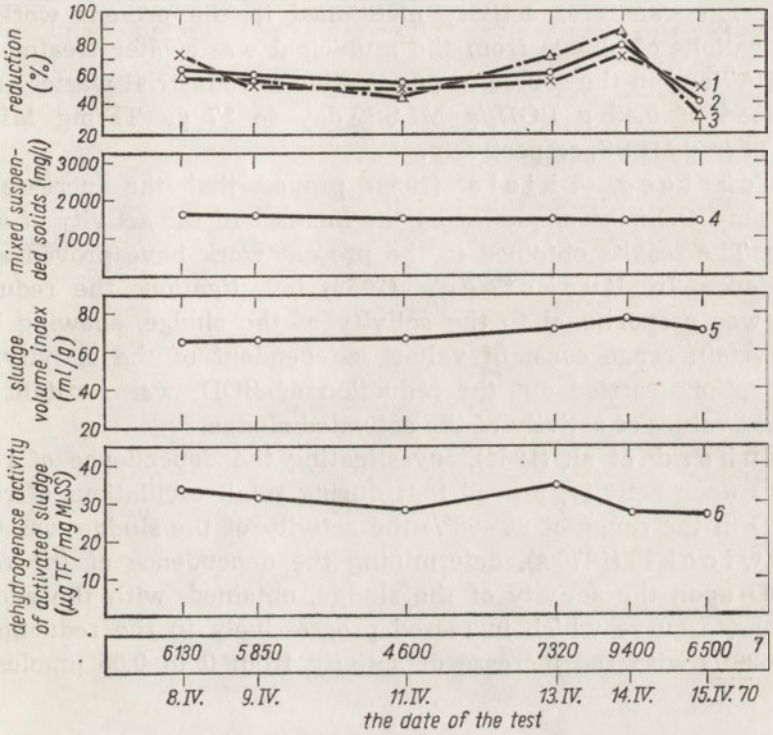

Fig. 4. The investigations of the process of purification of municipal wastes using the activated sludge method in the wastewater-treatment plant "ZASPA" in Gdańsk (April 8–15). The reduction of the basic pollution indicators. 1 — BOD_5, 2 — COD-permanganate, 3 — suspension, 4 — mixed liquor suspended solids, 5 — sludge volume index, 6 — dehydrogenase activity of the activated sludge, 7 — volume load of sludge

4. DISCUSSION

Recently dehydrogenase activity has become the subject of investigations.

Bucksteeg (1966) presents dehydrogenase activity as a function of the load by organic substances and the mixed liquor suspended solids. Effenberger (1967) shares his opinion but doubts whether this dependence is conditioned by the composition of sewage and the changes of sludge biocoenosis. Ford et al. (1966) did not observe a distinct correlation between the mentioned parameters.

Przewłocki (1970 b) conducting his investigations on municipal sewage expressed this dependence by the equation $y = 0.26x$, where y

stands for the activity expressed in μmoles TF/mg MLSS and x is the load of the sludge in g BOD_5/g MLSS · day. The above linear dependence defines that at a load of 0.45 g BOD_5/g MLSS · day the sludge activity equals 0.11 μmoles TF/mg MLSS which corresponds to 33 μg TF/mg MLSS. The values of activity mentioned in the present work in the investigations of sludge from the municipal wastewater treatment plant "ZASPA" and in the course of purification of industrial wastes amounted at a load of 0.45 g BOD_5/g MLSS · day to 27 μg TF/mg MLSS and 16 μg TF/mg MLSS, respectively.

Bucksteeg, Thiele (1959) proved that the increase of the reduction COD is accompanied by the increase of the activity of activated sludge. The results obtained in the present work have proved a similar dependence. In Bucksteeg's (1959) investigations the reduction of BOD_5 was proportional to the activity of the sludge, showing however in a certain range constant values independent of the activity. In the investigations carried out the reduction of BOD_5 was constant for the obtained values of activity of the activated sludge.

Lenhard et al. (1964), investigating the dependence of reduction of COD upon activity, proved that during small oscillations of reduction of COD in the range of 90—98% the activity of the sludge was variable. Przewłocki (1970 a), determining the dependence of the reduction of COD upon the activity of the sludge, obtained, with the addition of zinc ions, a curve which increased progressively in the reduction range of 40—90% with the increase of activity from 0 to 0.06 μmoles TF/mg

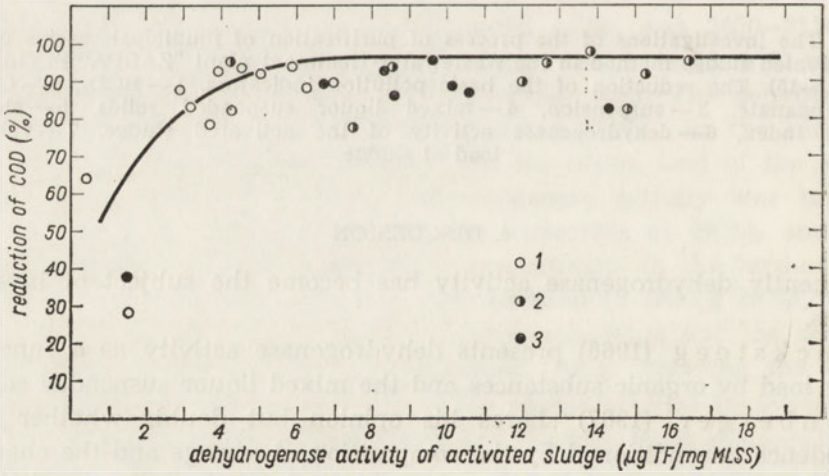

Fig. 5. The investigations on the process of purification of industrial wastes using the activated sludge method. The dependence of COD reduction on dehydrogenase activity of the activated sludge. 1 — aeration tank No. I (Febr. 17, 1969 — Febr. 26, 1970), 2 — aeration tank No. I (April 22 — December 1), 3 — aeration tank No. I A (April 29, July 6)

MLSS. This corresponds to 0—18 μg TF/mg MLSS. The results obtained in this work (Fig. 5) are similar to the results obtained by P r z e w ł o c k i (1970 a, b). The curve of the dependence of the reduction of COD upon activity has a similar character, it differs however in its range.

The character of the dependences discussed by the above mentioned authors and obtained in this work is similar. The differences occurring in the absolute values may result, among others, from using different types of wastes in the purification process (synthetic wastes, municipal and industrial wastes).

On the basis of the results of investigations of the purification process of industrial wastes one can present the following conclusions:

1. Increased numbers of filamentous bacteria of the Sphaerotilus genus, which cause the bulking of the activated sludge influence the rise of the sludge volume index, and the dehydrogenase activity of organisms taking part in the process of sewage purification does not increase but shows slight fluctuations (Fig. 2, 3).

2. The reduction of BOD_5 is high and does not depend upon the obtained values of the dehydrogenase activity of sludge. At the minimum and maximum values of sludge activity the obtained percentages of reduction of BOD_5 are similar (Fig. 2).

3. The maximum reduction of COD (95%) takes place at an activity greater than 5 μg TF/mg MLSS (Fig. 5).

4. The reduction of total Kjeldahl nitrogen usually increases with a rise of the activity of activated sludge. At an activated sludge activity

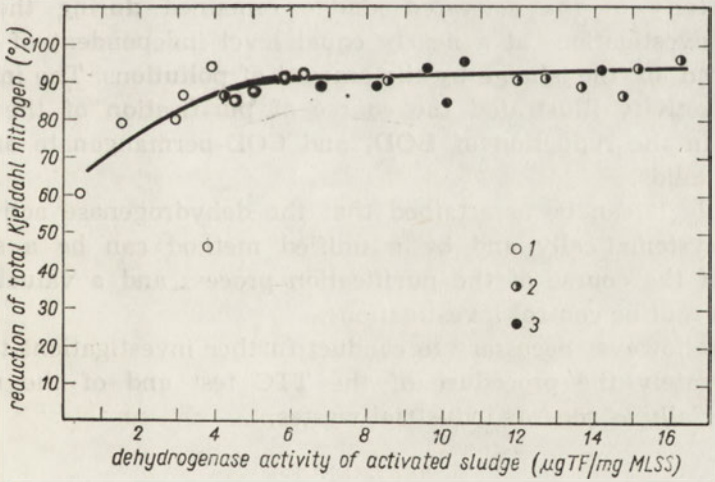

Fig. 6. The investigations on the process of purification of industrial wastes using the activated sludge method. The dependence of total nitrogen reduction on dehydrogenase activity of the activated sludge. 1 — aeration tank No. I (Febr. 17, 1969 — Febr. 26, 1970), 2 — aeration tank No. I (April 29 — July 1), 3 — aeration tank No. I A (April 29 — Dec. 6)

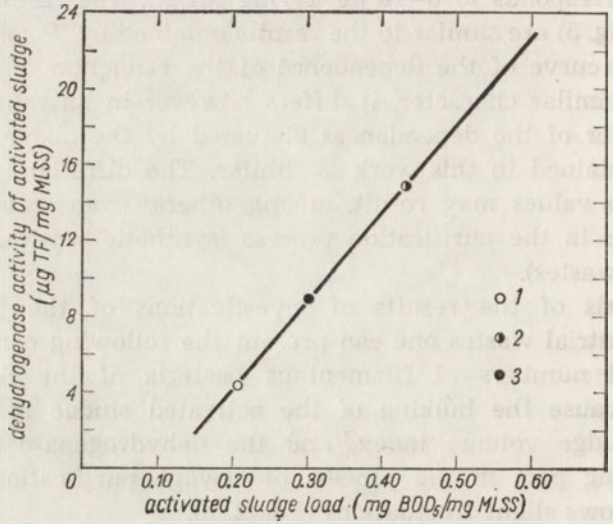

Fig. 7. The investigations on the process of purification of industrial wastes using the activated sludge method. The dependence of dehydrogenase activity on sludge load. 1 — aeration tank No. I (Febr. 17, 1969 — Febr. 26, 1970), 2 — aeration tank No. I (April 29 — July 1), 3 — aeration tank No. I A (April 29 — Dec. 6)

of 6 μg TF/mg MLSS approximately 92% of the total Kjeldahl nitrogen was reduced (Fig. 6).

5. A linear dependence exists between the load BOD of the activated sludge and its dehydrogenase activity (Fig. 7).

Summing up the obtained results of investigations of the process of purification of municipal sewage, one can ascertain that the dehydrogenase activity of the activated sludge remained during the whole period of investigations at a nearly equal level independent of changes of BOD load of the sludge by the amount of pollutions. The individual values of activity illustrated the course of purification of the sewage expressed in the reduction of BOD_5 and COD-permanganate and total suspended solids.

Generally it can be ascertained that the dehydrogenase activity determined systematically and by a unified method can be a sensitive indicator of the course of the purification process and a valuable supplement to routine control investigations.

It seems however necessary to conduct further investigations to define more accurately the procedure of the TTC test and of the research work, especially as regards industrial wastes.

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THE BOD COURSE OF SEWAGE ACCORDING TO THE QUANTITY AND QUALITY OF THE BACTERIAL SEED

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ABSTRACT

The investigations concerned the effect of the quantitative and qualitative composition of bacterial seed on a determined value of BOD; the method of dilutions and Warburg's manometric method were used. The degree of biodegradation of sewage was checked by measuring the daily decrease of COD determined by the dichromate method. In one case bacteria for inoculations were taken from municipal sewage, in another case — they were a mixture of definite species of aerobic bacteria. It was established that the height of the determined value of BOD might oscillate considerably according to the initial number of bacteria in the sample. If this number was less than 10^6 cells in one ml the phase of stagnation was prolongated. If the initial number was 10^6 cells in one ml, a higher rate of the reaction was obtained during the first 24 hours of observation than with a small number of cells.

1. INTRODUCTION

Though the determination of the biochemical oxygen demand (BOD) is not an ideal index of the organic content of sewage, still it has been the most frequent method used for this purpose. It allows to determine not only the concentration of the dissolved organic substances which can be oxydized biochemically but it also permits to evaluate the kinetics of the biochemical reactions occurring in the given environment. The proper course of the reaction depends on such factors as time, temperature, pH, the kind of medium, the presence and concentration of the mineral nutrient substances, and, above all, on the presence of a group of heterotrophic microorganisms appropriate in respect of quantity and quality (Gellmann, Heukelekian 1951, Busch 1958, Kołaczkowski et al. 1965, Malina et al. 1967). The function of bacteria in the BOD process and the dependence of the result of BOD_5 on the number of bacteria have frequently been the object of investigations (Hoover et al. 1953, Lewis, Busch 1965). Some of the authors have argued the dependence of the height of the result on the initial number of bacteria in the samples (Tidwell, Sorrels 1955, Busch 1958, Kołaczkowski et al. 1965), while others have stated that the number of bacteria at the beginning of the experiment had no influence on the result since the optimum conditions maintained during the examination were favourable to a fast development of bacteria (Schuller 1961).

Besides the dependence of the process on the number of bacteria one should also take into account the influence of the qualitative composition of the seed, i.e. the presence of a proper number of bacteria species capable of oxydizing organic substances (Tidwell, Sorrels 1955, Lee, Oswald 1959 a, b). A further condition of attaining a proper activity of the seed is its adaptability to all kinds of medium and the production of proper adaptation enzymes.

Another problem is the possibility of reducing the 5-day period of BOD

observation but with the quarantee of obtaining reliable results. Zehnpfennig, Nichols (1953), by using appropriate bacteria seed and by removing protozoa, have obtained, with the method of dilutions, more reproducible results for BOD_2 than for BOD_5. Caldwell, Langelier (1948) as well as Schuller (1961), using the manometric method, have suggested the possibility of reducing the observation period to 24 hours, at a higher temperature of incubation: at 25°C and even at 30°C.

The aim of the present paper was to observe the effect of the initial number of bacteria in the sample on the course and the obtained result of BOD.

In further investigations the possibility of using an inoculation of a given qualitative composition was considered, as this would make it easier to reproduce always the same seed and to introduce uniform conditions of observation. An attempt was made to chose a method that would make it possible to reduce the period of observation.

2. METHODS

"Artificial sewage" after Pasver (1955), was used for investigations in order to maintain always the same conditions of the experiments. The seed for the first series of investigations was prepared of a mixture of bacteria from municipal sewage. The seed used in the second series of experiments was a mixture of pure tribes of aerobic bacteria typical of water environment and sewage — *Escherichia coli, Pseudomonas aeruginosa, Alcaligenes faecalis, Serratia marcesceus, Proteus vulgaris* and *Bacillus cereus*. The tribes were obtained from the collection of the State Institute of Hygiene, Research Department of Serums and Vaccines, Warsaw. A 48-hour culture of bacteria, grown on "artificial sewage" at 20°C was centrifuged at 8000 g and washed twice with buffered water of 7.2 pH.

Observations of the process of biochemical oxydation were carried out with the use of three different methods: the determination of BOD by the method of dilutions (Standard method 1965); the determination of BOD by the manometric method with the use of Warburg's respirometer (Umbreit et al. 1964); the determination of BOD by defining the loss of the organic matter. The loss was defined by determining the oxydizability by the potassium dichromate method (COD), of the shaken sewage sample, every day during five days (Kołaczkowski et al. 1965).

At the same time the quantitative changes of the bacteria were checked and their number was determined on nutrient agar at the temperature of 20°C.

3. RESULTS

It was established that the level of BOD_1, BOD_2 and BOD_5, by all of the used methods, depended on the number of bacteria contained in the sewage. The mean results of BOD_1, BOD_2 and BOD_5 have been presented in Fig. 1; they were obtained by the method of dilutions and of inoculation from a mixture of bacteria cultivated on municipal sewage. The initial number of cells in the dilution water was increased from $1 \cdot 10^3$ to $5 \cdot 10^5$ cells in 1 ml. The value of BOD for identical sewage were the higher the greater the initial number of bacteria was.

The results of measurements carried out by the manometric method have been presented in Fig. 2. As it results from the run of curves, the use of the bacteria seed from sewage in a number of $1 \cdot 10^{10}$ cells/ml was almost immediately followed by violent respiration which indicated the shortening of the lag phase characterized by slight metabolic activity. This lag phase was prolongated when the initial number of bacteria was lower than 10^6.

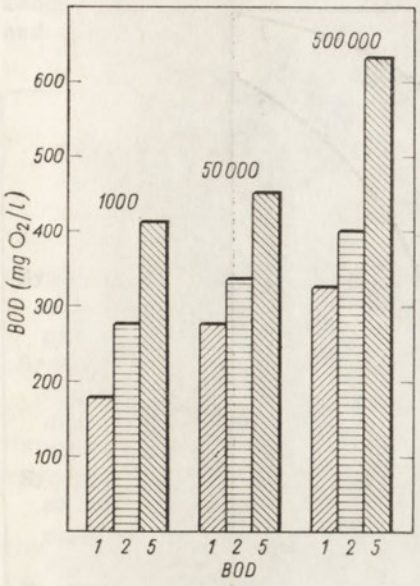

Fig. 1. Effect of initial number of bacteria on BOD_1, BOD_2 and BOD_5 values determined by the dilution method. Numbers denote the number of bacterial cells isolated from municipal sewage in one ml of dilution water

Figure 3 shows the process of BOD after an amount of 800 mg/l of phenol had been introduced into the sewage. No stopping of the multiplication process of microbes as the effect of phenol, was observed, but there occurred a reduction of their respiratory activity. This activity

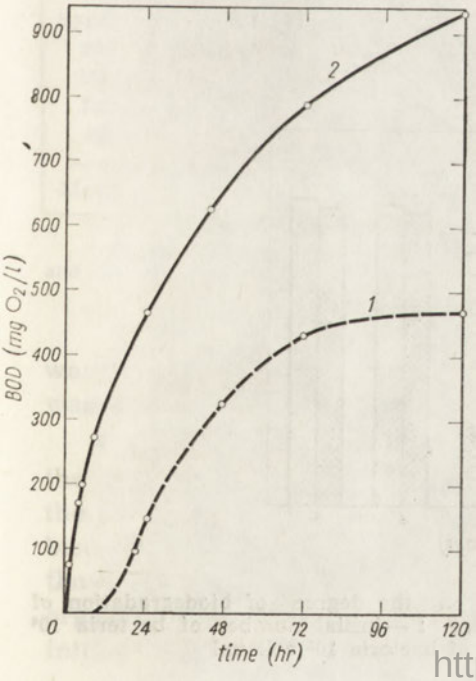

Fig. 2. Effect of initial number of bacteria on the BOD process determined by Warburg's method. Seed: bacteria from sewage. 1 — 2.10^5 cells/ml, 2 — 1.10^{10} cells/ml

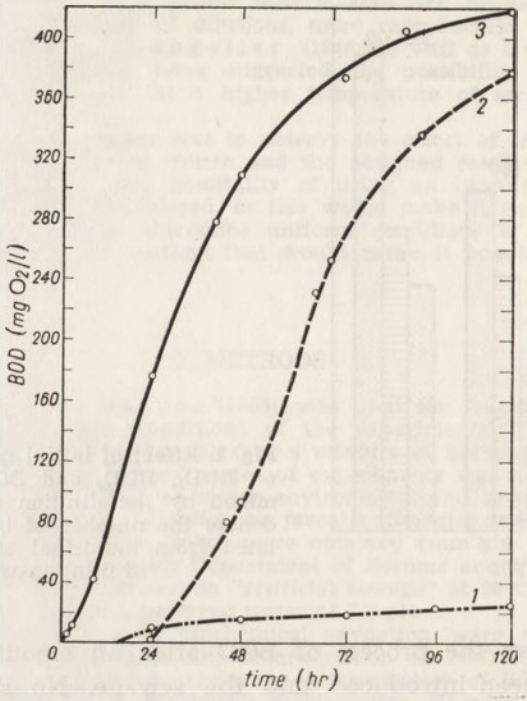

Fig. 3. Effect of initial number of bacteria on the BOD process determined by Warburg's method in sewage containing 800 mg/l of phenol. Seed: bacteria from sewage. 1 — initial number of bacteria 80 cells/ml, 2 — initial number of bacteria $6.5 \cdot 10^5$ cells/ml, 3 — initial number of bacteria $1.9 \cdot 10^7$ cells/ml

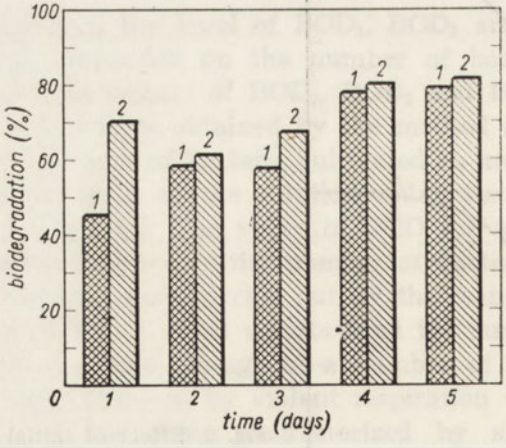

Fig. 4. Effect of initial number of bacteria on the degree of biodegradation of medium. Seed isolated from municipal sewage. 1 — initial number of bacteria 10^4 cells/ml, 2 — initial number of bacteria 10^5 cells/ml

Table I. Comparison of BOD_1 and BOD_5 values determined by Warburg's method and of the oxydization degree after 1 and 5 days, determined by the dichromate method (COD)

Kind of sewage and seed	Warburg's method			Oxydizability method (COD)		
	BOD_1	BOD_5	$\frac{BOD_1}{BOD_5}$ (%)	Oxidization (%) after 1 day	Oxidization (%) after 5 days	$\frac{COD_1}{COD_5}$ (%)
Synthetic sewage inoculated with municipal sewage	463	943	49.2	70	80.6	87.5
Synthetic sewage inoculated with a definite composition of species	180	323	55.8	31	42.5	73.1
Synthetic sewage with 80 mg/l of phenol inoculated with municipal sewage	321	497	64.8	14.8	20.04	72.5
Synthetic sewage with 80 mg/l of phenol inoculated with a definite composition of species	148	237	62.4	56.3	77.3	72.8
Synthetic sewage with 800 mg/l of phenol inoculated with municipal sewage	177	418	42.3	12.7	67.5	18.8*
Synthetic sewage with 800 mg/l of phenol inoculated with a definite composition of species	140	237	59.0	5.6	9.0	62.2*
Mean value			55.0			76.5

* Oxydization was stopped because of the toxic action of the phenol concentration and the results were omitted in calculation of mean values.

was found to be particularly weak when the initial number of bacteria was 80 cells/ml.

A confirmation of the observations discussed above was afforded by the results obtained while determining BOD through the definition of the loss of organic substance in the sewage, as this substance underwent biodegradation due to bacteria contained in the sewage. The results of those observations have been presented in Fig. 4. The greatest loss of organic substance was noted after the first 24 hours (70%) with the initial number of bacteria amounting 10^6 cells/ml. When the initial

number of bacteria was lower — 10^4 cells/ml — the decrease of the medium, after 24 hours, was only 45%. The difference of the degree of biodegradation of the sewage was smaller in the following days of observation.

In order to compare the results of one-day and five-day BOD, the values of BOD_1 and BOD_5 determined by the Wartburg manometric method have been presented in Table I together with the results showing the degree of oxydization of the sewage after one day and after five days. The results of BOD_1 amounted averagely to 55% of the values of BOD_5. Observations of the loss of organic substance carried out parallelly have shown an average of 76% of oxydization of the medium after 24 hours as compared with the degree of mineralization after 5 days. A high loss of the organic substance, by an appropriately high number of bacteria and a high value of BOD_1 suggest that the Wartburg manometric method might be used to obtain a reliable BOD result after 24 hours.

The comparison of the results obtained in the author's experiments for BOD_1 and BOD_5 defined by Wartburg's method and of BOD_5 values calculated by the method proposed by Schuller (1961) have been presented in Table II. In order to calculate BOD_5 a determined value of BOD_1 was assumed as well as a conversion coefficient obtained from the relation of experimentally determined values $BOD_5 : BOD_1$. As it results from the presented values, a relatively high conformability was obtained, of the calculated and the experimental BOD_5 results. The

Table II. Comparison of BOD_5 values experimentally obtained with BOD_5 values calculated from BOD_1

Experimental BOD (mg O_2/l)		$\frac{BOD_5}{BOD_1}$ (exp.)	Calculated BOD_5 ($BOD_1 \cdot 1.64$)	Difference (%)
BOD_1	BOD_5			
177	299	1.68	290	−3.0
185	306	1.65	303	−1.1
207	325	1.57	340	+4.5
208	329	1.58	341	+3.6
218	324	1.48	357	+9.3
133	245	1.84	219	−10.7
135	250	1.87	218	−12.6
145	236	1.62	234	−0.8
146	238	1.63	239	+0.4
150	239	1.59	246	+2.8
142	223	1.57	233	+4.4
151	229	1.51	248	+7.7
150	268	1.78	246	−8.3
	Mean	1.64		

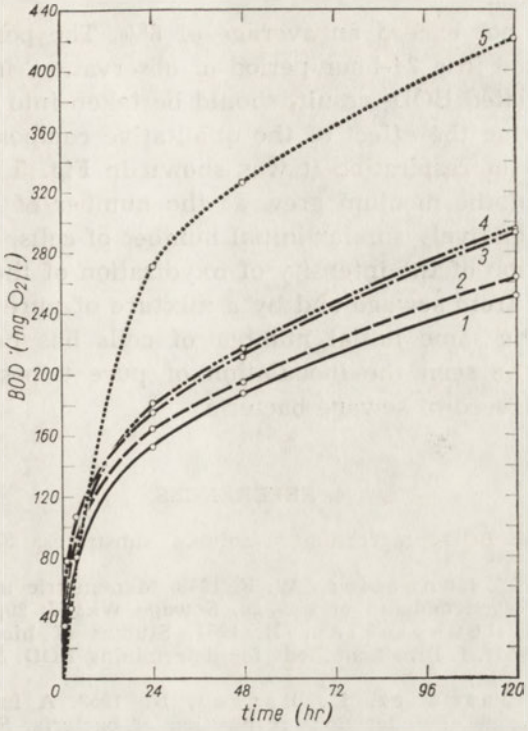

Fig. 5. Effect of qualitative composition of bacteria seeds on BOD value. Initial number of bacteria $5 \cdot 10^{18}$ cells/ml. 1 — *E. coli* — *P. aeruginosa*, 2 — *E. coli* — *P. aeruginosa* — *A. faecalis*, 3 — *E. coli* — *P. aeruginosa* — *A. faecalis* — *Serratia marcescens*, 4 — *E. coli* — *P. aeruginosa* — *A. faecalis* — *S.marcescens* — *Proteus vulgaris*, 5 — *E. coli* — *P. aeruginosa* — *A. faecalis* — *S. marcescens* — *P. vulgaris* — *B. cereus*

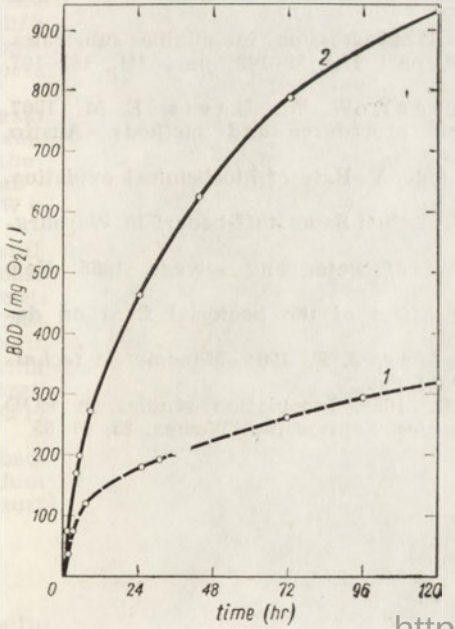

Fig. 6. Effect of quality of bacteria seed on BOD process determined by Warburg's method. Initial number of bacteria $1 \cdot 10^{19}$ cells/ml. 1 — inoculation of mixture of six bacteria species, 2 — inoculation from municipal sewage

divergences did not exceed an average of 6%. The possibility of using Warburg's method in a 24-hour period of observation, in order to obtain a reliable, calculated BOD_5 result, should be taken into consideration.

When analyzing the effect of the qualitative composition of the seed on the intensity of respiration it was shown in Fig. 5 how the activity of oxydization of the medium grew as the number of species increased with an approximatively similar initial number of cells.

The comparison of the intensity of oxydization of the medium by the seed of bacteria from sewage and by a mixture of pure tribes of aerobic bacteria with the same initial number of cells has been presented in Fig. 6. As can be seen the inoculation of pure tribes has shown less activity than the seed of sewage bacteria.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	21	1	153-163	1974

S. SPANDOWSKA

THE MECHANISM OF THE SELECTIVE DISTRIBUTION OF BACTERIA IN THE FILTER LAYER IN THE PROCESS OF THE ARTIFICIAL WATER INFILTRATION

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ABSTRACT

The changes of different physiological groups of bacteria condensation as well as of the chemical composition of water infiltrating in the 3-m layer of soil with 0.1–0.25 mm granulation have been determined. The bacteria activity was the highest in the infiltration water up to the depth of 150 cm. The complete elimination of coli bacteria group was found at this depth. The creation of suitable oxygen conditions can speed up the process of mineralization.

1. INTRODUCTION

Lately, a lot of attention has been dedicated to the problem dealing with the reutilization of the once used water by means of its artificial infiltration, because of the perspective of the ground water resources exhaustion because of their constantly growing utilization. The special geological layer, being situated under the filter reservoir, has been used as a slow sand filter. A lot of simultaneous and different physical, chemical, but first of all biological phenomena, closely connected one with another and having decisive influence of the high quality ground water, occur in this layer during the water infiltration.

On the basis of the accessible literature, it is seen that most of the biological examinations performed during the water infiltration dealt mainly with observation of the translocation possibility of the microorganisms, that were considered as the index of the sanitary water pollution (Prescott et al. 1950, Buttler et al. 1954, Baars 1957, Kanz 1960, Frank 1963, Švorcowa 1964, Weisman 1964, Georgescu 1965).

The biochemical processes occurring in the water during its infiltration are still little known. Up to now, the intensity of the biological transmutation has been observed only on the basis of the physical and chemical indications in the infiltrated water (Bettaque 1958, Morgan, Gilcreas 1960, Klapper 1962, Frank 1963, Schmidt 1963, Huismann, van Haaren 1966). The reports dealing with the distribution and activity of particular microgroups in the filter layer during the process of the pollution biodegradation in the course of the water infiltration, are considerably rare (Calaway 1957, Meissner 1958, Morgan, Gilcreas 1960).

In the present observations we have tried to watch the separate groups of bacteria appearing in the infiltrating water as well as their numerical relation and functions in the environment that differs very much from the one existing in the surface water reservoirs.

2. METHODS

The examinations have been carried out at the Poznań water supply intake, situated in the proglacial Warta River valley. The river water is pumped into 32

specially built infiltration ponds. (Fig. 1 — ponds No. 1–18 and 3a–18a), located in the three rows being parallel to the river as well as one to another. The water, infiltrating from the ponds into the ground, gathers in the working wells, that are

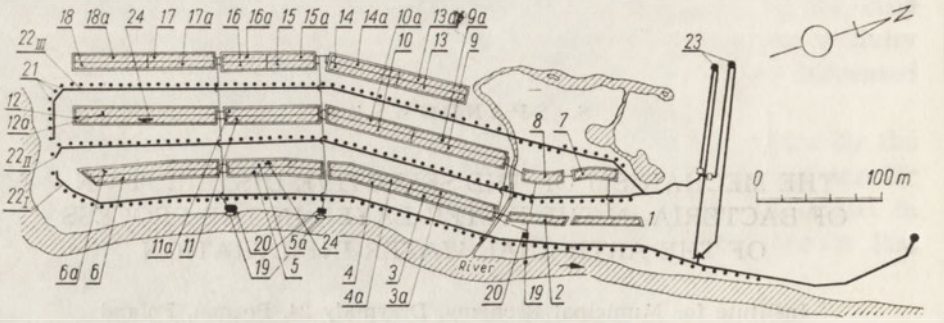

Fig. 1. Infiltration system. 1–18, 3a–18a — infiltration ponds, 19 — shore intake, 20 — pumping station, 21 — battery of wells, 22 (I, II, III) — siphons, 23 — collecting tanks, 24— sampling stations

located in the row along the ponds, in the distance of about 70–80 m. From there, the water is supplied to the main well (Fig. 1 — 23), by means of the siphon system (Fig. 1 — 22).

The main observation post was situated on pond No. 12, with the area of 26,000 m², located in the middle row (Fig. 1 — 24). Special vessels were installed in the pond bottom in the point consituting the half-height of the pond. The sampling of the infiltrating water from the depth of 10, 20, 30, 50, 75 and 100 cm (Fig. 2 — a) was possible due to the above mentioned vessels with the help of the specially

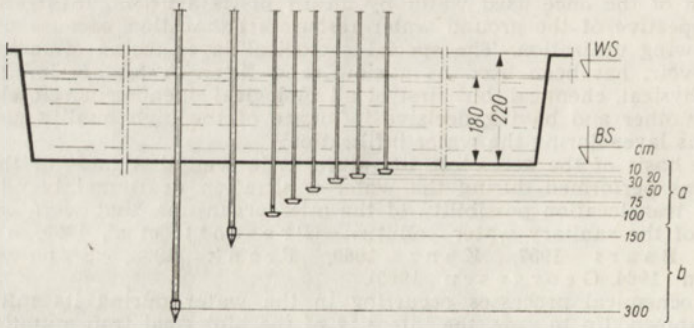

Fig. 2. Sampling sites of the infiltration water. WS — water surface, BS — bottom surface, a — water collection by vessels, b — water collection by pipes

inserted S-shaped pipes. The water sampling from the depth of 150 and 300 cm was possible due to the pipes (2 inches in diameter) driven into the bottom (Fig. 2 — b). On the second post, situated on pond No. 5 a (Fig. 1), the water sampling was possible only to the depth of 100 cm. The ground layer, being under the pond, consisted mainly of sand with the majority of fraction with the grain diameter from 0.10 to 0.25 mm, and with rather even determinant diameter (d_{10}) and the coefficient of inconformity [1] varying from 1.6 to 2.4.

[1] The ratio of diameters $d_{60} : d_{10}$ is called the coefficient of conformity; in reality being a coefficient of inconformity (Fair, Geyer 1960).

The filtration rate was lowering during the work of the pond, for example after a month-long work beginning with the moment of its filling with water, it amounted 1.2 cm/hr up to the depth of 25 cm, but when the depth was increasing further on up to 150 cm, then filtration rate amounted 4–5 cm/hr.

The pond water samples and infiltrating water samples were collected all the year round, each month, if it was possible (Table I).

Table I. The scheme of sampling in 1968

Pond No.	Month	The period of exploitation	Water level (cm)
12	Febr.	5 weeks	125
12	March	15 weeks	175
12	April	18 weeks	180
12	May	12 days after cleaning	150
12	July	2 weeks after cleaning	150
12	August	8 weeks after cleaning	150
5a	Oct.	5 days	120
5a	Nov.	5 weeks	170

The total number of heterotrophic bacteria was estimated with help of standard plate count and the test for presence of coliform colonies was performed by membrane filter technique. The bacteria, making simple organic multicarbon compounds rot, were estimated by means of tube-fermentating method. Special attention was paid at the group of bacteria participating in the biodegradation of nitrogen organic matter as well as in oxygenation or reduction of the mineralization products. While estimating different bacteria groups, the standard plate count was used for the estimation of colonies showing proteolytic features, multiple tube technique was used for the estimation of ammonification and denitrifying bacteria number, and membrane filter technique was used for the estimation of nitrifying bacteria number.

The biological activity of the bacteria was evaluated on the basis of simultaneous observations of the changes taking place in the chemical composition of infiltrating water.

3. RESULTS

In the first phase of observations the possibilities of bacteria penetration and their development in the water during infiltration were being analysed.

Besides other organisms, a very numerous bacterial flora gathers in the ground layer of the pond water as well as in the bottom sediment, because of the environment full of nourishing substances. On one hand such conditions are favourable to the bacteria development, but on the other hand numerous higher organisms, inhabiting the mud, feed on bacteria. In such a way, natural "biological barrier" comes into existence (K n o r r et al. 1956, K n o r r 1960). This barrier greatly eliminates some groups of bacteria. But considerable part of bacteria, thanks to their protective qualities, reaches greater depth together with the infiltrating water

On the basis of the data presented in Fig. 3, it is evident that the number of bacteria determined on the nutritive agar at 20°C in the infiltrating water taken from the ground 10—100 cm deep, was remaining manyfold higher in number (from $1 \cdot 10^3$ to $6.2 \cdot 10^4$ cells/ml) than in the pond water (from $1.2 \cdot 10^2$ to $2.8 \cdot 10^3$ cells/ml). At the depth of 150 cm, the bacteria number has evidently decreased. The bacteria number, determined after the incubation on the nutritive agar at 37°C (Fig. 4), has also increased in the infiltrating water at the more shallow layers, while comparing it with the number found in the pond water. The next decrease of their number was also seen at the depth of 150 cm.

Coli bacteria group (Fig. 5) has disappeared in the water taken from the layer 50—100 cm deep, but at the depth of 150 cm, they have not been found at all in 100 ml of water.

The bacteria, infiltrating together with the infiltration water, could influence the changes of the chemical composition of the water. While water being filtrated, organic substratum gradually used by bacteria, and during formation of partially or completely mineralized products of metabolism, new physico-chemical conditions have been formed as the depth was increasing. The above mentioned conditions influenced selectively the changes in the qualitative composition of the microflora as well as the development dynamics of definite bacteria groups. Simultaneous bacteriological and chemical analyses of infiltration water performed in the second phase of observations, showed the interdependence between the quantitative changes inside the bacteria groups and the intensity of changes of physico-chemical features of water. The course of changes taking place in qualitative composition of bacteria groups participating in transmutation of nitrogen compounds was presented in Fig. 6A, but the parallel analysis of changes occurring in the composition of nitrogen compounds recorded in March at the 3°C temperature of infiltration water was presented in Fig. 6 B. The number of proteolytic bacteria was gradually decreasing to 3 cells/ml of water at the level of 150 cm (Fig. 6A, curve 1). At the same time it was possible to notice the vivid decrease of organic nitrogen quantity from 4.4 μg/ml in the pond water to 1.6 μg/ml in the infiltration water at the depth of 150 cm (Fig. 6 B, curve 6). The group of ammonificating bacteria in great number in the pond water was found in similar number even at the depth of 100 cm (Fig. 6 A, curve 2). At the same time the increase up to 0.5 μg/ml of ammonia nitrogen occurred at the depth of 75—100 cm (Fig. 6 B, curve 8). Here the nitrifying bacteria began to act more intensively, because the ammonia, liberated in the process of the ammonification, constituted the energy source for them. The number of nitrifying bacteria amounted to 10^3 cells/ml of water at the depth of 300 cm (Fig. 6 A, curve 3). At the end it was possible to notice: the decrease of ammonia nitrogen in the water from 0.2 μg/ml to 0.05 μg/ml (Fig. 6 B, curve 8),

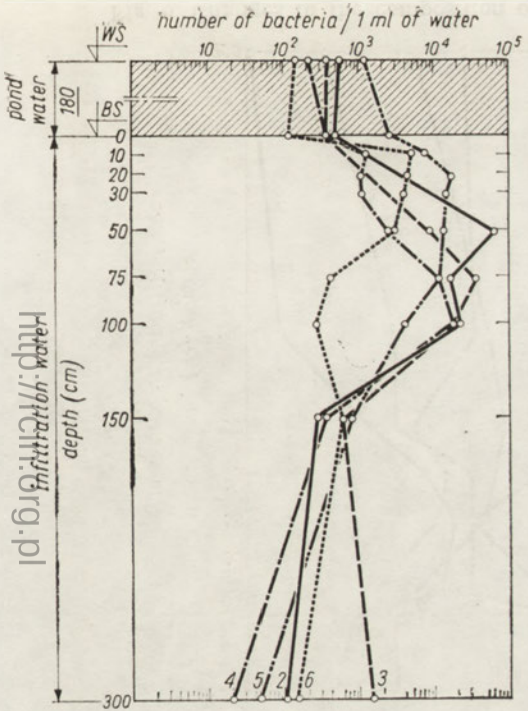

Fig. 3. Number of bacteria in infiltration water at different depths (cultivated on nutrient agar at 20°C). WS — water surface, BS — bottom surface, 2 — March, 3 — April, 4 — May, 5 — July, 6 — August

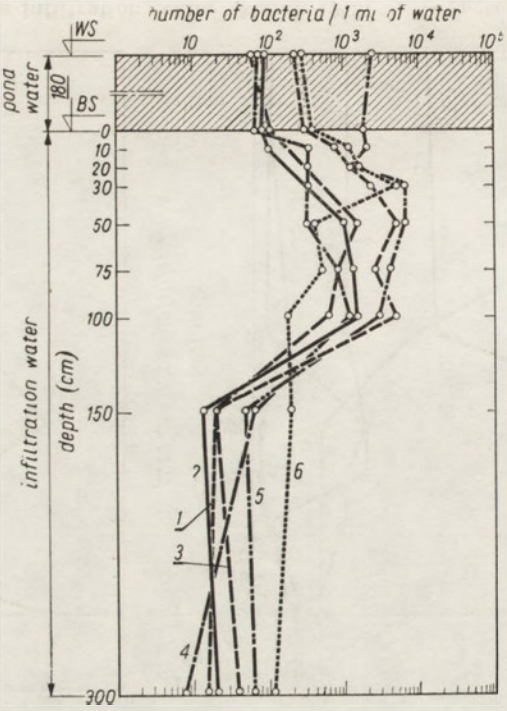

Fig. 4. Number of bacteria in infiltration water at different depths (cultivated on nutrient agar at 37°C). WS — water surface, BS — bottom surface, 1 — February, 2 — March, 3 — April, 4 — May, 5 — July, 6 — August

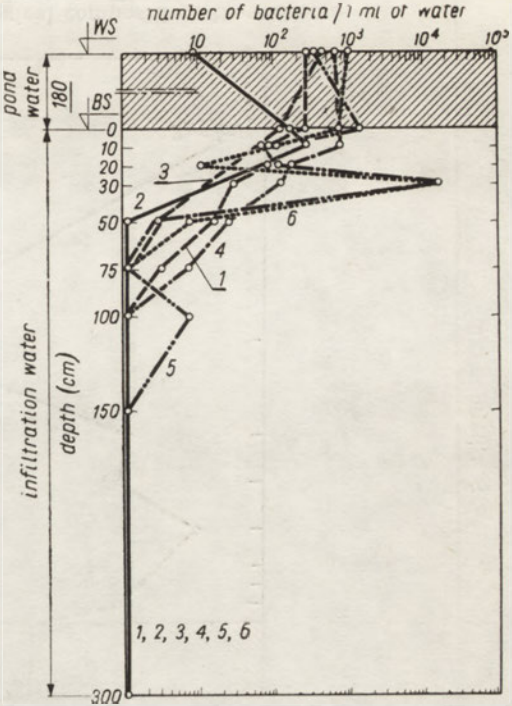

Fig. 5. Number of coliform bacteria in infiltration water at different depths. 1 — February, 2 — March, 3 — April, 4 — May, 5 — July, 6 — August

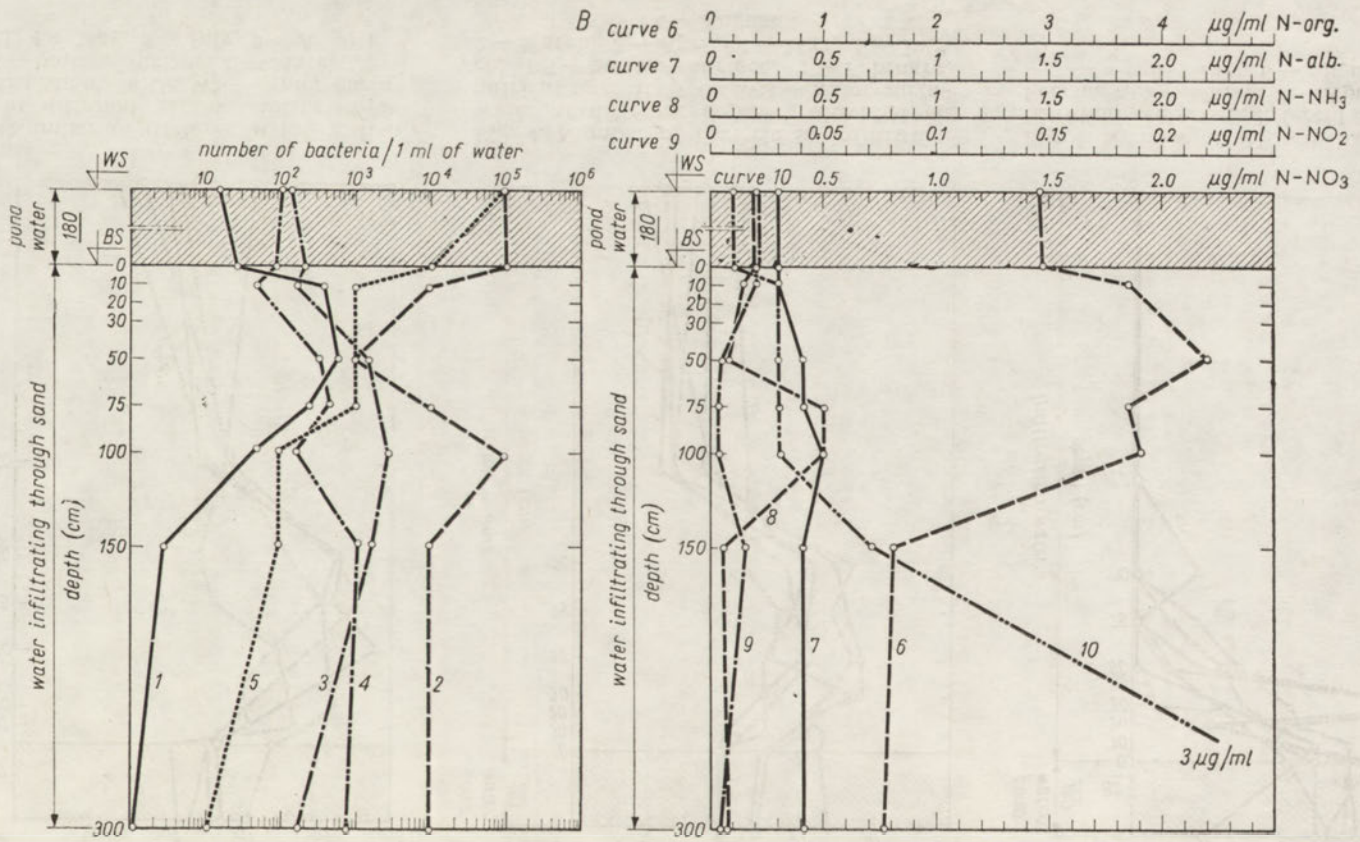

Fig. 6. Changes in the composition of the infiltration water (March 1968). A — bacteriological composition, B — chemical composition. 1 — proteolytic bacteria, 2 — ammonification bacteria, 3 — nitrifying bacteria NH_3-NO_2, 4 — nitrifying bacteria NO_2-NO_3, 5 — nitrates reducing bacteria, 6 — organic nitrogen, 7 — protein nitrogen, 8 — N-NH_3, 9 — N-NO_2, 10 — N-NO_3

the persistance of low concentration of nitrite nitrogen (Fig. 6 B, curve 9) and marked increase of nitrate amount (Fig. 6 B, curve 10) up to 3 μg/ml at the depth of 300 cm.

An important factor, setting conditions for such a course of the process, was the presence of oxygen in the infiltration water (Fig. 7). In March 1968, 45% of concentration (6 μg O_2/ml) was noticed in the infiltration water at the depth of 300 cm (Fig. 7, curve 2). During the summer period (in July 1968 at the temperature of water 24—26°C and the oxygen concentration of air in the pond water>100%, 12 μg O_2/l) a very fast disappearance of oxygen took place in the infiltration water. It was noticed only 1.2 μg O_2/l equalling 14% of concentration at the depth of 10 cm, but at the depth of 300 cm the complete absence of oxygen (Fig. 7, curve 5). In such conditions, in spite of the considerably high amount of bacteria, with proteolytic features (>10^3 cells/ml), that were found in the infiltration water at the depth of 20 cm and 300—600 cells/ml of water up to the depth of 75 cm (Fig. 8 A, curve 1), only the decrease of the content of the albumin nitrogen up to 0.8 μg/ml was noted at the depth of 100 cm (Fig. 8 B, curve 7). But organic nitrogen concentration persisted

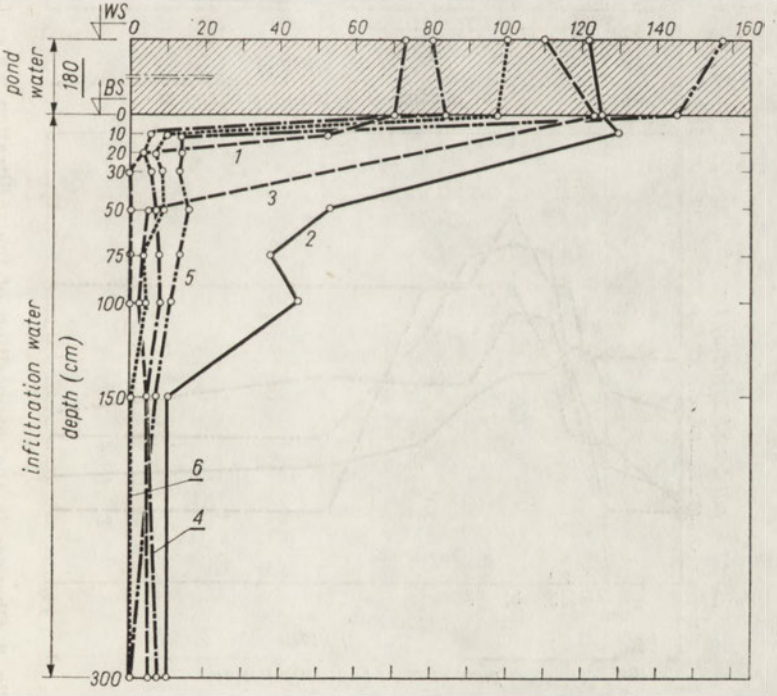

Fig. 7. Oxygen content (% of air saturation). 1 — February, 2 — March, 3 — April, 4 — May, 5 — July, 6 — August

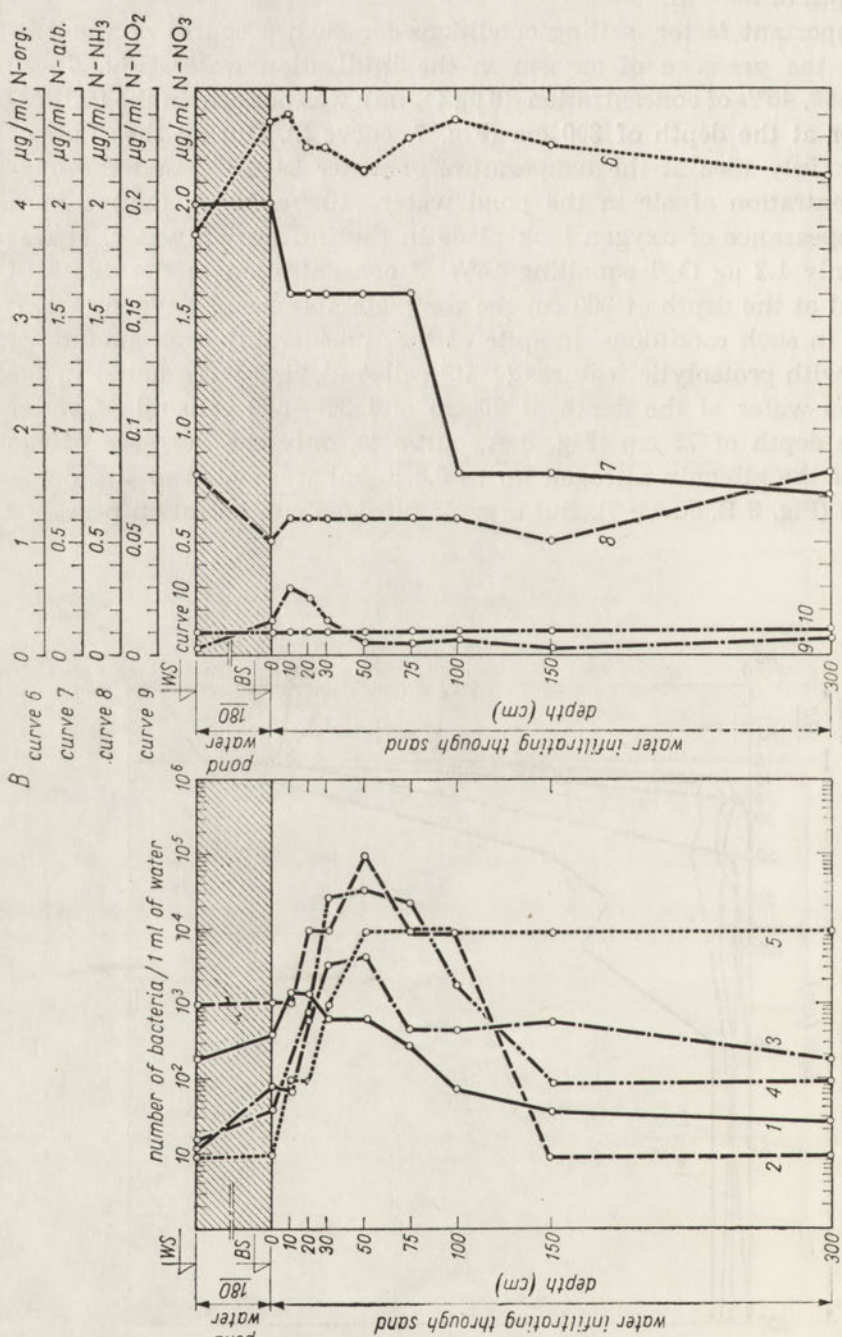

Fig. 8. Changes in the composition of the infiltration water (July 1968). A — bacteriological composition, B — chemical composition. 1 — proteolytic bacteria, 2 — ammonification bacteria, 3 — nitrifying bacteria NH_3-NO_2, 4 — nitrifying bacteria NO_2-NO_3, nitrates reducing bacteria, 6 — organic nitrogen, 7 — protein nitrogen, 8 — N-NH_3, 9 — N-NO_2, 10 — N-NO_3

at about 4 μg/ml of water up to the depth of 300 cm (Fig. 8 B, curve 6), and the ammonia nitrogen in the amount of 0.6 μg/ml of water (Fig. 8 B, curve 8). The parallel observations of bacteria amount showed that the amount of nitrifying bacteria considerably decreased up to 190 cells/ml in the infiltration water at the depth of 300 cm (Fig. 8 A, curve 4). The low concentration of nitrate nitrogen (0.1 μg/ml) (Fig. 8 B, curve 10) as well as the persisting amount of ammonia nitrogen (0.6 μg/ml of water at the depth of 300 cm) (Fig. 8 B, curve 8) did not point at activity of this bacteria group. The number of denitrifying bacteria has increased up to 10^4 cells/ml of water beginning with the depth of 50 cm and it was keeping in the same amount up to the depth of 300 cm (Fig. 8 A, curve 5).

The environment, changing because of the processes taking place inside it, influenced, except the absorption ability of the soil, the gradual bacteria elimination, showing certain order in disappearance of definite bacteria groups. The first to disappear was the group of coli bacilliform at the depth of 50–100 cm (Fig. 5). The next to disappear were the groups of bacteria putrefying simple multicarbon compounds at the depth of 100 cm. The number of bacteria with proteolytic features has been decreasing up to the depth of 150 cm. The amount of ammonification bacteria has been decreasing in the water samples taken at the depth from 150 to 300 cm. The nitrifying bacteria could survive in the bigger amount in the water at the depth of 300 cm than in the pond water although their activity "depended" on the oxygen amount in the infiltration water.

4. DISCUSSION

The microorganisms which could penetrate through the so-called "biological barrier" could survive, multiply and influence actively the changes of chemical composition of infiltration water.

Definite bacteria groups began to perform in succession peculiar them function of organic substances biodegradation. The most active bacteria have been found in the infiltration water up to the depth of 150 cm, that was connected with the great amount of bacteria living in this layer. The intensity of changes, occurring in the chemical composition of the infiltration water, testifies the biological activity of the examined bacteria groups. Their activity depended not only on the amount but also oxygen conditions as well as suitable substratum. It seems that the creation of suitable oxygen conditions in the infiltration layer can speed up the process of mineralization, that would be of great importance in the case of water intakes not having area big enough to guarantee long-time flow of infiltration water in the ground.

From the sanitary point of view it is possible to consider 150 cm layer with given granulation to be sufficient for the complete elimination of coli bacilliform.

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J. MALESZEWSKA

DEGRADATION OF METHYL PARATHION BY MICROORGANISMS OCCURRING IN SURFACE WATER AND SEWAGE

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ABSTRACT

The disintegration of 0,0-Dimethyl 0-p-nitrophenyl phosphorothioate by bacteria occurring in surface water and sewage was investigated. The study concerned isolated strains from water and sewage and preserved strains. It was established that the degradation of pesticide was due to *Bacillus cereus* preserved strain and to *Bacillus sp.* 8, isolated from sewage. The rate of biodegradation was accelerated by the presence of additional organic compounds in the substratum.

1. INTRODUCTION

The necessity of intensive development of various branches of economy is the result of increasing number of population in the world. This brings about a series of secondary, unfavourable phenomena, of which one of the most important is progressing chemical pollution of the environment. The menace to environment by pesticides is particularly essential, considering the growing production and utilization of these means, especially lately. Pesticides, infiltering surface waters may exert numerous noxious changes within aquatic biocenoses, of plants and animals. They may disturb autopurifying processes and induce difficulties in utilization of surface waters for communal aims, especially as the influence of longstanding activity of low concentrations of these compounds on human organisms is not sufficiently elucidated as yet.

In this connection the elimination of pesticide remains from the environment is of first-rate importance. The period of durability of pesticides within the soil as well as in water depends upon a series of physical, chemical and biological processes, such as photoinactivation, sorption and partly disactivation by colloidal substances, evaporation, oxigenation, reduction, hydrolysis, intake by plants and degradation by microorganisms.

Pesticides belonging to the group of phosphorus compounds, of which Methyl Parathion is representative (0,0-Dimethyl 0-p-nitrophenyl phosphorothioate) are much easier subject to degradation in a biological way than chlorinated hydrocarbons. Though many phosphoroorganic pesticides are more toxic than chlorinated hydrocarbons, they are considered as less dangerous because of the rapidity of their degradation for the environment, and are lately produced in large amounts, and their use increases systematically. The data from the literature, however, indicate that many pesticides from the group of phosphoroorganic compounds may, under some conditions, remain in aquatic environment as well as in the soil for long periods of months and even years (Alexander 1964).

In recent years a series of laboratory studies were carried out on the biological degradation of these compounds under the effect of microorganisms, which indicate that the process of biodegradation of pesticides of the phosphoorganic group is a complex one and depending on many factors. Achmed, Casida (1958) investigated the degradation of some pesticides of the group of phosphoorganic compounds brought about by the algae Chlorella, yeast of the Torulopsis genus

as well as by bacteria *Pseudomonas fluorescens* and *Thiobacillus thiooxidans*. They have revealed that microorganisms demonstrated their activity in relation to a series of investigated compounds.

The disintegration of Dipterex by some genus of fungi: *Aspergillus niger, Penicillium notatum* as well as *Fusarium sp.* were investigated by Zajed et al. (1965). Matsumura, Boush (1966) studied the disintegration of Malathion by *Trichoderma viride* and bacteria of the Pseudomonas genus.

The degradation of phosphoroorganic insecticides (with special stress put on Sumithion) by *Bacillus subtilis* was investigated by Yasuno et al. (1965), as well as by Miyamoto et al. (1966), who also denoted some transitional products, developing during the biodegradation of these compounds. The desintegration of Parathion into chemically pure compounds and of the commercial substance called Wofatox and containing Methyl Parathion were treated by Nauman (1967) who established that a series of bacteria isolated from the soil developed further in the presence of 10% Methyl Parathion, and also that a more rapid disappearance of the pesticide took place in a grafted substratum.

Ware, Roan (1970) cite the results of the paper of Hirakoso et al. who investigated the degradation of a series of phosphoroorganic insecticides by some kinds of bacteria isolated from surface water.

In the majority of cited data the degradation of insecticides was investigated within substrata containing additional organic compounds, in the shape of nutrients, yeast extract as well as peptone.

The aim of the present study was to investigate the possibility of Methyl Parathion degradation by bacteria occurring in surface water as well as in sewage, in a mineral substratum and in one containing additional organic compounds, with special consideration of the effect of additional compounds on the rate of this process.

2. MATERIAL AND METHODS

In the study two kinds of substances were applied: chemically pure Methyl Parathion, and its form used in practice Wofatox for dusting, containing 1.5% of Methyl Parathion, as active substance in a mineral carrier.

For isolation of bacterial strains which could be able to desintegrate Methyl Parathion a mineral substratum as well as substratum enriched in peptone which contained pesticide in form of Wofatox for dusting was used. The substrata were inoculated with water from the Vistula River as well as from municipal sewages, after mechanical purification.

The cultures were incubated at 25°C for a week. From cultures which presented signs of growth, seeds were transplanted onto nutritious agar in order to isolate strains for further investigations. The isolated strains were sown onto a substratum with Methyl Parathion, in order to determine the disintegration ability of the pesticide. The strains chosen for particular investigations of Methyl Parathion degradation were submitted to biochemical and morphological tests for identification (Breed et al. 1957).

Moreover the degradation of Methyl Parathion was tested by means of preserved strains: *Pseudomonas fluorescens, P. aeruginosa* as well as *Bacillus cereus* from the museum of PZH.

For testing Methyl Parathion degradation by pure strains of bacteria, a mineral substratum was applied, in which the insecticide was the only source of carbon, and also a mineral medium with Methyl Parathion, enriched with additional organic compounds: glucose, and some amino acids (alanine, serine, treonine, arginine, lysine, asparagine, tyrosine and tryptophan).

The culture of investigated strains of media with Methyl Parathion was done in a flask, to which was added 50 ml of medium. The media were inoculated with a suspension of bacteria, prepared from a 24 hr culture of nutritious agar.

The degree of degradation of Methyl Parathion by bacteria was determined in cultures incubated at 25°C. In the case of *P. aeruginosa*, a temp. of 37° was also applied. Cultures were kept for 8 to 30 days.

For each strain 2 to 3 series of investigations were performed. For each series of investigations — in order to determine the degree of chemical degradation — flasks were prepared, containing the medium used for the given series with Methyl Parathion, not inoculated with bacteria. These flasks were kept at the temperature of the cultures and used as controls.

Additional investigations were performed, in order to accelerate the degradation

of Methyl Parathion, by adapting the bacteria to utilization of that compound. Adaptation was performed in several successive stages. In these experiments, mineral media were used with added peptone, whose concentration was reduced in successive stages of adaptation, increasing at the same time the concentration of the insecticide. Inoculum for each stage was obtained by centrifuging the bacteria cells from the culture in which a reduction of Methyl Parathion by 50 to 100% was observed.

Two methods of Methyl Parathion labelling were applied:

1. The colorimetric method after Helrich (1965), appropriate to the discovery of insecticide in water (Łuczak, Gawrych 1965). By this method, Methyl Parathion is extracted by ethyl ether and hydrolyzed by potassium hydroxide in 50% ethyl alcohol. During hydrolysis of Methyl Parathion with KOH solution, potassium p-nitrophenol is liberated, giving a yellow hue to the solution. The colorimetric denotation was performed by using standards with p-nitrophenol on a photocolorimeter "Spekol".

2. The method of thin-layer chromatography, used in quantitative discovery of Methyl Parathion in soil samples (Obuchowska, Cywińska-Smoter 1971).

3. RESULTS

From media inoculated with water from the Vistula and from municipal sewage, a total of 20 bacteria strains was isolated. The investigations revealed the degradation of p-nitrophenol (formed during hydrolysis of Methyl Parathion) by 4 strains, one of which came from the Vistula and three from municipal sewage. Only one of them presented the capacity to disintegrate Methyl Parathion, which had been isolated from a medium inoculated with sewage. For detailed investigations on Methyl Parathion degradation, two strains were chosen: the strain isolated from municipal sewage, marked 7/1, belonging to the genus Pseudomonas, endowed with the capacity to disintegrate p-phenol, and the strain 8, as the only one in whose presence the degradation of Methyl Parathion could be established. This strain, marked as *Bacillus sp.* 8 presented biochemical and morphological features typical for *B. cereus.*

In series of investigations with strains of the genus Pseudomonas, Methyl Parathion was added to the medium in the amount of 100 mg/l.

Bacteria of the genus Pseudomonas isolated from municipal sewage (strain 7/1) did not disintegrate Methyl Parathion, either in mineral medium or in the presence of additional organic compounds. The preserved strains — *Pseudomonas fluorescens,* as well as *P. aeruginosa* — proved also to be unable to degrade insecticide in mineral medium. In the course of 3 to 4 weeks incubation periods the rate of Methyl Parathion discovered in inoculated media did not differ from the amounts found in control media, which did not contain any bacteria.

The enrichment of medium did not effect the degradation of Methyl Parathion by means of *P. aeruginosa.* The values of insecticide concentration obtained after 21 days of culture approximated those from control media.

In the case of *P. fluorescens* the enrichment of the medium with peptone in the initial phase of incubation of 6 to 7 days, had no effect

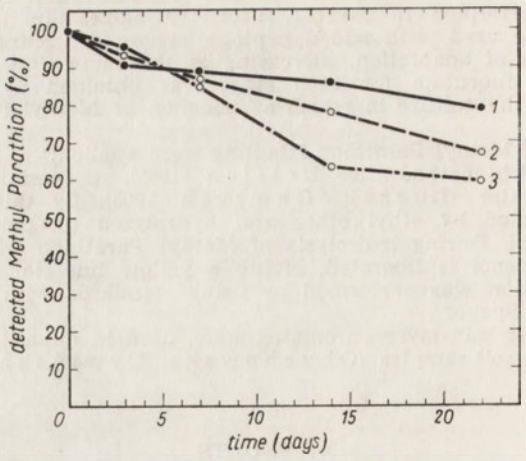

Fig. 1. Methyl Parathion detectable in a medium with 0.5% peptone depending on incubation time. 1 — not inoculated medium, 2 — medium inoculated with *P. fluorescens* (inoculum $3 \cdot 10^6$ cells), 3 — medium inoculated with *P. fluorescens* (inoculum $3 \cdot 10^9$ cells)

on the rapidity of insecticide degradation (Fig. 1). In the further 7 days of cultures of *P. fluorescens*, an insignificant drop of insecticide content, which indicated a process of biochemical degradation was noted. The increase of inoculum from $3 \cdot 10^6$ to $3 \cdot 10^9$ only insignificantly affected the degradation of Methyl Parathion by *P. fluorescens*.

The capacity of bacteria of the genus Bacillus to utilize Methyl Parathion as the only source of carbon was initially investigated in a mineral medium. Methyl Parathion was applied in amounts of 100 mg/l, the medium was inoculated with bacteria suspension, containing about

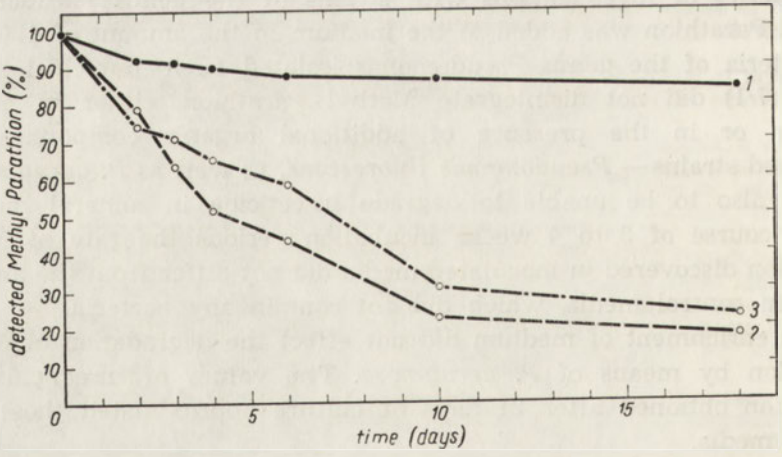

Fig. 2. Methyl Parathion detectable in a mineral medium depending on incubation time. 1 — not inoculated medium, 2 — medium inoculated with *B. cereus*, 3 — medium inoculated with *Bacillus sp.* 8

10^6 cells in 1 ml. The rate of degradation of Methyl Parathion was rather slow under such conditions (Fig. 2). After 30 days of the experiment, there still remained within the cultures about 50% of the outset quantity of insecticide. The curve in Fig. 2, illustrating the course of degradation present analogous tendencies of disintegration rate of the investigated compound, as well in cultures of the museum strain of *B. cereus*, as in cultures of *Bacillus sp.* 8.

Investigations on the effect of additional organic compounds on rapidity of Methyl Parathion degradation by bacteria of Bacillus genus were started by enrichment of the mineral medium with glucose. In the mineral medium, containing 0.05% KNO_3 as well as 0.1% glucose the degradation of Methyl Parathion ran merely by way of chemical hydrolysis, similarly as in control media.

In the second phase of investigations, potassium nitrate was replaced by ammonium phosphate. In the presence of 0.02% $NH_4H_2PO_4$ and 0.1% glucose, after 10 days of incubation there followed a drop of insecticide content in the cultures of *B. cereus* to 51% of initial value (Fig. 3).

Further experiments concerned the disintegration of Methyl Parathion in a medium containing 0.05% and 0.5% of peptone Proteose. At a content of 0.05% peptone, the most rapid degradation of Methyl Parathion took place during the 4 initial days of incubation (Fig. 4). In cultures of *B. cereus* after this time there still remained about 50% of added insecticide, and in cultures of *Bacillus sp.* 8 — about 60%. Further degradation, though slower, was observed between the 4th and 6th day. Investigations performed after 18 days of incubation did not present any further decrease of Methyl Parathion content in the cultures.

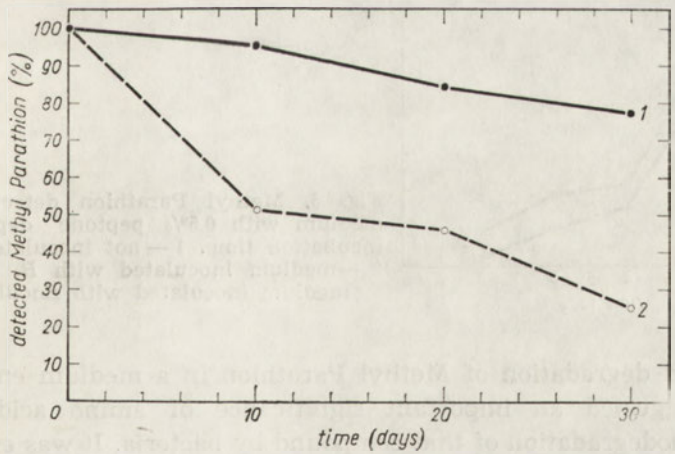

Fig. 3. Methyl Parathion detectable in a medium with 0.02% $NH_4H_2PO_4$ and 0.1% glucose depending on incubation time. 1 — not inoculated medium, 2 — medium inoculated with *B. cereus*

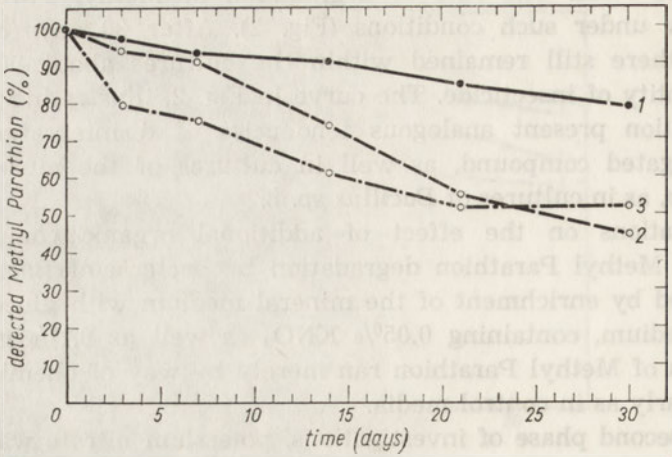

Fig. 4. Methyl Parathion detectable in a medium with 0.05% peptone depending on incubation time. 1 — not inoculated medium, 2 — medium inoculated with *B. cereus*, 3 — medium inoculated with *Bacillus sp.* 8

The increase of peptone up to 0.5% greatly accelerated the process of Methyl Parathion disintegration by bacteria of the Bacillus genus (Fig. 5). The highest degradation could be noted on the first 4 days of the experiment. After that period the content of insecticide dropped from 83.6 mg/l (100%) to 15.0 mg/l (17.0%) in the culture *Bacillus sp.* 8, and to 18.8 mg/l (22.4%) in the culture of *B. cereus*.

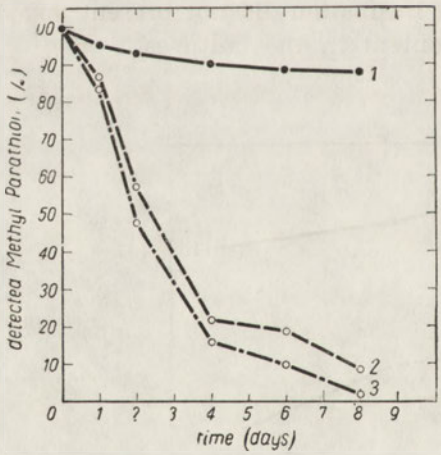

Fig. 5. Methyl Parathion detectable in a medium with 0.5% peptone depending on incubation time. 1 — not inoculated medium, 2 — medium inoculated with *B. cereus*, 3 — medium inoculated with *Bacillus sp.* 8

The rapid degradation of Methyl Parathion in a medium enriched in peptone suggested an important significance of amino acids in the process of biodegradation of that compound by bacteria. It was established that in the presence of all investigated amino acids occurred the degradation of Methyl Parathion by *B. cereus*, though this process did

not follow a similar course in every culture containing particular amino acids (Table I). The most rapid degradation was observed in the presence of serine, treonine, asparagine and alanine. In cultures containing triptophane and lysine, after 28 days of culture there still was found about 50% of the initial amount of Methyl Parathion.

Table I. The adaptation of *B. cereus* to use Methyl Parathion as a source of carbon

Adaptation stage	Initial Methyl Parathion content (mg/l)	Peptone concentration (%)	Inoculum	Incubation time (days)	Detectable Methyl Parathion	
					mg/l	%
I	0.98	0.5	$2.8 \cdot 10^6$	6	0.0	0.0
II	4.3	0.1	$3.0 \cdot 10^6$	6	0.3	7.4
III	9.3	0.05	$2.8 \cdot 10^6$	6	6.6	70.9
				14	5.0	54.3
				26	4.7	51.0
IV	64.4	0.01	$3.2 \cdot 10^6$	6	52.0	80.7
				14	45.4	70.4
				26	34.0	52.7

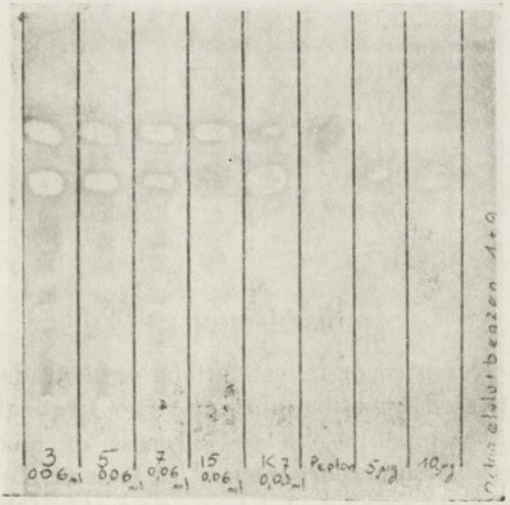

Fig. 6. Chromatogram of Methyl Parathion detectable in *B. cereus* culture. Chromatogram was developed in ethyl acetate + benzene system (1 : 9) and identified using fluoresceine and bromine vapours. 3 — 3 ml extract of 3 days culture, 5 — 0.06 ml extract of 5 days culture, 7 — 0.06 ml extract of 7 days culture, 15 — 0.06 ml extract of 15 days culture, K_7 — 0.03 ml extract of not inoculated medium after 7 days incubation (control), peptone — extract of *B. cereus* culture on a medium with peptone but without Methyl Parathion, 5 µg, 10 µg — Methyl Parathion standards

The degradation of Methyl Parathion by *B. cereus* in a medium containing 0.5% of peptone was confirmed by thin-layer chromatographic test (Fig. 6). In cultures of *B. cereus* could be observed a rapid drop of Methyl Parathion, especially within the first three days of incubation. After that time, the amount of developing metabolites did not undergo notorious changes. After 7 days there still were found in the cultures a little less than 10% of the outset amount of insecticide which was 50 mg/l.

The tests were performed in order to accelerate the degradation of Methyl Parathion by *B. cereus* in a mineral medium or in a medium with slight additional amount of organic compound, by subculture of the strain in media containing insecticide (Table II). The reduction of peptone concentration, independently of subculture in media with Methyl Parathion induced the remaining in the culture of important amounts of insecticide.

Table II. Methyl Parathion degradation by *B. cereus* in a medium with 0.1% of amino acids. The initial Methyl Parathion concentration 47.6 mg/l (100%)

Amino acid	Incubation time (days)			
	14		28	
	Detectable Methyl Parathion			
	mg/l	%	mg/l	%
Serine	7.5	15.7	0.3	0.6
Treonine	7.5	15.7	0.3	0.6
Asparagine	11.2	23.5	0.2	0.4
Alanine	11.2	23.5	0.2	0.4
Arginine	15.0	31.5	1.8	3.7
Tyrosine	21.9	46.0	18.5	38.8
Tryptophan	24.6	51.6	20.8	43.6
Lysine	30.2	63.4	22.7	47.6

4. DISCUSSION

The results here presented of investigations on degradation of Methyl Parathion were confirmed by other authors, who stress the significance of additional organic compounds in the process of biodegradation of a series of pesticides by microorganisms (Alexander 1964, Ko, Lockwood 1968, Anderson, Lichtenstein 1971). The results obtained permit to suppose that microbiological degradation of some phosphoroorganic insecticides in surface water may be in some cases a long-lasting process and depend on many factors. This is shown in many studies, bearing on the durability of these compounds in water (Faust, Suffet 1966, Łuczak, Gawrych 1966). The reason

of that durability of pesticides in aquatic medium may be found as well in the much poorer quality and quantity of microflora of surface waters as compared with conditions found in the soil, as — frequently — in the lack of sufficient amounts of easily reached by microorganisms organic compounds (Schwartz 1967). These compounds, as proved by data from the literature and also by the present investigations may be of essential significance for disintegration processes of a series of insecticides, resistant to biodegradation.

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INFILTRATION OF PESTICIDES FROM SOIL INTO SURFACE WATERS

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ABSTRACT

The phenomena connected with diffusion of pesticides used in agriculture into the surface waters have been discussed. The pesticide quantities reaching the water bodies depend on many factors, such as: chemical composition of a formulated product, its application, climatic conditions, configuration of the ground, structure of soil and its physico-chemical properties. One of the more important factors is sorption of pesticides in the soil. Microorganisms participate significantly in pesticide deactivation and metabolization, and their activity is also controlled by the ecological factors.

1. INTRODUCTION

The phenomenon of pesticides diffusion into the surface waters is of a complex character due to heterogeneity of soil environment and variety of the formulated products, in use. Therefore, in the present study pesticides will be generalized and all details as regards particular formulated products will be left out. Moreover, there are more and more of new complex products that are frequently put to use as a composite unity and in consequence they may give synergic biological effects caused by variability of physical and chemical characteristics of these products.

Concentration of the infiltrating into the surface waters, pesticides is of a wide range. It is a well known fact that it may reach a very high level. However, as long as their concentration does not exceed the level of biological detoxication ability, pesticides may disappear in a relatively short time from the water. For instance, data given by some authors (Faust, Suffet 1966) show that the halogenate insecticides that had infiltered into the lakes from the shore area have disappeared in the course of several days; they have remained, however, in the bottom sediments adsorbed by mud particles and they were detectable in there during a period of 16 months. Pesticides are concentrating also on the solid particles suspended in the water.

Pesticides pentrate into the waters by various means. Sometimes it is brought about by carelessness or ignorance of the users of those products, washing up the empty pesticide containers in the open water bodies or burying the containers and left-over pesticides into the ground (e.g. a quite well known instance of a village being deprived of potable water for many years as result of burying the DDT in its precincts, and this substance remains effective as long as up to 12 years). To remove the cause of that sort of measures would be quite sufficient.

On the contrary, the problem of pollution connected with natural factors is a much more difficult matter to deal with, even then when the whole operation is carried out correctly and in accordance with the rules. Here, the main causes of contamination are as follows: 1. dissemination of pesticides sprayed from an air-plane; 2. washing off from the plants and the surface of the soil by rain; 3. penetration through drain pipes or soil into subsoil water; 4. drifting from

drainage ditches. It is rather difficult to act directly against that kind of contamination, nonetheless, it is possible to foresee to what extent various pesticides might permeate to ground and surface waters and on what factors it depends.

Soil is an environment where structure and biological activity of the formulated chemical products undergo varied biochemical transformations. Owing to that circumstance, pesticides can pass through that environment in unchanged state or in a completely different form. Thus, the pollution of waters with pesticides introduced into the soil depends on the degree of their retainment, deactivation, or metabolization in the soil. The passage of pesticides through the soil or their retainment is conditioned by the following factors: climatic conditions (weather changes), topographic conditions (configuration of the ground), soil structure and physico-chemical properties connected with its structure, rate of pesticides metabolization by soil bacteria depending on chemical structure of pesticides, physical and chemical properties of pesticides.

2. CLIMATIC CONDITIONS AND CONFIGURATION OF GROUND

Pesticides sprayed over the plants or on the soil surface may easily drift into the surface waters especially in case of a rolling land and an opportune distribution of rainfalls. It happens sometimes that rain coming shortly after a spraying operation washes off the whole dose of pesticide into nearby water bodies. There might also occur a well known phenomenon of fish dying out in the mountain streams as result of the outflow of the chemical products used for treatment of meadows and pastures.

3. SOIL STRUCTURE AND ITS CHEMICAL AND PHYSICAL PROPERTIES

Pesticides introduced into the soil are circulating together with water and might penetrate into drain pipes and get into subsoil moisture and from there flow out to the surface. Soil with well-developed crumb structure shows a high degree of porosity. Likewise, light soil with a small content of colloidal silt is more pervious and allows the passage of water with dissolved or suspended pesticide particles more easily. In heavy soils pesticides are retained at the depth of hardly a few centimeters under the surface; remaining there for a longer time they constitute a potential threat to the environment (obviously the impending harm depends on the degree of their concentration).

The form of the applied pesticide is not a negligible matter; products of granular structure are the most difficult to be washed out, next come emulsions, while dusts and solutions are most easily washed out. Preparates of low solubility remain in soil for a longer time than the other ones, therefore they might be harmful to the surface waters, since they dissolve gradually and pass through to the water arteries. The effects of more soluble preparates are quicker and stronger and at the same time of a shorter persistance.

It could be presumed that the amount of pesticides and their speed in passing through the soil should be directly proportional to its solu-

bility. That interdependence, however, is not as simple as that. It has been frequently observed that the more soluble herbicides are shifting in the soil less easily than those less soluble. Thus, solubility of the preparates plays a secondary role. For instance, paraquat and diquat are easily dissolved in water but as soon as they get into the soil they become deactivated and are not washed out. Whereas monuron, thought it is a product of a relatively low solubility, spreads out in the soil along with the moving water. This apparent self-contradiction may be explained by the phenomenon of sorption; paraquat is adsorbed by soil strongly, and monuron much more weakly.

4. SORPTION OF PESTICIDES IN THE SOIL

Sorption, physical, chemical, and biological, is an essential factor in conditioning the diffusion of pesticides in soil. Many pesticides are strongly bound by the complex sorption of the soil and it is of a substantial significance for their activity, persistance and rate of their diffusion in soil. The sorptive capacity of soil is, in general, great — therefore the prescribed doses of pesticides are saturating only a small amount of the complex and theoretically they could be adsorbed completely. In that case their persistance in the soil is of many years standing and their infiltration into water is slow. However, not all the pesticides are adsorbed in the same degree, nor all the substances forming the sorptive complex are binding pesticides to the same extent; e.g. the successive order of appearance of the herbicides of urea derivatives on the surface of the sorptive complex is as follows: urea, monuron, diuron, linuron, neburon, chloroxuron.

The sorptive complex in soil consists of clay-minerals, such as: montmorillonite, kaolinite, silt, bentonite, with a high surface exchange--activity of cations. Moreover, a high exchange-activity of the surface is also shown by organic substances, humus organomineral complex, and products from cellulose and lignin decomposition. Data given by Swiss authors (Geissbühler et al. 1963) that urea derivatives are very strongly adsorbed by bentonite and much less strongly by cellulose, at that, about 50% of the adsorbed preparation undergoes desorption quickly and the remaining part — more slowly.

Consequently, soils with a high content of clay-minerals and especially those rich in organic substance retain larger amounts of pesticides, than the other ones with scarce organic substances, and in effect they hamper their mobility. Ward, Holly (1966) believe that sorption is inversely proportional to herbicides solubility and correlates above all with the organic substance contents in the soil (it has been confirmed, by way of example, on triazine herbicides).

The sorption process of herbicides depends on their electric charge.

Since the sorptive complex in soil has, in most cases, a negative electric charge, pesticides with negative charge are retained in the soil either very weakly or not at all. For instance, inactivation of paraquat and diquat in the soil is explained by their positive charge and irreversibility of sorption.

Soil reaction and periodical changes in acidity have a marked effect on sorption phenomena. S-triazine sorption on montmorillonite increases with a decrease in pH value; ametryne is most strongly adsorbed at 4.0 pH. Parathion, dicamba, picloram, fenac, 2,4-D, are also more strongly adsorbed at a low pH value. An increase in pH value decreases sorption and sets pesticides in motion.

The presence of cations in the soil and saturation of the surface of active complexes with them is a factor of substantial importance for setting in motion or retainment of the pesticides. Basing on the example of parathion it is possible to demonstrate the concurrence of cations and parathion at the entering on the sorptive surface. Cations are ranging in a decreasing order, as follows: $H^+ > Ca^{++} > K^+ > Na^+$. Some other authors have indicated, likewise, that prometryne sorption was decreasing in the presence of $CaCl_2$. In some other preparates the situation was reversed e.g. fluorometon sorption increased with the soil saturation with Ca^{++}.

Pesticides activity in the soil is affected by temperature. With the increasing temperature their solubility and the ability of passing through the soil are also increasing. Moreover, their sorption increases at higher temperatures, e.g. atrazyne and ametryne combine much more strongly at 25°C than at 10°C; the amount of atrazyne combined by 200 mg of humic acid at 25°C is fourfold greater than that at 3°C. Consequently, there are also changes in the amount of used calories, ranging from 8 to 15 kcal/mol.

Desorption is a phenomenon of no less importance for the retainment of pesticides in the soil. Sorption in soil may be irreversible, but more often than not it is reversible and then preparates pass back into solution. Hance (1967) has measured the time of the attainment of equilibrium between sorption and desorption in monuron, linuron, atrazyne, and chlorpropham, by viarious adsorbents. The time of sorption lasted about 24 hrs or less. Desorption process was, in general, slower and lasted about 72 hrs. Despite reversibility of desorption, a part of herbicides remained on the adsorbents. The degree of desorption depends, among other factors, on the character of the adsorbent.

Hartley (1967) gives some data indicating the significance of herbicides solubility in the attainment of sorption — desorption equilibrium. In the course of desorption pesticides with higher degree of solubility will be spread over quicker along with water movements.

There is a supplementary factor occurring in the described phenomena,

i.e. seasonal drying of the soil. In that case, pesticides cannot get off the surface of the sorptive complex and they become movable only after the soil is saturated with water, again.

5. MICROBIOLOGICAL TRANSFORMATIONS OF PESTICIDES IN SOIL

Pesticides udergo chemical and biological transformations in the soil. The main kinds of reactions leading to their decomposition are, as follows:

dehalogenation — a characteristic reaction at the aliphatic acids detoxication,

separation of alkyl groups — at decomposition of many herbicides e.g. simazine, diuron, trifluralin, etc.,

hydroxylation of aromatic nucleus,

disruption of the carbon chain,

oxidation,

reduction occurring in anaerobic conditions, that process is not well known, yet.

In most cases, transformations of pesticides in soil are leading to the formation of compounds easier dissolved in water than the preparate itself. Detoxication of those preparates occurs simultaneously, though not as a general rule. There are some well known cases of formation of some toxic products of the metabolism of pesticides (e.g. at the biological decomposition of carbamates — Bartha 1968). The role of microorganisms in those processes is rather complicated; soil bacteria while metabolizing pesticides are subjected themselves to the action of pesticides. Products from metabolism of microorganisms and pesticides may also react against each other and give as result a synergetic biological effect.

The rate of biological processes depends on ecological factors. In general, those factors that are stimulating the microorganisms development are simultaneously accelerating metabolization of pesticides in soil. Fluctuations of the reaction are either stimulating or inhibiting decomposition of the particular pesticides in dependence of the requirements of a group of microorganisms responsible for that process. That reaction may also change in a decisive manner the course of the chemical decomposition of pesticides. Lode (1967) while presenting the course of the TCA decomposition in various soils indicates its dependence on the mechanical composition and the pH value. In sandy soils decomposition occurred much more quicker, this being conditioned by the arrangement of air-water relations favourable for microflora. Acidification of soil, unfavourable for bacteria, inhibited that process.

There is a question — whether it is possible to avoid or at least to limit the infiltration of pesticides from the soil into the surface waters.

Pesticides are more active in water; plants and microorganisms are more sensitive to the effect of pesticides in water environment than in soil. For that reason, residues of pesticides which in soil do not produce any visible changes might cause in water undesirable phenomena.

Human intervention to deal with those phenomena is possible but not easy and must rely on a deep knowledge of the ecology of soil environment. It indicates the way of a proper formation of the soil environment leading to a good soil structure where air-water relations will give optimal conditions for the development of living organisms — animals, plants, and microorganisms. Abundant micro- and mesofauna, micro- and macroflora form a reserve of organic substances and organo--mineral complex with a great sorptive capacity. In such conditions it is possible to attain a strong sorption of pesticides and not letting them to be washed off.

Soils with a good structure and rich in organic substance are usually biologically active due to the abundant microflora. Owing to this, detoxication of pesticides can progress very quickly. Those features are characteristic for soils with high agricultural cultivation and high productivity. Thus, the better soil the greater freedom in prophilactic plant protection and treatment with chemical products and at the same time a less danger of potential contamination of soil environment.

It is also necessary to be well-acquainted with pesticides properties to be able to make a suitable selection of preparates for soil treatment. In general, those pesticides are put to use that are effective in action of short duration, and are not left over in soil as imminent residues that might be set in motion in the wrong time and place.

Doubtlessly, the time and manner of introducing pesticides into the soil is also important since their utilization and retainment in soil depend on those factors, as well.

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COMPARATIVE STUDIES ON THE DETERMINATION OF TOXICITY OF SOME PESTICIDES

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ABSTRACT

The toxicity of DDT, Cupritox-50 and Simazine was investigated with the parallel use of the BOD test, the mortality test and Kalabina's method. It was established that the two latter methods allow to determine precisely the toxicity of pesticides, while the BOD test does not always give representative results.

1. INTRODUCTION

The necessity of improving and creating suitable living conditions has caused the introduction of pesticides as means of protection of animal and vegetal production and also to fight against numerous infections.

For that purpose thousands of various chemical agents are sown every day, mainly in order to destroy insects (insecticides), weeds (herbicides) and microorganisms (bacteriocides and fungicides). However the action of those agents has frequently proved non-selective since they cause not only the mass destruction of the organisms against which they are meant but also, quite often, of useful organisms, and they may have an effect of people. Pesticides, washed by rain water, carried by the wind, accumulated in plants and animal tissues, get into the human organisms and may cause chronic diseases.

Inland and sea waters, in particular, nowadays contain considerable quantities of pesticides owing to which these waters are not suitable for normal use and may have a toxic effect on organisms using them.

In order to determine the degree of pollution of the water medium with pesticides, Taylor, Rybiński (1966) have been proposed to introduce the conception of "indices of danger" with regard to fish and mammals.

The variety of preparations used is very large; there still prevail insecticides, mostly chlorinated hydrocarbons. These compounds usually posses a high toxicity and durability (Taylor, Rybiński 1966, Tielecke 1967).

DDT (dichlordiphenyltrichlorethane) has proved to be the most dangerous for the environment and therefore it has been lately eliminated in many countries (Byrdy 1971). This compound can be cumulated in plant and animal tissues and thus it gets into the food chain while its amount increases in the particular links (Gaufin et al. 1962, Bridges et al. 1963, Yakubova, Bashirov 1966, Nicholson 1967, Dmoch et al. 1969).

For that reason investigations on new agents replacing DDT are in progress also in Poland (Fulde 1971, Byrdy 1971), but DDT accumulated in the environment and used in some mixtures may still act for a long time.

Among fungicides a copper preparation, the copper oxidochloride — Cupritox-50, is most commonly used in agriculture and fruit-growing.

Cupritox-50 is settled from water as a very fine suspension characterized by a particularly durable adhesiveness to plants, which increases the danger of its presence in the water environment (Popov 1959). Copper is the main active substance in this preparation (Borecki et al. 1965).

The toxic effect of copper ions on plant organisms ranges from 0.05 mg/l Cu^{++}

to several miligrams per litre (Bringmann, Kühn 1959) and for lower water organisms, according to Weber (1957), the limit concentration is 0.1 mg/l Cu^{++}. Those data are numerous and inevitably divergent as other ingredients of natural waters reduce or intensify the effect of the given salts.

Another, less known, pesticide of the group of triazine herbicides is the Simazine (Melnikov 1959). This preparation, if used in doses over 3–5 mg/l, spreads on the surface of the water and is adsorbed on dipped objects and plants. With a concentration of only 0.02 mg/cm² there appears a uniform film (Mazaev 1964). In doses up to 500 mg of active ingredient per litre (mg A.I./l) it does not prevent the development of *Pseudomonas fluorescens, Bacillus cereus* and *Sphaerotilus natans*. Instead, for fish and small water organism toxic doses range within large bounds, even up to 3000 mg/l (Solski 1968). It is probable that this also depends on other ingredients of the water, both mineral and organic, primarily on suspended matter and — as for fish — on the mucus produced for protection.

The purpose of the presented experiments was the comparative determination of the toxicity of three pesticides i.e. of the insecticide DDT, of the fungicide Cupritox 50 and of the herbicide Simazine.

2. METHODS

In order to establish the toxicity of the three chosen pesticides with regard to biochemical processes and water organisms the following methods were used: the determination of the biochemical oxygen demand during 20 days (BOD_{20}); the determination of the toxic action of the studied products on *Paramecium caudatum*, as index organisms; the determination of toxicity by the method proposed by Kalabina (1945).

The pesticides were used in the form of formulated products: DDT as "Azotox 33" (liquid) produced by Z. Ch. "Azot" in Jaworzno; Cupritox as "Miedzian-50" produced by Z. Ch. "Gamrat" in Jasło; and Simazine as "Simazin-50" Geigy. The content of active substance in these products was determined and all the results of experiments were referred to the particular doses of this substance.

BOD_{20} was determined by the method of diluting the particular doses of pesticides in reference to this process in control samples. The diluent water used in this process, beside careful oxigenation, contained all the microelements necessary to the development of microorganisms. Sewage sediments were used for inoculations. Oxygen and the occurrence of nitrates in the samples were alternately determined at two-day intervals.

When *Paramaecium caudatum* was used as an index organisms for the study of toxicity, an appropriate number of specimens were first grown through reproduction from single organisms on hay or lettuce infusion with Knopp mineral solution added. The solutions were prepared on tap water of 7.0 miliequivalents of hardness, at a temperature of 18–20°C. The experiments were carried out on Petri dishes of 6 cm of diameter; 10 ml of pesticide solution were used for 2 ml of the culture containing a dozen or so of specimens, the behaviour of which was observed (magnified 10×).

DDT in "Azotox-33" was used in concentration of 20–750 mg of active ingredient per litre; Cupritox in "Miedzian-50" — in quantities of 0.1–10.0 mg/l Cu^{++}; and "Simazine-50" — in concentration of 30–6000 mg/l. The time of observation was 48 hours.

The determination of toxicity by Kalabina's (1945) method consisted in systematic twenty-day observations of changes occurring in specially prepared water inoculated with sewage.

The process was checked for pH, for the oxidizability, for mineral nitrogen (ammonia, nitrite, nitrate) and for the number of bacteria on nutrient broth agar (after 24 hours, at 27°C of temperature) in two-day intervals. The investigated solutions contained pesticides in concentration selected according to the experiments carried out by the methods described above. Analogical processes were parallelly observed in the control sample which did not contain any toxic substances.

3. RESULTS

The determination of BOD as test of toxicity with reference to DDT (Table I) has shown that doses of 10–200 mg/l did not hamper this

Table I. BOD_5 and BOD_{20} for the following pesticides: DDT contained in "Azotox 33", "Cupritox-50" Simazine-50 (mean results of 3–7 series of determinations)

DDT			"Cupritox-50"			Simazyna-50		
Dose (mg/l)	BOD_5 (mg O_2/l)	BOD_{20} (mg O_2/l)	Dose (mg Cu^{++}/l)	BOD_5 (mg O_2/l)	BOD_{20} (mg O_2/l)	Dose (mg/l)	BOD_5 (mg O_2/l)	BOD_{20} (mg O_2/l)
0.0	1.3	3.0	0.0	1.3	3.1	0	1.3	3.1
10.0	4.6	full consumption of dissolved oxygen after 3—11 days	0.1	1.3	3.3	500	1.6	4.7
20.0	5.1		1 0	0.6	3.1	1000	2.3	7.3
50.0	8.9		5.0	0.1	1.7	2000	2.6	8.3
100.0	9.0		10.0	1.1	0.9			
150.0	8.9							
200.0	9.0							

process since both the absolute value of BOD_5 which grew, as the quantity of this product in the solution — compared with the control sample increased, and BOD_{20}, where oxygen was consumed in 3–11 days, prove that this process was not affected.

Such results might be also explained both by a weak action of DDT due to its poor solubility and by the influence of the diluent and the emulsifying substances of unknown composition which may reduce or intensify the effect of the actual product.

Instead, copper contained in the product "Cupritox-50" visibly hindered the process of the biochemical oxygen demand (Table I) in doses just over 1.0 mg/l Cu^{++}, which has been earlier established with other copper salts (Zdybiewska, Strutyńska 1963).

Simazine, in concentrations up to 2000 mg/l, did not hamper the BOD process since its absolute value rose as compared with the control sample. However these differences were not proportional to the concentration and did not evidence the degree of its dissolution.

The use of *Paramaecium caudatum* as index organism with regard to DDT (Table II) has made it possible to state that this preparation,

Table II. Toxicity of DDT contained in "Azotox-33 (liquid)" used with *Paramaecium caudatum* as index organism (mean results of 6 series of experiments)

DDT dose (mg A.I./l)	Kind of dose	Time of action *t* (hr)	% of living organisms after *t*
20	safe	48	100
40	safe	48	100
60	noxious	48	85
75	noxious	48	80
100	noxious	48	55
150	noxious	4	50
200	noxious	2	50
300	noxious	1	40
400	noxious	0.5	28
450	noxious	0.5	25
500	mortal	2	0
550	mortal	0.5	0
600	mortal	0.25	0
750	mortal	0.25	0

in quantities 20–40 mg of active ingredient per litre, has no toxic effect on these organisms, which can be proved by the fact that after 48 hours of observation they all were still alive (Table II, entry 1–2). Higher concentrations up to the dose of 200 mg/l caused the death of more and more specimens so, that after 48 hours, with this dose used, there only remained 50% alive. An immediate death of all the organisms occurred by doses higher than 500 mg/l of DDT.

Using the data obtained to calculate "safe concentration" according to Turnbull's formula:

$$S = \frac{48\ \text{Tlm} \cdot 0.3}{(24\ \text{Tlm}/48\ \text{Tlm})^2}$$

where: 24 Tlm — concentration of investigated substance which, in 24 hours, causes the death of 50% of test organisms, 48 Tlm — concentration of investigated substance which, in 48 hours, causes the death of 50% of index organism, it was established that with DDT "safe concentration" equals 30 mg/l.

It results from similar experiments carried out with "Cupritox-50" (Table III) that its doses, ranging from 0.1 to 0.3 mg/l Cu^{++} did not affect noxiously the organisms under observation during the 48-hour period (Table III, entry 1—3). A further increase of the dose caused

Table III. Toxicity of "Cupritox-50" with reference to *Paramaecium caudatum* as index organism (mean results of 6 series of experiments)

Cupritox dose (mg Cu^{++}/l)	Kind of the dose	Time of action t (hr)	% of living organisms after t
0.1	safe	48	100
0.2	safe	48	100
0.3	safe	48	100
0.4	noxious	48	98
0.5	noxious	48	90
1.0	noxious	48	80
2.0	noxious	48	50
3.0	noxious	4	40
4 0	noxious	2	45
5.0	noxious	4	40
6.0	noxious	1	15
6.1	noxious	1	5
6.2	noxious	1	5
6.3	mortal	2	0
6.5	mortal	1	0
7.0	mortal	0.5	0
10.0	mortal	immediately	

a greater decrease of Paramaecium till finally, with the dose of 10 mg/l Cu^{++} all the organisms were immediately killed. Here the "safe concentration" of this product equalled 0.3 mg Cu^{++}/l.

Observations concerning the influence of Simazine (Table IV) on *Paramaecium caudatum* have shown that with a dose of 30—100 mg/l of this product all the organisms were alive after 48 hours. An increase of the doses caused the growth of the percentage of dead organism

until, with a dose of 5000 mg/l all the specimens were immediately killed. In this case the "safe concentration" was ca. 100 mg/l of Simazine.

Table IV. Toxicity of "Simazin-50" Geigy with reference to *Paramaecium caudatum* as index organism (mean results of 6 series of experiments)

Simazine dose (mg/l)	Kind of dose	Time of action t (hr)	% of living organism after t
30	safe	48	100
50	safe	48	100
100	safe	48	100
125	noxious	48	95
200	noxious	4	85
250	noxious	4	80
500	noxious	4	55
1000	noxious	2	50
1500	noxious	1	25
2000	noxious	1	20
3000	noxious	0.5	10
4000	mortal	0.5	0
5000	mortal	immediately	0
6000	mortal	immediately	0

Kalabina's (1945) method used to state the toxicity of the investigated products has shown that with a 150 mg/l dose of DDT (Table V), after 14 days, there occurred but a slight set-back of nitrification and a decrease of the number of microorganisms, as referred to the control sample. The increase of the DDT dose up to 300 and 450 mg/l caused further hampering of the processes which could be noticed both in a visible check of nitrification and in the effect on the bacteria occurring in the solutions i.e. in a visible reduction of their number, immediately after the product was introduced as well as during its further action (Table V).

These results largely agree with the effects observed with *Paramaecium caudatum* used as index organism.

Further experiments carried out by the same method with "Cupritox-50" (Table VI) have shown that this product acted slightly in 0.1 and 0.5 mg/l concentration but had a visible effect in a dose of 1.0 mg Cu^{++}/l.

For Simazine (Table VII) a dose of 500 mg/l slightly hampered the nitrification process and somewhat reduced the development of microorganisms. However, 1000 and 2000 mg/l doses of this product checked a further reduction of nitrification effects in a very small degree but more visibly they reduced the development of microorganisms.

To sum up the results of the experiments described above it should be stated that the method of recording toxicity with the use of *Paramaecium caudatum* as well as Kalabina's (1945) method which

Table V. Effect of DDT contained in "Azotox 33" (liquid) on indices of water pollution, investigated by K a l a m i n a's (1945) method (temp. 19–21°C)

Time (days)	pH	Permanganate oxygen demant (mg O_2/l)	N-NH_3 (mg/l)	N-NO_2 (mg/l)	N-NO_3 (mg/l)	Number of colonies from 1 ml
			Control of process			
immediately	7.8	60.0	0.08	0.03	0	1300
2	7.5	50.0	0.06	0.07	0	1180
4	7.7	44.5	0.06	0.10	0	1000
6	7.7	38.0	0	0.30	0	900
8	7.5	32.0	0	0.45	0	800
10	7.7	28.0	0	0.70	0	700
12	7.8	23.0	0	0.90	1.00	650
14	7.7	20.0	0	1.10	1.50	650
16	7.8	18.0	0	1.10	1.50	—
18	7.8	17.0	0	1.30	1.85	—
20	7.8	16.0	0	1.35	2.00	—
			Dose 150 mg DDT/l			
immediately	7.6	71.0	0.08	0.05	0	1100
2	7.6	65.0	0.07	0.08	0	930
4	7.8	60.0	0.06	0.09	0	900
6	7.5	56.0	0.05	0.12	0	790
8	7.6	51.5	0.03	0.12	0.08	710
10	7.5	47.0	0	0.17	1.00	600
12	7 6	43.5	0	0.20	1.20	580
14	7.7	39.0	0	0.25	1.50	550
16	7.7	34.0	0	0.40	1.30	—
18	7.6	27.0	0	0.70	1.30	—
20	7.7	25.0	0	0.80	1.50	—
			Dose 300 mg DDT/l			
immediately	7.4	65.0	0.10	0.08	0	1000
2	7.5	58.0	0.10	0.10	0	900
4	7.8	53.0	0.09	0.15	0	800
6	7.6	48.0	0.07	0.20	0	620
8	7.5	43.5	0.07	0.25	0	550
10	7.8	39.0	0.60	0.40	0	480
12	7.7	36.0	0.80	0.50	0	550
14	7.6	31.5	0	0.50	0.4	400
16	7.8	27.0	0	0.50	0.5	—
18	7.7	25.0	0	0.60	0.5	—
20	7.6	21.5	0	0.40	0.6	—

Time (days)	pH	Permanganate oxygen demand (mg O_2/l)	N-NH_3 (mg/l)	N-NO_2 (mg/l)	N-NO_3 (mg/l)	Number of colonies from 1 ml
			Dose 450 mg DDT/l			
immediately	7.4	60.0	0.10	0.09	0	900
2	7.7	50.0	0.08	0.11	0	800
4	7.4	45.0	0.08	0.11	0	670
6	7.5	40.5	0.06	0.14	0	700
8	7.6	37.0	0.05	0.12	0	580
10	7.5	33.0	0.03	0.18	0	390
12	7.6	29.5	0.01	0.20	0	300
14	7.7	24.5	0	0.30	0.3	250
16	7.6	22.0	0	0.30	0.3	—
18	7.7	20.0	0	0.25	0.4	—
20	7.6	17.5	0	0.20	0.45	—

Table VI. Effect of "Cupritox-50" on water pollution indices, investigated by Kalabina's (1945) method (temp. 19–21°C)

Time (days)	pH	Permanganate oxygen demand (mg O_2/l)	N-NH_3 (mg/l)	N-NO_2 (mg/l)	N-NO_3 (mg/l)	Number of colonies from 1 ml
			Control of process			
immediately	7.8	60.0	0.08	0.03	0	1300
2	7.5	50.0	0.06	0.07	0	1100
4	7.7	44.5	0.06	0.10	0	1000
6	7.6	38.0	0	0.30	0	880
8	7.5	32.0	0	0.45	0	720
10	7.7	28.0	0	0.70	0	670
12	7.8	23.0	0	0.90	1.00	610
14	7.7	20.0	0	1.10	1.50	590
16	7.8	18.0	0	1.10	1.50	—
18	7.8	17.0	0	1.30	1.85	—
20	7.8	16.0	0	1.35	2.00	—
			Dose 0.1 mg Cu^{++}/l			
immediately	7.5	61.0	0.05	0.01	0	1220
2	7.6	51.0	0.05	0.08	0	1000
4	7.8	46.0	0.04	0.10	0	930
6	7.5	40.0	0	0.12	0	840
8	7.6	34.0	0	0.25	0	770
10	7.6	29.0	0	0.4	0	700
12	7.5	23.0	0	0.6	1.0	620
14	7.5	20.0	0	0.9	1.0	560
16	7.7	18.0	0	1.0	1.5	—
18	7.6	17.0	0	1.0	1.2	—
20	7.7	17.0	0	1.0	1.4	—

Time (days)	pH	Permanganate oxygen demand (mg O_2/l)	N-NH_3 (mg/l)	N-NO_2 (mg/l)	N-NO_3 (mg/l)	Number of colonies from 1 ml
			Dose 0.5 mg Cu^{++}/l			
immediately	7.4	55.0	0.08	0.05	0	1250
2	7.5	47.0	0.06	0.1	0	1050
4	7.8	43.0	0.06	0.1	0	900
6	7.6	38.0	0	0.2	0	800
8	7.5	31.5	0	0.2	0	680
10	7.8	27.0	0	0.3	0.8	600
12	7.7	24.0	0	0.5	1.0	530
14	7.7	20.0	0	0.5	1.2	500
16	7.8	18.0	0	0.4	1.3	—
18	7.7	16.0	0	0.6	1.3	—
20	7.8	15.0	0	0.5	1.4	—
			Dose 1.0 mg Cu^{++}/l			
immediately	7.5	50.0	0.08	0.09	0	1200
2	7.8	40.0	0.06	0.12	0	970
4	7.5	39.0	0.04	0 10	0	900
6	7.6	34.0	0	0.15	0	740
8	7.7	29.0	0	0.10	0	610
10	7.6	26.0	0	0.2	0	550
12	7.7	23.0	0	0.2	0	500
14	7.6	21.0	0	0.3	0.3	470
16	7.7	19.0	0	0.2	0.4	—
18	7.7	18.0	0	0.15	0.4	—
20	7.6	18.0	0	0.2	0.5	—

Table VII. Effect of "Simazine-50" Geigy on water pollution indices, investigated by Kalabina's (1945) method (temp. 19–21°C)

Time (days)	pH	Permanganate oxygen demand (mg O_2/l)	N-NH_3 (mg/l)	N-NO_2 (mg/l)	N-NO_3 (mg/l)	Number of colonies from 1 ml
			Control of process			
immediately	7.8	60.0	0.8	0.02	0	1300
2	7.5	50.0	0.6	0.07	0	1100
4	7.7	44.5	0.6	0.10	0	960
6	7.6	38.0	0	0.30	0	800
8	7.5	32.0	0	0.45	0	700
10	7.7	28.0	0	0.70	0	650
12	7.8	23.0	0	0.90	1.00	600
14	7.7	20.0	0	1.10	1.50	600
16	7.8	18.0	0	1.10	1.50	—
18	7.8	17.0	0	1.30	1.85	—
20	7.8	16.0	0	1.35	2.00	—

Time (days)	pH	Permanganate oxygen demand (mg O_2/l)	N-NH_3 (mg/l)	N-NO_2 (mg/l)	N-NO_3 (mg/l)	Number of colonies from 1 ml
			Dose 500 mg/l			
immediately	7.4	56.0	0.06	0.03	0	1300
2	7.6	51.0	0.04	0.08	0	1070
4	7.8	46.5	0.03	0.10	0	900
6	7.6	42.5	0.03	0.13	0	750
8	7.7	35.0	0.01	0.20	0	700
10	7.6	30.5	0	0.30	0.05	700
12	7.5	26.5	0	0.50	1.00	680
14	7.4	23.0	0	0.70	1.20	650
16	7.7	19.5	0	0.90	1.40	—
18	7.6	17.0	0	1.00	1.20	—
20	7.6	13.0	0	1.05	1.20	—
			Dose 1000 mg/l			
immediately	7.4	52.0	0.06	0.04	0	1200
2	7.5	48.0	0.03	0.10	0	1000
4	7.7	43.0	0.03	0.20	0	800
6	7.6	40.0	0.02	0.30	0	700
8	7.5	35.0	0	0.30	0	630
10	7.8	29.5	0	0.40	0.8	560
12	7.7	23.5	0	0.50	1.0	520
14	7.6	19.0	0	0.40	1.3	480
16	7.7	17.0	0	0.50	1.4	—
18	7.7	14.5	0	0.60	1.5	—
20	7.8	12.0	0	0.60	1.4	—
			Dose 2000 mg/l			
immediately	7.5	45.0	0.08	0.03	0	1100
2	7.7	40.0	0.07	0.08	0	900
4	7.6	37.5	0.04	0.10	0	750
6	7.7	32.0	0.02	0.12	0	600
8	7.8	25.5	0	0.10	0	500
10	7.6	20.0	0	0.10	0	400
12	7.7	18.5	0	0.30	0.7	360
14	7.5	15.5	0	0.25	0.9	300
16	7.6	12.0	0	0.20	0.7	—
18	7.6	9.0	0	0.20	1.0	—
20	7.7	7.5	0	0.10	1.0	—

permits not only to state the toxic effect of a substance on microorganisms but to determine its influence on nitrification processes which play a first-rank role in the processes of biological purification of sewage and self-purification of water — that those methods proved to be most useful.

Instead, the method of determining BOD_{20} as long and depending on the quantity and the quality of the microorganisms, which take part in the process of the biochemical dissolution of organic substances not always subject to such dissolution, seems to be less useful for that purpose.

Thus, basing on the results of the present work it can be stated that:

1. The method of determining BOD_{20}, used for "Cupritox-50" has made it possible to determine the limit concentration of copper ions which still would not check the process. However, in preparations consuming oxygen such as the products containing DDT and Simazine, the results were not typical, for it was impossible to distinguish the process of immediate oxygen consumption from the simultaneous check of biochemical processes.

2. The determination of toxicity with the use of *Paramaecium caudatum* as index organisms permitted to establish the toxic concentration of the particular pesticides and their "safe concentration".

3. The determination of toxicity by Kalabina's (1945) method has confirmed the results obtained by the use of *Paramaecium caudatum* and, at the same time, since a more complicated biocenose was under observation (samples inoculated with municipal sewage), it permits to foresee the possibilities of introducing biochemical processes of purification of sewage in the presence of the investigated substances, used in appropriate concentration.

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