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ASSESSMENT OF THE WATER QUALITY OF THE DON RIVER DOWNSTREAM FROM ITS CONFLUENCE WITH THE TEMERNIK RIVER BASED ON THE SAPROBITY INDEX AND BIOASSAY DATA

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Abstract. The Don River is one of the largest rivers in the East European Plain. The right tributary of the Don River—the Temernik River—flows through the metropolis of Rostov-on-Don and affects the water quality of the main watercourse. This study was aimed at the assessment of the organic pollution and toxicity of water in two horizons (0.3 and 9.0 m) of the Don River downstream from its confluence with the Temernik River using the saprobic index S and biotesting. Observations were carried out for 7 months in 2019. The data on the quality of the Don River water have been obtained from the combined results of two biological methods for the first time. The dynamics of changes in the quality of the river water in the course of 7 months is presented. The samples for the investigation were collected concurrently. Comparison of the water quality in two horizons (surface and deep) of the river section based on the data obtained using two biological methods has been conducted for the first time. The saprobity results for the phytoplankton indicated the presence of organic water pollution at both levels of the Don River during all 7 months of the study. The saprobity index values ranged from 2.0 to 3.6. Water quality was characterized as ranging from moderately polluted to polluted. This corresponds to α - β -mesosaprobic level. The maximum level of organic pollution according to the saprobity index was recorded in September for both horizons. Saprobity increases with an increase in the proportion of blue-green algae in the phytocenosis. The relative abundance of blue-green algae ranged from 45.83 to 77.77 %. The surface horizon had higher organic pollution during the warm period (May–September), and for the deep horizon it was higher during the cold period (April, October, and November). The toxic effect of the river water was manifested in the inhibition of the growth of *Chlorella vulgaris*. Water toxicity over the investigated period (7 months) was inconsistent. In each season, it only appeared for one month. Water toxicity at the both horizons was recorded in spring (April), summer

(August), and autumn (November). High saprobity and toxic effect of the water did not always coincide. The concurrent presence of these characteristics is typical for the deep horizon. Toxicity fluctuations are apparently associated with the temporal pattern of the Temernik River pollution by the metropolis waste waters and with the measures for the Temernik River improvement taken by the municipal authorities.

Keywords: phytoplankton, saprobity index S, organic pollution, bioassay, toxicity, Don River, Temernik River, water horizon

ОЦЕНКА КАЧЕСТВА ДОНСКОЙ ВОДЫ НИЖЕ МЕСТА ВПАДЕНИЯ РЕКИ ТЕМЕРНИК ПО ИНДЕКСУ САПРОБНОСТИ И ДАННЫМ БИОТЕСТИРОВАНИЯ

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Аннотация. Река Дон — одна из крупнейших рек Восточно-Европейской равнины. Правый приток Дона — река Темерник — протекает через мегаполис Ростов-на-Дону и оказывает влияние на качество воды основного водотока. Целью исследования была оценка органического загрязнения и токсичности вод двух горизонтов (0,3 и 9,0 м) реки Дон ниже по течению от места впадения в нее реки Темерник с использованием индекса сапробности S и биотестирования. Наблюдения проводили в течение 7 месяцев 2019 г. Впервые получены данные о качестве донской воды по совокупности результатов двух биологических методов. Представлена динамика изменения качества речной воды за семимесячный период. Исследования проводились в синхронно отобранных пробах. Впервые проведено сравнение качества воды двух горизонтов (поверхностного и глубокого) створа реки по данным двух биологических методов. Результаты сапробности по фитопланктону свидетельствуют о наличии органического загрязнения воды на обоих горизонтах р. Дон во все 7 месяцев исследования. Диапазон значений индекса сапробности составлял 2,0–3,6. Качество воды характеризовалось как находящееся в пределах от умеренно загрязненного до загрязненного. Это соответствует α - β -мезосапробному уровню. Максимальный уровень органического загрязнения по индексу сапробности отмечен для обоих горизонтов в сентябре. Сапробность увеличивалась с увеличением доли синезеленых водорослей в фитоценозе. Относительная численность синезеленых водорослей колебалась в пределах от 45,83 до 77,77 %. Более высокий уровень органического загрязнения в теплый период (май–сентябрь) был свойственен поверхностному горизонту, а в холодный период (апрель, октябрь и ноябрь) — глубокому горизонту. Токсическое действие речной воды проявлялось в угнетении роста *Chlorella vulgaris*. В течение 7 месяцев исследований токсичность воды была неравномерна. Она проявлялась только в одном месяце каждого сезона. В воде обоих горизонтов токсичность была отмечена весной — в апреле, летом — в августе, осенью — в ноябре. Наличие высокой сапробности и токсического действия воды не всегда совпадали. Больше число совпадений этих показателей характерно для глубокого горизонта. Колебания токсичности, вероятно, связаны с разным временным характером загрязнения реки Темерник сточными водами мегаполиса и с мероприятиями по оздоровлению р. Темерник, проводимыми администрацией города.

Ключевые слова: фитопланктон, индекс сапробности, органическое загрязнение, биотестирование, токсичность, река Дон, река Темерник, горизонт

INTRODUCTION

The Temernik River is a plain river and the right tributary of the Don River, belonging to the category of small rivers. Along with the Don River, it is one of the main watercourses of Russia and the second largest and third longest one in the country. The Temernik River passes two thirds of its course within the city of Rostov-on-Don. Domestic and industrial waste waters received by the Temernik River upon its passing through the metropolis have an adverse effect on the Don River water, causing the transformation of phytocenoses, which leads to intensive blooming of blue-green algae (cyanobacteria) [1, 2]. Active algal blooms usually lead to such a serious problem as the eutrophication of a river. The consequence of eutrophication, in addition to the increase in the productivity of aquatic communities, is the lag of decomposition processes. As a result, organic materials accumulate in the river water, which in turn leads to a significant increase in the levels of anthropogenic disturbances of aquatic ecosystems.

Beside eutrophication, there is another risk, which is toxicity. Toxicity is a biological characteristic. It is associated with the presence of pollutants of various chemical types in water, which have a negative impact on the hydrobiota [3].

One of the main components of aquatic ecosystems is the first trophic link, namely phytoplankton. The study of such a sensitive component (phytoplankton) is crucial for the assessment of the aquatic environment, particularly its pollution and toxicity.

Several studies of the phytoplankton of the Don River at the confluence with the Temernik River have been carried out. In the case of wastewater presence in addition to the eutrophication process, all these studies showed that the impact number of saprobiotic populations increased, the biomass of phytoplankton, especially green algae, decreased, and species from the Chrysophyta group disappeared [2]. In this study, saprobiotic species coexisted, indicating α - β -mesosaprobic conditions [4].

The data on the state of the Don River in the city of Rostov-on-Don, as well as the data obtained using biological methods (investigation of the planktonic cenoses of the Don River and the results of toxicity analysis obtained with the bioassay method), indicated an improvement in the ecological and toxicological situation since the 90s [4]. On the other hand, the data related to the content of certain chemicals in the water showed that their maximum allowable concentrations

were exceeded, often by 6–7 times. However, in general, the water quality, according to the classifications provided by analytical methods, also had a positive trend in recent years as it went from “very polluted” (3B) in 1988 to “moderately polluted” (3) in 2006 [4].

Bioassay, in addition to its wide applications in the conventional studies of various physical and chemical properties of water, is used for water quality control in the USA, Canada, and the European Union [5]. Bioassay usually uses indicator organisms that respond specifically to water pollution. The bioanalysis cannot specify which pollutant is responsible for the change in the biological activity of the selected bioindicator [6].

Interactions between substances present in water also affect the biological activity of bioindicators. As a consequence, the toxic effect on the body of a bioindicator is used as a measure of water pollution. Therefore, it can be said that it is impossible to assess the toxic effects in the environment using standard physicochemical methods only [1, 6].

Evaluation of endpoints was conducted using microalgae; acute and chronic tests involved identification of esterase inhibition, ATP energy loss, and growth inhibition. Many bioassays of algae are used to assess the toxicity of organic pollutants, herbicides, oil dispersants, wastewater, leachate from solid waste, groundwater, and organic extracts [6]. Phytoplankton organisms belonging to various species were used as test subjects. The most accurate results were obtained by a set of test indicators: the concentration of chlorophyll *a* and the growth rate of microalgae, presented in R 52.24.808-2014 [7].

The water at the mouth of the Temernik River and the Don River, downstream from the confluence with the Temernik River, was characterized by the highest degree of toxicity [8–10]. The results based on a set of bioassays have been confirmed by the analytical data of water pollution. High degree of toxicity and low quality of the Temernik River water in 2019 were consistent with hydrochemical data. According to the values of the Relative Combinatorial Water Pollution Index, the river water was rated as belonging to 4 “c” class, namely “very polluted” [10].

The main objectives of this study are to evaluate two biological methods using phytoplankton—bioassay and saprobity index—and identify the dynamics of water quality in two horizons of the Don River at the sampling station located downstream from the confluence with the Temernik River.

MATERIALS AND METHODS

Investigated area

The Don River is a basic source of drinking water and fish, as well as a recreational area for the population of Rostov-on-Don and downstream settlements. The river water is used for different purposes; the investigated area is also characterized by active navigation. The Temernik River basin is located within the city of Rostov-on-Don. This river receives heterogeneous waste waters from 155 facilities and transfers them to the Don River.

The water temperature in the river varied considerably. In April, it ranged from 10.6 to 11.2 °C, from May to September it was in the range of 18.1–24.9 °C, and in October–November it was in the range of 9.9–16 °C.

Sampling

The investigation of water quality was carried out in two horizons of the Don River at the sampling station, located 0.5 km downstream from the confluence with the Temernik River (Fig. 1). Assessment of the state of the Don River at the confluence with the Temernik River was carried out based on the saprobity (S) as an indicator of organic pollution and the bioassay data used to evaluate toxicity. Water and phytoplankton

sampling was conducted concurrently at the Horizon 1 (depth 0.3 m) and the Horizon 2 (depth 9.0 m). Samples were taken during seven months of 2019: in April, May, July, August, September, October, and November.

Water and phytoplankton samples from the both horizons (0.3 and 9.0 m) were taken using Rutner's bathometer according to [11]. This method of phytoplankton sampling is the most reliable both for the quantitative account of phytoplankton and for obtaining a qualitative characterization of the sample. Phytoplankton samples were preserved with 40 % formalin and had the end concentration of 2 %. Water samples for bioassay were not fixed in any way.

Several slides of phytoplankton from each sample were prepared in order to identify and determine the species composition of phytoplankton community and the relative proportions of each taxonomic group in the sample. Identification of species was performed using an optical microscope, according to the [12–17].

The total abundance of microalgae was calculated from the sum of the monthly densities of each species. Then the relative abundance (percentage of the total abundance) of blue-green algae species was calculated for each month.



Fig. 1. Location of the sampling station in the Don River within Rostov-on-Don borders, Russia

Рис. 1. Расположение створа исследований на р. Дон в пределах г. Ростов-на-Дону, Россия

Definition and calculations:

1. The number and frequency of occurrence of phytoplankton species were analyzed to identify any link between the composition of phytoplankton communities and organic pollution at the sampling station. The saprobity index (S) was estimated based on the formula proposed by Pantle and Buck [11], as shown below:

$$S = \frac{\sum(s \cdot h)}{\sum(h)},$$

where s is the significance indicator of each species (according to the lists of saprobiotic species) given in appendix 1 of the work, and h is a value that is found from a six-step scale of frequency values and determines the relative number of species [18].

2. Evaluation of water toxicity by bioassay.

The studies carried out using the bioassay methods were stipulated by the documents within the framework of the Federal Service of Russia for Hydrometeorology and Environmental Monitoring, which also includes the recommendations for the assessment of the toxicological state of water bodies [7, 19]. Water toxicity was assessed using *Chlorella vulgaris* Beijer, a representative of green microalgae, as a test subject.

The increase in the number of algal cells—a growth coefficient—served as the indicator of the toxicity of the sample. The degree of toxicity was assessed by the presence of a subacute toxic effect (sACT) in the test subjects affected by the water.

Experimental studies were carried out in the laboratory environment in the Hydrochemical Institute. The studies involved a test with 72 hours of exposure. The increase in the number of microalgae was used as a test value. A series of dechlorinated tap water samples was used as a control. All experiments were performed in triplicate. The criterion for the toxicity of the sample was the difference in toxicity values by 25 % or more, estimated as an increment in comparison with the water from the control sample.

RESULTS AND DISCUSSION

An analysis of the qualitative characteristics of phytocenoses at the investigated section of the Don River over the course of 7-month study showed that many phytoplankton species are indicators of organic water pollution.

Change in saprobity index. In spring, the saprobity index had the highest value of 2.71 (polluted) at the Horizon 2 in April. In summer, the highest value

was recorded in August—2.97 (polluted) and 2.93 (polluted) at the Horizons 2 and 1, respectively. The highest value throughout the entire course of investigation was recorded in the autumn season. In September of 2019, the same maximum value of 3.61 (polluted) was recorded for the both horizons. In the warm period from May to September, an increase in the saprobity index was recorded at the both horizons. During these warm months, the surface Horizon 1 was more polluted.

In the early spring season and the last two autumn months with lower temperatures, organic pollution was typical for the deep horizon.

At the investigated horizons, organic pollution, based on the values of the saprobity index, varied from 2 (moderate) to 3.61 (contaminated). The minimum value of the saprobity index was recorded at the Horizon 1 in April and October of 2019 (Fig. 2).

The increase in the pollution according to the saprobity index was associated with an increase in the abundance of blue-green algae. The mass development of a species belonging to this division of algae indicates the presence of organic pollution in the water. During summer, blue-green algae prevailed in terms of their abundance, which ranged from 45.83 to 76.47 % (Fig. 3).

The abundance of blue-green algae drastically increased during the summer months, with the highest values in August—by 76.47 % at the Horizon 1. For the autumn season, the highest values of the relative abundance of blue-green algae were recorded in September—up to 77.77 % at the Horizon 2. The lowest relative abundance of blue-green algae was recorded in spring, particularly in April (Fig. 3).

Change in water toxicity. Water toxicity varied throughout the entire study period (7 months). It is notable that, in each season, the toxicity of the water manifested only for one month. Thus, the water at the both horizons had a toxic effect in April, August, and November (Fig. 4).

Discussion. Our data on the toxicity of the water at the horizons were compliant with those previously obtained [8, 10]. The increasing trend for the inhibitory effect was recorded for the Horizon 2 based on the saprobity index and bioassay data [8].

Quality of synchronously taken samples of the river water was investigated using two biological methods. In the Don River, the mass development of such α -mesosaprobionts as *Euglena acus* and *Stephanodiscus hantzschii* was recorded at the both

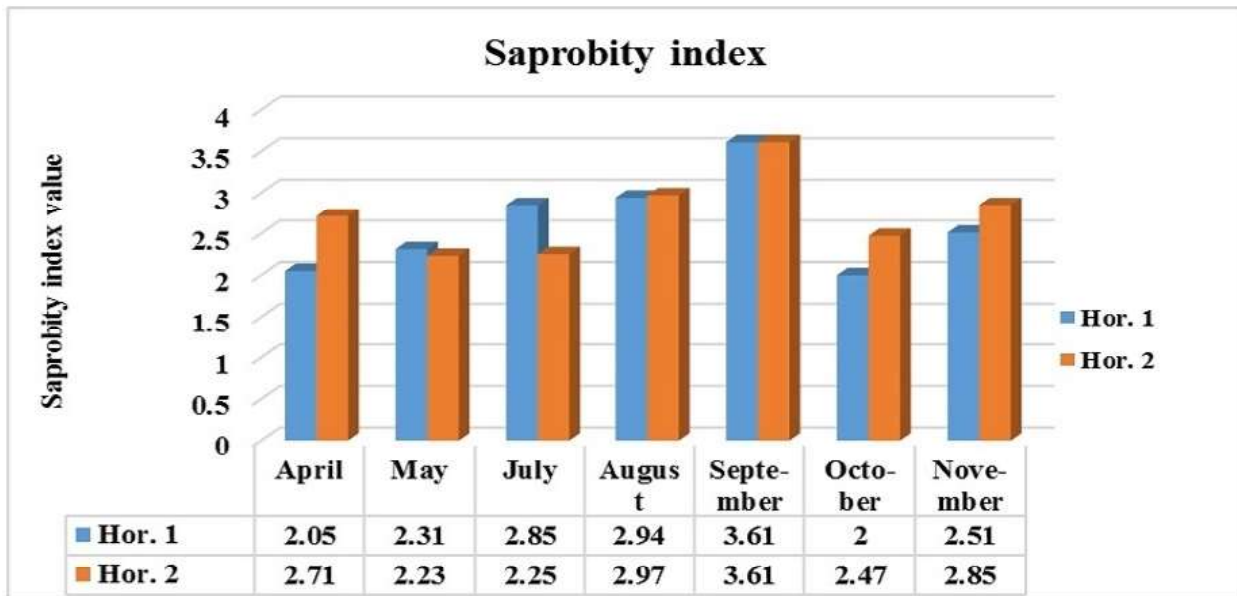


Fig. 2. Monthly variations of the saprobity index (S) at two horizons of the sampling station in the Don River
 Рис. 2. Ежемесячное изменение индекса сапробности S на двух горизонтах створа р. Дон

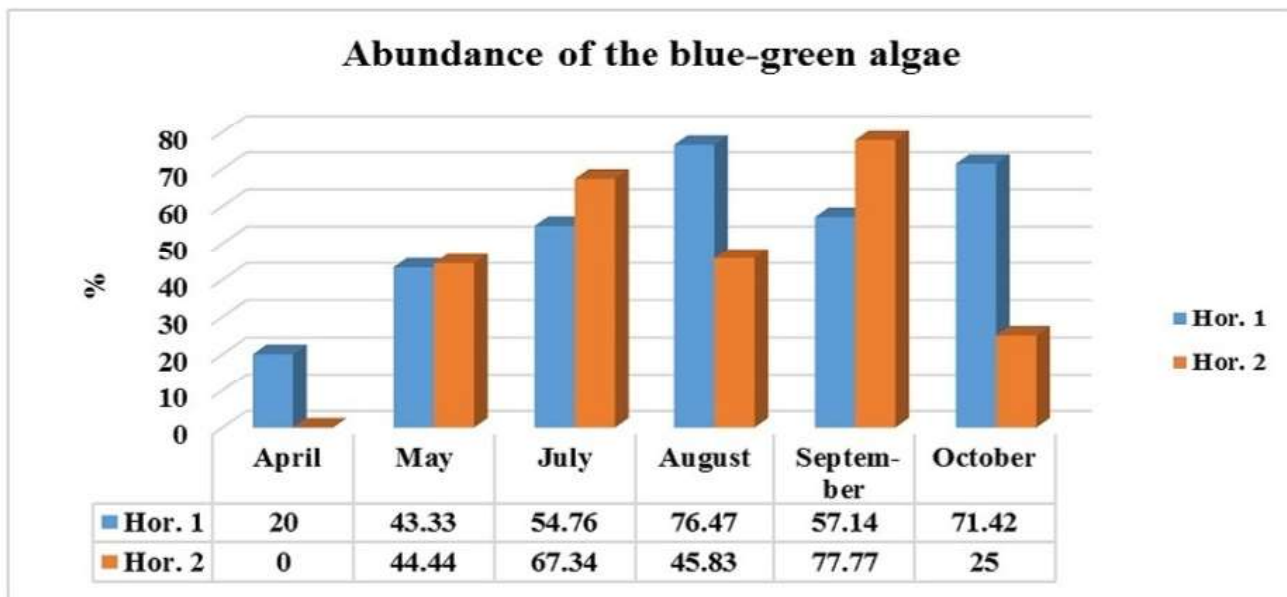


Fig. 3. Monthly variation of the relative abundance of the blue-green algae at the two horizons of the sampling station in the Don River
 Рис. 3. Ежемесячное изменение относительного обилия синезеленых водорослей на двух горизонтах створа р. Дон

horizons. The average value of the saprobity index was 2.61 for the both horizons, which defined the water as polluted.

The relationship between the organic pollution (phytoplankton saprobity) and the water toxicity at the

deep horizon was revealed (Table). The results of two biological methods provide more complete information about water quality.

The presence of high saprobity (pollution) and toxic effect of the water did not always coincide. A

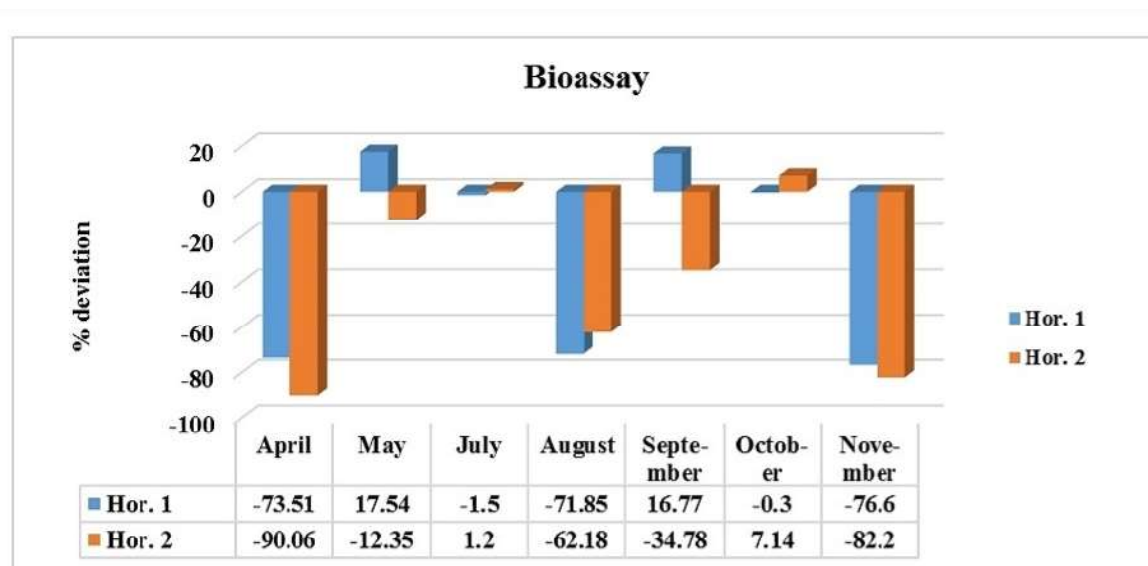


Fig. 4. Monthly change in the deviation of *Chlorella vulgaris* growth coefficient from the control for the two horizons of the sampling station in the Don River based on the bioassay data

Рис. 4. Ежемесячное изменение отклонения коэффициента прироста *Chlorella vulgaris* от контроля для двух горизонтов створа р. Дон по данным биотестирования

Water quality at the horizons of the sampling station in the Don River based on the saprobity index and bioassay data
 Качество воды горизонтов створа р. Дон по данным индекса сапробности и биотестирования

Month Месяц	Horizon 1 / Горизонт 1		Horizon 2 / Горизонт 2	
	Saprobity index Индекс сапробности	Toxicity Токсичность	Saprobity index Индекс сапробности	Toxicity Токсичность
April Апрель	moderately polluted умеренно загрязненные	subacute подострая	polluted загрязненные	subacute подострая
May Май	moderately polluted умеренно загрязненные	no subacute нет подострой	moderately polluted умеренно загрязненные	no subacute нет подострой
July Июль	polluted загрязненные	no subacute нет подострой	moderately polluted умеренно загрязненные	no subacute нет подострой
August Август	polluted загрязненные	subacute подострая	polluted загрязненные	subacute подострая
September Сентябрь	polluted загрязненные	no subacute нет подострой	polluted загрязненные	subacute подострая
October Октябрь	moderately polluted умеренно загрязненные	no subacute нет подострой	moderately polluted умеренно загрязненные	no subacute нет подострой
November Ноябрь	polluted загрязненные	subacute подострая	polluted загрязненные	subacute подострая

closer relationship between these characteristics has been identified at the Horizon 2 (the deep one). The surface horizon in a navigable river is, of course, more variable. Toxicity fluctuations are probably associated with the temporal pattern of pollution—in particular, with the irregular inflow of the polluting substances to the Temernik River from the city of Rostov-on-Don.

The decrease in the toxicity of the Don River water in the investigated area during some months can be considered as a positive sign for the aquatic environment of the Temernik River. The city administration has developed a multi-year program aimed at the improvement of the Temernik River water properties [2].

The assessment of water quality by a set of biological methods facilitates the evaluation of the ecotoxic status of aquatic ecosystems, providing the additional data.

The obtained results are important for a better understanding of the mechanisms of maintaining the stability of a river ecosystem in order to predict the processes of pollution and purification.

CONCLUSION

Analysis of the results of the water quality investigation at two horizons of the Don River at the sampling station, located 0.5 km downstream from the confluence with the Temernik River, conducted using two biological methods (phytoplankton saprobity and bioassay), has led to the following conclusions:

1. The results of saprobity assessment of phytoplankton indicate the presence of organic water pollution at the both horizons of the Don River during all 7 months of the study. The values of saprobity index ranged between 2.0 and 3.6. Water quality was characterized as ranging from moderately polluted to polluted. This corresponds to α - β -mesosaprobic level.

2. The maximum level of organic pollution based on the saprobity index was recorded in September for both horizons. It was confirmed that an increase in the proportion of blue-green algae in the phytocenosis indicates an increase in the saprobity of river water.

3. Higher organic pollution during the warm period (May–September) was typical for the surface horizon, while in the cold period (April, October, and November), the deep horizon was characterized by increased toxicity.

4. The toxic effect of the river water was manifested in the inhibition of *Chlorella vulgaris* growth. For each season, a subacute toxic effect of water at the both

horizons was recorded only during one month: April in spring, August in summer, and November in autumn.

5. The deep horizon was characterized by a greater number of the cases when high saprobity and water toxicity were present simultaneously.

The reason for the slight decrease in the water toxicity at two horizons in some months can be explained by the measures taken to stop the discharge of untreated sewage into the Temernik River.

The results of this investigation showed that the simultaneous application of two biological methods provides the possibility to obtain more complete information about the quality of river waters.

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