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EXPERIMENTAL ARTICLES

Monitoring of the Moskva River Water Using Microbiological Parameters and Chlorophyll *a* Fluorescence

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Abstract—The results of investigations of three Moskva River sites with different degrees of pollution using a complex of microbiological characteristics and the parameters of chlorophyll *a* fluorescence are presented. We determined that the bacterioplankton seasonal dynamics at less polluted waters (Tushino and Vorob'evy Gory) were similar but differed significantly from one in more polluted waters (Dzerzhinskii). The number of bacteria with active electron transport chain, as well as their share in the bacterioplankton structure, was higher in the water of Dzerzhinskii (average annual values of 0.23×10^6 cells/mL and 14%) than that in the less polluted water of Tushino and Vorob'evy Gory (0.14×10^6 cells/mL; 6% and 0.15×10^6 cells/mL; 7%, respectively). From April to October, the content of chlorophyll *a* and its photosynthetic activity were the highest in Tushino. In Dzerzhinskii, during spring the increase in photosynthetic activity commenced earlier and was more intensive that the increase in chlorophyll *a* content; i.e., the increase in phytoplankton biomass was temporarily suppressed. We suggest association of this phenomenon with suppression of organic matter synthesis by phytoplankton due to the high water pollution in Dzerzhinskii. The second autumn peak of chlorophyll *a* content, which was typical of clear water and was observed in Tushino, did not occur in Dzerzhinskii. We recommend combined application of these microbiological parameters and characteristics of chlorophyll *a* fluorescence for further monitoring.

Keywords: monitoring of water pollution, bacterioplankton, active bacteria, chlorophyll *a* fluorescence, Moskva River

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The first quantitative study of bacterial population in Moskva River water was carried by Stroganov et al. in 1911 (Stroganov, 1913). The next detailed study was carried out in 1944. Since 1950, the river was surveyed regularly in different seasons (Il'inskii et al., 1998; Popova, 1972; Shchegolkova et al., 2012). Investigation of the state and productivity of aquatic ecosystems affected by the megalopolis remain important in view of considerable expansion of its borders. Assessment of the informativeness of the major hydrobiological parameters, including the microbiological ones, is therefore required. Bacterio- and phytoplankton are the main groups responsible for the basic processes of degradation and production of organic matter (OM). Such parameters as total bacterioplankton abundance, numbers of metabolically active cells, and numbers of saprotrophic microorganisms reflect the processes of OM decomposition in an ecosystem. The content of chlorophyll *a*, the major photosynthetic pigment, determines the patterns of phytoplankton development and spatial distribution, while the parameters of chlorophyll *a* fluorescence may be used for rapid assessment of its photosynthetic activity (Matorin et al., 2004; Matorin and Rubin, 2012; Shchegolkova et al., 2012). Such comprehensive studies using up-to-date methods have not been carried out in the Moskva River in recent years.

The goal of the present work was to carry out comprehensive investigation of the yearly dynamics of microbiological parameters, as well as of chlorophyll *a* content and fluorescence in the surface layer of three sites of the Moskva River affected to a different degree by a megalopolis and to assess the informativeness of selected hydrobiological parameters.

MATERIALS AND METHODS

The study was carried out at three stations along the Moskva River flow: in Tushino (st. Tushino) where river waters enter the city boundaries, in the central part of Moscow (st. Vorob'evy Gory), and beyond Moscow border, in the town of Dzerzhinskii (st. Dzerzhinskii).

The samples were collected monthly (except for February 2010) from October 2009 to December 2010. The sampling locations were 3 to 5 m from the shore at

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depths of at least 1 m. When ice cover existed, the samples were collected through holes bored in the ice. For microbiological analysis, the samples were collected with a bottle bathometer with a 250-mL sterile vial. The samples for analysis of chlorophyll *a* concentrations and phytoplankton fluorescence were collected in nonsterile nontransparent 1-L plastic bottles and transported in a cooling bag within 1 h after sampling for analysis.

Total numbers of bacteria (TBN) were determined by epifluorescence microscopy of acridine orange-stained cells (Il'inskii, 2006).

Bacteria with the active electron transport chain (CTC + B), i.e., metabolically active microorganisms, were enumerated using 5-cyano-2,3-ditolyl tetrazolium chloride (CTC). The fluorescent stain was added to the final concentration of 5 mM. The CTC working solution was prepared as described previously (*Methods in Stream Ecology*, 2006). Water samples were incubated with CTC for 4 h under the conditions close to in situ ones. The share of CTC + B in the heterotrophic bacteriocenosis was calculated as its percentage of TBN.

The number of saprotrophic bacteria (SB) was determined by the most probable number method in the modified liquid ZoBell medium 2216E (Aaronson, 1970) without NaCl. McCrady statistical tables were used to quantify the results.

Chlorophyll *a* content in the water was determined by fluorometry (Holm-Hansen, 2005) using a MEGA-25 certified fluorometer developed at the Department of Biophysics, Biological Faculty, Moscow State University (Matorin and Rubin, 2012). Water samples (300 mL) were filtered through GF/F glass fiber filters (Whatman) 1–2 h after sampling. Chlorophyll *a* was extracted from the filters with 90% acetone for 18–24 h in the dark at 4°C, and fluorometry of the extract was carried out.

Constant (F_0) and maximal chlorophyll *a* fluorescence (F_M), as well as the relative yield of variable fluorescence (F_V/F_M), which is a measure of the maximal quantum efficiency of photosystem 2 (PS2), were determined in intact, darkness-adapted water samples. The latter parameter reflects the efficiency of the primary photosynthetic processes (F_V/F_M) and is a dimensionless energetic characteristic of the organism, which is analogous to performance and does not depend on the species.

Statistical treatment of the data was carried out using the STATISTICA-10 software package. Spearman index of cograduation was used for correlation analysis.

RESULTS AND DISCUSSION

Short characterization of the ecological state of the sampling sites. Three areas differing in the degree of contamination have been historically determined along the flow of the Moskva River within the city. The first area is located at the inflow of the river into the city near Tushino and is traditionally considered the cleanest one. The second area is in the central part of the city (within the Garden Ring), where water quality (the content of heavy metals and oil products) varies both seasonally and along the river flow. The third area is located at the outflow of the river from the city, where the Kur'yanov water treatment plant (KWTP) is located. Its water discharge results in elevated concentrations of biogenic elements (ammonium, nitrites, and phosphates) (Popova, 1972). According to the 2010 Mosecomonitoring report, at the inflow to the city the quality of Moskva River water was in agreement with the standards for the public water bodies, so the water at this area was characterized as "conditionally pure." In the central part of the city, in 2010 the content of suspended matter, ammonium, and oil products was higher than at the inflow into the river. At its outflow, the river is affected by KWTP water discharge, and the concentrations of biogenic matter increase considerably (in 2010 the average concentrations of ammonium, nitrite, nitrate, and phosphate increased 7.7, 3, 5, and 4 times, respectively). As a result, water in this area was characterized in 2010 as "weakly contaminated" (*Doklady o sostoyanii okruzhayushei sredy v gorode Moskve*).

Water temperature at these stations varied significantly during the investigation period.

During our study, ice cover at st. Tushino was formed in mid-November 2009. During winter, ice thickness varied from 2 to 40 cm. Ice cover was preserved until March 2010. Water temperature under the ice was below 1°C and varied from 0.00 to 0.4°C. During the period from April to October, when the ice cover was absent, water temperature in the surface layer of the river varied from 6 to 30°C (Table 1).

In the city center, at st. Vorob'evy Gory, ice was formed only during strong frosts and existed for a short time. During our observations, solid ice cover ~2 cm thick was observed only in December 2010. The temperature of the surface water layer during the hydrological winter (from November to March) varied from 0.4 to 2°C. From April to October, it varied from 5 to 28°C (Table 2).

At the site where the river left the city (st. Dzerzhinskii), water temperature during the hydrological winter (November 2009 to March 2010) varied from 0.5 to 3.5°C; i.e., it was somewhat higher than at st. Vorob'evy Gory and considerably higher than at st. Tushino. The spring increase in the temperature of the surface water layer began at st. Dzerzhinskii in early March, while at stations Tushino and Vorob'evy Gory it began a month later, in early April (Tables 1–3). Thus, at st. Dzerzhinskii in 2010 both higher water temperature in winter and its earlier increase in spring were observed.

Chlorophyll *a* content. Chlorophyll *a* content at st. Tushino varied from the minimum of 0.26 μg/L in

Table 1. Microbiological parameters, chlorophyll *a* content, water temperature, and ice cover: state of Moskva River at Tushino station in October 2009–December 2010

Date	Total bacterial number, 10^6 cells/mL	Bacteria with active electron transport chain, 10^6 cells/mL	Share of bacteria with active electron transport chain in the total bacterial number, %	Saprotrophic bacteria, cells/mL	Ratio of the total bacterial number to the number of saprotrophic bacteria	Chlorophyll *a* content, μg/L	Water temperature, °C	Ice thickness, cm
29.10.2009	1.32 ± 0.01	0.11 ± 0.001	8.88	25000	53	2.73 ± 0.55	9	None
17.11.2009	2.64 ± 0.01	0.01 ± 0.001	0.49	60000	44	1.59 ± 0.32	0.3	3
24.12.2009	1.82 ± 0.01	0.07 ± 0.001	3.57	25000	73	0.62 ± 0.12	0.2	18
22.01.2010	2.53 ± 0.01	0.05 ± 0.001	2.55	25000	101	0.26 ± 0.05	0.3	35
03.03.2010	1.84 ± 0.01	0.12 ± 0.001	5.26	25000	74	0.34 ± 0.07	0.3	40
05.04.2010	4.79 ± 0.01	0.55 ± 0.004	35.22	2500000	2	1.65 ± 0.33	6	None
27.05.2010	4.06 ± 0.02	0.11 ± 0.001	1.63	6000	676	5.68 ± 1.14	18	None
29.06.2010	3.97 ± 0.02	0.28 ± 0.002	9.49	4500	882	24.86 ± 4.97	27	None
23.07.2010	2.55 ± 0.01	0.09 ± 0.001	2.99	950	2684	11.77 ± 2.34	27	None
21.08.2010	2.91 ± 0.01	0.13 ± 0.001	5.00	4500	647	10.12 ± 2.02	30	None
01.09.2010	2.94 ± 0.01	0.11 ± 0.002	4.06	9500	310	7.19 ± 1.44	16	None
13.10.2010	1.62 ± 0.01	0.09 ± 0.001	5.29	25000	65	14.93 ± 2.99	10	None
10.11.2010	4.55 ± 0.01	0.09 ± 0.002	2.63	95000	48	3.36 ± 0.67	0.4	2
09.12.2010	4.01 ± 0.03	0.10 ± 0.001	2.96	95000	42	1.36 ± 0.27	0.2	20
Average	2.97	0.14	6.43	207175	407	6.18	10.34	

Table 2. Microbiological parameters, chlorophyll *a* content, water temperature, and ice cover: state of Moskva River at Vorob'evy Gory station in November 2009–December 2010

Date	Total bacterial number, 10^6 cells/mL	Bacteria with active electron transport chain, 10^6 cells/mL	Share of bacteria with active electron transport chain in the total bacterial number, %	Saprotrophic bacteria, cells/mL	Ratio of the total bacterial number to the number of saprotrophic bacteria	Chlorophyll *a* content, μg/L	Water temperature, °C	Ice thickness, cm
17.11.2009	1.73 ± 0.01	0.04 ± 0.001	3.85	6000	288	–*	3	None
24.12.2009	1.20 ± 0.08	0.14 ± 0.001	22.17	60000	20	0.17 ± 0.03	1	None
22.01.2010	3.80 ± 0.01	0.04 ± 0.001	2.92	25000	152	0.09 ± 0.02	0.4	None
03.03.2010	1.61 ± 0.01	0.06 ± 0.001	2.89	600000	3	0.10 ± 0.02	0.4	None
05.04.2010	4.68 ± 0.01	0.58 ± 0.003	19.08	2500000	2	1.70 ± 0.34	5	None
27.05.2010	4.21 ± 0.02	0.11 ± 0.001	2.36	250000	17	1.34 ± 0.27	19	None
29.06.2010	4.96 ± 0.01	0.22 ± 0.002	3.92	25000	198	11.56 ± 2.31	26	None
23.07.2010	1.44 ± 0.00	0.15 ± 0.001	6.58	4500	320	16.77 ± 3.35	27	None
21.08.2010	2.28 ± 0.01	0.20 ± 0.001	9.62	4500	507	8.9 ± 1.79	28	None
01.09.2010	2.66 ± 0.01	0.15 ± 0.001	9.22	95000	28	3.39 ± 0.68	17	None
13.10.2010	4.49 ± 0.02	0.07 ± 0.001	1.33	95000	47	1.64 ± 0.33	10	None
10.11.2010	3.45 ± 0.01	0.14 ± 0.002	5.64	9500	363	1.15 ± 0.23	3	None
09.12.2010	3.57 ± 0.02	0.03 ± 0.001	1.45	9500	376	0.57 ± 0.11	0.2	2
Average	3.08	0.15	7.00	283385	179	3.95 ± 0.71	11.42	

Table 3. Microbiological parameters, chlorophyll *a* content, water temperature, and ice cover: state of Moskva River at Dzerzhinskii station in October 2009–December 2010

Date	Total bacterial number, $10^{6\,l}$cells/mL	Bacteria with active electron transport chain, 10^6 cells/mL	Share of bacteria with active electron transport chain in the total bacterial number, %	Saprotrophic bacteria, cells/mL	Ratio of the total bacterial number to the number of saprotrophic bacteria	Chlorophyll *a* content, μg/L	Water temperature, °C	Ice thickness, cm
29.10.2009	1.32 ± 0.01	0.13 ± 0.002	9.85	6000	220	0.59 ± 0.12	10	None
17.11.2009.	2.30 ± 0.01	0.14 ± 0.002	6.09	250000	9	0.42 ± 0.08	6	None
24.12.2009	3.54 ± 0.02	0.07 ± 0.001	2.32	60000	59	0.52 ± 0.10	3	None
22.01.2010	3.54 ± 0.02	0.09 ± 0.001	4.25	600000	6	0.24 ± 0.05	0.5	None
03.03.2010	2.77 ± 0.02	0.24 ± 0.002	13.51	250000	11	1.64 ± 0.33	3.5	None
05.04.2010	3.59 ± 0.02	0.54 ± 0.003	38.17	600000	6	1.71 ± 0.34	4	None
27.05.2010	3.27 ± 0.01	0.23 ± 0.002	19.01	250000	13	3.21 ± 0.64	15	None
29.06.2010	3.58 ± 0.01	0.33 ± 0.002	26.19	25000	143	7.65 ± 1.53	25	None
23.07.2010	2.66 ± 0.01	0.13 ± 0.002	9.92	950	2800	16.30 ± 3.26	27	None
21.08.2010	3.12 ± 0.01	0.19 ± 0.001	12.75	4500	693	13.25 ± 2.65	28	None
01.09.2010	2.15 ± 0.01	0.28 ± 0.007	23.14	95000	23	14.85 ± 2.97	16	None
13.10.2010	3.14 ± 0.02	0.39 ± 0.004	6.74	95000	33	2.13 ± 0.43	11	None
10.11.2010	4.26 ± 0.015	0.20 ± 0.002	6.66	25000	171	1.38 ± 0.28	5	None
09.12.2010	4.05 ± 0.017	0.28 ± 0.003	11.21	25000	162	0.67 ± 0.13	3	None
Average	3.09	0.23	14.13	163318	311	4.61 ± 0.03	11.62	–

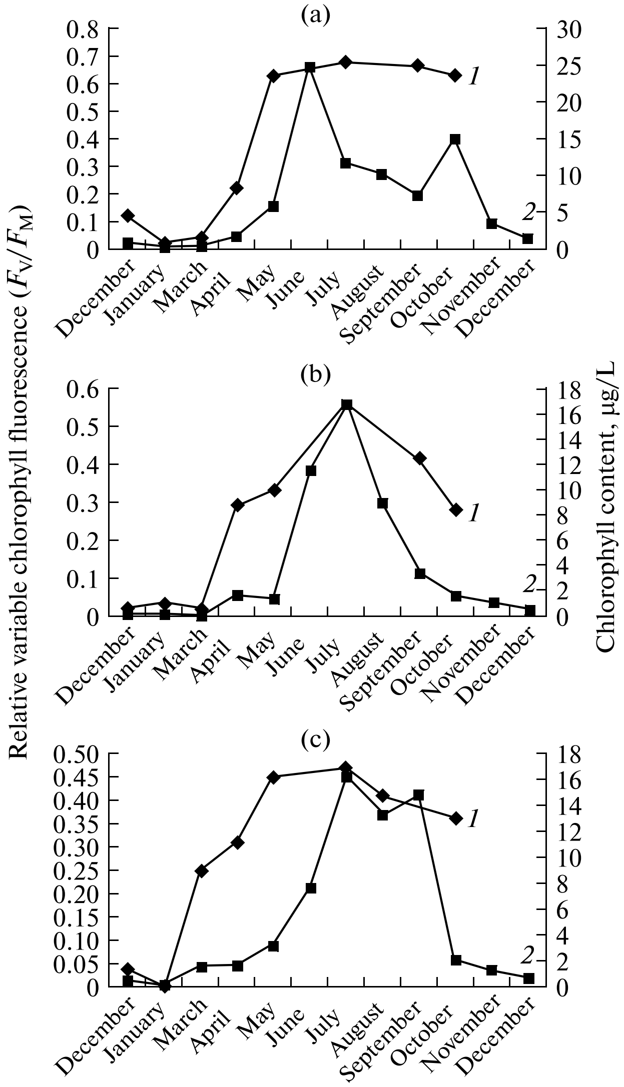

Fig. 1. Dynamics of the F_V/F_M parameter of relative variable fluorescence (*1*) and chlorophyll *a* content, μg/L (*2*) in the Moskva River surface layer: Tushino station, October 2009–December 2010 (a); Vorob'evy Gory station, December 2009–December 2010 (b); and Dzerzhinskii station, October 2009–December 2010 (c).

January to the maximum of 24.86 μg/L in June 2010 (Table 1, Fig. 1a). The average chlorophyll *a* content during the sampling period was 6.18 μg/L. From October to January 2009 chlorophyll content at st. Tushino decreased tenfold, from 2.73 to 0.26 μg/L. It began to increase noticeably only in April, immediately after thawing of ice and increase in water temperature to 6°C (Table 1). Chlorophyll *a* content peaked in June (24.86 μg/L) and then decreased gradually to 7.19 μg/L in September. During the first ten days of October 2010, at st. Tushino, a second chlorophyll *a* cpeak was recorded, which was lower than the summer one (14.93 μg/L) (Fig. 1a). However, in November 2010, after formation of ice cover began and water temperature decreased below 1°C, chlorophyll *a* content in st. Tushino water decreased sharply, to 3.36 μg/L, while a month later, in December 2010, below 10 cm of ice and at water temperature 0.2°C, it dropped to 1.36 μg/L. Interestingly, a year before, in October 2009, no pronounced second peak of chlorophyll *a* was observed, and its content was much lower than in October 2010.

At st. Vorob'evy Gory, chlorophyll *a* content varied from 0.09 in January to 16.77 μg/L in July (Fig. 1b). The average chlorophyll *a* content for the observation period was 3.95 μg/L, which was almost half of the value for st. Tushino. The summer increase in chlorophyll *a* content at st. Vorob'evy Gory began in April, similar to st. Tushino. Unlike st. Tushino, a sharp increase in chlorophyll *a* content began in June, rather than in May, while the maximum was observed in late July, rather than in June. From June to August, chlorophyll *a* content was high (8.95 to 16.77, with the average of 12.43 ± 3.98 μg/L). It decreased gradually from August to December, reaching 0.57 μg/L. The period of low chlorophyll *a* content (0.09 to 0.17, with the average of 0.15 ± 0.05 μg/L) continued from December 2009 to March 2010. Chlorophyll *a* concentration during the observation period indicated lower phytoplankton abundance at st. Vorob'evy Gory compared to st. Tushino. Moreover, the second peak of phytoplankton abundance observed at the river inflow into the city (st. Tushino) in October 2010 was not revealed at st. Vorob'evy Gory.

Chlorophyll *a* content in the Moskva River water at its outflow from the city (st. Dzerzhinskii) varied form 0.24 μg/L in January to 16.30 μg/L in July 2010. The average chlorophyll *a* content for the observation period was 4.61 μg/L. While it decreased from 0.59 to 0.24 μg/L from October 2009 to January 2010, chlorophyll *a* content in October and December was practically the same. The spring increase of chlorophyll *a* content at st. Dzerzhinskii began in March (figure c), rather than in April, as at the other two stations. High values of chlorophyll *a* content (7.65–16.30 μg/L, with the average of 13.01 ± 3.78 μg/L) continued from June to September and was a month longer than at the other two stations. No pronounced peak of chlorophyll *a* content was observed in autumn. Decrease in water temperature from 16°C in September 2010 to 11°C in October 2010 was accompanied by chlorophyll *a* content decreasing from 14.85 to 2.13 μg/L. It decreased further, reaching 0.62 μg/L at the end of observations in 2010.

Thus, the Moskva River surface water layers at st. Dzerzhinskii were characterized by earlier onset of the spring phytoplankton development (a month earlier than at Tushino and Vorob'evy Gory) and by the longest period of its summer abundance (from June to September).

For all three areas of the river, significant direct correlation between chlorophyll *a* content and water temperature was revealed. For the water at st. Tushino, R was 0.8 at $p < 0.01$, while for st. Vorob'evy Gory and st. Dzerzhinskii $R = 0.9$ at $p < 0.01$.

Assessment of activity of environmental phytoplankton by the parameters of chlorophyll *a* fluorescence. The value of relative variable fluorescence (F_V/F_M), which reflects the maximum quantum yield of PS2 (Matorin and Rubin, 2012) was used to assess the potential photosynthetic activity of phytoplankton. In natural aquatic ecosystems rich with mineral nutrients, F_V/F_M may be as high as 0.6–0.7. Stress factors, including contaminants, may result in a decrease of this parameter. Its value for dead cells is zero.

Similar to chlorophyll *a* content, the effect of the seasonal factor may be seen in the dynamics of the phytoplankton photosynthetic activity expressed as the F_V/F_M ratio (figure). In winter, phytoplankton activity was extremely low at all three stations (F_V/F_M below 0.01). These low F_V/F_M values were probably associated with intense cooling of the surface water layers, accompanied by the shift from the autumnal to the winter phytoplankton complex. Pronounced F_V/F_M minima were recorded only in Tushino and Dzerzhinskii in January, while the lowest value of this parameter for st. Vorob'evy Gory was observed from December 2009 to March 2010

During spring (March–April), activity of phytoplankton increased drastically, most probably due to the seasonal increase of water temperature. At st. Dzerzhinskii it reached 3.5°C by March, and the F_V/F_M value for the relevant sample was 0.25, while in Tushino and Vorob'evy Gory F_V/F_M values for March were only 0.04 and 0.02 at water temperatures of 0.3 and 0.4°C, respectively. In May, F_V/F_M values for the water samples varied within the range from 0.33 to 0.63, with the highest F_V/F_M, indicating the highest phytoplankton activity observed at st. Tushino.

Importantly, the decrease of phytoplankton abundance measured as chlorophyll content was recorded somewhat later than in the case of F_V/F_M measurement. This finding confirms that emergence of the cells with high activity of their photosynthetic apparatus and therefore with the high F_V/F_M values predates the bloom caused by increasing phytoplankton numbers. We have previously reported a similar effect for Lake Baikal (Matorin and Rubin, 2012).

In summer, the highest F_V/F_M values were observed at all stations, occurring in June at Tushino (F_V/F_M = 0.68) and in July at Vorob'evy Gory and Dzerzhinskii (0.56 and 0.47, respectively). This photosynthetic activity, which is relatively high for the river phytoplankton, indicated that in summer the phytoplankton at all stations was not limited by the main environmental factors and adapted well to high water temperature (up to 30°C). After the summer maxima, the photosynthetic activity decreased at all three stations, with this decrease most pronounced at st. Vorob'evy Gory and least pronounced at st. Tushino.

Interestingly, the F_V/F_M maximum for the summer water samples was lowest at st. Dzerzhinskii (0.47), probably indicating the negative effect of contamination arriving into the river from the megalopolis and of KWTP waste on potential activity of photosynthesis by phytoplankton. At the same time, the highest F_V/F_M values reported in summer for st. Tushino suggest that the conditions for development of the phytoplankton community were more favorable than at the other two stations.

Total abundance of bacterioplankton. During the period of observation, TBN in the surface water layers at st. Tushino from the minimum (1.32×10^6 cells/mL) in October 2009 to the maximum (4.79×10^6 cells/mL) in April 2010. The annual average TBN was 2.97×10^6 cells/mL. Low TBN values (from 1.32 to 2.53, on average $2.03 \pm 0.55 \times 10^6$ cells/mL) were found from late October 2009 to March 2010 (Table 1). In April, after thawing of the ice cover and heating of the water to 6°C, accompanied by the spring increase in the phytoplankton photosynthetic activity and in chlorophyll *a* content, a drastic increase in bacterial abundance (up to 4.79×10^6 cells/mL) was registered at st. Tushino. The spring–summer period of high bacterioplankton abundance (TBN from 3.97 to 4.79×10^6 cells/mL, on average $4.27 \pm 0.45 \times 10^6$ cells/mL) continued from April to late June. In July TBN decreased to 2.55×10^6 cells/mL and then fell further, to the minimum in October (1.62×10^6 cells/mL). In general, TBN values at st. Tushino were low, varying from 1.62 to 2.94×10^6 cells/mL with the average of $2.50 \pm 0.62 \times 10^6$ cells/mL. In November, after freeze-up, bacterial abundance increased again to 4.55×10^6 cells/mL and remained high in December 2010 (4.01×10^6 cells/mL).

Thus, two maxima were revealed for the surface layers of the river at st. Tushino in 2010: spring–summer (from April to June) and autumn–winter (from November to December). In 2009, TBN values in autumn and winter were very low, indicating its considerable fluctuations from year to year.

In the surface water layers at st. Vorob'evy Gory, TBN varied throughout the year from 1.20×10^6 cells/mL in January to 4.96×10^6 cells/mL in June 2010, with the annual average of 3.08×10^6 cells/mL. At this station, similar to st. Tushino, the lowest TBN values ($1.20–3.80 \times 10^6$ cells/mL, on average $2.09 \pm 1.17 \times 10^6$ cells/mL) were revealed in winter–spring, from November 2009 to March 2010 (Table 2). At st. Vorob'evy Gory, similar to st. Tushino, the number of bacteria increased drastically in April (to 4.68×10^6 cells/mL), simultaneously with the onset of an increase in chlorophyll *a* content, and remained high until the end of June (average value for this period was

$4.62 \pm 0.38 \times 10^6$ cells/mL). In July, during the period of the highest chlorophyll *a* content, TBN decreased to 1.44×10^6 cells/mL. In August, simultaneously with the continuing decrease in chlorophyll *a* content, bacterioplankton numbers increased somewhat (to 2.28×10^6 cells/mL), while in September TBN was already 2.66×10^6 cells/mL. The average TBN value for the period from July to September was $2.13 \pm 0.62 \times 10^6$ cells/mL. In October 2010 TBN at st. Vorob'evy Gory increased again to 4.49×10^6 cells/mL. Similar to st. Tushino, TBN at this station in November and December remained high (3.45 and 3.57×10^6 cells/mL, respectively). Similar to st. Tushino in 2010, in the surface water layers of st. Vorob'evy Gory two periods of maximal bacterioplankton abundance were revealed: spring–summer and autumn–winter. At the same time, bacterial abundance at st. Vorob'evy Gory in November–December 2009, similar to st. Tushino, was 2–3 times lower than in the same period in 2010.

Thus, the yearly dynamics of bacterioplankton abundance in the upper water layers at the river inflow into the city (Tushino) and in the center of the city (Vorob'evy Gory) exhibited considerable similarity.

TBN values in the upper water layers at st. Dzerzhinskii varied somewhat less than at stations Tushino and Vorob'evy Gory, from 1.32 to 4.26×10^6 cells/mL, with the average for all the period of observation 3.09×10^6 cells/mL (Table 3). In March, when chlorophyll *a* content increased in the water at st. Dzerzhinskii, TBN decreased to 2.77×10^6 cells/mL, compared to 3.54×10^6 cells/mL in January, and increased again in April to 3.59×10^6 cells/mL. Unlike stations Tushino and Vorob'evy Gory, at st. Dzerzhinskii no pronounced TBN maximum was detected in April. In spring–summer (from April to June) TBN varied from 3.27 to 3.59×10^6 cells/mL, with the average of $3.48 \pm 0.18 \times 10^6$ cells/mL. In summer–autumn (from July to September), when chlorophyll *a* content peaked, TBN fluctuated from 2.15 to 3.12×10^6 cells/mL, with the average of $2.64 \pm 0.49 \times 10^6$ cells/mL. During the drastic drop in chlorophyll *a* content in October, bacterial abundance increased to 3.14×10^6 cells/mL. In November TBN reached its maximum at 4.26×10^6 cells/mL. High bacterial numbers persisted at st. Dzerzhinskii in December (4.05×10^6 cells/mL).

Thus, in general, the seasonal dynamics of bacterioplankton abundance in the upper river layers at stations Tushino and Vorob'evy Gory exhibited considerable similarities and differed significantly from that at st. Dzerzhinskii. The April maximum of bacteria abundance revealed at stations Tushino and Vorob'evy Gory was not recorded at st. Dzerzhinskii, where the highest TBN value was found in November. Inverse correlation was found between TBN and chlorophyll *a* content ($R = -0.63$ at $p < 0.01$) at st. Dzerzhinskii during the period from December 2009 to December 2010, which indicated antiphased dynamics of these parameters. While at stations Tushino and Vorob'evy Gory increasing bacterial numbers were sometimes also observed at decreasing chlorophyll *a* content, no significant relationship between these parameters was determined.

Abundance of bacteria with the active electron transport chain. Bacteria with the active electron transport chain (CTC + B) are heterotrophic microorganisms actively functioning in the water at the time of sampling. Their number may be used therefore to assess the activity of heterotrophic processes.

At st. Tushino the number of metabolically active bacteria (CTC + B) varied during the year from the minimum of 0.01×10^6 cells/mL in November 2009 to 0.55×10^6 cells/mL in April 2010 and on average was 0.14×10^6 cells/mL (Table 1). An increase in the CTC + B number began in March, when the ice cover was 40 cm thick and chlorophyll *a* content in the water was low (Table 1). It reached the maximum (0.55×10^6 cells/mL) in April, before the onset of the spring phytoplankton bloom. In May, however, the numbers of active bacterioplankton decreased sharply to 0.11×10^6 cells/mL, i.e., almost 5 times lower than in April. While in June, at the time of the highest chlorophyll *a* content in the water a st. Tushino, CTC + B numbers increased to 0.28×10^6 cells/mL; it then decreased 0.09×10^6 cells/mL in July and remained almost stable until December (on average 0.10×10^6 cells/mL). At st. Tushino, a direct correlation was revealed between CTC + B numbers and TBN at st. Tushino ($R = 0.55$ at $p < 0.01$).

The share of CTC + B from TBN varied from 0.49% in November 2009 to 35.22% in April 2010, being on average 6.43% for the period of observation (Table 1).

At st. Vorob'evy Gory CTC + B numbers varied within about the same range: from 0.03×10^6 cells/mL in December 2010 to 0.58×10^6 cells/mL in April (on average 0.15×10^6 cells/mL) (Table 2). Similar to st. Tushino, CTC + B increased sharply in April (to 0.58×10^6 cells/mL). In May, however, the active fraction of bacterioplankton decreased to 0.11×10^6 cells/mL. CTC + B number increased in June to 0.22×10^6 cells/mL against the background of high chlorophyll *a* content in the water. From July to December CTC + B varied from 0.03×10^6 cells/mL to 0.20×10^6 cells/mL, being on average $0.12 \pm 0.06 \times 10^6$ cells/mL.

The share of active bacterioplankton from TBN at st. Vorob'evy Gory varied from 1.33% in October 2010 to 22.17% in December 2009, with an average of 7% (Table 2).

Thus, at the stations Vorob'evy Gory and Tushino the seasonal dynamics of CTC + B abundance was somewhat similar: high numbers of metabolically active bacteria in April, a decrease in May, and a small increase in June and August.

In the upper layers of river water at st. Dzerzhinskii, the number of active bacteria varied from 0.07 in December 2009 to 0.54×10^6 cells/mL in April 2010, being on average 0.23×10^6 cells/mL (Table 3). The annual average CTC + B number at st. Dzerzhinskii was higher than at stations Tushino and Vorob'evy Gory. At st. Dzerzhinskii the number of active bacteria began to increase in January and peaked in April simultaneously with the increase in chlorophyll *a* content and water temperature (0.54×10^6 cells/mL), similar to stations Tushino and Vorob'evy Gory. While in May CTC + B decreased considerably at st. Dzerzhinskii, similar to the other two stations (in this case, more than twofold, to 0.23×10^6 cells/mL), in June CTC + B increased again to 0.33×10^6 cells/mL. From July to December 2010 the number of active bacteria varied from 0.13×10^6 cells/mL in July to 0.39×10^6 cells/mL in October (on average for this period $0.24 \pm 0.09 \times 10^6$ cells/mL). Thus, three peaks of CTC + B abundance were pronounced at st. Dzerzhinskii (in April, June, and October).

The share of metabolically active bacteria from TBN at st. Dzerzhinskii varied from 2.32% in December 2009 to 38.17% in April 2010, being on average 14.13% (Table 3).

Thus, the yearly dynamics of CTC + B abundance at all three stations had certain similarities: the number of active bacteria began to increase in March, and the peak occurred in April. However, both the numbers and share of active bacterioplankton at st. Dzerzhinskii were usually higher than at stations Tushino and Vorob'evy Gory. Moreover, at st. Dzerzhinskii both an earlier increase in CTC + B abundance (in January–March, i.e., a month earlier than at the other two stations) and a second high peak of abundance of metabolically active microorganisms in October (0.39×10^6 cells/mL), which was not found at other two stations, were observed.

Numbers of saprotrophic bacteria. Since SB use easily utilizable organic matter as a substrate, abundance of such bacteria is one of the criteria of saprobity of water reservoirs. At st. Tushino, SB numbers varied from 950 cells/mL in July to a single maximum of 2.50×10^6 cells/mL in April (Table 1), being on average for the year 0.207×10^6 cells/mL. Low SB numbers, from 950 to 6000 cells/mL, were observed in summer (from May to August), when chlorophyll *a* content in the water was high. High values (95000 cells/mL) were revealed during the ice cover period in November and December 2010. The ratio between TBN and SB numbers varied during the period of observation from 2 in April to 2684 in July (Table 1). According to GOST 17.1.2.04-77, the water at st. Tushino during the hydrological winter (December 2009 to March 2010) may be characterized as α-mesosaprobic based on the seasonal average TBN/SB ratios (the average TBN/SB for this period was 83). In April, immediately after ice thawing and arrival of large amounts of nutrients, surface water layers at st. Tushino were characterized as polysaprobic, or polluted. In spring–autumn (May to September) the water was classified as β-mesosaprobic with a tendency to oligosaprobity. In October–December, the water at st. Tushino was classified as α-mesosaprobic.

SB numbers in the surface layers at st. Vorob'evy Gory varied from 4500 cells/mL in July–August to 2.50×10^6 cells/mL in April (on average 0.28 cells/mL, which was slightly higher than the average SB number for st. Tushino). During the period of observation, the TBN/SB ratio varied from 2 in April to 507 in August. According to GOST 17.1.2.04-77, based on the average TBN/SB ratios, the river water at st. Vorob'evy Gory in winter–spring (December 2009 to May 2010) was polysaprobic or polluted, while in June–December 2010 it was classified as β-mesosaprobic or contaminated.

At st. Dzerzhinskii SB numbers varied from the minimum of 950 cells/mL in July to the maximum of 0.6×10^6 cells/mL in April and January (Table 3), with the annual average of 0.16×10^6 cells/mL, which was considerably below the yearly average values for stations Tushino and Vorob'evy Gory (0.207 and 0.28×10^6 cells/mL, respectively; Tables 1 and 2). However, the TBN/SB ratio at st. Dzerzhinskii varied from 6 in January and April to 2800 in July. According to the average TBN/SB ratio, water at this site may be characterized as hypersaprobic or highly polluted during the period from December 2009 to May 2010 and as α-mesosaprobic or contaminated in June–December.

Thus, the investigated parameters of the overall microbiological state (total bacterial number, number of bacteria with the active electron transport chain, and number of saprotrophic bacteria) and the parameters characterizing the state of phytoplankton (chlorophyll *a* content and fluorescence) provide for adequate assessment of the ecological situation at different sites along the Moskva River. For example, at the Tushino and Vorob'evy Gory sites the spring increase of chlorophyll *a* content began in April, while at Dzerzhinskii it began a month earlier, in March, probably due to higher temperatures of the surface water layer at this site. Chlorophyll *a* content and its photosynthetic activity in April–October were the highest at Tushino (on average 10.89 μg/L and 0.56, respectively). At stations Vorob'evy Gory (on average 6.4 μg/L and 0.37, respectively) and Dzerzhinskii (on average 8.4 μg/L and 0.40, respectively), the values were considerably lower. At stations Tushino and Vorob'evy Gory, increased phytoplankton activity in April was associated with increased chlorophyll *a* content (figure). In Dzerzhinskii an increase in photosynthetic activity began earlier and was more intense than the increasing of chlorophyll *a* content; i.e., a considerable temporary delay of the growth of phytoplankton biomass was observed, corresponding to its increasing activity (Fig. 1c). This delay indicated suppressed synthesis of organic matter by phytoplankton, probably due to contamination of the river at st. Dzerzhinskii. At this station the second autumnal peak of chlorophyll *a* content, typical of the regions with relatively clean water and occurring at st. Tushino, was also not detected. Moreover, at this station a prolonged period of summer chlorophyll *a* abundance (from June to September), characterizing contaminated water bodies (Mineeva, 2004), was observed.

The average yearly TBN values for stations Tushino, Vorob'evy Gory, and Dzerzhinskii were quite close (2.97 ± 1.13; 3.08 ± 1.33, and 3.09 ± 0.79 × 10^6 cells/mL, respectively). At Tushino and Vorob'evy Gory, however, more pronounced seasonal changes in bacterioplankton abundance were observed, unlike st. Dzerzhinskii, where TBN variations were less pronounced. At all three stations, a sharp increase of phytoplankton abundance was revealed in April, at the time of snow melting and increasing water temperature. It was probably associated with the spring inflow of allochthonous matter into the river with melt water. However, even this spring outbreak of bacterioplankton abundance was considerably more pronounced in less contaminated parts of the river (stations Tushino and Vorob'evy Gory) than at st. Dzerzhinskii.

At st. Dzerzhinskii, increased chlorophyll *a* cconcentration in March was accompanied by a TBN decrease, while during the period of the highest chlorophyll *a* content (June–September), low TBN values were revealed. Development of heterotrophic bacteria is probably delayed in contaminated parts of the river in the presence of abundantly growing phytoplankton. A similar observation was made previously by Razumov, who associated it with release of bactericidal compounds by phytoplankton (Razumov, 1962). Moreover, the phytoplankton of contaminated areas was probably dominated by cyanobacteria producing exudates toxic to bacterioplankton (Novozhilova, 1958). However, even considering the hypotheses mentioned above, it should be concluded that the interaction between phytoplanktonic algae and bacteria is still open to discussion and requires further investigation.

Both the numbers of bacteria with an active electron transport chain (CTC + B) and their share within TBN were considerably higher at st. Dzerzhinskii (annual average values 0.23 × 10^6 cells/mL and 14%) than at the least contaminated stations at Tushino and Vorob'evy Gory (0.14 × 10^6 cells/mL; 6% and 0.15 × 10^6 cells/mL; 7%, respectively). Seasonal variations of CTC + B at all three stations shared considerable similarity: this parameter peaked in April, and a less pronounced increase in CTC + B abundance occurred in late June. At later period's abundance of active bacterioplankton decreased gradually at stations Tushino and Vorob'evy Gory, while at st. Dzerzhinskii another CTC + B increase occurred in October.

Direct correlations between CTC + B abundance and chlorophyll *a* content at all three stations (R = 0.76 at $p < 0.01$), as well as between the number of metabolically active bacteria and water temperature (R = 0.73 at $p < 0.01$ for st. Vorob'evy Gory and $R = 0.46$ at $p < 0.01$ for st. Tushino) make it possible to suggest that the metabolically active fraction of bacterioplankton depends considerably upon the phytocenosis state and the temperature. We reported previously the correlation between CTC + B and chlorophyll *a* content for three lakes in Kosino (Il'inskii, 2013). The correlation between these parameters was also reported by other authors for other aquatic environments (Del Giorgio and Scarborough, 1995; Sommaruga and Conde, 1997). However, no such correlation was revealed for the Moskva River water at st. Dzerzhinskii. In hypertrophic water bodies, metabolically active bacteria may probably depend on other sources of dissolved organic matter, apart from phytoplankton exudates, while water temperature and the overall state of bacteriocenoses may cease to be the governing factors (Robarts and Sephton, 1988). The share of metabolically active bacteria in the Moskva River bacterioplankton was low, with the average for all three stations of 9.24% and variation from 0.49 to 38.17%. The highest values of this parameter were determined at st. Dzerzhinskii, at the exit of the river from the city. At this station the CTC + B share within TBN varied from 2.32 to 38.17%, with the average of 14.13%. Other authors reported the share of active bacteria within freshwater bacterioplankton in moderate latitudes to vary from 2.5 to 20% (Sondergaard and Danielsen, 2001). In most water bodies of the Upper and Middle Volga, the average fraction of metabolically active cells was reported to be less than half the total bacterioplankton abundance determined by the standard epifluorescence microscopic technique (Kopylov and Kosolapov, 2008).

The highest annual average SB number was revealed at st. Vorob'evy Gory (283 385 cells/mL, variation from 4500 to 2 500 000 cells/mL), then at st. Tushino (201175 cells/mL, variation from 950 to 2 500 000 cells/mL), and the lowest number at st. Dzerzhinskii (163 318 cells/mL, variation from 950 to 600 000 cells/mL). However, the TBN/SB ratio indicated that the water at st. Dzerzhinskii was most heavily contaminated. According to the average TBN/SB ratio from December 2009 to May 2010, the water at this area may be characterized as hypersaprobic (strongly polluted), while from June to December 2010 it was α-mesosaprobic (contaminated). Importantly, development of saprotrophic bacteria at st. Dzerzhinskii exhibited an inverse correlation with chlorophyll *a* content ($R = -0.4$ at $p < 0.01$).

Thus, the microbiological parameters used, together with the parameters of abundance and activity of the phytoplankton community, supplement the available hydrochemical data and may be efficiently used for further monitoring of the state of the Moskva River.

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