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Seasonal Dynamics of Phytoplankton and Bacterial Plankton Characteristics in Esil River

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Water from Esil River was sampled in March-November 2013 and studied. Hydrochemical samples were used to determine chemical pollutants and water quality class. Hydrobiological water samples were used to define: 1) total bacterialcount, share of heterotrophic bacteria, bacterial production; 2) qualitative and quantitative characteristics of phytoplankton: species diversity, primary daily productivity. The outcomes were compared by their seasonal dynamics.

Key words: Hydrochemistry, bacterial plankton, phytoplankton, primary daily production, trophicity.

The Esil River is of high recreational, economic and natural importance for Astana and areas located in its basin. The water quality is bound to deteriorate with the development of the city and the region, as the main source of river water contamination is rain water run-off. Therefore, studies focusing on the impact of pollutants onto the activity of aquatic organisms and reserve self-purification capacities of the river are highly relevant.

Aquatic microorganisms are a vital element of aquatic ecosystems; they transform organics and minerals. Heterotrophic saprophile bacteria play the leading role in these processes; theyingest easily degradable organic matters. Many of these bacteria are capable of devouring quite a number of contaminants[1, 2].

Microorganisms, including the algae, are important for biological productivity of water bodies. Bacterial plankton, typically, plays a secondary role of decomposing agent in “biogenic elements” and “dead organic matter"[3]. However, as stated in this paper, experimental research using radioactive carbon isotope demonstrated that significant amount of organic matter is produced by phytoplanktonin dissolved form and iteasily transforms into the living matter of bacteria[1, 4]. Release of highly labile organic matter (irrespective of the process mechanism) is closely related to its consumption by heterotrophic microorganisms. Turnover time for phytogenic substrates is only several hours therefore they are not accumulated in the external environment.

As the water bodies contain predominantly conservative dissolved organic

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matter (DOM), it creates a deceptive impression about this absolutely "dead" fraction of organic matter as the main energy source for aquatic bacteria. In fact, the efficiency of use of dissolved organic matter is extremely low;relatively small fraction of labile DOM, which is continuously replenished by phytoplankton production, is a more efficient energy source for heterotrophic microorganisms[4, 5].

Goal

Study the seasonal trends invariations of quantitative indicators and ratio of productivity of phytoplanktonandbacterial planktonfor Esil River.

METHODS

For hydrochemical and microbiological analysis, the water from Esil River was sampled 3 km upstream Astana, close to Telman village.

Gauging station coordinates

Latitude: 51°52 46.693 N (51.096303)

Longitude: 71°282 29.173 E (71.474769)

The adjacent area is hilly steppe plain built-up with suburban constructions. The river valley is wide; slopes gradually merge with the adjacent area. The vegetation is represented by mat-grass and sheep fescue. The riverbanks are 2-3 m high, the left one is gently sloping (20-25^0), the right one is steep (40-45^0). The river bed is of sand and silt.

The following analyses were made: complex chemical analyses of 9 samples, 9 quantitative and 9 qualitative analyses of phytoplankton, 54 bacteriological analyses, and 18 measurements of primary and bacterial production.

Hydrochemical samples

The following elements were analyzed in the water: oxygen, aliovalent metals (Fe, Cu, Mg, Ca, Mn, Zn), nitrates, nitrites, chlorides, sulfates, phosphates, ammonium salt, total hardness of water, oil products, synthetic surface-active substance (SSAS), biological oxygen demand (BOD_5).

Based on the results, the hydrochemical water pollution index (WPI) was determined[6]:

$$WPI=\frac{1}{n}*\sum_{i=1}^{n}\frac{C_i}{MAC_i}$$

where: C_i – component concentration; n – number of indicators used for index calculation, n = 6; MAC_i – the set value of maximum allowable concentration for the specific type of water body. Using WPI, the water pollution degree may be rated by 7 classes[6].

Total Bacterial Count (TBC) was determined by direct standard light microscopic method by A.S. Razumov(1932) [7] using MBB-IAmisroscope. The cells were stained witherythrosine.

The quantity of heterotrophic bacteria, growing onMPA (meat-and-peptone agar)was determined in the water samples (dilution 1:100 and 1:1000). The colonies were counted in 7 days for MPA plates, and for plates with diluted agar (MPA:10) –in 15 days.

Daily primary phytoplankton production was calculated by closed-bottle method in oxygen modification (G.G.Winberg,1960and A.P. Sadchikov,2003)[8, 9]. Phytoplankton sample is put into glass-stoppered production bottles (6 per 1 sample). Oxygen in 2 bottles is measured right after the filling. The remaining bottles are exposed to light (2 bottles) and kept in dark place (2 bottles) for 8 hours. After the exposure, 1 mL of 32% NaOH+10% KJ and 1 mL 32% $MnCl_2$ is added to each bottle in succession to bind the dissolved oxygen. The bottles are closed, mixed, and kept in dark place for 2 hours, for sedimentation. Then 1 mL of concentrated $H_2SO_4$is added and mixed. The dissociatediodine is titrated against 0.01N thiosulfate solution $Na_2S_2O_3$to slightly yellow color. Then 1 mL of 0.2% starch solution is added, the liquid is further titrated until the blue color fades away. 50 mL is taken for titration purposes.

Oxygen concentration in the water is calculated by the following formula:

O_2 (mg/L) = n×K×0.08×1000/ (V_1-V_2),

where: n – amount of thiosulfate used for sample titration (mL); K – correction tothiosulfate titration; V_1- production bottle volume; V_2 – volume of chemical reactants added to fix the oxygen (mL); 0.08 – coefficient for conversion of thiosulfate into mg of oxygen; 1000 – conversion ofmLinto liters.

Photosynthesis and respiration processes take place in thelight bottles. No photosynthesis happens in the dark bottles, oxygen is only consumed for respiration. Net gross production (photosynthesis):

D=I-D(destruction = initial oxygen minus oxygen in dark bottle),
P= L-D (photosynthesis = oxygen in light bottle minus oxygen in dark bottle).

For converting O_2concentration into energy units, we usedthe balance equation of photosynthesis: 1 kcal = 0.2849 mg/L O_2.

The bacterial planktonbiomass was determined by A.G. Rodina's method [10],rate ofbacterial planktonreproduction was calculated by A.S. Razumov's method [7]. The latter assumes preliminary removal of bacteria-consuming zooplankton, by filtering the water samples through a filter that passes bacteria only. Generation number H is calculated by the following formula:

$$H = \lg B_f - \lg B_i / t \times \lg 2$$

Where: B_i- initial bacteria quantity;B_f- final quantity of bacteria; t - exposure time.

Reciprocal variable d = t/H - time for one generation. If t = 1, then H =1/d, and d= 1/H.

G.G. Winberg equation is used to calculate the bacterial production:

$$P_T = 0.6931/ d \times B_o$$

If time unit is a day, then daily production will be

$$P_T = 0.6931\ H \times B_o$$

In our paper we used average daily bacteria concentration instead of B_o.

RESULTS

Hydrochemical characteristics of Esil River over the period of March to November 2013 showed that the river experiences the highest contamination in March and April. MAC was exceeded for sulfate ions (6.36 timeshigher than MAC in average), magnesium (2.26 times higher than MAC in average), copper(3 times higher than MAC in average) and especially manganese (up to 33.26 times higher than MAC). The impurities concentration mainly grows due to natural reduction of water content in the river after winter time.

Based on the examined components, inspring the water pollution index was high (WPI4.54 and up), which is Class 5 pollution.

Table 1. Esil River Water Pollution Indexfor March-November 2013

Ingredients and parameters	Average concentration, mg/L	MAC repetition factor	Water Pollution Index	Water Quality Parameter
Oxygen	7.24	0.73	2.6	Pollution Class 4
BOD_5	1.81	0.6		
Sulfates	288	2.88		
Manganese	0.06	6.48		
Copper	0.005	4.72		
Zinc	0.014	1.41		

Table 2. Quantitativeparameters ofbacterial planktonin Esil River in the vicinity of Astana in March-November 2013

Month	Total bacterial count,mio cells/mL	Number of heterotroph bacteria, cells/mL
March	2.210±0.013	14500±2180
April	11.46±2.34	19125±3270
May	4.410±0.084	16000±3120
June	5.56±0.14	14230±2280
July	5.66±0.12	17000±2180
August	8.12±1.235	22000±4228
September	1.95±0.096	8950±1347
October	3.32±0.38	11880±3120
November	0.95±0.081	11520±1347

In summer months of June and July, BOD_5goes up to 3.1mg_2/L.

Unlike spring and summer periods, in July – September, WPI did not increase; it went down to 0.7 in average, which is Class 2 pollution. Namely, MAC in Esildecreased for ammonia salt (to 0.002 MAC), magnesium (to 1.15 MAC), nitrites (to 1.12 MAC), oil products (to 1.98 MAC),iron (to 2.0 MAC),SSAS (to 3.8 MAC). However, the suphate (3.5 MAC) and copper (4.3 MAC) concentrations remain high.

In late autumn, October and November, hydrochemical composition in the river experiences some increase in nitrites (up to 2MAC), sulphates(4 MAC in average), copper (up to 4.6 MAC), manganese (up to5MAC). During this period, the water body was rated Class 4 – polluted (WPI 2.3).

Therefore, the results of the chemical assay demonstrate stable increase inthe content ofmagnesium, nitrites, oil products, iron and considerable excess of SSAS, sulphates and copper throughout the months of observation.

Summarizing the water quality in Esil River by its hydrochemical characteristics over the research period (Table1), it may be concluded that average annual water pollution index in Esil River for 2013 is 2.6, which is Class 4 pollution level (polluted). The major contamination occurs in early spring and late autumn.

Quantitative indicators of bacterial plankton and count ofheterotrophic bacteria.

The results of bacterial count from March to November are given in Table 2.Total bacterial count in the river over the season varied from2.210±0.013 mio cells/mLup to11.46±2.34 mio cells/mL.This conforms toeutrophic type of water body[11].

In spring,the lowest average bacterial count was observed in March–2.210±0.013 miocells/mL.Heterotrophs accounted for 14500±2180 cells/mL (Figure1, 2).

In April we saw sharp increase in bacterial count (Figure2):TBCup to11.46±2.34 miocells/mL, among them heterotrophic bacteria up to19125±3270 cells/mL.Then bacterial count dropped in May (TBCup to 4.410±0.084 miocells/mL and heterotrophic bacteriaup to16000±3120 cells/mL).

In summer (June and July) average bacteria count increased vs. May: TBCup to5.66±0.12 miocells/mL,among them heterotroph bacteria 17000±2180 cells/mL.

The next peak of average bacteria count was noted in August amounting to 8.12±1.235 mio cells/mL.The saprophile ratio increased accordinglyto 22000±4228 cells/mL.

As the weather cooled down, in September and October, the total bacterial count dropped to1.95±0.096miocells/mL. In October, minor increase was observedup to3.32±0.38 miocells/mL, the bacterial count goes down in November to0.95±0.081 miocells/mL.With the total bacterial count, the heterotroph organisms decreased gradually: 8950±1347cells/mL–in September, 11880±3120cells/mL–in October, 11520±1347cells/mL – in November.

The heterotroph count to total bacterial count ratio grows, despite the fact that total bacterial count is increasing slower or remains the same. This suggests that self-purificationcapacity of the water body remains high, though some chemical ingredients are higher than MAC[1, 12].

Average total bacterial count throughout the season was 4.85mio cells/mL, average number of heterotroph organisms - 15022cells/mL. Average heterotroph count to total bacterial count ratio(Razumov Coefficient) was 0.31%, indicating that Esil River in the city is not a clean water body[2, 7].

Comparison of the results with the outcomes of other authors[13, 14] proved the seasonaltrend: there are two peaks of increased bacterial count, first in spring during snow melting and afterwards, and the second one in autumn. In our research, we have seen the first bacterial count peak in April and summer, with drop down in May. The second autumn peak was recorded in August.

Table 3. Primary Production (P) vs. Bacterial Production (Pb)

Month	Pkcal/m^2	Pbkcal/m^2	Pb/P
March	66.6	177	2.66
April	139	204	1.47
May	131	116	0.88
June	248	24.5	0.099
July	286	36.4	0.13
August	311	166	0.53
September	264	108	0.41
October	112	93	0.83
November	45	65	1.44

The quantity of heterotroph bacteria went up and down with the total bacteria count (Figure 1,2).

Total Bacterial Count, millioncells/mL

Phytoplankton Study

In the beginning of vegetation season (March-April) diatomic algae prevailed in quantity (59.6%) and biomass (84.1%). The most common species: *Navicula, Nitzshia, Stephanodiscus, Synedra*. The second group in the general phytoplankton biomass isgreen algae (quantity– 11.5%, mass – 5.3 %), including mainly protococcus. The following species prevailed: *Lambertia, Chlorococcum,Ankistrodesmus, Phacotus, Chlamydomonas*. Blue-green algae(*Oscillatoria, Gloecapsa, Anabaena*) were rare (0.03 % by quantity and 0.02% by mass). Euglena algae were represented by only one species, Trachelomonas, and did not affect the size of total biomass of the riverphytoplankton.

During the season of higher contamination of the water body (in early June, the content ofcopper, iron, SSAS, oil products increased),more halophile and indifferent algae appeared(24 types).

Halophobous types of algae, *Navicula cuspidate* Kütz., *Navicularadiosa*Kütz., *Naviculahumerosa* Breb., *Girosigma Spenseri* (W.Sm.), *Surirella Capronii* Breb., *Cosmariumpunctulatum* Breb., *Merismopediatenuissima* Lemm, were more common in July and September. This is an indirect evidence of absence of copper, iron, SSAS, oil products.

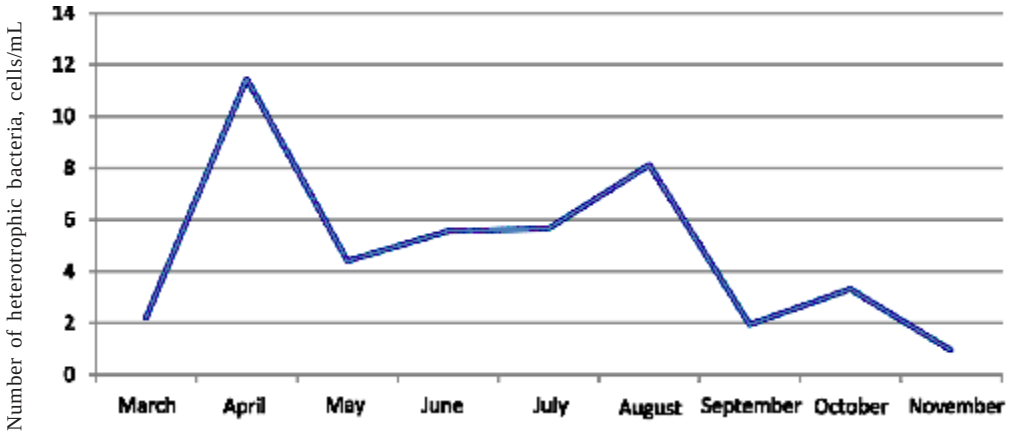

Fig. 1. Esil River - Total Bacterial Count Trend, mio cells/mL

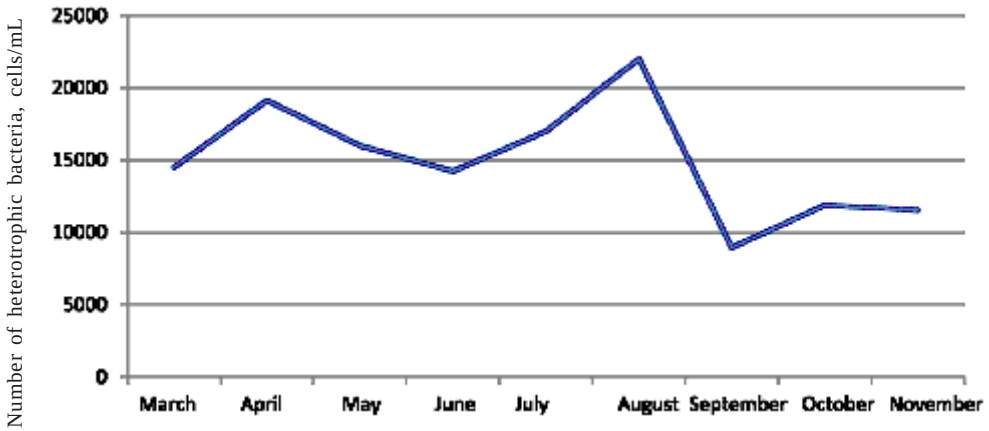

Fig. 2. Esil River – Heterotrophic Bacteria Count Trend, cells/mL

Phytoplankton biomass increased from 0.16 gr/m^3in the beginning of season to15.62 gr/m^3 in August. Biomass started to decrease in September due to lower water temperature. When the phytoplankton biomass was the biggest, the green algae number increased significantly (44 % by quantity, 26.5% by mass), euglena algae (26% by quantity, 31%by biomass), mainly of Euglena species. Share ofdiatomic algae went down to 26.3% by quantity and 37.3% by biomass.

Energy in autochthon and allochthon organic matter determines the size of production of heterotrophic organisms in water bodies located at various levels of trophic chain.Bacterial production, along with the primary phytoplankton production, defines the stock of initial organic matter which serves as a base for the trophic system in the water bodies.

With the considerable intake of allochthon organic matter, the heterotrophic organisms are mainly produced due to the energy transformed by the bacterial plankton. And vice versa, when lessallochton organics enter the system, almost all heterotrophic production is synthesized with the energy contained in the primary production which is generated in the process of phytoplankton photosynthesis.

Measurements of primary daily phytoplankton production and daily bacterial production (Table3)indicated that right after the ice clearance in EsilRiver the bacterial production is 2.66 times higher than phytoplanktonproduction in March and 1.47 times higher in April. In summer time, the primary production is higher. Though in August the Pb/P ratio goes up again and reaches 0.53, primary production remains higher than bacterial production through October. Only in November the bacterial production exceeds phytoplankton production again. The peak bioproductivity for both phytoplankton and bacterial planktonis in July, but bacterial plankton showed decrease in bioproductivity in June, whereas phytoplanktonproduction kept gradually increasing from 66.6 kcal/m^2 in March up to 311 kcal/m^2 in August.

So the seasonal trend for daily production in Esil River from March to November showed that heterotrophic metabolism is strong in cold periods (March, April, November), and autotrophic metabolism is typical for warm months.

The obtained results on seasonal trends can be explained by the fact that in early spring the river receives the melt water with chemical pollutants accumulated over the winter period and allochton organic matter, these factors crease conditions for predominant development of bacteria, namely heterotrophic bacteria. Chemical contamination determines dominance of halophilic resistant species of algae, from among diatomic algae. In warm months, with lower pollutant discharge and less allochton organic matter, the dominant composition of phytoplankton changes, its daily production goes up and the water body ecosystem functions in autotrophic style. As the temperature in the river decreases, the primary productivity drops and organic matter starts to accumulate due to the death of aquatic organisms. This causes the second peak in total bacterial count in August. This is also critical for gradual enhancement of heterotrophic metabolism, which then prevails in November.

CONCLUSIONS

1. Esil River is rated Class 4 – polluted (WPI 2.6) based on the hydrochemical characteristics over the period of March to November 2013.Namely, the content of sulphates, copper, magnesium, nitrites, SSAS, oil products, iron is increasing stably. The most polluted water was detected in March-April (WPI 4.54), the river water was relatively clean in June-July (WPI 0.7).
2. Total bacterial count in the river is typical for eutrophic water body.
3. Based on the quantitative dynamics ofbacterial planktonin Esil River over the period of March to November, the maximum average bacterial count was observed in April and August, consequently increasing the share of heterotrophic organisms.
4. Average ratio of heterotrophic organisms to total bacteria count was 0.31%, which also proves that Esil River is polluted. However, as the ratio of heterotrophic organisms to total bacteria count is increasing, it may be assumed that potentially the self-purification capacity of the water body remains high.
5. Species diversity of fitoplankton varies

through the vegetative season from dominant diatomic algae in spring to gradual domination of green algae and euglena algae in summer and autumn months. The weight ratio of these algae varies accordingly. Besides, the dominant algae types changed depending on the resistance to water pollution.

6. Based on the comparison of primary daily production of phytoplankton and daily bacterial production in Esil River, it may be concluded that in cold months (March, April, November) heterotrophic metabolism prevails, whereas autotroph metabolism is typical for the warmer months.

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