Dynamics of fouling of plastic waste fragments by microorganisms in the Gulf of Finland

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Abstract. In recent years, the problem of the aquatic environment pollution with synthetic polymers - plastic and microplastics - has become more urgent than ever, which causes an increasing interest of the scientific community in this topic, both in our country and abroad. The development of organic synthesis chemistry at the beginning of the last century led to the emergence of synthetic polymers – plastics; the chemical properties of plastic, making it an inexpensive, durable, and useful material, led to its widespread, mass distribution, but they are also the cause of difficulties with its disposal – the decay, decomposition, and disposal of many types of plastic takes decades, which much lower than the production rate of new plastic. As it is known, plastics are materials that are synthetic or natural high-molecular compounds (polymers); the term "microplastic" refers to particles of synthetic polymers or plastic copolymers (less than 5 microns), they are insoluble in water and non-degradable. Such characteristics of microplastic components as the area, the particle surface relief, the type of polymer will affect the biofilm formation rate. This indicator also is influenced by the flow rate, the bio-productivity of the waters, and the position of the particles in the water column.

1 Introduction

One of the most urgent environmental problems of the last decade of our century, causing genuine interest of the world scientific community, is the problem of accumulation in the aquatic environment of a relatively new pollutant - microplastics.

The XX century is a time of technological revolutions and it is called the "century of plastic" for reason, like the beginning of the current century, because of the rapid development of this material production and its widespread use in all spheres of human life. Plastic has gone from the creation of the first fully synthetic materials to the invention of biodegradable plastics.

In view of its cheapness, strength, and durability, back in the 50s of the twentieth century, plastic products began to displace paper, glass, and metal materials previously used for the production of containers, packaging of consumer goods, single-use goods. The "concept of single-use" generated by this fact led to the appearance of a huge amount of plastic waste, the accumulation of plastic garbage, and, as a result, to environmental

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pollution with plastic components that began to be found everywhere - in soil, water, and even living organisms.

But the chemical characteristics that made plastic an inexpensive, durable, and useful material at the same time gave rise to problems of its disposal; for the decay of many types of plastic, it takes years and even decades. In the Russian Federation, about 750 thousand tons of plastic waste are generated per year; in the UK, the volume of this waste reaches 7-8 million tons. Recycling at the same time affects 50% of plastic waste in Europe, while in the Russian Federation this indicator is no more than 4%. About 10% of plastic waste ends up in the World Ocean [9]. To date, about 165 million tons of plastic are circulating in the seas and oceans.

Plastics are materials that are synthetic or natural high-molecular compounds (polymers). Complex materials consisting of polymers and other materials are called plastics (for example, fiberglass/glass-fiber-reinforced plastic). Despite the mass production of a large number of types of plastic in the world, the following four types are leading in the market: polyethylene terephtalate (PET), polyvinyl chloride (PVC), polyethylene (PE), polypropylene (PP); polyurethane (PU), and polystyrene (PS) are also produced and used in large quantities.

The main sources of the aquatic environment contamination are both large plastic waste and smaller fragments of plastic, such as microplastics. Microplastic elements, getting into all parts of the World Ocean, become food for many organisms and moving along the food chains of the aquatic environment, accumulate in the organisms of hydrobionts. In addition, getting into reservoirs, the surfaces of plastic debris are colonized by representatives of various systematic and ecological groups, in particular by microorganisms with the formation of biofilms. Biofilms have a complex organization, these are aggregates of resident (transferred from the free-floating, planktonic stage) microorganisms surrounded by an extracellular matrix synthesized by them (mucus).

The biofilm formation leading to biofouling successively passes through four separate stages: deposition of dissolved organic molecules, colonization by bacterial cells, colonization by unicellular eukaryotic organisms, and attachment of larvae and spores.

An increase in the total particle density due to the biofilm formation leads to immersion to the bottom and entry into the bottom sediments. This ensures the vertical migration of microplastics in the aquatic (oceanic) environment [10,13].

The type of synthetic polymer, the fragment area, the flow velocity, and the fragment relief are the indicators that affect the rate of biofilm formation. In addition, it is directly related to the bioproductivity of the waters and the fragment position in the aquatic environment thickness. For example, plastic floating on the surface leads to the formation of biofilm in a few weeks or even days; biofilm on microplastics immersed in the water column is formed much slower [10].

Particle aggregation is another factor in the migration of microplastic fragments in the aquatic environment. Most often, aggregates include representatives of phytoplankton, microorganisms, and other organic residues that bind to particles of synthetic polymers, playing a key role in the movement of microplastic elements in the water column, and also cause their bioavailability. Up to 5300 aggregates can occur in a liter of seawater [14].

The formation of biofilms is one of the conditions for pathogenicity of microorganisms. The formation of biofilms, the presence of bacteria inside biofilms gives them advantages over existing as separate cells – such as high survival and resistance to damaging factors. According to numerous publications, a number of microorganisms, for example, E. coli, acquire high resistance, practically immunity to antibiotics due to the low level of metabolic activity.

The results of recent studies show that fragments of synthetic polymers become a place of accumulation and reproduction of microorganisms that are important in sanitary terms, many of which may be resistant to the effects of antibiotics, which is a big problem for representatives of the aquatic environment and, most importantly, a threat to human health. Thus, the total bacterial contamination (the number of saprophytic bacteria, TMC) and bacteria of the E. coli group (CB), which are in associative relationships with plastic waste particles, can be convenient indicators of the well-being of reservoirs in sanitary terms.

Wastewater can become a source of microorganisms resistant to antibiotics. Microplastic and nanoplastic particles can be in wastewater and get into the environment therefrom. Plastic waste particles can have biofilms on them from the very beginning, which are phylogenetically different from the seawater microbiome and the biofilm microbiome.

Pollution of wastewater with plastic waste is one of the most pressing problems for the Baltic Sea. A separate interest for Russian scientists is the Gulf of Finland of the Baltic Sea and its shallow-water part – the Neva Bay [3,7,5].

Located in the eastern part of the Baltic Sea, the Gulf of Finland is actively involved in economic and recreational purposes.

The presence of plastic waste is observed almost throughout the Russian part of the Gulf of Finland. A large number of different pollutants, including plastic, get here together with the flow of many rivers, and in particular with the flow of the Neva River, as well as with the wastewater of urbanized territories [1].

The Neva Bay is not a deep flowing isolated reservoir, since it is separated from the Gulf of Finland by the Gorskaya https://ru.wikipedia.org/wiki/%D0%93%D0%BE%D1%80%D1%81%D0%BA%D0%B0%D1%8F_(%D0%A1%D0%B5%D1%81%D1%82%D1%80%D0%BE%D1%80%D0%B5%D1%86%D0%BA) — Kronstadt — Bronka line with a complex of dams (St. Petersburg flood protection barrier). In some areas of the Neva Bay, a number of sanitary-significant indicators do not correspond to the MPC. The consequence of stagnant phenomena of the coastal part of the Neva Bay is the accumulation of pollutants and microflora [4,8].

In such conditions, the association of microorganisms that are important in sanitary terms in the composition of biofilms can cause a decrease in the sanitary well-being of the water area.

In connection with the above, the main purpose of this study was to study the rate of formation of biological films of various origin on plastic particles and the presence of sanitary microorganisms of the Escherichia coli group on them on the territory of the Neva Bay of the Gulf of Finland.

2 Materials and Methods

Samples were taken in the period from June to September 2021 in the Neva Bay area of the Gulf of Finland. The selection was carried out at three points: the northeastern part of Kotlin Island, the city of Sestroretsk near the northern part of the dam, the city of Lomonosov.

To study the formation of biofilm on the main types of plastic, a modified Lobelle and Cunliffe technique was used [12].

From samples of various types of sterile plastic with a size of 10X10cm, a series of 10 pieces was formed and transported under aseptic conditions to the experiment site. Then the samples were attached to weighted carriers, which were suspended 0.5 m below the surface level of the reservoir. A series of plastics PA, PS, PE, PET, PVC were used, which were attached to each carrier.

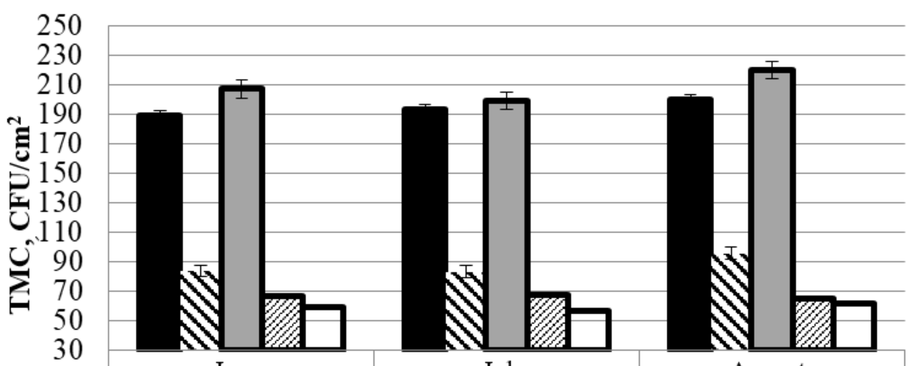
At each location, the experiment was duplicated in three repetitions. At intervals of every two weeks, a fragment was separated from a series of samples, which was placed in a sterile glass container, delivered to the laboratory within two hours for research.

The analysis of microorganisms was carried out under aseptic conditions using the wash method (MR 4.2.0220-20. 4.2. "Methods of control. Biological and microbiological factors. Methods of sanitary-bacteriological investigation of microbial contamination of environmental objects. Methodological recommendations"). The bacteriological study of microbial contamination of environmental objects includes the determination of the bacteria of the Escherichia coli group (CB) and the total bacterial contamination (total microbial count, TBC). To identify the CB, we used seeding of washes on the Kessler nutrient medium and on a dense differential diagnostic selective Endo medium. The latter was kept in an incubator at a temperature of (37 1)°C for 24 hours. To analyze the total bacterial contamination (TMC), 1.0 cm^3 of the wsh liquid was placed in a Petri dish and poured with molten nutrient agar (MIA), then incubated at a temperature of (30 1)°C. The preliminary count of the grown colonies was made after 48 hours, and the final one was made after 72 hours.

3 Results of the study of the primary microbiological succession formation

During the summer period of 2021, the dynamics of biofilm formation on fragments of samples of various types of plastic was studied. 5 different types of plastics are used: PVC, PA, PS, PE, PET.

The graph in Fig. 1 reflects the dynamics of plastic colonization by saprophytic bacteria (TMC) on samples taken in the water area near the city of Kronstadt. The largest number of saprophytic microorganisms was recorded on PA and PVC fragments, the smallest was noted on PET (3-3.5 times fewer microorganisms than on PVC).

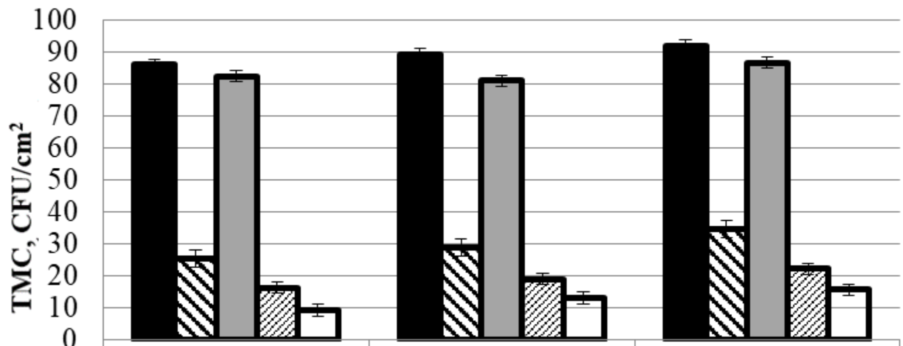


	June	July	August
PVC	188,9	193,6	200
PA	84	83,3	95,9
PS	207	199,3	219,6
PE	66,6	68	65,6
PET	59,6	57,3	62,3

Fig. 1. Dynamics of growth of microorganisms on plastic samples in the water area near the city of Kronstadt.

The diagram below (Figure 2) reflects the dynamics of colonization by saprophytic bacteria (TMC) of plastic fragments from the location of the water area near the city of

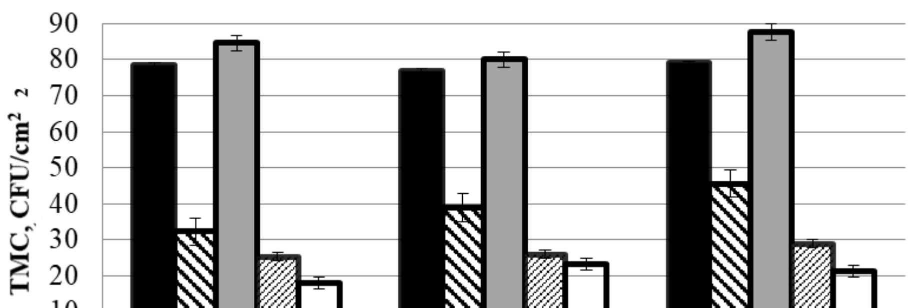
Sestroretsk. Here, PVC samples also show the largest number of saprophytic microorganisms, the level on PET is 6-9 times lower than on PVC.



	June	July	August
PVC	86	89,3	92
PA	25,3	29	34,6
PS	82,3	81	86,6
PE	16,3	19	22,3
PET	9,3	13	15,6

Fig. 2. Dynamics of growth of microorganisms on plastic samples in the city of Sestroretsk.

The following diagram shows a graph of the dynamics of fouling with saprophytic bacteria (TMC) of plastic fragments in the water area near the city of Lomonosov. The largest number of saprophytic microorganisms was recorded on fragments of PA and PVC, the smallest was noted on PET (2-4 times fewer microorganisms than on PVC).



	June	July	August
PVC	78,6	77	79
PA	32,3	39	45,6
PS	84,6	80	87,6
PE	25,3	26	28,9
PET	18	23,3	21,3

Fig. 3. Dynamics of growth of microorganisms on plastic samples in the city of Lomonosov.

Two weeks after placing plastic objects in reservoirs, microorganisms appeared at each station. The highest TMC level was noted on washes from fragments of all types of plastic samples in the area of the city of Kronstadt. The reason for such contamination may be an increased concentration of microorganisms in the water of this part of the water area, which is associated with its hydrological and hydrochemical conditions.

The total microbial count (TMC) in samples taken in the water area of the nearby towns of the city of Sestroretsk and Lomonosov is comparable. A similar horizontal distribution of microorganisms in the water area was observed during the entire research period. On the samples of most series during the experiment, the number of microorganisms was insignificant, but increased.

The maximum values of the total microbial count (TMC) on samples of all types of plastic were observed in August. The largest number of colonies of microorganisms relative to other plastic samples were registered on PVC and PS fragments. PET samples showed the smallest number of colonies. Also, the smallest CFU amount was noted on the PET fragments.

Tables 1-3 reflect the analysis of the CB detection at various locations of the water area.

Table 1. Results of inoculations for CB in the area of the city of Kronstadt.

Month	Number of colonies on plastic samples				
	PVC	PA	PS	PE	PET
June	12,6 ± 0,8	8,6 ± 0,8	8,6 ± 0,3	1,0 ± 0,3	0
July	14 ± 1,5	7,6 ± 1,4	8,6 ± 1,2	1,3 ± 0,8	0
August	13,6 ± 0,8	9,3 ± 0,8	11 ± 1,1	2,3 ± 0,6	1,0 ± 0,5

Table 2. Results of inoculations for CB in the area of the city of Sestroretsk.

Month	Number of colonies on plastic samples				
	PVC	PA	PS	PE	PET
June	5,3 ± 0,6	1,3 ± 0,6	4,0 ± 0,5	0	0
July	6,0 ± 0,5	2,0 ± 0,5	6,3 ± 0,8	1,0 ± 0,5	0
August	6,3 ± 1,2	2,3 ± 1,2	10 ± 0,5	0	1,0 ± 0,5

Table 3. Results of inoculations for CB in the area of the city of Lomonosov.

Month	Number of colonies on plastic samples				
	PVC	PA	PS	PE	PET
June	7,3 ± 1,2	4,3 ± 0,6	8,3 ± 0,3	0	0
July	10 ± 0,5	5,0 ± 1,1	8,3 ± 1,2	0	0
August	9,3 ± 1,4	5,6 ± 0,8	10 ± 2,0	1,3 ± 0,3	0

In the area of the city of Kronstadt, as in the case of the total microbial count, the largest number of CB colonies was noted. The largest number of colonies was recorded on samples of PVC and PS, on samples of PET, a smaller number of colonies of CB was noted in comparison with samples of other types of plastic.

In the water area near the cities of Sestroretsk and Kronstadt, Escherichia coli group bacteria began to be recorded only in August, in the area of the city of Lomonosov, Escherichia coli group bacteria (CB) were not detected on PET samples.

The reasons for the greater contamination of PVC and PS samples relative to PET and PE are explained by the peculiarities of the surface structure of these materials. PET and PE differ in a relatively smooth and fairly smooth surface structure, and the surfaces of PVC and PS are porous, with a non-uniform relief. Microorganisms accumulate in significant quantities on samples with a relief, uneven surface.

The seasonal dynamics in general duplicates the TMC dynamics.

CB CFU is on average 17 times less than CFU of saprophytic bacteria.

Summarizing, we can conclude that microorganisms easily contaminated plastic samples. Despite the lower level of CB, lower than TMC, the presence of microorganisms of this group on plastic fragments indicates that the samples may be accumulators and sources of the spread of pathogenic microorganisms.

Strengthening the control of the composition of effluents, exclusion or significant reduction of the ingress of plastic debris samples of various types and sizes into reservoirs are measures necessary to preserve and maintain a satisfactory sanitary condition of the waters of the studied water area. It is also necessary to deepen bioindication as a direction in monitoring the state of natural objects.

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