

Local Monitoring of Saprotrophic Bacterial Complexes of Urban Soils in Syktyvkar in 2019 and 2020

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Abstracts—Abundance and diversity of cultivated bacteria in saprotrophic soil complexes were monitored for two years on the territory of one of the large industrial cities of the European North of Russia, Syktyvkar. The analysis was carried out before and after quarantine due to the COVID-19 pandemic. The city of Syktyvkar is characterized by a high population and intensive anthropogenic pressure. According to the total indicators of the current state of the environmental components, it should belong to the tense category, by environmental standards. Studies were conducted in 2019–2020. Topsoils (0–10 cm) of urbanozems and horizon A in the park area in the urban space, as well as zonal undisturbed podzolic soil, were analyzed. Comparison of the two-year monitoring results demonstrated a marked increase in the number and diversity of saprotrophic bacterial complexes in 2020 in both urban and control soils (topsoil, 0–10 cm). Due to a sharp and prolonged decrease in the anthropogenic impact on the environment during quarantine measures, content of the bacteria of the families Enterobacteriaceae including sanitary-indicative (*Escherichia coli*, *Enterococcus faecalis*) and opportunistic and allergenic (*Enterobacter agglomerans*, *Citrobacter europaeus*, *Klebsiella oxytoca*, *Serratia marcescens*, etc.) in the urbanozems decreased. This can be considered as a manifestation of the soil's ability to “self-remediate” during a decrease in anthropogenic impact on the environment.

Keywords: urban soils, COVID-19, Syktyvkar, saprotrophic bacterial complex, sanitary-indicative bacteria, Enterobacteriaceae, *Escherichia coli*, *Enterococcus faecalis*

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INTRODUCTION

The city is a complex urban ecosystem. Maintaining its stable functioning and the “health” of the soil as a significant component of the ecosystem are the most important tasks, without which human life is impossible [11]. In recent years, due to increased interest in the problems of environmental safety and sustainable functioning of urban ecosystems, domestic and foreign works devoted to the study of soil biota more and more often consider issues of structural and functional transformations of communities of soil microorganisms due to anthropogenic influences of different quality and intensity. Household soil pollution is considered as one of the most serious instances for the violation of the “purity” of the city [1, 7, 12, 21]. Our study of urban soils performed in 2019 in southern cities of Russia with different intensities of anthropogenic impact (Sochi, Simferopol, Krasnodar, and Maykop) showed that in the 0–10 cm layer on the territory of the largest (Krasnodar) and large (Simferopol, Sochi) cities, bacterial complexes changed and representation of the Enterobacteriaceae and Enterococcaceae families increased [4]. The more intense the anthropogenic impact on soils (high population, tour-

ist load, etc.) led to the more pronounced changes in their structure [19]. Predominance of the bacteria of the Enterobacteriaceae families in soil saprotrophic bacterial community under these conditions results in an increase in the number and taxonomic diversity of sanitary indicative microorganisms. Maximum content of *Escherichia coli*, *Enterococcus faecalis*, *Clostridium perfringens*, which pose the greatest threats to human health, has been detected in the urban soils of Sochi. In this case, the deformation of the natural biocenosis under the influence of anthropogenic factors was the most pronounced. Along with sanitary indicative microorganisms, potentially pathogenic bacteria of the genera *Klebsiella*, *Enterobacter*, *Citrobacter*, and *Serratia* were found in urban soils, some species of which can cause intestinal and allergic diseases [4, 7].

The study of the diversity of both bacterial and, in general, microbial complexes of urban soils is of considerable interest not only from the point of view of fundamental science, but also in practical terms, due to the important role of microorganisms in creating and maintaining the stability of urban ecosystems, as well as from the point of view of studying microorganisms that can directly or indirectly have a harmful

Table 1. Characteristics of soils

Soil	Site
Urbanozemthin sandy loamy urbanostratozem on technogenic sediments, underlain by texture-differentiated profile-gleyed light loamy agrozem on water-glacial deposits	Central district (courtyard of the Institute of Biology of Komi Science Centre of the Ural Branch of the Russian Academy of Sciences)
Urbanozemagro soddy-podzolic urban-stratified unsaturated extremely fine medium loamy soil on cover loam	District Orbita (station of young naturalists, zoo)
Postagrozemtexture-differentiated medium-arable profile-gleyed light loamy on water-glacial sediments	District Orbita (station of young naturalists, territory fenced off for a park)
Undisturbed zonal (control)podzolic contact deeply clarified profile-gleyed light and heavy loamy on moraine deposits	Central district (territory of the station of the Institute of Biology of Komi Science Centre of the Ural Branch of the Russian Academy of Sciences)

effect on public health [23]. Further analytical work on the study of microbial complexes of urban soils is also necessary for the development of effective hygienic and environmental measures (recommendations) to improve the state of the urban environment. It is obvious that the complex characterization of the community of cultivated saprotrophic bacteria with a detailed analysis of the genus and species composition of the Enterobacteriaceae family is a very promising direction for bioindication of the current state of the urban ecosystem [13].

Thus, obtaining representative results of studying the taxonomic diversity of the complex of cultivated saprotrophic bacteria, including the assessment of the participation of the sanitary indicative species *Escherichia coli* and *Enterococcus faecalis*, in urban soils of different cities of Russia, differing in geographic location, climatic features and intensity of anthropogenic load, is an urgent task.

The goal of this work was to characterize the bacterial communities of urban soils on the territory of Syktyvkar. The capital of Komi is characterized by a high population and an intense anthropogenic load, and, according to the totality of the current state of indicators of environmental components, it belongs to the tense category of environmental standards [14].

MATERIALS AND METHODS

Soils of the territory of the city and its outskirts were studied (Table 1). Urbanozems occupy the historical part of the city including courtyards of educational and scientific organizations with the main buildings built in 1950–1970s. In their profiles, there are inclusions of household and building materials, and the soils have signs of zoning and the specifics of the deposits on which they were formed. As a control, we studied the zonal soil of the nearby forested undisturbed territory and the urban soil from the city park with a low household anthropogenic load.

Sampling was carried out in 2019 and 2020. In 2020, it was carried out immediately after the termination of

quarantine measures in connection with the first wave of the epidemic in late June–early July. In total, from the 0–10 cm layer of urban soils, where the anthropogenic influence is most noticeable, the upper layer of the urban park A1 (0–10 cm) and urban zonal A1 (2–7 cm) soils, 23 mixed samples were collected and analyzed. Detailed designations of soils were given in accordance with the Classification and Diagnostics of Soils of Russia (2004) and the project of a group of authors for the introduction of urban soils into this classification [10] (Table 1).

Determination of the number and taxonomic structure of the cultivated saprotrophic bacterial complex (SBC) at the genus level was carried out by the classical culture method. We used agar glucose-peptone-yeast medium (GPY), which allows one to isolate up to 50 genera of aerobic and facultative anaerobic bacteria from the soil [2, 5, 6]. The SBC number was expressed as the number of colony-forming units per gram of absolutely dry soil (CFU/g).

Bacteria were identified to the genus based on phenotypic (micromorphological, physiological–biochemical, and some chemotaxonomic) characteristics according to the key for identifying soil bacteria and generally accepted keys [9]. Genus and species identification of representatives of dominant taxa was refined based on phenotypic characteristics using the analysis of the variable sequence V3–V4 regions of the 16S rRNA gene using the BLAST program [8]. DNA from pure cultures of bacteria was isolated using the PrepMan Ultra Sample Preparation Reagent kit according to the manufacturer's recommendations. PCR products of V3–V4 variable regions of the 16S rDNA gene were sequenced according to MicroSeq 500 16S rDNA Bacterial Identification Kit's Protocol (Thermo Fisher) using standard primers fD1/rD1 (forward primer (fD1)-5'-AGAGTTT-GATCCTGGCTCAG-3' and reverse primer (rD1)-5'-AAGGAGGT GATCCAGCC-3') [15]. Capillary electrophoresis was performed using ABI Prism 3130 genetic analyzer. MicroSeq ID v.2.0 Software and validated MicroSeq ID 16S rDNA 500 Library v.2.0 were

used to analyze the obtained electrophoretograms and nucleotide sequences. Sequence analysis of the 16S rRNA gene was carried by Syntol Research and Production Company (Moscow). A total of 213 strains were identified.

For statistical processing of data on the number and relative abundance of bacteria, the STATISTICA 8 software (StatSoft, United States) was used.

RESULTS AND DISCUSSION

The number of SBCs in urban soils and control soils varied over the years of the study. In urban soil, both in 2019 and in 2020, it was higher than in the upper layer of the urban control soil A1 (Fig. 1). The results of two-way analysis of variance of data for the years indicated showed that the number of saprotrophic soil bacteria most reliably depends on the year of sampling ($F = 156.99$; $p < 0.01$) and, to a lesser extent, on the soil type ($F = 12.52$; $p < 0.01$).

Representatives of seven genera of the family Enterobacteriaceae and 18 genera of other saprotrophic bacteria were isolated from the studied soils over two years.

The taxonomic structure of the saprotrophic bacterial complex of urbanozems was characterized by specific features. In 2019, representatives of the family Enterobacteriaceae and bacteria of the genus *Arthrobacter* were predominant. The dominance of enterobacteria indicates significant household pollution of the urban environment [7]. In 2020, not only representatives of this family, but also of the genus *Pseudomonas* were among the dominant group microorganisms of urban soil. In general, in 2020, the taxonomic composition of saprotrophic bacteria in all studied soils (urban soil, soil of a city park, and undisturbed zonal soil) was distinguished by a large genera diversity in comparison with the previous year, primarily due to an increase in the number of the groups with medium and minor abundance (Table 2). In addition, the higher abundance and diversity of SBCs in 2020 may be associated with a prolonged period of decrease in the intensity of negative anthropogenic impact on the environment, including impact on the soil, due to quarantine measures.

It is known that signs of recovery can appear in different groups of organisms after the cessation of a negative anthropogenic impact [16, 17, 20]. We were able to identify such changes in the structure of bacterial complexes of soils with a greater or lesser degree of anthropogenic disturbance in the city of Syktyvkar.

During the two-year research, 106 strains of representatives of the families Enterobacteriaceae were isolated from urban soils and identified to species; 9 and 11 species were detected in 2019 and 2020, respectively (Table 3). However, taxonomic diversity differed in different years of the study. In 2019, species of the genera of these families that are opportunistic and aller-

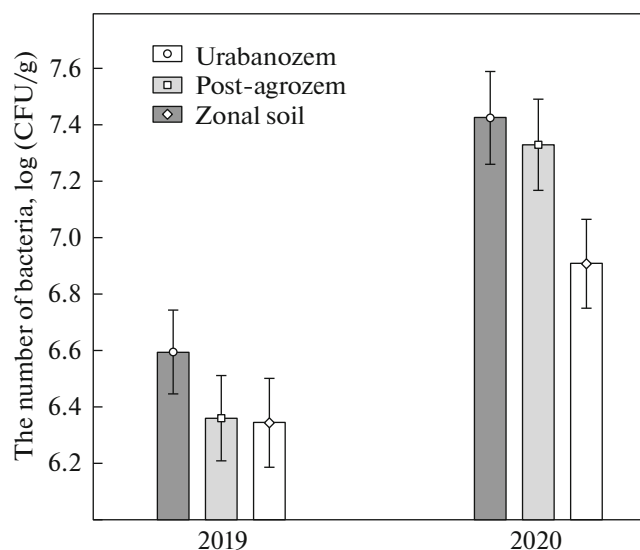


Fig. 1. The number of microorganisms of the saprotrophic bacterial complex (CFU/g) in the 0–10 cm layer of soils (mean values and error of the mean are shown).

genic for humans prevailed (*Escherichia coli*, *Enterococcus faecalis*, *En. faecium*, *En. durans*, and *Enterobacter agglomerans*), while, in 2020, phytopathogenic and saprotrophic species (*Klebsiella planticola*, *Erwinia herbicola*, and *Erwinia salicis*) were predominant (Table 3). In urbanozem samples collected in 2020, the proportion of sanitary-indicative bacteria *E. coli* and *En. faecalis*, which occupy leading positions as indicators of fecal contamination and pose the greatest threat to human health, primarily to persons with a reduced immune status, was lower. In 2019, the share of these species in and enterococcus soils was $>70\%$ among all isolated enterobacteria ($1.2 \pm 0.13 \times 10^6$ CFU/g), in 2020, it was $<10\%$ ($5.8 \pm 0.41 \times 10^5$ CFU/g). Despite the fact that during both years of research, the permissible values of the presence of *E. coli* and *En. faecalis* significantly exceeded assigned standards (MU 2.1.7.730-99. Hygienic assessment of soil quality in populated areas), a decrease in their number indicated an improvement in the epidemic state of urban soil.

Representatives of the family Enterobacteriaceae are characterized by active horizontal gene transfer. The higher the diversity of potentially pathogenic representatives of Enterobacteriaceae, the greater the possibility of transfer of pathogenicity (virulence) plasmids between the strains [22]. As a result, the increased load on the soil as a “bacterial” filter that disinfects pathogens and their toxins makes the urban soil and the environment as a whole more dangerous for humans.

The high number of population, as well as domestic and homeless animals in cities, is accompanied by an increase in the amount of household waste, areas of their disposal and the amount of excrement entering

Table 2. The structure of the cultivated saprotrophic bacterial complex of soils

Soil	Predominant microorganisms	Subdominant microorganisms	Groups of microorganisms with medium abundance and minor components
2019			
Urbanozem	families Enterobacteriaceae, <i>Arthrobacter</i>	<i>Myxococcus</i>	<i>Bacillus</i> , <i>Rhodococcus</i> , <i>Streptomyces</i> , <i>Pseudomonas</i> , <i>Aquaspirillum</i>
Postagrozem	<i>Pseudomonas</i> , <i>Cytophaga</i>	<i>Bacillus</i>	<i>Micrococcus</i> , <i>Myxococcus</i> , <i>Streptomyces</i> , <i>Azorhizophilus</i> , <i>Flavobacterium</i> , <i>Beijerinckia</i>
Undisturbed zonal soil	<i>Pseudomonas</i>	<i>Bacillus</i> , <i>Cytophaga</i>	<i>Cellulomonas</i> , <i>Flavobacterium</i> , <i>Streptomyces</i> , <i>Micrococcus</i>
2020			
Urbanozem	families Enterobacteriaceae, <i>Pseudomonas</i>	<i>Arthrobacter</i>	<i>Flavobacterium</i> , <i>Streptomyces</i> , <i>Bacillus</i> , <i>Myxococcus</i> , <i>Cytophaga</i> , <i>Micrococcus</i> , <i>Rhodococcus</i> , <i>Acinetobacter</i> , <i>Aquaspirillum</i> , <i>Polyangium</i>
Postagrozem	<i>Pseudomonas</i> , <i>Flavobacterium</i>	<i>Streptomyces</i>	<i>Bacillus</i> , <i>Beijerinckia</i> , <i>Cellulomonas</i> , <i>Cytophaga</i> , <i>Ensifer</i> , <i>Micrococcus</i> , <i>Myxococcus</i> , <i>Nesterenkonnia</i> , <i>Azorhizophilus</i> , <i>Aquaspirillum</i>
Undisturbed zonal soil	<i>Pseudomonas</i> , <i>Flavobacterium</i>	<i>Comamonas</i>	<i>Bacillus</i> , <i>Cellulomonas</i> , <i>Cytophaga</i> , <i>Ensifer</i> , <i>Nesterenkonnia</i> , <i>Streptomyces</i> , <i>Micrococcus</i> , <i>Aquaspirillum</i>

Predominant relative abundance > 30%; subdominant 20–30%; groups of average abundance 10–20%; minor components < 10% [6].

the soil. Household and fecal waste are the main sources of soil polluting substances, as well as pathogenic, opportunistic, and allergenic microorganisms and their toxins [3, 4, 7].

Table 3. Relative abundance of Enterobacteriaceae species

Bacterial species	Year	
	2019	2020
<i>Escherichia coli</i> *	50.81	9.66
<i>Enterococcus durans</i>	6.04	–
<i>En. faecalis</i> *	19.44	0.18
<i>En. faecium</i>	9.06	0.14
<i>Enterobacter agglomerans</i>	5.14	4.23
<i>En. tabaci</i>	–	4.85
<i>Erwinia carotovora</i>	–	3.06
<i>Er. herbicola</i>	–	5.34
<i>Er. salicis</i>	–	36.21
<i>Citrobacter europaeus</i>	0.63	–
<i>C. freundii</i>	0.43	–
<i>Klebsiella oxytoca</i>	3.61	–
<i>K. planticola</i>	–	31.09
<i>Serratia marcescens</i>	4.84	2.15
<i>S. nematodiphila</i>	–	3.09

* Sanitary indicative species of microorganisms; “–” not detected.

Such observations were recorded not only for bacteria, but also for ecologically significant groups of other organisms. Long-term studies of the microbiota of urban soils in the Moscow oblast, where complexes of soil mycelial and yeast fungi were the objects of investigation, revealed similar trends in the transformation of the species structure of communities. Compared with the zonal ones, there was a decrease in the content of fungal mycelium and an increase in that of spores, the proportion of dark-colored species of filamentous and yeast fungi, as well as a high level of pathogenic and allergenic species [12, 18].

Thus, in the current nonstandard situation caused by the pandemic, a unique opportunity allowed us to register how a sharp decrease in anthropogenic load on the environment as a whole affects the soil microbiota. Based on the work performed, it was possible to propose the use of such an indicator as the abundance of the cultivated saprotrophic bacterial complex and the taxonomic diversity of the family Enterobacteriaceae as a model for assessing the potential of soil biota for self-remediation.

CONCLUSIONS

The taxonomic composition of the cultivated saprotrophic bacterial complex of the studied soils (urbanozem, the soil of the city park, undisturbed zonal) in 2020 was characterized by a greater abundance at genus level compared to 2019, primarily due to an increase in the diversity of bacteria of groups with medium abundance and minor components.

This is due to a decrease in anthropogenic impact on the environment in 2020 after a long term (more than three months) quarantine in connection with the first wave of the pandemic.

A detailed study of the diversity of the families Enterobacteriaceae demonstrated that the share of pathogenic, opportunistic and allergenic, as well as the most dangerous for humans sanitary indicative species (*E. coli* and *En. faecalis*) in 2020 compared to the that in 2019 decreased markedly. This fact can be considered a probable evidence of the “self-remediation” of the soil in conditions of a decrease in anthropogenic impact on the environment after quarantine. A sharp and prolonged period of decline in the intensity of negative anthropogenic impact demonstrated the presence of a sufficient reparative potential of the cultivated complex of saprotrophic bacteria in the urbanozems of Syktyvkar.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interest.

Statement on the welfare of humans or animals. This article does not contain any studies involving animals performed by any of the authors.

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