Bacterial complexes of urbanozems of some southern cities of Russia

A M Glushakova¹², L V Lysak¹, A B Umarova¹ and I A Maksimova*¹

¹Faculty of Soil Science, Lomonosov Moscow State University, GSP-1, Leninskie Gory, Moscow 119234, Russian Federation
²I. Mechnikov Research Institute of Vaccines and Sera, 5A, Malyi Kazennyi Pereulok, Moscow 105064, Russian Federation

*Email: maximova.irina@gmail.com

Abstract. Urban areas characterized by different intensity of anthropogenic impact on the territory of some southern cities (Krasnodar, Sochi, Simferopol and Maykop) of Russia were studied. Obtained data on the diversity of bacterial complexes of urban soils indicate that in urban areas with high anthropogenic load (Sochi, Simferopol, Krasnodar) there is a significant transformation of the bacterial complex towards increased representation of the family Enterobacteriaceae. In addition to sanitary microorganisms (E. coli, En. faecalis) in the soils of these cities revealed the presence of bacteria of genera Klebsiella, Enterobacter, Citrobacter, Serratia, some species of which can cause intestinal and allergic diseases. Sulphite-reducing clostridium spores, including Clostridium perfringens, have also been found in Sochi urban areas.

1. Introduction

Urban soils differ significantly from zonal soils in chemical, physical and biological properties. This affects the performance of the most important environmental functions by urban soils [1, 2].

Permanent and obligatory inhabitants of soils are microorganisms that play an important role in the circulation of matter in nature. They also determine the state of other components in the ecosystem [3]. The soils are populated by different taxonomic and ecological trophic groups of microorganisms (cellulolytics, nitrogen fixers, ammonifiers, nitrifiers, etc.). In urban soils bacterial complexes undergo redistribution of taxa. Of note is the increase in the Enterobacteriaceae (Escherichia, Enterobacter, Klebsiella) content under household pollution of the soil. Among them many species are pathogenic, opportunistic, or allergenic that can pose threat to human health [4]. However, a comprehensive assessment of changes in bacterial community together with an assessment of the sanitary state of urban soils have not yet been carried out. Obviously, such a complex research can become a promising system for further monitoring and bioindication of the urban environment as a whole [5].

The aim of this work was to describe bacterial communities of urbanozems on the territory of the southern cities of Sochi, Simferopol, Krasnodar and Maykop that vary in the intensity of anthropogenic load.
2. Materials and methods

Samples of urbanozems collected in Krasnodar, Maykop, Simferopol and Sochi were studied. The soils were represented by urbanozems with inclusions of household and constructional materials, and at the same time they had signs of zonality and attributes of the deposits on which these soils were formed. In Krasnodar, they were represented by urbo-chernozem on carbonate loess-like loams (WRB – Calcic Chernozem (Technic)), in Maykop – by technogenic urbo-stratozem underlain by the alluvium of river terraces (WRB – Urbic Technosol), in Simferopol – by dark humus on technogenic deposits (WRB – Urbic Technosol), in Sochi – by urbo-stratozem underlain by concrete slabs (WRB Isolatic Urbic Technosol). As one of the controls less disturbed soils of botanical gardens located in the central districts of Krasnodar, Simferopol, and Sochi were studied. They were represented by the following soils: heavy loamy chernozem on carbonate loess-like loams (WRB – Calcic Chernozem), dark humus stratozem on crushed stone deluvium (WRB – Skeletic Regosol) and gleyic pebble cheltozem (WRB – Stagnic Alisol), respectively. The undisturbed suburban soils a few kilometers from the city line were chosen as another control. For Krasnodar, such control soil was represented by agro-chernozem on carbonate loess-like loams (WRB – Calcic Chernozem), for Maykop – dark merged soil on the sediments of the river terrace (WRB – Leptic Vertisol), and for Simferopol – agro-chernozem on the gravel deluvium (WRB – Gleyic Chernozem).

Soil sampling was carried out in 2019 in the summer-autumn period at depths of 0–10, 30–50 and 60–70 cm. In total, 49 mixed samples were analyzed.

The abundance and taxonomic structure of cultivated saprotrophic bacteria at the genus level were determined by the classical method of plating. The calculation was made per gram of absolutely dry soil. The standard PYG medium was used. Identification of bacteria to the genus level was carried out based on phenotypic features according to the key for identification of soil bacteria and generally accepted reference books [6], Bergey’s manual of systematic bacteriology. Statistical processing of data on the absolute and relative abundance of bacteria was carried out using the programs Microsoft Office Excel 2010 and Statistica 8.0. The determination of clinically significant microorganisms was carried out in accordance with the guidelines of the Federal Center of the Federal Service for Sanitary and Epidemiological Surveillance of the Ministry of Health of Russia (MU 2.1.7.730-99 Hygienic assessment of soil quality in populated areas). In soil samples, the abundance of the Escherichia coli group and Enterococcus, the presence of sulfite-reducing Clostridia spores (including Clostridium perfringens) in accordance with the methodological recommendations (Methods of microbiological control of the soil. Methodological recommendations No. FC/4022-2004) were determined.

The identification of enterobacteria to the species level was carried out based on the analysis of the v3v4 variable sequence of the 16S rRNA region. A total of 108 strains were identified.

3. Results

The abundance of cultivated saprotrophic bacteria in all the studied soils was greatest at a depth of 0–10 cm in horizons A1 and U1. In the upper humus horizons A1 and in the horizon U1 of urbanozems, it comprised 10^6 CFU/g. In urbanozems, the abundance of bacteria in the horizon U1 was slightly higher than in horizons A1. In Sochi, the abundance of bacteria in the horizon U2/U3 was significantly (by an order of magnitude) higher than in the corresponding horizon of other studied urbanozems and in the horizon A1/AB of control soils. As expected, the deeper in the profile of control soils and urbanozems, the lower the abundance of bacteria was. Minimum abundance values were recorded at depths of more than 60 cm (horizons AB/B and U3/U5).

Thus, the distribution of bacteria within the soil profile was generally consisting in a decrease in the abundance with transition from the upper to the lower horizons.

The taxonomic structure of bacterial complex in urbanozems had specific features. In the upper horizons, representatives of Enterobacteriaceae and Arthrobacter dominated, which indicates the presence of household and constructional pollution. The structure of the saprotrophic bacterial complex in urban and control soils was characterized by a similar composition in all cities studied. It was close to the general ecological patterns in the distribution of taxonomic diversity of bacteria in soils (table 1).
Table 1. Saprotrophic bacteria complexes structure of urbanozems, soils of botanical gardens and suburbs in the studied cities.

| Soil                  | Soil by profile | Dominants                        | Subdominants                  | Mid-relative abundance group |
|-----------------------|-----------------|----------------------------------|--------------------------------|-----------------------------|
| Urbanozems            | U1, 010 cm      | Enterobacteriaceae               | Bacillus, Myxococcus          | Rhodococcus                 |
|                       | U2/U3, 3050 cm  | Arthrobacter                     | Myxococcus                    |                             |
|                       | U3/U5, > 60 cm  | Arthrobacter                     | Rhodococcus                   |                             |
| Soils of botanical    | A1, 010 cm      | Pseudomonas                      | Myxococcus                    | Streptomyces                |
| gardens               | A1/AB, 3050 cm  | Bacillus                         | Cellulomonas                  |                             |
|                       | AB/B, > 60 cm   | Bacillus                         | Streptomyces                  |                             |
| Undisturbed suburban  | A1, 010 cm      | Pseudomonas                      | Cytophaga                     | Streptomyces                |
| soils                 | A1/AB, 3050 cm  | Bacillus                         | Cellulomonas                  |                             |
|                       | AB/B, > 60 cm   | Bacillus                         | Cytophaga                     |                             |

Determination of clinically significant species *Escherichia coli* in urbanozems (table 2) revealed the minimum excess (< 10 CFU/g) in the horizon U1 (34 CFU/g) in Krasnodar, the maximum excess – in Sochi in the horizons U1 (96 CFU/g) and U2 (82 CFU/g), and in Simferopol in the horizons U1 (68 CFU/g) and U2 (46 CFU/g) as well. The clinically significant *Enterococcus faecalis* was characterized by the minimum excess (14 CFU/g) in Krasnodar in the horizon U1 and the maximum excess in Sochi (38 CFU/g) in the horizons U1 and U2 (15 CFU/g). The perfringens titer (the smallest amount of soil in grams, in which a single viable *C. perfringens* cell is identified) for all the studied urbanozems, with the exception of the urbanozem in Sochi (U1) did not exceed permissible normative values (≤ 0.01 g). In Sochi, it comprised 0.02 g. Deeper in the profile (depth over 60 cm) in urbanozems and in all horizons of control soils clinically significant microorganisms were not found. Excessive values of clinically significant microorganisms indicate that either a recent or long-ago (perfringens titer) fecal contamination of urbanozems took place.

The content of clinically significant microorganisms in urbanozems in Sochi and Simferopol allows to assess their sanitary condition as moderately hazardous [Methods of microbiological control of the soil. Methodological recommendations No. FC/4022–2004] in the horizon U1 at a depth of 0–10 cm. It should be noted that in Sochi the values of sanitary indexes were especially high. This is a result of both a significant population density and a high anthropogenic load on the urban environment in the largest resort city of Russia.
Table 2. The content of clinically significant microorganisms in the studied urbanozems.

| Soil by profile | Enterococcus Index, CFU/g | E. coli Index, CFU/g |
|----------------|---------------------------|---------------------|
| **Sochi**      |                           |                     |
| U1 0–10 cm     | 38±0.22                   | 96±0.37             |
| U2 30–50 cm    | 15±0.54                   | 82±0.41             |
| U3 > 60 cm     | n.d.*                     | n.d.                |
| **Simferopol** |                           |                     |
| U1 0–10 cm     | 16±0.76                   | 68±0.37             |
| U1 30–50 cm    | 9±0.11                    | 46±0.43             |
| U3 > 60 cm     | n.d.                      | n.d.                |
| **Krasnodar**  |                           |                     |
| U1 0–10 cm     | 14±0.11                   | 34±0.19             |
| U2 30–50 cm    | 2±0.10                    | 4±0.12              |
| U3 > 60 cm     | n.d.                      | n.d.                |
| **Maykop**     |                           |                     |
| U1 0–10 cm     | n.d.                      | n.d.                |
| U2 30–50 cm    | n.d.                      | n.d.                |
| U3 > 60 cm     | n.d.                      | n.d.                |

* n.d. – not detected.

A high city population is accompanied by an increase in the amount of household waste, zones for their disposal in the urban environment and the amount of excrements of domestic animals entering the soil. It is household and fecal waste that is the main source of soil pollutants, as well as allergenic, pathogenic and opportunistic microorganisms [7–12].

The species identification of isolated clinically significant microorganisms conducted by sequencing the 16S rRNA gene also demonstrated the presence of opportunistic species in the studied urbanozems in Sochi, Simferopol and Krasnodar. In addition to E. coli and En. faecalis which occupy leading positions as indicators of fecal contamination and pose the greatest threat to human health, among the isolated bacteria there were representatives of the genera Klebsiella, Enterobacter, Citrobacter, Serratia (table 3). A significant number of species of these genera can also cause intestinal and allergic diseases, mainly in people with low immune status.

Table 3. The relative abundance (%) of E. coli, En. faecalis and other bacteria isolated from the studied urbanozems.

| Genera and species of bacteria | Sochi | Simferopol | Krasnodar | Maykop |
|-------------------------------|-------|------------|-----------|--------|
| E. coli                       | 58.7  | 52.5       | 26.5      | –      |
| E. faecalis                   | 11.5  | 10.0       | 8.0       | –      |
| E. faecium                    | 5.3   | 4.7        | 2.2       | –      |
| E. durans                     | 3.2   | 1.3        | 1.1       | –      |
| Klebsiella spp.               | 7.1   | 11.2       | 11.4      | –      |
| Enterobacter spp.             | 5.1   | 10.5       | 12.1      | –      |
| Citrobacter spp.              | 3.2   | 6.1        | 4.1       | –      |
| Serratia spp.                 | 2.2   | 1.2        | –         | –      |
| Others                        | 3.6   | 2.6        | 34.6      | 100.0  |
The spores of sulfite–reducing *Clostridia* found in the horizon U1 of the city of Sochi and represented mainly by the species *Clostridium perfringens* indicate fecal contamination that occurred a long time ago.

The soil as the habitat of microorganisms is known to be closely interconnected with air and water environments. It is obvious that an urbanozem with a high content of clinically significant opportunistic and pathogenic microorganisms will have a negative effect on other environments.

4. Conclusion
To study the diversity of microbial complexes in urbanozems is of considerable interest not only from fundamental science point of view. But also, in practical terms due to the important role that microorganisms play in formation and maintenance of the sustainability of urban ecosystems.

Acknowledgements
This research was conducted as a part of the project "Soil microbiomes: genomic diversity, functional activity, geography and biotechnological potential" No. 121040800174-6 and was also supported by a grant from the Russian Foundation for Basic Research No. 19-29-05252 mk.

References
[1] Lysak L V and Lapygina E V 2018 The diversity of bacterial communities in urban soils *Eurasian Soil Sci.* 51(9) 1050–6
[2] Braun B, Bockelmann U, Grohmann E and Szewzyk U 2006 Polyphasic characterization of the bacterial community in an urban soil profile with in situ and culture-dependent methods *Appl. Soil Ecol.* 31(3) 267–79
[3] Dobrovol’skaya T G, Zvyagintsev D G, Chernov I Yu, Golovchenko A V, Zenova G M, Lysak L V, Manucharova N A, Marfenina O E, Polyanskaia L M, Stepanov A L and Umarov M M 2015 The role of microorganisms in the ecological functions of soils *Eurasian Soil Sci.* 48(9) 959–67
[4] Lysak L V and Sidorenko N N 1997 Species diversity of rhodococci in urban soils *Microbiol.* 66(4) 480–1
[5] Terekhova V A 2011 Soil bioassay: problems and approaches *Eurasian Soil Sci.* 44(2) 173–9
[6] Lysak L V, Dobrovol’skaya T G and Skvortsova I N 2003 Methods for assessing bacterial diversity of soils and identification of soil bacteria (Moscow: MaxPress) p 120 (in Russian)
[7] Lysak L V 2010 Bacterial communities of urban soils extended *Thesis doctor of science (biology) dissertation* (Moscow: MSU) p 46 (in Russian)
[8] Zalar P, Novak M, de Hoog G S and Gunde-Cimerman N 2011 Dishwashers – a man-made ecological niche accommodating human opportunistic fungal pathogens *Fungal Biol* 115 997–1007
[9] Horner-Devine M C, Carney K M and Bohannan J M 2004 An ecological perspective on bacterial biodiversity *Proc. R. Soc. Lond.* 271 113–22
[10] Karlen D L, Andrews S S and Doran J W 2001 Soil quality: current concepts and applications *Advanc. Agron.* 74 1–39
[11] Puskas I and Farsang A 2009 Diagnostic indicators for characterizing urban soils of Szeged, Hungary *Geoderma* 148(3-4) 267–81
[12] Andreoni V, Cavalca L, Rao M A, Nocerino G, Bernasconi S, Dell’Amico E, Colombo M and Gianfreda L 2004 Bacterial communities and enzyme activities of PAHs polluted soils *Chemosphere* 57 401–12