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Article

Analysis of the quantitative composition of microbial soil communities of Khabarovsk city using "Izyskatel DV" software

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Abstract. The results of the analysis of soil samples taken in various functional zones of Khabarovsk in 2022–2023 using the specialized software for environmentalists “Izyskatel DV” are presented. An analysis of the number of microscopic soil fungi (micromycetes), actinomycetes and bacteria demonstrates that soils of the Khabarovsk industrial zone are characterized by a predominance of dark-colored micromycetes and actinomycetes, while soils of the residential zone by bacteria presence. The possibility of effective application of the developed software product is shown not only for chemical, but also for microbiological indicators of soil pollution. It also enables to identify soils with the unique quantitative and qualitative composition of the microbiota, with minimal time.

Keywords: soil microorganisms, transformed soils, database, data array analysis

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Научная статья

Анализ количественного состава микробных сообществ почв г. Хабаровска с использованием программного обеспечения «Изыскатель ДВ»

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Аннотация. Представлены результаты анализа почвенных проб, отобранных в различных функциональных зонах г. Хабаровска в 2022–2023 гг., с помощью специализированного программного обеспечения для экологов «Изыскатель ДВ». Анализ количества почвенных микроскопических грибов (микромицетов), актиномицетов и бактерий продемонстрировал, что для почв промышленной зоны Хабаровска характерно преобладание количества темноокрашенных микромицетов и актиномицетов, а для почв жилой зоны – бактерий. Показана возможность эффективного применения разработанного программного продукта не только для химических, но и микробиологических показателей загрязнения почв. С его помощью также стало возможным выявление почв, обладающих уникальным количественным и качественным составом микробиоты, при минимальных временных затратах.

Ключевые слова: микроорганизмы почв, преобразованные почвы, база данных, анализ массива данных

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Introduction

Influenced by urbanization, all components of the natural environment undergo transformations. Atmospheric air, surface and underground water sources, soils change, as a rule, under the influence of substances alien to this system.

The main anthropogenic pressure from the very beginning of almost any economic activity falls on soils, which serve as a key depositing medium and experience chemical and mechanical effects. Soils are a complex special natural formation that combines elements of both living and inanimate nature. Wildlife in the soil complex is represented by soil biota, consisting of macro-, meso- and microbiota. The soil microbiota is the most diverse community in terms of species, mainly determining the qualitative characteristics of the entire soil complex.

Under the influence of urbanization, soils transform resulting in waterlogging, salinization, erosion, pollution by organic and inorganic substances, which inevitably affects the soil microbiota. At the same time, urban soil is transformed unevenly depending on the type of its use (Mirchink, 1988).

Much attention has been paid by researchers to the development of a biotic concept for environmental quality control of natural and man-made ecosystems since sanitary and hygienic standards based on chemical methods of soil analysis are insufficient for a comprehensive assessment of the ecological state of soils (Astanin et al., 2024). In recent years, research in the field of biological control has been experiencing a period of particularly active development.

Bioindication is one of the most promising methods for determining the ecological state of a biocenosis, which makes it possible to identify the unique features of the environment under study. Plants and animals are usually employed as bioindicators in situ, but their use can only approximate environmental pollution (Shumilova, 2012). Representatives of fungi and bacteria give completely different results. They demonstrate significantly higher accuracy in predicting the state of the environment, ensuring determination both chemical pollution and physical characteristics of the studied soils. In particular, the study of the bioindication significance of mycological and microbiological indicators (quantitative and qualitative) reveals the anthropogenic impact on soils of the urban environment (Nazarenko and Svistova, 2016).

The phenomenon of accumulation of melanin-containing fungi has been repeatedly noted for soils of urban (urbanozems) and man-made territories. The increase in the proportion of dark-colored fungi is probably due to the known resistance of some species to a number of pollutants, such as radionuclides, heavy metals, etc. Consequently, the predominance of forms with dark-colored mycelium in the structure of soil micromycete populations (more than 60-70%) is an indicator of chemical contamination of soil (Zhao et al., 2017).

Mycoindication is actively used to assess the condition of soils under their chronic and acute contamination with substances of various chemical nature. Disturbances in the structure and composition of soil microbiocenoses appear earlier than changes in physico-chemical properties of soil and even when the content of pollutants is below the maximum permissible concentration (Khabirova et al., 2022).

To date, a large amount of materials has been accumulated on the study of microbiological activity of agricultural soils since the qualitative and quantitative characteristics of microbial communities directly affect soil fertility (Emtsev and Mishustin, 2020; Khabirova et al., 2022). At the same time, much less attention is paid to soils of the urban area, especially in the Russian Far East; in particular, in Khabarovsk, such work was carried out for the first time at the Institute of Water and Environmental Problems of the Far Eastern Branch of the Russian Academy of Sciences (Kiriyenko and Imranova, 2008).

In the course of studies of the quantitative and qualitative composition of the soil microbiota, it is necessary to process a data bulk when calculating the amount of CFU/g of absolutely dry soil. At the same time, the work is carried out with sufficiently large numbers (from tens of thousands to millions), which can lead to accidental errors. To minimize the likelihood of such errors, the software «Izyskatel DV» has been developed, which is an ecosystem and includes a set of programs and plug-ins (Barkhatov, 2023). In addition to performing calculations searching for statistical errors, and outputting final values, this software has the ability to visualize source data in the form of maps and various dependency graphs. With its help, it is possible to output the source data in several formats:

• all points on the interactive map at the sampling location, the point size corresponds to the weight of the indicator;

• points of maximum and minimum values on the interactive map;

• points filtered by certain characteristics – functional zone, soil type, year of sampling;

• the final report in PDF format with a complete list of indicators, coordinates of sampling points in the WGS-84 and MSC system, a description of soils and areas of the studied territories, graphs of the dependence of indicators on the user's choice.

In addition to the microbiological parameters of soils, the «Izyskatel DV» software allows you to work with any indicators of other natural environments: air, water, bottom sediments. The inherent advantages of this software are the simplicity of the interface and accessibility, as it is developed on the basis of the Microsoft Excel program.

At the moment, the practical significance of the software «Izyskatel DV» lies in the formation of databases based on design and working documentation within the framework of engineering and environmental surveys, combined in the ecosystem of the software «Izyskatel DV» (Barkhatov, 2023). Its advantage is in the reliability of user databases created from the engineering survey reports, as these reports have passed government examinations at various levels. You can learn more about the program in the work "Creation of specialized software for environmentalists «Izyskatel DV». Another advantage of this software is the ability to visualize tabular data, perform analytics on indicators, and make various kinds of samples that similar databases do not provide (Barkhatov, 2023).

Thus, the «Izyskatel DV» allows to

1) Systematize the scattered data on the granulometric and chemical composition of soils of the Far Eastern Federal District.

2) Calculate the total soil pollution and pollution indices.

3) Perform visualization by localities and sampling points with the use of estimated parameters.

4) Generate a report with an analytical reference of the point for the parameter under study.

The purpose of this work is to assess the state of mycological communities of soils in various functional zones of the city of Khabarovsk using the specialized software «Izyskatel DV».

Materials and methods

Sampling was carried out in 2022–2023 in three main functional zones of Khabarovsk: residential, industrial and recreational. Five samples were collected in each zone.

The combined sample was obtained as ten point samples taken from a depth of up to 15 cm by the envelope method. The selection was made according to GOST 17.4.4.02-2017[1] with a sterile spatula in sterile plastic bags (Zvyagintsev, 1980). Sterilization was carried out by chemical method.

Recreational areas in Khabarovsk were represented by green areas of the parks: Muravyov-Amursky Park, Dynamo Park, Severny Park (a green area with artificial plantings), Severny Park (an economically undeveloped part), Gagarin Park. The selected sampling sites were characterized by a fairly thick lower vegetation layer, as well as the presence of an upper layer of typical urban plantings. Growing communities are characterized as euhemerotic (Roslikova, 2019).

The residential area consisted of green areas at 158, Tikhookeanskaya St. and 43A Dikopoltseva

[1] GOST 17.4.4.02-2017. Soils. Methods of sampling and preparing samples for chemical, bacteriological, and helminthological analysis.

St., as well as 3 playgrounds in the Bolshaya Vyazemskaya microdistrict in the territories adjacent to kindergartens and schools. Similar to urban areas, the vegetation of residential areas is represented by eugemic communities (Roslikova, 2019).

The industrial zone of Khabarovsk is represented by territories subject to active anthropogenic impact. Sampling sites were located within:

1) the green buffer zone adjacent to the fence of the Khabarovsk Oil Refinery on Metallistov St.; only a low-power grass tier is present here;

2) the territory that has been clogged with construction debris and 15 year litter from roads nearby 38A, Voronezhskaya St.; vegetation is absent;

3) the local accumulation of waste at the private sector within the boundaries of the Lukashov nursery; vegetation is absent;

4) the territory of the existing ash dump of Khabarovsk CHPP №3; an area with assimilated vegetation represented by synanthropic grasses;

5) the territory of an abandoned poultry farm at 8 Yuzhnaya St.; the grassy and woody tiers are expressed, represented by urban synanthropic self-seeding.

A map of the location of sampling points is given in Fig. 1. Table 1 shows the characteristics of the selected soil samples, compiled based on the results of a field reconnaissance survey, as well as subsequent laboratory processing. The soil type was determined in the field by rolling according to the method of N.A. Kachinsky (Ganzhara, 2002).

In this work, the classical Koch method was used – the determination of the number of cells by seeding on dense nutrient media (Segi, 1983). This method is widely used to calculate the number of viable cells of cultured microorganisms in various natural substrates and in laboratory cultures. Each colony on a nutrient plate grows from a single colony-forming unit (CFU), which can be a bacterial, yeast cell, spore, mycelium of an actinomycete or micromycete (fungus). Thus, the obtained number of colonies that grew after sowing on a dense nutrient medium of a certain volume of the studied soil extract allows us to judge the initial content of colony-forming units of cultivated microorganisms in it.

A soil water extract was prepared from fresh unpolished soil samples. The soil suspension (10 g of soil per 90 ml of water) was mixed for 10 minutes and sown from the soil extract onto dense nutrient media according to the method of serial dilution. To count the number of bacteria and fungi, 1:100000 and 1:1000 dilutions were used. Since each soil sample has field moisture, the results are recalculated per 1 g of dry soil (Sagi, 1983; Zvyagintsev, 1980).

For cultivation of microorganisms, we used 5 types of solid nutrient media on agar: fish peptone agar (universal for most types of aerobic bacteria, as well as actinomycetes), fish peptone agar diluted 10 times (universal for most types of aerobic bacteria), Ashby medium (for the cultivation of nitrogen-fixing bacteria), starch-ammonia agar (as a universal medium for bacteria and actinomycetes), Chapek-Doks medium (for the cultivation of mold fungi) (Mirchink, 1988; Sagi, 1983).

The weight method, GOST 28268-89[2], was used to determine soil moisture. The determination of the acidity of soil samples was carried out using the Infraspak-Analyte-Anion 7000 pH meter, Russia.

Results and discussion

The amounts of CFU/g a.d.s. obtained as a result of laboratory studies for different groups of microorganisms, averaged over three replications and nutrient media, are shown in Table 2. However, such data series in tabular or graphical representation are inconvenient for conducting analytics. For a more visual presentation of the results, we used a specialized application for environmentalists «Izyskatel DV» developed for processing, visualizing and analyzing user databases within the framework of environmental monitoring of environmental components.

To use the program, input the initial data, including the results of field and laboratory studies. Descriptions of the test site, sampling conditions and the type of study soils are recorded in a field log during field works. Then the information is transferred to the appropriate columns in the input data field. According to the results of laboratory tests, the moisture measurement data of the soil sample and the number of counted colonies are recorded. Fig. 2 shows the main plug-in screen «Soils-Khabarovsk» of the Ecologist program, which is part of the ecosystem «Explorer of the Far East».

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[2] GOST 28268-89. Soils. Methods for determining moisture content, maximum hygroscopic moisture content, and wilting point of plants.

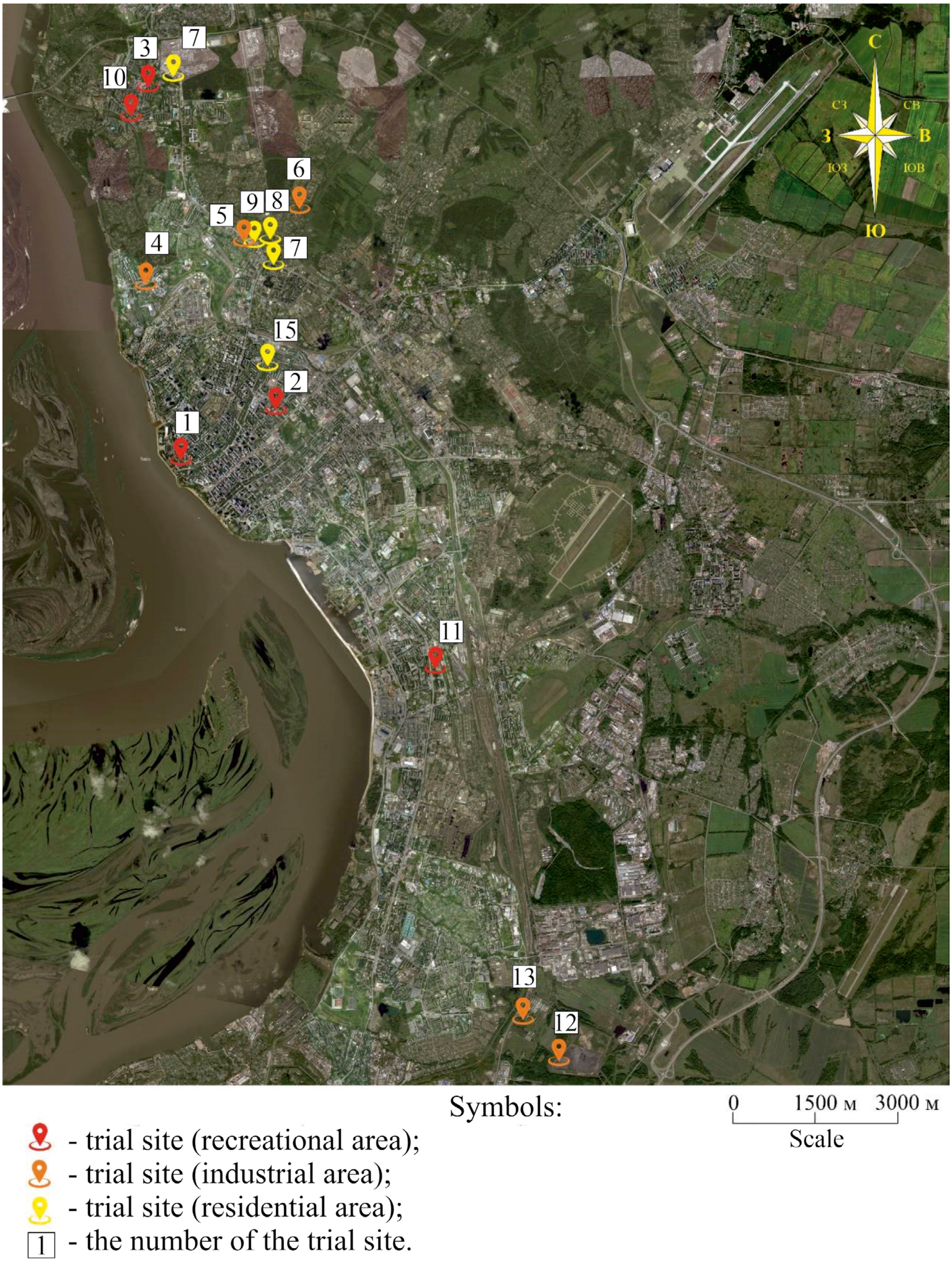


Fig. 1. Map of soil sampling points.

Table 1. Characteristics of the selected soil samples.

Sample no.	Location	Soil type	Color	Inclusions	Date of sampling	pH
Recreational area						
1	Muravyov-Amursky Park	Light loam	Brown	Thin filamentous roots	16.06. 2022	6.5
2	Dynamo Park	Light loam	Dark brown	Clay inclusions	16.06. 2022	7.5
3	Severny Park (developed part)	Medium loam	Light brown	Clay inclusions	16.06. 2022	7
10	Severny Park (undeveloped part)	Clay	Red	Large clay inclusions, representatives of the soil mesofauna	05.05. 2023	8
11	Park named after Gagarin	Medium loam	Brown	Filamentous roots, large stones	06.05. 2023	8
Industrial area						
4	Oil refinery factory, Metallistov St.	Sandy loam	Brown	Large stones, crushed stone, roots	22.06. 2022	7.5
5	CK Galaktika, Chekhov 22	Heavy loam	Light brown	Crushed stone, grus, crushed concrete, brick	22.06. 2022	7.5
6	The Lukashov Nursery, clutter	Medium loam	Light brown	Crushed stone, grus, crushed concrete, brick	22.06. 2022	7
12	TPP-1 ash dump	Sandy loam	Black	Ash and slag layer at a depth of 3 cm from the soil surface	05.05. 2023	7.5
13	Poultry farm, Severnaya St., 8	Sandy loam	Black	Crumbs of plastic waste, river shells, grus	05.05. 2023	7.5
Residential area						
7	Chekhov St. 1a	Heavy loam	Dark brown	Dense root system	27.06. 2022	7
8	Bolshaya St. 95a	Sandy loam	Black	Large lobed roots	27.06. 2022	8
9	Chekhov St. 22	Sandy loam	Dark gray	Rare roots, representatives of the soil mesofauna	27.06. 2022	8
14	Tikhookeanskaya 158	Medium loam	Gray	Crushed stone, representatives of the soil mesofauna	08.05. 2023	7
15	Kim Yu-jong 45a	Medium loam	Brown	Food and household waste	11.05. 2023	7

Table 2. The number of CFU/g of absolutely dry soil (average ± error of average) in the soils of Khabarovsk.

Sample no.	million CFU/g				thousand CFU/g	
	Amylolytic bacteria	Nitrogen fixing bacteria on low amounts of nitrogen	Heterotrophic bacteria	Actinomycetes	Pigmented micromycetes	Micromycetes
Recreational area						
1	0.56 ± 0.2	0.64 ± 0.3	0.7 ± 0.1	0.3 ± 0.1	1.19 ± 0.1	15.1 ± 2.6
2	12.44 ± 2.5	7.22 ± 0.6	12.17 ± 1.6	1.11 ± 0.1	2.38 ± 0.5	7.13 ± 0.6
3	0.95 ± 0.2	1.2 ± 1.2	1.34 ± 0.2	0.32 ± 0.1	2.38 ± 0.5	7.02 ± 1.9
10	7.02 ± 0.3	8.5 ± 1.1	6.88 ± 0.3	0.23 ± 0.2	2.67 ± 0.5	6.93 ± 0.9
11	2.52 ± 0.4	5.82 ± 1.3	2.6 ± 0.9	0.32 ± 0.1	1.34 ± 0.3	3.91 ± 1.7
Industrial area						
4	1.88 ± 0.7	1.03 ± 0.4	3.6 ± 0.6	0.96 ± 0.1	3.72 ± 0.7	7.71 ± 3.4
5	3.31 ± 1.8	4.27 ± 1.8	7.86 ± 0.9	0.28 ± 0.2	2.48 ± 0.5	5.51 ± 1.8
6	1.68 ± 0.6	1.3 ± 0.5	3.59 ± 0.9	0.18 ± 0.1	0	1.89 ± 0.6
12	11.24 ± 0.9	10.28 ± 2.9	8.57 ± 1.1	0.51 ± 0.1	3.85 ± 0.8	7.37 ± 2.9
13	2.4 ± 0.3	7.78 ± 1.9	4.28 ± 0.3	3.11 ± 0.6	21.84 ± 1.5	29.79 ± 5.1
Residential area						
7	5.25 ± 3.6	1.66 ± 0.5	4.48 ± 0.7	0.24 ± 0.1	0	3.95 ± 2.1
8	2.25 ± 1.4	1.15 ± 0.3	2.99 ± 0.1	0.59 ± 0.2	2.07 ± 0.3	4.94 ± 1.1
9	5.4 ± 0.9	3.79 ± 0.8	3.91 ± 0.1	1.42 ± 0.3	2.07 ± 0.3	4.74 ± 1.3
14	13.87 ± 1.2	6.98 ± 0.9	37.15 ± 0.1	0.32 ± 0.1	2.52 ± 0.6	7.02 ± 0.6
15	8.97 ± 0.7	14.97 ± 1.3	8.08 ± 1.4	0.73 ± 0.1	2.52 ± 0.5	6.41 ± 2.4

In the right half of the main screen there is a map of Khabarovsk with marked soil sampling points, and in the left there is a list of filters for selection of required indicators. The size of the point is directly proportional to the value of the selected indicator (in this case, the number of CFU/g of soil). It can be seen that the value of this indicator is maximal at point 13, therefore, it is necessary to analyze its specificity. Using the "Analytics" button, an extract is automatically generated at point 13, which includes a description of this point (Fig. 3). The extract provides the basic information about the location of the sampling point, the date and time of sampling, and soil description. As can be seen, the soils of point 13 contain a variety of man-made inclusions, as well as a light mechanical composition with an alkalinized environment that is unusual for the natural composition of this area. Thus, it can be concluded that the studied soils are highly disturbed.

Fig. 3 shows the information on the amount of CFU/g of a.d.s. at point 13. Previously operating poultry farm is not currently functioning in the study area. There is a pronounced dominance of the number of actinomycetes and micromycetes (the maximum in this indicator among all points) and suppression of bacterial growth along with an alkaline reaction of the medium (8 pH units). This also indicates a violation of the ecological balance in the soils of the studied area, which may be due to some factors specific to this type of enterprise, for example, the introduction of fresh bird droppings into soil, which has a pH level close to 7.0 (Saken and Fatkulin, 2020).

In addition, there is a high content of dark-colored (pigmented) micromycetes in the soils of the industrial zone (Suchkova, 2023). This pattern is confirmed by the works of other authors (Tatarinov and Dolgin, 2010; Zhao and Shirokikh, 2016). A point with an extremely high content of dark–colored micromycetes was identified – no. 13. A high content of pigmented actinomycetes was also noted at this point compared to other soils (the amount was not taken into account).

The software «Izyskatel DV» enables the data samples' formation from a common array, number and type-based ranking of microorganisms by categories (city, district, functional zones), determination

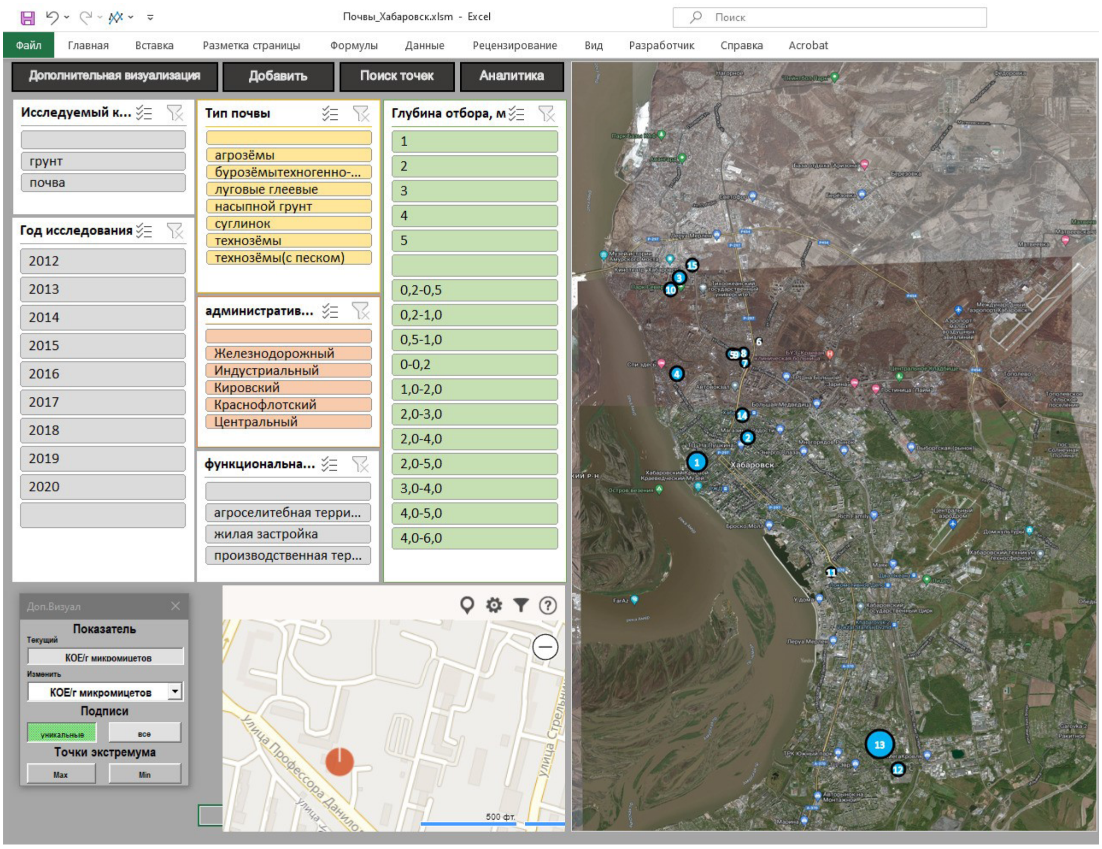
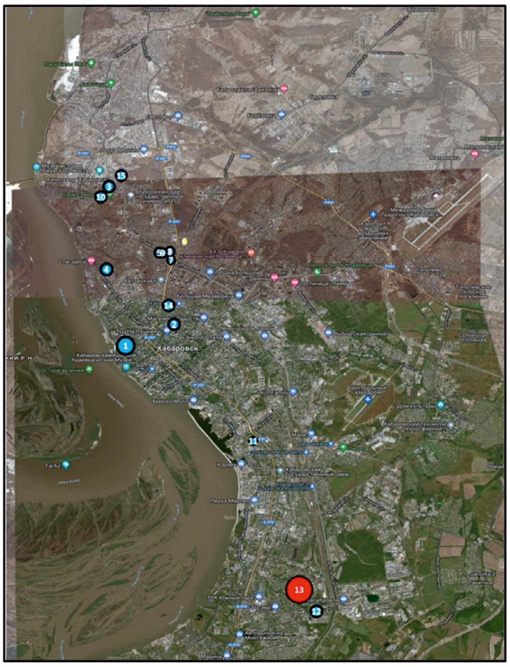


Fig. 2. Main screen of the plug-in "Soils-Khabarovsk" of the Ecologist program, ecosystem "Prospector DV".

Выписка по точке 13 06.12.2023

Местоположение:

Точка 13 расположена на территории населенного пункта (г. Хабаровск) по адресу: р-н индустриальный, ул. Северная д. 8.



● - исследуемый объект

Дата отбора: 05.05.2023
Время отбора: 14:25

Метеусловия на момент отбора:

Метеорологические условия			
Облачность	Температура воздуха	Влажность воздуха	Скорость
Малооблачно	12ºС	40%	2 м/с

Объект изысканий:

Район заброшенной птицефабрики

Описание почв:

Почвы представлены урбаноземами супесчаного характера, профиль нарушен. Отмечаются техногенные включения, такие как: крошка пластиковых отходов, речные ракушки, дресва, рНводн. 8.

Координаты точки:	N48°23'00,8712" ; E135°08'07,2755"
Координаты (десятич.):	48,38357 ; 135,135354
Функциональная зона:	промышленная зона

Ниже в таблице 1 представлены показатели по точке:

Таблица 1

Параметр					
km, млн. КОЕ/г				km, тыс. КОЕ/г	
Бактерии азотфиксаторы	Бактерии азотфиксаторы на малом количестве азота	Бактерии гетеротрофы	Актино-мицеты	Пигментирован-ные микромицеты	Микроми-цеты
Значение km в исследуемой точке 13, млн. КОЕ/г					
2,4±0,3	7,78±1,9	4,28±0,3	3,11±0,6	21,84±1,5	29,79±5,1
Максимальное значение в общем диапазоне точек (1-15)					
13,87±1,2	14,97±1,3	37,15±0,1	3,11±0,6	21,84±1,5	29,79±5,1
Минимальное значение в общем диапазоне точек (1-15)					
0,56±0,2	0,64±0,3	0,7±0,1	0,18±0,1	0	1,89±0,6

На графиках (рисунок 1) представлены минимальные, максимальные и фактические значения (13) km, КОЕ/г микромицетов, бактерий, актиномицетов по диапазону точек (1-15), фактические значения представлены по точке 13.

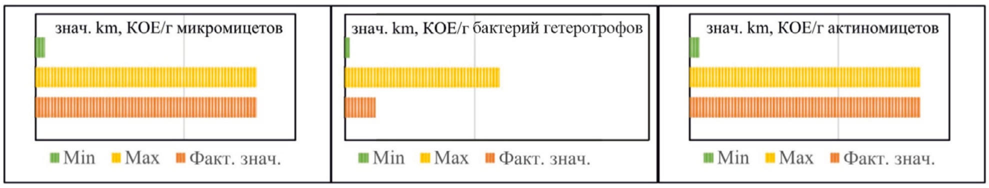
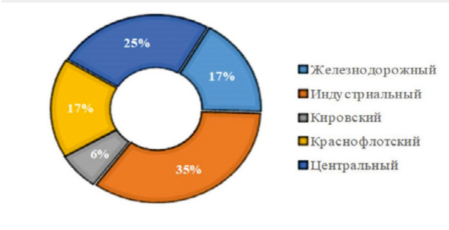


Рисунок 1 - графики максимальных, минимальных и фактичечих значений km, КОЕ/г

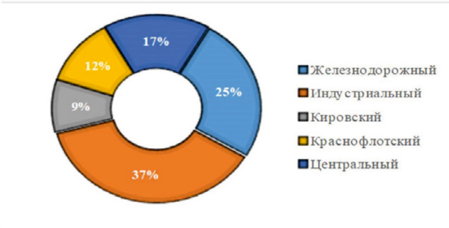
Fig. 3. Report on point 13, of the plug-in "Soils-Khabarovsk".

Общая аналитическая справка по диапазону 1-15 06.12.2023



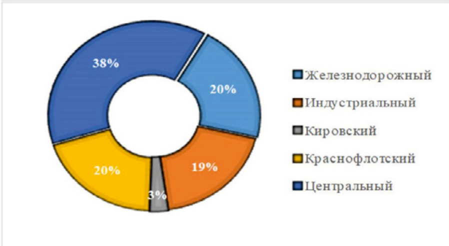
Сумма km, тыс. КОЕ/г микромицетов по районам

район	Сумма km
Железнодорожный	2,040
Индустриальный	4,106
Кировский	0,771
Краснофлотский	2,036
Центральный	2,925



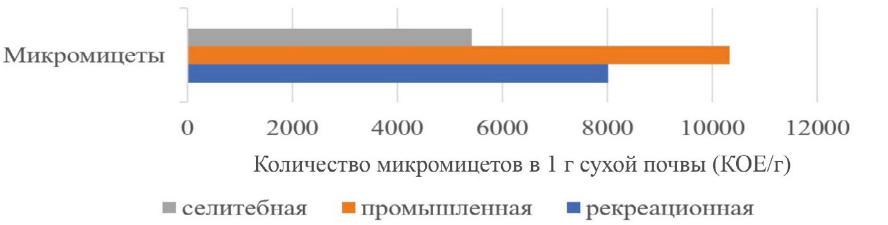
Сумма km, млн. КОЕ/г бактерий гетеротрофов по районам

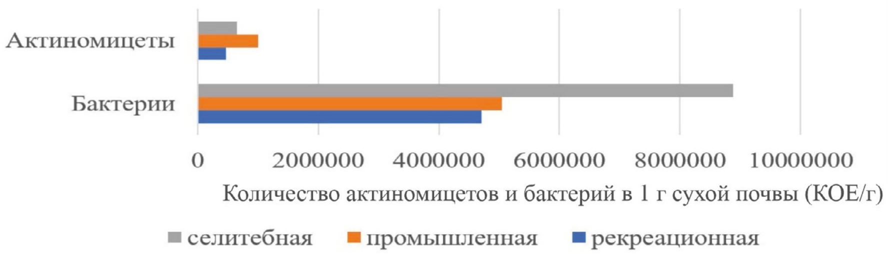
район	Сумма km
Железнодорожный	2,645
Индустриальный	3,931
Кировский	0,957
Краснофлотский	1,239
Центральный	1,735



Сумма km, млн. КОЕ/г актиномицетов по районам

район	Сумма km
Железнодорожный	6,028
Индустриальный	5,649
Кировский	0,805
Краснофлотский	5,813
Центральный	11,288





Вывод по графику: по величине средних значений численности актиномицетов и микромицетов на первом месте стоит промышленная зона. Это связано с угнетением бактериального сообщества на данных территориях вследствие химического загрязнения и физического воздействия – уплотнения почвы, переувлажнения или высушивания, угнетения или полного отсутствия растительности. При этом доступные питательные вещества поглощаются более устойчивыми пигментированными микромицетами.

Fig. 4. Report on the range of points 1-15, of the plug-in "Soils-Khabarovsk".

of points of extremes and building a series of decreasing numerical values. The processing results, along with the obtained conclusions, are generated into a summary report that can be uploaded in PDF format (Barkhatov, 2023).

Fig. 4 shows the ranking of the number of CFU/g microorganisms by administrative districts of Khabarovsk, built using the software «Izyskatel DV». As can be seen, the maximum number of CFU/g of micromycetes and bacteria falls on the Industrial Area, and actinomycetes in the Central Area. The sample is based on the database «Microorganisms-Khabarovsk», consisting of 15 points selected in the period from 2022 to 2023.

According to the degree of decrease in the average number of microorganisms for actinomycetes and micromycetes, the industrial zone is in the first place; for actinomycetes, the residential zone is in the second place, and for micromycetes – the recreational zone (Fig. 4), that coincides with the results of other authors (Kirienko and Imranova, 2008; Nazarenko and Svistova, 2016; Shumilova, 2012). For bacteria in a similar row, residential areas are in the first place, industrial in the second and recreational in the third.

Conclusions

1. Using the software «Izyskatel DV», ranked rows were compiled in descending order of abundance in various zones of Khabarovsk: for bacteria, it is residential > industrial > recreational; for actinomycetes, industrial > residential > recreational; for micromycetes, industrial > recreational > rural.

2. The software «Izyskatel DV» is fully suitable for analyzing the data arrays obtained from microbiological analysis. This tool significantly increases the possibilities for the presentation and interpretation of numerical data.

3. The revealed anomalous points (no. 13) in the content of dark-colored micromycetes and actinomycetes indicate a violation of the ecological state of the territory and require further study.

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