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An analysis of actinomycete complex structure in Cambisoil of South-East Baltic for the purposes of environmental monitoring

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Abstract

This article presents soil analysis of the microflora of natural and urban ecosystems that are exposed to the conditions of antropogenic pollution. Methodological basis for this work was founded upon the qualitative evaluation of different groups of microorganisms and upon systemic classification of soil actinomycetes that were extracted from antropogenically altered cambisoils. This research was conducted in 2015, on the territory of Kaliningrad Region, Russia, in South-Eastern Baltic Region. As a result of this study, we gathered material that was indicating prevalent role of actinomycetes in the structure of soil microbiocenosis. We also detected pigmented types of actinomycetes that were resistant towards antropogenic pressures as well as actinomycetes that served as indicators of negative changes in the environment. Results of this study will serve as a foundation for warehousing data on soil microbiological monitoring and for planning future events focused on bioremediation of polluted soils.

1. Introduction

The Kaliningrad region is Russia's westernmost territory situated in Central Europe on the south-eastern coast of the Baltic Sea. It is an exclave of Russia, as it has no land border with the main territory of the country (fig.1).

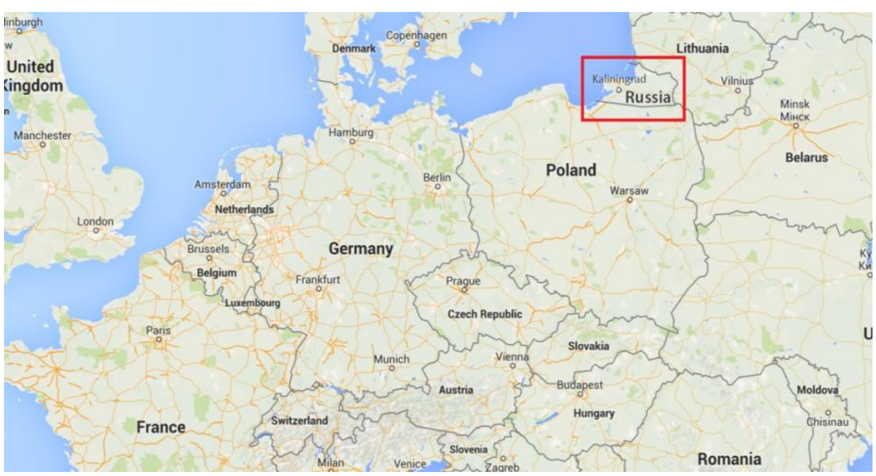

Figure 1: Geographical location of the Kaliningrad region on the Baltic Sea

The region outperforms the neighbouring territories in terms of population density, urbanisation, economic exploitation of lands, and the number of motor vehicles [1].

Almost half of the region's population live in the city of Kaliningrad. A high concentration of urban residents, transport, and industrial facilities on a rather small area is permeated with environmental risks. They relate to substantial air contamination, unsatisfactory quality of drinking water, the contamination of surface water, groundwater, and soils, and accumulation of significant amounts of industrial and household waste [2].

One of the most contaminated aspects of the environment is the soil. Exposed to significant anthropogenic pressure, it accumulates toxic compounds and becomes a source of environmental damage for plants, animals, and humans [3, 4, 5, 6]. Thus, comprehensive studies on urban soils are an important stage in developing measures to limit the consequences of anthropogenic pressure [7].

Soil microorganisms are very susceptible to anthropogenic pollution. Due to their abundance, diversity of their functions, and specific reactions to changes in the environment, they are used as indicators of the environmental condition [8]. Quantitative and qualitative changes in microorganism clusters allow us not only to evaluate ecological situation on the territory of conducted study during present time, but also to make predictions for the future environmental scenarios [9, 10]. Identification of autochthnous microorganisms, resistant to a variety of types of pollutants will help us to harness their use in making our environment cleaner [11, 12].

An inseparable part of the soil microflora are actinomycetes – spore-forming gram-positive bacteria capable of branching mycelium formation. They are extensively studied by specialists in microbiology, biochemistry, genetics, and related areas. This is explained by that fact that actinomycetes produce biologically active compounds, including antibiotics, enzymes, vitamins, and other substances widely used in medicine and the national economy [13, 14]. Their primary environmental role is decomposing complex stable substrates inaccessible to other microorganisms.

A relevant research area is studying actinomycete complexes in the soils of urban ecosystems. An assessment of biodiversity and morphological and physiological characteristics is of crucial significance in solving problems relating to the contribution of mycelial prokaryotes to the environmental functions of urbanised soils [15, 16].

This work aims to study actinomycetes in the soils of the Kaliningrad region differently affected by human-induced pressure. As a result, the content and systematic status of actinomycetes found in the human-impacted soils were identified.

This Cambisoil focused study characterized different levels of anthropogenic pressures in the city of Kaliningrad and the Kaliningrad region.

2. Methods

The soil samples for microbiological examination were taken in the summer 2015. The sterile samples were collected using the envelope method at a depth of 10 cm [17]. The average sample was obtained through mixing five isolated samples taken from the same site. During sampling, soil temperature was measured at the surface and at a depth of 10 cm, using a mobile pH meter with a built-in temperature probe. For measuring soil moisture, the samples were collected into metal containers. The soil samples were delivered for microbiological examination to the Immanuel Kant Baltic Federal University's Microbiology and Biotechnology Laboratory.

The inoculation and cultivation of microorganisms was conducted in a moist soil, where natural moisture was identified using the thermostatic gravimetric method [17]. The samples in the containers were weighed and placed in a drying chamber for complete drying at 105-110°C during six hours. After that, repeated weighing and moisture calculations were performed.

For the growth and differentiated categorization of soil microorganisms, we used methods of depth sowing, employing a variety of soil suspensions with different levels of concentration, as well as the following stimulating mediums : meat-peptone agar (MPA) for producing ammonifying microorganisms, a mix of MPA with yeast-malt agar (YMA) for spore-forming bacteria, Czapek solution for microfungi and starch-and-ammonia agar for producing actinomycetes [18]. Microorganisms were grown in an autoclave at a temperature of 28°C. Incubation time for ammonifying microorganisms, spore-forming bacteria, мicromycetes consisted of 5 days and for actinomycetes of 21 days. At the end of the incubation period, we counted the number of colonies that grew in the rich cultures in a Petri dish, to determine the colors of aerial and substrate actinomycete mycelium.

The colour of aerial mycelium was observed under natural lighting conditions. In most cases, the colour was a combination of several shades of grey, red, light beige, brown, or white. The colour of substrate mycelium was observed on the other side of the colony. It was determined by the effect of different pigments – yellow, orange, green, red, purple, brown, black, and others. Faint colouring did not have diagnostic significance, such

mycelium was characterised as colourless. After describing the colonies, actinomycete sections were identified by the colour of aerial mycelium and the series by the colour of the substrate mycelium [13].
The number of microorganisms was recorded in colony-forming units (CFUs) per 1 g of dry soil [17]. The data obtained were statistically processed using the Microsoft Excel software and presented as arithmetic means with a notation of standard deviations.

3. Experimental

Cambisoils are of crucial significance to agriculture. These soils are found in deciduous forests in moderately warm and humid regions of the subboreal zone in Western and Central Europe and the Far East. In Russia, cambisoils occur in the Kaliningrad region and the Primorsky region, in the south of the Khabarovsk region, and in the Amur region. Cambisoils of the Kaliningrad region are not only found in the natural conditions but also used for growing grain, non-food, and forage crops and vegetables. They also occur in the city of Kaliningrad and, therefore, they experience strong anthropogenic pressures.
Five sampling sites were selected for studying actinomycete complexes. The area of each key site consisted of 100 м^2. Their environmental characteristics are shown in table 1.

Table 1. Environmental characteristics of sampling sites

Sampling site location	**Phytocoenosis characteristics**	**Soil type**	**Soil moisture, %**	**Soil temperature** at a depth of 10 cm/at the surface, **C**°	**pH of the soil medium**
1 N54º56.383' E020º27.481' near the village of Klintsovka (control)	Spruce-beech-wood sorrel forest, underbrush: hazel, ash, young beech	Cambisoil, medium sandy clay on moraine sandy clay.	12.2	17.5/19.0	5.44
2 N54º52.230' E020º29.327' near the village of Melinkovo	Agricultural phytocoenosis. Winter rye mixed with vetches, camomile, and the silverweed	Cambisoil, cultivated medium sandy clay on moraine sandy clay	10.0	18.0/19.4	7.41
3 N 54º46.110' E 020º26.822' Kaliningrad, Belanova street	Hornbeam grove (hornbeam, oak, maple) in the city	Gleyic cambisoil	15.5	19.0/19.7	7.27
4 N54º40.629' E020º36.888' an airfield in Kaliningrad	Multigrass meadow dominated by the tansy, the common wormwood, sweet-clover, and individual *Acacia* plants	Cambisoil, human-impacted medium sandy clay	11.0	18.6/20.4	7.26
5 N54º40.551' E020º37.295' Kaliningrad, Emelyanova street	Roadside lawn, dominated by nettles, buttercups, *Glechoma*, plantain, meadow-grass, and individual lime trees	Cambisoil, human-impacted light sandy clay	4.6	16.7/19.0	7.24

These sites differ in both the level of anthropogenic pressure and physiochemical parameters. Site 1 – beech and woodsorrel spruce forest located in an environmentally pristine area on the Kaliningrad peninsula – was chosen as the control. Site 2 – an agricultural phytocenose – was most strongly affected by human occupation, namely, agricultural works, such as ploughing, harrowing, fertilisation, etc. The urban sites were selected in different functional zones of Kaliningrad characterised by different levels of anthropogenic pressure. The hornbeam forest (site 3) was situated on the north-western periphery of Kaliningrad in a recreational area, the grass meadow (site 4) in a residential area, and the most human-impacted site 5 – a roadside meadow – near a motorway. Site 5 was affected by trampling, pet walking, and traffic.

The physiochemical parameters of sampling sites were changing as follows. The soil moisture ranged from 4.6% on the roadside meadow to 15.5% in the hornbeam forest at the sampling temperatures of 16.7˚C and 19.0˚C respectively. The pH values in human-impacted soils ranged insignificantly from 7.24 on the roadside meadow to 7.41 in the agricultural phytocenose. In the control – the beech-woodsorrel spruce forest – it was only 5.44.

4. Results and discussion

The study of the soil microflora in Kaliningrad and Kaliningrad Region demonstrated that content ratio of different groups of microorganisms found in area's anthropologically altered soils was not the same and had significant quantitative deviations (Table 2).

Table 2. Microorganism content in Cambisoils in Kaliningrad and the Kaliningrad region

Sampling site	Quantitative content of microorganisms (CFU, thousand/g)			
	Ammonifying microorganisms	Spore-forming bacteria	Micromycetes	Actionmycetes
1 Beech-woodsorrel spruce forest (control)	315.6±79.8	1.0±0.4	3.2±1.1	14184.4±3198.6
2 Agricultural phytocoenosis	291.7±107.6	5.2±2.1	16.6±7.8	8123.5±2787.8
3 Hornbeam grove	616.6±95.9	3.8±1.5	4.5±1.1	5605.6±1494.4
4 Multigrass meadow	457.0±58.9	2.1±0.8	9.0±2.2	2525.0±624.8
5 Roadside lawn	82.6±41.3	0.7±0.3	14.4±9.4	8933.6±2147.2

Based on the data presented in Table 2, we can conclude that the quantity of ammonifying microorganisms that could absorb organic forms of nitrogen, varied from $82.6x10^3$ CFUs/g in the lawn soil along the motor road to $616.6x10^3$ CFUs/g in the Hornbeam grove; content of spore-forming bacteria varied from $0.7x10^3$CFUs/g to $5.2x10^3$ CFUs/g. Coincidentally, the least amount of spore-forming bacteria, just like in the case of the ammonifying microorganisms, were found in the lawn on the side of the motor road. The highest content of spore-forming bacteria was detected in agricultural phytocoenosis of cultivated medium clay Cambisoil. Quantitative data focused on micromycete content showed that in the soil samples taken in the Beech-woodsorrel spruce forest, general quantity of microfungi consisted of $3.2x10^3$ CFUs/g. This quantity is minimal compared to the quantity of micromycetes taken from the soil on other sites. The maximum amount of micromycetes in agricultural phytocoenosis was $16.6x10^3$ CFUs/g. At the same time, the amount of actinomycetes in studied soil varied from 2.5 on a multigrass meadow to 14 in the Beech-woodsorrel spruce forest.

Figure 2 gives a graphic representation of the combined content of different groups of microorganisms, site by site. Based on the data given in Figure 2, it is clear that the combined content of microorganisms in anthropogenically altered soils was reduced from: Cambisoil medium sandy clay on moraine sandy clay in the Spruce-beech-wood sorrel forest to cambisoil cultivated medium sandy clay on moraine sandy clay in Agricultural phytocoenosis to Gleyic Cambisoil in the hornbeam grove to Cambisoil, human-impacted medium sandy clay in the miltigrass meadow.

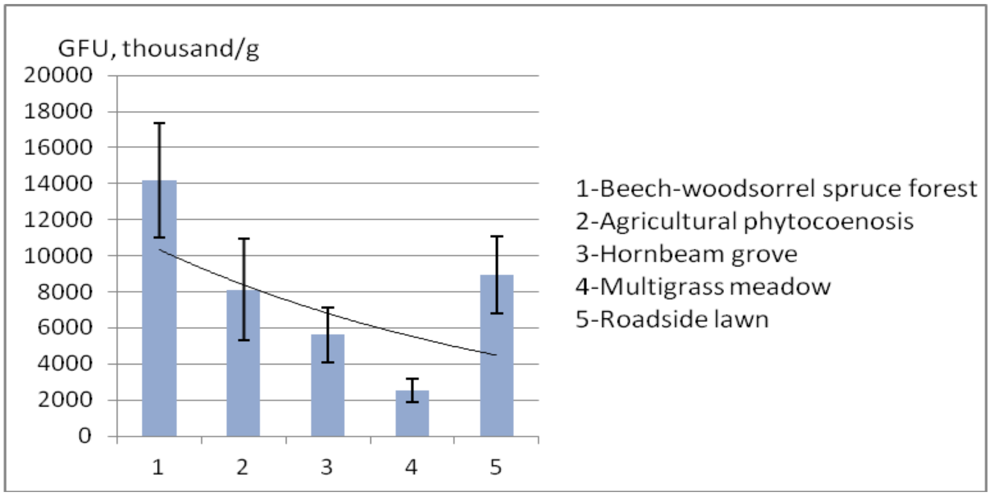

Figure 2: combined content of different groups of microorganisms, site by site

The analysis of actinomycete content in Cambisoils showed that they dominated over other groups of microorganisms – bacteria and micromycetes (fig. 3), which was confirmed by the earlier obtained data on the prevalence of actinomycetes in the soils of natural and urban ecosystems in Kaliningrad and the Kaliningrad region [9].

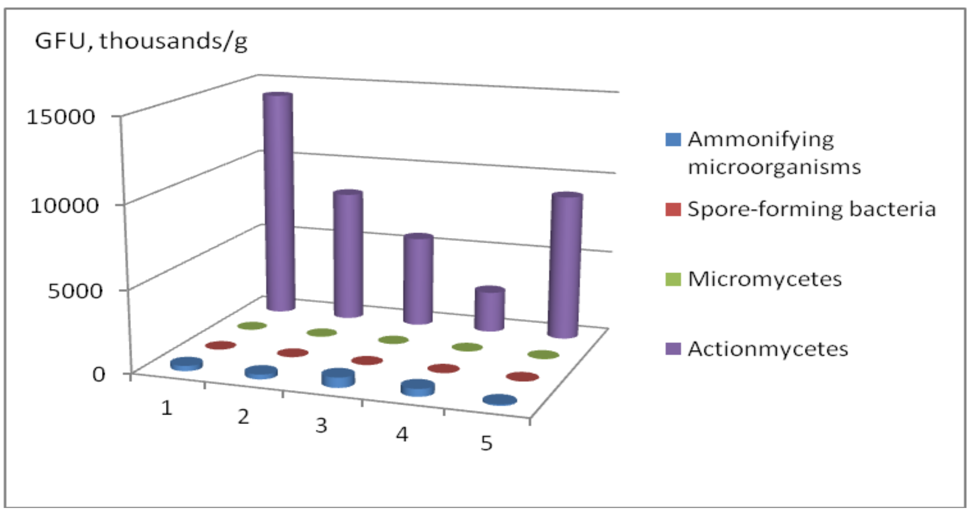

Figure 3: Structure of the microbiocenosis of the anthropologically altered soils taken from the key study sites in Kaliningrad and Kaliningrad Region (1 – beech-woodsorrel spruce forest, 2 – agricultural phytocoenosis, 3 – hornbeam grove, 4 – multigrass meadow, 5 – roadside lawn)

The colour of aerial and substrate mycelium was identified to arrange actinomycetes by taxonomic groups. Using the Gauze identification guide, actinomycetes were arranged into sections and series. Based on the data obtained, a qualitative analysis of actinomycetes was conducted (fig. 4 – 8).

The data on the structure of actinomycetes in the soils of the beech-wood sorrel spruce forest are shown in figure 4. This soil is dominated by the actinomycetes of the *Albus Albus* section and series (50%), followed by *Albus Albocoloratus* (25%), *Helvolo-flavus Flavus* (21%), and *Cinireus Chromogenes* (4%).

Figure 5 shows the structure of actinomycetes in the soil of the agricultural phytocoenosis. The figure shows that the soils of the agricultural phytocoenosis was dominated by the species of the *Albus Albocoloratus* section and series, which account for 50% of all actinomycete species observed at the site. The actinomycetes of the *Imperfectus* section without aerial mycelium were of common occurrence. Their proportion in the structure of soil actinomycetes of the agricultural phytocoenosis was 39%. The percentage of actinomycetes of the *Albus Albus* section and series, which are common in the control, accounted for 11% in the agricultural phytocoenosis.

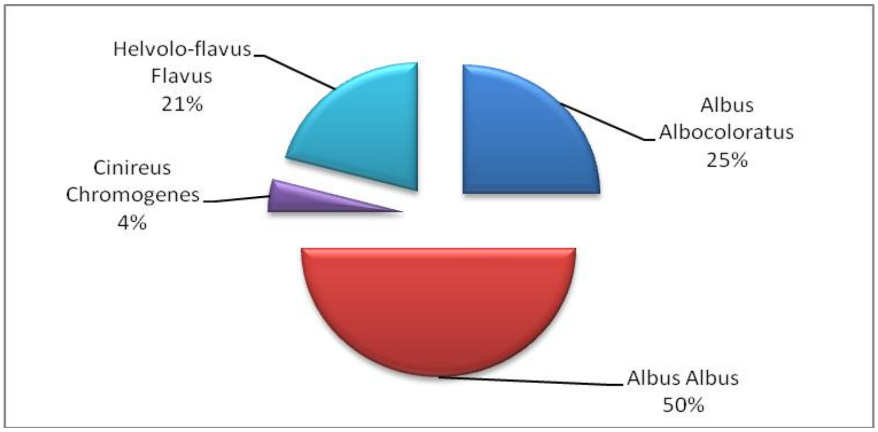

Figure 4: Actinomycete structure in the soil of the beech-woodsorrel spruce forest

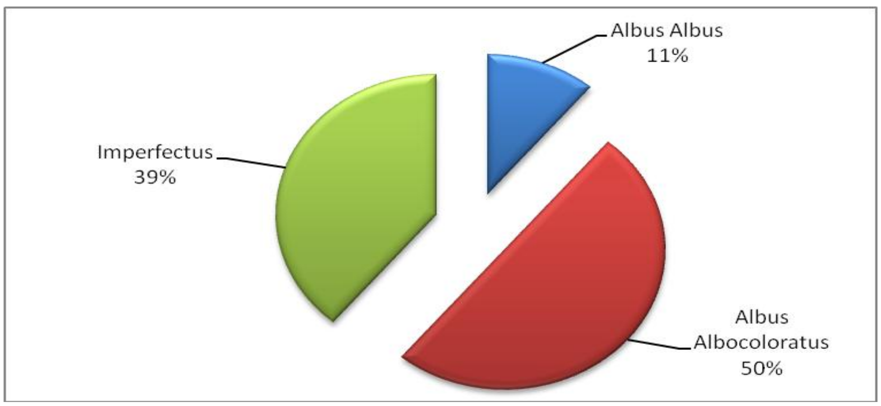

Figure 5: Actinomycete structure in the agricultural phytocoenosis

A wider species diversity of actinomycetes was observed in the hornbeam grove (fig. 6). This site was dominated by the actinomycetes of the *Albus Albocoloratus* section and series, which accounted for 54%. The proportion of the *Cinireus Chromogenes* section and series was 20%, whereas *Helvolo-flavus Flavus* and *Helvolo-flavus Helvolus* accounted for 13% each in the structure of the actinomycete complex of the hornbeam grove.

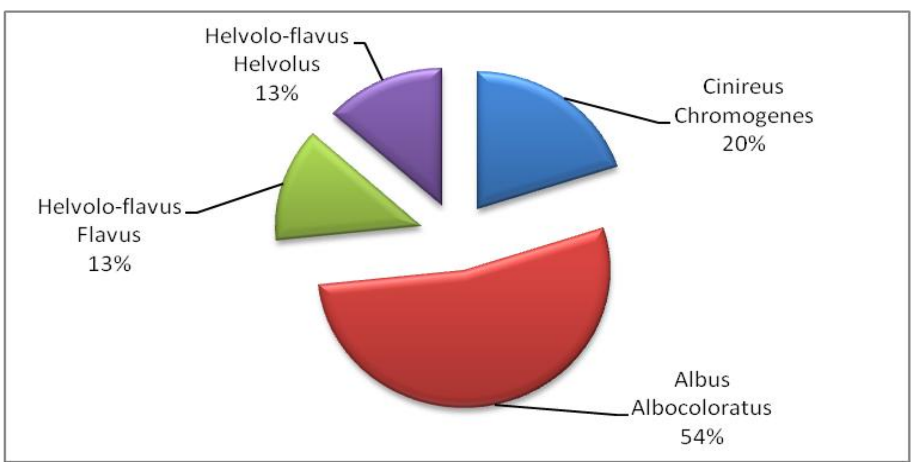

Figure 6: The structure of soil actinomycetes in the hornbeam grove

The soil of the multigrass meadow found in Kaliningrad did not demonstrate a wide diversity of actinomycetes (fig. 7). They are represented by two large groups of the *Albus Albocoloratus* and *Helvolo-flavus Flavus* section and series – 57% and 43% respectively.

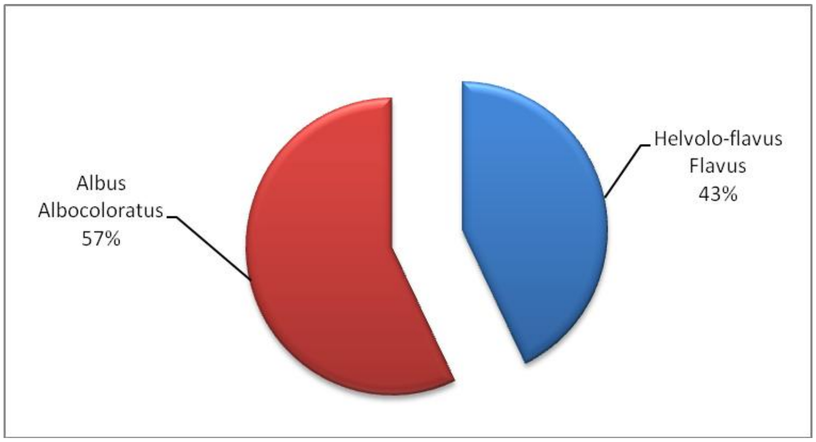

Figure 7: Actinomycete structure in the soil of the multigrass meadow

Figure 8 shows the diversity of actinomycetes in the Cambisoil of the roadside lawn (fig. 8). The figure suggests that the structure of the actinomycete complex of the roadside lawn is dominated by the species of the *Albus Albocoloratus* section and series and the *Imperfectus* section, which account for 36% and 32% respectively. The actinomycetes of the *Roseus Ruber* accounted for 18% and those of the *Albus Albus* section and series for 14%.

A high content of the actinomycetes of the *Albus Albus* section and series was observed at the control site. As the anthropogenic pressure grew, their proportion in the actinomycete complex structure of human-impacted soils reduced.

The observed reduction is possibly connected to the increased amount of pollutants in the soil, particularly heavy metals which represent the main contamination agents in the environment of our city [18]. We assume that high levels of lead, copper and zinc in the soil of Kaliningrad [19] could have negatively affected the growth and development of Albus Albus sensitive sections and series.

The species of the *Albus Albocoloratus* section and series were found at all sites except the control. As the anthropogenic pressure grew, their proportion in the soil actinomycete complex increased. The actinomycetes of the *Imperfectus* section, which has a defective reproductive function and is characterised by the absence of aerial mycelium, were observed in the soils of the agricultural phytocoenosis and at site 5 (the roadside lawn) most strongly affected by human-induced pressure. The latter manifests itself in the effects produced by fertilizers and herbicides introduced into the farmland soil or by different pollutants typical of the transportation sector.

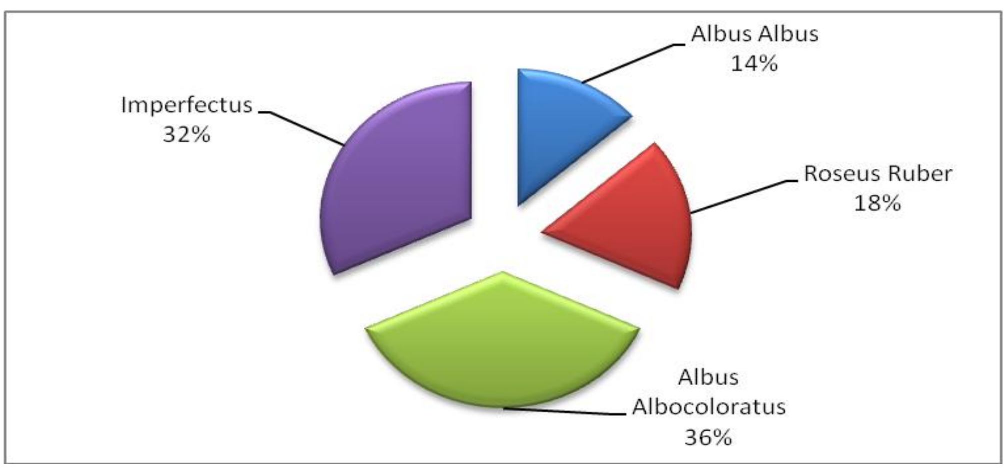

Figure 8: The structure of actinomycetes in the soil of the roadside lawn

The relative abundance of yellow-pigmented species of the *Helvolo-flavus* section ranged from 21% in the control to 43% in the multigrass meadow soil. The species of the *Roseus Ruber* section and series were found only in the heavily human-impacted soil of the roadside lawn. Overall, higher diversity of actinomycetes in urban soil, is clearly dictated by considerable substrate heterogeneity of city soil, the peculiarities of the microclimate and the variety of ways to introduce actinomycete spores. The increase in the proportion of pigmented actinomycete species was indicative of their resilience to a combination of the negative factors of the urban environment [15]. The emergence of the *Imperfectus* section suggests the low sustainability of urban ecosystems [20].

Conclusions

In conclusion, as a result of conducted studies that focused on actinomycete complex systems found in anthropogenically altered Cambisoils on the territory on South Eastern Baltic Region, it has been confirmed that actinomycetes dominate other forms of microorganisms on all key study sites. A higher degree of anthropogenic alterations has been noted to be in direct relationship with the increase of the pigmented species of the *Albus Albocoloratus* as well as *Helvolo-flavus Flavus* section and series, and with the emergence of the *Roseus Ruber* actinomycetes as well as the *Imperfectus* section with a defective reproductive function. These parameters can be used as the indicators of the quality of soil environment for the purposes of microbiological soil monitoring in natural and urban ecosystems. Pointing out stable types of actinomycetes that are not susceptible to anthropological pollution, will allow us to come up with new ways to use in the process of contaminated soil remediation and improvement of the ecological situation around the Baltic Sea.

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