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Assessment of soil microbiota in the area of the gas pipeline

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Abstract. The article presents the results of the evaluation of the soil microbiota in the area of the above-ground gas pipeline. The analyzed areas collected material of the rhizosphere (root soil, soil from the roots) and rhizoplan (root structures) of wormwood. Based on the microbiological study of soil microbiota, the cultural and morphological properties of microorganisms are determined. In a soil microbiota taken from the marginal area of the field under study, a microorganism of *Azomonas* genus is presumably identified. Microorganisms of *Acinetobacter* genus, *Agrobacterium* genus, and *Bradyrhizobium* genus are presumably identified in the soil, which is under the anthropogenic impact of the above-ground gas pipeline. These microorganisms provide the nutrients of the plant and as a consequence contribute to the natural restoration of the soil.

1. Introduction

At present, the pipeline construction capacity is increasing, as it is the most common form of gas transportation. In accordance with Federal Law of the Russian Federation No 69-FZ "On Gas Supply in the Russian Federation", the Decree of the Government of the Russian Federation dated September 10, 2016 No 903, the regional program "Gasification of Housing and Communal Services, Industrial and Other Organizations of the Volgograd Region for 2017-2021 is being implemented" (Resolution of November 21, 2017 No. 769. Overhead gas pipelines are being laid in compliance with building codes and regulations, which ensures their mechanical strength and stability. However, this does not exclude the negative soil and plants. The soil is transformed, its agrophysical and agrochemical properties deteriorate, which determine its water-air and thermal regimes, which affects the possibility of spatial growth of the root systems of plants [1-2, 3]. The soil is disturbed by the ratio of carbon and nitrogen, causing oxygen deficiency. The result of soil pollution in the passage area of the pipeline is the death of plants. Restoration of soil and plant integrity occurs within 2-3 years. At the same time, an important role is assigned to soil microorganisms.

The purpose of the work is to study soil microbiota in the area of passage of the above-ground gas pipeline, and to describe the morphological and cultural properties of microorganisms.

2. Materials and methods

The studies were carried out on the territory of agricultural land of the Nizhne-Volzhsky Scientific Research Institute - a branch of the FGB NU "Federal Research Center for Agroecology, Integrated Land Reclamation and Protective Forestation of the Russian Academy of Sciences". Plots of the field located along the Rakutin Avenue in the village of the Oblast Agricultural Experiment Station of the Gorodishchensky District of the Volgograd Region (longitude 48°88.0075' North, latitude 44°37.6880'



East) acted as analyzed plots. The analyzed section of the field, through which the overhead gas pipeline directly passes - section No. 1; marginal plot of the field (ecotone) - plot number 2.

Taking into account the fact that soil fertility, in addition to soil and agrochemical indicators of soil, can be judged by plants, dominantly growing on it and actively interacting with soil microbiota, we collected rhizosphere material in the analyzed areas (root soil, soil from roots) and rizoplan (directly root structures) Wormwood - *Artemisia absinthium* L.

Samples packed in sterile glassware were delivered to the laboratory on the day of material selection. Samples were allowed to be stored for no more than 24 hours at a temperature of 4-5 ° C. The study analyzed 3 types of microbiota samples: Sample No. 1 - soil microbiota near the roots, Sample No. 2 - soil microbiota from the roots, Sample No. 3 - grinded root microbiota, which was prepared under sterile conditions in a laminar box.

Sample No. 1 was obtained by shaking the soil from the plant root. 10 g of the sample was placed in a sterile flask with 100 ml of sterile distilled water.

Samples No. 2 and 3 were prepared as follows: 1) a sterile cut off root was placed in a sterile flask containing 100 ml of sterile distilled water; 2) for 20 minutes on the electromagnetic stirrer (maximum speed) washed the soil directly from the roots. The resulting soil extract was sample number 2; 3) observing the sterility of the conditions of the washed root, the remaining water was removed using sterile filter paper, then it was crushed, ground in a mortar with the gradual addition of sterile distilled water 100 ml; 4) for 10 minutes on an electromagnetic stirrer (maximum speed) a water suspension was obtained - sample No. 3.

The decrease in the concentration of microbial cells to 10⁷ in samples No. 1, 2, 3 was obtained by the dilution method of 1:10 in sterile saline. Sowing 0.1 ml of bacterial suspension was carried out on Petri dishes with dense nutrient medium with meat hydrolyzate (MPA). The repetition rate is 3. Next, the samples were incubated for 24 h at t 20–21 ° C in a thermostat and 48 h at room temperature for pigmentation. After that, the grown colonies were counted and the morphological and cultural properties of the isolated microorganisms were studied [5].

3. Results and discussion

The analysis of the soil microbiota of the field under study (regional plot No. 2) made it possible to reveal the presence of identical microorganisms in samples No. 1, 2, 3. The cultural characteristics of these microorganisms are the following: the size of the colonies is 6 to 9 mm; the shape of the colonies is regular, round; the edge contour is smooth; the color of the colonies is milky-beige, beige-mustard; the structure of the colonies is represented by fine granularity, homogeneous or non-uniform and transmits light; consistency - oily, nozzle-like, reaches for a loop and is easily smeared; the relief of the colonies is convex, drop-shaped with elements of the raised or depressed center; profile - convex; surface - shiny, wet, S - shape. The morphology of the isolated microorganisms is gram-negative cocci. Biochemical analysis allowed us to establish a positive reaction to catalase, the presence of a capsule and the absence of spores, as well as a positive reaction to cytochrome oxidase, detected in microorganisms isolated from a suspension of crushed root. All the above listed allowed identifying these microorganisms as nitrogen-fixing (*Azomonas*) [6].

Analysis of the soil microbiota in samples No. 1, 2, 3 taken directly at the site of anthropogenic impact (passing the above-ground gas pipeline) allowed us to establish various microorganisms according to their cultural and morphological properties (table 1).

Table 1. Soil microbiota under the elevated gas pipeline.

Cultural and morphological properties of microbes	Soil near the roots	Soil from the roots	Soil from the root of the crushed
The size	8 mm	3 mm	7 mm
The form	Not correct, ameboid	Round	Correct, round
Edge contour	Fistionchaty	Smooth	Smooth

Colony color	Milky white	Milky white	Colorless
Structure	Not uniform, light does not let in	Uniform, light does not pass	Fine-grained, the light passes
Consistency	Oily	Oily	Oily
Relief	Indented center	Slightly convex	Indented center
Profile	Crater shaped	Flat	Crater shaped
Surface	Matt, dry, folded, R-shape	Wet, glossy, S-shape	Shiny, wet, S-shape
Morphology	Sticks, large, long, with round ends, bipolar gram-negative	Sticks, short, with round ends, gram-negative	Sticks, short, oval, bipolar, gram-negative
Catalase Reaction	Vials of gas, strong positive reaction	Vials of gas, strong positive reaction	Vials of gas, strong positive reaction
Reaction gins	No capsule	No capsule	No capsule
Reaction to nitroreductase	The color has not changed, the reaction is negative	The color has changed, the reaction is positive	The color has not changed, the reaction is negative
Reaction to cytochrome oxidase	Colony coloration blue, positive reaction	Colony coloration blue, positive reaction	Colony coloration blue, positive reaction
Coloring according to Ozheshko	No spores	No spores	No spores
Estimated genus of microorganism	Acinetobacter	Agrobacterium	Bradyrhizobium

According to our research, isolated microorganisms are classified as chemoorganotrophs, associative nitrogen fixers, intracellular symbionts. They are able to capture atmospheric nitrogen, which is necessary for plants.

4. Conclusion

The development of the oil and gas industry in Russia is a priority state task. Special attention is paid to the gas transmission system. The negative impact on the environment occurs during the construction of gas pipelines and later during their operation. The negative effects of the gas transmission industry on the environment include a change in the physicochemical properties of the soil, a violation of the soil-plant integrity.

Volgograd region is an industrial, agricultural region. The construction and conduction of gas pipelines complicates the work on the cultivation of fields, which can lead to the devastation of soil resources.

The most common plant in the Volgograd region is Wormwood perennial herb (*Artemisia absinthium* L.). Wormwood is well distributed by seeds and rhizomes, being a root-showering plant. Bitter wormwood is practically not eaten by domestic animals, which explains its rapid spread and settlement on the technologically disturbed lands. Taking into account the specificity of the Volgograd lands (mainly chestnut lands) and climatic conditions (drought, lack of moisture), it is the bitter wormwood that best contributes to the formation of the soil microbiota. In this connection, this plant has become the source material for the study of the soil microbiota of the rhizosphere and the rhizoplan in the area of passage of the above-ground pipeline.

The results of the study of soil microbiota allowed determining various microorganisms, depending on the location of the sampling. So, in the soil microbiota, taken directly under the elevated gas pipeline, microorganisms are identified and suspected to be identified: Acinetobacter, Agrobacterium, Bradyrhizobium. In the soil microbiota of the marginal portion of the field, only one microorganism of the genus Azomonas is identified and supposedly identified.

We found that man-made impact predetermines an increase in the soil of various types of microorganisms that are producers of the most important plant nutrients. The microorganisms isolated during the work can fix molecular atmospheric nitrogen, converting it into plant-digestible forms, and tolerate adverse environmental circumstances, which makes them the main soil agents. Soil fertility depends on the soil microbiota, and the growth of bitter pollen contributes to the natural restoration of the soil in the Volgograd region.

To solve the ecologically important task of preserving soil fertility, soil and plant integrity and the soil resource in general, biomonitoring of the soil cover condition in various places of the gas transmission system (pipeline, compressor, gas distribution stations) is necessary in order to improve the technology of microbial biomass production and reduce the potential risk of gas transmission systems on the environment.

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References

- [1] Zakharchenko A V 2005 Natural restoration of anthropogenically changing soils *Bulletin of Tomsk State Pedagogical University* **7 (52)** 115-22
- [2] Berkeley P *et al.* 1997 *The determinant of bacteria Burgi* ed J Holt *et al.* (Moscow: World)
- [3] Chernichkin R V and Snakin V V 2015 The risk of contamination of soil and surface water during the construction of trunk pipelines *Problems of risk analysis* **12(5)** 28-33
- [4] Maksimov I V, Abizgildina R R and Pusenkova L I 2011 Stimulating plant growth microorganisms as an alternative to chemical means of protection against pathogens *Applied biochemistry and microbiology* **47(4)** 373-85
- [5] Zenova G M, Stepanov A L, Likhacheva A A and Manucharova N A 2002 *Workshop on soil biology* (Moscow: Publishing House of Moscow State University)
- [6] Rybakov D S and Kevlich V I 2017 Trace elements of technogenically polluted soils of the central part of Petrozavodsk *Soil Science* **6** 718-30