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The Influence of Soil Contamination of The Black Sea Coast of The Caucasus By Heavy Metals and Oil on The Abundance of *Azotobacter* Genus Bacteria.

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ABSTRACT

It is advisable to determine the level of soil resistance to chemical contamination using biodiagnostic methods. One of the biological indicators of the soil condition is the abundance of *Azotobacter* genus bacteria. As shown by the conducted study, when contaminating soils of the Black Sea coast of the Caucasus by heavy metals and oil, the abundance of *Azotobacter* genus bacteria is significantly reduced. In most cases, the degree of reduction in the values of biological indicators is directly dependent on the concentration of contaminants in the soil. It is established that in terms of the degree of negative impact on the abundance of nitrogen-fixing bacteria, heavy metals form the following series: *Cr > Pb > Cu = Ni*. Among the soils of the Black Sea coast of the Caucasus, southern chernozems are the most resistant to contamination by Cr, Cu, Ni, Pb and oil, whereas the brown forest soils are least resistant to contamination.

Keywords: chrome, nickel, lead, copper, oil, nitrogen-fixing bacteria, stability, contamination.

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INTRODUCTION

The natural conditions of the Black Sea coast of the Caucasus are extremely diverse. Change of moisture conditions, seasonal heterogeneity, the presence of mountains and plateaus form a diverse range of natural areas and landscapes. This region has soils, which are unique for Russia, found only in the Black Sea region, such as the humid subtropical yellow soils, brown soils of dry subtropics, and the southern chernozems (brown) of Taman [1, 2].

The current pace of development of tourism and recreation infrastructure on the Black Sea coast of the Caucasus (the construction of new resorts, Olympic facilities, airports, highways, pipelines, etc.) constantly strengthen the anthropogenic load on the environment. At that, increase of chemical contamination of soils remains one of the most pressing problems. Due to the significant differences in ecological and genetic properties of the tested soils, such as the amount of humus, the intensity of biological processes, the reaction of soil medium, absorption capacity, etc. [3], the soil of the Black Sea coast of the Caucasus vary greatly in terms of resistance towards anthropogenic impact.

It is advisable to determine the level of soil resistance to chemical contamination using biodiagnostic methods. Biological indicators are the first responders to external stimuli reflecting a negative change at the initial stages of their appearance [4].

Currently, a growing number of researches are dealt with the study of microorganisms, which are indicators of biological parameters of the soils when exposed to different contaminants. One of the biological indicators of the soil condition is the abundance of *Azotobacter* genus bacteria [5].

The objective of the present study was to investigate the impact of soil contamination of the Black Sea coast of the Caucasus by heavy metals and oil on the abundance of *Azotobacter* genus bacteria.

METHODS

Contamination of soil by heavy metals (HM) and oil was modeled in the laboratory conditions.

Table 1: The sampling locations of soils of the Black Sea coast of the Caucasus and their ecological and genetic characteristics

Soil	Sampling point	Coordinates	Humus content, %	pH	Granulometric texture
Southern chernozem	Temryuk district, Taman	45°10’51.73”N36°41’30.47”E	3.2	7.7	Heavy loamy texture
Typical brown soil	Anapsky district, SNR1 "Utrish"	44°46.764 E. 37°31.702 N	9.3	7.2	Heavy loamy texture
Cinnamonic calcareous soil	Anapsky district, SNR1 "Utrish"	44°47.139 E 37°24.971 N	15.0	7.0	Medium loamy texture
Cinnamonic leached soil	Anapsky district, SNR1 "Utrish"	44°45.880 E 37°26.958 N	6.8	7.1	Heavy loamy texture
Acid brown forest soil	Tuapse district, Gorsky village	44°23.342' N 038°43.894' E	1.3	4.4	Heavy loamy texture
Acid brown forest bleached soil	Sochi, Lazarevsky district, Sochi National Park	43°52.048' N 039°24.214' E	1.7	4.1	Light loamy texture
Typical sod carbonate soil	Tuapse district, Jubga village	44°19.624' N 038°41.636' E	5.4	7.5	Heavy loamy texture
Leached sod carbonate soil	Sochi, Hostinsky district, Caucasus reserve, Box Tree and Yew Grove	43°31.683' N 39°52.412' E	4.8	6.9	Heavy loamy texture
Yellow soil	Sochi, Adler district	43°27.445' N 039°56.952' E	3.2	5.2	Heavy loamy texture

[1]SNR - State Nature Reserve

All the major soils of the Black Sea coast of the Caucasus, namely, southern chernozem, cinnamonic leached soil, typical brown soil, cinnamonic calcareous soil, acid brown forest soil, acid brown forest bleached soil, typical sod carbonate soil, leached sod carbonate soil, and yellow soil were used as a research objects.

These soils occupy the main territory of the Black Sea coast of the Caucasus, though differ in terms of contents of carbonates, the alkaline-acid and redox conditions, amount of humus, biological activity and other properties [6-7]. Ecological and genetic properties of the studied soils are shown in Table 1.

Samples for the study were taken from the top soil of 0-10 cm, containing most of soil contaminating substances [8].

The contaminants included Cr, Cu, Ni, Pb and oil. Heavy metals were introduced into the soil in quantities of 1, 10, 100 MPC (maximum permissible concentration). These HM are interesting in terms of comparison, since their MPC is 100 mg/kg of soil. We used MPC values developed in Germany [9]. First, because the MPC values of total (gross) amount of copper and nickel in Russia are lacking. Second, the "Russian" MPC of lead often cannot be used in practice because its value is less than the actual content of this element in many types of soil.

Besides, the MPC of the oil content in the soil has not been developed either. Therefore, to express the oil concentration in the soil we used its percentage composition.

The heavy metals were introduced into the soil in quantities of 1, 10, 100 MPC (100, 1000 and 10000 mg/kg, respectively), while the oil – in the proportion of 1, 5, 10% by weight of the soil. Heavy metals contents in soil for up to 100 MPC and even more is often found in the areas of metallurgical, chemical and fuel-producing industries. Soil contamination up to 10 MPC, in addition to mentioned sources, is generally caused by vehicles and/or as a result of agricultural activities related to the use of fertilizers, pesticides, and seed dressers. Soil contamination by oil up to 10% by weight of the soil and more is commonly found in areas of oil production, transportation and refining [10].

We used also the oxides of heavy metals: $CrO3$, CuO, NiO, and PbO. First, a significant proportion of HM enters the soil in the form of oxides [11]. Secondly, the use of HM oxides allows excluding effects of related anions on soil properties, as it occurs when introducing metal salts.

The soil was incubated in vegetation vessels at room temperature (20-22°C) and optimum moisturization (60% of normal field capacity) in triplicates.

The abundance of *Azotobacter* genus bacteria was determined by the lumps fouling method in the Ashby medium [12].

To check the received data for fidelity we have conducted variance analysis determining the least significant difference (LSD).

RESULTS

As a consequence of the study, it was found that the contamination of the soils of the Black Sea coast of the Caucasus by Cr, Cu, Ni, Pb and oil usually reduces the abundance of nitrogen-fixing bacteria. An increase in the abundance of *Azotobacter* genus bacteria was observed only in cinnamonic calcareous soil at small doses (1%) of contamination by oil (Table 2). In most cases there was a direct dependence between the content of contaminant and the damage factor of the studied soil indicator.

Table 2: The impact of chemical contamination of soils of the Black Sea coast of the Caucasus on the abundance of *Azotobacter* genus bacteria, % of control

Element	The dose of the contaminant				
	Control	1 MPC (1 %)	10 MPC (5%)	100 MPC (10 %)	HCP_{05}
Southern chernozem					
Cr	100	100	69	7	10
Cu	100	100	92	88	13
Ni	100	100	100	93	9
Pb	100	100	85	81	12
Oil	100	100	91	86	7
HCP_{05}		12	12	14	
Typical brown soil					
Cr	100	27	18	7	5
Cu	100	73	57	42	9
Ni	100	89	76	35	10
Pb	100	82	68	32	10
Oil	100	95	77	59	11
HCP_{05}		10	10	7	
Cinnamonic leached soil					
Cr	100	16	11	0	4
Cu	100	100	96	37	11
Ni	100	66	68	42	9
Pb	100	95	55	32	10
Oil	100	50	55	37	8
HCP_{05}		8	8	6	
Cinnamonic calcareous soil					
Cr	100	14	8	5	4
Cu	100	96	64	50	10
Ni	100	97	67	38	10
Pb	100	40	39	8	6
Oil	100	109	101	95	14
HCP_{05}		9	9	8	
Acid brown forest soil					
Cr	100	82	0	0	6
Cu	100	93	32	0	8
Ni	100	70	41	19	8
Pb	100	91	46	29	9
Oil	100	67	35	18	7
HCP_{05}		9	5	4	
Acid brown forest bleached soil					
Cr	100	49	29	0	6
Cu	100	77	54	15	8
Ni	100	78	47	10	8
Pb	100	77	46	21	8
Oil	100	69	38	25	8
HCP_{05}		9	6	3	
Typical sod carbonate soil					
Cr	100	49	45	12	7
Cu	100	88	55	26	9
Ni	100	91	46	38	9
Pb	100	55	38	24	7
Oil	100	61	50	41	9
HCP_{05}		8	6	6	
Leached sod carbonate soil					
Cr	100	48	35	16	6
Cu	100	77	48	23	8
Ni	100	81	67	25	9
Pb	100	86	51	22	9
OIl	100	60	53	36	8
HCP_{05}		9	8	5	
Yellow soil					
Cr	100	37	11	0	5
Cu	100	94	52	29	9
Ni	100	94	59	21	9
Pb	100	95	59	34	10
Oil	100	93	87	68	12
HCP_{05}		11	9	7	

The reason for the negative impact of HM on biological objects is the fact that they are joined to the sulfhydryl groups of proteins, disrupting the synthesis of proteins, including enzymes, and also changing the penetrating ability of biological membranes [13].

The impact of oil on changes of soil properties is due to the presence in oil of HM, aromatic hydrocarbons and phenols, which have toxic effects on soil biota and enzyme activity. Besides, the oil plugs the channels and pores of the soil, binds the soil particles, breaking the moisture exchange and changing their water-physical properties [14].

Since all four investigated HM have the same MPC equal to 100 mg/kg, it is possible to compare correctly their toxic effects in relation to the studied biological parameters. The obtained results indicate that the chrome had the most significant negative impact. Lead, copper and nickel showed the least strength of the impact.

In terms of the negative effect on the change in abundance of *Azotobacter* genus bacteria, HM form the following sequence generalized for different types and subtypes of soils of the Black Sea coast of the Caucasus:

Cr (44) > Pb (66) > Cu (71) = Ni (71).

A similar pattern was observed in studies with other soils of the South of Russia, namely chernozems, chestnut soils, brown and grey forest soils, brown semi-desert soils, subalpine soils, sandy soils, etc. [15, 16].

During the study, a comparative analysis was conducted in terms of resistance of the *Azotobacter* genus bacteria to contamination by HM and oil for the major soils of the Black Sea coast of the Caucasus (the soils are arranged by decreasing of their resistance): southern chernozem (90) > typical brown soil (67) = yellow soil (67) ≥ cinnamonic calcareous soil (66) ≥ cinnamonic leached soil (63) ≥ typical sod carbonate soil (61) = leached sod carbonate soil (61) ≥ acid brown forest bleached soil (57) ≥ acid brown forest soil (56). Values in parentheses correspond to integrated indicator of soil conditions (IISC) (relatively to 100% for uncontaminated soil, averaged for three doses of contaminants).

The presented sequence is determined by environmental and genetic properties of the studied soils, primarily by granulometric texture, alkaline-acid and redox conditions, organic matter content, and biological activity.

The greatest buffering capacity to the contamination by HM and oil was shown by southern chernozem. Its heavy granulometric composition provides high absorption capacity. High humus content leads to the binding of HM into organometallic compounds. Weakly alkaline environment helps to fix cation forming metals.

Less resistant to contamination by HM was shown by brown and sod carbonate soils. Typically, these soils are characterized by heavy loam granulometric composition, high humus content, neutral or slightly alkaline reaction of the soil medium and other properties that contribute to the consolidation of HM. Good oxidizing conditions in these soils and the relatively high biological activity contribute to the oil degradation.

Brown forest soils have shown the least resistance to chemical contamination. They are distinguished by relatively low humus content, acidic reaction of soil medium, low enzymatic activity and, as a consequence, high mobility of HM and a low decomposition rate of oil.

The study has shown that the indicator of the biological status of soils, such as the abundance of *Azotobacter* genus bacteria can be recommended for use in monitoring, diagnostics and standardization of chemical contamination on the soils of Black Sea coast of the Caucasus.

CONCLUSION

Contamination of basic soils of the Black Sea coast of the Caucasus by the oil, chromium, nickel and lead decreases the abundance of *Azotobacter* genus bacteria. In terms of damage factor on the abundance of bacteria of the *Azotobacter* genus in the soils of the Black Sea coast of the Caucasus, heavy metals form the following generalized sequence:

$$Cr > Pb > Cu = Ni.$$

The degree of inhibition of the abundance of *Azotobacter* genus bacteria of the soils of the Black Sea coast of the Caucasus depends on the nature of the contaminant, its content in the soil and genetic properties of soils determining their resistance to contamination.

In most cases a direct correlation was observed between the content of the contaminants in the soil and the reduction in the abundance of *Azotobacter* genus bacteria.

In terms of resistance level to contamination by HM and oil, soils of the Black Sea coast of the Caucasus form the following sequence: southern chernozem > typical brown soil = yellow soil ≥ cinnamonic calcareous soil ≥ cinnamonic leached soil ≥ typical sod carbonate soil = leached sod carbonate soil ≥ acid brown forest bleached soil ≥ acid brown forest soil.

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