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# Enzymatic activity as a diagnostic indicator of soil pollution in Solovetsky settlement

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**Abstract.** This article presents the catalase activity as a diagnostic indicator of soil contamination by oil products and heavy metals by the example of the Solovetsky soil. 44 soil samples were taken during two summer expeditions launched by M. Lomonosov Northern (Arctic) Federal University in 2016 and 2017. The average content of organic matter (in terms of carbon) is 10%. The highest amount of carbon is found in peated, upper horizons (litter), ranging from 20 to 48%, and imbedded sandy layers – from 0.1% to 5%. The analyzed soils are slightly acidic and therefore are favorable for enzymes activity. Some of the soils in Solovetsky settlement were found to be contaminated with heavy metals and petroleum products, the latter affecting the catalase activity in oppositely directed ways. High levels of petroleum products lead to a marked decrease in the enzyme activity. The high content of heavy metals is likely to cause an increase in enzymatic activity, triggered by the protective mechanisms in microorganisms in response to the increase in heavy metals content. This leads to enhanced formation of reactive oxygen compounds, for the disposal of which catalase is responsible. Our study has confirmed the potential of catalase activity to be used as an indicator of the state of soils in Solovetsky settlement, known to be exposed to various types of pollution.

## 1. Introduction

In the domain of international cooperation, the protection of the Arctic environment constitutes a special area, which is to be taken when planning and implementing projects in the area. As a result of economic, military and other activities in the Arctic, there has been a qualitative increase in the release of various pollutants into the environment. Water and air pollution, expansion of anthropogenic landscapes, and unregulated exploitation of biological resources lead to rapid environmental degradation. One of the measures to implement the state policy in the field of environmental safety for the Russian Arctic zone is to establish special regimes for the use of natural resources, including, among other things, monitoring and reclamation of natural landscapes. These measures should be dealt with through coordinated work among the northern areas' regional authorities and federal ones. One of the most important contributors to environmental safety is the network of specially protected natural areas [6].

The Solovetsky Archipelago, with its historical and cultural heritage and unique natural landscape, is one of Russia's most well-known historical, cultural and natural heritage sites. Together with its



monuments, the churches of the Solovetsky Islands' Museum-Reserve, are on UNESCO World Heritage List, included UNESCO General Assembly Resolution N 632 of December 14, 1992. Currently, the Solovetsky Archipelago and the five-kilometer water area of the White Sea are included in the specially protected area – the Federal State Cultural Institution "Solovetsky State Historical-Architectural and Natural Museum-Reserve".

Today, the environment of the Solovetsky Archipelago is exposed to a significant anthropogenic impact, the monitoring and scientific exploration efforts remaining insufficient [7]. One of the main tasks that must be considered when it comes to tourism development is that of natural heritage preservation, associated the limiting the anthropogenic impact on the natural environment of the Solovetsky Archipelago. The increasing recreational load on soil cover makes it necessary to maintain soil monitoring on a regular basis. This monitoring covers so far only Bolshoy Solovetsky Island – through a number of scientific and training programs run by NArFU. The studies into soil ecology have shown that it is necessary to evaluate changes in their integral characteristics, one being biological activity. The integral characteristics of the Solovetsky soil biological activity, compared with physicochemical parameters, have several advantages – high sensitivity; rapid responsiveness to external influences; early manifestation of negative impact; availability of bio-indicators of exposure [4,5].

Quantitative measurements of biological activity target the effects of oil spills and excessive recreational load on the ecological paths in specially protected areas; and the biological activity of the soil and vegetation cover in anthropogenically-disturbed Arctic areas and their potential for restoration.

Changes in the number of microbial populations can be caused by deterioration of air regime or accumulation of toxic substances. In soil, immobilized (fixed) enzymes show high resistance levels in conditions of suppressed microbiota activity. So, the metabolism in the soil can remain relatively unchanged for a long time. Scientific studies indicate that a change in enzyme activity may signal an anthropogenic effect and occurrence of stress levels in the soil [9].

The catalase enzyme belongs to the class of oxidoreductases, responsible for catalyzing redox reactions, and is considered to be a characteristic indicator of biological activity in the soil. Its role in the soil-absorbing function is that of destructor of hydrogen peroxide, which is formed during the respiration processes of microorganisms and transformation of organic residues of vegetation.

As a diagnostic indicator of soil condition, catalase activity has proven very informative. Catalase enzyme is sensitive to the effects of the majority of factors and can therefore be used in assessing soil contamination with petroleum products and heavy metals [10].

Our research aims to measure the catalase activity and assess the possibility of using is as a diagnostic indicator of soil contamination with petroleum products and heavy metals by the example of Solovetsky soil.

## 2. Materials and methods

Soil samples were taken in Solovetsky settlement during two summer expeditions organized by NArFU in 2016 and 2017. The sampling and sample preparation process followed the regulations [2]. The soil samples were dried to air-dry condition and sieved (1 mm mesh diameter). This article presents the analysis results of 44 soil samples. The sampling sites and their coordinates are given in Table 1.

**Table 1** – Soil sampling sites.

Site	Coordinates	Sampling year	Number of sub-sites	Number samples taken
Gas station	N65.01'225" E 035.43'197"	2016	5	19
Diesel station	N65.01'181" E 035.43'005"	2017	4	10
Dry dock	N65.01'220" E 035.42'301"	2016 2017	5 4	6 5
Reference	N65.04'252" E 035.41'121"	2017	1	4

To identify the areas exposed to excessive anthropogenic load, a list of potential sources of pollution has been compiled. On the territory of Solovetsky, they include:

1. diesel power station – the one source of electricity for Solovetsky, built in the 1930s on the shore of the Syvatoe Lake. Its diesel fuel storage is located at a distance from the building of the power plant. A fuel leak occurred from the storage on April 25, 2011;
2. gas station, with gasoline (A-92) and diesel fuel storage tanks. Samples were taken of the soils around the station within the sanitary protection zone, not exceeding 50 m from the source; and
3. the sea water dock (dry dock), hydraulic structure actively used until the 1950s as a ship repair dock and currently as a parking place for small vessels. Samples were taken of the soils seasonally flooded with sea water.

The reference samples were of the soils several kilometers away from the settlement, where there is no anthropogenic impact.

The method of Stefanik and Dumitru was used to determine the enzymatic catalase activity [3]. The method is based on the ability of hydrogen peroxide to react with titanium (IV) salts in the presence of dilute sulfuric acid, forming a complex compound of yellow color. The optical density of the resulting solution reflects the amount of hydrogen peroxide not destroyed by catalase. The enzyme activity level was calculated according to the residual amount of the substrate, taking into account the possibility of non-enzymatic, peroxide cleavage.

The chemical analysis of the soil samples was carried out in the Laboratory of Biogeochemical Research at the Department of Chemistry and Chemical Ecology, NArFU Higher School of Natural Sciences and Technology. The granulometric composition of the soils was determined by elutriation [14]; the acidity of soil solution (pH of aqueous extract) according to GOST 26423-85; and the content of organic matter according to GOST 26213-91. Mass fractions of petroleum products were determined using the fluorometric method according to PND F 16.1.21-98 on a Fluorat-02 liquid analyzer. The biogenic elements content in the aqueous soil extract was determined by method of high-performance liquid chromatography in NArFU's Shared Use of Equipment Center "Arktika on liquid chromatograph LC-20 according to PND F 16.1: 2: 2.3: 2.2.69-10 (anions) and M 03.08-2011 (cations), with the financial support from the Russian Ministry of Education and Research (project code RFMEFI59414X0004). The total content of metals (Co, Ni, Mn, V, Pb, Zn, Cu, Fe, Cr, Ti, Sr) was evaluated based on X-ray fluorescence analysis (XRF) according to M 049-P/04 using SPECTROSCAN-MAX. The data were processed by standard methods of mathematical statistics using Microsoft Excel 10.0 software.

### 3. Results and discussion

Bolshoy Solovetsky Island is situated in the subzone of gley-podzolic and podzolic illuvial-humus soils of the northern taiga. Given its small area, the soil cover is heterogeneous. The soil characteristics are mainly defined by the terrain and nature of soil-forming rocks (sands). The soil profile is differentiated by particle size distribution: the upper part is lighter and the lower one is heavier, which can be due to the soil-forming processes and the heterogeneity of the soil-forming rock. Here, in conditions of ample

drainage, the processes of transformation and migration of substances occur in sand in acidic, profoundly washed, oxidized environment. Practically no gleization was observed. Boggy soils occur on slopes and depressions [8].

The illuvial-humus podzols are very acid throughout the entire profile, with maximum acidity occurring mostly in the coarse-humus (surface) horizon and the pH of the aqueous extract increasing slightly down the profile. The average pH of the aqueous extract for all samples was 5.67. It tends to vary slightly from the surface and all the way down the profile from 5.66 to 5.90. A weak acid area is characteristic of the soils under analysis. Such conditions are favorable for the activity of enzymes [12].

The organic matter content is greatest in the coarse-humus horizon, decreasing to 2%-3% in the eluvial horizon and to 1% or lower in sandy soils. The average content of organic matter in terms of carbon was measured 10%, being maximum in the peated, upper horizons (litter), where it ranges between 20% and 48%. In sandy layers with different inclusions, the content of organic matter varies from 0.1% to 5%. The soils with high organic matter content show an increased enzymatic activity. Since hydrolytic processes with invertase, phosphatases and amidases occur more intensely, the soil becomes enriched with humus and biogenic elements. The following ratios of exchange cations (%) are favorable for the action of enzymes: calcium – 60%-80%, magnesium – 10%-30%, potassium – 3%-8%, sodium  $\leq$  5% [12].

**Table 2** – The average content of exchangeable cations in the soils of Solovetsky (%).

Site	Sampling year	Calcium	Magnesium	Potassium	Sodium
Gas station	2016	22	8	29	41
Diesel station	2017	28	10	34	28
Dry dock	2016	18	5	29	48
	2017	59	7	33	11
Reference	2017	13	3	52	32

As can be seen from Table 2, no optimal ratio of exchange cations was not observed on any of the sites. The high concentrations of chloride ions and sodium cations are found in dry dock soils and are due to their proximity to the sea bay.

The content of biogenic elements varies greatly in the soils of Solovetsky. The average content of ammonium ions is 8 (from 3 to 12) mg/kg in the soils of dry dock; and about 7.5 (from 3 to 19) mg/kg around the diesel station with the optimum amount of 10-20 mg/kg. In the reference soils, the values did not exceed the error of determination. The soils around the gas station contained the average of 44 (from 2 to 178) mg/kg of ammonium ions; a significant content of phosphates (up to 800 mg/kg); and the minimal content of nitrates. Given the absence of agricultural fields near the sampling sites, it can be assumed that mineralization of organic matter is the main source of ammonium and phosphate intake. The maximum amount of organic matter (in terms of carbon - up to 48%) is noted.

In our study, the degree of soil contamination with heavy metals and oil products was assessed based on the order by which their concentrations exceeded maximum permissible concentrations (MPC) (Table 3).

**Table 3** – Soil pollution indicators [1].

Element / compound	Content (mg / kg) corresponding to the level of contamination				
	Level 1, acceptable	Level 2, low	Level 3, average	Level 4, high	Level 5, very high
Lead	<32	from 32 to 125	from 125 to 250	from 250 to 600	> 600
Zinc	<87	from 87 to 500	from 500 to 1500	from 1500 to 3000	>3000
Copper	<53	from 53 to 200	from 200 to 300	from 300 to 500	> 500
Cobalt	<50	from 50 to 50	from 50 to 150	from 150 to 300	> 300
Nickel	<85	from 85 to 150	from 150 to 300	from 300 to 500	> 500
Oil and petroleum products	<1000	from 1000 to 2000	from 2000 to 3000	from 3000 to 5000	>5000

**Table 4** – Gross (median) content of heavy metals and oil products (mg/kg).

Site	Sampling year	Pb	Zn	Cu	Co	Ni	Oil products
Gas station	2016	20	16	17	3	13	35
Diesel station	2017	21	32	24	5	21	544
Dry dock	2016	4580	508	36	3	35	1074
	2017	95	125	32	1	30	176
Control	2017	7	7	31	6	30	69

As can be seen from Table 4, the soils had a very high level of metals contamination in some areas. This is true primarily about zinc and lead, which confirms the presence of anthropogenic load on the soil of Solovetsky.

On the reference site, a slight excess of the MPCs for these metals has been noted in the surface horizon. This excess is insignificant, within the limits of determination error, for lead, but it is as high as 1.4 for zinc. The slight degree of pollution of the reference site can be explained by atmospheric transfer of pollutants and geographical features.

In the soils from the gas station, there was a significant excess of MPC for lead – by 1.3–7.0 times – which characterizes these soils as medium and highly polluted. The likely source of pollution with lead is ethyld gasoline, prohibited in Russia since 2002. The 2-fold excess of lead MPC in the soils around the diesel station is probably due to the influence of the extensively used unpaved road. The source of zinc contamination in these areas is not pronounced clearly.

Of all the sites, the most anthropogenically loaded is the dry dock. Polymetallic pollution of various levels was observed in the soils around it. At some points a very high level of pollution was observed for lead (300-times higher than MPC). Zinc pollution was found moderate (11-times higher than MPC). Copper and nickel concentrations slightly higher than their MPCs were found on one sub-site.

In addition to polymetallic pollution, the dry dock area was found to be polluted with petroleum products. This is due to the fuels and lubricants for the small vessels engines. Periodic floodings of the dry dock can lead to their spread over the soil cover. In 2016, the content of oil products in the surface horizons was estimated moderate (about 2000 mg/kg). In 2017, a higher level of pollution was observed (up to 3000 mg/kg). On the dock slopes, the content of petroleum products was less than 140-200 mg/kg, and on the top of the slope only 44 mg/kg.

Along the perimeter of the gas station, the content of petroleum products was small and did not exceed 180 mg/kg.

Previously, spills occurred around the diesel station (in particular of diesel fuels), which affected the state of the soils. It was not possible to assess pollution levels in the area affected because of its protected status. But, the content of petroleum products measured in the surface horizon at a short distance from the affected area ranges from 340 mg/kg (permissible level of pollution) to 13340 mg/kg (very high level). In the control soil, the content of oil products is below the permissible level of pollution.

Thus, our analysis of the soil samples taken in Solovetsky settlement has confirmed the presence of contamination with heavy metals and oil products. The level of soil contamination can be assessed by studying enzymatic activity. Any significant deviation from the average background values of the enzymes activity in the soil, indicates high probability of anthropogenic impact. At the same time, not every anthropogenic impact can cause negative consequences for the soil. The buffer capacity and self-cleaning ability of soils can again be assessed by analyzing the enzyme activity. The average catalase activity was 12 mg H<sub>2</sub>O<sub>2</sub> per 1 hour per 3g of soil, with tendency to decrease down the soil profile.

Firstly, this can be explained by the fact that the soil composition varies greatly (from the peat to the sand fraction). Secondly, since the greatest amount of humus occurs in the upper horizon, the organic matter transformations occur most intensely (the enzyme in question (n=43) shows direct correlation with the content of organic matter (r=0.36), the density of their connection being moderate). Thirdly, catalase is synthesized mostly by aerobic microorganisms, whose population decreases with depth as

the oxygen content decreases [11]. Therefore, only surface soil horizons were analyzed to determine the effect of pollutants on catalase activity.

In the surface horizon of the reference soil sample, catalase activity was measured ( $19 \pm 1$ ) mg H<sub>2</sub>O<sub>2</sub> per 1 hour per 3 g soil. Previous studies have found that fluctuations in the natural abiotic factors (temperature and humidity) can lead to a change in catalase activity within 28% [14]. We excluded from our analysis all fluctuations ranging from 14 to 24 mg of H<sub>2</sub>O<sub>2</sub> per 1 hour per 3 g of soil. Only a few of analyzed samples showed deviation from this range.

In soils of the dry dock, there is an increase in enzyme activity to 29 mg H<sub>2</sub>O<sub>2</sub> per 1 hour to 3 g relative to the reference samples. This can be explained by the protective mechanisms that microorganisms activate in response to the influence of heavy metals on their vital processes by increasing the absorption of oxygen. The biochemical reactions involving molecular oxygen are accompanied by the formation of toxic forms of oxygen, including hydrogen peroxide, for the disposal of which more catalase is synthesized.

For the diesel station, which is characterized by higher levels of oil pollution, on the contrary, a decrease in the enzyme activity to 3 units was observed. This fact can be associated with the decrease in aeration due to the barrier in the form of film that intake of sufficient quantities of oxygen. As noted, catalase is most active under aerobic conditions.

On other sites, only small deviations from the reference value of the enzyme activity were observed. The scale of the negative influences can be assessed by monitoring the area for enzyme activity within 1 to 3 months. It should be noted also that in order to obtain more reliable data, it is necessary to use more reference soil samples so that the enzymatic activity could be evaluated more objectively.

#### 4. Conclusion

Our studies have shown the presence of at least two types of soil pollutants in Solovetsky settlement – heavy metals and oil products. Their high concentrations affect the enzyme activity in oppositely directed ways. While high levels of petroleum products lead to a marked decrease in the enzyme activity, high levels of heavy metals may cause enzymatic activity to increase. This may be due to the protective mechanisms activated by microorganisms in conditions that are stressful to them, leading to enhanced generation of reactive oxygen compounds. Thus, our study has confirmed the potential of catalase activity to be used as an indicator of the state of the soils in Solovetsky settlement.

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