Sources of technogenic pollution influence on phytotoxicity of arctic soils (B. Solovetsky island)

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Abstract. Phytotoxicological evaluation of Arctic soils nearby hydrocarbon storage on Bol’shoy Solovetskiy island has been performed in this study. Totally 6 soil samples (5 contaminated samples and a control sample) were analyzed. Phytotesting was performed on cress (Lepidium sativum L.). Germination, root length, stem length, total biomass were estimated. The data was combined with data from respiratory activity assay of soils. Phytotoxicological impact of contaminated samples has been affirmed by both effects: developmental inhibition of test-culture and stimulating effect on it, according to homeresis phenomena. Respiratory activity of all analyzed samples did not exceed 52.5% of a control sample. Integrated biotesting system is recommended as preliminary screening analysis of soils, exposed to highly toxic contaminants.

1. Introduction

Arctic and subarctic soils are highly sensitive to anthropogenic influence due to human economic activities. Low average annual temperatures, permafrost and the other factors limit the rates of organic matter transformation processes in soils, influence on microbial biodiversity and its amount. These factors all together slow down remediation processes from toxic components in soils. On this evidence monitoring and estimation of potential contaminant sources in Arctic and Subarctic soils is of a great importance.

For evaluation of soil ecological state, the most important contaminants are traditionally analyzed by chemical methods. These contaminants are heavy metals, hydrocarbons, polycyclic aromatic hydrocarbons and so on. The majority of the methods are based on extraction of contaminants and their concentration measurement in soils. Although, estimated values do not always allow to evaluate toxic effects, performed by these contaminants, due to complex mixtures and interactions between its components in soil matrices. Biotesting methods are common for a complete and reliable ecological estimation and its condition monitoring. Alive organisms have an ability to react immediately on chemical substances and their mixtures, providing a consequent signal only on their biologically active forms. Biological test is a method for integral estimate of overall contaminants impact on organism, but not of a separate one. Basically, it is the sufficient advantage of biotesting method to use it for toxicological estimation of soils for ecological monitoring [1]. Simplicity and low price of the tests allow to use them as an estimation screening method of soils ecological state.

A range of test-objects is used in biotesting of soils. They are plants - common autotrophic biomass producer in trophic ecosystems [2], soil bacteria [3], fungi and protozoa - crucial components in organic matter transformation in soils.
The objective of the study was to evaluate the influence of anthropogenic pollution source on phytotoxicity of soils on B. Solovetskiy island. The experiment was performed as a combined analysis of phytotoxicity on cress (*Lepidium sativum* L.) and total respiratory activity estimations of soils.

2. Materials and methods

2.1. Sampling and analyses of soils

Soils samples were collected in August 2018 on Bol’shoy Solovetskiy island. Sampling points and coordinates are represented in figure 1.

![Figure 1. Sampling points on Bol’shoy Solovetskiy island.](image)

Samples were collected close to infrastructure objects, related to hydrocarbon storage. The sampling points were chosen for the experiment because these territories are in a high risk area of hydrocarbon contamination, due to its utilization and potential unexpected occurrences. The control sample was collected from the area with common vegetation cover and with far distance from technogenic objects, related to hydrocarbon usage or storage. Totally there were 6 samples of soils, collected from 0-20 cm layer.

Soil samples were cooled down for further processing. All samples were homogenized manually. During homogenization plant fragments and other premises with particle size more than 5 mm were eliminated. Sample humidity was estimated by drying of 20 g soil at 105 °C for 24 hours (Standard ASTM D2216, ASTM, 2010). Soil organic matter content was detected as ignition loss (at 550 °C for 4 hours [4]).

2.2. Phytotoxicity studies

Cress seeds were chosen as a test-object (*Lepidium sativum* L.), due to recommendations OECD (1984), US EPA (1982) and US FDA (1987). According to these recommendations cress is a plant, recommended for standard biological testing of environmental objects. Controlled parameters were seed germination, root elongation, shoot elongation and total biomass production in a sample (total root and shoot weight).
The amount of 20 seeds was placed into plastic glass, filled with soil sample, and moistened. The maximum depth for the seed was 5 mm. Plastic glasses were covered for the germination process. Samples were analyzed in three replicates each.

For the germination test, plastic glasses with seeds and soil samples were placed in a plant growth chamber (Binder KBWF 240) for 72 hours at 18 ± 1 °C, humidity 80 %, without light. Soils were regularly moistened with distilled water. At 72 hours the amount of sprout seeds was established. The germination test for the seeds was performed with 50 seeds from one batch, placed on moistened sterile filter paper in Petri dish. The seeds germination was 100%. The germination test was held at 18 ± 1 °C, humidity 80 %, without light. The amount of germinated seeds was established within 72 hours.

Consequently, cress sprouts from germination test were grown for 7 days at 25 ± 1 °C, with humidity of 80 %, and 10-hour light regime. The sprouts were regularly moistened with distilled water. Samples with sprouts at the end of the experiment are represented on figure 2.

![Figure 2. Cress-sprouts in soil samples.](image)

When the experiment was finished, the spouts were accurately separated from soil and washed out in water bath. Sprouts were analyzed for root and stem lengths. Sprouts from each of the replicate were placed together, conditioned at 30 °C for 3 days, and its biomass was evaluated.

2.3. Evaluation of respiratory activity of soils
Respiratory activity of the soils was estimated for each sample individually. Soil sample (with humidity 60%) of 15 g dry matter was placed in multilayer sterile cotton bag and tied up. Bags were conditioned above water for 24 hours at 17 °C. Moistened sample was placed in 250 mL flask by hanging it up above 20 mL of 0.05M NaOH. Flask was closed tightly, hermetized and placed in thermostat at 25 ± 0.5 °C for 48 hours. When the time expired, bag with the soil was revealed from the flask and its content was titrated against 0.05M HCl. Relative respiratory activity was estimated on 1 g of organic matter, based on ISO 14240-1 with few modifications.

2.4. Statistical analysis of results
Statistical analysis of the obtained data from phytotesting and respiratory activity was performed by MS Excel.
3. Results and discussion

3.1. Phytotoxicity of soil samples

The soils, which were chosen for the experiment are subjected to hydrocarbon and its premises contaminations. Previously, cress was established as a sensitive method for evaluation of soil contamination with heavy metals [4, 5] and hydrocarbons contamination [6]. Due to previous studies, biotesting on cress is an adequate and representative method for evaluation soils, contaminated with comparable contaminants complexes.

Results of soils evaluation, germination test, root length, stem length and total biomass are represented in table 1.

| Sample | Organic matter (%) | Germination (%) | Average root length (mm) | Average stem length (mm) | Biomass (g) |
|--------|--------------------|-----------------|--------------------------|--------------------------|-------------|
| Control | 3                  | 92              | 63.9±19.4                | 35.9±9.6                 | 0.364       |
| #1     | 4                  | 98              | 90.8±23.8                | 43.9±6.8                 | 0.104       |
| #2     | 92                 | 0               | 0                        | 0                        | 0           |
| #3     | 72                 | 7               | 2.8±1.3                  | 17.8±1.7                 | 0.015       |
| #4     | 68                 | 18              | 5.8±3.7                  | 16.2±3.8                 | 0.020       |
| #5     | 6                  | 92              | 60.8±24.1                | 47.1±8.9                 | 0.109       |

To compare germination data, it is worth to mention that germination for samples #2, #3 and #4 is less than 50 %, which says about inhibitory impact on seed germination in these samples. Sample #2 has the strongest inhibitory effect, due to 0% of germinated seeds in all three replicates. Toxic effect within this sample is established and proved.

Mañas et al. mentioned in his paper that even with the high number of germinated seeds, root length could be inhibited [7]. Low relationship between seed germination and root length was established in other papers [8]. Based on this fact, root length was evaluated for complete phytotoxic estimation. Root lengths for samples #2, #3 and #4 are much smaller comparing to control sample. Root length in sample #4 was 10% of root length in control sample, while root length in sample #3 - 4%. Consequently, results from germination test and root length test proved high toxic effect for soils from samples #3 and #4. High organic matter in the samples with phytotoxic effect could be one of the reasons for higher toxicity as well. To take in account organic matter content in contaminated soils and their toxic effects, there is the following relationship: phytotoxicity increases together with organic matter increase. This fact was established previously in papers [9, 10], where authors explained that organic substances in soils (for example, gumates) sustain toxicants retention and accumulation in soils, especially heavy metals.

Based on data from root length and stem length of sprouts, it was established that soils with determined toxic effect, had stem length longer comparing to root length. Root length/stem length ratio was 0.16 and 0.37 for samples #3 and #4 respectively. Samples #1, #5 and control has the opposite relationship, where root length was bigger comparing to stem length. Root length/stem length ratio for these samples was in range of 1.3...2.0.

Samples without toxic effect (#1, #5 and control), due to germination test and root length estimation, had common characteristics upon organic matter. It was in a range of 3 to 6% for the samples. Germination test values were close, and above 90%. However, root length and stem length in sample #1 was higher comparing to control sample (by 42 and 22% respectively). Sample #5 had almost the same root length value as control, but its stem length was 30% longer comparing to control. Common effect of growth enhancement for Lepidium sativum L. on soils, contaminated with heavy metals, was established in other studies [11, 12]. The author explained this phenomenon by hormesis effect - a stimulating action of small doses of toxicants on higher plants. Due to Hagner, this effect is
an important signal \[4\], which could affirm rather low concentrations of biologically available forms of toxicants in soils. Although, contaminants with low concentrations could highly affect organisms in case of long-term exposure or due to bioaccumulation of them. Hormesis effect might occur in our samples as well.

Biomass productivity is also one of the important parameters, characterizing their growth conditions. Influence of soil contamination on biomass productivity was established in papers \[4, 13\]. To confirm hormesis effect, biomass values for samples (#1, #5 and control) were compared. It was established total linear length of sprouts (root length and stem length) from control sample was lower compared to the same value of sample #1 and #5 by 8 and 35% respectively. However, total biomass of control sample is more than 3 times higher compared to total biomass of sample #1 or sample #5. Intensification in total length of a plant and biomass decrease could be a consequence of high doses of toxicants with concentrations, which do not maintain strong inhibition effect.

3.2. Respiratory activity of soils

Soil upper layers primarily assume contaminants effect. They are well-aired layers, inhabited with various soil microbiota: bacteria, fungi, algae, protozoa and animals. Many microbial communities are indicators of soil ecological sustainability \[14-16\]. For this reason scientists investigate their biodiversity, perform its quantitative assay, its metabolic enzyme activity assay or others. There are integral methods for complex evaluation of general community state. The principle of the method is that all creatures are part of trophic chains. They continuously decompose organic matter and transform it. One of the most important products in this process is carbon dioxide, which is a product of respiratory activity of aerobic organisms. The higher intensity of aerobic metabolism in soils - the higher carbon dioxide level. This relationship provided a basis for respiratory activity experiment for soils.

Values of respiratory activity in soils are represented on figure 3.

![Figure 3. Respiratory activity of soil samples.](image)

Results of integral evaluation of aerobic metabolism in soil microbiota showed that all testing samples had rather low respiratory activity comparing to control samples. Specific activities divided by organic matter for samples #1, #2, #3, #4 and #5 were from 20 to 52.5% higher comparing to control.

The respiratory activity data confirmed phytotesting results on Lepidium sativum L. Even soil samples, which didn’t represent high phytotoxic effect on plants, had low respiratory activity. It is an
additional supporting point in term of hypothesis about homeresis effect in samples #1 and #5, where there was rather low soil contamination.

4. Conclusion
There was a phytoxicological estimation of Arctic soils in the zones with high risk of contamination. It was established that soil samples, collected nearby hydrocarbon storage place on Bol’shoi Solovetskiy island, had phytotoxic premises. Obtained data was confirmed with results from phytotesting on Lepidium sativum L. and results from soil respiratory activity test, which had mutual effect. Different level of phytotoxic action was established through two different types effects: growth inhibition of test-culture and stimulating effect due to homeresis. Respiratory activity of all samples was not higher than 52.5% comparing to control sample.

It is established, that integrated biotesting system is recommended as a preliminary screening analysis of soils, exposed to highly toxic contaminants. In time analysis of soils, which are with a high risk of being exposed to anthropogenic contamination, could allow to determine contamination fact and take soil remediation actions simultaneously. For complete analysis of ecological state of contaminated soils and their further recultivation, it is recommended to complete biotesting with more complicated and up-to-date physico-chemical methods.

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