

## Biotesting of radioactively contaminated forest soils using barley-based bioassay

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**Abstract.** Findings from radioactivity and phytotoxicity study are presented for soils from nine study-sites of the Klintsovsky Forestry located in the Bryansk region that were radioactively contaminated after the Chernobyl accident. According to the bioassay based on barley as test-species, stimulating effect of the soils analyzed is revealed for biological indexes of the length of barley roots and sprouts. From data on <sup>137</sup>Cs specific activities in soils and plant biomass, the migration potential of radionuclide in the "soil-plant" system is assessed as a transfer factor. With correlation analysis, an impact of <sup>137</sup>Cs in soil on the biological characteristics of barley is estimated.

### 1. Introduction

As a result of the Chernobyl accident, the highest radioactive contamination of soil (in particular, forest soils) occurred in the Tula, Kaluga, Bryansk and Orel regions, representing more than 30% of the total area of forest in these regions. Contaminated soils have become one of the most important sources of radioactive impact on plants and other living organisms in forest ecosystems.

Nowadays there are a number of modern techniques to assess soil toxicity, but their application is usually limited due to their high labor intensity and cost. An effective and relatively low-cost approach to estimate toxicity of radioactively contaminated soils is based on an application of bio-testing methods using higher plants test-systems, such as barley.

Thus, this work was aimed at an estimation of biological effects in the test-species (barley) from the radioactively contaminated soils from the Klintsovsky Forestry located in the Bryansk region.

### 2. Materials and methods

Soil samples for the study were taken in 9 study-sites within the techniques forestry (the Bryansk region) that was radioactively contaminated after the Chernobyl accident [1]. As a control sample, forest soils were taken in the "Ugra" National park, near the Sukovka village (NL 54°51' HP 35°07').

The specific activities of <sup>137</sup>Cs in soils and in barley were measured by gamma spectrometry (table 1). Review of published data shows that in the south-west of the Bryansk region the specific activities of <sup>137</sup>Cs in soils decreased with a factor of 7-13 times comparing to 1986 [2].

Transfer factor (*TF*) was calculated as a ratio of the radionuclide specific activities in plant mass ( $A_{plants}$ , Bq/kg) to the specific activities of soil ( $A_{soil}$ , Bq/kg) [3]:



$$TF = \frac{A_{plant}}{A_{soil}} \quad (1)$$

**Table 1.** Specific activities of  $^{137}\text{Cs}$  in forest soils and in barley and results of  $TF$  calculation.

№	Study-site	Soil type	Specific activity of $^{137}\text{Cs} \pm \Delta$ , Bq/kg		$TF$
			in soil	in barley	
1.	Baturovka 6	sand	$8214 \pm 220$	$1793 \pm 661$	0.22
2.	S/H 19	sand	$274 \pm 15$	$500 \pm 41$	1.82
3.	Petrova Buda	sand	$1306 \pm 116$	$729 \pm 141$	0.56
4.	Pravda 9	sandy loam	$288 \pm 14$	$500 \pm 30$	1.74
5.	Ushherp'e 21	sandy loam	$2730 \pm 180$	$548 \pm 199$	0.20
6.	Ushherp'e 81	sandy loam	$1352 \pm 118$	$500 \pm 60$	0.37
7.	Krasnaya gora 44	light loam	$607 \pm 50$	$400 \pm 46$	0.66
8.	Ushherp'e 10	light loam	$1206 \pm 114$	$650 \pm 50$	0.54
9.	Ushherp'e 64	light loam	$1378 \pm 113$	$1793 \pm 661$	0.29

For biotesting, soil samples ( $m = 500 \text{ g}$ ) are placed in special containers and moistened to 60% of the total field capacity. In the prepared soils, barley seeds (not less than 40-50) are planted. Containers are placed into a climate chamber (Panasonic MLS) with automatically controlled parameters: temperature (day time -  $21 \text{ }^{\circ}\text{C}$ , night -  $16 \text{ }^{\circ}\text{C}$ ) and illumination (day - 16 hr, night - 8 hr). Exposure period is 14 days (Figure 1).



**Figure 1.** Sprouting barley seeds in climate chamber (Panasonic MLS).

At the end of exposure, plants are carefully pulled off the soil, washed with tap water, and length of barley roots and sprouts are measured. Toxicity of the soils tested is estimated from a difference between control and impacted samples; the Student t-test was used as a statistical criteria.

Soil phytotoxicity level ( $PE$ ) was calculated as:

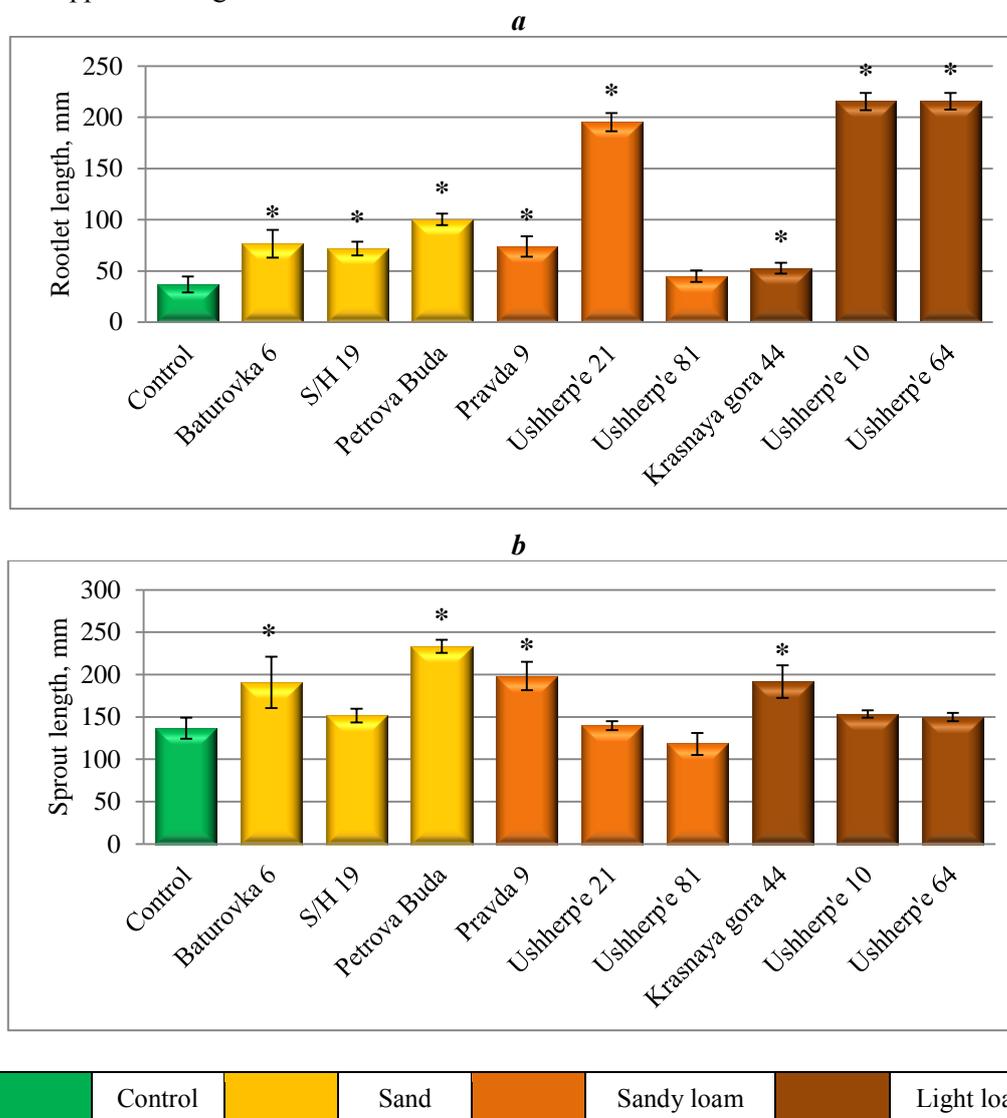
$$PE = \frac{TF_{experiment} - TF_{control}}{TF_{control}} \cdot 100\% , \quad (2)$$

where  $PE$  – index of phytotoxicity, %;  $TF_{experiment,control}$  – test-function of biological indexes: length of barley roots and sprouts (mm) in the experimental and control soil samples, accordingly [4].

### 3. Results and discussion

The data obtained at barley seeds' germination on soils from the Klintsovsky Forestry are shown in Figure 2a. According to the bioassay, the biological indicator of barley rootlet length showed the stimulation effect compared to the control sample. Root length at the test-species germination on all the soil samples from the radioactively contaminated study-sites (except for Ushherp'e 81) was significantly higher ( $\alpha < 0.05$ ) than in control.

With second treat, the barley sprout length, the stimulation effect was also revealed for barley plants grown on radioactively contaminated soils as compared with the control soil sample (Figure 2b). Statistically significant increase in sprout growth was found in soil samples collected from Baturovka 6, Petrova Buda 3, Pravda 9 and Krasnaya gora 44 study-sites, in all other samples the differences appeared insignificant.



**Figure 2.** Length of roots (a) and sprouts (b) at barley seeds germination on radioactively contaminated soils from the Klintsovsky Forestry located in the Bryansk region.

\* - difference from control is significant,  $\alpha < 0.05$

Data from phytotoxicity assessment are presented in table 2. Based on an integral assessment study of soils phytotoxicity with the "length of barley roots" test-function, a significant stimulation of root growth in terms of *PE* is concluded as this index increases by a factor of 1.1–4.8 (table 2). The soil samples tested can be ranged by a degree of phytotoxicity manifestation as follows: Pravda 9 < Baturovka 6 < Petrova Buda 3 < Ushherp'e 21 < Ushherp'e 10 < Ushherp'e 64. Phytotoxicity estimation with the "length of barley sprouts" test-function showed that the most pronounced stimulating effect is registered in the following samples: Baturovka 6, Petrova Buda 3, Pravda 9 and Krasnaya gora 44; in soils from all other study-sites phytotoxicity effect of stimulation is not significant.

**Table 2.** Integral assessment of soil phytotoxicity in samples tested.

Study-site	Root		Sprout	
	< length >± Δ, MM	<i>PE</i> , %	< length >± Δ, MM	<i>PE</i> , %
Control	36.9 ± 7.8		136.7 ± 12.4	
Baturovka 6	76.6 ± 13.5	107.5	190.6 ± 30.4	39.7
S/H 19	71.9 ± 6.8	94.9	151.5 ± 8.2	11.1
Petrova Buda	100.2 ± 5.6	171.5	233.3 ± 7.8	70.9
Pravda 9	44.9 ± 5.6	21.7	118.1 ± 13.0	-13.4
Ushherp'e 21	195.4 ± 8.9	429.1	139.7 ± 5.3	2.4
Ushherp'e 81	73.9 ± 10.1	100.2	198.3 ± 16.9	45.3
Krasnaya gora 44	52.8 ± 5.4	42.9	191.8 ± 19.3	40.5
Ushherp'e 10	215.8 ± 8.1	484.4	149.8 ± 5.0	9.8
Ushherp'e 64	215.3 ± 8.5	483.3	153.4 ± 4.5	12.5

To reveal a relationship of biological indexes of barley (the length of roots and sprouts) from the <sup>137</sup>Cs specific activity in soil, a linear correlation analysis was performed. Findings obtained show that soil contamination with <sup>137</sup>Cs-137 has had a significant influence ( $\alpha < 0.05$ ) on the length of barley sprout only in light loam soils. The correlation between the transfer factor of <sup>137</sup>Cs in the "soil-plant" system and the bioassay indexes is not revealed. An absence of the relationship between these indexes can be explained by limited data samples received for the radionuclide concentration in every type of soil tested.

#### 4. Conclusions

In studies of soils sampled in the Klintsovsky forestry and laboratory barley-based bioassay, the specific activities of <sup>137</sup>Cs are measured in the "soil-plant" system and the transfer factor is calculated for the radionuclide uptake by the test-species, which amounts to 0.20-1.82. The biological effect of radioactively-contaminated soils for barley is analyzed with indexes of length of roots and sprouts as well as phytotoxicity index. Findings obtained indicate that nearly for all soils tested (except for the Ushherp'e 81) the stimulating effect for growth processes in barley roots are detected, and also in many soil samples the stimulation of above-ground part of the plants is observed. A reliable correlation between the specific activity of <sup>137</sup>Cs in soil and biological indexes in barley is installed for only one type of the soils tested – light loam.

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