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ECOLOGICAL SAFETY

Ecotoxicological Assessment of Roadside Soils in Areas along Leningradskoe Highway Using Laboratory Phytotesting

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Abstract—Roadside soils along Leningradskoe Highway are ecotoxicologically assessed by the laboratory phytotest method based on two test cultures. The research was carried out along a 300-m-long transect established perpendicular to the highway. The toxicity of soils, determined according to inhibition of the growth parameters of *Lepidium sativum* L. (cress) and *Hordeum vulgare* L. (rye), has been revealed in the majority of the studied area and is characterized by a bimodal distribution: it is maximum at a distance of several meters from the highway (dangerously and moderately toxic soils), sharply decreases at a distance of 7 m from it, and gradually increases again towards the end of the studied zone (moderately toxic soils). The degree of phytotoxicity is differentiated depending on the test culture variant: *H. vulgare* was characterized by a lower sensitivity to pollutants and did not reveal toxicity in the 7–25 m zone from Leningradskoe Highway, while *L. sativum* revealed toxicity of soils throughout the investigated area. Among the analyzed test parameters, root and seedling length were the most sensitive to pollutants, while seed germination was uninformative.

Keywords: ecotoxicological assessment, phytotesting, toxicity, rye, cress, root length, seedling length, seed germination, highway, road, roadside area, soil, oil products, chlorides

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INTRODUCTION

High road density and intense traffic flows are essential characteristics of modern urban ecosystems. Soils and ground along main highways are zones of high environmental risk, since they receive a wide range of pollutants resulting from automobile exploitation. The dominant pollutants of roadside areas are oil products (OPs), soot, heavy metals (HMs), polycyclic aromatic hydrocarbons (PAHs), and salt-based deicing agents (DA). They are formed due to the incomplete combustion of motor fuel (PAHs and soot) and its leakage (OPs); corrosion of motor vehicle bodies, radiators, and brake shoes (HMs); and the abrasion and pulverization of tires due to interaction with road beds (HMs and PAHs) [13]. The use of DAs in roads and adjacent areas causes saline contamination of soils. PAHs and HMs are compounds highly toxic to living organisms, have a low rate of degradation, and persist in soils for many years. OPs generally indirectly influence living organisms; they change soil physical and chemical properties: density, moisture, and air and redox regime. High concentrations of DA salts can cause tissue necrosis in plants and their subsequent death [15].

The classical method for ecological assessment of soils in transport zones, which is based on the quantitative pollution parameters, such as maximum permissible concentrations (MPCs) and maximum permissible levels (MPLs), has a number of shortcomings: (1) time-consuming analytical identification of a wide range of pollutants of different classes; (2) absence of standards for a number of compounds; (3) failure to take into account the effect of combined action of pollutants, as well as the influence of new toxic compounds in the transformation of pollutants that have entered soils. An alternative to quantitative estimates of pollutant contents is biotesting, the results of which yield an integral estimate of the ecological state of soils.

One of the biotesting methods for assessing urban ecosystem soils is phytotesting [1, 11]. It is based on the sensitivity of plants (test cultures) to exogenous

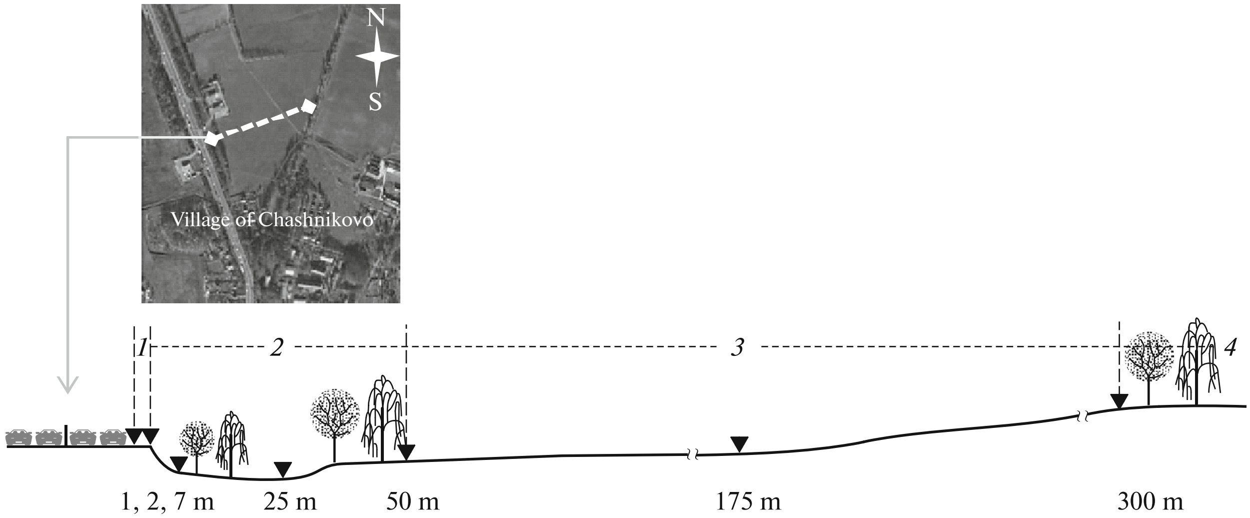

Fig. 1. Sketch of study area: *1* roadside verge (no vegetation); *2* narrow gully with two rows of windbreaks; *3* arable fields with perennial grasses; *4* windbreak between fields.

effects, which is reflected in their growth and morphological characteristics (test parameters): seed germination, root length, seedling length (the aboveground plant part), and plant biomass. The toxicity of the object under study is assessed by the decrease in the quantitative characteristics of the test parameters compared to a control variant (unpolluted soil) [12]. A high informative value, good survivability of test cultures, and easy implementation and rapidity of the method are responsible for the high interest in its application, which is reflected in the exponential growth in the number of publications using laboratory phytotesting over the past 20 years.

The soils and ground of transport zones attraction the particular attention of researchers worldwide due to their environmental hazard and related risks for human health. In Russia, these studies are mainly aimed at monitoring the quantitative content of pollutants, while there are few studies based on phytotesting for ecotoxicological assessment of roadside soils; in addition, they are implemented in a simplified form using one test culture, while international standards require the simultaneous presence of no less than one monocotyledon and one dicotyledon [8].

The objective of this study was to assess the environmental toxicity of roadside soils with a case study of a section of Leningradskoe Highway in Moscow oblast using the laboratory phytotesting method, which is based on international recommendations and the simultaneous use of two test cultures.

MATERIALS AND METHODS

The research was carried out in Solnechnogorsk district, Moscow oblast, at the Chashnikovo Training and Experimental Soil Ecological Center, Moscow State University. The area is characterized by a temperate continental climate that differs from the Moscow climate by a slightly lower average annual temperature and higher amount of precipitation. The average annual temperature is 3.2°, the average annual precipitation is 650 mm, and western winds prevail.

We studied soils and ground along Leningradskoe Highway, which is part of the M10 Federal Highway connecting Moscow and St. Petersburg (25 km from the Moscow Ring Road). The highway in this area has two traffic lanes on either side and a capacity of 48 600 vehicles per day.

The study object is topsoil (0–3 cm) exposed to maximum anthropogenic load. A 300-m-long transect was laid perpendicular to the highway, and experimental plots with dimensions of 0.5 × 0.5 m were delineated along this transect at distances of 1, 2, 7, 25, 50, 175, and 300 m from the road. Samples were collected in May 2017 after spring snowmelt. The topsoil horizon was taken in each plot with a shovel; it was then air-dried at 20–25° in a dark room, mixed, sieved through a screen with a mesh diameter of 2 mm, and stored in glassware at room temperature with no exposure to light.

Along the transect, the landscape of the study area varies with distance from Leningradskoe Highway: there is no vegetation in the immediate vicinity of the road (in the roadside verge) (0–2 m) (Fig. 1(1)); the roadside verge changes to a narrow gully (2–50 m) that includes two rows of windbreaks of birch and hazel (Fig. 1(2)); in turn, the gully changes to a field with perennial grasses (50–300 m) and the area gradually rises with distance from the second windbreak (Fig. 1(3)); the third birch and hazel windbreak is at the end of the field (Fig. 1(4)). There is a legume–grass mixture in

Table 1. Characteristics of roadside soils along Leningradskoe Highway

Soil characteristics	Distance from road, m						
	1	2	7	25	50	175	300
pH_{wat}	8.7	8.2	7.7	7.1	6.8	6.9	7.1
C_{org}, %	2.2	2.0	3.4	2.4	1.8	2.1	2.6
Electric conductivity, μS cm^{-1}	60	50	150	90	50	40	60
Grain size distribution	Sandy loam	Sandy loam	Light loam	Light loam	Light loam	Light loam	Light loam
Density, g cm^{-3}	1.33	1.26	0.82	0.86	0.76	0.63	0.85
Full water capacity, wt %	33.1	33.2	46.9	50.0	57.4	57.9	48.3
Oil products, mg kg^{-1}	10292	8484	3167	219	77	84	90
Chloride ions, mg kg^{-1}	52.4	99.5	104.7	26.2	15.7	68.1	20.9

the field that has been cultivated here for 10 years without fertilizers and is tilled annually.

The soil cover in the study area is represented by chemotechnozems in the roadside verge zone (0–2 m from the road) and by podzolic-gleyic (7–25 m) and cultivated soddy-podzolic (50–300 m) soils.

Phytotesting was carried out simultaneously using two test cultures: a monocotyledon culture (rye *Hordeum vulgare* L.) and a dicotyledon culture (cress *Lepidium sativum* L.). The choice of these is explained by their proven sensitivity to a wide range of pollutants, by favorable growth conditions on zonal soils and climatic conditions in Moscow oblast, and by the fact that both cultures are included in the list of internationally recommended plants (they are presented in the OECD [19] and USEPA [18] methods) for use in laboratory phytotesting. The test parameters of plant growth and development for assessing soil toxicity include root length, seedling length (aboveground plant part), and seed germination. Phytotesting was carried out in three replicates for each test culture. The process was implemented at the laboratory in accordance with the principles provided in technique [4]. Fifty grams of air dry soil were preliminarily sieved through a screen with a mesh diameter of 2 mm and each put in a sterile Petri dish with a diameter of 10 cm. Distilled water was evenly added dropwise to the sample surface in an amount of 60% of full soil water capacity (Table 1). When samples were evenly moistened (after 1 h), seeds were put on the soil surface: 12 rye seeds and 20 cress seeds per dish. The difference in the number of seeds is due to the differences in their size. With allowance for the three replicates of the experiment, the total number of seeds for studying each soil sample was 36 seeds for rye and 60 seeds for cress. Petri dishes with seeds were covered, put in sealed plastic bags to prevent water evaporation, and incubated at 22° in darkness for 7 days. At the end of the exposure time, seed germination was assessed in each dish. Plant seedlings were then washed of soil and placed on paper tissues, where the length of the main root and aboveground part was measured with a ruler. The toxicity of soils was estimated by the level of variation in plant growth and development parameters (root length, germination, length of the aboveground part) in the test samples compared to a control sample according to formula $N(\%) = (A - B) \times 100/A$, where A is the mean value of the parameter in the control sample and B is the mean value of the parameter in the test sample. Depending on the level of variation in root length and seed germination compared to the control (%), the following degrees of soil toxicity have been distinguished [4]: $0 < N \leq 20$ (degree V), almost nontoxic, $20 < N \leq 50$ (degree IV), low-toxic, $50 < N \leq 70$ (degree III), moderately toxic, $70 < N < 100$ (degree II), dangerously toxic, $N = 100$ (degree I), and highly dangerous toxic. An artificial soil prepared in accordance with the ISO standard [16] in the form of a mixture was used as a standard (its mass content was as follows: 10% of sphagnum peat, 20% of kaolinite clay, 69% of fine silica sand, and 1% of calcium carbonate) to obtain a pH-neutral soil.

The content of OPs in soils is determined by infrared spectrometry according to [7]. The method is based on extraction of OPs from soil using carbon tetrachloride (CTC), followed by purification of the extract of low-polar compounds on a chromatographic column with aluminum oxide as the sorbent. The soil (5 g) was filled with 10 mL of CTC and stirred on a rotator for 1 h; the resulting solution was filtered. The procedure was repeated three times, thereby providing exhaustive extraction. Five milliliters of the resulting extract was then purified with aluminum oxide on the chromatographic column. OPs were quantitatively determined on a KN-3 concentration

Table 2. Degree of toxicity of roadside soils along highway

Test culture	Distance from road, m						
	1	2	7	25	50	175	300
Hordeum vulgare	II	II	–	–	IV	IV	IV
Lepidium sativum	II	III	IV	IV	IV	IV	IV

(I) Highly dangerous toxic; (II) dangerously toxic; (III) moderately toxic; (IV) low-toxic; (V) almost nontoxic.

meter (SIBEKOPRIBOR, Russia) by the intensity of absorption of C–H-bonds of methylene ($-CH_2-$) and methyl ($-CH_3$) groups in the infrared spectrum (λ = 3.40 μm). The ternary OP mixture was used as a standard in CTC and consisted of normal alkane, isoalkane, and benzene at a ratio of 37.5 : 37.5 : 25.0 (%) (GSO no. 7554-99).

The content of chloride ions in the soil was determined according to the Mohr argentometric titration method [10]. An aliquot was collected from a water extract prepared at a soil : solution ratio of 1:5 and then titrated with a silver nitrate solution. The minimum relevant titration volume was 0.03 mL (1 drop).

The pH of the soil suspension was determined at a soil : water ratio of 1 : 2.5 with an HI 8314 pH meter (Hanna Instruments, Germany) with an HI 1230 combination electrode [3].

The organic carbon content was determined according to Tyurin's method modified by Nikitin with a volumetric finish. The method is based on the oxidation of organic matter (OM) with a sulfuric solution of potassium bichromate, the excess of which is titrated with Mohr salt. The carbon content is indirectly determined by the amount of sulfochromic acid which was used for OM oxidation. This method establishes the oxidability of humus in terms of the amount of carbon, assuming that the average OM composition corresponds to the formula $C_x(H_2O)_y$ [9]. The sample weight varied from 0.1 to 0.5 g, depending on the assumed soil OM content according to the scale provided by the technique. Ten milliliters of potassium dichromate diluted with sulfuric acid (1 : 1) was added to the soil in each flask, and the flasks were then placed in a drying cabinet heated to 140°, where they were exposed for 30 min, followed by their cooling to room temperature and titration.

The electric conductivity of the soil suspension was measured at a soil : water ratio of 1 : 5 using a DiST 4 WP conductivity meter (Hanna Instruments, Germany) [10]. The soil density was determined by the drilling method; the water saturation capacity was determined by the tube method; the grain size distribution was determined under field conditions using the plastic limit test [2].

The results of the study were statistically processed in Microsoft Excel 2015.

RESULTS AND DISCUSSION

The effect of operation and maintenance of Leningradskoe Highway is reflected in the characteristics of roadside soils and ground and manifested as an increase in pH, overconsolidation, and low water saturation capacity compared to unpolluted zonal soils (Table 1). The increase in organic carbon content on the roadside (1–2 m), where there is no vegetation, is explained by the presence of soot due to the incomplete combustion of motor fuel, which is particularly characteristic of diesel transport; high C_{org} values in the 7–25 m zone are due to the washing of OM-enriched fine particles from roadside ground, as well as to their movement downslope. It was previously shown that the presence of OPs barely influences the amount of organic carbon [20]. The electric conductivity of soils is characterized by low values and the local maximum in the 7–25 m zone corresponds to the lowest values of the ground elevation, where the accumulation of salts washed from the road surface is the most probable.

The toxicity, determined by inhibition of the growth parameters of *L. sativum* and *H. vulgare* on the studied soils compared to the control unpolluted soil, was revealed in the majority of the area. The degree of soil toxicity is characterized by a bimodal distribution with distance from the highway: the highest intensity of inhibition of plant growth parameters (by 60–80%) was revealed at a distance of several meters from the road; it significantly decreases at a distance of 7 m from the highway and again increases to 30–40% at a distance of 175–300 m from the highway (Fig. 2). The root and seedling length parameters were the most sensitive to the chemical pollution of soils, while seed germination was uninformative.

Despite similar patterns in the growth characteristics of both test cultures, they had some differences from each other. Unlike *L. sativum*, *H. vulgare* showed no inhibition of the growth parameter in soils at a distance of 7–25 m from the highway. We assume that this is due to a lower sensitivity of monocotyledon cultures to the set of roadside pollutants than the sensitivity of dicotyledon cultures. This effect of the selective sensitivity of monocotyledon and dicotyledon plants has been described by a number of researchers during the phytotesting of different substances, including OPs, HMs, and PAHs [5]. This yields different results in determining soil toxicity, depending on the variant of the test culture used. It should be noted that the nutrient stock is higher in rye seeds than in cress seeds; therefore, rye may be less sensitive to negative envi-

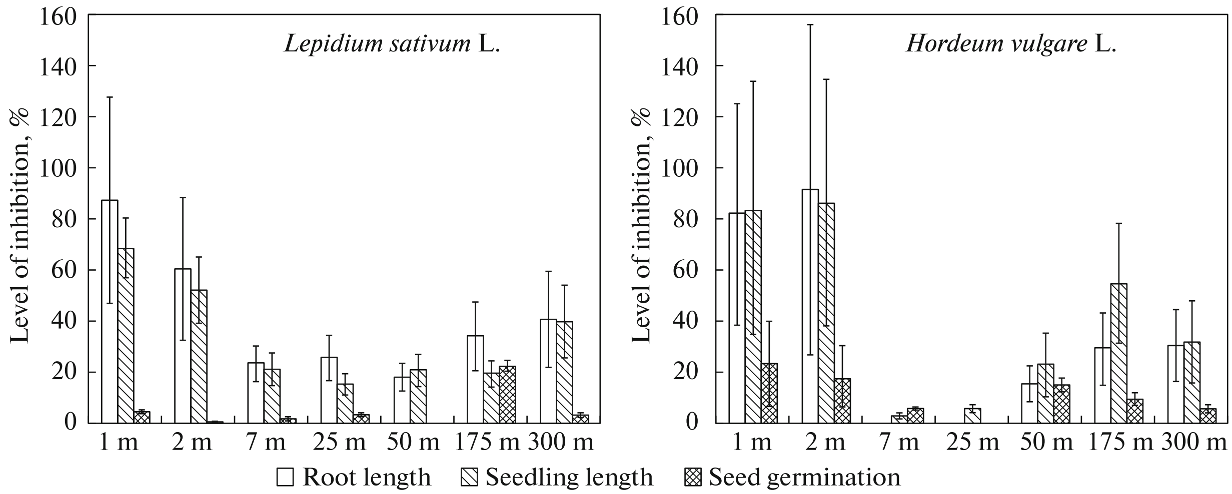

Fig. 2. Inhibition of growth parameters of test cultures in roadside soils along Leningradskoe Highway compared to unpolluted soil.

ronmental factors than *L. sativum* at early germination stages.

When *H. vulgare* is used as a test culture, the soils are determined as dangerously toxic in 1–2 m zone from the highway, nontoxic in the 7–25 m zone, and low-toxic in the 50–300 m zone; according to phytotesting based on *L. sativum*, the soils are determined as dangerously and moderately toxic in the 1–2 m zone and low-toxic in the 7–300 m zone (Table 2). A considerable number of plant seeds and experimental replicates provided statistically significant soil toxicity results.

Let us consider the features of the spatial distribution of pollutants in soils. It is known that, along with heavy metals, OPs are quantitatively dominant among the set of pollutants in roadside soils. In addition, the trends of the spatial distribution of OPs are very similar to those for HMs [14, 21]. Pollution with HMs in the soils of the study area was revealed in the 0–30 m zone from the highway [6]. The maximum OP concentrations (Table 3) established for soils in Moscow (300 mg/kg), which are one order of magnitude higher than the permissible values, were revealed at a distance of 0–7 m from the highway.

The content of OPs decreases two to three times at a distance of 7 m from the highway compared to their content in the roadside verge and sharply decreases to the background values at a distance of 25 m, which are characteristic of natural soddy-podzolic soils and remain constant with distance from the highway along the transect. This spatial distribution of OPs (intensive pollution of the roadside and a sharp decrease in concentrations within 10 m) is typical of soils along highways [14]. This is due to the features of their entry into soils: they migrate with rainwash from the road surface during rains and snowmelt, as well as with vehicle-produced splashing and aerosols.

Analysis of the content of chloride ions contained in the vast majority of DAs used in Moscow and Moscow oblast showed low amounts in soils, which may be due to the low use of DAs in this study area along Leningradskoe Highway, as well as to the absence of their use in roadside areas.

The maximum OP concentrations revealed at a distance of several meters from the highway are well correlated with high soil phytotoxicity values in this zone. The decrease in their content by two to three times at a distance of 7 m from the road is also consistent with the decrease in soil toxicity in this zone compared to the roadside. However, despite the further sharp decrease in the OP concentration to the background level in the 25–300 zone m, phytotoxicity does not decrease. It can logically be assumed that this is due to the presence of additional pollutants in soils.

Table 3. Content of some pollutants in roadside soils along highway

Pollutants	Distance from road, m						
	1	2	7	25	50	175	300
Oil products, mg kg^{-1}	10292	8484	3167	219	77	84	90
Chloride ions, mg kg^{-1}	52.4	99.5	104.7	26.2	15.7	68.1	20.9

The bimodal pattern of phytotoxicity observed for both test cultures may coincide with the spatial distribution of PAHs in roadside areas. While contained in nanoconcentrations, these compounds can be transported by air over considerable distances. It is known from the published data and our own studies that, depending on the road configuration and topographic features, the amount of PAHs may be considerable at a distance of up to several hundred meters from the highway [17, 22, 23].

CONCLUSIONS

(1) The use of phytotesting for ecotoxicological assessment of roadside soils with integrated pollution is relevant due to the sensitivity of higher plants to pollutants.

(2) Root and seedling length are the most sensitive among the analyzed test parameters, while seed germination is noninformative.

(3) The distribution of the toxicity of roadside soils for Leningradskoe Highway along the transect is bimodal: it is maximum at a distance of several meters from the highway (dangerously and moderately toxic soils), sharply decreases at a distance of 7 m from it, and gradually increases again towards the end of the studied zone (moderately toxic soils).

(4) The phytotoxicity results are differentiated depending on the variant of the test culture used: *H. vulgare* has a lower sensitivity to pollutants and does not reveal toxicity in the 7–25 m zone from the highway, while *L. sativum* demonstrates toxicity in soils throughout the studied area.

(5) The simultaneous use of monocotyledon and dicotyledon cultures under conditions of the integrated soil pollution increases the accuracy of the method.

(6) The example of Leningradskoe Highway showed that roadside soils along a main highway exhibit toxicity even at a distance of 300 m from the road. Fields should not be cultivated near main highways, since this may involve ecological risks, human health risks, a decrease in the profitability of agricultural machinery, and a decrease in harvest yield.

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