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Ecotoxicological effects of traffic-related pollutants in roadside soils of Moscow

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ABSTRACT

The objective of this research is to find correlations between traffic-related contaminants in the roadside soils and their ecotoxicity. The study was conducted in Moscow in the vicinity of a highway of 125 000 vehicles per day. The topsoils (0–3 cm depth) were sampled perpendicular to the road at 1-, 6-, 10-, 18- and 50-m distances from the roadbed. Total petroleum hydrocarbons (TPH), polycyclic aromatic hydrocarbons (PAH), heavy metals (HM) in total and phyto-available forms, and deicing salts (DS) were determined. A battery of soil-contacting organisms was tested: phytotoxicity of rye (*H. vulgare* L.) and garden cress (L. *sativum* L.); *E. foetida* earthworm growth rate and mortality; basal and substrate-induced respiration activity, nitrogen fixation and the denitrification activity of the soil microbial complex. To determine the possible risk to aquatic ecosystems, the algal toxicity test (*S. quadricauda*) was provided. Correlations between "chemical" data and intensity of "biological" effects were analyzed.

Concentrations of most contaminants declined to the background values with distance from the road increase. However, the toxicity of roadside soils was obtained for all examined organisms within the whole 50 m zone. Live organisms exhibited different sensitivities to roadside soils pollution. The intensity of inhibition effects decreased in order: higher plants > earthworms and microorganisms > algae. The risk for aquatic ecosystems was assessed as low. Higher plants toxicity correlated with TPH, PAH, some HM, and DS; earthworm toxicity correlated with TPH, some PAH, HM, and DS; microorganism toxicity correlated with TPH and DS; algae had no observed correlations with contaminants. TPH and DS were general ecotoxicants affecting all organisms. Higher plants may be considered the PAH indicators and earthworms as HM indicators. A set of higher plants and earthworms may be recommended as the reduced test-battery of relevant organisms for cost-effective assessment of the toxicity of roadside soils.

1. Introduction

Motor transport is reported as the key source of environmental pollution in megacities (WHO, 2005). Motor transportation results in a wide range of contaminants, among which polycyclic aromatic hydrocarbons (PAH), heavy metals (HM), total petroleum hydrocarbons (TPH) are the most commonly found. Moscow is on the list of the top cities worldwide for the heavy traffic loads and limited road capacity. About 700,000 vehicles are in constant use in a city of 2500 square kilometers. The continuous growth of vehicles number and the presence of cars in home driveways cause the widespread distribution of pollutants in urban ecosystems of megalopolises.

Traffic-related contaminants spread to the surrounding environment in air as dust particles or aerosols, or via road runoff and spray waters (e.g., from street cleaning vehicles). The most intensive accumulation of pollutants occurs in roadside soils because of chemical sedimentation from air pollutants or from contaminated waters. The 10-m zone perpendicular to the roadside is widely reported as the area of the highest contamination, and this can extend as far as 50 m (Werkenthin et al., 2014) or even 250 m if fine particulate matter is dispersed (Zechmeister et al., 2005).

Studying the ecological condition of soils in urban roadside areas is extremely important because these are the most polluted areas in the urban environment. Environmental assessment of soils is usually based

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on a chemical approach – determination of concentration loads for known pollutants and comparing them with established allowable limits. This chemical approach has some limitations. Chemical information does not provide an exhaustive picture of all possible biological effects of toxicants, or the synergistic or antagonistic relationships between contaminants in such complex multicomponent systems as soils; the pollutants can be characterized by different availabilities in relation to living organisms depending on soil properties; and new contaminants can appear in the transformation process of known chemical substances. As a result, pollutants, even in small and poorly detectable concentrations, can have a significant negative impact on living organisms; therefore, compounds in certain soil conditions that are highly hazardous may be related to poor bioavailability. Thus, to assess the actual environmental risk associated with soil contamination, chemical analysis should be accompanied by ecotoxicological studies on organisms of different trophic levels (including producers, consumers, and reducers), living in the liquid and solid phases of soils. This "biological" approach is increasingly recognized by scientists (Lors et al., 2011; Hagner et al., 2018) and is included in environmental practices in the United States and some European countries.

Despite there being many published studies related to roadside chemical conditions (Yang et al., 1991; Wawer et al., 2015; Li et al., 2015), very little is known about their ecotoxicological state. Thus, bioassay studies are especially important for comprehensive assessment of these ecosystems. The more complicated task is to discover the links between contaminant content and the ecotoxicological effects on living organisms. This would allow for prediction of biological effects based on chemical contamination levels; to learn the most ecotoxicologically sensitive organisms; to learn whether any “super toxicants” exists and to recommend a set of organisms that can be used for cost-effective bioassays.

The objectives of this research are the following: 1) to determine the content and distribution of traffic-related contaminants of different classes (TPH, PAH, HM, DS) in roadside soils at different distances perpendicular to the road 2) to investigate the toxicity of soils by studying the reaction of a complex of living organisms of different trophic levels: higher plants and microalgae (producers), earthworms (consumers), and microorganisms (decomposers) 3) to identify the links between the distribution of pollutants in soils along motorways and the toxicity of soils to living organisms through a correlation analysis of soil datasets.

2. Materials and methods

2.1. Study area and sampling procedure

The research area is situated in the western administrative district of Moscow. The average temperature is − 7.7, 5.6, 17.1, and 5.0 °C and the precipitation level is 45, 43, 82, and 61 mm per month for winter, spring, summer, and autumn, respectively. Manufacturing plants are concentrated in the southeast of Moscow, which minimizes the possibility of their emission from contaminating the studied area because the prevailing winds are westerly.

The research was conducted on soils located along Minskaya Street, a 4-km highway with asphalt pavement that was constructed in 1958. The road runs from northwest to southeast. It is a six-lane road, with three lanes in each direction. The traffic intensity is approximately 125 000 vehicles per day.

The roadside soils were examined along a 50-m transect perpendicular to the roadbed (Fig. 1). The transect crossed the following landscape forms: a lawn of perennial grasses, an asphalt walkway, a lawn of perennial grasses, a shrub hedge, and an area of a park with deciduous shrubs and trees. The study area sloped up from the roadbed towards the hedge with a difference in height of about 0.5 m between the two points). Afterwards, the terrain was flat. The next nearest road was 3 km from Minskaya Street. Soil sampling was conducted at 1, 6, 10, 18, and 50 m from the roadbed in April after the spring melting period. Starting April, DS were not applied on the road until the new snow in November. At every perpendicular distance, three sampling sites (0.5 × 0.5 m) were positioned 30 m apart. The topsoils (0–3-cm depth) were excavated with a hand shovel from each sampling site. All samples were air dried, sieved through a 2-mm sieve, and stored at 23 °C in the dark. The properties of the studied soils are presented in detail in Table 1. A reference sample was taken at 150 m from the roadside and checked for the pollutant content to verify that there were no pollutants. The reference sample was of close chemical and physical properties to the examined soils, with the only difference being the contaminant contents.

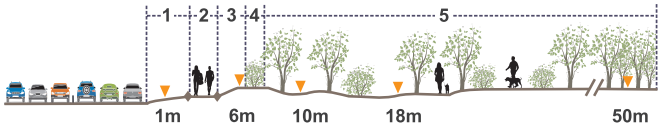

Fig. 1. Transect showing the roadside landscape of Minskaya Street: 1 – a lawn of perennial grasses; 2 – an asphalt walkway; 3 – a lawn of perennial grasses; 4 – a shrub hedge; 5 – an area of park with deciduous trees and shrubs.

Contaminated topsoils are regularly replaced with unpolluted soils as a specific urban soil management practice in Moscow. This supports plant growth and prolongs contaminant sorption in the soil and removal from the atmosphere. The Minskaya Street roadside soils that are designated for replacement are within 6 m of the roadbed and were last changed 3 years prior to this study. The soils greater than 6 m are in the park zone, which was organized in 2003 and are not part of the regular replacement practice.

2.2. Chemical analysis

2.2.1. Contaminant analysis

TPH were extracted from 5 g of soil using 50 mL carbon tetrachloride. We separated 5 mL of extract from the polar compounds with an aluminum oxide column, and TPH contents were detected by spectrometry (λ = 3.4 mkm) using an infrared concentrator KH-3 (SIBECOPRIBOR, Russia) according to the standardized technique (Federal Environmental Regulations of Russia, 2005).

PAH were initially extracted from 2 g of soil using 50 mL of methylene chloride and separated by high-performance liquid chromatography (HPLC) using an Agilent 1200 (USA) with a fluorescence detector. We analyzed 13 PAH types of the 16 presented in the Priority Pollutants List (US EPA, 2014): fluoren (FLU), acenaphthene (AFT), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLT), pyrene (PYR), benz[a]anthracene (B[a]A), chrysene (CHR), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo[a]pyrene (B[a]P), dibenz[ah]anthracene (DB[ah]A), and benzo[ghi]perylene (B[ghi]P).

Cu, Zn, and Pb were detected by inductively coupled plasma spectrometry (ICP-MS) using an Agilent 7500a (USA). In 0.5 g of soil sieved through a 1-mm sieve, the total HM content was determined using microwave acid digestion with 12 mL of aqua regia. The digested soil was transferred to 100-mL flasks and filled to the mark with deionized water. Phytoavailable HM (Cheng et al., 2011) were extracted from 5 g of soil sieved through a 1-mm sieve using 50 mL of ammonium acetate buffer (NH4OAc) at 4.8 pH. The suspension was intensively shaken for 1 h, then filtered through ash-free cellulose with a 5–8-μm pore diameter.

The deicing salts (DS) as Cl^- anions were analyzed in a water extract of soil (soil/water ratio, 1:10) filtered through a 0.45-μm sieve, and contents were detected using an anion liquid chromatograph with an ICS-2000 conductometric detector (Dionex, USA).

2.2.2. Soil property analysis

Electrical conductivity (EC) was detected in suspension (soil/water

Table 1
Characteristics of roadside soils (standard deviations are given in parentheses).

Parameter	Distance from the road, m					
	1	6	10	18	50	150 (reference)
pH_{H20}	7.9 (0.1)	7.7 (0.1)	7.5 (0.1)	7.0 (0.2)	7.2 (0.0)	6.8 (0.0)
Total carbon, %	5.82 (0.17)	5.76 (0.59)	3.65 (0.06)	3.87 (0.37)	3.52 (0.35)	3.50 (0.09)
Total nitrogen, %	0.25 (0.06)	0.26 (0.02)	0.27 (0.02)	0.29 (0.01)	0.30 (0.01)	0.29 (0.01)
EC, $\mu S\,cm^{-1}$	398 (99)	290 (68)	99 (4)	89 (13)	91 (15)	72 (3)
Grading	loamy sand	loamy sand	sandy loam	sandy loam	sandy loam	sandy loam
Density, $g\,cm^{-3}$	1.03 (0.11)	1.05 (0.10)	1.13 (0.08)	1.14 (0.06)	1.15 (0.09)	1.16 (0.05)
Moisture capacity, wt%	33.1 (4.5)	33.2 (3.6)	36.5 (3.0)	42.3 (2.1)	46.5 (3.1)	46.0 (2.2)
P_2O_5, $mg\,kg^{-1}$	224.00 (8.46)	334.76 (42.05)	446.53 (27.98)	622.51 (161.51)	596.12 (201.81)	278.8 (30.45)
K_2O, $mg\,kg^{-1}$	942.16 (32.48)	564.55 (178.31)	244.99 (22.64)	384.38 (66.34)	412.99 (162.07)	365.36 (46.18)
$N\text{-}NH_4$, $mg\,kg^{-1}$	5.18 (3.26)	17.16 (12.65)	8.70 (5.47)	19.62 (6.46)	9.50 (6.35)	27.74 (10.01)
$N\text{-}NO_3$, $mg\,kg^{-1}$	1.97 (0.97)	6.26 (3.04)	3.80 (1.52)	2.36 (2.56)	16.89 (2.77)	10.00 (1.54)
Na^+, $mmol\,100\,g^{-1}$	2.25 (0.54)	0.22 (0.03)	0.69 (0.15)	0.28 (0.01)	0.20 (0.01)	0.46 (0.05)
Ca^{2+}, $mmol\,100\,g^{-1}$	6.47 (0.14)	7.93 (3.33)	2.74 (0.81)	3.44 (3.01)	8.33 (3.51)	6.23 (0.21)
Mg^{2+}, $mmol\,100\,g^{-1}$	0.4 (0.07)	0.86 (0.28)	0.34 (0.10)	0.28 (0.24)	0.95 (0.13)	1.09 (0.16)
$HCO3^-$, $mmol\,100\,g^{-1}$	0.67 (0.14)	0.75 (0.00)	0.58 (0.14)	0.58 (0.14)	0.50 (0.00)	0.08 (0.01)

ratio, 1:5) using a DiST 4 WP conductometer (Hanna Instruments, Germany). Total soil carbon and nitrogen were measured using an element analyzer Vario III (Elementar, Germany) via sample combustion in an oxygen medium at 1150 °C. Soil pH was determined in suspension (soil/water ratio, 1:2.5) using an HI 8314 portable ion meter (Hanna Instruments, Germany). The soil density and moisture capacity were determined gravimetrically; the soil texture was detected under field conditions (Vadyunina and Korchagina, 1986). More details on soil properties analysis are presented in our previous research (Nikolaeva et al., 2017). Determination of mobile phosphorus and potassium, ammonium, nitrates, sodium, calcium and magnesium, bicarbonate ions followed the National Standards of the Russian Federation (2011; 1985d; 1985c; 1986; 1985b; 1985a, accordingly).

2.2.3. Quality assurance and quality control

The climatic conditions of laboratory working environment matched the instrument instructions. Only high purity reagents were applied for the analysis.

The detection limit was $3\,\mu g\,kg^{-1}$ for FLU, AFT, PHE, ANT, and FLT and 0.3 for PYR, B[*a*]A, CHR, B[*b*]F, B[*k*]F, B[*a*]P, DB[ah]A, and B[ghi]P; $0.1\,mg\,kg^{-1}$ – for HM; $50\,mg\,kg^{-1}$ – for TPH. To ensure analytical precision of PAH detection, we included reference soil sample in each cycle of measurements. The indicated values did not exceed 5% variance for all individual components.

The following standards were applied for accuracy detection measurements: PAH calibration mix (CRM 47940) of Sigma-Aldrich – for PAH; the Agilent Initial Calibration Verification Standard 5183–4682 - for HM. The Russian State Standard Sample № 7554–99 (Ecoanalytica, Moscow) was used for TPH analysis and calibration procedure. Quality control samples were applied several times per instrumental run being incorporated every 30 samples. The accepted deviation between the measured and standard value was 5%. The blank samples made 4% of all analyzed samples. The quality criterion for blank samples was the measured values should be below detection limits.

Calibration curves were prepared using 7 concentrations of reference materials. Accepted correlation coefficients were 0.99.

2.3. Bioassay procedures

2.3.1. Soil bioassay

2.3.1.1. Higher plant bioassay. Higher plant bioassays were performed according to Federal environmental regulations of Russia (2009). Seed emergence capacity and the length of seedling roots and sprouts of *Hordeum vulgare* L. (*H. vulgare*) as monocotyledonous plant (produced by LLC "Gardenzone", Russia) and *Lepidium sativum* L. (L. *sativum*) as dicotyledonous plant (produced by LLC Group of companies "Gavrish", Russia) were determined. The bioassay was performed in three replicates for each soil sample and test culture. Test validity criterion was 100% of germinated plants in the control variant. Sterilized plastic Petri dishes (diameter, 10 cm) were filled with 30 g of air-dried soil sieved through a 2-mm sieve. Distilled water was added to soil dropwise following 60 w%. of their moisture capacity. After 1 h, when the soil became evenly moistened, seeds were placed on the surface of soil at equal distance from each other. Twelve seeds per Petri dish were added for rye and 20 for cress owing to the difference in seed size. Then, the Petri dishes were covered with lids and placed to plastic zip-lock packages to prevent water loss due to evaporation from soil surface. The packages were stored in darkness for 7 days at 25 °C. After the incubation period, the number of germinated seeds was counted. Then, the soil was washed from the seedlings and they were placed on paper sheets. Root and sprout length was measured manually with a ruler.

Soil toxicity was assessed based on the toxicity index calculated by the formula [(A-B)/A] x 100, where: A is the analyzed parameter (seed germination or root/sprout length) on the control soil and B is the analyzed parameter on the examined soil.

2.3.1.2. Earthworm bioassay. *Eisenia foetida* (*E. foetida*) earthworm bioassay was performed in accordance with the OECD 207 (1984) regulations. Earthworms were obtained from the collection of the laboratory of environmental chemistry in All-Russian Research Institute of Phytopathology. Adult earthworms of the same age with individual weights from 200 to 270 mg were used. Before the experiment, earthworms were kept for 3 days on moistened fine-grained sand (250 μm) to clear the intestines of the original substrate. The tests were conducted in 1-L polypropylene cylindrical jars (diameter, 11 cm). The jars were equipped with screw lids to prevent escape and minimize water evaporation. Each lid was punched with 100 0.5-mm holes of for ventilation. A total of 500 g of dry soil sieved through a 2-mm sieve was placed in each jar. Soils were moistened by adding deionized water to obtain 60 w% of the maximum water holding capacity and the medium was mixed. Ten adult earthworms were added to each jar. Tests were performed with three replicates for each sampling location and control soil. The jars covered with lids were incubated at 20 °C, 80% relative humidity and 400–800 lx of constant light. Every 2 days, the moisture content was checked gravimetrically and corrected by adding water. *E. foetida* mortality and weight gain were assessed at experimental days 7 and 14. The worms were washed with deionized water prior to weighing and excess water removed by placing the worms briefly on filter paper. *E. foetida* mortality was calculated using the formula [(A-B)/A] × 100, where: A is the number of earthworms in the control soil, and B is the number of earthworms in

examined soil. The growth rate (weight gain) was evaluated as $[(W_{7(14)}-W_i)/W_i] \times 100$, where W_i is the earthworm weight at the beginning of the experiment, $W_{7(14)}$ is the earthworm weight at day 7 (day 14).

2.3.1.3. Effects on microorganisms

2.3.1.3.1. Soil basal respiration (SBR). 2 g of air-dried 2-mm sieved soil was placed in 15-mL glass vessels, moisturized with sterile distilled water following 60 w% of soil moisture capacity, and then preincubated for 5 days at 25 °C in desiccator. BSR was measured as the rate of CO_2 produced (CO_2 emission) from a 2-g sample during 24 h of incubation at 25 °C using a M-3700 gas chromatography (Meta-Chrom, Russia) with a katharometer (column length, 3 m; filler material Polysorb-1, transmitter gas, He) (Gardini et al., 1991).

2.3.1.3.2. Substrate-induced soil respiration (SIR). A total of 1 g of preincubated soils (described above) in 15-mL glass vessels were supplemented with 0.1 mL of glucose solution (10 mg glucose g^{-1} soil) and sealed. After 5 h of incubation at 22 °C, the amount of carbon dioxide produced was measured using the gas chromatography (Ananyeva et al., 2008a).

Nitrogen fixation rate (NFR) in soil was determined in aerobic conditions by an acetylene reduction assay using gas chromatography (Hardy et al., 1973). In this method, acetylene is reduced to ethylene (C_2H_4) due to nitrogenase enzyme activity and ethylene formation is calculated. After a pre-incubation period, 5 g of soil samples were placed in glass vials (15-mL volume), 1 mL of 10% glucose solution was added to soil and incubated at 25 °C for 24 h to initiate active growth of microorganisms. Then, the flasks were closed tightly with rubber plugs and 1 mL of the gas in each flask was replaced with acetylene via a syringe. The flasks were incubated at 25 °C for a further 1 h and a 1-mL sample of the gas was collected from each flask and analyzed using a Kristall 2000 chromatography (Meta-Chrom, Russia) with a flame ionization detector (column length, 1 m; filler material, Porapak N 80/100 [Sigma-Aldrich, USA] transmitter gas N_2).

Denitrification rate (DR) was determined as N_2O produced during incubation at 25 °C for 24 h using the Kristall 2000 chromatography with an electron capture detector. A total of 5 g of soils was placed in 15-mL flasks, then 1 mL of water solution containing 2.5 mg of glucose and 0.8 mg of KNO_3 per gram of soil substrate was added. For creating anaerobic conditions, an additional 2 mL of water was applied. The flasks were closed with rubber plugs, the air inside the flask was replaced with argon (argon at a high-pressure was forced through the flask for 40 s and the air volume was displaced through a vent) to produce anaerobic conditions. Then, 1 mL acetylene was added (Yoshinari et al., 1977).

2.3.2. Aquatic bioassay

2.3.2.1. Algal bioassay. The green axenic alga *Scenedesmus quadricauda* was used for bioassay according to ISO 8692:2012, OECD 201 (2011) and the Federal environmental regulations of Russia (2007). The algal culture was obtained from the collection of the Department of hydrobiology of Faculty of biology in Lomonosov Moscow State University. The stock culture of algae was cultivated on the Uspenskiy medium at 22–24 °C. For the experiment, a 3–4-day culture (at the exponential growth stage) was used. Aqueous extracts of examined soils were prepared in the weight ratio 1:4 (soil containing hygroscopic water to distilled water at pH 7.0–7.5). The suspensions were intensively shaken for 2 h at 120 rpm, then sedimented for 30 min and filtered through ash-free cellulose with a pore diameter of 5–8 μm. Mineral nutrients were added to the filtrates to prepare Uspenskiy medium (Terekhova et al., 2018). Pure Uspenskiy medium was applied as the control. Stock solution of algae was added to all filtrates and the control to achieve a concentration of 50,000 cells mL^{-1} at the beginning of experiment. Algae were cultivated in 150-mL glass flasks for 72 h under 18 h light (5000 lux) and 6 h darkness and at 22–24 °C. Experiments were carried out in triplicate. The toxicity of aqueous extracts was assessed via the change in fluorescence intensity of chlorophyll relative to the control using Fluorate-05–2 M (Lumeks, Russia). The measurements were realized in the suspension as the density of algae was high and additional extraction of chlorophyll was not required. The intensity of chlorophyll fluorescence was registered at 680 nm excited at 420 nm. The toxicity index was calculated as: $[(B-A)/A] \times 100$, where B is the fluorescence intensity of chlorophyll on the examined soil and A is the fluorescence intensity of chlorophyll on the control soil.

2.3.3. Quality assurance and quality control

100% seed germination was achieved for both test-cultures in control variant, meeting the validity criteria of the higher plant bioassay (OECD, 2006). To increase results precision a total of 60 L. *sativum* and 36 *H. vulgare* plants were assessed to characterize soils in each sampling location instead recommended minimum in 30 plants of each species. *H. vulgare* and L. *sativum* test-cultures were selected for the following reasons: their sensitivity to HM, TPH, PAH, DS has been proven by research (Arambašić et al., 1995; Yixian, 1997; Smreczak and Maliszewska-Kordybach, 2003; Ali et al., 2004; Oleszczuk, 2008); they are among the recommended cultures in the guidelines of different regulating organizations (OECD, 2006; US EPA, 2012); and they are tolerant for growth in soils under local climatic conditions.

Conditions for the validity of the earthworms bioassay were obtained - the mortality in the control variants did not exceed 10% (OECD, 1984), and made 0% at day 7 and day 14. All the worms applied were of standard age and weight.

For the algal test to be valid, the following performance criteria were used: the fluorescence intensity in the control cultures should have increased by a factor of at least 10 within the 72-h test period; the mean coefficient of variation for section-by-section specific rates of fluorescence intensity in the control cultures must not exceed 20% (FR, 2007; OECD, 2011). Potassium dichromate as a reference substance was used to check the test procedure. We tested the reference substance before the test procedure start to ensure its quality.

2.4. Data analysis

All the experiments were performed with 3 replicates for each sampling location and the results were statistically processed. Standard deviations and correlations were determined using Microsoft Excel 2010 and R 3.1.3 software. Principal components analysis (PCA) was carried out using XLSTAT Software 2014. An aggregate of 55 parameters for the five soil sampling locations were incorporated into the PCA. These parameters included data on soil properties, traffic-related pollutants, and ecotoxicological effects of soils on organisms.

3. Results and discussion

3.1. Soil properties

The examined soils were characterized by properties typical for urban artificial landscapes in Moscow (Table 1). They were rich in organics because they contained peat, which is a source of carbon, and characterized by an alkaline pH and relatively low density. We considered that increased levels of background nitrogen, phosphorous and potassium were related to the urban activity effect described by many authors as the key characteristic of city areas (Zhao and Xia, 2012; Forman, 2014). We attributed the elevated levels of these elements as the distance from the roadside increased to the older age of the topsoil in the 18- to 50-m zone compared with the 1- to 10-m zone. The roads caused an increase in the EC values in the 1- to 10-m zone. Nevertheless, most of the obtained values of the soil characteristics met the permissible levels set for artificial soil-like substrates in Moscow (Resolution of the Moscow Government on Improving Quality of Urban Soils, 2004).

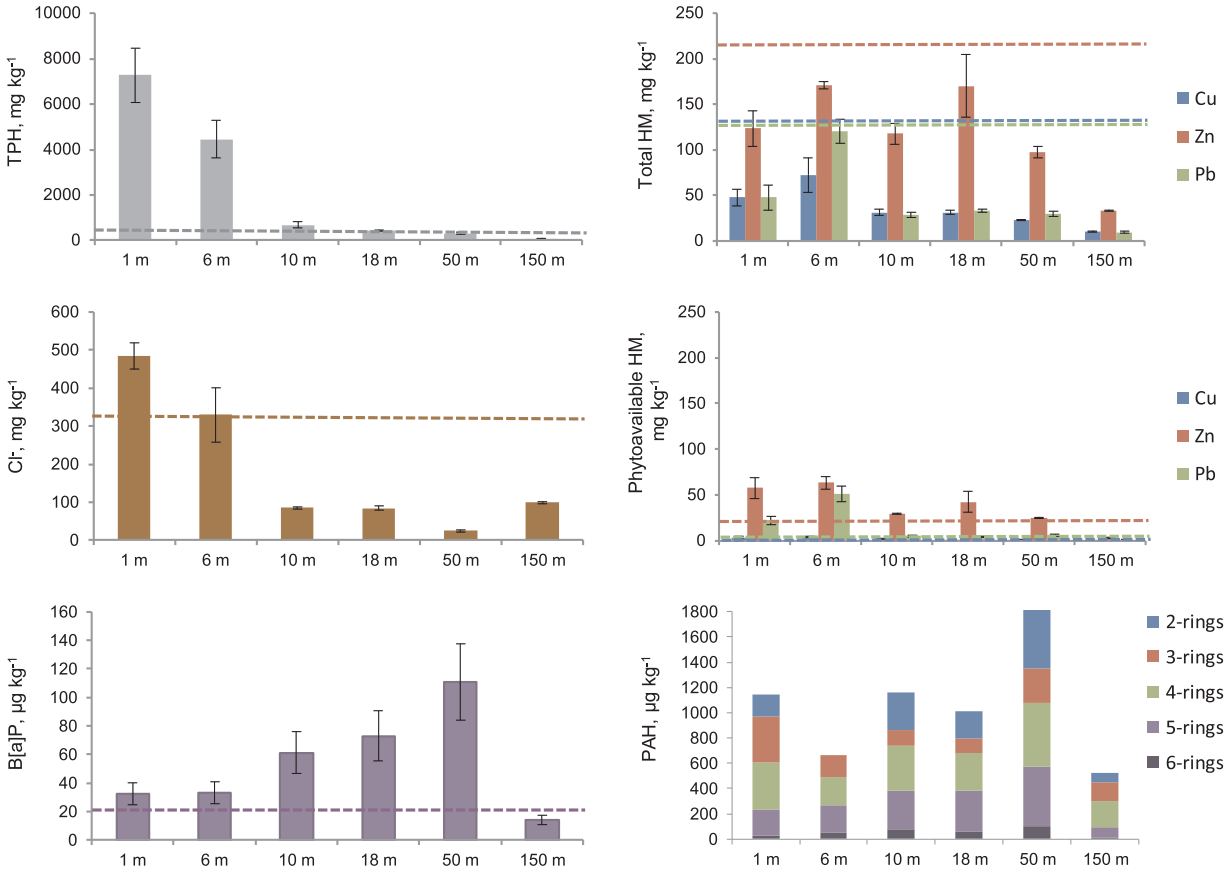

Fig. 2. Distribution of contaminants in topsoils based on distance from the road. Dotted lines indicate permissible levels of contaminants in Russia. Total PAH is not regulated in Russia.

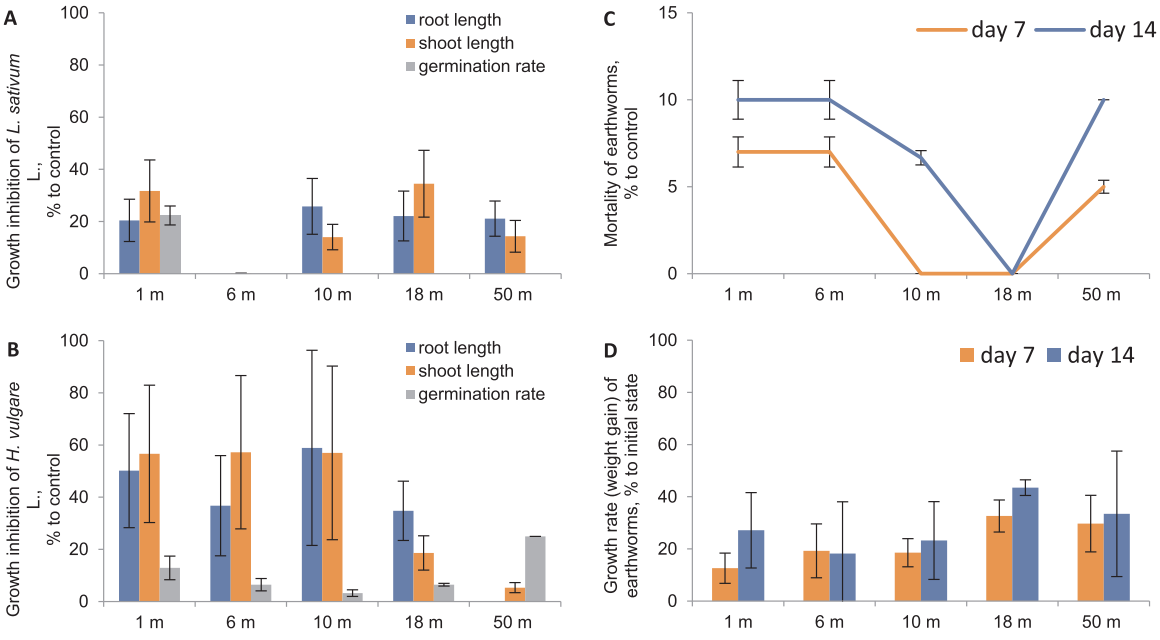

Fig. 3. Ecotoxicological effects of roadside soils to higher plants and earthworms: growth inhibition of *H. vulgare* L. (A) and L. *sativum* L. (B); mortality of *E. foetida* (C) and inhibition of growth rate (D) at days 7 and 14.

3.2. Contaminant distribution

The highest concentrations of the most of contaminants (TPH, HM, DS) occurred within 1–6 m from the highway (Fig. 2). TPH and phytoavailable HM were significantly above the permissible levels set for Moscow soils by an order of magnitude, while HM total and DS did not exceed standards. The contaminants declined intensively following the distance from the road increase meeting the background levels within 18–50 m roadside zone.

PAH distribution increased with distance from the road

contradicting the classical declining trend. We attributed this to the lower age of soils within 1–6 m zone compared with the 10–50 m zone, and the presence of the dense tree line beyond the 50-m position filtering sediment and fine PAH-containing particles that had been transported from the road. The mean concentrations of total PAH along the 50-m transect were about 10 times higher than the background levels in typical park zones in Moscow and also exceeded the worldwide background values of total PAH in unpolluted areas (5–100 μg kg^{-1}) according to the Canadian Council of Ministers of the Environment CCME (2010). Mean concentrations of B[*a*]P (Fig. 2) were 62 ± 33 μg kg^{-1}, which was three times higher than the permissible level set in Russia (20 μg kg^{-1}) according to the Russian sanitary tresholds (1987). In group composition 3,4, and 5-rings PAH were prevailing, concentration of 6-rings ones was minimal in all sampling locations. It was found the common character of ratio between different PAH groups. Among individual PAH, the key contributors were PHE and FLT, which are markers of vehicle emissions (García-Alonso et al., 2003), and B[b]F.

3.3. Bioassays and correlations with contaminant concentrations

3.3.1. Higher plant bioassay

Toxicity was indicated for all the soils within the 1- to 50-m roadside zone, but *H. vulgare* and L. *sativum* performed it differently (Fig. 3. A, B). Despite both test cultures demonstrated toxicity within the 50-m roadside zone, its intensity and characteristics differed.

H. vulgare was more sensitive to soil pollution, performing 1.5–2 times higher toxicity than L. *sativum*. A difference in toxicity values also depended on the test parameter analyzed. Toxicity related to shoot and root length was significantly higher than seed germination for both test cultures.

H. vulgare showed a decreasing trend of toxicity related to shoot and root length from 1 to 50 m, which was in line with the decreasing trend of TPH, HM, and DS concentrations in the examined soils. Toxicity related to the germination rate was of gradual decrease in the 1- to 10-m zone and increased in the 18- to 50-m zone. This trend was in line with the PAH concentration distribution and strong correlations were found between germination-related toxicity and different PAH groups (2–5 rings) (Table 2). We can suppose that the germination rate parameter showed a high correlation in our study with PAH only (rather than other contaminants) and may be applied as the marker for PAH contamination in roadside soils.

L. *sativum* showed a flat distribution of shoot- and root-related toxicity with distance from the road, except for the 6-m location where no inhibition of any growing parameter was observed. Soils in this position had the lowest PAH content compared with other locations, and the group of 3-rings PAH was not presented there. Moreover, FLU, which has been reported to have the highest toxic potential among other PAH in higher plants (Somtrakoon and Chouychai, 2013), was also not detected at 6-m location. Strong correlations were found between PAH and the root length parameter while shoot length did not demonstrate these correlations (Table 2). Germination rate inhibition was observed only at the 1-m location.

3.3.2. Earthworm bioassay

Sensitivity of earthworms to TPH (Ramadass et al., 2015), DS (Owojori et al., 2009), HM (Spurgeon and Hopkin, 1996) and PAH (Eijsackers et al., 2001) in monocontaminated soils is widely confirmed, while their reactions in polycontaminated roadside soils have not been studied.

E. foetida earthworms were sensitive to the contaminants in soil within the 50-m roadside zone. Mortality of *E. foetida* did not exceed 10% of the control at day 14 (Fig. 3. C). Despite this, earthworms showed clear differences in growth rates between sampling locations (weight gain rate) (Fig. 3. D). This parameter was of high sensitivity and was correlated with mortality at day 14 ($R = -0.73$, $p < 0.05$).

Table 2
Correlation coefficients between ecotoxicological effects and contaminants concentrations in topsoils within 50-m roadside zone.

			PAH						HM total			HM phyto-available			DS
		TPH	PAH 2	PAH 3	PAH 4	PAH 5	PAH 6	Pb	Cu	Zn	Pb	Cu	Zn	Cl^-
Higher plants	*L. sativum* L. Root length growth inhibition, % to control	–	0.75	–	0.64	–	–	–	–	–	–	–	–	–
	L. sativum L. Shoot length growth inhibition, % to control	–	–	–	–	–	–	–	–	–	–	–	–	–
	L. sativum L. Germination rate, % to control	0.80	–	–	–	–	–	–	–	–	–	0.65	0.66	0.83
	H. vulgare L. Root length growth inhibition, % to control	–	–	–	–	–	–	–	–	–	–	–	–	–
	H. vulgare L. Shoot length growth inhibition, % to control	0.65	–	–	–	–	–	–	0.67	–	0.56	0.81	0.60	0.69
	H. vulgare L. Germination rate, % to control	–	0.64	0.68	0.83	0.66	–	–	–	–	–	–	–	–
Algae	*S. quadricauda* fluorescence intensity inhibition, % to control	–	–	–	–	–	–	–	–	–	–	–	–	–
Earthworms	*E. foetida* mortality (day 7), % to control	0.87	–	0.68	–	–	–	0.70	0.76	–	0.81	0.85	0.77	0.85
	E. foetida weight gain rate (day 7), % to initial state	− 0.80	–	–	–	–	–	–	–	–	–	− 0.79	− 0.53	− 0.79
	E. foetida mortality (day 14), % to control	0.57	–	0.73	–	–	–	–	–	–	0.54	0.55	–	0.50
	E. foetida weight gain rate (day 14), % to initial state	–	–	–	–	–	–	− 0.63	− 0.67	–	− 0.68	− 0.75	–	–
Micro-organisms	Basal respiration, μg CO_2-C g^{-1} h^{-1}	–	–	–	–	–	–	–	–	–	–	− 0.58	− 0.50	− 0.53
	Substrate-induced respiration, μg CO_2-C g^{-1} h^{-1}	− 0.77	–	–	–	–	–	–	–	–	–	− 0.70	–	− 0.78
	Nitrogen fixation, ng C_2H_4 g^{-1} h^{-1}	–	–	–	–	–	–	–	–	–	–	–	–	–
	Denitrification, μg N_20 g^{-1} h^{-1}	− 0.88	–	–	–	–	–	–	–	–	–	− 0.70	− 0.71	− 0.90

*Values below 0.50 are marked as "-".
**PAH 2 - the group of 2 rings PAH.

E. foetida mortality and growth rate values showed correlations with the intensity of soil contamination within the 50-m roadside zone, demonstrating links with a spectrum of pollutants (TPH, PAH 3, HM, and DS) (Table 2). Multiple correlations were observed between *E. foetida* and different HM. The sensitivity of earthworms to HM has been reported in many studies and their effects are different depending on individual HM. In this research, we found strong correlations between mortality (at day 7) and both HM forms in the studied roadside soils for all metals (Cu, Zn, Pb) except for total Zn. Inhibition of the weight gain rate parameter (at day 7) showed correlations with phyto-available Cu and Zn only and links with any total metals were not found. Thus, detection of phyto-available HM forms allowed us to discover more correlations and should be recommended for analysis in ecotoxicological studies in parallel with HM total determination.

The trend of the growth rate parameter was opposite to the mortality parameter. A higher growth rate corresponded with a lower mortality, and an absence of contaminant-related mortality at the 18-m location was associated with a maximum of growth rate (at day 14). Parallel execution of both ecotoxicological parameters allowed us to conclude that *E. foetida* mortality was driven by the inhibition effect of traffic-related contaminants on earthworm growth.

3.3.3. Effects on microorganisms

Most parameters of microorganism functioning were characterized by a linear growth of activity as the distance from the road increased (Fig. 4). Activity rates near the road (1–10 m) compared with those at the 50-m location were inhibited.

The SIR intensity (which characterizes the maximum basal metabolic activity of the community) had a smooth linear decline as the distance from the road decreased, showing a 45% loss within the 50-m roadside zone (the differences between locations were reliable, $p < 0.05$). According to absolute values, the determined SIR activity in the 18- to 50-m zone approximately corresponded to the levels of Albeluvisols (Iuss Working Group, 2015) – natural soils of Moscow (Ananyeva et al., 2008b).

As with SIR, the magnitudes of DR declined as the distance from the road decreased, showing a 30% loss within the 50-m roadside zone (with significant differences between locations, $p < 0.05$). In terms of absolute values, DR corresponded to the levels of Albeluvisols. We address the leading role in the inhibition of DR and SIR to TPH and DS contaminants as the very strong correlations between their concentrations and DR were found (Table 2).

The SBR decreased slightly as the distance from the road decreased (the differences were significant, $p < 0.05$), and did not exceed 1 mg CO_2 -C g^{-1} h^{-1}. Urban soils of lawns and parks far from the roads are usually characterized by higher rates of respiration, and we report the overall inhibition of SBR. However, the absence of radical changes in SBR near the road and at a 50-m distance indicates the good adaptation of the soil microbiome to roadside pollution. Probably only microorganisms that are physiologically active and have mechanisms of resistance to toxicants perform the SBR.

The magnitude of the NFR did not show a reliable trend with distance from the road, and no correlations to any contaminants were observed. This parameter of soil microbial complex functioning seems to be of lower sensitivity to other parameters researched.

In general, the proximity to a road with heavy vehicle traffic significantly reduces the integral indicators of soil microbial activity by 20–45% with the exception of NFR. The sensitivity to traffic-related contaminants increases in the order of SBR – DR – SIR. At the same time, the inhibition of ecotoxicological parameters is linear, but not dramatic. Even in the close vicinity of the road, activity remains at the level of typical anthropogenically disturbed local soil at a moderate impact level.

3.3.4. Algal bioassay

Roadside soils did not exhibit significant toxicity in algae. Fluorescence intensity inhibition did not exceed 18% compared with the control and the maximum effect was determined at 50 m from the highway. No relationship between toxicity data and distance from the road was found.

Algae bioassay was applied for toxicity evaluation of soil liquid phase and risk assessment of potential contamination for water bodies. The low values of algae toxicity that were observed in several locations were expected because most traffic-related contaminants are not transferred from soil to their water extracts because of hydrophobic properties (TPH, PAH) and strong sorption in the soil matrix and organic matter (HM). Only DS have a high potential to be presented in water extracts. Toxicity determined on algae did not exhibit correlations with any traffic-related contaminants as the distance from the road increased (Table 2). Nevertheless, we cannot postulate that determining soil toxicity using algae studies is not informative for contaminated roadside soils because algae may reflect the sorption

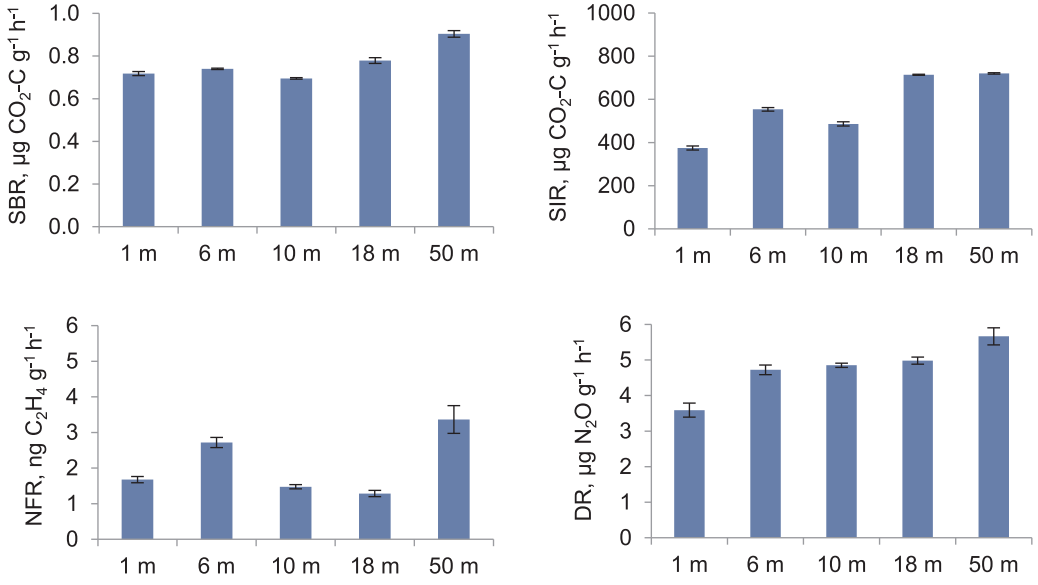

Fig. 4. Parameters of microbial complex functioning in roadside soils: actual (SBR), substrate induced respiration (SIR); nitrogen fixation (NFR) and denitrification (DR) activity.

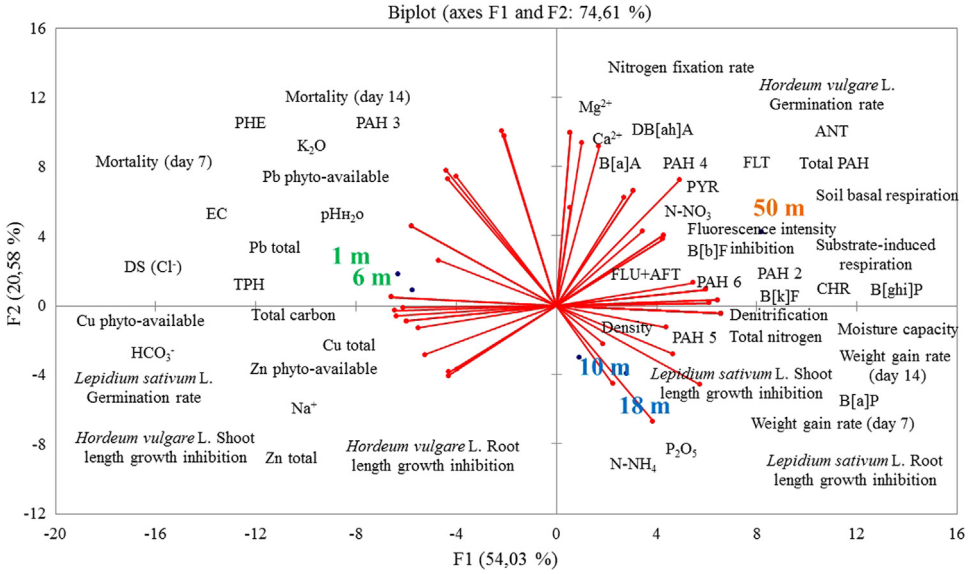

Fig. 5. Biplot for five soil sampling locations at different distances from the road in principal components 1 and 2.

capacity potential for soils and the risk of contaminants migrating to water systems. In our study, all the pollutants seemed to be fixed in the soil and the probable risk for water ecosystem pollution at the time of the research was low.

3.4. PCA

PCA yielded four principle components (PC) and confirmed differences between five soil sampling locations, dividing them into three groups (Fig. 5). Group 1: 1- and 6-m sampling locations with negative loadings on PC1. Grouping of these locations is based on the similarity of parameters related to the physical and chemical soil properties and some nonorganic traffic-related pollutants. Group 2: 10- and 18-m sampling locations with positive loadings on PC1 and negative loadings on PC2. Grouping of these locations is based on the similarity of parameters related to the nitrogen cycle and ecotoxicological effects of higher plants and earthworms. Group 3: 50-m sampling location with positive loadings on PC1 and PC2. This separation is driven mainly by organic traffic-related pollutants and ecotoxicological effects such as SBR and SIR, and the fluorescence intensity inhibition of algae.

4. Conclusions

The spatial distribution patterns of most contaminants decreased with distance from the road, reaching the background values in the 18- to 50-m zone. Despite this, toxicity of roadside soils was obtained for all examined organisms in direct contact with soil medium within the whole 50-m zone.

We recommend applying higher plants as the best organisms for examining roadside soil toxicity because of their displaying the highest toxicity and links with the maximal quantity of contaminants. It is necessary to highlight that their ecotoxicological parameters should be analyzed as a whole. If root length and germination rate are classical parameters and included in different guidelines (Nikolaeva and Terekhova, 2017) shoot length is of lower importance. Earthworms should also be included in toxicity assessment as they are the most sensitive to HM among all the organisms. Like higher plants, earthworms also showed correlations with DS and TPH, and less so with PAH. Despite similar correlations to contaminants, both ecotoxicological parameters (mortality and growth rate) should be assessed as the growth rate showed greater sensitivity to contaminants than mortality. Ecotoxicological parameters of soil microbial complex SIR and DR acted as good indicators of TPH and DS and demonstrated quite high toxicity. Integration of algae in the biotest battery is questionable based on results of this research because they did not demonstrate toxicity in the most contaminated part of the study area and demonstrated no links to pollutants. Nevertheless, we assume their application is useful if we consider the potential risk for aquatic ecosystems due to contaminant migration.

Biological methods are a prospective tool for assessing polycontaminated systems of roadside soils. This study showed that application of a battery of bioassays revealed soil toxicity at distant locations (18–50 m from the road), which were within allowable contamination levels. Chemical analysis must be accompanied by biological studies for comprehensive ecological assessment of roadside soils. Higher plants and earthworms may be recommended as the reduced test-batteries of relevant organisms for cost-effective assessment of the toxic potential of roadside soils.

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Conflict of interest

There is no conflict of interests between the authors of this article.

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