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Research Paper

Linking pollution of roadside soils and ecotoxicological responses of five higher plants

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ABSTRACT

This research studies a typical landscape of an agricultural area separated from the road by a ditch with trees. Soils were sampled at 1, 2, 7, 25, and 50 m from the road. The concentrations of polycyclic aromatic hydrocarbons (PAH), total and phyto-available heavy metals (HM), total petroleum hydrocarbons (TPH), and de-icing salts (DS, Cl^-) were determined using standard techniques. A set of higher plants (*Lepidium sativum* L., *Sinapis alba* L., *Raphanus sativus* L., *Hordeum vulgare* L., *Avena sativa* L.) was applied for toxicity evaluation of soils. The objective of this research is to find correlations between pollution of roadside soils and their phytotoxicity. HM, TPH and DS contamination of soils was observed in the 0–25 m zone, and PAH contamination was found up to the 50 m. Soil toxicity was declining from the road to the 50 m. Phytotoxicity related to majority of plants performed correlations with the same set of contaminants: TPH, 2-rings PAH, phyto-available Zn, Cu, Pb, and total Zn. No any correlations demonstrated *Avena sativa* L., being not applicable for ecotoxicological assessment of roadside soils. Despite the phytotoxicity was generally in line with contaminants loads, surprisingly low values were indicated in the ditch characterized by the strong pollution. We attribute this to the contrasting properties of soils there – the higher content of organics and clay. Sensitivity of plants to roadside pollution decreased in the row *Lepidium sativum* L. > *Hordeum vulgare* L. > *Sinapis alba* L. > *Raphanus sativus* L. The most reliable test-parameters for toxicity estimation were the root and the shoot length, germination rate was not informative indicating low phytotoxicity values. The research showed the importance of the right choice of test-cultures and test-parameters to judge phytotoxicity correctly. Linking the contaminants loads and phytotoxicity effects is valuable for comprehensive ecotoxicological assessment.

1. Introduction

The negative effects of automobile transportation on the environment and human health are broadly recognised (WHO, 2005). Motor vehicles generate a wide range of contaminants, among which polycyclic aromatic hydrocarbons (PAH), heavy metals (HM), total petroleum hydrocarbons (TPH), and de-icing salts (DS) are the key contributors. PAH are characterised by proven genotoxic, carcinogenic and teratogenic health effects (Grimmer, 1983; WHO, 2000). Due to their low solubility, high hydrophobicity, and photochemical stability, PAH tend to accumulate in the environment. The incomplete combustion of fossil fuels is the key source of PAH related to automobile traffic. Additional emissions occur as a result of the abrasion of rubber tires, asphalt road surfaces, and brake linings (Takada et al., 1991). The adverse effects of HM on human health include the ability to promote blood, neurological, and respiratory diseases, as well as cancer (WHO, 2007; Laumbach and Kipen, 2012). They are mainly released into the environment from the corrosion of automobile bodies, radiators and brakes, tire dust, asphalt pavement abrasion, and fuel combustion related to Pb (Bohemen and Janssen Van de Laak, 2003). HM are characterised by high persistence in the environment, as they are of low leachability under oxidative conditions (Popescu et al., 2013) and cannot undergo microbial or chemical degradation (Briffa et al., 2020). The application of DS also contributes to environmental pollution, causing plant tissue necrosis in high doses (Gałuszka et al., 2011). TPH, which are mainly originating from fuel leakages, affect live organisms by indirectly changing the properties of contaminated media.

The soils and ground along roads act as a sink for contaminants,

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which are transported to them *via* aerial migration or *via* the infiltration of road runoff and spray waters. Contamination of roadside areas, which is associated with high environmental and human health risks, raised numerous worldwide researches in the world (Zehetner et al., 2009; Kluge and Wessolek, 2012; Wawer et al., 2015; Shamali De Silva et al., 2016; Leopold et al., 2018). Generally, 3 areas of roadside pollution are observed in roadside soils (Werkenthin et al., 2014): a 0–2 m area that is mostly affected by runoff water from the road and spray water; a 2–10 m area that is dominated by splash water and partly influenced by runoff water, depending on the inclination of the slope; and a 10–50 m area that is contaminated *via* airborne pollutants transported from the road.

Despite the existence of extensive published data, further research is still relevant, as the contamination of surface soils may differ depending on the complex of factors, including climatic conditions, local relief, vegetation type, road configuration, traffic intensity, vehicle type, and soil parameters (Ahmed et al., 2016). Particularly, the publications on a wide range of pollutants in roadside soils of Russia are limited. The existed ones relate to urban soils, but they are not presented for agricultural soils, which are the object of study in this work. It is not uncommon for roadside areas to be dedicated to agricultural production in suburbs of big cities. Information on traffic-related contaminants distribution is especially important for agricultural lands placed in the vicinity of roads, as the pollutants may accumulate in crops and pose negative effects on food safety.

Contrasting to chemical data, there is much less information available on the environmental toxicity of roadside soils. Pollutants, depending on the properties of soils and climatic conditions, can exhibit different toxicity (Hagner et al., 2018) and the relationship between the total concentration of contaminants in soil ant its toxicity may be not direct (Delerue et al., 2019). A good practice to estimate the actual hazard of pollution for the environment is the accompanying of chemical analysis by biological tests. It is especially important for poly-contaminated systems where interactions between contaminants is an additional factor of ecotoxicological uncertainty. Among different organisms, applied in bioassays practices, higher plants are among the most suitable organisms for terrestrial ecosystems toxicity evaluation (Lors et al., 2011). In our previous research (Nikolaeva et al., 2017) higher plants were found to be the most sensitive to traffic-related contaminants among the battery of organisms of different trophic levels. One of the methodological problems in the procedure of phytotesting is not uniform responses of different higher plants to pollutants (Czerniawska-Kusza et al., 2006). Quite little is known about the individual sensitivity of higher plants to traffic-related contaminants. The insights in this field will help to provide comprehensive ecotoxicological assessment of roadside soil media.

Objective of this article is to find out correlations between phytotoxicity of soils and loads of traffic-related contaminants.

2. Materials and methods

2.1. Research area and soil sampling

The research was conducted in the Moscow region, 25 km to the north of the city. The study area experiences temperate continental climate conditions. The average winter and summer temperature are − 5 °C and + 21 °C, and the precipitation levels are 50 mm and 83 mm per month, respectively. The prevailing winds are south-westerly. The natural and arable Albeluvisols (WRB, 2014) are predominant soils there.

The study was conducted in the vicinity of Leningradskoe Highway. This road is a part of the M10 Federal Highway, connecting Moscow and Saint Petersburg, and a part of the E105 European Highway. At the research site (56.038447, 37.162215) the road has operated under heavy traffic for over 50 years. It is known, no any works disturbing roadside soils at the area of study had been carried out for the last 10 years at least. The site is characterised by traffic of 50 000 vehicles per day, 2 lanes for each direction, and asphalt pavement.

The roadside soils were examined *via* 3 parallel 175 m transects spaced 100 m apart. The transects were perpendicular to the road and located on its downwind side. No any disturbance of studied soils were The landscape along the transects with increasing distance from the road was as follows (Fig. 1): 1 - flat sandy roadside with no vegetation (0–2 m from the roadbed), 2 - relief depression (the ditch) with 2 lines of wind breaking hedges of broad-leaf trees (2–50 m); 3 - arable field sloping upwards (50 – 175 m).

The sampling campaign was undertaken in May after the end of natural snow melting. To study the surface distribution of contaminants, topsoils (0–3 cm depth) were sampled at the distances of 1, 2, 7, 25, 50 m from the road in each of the 3 transects. The reference sample was at the distance of 175 m from the roadbed. At each distance, the topsoil layer was excavated with a hand shovel from a 0.5 × 0.5 m site. The properties of soils are given in Table 1.

2.2. Methods

2.2.1. Contaminant analysis

TPH were extracted from 5 g of soil using 50 mL carbon tetrachloride. We separated polar compounds from 5 mL of extract with an aluminium oxide column, and detected the TPH contents by spectrometry ($\lambda = 3.4$ mkm) using an infrared concentrator KH-3 (SIBECOPRIBOR, Russia) according to the standardised 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 1260 system (USA) with a fluorescence detector. We analysed 13 PAH types of the 16 presented in the Priority Pollutants List (US EPA, 2014): fluorene (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, Pb, and Ni were detected by inductively coupled plasma spectrometry (ICP-MS) using an Agilent 7500a system (USA). The total HM content was determined in 0.5 g of soil sieved through a 1 mm sieve, 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 deionised water. Phyto-available HM were extracted from 5 g of soil sieved through a 1 mm sieve, using 50 mL of ammonium acetate buffer (NH_4OAc) at pH 4.8. The suspension was vigorously shaken for 1 h, then filtered through ash-free cellulose with a 5–8 μm pore diameter.

The chloride ion concentration in the soil was determined according to the Mohr argentometric titration method (Vorobyeva, 2006). 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).

2.2.2. Soil property analysis

The organic carbon content was determined by the Tyurin method,

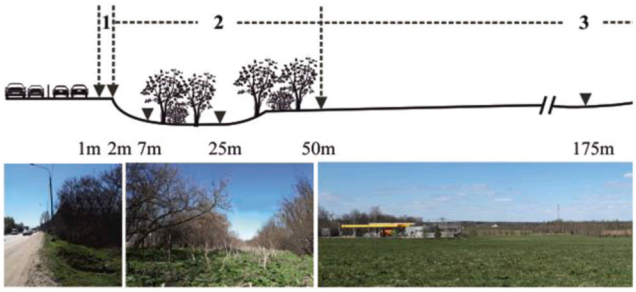

Fig. 1. Roadside territory layout across the Leningradskoe Highway.

Table 1
Characteristics of roadside soils.

Parameter	1 m	2 m	7 m	25 m	50 m	175 m
pH_{H20}	8.7	8.3	7.7	7.2	6.9	6.9
C organic, %	2.1	1.9	2.8	2.4	1.9	2.3
EC, μS cm^{-1}	80	55	95	85	40	55
Grading	loamy sand	sandy loam	medium loam	sandy loam	sandy loam	sandy loam

Comment: standard deviation does not exceed 2.8% from average values for pH_{H20}, 33.9% - for C organic, 38.6% - for EC.

modified by Nikitin (National Standards of the Russian Federation, 1991; Orlov and Grishina, 1981). The method is based on the oxidation of soil organic matter by potassium dichromate sulfuric acid solution and further titration of sulfuric acid excess by silver nitrate.

Electrical conductivity (EC) was detected in suspension (soil/water ratio, 1:5) using a DiST 4 WP conductometer (Hanna Instruments, Germany). Soil pH was determined in suspension (soil/water ratio, 1:2.5) using an HI 8314 portable ion metre (Hanna Instruments, Germany). The soil density and moisture capacity were determined gravimetrically; the soil texture was detected under field conditions (Vadyunina and Korchagina, 1986).

2.2.3. 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 *Lepidium sativum* L. (L. *sativum*), *Sinapis alba* L. *(S. alba), Raphanus sativus* L. *(R. sativus)* as dicotyledonous plants and *Hordeum vulgare* L. (*H. vulgare*) and *Avena sativa* L. (*A. sativa*) as monocotyledonous plants 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. 12 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 ziplock 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 were 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 analysed parameter (seed germination or root/shoot length) on the control soil and B is the analysed parameter on the examined soil.

2.2.4. Quality assurance and quality control

The climatic conditions of the laboratory working environment matched the instrument instructions. Only high purity reagents were used for the analysis. The limits of detection were 3 μg kg^{-1} for FLU, AFT, PHE, ANT, and FLT; 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; and 50 mg kg^{-1} for TPH. To ensure analytical precision of PAH detection, we included a reference soil sample in each cycle of measurements. The indicated values did not exceed 5% variance for all the individual components. The following standards were used to ensure the accuracy of the detection measurements: the PAH calibration mix (CRM 47940) from Sigma-Aldrich was used for PAH and the Agilent Initial Calibration Verification Standard 5183-4682 was used for HM. The Russian State Standard Sample No 7554-99 (EcoAnalytica, Moscow) was used for TPH analysis and calibration procedure. Quality control samples were measured several times per instrumental run, incorporated after every 30 samples. The accepted deviation between the measured and standard value was 5%. The blank samples comprised 4% of all the analysed samples. The quality criterion for blank samples was that measured values should be below detection limits. Calibration curves were prepared using 7 concentrations of reference materials. The correlation coefficients were considered acceptable at 0.99.

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, 45 S. alba, 45 R. sativus* and 36 *H. vulgare* plants were assessed to characterize soils in each sampling location instead recommended minimum in 30 plants of each species. The test-cultures were selected for the following reasons: their sensitivity to HM, TPH, PAH, DS has been proven by research; 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.

2.2.5. 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 10.

Cluster analysis was conducted in Phyton 3.7 with libraries Scipy and Matplotlib.

3. Results

3.1. Surface distribution of traffic-related contaminants

3.1.1. PAH

The mean values of total PAH within the 175 m transect varied from 190 to 5,557 μg kg^{-1} (Table 2).

PAH were distributed with increasing distance from the road as follows: high levels were found at 1–2 m, followed by a maximum concentration at 7 m, and decreased concentrations that met Moscow-region background values were observed at the 175 m location and beyond (Fig. 2A). Concentrations exceeding the standards for B[a]P (20 mg kg^{-1}), the only individual PAH standardised in Russia (Russian Sanitary Thresholds No. 42-128-4433-87 (1987)), were found in the zone of 1–25 m. The maximum excess, greater by more than an order of magnitude, was observed at the 7 m location (Fig. 2B).

The diagnostic ratio analysis (B[b]F/B[k]F > 0.5, B[a]P/B[ghi]P between 0.5 and 0.6 (Ravindra et al., 2008), FLT/(FLT + Pyr) between 0 and 1 (Yunker et al., 2002)) and the predominance of PHE, FLT and B[b]F among PAH identified traffic emissions as the main source of pollution for the whole study site. The distribution patterns of most individual PAH were of similar character at different distances from the road, but at 7 and 25 m, a fractionation happened; that is, the proportion of B[ghi]P and B[a]P among total PAH sharply increased (Table 2).

3.1.2. HM

The concentration of total HM (Fig. 2D) varied from 8.6 to 13.9 for Ni, from 9.3 to 40.5 for Cu, from 41.5 to 107.2 for Zn, and from 7.4 to 16.2 mg kg^{-1} for Pb. These values were significantly lower than the permissible levels set in Russia (State Hygienic Standard of Russian Federation 2.1.7, 2511-09, 2009). The maximum concentration of HM was found in the 1–7 m roadside zone, and the concentrations decreased with distance from the road to 175 m. Starting at a 25 m distance from the road, the concentrations of Cu, Zn, Ni and Pb met background values.

The distribution patterns of phyto-available HM and total HM along the transect were similar (Fig. 2E). The concentrations of these contaminants were 0.3–6 mg kg^{-1} for Ni, 0.2–4.4 mg kg^{-1} for Cu, 2.5–49.6 mg kg^{-1} for Zn, and 0.4–2.7 mg kg^{-1} for Pb. In contrast to the total HM, the concentrations of all phyto-available HM, excluding Pb, in

Table 2
Mean concentrations of PAH in surface soils and proportion of each individual PAH in the total content.

PAH	1 m		2 m		7 m		25 m		50 m		175 m	
	μg kg^{-1}	Share, %	μg kg^{-1}	Share, %	μg kg^{-1}	Share, %	μg kg^{-1}	Share, %	μg kg^{-1}	Share, %	μg kg^{-1}	Share, %
NAPH	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0
FLU	**6**	1	**4**	0	**4**	0	**1**	0	**0**	0	**0**	0
ANAPH	**6**	1	**4**	0	**4**	0	**1**	0	**0**	0	**0**	0
ANT	**12**	1	**12**	1	**65**	1	**6**	1	**3**	1	**3**	2
PHE	**136**	13	**102**	11	**268**	5	**33**	6	**15**	5	**18**	9
FLT	**128**	12	**104**	12	**863**	16	**86**	15	**49**	17	**29**	15
PYR	**158**	15	**134**	15	**651**	12	**79**	14	**44**	16	**29**	15
B[a]A	**114**	11	**92**	10	**426**	8	**53**	9	**28**	10	**18**	10
CHR	**106**	10	**78**	9	**394**	7	**43**	8	**24**	8	**16**	8
B[b]F	**167**	15	**159**	18	**805**	14	**87**	16	**44**	15	**33**	18
B[k]F	**20**	2	**17**	2	**259**	5	**28**	5	**12**	4	**9**	5
B[a]P	**22**	2	**26**	3	**499**	9	**39**	7	**17**	6	**10**	5
DB[ah]A	**151**	14	**124**	14	**193**	3	**22**	4	**23**	8	**10**	5
B[ghi]P	**51**	5	**46**	5	**1,127**	20	**80**	14	**23**	8	**15**	8
Total	**1,076**		**902**		**5,557**		**558**		**284**		**190**	

Comment: standard deviation does not exceed 7% for samples at the 1–2 m locations, 39% at the 7 m, 28% at the 50–175 m

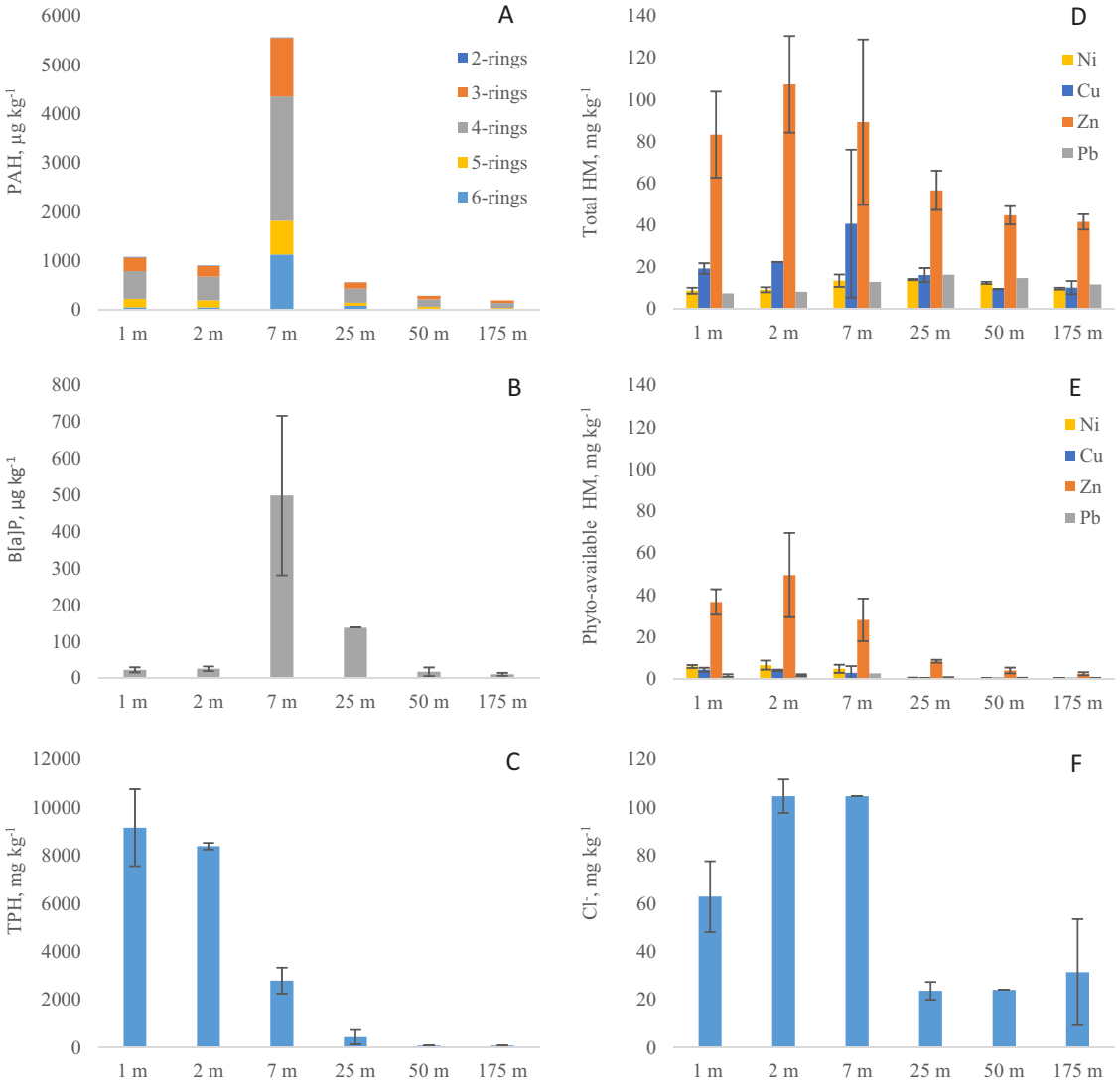

Fig. 2. Surface distribution of traffic-related contaminants in roadside soils: A – polycyclic aromatic hydrocarbons (PAH); B – benzo[a]pyrene (B[a]P); C – total petroleum hydrocarbons (TPH); D – total heavy metals (total HM); E – phyto-available heavy metals (phyto-available HM); F – de-icing salts as chloride-ion (Cl^-)
Comment: permissible levels of contaminants set in Russia are as follows: Ni - 80/4, Cu - 132/3, Zn- 220/23, Pb- 130/6 mg for total/phyto-available HM forms; TPH – 1,000 mg kg^{-1}; Cl^- - 330 mg kg^{-1}; B[a]P – 20 μg kg^{-1}; total PAH are not regulated in Russia.

the 1–7 m roadside zone were above the permissible levels established in Russia (State Hygienic Standard of Russian Federation 2.1.7, 2041-06, 2006).

3.1.3. TPH

The surface distribution of TPH exponentially decreased from 8,287 to 57 mg kg^{-1} as distance from the road increased (Fig. 2C). The maximum value was observed in the 1–2 m roadside zone, exceeding the norms established in Russia (1,000 mg kg^{-1}) by an order of magnitude; at 50 m and beyond, the background values characterised for Albeluvisols of Moscow region were achieved.

3.1.4. DS

The Cl^- concentrations were characterised by quite low values from 105 to 27 mg kg^{-1}. The maximum values in surface soils were found in the 2–7 m zone, and decreased with increased distance from the road (Fig. 2F).

3.2. Higher plant bioassay and correlations with contaminants distribution

All the test-cultures indicated the toxicity of roadside soils but its intensity and trend with distance from the road increase was different (Fig. 3). Following toxicity intensity, 2 groups of higher plants are clearly observed – L. *sativum* and *H. vulgare* (Fig. 3A, D), indicated high toxicity, and *S. alba, R sativus, A. sativa* (Fig. 3B, C, E) showed 2–3 times lower toxicity.

L. *sativum* and *H. vulgare* performed the common character of toxicity intensity along the transect: it was strong within 1–2 m roadside zone and several times lower – within the 7–50 m roadside zone. Analogical trend along the transect but of lower values indicated *S. alba.* Toxicity indicated by *R. sativus* and *A. sativa* did not depend on distance from the road.

The phytotoxicity trend along the transect was generally in line with the distribution of the majority of contaminants for *H. vulgare,* L. *sativum* and *S. alba*, but at the 7 m location it was contradicting – the lowest phytotoxicity was found despite the high concentration of pollutants. L. *sativum* and *H. vulgare* phytotoxicity intensity correlated with TPH, mobile HM (Cu, Ni, Zn), total HM (Zn) and 2-rings PAH concentrations (Table 3).

R. sativus correlations were also the same but only for the root length test-parameter. *S. alba* performed correlations with the same set of contaminants but they were strong ($R > 0.5$) for the phyto-available HM and TPH only. *A. sativa* did not show any correlations with the contaminants, excluding the correlation of germination rate inhibition with TPH.

The hierarchical cluster analysis of toxicity levels indicated by different test-parameters (Fig. 4) revealed 4 main clusters: 1 - *H. vulgare* and L. *sativum* shoot and root length; 2 - L. *sativum* and *R. sativus* shoot and root length, and *H. vulgare* germination rate; 3 - germination rate of all species except *H. vulgare*; 4 - *A. sativa* shoot and root length.

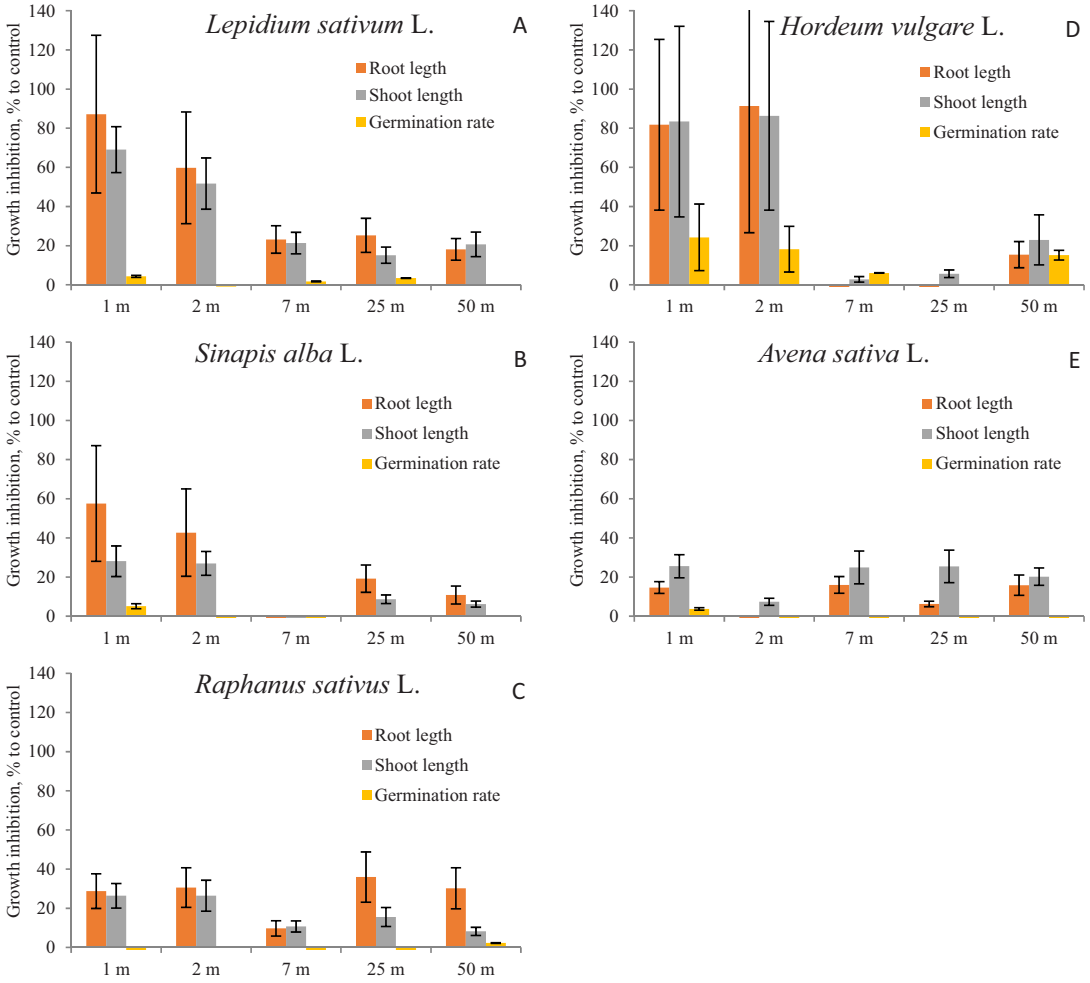

Fig. 3. Ecotoxicological responses of higher plants to pollution of roadside soils: A – *Lepidium sativum* L., B – *Sinapis alba* L.; C – *Raphanus sativus* L.; D – *Hordeum vulgare* L.; E – *Avena sativa* L.

Table 3
Correlation coefficients between phytotoxicity intensity and contaminants concentrations within 50 m roadside zone.

Plant	Phytotoxicity	PAH					HM total				HM phyto-available						
		PAH 2	PAH 3	PAH 4	PAH 5	PAH 6	Ni	Pb	Cu	Zn	Ni	Pb	Cu	Zn	TPH	DS (Cl^-)	C org.
L. *sativum* L.	Root length growth inhibition, % to control	0.79	–	–	–	–	–	–	–	0.56	0.74	–	0.82	0.74	0.94	–	–
	Shoot length growth inhibition, % to control	0.80	–	–	–	–	–	–	–	0.59	0.78	–	0.85	0.77	0.96	0.50	–
	Germination rate, % to control	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S. alba L.	Root length growth inhibition, % to control	–	–	–	–	–	–	–	–	–	–	–	0.54	0.53	0.78	–	-0.65
	Shoot length growth inhibition, % to control	–	–	–	–	–	–	–	–	–	–	–	0.54	0.57	0.78	–	-0.72
	Germination rate, % to control	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
R. sativus L.	Root length growth inhibition, % to control	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	-0.68
	Shoot length growth inhibition, % to control	0.66	–	–	–	–	–	–	–	0.67	0.73	–	0.78	0.81	0.9	–	–
	Germination rate, % to control	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	-0.71
H. vulgare L.	Root length growth inhibition, % to control	0.61	–	–	–	–	–	–	–	0.61	0.72	–	0.76	0.79	0.92	–	-0.66
	Shoot length growth inhibition, % to control	0.62	–	–	–	–	–	–	–	0.59	0.71	–	0.76	0.77	0.92	–	-0.67
	Germination rate, % to control	0.58	–	–	–	–	–	–	–	–	0.57	–	0.63	0.55	0.76	–	-0.69
A. sativa L.	Root length growth inhibition, % to control	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Shoot length growth inhibition, % to control	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.60
	Germination rate, % to control	0.54	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Comment: correlation coefficients below 0.50 in absolute value are marked as "–".

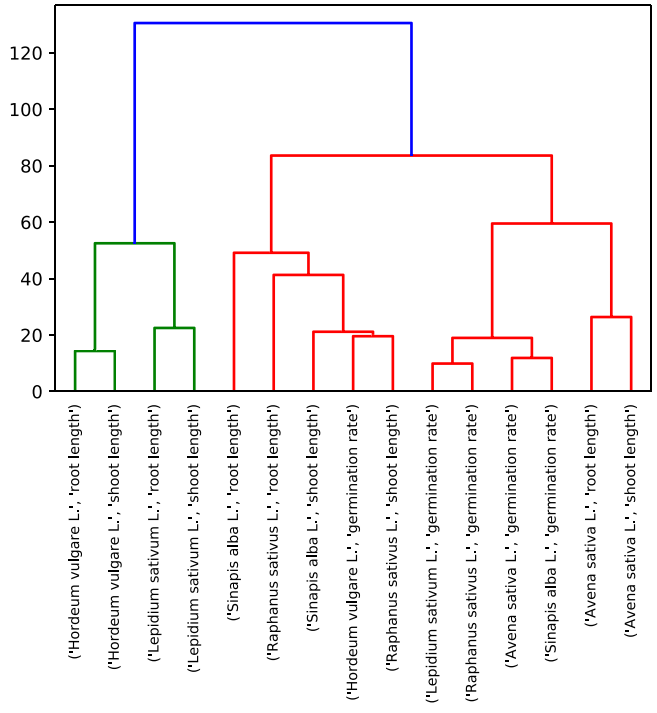

Fig. 4. Dendrogram of higher plants test-parameters performance.

4. Discussion

4.1. Surface distribution of traffic-related contaminants

The distribution of all the contaminants in the surface soils along the transect was strongly affected by the roadside area design. The maximal concentrations of PAH and DS were shifted away from the roadside by 7 m; the concentrations of HM were equal within the 1–7 m zone and exhibited maximal values. Although the TPH concentrations declined from 1 m to farther locations, their concentration at 7 m was unusually high. We can conclude that the terrain, sloping down from the roadbed, promotes the migration of contaminants with runoff to the depression located at 2–25 m distance from the road (Fig. 1). The accumulation of contaminants at 7 m happened due to its position near the hedge of trees and shrubs, where airborne particles migrated from the road and became sedimented. The filtering role of tree belts along roads is well known (Zehetner et al., 2009).

We would like to highlight the extremely high concentrations of PAH found at 7 m (5,557 μg kg^{-1}), which exceeded the maximum values observed in roadside soils of typical highway margin designs in Moscow, reported as 1,820 μg kg^{-1}, by several times (Nikolaeva et al., 2017). Such PAH accumulation is probably promoted by the inhibition of the biodegradation of these PAH due to high values of Cl^- and TPH at 7 m, which could have a negative influence on soil microorganisms. The landscape also affected the patterns of individual PAH distribution along the transect. We suggest that increases in the proportions of B[ghi]P and B[a]P at the 7 and 25 m locations are due to their preferential sedimentation by the hedge of trees and shrubs, as these compounds have the highest molecular weights. In addition to deriving from a fuel combustion source, B[ghi]P and B[a]P are known to be the key PAH components in automobile tires (Sadiktsis et al., 2012). Thus, the highest share of these PAH in the “drainage zone” may also be driven by the flushing of tire abrasion products from the road surface to the

depression.

Among traffic-related HM, the predominance of Zn was observed to be consistent with other studies. We relate this predominance of Zn to the many sources of Zn origin – corrosion of automobile bodies and galvanised metallic structures (traffic signals, dividing strips) (Junaid et al., 2013), as well as tire abrasion (Bohemen and Janssen Van De Laak, 2003). The distribution patterns of total HM revealed 2 groups of metals based on behaviour: Zn and Cu, which significantly exceeded background values in the 1–7 m roadside zone, and Ni and Pb, which were constant along the whole transect at the background value level. Among the phytoavailable metals, a different pattern was observed: all the HM showed higher concentrations in the 1–7 m zone and declined farther from the road, revealing the contamination of the 1–7 m zone. According to Russian standards, the total HM did not exceed the permissible levels; however, phytoavailable HM did exceed these norms in the 1–7 m zone for all the metals except Pb. According to Morin et al. (1999), HM form stable complexes with soil components over time. It is likely that under intense loads of HM, most of the sorption positions in soil are occupied and the process of binding HM with soil is not intense. HM mobility may also be promoted by the high levels of Cl^- present in topsoils of the 1–7 m roadside zone compared to the 25–175 m area (Fig. 2F). Li et al. (2015) reported that application of NaCl causes soil dispersion and formation of mobile metal complexes in soils.

TPH are mainly attributed to fuel leakages and are usually found in the several-metre-wide areas near roads (Dierkes and Geiger, 1999), but in this study, the area of contamination by TPH was found to be wider, reaching background values reported by Lisovitskaya and Mozharova (2013) within 25–50 m from the road. The sharp downward slope of the ground surface from the roadside promotes TPH migration at farther distances due to horizontal migration through surface relocation of polluted soil particles by rain, snowmelt and spray from road cleaning machines.

Cl^- is the key component of DS applied in the Moscow region. Despite the quite low measured values of DS, the road does influence these values, as the Cl^- concentrations decrease from the 1 m to the 25 m sites. The values found in surface soils starting at 25 m from the road and beyond are close to the average background concentrations of Cl^- (21 mg kg^{-1}) reported for the Moscow region (Nikiforova et al., 2014). The observed maximal values (105 mg kg^{-1}) are significantly lower compared to roadside soils within the city of Moscow. We attribute this fact to the lower DS loads at the study site compared to city areas. As pedestrian ways are absent along the studied section of Leningradskoe Highway, DS are directly applied only on the road surface and not on roadside areas. Currently, the observed Cl^- concentrations do not pose any risks for growing plants at these sites. We expect further decrease of DS in the summer-autumn period due to rains (the soil sampling was done in May).

The distribution of traffic-related contaminants from 7 to 175 m shows a classic declining trend, and the most intense decrease happens behind the first of two hedges at the distance of 25 m from the road for HM, TPH and DS, where concentrations reach background values. The zone of PAH pollution is extended by 50 m due to possible transport of the finest particles, which are the key carriers of PAH. Thus, the design of the roadside area, with a depression near the road and the presence of 2 forest belts, significantly reduces the risks of farming in the area of 50–175 m, where a field of perennial grasses is currently located at our sampling site. Nevertheless, we should note that the content of B[a]P in this zone (10–18 μg kg^{-1}) is quite close to the maximum permissible levels set in Russia (20 μg kg^{-1}), probably due to the long history of road operation.

4.2. Higher plant bioassay and correlations with contaminant distribution

The highest levels of phytotoxicity discovered in 1–2 m roadside zone were in line with the highest loads of contaminants there. The surprisingly low phytotoxicity at the 7 m location, contaminated level like soils at the 1–2 m location and even more contaminated by PAH, can be explained by the contrasting soil properties of this position – the maximal content of organic matter compared the other positions along the transect and clay texture (Table 1). The similar effect was observed by Czerniawska-Kusza et al. (2006), reported influence of soil organic matter content and grain-size distribution on plant responses to toxicants. We found the strong inverse correlation between phytotoxicity of all studied soils and their organic matter content (R = 0.5–0.7, p-level 0.05) (Table 3). Organic matter is known to decrease availability of contaminants – by means of HM complexation with humic acids, sorption of PAH and TPH. Silt particles are also responsible for the mineral sorption of contaminants, decreasing their availability for plants. Starting 25 m from the road, despite the contaminants met background values, the low toxicity (within 20%) still revealed there and far in the 50 m location what may be the effect of the joint influence of a complex of contaminants in low concentrations.

The interesting finding is the uniform character of correlations between the toxicity intensity and contaminants loads. All the higher plants, excluding *A. sativa,* performed correlations with the common set of contaminants – 2-rings PAH, total Zn, phyto-available Cu, Zn, and Ni, and TPH along the transect (Table 3). This result related to PAH, corresponds to the finding of Henner et al. (1999), reported high molecular weight PAH (3 ± 5 rings) as not toxic for higher plants. No correlations were found with Cl^- for the most of plants. We attribute this to the relatively low DS concentrations which are safe for plants in the found diapason (Nikolaevskiy et al., 1998). The similar phytotoxicity character and common correlations with contaminants distribution performed by different test-cultures gives an evidence that higher plant bioassay is a working tool for the ecotoxicological assessment of polycontaminated roadside soils. However, it is important to take into account the different absolute values of phytotoxicity of studied plants. Their sensitivity decreased in the row L. *sativum* and *H. vulgare* > *S. alba* > *R. sativus*. The highest inhibition was characterised for the root and the shoot length parameter but the seed germination was inhibited 5 – 10 times lower. Moreover, the toxicity values related the seed germination parameter of some test-cultures (L. *sativum, S. alba, R. sativus*) did not perform any correlations with the contaminants, being not informative.

The hierarchical cluster analysis of toxicity levels indicated by different test-parameters (Fig. 4) showed that the germination rate for all the test-cultures is allocated to a separate group from the shoot and the root length test-parameters. Assuming this, we do not recommend the germination rate as the target parameter for application in ecotoxicological assessment of roadside soils. The use of the root and the shoot length inhibition of *H. vulgare* and L. *sativum* as the most sensitive test-cultures among the studied set of higher plants is highly recommended; the root and the shoot length of *S. alba* and the shoot length of *R. sativus* is also relevant; *A. sativa* is not recommended.

5. Conclusions

The distribution of all the contaminants in the surface soils along the transect was of complex character with specifics driven by roadside area design. The determined concentrations of traffic-related contaminants are generally consistent with worldwide averages in roadside soils for TPH, DS and HM, but much higher PAH levels are found in the tree-lined ditch. A drainage system consisting of a ditch, which can intercept road runoff, splashes and sprays, in combination with tree hedges provides a defence for agricultural lands located behind it and seems to be an effective strategy for ecological product safety. Higher plants bioassay performed as the meaningful approach for ecotoxicological assessment of polycontaminated roadside soils: the most of test-cultures showed the common toxicity trends and revealed the correlations with a set of contaminants. But not only contaminants level determines the phytotoxicity intensity. Despite the high levels of contaminants in the ditch, the higher plants bioassay indicted the low phytotoxicity there probably due to the high organic matter and clay particles content. It gives

evidence that negative toxic effects of traffic-related contaminants are also regulated by soil properties. It is necessary to put attention to the right choice of test-cultures and judge it on the right test-parameters. Particularly it was shown that root length and shoot length parameters may be recommended for the practices of phytotoxicity assessment, while germination rate was not informative. Linking the contaminants loads and phytotoxicity effects may be a good practice on the way to the right choice of test-cultures for comprehensive ecotoxicological assessment of polycontaminated media.

CRediT authorship contribution statement

Olga Nikolaeva: Conceptualization, Writing - original draft, Writing - review & editing, Methodology, Investigation, Data curation, Formal analysis, Validation, Visualization, Resources, Project administration, Supervision. **Mikhail Karpukhin:** Methodology, Investigation, Formal analysis, Validation, Writing - review & editing, Resources. **Rostislav Streletskii:** Methodology, Investigation, Formal analysis, Writing - review & editing. **Marina Rozanova:** Methodology, Investigation, Formal analysis, Writing - review & editing. **Olga Chistova:** Investigation. **Nadezhda Panina:** Investigation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Olga Nikolaeva, Mikhail Karpukhin, Rostislav Streletskiy, Marina Rozanova, Olga Chistova, and Nadezhda Panina as the authors of research article “Linking pollution of roadside soils and ecotoxicological responses of five higher plants” have no any conflicts of interests.

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Conflict of interests

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

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