Ecological State Assessment of Urban Soils by Bioassay

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Abstract The aim of this study was to assess the ecological state of the soil of a megalopolis (St. Petersburg) located in the North-West of the Russian Federation using bioassay in order to create a set of the most sensitive biotests. For this, 4-year monitoring studies of the soil quality in different functional zones of the city were carried out using the bioassay, physicochemical, and chemical analyses. A set of express biotests that allow for an integral ecotoxicological assessment of urban soils was developed and tested for the first time. The developed block of biotests consists of test organisms that are representatives of the main levels of the trophic chain: higher plants (Triticum vulgare L.) for producers, hydrobionts (Paramecium caudatum) for consumers, and natural soil microbiocenosis for decomposers. All the test cultures revealed the toxicity of urban soils; they were characterized by different sensitivities to toxicants. Our result showed that a correct assessment of the ecological state of urban soils is possible on the basis of the combined use of eluate (water extract) and contact bioassays. Biotests make it possible to record negative phenomena in urban soils, which occur even under low human-induced loads. The research data confirm the necessity and effectiveness of the study of the soils’ state using a battery of bioassays for inclusion in the monitoring system of urban soils.

Keywords Toxicity · Bioassay · Urban soil · Test culture · Phytotesting

1 Introduction

The anthropogenic pollution of the soil is a global environmental problem. As a result of anthropogenic activity, a variety of organic and inorganic pollutants enter the soil and negatively affect its normal functioning (Baderna et al., 2015; Cachada et al., 2016; Swartjes, 2011). In turn, ecological disturbances of the soil lead to serious changes and degradation of the entire natural complex, which ultimately creates a public health threat (Steffan et al., 2018). Therefore, at present, one of the most important global problems in the development of new biotechnologies is the search for effective and reliable methods of environmental control of anthropogenically contaminated soils (Environmental risk assessment of soil contamination, 2014). Such polluted objects include the soil of cities where a population is higher than 1 million inhabitants.

The soil in the city is the main environment that retains various pollutants, both directly deposited...
from the air and washed away from green spaces. It is an accumulator of many toxic compounds and, at the same time, a geochemical barrier to the migration of pollutants from the atmosphere to ground and surface waters. In fact, urban soils are a depositing medium for almost all pollutants (Rotting et al., 2006; Vodyanitskii 2011). They contain increased amounts of carbonates, sulphates, heavy metals, polycyclic aromatic hydrocarbons, petroleum products, pesticides, and other pollutants. In Europe and Russia, the soils of many cities are in unsatisfactory condition, which is recognized by many researchers (Kosheleva et al., 2018; Mills et al., 2020; Stroganova et al., 1997).

At the same time, it is incorrect to use only pollution indicators to assess soil quality: several indicators adopted in Russia, such as MPC (maximum permissible concentration) and APC (approximate permissible concentration), which are developed for agricultural soils and the MPC levels are commonly exceeded in soil from the large industrial Russian cities (Gorbov et al., 2015; Rybakov and Kevlich 2017). It is also necessary to take into account the important fact that the indicators of MRS and ARS do not adequately reflect the ecological state of urban soils. It is necessary to develop a set (block) of sensitive biotests for an objective assessment of the ecological state of urban soils.

In addition, the standards for permissible levels of soil contamination in different countries can differ tens and even hundreds of times. This makes it impossible to carry out an objective comparative assessment of the soil quality of the cities in different countries from the point of view of their ecological well-being. It is also necessary to take into account new substances that are formed in urban soils that can have a significantly higher toxic effect and create a greater danger to biota and human health than the original pollutants. The fact is that possible synergetic or additive effects can be induced by pollutants in the soil.

The soil landscape is an indicator of the ecological and sanitary state of the city; therefore, it is important to carry out regular soil monitoring using both chemical and biological indicators. However, pollution control, based on chemical and physicochemical research methods, cannot cover the whole variety of pollutants. The results of those methods only show certain concentrations of recognizable chemicals.

Therefore, to assess the environmental risk of anthropogenically polluted environments, many researchers recommend using the integrated “Triad” approach, summarizing the results of chemical, environmental, and toxicological studies (Klimkowicz-Pawlas et al., 2019; Sorvari et al., 2013; Terekhova et al., 2014). The “triadic” method was included in the legislation of some European countries and it is currently used in the assessment of contaminated soils, including in urban areas (Alvarenga et al., 2012; Critto et al., 2007; Karczewska & Kabala, 2017; Perrodin et al., 2011). When assessing contaminated soils, the Ecotoxicological Classification Risk Index for Soil (ECRIS) is also applied, a new classification system specific for soil risk assessment, which gives a comparative indication of the risk linked to environmental contamination by any chemical (Senese et al., 2010).

In this regard, in the scheme of soil control in urban areas, along with the chemical analysis, it is necessary to include ecotoxicological studies carried out using bioassay, for example, 86-chemical-based risk assessment and in vitro models of human health effects induced by organic pollutants in soils from the Olona Valley (Baderna et al., 2013). At present, bioassay is considered one of the most effective methods for the integral assessment of the toxicity of anthropogenically disturbed soils. In Russia, ecotoxicological studies of contaminated areas were carried out using bioassay. These studies are also an obligatory element in the system of environmental soil monitoring. Bioassay is carried out under controlled laboratory conditions by registering changes in biologically important indicators (test functions) of living organisms resulting from the pollution impact in the investigated soils. The reliability of the bioassay results of the object under study depends on the correctly selected test organisms and their test functions (Pukalchik et al., 2019). Due to the different sensitivities of organisms to toxicants in ecotoxicological studies, it is recommended to use test cultures of different taxonomic groups (Mónok et al., 2020; Van Gestel et al., 2001). Therefore, the most important link in bioassay to obtain environmentally significant results is the development of a set (block) of sensitive biotests to various research objects (Blaise 2000; Olkova, 2018; Terekhova et al., 2018; Bardina et al., 2020).
The objective of this study was to carry out a comparative ecotoxicological assessment of the soil of various functional zones of St. Petersburg, Russian metropolis, using bioassay in order to create a set of the most sensitive biotests on daphnia (*Daphnia magna* S.), ciliates (*Paramecium caudatum*), seeds (*Triticum vulgare* L.), and natural microbiocenosis and for their use in monitoring urban soils.

2 Materials and Methods

2.1 Study Sites

Scientific research on monitoring the ecological state of urban soils in the city of St. Petersburg has been carried out for 4 years. St. Petersburg is located in the North-West of the Russian Federation, in the eastern waters of the Baltic Sea and in the delta of the Neva River, at an altitude of 12 m above sea level. The geographical coordinates of the city are 59°57′ N and 30°19′ E. Stockholm, Helsinki, and Oslo are located at this northern latitude with similar climatic parameters. But the anthropogenic load on the soils of St. Petersburg is much higher compared to that of the cities of Northern Europe due to the larger area of the city and the larger population. The population of St. Petersburg since the 80 s of the last century has exceeded 5 million inhabitants; area 1439 km². Northern maritime climate makes soils more vulnerable to pollution.

The most radical changes in the soil of St. Petersburg took place in a relatively short period of time (300 years), when the point nature of soil destruction in the city turned into an areal one (Aparin et al. 2015). High rates of population growth and industrial development, especially in the late nineteenth and twentieth centuries, contributed to the rapid degradation and pollution of urban soils.

The soils of many cities are a very difficult object for bioassay, due to the diversity and large number of pollutants in them. Most of the pollutants are concentrated in the upper soil layer, where their gradual accumulation takes place. The degree of anthropogenic pressure on the soil in cities depends on the nature of its use (the so-called functional zones). The strongest influence of pollutants is experienced by the soils of industrial zones, motor roads, and driveways, and the lowest influence by the soils of city parks, gardens, and recreation.

The study was carried out at six sites located in different parts of the city (Fig. 1) and in different functional zones: industrial (plot 1), residential (plots 2, 3, 4, 5), recreational (plot 6).

Plot 1. Territory of the CHP plant with a capacity of 301.1 MW (Fig. 2).

Plot 2. Lawn along one of the main heavily trafficked roads in St. Petersburg towards the airport. Coordinates: 59°47′25″ N 30°19′42″ E (Fig. 3).

Plot 3. Lawn in the center of the square, which is the central road transport ring of the city district. Coordinates: 59°87′63″ N 30°25′76″ E (Fig. 4).
Plot 4. The boulevard lawn is located between the pedestrian part and the road, at the entrance to the metro station. Regular seasonal use of de-icing agents is typical. Coordinates: 59°94′6″ N 30°35′92″ E (Fig. 5).

Plot 5. Lawn is located in the center of the square, which is a transport interchange in the historic district of the city. Coordinates: 59°52′34″ N 30°15′27″ E (Fig. 6).

Plot 6. City garden with an area of 11.3 ha, founded in 1920. Coordinates: 59°53′30″ N 30°16′37″ E (Fig. 7).

At monitoring sites along transport routes and in the city garden, the soil is represented by anthropogenically deeply transformed soils—urbanozem, in the industrial zone—by urbanostratozem (Technosols in the system WRB). Urbanozems are genetically independent soils with features both of zonal
soils and specific properties. They are characterized by a thick humus surface layer (at least 40 cm) formed by mechanical mixing and containing an admixture of anthropogenic inclusions—construction and domestic waste, industrial waste, etc. The humus composition of the upper layer of urban soils under automorphic conditions usually reflects zonal conditions. Urbosтратozems are urban soils that are formed when new portions of various materials containing urban artifacts are constantly or periodically supplied to the surface (Prokof’eva et al., 2014).

The area of the monitoring sites was 20 m². The configuration of the sites depended on the landscape and was presented in two sizes: (a) 10 m × 2 m; (b) 4 m × 5 m.

Soil samples were taken every year, three times during the growing season (spring in May, summer in July, autumn in October). A mixed soil sample from the plots was prepared in the field from 20 individual samples taken with a titanium drill from a depth of 0–5 cm and 5–20 cm. The samples were combined into one mixed sample in order to increase the representativeness of analytical samples for objective laboratory study. When carrying out contact bioassay of urban soils, natural soils similar in grain size composition and content of organic matter, 50–100 km away
from sources of human-induced pollution, served as controls. The results of the chemical analyses of the control soils showed that the concentrations of heavy metals in them (Pb, Zn, Cu, Cd, Ni) did not exceed the background values of these elements for the soils of the region. In total, 144 soil samples were examined over a 4-year period.

2.2 Physical–Chemical and Chemical Analysis

Standard physicochemical and chemical analyses of the studied soils were carried out according to the national standard methods and included the following: determination of pH by potentiometric method in a water extract in a ratio of soil:distilled water 1:5 on an HI99121 device from Hanna in accordance with GOST 26,423–85, specific electrical conductivity by conductometric method in a water extract in a ratio soil:distilled water 1:5 on a HI 98,311 device from Hanna in accordance with GOST 26,423–85, sodium and potassium content in a water extract by flame photometric method on the FPA-2–01 photometer in accordance with GOST 26,423–85 in a water extract, and chlorine content in a water extract by titrimetric analysis: the content of water-soluble calcium and magnesium in the water extract 1:5—by the complexometric method; the content of mobile phosphorus and potassium in the extract soil extraction solution 0.2 mol/dm³ 1:5—by spectrophotometric method according to GOST 54,650–2011. In the selected soil samples, the content of gross forms of heavy metals belonging to the 1st and 2nd hazard classes (Pb, Zn, Cu, Cd, Ni) was also determined by the method of spectrometry with inductively coupled plasma on mass spectrometer ICMS-2030 (Shimadzu).

All analyses were performed in a certified laboratory.

The cumulative pollution index ($Z_c$) is widely used in ecological and geochemical indication of soil pollution; it is the sum of the concentration coefficients of toxicants relative to the background level (Nevidomskaya et al., 2020). $Z_c$ is calculated using the following formula (1):

$$Z_c = \sum K_c - (n - 1)$$  

(1)

where $K_c = C_i / C_{backgr}$ is the concentration coefficient of the $i$th chemical element;

$C_i$ is the actual content of the $i$th chemical element in the soil, mg kg$^{-1}$;

$C_{backgr}$ is the background content of the $i$th chemical element in the soil, mg kg$^{-1}$;

$n$ is the number of accounted chemical elements with $K_c > 1$.

The values characterizing the total pollution $Z_c$ according to the degree of danger have the following ranges: $Z_c < 16$, low level; $16 < Z_c < 32$, medium; $32 < Z_c < 64$, high; $64 < Z_c < 128$, dangerous; $Z_c > 128$, extremely dangerous.

The $Z_c$ indicator takes into account the multi-elemental nature of the territory pollution with heavy metals and it is used to assess the ecological state of urban soils (SANPIN 1.2.3685–21). In our case, the average content of heavy metals in the suburbs of St. Petersburg was taken as a background.

2.3 Bioassays

Ecotoxicological studies of the acute toxicity of contaminated soils were carried out using eluate and contact bioassays.

There are different methods of soil biotesting using aquatic organisms (Pukalchik et al., 2019). Eluate bioassay was carried out by us using two biotest organisms: daphnia ($Daphnia magna$ Straus) and ciliates ($Paramecium caudatum$).

Daphnia are considered one of the most sensitive test organisms to the toxic effects of pollutants. Water extracts from soils were prepared using cultivation water in accordance with the requirements of the toxicity measurement technique: four parts of water were added to one part of the soil. The criterion for acute toxicity on daphnia was the death of more than 50% of individuals in the tested samples within 48 h, compared with the control, A, %, (PND, 2014).

Ciliates $Paramecium caudatum$ served as another test organism for eluate bioassay. In terms of their sensitivity to the effects of toxic substances, ciliates are close to the sensitivity of human and animal tissues; therefore, they are widely used in bioassay (Mai et al., 2014; Norikazu et al., 2003).

The determination of the aquatic environment toxicity is based on the ability of ciliates to respond to the substances in the aquatic environment that pose a danger to their vital activity and to move directionally along the concentration gradient (in the direction of concentration changes) of these
substances (chemotactic reaction), thus avoiding their harmful effects (FR.1.39.2015.19243). The toxicity criterion for the test on ciliate is the toxicity index \((T)\)—a dimensionless value that takes values from 0 to 1 in accordance with the degree of toxicity of the studied sample. The toxicity index is determined by the following formula (2):

\[
T = \frac{I_{\text{cont.}} - I_{\text{exp.}}}{I_{\text{cont.}}}
\]  

(2)

where \(I_{\text{cont.}}\) and \(I_{\text{exp.}}\) are the average instrumental indication for control and studied samples, respectively.

By the value of the toxicity index \(T\), the samples are classified into 3 groups according to the degree of toxicity: I, permissible degree \((0 < T \leq 0.40)\); II, moderate degree \((0.40 < T \leq 0.70)\); III, high degree \((T > 0.70)\).

The literature provides data on the high efficiency of contact bioassays for soil assessment (Leitgib et al., 2007; Manzo et al., 2008; Pukalchik et al., 2019). In our studies, during contact bioassay, seeds of higher plants and microorganisms were used as test organisms.

To determine the degree of toxicity of urban soils, we used contact (substrate) phytotesting developed at the St. Petersburg Research Center for Ecological Safety of the Russian Academy of Sciences (Kapelnkina et al., 2009). In this method, soft wheat \((Triticum vulgare\) L.) is used as a test culture. This technique allows to diagnose the level of toxicity of contaminated soils on the basis of a decrease in seed germination \((N_1, \%)\) and suppression of roots \((N_2, \%)\) in comparison with the control sample: V, practically non-toxic \((0 < N_1 \leq 20\%, 0 < N_2 \leq 20\%); IV, slightly toxic \((0 < N_1 \leq 20\%, 20\% < N_2 \leq 50\%); III, moderately toxic \((20\% < N_1 \leq 70\%, 50\% < N_2 \leq 70\%); II, dangerously toxic \((20\% < N_1 \leq 70\%, 50\% < N_2 \leq 70\%); I, highly hazardous toxic \((N_1 = N_2 = 100\%). The natural complex of microorganisms contained directly in the studied soils was adopted as a test organism for the second type of contact bioassay. Contact microbial tests currently used in Russia are based on assessing the effect of contaminated soil on an artificially introduced test microorganism (Galitskaya and Selivanovskaya, 2009). However, the soils themselves contain a significant amount of viable microflora. This allows the use of a natural complex of microorganisms contained directly in the soils as a test culture in soil bioassay.

The most important indicator of the microbiological state of soils is the characteristic of the functional abilities of soil microorganisms. Functional properties and their diversity determine the intensity of the most important biochemical processes in the soil and the stability of the ecological balance that has developed in the soil. An integral indicator of the intensity of microbiological processes taking place in soils in the aggregate is their release of carbon dioxide, or, in other words, soil or microbial respiration.

Within the 3 years, soil samples for microbiological studies were taken three times during the growing season (spring, summer, and autumn), and then in the summer of the fourth year of observation. The level of microbial respiration was established under controlled laboratory conditions by the production of CO\(_2\) by the adsorption method (Alef, 1995). Soil samples were preliminarily moistened with distilled water to 60% of full moisture capacity and composted at room temperature. The criterion for assessing the toxicity of urban soils was determined on the basis of statistically significant changes in the level of their microbial (soil) respiration in comparison with the control samples. The soil state is considered ecologically dangerous if the decrease in the microbial respiration level, in comparison with the control, exceeds the critical threshold for the stability of soil ecosystems, which is a loss of no more than 30% of the bioorganic potential (Yakovlev & Evdokimova, 2011).

When carrying out contact bioassay of urban soils, natural soils similar in grain size composition and content of organic matter, 50–100 km away from sources of human-induced pollution, served as controls. The heavy metal values in control soils were at the background level of the region.

### 2.4 Statistics

All determinations were carried out in four replicates. The results were processed by analysis of variance using MS Excel and Statistica 10. The reliability of differences between the mean values was compared using the Student test in the variant of grouping samples with the least significant difference (LSD) at a significance level of 5% \((P < 0.05)\). The tables show
mean values ± standard deviations; values with different letters differ significantly.

3 Results and Discussion

Our research has confirmed that urban soils differ significantly from their natural counterparts in chemical and physicochemical parameters as a result of anthropogenic impact (Ajmone-Marsan et al., 2019). Urban soils are formed by the imposition of anthropogenic impact on natural processes of soil formation. As a result, soils are formed (urbanozem, urbostratozems, etc.), which, in terms of biological and physicochemical parameters, differ significantly from their natural analogs (Stroganova & Agarkova, 1993). Compared to zonal soils, they are characterized by a disturbed profile, strong compaction, an alkaline environment reaction, and pollution with a wide range of various toxic substances—heavy metals, oil products, pesticides, PAHs, de-icing agents, and others (Yang & Zhang, 2015).

One of the important indicators of anthropogenic impact on the urban soils is the pH of the aqueous solution. The completed 4-year monitoring revealed that urbanozems (plots 2–6) had a neutral or slightly alkaline environment ($\text{pH}_{\text{H}_2\text{O}}$ varied in the range of 6.1–7.9). Fluctuations in pH over the years were insignificant. The highest pH values ($\text{pH}_{\text{H}_2\text{O}}$ 8.4) were recorded in the summer during all the years of observation in the urbostratozem (plot 1), where construction and household waste was stored. According to Russian standards, the soil condition at plot 1 was degraded in terms of the acid–base regime (Hygienic evaluation of soil in residential areas, 1999).

Another integral indicator of the physical and chemical soil state is its electrical conductivity. This indicator correlates with such important soil properties as moisture content, the composition of the soil solution, the granulometric and aggregate texture, etc. (Friedman, 2005). The value of electrical conductivity determines, among other things, the important soil characteristic of its salinity. Excessive salt content can contribute to the appearance or enhancement of soil phytotoxicity as a result of an increase in the osmotic pressure of soil solutions and the inhibitory effect of ions. Soils are considered saline if the conductivity exceeds 2 mS (mS cm$^{-1}$). In our work, the electrical conductivity of the studied soils did not exceed 1.04 mS; they were not salted.

In the spring, in all studied soils, there was an increase in the content of water-soluble forms of calcium (up to 16 mg 100 g$^{-1}$), magnesium (up to 8 mg 100 g$^{-1}$), sodium (up to 25 mg 100 g$^{-1}$), and potassium (up to 25 mg 100 g$^{-1}$) due to the salt transfer of de-icing agents into the soil composition. By autumn, the content of these cations in the upper soil layers decreased significantly.

Urban soils are enriched in mobile forms of nutrients, primarily phosphorus and potassium (Pouyat et al., 2015; Yang & Zhang, 2015). In the studied soils, the content of mobile potassium varied from medium to very high values (10.35–110.64 mg 100 g$^{-1}$). The availability of mobile phosphorus was high and very high (up to 130 mg 100 g$^{-1}$). In this regard, we can talk about phosphorus pollution of the studied urban soils.

The main pollutants of urban soils are heavy metals that accumulate in the upper soil layer (Wei & Yang, 2010; Osma et al., 2012; Vodyanitskii 2013; Burghardt et al., 2015; Kostic et al., 2019).

The studied soils in the majority of cases were characterized by an increased content of heavy metals, compared with the regional background. The priority soil pollutants among heavy metals at the roadside sites were Pb > Cu > Zn, at the industrial site (plot 1): Cu > Cd > Zn > Pb. Thus, almost all studied urban soils were contaminated with heavy metals to one degree or another (Table 1).

However, only at plot 3, located along a motor road with heavy traffic, in the surface layer of 0–5 cm, a moderate degree of soil pollution with heavy metals ($Z_c = 20.1$) was revealed with an ecological-geochemical indication of soil pollution using the total pollution indicator $Z_c$, which takes into account the polyelemental nature of the territory pollution with heavy metals.

3.1 Eluate Bioassays

The study of the water extract using the test culture *Daphnia magna* Straus revealed acute toxicity in soils (A > 50%) only in the third year of observation in spring on plots 5 and 6, where de-icing agents were used in large volumes (Fig. 8). In the summer and autumn of the same year, the acute toxicity of the aqueous extract from the soils of industrial plot 1 was
established. In the rest of the observation periods, the toxicity of aqueous extracts from all studied soils using this culture was not detected. This may be due to the presence of P, K, Ca, and Mg ions in aqueous solutions, which have a protective effect for daphnia (Mertens et al., 2007; Pukalchik et al., 2019).

A moderate toxicity degree ($0.42 < T \leq 0.63$) was detected in spring and summer at plot 1 on another
Comparing the results obtained by the two methods of eluate bioassay of urban soils, one can state that the ciliates are more sensitive as a test culture than daphnia. The results obtained agree with the data of other researchers, who note the high sensitivity of the aquatic organism *Paramecium caudatum* to water-soluble forms of heavy metals and organic toxicants in the soil (Olkova et al., 2016).

**Fig. 9** Bioassay results of the soil for *Paramecium caudatum*, third year of observation, summer season

**Fig. 10** Bioassay results of the soil for *Paramecium caudatum*, fourth year of observation, summer season
3.2 Contact Bioassays

The priority biotic indicator of soil health is the state of higher plants growing on them. Therefore, phytotesting is the most important component of the whole complex of ecotoxicological studies of soils (Vasilyeva et al., 2020).

In the spring, a moderate degree of toxicity at plots 1 and 2 and a low degree of toxicity at plots 3 and 6 were detected in the first year of observation, using the contact phytotesting (Table 2). In the soils of plots 4 and 5, phytotoxicity was absent, which was due to the addition of clean soil by gardening workers. In summer, weak phytotoxicity was recorded at plots 1, 4, and 6. In autumn, moderate phytotoxicity was present only in the soil of plot 1.

In the second year of observation, a moderate degree of toxicity was recorded in the spring at plots 1 and 6 and a weak one on the remaining plots 2–5 (Table 3). In summer, weak phytotoxicity was observed at all sites, except for plot 1, where it was absent. In autumn, phytotoxicity (slight degree) was found only in the soil of plot 1.

In the third year of the study, a moderate degree of phytotoxicity was revealed at plots 1 and 3, and a weak one at plots 4 and 6 in the spring (Table 4). In summer, moderate phytotoxicity was observed only at industrial plot 1. In autumn, moderate phytotoxicity appeared at three plots at once—1, 2, and 4.

On the 4th year of observation in the summer season, a moderate degree of toxicity was still observed at site 1 (Table 5). In the soils of the remaining plots, phytotoxicity was absent.

The studies carried out by the contact bioassay indicated that phytotoxicity was present in the upper layers of the studied soils in spring. The soil toxicity increase in the spring could be due to several reasons: first, the increased content of soluble salts caused by excessive use of de-icing agents. Second, snow melting is an important factor of soil pollution in spring. Snow is a deposit medium with high sorption properties, and toxic substances accumulate in it during the winter (Coldsnow & Relyea, 2018; Oberts, 2003). This can lead to the higher spring phytotoxicity of urban soils (Bardina et al., 2017; Kostka et al., 2019; Šerá, 2017).

A decrease in the degree of phytotoxicity, or no record of it at all, with the exception of industrial site 1, was observed at most sites in the summer. This can be caused by the leaching of contaminants into the underlying soil layers. In the autumn period, phytotoxicity was observed in the soil at industrial plot 1, where the anthropogenic impact was the strongest (emissions from thermal power plants and vehicles, household and construction waste). In addition, moderate phytotoxicity was recorded at sites 2 and 4 in the third year of observation, which may have been due to the consequences of an extremely hot and dry summer.

The results obtained agree with the data of other authors, who also, using bioassay, recorded fluctuations in the content of toxicants in the upper layers of urban soils, depending on meteorological conditions and the season (Westerhoff et al., 2018).

It should be noted that, over the entire observation period, among the studied soils, phytotoxicity was most often detected in the soil of plot 1. In most cases, the phytotoxicity of this soil was characterized as moderate.

The state of soil microbiota, along with higher plants, is the most important indicator of soil quality. This is due to the exceptional role that microbial communities play in the cycle of biogenic elements in the biosphere and in maintaining ecological balance in soils. In addition, microorganisms are sensitive indicators of the ecological state of soils. In this regard, soil microorganisms are optimal test cultures for soil bioassay (Terekhova, 2011). The results of studying the microbiological state of urban soils for the first year of observation are presented in Fig. 11.

It was found that in the spring of this year, almost all the studied soils showed toxicity in relation to microorganisms. This is evidenced by a significant decrease in the intensity of microbial respiration in soils in comparison with the control. Respiration decreased most of all in the soil of industrial site 1 (−42%), as well as in the soils of lawns (plots 2 and 4) located along busy motor roads (by 35% on average). This level of decrease in the biological indicator is considered ecologically dangerous, since it exceeds the critical threshold for the stability of soil biosystems, which is a loss of no more than 30% of the bioorganic potential from the control level (Gorobtsova et al., 2016; Yakovlev & Evdokimova, 2011). The microbial activity of the soils of plot 5 (−28.0%) and plot 3 (−25.6%) was disturbed to a lesser degree. A slight decrease in respiratory activity was recorded in the soil at plot 6 (city garden).
Table 2  Results of contact bioassay on seeds of *Triticum aestivum* L., first year of observation. *Degrees of sample toxicity are as follows: III, moderately toxic; IV, slightly toxic; V, practically non-toxic. Different letters represent significant differences between samples on plots (LSD-test, $P \leq 5$)

| Plot | Sampling depth, cm | Observation time | | | | |
|------|-------------------|------------------|---|---|---|---|
|      |                   |                  | Spring | Summer | Autumn | |
|      |                   | Germination rate ($N_1$) | Root length ($N_2$) | Degree of toxicity | Germination rate ($N_1$) | Root length ($N_2$) | Degree of toxicity | Germination rate ($N_1$) | Root length ($N_2$) | Degree of toxicity* |
| 1    | 0–5               | −14.0            | −55.3 | III | −23.7 | +10.0 | IV | −31.3 | −31.2 | III |
|      | 5–20              | −19.0            | −36.0 | IV  | −5.2  | −1.7  | V  | −8.4  | −26.2 | IV  |
| 2    | 0–5               | −17.8            | −54.8 | III | −5.2  | +12.3 | V  | −0.8  | −0.5  | V   |
|      | 5–20              | −14.0            | −44.3 | III | +5.3  | +4.7  | V  | +1.8  | −17.3 | V   |
| 3    | 0–5               | −7.7             | −28.3 | IV  | +5.3  | +18.4 | V  | −0.8  | −1.6  | V   |
|      | 5–20              | −7.7             | −2.8  | V   | +5.3  | −10.3 | V  | −0.8  | −6.2  | V   |
| 4    | 0–5               | −10.1            | −4.2  | V   | +2.6  | −36.8 | IV | +1.8  | +6.2  | V   |
|      | 5–20              | −12.8            | −8.3  | V   | +5.3  | +6.0  | V  | +1.8  | −3.3  | V   |
| 5    | 0–5               | −1.3             | −16.9 | V   | −2.6  | −14.0 | V  | −0.8  | +18.2 | V   |
|      | 5–20              | −7.7             | −14.2 | V   | −2.6  | +3.7  | V  | −3.4  | −14.4 | V   |
| 6    | 0–5               | −10.1            | −39.1 | IV  | +2.6  | −27.6 | IV | −5.9  | −10.2 | V   |
|      | 5–20              | −7.7             | −32.9 | IV  | +5.3  | +6.0  | V  | +1.8  | +6.1  | V   |
Table 3  Results of contact bioassay with seeds of *Triticum aestivum* L., second year of observation. *Degrees of sample toxicity are as follows: III, moderately toxic; IV, slightly toxic; V, practically non-toxic. Different letters represent significant differences between samples on plots (LSD-test, *P* ≤ 5)*

| Plot | Sampling depth, cm | Observation time | Germination rate (*N*1) | Root length (*N*2) | Degree of toxicity |
|------|--------------------|------------------|-------------------------|--------------------|-------------------|
|      |                    | Spring           |                         |                    |                   |
|      |                    |                  | Germination rate (*N*1) | Root length (*N*2) | Degree of toxicity |
| 1    | 0–5                |                  | +2.8                    | −66.0              | III               |
|      | 5–20               |                  | +2.8                    | −50.4              | III               |
| 2    | 0–5                |                  | −2.6                    | +0.7               | V                 |
|      | 5–20               |                  | −13.5                   | −28.4              | IV                |
| 3    | 0–5                |                  | +5.4                    | −41.9              | IV                |
|      | 5–20               |                  | +5.4                    | −11.3              | V                 |
| 4    | 0–5                |                  | 0                       | −25.1              | IV                |
|      | 5–20               |                  | 0                       | +3.7               | V                 |
| 5    | 0–5                |                  | +2.8                    | −42.2              | IV                |
|      | 5–20               |                  | −5.4                    | −27.2              | IV                |
| 6    | 0–5                |                  | −5.4                    | −65.8              | III               |
|      | 5–20               |                  | +8.2                    | −30.8              | IV                |
|      |                    | Summer           | −15.0                   | +6.8               | V                 |
|      |                    |                  | −20.0                   | +4.7               | V                 |
|      |                    |                  | −20.0                   | +30.0              | IV                |
|      |                    |                  | −17.5                   | +19.7              | V                 |
|      |                    |                  | −2.5                    | +0.8               | V                 |
|      |                    |                  | −12.5                   | −23.4              | IV                |
|      |                    |                  | −7.5                    | −22.8              | IV                |
|      |                    |                  | −20.0                   | −26.7              | IV                |
|      |                    |                  | −15.0                   | +19.5              | V                 |
|      |                    |                  | −15.0                   | −29.2              | IV                |
|      |                    |                  | −10.0                   | +10.9              | V                 |
|      |                    |                  | −10.0                   | −20.4              | IV                |
|      |                    | Autumn           | −15.4                   | −6.6               | V                 |
|      |                    |                  | −20.5                   | +6.2               | IV                |
|      |                    |                  | −2.5                    | +5.8               | V                 |
|      |                    |                  | +5.4                    | +4.9               | V                 |
|      |                    |                  | +5.6                    | −10.5              | V                 |
|      |                    |                  | +5.4                    | −11.3              | V                 |
|      |                    |                  | +2.9                    | +3.5               | V                 |
|      |                    |                  | +6.5                    | −6.2               | V                 |
|      |                    |                  | +2.5                    | +3.8               | V                 |
|      |                    |                  | +8.2                    | +3.7               | V                 |
|      |                    |                  | +8.5                    | −10.5              | V                 |
Table 4 Results of contact bioassay with seeds of *Triticum aestivum* L., third year of observation. *Degrees of sample toxicity are as follows: III, moderately toxic; IV, slightly toxic; V, practically non-toxic. Different letters represent significant differences between samples on plots (LSD-test, *P* ≤ 5)

| Plot | Sampling depth, cm | Observation time | Germination rate ($N_1$) | Root length ($N_2$) | Degree of toxicity | Germination rate ($N_1$) | Root length ($N_2$) | Degree of toxicity |
|------|-------------------|-----------------|--------------------------|-------------------|--------------------|--------------------------|-------------------|--------------------|
|      |                   | Spring          |                          |                    |                    | Summer                   |                    |                    |
| 1    | 0–5               | −2.8            | −15.5                    | V                  | −0.4               | +11.9                    | V                  | −32.4              | +28.9              | III                |
|      | 5–20              | −27.8           | +16.6                    | III                | −21.1              | +58.5                    | III                | −29.8              | +54.2              | III                |
| 2    | 0–5               | −13.9           | −19.9                    | V                  | −10.2              | +22.1                    | V                  | −43.2              | −10.8              | III                |
|      | 5–20              | −19.4           | +11.3                    | V                  | −10.5              | +38.4                    | V                  | −16.1              | +15.3              | V                  |
| 3    | 0–5               | −33.3           | −39.8                    | III                | −7.9               | +38.2                    | V                  | −16.1              | +2.0               | V                  |
|      | 5–20              | +2.8            | −32.3                    | IV                 | −13.2              | +8.0                     | V                  | −16.2              | +1.6               | V                  |
| 4    | 0–5               | −13.9           | −30.1                    | IV                 | −13.2              | +8.8                     | V                  | −56.8              | +3.2               | III                |
|      | 5–20              | −11.1           | −17.7                    | V                  | −15.8              | +24.8                    | V                  | −51.3              | −28.1              | III                |
| 5    | 0–5               | −5.6            | +19.9                    | V                  | −7.9               | −4.6                     | V                  | −10.5              | −12.3              | V                  |
|      | 5–20              | −2.8            | +34.5                    | V                  | −18.9              | +28.1                    | V                  | −5.7               | +12.3              | V                  |
| 6    | 0–5               | −2.8            | +48.3                    | V                  | −15.8              | −19.8                    | V                  | −2.8               | +10.5              | V                  |
|      | 5–20              | −19.4           | −21.6                    | IV                 | −15.8              | +3.6                     | V                  | −3.5               | +13.2              | V                  |
St. Petersburg is located in a cold humid climate zone, which is characterized by significant snow cover in the winter season. Therefore, the spring suppression of microflora in soils located along transport roads could be due to excessive use of de-icing agents and snow melting. In spring, as a result of snow melting, large amounts of pollutants enter urban soils, which leads to a decrease in their biological activity, in particular the intensity of microbial respiration (Ivanova et al., 2015). It should be emphasized that over the 3-year period of our observations, it was the first year of observation that was characterized by the greatest amount of snowfall, which fell from January to March.

Respiratory activity in the soils of St. Petersburg in summer not only recovered to the control level, but in some cases significantly exceeded it. Thus, the respiration of the lawn soils on sites 3, 5, and 6 was higher than in clean undisturbed soil. The increase in the biological activity of urban soils in the summer after the spring recession can be explained by the leaching water regime characteristic of regional soils, in which pollutants are washed out into the underlying soil layers.

It is necessary to take into account the high adaptive capacity of soil microorganisms, which manifests itself in the rapid development of species resistant to pollution and allows the population to return to a state of equilibrium after external influences (Dobrovol’skaya et al., 2015). Shchepeleva et al. (2017) reported higher summer values of carbon dioxide emissions from urban soils than in spring and autumn. The scientific literature also contains information on the minimum values of CO₂ emissions from urban soils in early spring, after winter snowmelt. Furthermore, during the growing season—from

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Table 5 Results of contact bioassay with seeds of *Triticum aestivum* L., fourth year observation. *Degrees of sample toxicity are as follows: III, moderately toxic; V, practically non-toxic. Different letters represent significant differences between samples on plots (LSD-test, *P* ≤ 5)

| Plot | Sampling depth, cm | Observation time | Germination rate (*N*₁) | Root length (*N*₂) | Degree of toxicity * |
|------|-------------------|-----------------|-------------------------|-------------------|---------------------|
|      |       | Summer          |                         |                   |                     |
| 1    | 0–5   | −13.2           | −52.0                   | III               |
|      | 5–20  | −21.0           | −51.4                   | III               |
| 2    | 0–5   | −10.5           | −6.6                    | V                 |
|      | 5–20  | −2.6            | −19.0                   | V                 |
| 3    | 0–5   | −5.3            | +3.2                    | V                 |
|      | 5–20  | −18.4           | −18.2                   | V                 |
| 4    | 0–5   | −20.0           | −9.0                    | V                 |
|      | 5–20  | −7.9            | −11.1                   | V                 |
| 5    | 0–5   | 0               | +4.3                    | V                 |
|      | 5–20  | −19.7           | +3.7                    | V                 |
| 6    | 0–5   | −15.8           | +4.7                    | V                 |
|      | 5–20  | −7.9            | +0.1                    | V                 |

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Fig. 11 Respiration rate of the upper horizons of urban soils (mg CO₂ 100 g⁻¹ dry soil, day), 1st year of observation
early spring to early autumn, a gradual increase in soil respiration was noted (Ivanova et al., 2015).

In autumn, the intensity of soil respiration, varying depending on the type of functional zone, was noticeably lower than in summer. Toxicity was again revealed in the soils of the industrial zone at site 1 and in the roadside lawn at site 4. At the same time, the differences in the amounts of carbon dioxide emitted from urban soils and the control sample, compared with the previous observation periods, were largely erased and did not exceed ±24%.

In the ensuing years, similar trends were observed in the spatial and seasonal dynamics of the toxicity of urban soils, determined by the intensity of microbial respiration. In the second year of observation, toxicity was detected at two sites: plot 1 and plot 4 (Fig. 12).

The toxicity values were maximal in the spring, when the decrease in carbon dioxide emissions compared with the control was 26% and 54%, respectively. In summer, the microbiological activity of all the studied soils either did not differ from the control sample or slightly exceeded it. In autumn, toxicity reappeared in the soils of industrial site 1 and the lawn at site 4. However, its values were significantly lower than in spring.

In the third year of observation, as in previous years, soil toxicity for microorganisms during the growing season was maximum in spring (Fig. 13).

The highest toxicity in spring, determined by a decrease in the intensity of microbial respiration, compared with the control, as well as in other years, was characterized by the soil of industrial site 1 (−43.6%) and the soil of the lawn at site 4 (−32.3%). Roadside soil toxicity was identified at site 5 (−24.7%). However, this level of toxicity is considered acceptable, since it is lower than the critical threshold of the stability of the soil biosystem, which is a loss of more than 30% of the integral biological indicator of the control value (Gorobtsova et al., 2016; Yakovlev & Evdokimova, 2011). In the summer of this year, as in the first year of observation, the respiratory activity of the studied soils was higher than in the control. In summer and autumn, at industrial site 1, a decrease in respiratory activity in the soil was still observed, by 42.8% and 38.1%, respectively. In autumn, the respiration of soils in the zone of influence of transport roads, as in other years, decreased and did not significantly differ from the control level.

Unlike other studied urban soils, the soil of industrial plot 1 was characterized by consistently low values of microbial respiration throughout the entire growing season of the third year of observations. On average, the decrease in the amount of carbon dioxide emitted by this soil in comparison with the control was 41.5%.

In the summer of the fourth year of observation, a toxicity hazardous to microorganisms was recorded in the soils of industrial plot 1 (−31.0%) and the lawn of plot 4 (−36.7%).
A lower respiratory activity of the soil at industrial plot 1, as compared to the areas along motor roads, was observed in most cases during all the years of observation. This fact, most likely, was due to the additional anthropogenic load on the soil of the industrial site: soil over packing and waste (more than 30%). Our researches showed us that strong soil compaction leads to the creation of microaerophilic or even anaerobic conditions in the upper soil layer, especially in spring during snow melting and prolonged rains. Such a sharp violation of gas exchange and moisture in the studied soil could inhibit the vital activity of the aerobic microflora, as a result of which the intensity of carbon dioxide emission decreased (Smagin et al., 2006; Stoma et al., 2020).

The results of 4 years of research indicate that among all studied urban soils, the garden soil was characterized by the best microbiological state (plot 6). The intensity of microbial respiration in this soil in the majority of cases did not significantly differ from the control. Therefore, we can conclude that the soil of the garden is in a zone of low ecological risk, the degree of its pollution is characterized as low, and possible processes of degradation of microbocenoses are quickly reversible.

3.3 Results of the Soil Toxicity

Bioassay results of urban soils show that the data of eluate and contact bioassays do not always coincide. This is most likely due to the different compositions of toxicants in the analyzed samples. Eluate bioassay allows assessing the toxicity of soil and groundwater due to the soluble forms of pollutants and contact bioassay due to the toxicity of the solid phase of soils.

The sensitivity of biotests used to identify the toxicity of urban soils varied and decreased in the following order: inhibition of seed growth of higher plants (37.8%) > inhibition of respiration of microorganisms (18.3%) ≥ chemotaxis of ciliates (18.3%) > mortality of daphnia (5.0%). It should be noted that the bioassay results of the same soil did not always coincide in terms of observation. This phenomenon, most likely, was caused by the dynamics of the content of pollutants in the soil.

It was found that contact bioassays are characterized by a higher sensitivity to the toxicants in soils, compared to bioassay of water extracts. Other researchers came to a similar conclusion (Hubálek et al., 2007; Pukalchik et al., 2019).

The results of the conducted studies allow us to conclude that among the studied samples the soil of plot 1 (the territory of the CHP plant) is in the zone of the highest ecological risk, and the soil of the garden is located in the zone of the lowest ecological risk (site 6). Among the soils of roadside strips of motorways, the soil of plot 4 was distinguished by the highest toxicity (Table 6).
Four-year ecotoxicological studies were carried out in St. Petersburg, a large city located in the North-West of the Russian Federation, in the eastern waters of the Baltic Sea and in the delta of the Neva River. Stockholm, Helsinki, and Oslo are located at this northern latitude with similar climatic parameters. But the anthropogenic load on the soils of St. Petersburg is much higher compared to that of the cities of Northern Europe due to the larger area of the city and the larger population.

The soils of different functional zones of St. Petersburg were studied using the methods of eluate and contact bioassays. These zones were an industrial zone (the territory of a thermal power plant), a residential zone (lawns along motor roads), and a recreational zone (city garden).

Biological analyses have shown varying degrees of toxicity in urban soils. The littered and overcompacted
soil of the monitoring site on the territory of the CHP plant has the highest toxicity among the studied soils. According to the bioassay results, the ecological state of the soil of the city garden was the best among the studied objects. This soil was in a zone of low ecological risk, the degree of its pollution was characterized as weak, and the processes of degradation of microbionics were rapidly reversible. Among the roadside soils, the most toxic was the lawn soil along roads with excessive use of de-icing agents at plot 4.

The nature of the seasonal dynamics of the ecotoxicological state of urban soils was determined by the degree of anthropogenic load and weather conditions, and also depended on the type of test organism. The toxicity of the studied soils had a pronounced seasonal dynamic for microorganisms. In all years of observations, it was the highest in spring, decreased in summer, and increased again in autumn in most cases.

Our data indicate that the test organisms that we used in bioassays demonstrate different sensitivities to toxicants. The sensitivity in ciliates was higher than that of daphnia in the eluate bioassay. The phytotest was more sensitive than the test on microorganisms in contact bioassay. In general, the test organisms made up a range sensitivity: higher plants > microorganisms ≥ ciliates > daphnia.

As a result of the studies carried out, a battery of bioassays was developed and tested, which allows the recording of negative phenomena in soils even under weak anthropogenic loads. The applied battery of bioassays consisted of test organisms—representatives of the main levels of the trophic chain: producer higher plants—Triticum vulgare L. for producers, hydrobionts—Paramecium caudatum for consumers, and natural microbionics for decomposers contained in soils. The developed battery of bioassays did not include the representative of aquatic organisms Daphnia magna St. due to the low sensitivity of this test culture to the toxicity of urban soils.

It was found that the contact bioassays used were characterized by a higher sensitivity to the toxicants in soils, compared with bioassay of water extracts. However, despite this, we believe that a correct assessment of the ecological state of urban soils is possible only on the basis of the combined use of eluate and contact bioassays.

So, the compiled set of biotest systems for assessing the acute toxicity of urban soils, taking into account their contamination with a wide range of pollutants, should include standardized methods of eluate bioassay using aquatic organisms (ciliates) as test organisms, contact bioassay using seeds of higher plants (wheat), and natural complex of soil microorganisms. The proposed methods are characterized by rapidity, sensitivity, and a high degree of efficiency in order to assess the ecological state of urban soils in different functional zones.

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Declarations

Conflict of Interest The authors declare no competing interests.

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