Water Air Soil Pollut (2017) 228: 351
DOI 10.1007/s11270-017-3521-3

Applying Bioassay Methods for Ecological Assessment of the Soils from the Brownfield Sites

Tamara V. Bardina · Marina V. Chugunova · Valery V. Kulibaba · Yulia M. Polyak · Victoria I. Bardina · Lyudmila P. Kapelkina

Received: 19 May 2017 / Accepted: 3 August 2017 / Published online: 25 August 2017

Abstract Biological organisms, used as test objects in pollution tests may be as good, or even more so, in detecting soil contamination, than chemical analyses. In this study, we used five bioassay methods, together chemical and physical-chemical tests, for comprehensive environmental assessment of contaminated soils located at the industrial waste storage sites in North-West Russia. Examined soils have been contaminated with various toxic pollutants at various times in the past. The level of contamination by Hg, Pb, Cd, Zn, Co, As, Cr, Cu, Mn, V, and As in studied soils varied depending on a site type. The concentrations of these elements were 20 to 43 times higher than the regional geochemical baseline at all sites. The organic pollutants (3,4-benzo(a)pyrene and polychlorinated biphenyls) were found at some sites. Ecotoxicological studies were carried out using test organisms from different taxonomic groups: ciliates *Paramecium caudatum* Ehrenberg, green algae *Scenedesmus quadricauda* (Turp.) Brebisson, seeds of common oat *Avena sativa* L.,wheat *Triticum aestivum* L., and a natural community of microorganisms. All the employed bioassays revealed some of the aspects of contamination, supported or supplemented each other's estimates, and gave excellent performance at the sampling sites.

T. V. Bardina (✉) · M. V. Chugunova · V. V. Kulibaba · Y. M. Polyak · V. I. Bardina · L. P. Kapelkina
Scientific Research Centre for Ecological Safety of the Russian Academy of Sciences (SRCES RAS), 19711018, Korpusnaya str, St. Petersburg, Russia
e-mail: bardinatv@mail.ru

Keywords Test organism · Toxicity · Contact bioassays · Integrative soil evaluation · Brownfield sites

1 Introduction

Areas of the past ecological damage, or brownfields, represent one of the most relevant ecological problems; remediation of such lands is growing more and more important. Brownfields are decommissioned lands, previously used as industrial waste sites, landfills, and dumps, as well as other contaminated areas that were previously in use. The disturbance loads and scopes of the brownfields vary from rare global- and nation-scale ecological disaster areas, to common regional and local long-term waste deposit sites. Conservation agencies of many countries fight the effects of these areas on the surrounding ecosystems, and such efforts often gain state-level priority. Supporting measures include compiling of the inventories and data bases of the brownfields, flexible remediation rules determine the sites suitable for various functions, remediation measures themselves, as well as the estimation instruments, are constantly developed. In the Russian Federation, the effects of brownfields are very pronounced, and liquidation of these sites is an acute problem (Saraev et al. 2015). During the last two decades, brownfields became the veritable source of ecological damage, representing danger to the health of the people living nearby. In Russia, the problem is now being addressed by a specialized state programme aimed at mitigation of the past environmental damage.

As the primary pollution by heavy metals is decreasing globally, the brownfield sites are becoming the major source of secondary pollution (Vodyanitskii 2013). It is well-known that the upper soils are particularly susceptible to the toxic contamination and accumulation of contaminants (Wolterbeek and Verburg 2001; Baran et al. 2014), and heavy metals are the main pollutants of the brownfield soils.

There are legally defined maximum permissible concentrations (MPCs) that specify the levels of pollutants in soils. At brownfield, as well as other sites, chemical tests are traditionally used to measure such concentrations (Remon et al. 2005; Hu et al. 2013). However, the list of pollutants and methods for their detection, specified in normative regulations, is not comprehensive, which undermines the accuracy of the environmental assessment (Maxam et al. 2000; Ahtiainen et al. 2002). MPCs are country-specific (e.g., SANPIN 2.1.7.1287-03; GN 2.1.7.2041-06 (2006); GN 2.1.7.2511-09 (2009). are only used in the Russian Federation); a unified, internationally accepted levels are not, as yet, developed. Additionally, existing soil quality standards and MPCs are controversial in Russia (Kapelkina 2013): they consider neither the soil type and resilience nor intended land use. For instance, MPCs are the same for agricultural and industrial soils, and thus may differ from levels defined in other countries by one or two orders of magnitude. Public health validation of the permissible concentrations of the chemicals derived from four experimentally defined parameters, i.e., their ability to enter (i) the plant tissue, (ii) ground water, (iii) the atmosphere, and (iv) a generalized sanitary hazard index (the effects of the chemical on the self-purification and biological activity of the soil). The smallest of these four levels is set as a minimum permissible concentration. For some chemicals, only provisional, approximate permissible concentrations (APCs) exist, derived from pH and particle-size composition of the soil. Both measures are unsuitable for industrial lands. Useful regulatory standards have to be based on the influence of the pollutants on the public health. Additionally, present MPCs and APCs do not consider the age of contamination, although it is known that, e.g., mobility of the heavy metals falls with time, thus reducing their hazard level (Vodyanitskii 2013).

Another measure of the soil quality is an integrated contamination index Zc (Saet et al. 1990), which aggregates pollution levels of several individual heavy metals. This index provides an easy-to-interprete, clear-cut instrument for classification of soil contamination levels compared to the background. Value ranges of this index delineate five such categories: uncontaminated soils, permissible contamination level, moderately hazardous, hazardous, and extremely hazardous contamination (SANPIN 2.1.7.1287–03). This index, although widely used even in the state-level ecological regulations, is far from satisfactory. Its main problem lies in sensitivity to the number of the chemical elements used in the analysis: as this number increases, so does the resulting value of Zc (Smagin 2013).

A modern approach to ecological evaluation of the soil quality should take into account biotic parameters. Hence, many researchers call for interdisciplinary approach to brownfield assessment that involves chemical testing and toxicological and bioassay methods (Linkov et al. 2006; Alvarenga et al. 2012; Ribe et al. 2012; Feng et al. 2016; Voronich et al. 2016; Filenko 2007).

Bioassay is a laboratory method for determining the quality of environmental entities with the use of test organisms. The unique advantage of bioassay is its ability to register the total toxic impact of multiple pollutants on the living organism (Terekhova 2011; Olkova 2014). For instance, several chemical pollutants may be present in concentrations below permitted levels, but still have negative effect on the ecosystem, and bioassay methods can reveal this integral influence much better than chemical testing (van Gestel et al. 2001; Lors et al. 2011). Besides, bioassay may function as a part of the TRIAD diagnistic complex (Chapman Dagnino et al. 2008; Terekhova et al. 2014). Another benefit of the bioassay method lies in its ability to predict upcoming negative changes in the ecosystem before they take effect.

In their existing form, bioassays already form a reliable source of the integral assessment of brownfields, and deserve further development as a part of environmental monitoring for the contaminated sites (Bardina et al. 2014a, b, c, 2016). Still, as they gain popularity in modern ecological studies, biotesting methods encounter diverse demands. They need to be accurate, quick, cost-effective, and produce repeatable, reproducible results (Filenko and Terekhova 2016). To ensure the latter, one must employ certified test cultures and procedures.

Existing bioassays predominantly analyze soil eluate (i.e., aqueous extract), while contact methods are in great demand. Additionally, reliable transition is needed from the results of acute toxicological test to predicting the outcome of more prolonged, and costly (Solomon et al. 2008), chronic experiment.

The success rate of any particular variant of a bioassay closely depends on local conditions, including the landscape features, mesoclimate, soil, and geochemistry, as well as specific traits of the test objects. On the other hand, no single species can be a universal indicator of the environmental health of a soil (Broos et al. 2005; Foucault et al. 2013). Several species from various trophic levels: producers, consumers, and decomposers, should be used for comprehensive environmental assessment. Thus, important and highly relevant areas of research include the following: (1) identifying the applicability of various bioassay systems for specific objects, (2) regional geo-environmental adaptation of bioassay methods, and (3) developing comprehensive panels of the biotests to be used together (Manzo et al. 2008; Matejczyk et al. 2011).

The aim of the present study is to test the usefulness of several dissimilar bioassay methods on four brownfield sites, contaminated by various waste types. The investigation objectives are to determine the physical-chemical and chemical parameters of the soil samples from various brownfield sites; to apply various acute eluate and contact soil bioassays, in order to compare the resulting estimates; and to evaluate the integral sensitivity of applied set of test systems for the soils from the areas suffering the long-term storage of the solid wastes.

2 Material and Methods

2.1 Study Sites and Sampling

The study was carried out at four brownfield sites in North-West Russia. These sites are located in four different geomorphological subareas, each with its own characteristic soil type. All four sites suffered ecological damage in the near past, each site being contaminated by different combinations of toxic substances.

Site 1 is an area about 6 ha, located in the Neva lowland near the coastal zone of Lake Ladoga (59° 43′ 44″ N 31° 36′ 59″ E, Fig. 1), with *Podzolics surfacely-gleyic* (*Luvic Stagnosols Dystic of WRB, 2006*) soils typical for the locality. Hydrological conditions of the terrain include a weak surface runoff and significant groundwater infiltration. The site itself was used for storage of industrial waste, municipal solid waste, wood processing waste, agricultural waste and construction debris between 1980 and 2005. The layer of topsoil at the site has been partially destroyed. The area was covered with intermittent shrubbery and scattered small-volume dumps of the mixed waste. We took five soil subsamples from one $10m^2$ plot from the depth of 0–20 cm and joined them to form a single mixed sample.

Site 2 is an 8-ha area, located near Lake Lublinskoye (60° 20′ 11″ N 29° 53′ 59″ E). Surrounding terrain is terraced, gently rolling, with sandy *Podzolics illuvial-ferrugenous (Carbic Podzols)* soils. Hydrological conditions of the terrain include an underground runoff to the lake. The site has been used as mixed waste deposit in 1990s. The area was covered with scattered small-volume dumps of the mixed waste. We took five subsamples of soil from one 10-m^2 plot from the depth of 0–20 cm, and joined them to obtain one mixed sample.

Site 3 is a brownfield of 150 ha, lying on the bank of Voronka River (59° 43′ 12″ N 29° 18′ 23″ E). The surrounding landscape is an Ordovic plato, with developed limestone karst processes and *Sod-calcareouses (Redzic leptosols eutric)* loam soil. Hydrological conditions are characterized by a weak surface runoff in the direction of the nearby swamp and river, as well as a significant groundwater infiltration. The study site is contaminated by construction debris and industrial and mixed wastes, which were stored for 30 years, between 1970 and 2000. We took a total of five samples from each of two 10-m^2 plots with different contaminants: (1) industrial waste and (2) mixed waste. Samples were taken separately from 0 to 5 cm and 5 to 20 cm (SANPIN 2003).

Finally, site 4 is a 6.7-ha industrial waste dump and surrounding territory, located near lake Ladoga on the bank of the Neva River (59° 40′ 22″ N 31° 01′ 04″ E). Typical landscapes of the locality are gently rolling hills, with *Sod-podzolics illuvial-ferrugenous (Umbric Albeluvisols Abruptic)* soils on higher grounds and *Podzols illuvial-ferrugenous (Carbic Podzol)* soils in depressions. Hydrological conditions of the terrain include a noticeable lateral and vertical migration of water. The site of the dump was used for long-term unregulated storage of industrial wastes of sulfuric acid production. They have a complex composition, with prevalence of iron and other metals typical for the sulfide iron ores, and also include burned pyrite, industrial slag and secondary metabolites. The depositing started in 1965 and stopped in 1978, but the existing waste was never removed. Surrounding land was also contaminated over the years because of both surface runoff and groundwater

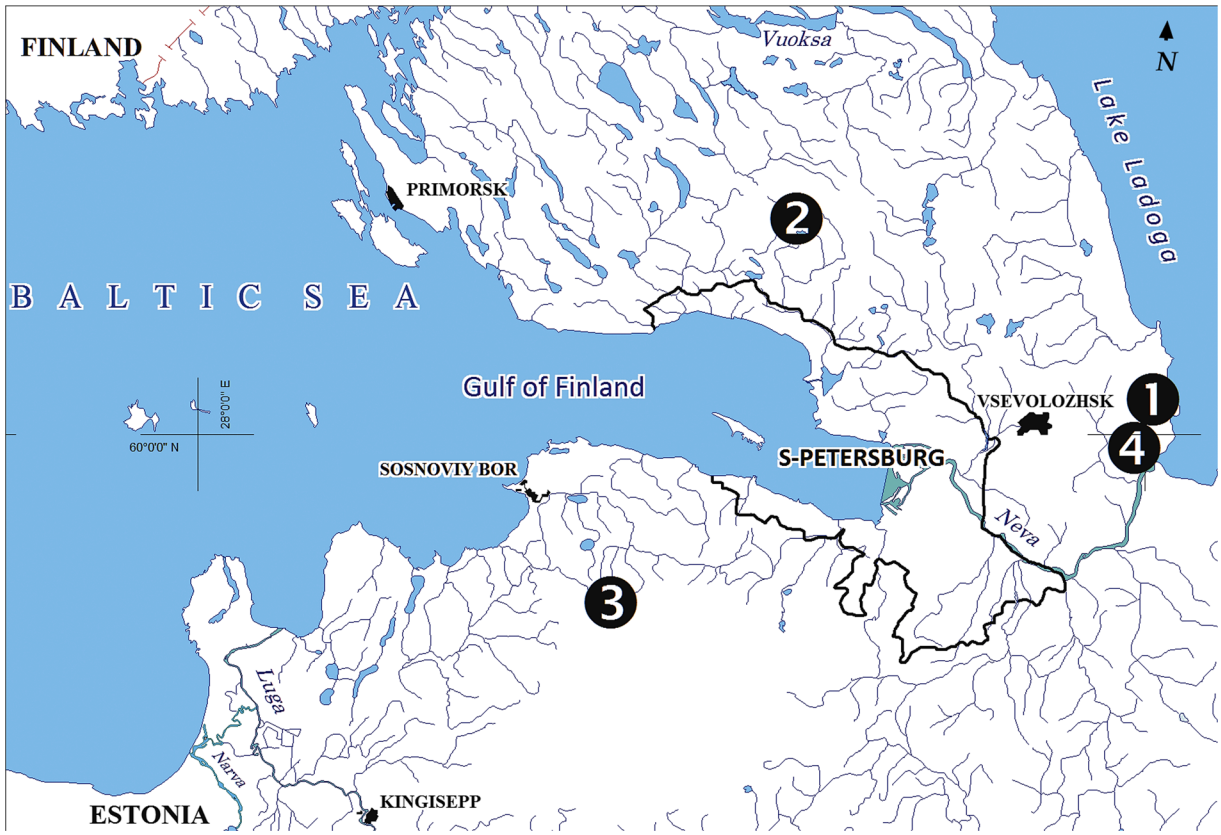

Fig. 1 Map of the study sites (S1, S2, S3, S4). Scale 1:1,500,000

infiltration of dissolved pollutants (Saraev et al. 2015). For sampling, we selected five 10-m^2 plots, located about 10 m away from the edge of the existing dump, in the south (1), south-east (2), east (3), north (4) and south-west (5) of the contaminated site 4. Again, we delineated two separate sampling depths: 0–5 cm and 5–20 cm. From each depth, five subsamples were taken within one plot and joined in a single mixed sample.

2.2 Physical-Chemical and Chemical Methods

We employed a range of physical-chemical and chemical tests, including measurements of pH and conductivity of the soil extracts. Heavy metals, arcenic, and phosphorous were determined by inductively coupled plasma mass spectrometry. Concentration of polychlorinated biphenyls, oil products, and chlorinated pesticides (HCH, DDT) was measured using gas chromatographic method with electron-capture detector (GC-2010, Shinadzu, Japan). Concentration of 3,4-benzo(a)pyrene was measured using high-performance liquid chromatography with fluorescence detection method (Fluorat-02, Russia). To estimate the integrative level of chemical contamination, we calculated the total index of pollution Zc:

$$Zc = \sum C_s \Big/ C_b - (n-1),$$

where C_s is the concentration of a chemical substance in the sample (mg/kg), C_b is the regional background concentration (mg/kg), and n is the number of substances. Zc values below 16 indicate permissible contamination level, values in the range between 16 and 32 – moderately hazardous, values from 32 to 128 – hazardous and and Zc values above 128 indicate extremely hazardous contamination (SANPIN 2003).

2.3 Eluate Bioassay Methods

There are two major approaches to biotesting of the soil toxicity. The first uses the aqueous extract, or eluate, of the soil sample, with various aquatic organisms (algae,

ciliates, daphnia, etc.) as test organisms. The second approach is contact bioassay, where the test organism contacts directly with the untreated test sample. This type of analysis helps to establish the level of impact of solid pollutants (Voronich et al. 2016; Bardina et al. 2016; Selivanovskaya and Galitskaya 2006). Here, both types of biotests were used, and final soil toxicity was estimated based on the most sensitive result.

We prepared eluates by mixing 10 parts of distilled water with 1 part of the sampled soil for 24 h to allow complete extraction. These were used in three types of bioassays: with ciliates *Paramecium caudatum* Ehrenberg (consumers), green algae *Scenedesmus quadricauda* (Turp.) Brebisson and seeds of common oats *Avena sativa* L. (producers). Daphnia were not employed, since they are intolerant to the acidic reaction, pronounced in all sample extracts from site 4. We did not attempt to neutralize the eluate, since this process alters the chemical composition of the sample and influence the measurements of toxicity (Filenko 2007).

We chose *P. caudatum* as a test species because these protists are sensitive to heavy metals (Eriksen 1990) and respond to the presence of dangerous substances by directional movement across the concentration gradient of these substances (chemotactic response). At the same time, *Paramecium* demonstrates negative geotaxis. When placed in a vertical test tube, these ciliates aggregate in the upper portions of the medium. This tendency is used in a biotest method, where a thickened medium with ciliate culture is placed at the bottom of the test tube, and examined eluate is poured on top (PND 2010). A stable interface boundary, which forms between the liquids, does not block ciliate movements between upper and lower zones. After 30 min, the ciliates redistribute themselves between two zones. The higher the toxicity of the sample, the smaller the proportion of ciliates in the upper zone of the test tube. We measured the concentration of the ciliates (cells/ml) and determined the sample toxicity index (T) with the following equation:

$$T = (I_c - I_s) \Big/ I_c$$

where I_c is the mean of the ciliate concentration in the control and I_s—in the analyzed sample.

Samples were classified into three groups of toxicity: (1) low (at $0.0 < T \leq 0.4$), (2) moderate ($0.4 < T \leq 0.7$), and (3) high degree of toxicity ($T > 0.7$). Sometimes, samples may contain harmless substances that are attractive for ciliates. In such cases, I_s may even exceed I_c, thus giving negative values of toxicity index. These results indicated the absence of toxicity and were treated as zeros.

To increase the sensitivity of detection, we prepared two subsamples: the first was used as is; the second was diluted with distilled water 100 times. The solutions were tested, and dilution rate, at which the toxic effects of the extract disappeared, was used to judge the sample quality.

The use of *S. quadricauda* in biotesting is based on the sensitivity of its growth rate. We used a standard protocol (Grigoriev and Tyutkova 2011), where two populations of algae were grown in a culture medium based on the eluate and on distilled water. After an interval of 45 h, their optical densities were measured and compared using the formula:

$$I = \left[(X_c - X_s) \Big/ X_c \right] \cdot 100\%,$$

where X_s and X_c are the average values of optical density in the sample and control, respectively. A growth rate decrease of 20% or more (growth suppression) and increase by 30% or more (growth stimulation), were used as threshold values of acute toxicity.

In all tests we used pure cultures of the *Scenedesmus* that were in the exponential phase of growth. To distinguish between toxicity levels of the soil extracts, we prepared three subsamples: the undiluted original eluate, and extracts diluted 10 and 100 times. All these were tested, and dilution rate, at which the toxic effects of the extract disappeared, was used to judge the sample quality. Samples that only exhibited toxicity in undiluted state were designated "weakly toxic." Samples that remained toxic when diluted 10 times but not 100 times were identified simply as "toxic." Finally, samples that remained toxic even if diluted 100 times were marked "highly toxic" (Grigoriev and Tyutkova 2011).

Finally, we used the seeds of common oat (*A. sativa*) as the test objects since phytotesting also proved useful in assessment of ecological conditions of soils (Terekhova et al. 2016). We planted 25 oat seeds in Petri dishes, added either distilled water (control), or undiluted soil extract, and placed the dishes into climatic chamber (20–23 °C). After 5 days, the root length of the

seedlings was measured and compared; each sample was tested in test replications. We determined the sample toxicity index (E) with the following equation:

$$E = \left[\left(X_c - X_s \right) \Big/ X_c \right] \cdot 100\%,$$

where X_s and X_c are the average values of root length of the seedlings in the sample and control, respectively. Eluate was judged toxic if the root length of the seedlings in the sample exceeded that of oats grown in the control by more than 20% (MR 2007).

2.4 Contact Bioassay Methods

The main advantage of contact bioassay lies in revealing the effect of solid pollutants as opposed to the dissolved ones (Terekhova et al. 2016). Methods employing seeds of the higher plants, as well as natural microbial communities, were suggested and developed in the recent years. Examples include international standards for plants ISO 11269-1, ISO 11269-2 (Fomin and Fomin 2001) as well as national standards (Kapelkina et al. 2009). The latter standard recommends barley (*Hordeum vulgare* L.) and common wheat (*Triticum aestivum*) as test cultures.

In the present study, we used the seeds of wheat, which is better adapted to the regional climate. Twenty seeds were placed into Petri dishes on the surface of soil sample (1-cm thick), moistened to 60% of moisture-holding capacity and allowed to germinate and grow for 5 days at the same humidity. For control, we planted the same number of seeds into clean, uncontaminated soils, similar in humus content and granulometric composition to each test sample. For each sample, we performed four replications of the test. In each case, we measured two response variables: the rate of germination and the length of the roots, which are the most sensitive parameters in phytotesting (Wang et al. 2001). We compared sample and control measurements of germination rate (N_1) and root length (N_2) separately, using the formula:

$$N = \left(M_c - M_s \right) \cdot 100 \Big/ M_c,$$

where M_c is the average value under control conditions and M_s is the average value of test sample. The degree of sample toxicity was judged by suppression of germination and reduction of the root length: V—practically non-toxic ($0 < N_1 \le 20$ and $0 < N_2 \le 20$); IV—slightly toxic ($0 < N_1 \le 20$ and $20 < N_2 \le 50$); III—moderately toxic ($20 < N_1 \le 70$ and $50 < N_2 \le 70$); II—highly toxic ($70 < N_1 < 100$ and $70 < N_2 < 100$); I—extremely toxic ($N_1 = 100$ and $N_2 = 100$).

The choice of controls is extremely significant in contact bioassays (Terekhova et al. 2016). To establish the background levels for comparison, one has to select the soils, which were not affected by anthropogenic activities, and which closely resemble the samples from the brownfields in physical and physical-chemical characteristics. To ensure this, we used control soil quantities, individually matched to samples from each of the tested sites.

Microorganisms are also sensitive indicators of the biological status of soils (Vodyanitskii 2013; Voronich et al. 2016). We used the natural community of microorganisms, present directly in the studied soils, for the second contact bioassay. To determine toxicity of each sample, we estimated biological activity of microbial community as manifested by soil respiration, which is one of the most important indicators of destruction processes caused by microorganisms (Anderson et al. 2011). Soil respiration was deduced from the intensity of carbon dioxide release, measured by modified adsorption method (Alef 1995). For this test, we used samples taken to the lab immediately upon collection (after no more than 48 h). Moist soil sample, equivalent to 1 g of soil dehuminified at 104 °C, was placed into an airtight glass jar for 24 h. CO_2, released during the incubation, was adsorbed in NaOH (0.02 mol/L), which was titrated with H_2SO_4 (0.01 mol/L). The jars with NaOH, but without the soil, were used to measure CO_2 levels in the air itself. The rate of the respiration was calculated in milligrams CO_2/ 100 g of dry soil/ 24 h. Uncontaminated soils (control), similar in humus content and granulometric composition to each test sample, were treated in the same way to obtain CO_2 release readings. Each test was repeated in four replications.

2.5 Statistics

To estimate central tendencies and range of measured biological characteristics, we calculated arithmetic means and standard errors (sample size n always equaled 4). To compare samples and controls, we used one-way analysis of variance (ANOVA) and Tukey's post hoc test. Differences

were considered significant at $p < 0.05$. Data analysis was performed with Statistica 10.0.

3 Results and Discussion

3.1 Physico-Chemical and Chemical Parameters of Soils

Soils at sites 1, 2, and 3 had close to neutral and neutral reaction (pH ranged from 5.9 to 6.9). Alkalization of these soils was caused by anthropogenic pollution. At site 4, soil had a more pronounced acidic reaction (pH 3.9–5.4) because of the acidic surface runoff from the waste dump and migration of the products of sulfur oxidation. The conductivity of all samples ranged from 0.05 to 0.12 mS/cm, indicating the absence of salination.

Some pollutants were common to all sites, e.g., all examined soils a had higher content of total phosphorus (428–699 mg/kg) compared to that of the background (302 mg/kg), due to anthropogenic pollution. Heavy metals were also ubiquitous toxicants (Table 1).

Heavy metals typically settle in the most fertile humic soil layer (0–20 cm), which also defines the crop ranges (Minkina et al. 2010). We expected that soils from different sites would be unequal in the levels of contamination with heavy metals. This is why measurements of Zc index were included into our study.

As expected, soils from various study sites differed in the level of contamination with heavy metals. Soil at the site 1 had the highest total index of pollution Zc of 65.5, and thus contamination of the area was classified as hazardous. Substances of hazard categories 1 and 2 (Pb, Cd, Zn, and Cu, respectively) were present at levels, exceeding maximum permissible concentration or tentative allowable concentration. Among organic pollutants, concentration of polychlorinated biphenyls was above MPC (0.16 mg/kg against 0.06 mg/kg).

At site 2, we found a low value of Zc (7.4), which placed the total heavy metal pollution of this soil into permissible category. Still, three elements: Pb, Cd and Zn, all from hazard category 1, slightly exceeded maximum or tentative permissible concentration. Polychlorinated biphenyls, at a level somewhat above threshold, were also found at site 2 (0.072 mg/kg).

Total heavy metal concentration at site 3 was judged permissible (Zc = 7.3). The main pollutants, however, were organic substances: 3,4 benzo(a)pyrene (0.085 mg/kg against MPC 0.02 mg/kg) and polychlorinated biphenyls (0.20 mg/kg against MPC 0.06 mg/kg). These substances are extremely toxic even in small concentrations and cause cell death.

Soil samples from site 4 had a Zc value of 29.9, or moderately hazardous total chemical pollution level. Among individual heavy metals, Pb (hazard category 1), Zn (hazard category 1), Cu (hazard category 2), and As (hazard category 1) were especially pronounced. The levels of organic pollutants did not exceed the established Russian standards. The concentration of oil products was below the background level of 180 mg/kg in all four study sites. Site 4 is located on the outskirts of an existing landfill, storing industrial waste. The landfill itself is a source of extreme environmental hazard, as revealed by a previous chemical study of the area: the landfill soil had high level of total heavy metal pollution (Zc = 607) (Bardina et al. 2014a, b, c). Aside from this, complex biochemical processes of waste decomposition take place over long periods of time, accompanied by formation of toxic organic and inorganic compounds. As a result, specific anthropogenic soils, sometimes extremely hazardous and toxic, are formed in the landfill body. Strong contamination of the surrounding territories (exemplified by site 4) happens due to sliding of the substrate from the landfill and, perhaps, other migration mechanisms.

Thus, chemical testing revealed strong heavy metal pollution and mild organic pollution at site 1, no total contamination by heavy metals at sites 2 and 3 (although individual element might have exceeded the limits) and significant presence of organic toxicants at site 3, and notable contamination with heavy metals but no organic pollution at site 4. Pb, Zn, Cr, Cu, Mn, and V demonstrated the most significant excess over their regional geochemical baseline at all sites.

3.2 Ecotoxicological Evaluation of Soils with Eluate Tests

The choice of an appropriate test organism for a bioassay, and the resulting usefulness of the procedure, largely depend on the chemical composition of

Table 1 Concentrations of organic and inorganic contaminats in the soils

Site	Element content, mg/kg							
	Hg	Pb	Cd	Zn	Ni	Co	Cr	Cu
1	0.09 ± 0.01a	271.1 ± 28.1a	7.31 ± 1.24a	406.2 ± 41.0a	18.1 ± 1.8a	8.73 ± 0.91a	44.1 ± 4.6a	112.5 ± 16.8a
2	0.04 ± 0.01b	61.7 ± 6.0b	0.9 ± 0.1b	93.1 ± 11.1b	9.5 ± 1.0b	4.18 ± 0.42b	31.6 ± 3.3b	23.5 ± 2.4b
3	0.06 ± 0.01 b	7.4 ± 0.8c	0.17 ± 0.02c	100.4 ± 12.0b	14.2 ± 1.4c	7.64 ± 0.77a	42.1 ± 4.3a	19.0 ± 1.7b
4	< 0.01c	85.1 ± 9.3d	0.35 ± 0.05d	214.9 ± 25.0c	4.83 ± 0.05d	11.8 ± 1.12 ac	18.7 ± 2.0c	87.2 ± 7.8a
Hazard category	1	1	1	1	2	2	2	2
MPC	2.1	32	–	–	–	–	–	–
TAC	–	–	0.5	55	20	–	–	33
Regional background level	0.03	19.11	0.17	43.10	15.30	4.10	12.50	18.0

Site	Element content, mg/kg			Zc	Organic substance content, mg/kg		
	Mn	V	As		3,4 Benzo(a)pyrene	Polychlorinated biphenyls	Oil products
1	519 ± 48.2a	36.4 ± 2.9a	2.77 ± 0.06a	65.5	< 0.001	0.160	50.0
2	333 ± 23.2b	31.6 ± 2.5a	2.11 ± 0.04b	7.4	< 0.001	0.072	158.0
3	630 ± 48.3c	44.0 ± 3.9b	4.46 ± 0.09c	7.3	0.085	0.200	110.0
4	170 ± 9.8d	15.6 ± 1.4c	52.6 ± 1.2d	29.9	< 0.005	< 0.001	74.2
Hazard category	3	3	1	NA	1	–	–
MPC	1500	150	2	NA	0.02	0.06	
TAC	–	–	–	NA	–	–	–
Regional background level	117.7	16.2	2.62		–	–	180.0

Data are given as an average value ± standard error. Different letters represent significant differences between samples on rows (LSD test, $p \leq 5$)

Zc total contamination index, *MPC* maximum permissible concentration, *TAC* tentative allowable concentration, *NA* not applicable

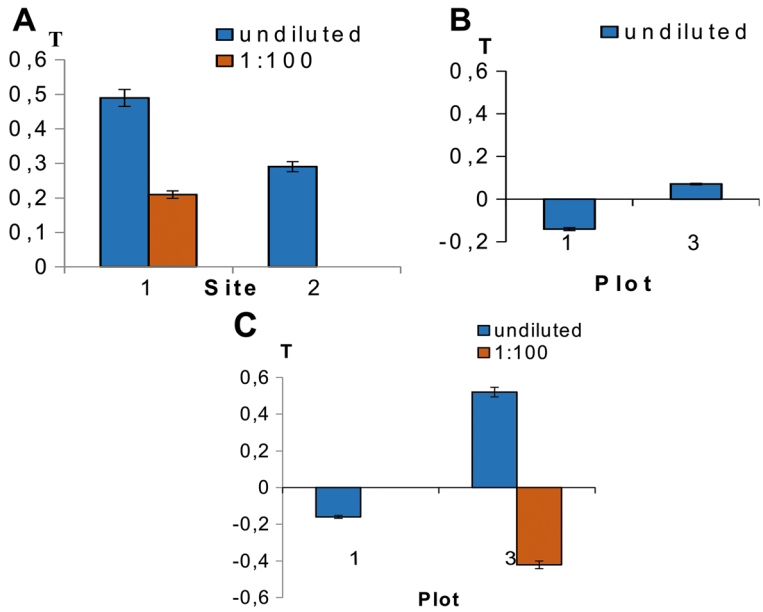

Fig. 2 Toxicity index of soil extracts, measured with *Paramecium caudatum* biotest. **a** Sites 1 and 2 (depth 0–20 cm). **b** Site 3 (depth 0–5 cm). **c** Site 3 (depth 5–20 cm)

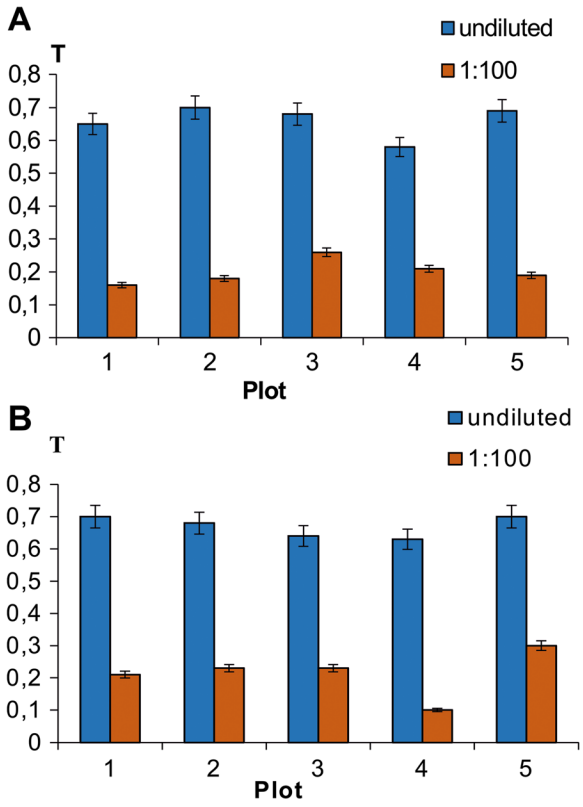

Fig. 3 Toxicity index of soil extracts from site 4, measured with *Paramecium caudatum* biotest. **a** Samples from depth 0–5 cm. **b** Samples from 5 to 20 cm

the studied samples (Filenko and Terekhova 2016). Thus, we kept in mind the varying sensitivity of hydrobionts while choosing the organisms used in the tests. One of the most common species, used to evaluate aqueous solutions is a crustacean *Daphnia magna straus* 1820. They are easily cultured and demonstrate high sensitivity to various pollutants (Terekhova 2011). Unfortunately, these animals are best used for testing the solutions with pH about 7.0–8.3 (ISO 6341: 2012). Since soil samples from site 4 were overly acidic, we could not employ *Daphnia* directly. Using additional substances to bring the pH to the recommended range was also deemed undesirable, as that would necessarily affect the chemical composition of the sample (Terekhova 2011). Therefore, we chose other test species: *P. caudatum, S. quadricauda* and *A. sativa* L.

Results of bioassay with *P. caudatum* as test organism are given in Figs. 2 and 3. Soil extracts from site 1 were moderately toxic (effect disappeared at ×100 dilution). Samples from site 2 (Fig. 2a) and the upper soil horizon (0–5 cm) of site 3 (Fig. 2b) were not toxic to ciliates. Samples from 5 to 20 cm were found toxic, with negative effect on ciliates disappearing only at ×100 dilution (Fig. 2c). Finally, the soils at site 4 also had pronounced toxic effect, which disappeared only at ×100 dilution (Fig. 3). Biotests using *P. caudatum* were among the quickest and least expensive, although very sensitive to heavy metal contamination (Terekhova 2011).

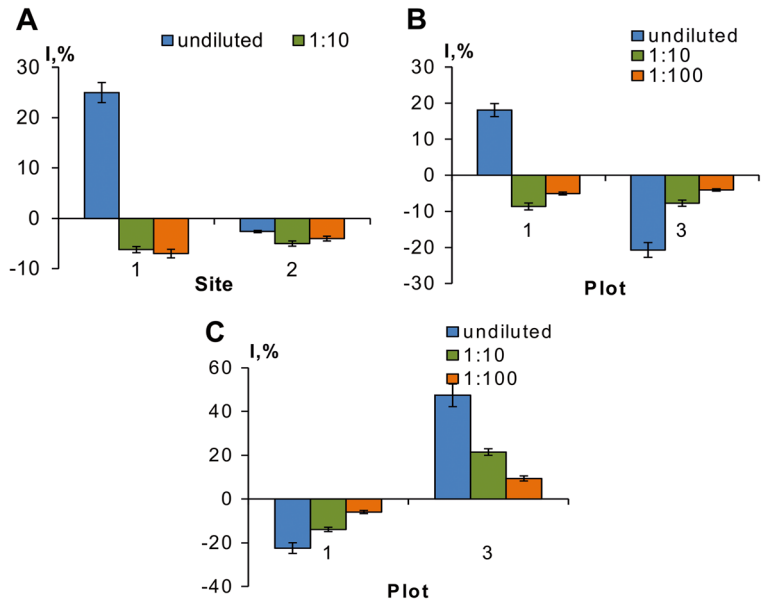

Fig. 4 Growth inhibition (*I*) of green algae *Scenedesmus quadricauda*. **a** Samples from sites 1 and 2 (0–20 cm). **b** Samples from site 3 (0–5 cm). **c** Samples from site 3 (5–20 cm)

The results of biotesting of soil extracts using green algae *S. quadricauda* are presented on Figs. 4 and 5. The sample from site 1 had low toxicity, which disappeared at ×10 dilution (Fig. 4a). Soil from site 2 was non-toxic, while site 3 had mixed status. Samples from plot 1 were not toxic to algae, while plot 2 demonstrated no effect at depths 0–5 cm (Fig. 4b) and a pronounced effect at greater depths (5–20 cm), disappearing only with ×100 dilution (Fig. 4c).

At site 4, all of the examined soil extracts had an acute toxic effect on *Scenedesmus;* there were no differences between the toxicity of the horizons 0–5 cm (Fig. 5a) and 5–20 cm (Fig. 5b) (p values > 0.1). Both undiluted and diluted 10 times eluates from plots 1, 2 and 3 completely suppressed algal growth ($I = 100\%$). With a further dilution of extracts (×100) toxic effect disappeared. At site 4, soil extracts from plot 4 had the least inhibitory effect on the algae: undiluted eluate suppressed population growth by 27%, and the toxic effect disappeared at ×10 dilution. Finally, extracts from plot 5 had notable toxic effect: undiluted eluate completely inhibited the growth of algae, and the toxic effect was still present in ×10 dilution (but not in ×100).

Biotests with seeds of common oat *A. sativa* L revealed no toxic effect at sites 1 and 2 (Table 2), while sites 3 and 4 had reduced the root growth in seedlings above the critical level. However, no dramatic effects, such as complete suppression of germination and growth, were observed in any of the samples. These results indicate that, at the studied sites at least, oat is

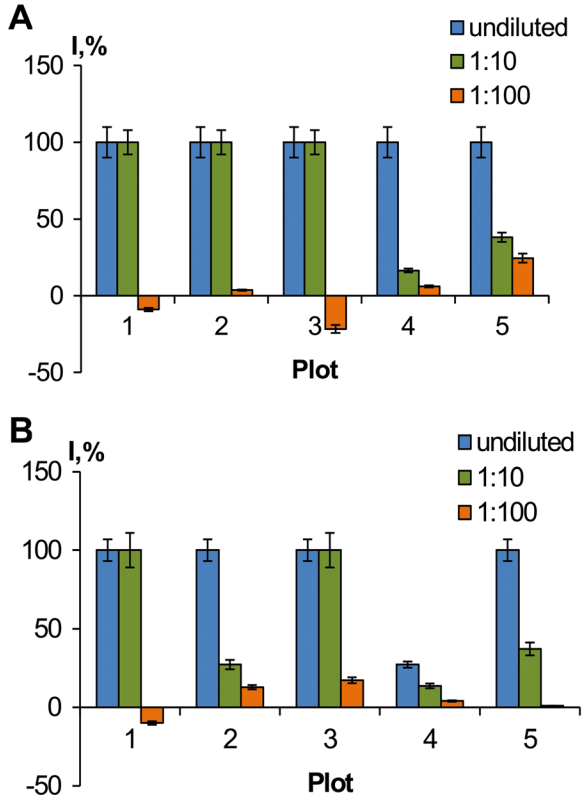

Fig. 5 Growth inhibition (*I*) of green algae *Scenedesmus quadricauda* in soil extracts from site 4. **a** Samples from depth 0–5 cm. **b** Samples from 5 to 20 cm

Table 2 Results of eluate biotest with the seeds of *Avena sativa* L.

Plot	Sampling depth, cm	Root length, mm	Toxic effect, E_t %	Reaction of the test organisms
Site 1				
Control	0–20	50.1 ± 2.7a	–	No reaction
1	0–20	59.6 ± 2.7b	+18.9	No reaction
Site 2				
Control	0–20	50.1 ± 2.7a	–	No reaction
1	0–20	53.4 ± 6.6a	+6.6	No reaction
Site 3				
Control	0–20	72.8 ± 0.6c	–	No reaction
1	0–5	51.8 ± 6.9d	−28.8	Growth supression
	5–20	64.4 ± 5.4c	−11.5	No reaction
2	0–5	74.4 ± 2.6c	+2.6	No reaction
	5–20	52.5 ± 10.1d	−27.9	Growth supression
Site 4				
Control	0–20	46.1 ± 3.7e	–	No reaction
1	0–5	26.7 ± 0.1f	42.1	Growth supression
	5–20	20.4 ± 0.6g	55.7	Growth supression
2	0–5	14.8 ± 0.2j	67.9	Growth supression
	5–20	22.7 ± 0.8g	50.8	Growth supression
3	0–5	16.3 ± 1.6j	64.6	Growth supression
	5–20	20.2 ± 1.9g	56.2	Growth supression
4	0–5	34.1 ± 5.7e	26.0	Growth supression
	5–20	38.3 ± 4.4e	16.9	No reaction
5	0–5	12.1 ± 3.6j	73.8	Growth supression
	5–20	18.5 ± 1.8 g	59.9	Growth supression

Data are given as average ± standard error. Different letters represent significant differences between samples on plots (LSD test, $p \leq 5$)

more resistant to contamination than ciliates and algae.

The highest toxicity was seen at site 4 and the lowest at site 2 (Table 5). Results of the ciliate and algae bioassays correspond with the results of chemical testing for contamination with heavy metals: both methods revealed sites 1 and 4 as polluted, although the reported severity of danger varied. Such partial agreement supports the established view on ciliate sensitivity to heavy metals (Voronich et al. 2016; Bardina et al. 2014a, b, c). However, chemical test results not always got in line with results of bioassays: mass spectrometry showed the site 1 as the most polluted by heavy metals, while bioassays consistently indicated the site 4 as the most hazardous. This may be due to an acidic environment at site 4 and high lability of heavy metals in acidic conditions.

Higher toxicity of soil samples from greater depth (5–20 cm vs 0–5 cm) at site 3 may be explained by downward migration of soluble forms of toxicants happening alongside water infiltration. Generalized relative sensitivity of the three eluate biotests may be summarized as: *P. caudatum* = *S. quadricauda* > *A. sativa.*

3.3 Ecotoxicological Evaluation of Soils with Contact Bioassay

We performed two types of contact biotests, using grains of *Trinicum aestivum* and natural microbial community.

Results of the bioassays with common wheat as test organism are given in Table 3. At site 1, we found moderate degree of toxicity; at site 2, the measured toxicity was low. The soils from site 3 were not toxic to wheat seeds. Indeed, our previous results indicate that at least 1 mg/kg of benzo(a)pyrene is needed to elicit a response (Bardina et al. 2016).

Table 3 Results of contact bioassay with seeds of *Triticum aestivum* L.

Plot	Sampling depth, cm	Germination rate, %	Germination rate (N_1)	Root length, mm	Root length (N_2)	Degree of toxicity
Site 1						
Control	0–20	83.3 ± 3.3a	–	25.4 ± 1.5a	–	–
1	0–20	60.0 ± 2.1b	−27.9	27.5 ± 1.8a	+8.3	III
Site 2						
Control	0–20	83.3 ± 3.3a	–	25.4 ± 1.5a	–	–
1	0–20	81.7 ± 3.0a	−1.9	18.1 ± 0.8b	−28.7	IV
Site 3						
Control	0–20	95.5 ± 3.5c	–	26.5 ± 1.0c	–	–
1	0–5	90.0 ± 3.5c	−5.3	32.2 ± 4.6c	+21.4	V
	5–20	95.0 ± 0c	0	46.3 ± 3.1d	+74.4	V
h2	0–5	87.5 ± 3.3c	−7.9	40.8 ± 3.4d	+53.6	V
	5–20	87.5 ± 1.8d	−7.9	43.0 ± 3.2d	+62.1	V
Site 4						
Control	0–20	87.5 ± 1.8d	–	19.7 ± 0.5e	–	–
1	0–5	32.5 ± 5.3e	−62.9	1.9 ± 0.4f	−90.4	II
	5–20	0.5 ± 0.4f	−99.4	0.5 ± 0.4g	−97.5	II
2	0–5	30.0 ± 3.5e	−65.7	2.3 ± 0.9f	−88.3	II
	5–20	67.5 ± 8.8g	−22.9	3.0 ± 0.5f	−84.8	II
3	0–5	52.5 ± 5.3h	−40.0	4.4 ± 0.1h	−77.7	II
	5–20	55.0 ± 7.1gh	−37.1	4.2 ± 0.1h	−78.7	II
4	0–5	75.0 ± 3.5g	−14.3	9.2 ± 0.1i	−44.2	IV
	5–20	95.0 ± 3.5i	+8.6	10.4 ± 1.4i	−47.9	IV
5	0–5	17.5 ± 1.8j	−80.0	2.4 ± 0.8f	−87.8	II
	5–20	32.5 ± 5.3e	−62.8	2.3 ± 0.2f	−88.3	II

Data are given as average ± standard error; degrees of sample toxicity are as follows: II—highly toxic, III—moderately toxic, IV—slightly toxic, and V—practically non-toxic. Different letters represent significant differences between samples on plots (LSD-test, $p \leq 5$)

Finally, most plots from site 4 had a dangerous level of toxicity. The single exception was plot 4, where the degree of phytotoxicity was low. Presumably, it is a result of uneven secondary contamination of the lands around the industrial waste dump at the heart of site 4. Acidic reaction of the surroundings facilitates the transition of the total forms of heavy metals into active forms, thus increasing phytotoxicity of the soil. Our earlier studies also found direct correlation between the response of *T. aestivum* and concentration of heavy metals (Bardina et al. 2014a, b, c). The most sensitive parameter, measured here as well as in other studies, was the root length, while the overall rate of germination was less affected (Isak et al. 2013; Terekhova et al. 2016).

Table 4 presents the results of microbial contact bioassay. The respiratory activity of microorganisms from most samples was significantly lower than the baseline. At sites 2, 3 (plot 1), and 4, the respiration rate was 30–70% less than a threshold value. It is known, that a critical level of soil system stability is the loss of not more than 30% of microbial biological activity (Yakovlev and Evdokimova 2011). Thus, we found a widespread and significant degradation of microbial communities in the studied soils, which, in turn, indicates low resistance of the soils as a whole to the toxic effects of pollutants. At site 3 (plot 2), the rate of reduction in biological activity did not exceed 30%; thus, degradation of microbial communities at this location may yet be reversible. However, site 1 is the most notable exception. At this site, we found no

Table 4 Respiration rate of soil microbial communities

Plot	Sampling depth, cm	Soil respiration rate, mg CO_2/100 g/day	Soil respiration (sample vs control), %
Site 1			
Control	0–20	29.4 ± 0.7a	–
1	0–20	30.2 ± 1.9a	+2.7
		Site 2	
Control	0–20	31.0 ± 1.6b	–
1	0–20	10.7 ± 0.6c	−65.5
		Site 3	
Control	0–5	10.1 ± 1.0d	–
	5–20	8.0 ± 0.4e	–
1	0–5	6.3 ± 0.4f	−37.6
	5–20	5.5 ± 0.1g	−31.3
2	0–5	7.5 ± 0e	−25.7
	5–20	6.1 ± 0.2f	−23.8
		Site 4	
Control	0–5	23.2 ± 0.9h	
	5–20	16.4 ± 1.5i	
1	0–5	8.8 ± 0.1j	−62.1
	5–20	6.6 ± 0.4k	−59.8
2	0–5	8.6 ± 0.5j	−62.9
	5–20	7.1 ± 0.1k	−56.7
3	0–5	12.5 ± 0.1e	−46.1
	5–20	20.3 ± 0.7h	−58.7
4	0–5	8.7 ± 0j	−62.5
	5–20	30.8 ± 1.8m	−37.4
5	0–5	14.4 ± 0.8i	−37.9
	5–20	11.6 ± 0.5e	−29.3

Data are given as average ± standard error. Different letters represent significant differences between samples on plots (LSD test, $p \leq 5$)

differences in biological activity of microbial communities of the control and test sample. We believe that the microorganisms at this locality are resistant to the existing toxicity (although chemical testing found this site as the most polluted with heavy metals). This apparent contradiction is resolved by noting that in a polluted locality which retains its vegetation cover, heavy metals lose their mobility and even enhance soil respiration and CO_2 production (Vodyanitskii 2013).

Results of two contact bioassays mostly agree with each other (Table 5). The two exceptions were sites 1 and 3. Soil sample from site 1 was moderately toxic to wheat but not toxic for microbial community. The latter may be caused by adaptation of the microorganisms to long-term contamination. Soils from site 3 demonstrated toxicity for microbial community but were not toxic for wheat seeds. This is consistent with our previous experiments, where response of wheat to pollution with benzo(a)pyrene was observed only when the content of this pollutant in loamy soils exceeded 50 MPC (Bardina et al. 2016). On the other hand, wheat is strongly sensitive to heavy metals (Bardina et al. 2014a, b, c), which is seen in complete agreement between estimates given by this test organism (Table 5) and chemical testing (Table 1).

At some sites, contact bioassay methods were found to be more sensitive to the presence of toxicants than eluate bioassays. For instance, soil toxicity at site 2 was found by contact methods, while eluate bioassays did not detect the toxicants (probably organic in nature, as indicated by chemical tests). Notwithstanding some differences in sensitivity, however, all the bioassays revealed a dangerous level of toxicity at site 4.

Table 5 Integrated estimates of the soil toxicity (across all employed biotests)

Plot	Sampling depth, cm	Eluate bioassay			Contact bioassay	
		Paramecium caudatum	*Scenedesmus quadricauda*	*Avena sativa*	*Triticum aestivum*	microbial community
Site1						
1	0–20	Moderately toxic	Mildly toxic	Non-toxic	Moderately toxic	Non-toxic
Site 2						
1	0–20	Non-toxic	Non-toxic	Non-toxic	Mildly toxic	Toxic
Site 3						
1	0–5	Non-toxic	Non-toxic	Toxic	Non-toxic	Toxic
	5–20	Non-toxic	Non-toxic	Non-toxic	Non-toxic	Toxic
2	0–5	Non-toxic	Non-toxic	Non-toxic	Non-toxic	Toxic
	5–20	Moderately toxic	Toxic	Toxic	Non-toxic	Toxic
Site 4						
1	0–5	Moderately toxic	Toxic	Toxic	Highly toxic	Toxic
	5–20	Moderately toxic	Toxic	Toxic	Highly toxic	Toxic
2	0–5	Moderately toxic	Toxic	Toxic	Highly toxic	Toxic
	5–20	Moderately toxic	Toxic	toxic	Highly toxic	Toxic
3	0–5	Toxic	Toxic	Toxic	Highly toxic	Toxic
	5–20	Toxic	Toxic	Toxic	Highly toxic	Toxic
4	0–5	Toxic	Mildly toxic	Toxic	Mildly toxic	Toxic
	5–20	Toxic	Mildly toxic	Non-toxic	Mildly toxic	Toxic
5	0–5	Toxic	Toxic	Toxic	Highly toxic	Toxic
	5–20	Toxic	Toxic	Toxic	Highly toxic	Toxic

4 Conclusions

The present study is focused on ecological evaluation of the brownfields using not only chemical, but also biologial testing methods. Chemical examination revealed that sited differed in pH reaction and identified heavy metals as primary pollutants. Various bioassay methods were more sensitive to the presence of toxicants than the methods of chemical analysis. In accord with previous publications, we found that reaction of different biotest systems depended on the chemical composition of the pollutants (Ram et al. 2004; Canna/Michaelidou et al. 2000). Various test species have unequal sensitivity to the wide range of toxicants. Aquatic organisms (protozoa and algae), used in eluate bioassay, were more sensitive than the seeds of higher plant. Both of the contact bioassays were sensitive to pollutants, since toxic effect of several samples was revealed more clearly with these methods than that with either chemical testing or eluate bioassays. In this study, we did not reveal any test method as superfluous: the wider the list of used methods, the larger the scope and reliability of pollution detection. Finally, we found that both eluate and contact bioassays are well-suited and should be used together for ecotoxicological assessment of brownfield soils. The latter finding agrees with the results for other types of objects (Terekhova 2011; Filenko and Terekhova 2016). We believe that compilation of a database of bioassay systems, suitable for testing brownfield soils, is an important step in remediation of these territories.

Acknowledgements The present study was supported by state research topic of SRCES RAS No. 012013600650.

Compliance with Ethical Standards The authors declare that they have no conflict of interest.

References

Ahtiainen, J., Valo, R., Järvinen, M., & Joutti, A. (2002). Microbial toxicity tests and chemical analysis as monitoring parameters at composting of creosote-contaminated soil. *Ecotoxicology and Environmental Safety, 53*, 323–329.

Alef, K. (1995). Soil respiration. In K. Alef & P. Nannipieri (Eds.), *Methods in applied soil microbiology and biochemistry* (pp. 214–219). London: Academic Press, Harcourt Brace & Company.

Alvarenga, P., Palma, P., de Varennes, A., & Cunha-Queda, A. C. (2012). A contribution towards the risk assessment of soils from the São Domingos Mine (Portugal): chemical, microbial and ecotoxicological indicators. *Environmental Pollution, 161*, 50–56.

Anderson, N.-H., Heinemeyer, O., & Weigel, H.-J. (2011). Changes in the fungal-to-bacterial respiratorial ratio and microbial biomass in agriculturally managed soils under free-air CO_2 enrichment (FACE)—a six-year survey of field study. *Soil Biology and Biochemistry, 43*, 895–904.

Baran, A., Czech, T., & Wieczorek, J. (2014). Chemical properties and toxicity of soils contaminated by mining activity. *Ecotoxicology, 23*, 1234–1244.

Bardina, T. V., Chugunova, M. V., Pnd, L. P., & Bardina, V. I. (2014a). Biological assesment of city soils toxicity in soil—ecological monitoring. *Ekology of Urban Areas, 2*, 87–91 (in Russian).

Bardina, T. V., Chugunova, M. V., Kulibaba, V. V., & Bardina, V. I. (2014b). Evaluation of the ecological state of the past environmental damage objects soils with bio-testing methods. *Regional environmental issues, 5*, 37–42 (in Russian).

Bardina, T. V., Chugunova, M. V., Kulibaba, V. V., Bardina, V. I. The use of biological methods for control of soils at industrial waste landfill. International symposium: "Environmental and engineering aspects for sustainable living". Hannover, 27–28 November 2014. Hannover, pp 26-27. ISBN 978-3-00-032886-2. ewg-board.eu/daten/euro-eco-2014 2014c.

Bardina, T. V., Kulibaba, V. V., Chugunova, M. V., & Bardina, V. I. (2016). Ecotoxicity diagnostics of soils of the past environmental damage industrial facilities with the help of the biotesting systems. *Regional environmental issues, 2*, 20–25 (in Russian).

Broos, K., Mertens, J., & Smolders, E. (2005). Toxicity of heavy metals in soil assessed with various soil microbial and plant growth assays: a comparative study. *Environmental Toxicology and Chemistry, 24*(3), 634–640.

Canna/Michaelidou, Nicolaou, A. S., Neopfytou, E., & Christodoulidou, M. (2000). The use of a battery of microbiotests as a tool for integrated pollution control: evaluation and perspectives in Cyprus. In G. Persoone, C. Janssen, & W. De Coen (Eds.), *New microbiotests for routine toxicity screening and biomonitoring* (Vol. 4, pp. 39–48). New York: Kluwer Academic/Plenum Publishers.

Chapman Dagnino, A., Seforzini, S., Dondviero, F., Fenoglio, S., Bona, E., Jensen, J., & Viarengo, A. A. (2008). Weight-of evidence approach for the integration of environmental triad data to assess ecological risk and biological vulnerability. *Integrated Environmental Assessment and Manaement, 4*, 314–326.

Eriksen, K. D. H. (1990). Cytosolic binding of Cd, Zn, and Ni in four polychaete species. *Comparative Biochemistry and Physiology, 95*, 111–115.

Feng, D., Teng, Y., Wang, J., & Wu, J. (2016). The combined effect of Cu, Zn and Pb on enzyme activities in soil from the vicinity of a wellhead protection area. *Soil and Sediment Contamination: An International Journal, 25*(3). doi:10.1080/15320383.2016.1130687.

Filenko, O. F. (2007). Biologicheskie metody v kontrole kachestva okruzhayuschej sredy. *Ekologicheskie sistemy i pribory, 6*, 18–20 (in Russian).

Filenko, O. F., Terekhova, V. A. (2016). Environmental purpose of bioassay: informative value and versatility. Biodiagnostics and assessment of environmental quality: approaches, methods, criteria and reference standards in ecotoxicology. Book of Abstracts of the International Symposium, October 25–28. Moscow, Russia, GEOS 3:232–238.

Fomin, G. S., Fomin, A. G. (2001). Pochva. Control' kachestva i ekologicheskoj bezopasnosti po mezhdunarodnym standartam (p. 305). Moscow: Handbook.

Foucault, Y., Durand, M.-J., Tack, K., Schreck, E., Geret, F., Leveque, T., Pradère, P., Goix, S., & Dumat, C. (2013). Use of ecotoxicity test and ecoscores to improve the management of polluted soils: case of a secondary lead smelter plant. *Journal of Hazardous Materials, 246-247*, 291–299.

van Gestel, C. A., van der Waarde, J. J., Derksen, J. G., van der Hoek, E. E., Veul, M. F., Bouwens, S., Rusch, B., Kronenburg, R., & Stokman, G. N. (2001). The use of acute and chronic bioassays to determine the ecological risk and bioremediation efficiency of oil-polluted soils. *Environmental Toxicology and Chemistry, 20*, 1438–1449.

GN 2.1.7.2041-06. (2006) predelno dopustimye koncentracii PDK himicheskih veshchestv v pochve. Moscow (in Russian)

GN 2.1.7.2511-09. (2009). orientirovochno-dopustimye koncentracii ODK himicheskih veshchestv v pochve. Moscow (in Russian)

Grigoriev, Y. S., Tyutkova, E. A. (2011). Method for measuring the acute toxicity of freshwater, waste water, soil-water extracts, sewage sludge and waste extracts to algae Scenedesmus (*Scenedesmus quadricauda* (TURP.) BREB.) (p. 32). Moscow (PND FT 14,1:2:4.17-2011K; FR.1.39.2011.097150) (in Russian).

Hu, Y., Liu, X., Bai, J., Shih, K., Zeng, E. Y., & Cheng, H. (2013). Assessing heavy metal pollution in the surface soils of a region that had undergone three decades of intense industrialization and urbanization. *Environmental Science and Pollution Research, 20*, 6150–6159.

Isak, R. S., Parveen, R. S., Rafique, A. S., & Alamgir, A. S. (2013). Phytotoxic effects of heavy metals (Cr, cd, Mn and Zn) on wheat (*Triticum aestivum* L.) seed germination and seedlings growth in black cotton soil of Nanded, India. *Research Journal of Chemical Science, 3*(6), 14–23.

ISO 6341: 2012. (2012). *Water quality determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea) acute toxicity test*. London: British Standards Institution.

Kapelkina, L. P. (2013). Problems of the soil pollutants standartization, standardization and regulation of environmental and soils quality and land management. In S. A.

Shoba, A. S. Yakovlev, & N. G. Rybalsky (Eds.), *A corresponding member of the Russian Academy of Sciences* (pp. 196–201). Moscow: NIA-Priroda.

Kapelkina, L. P., Bardina, T. V., Bakina, L. G., Chugunova, M. V., Gerasimov, A. O., Mayachkina, N. V., Galdiyants, A. A. (2009). Metodika vypolneniya izmerenij vskhozhesti semyan i dliny kornej prorostkov vysshikh rastenij dlya opredeleniya toksichnosti tekhnogenno zagryaznennykh pochv. FR.1.39.2006.02264 (p 19). Sankt-Peterburg (in Russian).

Linkov, I., Sattestrom, F. K., Kiker, G., Batchelor, C., Bridges, T., & Ferguson, E. (2006). From comparative risk assessment to multi-criteria decision analysis and adaptive management: recent development and applications. *Environment International, 32*, 1072–1093.

Lors, C., Ponge, J.-F., Aldaya, M. M., & Damidot, D. (2011). Comparison of solid and liquid-phase bioassays using ecoscores to assess contaminated soils. *Environmental Pollution, 159*, 2974–2981.

Manzo, M., Nicola, F. D., Picione, F. D. L., Maisto, G., & Alfani, A. (2008). Assessment of the effects of soil PAH accumulation by a battery of ecotoxicological tests. *Chemosphere, 71*, 1937–1944.

Matejczyk, M., Grazyna, A. P., Nałecz-Jawecki, G., Ulfig, K., & Markowska-Szczupak, A. (2011). Estimation of the environmental risk posed by landfills using chemical, microbiological and ecotoxicological testing of leachates. *Chemosphere, 82*, 1017–1023.

Maxam, G., Rila, J.-P., Dott, W., & Eisentraeger, A. (2000). Use bioassays for assessment of water-extractable ecotoxic potential of soil. *Ecotoxicology and Environmental Safety, 45*, 240–246.

Minkina, T. M., Motusova, G. V., Nazarenko, O. G., & Mandzhieva, S. S. (2010). *Heavy metal compounds in soil: transformation upon soil pollution and ecological significance* (p. 188). New York: Nova Science Publishers, Inc.

MR. (2007). (Obosnovanie klassa opasnosti otkhodov proizvodstva i potrebleniya po fitotoksichnosti.) MR 2.1.7.2297–07. Moscow: Federal'nyj centr gigieny i ehpidemiologii Rospotrebnadzora, 2008 (in Russian).

Olkova, A. S. (2014). Biotestirovanie v nauchno-issledovatelskoj i prirodookhrannoj praktike Rossii. *Uspekhi sovremenno jbiologii, 134*(6), 614–622 (in Russian).

PND. (2010). Metodika opredeleniya toksichnosti prob pochv, donnykh otlozhenij i osadkov stochnykh vod ekspress-metodom s primeneniem pribora "Biotester". (FR.1.31.2005.01882 (red.2010) i PND F T 16.2:2.2–98 (red. 2010). Saint-Petersburg: OOO «SPEKTR-M» (in Russian).

Ram, C., Pandey Praveen, K., & Archana, S. (2004). Comparative toxicological evaluation of untreated and treated tannery effluent with Nostoc muscorum L. (algal assay) and microtox bioassay. *Environ. Monit. and. Assess., 95*(1–3), 287–294.

Remon, E., Bouchardon, J.-L., Cornier, B., Guy, B., Leclerc, J.-C., & Faure, O. (2005). Soil characteristics, heavy metal availability and vegetation recovery at a former metallurgical landfill: implications in risk assessment and site restoration. *Environmental Pollution, 137*, 316–323.

Ribe, V., Auleniusa, E., Nehrenheima, M., Martellb, U., & Odlarea, M. (2012). Applying the triad method in a risk assessment and metal industry site. J. *Hazardous Materials, 207–208*, 15–20.

Saet, Y. U. U., Revich, B. A., Yanin, E. P., & Dr, I. (1990). *Geohimiya okruzhayushchej sredy* (p. 335). Moscow: Nedra (in Russian).

SANPIN (2003). (Sanitarno-epidemiologicheskie trebovaniya k kachestvu pochvy.) SANPIN 2.1.7.1287-03 (p. 19). Moscow: Federal'nyj centr gigieny I ehpidemiologii Rospotrebnadzora, 2005 (in Russian).

Saraev, A. K., Simakov, A. E., Pitulko, V. M., Kulibaba, V. V., Tokarev, I. V., & Tezkan, B. (2015). Use of new radiomagnetotelluric sounding technique for inventory and assessment of buried objects of past environmental damage in soils and groundwater. *Regional Ecology, 1*(36), 7–11 (in Russian).

Selivanovskaya, S. Y., & Galitskaya, P. Y. (2006). Otsenka toksichnosti pochv s ispolzovaniem kontaktnogo metoda biotestirovaniya. *Toxicological Revien, 4*, 12–15 (in Russian).

Smagin, A. V. (2013). A resource characterization of soil and its relation to standardization and regulation of environmental quality. In S. A. Shoba, A. S. Yakovlev, & N. G. Rybalsky (Eds.), *A corresponding member of the Russian Academy of Sciences* (pp. 61–81). Moscow: NIA-Priroda.

Solomon, K. R., Brock, T. C. M., De Zwart, D. et al. (2008). Extrapolation in the context of criteria setting and risk assessment. In *Extrapolation Practice for ecotoxicological effect characterization of chemicals* (pp. 1–32). SETAC Press & CRC Press, Boca Raton.

Terekhova, V. A. (2011). Soil bioassay: problems and approaches. *Eurasian Soil Science, 44*, 173–179.

Terekhova, V. A., Pukalchik, M. A., & Yakovlev, A. S. (2014). The triad approach to ecological assessment of urban soils. *Eurasian Soil Science, 47*(9), 952–958.

Terekhova, V. A., Voronina, L. P., Nikolaeva, O. V., Bardina, T. V., Kalmatskaya, O. A., Kiryushina, A. P., Uchanov, P. V., Kreslavskij, V. D., & Vasileva, G. K. (2016). Primenenie fitotestirovaniya dlya resheniya zadach ekologicheskogo pochvovedeniya. *Bulletin «Use and protection of natural resources of Russia», 3*, 37–41 (in Russian).

Vodyanitskii, Y. N. (2013). Contamination of soils with heavy metals and metalloids and its ecological hazard (analytic review). *Eurasian Soil Science, 46*, 793–801.

Voronich, S. S., Grebenkin, N. N., Royeva, N. N., Zaitsev, D. A., Baranov, A. N., Pakhomov, D. E., & Khlopayev, A. G. (2016). A modern concept of the system of ecological monitoring of the industrial regions of Russia. *Regional environmental issues, 2*, 14–19 (in Russian).

Wang, X., Sun, C., Gao, S., Wang, L., & Shuokui, H. (2001). Validation of germination rate and root elongation to assess phytotoxicity with Cucumissativus. *Chemosphere, 44*, 1711–1721.

Wolterbeek, H. T., & Verburg, T. G. (2001). Predicting metal toxicity revisited: general properties vs. specific effects. *Sci Total Environ, 279*, 87–115.

Yakovlev, A. S., & Evdokimova, M. V. (2011). Ecological standardization of soil and soil quality control. *Eurasian Soil Science, 44*, 534–546.