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Applying Bioassays for Investigation of Soils from Suburban Green Sites

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Abstract **–** Urban green sites have many environmental and social benefits. As soils are key elements of these sites, investigation of their characteristics is highly recommended. In the present study, six different bioassay methods were used, together with physico-chemical and chemical measurements, to investigate the soil quality in suburban green sites in Budapest, Hungary. The bioassays were carried out using test species from different taxonomic groups: *Azomonas agilis* and *Pseudomonas fluorescens* (bacteria), *Sinapis alba* and *Lactuca sativa* (plants), *Folsomia candida* and *Eisena fetida* (invertebrates) were also used. All the performed bioassays showed some extent of toxicity due to the contact with certain soil samples, however the test organisms demonstrated varying sensitivity. According to the results, dehydrogenase activity of *P. fluorescens*, germination rates of the tested plants, and reproduction of invertebrates were the most sensitive endpoints. Toxicity of soil samples could be partly explained by its Cd, Cr and Pb content, since levels of these metals were far above the natural background. Our results encourage the need to investigate the soil quality in suburban green sites, as well as combining different bioassay methods during soil examinations.

Keywords**:** Bioassay, Urban soil, Green site, Heavy metal.

1. Introduction

According to numerous studies, human activities are strongly influence soil characteristics in urban areas [1-4]. Urban soils are usually having high bulk density, high pH and carbonate content, and often contain some pollutants, which pose potential risks to the environment [2-4]. One of the main contaminants are heavy metals, which can be accumulated at relatively high concentrations in topsoils [2,5,6]. Since the anthropogenic pollution of heavy metals is expected to decrease globally, urban soils can be a major source of secondary metal pollution [7].

In recent years, more and more study has been carried out to assess the soil quality in different cities. Most of them are mainly concentrated on soils influenced directly by human activities (e.g. in industrial areas, brownfields or near heavy traffic) or soils, which come easily in contact with humans (e.g. in parks or children's playgrounds) [5,8,9]. Thus we have little information about the soil characteristics of green sites in suburban areas. However, studying these soils are also an important issue, since green sites provide essential benefits to urban inhabitants (e.g air purification, water and climate regulation), and also offering habitat for terrestrial communities [10,11].

Many researchers stated that biological parameters should be taken into account during the evaluation of soil quality, besides the traditional chemical methods [12-15]. Soil bioassays are efficient methods to estimate the potential danger of different impacts on the soil [15]. Moreover, with the use of these tests, useful information can be obtained in connection with the potential environmental risks of contaminated soils [12,15]. For appropriate characterization of soils, it is recommended to use a number of taxonomically different test species, which play different roles in soil ecosystem [16,17]. Standardized methods of bacterial, plant and soil animal bioassays are also available in the literature.

The aim of the present study was to apply different bioassay methods for investigation of soils originated from various suburban green sites. Our objective was also to compare the usefulness and sensitivity of these methods. The main physico-chemical and chemical parameters, including heavy metal contents of soils, were also determined. The study was conducted in Budapest city (the capital of Hungary), which has been inhabited since the ancient age, and it has a long industrial history. Since human activities had influenced soil for centuries, this city was very suitable for our research. Budapest has an area of 525 km^2, but only about 16 % of them is green area. Due to this low rate, it is very important to preserve these green sites in good condition, which is not possible without examining soils.

2. Material and methods

The study was carried out at six green sites (covered with grass vegetation), which were located in a suburban area of Budapest (Figure 1). In each site four composite topsoil samples (containing at least 10 subsamples) were collected from the 0-20 cm soil layer. Before the examinations, soils were homogenised, air-dried and sieved (2-mm mesh). Soil texture, pH, $CaCO_3$ content, humus content, and water soluble salt content were determined according to Hungarian standard methods [18,19]. Trace metal (Cd, Co, Cu, Cr, Ni, Pb, Zn) concentration of soil samples were measured after HNO_3+H_2O_2 digestion by atomic absorption spectrometry.

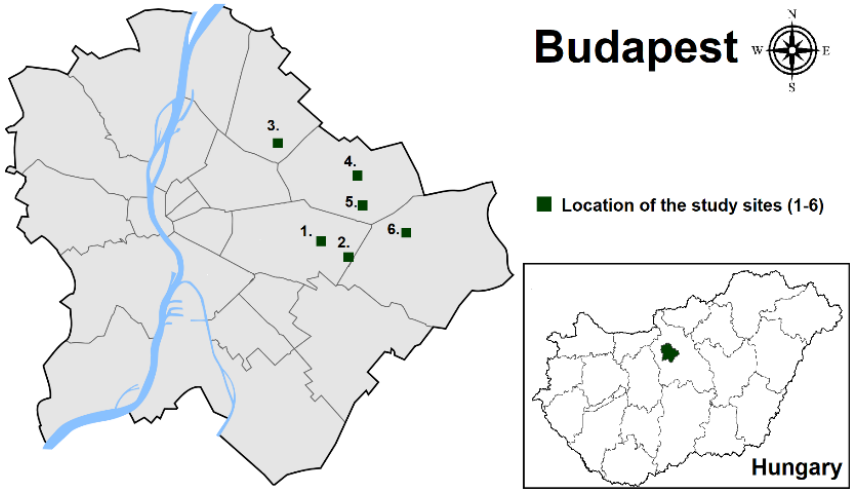

Fig. 1: Location of the study sites.

Applied bioassay methods were performed following previously published methods or OECD standards, with minor modifications (Table 1.).

Azomonas agilis bioassay: Dilution series (1 g, 0.5 g, 0.25 g, 0.125 g, 0.0625 g) from the soil samples were placed into test tubes and sterilized in autoclave. Then 2 ml stock solution was added to each test tube, which contained *A. agilis* suspension in liquid Fjodorov media and TTC (2,3,5-triphenyl-tetrazolium-chloride). After that, test tubes were homogenized and incubated in the dark at 28 ± 2 °C for 3 days. TTC is normally reducing to red-coloured triphenyl-formazan by microbial activity, however toxic substances in soils can inhibit this process. The inhibition was determined visually: no colour change mean 100 %, pink colour mean 50 %, while red colour mean 0 % inhibition. From these results IC_{50} values (concentration producing 50 % inhibition effect) were determined by a log-logistic dose-response model with GraphPad Prism 6 software.

Pseudomonas fluorescens bioassay: The test procedure was the same than with *A. agilis*. However, in this case, stock solution contained *P. fluorescens* suspension instead of *A. agilis*.

Plant bioassays: White mustard (*Sinapis alba*) and lettuce (*Lactuca sativa*) were used for plant assays. The tests were conducted by taking 30 g moistened soil into plastic pots (height: 40 mm, diameter: 120 mm). Then twenty-five seeds were placed into the soil surface, and the plots were incubated in the dark at 20 ± 2 °C. After 5 days germinated seeds were recorded, and the length of roots and shoots was measured with ruler.

Folsomia candida bioassay: Ten 9-12 days old juvenile springtails were transferred into the test vessels (275 ml) containing 30 g moistened soil samples. Then test vessels were kept for 4 weeks at 20 ± 2 °C temperature and 16/8 light/dark cycle. During this time, springtails were fed with granulated dried baker's yeast. At the end of the test, test vessels were flooded with distilled water and the floating adult and juvenile animals were counted.

Eisena fetida bioassay: Ten adult earthworms (between 0.3 and 0.6 g weight) were placed into test vessels (1000 ml), which contained 500 g moistened soil samples, and they were incubated for 4 weeks under the same condition than

in *F. candida* test. Earthworms were fed with oatmeal during the test. After 4 weeks the living adult worms are counted and weighted. After that, these earthworms were removed from soil, which is then incubated for 4 additional weeks. At the end of the second incubation, the number of juvenile animals were also counted.

For evaluation of the measured parameters in plant test, *F. candida* test and *E. fetida* test, artificial OECD soil (70 % % quartz sand, 20 % kaolinite clay and 10 % sphagnum moss, pH 6.0±0.5) was used as control soil. The results were interpreted by inhibition of samples (%) compared with the control.

Table 1: Summary of the applied bioassay methods.

Test organisms	**Endpoint of the test**	**Time**	**Interpretation of the results**	**Reference**
Azomonas agilis	dehydrogenase activity	3 days	IC_{50} value	[12,14]
Pseudomonas fluorescens				
Sinapis alba	germination, root and shoot elongation	5 days	Inhibition (%)	[20]
Lactuca sativa				
Folsomia candida	adult's survive and reproduction	4 weeks		[21]
Eisena fetida	adult's survive, adult's growth and reproduction	4 + 4 weeks		[22]

3. Results

3. 1. Physico-chemical and chemical measurements

The physico-chemical and chemical characteristics of the tested soils are shown on Table 2. Soil texture were clay loam or sandy clay loam in all sites, except for Site 6, where it was clay. Soil pH was close to neutral in all site. In Site 1 and 5 soils were weakly calcareous, while in the other sites they were moderately calcareous. Most of the soils contain relatively high amounts of humus, only soils from Site 2 and 3 could be categorized as moderately humus-rich. Water soluble salts content were varied between 0.04 % and 0.08 %, which values are fairly low.

Table 2: Main physico-chemical and chemical characteristics of the tested soils.

Parameter	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**
Texture	Sandy clay loam	Clay loam	Clay loam	Sandy clay loam	Sandy clay loam	Clay
pH	7.2	7.1	7.2	7.0	6.7	7.5
$CaCO_3$ content (%)	0.49	5.15	5.08	7.50	0.80	8.73
Humus content (%)	3.26	2.44	2.65	6.02	4.38	6.38
Water soluble salts content (%)	0.06	0.06	0.08	0.07	0.04	0.08
Cd content (mg kg^{-1})	1.54	1.62	2.74	2.71	2.33	1.92
Co content (mg kg^{-1})	7.67	9.20	14.56	14.1	14.49	8.68
Cr content (mg kg^{-1})	93.09	111.87	220.88	231.15	248.94	63.73
Cu content (mg kg^{-1})	38.63	28.96	29.77	31.64	29.94	37.37
Ni content (mg kg^{-1})	22.91	20.41	31.28	37.7	32.20	37.39
Pb content (mg kg^{-1})	173.00	198.88	292.80	209.5	220.69	209.98
Zn content (mg kg^{-1})	56.07	39.64	44.78	45.59	33.03	48.22

Trace metal contents of the tested soils were compared to the natural background values of Hungarian soils defined by [23]. Concentration of Cd, Cr and Pb were much higher than background values (0.5, 30 and 25 mg kg^{-1} respectively) in all soil, while Cu and Ni concentration were close to these values (30 and 25 mg kg^{-1}). In contrast, Co and Zn concentration of soils were below the background values (15 and 100 mg kg^{-1}).

3. 2. Bioassay methods

The results of the applied bioassays are summarized on Table 3. According to the results of bacterial bioassays, soil samples could be divided into 2 groups: samples from Site 1, 2 and 3 were more toxic to the dehydrogenase activity of bacterial test organisms than samples from Site 4, 5 and 6. Among the two test organisms, *P. fluorescens* was proved to be more sensitive than *A. agilis*, since IC_{50} values were less in its bioassay.

Results of plant bioassays showed, that soils from Site 1, 2 and 3 were also highly toxic to the germination and shoot elongation of *S. alba* and *L. sativa*. In addition to this, soils from Site 2 and 3 were also inhibited the root elongation of plants. Soils from Site 6 were also toxic, but only to the germination rate, while samples from Site 4 and 5 were not toxic to the test plants or the degree of their toxicity was low. Between the sensitivity of the two plant bioassay, there was no clear difference.

Interestingly, results of *F. candida* and *E. fetida* bioassays were not in correspondence with the previous results. In *F. candida* bioassay, the number of adult animals were much less in samples from Site 3 and 4 compared with control. Soils from Site 5 and 6 only moderately decreased the number of adults, the inhibition rates were 22.7 and 14.5 %. Soils from Site 1 and 2 not affect this parameter. Reproduction of *F. candida* was highly inhibited (more than 50 %) by samples from Site 3, 4, 5 and 6, while samples from Site 2 moderately inhibited it with an inhibition rate of 23.7 %.

The tested soils were not decreased remarkably the number of *E. fetida* adults, however in soils from Site 3, 5 and 6 adult's growth were slightly (between 13.3 and 17.0 %) inhibited. The reproduction of *E. fetida* were inhibited by all the tested soil samples. The most toxic samples to this parameter were originated from Site 5, the number of juveniles were decreased on average by 71 % in them compared with control.

Table 3: Effects of the tested soils on the test organisms in the applied bioassays.

Test organisms	**Endpoint of the test**	**Interpretation of the result**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**
Azomonas agilis	dehydrogenase activity	IC_{50}	0.83	0.84	0.73	1.17	1.37	1.09
Pseudomonas fluorescens			0.25	0.26	0.2	0.47	0.62	0.62
Sinapis alba	germination rate	Inhibition (%)	43.2	55.0	47.9	14.3	4.1	25.1
	shoot length		17.7	26.4	23.0	1.9	13.2	11.0
	root length		-4.3	41.7	25.7	-3.8	12.8	-1.8
Lactuca sativa	germination rate		42.2	57.8	50.6	17.7	4.8	37.1
	shoot length		30.4	47.8	24.1	13.7	17.5	6.5
	root length		8.9	35.4	22.3	0.2	11.3	-0.9
Folsomia candida	adult's survive		-7.3	8.2	62.7	42.7	22.7	14.5
	reproduction		-0.7	23.7	84.0	78.6	74.3	58.8
Eisena fetida	adult's survive		0.0	-2.6	7.9	5.3	5.3	2.6
	adult's growth		7.4	-4.1	13.3	-7.6	14.0	17.0
	reproduction		16.7	23.2	41.9	39.5	71.0	25.6

4. Conclusion

According to our results, general characteristics of urban soils (e.g. high pH, high carbonate content) were not observed observed in soil samples from suburban green sites. It was expected, since these sites had not been influenced directly by by human activities. On the contrary, trace metal concentrations of soils were far above the natural background levels in the in the case of Cd, Cr and Pb, which means that these metals are derived very likely from anthropogenic sources (e.g. atmospheric deposition and road traffic emissions) [5,6,24]. Cr is essential element for some physiological progress in low quantities, but Cd and Pb are without known biological function [6,25]. In excessive concentrations all three metal can be toxic to soil organisms [24,25].

Results of the bioassays indicated poor soil quality, since it showed that all tested soil had toxic effects on some test organisms, which could be partly explained by the high Cd, Cr and Pb content of samples. However, it is important to note, that other contaminants (which were not involved in chemical analyses) may also contribute to the toxicity of these soils. The sensitivity of the test organisms and the measured endpoints varied depending on the soil. It was concluded, that soil from Site 3 were toxic according to almost all the bioassays. Samples from Site 1 and 2 were more toxic to bacteria and plants, while samples from site 4, 5 and 6 were more toxic to the invertebrates. These differences between soil samples prove the need of using different test organisms in order to assess soil quality [12,15].

All in all, it was revealed, that soils from suburban green sites negatively affect various parameters of important soil organisms under laboratory conditions, which means that these soils may also harmful for terrestrial communities on sites. Therefore, in the future, much more attention should be given to the investigation of soil characteristics (especially biological parameters) in suburban green sites.

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