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Physico-Chemical and Ecotoxicological Characterizations of Suburban Soils

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Abstract *- The study aimed to evaluate the quality of some suburban soils in Budapest (Hungary) by integrating different physico-chemical and ecotoxicological approaches. Soils were sampled from six different sites and characterized for main soil properties (pH, $CaCO_3$, humus, and water-soluble salt contents) and total-extractable metal concentrations (Cd, Co, Cr, Cu, Ni, Pb, Zn). Ecotoxicological characterization was carried out using test species from different taxonomic groups: Azomonas agilis and Pseudomonas fluorescens (bacteria), Sinapis alba, and Lactuca sativa (plants), Folsomia candida, and Eisenia fetida (invertebrates). All ecotoxicological tests performed showed some degree of toxicity due to contact with certain soil samples, however, the test organisms showed different sensitivities. The results showed that the dehydrogenase activity of P. fluorescens, the germination rate of the studied plants, and the reproduction rate of the invertebrates were the most sensitive toxicological endpoints. Based on our correlation analysis, the outcome of ecotoxicological tests was influenced by the humus content and heavy metal concentration of the soil.*

Keywords: Ecotoxicology, Ecotoxicological tests, Heavy metals, Suburban soils, Urban soils

1. Introduction

According to various previous studies, human activities have a very strong influence on the soil characteristics of urban areas [1]–[4]. Urban soils generally have high bulk densities, high pH, and carbonate content, and often contain certain pollutants that pose potential risks to ecosystem members [2]-[4]. One of the main pollutants in these soils is heavy metals, which can be accumulated in topsoil at relatively high concentrations [2], [5], [6]. As anthropogenic pollution of heavy metals is not expected to decrease globally, urban soils may become major sources of secondary metal pollution [7].

In recent decades, many studies have been conducted to assess soil quality in different cities. Most of them focus on soils that are directly affected by human activity (e.g. soils in industrial or roadside areas), or on soils, which come easily in contact with humans, especially children's (e.g. soils in parks or playgrounds) [5], [8], [9]. However, there are only a few examples of studying suburban soils in the literature, so little data are available on the soil characteristics of these areas. Nevertheless, the study of these soils is also important, as they bring fundamental benefits to urban residents (e.g., air purification, water, and climate regulation) and provide habitat for terrestrial communities [10], [11].

Although traditional physico-chemical test methods are suitable for assessing soil quality, supplementing them with ecotoxicological tests can provide an even more accurate picture of the condition of soils [12]-[15]. These tests are primarily suitable for estimating the potential risks (especially toxicity) of various effects on soil and pollution [12], [15]. For proper assessment of soil quality, the use of several taxonomically distinct test species that play different roles in the soil ecosystem is recommended during the ecotoxicological tests [16], [17].

The main objective of our study was to assess the soil quality and toxicity of soils originated from various suburban green sites. To achieve this, we combined traditional physico-chemical methods and ecotoxicological tests. Another objective was to search for correlations between the results in order to identify

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which soil characteristics influence the outcome of ecotoxicological tests.

2. Material and methods

2.1. Study area

The study was conducted in the eastern part of Budapest (the capital of Hungary), which is located in Central Europe. The climate of the area is (humid) continental and the soils here are generally sandy soils. Budapest has been inhabited since the Roman age, and it has also a long industrial history. Due to the long-term human activities, the natural state of the soil is likely to have changed significantly. The city has an area of 525 km^2, however, only about 16 % of them are green areas, which are mainly located in the suburbs. Due to this relatively low rate, it is very important to preserve the green sites in good condition, which is not possible without monitoring suburban soils.

2.2. Study sites and soil sampling

The study was carried out at six sites, which were covered with grass vegetation (Figure 1.). All of them was park site or other recreational green areas. In each site, four composite samples were collected by mixing 10 subsamples taken randomly from the study site. All samples were originated from the upper layer of soil (0-20 cm).

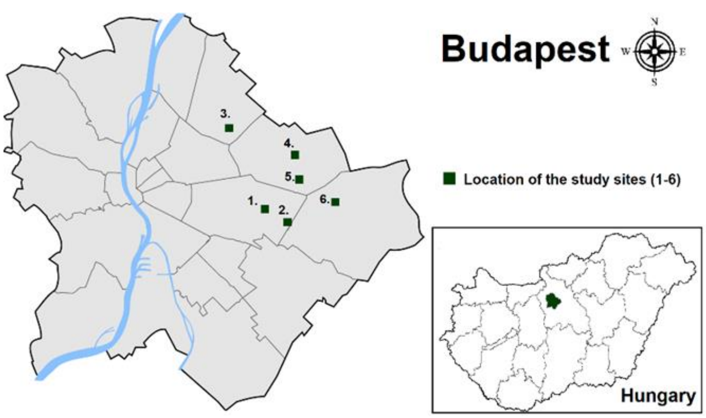

Figure 1. Location of the sampling sites.

2.3. Physico-chemical methods

All soil samples were homogenized, air dried, and sieved through a 2 mm diameter sieve before the examinations. Soil textures were identified by particle size distribution with a hydrometer. Soil pH was measured with a pH meter in an H_2O solution (1:2.5 soil/solution ratio), while the total salt (TS) contents were determined by a conductometer in another H_2O solution (1:5 soil/solution ratio). The humus (H_{ty}) contents were determined according to the Tyurin method, and the calcium carbonate ($CaCO_3$) contents were measured with a Scheibler calcimeter. Total-extractable metal (Cd, Co, Cu, Cr, Ni, Pb, Zn) concentrations were recorded by atomic absorption spectrometry (AAS) after HNO_3+H_2O_2 digestion.

2.4. Ecotoxicological methods

Six different test species were used during the ecotoxicological characterization of the soil samples: *Azomonas agilis* and *Pseudomonas fluorescens* (bacteria), *Sinapis alba,* and *Lactuca sativa* (plants), *Folsomia candida,* and *Eisenia fetida* (soil invertebrates). The two bacterial tests were performed using the methods of previous studies [12], [14]. Plant and invertebrate tests were based on various OECD guidelines [18], [19], [20]. For these tests standard OECD soil (consisting of 70 % quartz sand, 20 % kaolinite clay, and 10 % sphagnum moss) was applied as control soil. The main features of the ecotoxicological tests are shown in Table 1.

Table 1. Main features of the applied ecotoxicological tests.

Species	Endpoints	Time	Interpretation
A. agilis	dehydrogenase activity (DHA)	3 days	IC_{50} value*
P. fluorescens			
S. alba	germination rate, root elongation	5 days	Inhibition (% of control)
L. sativa			
F. candida	survival rate, reproduction rate	4 weeks	
E. fetida		8 weeks	

Note: **soil dose resulting in a 50 % inhibition effect of bacterial dehydrogenase enzyme activity (DHA)*

2.5. Statistical analyses of the results

Statistical analyses were performed using SPSS software. Kruskal-Wallis test ($p < 0.05$) was used to compare the results obtained from different sites. Spearman's correlation ($p < 0.05$) was used to determine if there was a correlation between physico-chemical and ecotoxicological data.

3. Results and discussion

3.1. Results of physico-chemical characterization

The texture of the soils was clay loam or sandy clay loam, except for Site 6, where it was clay. The other physico-chemical parameters are shown in Table 2. Soil pH was close to neutral at all sites, while TS contents were between 0.06 and 0.08 %, which values are relatively low. At sites 4 and 6, the amount of H_{ty} and

$CaCO_3$ in the soil was significantly higher than at some other sites. In general, the lowest heavy metal concentrations (excluding Cu and Zn) were observed at site 1, while the highest was observed at sites 3, 4, and 5. Cr concentrations at sites 3, 4, and 5 were significantly higher than at the other sites. And the Pb concentration was also significantly higher but only at site 3. Compared to previous studies, the concentrations of Cd, Cr, and Pb are relatively high in our soil samples [5], [6].

Table 2. Results of physico-chemical characterization of soil samples.

	Site number					
	1	**2**	**3**	**4**	**5**	**6**
pH	7.2 ±0.1 **AB**	7.1 ±0.4 **AB**	7.2 ±0.2 **AB**	7.0 ±0.1 **B**	6.7 ±0.2 **A**	7.5 ±0.1 **B**
TS* (%)	0.06 ±0.01 **A**	0.06 ±0.01 **A**	0.08 ±0.01 **A**	0.07 ±0.01 **A**	0.04 ±0.01 **A**	0.08 ±0.01 **A**
H_{ty}^+ (%)	3.3 ±0.5 **A**	2.4 ±0.3 **A**	2.7 ±0.5 **A**	6.0 ±0.4 **B**	4.4 ±0.5 **AB**	6.4 ±0.5 **B**
$CaCO_3$ (%)	0.5 ±0.5 **A**	5.2 ±1.2 **AB**	5.1 ±1.0 **AB**	7.5 ±0.6 **B**	0.8 ±0.6 **A**	8.7 ±1.1 **B**
Cd (mg kg^{-1})	1.5 ±0.3 **A**	1.6 ±0.4 **AB**	2.7 ±0.3 **B**	2.7 ±0.2 **B**	2.3 ±0.3 **AB**	1.9 ±0.3 **AB**
Co (mg kg^{-1})	7.7 ±0.9 **A**	9.2 ±0.8 **AB**	14.6 ±1.2 **AB**	14.1 ±0.4 **AB**	14.5 ±0.2 **B**	8.7 ±1.1 **AB**
Cr (mg kg^{-1})	93.1 ±6.1 **AB**	111.9 ±28.9 **B**	220.9 ±19.4 **C**	231.2 ±36.4 **C**	248.9 ±11.7 **C**	63.7 ±13.9 **A**
Cu (mg kg^{-1})	38.6 ±4.6 **A**	29.0 ±6.9 **AB**	29.8 ±1.8 **B**	31.6 ±1.6 **AB**	29.9 ±8.0 **AB**	37.4 ±3.0 **AB**
Ni (mg kg^{-1})	22.9 ±1.8 **A**	20.4 ±3.0 **A**	31.3 ±1.9 **AB**	37.7 ±3.0 **B**	32.2 ±2.8 **AB**	37.4 ±2.5 **B**
Pb (mg kg^{-1})	173.0 ±12.8 **A**	198.9 ±39.4 **AB**	292.8 ±31.5 **C**	210.0 ±29.2 **AB**	220.7 ±18.7 **B**	210.0 ±15.3 **AB**
Zn (mg kg^{-1})	56.1 ±13.1 **A**	39.6 ±5.7 **AB**	44.8 ±6.7 **AB**	45.6 ±5.9 **AB**	33.0 ±2.7 **B**	48.2 ±4.1 **A**

Data are shown as means ± sd (n=4). ***Notes***: **Total salt content, +Humus content. Different letters indicate significant differences between sites (Kruskal-Wallis test, $p < 0.05$).*

3.2. Results of ecotoxicological characterization

The results of the bacterial tests are shown in Figure 2. All samples were quite toxic to the test bacteria, even less than 1.5 g of soil inhibited the bacterial dehydrogenase enzyme activity by 50 %. Lower IC_{50} values were observed at sites 1, 2, and 3, however, there was no significant difference between sites according to the statistical analysis.

Among the two tests, the *P. fluorescens* test was found to be more sensitive than the *A. agilis* test, because then a lower soil dose caused the same inhibitory effect.

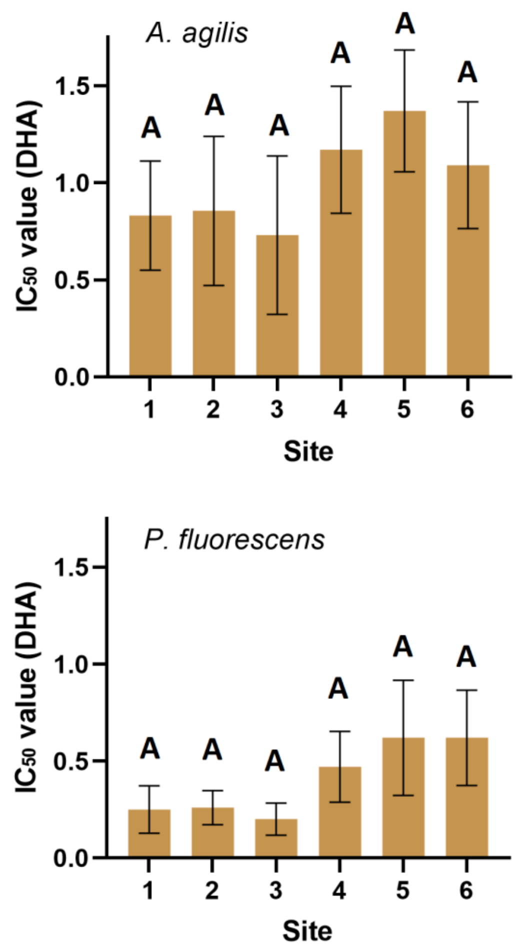

Figure 2. Results of the bacterial tests. ***Note***: *Different letters indicate significant differences between sites (Kruskal-Wallis test, $p < 0.05$).*

Results of plant bioassays showed that soils from Site 1, 2, and 3 were also highly toxic to the germination of the test plants (Figure 3.). These soils reduced the germination rate of the plants by about 50 % compared to the control. In contrast, the sample from site 5 was significantly less toxic to plants, decreasing plant germination by only about 5 %. The sample from sites 4 and 6 reduced this parameter by about 30 %.

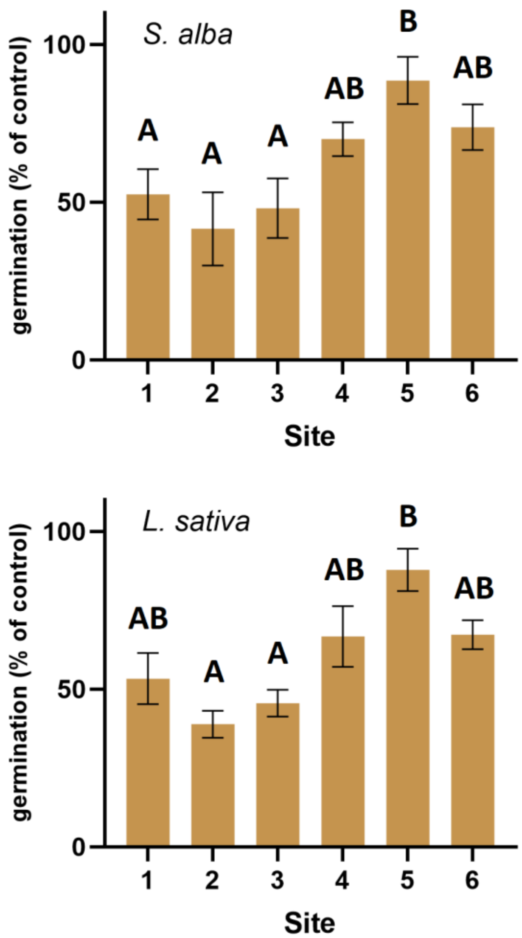

Figure 3. Germination of the test plants ***Note****: Different letters indicate significant differences between sites (Kruskal-Wallis test, $p < 0.05$).*

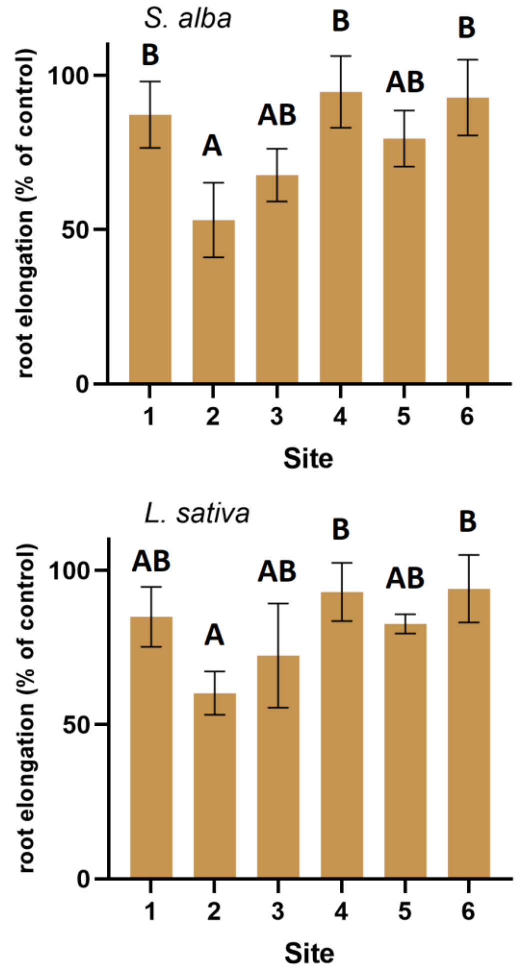

Figure 4. Root elongation of the test plants. ***Note****: Different letters indicate significant differences between sites (Kruskal-Wallis test, $p < 0.05$).*

The soil sample from site 2 was the most toxic to the root elongation of the test plants, reducing this parameter by about 40 % (Figure 4.). Soils from sites 4 and 6, on the other hand, did not or only slightly inhibit root elongation. The other samples caused between 10 % and 30 % inhibition.

Overall, there was no clear difference between the sensitivities of the two plant tests for either germination or root elongation.

Interestingly, the results of invertebrate tests were inconsistent with the bacterial and plant tests. In the *F. candida* test, the sample from site 3 proved to be the most toxic in terms of animal survival, killing more than 70% of the test animals compared to the control (Figure 5.). Soil from sites 4 and 5 killed nearly 50 %, while soil from site 6 nearly 40 % of *F. candida* individuals. The soil sample from sites 1 and 2 was significantly less toxic in this regard, killing less than 20 % of the test animals.

In contrast to the *F. candida* test, in the *E. fetida* test, soil samples did not have a large adverse effect on the survival of individuals. They decreased this parameter by less than 10 % compared to the control. Among the samples from different sites, there were no significant differences.

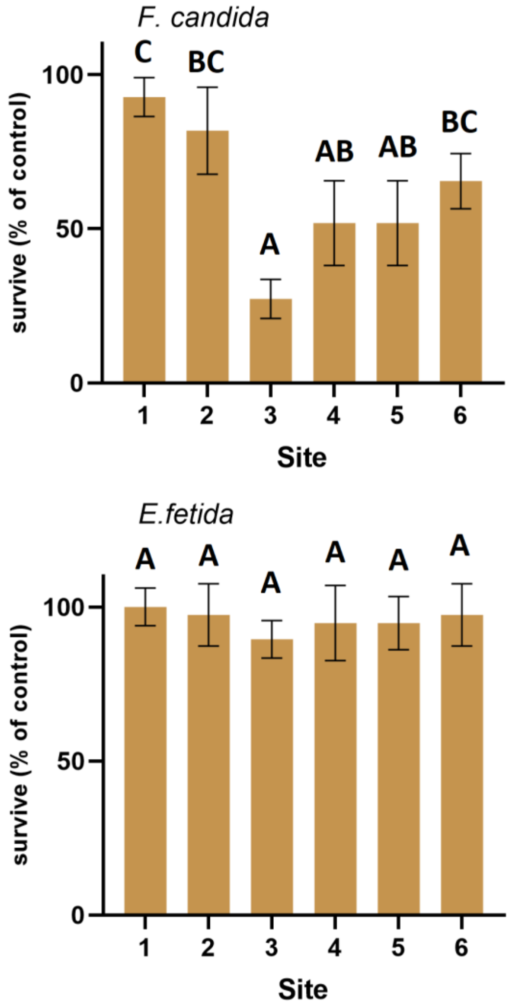

Figure 5. Survival of the test invertebrates. ***Note****: Different letters indicate significant differences between sites (Kruskal-Wallis test, $p < 0.05$).*

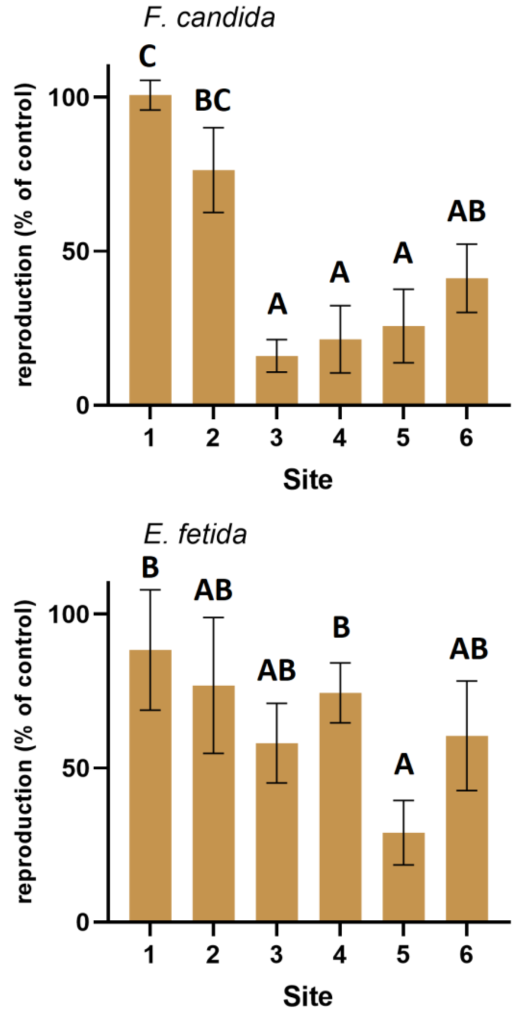

Figure 6. Reproduction of the test invertebrates. ***Note****: Different letters indicate significant differences between sites (Kruskal-Wallis test, $p < 0.05$).*

Reproduction was found to be a more sensitive endpoint than survival in both invertebrate tests (Figure 6.). Soil from sites 3, 4, and 5 reduced *F. candida* reproduction rate by about 80 %, while soil from site 6 also reduced by nearly 60 %. Samples from sites 1 and 2 did not prove to be very toxic in this respect either.

The soil sample from site 5 was also highly toxic to the reproduction rate of *E. fetida*, decreasing it by more than 70 %. This is significantly more than the result obtained for the sample from sites 1 and 4. The other five samples caused between 15 % and 40 % inhibition in reproduction.

Comparing the two invertebrate tests, it can be concluded that the *F. candida* test was more sensitive.

Results of the ecotoxicological characterization indicated poor soil quality since it showed that all tested soil samples had some degree of toxic effects on some test species. It can be concluded that the soil from Site 3 was relatively toxic in almost all ecotoxicological tests. Soils from Sites 1 and 2 were more toxic to bacteria and plants, while soils from sites 4, 5, and 6 were more toxic to the invertebrates. These differences demonstrate that it is not enough to include only one or two species in the studies when assessing soil quality and toxicity. The more species we use, the more accurate our results are likely to be [12][15]. Looking at the endpoints examined, the more sensitive were: DHA of *P. fluorescens*, germination rates of the test plants, and reproduction rates of test invertebrates.

3.3. Correlation between physico-chemical and ecotoxicological data

Based on Spearmen's correlation analysis, there was no significant relationship between the examined physico-chemical characteristics and the results of bacterial tests (Table 3.). The only exception was the TS contents of the soil, which was negatively correlated with the IC_{50} values obtained from the *A. agilis* test.

H_{ty} content of the soil samples was positively correlated with the germination rate of *S. alba* and the root elongation of both plants. This was expected, as it is well known that humus promotes plant growth. In contrast, the Pb concentration of soils was negatively correlated with the germination rate and root elongation of the test plants. Pb is a non-essential element for plants and, according to previous studies, causes significant damage to them in excessive concentrations [21], [22], [23]. This could also have happened in our study, as the Pb concentration of our soil samples was relatively high.

Table 3. Correlation between physico-chemical data and the results of bacterial and plant tests.

	Bacterial tests		Plant tests			
	IC_{50} value		germination rate		root elongation	
	A. agilis	*P. fluorescens*	*S. alba*	*L. sativa*	*S. alba*	*L. sativa*
pH	0.04	-0.04	0.10	0.21	-0.10	0.01
TS*	**-0.49**	-0.20	-0.26	0.31	0.08	0.02
H_{ty}^{+}	0.23	0.02	**0.48**	0.35	**0.53**	**0.55**
$CaCO_3$	0.06	0.15	-0.03	-0.14	0.13	0.20
Cd	0.34	0.22	0.25	0.15	-0.23	-0.09
Co	0.29	0.10	0.32	0.35	-0.02	0.03
Cr	0.27	0.09	0.33	0.37	-0.02	0.05
Cu	-0.04	0.09	0.30	0.06	0.42	-0.01
Ni	0.35	0.03	0.41	0.43	0.43	0.36
Pb	-0.31	-0.39	**-0.65**	**-0.66**	**-0.48**	**-0.51**
Zn	-0.31	-0.29	-0.42	-0.38	0.19	0.22

Data show Spearman's correlation coefficients. ***Notes****: *Total salt content, +Humus content. Bold numbers indicate a significant correlation (Spearman's correlation, $p < 0.05$).*

Soil heavy metal concentrations strongly influenced the survival rate and reproduction of *F. candida*.

Table 4. Correlation between physico-chemical data and the results of invertebrate tests.

	Invertebrate tests			
	survival rate		reproduction rate	
	F. candida	*E. fetida*	*F. candida*	*E. fetida*
pH	-0.11	-0.04	0.04	0.11
TS*	-0.26	0.07	-0.21	-0.08
H_{ty}^{+}	-0.14	0.09	-0.23	0.41
$CaCO_3$	-0.22	-0.06	-0.30	0.11
Cd	**-0.58**	-0.39	**-0.68**	0.34
Co	**-0.71**	-0.10	**-0.71**	0.36
Cr	**-0.55**	-0.21	**-0.61**	0.44
Cu	0.34	0.20	0.37	**-0.48**
Ni	**-0.51**	-0.02	**-0.58**	0.17
Pb	0.45	0.38	**0.53**	-0.19
Zn	0.21	0.17	0.27	**-0.48**

Data show Spearman's correlation coefficients. ***Notes****: *Total salt content, +Humus content. Bold numbers indicate a significant correlation (Spearman's correlation, $p < 0.05$).*

Concentrations of Cd, Cr, Co, and Ni were significantly correlated with these two parameters, and the correlation coefficients were also relatively high (between -0.51 and -0.71). This suggests that high soil concentrations of these metals may be the reason for the high toxicity of our samples to *F. candida*. The high sensitivity of this invertebrate to various heavy metals has been established in the past [24]. Interestingly, the Pb concentration of soils was positively correlated with the reproduction rate of *F. candida*.

None of the physico-chemical parameters examined were statistically related to the survival rate of *E. fetida*. However, the concentration of Cu and Zn in the soil negatively correlated with its reproduction rate. This means that the reproduction of *E. fetida* was inhibited by completely different heavy metals than that of *F. candida*.

4. Conclusion

In our research, we examined the quality and toxicity of suburban soils in Budapest using physico-chemical and ecotoxicological methods. It was revealed that some heavy metals (e.g. Cr, Cr, and Pb) presented in relatively high concentrations in soil samples, which may be the main reason for the observed toxicity of the samples. In fact, all soil samples examined were found to be toxic to at least one test species under laboratory conditions, which means that these soils could be also harmful to terrestrial communities on sites. This draws attention to the necessity of examining suburban soils.

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