

Original Research

Establishing Impact of the Long-Term Action of Waste Dumps with the Occurrence of Waste Tires on the Soil Environment

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Abstract

Long-term presence of waste tires in the soil brings negative effects and risks for the environment. Waste tires can be found in the open nature long after they have been left there. Their chemical composition and components are degradable and contaminate the environment. The research is focused on the evaluation of chemical changes in soils exposed to the long-term action (disposal) of waste tires and on the determination of the degree of phytotoxicity of such soils. Soil samples were collected from 4 sites in the territory of the Czech Republic. The samples were subjected to chemical analyses in an accredited laboratory. The analyses revealed markedly exceeded limits for heavy metals, namely Pb, Zn and Hg in some soil samples, as compared with the Decree No. 153/2016 Coll. Ranges of exceeded limits were from 40% to 26 566%. Then the samples were evaluated by means of microbiotests (acute phytotoxicity by means of Phytotoxkit test set, 72 hours) and also by means of long-term phytotoxicity test using the subchronic container test (28 days). The tests of phytotoxicity were made with the seeds of *Sinapis alba* L. and *Lepidium sativum* L. Results of subchronic container tests showed the percentage of germination (PG) in the soil samples ranging from 74.36%-90.48%. A sample with the PG value lower than 90% was evaluated as phytotoxic compared with the control substrate (100%). In the acute microbiotest, the soil samples exhibited values of root growth inhibition (IR) ranging from 5.12%-50.98%. Substrates with the IR value (%) over 0 showed inhibition/retardation of root growth in the tested plant, and the soil is considered to be phytotoxic.

Keywords: soil, chemical composition, heavy metals, phytotoxicity, inhibition

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Introduction

In earlier times but also today, waste tires (WT) were and still are disposed freely in the open nature into waste dumps which are in the regime of relic dumps or temporary waste dumps [1-2]. In many cases, these dumps represent unsecured areas where WT get into direct contact with the soil or aquatic environment where they are disturbed (their particles released) by temperature, light and precipitation, and a leachate then contaminates environment components [3-4].

There are approximately 1-1.5 billion WT coming to existence every year, of which ca. 0.6-1 million ends on waste dumps or are left behind in the open nature without any control. With the growing world population, the number is likely to grow further and we will have to face global challenges how to handle this waste which is at the same time a valuable commodity [5-8].

On waste dumps, WT lose their potential for the utilization of valuable resources contained in them [9]. Still some countries such as UK, USA and India dispose WT on waste dumps at more than 65%. This type of utilizable waste should be given priority in handling (reuse, recycling, pyrolysis or use for energy) which would prevent contact of WT with the environment to necessary minimum [1]. Legislative frameworks should be properly adjusted and regulations should be made stricter to reduce as much as possible the illegal disposal of WT, to contribute at the same time to the recycling of WT and to meet goals of circular economy [10].

Tires are very flexible and complex products in the shape of toroid, consisting of more than 45% of synthetic and natural rubber mixture, 25% of soot and 15% of softeners. The three main components are supplemented with a smaller but still significant representation of textile fibre, steel cord and other chemical components (vulcanizing agents, vulcanization accelerators and antidegradants) [11-12]. Their composition is unique with each manufacturer, and to be introduced onto the market they have to meet the most fundamental safety and comfort functions (direction control, load transmission, rolling resistance, adsorption etc.) [2, 13].

As to materials of which WT are composed, these are mostly non-degradable in the open nature, which is a problem in terms of waste amounts notwithstanding the risk of ignition and subsequent fire [11, 14]. WT disposed in the open nature catch water, also thanks to their shape, by which they function as fertile soil for disease-carrying mosquitoes and other animals [15-16]. With respect to their chemical composition and components, the issue of environment contamination arises due to the release of microparticles and the development of leachates over time [17]. WT contain among other things also the heavy metals (HMs) (Zn, Co, Pb, Cr, Hg, As etc.) [18] that are toxic and resistant to chemical or microbial degradation, and thus may persist in the soil even for several tens of years [19-21].

Toxicity of HMs is also problematic because of their accumulation and circulation in the environment [22].

In this study, the authors focused on sites with dumps containing WT of different age. A hypothesis was stated that WT have a negative and toxic impact on the environment. The goal of the study was: (a) chemically analysis of soil samples from the individual sites focused on HMs, (b) comparison of HMs values from the tested sites with the legislative limits, (c) determination of the acute degree of phytotoxicity of soils sampled from the respective sites, and (d) establishment of phytotoxicity using the subchronic container test.

Experimental

Research Sites – Dumps of Waste Tires

Research sites were dumps with a long-time occurrence of WT. There were four such waste dumps in the Czech Republic (CR) (Fig. 1): 1. Site 1 (Vysočina Region; 49.4741025N, 16.0971575E), 2. Site 2 (Plzeň Region; 49.4375319N, 13.2839694E), 3. Site 3 (Pardubice Region; 49.9261547N, 16.5523758E), 4. Site 4 (Liberec Region; 50.7354786N, 15.2731019E).

Waste dump 1 is an old uncontrolled dump of communal waste, construction waste and WT, which is located on the edge of the village boundaries, in the open nature near farmed areas. WT occurring in the dump are both in the soil environment and on the soil surface. The dump came to existence in about half of the 1990s. This means that WT have been there already more than 25 years. Surface area of waste dump 1 is about 450 m². Compared with the other sites, this dump contains the lowest amount of tires. Waste dump 2 is a site where only WT are dumped. They have been there for more than 7 years. They occur on the soil surface, not being secured, in a direct contact with the soil. Surface area of waste dump 2 is about 620 m². Waste dump 3 is a dump of WT where WT have been dumped more than 10 years. Similarly as on Site 2, WT are in a direct contact with the soil, occurring on the soil surface without any safety measures. It is a private property of the former agricultural enterprise. Surface area of waste dump 3 is about 440 m². Together with the waste dump 2, these two sites contain hundreds of WT on heaps. The last waste dump 4 is an old uncontrolled dump of waste from vehicles after their end of life (primarily metal constructions, plastic parts and auto glass) and WT located on a private property close to a watercourse. WT occurring on the dump are both in the soil and on the soil surface. The dump came to existence already in 1993 and is nearly 30 years old. Surface area of waste dump 4 is about 22 m². Compared with the waste dump 1, the number of WT is by several units higher.

The above-mentioned age of WT dumps was determined by authors based on the comparison of historic orthophoto maps and local surveys.

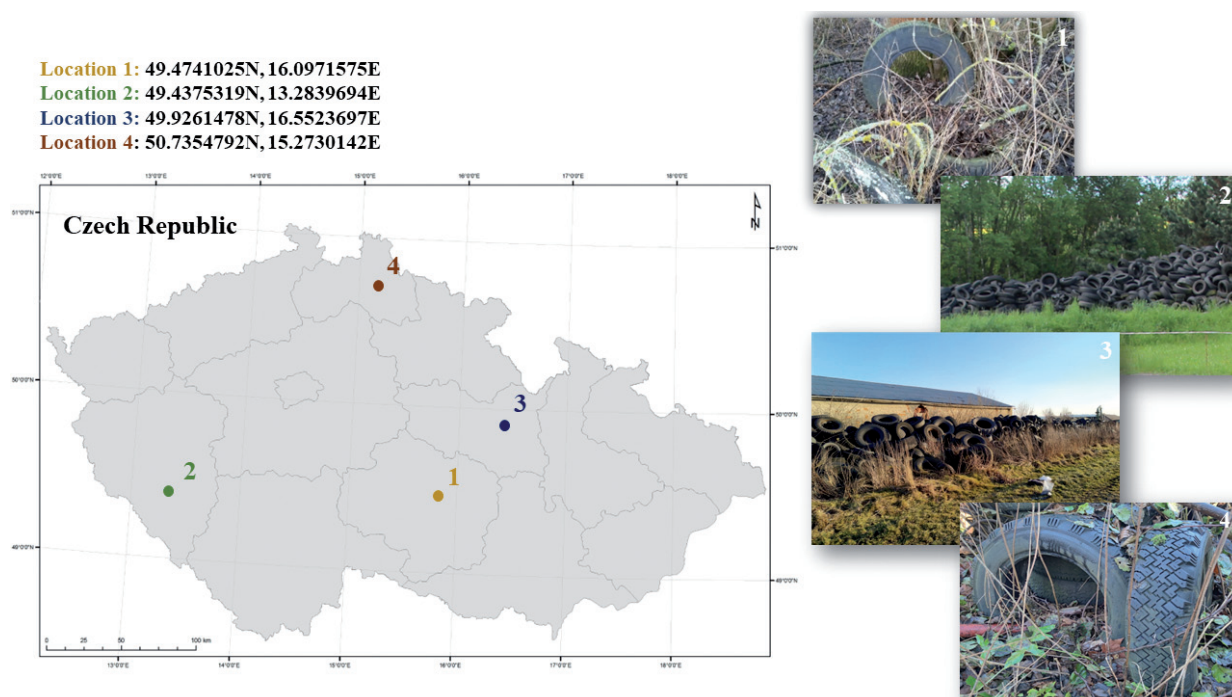


Fig. 1. Localization of sampling points with the occurrence of waste tires.

Soil Sampling

Soil samples from the respective dumps (1, 2, 3 and 4) were collected in line with the legislative regulation issued by the Ministry of the Environment of the Czech Republic Decree No. 153/2016 Coll. on the establishment of details concerning quality of agricultural land and on the change of Decree No. 13/1994 Coll., by which some details concerning the protection of agricultural land resources are governed, Annex 4, Procedure of Sampling, as amended. The Annex states also a minimum number of collected mixed soil samples in dependence on the size of surveyed area in hectares. Before the soil sampling, the minimum number of samples was determined empirically, the number being determined to min. 3 mixed collected samples (so-called broken samples) that were collected manually - see Table 1. This was including the collection of one reference sample which was at all times collected outside the area from which the mixed soil samples were collected, in order to capture the background state [23].

Sampling points on the four experimental sites were determined at random, respecting the principle of even distribution across the particular site. Soil samples from the WT dumps were labelled as follows: Site 1 (SWT1), Site 2 (SWT2), Site 3 (SWT3) and Site 4 (SWT4). Reference soil samples from Sites 1, 2, 3 and 4 were labelled S1, S2, S3 and S4, respectively. Soil samples collected from each site totalled 8 kg. They were collected from the topsoil layer 0.25-0.30 m in the period from July to November 2022. Tools for the collection of samples did not cause contamination

of samples, which prevented so-called cross-contamination. After collection, each sample had to be legibly marked. Samples were packed and stored under conditions of cleanliness and integrity of packaging and appropriate time for their storage was kept. The soil samples were placed into polyethylene bags and transported to the laboratory of Department of Applied Landscape Ecology, Mendel University in Brno at a temperature of 4°C to be prepared for analyses. Prior to testing, coarse fractions were removed from them by sifting through a sieve with an eye diameter of 2 mm.

Analysis of Soils and Determination of Heavy Metal Contents

Reference soil samples (S1, S2, S3 and S4) and soil samples collected from the dumps of WT (SWT1, SWT2, SWT3 and SWT4) were analysed for the

Table 1. Determination of the minimum number of collected mixed soil samples in dependence on the size of surveyed area [23].

Size of surveyed area (ha)	Number of mixed samples
≤ 0.0500	3
0.0501-1.0000	4
1.0001-10.0000	6
10.0000-30.0000	6 + 1 samples per each 2 ha over 10 ha
>30.0000	Individual plan of sampling

Table 2. Test methods.

Parameter	Identification of SOP test method	Accr.	UM (%)
Dry matter	GRA 03A: ČSN 72 0102, ČSN EN 14346:2007, ČSN EN 480-8	A	10%
Hg	AAS 06-07: ČSN EN ISO 16968, EN ISO 16968, ČSN 465735, ČSN EN 71-3:1996	A	20%
As	ICP 03B: ČSN EN ISO 17294-2, ČSN EN 13346:2001, ČS(1N) 46 5735, ČSN EN ISO 16968, ČSN EN 1388	A	20%
Se	ICP 03B: ČSN EN ISO 17294-2, ČSN EN 13346:2001, ČS(1N) 46 5735, ČSN EN ISO 16968, ČSN EN 1388	A	20%
Cd	ICP 03B: ČSN EN ISO 17294-2, ČSN EN 13346:2001, ČS(1N) 46 5735, ČSN EN ISO 16968, ČSN EN 1388	A	20%
Cr	ICP 04A: ČSN EN ISO 11885, ČSN EN 480-12, ČSN EN (1) 13346:2001, ČSN 465735, ČSN EN ISO 16968	A	20%
Co	ICP 04A: ČSN EN ISO 11885, ČSN EN 480-12, ČSN EN (1) 13346:2001, ČSN 465735, ČSN EN ISO 16968	A	20%
Zn	ICP 04A: ČSN EN ISO 11885, ČSN EN 480-12, ČSN EN (1) 13346:2001, ČSN 465735, ČSN EN ISO 16968	A	20%
Pb	ICP 04A: ČSN EN ISO 11885, ČSN EN 480-12, ČSN EN (1) 13346:2001, ČSN 465735, ČSN EN ISO 16968	A	20%

Note: UM (measurement uncertainty – is defined as a measurement uncertainty on a significance level of 95% with expansion coefficient $k = 2$; measurement uncertainty is expressed in accordance with EA-4/16); Accr. (Discerns standard operative procedures (SOP) within the scope of accreditation (A)).

presence of HMs in the testing accredited laboratory. Tested metals were Hg, As, Se, Cd, Cr, Co, Zn and Pb (mg kg^{-1}). They were determined using standard operative procedures (SOP). An overview of methods used for the respective HMs is presented in Table 2.

Measured values were evaluated and processed in the Microsoft Excel programme. Values of HMs (mg kg^{-1}) were compared with limits following out from the legislative regulation (Decree No. 153/2016 Coll. on the determination of details concerning quality of agricultural soil) and with values of concentration ranges in the soil for HMs (Pb, Cd, Cr, Hg and Zn), as published by Riley et al. [24] and Wuana and Okieimen [25].

Soil Phytotoxicity Bioassay

In the acute test, microbistest of soil toxicity determines the degree of soil toxicity as compared with higher plants within 72 hours. The phytotoxicity of soil samples was evaluated using the Phytotoxkit™ test. Seeds chosen for the experiment thanks to their high sensitivity and in line with the Phytotoxkit™ methodology were those of white mustard (*Sinapis alba* L.) (*S.alba*) and garden cress (*Lepidium sativum* L.) (*L.sat*). These two dicotyledonous species are indicators of soil contaminants. The presence of pollutant is indicated by dwarfed growth, retarded germination or slow growth. In this experiment, the authors decided to use the seeds of *S.alba* and *L.sat* based on their previous experience from other research studies that confirmed the seeds sensitivity [17, 26-29].

The reference soil samples (S1, S2, S3 and S4 control samples) and SWT1, SWT2, SWT3, SWT4 (soil samples exposed to WT) were subjected to tests of acute phytotoxicity at 100% concentration. The Phytotoxkit™ test set determines a decrease (or absence) of the so-called germination of seeds and growth of young roots after 3 days from the exposure of seeds to contaminated soil samples as compared with the reference soil samples. In this experiment, we used soil samples from a depth of 0.25-0.30 m; these samples were used as a so-called background. Saturation with water for the samples of tested soils was calculated according to the user manual for Phytotoxkit™ test. The measured amount of distilled water (calculated according to the rapid method for determining the water capacity of the test substrate) was poured onto the weighed sample volume with a graduated cylinder and mixed. The test sample was then placed in the sample box (the material is PET). Then filter paper was placed onto the samples and ten seeds of *S. alba* and *L. sat* were at put thereon, which were always placed at the same distance of 1 cm from the middle ridge of the test board. After closing, the test sample containers were inserted in vertical position in a holder into the incubator for 72 hours at a temperature of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$. After the incubation period, photographs of sample containers were taken and root lengths were measured (mm) using image analysis by software Tool 3.0 (UTHSCSA, San Antonio, TX, USA). The tests were made in three replications, i.e. altogether 48 tested sets. Inhibition of root growth (IR%) of *S.alba* or *L. sat* was calculated according to the following Equation (1) [30].

$$IR (\%) = \frac{(L_{S1} \text{ or } L_{S2} \text{ or } L_{S3} \text{ or } L_{S4} - L_{SWT1} \text{ or } L_{SWT2} \text{ or } L_{SWT3} \text{ or } L_{SWT4})}{(L_{S1} \text{ or } L_{S2} \text{ or } L_{S3} \text{ or } L_{S4})} \times 100 \quad (1)$$

Explanatory notes:

L_{S1} or L_{S2} or L_{S3} or L_{S4} give the mean value of plant species root lengths in the reference soil samples.

L_{SWT1} or L_{SWT2} or L_{SWT3} or L_{SWT4} give the mean value of plant species root lengths in the tested soil samples [26, 30-32].

The plots in this article shows the mean, whiskers representing the standard error, values with different letters (a;b) indicate a significant difference ($p < 0.05$) between variants. Differences between variants were analysed using the parametric LSD Fisher test (in the Czech software Statistica.pro). As according to the experimental methodology, three repetitions were performed and the data were subsequently processed.

Long-Term Test of Soil Phytotoxicity – Subchronic Container Test

Subchronic test of phytotoxicity determines ecotoxic effects of the soil on higher plants in 28 days. Seeds chosen for testing were those of *S.alba* and *L.sat*. In line with the methodology of ČSN EN 13432 [33], 0.2 kg (100%) of soil from waste dumps (SWT1, SWT2, SWT3 and SWT4) and reference soil samples (100%) (S1, S2, S3 and S4) was placed into terracotta containers. Onto the soil surface in each container 50 seeds of tested plants were placed, which were then covered with a layer of silica sand and moistened with distilled water (DW). Each sample was tested three times. The total number of containers used for testing was 48. The tests were made at a temperature of $22^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and relative moisture content 60-65% (in line with standard methodology). During the exposure, the samples were regularly moistened with DW. After the experiment, the results were evaluated, and compared with the control samples (S1, S2, S3 and S4) by calculating the percentage of germination (PG) according to the following Equation (2) [33].

$$PG (\%) = \frac{(N_{SWT1} \text{ or } N_{SWT2} \text{ or } N_{SWT3} \text{ or } N_{SWT4})}{(N_{S1} \text{ or } N_{S2} \text{ or } N_{S3} \text{ or } N_{S4})} \times 100 \quad (2)$$

Explanatory notes:

N_{SWT1} or N_{SWT2} or N_{SWT3} or N_{SWT4} is a value giving the number of germinated seeds/growing plants in the tested soil samples.

N_{S1} or N_{S2} or N_{S3} or N_{S4} give the number of germinated seeds/growing plants in the reference soil samples [17, 33].

Results of the subchronic test of soil phytotoxicity were evaluated according to the following criteria: PG (%) <90 classifies the soil sample as phytotoxic, PG (%) >90 and <110 classifies the soil sample as non-phytotoxic, with no impact on the plant species, and

PG (%) >110 classifies the soil sample as non-phytotoxic, with a stimulating effect on the plant species [34-36].

Results and Discussion

Results of Soil Analyses on Heavy Metal Contents

The soil samples collected from 4 sites were analysed for the contents of HMs. It appears that SM concentrations in different soil samples greatly differ from sample to sample. Results of HMs contents in the individual soils (S1-S4 and SWT1-SWT4) are presented in Fig. 2. The results were compared with legislative limits published in Decree No. 153/2016 Coll. on the establishment of details concerning the protection of agricultural land quality, for normal soils [23] (Fig. 3) and with the concentration ranges of HMs (mg kg^{-1}) As, Cd, Cr, Hg, Pb, and Zn in soils published by Riley et al. [24] and Wuana and Okieimen [25]. Further, the results were compared with intervention values for HMs (mg kg^{-1}) As, Cd, Cr, Hg and Pb in soils, according to Wuana and Okieimen [25].

Fig. 2 represents the contents of HMs (Hg, As, Se, Cd, Cr, Co, Zn and Pb) (mg kg^{-1}) in the tested soils from waste dumps 1-4 with the occurrence of WT (SWT1-SWT4), and compares them with soils collected outside the place where WT are stored (S1-S4). Site 1 (age 25 years) is a dump of WT in the order of units. The highest values of HMs (mg kg^{-1}) in this dump were recorded in Zn (106 mg kg^{-1}), Cr (30.5 mg kg^{-1}) and Pb (19.8 mg kg^{-1}); the lowest ones were measured in Hg (0.1 mg kg^{-1}) and Cd (0.35 mg kg^{-1}). The greatest differences in the values of HMs content between the control soil (S1) and the tested soil (SWT1) were recorded in Cd (by 75%) and Co (by 38%); the lowest differences were recorded in Se (by 9%) and Cr (by 13%). As the most important HMs (in terms of its highest content in the tire), Zn exhibited a difference of approximately 30%.

In Site 2 (age 7 years) and Site 3 (age more than 10 years), the dumps contain hundreds of WT on heaps. The highest values of HMs (mg kg^{-1}) in this dumps were recorded in Zn (SWT2 - 1250 mg kg^{-1} ; SWT3 - 170 mg kg^{-1}) and Cd (SWT2 - 5.38 mg kg^{-1} ; SWT3 - 3.74 mg kg^{-1}); the lowest values were measured in Hg (SWT2 - 0.26 mg kg^{-1} ; SWT3 - 0.1 mg kg^{-1}) and Se (SWT2 - 1.27 mg kg^{-1} ; SWT3 - 2.14 mg kg^{-1}). In both sites, the greatest differences in the values of HMs contents were recorded in Cd (in SWT2 by 1 181% as compared with S2; in SWT3 by 1 069% as compared with S3) and Zn (in SWT2 by 1 340% as compared with S2; in SWT3 by 221% as compared with S3). In Site 2, high differences were found also in the content of Pb (by 306%) and Hg (by 287%) between the control soil (S2) and tested soil (SWT2). On the other hand, the lowest differences between the control soil (S2) and the tested soil (SWT2) were recorded in the contents of Cr (by 6%) and As (by 15%). In Site 3, the lowest differences

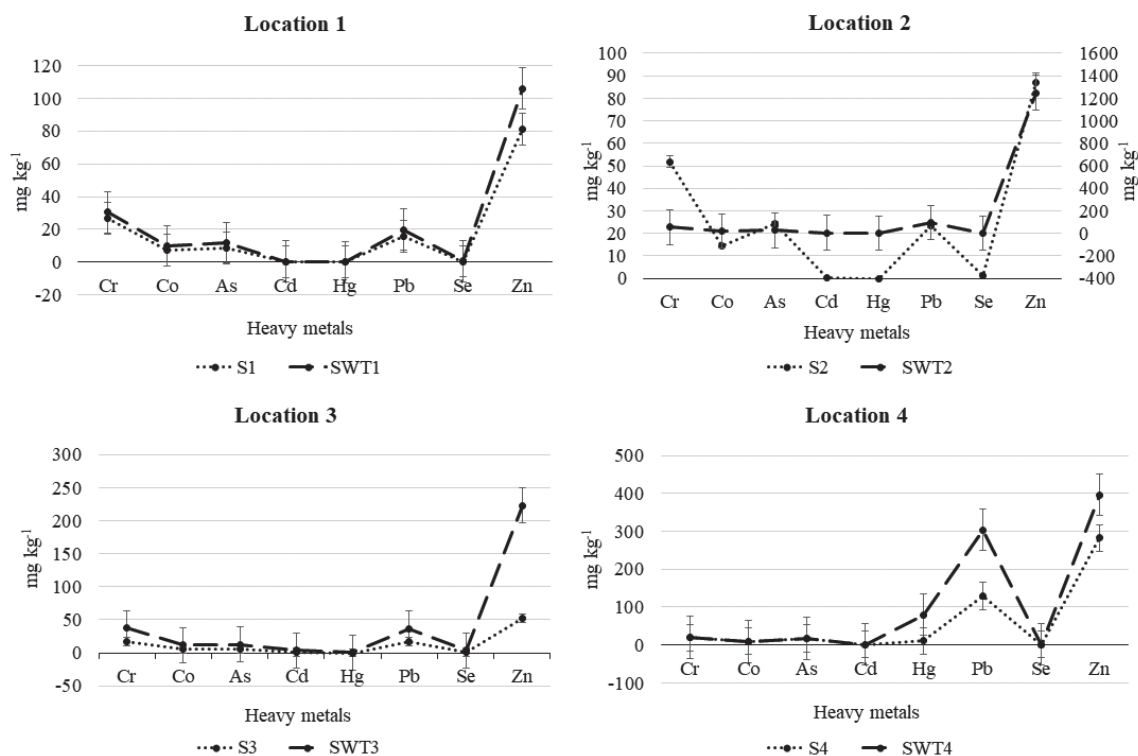


Fig. 2. Contents of HMs (Hg, As, Se, Cd, Cr, Co, Zn and Pb) (mg kg^{-1}) in the soils from waste dumps 1, 2, 3 and 4, standard error was within 1%.

in the contents of HMs between the control soil and the tested soil were recorded in As (by 14%) and Pb (by 20%).

The oldest WT dump (age 30 years) is Site 4 without about twenty WT. The highest values of HMs on this dump (mg kg^{-1}) were recorded in Zn (396 mg kg^{-1}), Pb (304 mg kg^{-1}) and Hg (80 mg kg^{-1}); the lowest contents of HMs were recorded in Se (1.05 mg kg^{-1}) and Cd (1.13 mg kg^{-1}). Here, the greatest differences of values between the control and tested soils were measured in the content of Hg (by 692%) and Pb (by 136%). The lowest differences in the values of HMs contents between the control (S4) and tested soil (SWT4) were recorded in Co (by 0.1%) and As (3%). Zn exhibited a difference of approximately 40%. On sites 1, 2, 3 and 4, the collected soil samples that had been in contact with WT showed higher values in all tested HMs. Soils exposed to different times of the impact of WT exhibited higher values of HMs.

Compared with the Decree No. 153/2016 Coll. (Fig. 3), neither the tested soils (SWT1-SWT4) nor the control soil samples (S1-S4) exceeded limits stipulated by legislation in Cr (90 mg kg^{-1}) and Co (30 mg kg^{-1}). The highest values of Cr were recorded in sample SWT2 (55.10 mg kg^{-1}) and the lowest ones in sample SWT4 (20.10 mg kg^{-1}). The highest values of Co were determined again in sample SWT2 (21 mg kg^{-1}) and the lowest ones in sample SWT3 (6.39 mg kg^{-1}). As to the contents of the other HMs: As, Cd, Pb, Hg and Zn - limits of some elements were exceeded in some soils even multiple times.

The highest content of As was measured in soil samples from waste dump 2 (SWT2 27.70 mg kg^{-1}), which indicates that the limit was exceeded by nearly 40%. In the other dumps (1, 3 and 4), the limits for As were not exceeded. The lowest values for As were shown by soils from waste dump 3 (SWT3 6.85 mg kg^{-1}).

Limits for the content of Cd in the soil was exceeded in a majority of waste dumps 2, 3 and 4 (SWT2 (5.38 mg kg^{-1}) by more than 975%, in SWT3 (3.74 mg kg^{-1}) by nearly 650%, and in SWT4 (1.13 mg kg^{-1}) by more than 125%). Soil samples from waste dump 1 did not exceed limits for the content of Cd (SWT1 0.35 mg kg^{-1}).

As to the content of Hg, soil samples from waste dump 4 (SWT4 80 mg kg^{-1}) exceeded the limits by 26 566%. Even the control soil sample S4 exhibited values exceeding the Hg limits in the soil (10.10 mg kg^{-1}). Soil samples from the other tested waste dumps (SWT1, SWT2 and SWT3) reached max. 1/3 of Hg limits in the soil (0.3 mg kg^{-1}).

Another tested element was Pb whose limits were exceeded in soil samples from waste dumps 4 and 2 (SWT4 304 mg kg^{-1} , by nearly 410%; SWT2 95.30 mg kg^{-1} , by nearly 60%). The control sample S4 (129 mg kg^{-1}) exhibited values exceeding Pb limits in the soil, too (60 mg kg^{-1}), they were however much lower than soil samples that were in contact with WT. The control sample S2 (23.50 mg kg^{-1}) did not exceed the legislative limit. The lowest values were recorded in waste dumps 1 and 3 (SWT1 19.80 mg kg^{-1} and SWT3

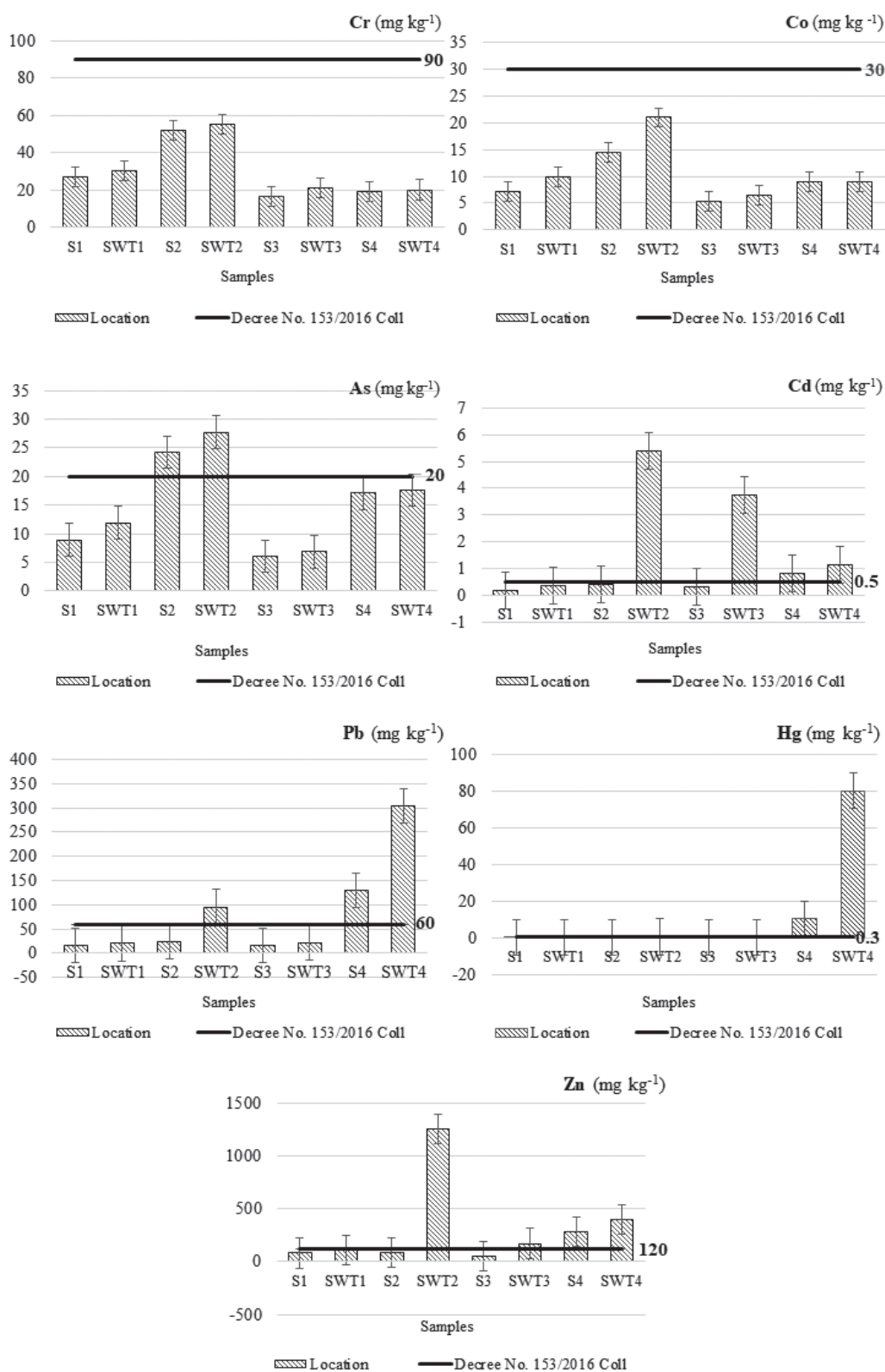


Fig. 3. Comparison of HMs (Hg, As, Se, Cd, Cr, Co, Zn and Pb) values (mg kg⁻¹) in the soil samples from surveyed waste dumps with limits established by legislation (Decree No. 153/2016 Coll.), standard error was within 1%.

20.20 mg kg⁻¹) that reached max. 1/3 of Pb limits in the soil (60 mg kg⁻¹). As to the fact that soil samples SWT4 (waste dump 4) showed limits of Pb and Hg exceeded multiple times, the authors state that the was the oldest

one, which means that the contact of WT with the soil was the longest one in terms of time and the soil environment was thus exposed to the impact of WT for the longest time.

The last assessed element was Zn (limit 120 mg kg⁻¹) whose limits were exceeded in waste dump 2 (SWT2 1 250 mg kg⁻¹, by more than 940%), in waste dump 4 (SWT4 396 mg kg⁻¹, by 230%) and in waste dump 3 (SWT3 170 mg kg⁻¹, by more than 40%). As to control samples, the legislative limit was exceeded only in S4 from waste dump 4 (282 mg kg⁻¹). Sample SWT1 from waste dump 1 (106 mg kg⁻¹) reached as the only one from the soil samples from waste dumps the lowest values and did not exceed the legislative limit. Yet, the value was not negligible.

The soils were also tested for the content of Se. However, Decree No. 153/2016 Coll., on the establishment of details concerning the protection of agricultural land quality, for normal soils does not stipulate limits for Se, and this is why the element was not compared with the Decree.

Riley et al. [24] and Wuana and Okieimen [25] state ranges of HMs concentrations in soils (mg kg⁻¹) for As (0.10 - 102 mg kg⁻¹), Cd (0.10-345 mg kg⁻¹), Cr (0.05-3 950 mg kg⁻¹), Hg (<0.01-1 800 mg kg⁻¹), Pb (1.00-69 000 mg kg⁻¹), and Zn (150-5 000 mg kg⁻¹). The ranges were compared with the concentration of HMs (mg kg⁻¹) measured in the soil samples from waste dumps 1-4. Upper limits of HMs concentration ranges stipulated by Riley et al. and Wuana and Okieimen were not exceeded by any tested soil samples. Soil samples SWT1 in terms of the amount of Zn (106 mg kg⁻¹) did not exceed even the lower limit of the concentration range Zn (150-5 000 mg kg⁻¹) (Fig. 3) [24-25].

Wuana and Okieimen inform there are intervention values established for some HMs (mg kg⁻¹): As (625 mg kg⁻¹), Cd (380 mg kg⁻¹), Cr (240 mg kg⁻¹), Hg (530 mg kg⁻¹) and Pb (210 mg kg⁻¹). The authors claim that when these values are exceeded, the functionality of soils is seriously jeopardized and there are also risks for the environment and human health [25]. Compared with the results (Fig. 3), the intervention value was exceeded in Pb (210 mg kg⁻¹) from waste dump 4 (304 mg kg⁻¹).

Other soils from the waste dumps (1, 2, 3, 4 - except for the intervention value in Pb) did not exceed these values in As, Cd, Cr, Hg and Pb as compared with the values stated by Wuana and Okieimen. The authors state at the same time that the soils are not endangered and there is no risk for the environment or humans [25]. However, if we proceed from the legislative limits [23], soil samples from waste dumps 2 (As, Cd, Pb and Zn), 3 (Cd and Zn) and 4 (Cd, Pb, Hg and Zn) exceeded in the case of these HMs the limits stipulated by legislation, and the soils are burdened with their above-limit occurrence. Zhao et al. [37] add that HMs and their accumulation in the soil cause biological toxicity putting into danger the agricultural ecosystem. HMs persist in the soil for a long time and are harmful to human health [37].

Wik and Dave [38] inform that products made of tires contain additives with HMs, including Zn and Pb. Zinc is commonly used in tires as activator while Pb is usually added as pigment, thermal stabilizer or for other properties [38]. Metals are usually added to polymer structure by physical mixing during production, and they may be released into the environment during degradation [39]. In conditions of the environment, half-time of rubber particles from tires in the soil is 16 months [40]. The tested waste dumps represent sites where soils have been exposed to metals released during the degradation of WT already for several years or tens of years. Kim et al. [41] arrived at a conclusion that tire particles can affect the soil environment by changing soil properties and leaching of chemical substances, thus having unfavourable impacts on soil organisms [41].

Soil Phytotoxicity Bioassay

Results of root growth inhibition (IR%) of *Sinapis alba* L. and *Lepidium sativum* L. in the acute test of soil phytotoxicity from waste dump 1 (SWT1), 2 (SWT2), 3 (SWT3) and 4 (SWT4) are shown in Fig. 4.

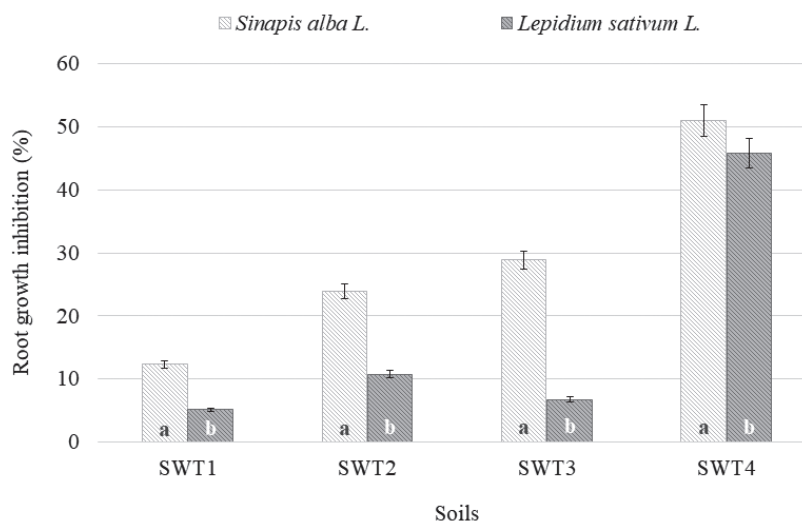


Fig. 4. Root growth inhibition of *Sinapis alba* L. and *Lepidium sativum* L. in the acute test of soil phytotoxicity.

The results of the acute test of soil phytotoxicity were assessed according to the following range that was stipulated by MicroBioTests Inc. [30]. When the percentage of IR shows minus values (IR (%) <0), the growth of roots in plant species is stimulated and the substrate is not considered phytotoxic. In the opposite case, when the percentage of IR exhibits plus values (IR (%) >0), the growth of roots is inhibited/retarded and the substrate is considered phytotoxic [30]. IR values (S.alba and L.sat) for soil samples from the tested waste dumps ranged from 5.12%-50.98%. All values indicate inhibited/retarded growth both in S.alba and L.sat. Soil samples that had been in long contact with WT are evaluated as phytotoxic. The authors state that the differences in IR values (%) might result from the different amounts of WT on the given sites but also due to the time for which the WT were in contact with the soil. During this time, due to the influence of abiotic factors (temperature, humidity, precipitation, soil moisture, soil reaction, etc.), contaminants are gradually released from the WT into the soil.

Waste dump 4 (SWT4) shows the highest percentage of growth inhibition/retardation in S.alba (50.98%) and L.sat (45.86%). It is at the same time the oldest site with the occurrence of WT (nearly 30 years). During those 30 years, HMs were gradually accumulating in the soil and contaminated it, particularly Pb, Hg and Zn which exceed the stipulated limits multiple times (Fig. 3). Yadav [42] informs that Pb is one of the most occurring toxic elements in the soil, which particularly disturbs photosynthetic processes and growth of plants. High concentrations of Hg are phytotoxic for plant cells. The problem of Hg in the soil is namely its accumulation in higher plants. High levels of Zn are toxic to plants, too, disturbing their metabolic functions and hence restricting the growth of roots and plants themselves [42].

Waste dump 1 (SWT1) exhibits the lowest percentage of inhibited/retarded growth of S.alba (12.31%) and L.sat (5.12%) in spite of the fact that it is the second

oldest site with the occurrence of WT (nearly 25 years). However, the concentrations of HMs on this site did not exceed limits stipulated by the Decree. Based on the results, the authors assume that the concentrations of HMs on Site 1 and the related lowest degree of toxicity to plants is given by the lower number of dumped WT.

Results of the inhibition/retardation of the growth of S.alba and L.sat on Sites 2 and 3, with respect to their amounts in/on the soil, range from 6.78-28.86%. These values again indicate soil phytotoxicity. At the same time, waste dumps 2 and 3 exceed also some limits for HMs, namely As, Cd, Pb and Zn (see Fig. 3).

Long-Term Test of Soil Phytotoxicity – Subchronic Container Test

Results of the long-term test of soil phytotoxicity (percentage values (PG%) of the growth of growing plants/germinated seeds of *Sinapis alba* L. and *Lepidium sativum* L. for 28 days) in samples from waste dumps 1 (SWT1), 2 (SWT2), 3 (SWT3) and 4 (SWT4) are presented in Fig. 5.

The plot shows the mean, whiskers representing the standard error, values with different letters (a;b) indicate a significant difference ($p < 0.05$) between variants, differences between the variants were analysed with the parametric LSD Fischer test.

PG of germinated plant species in samples SWT1, SWT2, SWT3 and SWT4 were evaluated in accordance with the standard ČSN EN 13432 after 28 days of experiment duration.

Soil phytotoxicity was demonstrated in samples SWT1 - L.sat (84.78%); SWT2 - S.alba (89.47%), L.sat (82.61%); SWT3 - S.alba (86.49%), L.sat (76.09%) and SWT4 - S.alba (83.33%), L.sat (74.36%). In SWT1 - S.alba (90.48%), phytotoxicity was not confirmed. Evaluation of this sample did not reveal any effect on S.alba. However, SWT1 - L.sat (84.78%) showed a PG decrease by 6.72%. Regarding possible deviations within the standard error, the authors maintain that

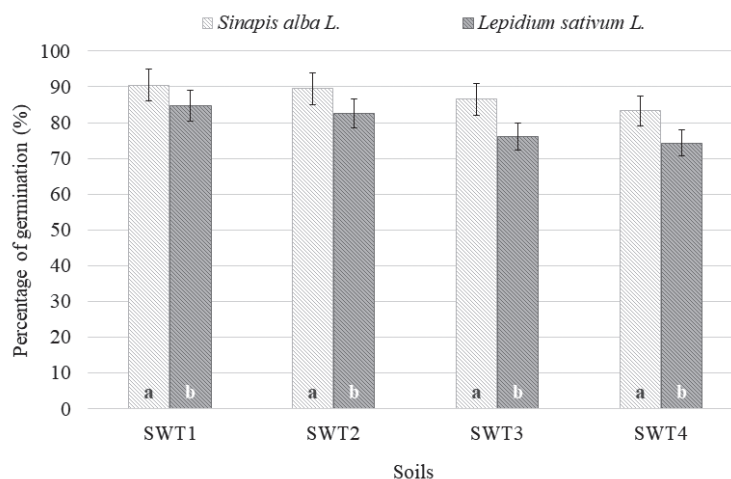


Fig. 5. Percentage values of the growth of growing plants/germinated seeds of *Sinapis alba* L. and *Lepidium sativum* L. for 28 days.

despite the rating scale, the soil sample SWT1 can be considered phytotoxic.

During the experiment, changes were observed on the germinated plants (*S.alba*, *L.sat*) in the control soils (S1-S4) as well as in the tested soils (SWT1-SWT4). Samples of tested soils SWT1-SWT4 showed inhibition, retarded growth and dwarfing of *S.alba* and *L.sat* plants. These samples exhibited necroses, chloroses and symptoms of plant mortality already in week 3. Soil samples from waste dumps 1-4 (S1-S4) exhibited signs of phytotoxicity.

Dose et al. [43] inform that rubber in tires may contain up to 5% of zinc weight, and its degradation may result in the release of this element, but also of other elements and substances from which the tires are produced [43]. Various studies [21, 44-46] mention that the causes are primarily excessive amounts of Zn, Hg, As and Cd. As compared with the control samples, preparation of plants (*S.alba* and *L.sat*) in the tested soils also revealed the dwarfed growth of roots which might have been caused by the accumulation of excessive Pb in the plants, affecting their metabolic functions [47].

Summary

It is estimated that Europe, USA and Japan produce together 5×10^9 kg of discarded tires per year [48]. It should be pointed out that during their use, tires release microplastic contaminants from treads [49-52]. Upon the end of their service life, waste tires can continue releasing microplastic materials due to climate changes even for several tens of years if not handled and stored appropriately [53-55]. Hartmann et al. [56] inform that the impact of microplastic materials from tires results in the retarded growth of edible parts of crops, as they observed in the roots of carrot and maize, spring onion, lettuce, Chinese cabbage, rice and wheat grain [56].

Tires represent a very valuable commodity produced of raw materials originating from diverse corners of the world [57-58]. A request of circular economy at the end of service life of tire is its maximum utilization [58], which has to do with the minimization of the amount of new raw materials (rubber harvesting, steel production, input chemical substances etc.) necessary for the manufacture of new tires. It is therefore necessary to update legislation which prevents/stops the free disposal of waste tires in the open nature [59]. Contamination with HMs has become one of the most serious environmental problems to be faced by the world [60]. Important is also the environmental awareness of customers, which should highlight the risks connected with the formation of relic landfills/waste dumps on the environment. Information about handling tires at the end of their service life and possibilities of their further use should thus be a mandatory component of purchasing new tires.

Conclusions

The aim of the study was to determine impacts of the long-term action of illegal landfills and waste dumps with the occurrence of waste tires on the soil environment. Chemical analysis of soil samples from individual experimental sites was used to establish the amounts of heavy metals in the soils. These were compared with legislative limits. It was found out that inorganic pollutants such as As and Pb that are not degradable in the soil exceeded limits in some soil samples on average by tens of percent. Conversely, Cr (mg kg^{-1}) which is also not degradable in the soil, and Co did not exceed limit values in the tested soils. Some values of Cd, Hg, Pb and Zn were very interesting. In soil samples collected from waste dump 4, the amount of Hg exceeded legislative limits by 26 566%; the amount of Pb exceeded them by nearly 410%; the amount of Zn exceeded them by 230% and the amount of Cd exceeded by more than 125%. A similar situation was observed in samples from waste dump 2, in which the values of Cd exceeded limits in the soil by more than 975%; the values of Zn by more than 940% and the values of Pb by nearly 60%. And in the end in soil samples collected from waste dump 3, the amount of Cd exceeded legislative limits by nearly 650% and the amount of Zn by more than 40%.

Results of acute toxicity showed that soils collected from the individual sites are phytotoxic. Differences in the values of root growth inhibition among the tested soils may be given by the different age of waste dumps as well as by the different amounts of dumped waste tires. The phytotoxicity of soils from all tested dumps was confirmed also by the test of subchronic phytotoxicity.

Results of our study indicate that the soil environment exposed to the effect of waste tires was adversely affected by the contact with them both as to higher occurrence of some heavy metals, and in terms of phytotoxicity. It does not have to mean necessarily that a greater amount of waste tires at the place of waste dump/relic landfill would logically increase the degree of toxicity as an important indicator is also the time for which the waste tires were in contact with the soil. Further research on the toxicity of used tires and on how to use/recycle the used tires effectively is necessary. The authors will continue this topic to devote.

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Conflict of Interest

The authors declare no conflict of interest.

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