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The Influence of Microplastics from Ground Tyres on the Acute, Subchronical Toxicity and Microbial Respiration of Soil

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Citation: Šourková, M.; Adamcová, D.; Vaverková, M.D. The Influence of Microplastics from Ground Tyres on the Acute, Subchronical Toxicity and Microbial Respiration of Soil. *Environments* **2021**, *8*, 128. https://doi.org/10.3390/environments8110128

Academic Editor: Gianniantonio Petruzzelli

Received: 28 October 2021
Accepted: 16 November 2021
Published: 18 November 2021

Abstract: As a rubber annular coat of rim wheels, tyres are inevitable parts of all vehicles in modern times. As to their composition, however, they represent a risk for the environment. During the use of tyres, tyre tread patterns become abraded, which results in its gradual wear and necessary replacement. These micro and nano particles are then gradually extracted into the environment, namely soils and waters. Our research study was focused on the assessment of subchronical phytotoxicity (pot trial with a mixture of substrate and predetermined ratio of abrasion products lasting 28 days) and biological tests (testing phytotoxicity of leaches with predetermined ratio of abrasion products on Petri dishes). The biological tests were comprised two plant species—seeds of white mustard (*Sinapis alba* L.) and garden cress (*Lepidium sativum* L.). In the mixtures of substrate with determined shares of abrasion products (5%, 25%, 50% and 75%), respiration of CO_2 was also established by means of soil microbial respiration (Solvita CO_2-Burst). Substrates with 5% and 25% abrasion proportions showed increased biological activity as well as increased CO_2-C emissions. The increasing share of abrasion products resulted in decreasing biological activity and decreasing CO_2-C emissions. The results of subchronical phytotoxicity ranged from 62% to 94% with values below 90% indicating substrate phytotoxicity. The results of biological tests focused on the phytotoxicity of tested samples exhibiting values from 35% to 70% with respect to the germination index with values below 66% indicating the phytotoxicity of tyre abrasion products.

Keywords: pot experiment; biological test; soil respiration; germination index; *Lepidium sativum* L.; *Sinapis alba* L.

1. Introduction

The global production of tyres grows every year by several million tonnes and is estimated to reach 2.7 billion pieces of tyres by 2022 [1]. Such a number of tyres represents a massive burden on the environment during their life cycle (mining, manufacture, transport, exploitation and disposal) [2,3]. The stage of exploitation induces environmental problems caused by tyre abrasion products and significantly contributes to most environmental impacts in the life cycle of tyres [2]. Farhan et al. [4] reported that the total amount of abrasion products from the global use of tyres entering the environment every year is up to 6 million tonnes. Microparticles of tyre abrasion products containing synthetic rubber are considered to be elastomers and are classified as microplastic materials [5–9]. Microplastic materials are polymers (fractions smaller than 5 mm and larger than 10 nm) comprising synthetic organic compounds occurring in the atmosphere, in waters and in soils as a part of their pollution [10–12]. The particles are not only dangerous mainly due to their circulation in the atmosphere and subsequent inhalation but also due to their cumulation in the food chain and dispersion at great distances where they can contaminate soils. All this may subsequently impair the growth of crops [10,13,14].

The biological functioning of soil is provided by CO_2 respiration of all micro-organisms and macro-organisms living therein. It is an important ecological process that is sensitive to climatic changes and determines the soil's C supply [15,16]. Healthy soil exhibits an increased respiration rate under moist and warm climatic conditions [16,17] and microbial activity [15]. Moreover, the photosynthesis of plants depends on available CO_2; therefore, its amount emitted from the soil directly affects the growth of plants close to the soil surface. Thus, soil respiration can provide a basic information about the condition of crops [18]. However, the abrasion of microplastic (hereinafter referred to as tyre abrasion) can significantly affect this respiratory activity and disrupt natural conditions in the soil environment. This study aimed at the establishment of respiration activity of soil that has been exposed to different shares of tyre abrasion products.

Another important task of the study was to test the phytotoxicity of tyre abrasion products. Phytotoxicity is any unfavourable impact on plants caused by growth conditions or by specific (phytotoxic) substances [19,20]. The testing of phytotoxicity dwells on the principle of using the capacity of substances to cause toxic effects, which result in serious biological injury or death after exposure to or contamination with such a substance. These tests are specific in the direct observation of reactions of plants (germination, dwarfing, decay, leaf fall and the like) after the application of a sample or substance [20,21]. The tests primarily make use of terrestrial plants that are used in screening the phytotoxicity of soils, landfill leachates, pesticides, composts, chemicals, etc. [20,22,23]. Being classified as microplastic material, the abrasion products from tyres, which permeate into the environment, is an important source of pollutants [5,11,24], and its ecotoxicological impacts should be investigated. In this study, we test phytotoxicity and the respiration of soil contaminated with abrasion products from tyre tread patterns (microparticles) produced during the common use of tyres.

The goals of this study were as follows: (i) to determine the degree of acute phytotoxicity from tyre abrasion products by means of biological tests, (ii) to determine the degree of subchronical toxicity of tyre abrasion products by means of pot trial and (iii) to determine the soil respiration activity at defined shares of tyre abrasion products.

2. Materials and Methods

For our study, we chose abrasion products from tyres released during their use, i.e., micro particles with fraction sizes ranging from 0.1 to 0.4 mm. The selected parameters of the tested abrasion products are listed in Table 1. The products were subjected to biological tests of acute toxicity and subchronical toxicity (pot experiment) in combination with the plant species of *Lepidium sativum* L. and *Sinapis alba* L. The Solvita CO_2-Burst set was used to establish the microbial respiration of soil (substrate mixture) containing predetermined shares of abrasion products both at the beginning of the pot experiment and at its end (after 28 days). A scheme of the experimental methodology used is presented in Figure 1.

Table 1. Selected parameters of the tested abrasion products.

Parameter	Unit	Result
Benzo(a)Pyrene	$mg \cdot kg^{-1}$	0.19
Benzo(e)Pyrene	$mg \cdot kg^{-1}$	0.79
Pyrene	$mg \cdot kg^{-1}$	19.00
Naphthalene	$mg \cdot kg^{-1}$	0.39

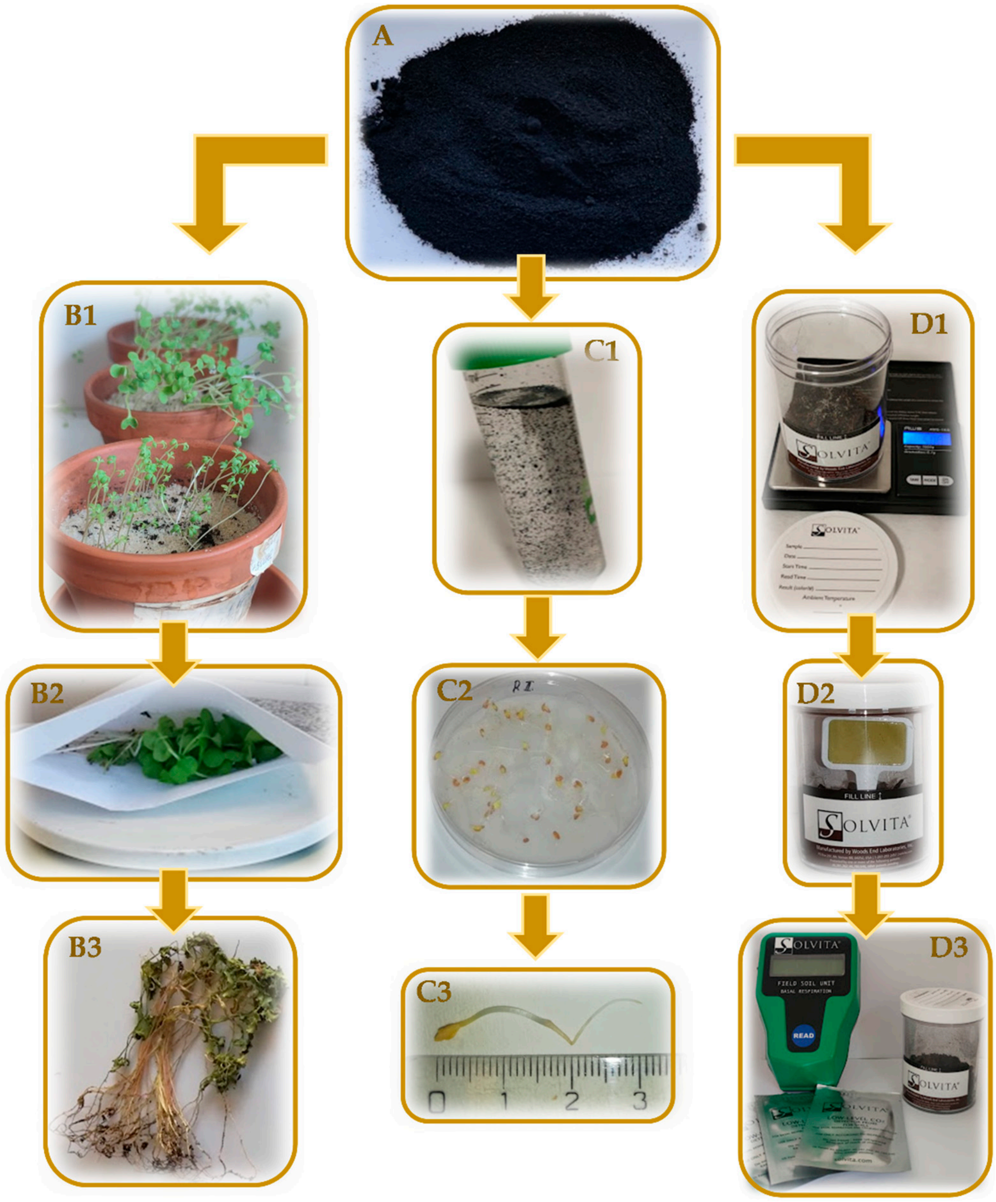

Figure 1. Scheme of phytotoxicity tests and measurement of soil biological activity. Images (**A**) abrasion product from tyres; (**B1**) experiment with subchronical toxicity; (**C1**) extract from tyre abrasion products; (**D1**) weighing and preparation of substrate and tyre abrasion products for measuring the soil respiration activity; (**B2**) wet biomass; (**C2**) biological tests of phytotoxicity; (**D2**) course of the measurement of soil respiration activity; (**B3**) dry biomass; (**C3**) measurement of partial roots and evaluation; (**D3**) evaluation of soil respiration activity.

2.1. Subchronical Toxicity of Abrasion Products from Tyres

The test for subchronical toxicity of tyre abrasion products was conducted in line with the methodology for establishing ecotoxic effects on higher plants described in ČSN EN 13432 [25]. The experiment consists in long-term exposures of *Sinapis alba* L. and *Lepidium sativum* L. seeds relative to the effect of abrasion products incorporated into the mixture of the substrate determined by European norms. The substrate consists of quartz sand (fine sand predominates, more than 50% by volume of particles with a diameter of 0.05–0.20 mm), standardized soil used for testing and peat (air dried and finely ground, without visible plant residues), which constitutes the examined soil. A total of 30 terracotta pots (Ø11 cm, height 9.7 cm) were used for the given experiment. Tyre abrasion products were applied into the soil at 5%, 25%, 50% and 75% (always in triplicates) and watered with 50 mL of DW 3–4 times a week. No artificial nutrients were applied to the soil during the experiment. A control sample consisted of substrate without the addition of abrasion products. The growth of plants/germinated seeds was monitored for 28 days. The experiment was conducted in laboratory conditions (temperature 20 ± 2 °C, relative humidity 60–62%). The experiment was ended and evaluated after the lapse of the given time. Inhibition of root growth (IR) was then calculated for *Lepidium sativum* L. and *Sinapis alba* L. (Equation (1)) with the following evaluation: phytotoxic (inhibition) IR (%) < 90, non-phytotoxic (no effect) 90 > IR (%) < 110 and growth stimulation IR (%) > 110 [26,27]. The obtained results were evaluated graphically and statistically:

Inhibition of root growth IR (%)

$$IR = (P_T/P_C) \times 100\ [\%] \tag{1}$$

- P_T—number of germinated seeds/plants growing in the tested substrate;
- P_C—number of germinated seeds/plants growing in the control substrate.

2.2. Determination of Biomass Weight from the Experiment of Subchronical Toxicity

The determination of subchronical toxicity was followed by the determination of biomass weight. Biomass (aboveground parts of the tested *Lepidium sativum* L. and *Sinapis alba* L. plants) was taken out from the containers, dissected and weighed (=wet biomass). The wet biomass was dried out at 22 ± 2 °C for 168 h and weighed again. Then, it was desiccated in an Ecocell drier at 60 °C and weighed on analytic digital scales (Precisa 4000 C); the measured values were recorded (=dry biomass) [28]. The obtained results were evaluated graphically and statistically.

2.3. Biological Test of Tyres Abrasion Products

The biological test of tyre abrasion products (Germination index (GI)) was performed by using tests of phytotoxicity in line with the methodology developed by ALS Czech Republic, Ltd. The seeds of *Lepidium sativum* L. and *Sinapis alba* L. were exposed to a short term (acute) influence of extracts from tyre abrasion products [29,30]. The aqueous extracts of tyre abrasion products were in predetermined shares (5%, 25%, 50% and 75%) that were diluted and applied onto filter paper at the bottom of a Petri dish (10 mL). Each variant had three repetitions. Then, 30 seeds of *Lepidium sativum* L. and *Sinapis alba* L. were evenly distributed onto the filter paper. The initial sample for establishing extract phytotoxicity was the one without the addition of tyre abrasion products (control sample). The samples were incubated in the controlled conditions of the Ecocell dryer with no access of light for 48 h at 26 ± 2 °C. The biological test ended with the measurement of partial lengths of *Lepidium sativum* L. and *Sinapis alba* L. roots at an accuracy of 1 mm [29,31,32]. Based on the results, the degree of extract phytotoxicity (GI) was established as follows: non-phytotoxic 66 < GI (%) < 100, phytotoxic 26 < GI (%) < 65 and highly phytotoxic GI (%) < 25. The calculation of GI (%) (Equation (4)) was evaluated according to the resulting parameters as follows: germination (G (%)) and elongation (L (%)) according to Equations (2) and (3) [26,29]. The obtained results were evaluated graphically:

Germination G (%)

$$G = (N_S/N_C) \times 100\ [\%] \tag{2}$$

- N_S—number of seeds germinated in the tested extract;
- N_C—number of seeds germinated in the control sample;

Elongation L (%)

$$L = (L_S/L_C) \times 100\ [\%] \tag{3}$$

- N_S—average root length in the tested extract;
- N_C—average root length in the control sample.

Germination index GI (%)

$$GI = (G \times L)/100\ [\%] \quad (4)$$

2.4. Respiration Activity of Soil with the Content of Tyre Abrasion Products

The respiration activity of soil was tested in compliance with standards of the CO_2 method from Solvita by using the Soil Master Kit. The substrate characterized in the part of the experiment with subchronical toxicity was weighed in the predetermined shares of tyre abrasion products (5%, 25%, 50% and 75%) into a closable glass (Solvita) and moistened with distilled water. The initial sample for establishing the soil respiration activity was the substrate without the addition of tyre abrasion products (control sample). The Solvita CO_2 soil probe was placed vertically into a glass and closed. The measurement was repeated three times for each share of tyre abrasion products (altogether 15 glasses) for 24 h at 20 ± 1 °C. Then, the probe was inserted into the digital colour reader, which evaluated colour levels and CO_2-C ($kg \cdot ha^{-1}$) emissions [33]. The measurement of respiration activity was repeated also at the end of the experiment with subchronical toxicity (i.e., after 28 days) in order to compare the long-term action of tyre abrasion products on the respiration (emissions) of the given substrate. The obtained results were evaluated graphically.

3. Results

3.1. Subchronical Toxicity of Abrasion Products from Tyres

The results of the experiment with subchronical toxicity are presented in Figure 2.

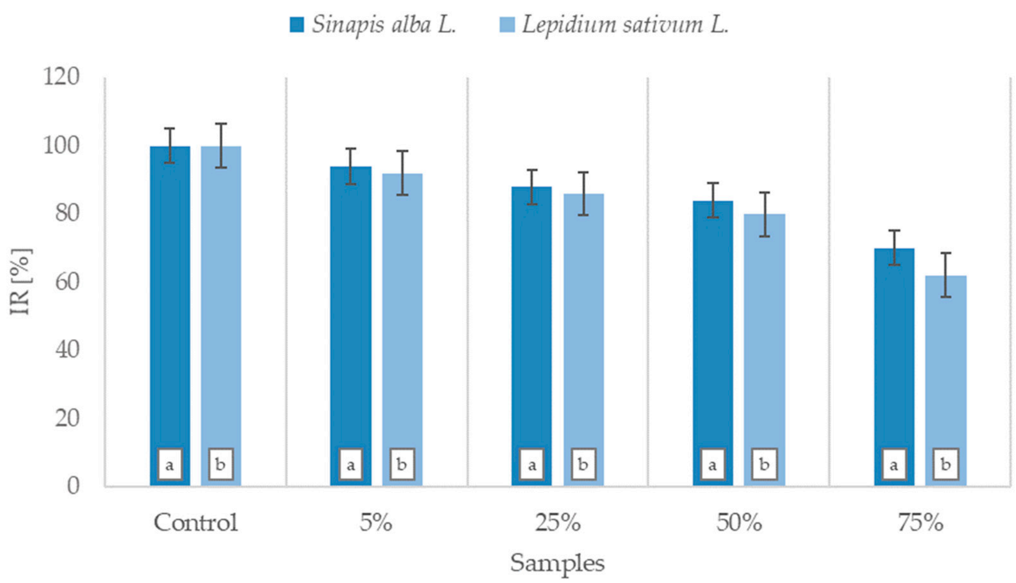

Figure 2. Percentage values of growth inhibition of germinated seeds/growing plants of *Sinapis alba* L. and *Lepidium sativum* L. in 28 days.

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

The values of IR of *Sinapis alba* L. and *Lepidium sativum* L. ranged from 62% to 94%; standard ČSN EN 13432 defines the value of germinated seeds/growing plants below 90% (compared with the values of control sample without the tyre abrasion products) as phytotoxic [25]. Baran and Tarnawski [27] classified growth inhibition values of germinated seed/growing plants below 90% as inhibiting, from 90% to 110% as having no effect (non-phytotoxic) and above 110% as growth stimulating [27]. The germination capacity classified as non-phytotoxic (with no effect) was demonstrated in substrates with 5% of tyre abrasion products both in the growth of *Sinapis alba* L. (IR = 94%) and in *Lepidium sativum*

L. (IR = 92%). *Lepidium sativum* L. is more sensitive to the presence of toxic substances and responded more sensitively to tyre abrasion products, with the number of germinated seeds/growing plants being lower in units than in *Sinapis alba* L. The other substrates with higher shares of abrasion products (25%, 50% and 75%) exhibited the values of germinated seeds/growing plants below 90% and were marked as phytotoxic.

3.2. Biomass Weight from the Experiment of Subchronical Toxicity

In the wet and dry biomass of *Lepidium sativum* L. and *Sinapis alba* L., the resulting weight was compared in individual samples of plants collected from the tested substrates with tyre abrasion products and from the control sample (Figure 3—wet biomass; Figure 4—dry biomass).

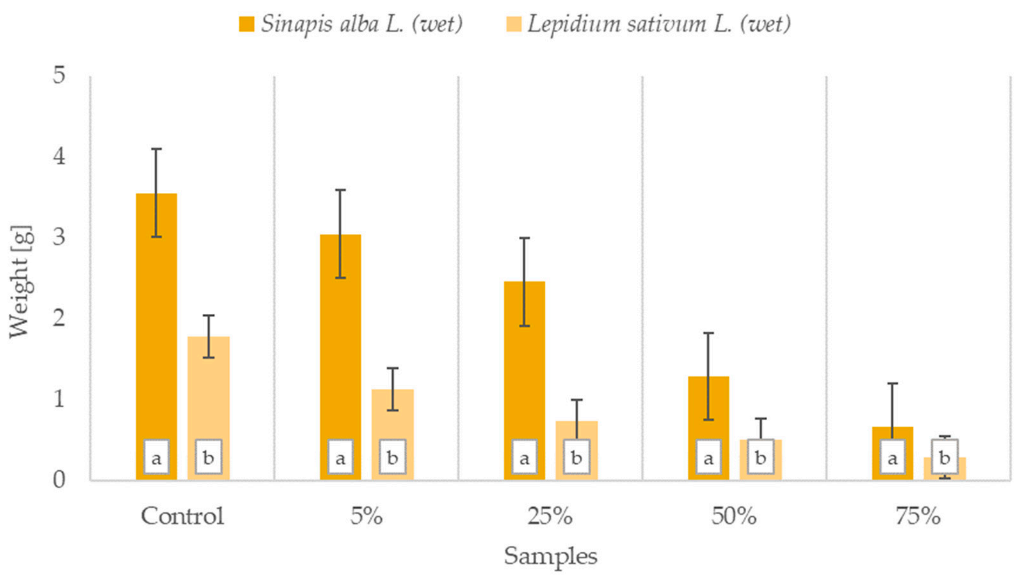

Figure 3. Weight of wet biomass of *Lepidium sativum* L. and *Sinapis alba* L.

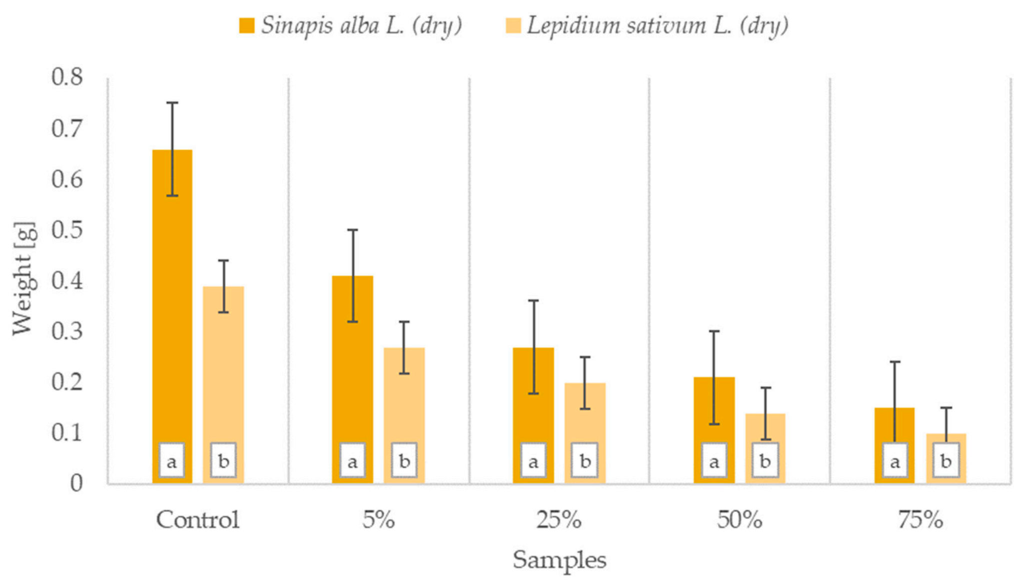

Figure 4. Weight of dry biomass of *Lepidium sativum* L. and *Sinapis alba* L.

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

The highest average wet biomass weight was recorded for a sample with 5% tyre abrasion (3.05 g for *Sinapis alba* L.; 1.13 g for *Lepidium sativum* L.). In contrast, the lowest average wet biomass weight was recorded for a sample with 75% abrasion content (0.66 g for *Sinapis alba* L.; 0.29 g for *Lepidium sativum* L). The highest average weight of dry biomass was recorded for the sample with 5% content (0.41 g for *Sinapis alba* L.; 0.27 g for *Lepidium sativum* L.). In contrast, the lowest average dry biomass weight was recorded for a sample with 75% tyre abrasion (0.15 g for *Sinapis alba* L.; 0.1 g for *Lepidium sativum* L.).

3.3. Biological Test of Tyre Abrasion Products

The results from biological tests of tyre abrasion products phytotoxicity are presented in Figure 5.

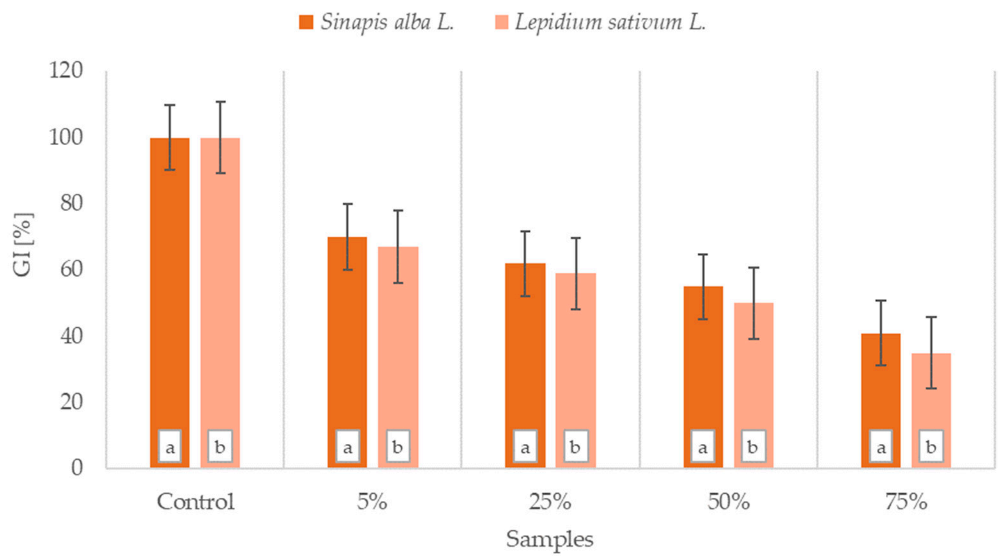

Figure 5. Values Germination index of *Lepidium sativum* L. and *Sinapis alba* L. in the biological test for determination of phytotoxicity.

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

Values of GI decreased with increasing shares of tyre abrasion products. The lowest level of phytotoxicity was recorded in the sample with the 5% share of abrasion products tested on *Sinapis alba* L. (GI = 70%). At the same time, this share of 5% was non-phytotoxic ($66 < GI (\%) < 100$). In contrast, the highest level of phytotoxicity was recorded in the sample with 75% of abrasion products tested on *Lepidium sativum* L. (GI = 35%). *Lepidium sativum* L. reacted again more sensitively to higher shares of tyre abrasion products, with the percentage of phytotoxicity (GI) being higher in units than in *Sinapis alba* L. The biological tests demonstrated phytotoxicity of tyre abrasion products from their share of 25% in the extract.

3.4. Respiration Activity of Soil with the Content of Tyre Abrasion Products

The results of CO_2 respiration are shown in Table 2 and in Figures 6 and 7.

Figure 6 represents a curve of soil biological activity—respiration in the test glass (Solvita) for 24 h for substrate with the predetermined shares of tyre abrasion products. The highest biological activity and, simultaneously, the highest CO_2 emissions released from the soil were recorded in the substrates with 5% and 25% tyre abrasion products. The lowest biological activity and, simultaneously, the lowest CO_2 emissions were recorded in the substrate with 50% and 75% tyre abrasion products.

Table 2. Interpretation of respiration in the experimental receptacle after 24 h (1 day) and upon the end of the experiment with subchronical toxicity after 672 h (28 days).

	Abrasion Products in Substrate	Gel Colour	Biological Activity	Estimated Emissions of CO_2-C (kg·ha^{-1})
After 24 h	0%	Colour 5.52 Light yellow	Very high	96.6
	5%	Colour 5.8 Light yellow	Very high	127
	25%	Colour 6.09 Light yellow	Very high	170
	50%	Colour 4.96 Yellow	Medium high	55.1
	75%	Colour 4.53 Yellow	Medium high	35.5
	Abrasion Products in Substrate	**Gel Colour**	**Biological Activity**	**Estimated Emissions of CO_2-C (kg·ha^{-1})**
After 672 h (28 days)	0%	Colour 5.46 Light yellow	Very high	90.5
	5%	Colour 5.9 Light yellow	Very high	148
	25%	Colour 5.94 Light yellow	Very high	186
	50%	Colour a 5.38 Light yellow	Very high	83.7
	75%	Colour 4.95 Yellow	Medium high	59.7

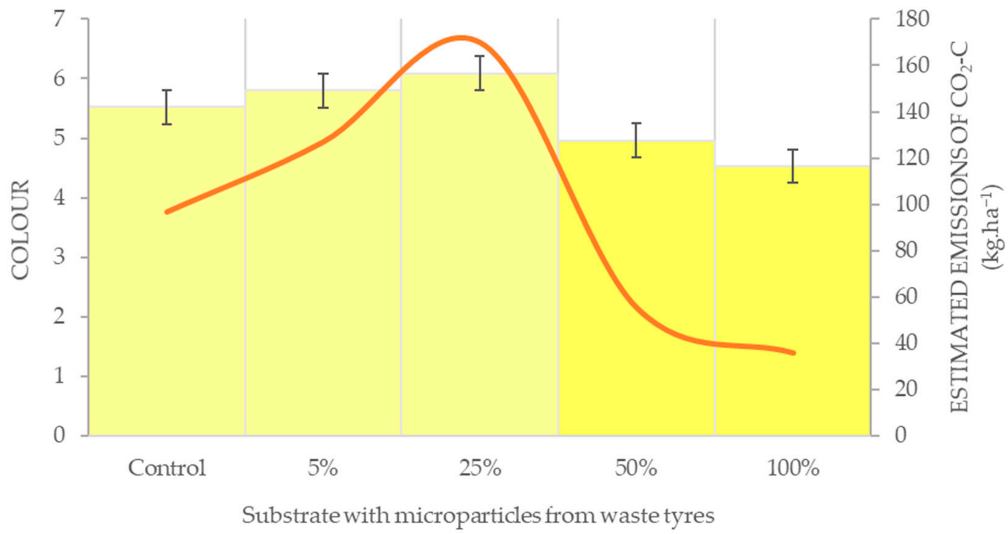

Figure 6. Interpreting the CO_2 respiration after 24 h.

Figure 7 represents a curve of soil biological activity—respiration in the test glass (Solvita) upon the end of the experiment for the substrate with predetermined shares of tyre abrasion products after the end of experiment with subchronical toxicity (after 672 h, i.e., 28 days). The highest biological activity and, simultaneously, the highest CO_2 emissions released from the soil were recorded in the substrates with 5% and 25% tyre abrasion products. The lowest biological activity and, simultaneously, the lowest CO_2 emissions released from the soil were recorded in the substrate with 50% and 75% of tyre abrasion products.

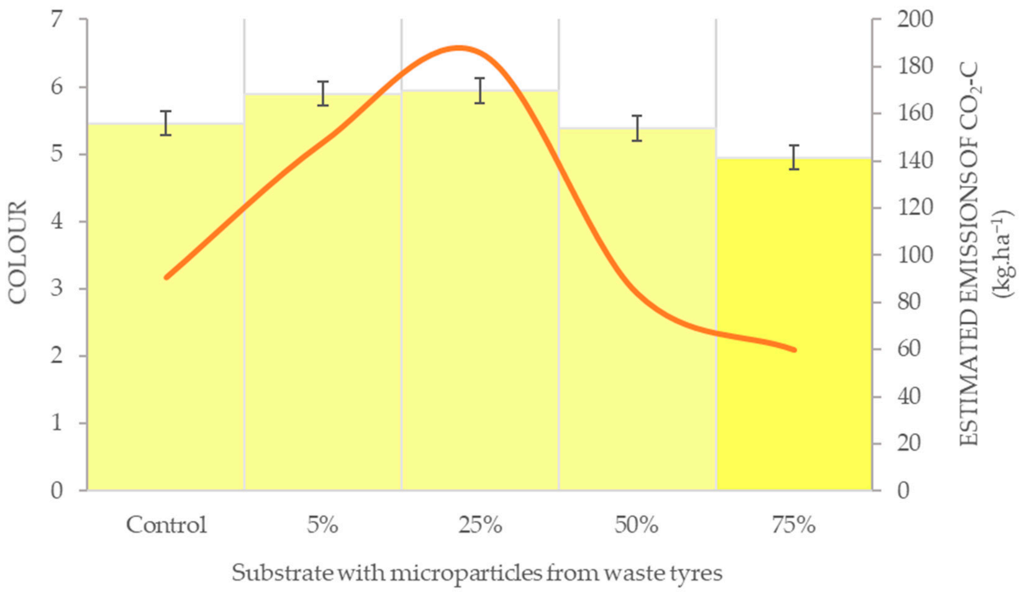

Figure 7. Interpreting the CO_2 respiration after 672 h (28 days).

4. Discussion

4.1. Subchronical Toxicity of Abrasion Products from Tyres

The tests of subchronical toxicity of tyre abrasion products were performed for 28 days as a pot experiment with the plant species of *Lepidium sativum* L. (LS) and *Sinapis alba* L. (SIA) and exhibited IR values from 62% to 94%. As the only sample, the substrate with 5% of abrasion products did not show phytotoxicity when compared with the control sample (IR_{LS} = 92%; IR_{SIA} = 94%). The other substrates with higher shares of tyre abrasion products (IR_{LS} for the shares of 25% = 86%, 50% = 80% and 75% = 62%; IR_{SIA} for the shares of 25% = 88%, 50% = 84% and 75% = 70%) exhibited phytotoxicity (inhibition of germinated seeds/growing plants) when compared with the control sample. The results of the experiment show that the increasing proportion of tyre abrasion had a significant impact in terms of phytotoxicity. In order to assess the effect of tyre abrasion, the parameters of GI (number of germinated seeds), plant biomass growth (wet and dry biomass weight) and IR were examined with respect to root growth stimulation. To the best of the authors' knowledge, studies have not yet been carried out to test the subchronic toxicity of tyre abrasion on *Lepidium sativum* L. and *Sinapis alba* L. However, a similar study was conducted by Leifheit et al. [34], where the authors state that microplastic materials from tyre abrasion products, particularly in high concentrations, affect the growth of plants, their reproduction, metabolism and mortality. Their experiment consisted in testing tyre abrasion products on leek (*Allium porrum* L.) at concentrations ranging from 0 to 160 mg·g^{-1} in containers with soil. In higher concentrations of tyre abrasion products, retarded growth and changes in plant anatomy and morphology were observed. The authors pointed out that the harmful effects are caused primarily by excessive zinc (Zn) values, which were several times higher than setup limits [34].

A similar study was conducted by Salonen et al. [35] who tested the toxicity of tyre abrasion products in soil on common rough woodlouse (*Porcellio scaber*) and white worm (*Enchytraeus crypticus*) in concentrations 0%, 0.02%, 0.06%, 0.17%, 0.5% and 1.5%. The authors claim that the reproduction of white worm decreased by 20% already in a concentration of tyre abrasion products of 0.02%. In a concentration of 1.5%, the reproduction of white worm was reduced by 38%, and the reproduction of common rough woodlouse dropped even more by 65%. In the tested soil, the authors found a wide range of compounds that are, however, not too harmful in low concentrations (0.02%). Higher concentrations of tyre abrasion products (1.5%) were observed to cause negative effects, especially due to high concentrations of Zn and other harmful substances acting for a long time [35].

4.2. Biomass Weight from the Experiment of Subchronical Toxicity

By comparing the weights of wet biomass in *Lepidium sativum* L. and *Sinapis alba* L., it was recorded that they were decreasing in the order of tenths up to units of grams with increasing shares of tyre abrasion products added to the substrate. The reduced weight was recorded in dry biomass too. The occurrence of tyre abrasion products in the soil results in the insufficient uptake of nutrients by plants, and their growth is considerably limited by heavy metals and other organic compounds. Higher values of dry and wet biomass were exhibited by substrate samples with the lowest shares of abrasion products.

Various studies [36–39] reported that heavy metals, which are among the other things also contained in tyres, are toxic to plants and can trigger oxidation stress, which results in the damage of cells. In their research, Councell et al. [40] warned that the wear of tyres results in an annual release of up to 7500 Mg of zinc (Zn) [40].

It is believed that a tyre contains approximately 0.8 $mg \cdot kg^{-1}$ of arsenic; 0.28–5 $mg \cdot kg^{-1}$ of cadmium; 0.4–10 $mg \cdot kg^{-1}$ of chromium; 0.9–25 $mg \cdot kg^{-1}$ of cobalt; 1–50 $mg \cdot kg^{-1}$ of nickel; 1–160 $mg \cdot kg^{-1}$ of lead; and 81–420 $mg \cdot kg^{-1}$ of aluminium. Nevertheless, the highest content is that of zinc (up to 10,000 $mg \cdot kg^{-1}$) in the form of zinc oxide (ZnO) or zinc sulphate (ZnS) [41]. Yadav [38] claims that a surplus of cadmium causes impaired function of photosynthesis and reduced uptake of nutrients in plants [38]. Thus, cadmium and zinc are significant triggers of phytotoxicity in higher plants [38,42,43]. Li et al. [44] tested the effect of cobalt in the soil on the total biomass weight of tomatoes, barley and oilseed rape. In their study, the authors found out that a higher concentration of cobalt in the soil significantly affects the growth of shoots and impairs the yield of plants [44].

Sheng et al. [45] performed a similar study in which they monitored changes in the weight of earthworms (*Eisenia fetida*) in the substrate with 5%, 25%, 50% and 100% proportions of microplastics from tyres (abrasion). The authors state that when *Eisenia fetida* is exposed to higher proportions of tyre abrasion, it increases their mortality and reduces their body weight [45].

4.3. Biological Test of tyre Abrasion Products

The biological test of phytotoxicity is based on the reaction of sensitive plant in the environment of aqueous extracts from tyre abrasion products. The degree of phytotoxicity, which is a direct reflection of the content of toxic substances [29,30], is expressed by means of GI.

Šourková et al. [29] tested phytotoxicity in a 100% concentration of extracts from the fractions of waste tyres (10 × 4 cm) in DW and in water from a recipient (PW) on the seeds of *Lepidium sativum* L. The values of extract from the fractions of tyres in the DW sample (GI = 49%) as well as in the PW sample (GI = 67%) were evaluated as phytotoxic at the end of the study [29]. Bouda and Formánková [30] claimed in their study that GI expressed in the percentage (%) of control sample (DW without the addition of tyre abrasion products) above 66% (GI > 66%), which indicates an extract with the determined share of tyre abrasion products as stable (non-phytotoxic/with no effect). GI values ranging from 26% to 65% (26% < GI > 66%) indicated an extract as phytotoxic, and GI values below 25% (GI < 25%) indicated an extract as highly phytotoxic [30]. Extracts from tyre abrasion products (GI_{LS} for the shares of 25% = 59%, 50% = 50% and 75% = 35%; GI_{SIA} for the shares of 25% = 62%, 50% = 55% and 75% = 41%) were classified as phytotoxic (26% < GI > 66%). The extract with 5% of tyre abrasion products tested on *Lepidium sativum* L. (GI = 67%) and *Sinapis alba* L. (GI = 70%) was classified as non-phytotoxic (GI > 66%). In their study from 2006, Plíva et al. [46] stated that GI values above 80% (GI > 80%) are indicated as stable (non-phytotoxic/with no effect) when compared with the control sample of extract without tyre abrasion products. GI values ranging from 60% to 80% (60 < GI > 80%) indicate the extract as phytotoxic, and GI values below 59% (GI < 59%) indicate the extract as highly phytotoxic [46].

Compared with results of the study published by Šourková et al. [29], it was demonstrated that an extract from tyre abrasion products itself indicates much lower GI values (%) than an extract from the entire tyre fraction. The 100% concentrations of extract from tyres in DW tested on *Lepidium sativum* L. exhibited values of GI = 49% [29]. In the current study, the GI values in the 75% concentration of extract from tyre abrasion products in distilled water (DW) for *Lepidium sativum* L. were 35% (in *Sinapis alba* L., they were 41%). As stated by Wik and Dave [47], tyre abrasion products from the tyre tread pattern represents much more serious impacts at the stage of tyre service life than expected. Microparticles from tyre abrasion products exhibit much greater leachability and are much more toxic than extracts prepared from complete tyres [47,48]. The biological tests of the phytotoxicity of extracts from tyre abrasion products demonstrated that even low concentrations have a significant impact on the metabolism of plants. High concentrations of extracts considerably disturb the defence system of plants and gradually cause their death [29].

4.4. Respiration Activity of Soil with Content of Tyre Abrasion Products

The study evaluated the respiratory activity of the soil after 24 h and 672 h (28 days) in contact with individual concentrations of tyre abrasion. After 24 h, the Solvita kit evaluated a light-yellow colour level for substrates with 5% and 25% abrasions, indicating soil with high biological activity with an excellent supply of organic matter. For substrates with 25%, 50% and 75% abrasion contents, the yellow colour level was evaluated, which indicates biologically very active soil with a high content of organic matter. After 28 days (672 h), the Solvita kit evaluated the light-yellow colour level for substrates with 5%, 25% and 50% abrasions, indicating soil with high biological activity with an excellent supply of organic matter. The yellow colour level was evaluated for the substrate with 75% abrasion, which indicates biologically very active soil with a high content of organic matter [33].

The results show that the application of 5% and 25% abrasion increases the biological activity of soil and increases CO_2-C emissions. Leifheit et al. [34] in their study also performed a container experiment in which they tested 0–160 mg·g^{-1} concentrations of tyre abrasion and their effect on soil respiration. The experiment was terminated by harvesting *Allium porrum* after 42 days. The authors found that tyre abrasion altered bulk density and soil aeration, which probably improved the conditions for microbial activity at higher abrasion concentrations [34]. Kreider et al. [49] reported that tyre abrasion is a significant source of C in the form of synthetic polymers and carbon blacks, which are easily leached and available to soil microorganisms [34,50]. Microplastic was reported to contain about 80% C, which is considered a global factor of change in anthropogenic activity [51,52]. However, tyre wear does not only contain synthetic polymers and carbon black [53] but is also a source of heavy metals, reactive additives and polycyclic aromatic hydrocarbons that accumulate in the soil. Gülser and Erdo [54] reported that soil respiration has gradually decreased with increasing distance from the road, depending on the heavy metals involved. Based on our results and in comparison with the authors' studies, we believe that low abrasion concentrations (5% and 25%) have a positive effect on soil respiration and microbial processes in the soil. However, we assume that the negative effects of abrasion will not manifest themselves in soil over time.

5. Conclusions

In our study, subchronical toxicity of tyre abrasion products were determined. It was found out that substrates with a 5% share of abrasion products exhibited a germination capacity of 92% in *Lepidium sativum* L. and 94% in *Sinapis alba* L. These two substrates were did not experience effects in terms of phytotoxicity. Other substrates with higher shares of tyre abrasion products (25%, 50% and 75%) exhibited growth inhibition (phytotoxic effect).

The end of the test with subchronical toxicity was followed by the test in which the effect of tyre abrasion products on the total weight of wet and dry biomass was determined in *Lepidium sativum* L. and *Sinapis alba* L. It was demonstrated that higher shares of abrasion products in soil reduced biomass weight. Plants reacted to the situation by reducing the uptake of nutrients; their growth was significantly limited due to heavy metals and other organic compounds and they gradually died.

Acute phytotoxicities of extracts from tyre abrasion products were established by means of biological tests on the seeds of *Lepidium sativum* L. and *Sinapis alba* L. It was demonstrated that extracts with higher shares of abrasion products (25%, 50% and 75%) were phytotoxic. The 5% extract did not exhibit any effects, and its phytotoxicity was not proven.

At the end of the study, biological activity of the soil (substrate) was evaluated, which was exposed to the effects of tyre abrasion products after 24 h (1 day) and 672 h (28 days). In substrates with higher abrasion rates, respiratory activity and CO_2 emissions increased over time at 5% and 25% concentrations. Higher abrasion concentrations (50% and 75%), in turn, gradually reduced respiratory activity.

This research study aimed to contribute to another evaluation of abrasion products from tyres arising during the phase of their use. Testing phytotoxicity in higher plants (*Lepidium sativum* L. and *Sinapis alba* L.) and the determination of soil biological activity provided further results for studying the negative effects of tyre abrasion products and, hence, microplastic particles on the environment.

Author Contributions: Conceptualization, M.Š., D.A. and M.D.V.; methodology, M.Š. and D.A.; validation, D.A., and M.D.V.; formal analysis, M.Š. and D.A.; investigation, M.Š.; data curation, D.A. and M.D.V.; writing—original draft preparation, M.Š., D.A. and M.D.V.; writing—review and editing, M.D.V.; visualization, M.Š. and M.D.V.; supervision, D.A.; project administration, M.D.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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