

Article

Biological Assessment of Soils Following Waste Tyre Fires and Potential Remediation—A Case Study

Markéta Škrabalová, Dana Adamcová * and Milada Šťastná

Department of Applied and Landscape Ecology, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic; marketa.skrabalova@mendelu.cz (M.Š.); milada.stastna@mendelu.cz (M.Š.)

* Correspondence: dana.adamcova@mendelu.cz (D.A.)

Abstract

Waste tyre fires are a significant environmental issue that leads to the release of toxic substances into the soil, particularly polycyclic aromatic hydrocarbons and heavy metals. These contaminants can adversely affect the physicochemical properties of the soil, its microbial activity, and plant growth. The aim of this study is to assess the degree of phytotoxicity in soils affected by tyre waste fires using acute and biological tests, while simultaneously measuring microbial respiration as an indicator of soil biological activity. Furthermore, the effectiveness of the application of a 5% dose of biochar as a remediation measure was evaluated. The results showed that leachates from contaminated soils exhibited phytotoxic effects, with growth inhibition of 26.94–28.12% and reduced seed germination of 55.6–55.9%. The application of biochar to the soil under study significantly reduced phytotoxicity-induced growth inhibition (20–23.11%) and improved seed germination rates (79.76–83.71%). Microbial respiration gradually increased following the application of biochar; after 28 days it was over 30% higher compared to soils without biochar amendment. This study confirms that biochar can be an effective amendment that improves the biological quality of soils impacted by tyre waste fires.

Keywords: biochar; phytotoxicity; germination index; root growth inhibition; soil respiration; waste

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1. Introduction

The storage and disposal of used tyres represent a major worldwide ecological challenge, as they rank among the fastest-growing forms of non-biodegradable waste on the planet, largely due to the rapid expansion of vehicular transport [1].

Tyres are composed of synthetic rubber polymers, reinforcing fillers, and chemical additives, including heavy metals (HMs) such as zinc (Zn) and copper (Cu), as well as organic compounds such as polycyclic aromatic hydrocarbons (PAHs) and other reactive additives [2–5].

Tyre fires are primarily initiated by human activities, whether accidental or deliberate. In recent decades, numerous tyre fire incidents have been reported [6–10], which have posed significant environmental hazards due to the emission of toxic chemical substances.

Open-air fires of waste tyre (WT) stockpiles present severe environmental risks [11–13]. During tyre combustion, complex mixtures of toxic substances are released into the

atmosphere, surface waters, and especially soils. Most notable are PAHs, HMs, partial degradation products of organic polymers, and volatile organic compounds (VOCs) [13,14]. These contaminants can alter the physicochemical properties of soil (e.g., pH, sorption capacity, and structure), limit nutrient availability, and disrupt microbial communities as well as plant growth [15,16]. Gaseous and particulate emissions can be transported over long distances, from metres to hundreds of kilometres, before deposition occurs [17].

Soil is an irreplaceable natural resource and one of the most valuable assets for sustaining life on Earth [18]. Therefore, an assessment of a contaminated site requires the application of biological assays that can directly evaluate the environmental risk and functional impacts on living organisms.

One promising soil remediation strategy involves the use of biochar. Biochar, produced through the thermochemical decomposition of biomass, is an environmentally friendly alternative to various carbon-based materials and can be used for carbon sequestration as a climate change mitigation measure [19]. In recent years, biochar has been extensively studied for use as a potential sorbent of contaminants and an agent that improves soil properties [20,21]. Biochar has attracted attention due to its beneficial effects on soil quality, including enhanced soil fertility, carbon storage, and contaminant immobilisation [22].

Its principal mechanisms of action include the increased sorption of organic contaminants (reducing their bioavailability), stronger binding of HMs, stabilisation of organic matter, pH regulation, and improved water-holding capacity. Furthermore, biochar provides suitable microhabitats for soil microbial communities [23], which may lead to reduced soil phytotoxicity and enhanced soil respiration. However, the effectiveness of biochar depends on its type, application rate, and the specific properties of the contaminated soil.

Current research increasingly focuses on interventions aimed at restoring soils in severely degraded areas [24–27]. Some studies have explored the use of biochar as a remediation amendment, including recommendations for its application [24], or have evaluated the effectiveness of biochar produced from biosolids for the remediation of petroleum-contaminated soils [25]. Other studies have concentrated on plant-based remediation strategies: Băbău et al. [26] investigated the rehabilitation of sterile mining spoil heaps, whereas Băbău et al. [27] examined the phytoremediation of spoil heaps using *Robinia pseudoacacia*.

While previous research has primarily focused on the remediation of soils contaminated with metals and organic compounds using biochar [24,25] or on plant-based remediation strategies [26,27], a clear gap remains in the literature regarding the assessment of soil phytotoxicity following waste tyre fires under real-world conditions. The present study addresses this gap by investigating the effect of a 5% biochar amendment in Cambisol soil affected by tyre fires, combining biological phytotoxicity assays with measurements of microbial respiration to provide a more integrated assessment of soil biological quality.

The objectives of this study were to (1) conduct microbiological bioassays of soils affected by WT fires; (2) determine the degree of phytotoxicity using seeds of selected plant species; (3) evaluate the development of microbial respiration (a) prior to the WT fire, (b) following the WT fire, and (c) following the WT fire with the addition of a biochar amendment; and (4) verify the effectiveness of a 5% biochar amendment in reducing phytotoxicity and enhancing soil biological activity.

2. Materials and Methods

For this study, a total of 20 kg of soil was collected in a single sampling event from a depth of 0–0.20 m at an elevation of 385 m a.s.l. in the Czech Republic (South Moravian

Region) at the geographic coordinates 49.4320075 N, 16.3813253 E (Figure 1). The soil sampling was conducted in accordance with the mandatory legislative Regulation No. 153/2016 Coll., which specifies the requirements for the protection of agricultural soil quality (Annex 4). This regulation provides empirical guidance on the minimum number of samples to be collected, depending on the size of the surveyed area (for the demarcated plot of 115 m², a total of three composite samples were collected) [28]. The soil type corresponds to Cambisol from lower elevations, typical of a temperate zone and characterised by a high level of biological activity [29]. The soil samples were placed in polyethylene bags and transported to the laboratory under cool conditions (4 ± 2 °C). During sampling, packaging, and storage, particular care was taken to maintain container cleanliness and appropriate holding times.

From the total quantity (20 kg), 10 kg of soil was stored in a separate sterile container and designated as a control soil (CS), serving as a reference for the soil conditions prior to WT combustion. The remaining 10 kg of soil was used for the experimental combustion of waste tyres under real-world conditions.

The experimental combustion was conducted under outdoor (real-world) conditions within a confined plot (2 × 2 m), isolated from its surroundings. The WT was cleaned, cut into pieces, and ignited using a gas burner under controlled conditions. After complete combustion, the fire was extinguished by a certified professional using distilled water (DW). After the fire was extinguished, soil samples were collected from directly beneath the burned tyre at the affected site, air-dried under laboratory conditions, sieved through a 2 mm mesh to remove coarse fractions, and subsequently subjected to testing. This post-fire soil sample is hereafter referred to as SAF (Soil After Fire).

To investigate post-fire changes in the soil (germination, phytotoxicity, microbial respiration) and evaluate potential remediation measures, a portion of the post-combustion soil (5 kg, half of the SAF samples) was amended through the application of 5% (*w/w*) biochar. This amended soil sample is referred to as SAFB (Soil After Fire with Biochar amendment). SAFB was included in the testing series. The application rate was selected based on previous biochar studies [30–33], which encompassed a variety of soil types and substrates. For example, Zhu et al. [30] applied a 5% biochar amendment to silt loam soil during rice cultivation, observing reductions in yield and alterations in the microbial community, whereas Guo et al. [31] applied 5% biochar to clayey sand (SC)–recycled concrete aggregate and reported significant improvements in plant growth, water retention, and the physical properties of the substrate. Bhat et al. [32] reported beneficial effects of biochar on sandy and medium-textured soils, while Antonangelo et al. [33] reported positive effects on agroforestry and forest soils with varying textures (from sandy to clayey). These findings support the appropriateness of a 5% application rate, although the ultimate effect depends on soil type and specific experimental conditions.

The biochar used in this study was produced as the final product of the pyrolysis of waste wood (pallets and wooden transport packaging) and is certified for use in organic production in accordance with Regulation (EU) 2018/848 of the European Parliament and of the Council. It is composed of 90% carbon and consists of a fine fraction with a particle size of 0–1 mm [34].

All soil samples were subjected to biological assays (48 h), acute phytotoxicity assays (72 h), and measurements of microbial respiration over a 28-day incubation period. The application of the high biochar dose was intended to evaluate the extent to which it could mitigate phytotoxic effects, increase seed germination rate, and enhance soil microbial respiration.

An overview of the experimental design is presented in Figure 1.

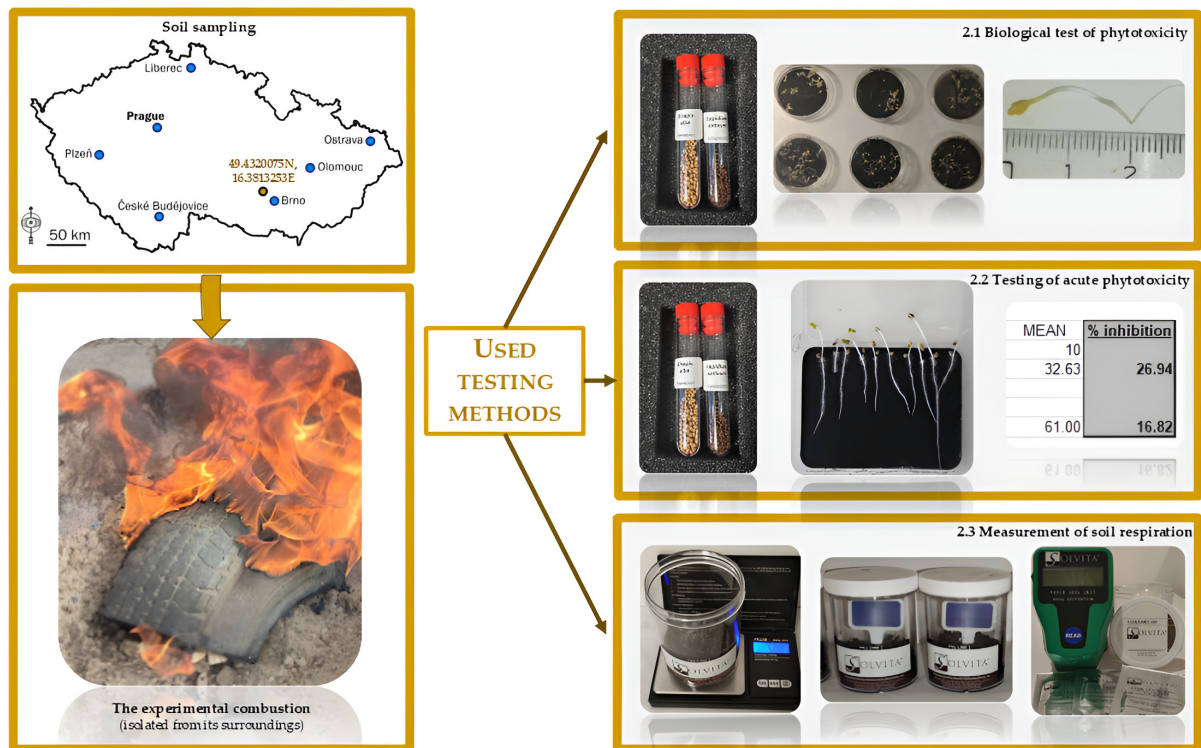


Figure 1. Schematic representation of soil bioassays (using *Sinapis alba* L. and *Lepidium sativum* L.) and measurement of soil respiration following waste tyre combustion under real-world conditions. The map included in the figure was adapted from Ref. [35].

2.1. Biological Test of Phytotoxicity of Soil Sample After Tyre Fire

The biological phytotoxicity bioassay was conducted following the procedure developed by ALS Czech Republic, s.r.o. [36], using *Sinapis alba* L. (white mustard) and *Lepidium sativum* L. (garden cress) as test species.

A sterilised filter paper was placed at the bottom of a sterilised Petri dish (Ø 85 mm), to which 10 mL of the prepared soil extract was applied. The extract was prepared according to the standard protocol at a temperature of 22 ± 2 °C. A representative soil sample collected from the area affected by the tyre fire was homogenised and sieved through a 10 mm mesh. A soil extract was prepared by mixing 50 g of the sample (on a dry weight basis) with 500 mL of distilled water, at a solid-to-liquid ratio of 1:10. The mixture was shaken on a rotary shaker for 1 h, then centrifuged (8 min, 4000–5000 rpm) and finally filtered through a 5 µm pore-size filter paper. Subsequently, 30 seeds were evenly distributed across the surface of the dish. The Petri dishes were placed in an incubator and incubated in the dark at 26 ± 2 °C for 48 h. Each treatment was performed in triplicate. After exposure, photographs were taken, and root lengths were manually measured to an accuracy of 1 mm [36].

To assess the degree of soil phytotoxicity, the germination percentage ($G(\%)$) was first calculated (Equation (1)) based on the number of germinated seeds in the test sample (Q_T) and CS (Q_C). Root elongation ($L(\%)$) was determined (Equation (2)) using the average root length of the test sample (L_T) and the CS (L_C). Finally, the germination index ($GI(\%)$) was calculated (Equation (3)) [36], and the results were interpreted according to the criteria in Table 1.

Germination percentage:

$$G(\%) = \frac{Q_T}{Q_C} \times 100 \quad (1)$$

Elongation:

$$L(\%) = \frac{L_T}{L_C} \times 100 \quad (2)$$

Germination index:

$$GI(\%) = \frac{G \times L}{100} \times 100 \quad (3)$$

Table 1. Range and interpretation of the germination index [37,38].

GI(%) Range	Interpretation
<25	Highly phytotoxic
26–65	Phytotoxic
>66	Non-phytotoxic

2.2. Testing of Acute Phytotoxicity of Soil After Tyre Fire

The acute toxicity bioassay evaluates the degree of sample toxicity towards higher plants over a 72 h exposure period. Phytotoxicity was determined using Phytotoxkit™ test kits (MicroBioTest Inc., Ghent, Belgium), with *S. alba* and *L. sativum* employed as test species, following the Phytotoxkit™ standard protocol [39]. Both dicotyledonous species are recognised as sensitive indicators of soil contaminants, capable of signalling the presence of pollutants in soil and are widely used in phytotoxicity assays [40]. These species are characterised by rapid germination and growth, ease of cultivation, and high sensitivity to stress factors. Furthermore, both species were also included in our previous studies, where their suitability for testing was repeatedly confirmed owing to their favourable traits and reliability [41–43].

The Phytotoxkit™ test system consists of two chambers. The lower chamber, which contains the soil under test and a pre-determined volume of distilled water (DW) adjusted to the sample's water retention capacity, and the upper chamber, which serves as the germination and root growth zone for the test seeds. Following hydration of the lower chamber, a filter paper was placed on the soil surface, and ten seeds of the same plant species were evenly distributed. As a control, an artificial OECD reference soil was used, consisting of 5% CaCO₃ and peat, 10% kaolinite clay, and 85% quartz sand [39].

Each sample (CS, SAF, SAFB) was tested in triplicate. Incubation was conducted in the dark in an incubator at 25 ± 1 °C for 72 h, in accordance with the Phytotoxkit™ methodology [39].

At the end of the incubation period, the test plates were photographed, and the germinated root lengths were measured (in mm) using UTHSCSA ImageTool 3.0 software (University of Texas Health Science Center, San Antonio, TX, USA) [39].

The percentage of root growth inhibition (*IR*(%)) was calculated using Equation (4), where *L_C* represents the mean root length in the control (OECD reference soil) and *L_T* the mean root length in the test sample. A positive *IR*% value indicates inhibition (phytotoxicity), whereas a negative value indicates stimulation of root growth (non-phytotoxicity) [39].

Root growth inhibition:

$$IR(\%) = \frac{L_C - L_T}{L_C} \times 100 \quad (4)$$

All procedures were performed in accordance with the Phytotoxkit™ standard protocol [39].

2.3. Measurement of Soil Respiration After Tyre Fire

The soil samples tested—CS, SAF, and SAFB—were analysed for basal soil respiration following the Solvita Natural Soil Respiration Test Kit, SOP Version: 2019:2.1, according to the standard procedure for testing of natural soil respiration [44].

An aliquot of 0.05 kg of soil was weighed into a 475 mL polystyrene incubation container and moistened with distilled water (DW) to the appropriate 70% field capacity [45]. A CO₂ detection probe was inserted vertically into the container, which was then tightly sealed. The test was conducted in triplicate for each sample (CS, SAF, and SAFB) and incubated at 21 ± 1 °C for 24 h. After each 24 h incubation period (a total of five incubation periods: 1st day, 7th day, 14th day, 21st day, and 28th day), the colour of the probe was recorded using the Solvita Digital Colour Reader (DCR), which is an integral component of the kit, and subsequently converted into CO₂ emission values (kg ha⁻¹) according to the Solvita conversion protocol. The test kit and DCR are manufactured by Woods End Laboratories, Inc., PO Box 297, Mt Vernon, ME 04352, USA [44].

3. Results

3.1. Biological Test of Phytotoxicity of Soil Sample After Tyre Fire

The results of GI(%) for *S. alba* and *L. sativum* obtained from the soil extract bioassay (CS, SAF and SAFB) are presented in Figure 2.

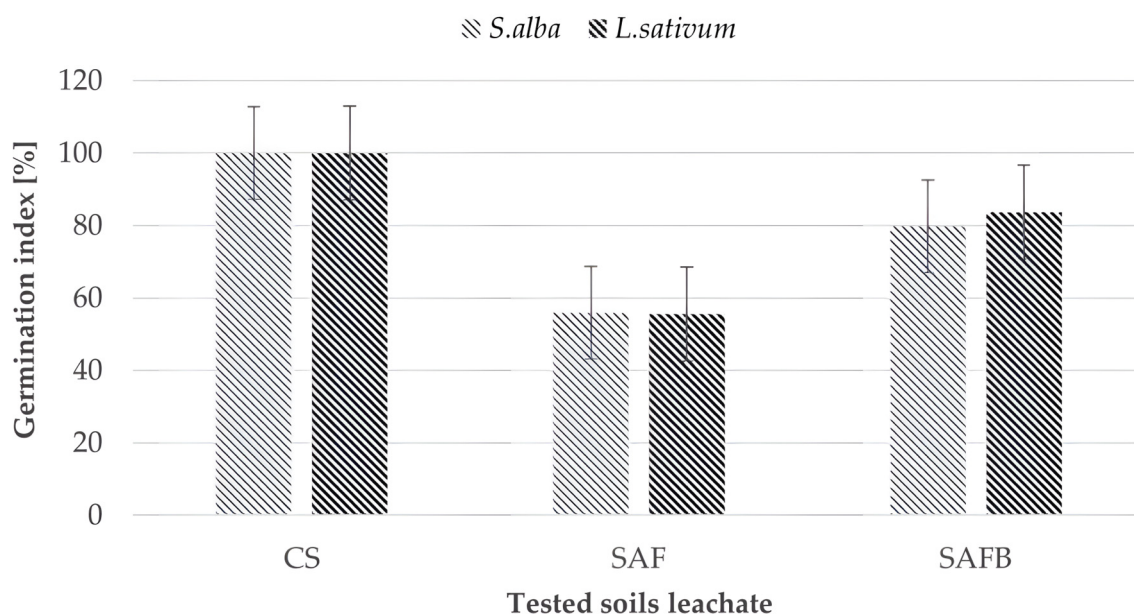


Figure 2. Germination index (%) of *Sinapis alba* L. and *Lepidium sativum* L. in soil extracts from Control Soil, Soil After Fire, and Soil After Fire with Biochar in the biological phytotoxicity bioassay.

The GI(%) values of the soil extracts (SAF and SAFB) are shown in Figure 2 as mean values, ranging from 55.63% to 83.71%. For both plant species tested, a marked increase in germination was observed in the SAFB variant compared to the SAF variant.

For *S. alba*, the GI(%) was 55.89% in the SAF extract, whereas the SAFB extract exhibited a significantly higher value of 79.76%, representing an increase of approximately 42.71%. A similar trend was observed for *L. sativum*, where the germination increased from 55.63% (SAF) to 83.71% (SAFB), corresponding to an increase of approximately 50%. By comparison, the CS extract exhibited a GI(%) value of 100% for both plant species.

These findings clearly indicate that the SAFB extract promoted higher seed germination in both test species and, according to Table 1, can be considered to be non-phytotoxic, whereas the SAF extract is classified as phytotoxic.

Although the differences between SAF and SAFB were not statistically significant ($p > 0.05$), the application of biochar resulted in a clear increase in $GI(\%)$ in both *S. alba* and *L. sativum*, indicating a biologically relevant improvement in soil conditions.

3.2. Testing of Acute Phytotoxicity of Soil After Tyre Fire

The results of $IR(\%)$ for *S. alba* and *L. sativum* obtained from the acute phytotoxicity bioassay of soils (SAF and SAFB) are presented in Figure 3.

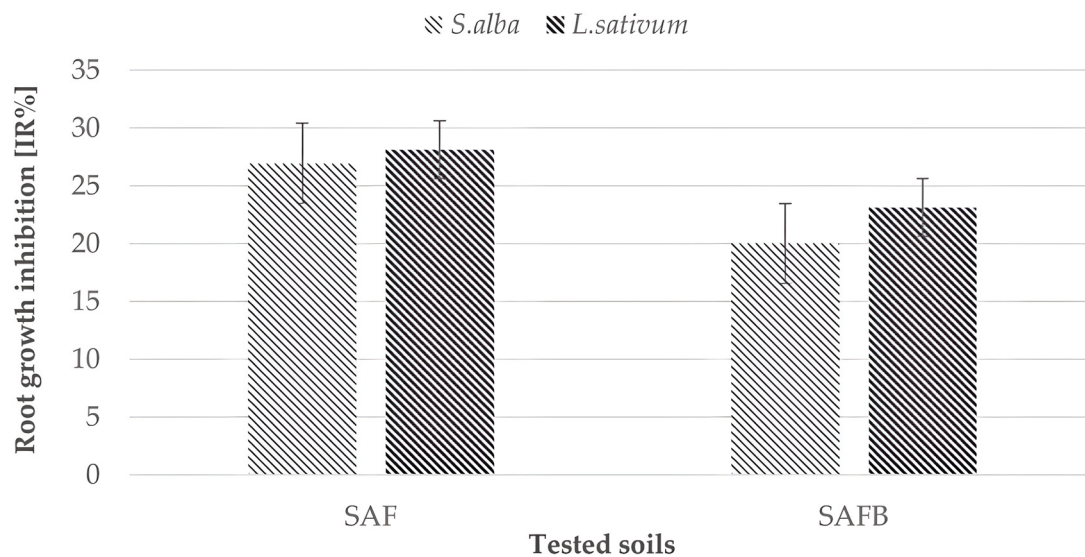


Figure 3. Root growth inhibition (%) of *Sinapis alba* L. and *Lepidium sativum* L. for Soil After Fire and Soil After Fire with Biochar soils.

The $IR(\%)$ values for the SAF and SAFB soils are shown in Figure 3 as mean values, ranging from 20.02% to 28.12%. These values indicate the inhibition (suppression) of root growth in *S. alba* and *L. sativum*. According to the Phytotoxkit™ interpretation, all the samples tested ($IR(\%) > 0$) were classified as phytotoxic [39].

In the soil variant amended with 5% biochar (SAFB), a decrease in $IR(\%)$ was observed compared to the SAF variant, by approximately 25.69% for *S. alba* and 17.82% for *L. sativum*.

Although the differences between SAF and SAFB were not statistically significant ($p > 0.05$), the application of biochar resulted in a clear reduction in $IR(\%)$ in both *S. alba* and *L. sativum*, indicating a biologically relevant improvement in soil conditions.

3.3. Measurement of Soil Respiration After Tyre Fire

The results of soil respiration testing, including gel colour, biological activity, and estimated CO_2-C emissions ($kg\ ha^{-1}$) for the CS, SAF, and SAFB soils in incubation containers at defined time intervals (1st, 7th, 14th, 21st, and 28th days), are presented in Table 2. The temporal progression of soil respiration is shown in Figure 4.

Table 2. Soil respiration activity of the Control Soil, Soil After Fire, and Soil After Fire with Biochar samples in incubation containers at specified time intervals.

Measurement Interval	Soil Sample	Gel Colour (Value)	Biological Activity
1st day	CS	LY (4.58)	Medium high
	SAF	LY (4.19)	Medium high
	SAFB	Y-G (3.45)	Ideal

7th day	CS	LY (4.72)	Medium high
	SAF	LY (4.83)	Medium high
	SAFB	LY (4.83)	Medium high
14th day	CS	LY (4.84)	Medium high
	SAF	LY (4.89)	Medium high
	SAFB	LY (4.91)	Medium high
21st day	CS	LY (4.58)	Medium high
	SAF	LY (4.08)	Medium high
	SAFB	LY (4.76)	Medium high
28th day	CS	LY (4.48)	Medium high
	SAF	LY (4.35)	Medium high
	SAFB	LY (4.75)	Medium high

The “ideal” activity that was only observed for SAFB on day 1 indicates a biologically active microbial population and good organic matter content. The CO₂ detection probe for the sample showing ideal activity turned yellow-green (Y-G). A “Medium high” activity, observed in all other soil samples, indicates highly active microbial populations with high organic matter content. The probe colour for medium high activity is light yellow (LY) [44].

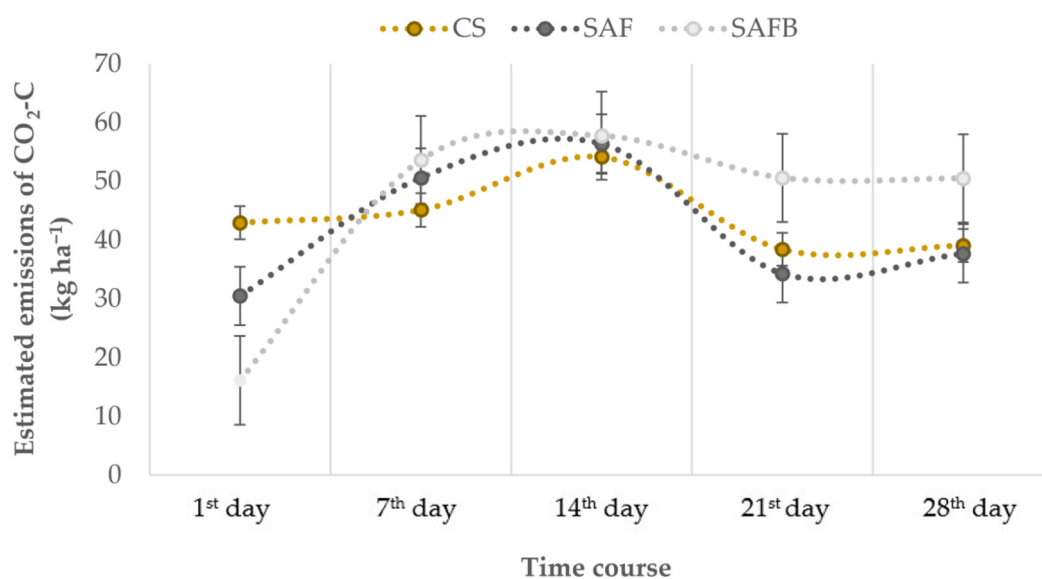


Figure 4. Temporal progression of soil respiration in Control Soil, Soil After Fire, and Soil After Fire with Biochar over the incubation period.

The soil respiration results, presented in Figure 4, ranged from 16.12 to 57.77 kg CO₂-C ha⁻¹ over the incubation period (1–28 days). According to the SOLVITA SOP (Version: 2019:2.1) [44], the SAFB sample exhibited ideal biological activity and a high organic matter content after 24 h. The remaining CS and SAF samples showed medium high biological activity throughout the measurement period, indicating high organic matter content.

Peak biological activity was observed on day 14 for all samples: CS = 54.17 kg CO₂-C ha⁻¹, SAF = 56.43 kg CO₂-C ha⁻¹, and SAFB = 57.77 kg CO₂-C ha⁻¹. The lowest activity was recorded after 24 h for SAF (30.49 kg CO₂-C ha⁻¹) and SAFB (16.12 kg CO₂-C ha⁻¹). For CS, the lowest respiration values were observed at the end of the experiment on day 21 (38.43 kg CO₂-C ha⁻¹).

At the start of the experiment (24 h), CO₂-C emissions were 30.49 kg ha⁻¹ for SAF and 16.12 kg ha⁻¹ for SAFB. By day 28, these values had increased to 37.73 kg ha⁻¹ (+23.75%) for SAF and 50.50 kg ha⁻¹ (+213%) for SAFB. At the end of the experiment, the SAFB sample exhibited approximately 33.85% higher CO₂-C emissions compared to SAF.

Statistically significant differences were observed at 24 h and 21 days ($p < 0.05$), whereas differences at 7, 14, and 28 days were not statistically significant ($p > 0.05$). Nevertheless, the application of biochar resulted in a clear increase in soil respiration in SAFB samples after 7–14 days, indicating a biologically relevant stimulation of microbial activity. In contrast, the SAF samples showed a transient peak at 14 days followed by a decline, which is consistent with the expected inhibitory effect of residual toxic compounds originating from tyre combustion. Therefore, the temporary increase in CO₂-C emissions in SAF does not contradict expectations; rather, it reflects an initial mobilisation of easily decomposable substrates before the effects of toxicity become dominant.

4. Discussion

4.1. Biological Test of Phytotoxicity of Soil Sample After Tyre Fire

The influence of biochar on the early growth stages of plants has rarely been investigated [46]. Seeds represent the initial phase of a new crop cycle, and successful germination is a critical determinant of crop establishment, productivity, and overall system resilience [47].

The eluates of the SAF exhibited phytotoxic effects towards the test species, *S. alba* ($GI = 55.89\%$) and *L. sativum* ($GI = 55.63\%$), according to the classification of Meena et al. [37] and Boutasknit et al. [38], which defines phytotoxicity as the GI range of 26–65%. In contrast, the eluates of the SAFB soils, which received a 5% biochar amendment, were classified as non-phytotoxic ($GI > 66\%$), with GI values of 79.76% for *S. alba* and 83.71% for *L. sativum* [37,38]. The addition of 5% biochar to post-fire soils (SAFB) resulted in an increase in the germination of 42.71% for *S. alba* and 50.48% for *L. sativum* compared to the unamended variant (SAF).

According to the classification reported by Sobarzo-Bernal et al. [48], the SAF eluates for both species (*S. alba* and *L. sativum*) would be considered moderately phytotoxic (GI 50–80%), posing a potential risk to sensitive plant species. Conversely, the SAFB eluates would be regarded as stable or non-phytotoxic ($GI > 80\%$), reflecting an improved soil environment supportive of germination and early seedling growth [48].

The observed improvement in germination following biochar application is consistent with findings reported by Ogunremi et al. [49], who demonstrated improved germination rates of *Zea mays* L. and *Pennisetum glaucum* (L.) R.Br. in contaminated soils amended with biochar. These authors also observed that higher biochar doses (up to 3%) yielded the most pronounced germination and seedling growth responses [49]. This effect has been attributed to the increased availability of essential mineral nutrients (Na, Mg, Ca, K, Cu, Fe), as described by Adekiya et al. [50], who further noted that such amendments enhance soil pH, porosity, and reduce the bioavailability of HMs.

Given biochar's capacity for nutrient and water retention, soil enrichment with biochar may play a pivotal role in promoting seed germination and early seedling growth [51–53].

4.2. Testing of Acute Phytotoxicity of Soil After Tyre Fire

The results obtained for $IR(\%)$ indicate that both SAF and SAFB soils exhibited phytotoxic effects ($IR > 0$) on the root growth of *Sinapis alba* L. and *Lepidium sativum* L. The mean $IR(\%)$ values ranged from 20.02% to 28.12%. The application of biochar (5% w/w in

SAFB soil) led to a marked reduction in phytotoxicity, of approximately 25.69% for *S. alba* and 17.8% for *L. sativum*, compared to the unamended soil (SAF).

This reduction can be attributed to several mechanisms that have also been confirmed by recent studies. For instance, Kang et al. [54] demonstrated that biochar application to soils contaminated with HMs enhanced plant growth by decreasing the extractability of metals and reducing their accumulation in plant shoots, while simultaneously alleviating oxidative stress [54]. Biochar has thus been recognised as a promising and efficient material for soil amendment [55].

Similarly, Wu et al. [56] reported that biochar derived from compost or plant residues, when applied at variable concentrations, significantly reduced the soil phytotoxicity associated with contaminant load, while improving the root morphology and physiological traits of plants.

Comparable findings were presented by Tumurbaatar et al. [14], who conducted standardised phytotoxicity tests using *Lactuca sativa* L. and *S. alba*. The authors attributed the observed phytotoxic effects to the excessive presence of pollutants in soils following WT combustion. These pollutants primarily include HMs such as Co, Cr, As, Cd, Pb, Hg, and Zn, commonly found in WT residues at elevated concentrations [43], as well as high levels of PAHs, including pyrene, fluoranthene, phenanthrene, chrysene, and benzo(a)anthracene among others [57].

Recently, biochar has also been extensively utilised as a carrier for slow-release fertilisers enriched with macro- and micronutrients, offering additional benefits for soil restoration and plant growth [58].

4.3. Measurement of Soil Respiration After Tyre Fire

The application of biochar to SAFB resulted in lower CO₂-C emissions within the first 24 h (16.12 kg ha⁻¹) compared to SAF (30.49 kg ha⁻¹), a reduction of approximately 47.13%. After 7 days, however, the CO₂-C emissions from SAFB (53.64 kg ha⁻¹) exceeded those from SAF (50.63 kg ha⁻¹) by nearly 6%. According to Yan et al. [59], biochar application gradually increases the soil organic carbon content (C), thereby stimulating the growth and activity of soil microorganisms. Cheng et al. [60] further reported that biochar immobilises hydrophobic contaminants that result from tyre combustion, reduces the concentration of freely dissolved substances, and improves overall soil condition. This is reflected in the pattern of enhanced soil respiration (Figure 4) following biochar application, which progressively increased from day 7 onward.

Elevated CO₂-C emissions were also observed in SAF, which exhibited higher values on days 7 and 14 compared to the CS. However, from day 21 onward the CO₂-C emissions from SAF (34.30 kg ha⁻¹) were lower than those from CS (38.43 kg ha⁻¹). This decline suggests the gradual influence of HMs and PAHs that accumulate in soils after WT combustion [61].

Zhu et al. [30] emphasised that biochar plays a significant role in soil nitrogen and carbon cycles, and that high application rates can substantially influence nutrient metabolism and biogeochemical processes in soil organisms. However, they also noted that high doses of biochar (e.g., 5% w/w) may have an adverse effect on plant growth due to potential chemical toxicity. Similarly, Zhang et al. [62] found a strong linear correlation between soil respiration rate and soil temperature, which was attenuated when high doses of biochar were applied.

Based on the present findings, it can be concluded that even a 5% biochar amendment in soils affected by WT fires has a positive effect on soil respiration and microbial processes, supporting its potential for use as a remediation agent for contaminated soils. Nevertheless, the appropriate application rate must always be carefully selected to ensure remediation efficiency. Dimitriadou et al. [63] reported that biochar demonstrates a strong

potential for soil remediation, improving fertility and crop productivity, and therefore can be successfully utilised as a soil restoration factor.

Further research is required to examine different biochar application rates and their respective ratios to optimise soil remediation strategies and assess long-term effects.

5. Conclusions

The findings of this study demonstrate that soils affected by waste tyre fires exhibit significant phytotoxicity, manifested by reduced seed germination and inhibited root growth in the tested species *Sinapis alba* L. and *Lepidium sativum* L.

The eluates of the Soil After Fire showed a germination index of 55.89% for *Sinapis alba* L. and 55.63% for *Lepidium sativum* L., corresponding to moderate phytotoxicity according to established classifications. Root growth inhibition was detected in both tested plant species (26.94% for *Sinapis alba* L. and 28.12% for *Lepidium sativum* L.), reflecting the toxic impact of residual contaminants, primarily polycyclic aromatic hydrocarbons and heavy metals, on early plant development. These phytotoxic effects are further supported by the temporal pattern of microbial respiration in Soil After Fire, which increased during the first fourteen days before subsequently declining (1st day: 30.49 kg CO₂-C ha⁻¹; 7th day: 50.63 kg CO₂-C ha⁻¹; 14th day: 56.43 kg CO₂-C ha⁻¹), indicating an initial microbial stimulation followed by a gradual suppression consistent with contaminant-related stress.

The application of a 5% dose of biochar resulted in a clear improvement of soil quality, reflected by reduced plant phytotoxicity, enhanced seed germination, and increased microbial respiration. Germination index values increased to 79.76% for *Sinapis alba* L. (+42.71%) and 83.71% for *Lepidium sativum* L. (+50%), while root growth inhibition decreased by approximately 25.69% and 17.82% for *Sinapis alba* L. and *Lepidium sativum* L., respectively, relative to the Soil After Fire variant. Although statistically significant differences were observed only for soil respiration at 24 h and 21 days ($p < 0.05$), the overall trends demonstrate a biologically relevant positive effect of biochar on soil conditions. Soil respiration further supported these findings: CO₂-C emissions in Soil After Fire with Biochar increased from 16.12 kg ha⁻¹ at 24 h to 50.50 kg ha⁻¹ at 28 days (+213%), while emissions in Soil After Fire rose from 30.49 kg ha⁻¹ to 37.73 kg ha⁻¹ (+23.75%). At the end of the experiment, Soil After Fire with Biochar exhibited approximately 33.85% higher CO₂-C emissions compared to Soil After Fire. These results collectively indicate stimulation of microbial processes, enhanced organic matter decomposition, and improved nutrient availability following biochar application.

Overall, the study confirms that biochar is an effective soil amendment for soils impacted by waste tyre fires. Its application mitigates phytotoxic effects, supports seed germination and root development, and enhances microbial functionality, thereby improving overall soil health and fertility. The results demonstrate measurable improvements in plant growth and microbial activity, indicating that biochar can counteract the inhibitory effects of post-fire contaminants. This highlights its practical relevance in post-fire soil management and amendment strategies.

Further research should investigate the long-term effects of different biochar types and application rates, their interactions with soil physicochemical properties, and their environmental stability and efficiency under real-world conditions. Such studies are essential to optimise amendment strategies and ensure the sustainable improvement of soils affected by tyre fires. The authors intend to address these aspects in future studies.

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