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Medium-Dependent Phytotoxic Effects of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) on Early Development in Selected Monocot and Dicot Plant Species: A Comparative Assessment

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Abstract

Per- and polyfluoroalkyl substances (PFAS) are human-made organofluorine compounds that resist physical, chemical, and biological degradation. Used widely since the 1940s, they are now ubiquitous in the environment. Despite restrictions or bans in many countries, the extreme persistence, mobility, and bioaccumulation of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS)—the PFAS most commonly found in soils and waters—still pose major ecological risks. Phytotests use plants as bioindicators to assess the biological impact of the tested agent on various physiological end parameters, such as seed germination or root length. To our knowledge, the current literature lacks direct evidence regarding the toxic effects of PFOA on *Sorghum bicolor* germination and early seedling development, nor does it adequately characterize the individual effects of PFOA or PFOS on *Lepidium sativum* and *Sinapis alba*. To address this gap, this study examined how and to what extent the type of growth medium to which PFOA or PFOS is added affects their phytotoxicity. Empirical evidence shows that matrix type strongly affects plant physiological responses to PFOA and PFOS, as indicated by changes in root elongation. Root growth was most inhibited in the liquid phase. Specifically, the application of PFOS at a dose of 1 mg L^{-1} caused a 20.2% inhibition of *Sorghum bicolor* root growth. In contrast, *Lepidium sativum* and *Sinapis alba* exhibited 22.1% and 21.1% inhibition, respectively, but only at a higher dose of 10 mg L^{-1}. Regarding PFOA, the strongest effect was observed at a dose of 10 mg L^{-1}, which inhibited the root growth of *Sorghum bicolor* by 9.8%, *Lepidium sativum* by 14.9%, and *Sinapis alba* by 14.3%. In the reference soil, toxicity was lower, likely due to the high sorption capacity of kaolin clay for PFAS, while humus soil better reflects natural ecosystem conditions. Plant sensitivity depended on species and PFAS type: *Sinapis alba* was most sensitive to PFOS (27.2% root growth inhibition at 0.1 mg L^{-1} in humus soil) and least sensitive to PFOA (16.7% root growth induction at 1 mg L^{-1} in reference soil), and *Sorghum bicolor* and *Lepidium sativum* showed similar response patterns.

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Keywords: poly- and perfluoroalkyl substances (PFAS); microbiotests; phytotoxicity tests; aquatic matrices; solid matrices; root growth inhibition; *Sorghum bicolor* (L.) Moench; *Lepidium sativum* L.; *Sinapis alba* L.

1. Introduction

The presence of anthropogenic organic contaminants in ecosystems is still an insufficiently recognized problem with broad environmental implications. A particular group among these substances is represented by per- and polyfluoroalkyl substances (PFAS) which are heterogeneous, fully synthetic chemical compounds. Their characteristic feature is the presence of a perfluorinated methyl group ($-CF_3$) and a methylene group ($-CF_2-$) [1,2]. PFAS are oleophobic due to the absence of van der Waals interactions and hydrophobic because local dipole moments are eliminated along the C–F chain [3]. Owing to their intrinsically low surface tension and amphiphilic molecular architecture, PFAS function as highly efficient surfactants [4]. Furthermore, the C–F bond has a high energy, 530 kJ mol^{-1}, making it one of the most durable in organic chemistry. Thanks to this strong bond, all PFAS compounds demonstrate high chemical stability and thermal resistance, as well as considerable resistance to biotic decomposition which has led to them being termed "forever chemicals" [5,6]. Such properties make PFAS suitable for many technical applications, and they have been in widespread use since the 1940s in both industry and consumer products [7]. They have been used, among others, as surfactants in fire-fighting foams and corrosion protection agents, and as hydrophobic and oil-resistant agents for impregnating textiles, leather goods and materials intended for contact with food and in the production of cosmetics. In the current literature, more than 200 uses, arranged into 64 categories, were identified for more than 1400 individual PFAS [8,9].

Due to their widespread use, PFAS are pervasive throughout the biosphere and the features that favor their use are currently increasing environmental pressure. Their considerable stability, combined with their mobility in the environment and high bioaccumulation potential, increasing with carbon chain length, have resulted in them being labelled as toxic substances that threaten the homeostasis of ecosystems. As such, the compounds have been classified as Persistent Bioaccumulative and Toxic (PBT), and the dissemination of PFAS in the environment is viewed with great concern [6]. Among the various types of PFAS, the most studied are those with C4 to C14 carbon chains containing carboxyl and sulfonic groups; of these, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) are the most frequent environmental contaminants in both soil and water, including sea water and drinking water [10,11]. Compared with numerous legacy persistent organic pollutants (POPs), PFAS are more frequently detected in aquatic compartments, a distribution pattern attributable to their comparatively high aqueous solubility and lower molecular mass [12]. Consequently, aquatic systems represent a principal pathway for the environmental transport and dispersal of PFAS [13]. Reported occurrences of PFOA and PFOS in aquatic ecosystems exert effects across all trophic levels, encompassing autotrophic organisms at the base of food webs such as microalgae, primary and secondary consumers (e.g., invertebrates and fish), and higher-order predators, including mammals [14–18]. Given the complex interactions within trophic networks, these pollutants may be translocated from aquatic ecosystems to terrestrial food webs, where their pronounced persistence promotes biomagnification [19,20]. It has been shown that the occurrence of PFAS in aquatic ecosystems constitutes not only a direct toxicological hazard but also alters the bioavailability and toxicity of co-occurring xenobiotics, thereby modulating their effects on exposed organisms [21]. Parallel to their aquatic transport, PFAS are extensively distributed within

terrestrial ecosystems. Soil matrices serve as the primary reservoir for PFOA and PFOS accumulation, particularly within agricultural and industrial lands, airports, military areas, and sites contaminated by firefighting foam runoff [22]. Due to their high mobility in the soil solution, these compounds are readily taken up from the soil via the root system and subsequently translocated to above-ground tissues in both crops and wild plants [23,24]. Consequently, their persistent accumulation in terrestrial flora poses direct phytotoxic risks and threatens the stability of terrestrial ecosystems. Although production and use of PFOA and PFOS are now restricted or banned globally, their presence in the environment remains problematic [25,26].

While the dose-dependent toxic effects of PFOA and PFOS on human and animal health have already been clearly documented in the literature [27,28], the assessment of the phytotoxicity of these compounds remains an insufficiently explored issue. Available data indicate that these substances induce multilevel negative effects, manifested as morphological, physiological, and biochemical changes, as well as modulation of gene expression profiles [29–32]. However, the dose ranges that cause measurable biological effects show considerable variation between individual studies. Furthermore, previous studies have been conducted using various types of growth media, including hydroponic systems, agar-solidified media, and different types of soils [23,32,33]. Literature data concerning phenotypic assessment of the impact of PFOA and PFOS on parameters such as seed germination, root elongation, seedling growth, and biomass accumulation reveal both inhibitory effects and hormetic responses to the examined PFAS [34–36]. It is suggested that the main factor determining the observed discrepancies in results may be the type of growth medium used, which directly determines the effective dose of the substance reaching the root system. To date, however, there is a lack of comparative studies verifying the impact of different matrices within a single, unified experimental setup, which makes it impossible to conclusively assess the modifying effect of the medium on the early growth of plants exposed to PFOA and PFOS.

Phytotests are research methods that use plants as bioindicators, enabling the assessment of the actual biological impact of the analyzed substance on selected endpoints, such as seed germination rate, root length or seedling biomass. In addition to evaluating the presence and toxicity of substances, they can be used to determine the bioavailability of xenobiotics in the analyzed medium. Furthermore, they make it possible to verify synergistic interactions between the xenobiotic and the medium with respect to the induced physiological effect in plants [37]. The results of biotests may also be useful in selecting plant species that exhibit tolerance to a specific pollutant, which can be of critical importance for identifying taxa intended for use in phytoremediation projects. Furthermore, the obtained results may constitute a significant complement to data acquired using classical analytical techniques.

This study presents the results of phytotests carried out in accordance with the adopted standards, in which the OECD guidelines were applied and which are also consistent with ISO 18763:2016 [38,39]. The selection of test species was based on the OECD recommendations, according to which at least three species should be chosen from those listed in the guideline. Consequently, three test plant species were selected: sorghum bicolor, garden cress, and white mustard. These higher vascular plants are widely utilized as bioindicators in standardized analytical procedures and commercial diagnostic assays [40,41]. These organisms serve to evaluate the phytotoxicity of solid and liquid matrices contaminated by both anthropogenic heavy metal ions and organic xenobiotics [42,43]. Among the selected species, garden cress exhibits exceptional sensitivity to polymetallic pollution, petroleum hydrocarbons, and phytotoxic fractions of sewage sludge, responding with a drastic inhibition of seed germination and root retardation [44,45]. In contrast, white mustard demonstrates a

high level of growth inhibition in acute toxicity assays when exposed to organic compounds and active pesticide ingredients, including herbicides [46]. The distinct physiological response of sorghum to chemical stressors stems from its classification as a monocotyledonous (monocot) plant; its specific root system morphology and distinct metabolic pathways determine a different sensitivity to selected toxicants compared to the concurrently evaluated dicotyledonous species. Despite their widespread use, to the best of our knowledge, the literature lacks information on the direct toxic effect of PFOA on the germination and early development of sorghum, as well as on the individual effects of PFOA or PFOS on garden cress and white mustard.

The aim of this study was to determine how different growth media influence the phytotoxicity of PFOA and PFOS during seed germination and early seedling development. To achieve this objective, parallel phytotoxicity tests were carried out using three types of media that differ significantly in their physicochemical properties, i.e., liquid medium, reference soil, and humus soil. On each of these media, the effects of selected doses of PFOA and PFOS on three plant species serving as bioindicators were assessed independently. The impact of the compounds under study was determined on the basis of parameters such as germination capacity and root length. Furthermore, the aim of the study was to assess which of the plant species selected for biotests shows the highest sensitivity to the presence of chosen PFAS compounds in the medium, which may have practical application as indicator plants, as well as to identify the parameter (or parameters) with the greatest sensitivity and potentially primary importance in assessing exposure to PFAS.

2. Materials and Methods

2.1. Reagents and Preparation of Stock Solution

Pentadecafluorooctanoic acid (PFOA; $C_8HF_{15}O_2$; CAS 335-67-1; 95.4%) and heptadecafluorooctanesulfonic acid (PFOS; $C_8HF_{17}SO_3$; CAS 1763-23-1; 35.2% solution in H_2O) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All stock solutions of the tested chemicals were prepared in deionised water using polypropylene (PP) containers to avoid adsorption on the glass surfaces [47]. To prepare the PFOA solution, an aliquot was first dissolved in methanol (20 µL of methanol per 1 mg of PFOA) and then topped up with deionized water. Consistent with the recommendations outlined in the OECD Guidance Document No. 23 [48], a preliminary experiment was conducted and no toxic effects on plants attributable to methanol were observed at the concentration tested.

2.2. Phytotoxicity Bioassays

The phytotoxic effect of PFOA and PFOS on seed germination and early growth of selected mono- and dicotyledonous plant species was determined using a Phytotoxkit™ commercial toxicity bioassay (MicroBioTest Inc., Gent, Belgium) compliant with ISO 18763:2016 [39,49]. Two types of microbiotest were used: (1) Phytotoxkit™ for the liquid phase, enabling a determination of the direct impact of chemical compounds without the introduction of these compounds into the soil, and (2) Phytotoxkit™ for the solid phase, used to assess the phytotoxicity of the soil spiked with the tested substance, and to determine its impact on seed germination and root growth. For the solid-phase Phytotoxkit™, two types of soil were used: artificial reference soil, recommended by the OECD [50] and compliant with ISO 11269-1:2012 and ISO 11269-2:2012 [51,52] and humus soil.

The liquid-phase Phytotoxkit™ test was prepared with deionized water (control) and PFOA or PFOS solutions at concentrations of 0.1, 1 and 10 mg dm^{-3} [53]. The solid-phase Phytotoxkit™ test was prepared with reference soil and with humus soil; the PFOA or PFOS solutions were used to spike the soil at final concentrations of 0.1, 1 and 10 mg kg^{-1} of soil dry weight; deionized water was used in the control variant. The tests were performed in

transparent polyvinylchloride (PVC) test plates, with 10 seeds of one species on each. Every experimental variant was repeated three times. The seed-containing plates were incubated for 72 h in a non-ventilated thermostatic chamber (Incucell, IC 111; BMT Medical Technology s.r.o., Brno, Czech Republic) maintained at 25.0 ± 0.5 °C under complete darkness. At the end of the incubation period, the plates with seedlings were taken out from the thermostatic chamber and the lids carefully removed. For morphometric root measurements, digital photographs at a resolution of 2592 × 4608 pixels were subsequently analyzed using ImageJ software (version 1.54, National Institutes of Health, Bethesda, MD, USA).

2.3. Preparation and Physicochemical Analyses of Soils

The reference soil (MicroBioTests Inc., Gent, Belgium) consisted of a specially prepared artificial soil intended for the Phytotoxkit™ microbiotest. It consisted of quartz sand (85%), kaolinite clay (10%) and sphagnum peat (5%).

A humus soil was prepared, consisting of a mixture of sandy soil (one part) and universal horticultural soil (two parts), i.e., a combination of high peat (sphagnum), low peat, natural organic compost, composted finely ground Bio-Bark and humic soil ("Skarb ogrodu", Ogro-Plant, Starcza, Poland). The sandy and horticultural soils were first thoroughly mixed, and then sifted through a 10 × 10 mm and a 2 × 2 mm metal sieve. Both the source soils and the prepared humus soil were tested for pH, salinity, specific gravity (γ), organic carbon (OC) content, as well as the copper, zinc, manganese and iron contents.

The pH was determined by potentiometry of air-dried fine soil samples in H_2O using a soil/solution ratio of 1:2.5 using a pH-meter (model PHG, Dramiński S.A., Olsztyn, Poland) [54]. Soil salinity, i.e., the salt content of the soil per unit volume, given in g dm^{-3} of water [55], was determined by conductometry using a salt metre (model CC-216, Elmetron, Zabrze, Poland); the metre measures the specific conductivity of a soil solution, with the salinity value given in grammes of NaCl per 1 dm^{-3} of water (g NaCl dm^{-3}). The measurement was performed using 12 g of soil in 30 cm^3 of distilled H_2O.

Although the specific gravity (γ) of solid particles, i.e., the ratio of the mass of a given volume of solids to the mass of the same volume of water at 40 °C, can be measured using various methods, the present study used a pycnometer [56]. In a typical soil mixture, different particles have different specific gravities; therefore, the specific gravity of an entire mixture is calculated as the mean specific gravity of all the solid particles present.

Organic carbon (OC) content was determined by the Tiurin oxidimetric method. This involves burning coal with a strong oxidant, potassium dichromate ($K_2Cr_2O_7$), in a 48% sulfuric acid (H_2SO_4) medium and the excess of the oxidant was back-titrated potentiometrically using a pH/mV meter (model N-5123, MERA-ELWRO, Wrocław, Poland) [57]. The content of trace elements (copper, zinc, manganese and iron) was determined using the Rinkis method after extraction in 1 mol dm^{-3} HCl and measured spectrophotometrically using a Spekol 11 spectrophotometer (Carl Zeiss Jena, Jena, Germany) [58].

2.4. Plant Material

The test used certified seeds (MicroBioTest Inc., Gent, Belgium) of three plant species as bioindicators: the monocotyledon sorghum bicolor (*Sorghum bicolor* (L.) Moench; SOS; batch number SOS 291023), and the dicotyledons garden cress (*Lepidium sativum* L.; LES; batch number LES 061223) and white mustard (*Sinapis alba* L.; SIA; batch number SIA 050124). All plant species selected in this study are listed among the recommended test species in the OECD Guideline for the Testing of Chemicals, Test No. 208, which is designed to assess the potential toxic effects of soil-incorporated chemical substances on seed germination and early seedling development [38].

2.5. Germination and Roots Growth

According to the Phytotoxkit™ test procedure and the ISO 11269-1:2012 guidelines [51], two indicators of plant response to the tested medium with added PFAS were evaluated compared to the corresponding control medium: (i) reduction in seed germination capacity and (ii) degree of inhibition of root growth. Furthermore, seeds were classified as germinated only when the length of the primary root exceeded 1 mm. These indicators have been presented in the form of parameters such as the percentage of germination inhibition (%GIH) and the percentage of root growth inhibition (%RGI).

%GIH in the analyzed seeds was calculated using the following formula:

$$\%GIH = \frac{\overline{X}_{GControl} - \overline{X}_{GTreated}}{\overline{X}_{GControl}} \times 100\% \quad (1)$$

where:

$\overline{X}_{GControl}$—mean number of germinating control seeds,
$\overline{X}_{GTreated}$—mean number of germinating treated seeds;
and %RGI was calculated as follows:

$$\%RGI = \frac{\overline{X}_{LControl} - \overline{X}_{LTreated}}{\overline{X}_{LControl}} \times 100\% \quad (2)$$

where:

$\overline{X}_{LControl}$—mean root length of control seedlings,
$\overline{X}_{LTreated}$—mean root length of treated seedlings.

An alternative criterion recommended in the Phytotoxkit™ procedure, and which we implemented in the present study, is the measurement of the length of the longest root on each test plate (L_{MAX}). This parameter is recommended as a simplified surrogate indicator compared to determining the length of all roots developing from germinating seeds.

2.6. Statistical Analysis

To evaluate the influence of the applied matrices on the modulation of the phytotoxic effects of selected PFAS on seed germination and early plant development, the following statistical approaches were employed:

(a) to eliminate variability arising from heterogeneous growth dynamics of the individual species on the tested matrices, a separate comparative analysis was performed for each species. This analysis examined changes in the %RGI, relative to the corresponding control, across all three matrices to which the individual doses of the selected PFAS were applied.

(b) Root length in seedlings exposed to different concentrations of a given PFAS was compared with that of the corresponding control. Statistical analyses were carried out separately for each plant species and matrix type over the full dose range of the specific PFAS.

(c) The suitability of using the L_{MAX} as a simplified phytotoxicity indicator was assessed, considering it as an alternative to the conventionally determined mean root length.

Prior to conducting parametric tests, the normality of the data distribution within the studied groups was evaluated using the Shapiro–Wilk test, and the homogeneity of variances was assessed with Levene's test. Data analysis was performed in three stages. (1) For the %RGI, the control group was used as the reference for calculations and was not directly included in the statistical model. Differences between matrix types, the effect of PFAS dose, as well as the interaction between these factors, were examined using two-way analysis of variance (two-way ANOVA). Owing to unequal group sizes, the Spjøtvold–Stoline modification of Tukey's post-hoc test was applied at this stage. (2) For the raw root length measurements, the effect of PFAS doses relative to the control was analysed using

one-way ANOVA followed by Dunnett's post-hoc test. (3) To evaluate the suitability of L_{MAX} root as a simplified toxicity indicator, Pearson's r correlation analysis was conducted between the mean root length on an individual plate and the L_{MAX}. This analysis was carried out in subgroups defined by plant species, matrix type, and individual PFAS compound ($N = 12$). The strength of the correlations was interpreted according to the Evans classification [59]. In all analyses, the significance level was set at $\alpha = 0.05$. If in the aforementioned cases the assumptions of normality and homogeneity of variance were not met, the non-parametric Kruskal–Wallis test was employed, followed by a post-hoc analysis using the multiple comparisons of mean ranks test, with appropriate adjustment of the significance level for multiple testing. All statistical procedures were performed using Statistica software (version 13.3, TIBCO Software Inc., Palo Alto, CA, USA).

3. Results

3.1. Physicochemical Properties of the Soil

The basic physicochemical properties of the reference soil and humus soil used in the Phytotoxkit™, and the sandy soil and universal horticultural soil acting as source soils are given in Table 1. The contents of selected elements found in humus soil prepared for phytobiotests are within the standards specified in the applicable Regulation of the Polish Minister of the Environment, which means that they do not have a negative impact on human health and the state of the environment [60].

Table 1. Physicochemical properties of the reference soil and humus soil used in the Phytotoxkit™, as well as the sandy soil and universal horticultural soil acting as source soils.

Properties	Unit	Reference Soil	Humus Soil	Sandy Soil	Universal Horticultural Soil
pH (in H_2O)		**6.0**	**7.1**	5.7	7.2
Salinity	g NaCl dm^{-3}	—	**1.77**	0.08	2.04
Specific gravity (γ)	kg dm^{-3}	**2.62**	**2.49**	2.64	1.96
Organic carbon (OC)	%	—	**11.26**	0.82	21.17
Cu	mg dm^{-3}	—	**1.30**	1.40	1.30
Zn	mg dm^{-3}	—	**11.7**	1.20	15.8
Mn	mg dm^{-3}	—	**5.00**	30.0	4.10
Fe	mg dm^{-3}	—	**148**	364	145

3.2. Evaluation of Phytotoxic Effects

In accordance with the Phytotoxkit™ protocol, the test is deemed valid when the plants in the negative control fulfill both of the following criteria: (i) the mean percentage of germinated seeds is at least 70% for each of the three test species, and (ii) the mean root length for each species is at least 30 mm, or at least 40 mm when the length of the longest root is used as the evaluation metric. All the above Phytotoxkit™ criteria have been met; therefore, the conducted tests are considered fully valid.

3.2.1. Germination

Significant differences in seed germination were not observed in any of the experimental treatments applied relative to the control samples. The incidental effects that occurred indicate the absence of a consistent plant response, expressed as inhibition of seed germination, to the application of PFAS within the range of doses selected for the experiment, irrespective of the medium employed. Detailed data regarding the number of germinated seeds, %GIH, as well as L_{MAX} for each plant species across all experimental variants are provided in Tables S1–S3 in the Supplementary Materials.

3.2.2. Root Growth

In the present experiment, the tested plants demonstrated distinct responses, evidenced by modifications in root length, which were dependent on both the type of test medium used and the type and dose of the applied PFAS compounds.

Sorghum bicolor (SOS)

Two-way ANOVA revealed a strong influence of medium type on PFOA-induced inhibition of SOS root growth ($F(2, 242) = 10.65$; $p < 0.001$), while PFOA concentration exerted a smaller yet still statistically significant effect ($F(2, 242) = 3.27$; $p = 0.040$). A significant interaction between medium type and PFOA concentration ($F(4, 242) = 4.06$; $p = 0.003$) indicated that PFOA toxicity on root elongation depended on properties of the growth medium. Tukey's HSD post hoc test showed that the most pronounced, statistically significant difference occurred between the liquid phase and the humus soil at the intermediate PFOA dose: SOS root growth inhibition in the humus soil was 22.5% ($p < 0.001$) higher than in the liquid phase (Figure 1A). Dunnett's test indicated a clear matrix-dependent effect of PFOA on SOS root growth. In the liquid phase, inhibition of SOS root growth relative to the corresponding control was observed only at the highest PFOA concentration (9.8%; $p = 0.044$), whereas in the reference soil it was already visible at the intermediate and highest dose levels (9.9%; $p = 0.038$ and 11.6%; $p = 0.012$ respectively), and in the humus soil at all applied doses (10.2%; $p = 0.011$; 21.2%; $p < 0.001$ and $p = 0.009$ respectively).

The impact of PFOS on SOS root growth inhibition varied across the tested types of medium, and the Kruskal–Wallis test confirmed statistically significant differences among the experimental treatments ($H(8, N = 241) = 95.25$; $p < 0.001$). The most pronounced differences in %RGI occurred between the treatments with liquid medium and those with humus soil (Figure 1B). Unlike PFOA, PFOS exerted a stronger inhibitory effect on root growth in the liquid medium than in humus soil, generating differences in the degree of root growth inhibition between these matrices of 25.8% ($p < 0.001$) and 28.2% ($p < 0.001$) at low and medium doses, respectively. However, in humus soil, exposure to the highest PFOS dose triggered a distinctly stronger SOS response compared with lower concentrations, resulting in stronger root growth inhibition by 32.4% ($p < 0.001$) and 30.6% ($p < 0.001$) compared with the low and intermediate doses, respectively, and by 26.6% ($p < 0.001$) relative to the reference soil with the same PFOS level. Statistically significant differences in SOS root length were also detected between PFOS-exposed variants and their corresponding controls (Dunnett's test). In the liquid-phase test, PFOS caused marked root shortening: root length dropped by 15.9% ($p < 0.001$), 20.2% ($p < 0.001$), and 15.5% ($p < 0.001$) at low, intermediate, and high concentrations, respectively, compared to the control. In addition, an 8% ($p = 0.035$) reduction in root length relative to the corresponding control was observed in the test with reference soil following the application of PFOS at an intermediate dose, whereas in humus soil exposed to the highest PFOS dose, roots were shortened by 22.5% ($p < 0.001$).

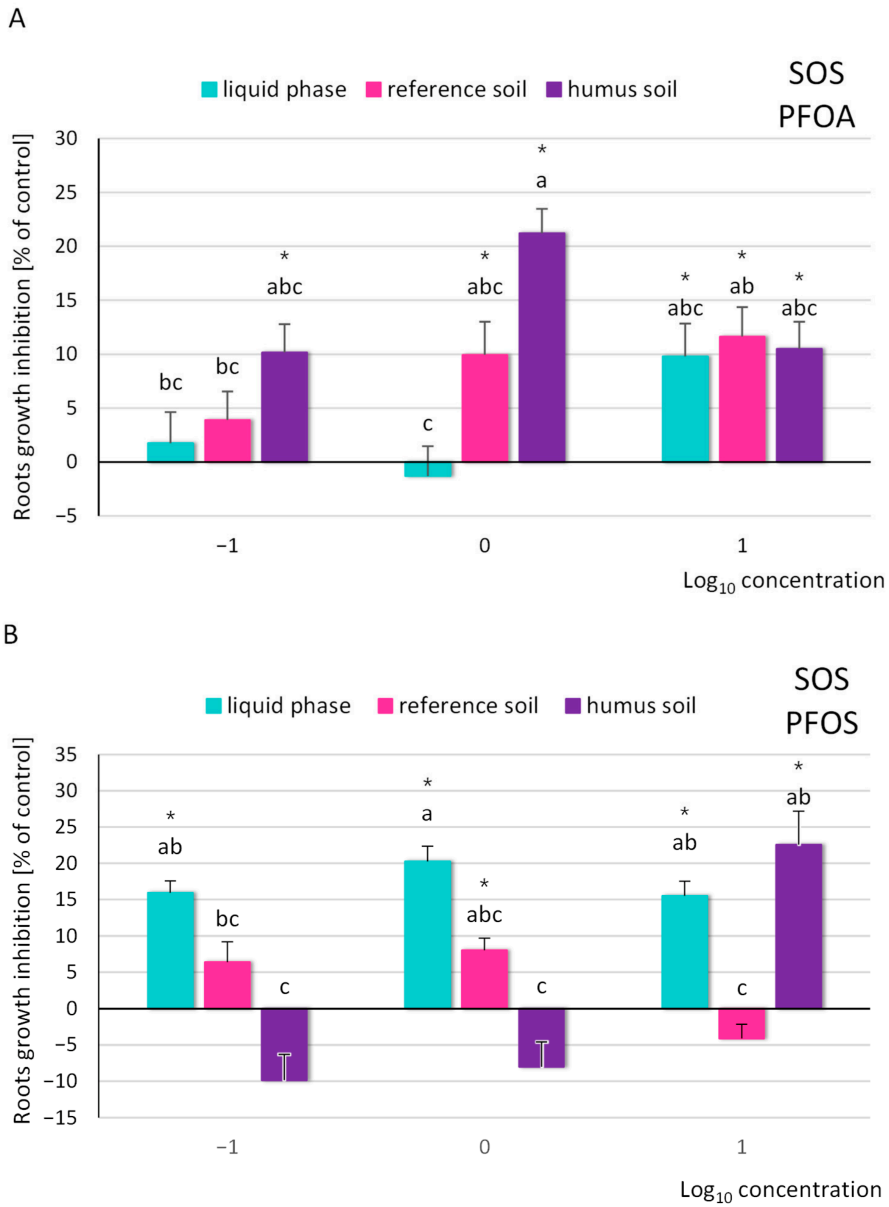


Figure 1. Medium-dependent inhibition of *Sorghum bicolor* (SOS) root growth following treatment with (**A**) perfluorooctanoic acid (PFOA) or (**B**) perfluorooctane sulfonic acid (PFOS). Data are presented as means ± SE. The *X*-axis shows log-transformed per- and polyfluoroalkyl substances (PFAS) concentration values. Different letters indicate statistically significant differences between groups (for (**A**): two-way ANOVA, with Tukey's HSD test; $p < 0.05$, for (**B**): Kruskal–Wallis followed by a post-hoc analysis using the multiple comparisons of mean ranks test; $p < 0.05$). Asterisks indicate significant differences between the treated variant and the corresponding control (one-way ANOVA, with Dunnett's test; $p < 0.05$).

Lepidium sativum (LES)

For the subsequent test species, LES, a two-way analysis of variance (ANOVA) showed that the type of used medium significantly differentiated the degree of root growth inhibition ($F(2, 239) = 8.90$; $p < 0.001$), while the PFOA concentration had a weaker, though still statistically significant effect ($F(2, 239) = 3.15$; $p = 0.045$). The highly significant interaction between these factors ($F(4, 239) = 9.83$; $p < 0.001$) indicates that the effect of PFOA concentration on root growth inhibition was strongly modulated by the properties of the medium. In liquid phase, PFOA showed its strongest toxic effect at intermediate and high concentrations, whereas in humus soil its strongest effect occurred at the lowest

dose (Figure 2A). A statistically significant difference between media at the same PFOA dose appeared only at the highest level, where %RGI in liquid medium was 14.4% greater than in humus soil ($p < 0.001$; Tukey's HSD test). A statistically significant inhibition of LES root growth was found in comparison with the control value, both in liquid medium and in reference soil, after the application of the intermediate (by 10.4%; $p < 0.001$ and 6.3%; $p = 0.008$, respectively), as well as the highest PFOA dose (14.9%; $p < 0.001$ and 6.8%; $p = 0.004$; Dunnett's post-hoc test). Moreover, in humus soil, the root length was significantly smaller than in the corresponding control sample (by 8.4%; $p = 0.004$) when the lowest PFOA dose was applied.

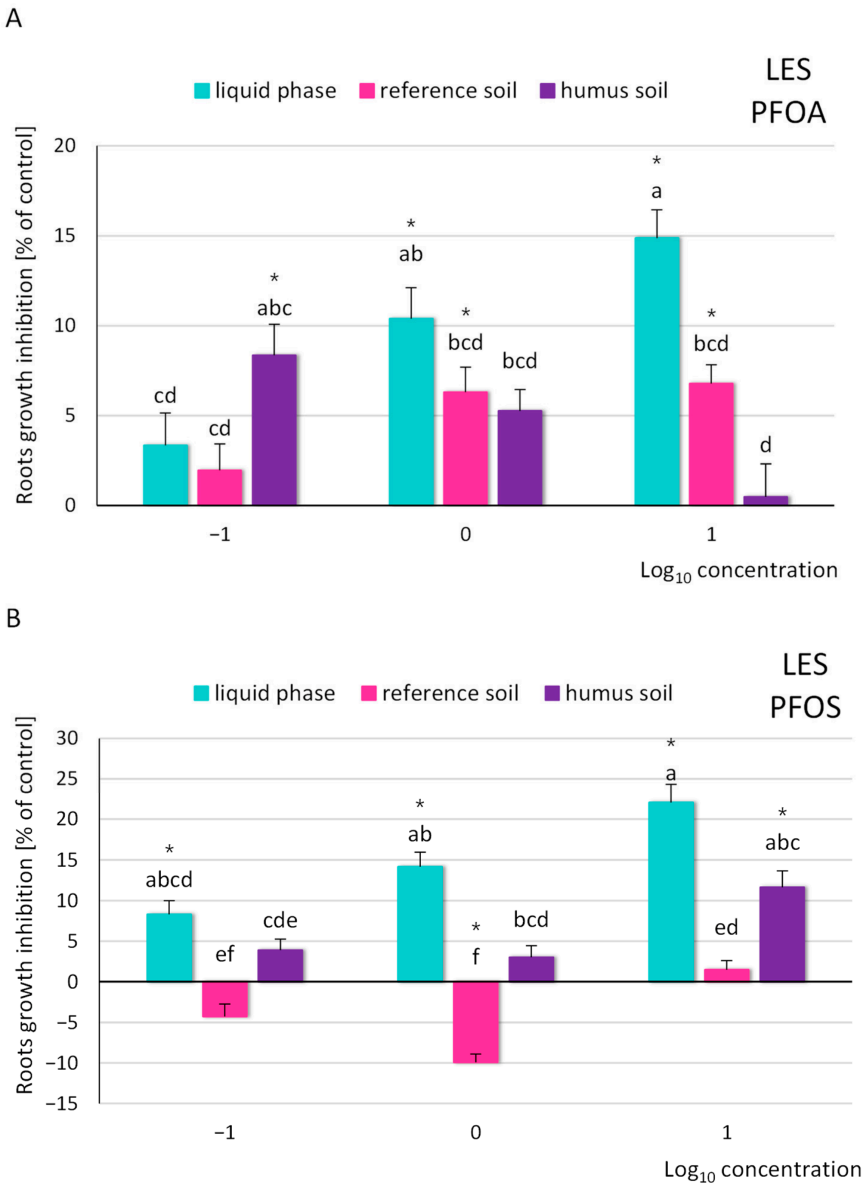


Figure 2. Medium-dependent inhibition of *Lepidium sativum* (LES) root growth following treatment with (**A**) perfluorooctanoic acid (PFOA) or (**B**) perfluorooctane sulfonic acid (PFOS). Data are presented as means ± SE. The *X*-axis shows log-transformed per- and polyfluoroalkyl substances (PFAS) concentration values. Different letters indicate statistically significant differences between groups (for (**A**): two-way ANOVA, with Tukey's HSD test; $p < 0.05$, for (**B**): Kruskal–Wallis followed by a post-hoc analysis using the multiple comparisons of mean ranks test; $p < 0.05$). Asterisks indicate significant differences between the treated variant and the corresponding control (one-way ANOVA, with Dunnett's test; $p < 0.05$).

The application of PFOS in the experiments conducted with LES confirmed a medium-dependent response of the plants to the applied substance. The Kruskal–Wallis test showed that both the applied matrix and the PFOS dose significantly differentiated the %RGI ($H(8, N = 248) = 134.18$; $p < 0.001$). In the liquid medium, along with the increase in PFOS concentration, a clear, gradually intensifying inhibition of root growth was observed, amounting to 8.3% ($p = 0.009$), 14.2% ($p < 0.001$), and 22.1% ($p < 0.001$) at the low, intermediate, and highest doses, respectively (Dunnett's test; Figure 2B). In contrast, in humus soil a statistically significant limitation of root growth relative to the control was only recorded at the highest PFOS dose and amounted to 11.6% ($p < 0.001$). A different response pattern was observed in the experiment conducted in reference soil, where application of the intermediate PFOS concentration resulted in a 9.9% ($p < 0.001$) stimulation of root growth compared to the control. This differentiated plant response accentuated the contrasts between the tested matrices, liquid medium and reference soil, for which the differences in root growth percentage were 12.6% ($p < 0.001$), 24% ($p < 0.001$), and 20.5% ($p < 0.001$) at low, intermediate, and high PFOS concentrations, respectively. In addition, statistically significant differences were also found between reference soil and humus soil after application of the intermediate and high PFOS doses, amounting to 12.9% ($p < 0.001$) and 10.2% ($p < 0.03$), respectively.

Sinapis alba (SIA)

Experimental trials conducted on selected media using the SIA test plant revealed clear differences between them, measured by the degree of root growth inhibition under the influence of PFOA and PFOS. Analysis with the Kruskal–Wallis test confirmed the presence of very strong, highly statistically significant differences ($H(8, N = 246) = 96.35$; $p < 0.0000$). At the lowest applied PFOA concentration, the liquid phase proved to be the most toxic: %RGI there was significantly greater—by 7.7% ($p = 0.002$) than in the reference soil and by 9.6% ($p < 0.001$) than in the humus soil (Figure 3A). The contrast became even more pronounced at the intermediate PFOA dose: on the liquid medium, %RGI was higher by 19.2% ($p = 0.0002$) and 20.9% ($p < 0.001$) than in the reference soil and humus soil, respectively. Only the highest PFOA dose partially altered this effect. At that point, a statistically significant difference was recorded only between the liquid phase and the humus soil—the liquid medium still had a stronger effect, causing 15.6% ($p = 0.002$) greater inhibition of root growth. Compared with the control group, SIA root growth was significantly suppressed, by 14.3% ($p < 0.0001$; Dunnett's test), but only at the highest PFOA concentration. Notably, at the intermediate PFOA level, root growth was significantly stimulated, increasing by 15% compared to the corresponding control in the reference soil ($p = 0.014$; Dunnett's test) and by 16.7% in the humus soil ($p = 0.03$; Kruskal–Wallis test).

A different pattern of inhibition of SIA root growth was found after the application of PFOS on the tested matrices. The Kruskal–Wallis test showed a highly significant effect of the combination of medium and concentration on %RGI ($H(8, N = 246) = 86.91; p < 0.0001$). The post hoc analysis showed that the effect of PFOS concentration is not uniform and depends strictly on the type of medium used. At the lowest concentration applied, SIA root growth in the trials conducted on humus soil was significantly more inhibited compared to the liquid medium (by 13%; $p = 0.001$) and the reference soil (by 17.4%; $p < 0.0001$; Figure 3B). At higher concentration, this trend was reversed. At the intermediate dose, the greatest %RGI was observed in the liquid phase, where it was 11.8% higher than in the humus soil ($p = 0.003$). At the highest PFOS concentration, the most pronounced and statistically significant inhibition of root growth was also detected in the liquid phase, exceeding that in the reference soil by 15.4% ($p < 0.0001$). A comparative analysis of the degree of inhibition of root length relative to the corresponding control sample (Kruskal–

Wallis test) revealed statistically significant differences between the tested medium types. Toxic effects of PFOS in the liquid medium were observed at all concentrations used, with root growth inhibition of 14.1% ($p < 0.0001$), 9.8% ($p < 0.0001$), and 27.2% ($p < 0.0001$) following application of the low, intermediate, and highest concentration, respectively. In the reference soil, inhibition of SIA root growth was recorded at the low (9.8%; $p = 0.0002$) and intermediate (13.6%; $p < 0.0001$) PFOS doses. In contrast, in the humus soil, an inhibitory effect on root growth was detected at the low (27.2%; $p < 0.0001$) and high (27.2%; $p = 0.001$) PFOS concentrations.

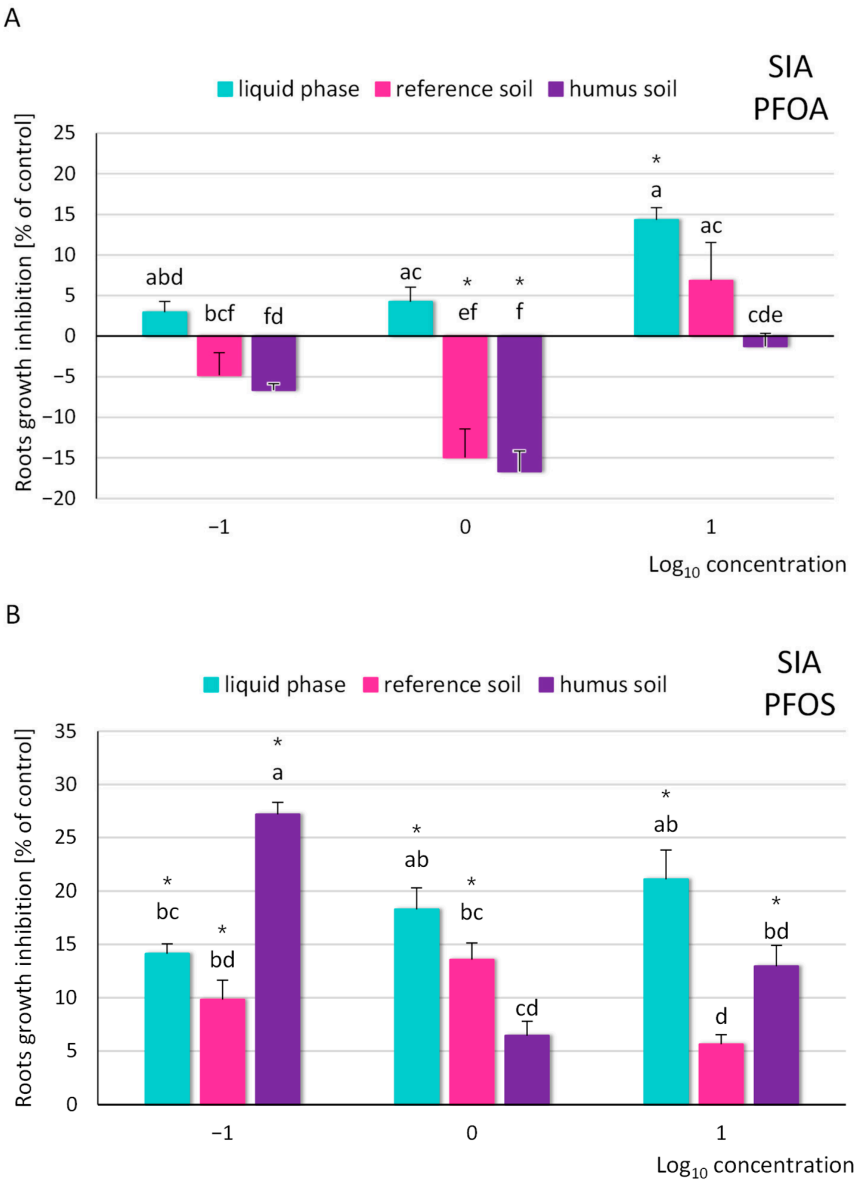


Figure 3. Medium-dependent inhibition of *Sinapis alba* (SIA) root growth following treatment with (**A**) perfluorooctanoic acid (PFOA) or (**B**) perfluorooctane sulfonic acid. Data are presented as means ± SE. The *X*-axis shows log-transformed per- and polyfluoroalkyl substances (PFAS) concentration values. Different letters indicate statistically significant differences between groups (Kruskal–Wallis followed by a post-hoc analysis using the multiple comparisons of mean ranks test; $p < 0.05$). Asterisks indicate significant differences between the treated variant and the corresponding control (for (**A**) liquid phase and reference soil: one-way ANOVA, with Dunnett's test; $p < 0.05$, for the remaining variants Kruskal–Wallis followed by a post-hoc analysis using the multiple comparisons of mean ranks test; $p < 0.05$).

3.2.3. Correlation Analysis of Root Length Parameters

The Pearson's *r* correlation analysis between the mean root length and maximum root length per individual plate in most experimental variants showed a significant positive correlation between the two parameters (Table 2).

Table 2. Pearson correlation coefficients (r) and coefficients of determination (R^2) between mean root length and maximum root length across all experimental variants (ns indicates no significant difference at $p > 0.05$).

Plant Species	Growth Medium	PFAS	Sample Size (N)	Correlation Coefficient (r)	Coefficient of Determination (R^2)	Significance Level (p)
SOS	liquid phase	PFOA	12	**0.856**	**0.733**	<0.05
		PFOS	12	**0.847**	**0.717**	<0.05
	reference soil	PFOA	12	**0.890**	**0.792**	<0.05
		PFOS	12	**0.850**	**0.723**	<0.05
	humus soil	PFOA	12	**0.891**	**0.794**	<0.05
		PFOS	12	**0.963**	**0.928**	<0.05
LES	liquid phase	PFOA	12	**0.989**	**0.979**	<0.05
		PFOS	12	**0.962**	**0.925**	<0.05
	reference soil	PFOA	12	**0.924**	**0.854**	<0.05
		PFOS	12	**0.926**	**0.858**	<0.05
	humus soil	PFOA	12	**0.870**	**0.756**	<0.05
		PFOS	12	**0.650**	**0.422**	<0.05
SIA	liquid phase	PFOA	12	**0.932**	**0.868**	<0.05
		PFOS	12	**0.852**	**0.726**	<0.05
	reference soil	PFOA	12	0.460	0.212	ns
		PFOS	12	0.557	0.310	ns
	humus soil	PFOA	12	**0.955**	**0.912**	<0.05
		PFOS	12	**0.995**	**0.990**	<0.05

In the case of the liquid phase, a very strong correlation was found between the endpoints analyzed ($r > 0.84$) for all species tested exposed to both PFOA and PFOS. The highest correlation coefficients were recorded for LES, for which, in the presence of PFOA and PFOS in liquid medium, the correlation coefficients were $r = 0.989$ and $r = 0.962$, respectively. The corresponding coefficients of determination (R^2) indicated that 97.9% and 92.5% of the variability in mean root length, respectively, was reflected in the values of maximum root length.

A more complex relationship was observed for the correlations determined in the variants with reference soil. High Pearson correlation coefficients (r) were observed in both the PFOA and PFOS assays for only two of the plant species examined; for SOS, correlation coefficient values of $r = 0.890$ and $r = 0.850$ were obtained, while for LES the values were as high as $r = 0.924$ and $r = 0.926$ for PFOA and PFOS, respectively (strong correlations in both cases). Only in the case of SIA was a statistically significant relationship not observed between the parameters analyzed in the tests carried out using the reference soil.

The use of humus soil created conditions in which the relationship between the two analyzed parameters was highly statistically significant. A particularly strong correlation was observed after the application of PFOS, where for SIA the correlation coefficient was

$r = 0.995$, and for SOS it was $r = 0.963$, with determination coefficient (R^2) values explaining 99% and 92.8% of the variability of the examined parameters, respectively. Similarly, a comparable strength of correlation relationships was observed after the application of PFOA, with correlation coefficients for SIA and SOS reaching $r = 0.955$ and $r = 0.891$, respectively. A variation in the correlation strength among the examined PFAS compounds was observed solely in the case of LES. Specifically, for the PFOA variants, a statistically significant and very strong correlation was maintained ($r = 0.870$), whereas following the application of PFOS, the correlation, although still statistically significant, decreased to a lower level ($r = 0.650$).

4. Discussion

The results obtained in this study clearly indicate that the type of medium used is a significant factor modulating the physiological response of plants to the presence of the examined PFAS. This factor is sufficiently critical that instances exist in which an identical dose of a given xenobiotic elicits either an inhibitory or a stimulatory response in root growth, contingent upon the medium to which it is applied.

The liquid phase containing PFOA or PFOS created conditions in which root growth was inhibited or did not undergo significant changes. Moreover, in none of the experimental variants utilizing the liquid phase was a growth-promoting effect observed, in contrast to the outcomes obtained with the other media tested. Such results can be partly explained by the fact that the roots of plants cultivated in this medium are directly exposed to the dissolved compounds present in the solution. In contrast to solid media, this system lacks particulate components capable of adsorbing, retaining, or otherwise modulating the bioavailability and effective concentration of toxic substances. A particularly pronounced inhibition of root growth was observed when PFOS was administered in the liquid phase, whereby all tested concentrations of this compound suppressed root elongation in each of the investigated species. Among the analyzed variants, the maximum %RGI value relative to the corresponding control was 22%. According to the toxicity classification system proposed by Persoone et al. [61] for natural waters and wastewaters, in which the reaction of the test organism should be classified as toxic when the percentage of the examined endpoint is equal to or higher than 20%, it can be concluded that PFOS applied in the liquid phase caused slight acute hazard (class II). Based on the results obtained in the liquid phase assays, SIA appeared to be the most sensitive species to PFOS exposure.

In the case of PFOA applied in the liquid phase, a clearly weaker plant response was observed, manifested as inhibition of root elongation. In LES, a significant reduction in root growth was detected at both intermediate and highest PFOA concentrations, whereas in SOS and SIA, inhibition of root growth occurred only at the highest concentration applied, and its extent did not exceed 15%. The possibility cannot be excluded that the observed differences between PFOA and PFOS in both the magnitude and extent of toxicity, as reflected by %RGI, are associated with distinct uptake mechanisms of these compounds in plants. Previous studies have shown that, owing to its higher aqueous solubility compared with PFOS, PFOA is transported predominantly by passive diffusion from the soil solution into the root interior (into the apoplast and cell sap), and subsequently translocated to the aerial plant parts via the transpiration stream [62]. PFOS, in contrast, exhibits a greater affinity for proteins and lipids located in the outer root tissues (binding to cell wall and membrane constituents), which leads to its accumulation at the root surface [63]. This localized enrichment may enhance the phytotoxic effects of PFOS on root tissues and result in a more pronounced inhibition of root elongation.

A markedly different toxicity profile with respect to the inhibition of root elongation in the test plants was observed when PFAS were applied in the reference soil. However, as

before, the strongest inhibition of root growth was found in SIA exposed to PFOS, the use of an intermediate dose resulted in a smaller decrease in root length (by 13.6%), while in the case of SOS the same dose caused only an 8% reduction in the value of this parameter. In the case of PFOA application to the reference soil, discrepancies were likewise observed relative to the responses obtained in the liquid phase, reflected in differing sensitivities of the test species to the effects of the xenobiotic under investigation. The greatest phytotoxic effect was recorded for SOS at the intermediate and highest application rates (maximum %RGI value of 11.6%), followed by LES under the same dosing conditions (maximum %RGI value of 6.8%). It is worth noting that in the variants with reference soil, especially after the highest dose was applied, the toxicity of a given xenobiotic towards root elongation was clearly lower than that observed in the aqueous phase; this was the case, for example, for PFOS in the tests with SOS, LES and SIA, as well as for PFOA in the test with LES.

Although PFAS sorption was not directly measured in the present study, the literature evidence suggests that interactions with kaolinite may have contributed to the lower phytotoxicity observed in the reference soil. One possible explanation is the presence of kaolin clay, which has been reported to exhibit substantial sorption affinity for PFAS compounds. Kaolinite is a clay mineral which, due to its specific crystalline structure and characteristic physicochemical properties, has attracted significant interest as a material with high application potential in environmental remediation processes, serving as an effective adsorbent [64]. From a structural standpoint, it is classified as a 1:1 layered aluminosilicate, in which each individual layer is composed of one aluminum octahedral sheet and one silicon tetrahedral sheet [65,66]. Importantly, hydroxyl groups located on the surface of the octahedral layer in kaolinite confer a hydrophilic character to it, while the surface of the silicon tetrahedra exhibits hydrophobic properties. It is suggested that the unique coexistence of hydrophobic and hydrophilic surface characteristics of kaolinite exerts a decisive influence on the mechanism and progression of PFAS adsorption [66]. This hypothesis is strongly supported by literature data indicating that adsorption of PFAS molecules containing a sulfonic or carboxylic functional group on the surface of kaolinite is up to ten times higher than in the case of other clay minerals, which as a rule are characterized by a high sorption potential [67]. The adsorption process on kaolinite proceeds particularly efficiently in the case of long-chain PFAS compounds, such as PFOA and PFOS, in contrast to short-chain PFAS, for which this process is significantly limited due to their higher mobility [68]. Moreover, it has been demonstrated that the adsorption of PFOS in kaolinite is substantially higher than that of PFOA [69]. In the context of phytotoxicity assessment, this may suggest a lower bioavailability and, consequently, a reduced phytotoxic potential of PFOS relative to PFOA when both compounds are present in a reference soil matrix. Nevertheless, as PFAS bioavailability was not directly determined in the present study, the observed differences should be interpreted with caution and may only indirectly reflect differences in bioavailability. When these findings are compared with the results obtained in the present study, a similar pattern emerges with respect to the effects of PFOA and PFOS on root elongation in SOS and LES: the toxic effect of PFOA in the reference soil exceeds that of PFOS. However, this relationship was not observed for SIA.

Another interesting effect observed after the application of the intermediate PFAS dose to reference soil was the stimulation of root growth, amounting to 9.9% for LES after the use of PFOS and as much as 15% for SIA following the application of PFOA. The phenomenon of stimulation of plant growth induced by exposure to low concentrations or doses of toxic substances is referred to as hormesis [70]. Recent literature in this domain suggests that the primary criterion for recognizing the occurrence of this phenomenon should be the directly measurable enhancement of plant growth, whereas other physiological parameters ought to be regarded only as auxiliary indicators [71]. The occurrence of hormetic

responses has been documented upon exposure to low concentrations of numerous environmental contaminants [72–74], including pollutants belonging to the group of PFAS. For instance, in hemp (*Cannabis sativa* L.), 14 days of exposure to 1 mg L^{-1} PFOA promoted shoot biomass [32]. Moreover, in common wheat (*Triticum aestivum* L.), PFOA exerted a promotive effect on seedling development and root elongation at concentrations below 0.2 mg kg^{-1}, whereas it significantly suppressed both root and shoot growth at concentrations exceeding 800 mg kg^{-1} [34]. The determinants of alterations in root growth following exposure to PFOA and PFOS have also been investigated in the model plant species *Arabidopsis thaliana* L. The results demonstrated that both compounds modulate phytohormonal activity, particularly affecting auxin biosynthesis and the associated signaling pathways, whereas PFOS additionally elicits an elevation in abscisic acid (ABA) levels and perturbs ABA-dependent signaling [75]. It cannot be excluded that the observed effects contribute to the induction of hormesis, regarded as a specific form of organismal acclimation in which auxins are postulated to play a role in modulating physiological responses. However, a key distinction must be made between a statistically significant growth stimulation at a specific concentration and a fully verified, non-monotonic dose–response pattern. Although the stimulatory responses observed in the present study are statistically significant and consistent with hormesis, the limited number of intermediate doses evaluated in our experimental design was not specifically intended to mathematically model or evaluate hormetic dose–response relationships. Therefore, dedicated experiments utilizing a broader range of narrow concentration gradients would be required to confirm the precise mathematical trend of this occurrence of a hormetic mechanism. Nevertheless, the molecular and physiological mechanisms underlying this phenomenon remain poorly characterized and warrant further comprehensive experimental investigation.

The application of humus soil in the phytotests resulted in the greatest differentiation of results. However, with few exceptions, the PFAS compounds tested did not exhibit any measurable toxic effects in most of the analyzed plant species–PFAS pairs; the situation was different for SOS exposed to both PFOA (21.2% RGI) and PFOS (22.5% RGI), as well as for SIA (27.2% RGI) exposed to PFOS. In these cases, this particular matrix contributed to the manifestation of the strongest toxic effects, expressed as inhibition of root elongation. On the other hand, application of PFOA at the intermediate dose to humus soil elicited the most pronounced stimulatory effect on SIA root growth, resulting in an increase of 16.7% relative to the corresponding control. Furthermore, in numerous instances, the plant response to the incorporation of the tested PFAS compounds into humus soil differed significantly from the response observed under exposure to the same PFAS compounds in the liquid phase (SOS: PFOA intermediate dose, PFOS low and intermediate doses; LES: PFOA high dose; SIA: PFOA intermediate and high doses; PFOS low and intermediate doses), as well as in the reference soil (SOS: PFOS high dose; LES: PFOS intermediate and high doses; SIA: PFOS low dose).

The attenuation of PFAS toxicity observed in humus soil may be associated with physicochemical properties known to influence PFAS partitioning and availability to plants. Previous studies have indicated that, in constraining the bioavailability of the examined xenobiotics, parameters that directly govern the sorption capacity of the soil matrix—such as soil structure, total organic matter content, particularly its hydrophobic fractions, and organic carbon content—may play a critical role. It has additionally been demonstrated that PFOS exhibits the highest degree of sorption to soil organic matter (61–98%), exceeding the corresponding values reported for PFOA and perfluorobutanoic acid (PFBA) [76]. It cannot be excluded that this specific property of PFOS also manifested in the experiments with the SOS and LES species, for which—excluding the highest tested concentration—the toxicity of PFOS with respect to root elongation was lower than that of PFOA when

both compounds were applied to humus soil. Furthermore, a comparable effect was also observed for these species in the tests with reference soil. This divergence observed at the highest dose may be attributed to the potential saturation of sorption sites within the soil organic matter matrix. It cannot be excluded that while the high organic matter content in humus soil effectively immobilizes PFAS at low and intermediate doses, the highest applied concentration may have exceeded the binding capacity of the soil's organic fraction for these compounds. Consequently, a threshold may have been reached above which the excess, unbound PFAS remains bioavailable in the soil solution, leading to a pronounced toxic response and distinguishing these treatments from the behavior observed in the reference soil. Phytotoxicity assays assessing the effects of PFOA and PFOS on root elongation in *Brassica chinensis* L. (currently classified as *Brassica rapa* subsp. *chinensis* (L.) Hanelt), conducted across different soil types, revealed that soil organic matter content shows the strongest correlation with the threshold toxicity values of PFOA and PFOS. Furthermore, the authors reported that cation exchange capacity is also an important determinant, indicating that sorption of PFOA and PFOS by the solid soil phase may represent a key mechanism mitigating their toxic effects [77]. In accordance with the OECD guidelines on the requirements for the properties of soil used for phytotoxicity tests, the soil organic matter content should not exceed 3%, and the organic carbon content should not be greater than 1.5% [38]. In humus soils, the values of these parameters frequently exceed the recommended thresholds; nevertheless, given the potential application of phytotoxicity tests to soils derived from real-world environmental samples, the implementation of such phytotests has substantial and direct practical relevance.

The suitability of using the maximum root length measured on a single plate as a time-efficient alternative metric for determining the phytotoxicity of PFOA and PFOS was assessed by calculating correlation coefficients between mean root length and maximum root length per plate. The results demonstrated a statistically significant and strong correlation between these parameters across all experimental variants, in both liquid phase and humus soil assays. Accordingly, the observed correlations support further evaluation of maximum root length as a simplified endpoint in PFAS phytotoxicity studies.

Although the 72 h exposure provides valuable insights into the acute phytotoxicity of PFOA and PFOS during early growth stages, short-term bioassays carry inherent limitations regarding chronic toxicity. Future research should focus on extended exposure periods and the evaluation of PFAS accumulation within mature plant tissues to more comprehensively reflect long-term environmental scenarios.

Furthermore, in a broader context, the species-specific differences in sensitivity observed in this study have important implications for assessing the ecological risks of PFAS contamination in terrestrial environments. The high sensitivity of certain species, such as *Sinapis alba* to PFOS, underscores their potential as effective bioindicators for the early detection and monitoring of environmental PFAS stress. Conversely, variations in tolerance among crops and wild plants suggest that PFAS pollution could selectively alter plant community structures and affect agricultural productivity. Therefore, ecological risk assessments should utilize a diverse battery of phytotests rather than relying on a single indicator species to accurately evaluate the impact of PFAS on terrestrial ecosystems.

5. Conclusions

In summary, the results obtained in this study clearly demonstrate that the type of medium employed constitutes a critical factor modulating the physiological response of plants, as evaluated through alterations in root elongation under exposure to PFOA or PFOS. Although no effect of these compounds on the percentage of germination inhibition (%GIH) was detected within the range of doses tested, early-stage root growth in seedlings proved to

be a sensitive endpoint. The strong correlation observed between mean root length and the length of the longest root on each plate (L_{MAX}) indicates that both parameters are equally robust and reliable quantitative descriptors of the phenomenon under investigation.

The strongest suppression of root growth occurred when the examined PFAS were supplied in the liquid phase, while their application in the reference soil and humus soil produced more variable effects. These results indicate that, at relatively low concentrations of long-chain PFAS, a distinct direct phytotoxic impact can be clearly detected in the liquid phase; however, the outcomes obtained with the reference soil should be interpreted with particular caution. This precaution is justified primarily by the composition of the reference soil, which contains kaolin clay, which has been reported to exhibit a strong sorption affinity for long-chain PFAS. Although some variability was observed in the phytotoxic responses to the tested PFAS in humus soil, the use of this matrix may be advantageous due to its closer approximation of environmentally relevant conditions and its practical applicability.

The observed effects were dependent both on the test plant species and on the specific PFAS applied. Among the evaluated species, SIA exhibited the highest sensitivity to PFOS, as evidenced by the most pronounced inhibition of root growth. Conversely, following exposure to PFOA, SIA demonstrated the least pronounced inhibitory effect on root growth relative to the other analyzed species. For the SOS and LES, which exhibited a relatively similar response pattern to the applied PFAS but differed from that of the SIA, no stimulation of root growth was detected following PFOA exposure, whereas in the SIA, no induction of root growth was observed after exposure to PFOS. Future studies should combine phytotoxicity assays with direct measurements of PFAS sorption, bioavailability, and plant uptake in different soil matrices in order to clarify the mechanisms underlying the medium-dependent responses observed in this study.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/w18212779/s1; Table S1. The percentage of germination inhibition (%GIH) and the length of the longest root (L_{MAX}) in of *Sorghum bicolor* (SOS) following exposure to PFOA or PFOS across all experimental variants. Table S2. The percentage of germination inhibition (%GIH) and the length of the longest root (L_{MAX}) in *Lepidium sativum* (LES) following exposure to PFOA or PFOS across all experimental variants. Table S3. The percentage of germination inhibition (%GIH) and the length of the longest root (L_{MAX}) in *Sinapis alba* (SIA) following exposure to PFOA or PFOS across all experimental variants.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Abbreviations

The following abbreviations are used in this manuscript:

PFAS	Per- and polyfluoroalkyl substances
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonic acid
POPs	Persistent organic pollutants
PFBA	Perfluorobutanoic acid
OC	Organic carbon
SOS	*Sorghum bicolor* (L.) Moench
LES	*Lepidium sativum* L.
SIA	*Sinapis alba* L.
%GIH	Percentage of germination inhibition
%RGI	Percentage of root growth inhibition
L_{MAX}	Length of the longest root
ABA	Abscisic acid
PBT	Persistent Bioaccumulative and Toxic

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