agriculture

MDPI

Article

Morphological, Histological, and Glyphosate Residue Analysis of *Helianthus annuus* L. Plants Treated with Glyphosate

Szilvia Kisvarga [1], Dóra Hamar-Farkas [1,*], Katalin Horotán [2], Katalin Inotai [3], Mária Mörtl [3], András Neményi [1], András Székács [3] and László Orlóci [1]

[1] Ornamental Plant and Green System Management Research Group, Institute of Landscape Architecture, Urban Planning and Garden Art, Hungarian University of Agriculture and Life Sciences (MATE), 1223 Budapest, Hungary
[2] Zoological Department, Institute of Biology, Eszterházy Károly Catholic University, 3300 Eger, Hungary
[3] Institute of Environmental Sciences, Hungarian University of Agriculture and Life Sciences (MATE), 1022 Budapest, Hungary
* Correspondence: farkas.dora@uni-mate.hu

Abstract: Several recent studies have shown that glyphosate and its metabolite, aminomethylphosphonic acid (AMPA), resist rapid degradation and, therefore, can accumulate in plants. Continuing our previous research, we aimed to investigate the effect of indirectly spraying glyphosate on leaves and soil on non-target plants in the case of *Helianthus annuus* L. The plants were treated with glyphosate in their 5–6 leaf stages, the effects of which were assessed two weeks later from a morphological and histological point of view, as an evaluation of the residues of glyphosate and its metabolite, AMPA. They had an effect on both treated groups. In the case of the morphological parameters (plant height, number of leaves, and fresh and dried root and green mass), the data of the treated plants were statistically lower than in the case of the control group. The epidermis and the transport tissue system were damaged, and tissue death was observed in plants exposed to glyphosate. Both compounds were detected in all plant parts (roots, stems, lower leaves, and upper leaves), well above the limit of detection (0.025 mg/kg) and limit of quantitation 0.075 mg/kg showing a statistical difference with the control plants. This proved that glyphosate is incorporated into the plant organism even when applied indirectly.

Keywords: glyphosate; AMPA; *Helianthus*; sustainability; toxicology

Citation: Kisvarga, S.; Hamar-Farkas, D.; Horotán, K.; Inotai, K.; Mörtl, M.; Neményi, A.; Székács, A.; Orlóci, L. Morphological, Histological, and Glyphosate Residue Analysis of *Helianthus annuus* L. Plants Treated with Glyphosate. *Agriculture* **2023**, *13*, 1014. https://doi.org/10.3390/agriculture13051014

Academic Editor: Xingang Liu

Received: 13 April 2023
Revised: 28 April 2023
Accepted: 3 May 2023
Published: 5 May 2023

1. Introduction

1.1. The Impact of Pesticides on the Environment

Environmental problems have always received significant attention from the scientific community [1]. Due to their stationary nature, plants must withstand a variety of abiotic stresses, including heavy metals, salt, drought, nutrient deficiency, light intensity, pesticide pollution, and extreme temperatures. These stressors result in serious changes in crop quality and quantity worldwide [2], as does contact with pollutants that also burden the environment [1]. Environmental pollutants are toxic substances that can enter the environment from both anthropogenic and natural sources [3]. These substances include a wide range of chemical or physical substances released into the environment that can contaminate the abiotic components of ecosystems [3,4].

1.2. The Effect of Glyphosate and Its Metabolites on the Living Environment

Glyphosate (*N*-(phosphonomethyl)glycine) is one of the most widely used broad-spectrum organophosphorus herbicides [5]. The parent compound was introduced by Monsanto Corporation under the brand name 'Roundup' in 1974 [6–8]. Glyphosate is the most widely used herbicide in the world [9], which targets 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) and inhibits the biosynthesis of aromatic amino acids in

the shikimate pathway [10–12]. In turn, glyphosate is a non-selective herbicide, an active ingredient without any available analogues in its mode of action category. Inhibition of EPSPS by glyphosate delays the synthesis of essential secondary metabolites and proteins; it also inhibits vital energy pathways in soil microbes and plants [13–15]. It also has a significant impact on non-target plants and aquatic communities [16]. Today, agricultural activities are highly dependent on the use of glyphosate-based herbicides, and the global consumption of these products has increased a hundredfold since the introduction of genetically modified crops in the mid-1990s [7,17]. It is a widely used herbicide against perennial and annual weeds in agriculture, and in forestry, home gardens and urban areas [18]. However, only a small part of the total applied glyphosate serves the actual purpose, and most of it ends up in the environment, which reduces crop yields and endangers human and animal life. Glyphosate can reach non-target plants via different routes, such as spraying, release through the tissue of treated plants, and dead tissue of weeds [19]. Low (10 μg/L) and sublethal concentrations of glyphosate were detected in plants within 20 m of the glyphosate application site [20], also in *Urochloa decumbens* plants [21]. As a consequence of this non-targeted exposure, glyphosate residues are also detected in various food plants and foods [19].

In addition to glyphosate, more and more scientific publications are drawing attention to the toxicity of aminomethylphosphonic acid (AMPA). AMPA is the main metabolite from the breakdown of glyphosate; since its accumulation has been demonstrated in various samples (e.g., soil, water, food, and human bioaccumulation), understanding its toxicity and environmental persistence is as important as that of glyphosate [22]. AMPA is a key and most persistent metabolite of glyphosate that is commonly found in sediment, surface, and groundwater [8]. A higher proportion of agricultural land use close to the inhabited environment can increase exposure to AMPA, which can also be detected in urine [23]. Based on the literature findings, approximately 30% of the world's cropland is contaminated at low levels with glyphosate, while 93% of the world's cropland is contaminated with AMPA [24]. Much information has not yet been revealed about the ecotoxic effects of AMPA [25,26]. Therefore, to better assess the risk of contamination with AMPA, the ecotoxicity and biodegradation pathways and kinetics of this metabolite need to be elucidated [24].

The results, therefore, indicate that glyphosate and its metabolites may have indirect, long-term consequences for non-target plants and microbes in environments where glyphosate degradation is delayed due to seasonally cold climate conditions [11]. In some tissue types of forest plant species, residues persisted for up to 12 years, and root tissues generally retained glyphosate residues longer than shoot tissue types [27]. The samples examined from the colder, more northerly biogeoclimatic zone retained significantly higher glyphosate levels for a longer time than the samples collected from the warmer biogeoclimatic zone. Glyphosate residues in the soil also alter the composition of endophytic microbial communities, both for bacteria and fungi [28]. In addition, they can also appear in organic manure and can be taken up by plants (*Festuca pratensis*, *Fragaria* × *vescana*), so glyphosate can also spread with organic manure, which counteracts the proper development of plants [29]. The amount of glyphosate in several urban soils is above the defined dissolution level, which also poses a threat to living waters. Moreover, its carcinogenic effect is not a negligible factor [30]. Outbreaks of many animal and plant diseases have been linked to the accumulation of glyphosate in the environment. The long-term effects of glyphosate have been underestimated, and new standards will be needed for residues in plant and animal products and in the environment [31].

In agricultural practices and home gardens, glyphosate is an important herbicide. However, new research shows that it is essential to identify the most environmentally and toxicologically sensitive scenarios. This is necessary to guide the future use of glyphosate so that it remains useful, ensuring minimal environmental pollution and no negative health consequences [24].

The literature data show that glyphosate and its metabolites are hazardous to the environment and can be detected not only in the target plant but also in the surrounding plants, so there is a high chance that toxic substances will enter the food chain with the use of glyphosate. In our glyphosate tests with sunflowers [32], which began in 2021, we established that glyphosate could appear in the target plants even if the target plants were not treated with the chemical directly. We continue these studies with a variety of the *H. annuus* species, as this species is an important crop not only in the food industry, but also in the field of ornamental plant application. Continuing our previous research, we aimed to investigate the effect of indirectly spraying glyphosate on leaves and soil on non-target plants in the case of sunflower plants. We supplemented our analysis with morphological and histological measurements. Thus, new research may prove that glyphosate and its metabolites can appear in the plant organism even if they did not receive glyphosate treatment directly or did not perform a role as a target plant in the experiment. Based on our results and other studies [6,17,33], it would be worthwhile to reconsider the extent of glyphosate use in the future and take this factor into account—not only in relation to food plants but also in relation to plant material for green space management.

2. Materials and Methods

2.1. Materials Used for the Experiment

For our experiments, we used a commercially available non-glyphosate-resistant GK Milia CL sunflower hybrid variety, which was bred by Gabonakutató Nonprofit Közhasznú Kft. (Szeged, Hungary) [34]. This early variety has a high oil content and tolerates abiotic stresses well. It can be treated with Clearfield® herbicide technology, certified in 2018 [35]. The seeds have not been pretreated or coated. The variety was also used successfully in previous tests with glyphosate exposure, the variety is susceptible to the active ingredient [32], but several literature references point to the sensitivity of *H. annuus* to glyphosate [36–40].

Glialka manufactured by Monsanto Company (St. Louis, MO, USA) and owned by Bayer GMBH (Monheim am Rhein, Germany), was used for treatment. This is a fully effective weed killer with a glyphosate content of 360 g/L. The active ingredient of the chemical used is glyphosate, the effects of which are also described in the literature [19,20,41–43]. The plants were sown from seed on 9 May 2022 in outdoor conditions under natural lighting. The temperature was not altered—there was no cooling or heating—thus striving to imitate the natural conditions of upbringing and cultivation. The plants grew under an average temperature of 8–12 °C at night and 15–20 °C during the day in Budapest, Hungary. On 13 June, glyphosate treatment was applied at a concentration of 0.1 mL/L.

2.2. Description of the Experiment

Our experiment was conducted at the Budatétény (Budapest, Hungary) research station of the Institute of Landscape Architecture, Urban Planning, and Garden Art of the Hungarian University of Agriculture and Life Sciences in 2022. The seeds were sown in 20 L pots containing Klassman-Deilmann TS3 Fine, Geeste, Germany soil with the following parameters: pH (H_2O) 6, N 140 mg/L, P (P_2O_5) 100 mg/L, K (K_2O) 180 mg/L, Mg 100 mg/L, S 150 mg/L. The plants were regularly watered during the growing season. One seed was placed in the containers. Seeds were sown in more containers than planned so that by the end of germination, there would be a sufficient amount of experimental plants. The control group (referred to as 'Control') received only water, no nutrients, and no other influencing substances. The plant growing conditions were identical to the treated plants. The plants were grown outdoors. The treated groups were raised in isolation from each other in the experimental area of the Research Institute. The containers were raised on a sterile, artificial surface, preventing them from escaping into the environment and excluding the possibility that other chemicals from the soil could enter the soil of the containers. Glyphosate treatment was administered once when the plants were in the 5–6 leaf stage. The treatment—imitating the application of chemicals in the field—was sprayed on the soil on the edge of the container and drifted onto the plant from a distance

of 0.5 m in slightly windy weather (2–3 km/h). Glyphosate treatment was administered once the plants were in the 5–6 leaf stage. The treatment—imitating the application of chemicals in the field—was sprayed on the soil on the edge of the container and drifted onto the plants from a distance of 0.5 m in a slightly windy weather (2–3 km/h). The applied glyphosate could reach the plant and the soil surface. To separate this, two groups were formed. In one group, the parts of the plant above the soil surface were covered with foil to prevent glyphosate from getting on the plant ('Soil' group). Another group was also created, where the soil surface around the plant was covered during the treatment ('Leaf' group). Cultivation in a container is of particular importance, taking into account environmental protection aspects (spraying on open ground can enter the ecosphere). At that time, the plants were 5 weeks old, as in our previous experiment in 2021 [32]. Two weeks passed between the glyphosate treatment and the final assessment. In addition to the control treatment, we used the agent at a concentration of 1.0 L/ha—relying on the manufacturer's recommendations and the results of our previous measurements, and the conclusions drawn from them. Afterward, the plants were subjected to morphological, histological, and laboratory assessments.

2.3. Morphology

The morphological measurements were made as follows: (a) Height: height measured from the ground surface to the highest point of the plant. (b) Number of leaves: the number of leaves on the plant at the time of the survey. (c) Fresh and dried green mass refers to the total mass of the plant above the soil surface (leaves, petioles, and shoots) cut at the root neck. This was completely dried and then weighed. (d) Fresh and dried root mass: the mass of the entire root system, calculated from the root neck, cleaned of soil parts below the soil surface. This was completely dried, then weighed. Average values were calculated from these data.

A simple manual measuring tape was used for the measurement. A Kern PCB 6000-1 analytical laboratory balance was used for mass measurements. The drying of the green plant parts and the root system took place in a VEB MLW Labortechnik Ilmenau drying cabinet for 120 h at 40 °C.

2.4. Histology

For the histological examination of sunflower plants, stem cross-sections were examined. For this, cross-sections from three points of the stem were made: above the root neck, from the middle, and from the upper part of the stem. The cutting was completed manually with a scalpel, so several sections were made from each part of the stem; this was necessary because the thickness of the section cannot be precisely controlled in the case of manual cutting, and the samples cannot be examined under a microscope.

The cut part of the stem was placed in a watch glass filled with distilled water, then transferred to the slide with a soft-bristled brush. After that, we dropped water on it and covered it with a coverslip, and examined it under the microscope. The Euromex bScope BS.1153-PLi microscope was used for the light microscopic examinations.

The cut samples were examined with a PLi 4/0.1 lens; this magnification allows adequate examination of the structure of the stem and any changes that may occur in it. Using a Levenhuk M1400 plus camera and the Topuview program to make the microscopic images enabled the taking of high-resolution images. The subsequent color correction of the photographs was carried out with the Gimp program.

2.5. Sampling and Sample Preparation Prior to GC-MS Analysis

Sampling for the analytical measurements was carried out 42 days after seeding the plants (14 days after the treatments with glyphosate). Six plants were processed by dissecting each one to obtain corresponding plant matrices (stem, leaf, and root). The root was carefully dug around, and the plants were lifted out together with the soil ball attached to the root, which was immediately put in a plastic bag. The shoot above the ground was

cut off at the root neck, and it was also put in a bag so that it would not dry out until it was transported to the laboratory.

The soil was sampled from the part around the root zone. The sample was stored at −80 °C until processing. Soil, stem, and leaf masses were measured prior to and after drying. The leaves at the top of the shoot and the leaves in the lower part of the stem were selected separately. The roots were cleaned of larger pieces of soil and grains with the help of a brush.

Soil samples were dried in a drying oven at 105 °C for 24 h until constant mass.

Stems and leaves were chopped with a chopper and then lyophilized (Christ Alpha 1-4 LD Freeze dryer, Osterode am Harz, Germany). The roots were also cut up with pruning shears prior to lyophilization.

Individual samples obtained after lyophilization were combined for all four sample types (soil, stem, leaf, and root). Combined lyophilized samples were stored at 4 °C until chemical analysis (but not exceeding one week).

2.6. Residue Analysis by GC-MS

The instrumental analytical method published earlier [32] was used for the measurements, with some modifications. A total of 10 mL of acetonitrile was added to 1 g of dried sample in a 50 mL centrifuge tube. These samples were intensively shaken for one hour by an Ohaus (Parsippany, NJ, USA) digital vortex mixer at 1100 rpm, then centrifuged (3000 rpm, 15 min, at 25 °C). Three mL of the supernatant was removed and filtered through a syringe filter (0.45 µm). Then, the extract was evaporated to the dryness at 60 °C in a nitrogen stream.

Derivatization of the compounds was carried out according to the earlier published procedure [32]. Briefly, 60 µL of dry pyridine was added to the dried residue, and after 5 min 100 µL of silylating agent, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) was added. This mixture was heated at 60 °C for 3 h, then measured by gas chromatography using mass spectrometric detection mode (GC-MS). GC-MS analysis was performed on a Varian Saturn 2000 workstation equipped with a Varian CP 8200 autosampler (Varian Inc., Walnut Creek, CA, USA). Quantification of the glyphosate and AMPA was performed by using external calibration. The chromatographic conditions were as before [32]. The estimated values of the limits of detection (LODs) were 0.025 mg/kg, and limits of quantification (LOQs) were 0.075 mg/kg determined by standard solutions containing both glyphosate and AMPA. Selected ions for single ion monitoring were 195, 211, and 226 for AMPA derivative and 268, 240, and 211 for silylated glyphosate derivative, respectively. External calibration based on ion intensities 211 and 240 for AMPA and glyphosate, respectively. Recoveries were determined from the results obtained for spiked control samples in triplicates at spiking levels of 0.2, 0.5, and 1.0 mg/kg for each matrix. Average recoveries for glyphosate for soil, roots, stems, and leaves at spiking level 0.5 mg/kg were 78 ± 6, 94 ± 7, 92 ± 8% and 87 ± 9%, respectively. For AMPA, the corresponding values were 83 ± 8, 96 ± 6, 93 ± 7%, and 92 ± 8% for soil, roots, stems, and leaves, respectively. The recoveries for spiking levels 0.1 and 1.0 mg/kg ranged from 62% to 94% for glyphosate, whereas 69 to 98% was determined for AMPA metabolite. The lowest values were measured for soil spiked at 0.2 mg/kg. Thus, the highest LOQs (0.120 mg/kg for glyphosate and 0.109 mg/kg for AMPA) were obtained for this matrix. The lowest LOD and LOQ values for glyphosate were 0.027 and 0.081 mg/kg for stem and root samples, respectively. For AMPA, these limits were 0.026 and 0.077 mg/kg for the same sample types, respectively.

2.7. Statistical Evaluation

The experiment and its repetitions, the measurement, and the evaluation of the measured data, as larger processes took place one after the other, were carried out simultaneously. The processing, comparison, and examination of measurable deviations of our results was carried out with the IBM SPSS Statistics 26 program, using the ANOVA model

during the morphological examination and the UNIANOVA method when analyzing the results of the chemical residue examination. In order to achieve a normal distribution of the data series, data transformation was performed on part of the data (root length, dry green, and root mass) using the Winsorization method, and the chi-square test was proved [44]. Shapiro–Wilk test was used for all other data. In all cases, the measured data were analyzed at a 95% reliability (significance) level. Having evaluated the Levene test, if the Sig. > 0.05, then Tukey, and if Sig. < 0.05, Games-Howell post hoc test was used.

3. Results

3.1. Morphological Evaluation

The morphological data show a significant difference between the treatments in all cases, with the exception of root length; that is, it can be concluded from the morphological properties that glyphosate has entered the plant organism. The height (52.508 cm) of the control group (Table 1 (a)) shows significantly higher values than the average height of the leaf-treated (47.1 cm) group ($p = 0.005$).

Table 1. Aggregated average data of *Helianthus annuus.* Different letters (a, b, c, or ab) indicate significantly different groups (horizontally): (a) Height of the treated groups (Games-Howell, $p < 0.05$). $p = 0.005$; (b) Number of leaves of the treated groups (Tukey, $p > 0.05$). $p = 0.017$; (c) Fresh shoot and leaf weight of the treated groups (Tukey, $p > 0.05$). $p = 0.000$; (d) Fresh root weight of the treated groups (Games-Howell, $p < 0.05$). $p = 0.000$; (e) Dried shoot and leaf weight of the treated groups (Games-Howell, $p < 0.05$). $p = 0.000$; and (f) Dried root weight of the treated groups (Games-Howell, $p < 0.05$). $p = 0.001$.

	Parameter	Control	Soil	Leaf	Unit
(a)	Height	52.508 ± 6.62 (b)	52.850 ± 3.58 (b)	47.100 ± 1.71 (a)	cm
(b)	Leaves number	16.83 ± 2.92 (b)	16.08 ± 2.68 (ab)	13.83 ± 1.85 (a)	pcs
(c)	Fresh shoot and leaf weight	48.91 ± 5.41 (c)	36.60 ± 7.58 (b)	25.02 ± 4.71 (a)	g
(d)	Fresh root weight	11.55 ± 2.62 (b)	8.48 ± 1.77 (a)	7.51 ± 1.06 (a)	g
(e)	Dried shoot and leaf weight	10.58 ± 0.98 (b)	9.07 ± 2.77 (b)	5.80 ± 0.68 (a)	g
(f)	Dried root weight	1.42 ± 0.42 (b)	1.23 ± 0.31 (b)	0.91 ± 0.08 (a)	g

The penetration of glyphosate through the leaves and shoots is stronger than in the soil-treated group.

A similar trend can be observed in the case of the leaf number values (Table 1 (b)), and the results are statistically different from each other ($p = 0.017$).

The glyphosate that affected the leaves directly affected the leaf number; this can be clearly seen in the results—the lowest average number of leaves can be observed in this group (13.83 pcs), while in the case of control (16.83 pcs) and in the case of the soil-treated group (16.08 pcs) we obtained higher values. Although glyphosate could reach the roots during the five-week cultivation period and translocate from the leaves and shoots, its effect was still barely noticeable. Thus, glyphosate reduced the values of plant height and number of leaves, which results are in accordance with those reported by Junior [45].

The results of fresh shoot mass (Table 1 (c)) and fresh root mass (Table 1 (d)) also fit this trend.

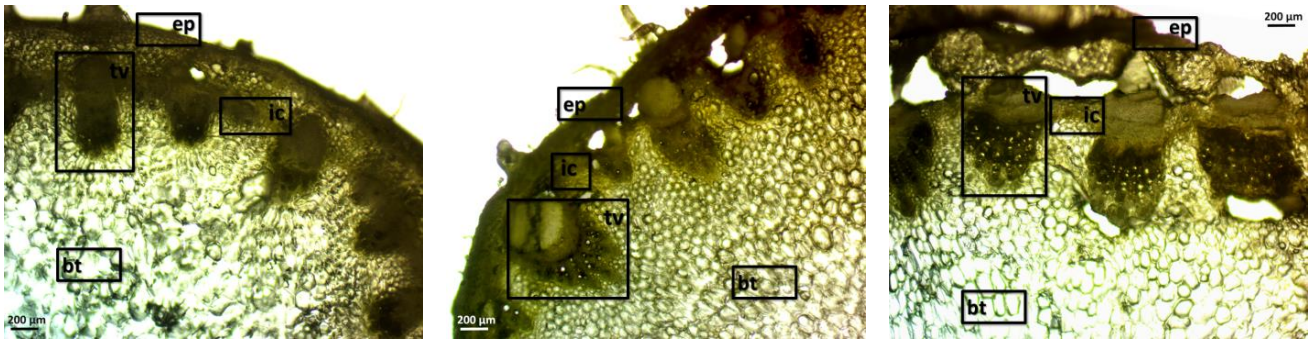
The highest fresh green mass (48.91 g) and fresh root mass (11.55 g) of the control plants differ significantly from the soil-treated fresh green mass values (36.6 g fresh green mass; 8.48 g average value of fresh root mass), and from the leaf-treated fresh green mass values (fresh green mass was 25.02 g; average value of fresh root mass was 7.51 g). The results of the dried green mass (Table 1 (e)) and root mass (Table 1 (f)) correlate with the results of the fresh mass measurement and also show significant differences between the tested groups for the dried green mass ($p = 0.00$) and the dried root mass ($p = 0.01$).

The morphological results are in accordance with the results of Smedbol et al. [46], as the amounts of glyphosate and AMPA in the leaves are higher than in the root system after

a single glyphosate treatment. We experienced similar results as Wood [47] in his findings that glyphosate and AMPA exert their effects by reaching the roots and moving towards the shoot and leaf tissues.

3.2. Histological Examination

The difference between the stem parts of the control and treated groups is also visible in the results of the histological examination. In the case of the parts above the root neck in the control group (Figure 1a), it is clearly visible that the epidermis is intact, the transport vessels are developed, and the interfascicular cambium is almost completely formed.



(**a**) (**b**) (**c**)

Figure 1. Microscopic images of the lower stem part of the treatments: (**a**) Control; (**b**) Leaf treated 1000 mg/kg; (**c**) Soil treated 1000 mg/kg. The abbreviations shown in the pictures mean the following: ep—epidermis, tv—transport vessels, ic—interfascicular cambium, bt—basal tissue (photos: Horotán, 2022).

The cells of the pith tissue can be clearly seen, but it is visible that the plant is still in a juvenile stage, as these cells are not yet fully formed. Overall, it can be said that the plant developed according to its stage of development.

The effect of glyphosate and AMPA can already be seen in the microscopic image of the examined individual of the leaf-treated group (Figure 1b). Although the epidermis is still intact, the cells fit closely together, and distortions can already be observed in the transport vessels. The fascicular cambium is formed, although directly below the epidermis, almost touching it, there is no basal tissue between them. The formation of the cambium ring has begun, but it is distorted. The tissue of the transport vessels has lightened—their function has decreased or ceased; the cambium can still be clearly observed. The development of the vessels is inhibited; this can be seen from the branched shape of the vessels. The cells of the pith tissue are still relatively developed, and the cell walls are strong and connected. As a result of the tissue necrosis observed under the epidermis, the formation of cavities can be observed.

The effect of glyphosate and AMPA can be observed even more strongly in a specimen examined with a microscope from the group that received soil treatment (Figure 1c). The epidermis has died or detached from the plant. The base tissue between the epidermis and the transport tissue is severely damaged; tissue necrosis can be observed in several places, as a result of which the stem has become hollow. The fascicular cambium is strong, but it was severely distorted and damaged due to the treatment.

3.3. Residue Assessment

In the residue evaluation, glyphosate and AMPA content were evaluated in root, stem, and leaf parts. A lot of matrix components appeared in the GC-MS chromatograms (Figure 2). The limit of quantification (LOQs) was 0.075 mg/kg for both glyphosate and AMPA, the control group did not contain glyphosate, so only the groups treated with glyphosate were evaluated. Samples were taken from all parts of each group, but only those results where a significant difference was found are presented.

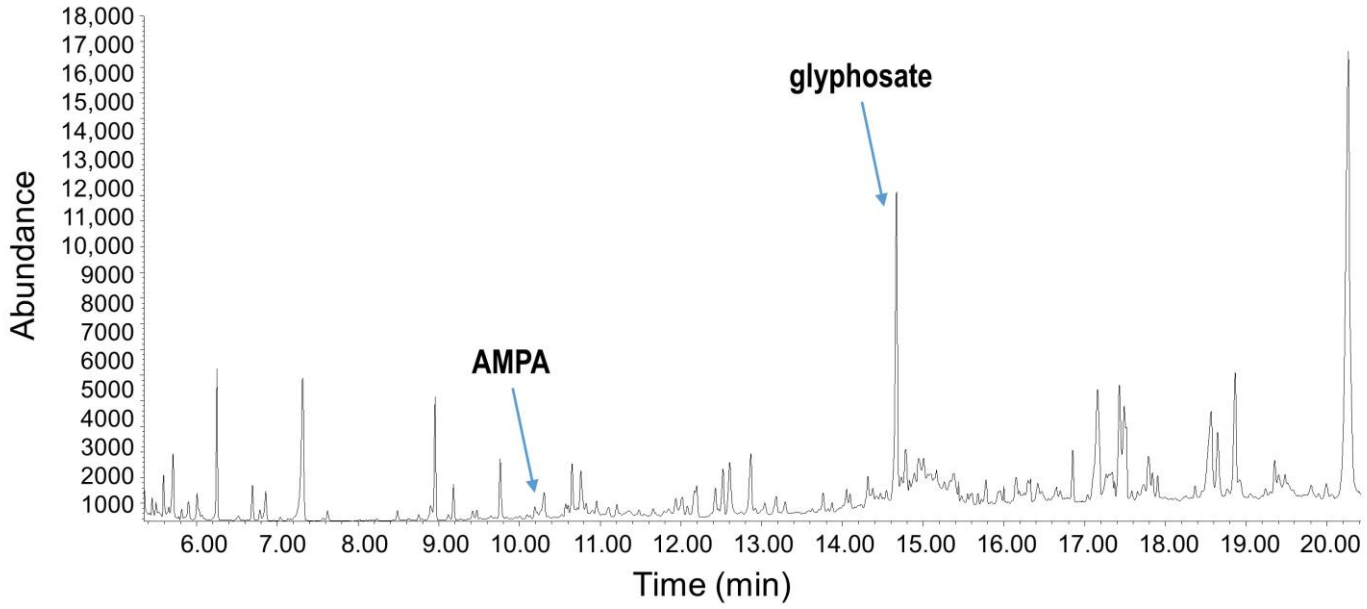


Figure 2. GC-MS chromatogram of trimethylsilyl derivatives of glyphosate and aminomethylphosphonic acid (AMPA) in a leaf sample extract.

3.4. Glyphosate Content

There was no statistically detectable difference in the leaves and stems, but it was detectable in all measurements (Figure 3), and in the case of the lower and upper leaves, the values exceeded 1 mg/kg.

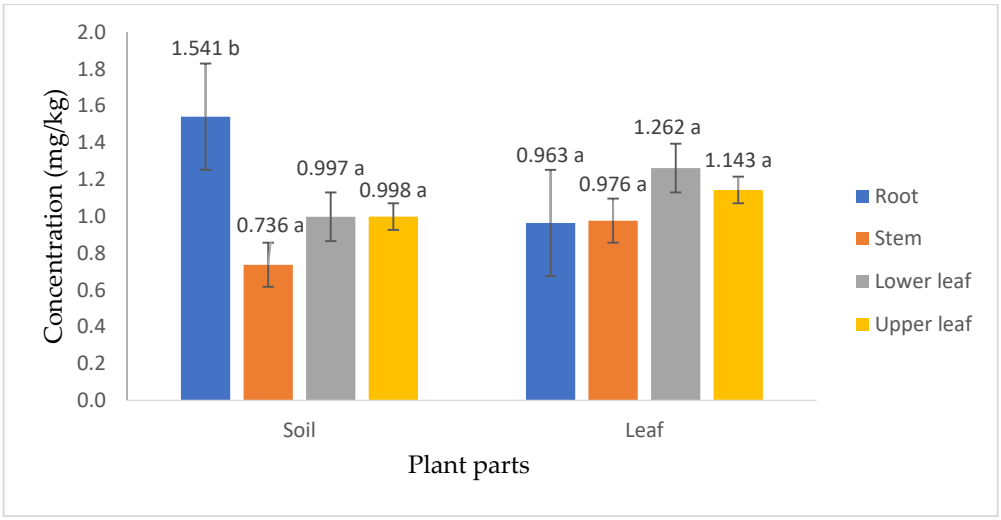


Figure 3. Glyphosate residue content in different plant parts of *Helianthus annuus*. Different letters indicate significantly different groups.

These values were 0.963 mg/kg and 0.976 mg/kg in the cases of the root system and the stem, respectively. The average glyphosate content of the foliar-treated group was 0.963 mg/kg, which is significantly different from the glyphosate content of the soil-treated group (1.54 mg/kg).

3.5. AMPA Content

The glyphosate metabolite compound was also significantly detectable in all plant parts (Figure 4), with even higher values than in the case of glyphosate, where it exceeded

1 mg/kg only in the case of leaves (Figure 3). In the case of AMPA, it always exceeds this value.

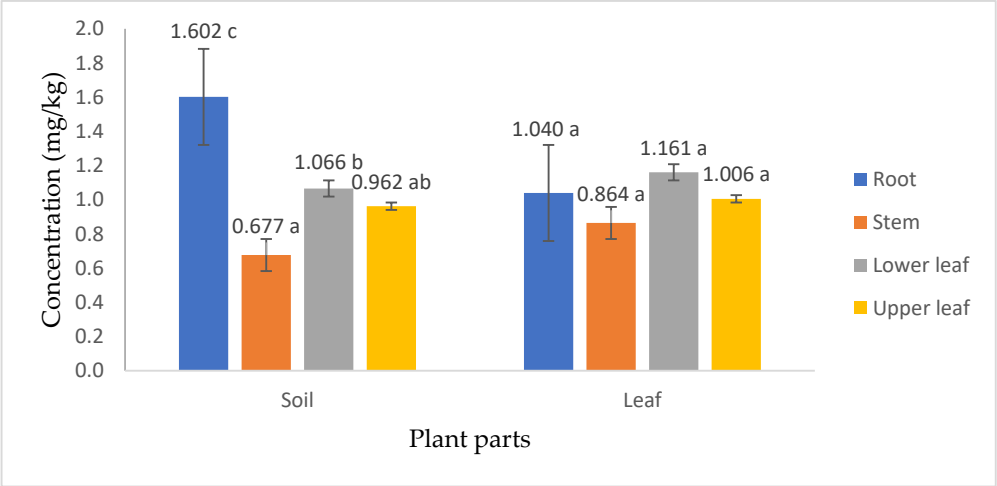


Figure 4. AMPA residue content in different plant parts of *Helianthus annuus*. Different letters indicate significantly different groups.

A significant difference was observed only in the root system between the two treatments. It can be seen that 1.04 mg/kg AMPA was found in the leaves of the foliar-treated group, while the value was 1.602 mg/kg for the soil-treated group.

In summary, it can be said that both glyphosate and AMPA content can be detected in the tested plants. In the case of the groups that received the soil treatment, it accumulated in the roots and differed significantly from the glyphosate and AMPA content of other plant parts, while in the case of the individuals that received the leaf treatment, there was no statistically detectable difference between the results of individual plant parts, the distribution of glyphosate and AMPA was uniform in the plant.

4. Discussion

Glyphosate is among the most widely used pesticides of our time—it is used both on food plants and ornamental plants in green areas. Opinions differ on the extent of the environmental burden caused by glyphosate. On the one hand, the global application of glyphosate exceeds 1000 kilotonnes per year, resulting in the emergence of glyphosate residues as a ubiquitous environmental pollutant. On the other hand, reported exceedance of the official maximum residue levels (MRLs) of glyphosate residues in food appeared to be very low (0.15% incidence) in the European Union in 2021 [48]. However, these exceedances included pseudo-cereals, the category sunflowers belong to. As for sunflower crops, an MRL for glyphosate residues has been specified for sunflower seeds only, even though other parts of the plant are also used as animal feed. In relation to the growing use of glyphosate, the European Food Safety Authority proposed to elevate the existing MRL in sunflower seeds (20 mg/kg) by 50% to 30 mg/kg [49]. Differences in opinion were observed for international organizations [50]. In addition to glyphosate, AMPA, as one of the most important metabolites, forms the basis of many studies. In this study, we have analyzed how *H. annuus*, as an important food industry and at the same time ornamental plant, reacts to glyphosate in the case that the active ingredient is only indirectly brought into the vicinity of the cultivated plant by the application of field weed control technology. We simulated these indirect conditions using two methods (soil and leaf). If insights are confirmed, glyphosate will be detectable in plant organs and tissues [47]. An important question was whether AMPA would appear alongside glyphosate. In Sun et al. [51], the appearance of glyphosate and AMPA was also expected if they became detectable indirectly. We complemented our chemical residue analysis with morphological and histological measurements.

The morphological results already answered the question, as the results of the control group reached significantly higher values for several parameters (plant height, number of leaves, fresh green mass, fresh root mass, dried green mass, and dried leaf mass). Glyphosate and AMPA have been reported to cause deformations, growth suppression, and other negative effects on plants, even though concentrations reaching non-target plants are typically very low, as noted by Timms and Wood [52]. The plants were finally evaluated at the age of 5 weeks, when they were still in a juvenile stage. Two weeks passed between the glyphosate treatment and the final assessment. The foliar-treated groups had lower morphological values in all cases than the soil-treated groups. The morphological measurements showed that the morphological results of each leaf-treated group were statistically different from those of the control group (Table 1). It can be concluded that the results of the control group were higher than those of the treated groups for all measured parameters. All this leads to the conclusion that glyphosate also drifted onto the leaves and reached the roots in the case of soil treatment. This correlates with the statement of Dill et al. [53] that the phloem promotes the translocation of glyphosate to the meristematic part of the roots and other parts of the plant. This movement of glyphosate through the phloem helps link the role of environmental conditions to translocation efficiency and plant development. Once it got into the roots and the leaves, its translocation started, as the whole plant organism was distorted and underdeveloped compared to the individuals of the control group. This result can be explained by the fact that the roots received glyphosate only indirectly, while the leaves received it directly. The results show that there was no difference between the dried plant parts (green parts, such as leaves, petioles and shoots, and the root system) between the plants of the soil treatment and the control group, which was detectable when measuring the fresh parts. This can be explained by the fact that the limited water absorption due to glyphosate (impeding the functioning of the transport tissue system) was detectable, and there was a statistical difference in the fresh plant parts. The moisture content of the dried parts was already similar—this no longer shows a statistically significant difference between the two groups. In the case of the leaf treatment, the plants of the group were damaged by glyphosate to such an extent that the statistical difference could be detected even in the dried state. The short duration of the growing period provided an opportunity for the short-term effects of glyphosate and AMPA transport as well.

Complementing these results with the histological measurements, our results confirm those reported by de Freitas-Silva [54] since the presence of the pesticide in the plant is also visible from the deformity of the epidermis, the transport tissues, and the basal tissue. Glyphosate did not reach the pith tissue two weeks after treatment, as reported by de Freitas-Silva [54]. It began to destroy the transport tissue system and the epidermis, as can be seen from the microscopic images of the survey. The herbicide amino acid also produces depletion [55], which was also shown by tissue death (Figure 1). This is also related to the findings of Dill et al. [53] that the herbicide passes through the cuticle to the apoplast, which then reaches the symplast, where the phloem transports it to the rest of the plant. The absence of 5-enolpyruvyl shikimate-3-phosphate synthase, an enzyme whose absence leads to senescence and death by affecting plant metabolic functions, is seen [56]. Another important effect of glyphosate, dehydration, can also be seen in the histological images. Cyclic disruption of photosynthesis causes plants to dry out—and this disruption is reflected in the entire tissue system [57]. The translocation mechanism of glyphosate, also described by Nguyen [58], applies in the experiment: glyphosate reached the tissues, such as the roots, through the phloem and finally destroyed the still developing parts of the plant—in this case, among other things, the central tissue. It can also be observed that the epidermis is stronger, and the fascicular cambium is more developed in the individuals of the glyphosate-treated groups than in the control group. The interfascicular cambium was fully formed, while this was not achieved in the control group. From all this, we can conclude, in accordance with Strandberg et al. [20], that herbicides used in sublethal doses can improve plant properties. The most intense effect was observed in the soil-treated

group, which can also be explained by the fact that the root system received two doses of glyphosate since on the one hand, it was taken up by the roots from the soil, and on the other hand, the glyphosate taken up by the leaves began to be stored in the roots, as can be found in the study of Wood [47] and Nguyen et al. [58]. The histological measurement can be considered an important part of the experiment because it shows the translocation mechanism of action of glyphosate, and it also shows that glyphosate destroys tissues and cells indirectly.

After that, we performed the glyphosate and AMPA residue measurements, where our assumptions were presented even more numerically: glyphosate and AMPA could be detected in the entire plant (root system, stem, lower leaves, and upper leaves). This value was very high, exceeding the LOD detection limit of 0.025 mg/kg in all cases. This is related to the measurements of Florencia et al. [59], according to which glyphosate and AMPA can be stored in plant tissues. The residue concentrations detected in our study were higher than some previously reported results, but they correlate perfectly well with the detection of Botten and Wood [27]. Among the investigated plant parts, however, there was only a significant difference between the two treatments in the case of the root system, while this difference did not appear in other plant parts. This is related to Wood's [47] assertion that the root system is particularly important for glyphosate measurements. Smedbol et al. [46] found that glyphosate uptake is higher in leaves and stems than in roots, then AMPA content begins to increase in roots. This finding correlates with our measured results. All of this can be dangerous for the environment in several ways, for example, it can easily enter the food chain. The plant is also often used as a green manure plant. In this case, Mamy et al. [60] also showed that the effect of glyphosate on plant residues when it entered the soil was significantly different from the effect of glyphosate applied directly to the soil. The persistence of glyphosate in soil increased with increasing amounts of plant residues and decreased with their distribution in the soil.

In summary, glyphosate and its metabolites can accumulate in the plant even if they are treated indirectly [61], and accumulation is very high in different plant parts. Both drift to the leaf and drift to the soil through its rhizosphere, result in the chemical appearing and accumulating in the plant organism. In addition to glyphosate, AMPA also appeared in the plant through both indirect application methods. This should be taken into account in the future, as it can enter the food chain in the case of food industry crops, and in species of green space management, it can also accumulate in public areas and increase the carcinogenic effect on the inhabitants of cities and settlements, in connection with the results of Meftaul et al. [30] and Haberkon et al. [62]. In populated urban areas, glyphosate is mostly used on ornamental plants. Ornamental plants are grown in parts of cities and towns where people are more concentrated—so a large number of residents can come into direct contact with glyphosate since the danger of drift is insurmountable here, and glyphosate gets into the air very easily. In addition, it can get easily into living waters and soil, where it can have further harmful effects on the environment and wildlife. It is worth considering the development and use of other alternatives that are less harmful to the environment, which could at least partially replace or replace glyphosate. Currently, there are already initiatives for this—in Budapest, for example, the use of glyphosate has been banned for several years, as is the case in more and more cities around the world. This process is expected to continue in the future.

5. Conclusions

The use of glyphosate is already a global issue. According to the opinion of a part of the scientific world, it does not impact the environment, but there are researchers who constantly publish about the dangers of glyphosate and its metabolites. As a result of our experiment, the indirectly applied glyphosate was detected in the tissues of young *H. annuus* plants two weeks after the treatment by morphological, histological, and residue measurements. The residue measurements showed not only glyphosate but also its important metabolite, AMPA. Since this total herbicide active ingredient is not only used in

food crops, and agriculture, but is also used in the cultivation of ornamental plants, this also raises important questions for the horticultural sector. Since municipal green space management also uses the chemical in several countries (for example, many settlements in Hungary), glyphosate can come into direct contact with the urban population and enter human and animal bodies. The reason for this is that the green areas are frequently visited by people, and the application currently rarely reaches only the target plants. In this case, not only the urban population but also the urban fauna, soil, and living water can be polluted. In the future, it is therefore worth thinking about what alternative weed control technologies can be introduced, at least in the settlements, which are less likely to affect the environment. Using these, the rate of glyphosate application could also be reduced. Many technologies are currently available, including biological herbicides or the hot foam technology, which Budapest has been using instead of glyphosate for several years now.

Author Contributions: Conceptualization, S.K., K.H., K.I., M.M., A.S. and L.O.; methodology, S.K., K.H., K.I., M.M., A.S. and L.O.; software, D.H.-F., K.H., K.I., M.M. and A.S.; validation, S.K., D.H.-F., A.N. and L.O.; formal analysis, D.H.-F., K.H. and A.N.; investigation, S.K., D.H.-F., K.H., K.I., M.M., A.S. and L.O.; resources, S.K., K.H., K.I., M.M., A.S. and L.O.; data curation, S.K., D.H.-F., K.H., K.I., M.M. and A.S.; writing—original draft preparation, S.K., D.H.-F., K.H., K.I., M.M. and A.S.; writing—review and editing, S.K., D.H.-F., A.N., M.M. and A.S.; visualization, D.H.-F. and K.H.; supervision, K.H., K.I., M.M., A.S., A.N. and L.O.; project administration, S.K. and D.H.-F.; funding acquisition, S.K., D.H.-F. and L.O. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the ÚNKP-22-4-II-MATE/1. new national excellence program and by the 2022-2.1.1-NL-2022-00006 "development of the agrotechnology national laboratory" program (grant agreement NKFIH-3524-1/2022) by the ministry for culture and innovation from the source of the national research, development, and innovation fund.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data is not publicly available.

Acknowledgments: We are grateful to our colleagues: Zsanett Istvánfi, Györgyné Gondos, Zsolt Lénárt, and Attila Janik for their help with measurements, and for giving us the opportunity to carry out this work.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Alengebawy, A.; Abdelkhalek, S.T.; Qureshi, S.R.; Wang, M.Q. Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. *Toxics* **2021**, *9*, 42. [CrossRef]
2. Sharma, A.; Kumar, V.; Shahzad, B.; Ramakrishnan, M.; Singh Sidhu, G.P.; Bali, A.S.; Handa, N.; Kapoor, D.; Yadav, P.; Khanna, K.; et al. Photosynthetic response of plants under different abiotic stresses: A review. *J. Plant Growth Regul.* **2020**, *39*, 509–531. [CrossRef]
3. Bhunia, P. Environmental toxicants and hazardous contaminants: Recent advances in technologies for sustainable development. *J. Hazard. Toxic Radioact. Waste* **2017**, *21*, 02017001. [CrossRef]
4. Chin, N.P. Environmental toxins: Physical, social, and emotional. *Breastfeed. Med.* **2010**, *5*, 223. [CrossRef] [PubMed]
5. Gill, J.P.K.; Sethi, N.; Mohan, A. Analysis of the glyphosate herbicide in water, soil and food using derivatising agents. *Environ. Chem. Lett.* **2017**, *15*, 85–100. [CrossRef]
6. Székács, A.; Darvas, B. Forty years with glyphosate. In *Herbicides—Properties, Synthesis and Control of Weeds*; Hasaneen, M.N.A.E.-G., Ed.; InTech: Rijeka, Croatia, 2012; pp. 247–284.
7. Székács, A.; Darvas, B. Re-registration challenges of glyphosate in the European Union. *Front. Environ. Sci.* **2018**, *6*, 78. [CrossRef]
8. Singh, S.; Kumar, V.; Gill, J.P.K.; Datta, S.; Singh, S.; Dhaka, V.; Kapoor, D.; Wani, A.B.; Dhanjal, D.S.; Kumar, M.; et al. Herbicide glyphosate: Toxicity and microbial degradation. *Int. J. Environ. Res. Public Health* **2020**, *17*, 7519. [CrossRef]
9. Ramula, S.; Kalske, A.; Saikkonen, K.; Helander, M. Glyphosate residues in soil can modify plant resistance to herbivores through changes in leaf quality. *Plant Biol.* **2022**, *24*, 979–986. [CrossRef]

10. Haslam, E. *The Shikimate Pathway: Biosynthesis of Natural Products Series*, 1st ed.; e-book; Elsevier: Amsterdam, The Netherlands, 2014.
11. Helander, M.; Pauna, A.; Saikkonen, K.; Saloniemi, I. Glyphosate residues in soil affect crop plant germination and growth. *Sci. Rep.* **2019**, *9*, 19653. [CrossRef]
12. Peillex, C.; Pelletier, M. The impact and toxicity of glyphosate and glyphosate-based herbicides on health and immunity. *J. Immun. Toxicol.* **2020**, *17*, 163–174. [CrossRef]
13. Sviridov, A.V.; Shushkova, T.V.; Ermakova, I.T.; Ivanova, E.V.; Epiktetov, D.O.; Leontievsky, A.A. Microbial degradation of glyphosate herbicides. *Appl. Biochem. Microbiol.* **2015**, *51*, 188–195. [CrossRef]
14. Ledoux, M.L.; Hettiarachchy, N.; Yu, X.; Howard, L.; Lee, S.O. Penetration of glyphosate into the food supply and the incidental impact on the honey supply and bees. *Food Control* **2020**, *109*, 106859. [CrossRef]
15. Fuchs, B.; Saikkonen, K.; Helander, M. Glyphosate-modulated biosynthesis driving plant defense and species interactions. *Trends Plant Sci.* **2021**, *26*, 312–323. [CrossRef] [PubMed]
16. Zulet González, A.; Fernández Escalada, M.; Zabalza Aznárez, A.; Royuela Hernando, M. Enhancement of glyphosate efficacy on *Amaranthus palmeri* by exogenous quinate application. *Pestic. Biochem. Physiol.* **2019**, *158*, 1–11. [CrossRef]
17. Richmond, M.E. Glyphosate: A review of its global use, environmental impact, and potential health effects on humans and other species. *J. Environ. Stud. Sci.* **2018**, *8*, 416–434. [CrossRef]
18. Zhang, C.; Hu, X.; Luo, J.; Wu, Z.; Wang, L.; Li, B.; Wang, Y.; Sun, G. Degradation dynamics of glyphosate in different types of citrus orchard soils in China. *Molecules* **2015**, *20*, 1161–1175. [CrossRef]
19. Rivas-Garcia, T.; Espinosa-Calderón, A.; Hernández-Vázquez, B.; Schwentesius-Rindermann, R. Overview of Environmental and Health Effects Related to Glyphosate Usage. *Sustainability* **2022**, *14*, 6868. [CrossRef]
20. Strandberg, B.; Sørensen, P.B.; Bruus, M.; Bossi, R.; Dupont, Y.L.; Link, M.; Damgaard, C.F. Effects of glyphosate spray-drift on plant flowering. *Environ. Pollut.* **2021**, *280*, 116953. [CrossRef]
21. De Moraes, C.P.; de Brito, I.P.; Tropaldi, L.; Carbonari, C.A.; Velini, E.D. Hormetic effect of glyphosate on *Urochloa decumbens* plants. *J. Environ. Sci. Health B* **2020**, *55*, 376–381. [CrossRef]
22. Martins-Gomes, C.; Silva, T.L.; Andreani, T.; Silva, A.M. Glyphosate vs. glyphosate-based herbicides exposure: A review on their toxicity. *J. Xenobiot* **2022**, *12*, 3. [CrossRef]
23. De Troeyer, K.; Casas, L.; Bijnens, E.M.; Bruckers, L.; Covaci, A.; De Henauw, S.; Hond, D.E.; Loots, I.; Nelen, V.; Verheyen, V.J.; et al. Higher proportion of agricultural land use around the residence is associated with higher urinary concentrations of AMPA, a glyphosate metabolite. *Int. J. Hyg. Environ. Health* **2022**, *246*, 114039. [CrossRef] [PubMed]
24. Ojelade, B.S.; Durowoju, O.S.; Adesoye, P.O.; Gibb, S.W.; Ekosse, G.I. Review of Glyphosate-Based Herbicide and Aminomethylphosphonic Acid (AMPA): Environmental and Health Impacts. *Appl. Sci.* **2022**, *12*, 8789. [CrossRef]
25. Sihtmäe, M.; Blinova, I.; Künnis-Beres, K.; Kanarbik, L.; Heinlaan, M.; Kahru, A. Ecotoxicological effects of different glyphosate formulations. *Appl. Soil Ecol.* **2013**, *72*, 215–224. [CrossRef]
26. Qu, M.; Wang, L.; Xu, Q.; An, J.; Mei, Y.; Liu, G. Influence of glyphosate and its metabolite aminomethylphosphonic acid on aquatic plants in different ecological niches. *Ecotoxicol. Environ. Saf.* **2022**, *246*, 114155. [CrossRef] [PubMed]
27. Botten, N.; Wood, L.J.; Werner, J.R. Glyphosate remains in forest plant tissues for a decade or more. *For. Ecol. Manag.* **2021**, *493*, 119259. [CrossRef]
28. Ramula, S.; Mathew, S.A.; Kalske, A.; Nissinen, R.; Saikkonen, K.; Helander, M. Glyphosate residues alter the microbiota of a perennial weed with a minimal indirect impact on plant performance. *Plant Soil* **2022**, *472*, 161–174. [CrossRef]
29. Muola, A.; Fuchs, B.; Laihonen, M.; Rainio, K.; Heikkonen, L.; Ruuskanen, S.; Saikkonen, K.; Helander, M. Risk in the circular food economy: Glyphosate-based herbicide residues in manure fertilizers decrease crop yield. *Sci. Total Environ.* **2021**, *750*, 141422. [CrossRef]
30. Meftaul, I.M.; Venkateswarlu, K.; Annamalai, P.; Parven, A.; Megharaj, M. Glyphosate use in urban landscape soils: Fate, distribution, and potential human and environmental health risks. *J. Environ. Manag.* **2021**, *292*, 112786. [CrossRef]
31. Van Bruggen, A.H.C.; Finckh, M.R.; He, M.; Ritsema, C.J.; Harkes, P.; Knuth, D.; Geissen, V. Indirect effects of the herbicide glyphosate on plant, animal and human health through its effects on microbial communities. *Front. Environ. Sci.* **2021**, *9*, 763917. [CrossRef]
32. Farkas, D.; Horotán, K.; Orlóci, L.; Neményi, A.; Kisvarga, S. New methods for testing/determining the environmental exposure to glyphosate in sunflower (*Helianthus annuus* L.) plants. *Sustainability* **2022**, *14*, 588. [CrossRef]
33. Fogliatto, S.; Ferrero, A.; Vidotto, F. Current and future scenarios of glyphosate use in Europe: Are there alternatives? *Adv. Agron.* **2020**, *163*, 219–278.
34. Gabonakutató Nonprofit Közhasznú Kft. (Szeged, Hungary). Available online: https://www.gabonakutato.hu/hu/vetomag/napraforgo/olajnapraforgo/gk-milia-cl (accessed on 5 December 2022).

35. Clearfield®Herbicide Technology. Available online: https://agriculture.basf.us/crop-protection/products/herbicides/clearfield.html (accessed on 5 December 2022).
36. Eker, S.; Ozturk, L.; Yazici, A.; Erenoglu, B.; Romheld, V.; Cakmak, I. Foliar-applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (*Helianthus annuus* L.) plants. *J. Agric. Food Chem.* **2006**, *54*, 10019–10025. [CrossRef] [PubMed]
37. Singh, V.; Etheredge, L.; McGinty, J.; Morgan, G.; Bagavathiannan, M. First case of glyphosate resistance in weedy sunflower (Helianthus annuus). *Pest Manag. Sci.* **2020**, *76*, 3685–3692. [CrossRef] [PubMed]
38. Ozturk, L.; Yazici, A.; Eker, S.; Gokmen, O.; Römheld, V.; Cakmak, I. Glyphosate inhibition of ferric reductase activity in iron deficient sunflower roots. *New Phytol.* **2008**, *177*, 899–906. [CrossRef] [PubMed]
39. Tesfamariam, T.; Bott, S.; Cakmak, I.; Römheld, V.; Neumann, G. Glyphosate in the rhizosphere—Role of waiting times and different glyphosate binding forms in soils for phytotoxicity to non-target plants. *Eur. J. Agron.* **2009**, *31*, 126–132. [CrossRef]
40. Shaner, D.L. Effects of glyphosate on transpiration. *Weed Sci.* **1978**, *26*, 513–516. [CrossRef]
41. Mesnage, R.; Defarge, N.; De Vendômois, J.S.; Séralini, G.E. Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. *Food Chem. Toxicol.* **2015**, *84*, 133–153. [CrossRef]
42. Gomes, M.P.; Smedbol, E.; Chalifour, A.; Hénault-Ethier, L.; Labrecque, M.; Lepage, L.; Juneau, P. Alteration of plant physiology by glyphosate and its by-product aminomethylphosphonic acid: An overview. *J Exp. Bot.* **2014**, *65*, 4691–4703. [CrossRef]
43. Duke, S.O.; Lydon, J.; Koskinen, W.C.; Moorman, T.B.; Chaney, R.L.; Hammerschmidt, R. Glyphosate effects on plant mineral nutrition, crop rhizosphere microbiota, and plant disease in glyphosate-resistant crops. *J. Agric. Food Chem.* **2012**, *60*, 10375–10397. [CrossRef]
44. D'Agostino, R.B.; Pearson, E.S. Testing for departures from normality. *Biometrika* **1973**, *60*, 613–622. [CrossRef]
45. Junior, W.R.C.; da Costa, Y.K.S.; Carbonari, C.A.; Duke, S.O.; Alves, P.L.D.C.A.; de Carvalho, L.B. Growth, morphological, metabolic and photosynthetic responses of clones of eucalyptus to glyphosate. *For. Ecol. Manag.* **2020**, *470*, 118218. [CrossRef]
46. Smedbol, E.; Lucotte, M.; Maccario, S.; Gomes, M.P.; Paquet, S.; Moingt, M.; Mercier, L.L.C.; Sobarzo, M.R.P.; Blouin, M.A. Glyphosate and Aminomethylphosphonic acid content in glyphosate-resistant soybean leaves, stems, and roots and associated phytotoxicity following a single glyphosate-based herbicide application. *J. Agric. Food Chem.* **2019**, *67*, 6133–6142. [CrossRef]
47. Wood, L.J. The presence of glyphosate in forest plants with different life strategies one year after application. *Can. J. For. Res.* **2019**, *49*, 586–594. [CrossRef]
48. Carrasco Cabrera, L.; Di Piazza, G.; Dujardin, B.; Medina Pastor, P. The 2021 European Union report on pesticide residues in food. *EFSA J.* **2023**, *21*, 7939. [CrossRef]
49. European Food Safety Authority (EFSA) Review of the existing maximum residue levels for glyphosate according to Article 12 of Regulation (EC) No 396/2005—Revised version to take into account omitted data. *EFSA J.* **2019**, *17*, 5862. [CrossRef]
50. Benbrook, C.M. How did the US EPA and IARC reach diametrically opposed conclusions on the genotoxicity of glyphosate-based herbicides? *Environ. Sci. Eur.* **2019**, *31*, 2. [CrossRef]
51. Sun, M.; Li, H.; Jaisi, D.P. Degradation of glyphosate and bioavailability of phosphorus derived from glyphosate in a soil-water system. *Water Res.* **2019**, *163*, 114840. [CrossRef]
52. Timms, K.P.; Wood, L.J. Sub-lethal glyphosate disrupts photosynthetic efficiency and leaf morphology in fruit-producing plants, red raspberry (*Rubus idaeus*) and highbush cranberry (*Viburnum edule*). *GECCO* **2020**, *24*, e01319. [CrossRef]
53. Dill, G.M.; Sammons, R.D.; Feng, P.C.; Kohn, F.; Kretzmer, K.; Mehrsheikh, A.; Bleeke, M.; Honegger, J.L.; Farmer, D.; Wright, D.; et al. Glyphosate: Discovery, development, applications, and properties. In *Glyphosate Resistance in Crops and Weeds*; Nandula, V.K., Ed.; Jihn Wiley & Sons, Inc.: Hoboken, NJ, USA, 2010; Volume 1, p. 344.
54. de Freitas-Silva, L.; de Araújo, T.O.; Nunes-Nesi, A.; Ribeiro, C.; Costa, A.C.; da Silva, L.C. Evaluation of morphological and metabolic responses to glyphosate exposure in two neotropical plant species. *Ecol. Indicat.* **2020**, *113*, 106246. [CrossRef]
55. Zhigailov, A.V.; Nizkorodova, A.S.; Sharipov, K.O.; Polimbetova, N.S.; Iskakov, B.K. Glyphosate treatment mediates the accumulation of small discrete 5′-and 3′-terminal fragments of 18S rRNA in plant cells. *Vavilov J. Genet. Breed.* **2023**, *162*, 93.
56. Mahendrakar, K.; Venkategowda, P.M.; Rao, S.M.; Mutkule, D.P. Glyphosate surfactant herbicide poisoning and management. *Indian J. Crit.* **2014**, *18*, 328.
57. Helander, M.; Saloniemi, I.; Saikkonen, K. Glyphosate in northern ecosystems. *Trends Plant Sci.* **2012**, *17*, 569–574. [CrossRef] [PubMed]
58. Nguyen, D.B.; Rose, M.T.; Rose, T.J.; Morris, S.G.; Van Zwieten, L. Impact of glyphosate on soil microbial biomass and respiration: A meta-analysis. *Soil Biol. Biochem.* **2016**, *92*, 50–57. [CrossRef]
59. Florencia, F.M.; Carolina, T.; Enzo, B.; Leonardo, G. Effects of the herbicide glyphosate on non-target plant native species from Chaco forest (Argentina). *Ecotoxicol. Environ. Safe* **2017**, *144*, 360–368. [CrossRef] [PubMed]
60. Mamy, L.; Barriuso, E.; Gabrielle, B. Glyphosate fate in soils when arriving in plant residues. *Chemosphere* **2016**, *154*, 425–433. [CrossRef] [PubMed]

61. Zhang, Z.; Rengel, Z.; Chang, H.; Meney, K.; Pantelic, L.; Tomanovic, R. Phytoremediation potential of Juncus subsecundus in soils contaminated with cadmium and polynuclear aromatic hydrocarbons (PAHs). *Geoderma* **2012**, *175*, 1–8. [CrossRef]
62. Haberkon, N.B.R.; Aparicio, V.C.; Mendez, M.J. First evidence of glyphosate and aminomethylphosphonic acid (AMPA) in the respirable dust (PM10) emitted from unpaved rural roads of Argentina. *Sci. Total Environ.* **2021**, *773*, 145055. [CrossRef] [PubMed]