Article

New Methods for Testing/Determining the Environmental Exposure to Glyphosate in Sunflower (*Helianthus annuus* L.) Plants

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Abstract: Glyphosate is still the subject of much debate, as several studies report its effects on the environment. Sunflower (GK Milia CL) was set up as an experimental plant and treated with glyphosate concentrations of 500 ppm and 1000 ppm in two treatments. Glyphosate was found to be absorbed from the soil into the plant organism through the roots, which was also detectable in the leaf and root. Glyphosate was also significantly detected in the plant 5 weeks after treatment and in plants that did not receive glyphosate treatment directly, so it could be taken up through the soil. Based on the morphological results, treatment with higher concentrations (1000 ppm) of glyphosate increased the dried mass and resulted in shorter, thicker roots. Histological results also showed that basal and transporter tissue distortions were observed in the glyphosate-treated plants compared to the control group. Cells were distorted with increasing concentration, vacuoles formed, and the cell wall was weakened in both the leaf-treated and inter-row-treated groups. In the future, it will be worth exploring alternative agricultural technologies that can reduce the risk of glyphosate while increasing economic outcomes. This may make the use of glyphosate more environmentally conscious.

Keywords: *Helianthus*; sunflower; morphological; sustainable; glyphosate; pesticide; residue; pollution; weed control; organic plant production

1. Introduction

Glyphosate (NA-(phosphono-methyl) glycine) was introduced commercially in 1974 [1] and is considered to be the most widely used herbicide in the history of agriculture [2,3], a postemergent, systemic, non-selective chemical [4,5] that poses a high risk to human health and the environment [4]. In 2014, farmers in the United States sprayed enough glyphosate to reach about 1 kg per hectare of cultivated land, amounting to nearly 0.53 kg/ha for all crops in the world [6]. In 2017, glyphosate accounted for 33% of herbicide sales in Europe. One-third of the sown area of annual crop systems and half of the area of trees received glyphosate annually. Glyphosate is widely used for at least eight agricultural purposes, including weed control, plant drying, sealing of cover crops, temporary grassland removal, and permanent grassland renewal [7]. It has been widely used for the past 40 years, with the assumption that side effects are minimal [8]. Since the mid-1990s, there have been significant changes in the timing and manner of application of glyphosate herbicides, resulting in a drastic increase in total application rates [6]. There are currently no public data on the use of glyphosate in Europe [7].

1.1. Glyphosate

Glyphosate is a highly potent broad-spectrum herbicide that targets 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS) [9]. The majority of soybeans (*Glycine max* L.) planted in the United States are resistant to glyphosate due to the introduction of a gene encoding glyphosate-insensitive 5-enolipyruvylsikimate-3-phosphate synthase [10]. Gene expression studies have also shown that the gene encoding EPSPS is maximally expressed in meristems, so glyphosate must migrate to plant growth sites to be effective [11]. Glyphosate was metabolized to the same extent in *Sorghum halepense* (L.) Pers. in both glyphosate-resistant and -sensitive populations, and there was no significant difference in the inhibition of 5-enol-pyruvylsikimate-3-phosphate synthase (EPSPS), nor in the basic activity [12].

It is used primarily against deep-rooted perennial weeds in agriculture, forestry, and wetlands and parks. The focus is on its agricultural use, and the possible presence of residues that also accumulate in crops and human and animal tissues. According to a World Health Organization (WHO) report, the residue content in plant organs is negligible [4], yet several studies point to the opposite [13]. The effect of low-dose glyphosate on animals and humans has recently been documented, suggesting changes and shifts in the composition of microbial communities in plants and the animal gut [8]. For spring wheat (*Triticum aestivum* L.), a number of laboratories are currently investigating both its accumulation and its toxicity in both animals and plants [14].

Glyphosate-resistant, genetically modified crops have been used since 1996 and are becoming more widespread worldwide [9]. Commercially available glyphosate-based formulations typically contain 41% or more of the active ingredient, while household formulations contain 1% glyphosate [15]. However, glyphosate can also have extensive, undesirable effects on nutrient efficiency, compromising agricultural sustainability. It weakens the defenses of plants against pathogens and pests as it also accumulates in meristem tissues [2]. The toxicity formulations vary due to the value being influenced by multiple factors. The toxicity of the preparation depends on the quality and quantity of surfactants. Experimental results suggest that the toxicity of the polyoxyethyleneamine (POEA) surfactant is greater than that of glyphosate alone or the average toxicity of most commercially available agents [15]. Few plant species are inherently resistant to glyphosate. In addition, measurements of glyphosate did not result in any evidence of resistance of weeds to the active substance in the field. This may be explained, among other things, by the lack of glyphosate residue in the soil [16]. Recent evidence, however, calls into question the inactivation properties and safety of glyphosate. Glyphosate can be retained and transported in soil [17].

Aminomethylphosphonic acid (AMPA) is the most frequently detected metabolite of glyphosate in plants [18]. Glyphosate and the metabolite AMPA accumulate in the environment [8]. Glyphosate-based herbicides have been found to cause abnormal growth structures and phenological changes in some agriculturally relevant plants, such as one or more morphological changes in anthers, anthers, pollen, or flowers, and to reduce the number of seeds in the glyphosate-resistant plant [19]. In soybean plants, glyphosate-containing formulations had no effect on chlorophyll content, the dry weight of roots and shoots, and the number of shoots, but reduced shoot biomass by 21–28% [20]. There is a significant difference in glyphosate and AMPA levels between some plant species [18]. In some first- and second-generation glyphosate-resistant *Glycine max* L., photosynthesis becomes inhibited [21]. The content of sikhate is twice as high for older leaves and 16 times as high for young leaves in non-glyphosate-resistant plants [22], with the highest sensitivity to glyphosate at the beginning of the vegetative stage [23]. This affects nutrient uptake, leading to a decrease in biomass. Its effect increases with increasing glyphosate levels [21]. The increasing glyphosate ratio showed a significant decrease in photosynthetic activity, accumulation of leaf tissue macro- and microelements, and decreased nutrient uptake in *Glycine max* L. individuals [24]. In *Glycine max* L. Merr. 'Williams' individuals, glyphosate also reduced chlorophyll content [25] but accumulated in nodules [26]. In *Lupinus albus* L. plants, nitrogenase activity was reduced 24 h after glyphosate treatment, even at the

lowest and sublethal doses (1.25 mM). In the longer term (5 days), the starch content of the shoots and the amount of sucrose synthase also decreased, while the sucrose content of the shoots increased [27]. In *Eichhornia* Kunth. plants, glyphosate was also detectable in the plants on day 14 after treatment [28]. Glyphosate also affects the mineral content. In plants of *Glycine max* L., glyphosate has been shown to interfere with the uptake and retranslocation of Ca, Mg, Fe, and Mn, most likely to bind and thus immobilize them. The decrease in iron, manganese, calcium, and magnesium concentrations in seeds by glyphosate is very specific and may affect seed quality [22]. *Gossypium hirsutum* L. has been shown to accumulate significantly more glyphosate in reproductive tissues than in vegetative tissues [29]. *Agropyron repens* (L.) Beauv. glyphosate treatment showed that offspring closer to the mother plant survived glyphosate treatment more easily than those farther away. This suggests that higher bud death near the rhizome peak is attributable to higher glyphosate accumulation [30].

Searching major scientific databases (Google Scholar, ScienceDirect) for "glyphosate," "herbicide use," and "pesticide use" leads to just a few articles reporting global sales of glyphosate. The lack of public data on the use of glyphosate has already been highlighted by several NGOs and researchers. Based on data and additional estimates, the total volume of glyphosate sold in the EU in 2017 was 49,427 tonnes [7].

1.2. Histological and Morphological Changes

The persistence of glyphosate residues in plant tissues varies depending on the species and plant tissue type [31]. In maize (*Zea mays* L.), glyphosate reduced the efficiency of photosynthesis, causing changes in leaf anatomy and stem physical properties, leading to a decrease in grain size [32]. In *Rosa acicularis* plants, 2 years after glyphosate treatment, the effect of the chemical was also detected in pollen, petals, and flower tissues [19]. It did not affect the number of flowers, but it did affect the cumulative number of flowers [33]. Glyphosate-treated tomato seedlings (*Solanum lycopersicum* L.) showed increased root growth and elongation of chloroplasts when seeds were sown in glyphosate containing soil and if sprayed with glyphosate [34]. Glyphosate has been shown to have a detrimental effect on the yield and growth of young cocoa plants [35]. In *Sida acuta* Burm.f. specimens treated with glyphosate, the stem was swollen and bent. The leaves turned yellow, while the roots were swollen and necrotic. Vegetative growth decreased, and the plants eventually withered [36]. In plants of *Salvinia cucullata*, glyphosate was ecotoxic [37]. In this plant species, concomitant use with copper may increase the ecological risk [38]. The active substance accumulation in *Lemna minor* plants was tenfold compared to the maximum acceptable residue level (MRL) after 7 days of glyphosate treatment [39]. In wheat (*Triticum aestivum* L.), the use of glyphosate did not affect the primary and secondary structure of the proteins [40]. *Chloris elata* Desv. specimens have been shown to form wax crystals around the stomas of older plants, which contributed to a decrease in glyphosate sensitivity [41]. The use of glyphosate and paraquat negatively affected the germination of *Vicia faba*, *Phaselolus vulgaris*, and *Sorghum bicolor* seeds, photosynthetic pigments, and amino acids [42].

1.3. Effects of Glyphosate on the Human Environment

Exposure to carcinogens is responsible for a number of human health problems [43]. We are increasingly facing the severe social and economic effects of environmental degradation worldwide [44], and seasonal changes also affect soil habitats [45]. The use of glyphosate causes potential health problems [46]. Decreases in plant diversity and numbers have been widely reported in the agricultural ecosystems of North America and Europe. Intensive use of herbicides within arable land and drift from neighboring habitats are partly responsible for the change [47]. Its toxicity is not limited to plant organisms but can be clearly demonstrated in human cells, so a human health risk analysis process for glyphosate should be developed in the future [48]. The rapid transport of glyphosate is well illustrated by the detection of glyphosate residues in bottled drinking water and

human urine in Mexico [49]. Glyphosate [50] is the most widely used pesticide in Indonesia. Occasionally, there are reports of contamination of drinking water sources with herbicides (including glyphosate) in Taiwan. Glyphosate is not yet a chemical in the assessment of Taiwanese drinking water quality standards [51]. Glyphosate and its metabolite, aminomethylphosphonic acid (AMPA), have recently been identified as potential contributors to the development of various diseases such as autism, Parkinson's and Alzheimer's disease, and cancer [52]. However, several researchers in Australia have a number of objections to the harmful effects of residues on human health. Glyphosate-based products are being intensively tested by governments at all levels. Some jurisdictions have already banned or restricted its use [53].

Glyphosate and AMPA are largely retained in the surface soil layer as residues [54], and AMPA persists longer than the parent compound of glyphosate [55]. It was also shown that the content of pesticides in water samples from reservoirs, including glyphosate, was higher during the agricultural season (1 April–15 September) than during the off-season [56]. Residual concentrations of herbicides and their metabolites in harvested *Zea mays* L. plants increased in direct proportion to increasing the application rate of herbicides [57]. Glyphosate was also detected in soil and rice grains in rice production [58]. The persistence of herbicides, including glyphosate, in the soil and its effect on soybean yield has also been studied in soybeans [59].

1.4. Genetic Changes Due to Glyphosate in Living Organisms

The vast majority of negative results in well-conducted bacterial reversion and in vivo mammalian micronucleus and chromosome aberration tests indicate that glyphosate and typical glyphosate-based formulations (GBF) are not genotoxic in these nuclear assays. Reports of positive results for endpoints of deoxyribonucleic acid (DNA) damage indicate that glyphosate and GBFs tend to induce DNA damage at high or toxic doses, but the data suggest that this is due to cytotoxicity rather than the GBF activity of DNA that may be associated with surfactants [60].

Glyphosate affects the composition and activity of the microbial community in the rhizosphere and increases protein metabolism and decreases amino acid synthesis [61]. Glyphosate can also genetically modify living organisms. Glyphosate resistance appears at different levels in giant ragweed (*Ambrosia trifida*). Introns show a higher expression pattern with data measured in resistant individuals following putative glyphosate treatment [62]. In *Conyza bonariensis* individuals, many genes are differentially expressed upon glyphosate treatment, so treatment involves a large number of genes [63]. Glyphosate inhibits the pathway of tryptophan biosynthesis in the apical bud of soybean (*Glycine max*) [64].

Transcripts in the microbial community were affected by glyphosate. Some cyanobacteria, such as *Synechococcus*, may use glyphosate as a source of P. In many metabolic pathways, genes are overexpressed under glyphosate stress [65]. Polymerase chain reaction (PCR) analysis of mammalian somatic cells has shown that glyphosate induces gene expression changes [66].

The aim of our study was to demonstrate that glyphosate does not degrade in soil and is incorporated into crops, even several weeks after glyphosate treatment. Glyphosate can also be taken up by the plant through the soil. The main question of our study was whether or not there was a significant difference between the amount of glyphosate absorbed through the soil and the amount of glyphosate applied to the plant through the leaf. This is demonstrated by morphological residue detection and microscopic examination. Our model plant was the sunflower (*Helianthus annuus* L.).

2. Materials and Methods

2.1. Materials Used

For our experiments we used a non-glyphosate-resistant GK Milia CL sunflower hybrid variety, which is a variety bred by Gabonakutató Nonprofit Közhasznú Ltd. (Szeged, Hungary) [67], and is also owned by the Ltd. GK Milia CL is a commercially available

sunflower variety that ripens early and tolerates abiotic stress well and can be treated with the 2018 certified Clearfield® weed control technology [68]. The seed was harvested in 2020, uncoated.

Glialka, manufactured by the Monsanto Company (St. Louis, MO, USA) and owned by Bayer GMBH (Monheim am Rhein, Germany), was used for the experiment. Total herbicide with active ingredient 360 g/L glyphosate.

2.2. Experimental Conditions

The experiment was carried out at the Budatétény Station of the Institute of Landscape Architecture, Urban Planning, and Garden Art of MATE in 2021 under greenhouse conditions. The seeds were sown in a plastic propagation tray measuring 59 cm × 29 cm × 7 cm. For this purpose, a medium suitable for growing seedlings in trays (Klassmann-Deilmann TS 3 Fine, Geeste, Germany) was used, the properties of which were as follows: pH (H_2O) 6, N 140 mg L^{-1}, P (P_2O_5) 100 mg L^{-1}, K (K_2O) 180 mg L^{-1}, Mg 100 mg L^{-1}, S 150 mg L^{-1}. The trays were lined with 40 seeds in sowing and 5 replicates in a randomized block arrangement. Germinating weeds were mechanically removed in half of the boxes, and no weed removal was performed in the other half. Fourteen days before sowing, the weeds were removed in the weed trays with the Glialka chemical. The following concentrations were used in each case: 500 ppm, 1000 ppm, and 2000 ppm.

The sunflower seeds were sown on 16 September 2021 in a glyphosate-free medium and the other part in a medium sprayed with Glialka. Plants sown in the herbicide-free medium were sprayed with Glialka at 3 weeks post-sowing. The amount of spray was applied in each case at a rate of 2 L/100 m^2 [68], in the 3 concentrations already mentioned. The plants were kept at 20 °C and only irrigation water was obtained during the experiment. The control group was seeded in a clean medium and received only irrigation water. Seedlings were evaluated at 5 weeks post-sowing. The following vegetative parameters were surveyed: Root and stem length, the fresh and dried weight of root and shoot, and the number of leaves. Plants that received a concentration of 2000 ppm were killed during the experiment and were therefore not evaluated further.

2.3. Histology

Histological samples were taken from three points of the measured plants: From the root collar, from the stem 1 cm above the root collar, and from the stem above the lowest leaf. The plants were cleaned with distilled water and pruned mechanically with a hand scalpel. A Euromex bScope BS.1153-PLi biological microscope with a compatible camera (Levenhuk m1400 plus) was used for the survey. Due to the pruning procedure, the oil immersion lens arrays could not be used, so due to the nature of the sections, PLi 4/0.1 lenses were used, which provided forty-fold magnification. The eyepiece was of the WF120×/20 type and size. The samples were not stained. The images were post-corrected with GIMP 2.10 (owned by Spencer Kimball, Peter Mattis).

2.4. Residue Testing

The test method of Gonclaves and Catrinck [69] was used for the measurements. The Varian GC/MS/MS 4000 instrument was used for the tests (Table 1), using the following materials:

- EPA 547 Glyposate solution 1000 µg/mL in H_2O; Sigma-Aldrich (St. Louis, MO, USA); Lot: LRAC4997.
- BFTFA + 1% TMCS; Sigma-Aldrich; Lot: BCCD0447.
- Acetonitrile; Merck (Budapest, Hungary); Lot: I09677130 829.
- Pyridine; Scharlau (Barcelona, Spain); Lot: PI0123.

Table 1. Chromatographic conditions used for residue testing [69].

Parameters	Values		
Column	Rxi-5Sil MS, 30.00 m, 0.25 mm ID, 0.25 μm		
Injector temperature:	280 °C		
Injected volume:	1 μL		
Split ratio:	splitless		
Carrier gas:	helium 5.0		
Carrier gas volume flow	constant, 2 mL/perc		
	MS settings		
Ion trap temperature	150 °C		
Ion source temperature	200 °C		
Transfer line temperature	220 °C		
Ionization	EI		
Measurement mode	SIS		
	SIS parameters		
Stored masses (m/z)		232	
		312	
		340	
Temperature (°C)	100		300
Scale of temperature increase (°C/min)	0.0		8.0
Time to maintain temperature (minutes)	0.00		0.00
Total time (minutes)	0.00		25.00

In the measurement procedure, approximately $1 \times g$ of sample was weighed to a centrifuge tube and 10 mL of acetonitrile was added (in the case of a smaller sample, proportions were kept). It was shaken for 1 h. It was then centrifuged for approximately $3000 \times g$ and filtered through a syringe filter. Then, 300 μL of this clear solution was taken and evaporated to dryness at 60 °C. After cooling, 60 μL of pyridine was added to the dry residue and, after waiting for 5 min, 100 μL of the N, O-bis (trimethylsilyl)trifluoroacetamide (BSTFA) + 1% trimethylsilyl chloride (TMCS) silylating agent was added. This mixture was heated at 60 °C for 30 min and then measured by Gas Chromatography—Mass Spectrometry. The procedure was the same when creating a calibration line.

2.5. Statistical Measurements

The samples were independent, and the correct experimental design and correctness of the sampling were ensured during the sampling. We used Microsoft Office 365 Excel to document our measurement data and Microsoft Office 365 Word to edit the text. The processing, comparison, and analysis of our measurable differences were performed with IBM SPSS Statistics 25 using a one-way analysis of variance (ANOVA). The measured data were analyzed with a 95% confidence level (significance) in all cases. When evaluating the Levene test, if probability value (*p*) > 0.05, then the Tukey test was used, and if $p < 0.05$, the Games-Howell test was used.

3. Results

3.1. Morphology

Performing morphological measurements was an important factor in the series of measurements. In this way, we can obtain an idea of the changes at the organ level that can occur in plants that receive glyphosate through leaves or roots. This also demonstrates that glyphosate is absorbed through the root.

3.1.1. Root Morphological Changes

Root length was significantly reduced in the treated groups (Figure 1). The control stock, which did not receive any form of glyphosate treatment, had an average root length of 11.56 cm at the time of final evaluation, which is significantly different from all treated

groups. For plants treated with a 500 ppm solution, the lowest value (1.873 cm) was measured in the group receiving the chemical through the root. There was no significant difference in root lengths between the 500 ppm leaf-treated and both 1000 ppm treatments.
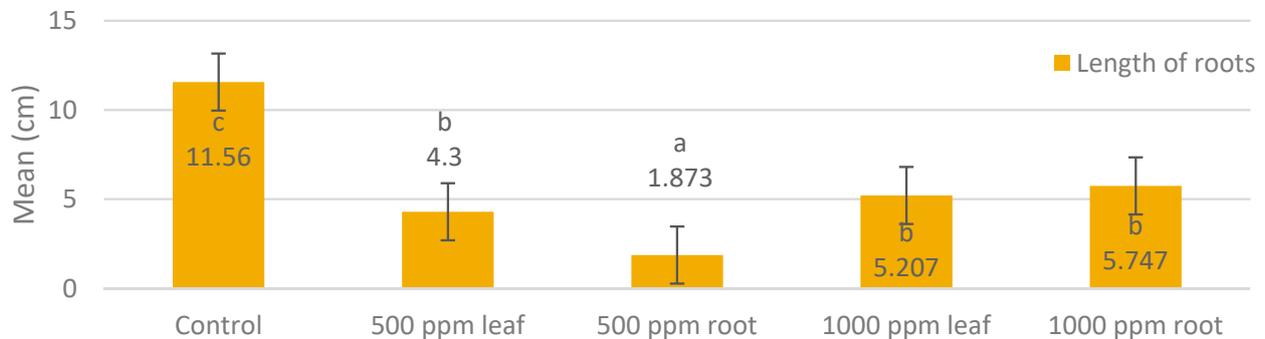

Figure 1. Length of roots on *Helianthus annuus* GK Milia CL after glyphosate treatment. Means with different letter are significantly different by Tukey's test at $p < 0.05$ (Budapest, 2021).

Comparing these data with fresh and dried root weights (Figure 2) it can be observed that the untreated group had the highest fresh root weight (0.7467 g), which was significantly different from the mean weight of the treated groups. The average fresh weight of the roots (0.6067 g) was also significantly higher in the 1000 ppm individuals treated through the root, but in this case, the root was also shorter. In the groups receiving the 500 ppm solution, the average fresh root weight of the leaf spray group was 0.41 g, from which the average fresh root weight of the glyphosate uptake (0.2253 g) was significantly different. The lowest fresh weight value was produced by the group receiving the leaf spray at a concentration of 1000 ppm (0.106 g). These data show that there are greater differences in the fresh weights of plants treated through the leaf and root than the higher concentration of glyphosate. For the groups receiving the 500 ppm solution, this difference, although showing some difference, is not significant. In summary, the effect of the 1000 ppm treatments, compared to each other, has already been shown in phenotypic properties. Compared to the control, much shorter, more fleshy roots were formed in sunflowers treated through roots at a concentration of 1000 ppm. Increased dry weight may indicate stress on the plant.
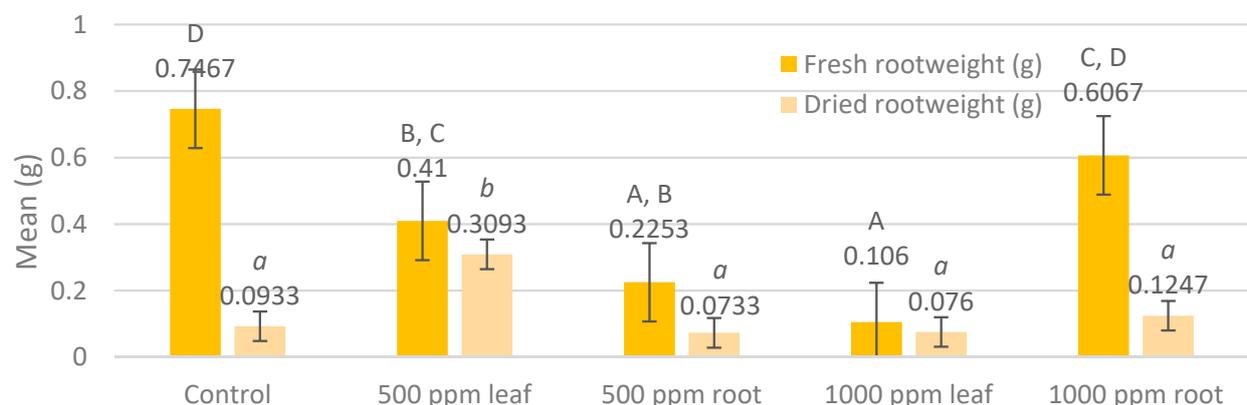

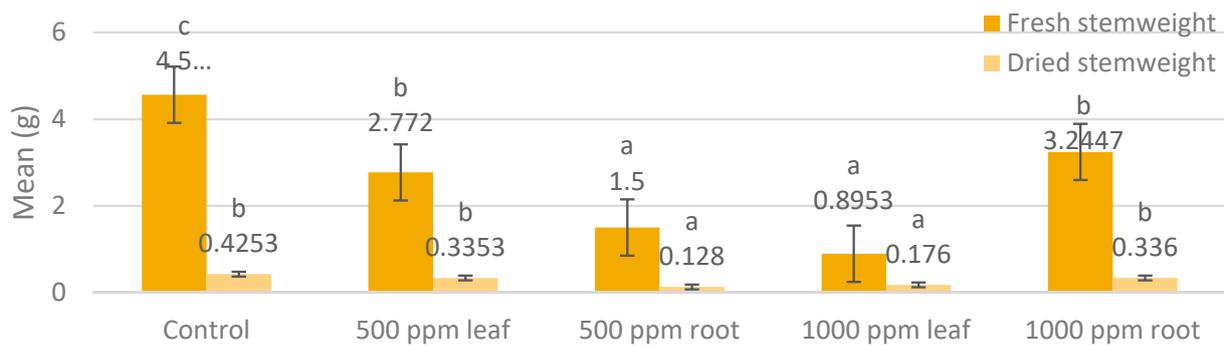**Figure 2.** Parameters of roots on *Helianthus annuus* GK Milia CL after glyphosate treatment. Means with different letter are significantly different by Tukey's test at $p < 0.05$ (Budapest, 2021).

3.1.2. Stem Morphological Changes

Examining the stem weight results (Figure 3), clearly visible that the highest fresh (4.564 g) and dry (0.4253 g) stem weights were measured in the untreated individuals. These data show a significant difference with the results of all treated groups. The results of the parameters were analogous to the values measured at the roots, so the results measured

in the group treated through roots with a concentration of 1000 ppm were significantly higher, both statistically, in the case of fresh (3.2447 g) and dried stems (0.336 g). This shows that absorption through the root is slower than in the case of leaf treatment with the same concentration solution, which produced almost the lowest measured average weight for fresh (0.8953 g) and dry weight (0.176 g). In the case of the treatment obtained with a solution concentration of 500 ppm, the opposite was observed, whereby the individuals receiving the leaf spray achieved a higher fresh and dried stem weight than the individuals treated through the root.

Figure 3. Rate of fresh and dried stem weight on *Helianthus annuus* GK Milia CL after glyphosate treatment. Means with different letter are significantly different by Tukey's test at $p < 0.05$ (Budapest, 2021).

3.2. Histology

Examining the stem cross-sections (Figure 4) it can be observed that the stem section shows a uniform picture in the control group (Figure 4c). The individual tissue areas are clearly visible and can be distinguished from each other. Epidermal cells close and are uniform. The location of the vascular bundles is regular and well separable from the basal tissues. The basal tissue cells are regular, and the cell wall is strong and uniform. No change or distortion is visible. As the glyphosate concentration increases, the deterioration and disintegration of the tissue structure can be clearly seen, which increases and becomes more visible with increasing concentration. Necrosis of pith cells started 4 days after treatment [70]. In the groups treated with a 500 ppm solution (Figure 4a,b), intercellular cavities (vacuoles) appear, which may indicate glyphosate-induced stress in young seedlings, as plant cell vacuoles are multifunctional organelles that play a central role in cell development strategies. They are involved in cellular responses to environmental and biotic factors that cause stress [71]. The transport vessel system is thinner, smaller in cross-section than the control group due to glyphosate, the vascular bundles are damaged, the shape of the parenchyma tissue cells changes, and the cell wall becomes thinner, which is also a phase of cell death. Glyphosate also has an effect on the disorganization of the transport tissue system [72]. This is characterized by the rupture of the plasma membrane [73]. Epidermal tissue cells adhere more loosely. The cell wall of the epidermis is thinner and not uniform in thickness.

In the groups treated with the 1000 ppm solution (Figure 4d,e), the process of cell death can be observed more and more strongly. In plants receiving this concentration, the tissues responded even more strongly. In these groups, the enlargement and disintegration of cells in the central parenchyma tissue and the weakened state of the transport tissue system became even more intense. These cells are necrotically disrupted, cell walls are not always separable, and cells are small in size. The vascular bundles were irregularly arranged, not delimited. The epithelial cells are small, do not adhere tightly, and the cell walls are weak, in several cases torn apart. Chloroplasts also disintegrated, which is also related to Lee's 1981 [74] finding that there was an inhibition of chlorophyll synthesis by glyphosate.
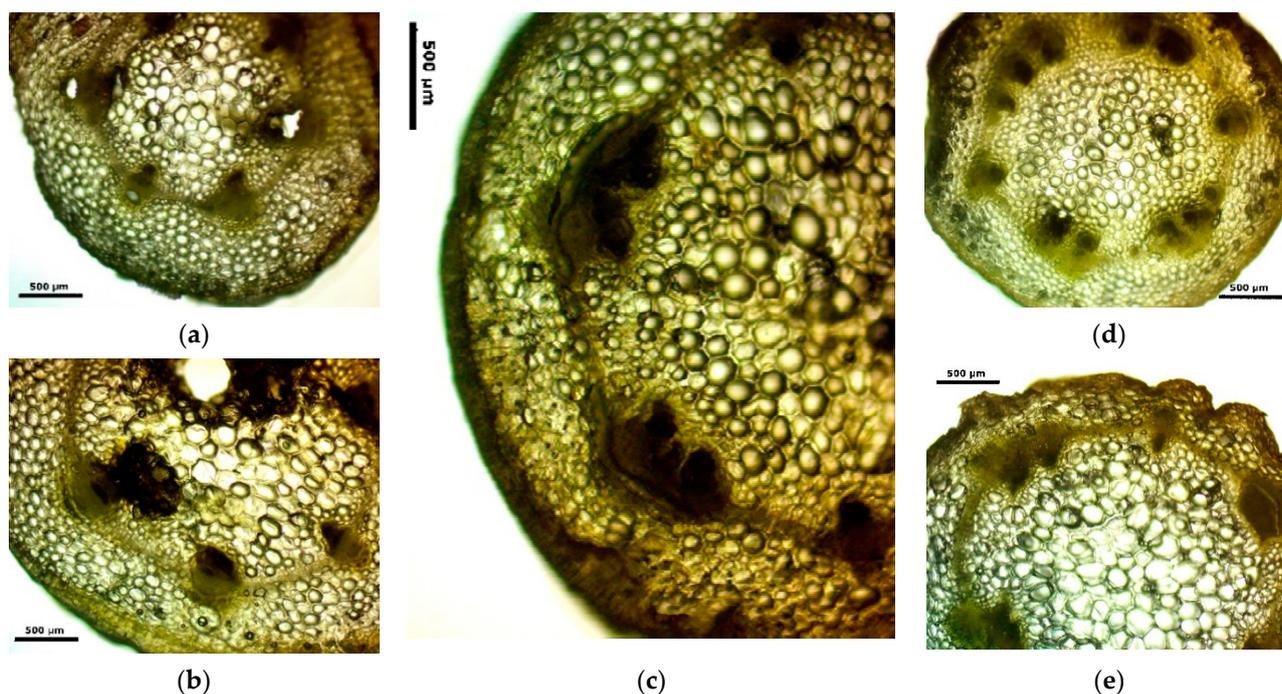
Figure 4. Root cross sections after glyphosate treatments: (**a**) 500 ppm concentration root treatment; (**b**) 500 ppm concentration leaf treatment; (**c**) control treatment; (**d**) 1000 ppm root treatment; (**e**) 1000 ppm leaf treatment (Budapest, 2021).

Comparing the histological changes caused by root and leaf treatments, it can be observed that the histological changes in the root-treated groups are weaker than in the leaf-treated groups, but the effect of glyphosate is also very pronounced in the untreated groups. A more advanced state of the glyphosate effect can be observed in the plants of the leaf-treated groups, which confirms our partial result presented earlier: The effect is stronger in the leaf-treated individuals and the absorption of the chemical is faster. This is confirmed by the worse condition of the cells, the thinner cell walls, and the greater disorder of the transport tissue system.

Overall, the effect of glyphosate can be observed in individual tissue areas, the effect of which is directly proportional to the increase in concentration.

3.3. Result of Residue Tests

In the case of residue measurements, the root and stem parts were evaluated separately (Figure 5). This was considered important because it allows the extent of glyphosate uptake by the root to be monitored even more. Glyphosate accumulated in both leaf-treated and root-treated plants when treated with 500 ppm and 1000 ppm solutions, respectively. The amount detectable for the latter is higher in both concentrations compared to the control treatment. We found that in plants treated with leaf spray, the residue value was significantly higher in the root than in the stem.
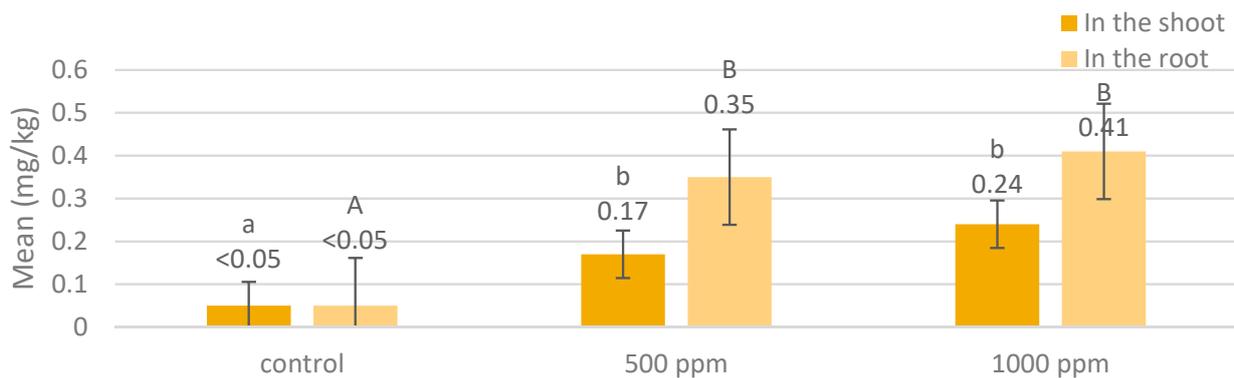
Figure 5. Glyphosate residue in leaf and root on *Helianthus annuus* GK Milia CL after glyphosate spray treatment on leaves. Means with different letter are significantly different by Tukey's test at *p* < 0.05 (Budapest, 2021).

In the groups treated through the root, it can be said that glyphosate residue was detectable in all cases (Figure 6). In the group treated with the 500 ppm solution, the residue measured in the roots (0.19 mg/kg), despite the lower concentration, is not statistically different from the results measured in the root at a concentration of 1000 ppm (0.2 mg/kg). However, when examining the shoots, there is a significant difference between the two concentrations. In the group treated with the 500 ppm solution, the residue detectable in the shoot was as low as in the control treatment (0.07 mg/kg), while in the group treated with 1000 ppm, it was more than twice as high. Overall, glyphosate was absorbed from the root into the plant organism, which was also detectable in the leaf and root.
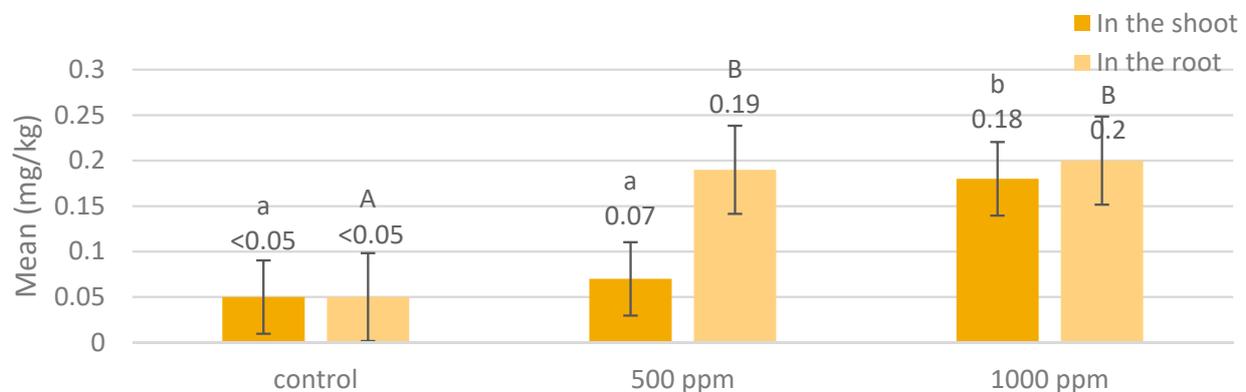

Figure 6. Glyphosate residue in leaf and root on *Helianthus annuus* GK Milia CL after glyphosate spray treatment on root. Means with different letter are significantly different by Tukey's test at *p* < 0.05 (Budapest, 2021).

4. Discussion

Glyphosate is a worldwide used herbicide [9] that, according to several studies, can both modify the gene pool of living organisms [60,63] and also have an adverse effect on the biosphere [46,47]. It may also be responsible for the development of Alzheimer's and Parkinson's disease [52].

Glyphosate is also a major herbicide in field crops. The sunflower (*Helianthus annuus* L.) cultivar GK Milia CL [67] is a very environmental stress-tolerant variety, which was used in our studies.

Although several studies have shown that glyphosate causes morphological changes in the plant [32], and glyphosate is rapidly degraded and is not transferred to other living organisms [4]. By preemergent treatment of a sunflower plant, we would have liked to investigate that glyphosate remains in the plant for several weeks and is detectable. The plants were not grown until seed ripening, so the topic of a future series of measurements

will be the morphological, histological changes, and residue results that can be detected in the pre-harvest state. In the present experiment, sunflower plants were surveyed at 5 weeks of age to model the composition and properties of green manure or fodder harvested in this condition as a result of prior glyphosate treatment. The measurements were also based on experiments by [23], in which the author explains that the highest sensitivity to glyphosate can be attributed to the beginning of the vegetative stages of plants. In our series of measurements, we sought to determine whether the treatment of non-chemically treated plants with adjacent row spacing and the treatment of plants in adjacent rows with glyphosate had an effect.

Sunflower plants were evaluated by morphological and histological methods and residue measurements were performed. Morphological measurements confirmed that glyphosate, both applied to the leaf and absorbed through the root, is evident in the morphological parameters. Our results were similar to those described by [36] and contradict the results of Reddy et al. that glyphosate has no effect on root and shoot dry weight but increases shoot biomass [20].

The morphological results confirmed Zobiole et al.'s [24] measurements that biomass decreases with increasing glyphosate levels. The presence of glyphosate caused morphological and histological degeneration in the plant in all cases, as we have shown in our results. Our results confirm the findings made by Gomes et al. [32] and contradict Khan et al.'s [34] findings. The appearance of glyphosate in the tissues can be detected as early as 48 h after treatment, and these effects can be observed more and more over time [75], as was observed in our measurements. Tissue change caused by glyphosate was observed over the entire surface of the stem cross-section in all tissue areas. All tissue areas were distorted, disordered, cells were destroyed, and in several cases, vacuum cell death occurred, which is also related to van Doorn's [73] work. These tissue and cellular changes can be observed in the case of root-treated plants as well as in the case of stem-treated plants, which is an excellent example of our accumulation of glyphosate in root-treated plants.

In the residue evaluation, we found that glyphosate levels were significantly detectable at week 5 post-preemergent treatment, exceeding Wang et al. [28] finding that glyphosate was detectable for 14 days after treatment. Higher glyphosate levels were measurable in the roots, similar to the findings of Pline et al. [29] and Claus and Behrens [30]. This is also related to Sesin et al.'s [31] findings that glyphosate accumulates in different amounts in different tissues and organs. The amount of glyphosate in the shoots and roots of sunflower was significantly different in several cases. All this proved that glyphosate could be detected in the plant more than 4 weeks after treatment, and that glyphosate in the soil was absorbed through the root in statistically detectable amounts.

5. Conclusions

One of the great challenges of the present is the continued supply of food to an ever-increasing human population. This goal can only be achieved effectively if good quality, high-content, vital plants are produced. The primary task to create this is to produce and maintain a weed-free area. The use of glyphosate, although currently one of the most effective herbicides in the world, raises a number of environmental issues. As a result of our series of measurements, it can be stated that glyphosate is present in the plant even if it is not in direct contact with glyphosate.

This poses a risk, even if the fodder plant is used for human or animal use. In the case of young plants, its use as a silage plant may be of even greater concern. Another dangerous area is its use as green manure, which also means its use at a young age, and this is related to Lee's [74] measurements that the residue is more detectable in young plants. This can be particularly dangerous for the above-mentioned uses in agriculture. Glyphosate may be present in the plant in an amount that can be determined by testing.

Glyphosate therefore accumulates in the plant even if it does not come into direct contact with the plant, rather only with the soil of the adjacent row spacing or with the crop or weed population on it. This is also related to Golt and Wood's [19] results.

The use of glyphosate is not expected to decrease in the future if the plant protection product remains on the market. Due to its decades-long use, glyphosate and its excipients have been shown to accumulate in the soil, water, and living organisms. Their decomposition process is slow and carries a high risk to the environment and human health. In general, glyphosate is currently one of the most effective and widely used herbicides, the extraction of which can have significant economic effects. Therefore, it is advisable to consider alternative remediation technologies that can be used to mitigate these risks while increasing economic outcomes. Thus, it can be an effective tool for environmental protection.

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