

## Article

# Hormesis, the Individual and Combined Phytotoxicity of the Components of Glyphosate-Based Formulations on Algal Growth and Photosynthetic Activity

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**Abstract:** The occurrence of the market-leading glyphosate active ingredient in surface waters is a globally observed phenomenon. Although co-formulants in pesticide formulations were considered inactive components from the aspects of the required main biological effect of the pesticide, several studies have proven the high individual toxicity of formulating agents, as well as the enhanced combined toxicity of the active ingredients and other components. Since the majority of active ingredients are present in the form of chemical mixtures in our environment, the possible combined toxicity between active ingredients and co-formulants is particularly important. To assess the individual and combined phytotoxicity of the components, glyphosate was tested in the form of pure active ingredient (glyphosate isopropylammonium salt) and herbicide formulations (Roundup Classic and Medallon Premium) formulated with a mixture of polyethoxylated tallow amines (POEA) or alkyl polyglucosides (APG), respectively. The order of acute toxicity was as follows for Roundup Classic: glyphosate < herbicide formulation < POEA. However, the following order was demonstrated for Medallon Premium: herbicide formulation < glyphosate < APG. Increased photosynthetic activity was detected after the exposure to the formulation (1.5–5.8 mg glyphosate/L and 0.5–2.2 mg POEA/L) and its components individually (glyphosate: 13–27.2 mg/L, POEA: 0.6–4.8 mg/L), which indicates hormetic effects. However, decreased photosynthetic activity was detected at higher concentrations of POEA (19.2 mg/L) and Roundup Classic (11.6–50.6 mg glyphosate/L). Differences were demonstrated in the sensitivity of the selected algae species and, in addition to the individual and combined toxicity of the components presented in the glyphosate-based herbicides. Both of the observed inhibitory and stimulating effects can adversely affect the aquatic ecosystems and water quality of surface waters.

**Keywords:** glyphosate; co-formulants; POEA; APG; algae; phytotoxicity; photosynthetic activity; hormesis; growth inhibition; combined toxicity



**Citation:** Klátyik, S.; Takács, E.; Barócsi, A.; Lenk, S.; Kocsányi, L.; Darvas, B.; Székács, A. Hormesis, the Individual and Combined Phytotoxicity of the Components of Glyphosate-Based Formulations on Algal Growth and Photosynthetic Activity. *Toxics* **2024**, *12*, 257. <https://doi.org/10.3390/toxics12040257>

Academic Editor: Luis Alberto Henríquez-Hernández

Received: 15 February 2024

Revised: 22 March 2024

Accepted: 27 March 2024

Published: 30 March 2024



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## 1. Introduction

The majority of various pesticide formulations have significant direct or indirect detrimental effects on the environment, particularly in surface waters due to their leaching, surface run-off from treated areas, drifting, foliar spray, and unintended overspray [1–3]. Non-selective glyphosate-based herbicides (GBHs) are no exemption from this trend [4,5]. Originally, these herbicides were exclusively applied for pre-emergence weed control. However, the introduction of glyphosate-tolerant genetically modified crops (not authorized for cultivation

in the European Union) and the adoption of pre-harvest desiccation practices in agriculture resulted in a substantial increase in the use of glyphosate-based formulations [6–8]. However, the approval of the active substance glyphosate has been renewed according to the current legislation subject to the specified conditions and restrictions. Based on the Commission Implementing Regulation (EU) 2023/2660, pre-harvest use of GBHs as desiccants to control the time of harvest or optimize threshing is not authorized [9,10].

Globally, more than 2000 commercial GBHs are used for chemical plant protection against weeds. Different salts of glyphosate (e.g., glyphosate isopropylammonium salt, glyphosate diammonium salt, or glyphosate trimethylsulfonium salt) are used as active ingredients in various GBHs to enhance the solubility of the parent compound, glyphosate [11,12]. In addition to the active ingredient, various co-formulants are also included in GBHs. The primary function of these co-formulants is to facilitate the effectiveness and bioavailability of the formulation by increasing the solubility, adsorption, and absorption of the active ingredient [13]. For example, POEA (a mixture of polyethoxylated tallow amines) as a formulating agent in GBHs enhances the penetration of glyphosate into the plant cell [14]. Various co-formulants presented in commercial pesticides were considered inactive components with regard to the required main biological effect of the formulation. However, numerous studies have indicated the high individual toxicity of co-formulants and the enhanced combined toxicity of the active ingredients and co-formulants in various commercial pesticide formulations compared to the individual toxicity of active ingredients [15–17]. Therefore, the use of POEA in GBHs has been banned in the EU due to the incriminating scientific evidence [18].

As a result of excessive global use, glyphosate has become a ubiquitous contaminant in aquatic ecosystems [19,20]. The appearance and concentration of glyphosate in the different environmental elements (e.g., soil, ground and surface waters) are highly influenced by several abiotic (e.g., hydrological conditions, pH, suspended materials), biotic (e.g., activity and composition of the microbial community), and climatic factors (e.g., rainfall frequency and intensity) [21–23], in addition to the condition of pesticide treatments (e.g., frequency and timing of the treatment) [22,24]. In the past, glyphosate was not included in standard pesticide monitoring programs. Thus, the environmental concentration of glyphosate and its metabolites were underestimated particularly in regions where pre-harvest desiccation practices are widespread or the cultivation of glyphosate-tolerant genetically modified crops occurs extensively. The primary metabolite of glyphosate, aminomethylphosphonic acid (AMPA), is more mobile in water than the parent compound [25,26] and is frequently detected in various environmental elements, including groundwater and surface waters [26–31]. However, it is important to note that the appearance of AMPA in environmental matrices (e.g., groundwater, influents, or sewage sludge) is not exclusively a result of glyphosate metabolism, as it can also originate from phosphonate detergents used in different softeners and cleaning agents [31,32]. According to the U.S. Geological Survey, the presence of glyphosate and/or AMPA was identified in 59% of the analyzed surface waters [33].

The level of glyphosate contamination can reach up to 5.2 mg/L in surface water, although mainly in streams near the treated agricultural fields and especially after heavy rains [27,34,35]. However, high variability can be observed in the detected glyphosate residue levels in various surface water samples [27]. In surface waters collected in Argentina, the average concentrations of glyphosate and AMPA were in the ranges of 17.5–35.2 and 0.6–2.1 µg/L, respectively [36]. However, maximum concentrations were up to 0.258 and 5.87 mg/L, respectively, in the analyzed groundwater and surface water samples [37]. Based on European monitoring programs, the level of glyphosate contamination in surface waters within the EU seems to be relatively lower, with typical glyphosate concentrations ranging between 0.05 and 0.85 µg/L, but residues are consistently detectable [27]. In water samples collected from Hungary, Switzerland, and Italy, the detected glyphosate contamination ranged from 0.035 to 96 µg/L [5,27,35,38–40].

Different GBHs manufactured with various co-formulants show different environmental behaviors (e.g., different half-lives and mobility in soil and water). After the pesticide treatments, the active ingredients and the co-formulants rapidly become separated in most cases. The half-life ( $DT_{50}$ ) of glyphosate in water varies from a few to 91 days [41]. Furthermore, the photo- and biodegradation of the active ingredient also occurred in surface waters [41,42], although limited information is available about the half-lives and the environmental fate of the co-formulants [20,43]. In general, most studies focus on the possible toxic effects on various aquatic organisms and the analytical possibilities of the qualitative and quantitative determination of the co-formulants including POEA and APGs. However, the presence of the GBH co-formulant POEA has been observed extensively in soils collected from agricultural fields of the mid-western states in the USA, where the cultivation of genetically modified glyphosate-tolerant crops is concentrated [44]. Additionally, studies have demonstrated the persistence of POEA in soil along with glyphosate and AMPA [44–46] and possible access to natural waterways [20,43,45]. Due to their low environmental impacts and biodegradability [47], APGs are commonly used as additives in pesticide formulations, personal skin products, and drugs [48,49]. The environmental concentration of co-formulants is generally not monitored [45]; therefore, the exact concentration of POEA and APGs in surface waters is not known. However, the presence of co-formulants such as POEA and APGs in the environment has been demonstrated (e.g., soil, sediment, wastewater) [43,49]. Typically, glyphosate and the co-formulants presented in the GBHs coexist in environmental matrices (e.g., soil and waters) and such co-exposure can affect various non-target aquatic organisms. The aquatic organisms and communities are highly exposed to water pollution [50], as their contact with xenobiotics in water is unavoidable. Recently, the possible combined toxic effects between active ingredients and co-formulants on the environment and non-target organisms are poorly understood.

The toxic effects of glyphosate and its formulations have been studied in numerous aquatic organisms, such as various algae species [51,52], crustaceans (e.g., *Daphnia magna*) [53], mollusks [54], fish [55], and amphibians [56]. Based on the results of ecotoxicological testing performed on a wide range of aquatic plant and animal organisms, the damage to different physiological and behavioral functions was demonstrated [20]. In aquatic ecosystems, algal communities constitute the primary producer level and the majority of biomass, playing a key role in the oxygen cycle of water and the atmosphere. They also have essential roles in aquatic food webs and nutrient transport processes [57,58]. In addition, some species (e.g., *Ankistrodesmus fulcatus*) can participate in the breakdown of organic pollutants and toxic compounds (e.g., tributyltin) [59]. However, the massive proliferation of certain cyanobacterial (e.g., *Anabaena flos-aquae*, *Microcystis aeruginosa*) [60] and green algae species (e.g., *Pleodorina indica*) can lead to deterioration of the water quality of surface waters [61]. The determination of the effects of herbicides used for chemical plant protection on algal species is crucial for the toxicological assessment of herbicide formulations. Various algal species are widely used for environmental biological monitoring [62] and bioremediation activities [63]. The different effects of glyphosate, its metabolite, co-formulants, and/or commercial herbicide formulations on green algae and cyanobacterial species are summarized in Table 1 partially based on our previous review about aquatic ecotoxicity of glyphosate, its formulations, and co-formulants [20].

Based on the results of ecotoxicological studies, the inhibitory effects of glyphosate and its formulation on various green algal (e.g., *Chlorella vulgaris*, *Scenedesmus incrassatulus*, *Pseudokirchneriella subcapitata*) [64,65] and cyanobacterial (e.g., *M. aeruginosa*) species were observed [66]. However, stimulated growth was observed at lower test concentrations after exposure to glyphosate and glyphosate-based formulations [64,65,67].

In addition, altered cell morphology, disrupted ultrastructure (e.g., damaged thylakoids and mitochondria) as well as altered biochemical and physiological parameters (e.g., antioxidant activity, lipid peroxidation) were also demonstrated in algae [52,68,69]. Additionally, differences were observed in the sensitivity of the investigated aquatic organisms, even with similar lifestyles, habitats, or identical taxa [70–72]. For example, the determined 72 h  $EC_{50}$

values are in the range of 24.7–166 mg/L [15,20,73] for *P. subcapitata*, while for *Desmodesmus subspicatus* higher values were calculated (72.9–166 mg/L) [41,52,74–76] during the ecotoxicological testing on the effects of glyphosate. Moreover, potential adverse effects of glyphosate and GBHs were indicated also on freshwater periphyton [77–79]. Moreover, the increased toxicity of Roundup was demonstrated on cyanobacterial and green algal species (*M. aeruginosa*, *Nitella microcarpa* var. *wrightii*) in the presence of POEA [80]. The 72 h EC<sub>50</sub> values for POEA in *P. subcapitata* ranged from 0.2 to 4.9 mg/L [15,81,82]. The negligible aquatic toxicity of APGs was demonstrated on *P. subcapitata* [83], but the toxicity of APGs highly depends on the length of the carbon chain [84,85].

In addition to growth inhibition, photosynthetic activity is a commonly used endpoint during the assessment of phytotoxic effects. The measurement of photosynthetic parameters ensures a non-invasive and rapid indication of harmful effects. A widely used method for the measurement of photosynthetic activity is the detection of induced chlorophyll-a fluorescence [86]. Recently, the measurement of photosynthetic activity is widely used in research on stress effects on plant organisms, and for characterizing the physiological state of plants [87–89]. In addition to herbicide active ingredients that directly inhibit photosynthesis (e.g., atrazine), additional active ingredients, including glyphosate, can also impact photosynthetic and respiratory processes by influencing various metabolic pathways [90,91]. The adverse effects of glyphosate on photosynthetic processes can be explained by the direct or indirect inhibition of plastoquinone biosynthesis [92,93]. Furthermore, the reduction in chlorophyll concentration [94] directly affects the rate of electron transport in the chloroplast [91]. Reactive oxygen species generated in mitochondria can further affect photosynthesis by inhibiting the respiratory electron transport chain as a result of glyphosate exposure. The generated free radicals leave mitochondria and enter the chloroplast, where they cause oxidative damage to the photosynthetic apparatus and reduce the activity of photosynthesis [94]. The phytotoxic effects of glyphosate and its herbicide formulation on photosynthetic activity have been studied on phytoplankton species [95,96]. The observed effects indicated damage to the photochemical efficiency of the PS II photochemical system [95]. However, increased growth, chlorophyll-a content, and photosynthetic activity were observed at lower concentrations [90].

The aim of this study was to assess the individual and combined acute phytotoxicity of the components of glyphosate-based formulations. During the comparison of toxic effects, glyphosate was tested in the form of pure active ingredient (glyphosate isopropylammonium (IPA) salt) and preparations (Roundup Classic and Medallon Premium) formulated with a mixture of polyethoxylated tallow amines (POEA) or alkyl polyglucosides (APG), respectively. In addition, the individual toxicity of the formulating agents (POEA and APG) was also investigated. During our study, standard algal growth inhibition assays were performed on different green algae species (*D. subspicatus*, *P. subcapitata*, *Scenedesmus obtusiusculus*) and a cyanobacteria (*A. flos-aquae*). Based on the results, the differences in the sensitivity of various algal species were also compared. In addition, we investigated the possible effects on the photosynthetic activity of *P. subcapitata* algae cells exposed to the components of Roundup Classic individually and in combination.

**Table 1.** Effects of glyphosate, its metabolite, co-formulants, and/or commercial herbicide formulations on green algae and cyanobacterial species.

Algae Species	Tested Substances	Test Concentrations	Test Period	Tested Parameters	Main Results	Reference
<i>P. subcapitata</i>	technical-grade glyphosate (GLY) acid, GLY-IPA <sup>a</sup> , Roundup, POEA <sup>b</sup>	dilution series	96 h <sup>c</sup>	growth inhibition	96 h IC <sub>50</sub> <sup>d</sup> = 3.92 mg a.e. <sup>e</sup> /L (POEA), 5.81 mg a.e./L (Roundup), 24.7 mg a.e./L (GLY acid), 41.0 mg a.e./L (GLY-IPA)	[15]
<i>P. subcapitata</i>	Roundup	4.7–60 mg/L	96 h	growth inhibition	96 h EC <sub>50</sub> <sup>f</sup> = 15.60 mg/L, damaged cell ultrastructure	[52]
<i>C. vulgaris</i>	GLY, AMPA <sup>g</sup>	0.05–50 mg/L, individual and co-exposures	7 d <sup>h</sup>	growth inhibition, pigment content, antioxidant activity	stimulated growth ( $\leq$ 0.5 mg/L), growth inhibition ( $\geq$ 5 mg/L), inhibitory effect ( $\geq$ 5 mg/L GLY and AMPA), altered pigment levels, increased antioxidant activity	[64]
cyanobacteria, <i>Chlorophycean</i> microalgae	GBH <sup>i</sup> (Faena)	1–100 mg/L	96 h	growth inhibition, antioxidant enzymes	IC <sub>50</sub> = 1.022–2.702 mg/L, affected antioxidant enzyme activity ( $\geq$ 0.74 mg/L)	[65]
<i>M. aeruginosa</i>	GLY	1–10 mg/L	9 d, enzyme assays: 24–48 h	growth inhibition, chl-a <sup>j</sup> content, antioxidant activity, cell apoptosis	reduced growth and chl-a content, increased antioxidant activity (1–2 mg/L), induced apoptosis	[66]
cyanobacterial strains	GLY	8.5–33.8 mg/L	15 d	growth inhibition, phosphate and phosphonate levels	species- and dose-dependent stimulatory effects, decreased phosphonate levels, concentration-dependent phosphate uptake	[67]
<i>S. vacuolatus</i>	GBH (Glifosato Atanor) with 2.5% of the surfactant (alkyl aryl polyglycol ether) pesticide adjuvants	0–8 mg GLY/l	96 h	growth, morphology, oxidative stress parameters	96 h IC <sub>50</sub> = 4.9 mg/L, metabolic and morphological changes ( $\geq$ 4 mg/L), oxidative damage ( $\geq$ 6 mg/L)	[68]
cyanobacterial species		dilution series	96 h	growth inhibition	substance- and species-specific effects	[71]
<i>N. microcarpa</i> var. <i>wrightii</i>	technical-grade GLY, GBH (Roundup), AMPA	GLY, Roundup: 0.28, 3.5, 6 mg/L; AMPA: 0.03 mg/L	7 d	photosynthetic rate, dark respiration rate, chl-a	higher toxicity of Roundup, stimulatory effect of AMPA	[80]
<i>P. subcapitata</i>	POEA	dilution series	96 h	growth inhibition	96 h EC <sub>50</sub> = 4.1–4.9 mg/L	[81]
<i>P. subcapitata</i>	MON 0818	dilution series	96 h	growth inhibition	96 h EC <sub>50</sub> = 0.21–1.61 mg/L	[82]
<i>C. vulgaris</i> , <i>Oophila</i> sp	APG <sup>k</sup>	dilution series	72 h	growth inhibition	negligible aquatic toxicity	[83]
<i>P. subcapitata</i>	APG	dilution series	72 h	growth inhibition	toxicity affected by the length of the carbon chain	[84]
green microalgae species	APG	0.26–6.8 mg/L	72 h	growth inhibition	72 h EC <sub>50</sub> = 0.32–2.7 mg/L	[85]
<i>M. aeruginosa</i>	GLY, Roundup	0.06–29.6 µg/L	21 d	cell number, chl-a, APA <sup>l</sup> activity	increased cell number and chl-a, inhibition ( $>$ 5.92 µg/L), GLY increased photosynthesis, concentration-dependent APA activity	[90]
freshwater microalgae	GLY	maximum tested concentration: 5.07 g/L	80 min	chl-a fluorescence, cell viability	concentration-specific effect on maximum quantum yield of PSII <sup>m</sup> ( $<$ 0.17 mg/L)	[95]
microalgal and cyanobacterial species	Factor 540R	10–1000 µg/L	48 h	growth inhibition, photosynthetic parameters	48 h EC <sub>50</sub> = 406–724 µg/L, modified photosynthetic response ( $\geq$ 10 µg/L)	[96]

<sup>a</sup> glyphosate isopropylammonium salt; <sup>b</sup> mixture of polyethoxylated tallow amines; <sup>c</sup> hour; <sup>d</sup> half-maximal inhibitory concentration; <sup>e</sup> acid equivalent; <sup>f</sup> 50% effective concentration; <sup>g</sup> aminomethylphosphonic acid; <sup>h</sup> day; <sup>i</sup> GBH: glyphosate-based herbicide; <sup>j</sup> chlorophyll-a; <sup>k</sup> alkyl polyglycoside; <sup>l</sup> alkaline phosphatase activity; <sup>m</sup> photosystem II.

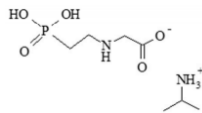
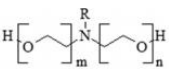
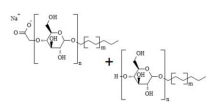


## 2. Materials and Methods

### 2.1. Standard and Reagents

Glyphosate IPA salt and the mixture of polyethoxylated tallow amines (POEA, under the tradename: Emulson AG GPE 3SS) were received from Lamberti SpA (Albizzate, Italy). The glyphosate-based Roundup Classic (Monsanto Europe S.A./N.V.) [97] and Medallon Premium (Syngenta) [98], in addition to the alkyl polyglucosides (APG, under the tradename: Plantapon LGC) were purchased from a public commercial source. The main chemical properties of the investigated herbicide active ingredient, formulations, and surfactants (POEA and APG) used in the investigated formulations can be found in Table 2. Based on the Material Safety Data Sheet (MSDS), Roundup Classic contains 41.5% glyphosate IPA salt and 15.5% POEA. In addition, Medallon Premium consists of 34% glyphosate diammonium salt and 10–20% APG. However, the selected formulations contain different salts of glyphosate, and the indicated concentrations of the active ingredient correspond to 360 g/L glyphosate acid concentration for both preparations. During the ecotoxicological testing, glyphosate was tested only in the form of glyphosate IPA salt. In the tested concentration ranges, the water solubility is not limited for any forms of glyphosate active ingredient, and in water, the salts of glyphosate quickly dissociate into ions that are also found in nutrient solutions and buffer solutions used in ecotoxicological studies.

**Table 2.** Composition and chemical characteristics of the investigated chemical substances.

Active ingredient (AI)					
Chemical Name	CAS No. <sup>1</sup>	Concentration of the AI	Physical Appearance	Chemical Structure	
glyphosate iso-propylammonium (IPA) salt	38641-94-0	62% (486 g/L glyphosate acid)	water-soluble emulsion		
Glyphosate-based formulations					
Product name	AI	Concentration of the AI	Co-formulants	Concentration of the co-formulants	Type of formulation
Roundup Classic	glyphosate IPA salt	41.5% (360 g/L glyphosate acid)	mixture of polyethoxylated tallow amines (POEA)	15.5%	liquid water-soluble concentrate
Medallon Premium	glyphosate diammonium salt(CAS 69254-40-6)	34% (360 g/L glyphosate acid)	alkyl polyglucosides (APG)	10–20%	liquid water-soluble concentrate
Co-formulants					
Product name	Co-formulant	Concentration of the co-formulant	Additives	Type of formulation	Chemical structure
Emulson AG GPE 3SS	POEA (CAS 61791-26-2)	100%	–	water-soluble emulsion	
Plantapon LGC	APG (Na-lauryl glucose carboxylate CAS 383178-66-3 + lauryl glucoside CAS 110615-47-9)	28.5–34.0%	water: 66–71.5%	water-soluble emulsion	

<sup>1</sup> Chemical Abstracts Service (CAS) Registry Number.

## 2.2. Selected Algae Monocultures

The selected algae species were obtained from public collections. The green algae *Pseudokirchneriella subcapitata*, Korshikov (NIVA-CHL1, previous name: *Selenastrum capricornutum*, current name: *Raphidocelis subcapitata*) was obtained from the alga collection of the Norwegian Institute for Water Research. The additional *Desmodesmus subspicatus*, Hegewald & Schmidt (CCAP 276/20) and *Scenedesmus bijugus* var. *obtusiusculus*, Schmidt (CCAP 276/25) green algae species, as well as the investigated filamentous cyanobacteria *Anabaena flos-aquae* (CCAP 1403/13D, current name: *Dolichospermum flos-aquae*), were derived from the Scottish Culture Collection of Algae and Protozoa. The batch culture of green algae species and the selected cyanobacteria were maintained in Zehnder-8 (pH = 6–7) [99] and Allen (pH = 6–7) [100] media, respectively. Fresh media were added to the algae cultures every two weeks, and they were maintained at  $20 \pm 2$  °C and illuminated in a 14:10 light/dark period with the use of cool-white fluorescence tubes (15  $\mu\text{mol}/\text{m}^2/\text{s}$ ). The sensitivity of the algae cultures was verified with the use of the reference substance (potassium dichromate,  $\text{K}_2\text{Cr}_2\text{O}_7$ ) before testing and was proven to be acceptable (72 h  $\text{EC}_{50} = 1.0 \pm 0.1$  mg/L) within the appropriate ranges ( $0.8 \pm 0.1$  mg/L for *D. subspicatus*;  $1.2 \pm 0.3$  mg/L for *P. subcapitata*) based on the relevant standard protocol [101].

The selected green algal and cyanobacterial strains are sensitive to changes in water quality, so they serve as excellent test organisms for the investigation of the toxic effects of aquatic pollutants. *P. subcapitata* and *D. subspicatus* are also considered reference species recommended by the related OECD guideline [102]. Additionally, the selected strains can be easily maintained under laboratory conditions, and are characterized by a fast reproduction and life cycle [103,104]. Based on the scientific literature, significant differences can be observed in the sensitivity of algal species to certain pollutants even within the same taxa [71]. To investigate and compare the sensitivity of taxonomically close and distant species to the applied treatments, three common representatives of freshwater green algae (Phylum: *Chlorophyta*) were selected. Two of the selected green algae species (*D. subspicatus* and *S. obtusiusculus*) belong to the same taxonomic family (*Scenedesmaceae*), thus the sensitivity can be compared also in the case of taxonomically very close species. With the use of *A. flos-aquae* representing a species of cyanobacteria known for its ability to form harmful algal blooms [60], the differences in the sensitivity to the effects of glyphosate can be evaluated for eukaryotic green algae cells and a prokaryotic cyanobacterium as well.

## 2.3. Algal Growth Inhibition Tests

The individual and combined phytotoxic effects of the components of the tested GBHs were evaluated in algal growth inhibition tests based on the OECD 201 guideline [102]. Growth inhibition tests were performed on three unicellular green algae species (*P. subcapitata*, *D. subspicatus*, *S. obtusiusculus*) and, in the case of the active ingredient glyphosate, the tests were also performed on the selected filamentous cyanobacteria (*A. flos-aquae*). The duration of the test was 72 h. During the tests, continuous and uniform cool-white illumination ( $104.9\text{--}14.9$   $\mu\text{E}/\text{m}^2/\text{s}$ ), optimal pH of algal media (pH = 6–7 for Zehnder-8 and Allen media, as well), controlled temperature ( $22 \pm 2$  °C) and stirring (continuous, 100 rpm) were ensured in a shaking incubator (Witeg WIS-10RL, Wertheim, Germany) [102]. The tested compounds were serially diluted, and five concentrations of the substance along with the control were investigated in three repetitions at each level. Each test was repeated three times for each investigated compound. The initial number of algae cells was  $10^5$  cells/mL in the tested and control groups with the fulfillment of the conditions for exponential growth during the entire exposure time. During the growth inhibition assays, the algal cell density was determined daily in the control group to monitor the required specific reproduction rate. The concentration ranges used in the algal growth inhibition tests were as follows for the different species: (1) *P. subcapitata*: glyphosate IPA salt: 22–352 mg/L, Roundup Classic: 3.5–56 mg/L, Medallon Premium: 45–720 mg/L, POEA: 0.5–8 mg/L, APG: 6.5–104 mg/L; (2) *D. subspicatus*: glyphosate IPA salt: 22–352 mg/L, Roundup Classic: 7–112 mg/L, Medallon Premium: 95–1520 mg/L, POEA: 0.8–13 mg/L, APG: 10–160 mg/L; (3) *S. obtusiusculus*:

glyphosate IPA salt: 22–352 mg/L, Roundup Classic: 15–240 mg/L, Medallion Premium: 125–2000 mg/L, POEA: 1.5–24 mg/L, APG: 30–480 mg/L; (4) *A. flos-aquae*: glyphosate IPA salt: 4.5–36 mg/L.

At the end of the experiments, we determined the amount of algal biomass in each control and treated group. Algal biomass was characterized by the measurement of optical density and chlorophyll-a content. The optical density of green algae cells was determined at a wavelength of 750 nm using a spectrophotometer (UV/VIS Camspec single beam M330, Camspec, Crawley, UK), also in three repetitions for each sample [101]. In addition to the measurement of optical density, the potential toxic effects were also evaluated based on the chlorophyll-a content of the samples in the tests performed on green algae species exposed to the POEA-formulated herbicide and its components. Due to the filamentous structure of *A. flos-aquae*, more reliable results were obtained with the measurement of the chlorophyll-a content. The correlation between the two test methods proved to be very high in the case of green algae species ( $R^2 > 0.998$ ). After the extraction process, the chlorophyll-a content of the samples was also determined using a spectrophotometric method in the three replicates [105]. In the performed tests, the coefficient of variation for section-by-section specific growth rates (days 0–1, 1–2, and 2–3) remained below 35% in the control groups. During the entire duration of the tests, the coefficient of variation of the specific growth rates did not exceed 7% in the parallel control cultures of *P. subcapitata* and *D. subspicatus*. In addition, more than 16-fold growth was detected in the control groups, thus the tests can be considered valid.

During the testing of individual and combined effects on algal growth, algae cells were exposed to glyphosate IPA salt and the tested surfactants (POEA and APG) individually and in the form of formulated herbicides. The individual and combined toxicity of the tested substances was evaluated by the determined 72 h EC<sub>50</sub> values. The 72 h EC<sub>50</sub> values were calculated based on the measured optical densities and chlorophyll-a contents as well. During the comparative study of the individual and combined toxicity, the 72 h EC<sub>50</sub> values determined for the tested GBH formulations were corrected with the nominal content of the active ingredient glyphosate and the surfactant as well, based on the MSDS (Table 2).

#### 2.4. Photosynthetic Activity Tests

The individual and combined effects of the components of Roundup Classic were assessed on the photosynthetic activity of *P. subcapitata* green algae. The photosynthetic activity was determined in the samples derived from the algal growth inhibition tests after the 72 h exposure. The measurements were carried out with a portable FluoroMeter Module (FMM) device based on the detection of laser-induced chlorophyll-a fluorescence [106]. The measuring principle of the instrument is based on the “Kautsky effect” [86]. Under dark conditions, the photochemical process of photosynthesis in plant cells temporarily ceases, and upon sudden high-intensity stimulation, typically by laser excitation, the chlorophyll molecules in the cells immediately begin to absorb light. However, the optimal conditions for photosynthesis develop more slowly, so only a small fraction of the energy of the light absorbed at the beginning is used in the process of photosynthesis. The excess light energy is re-emitted by the cells in the form of fluorescent light. After a few minutes, as the photosynthetic process resumes, the plant cell utilizes the absorbed light with higher efficiency, causing the intensity of fluorescent radiation to gradually decrease, stabilizing at a lower value [86,107].

During the 96-well microplate-based assay, the photosynthetic parameters were measured after a 10 min dark adaptation with the use of a special sample holder. After the dark acclimatization, the samples were excited with a laser diode (10 mW) at the wavelength of 635 nm. After excitation, the duration of the measurement was 5 min. During the measurements, the intensity of the fluorescent light emitted by the sample was detected at wavelengths of 690 nm and 735 nm [106,107]. The measurements were performed in triplicates. Photosynthetic activity of the control and treated groups was characterized by the observed ratio of fluorescence decrease (Rfd\*) and the proxy of quantum efficiency of



the algae photosystem PSII ( $F_v^*/F_p$ ). Here,  $F_p$  means the peak fluorescence value derived from the fluorescence induction curve using the FMM module, while  $F_v^*$  represents the variable fluorescence in terms of  $F_p$ . Essentially, the  $F_v/F_m$  parameter describes the impact of plant stress on photosystem II in a dark-acclimated state, where  $F_m$  is the maximum chlorophyll fluorescence under a saturating radiation pulse in such conditions [108,109]. As the maximum actinic level available with the FMM will not saturate PSII,  $F_p$  is used to distinguish it from  $F_m$ , which represents the maximum fluorescence value during continuous excitation under full saturation [88,108].  $Rfd^*$  corresponds to  $F_d/F_s$ , where  $F_s$  is the observed steady-state fluorescence and  $F_d$  indicates the fluorescence reduction from  $F_p$  to  $F_s$  [106,108]. Detailed explanations of different fluorescence parameters are summarized in Table 3. The effects on photosynthetic activity were compared based on the values detected at the wavelength of 690 nm [88].

**Table 3.** The main fluorescence parameters and quantities determined by FMM [106].

Fluorescence Parameter	Definition	Interpretation
$F_o$	observed	Non-variable (original) fluorescence intensity
$F_p$	observed	Peak fluorescence intensity, maximum fluorescence at a non-saturating light pulse
$F_v^*$	$F_p - F_o$	Variable fluorescence in terms of $F_p$
$F_v^*/F_p$	$F_v^*/F_p$	Proxy of quantum efficiency of photosystem II
$F_s$	observed	Steady-state (terminal) fluorescence
$F_d$	$F_p - F_s$	Fluorescence decrease in terms of $F_p$
$Rfd^*$	$F_d/F_s$	Fluorescence decrease ratio

### 2.5. Statistical Analysis

Based on the results of algal growth inhibition tests, 72 h  $EC_{50}$  values for both measured parameters (optical density and chlorophyll-a content) were determined using the ToxRat Pro 3.0 statistical software (ToxRat Solutions GmbH, Alsdorf, Germany). The additional statistical analyses were performed with the use of the R Statistical program 4.2.1. (R Development Core Team, Vienna, Austria). The effects of the individual and combined exposures, in addition to the differences between the determined 72 h  $EC_{50}$  values, and the detected parameters of photosynthetic activity ( $F_v^*/F_p$  and  $Rfd^*$ ) were evaluated with the use of general linear models. Before the statistical analysis, the normality of the data and the homogeneity of variance were checked by Shapiro–Wilk and Levene’s or Bartlett’s tests at the significance level of 0.050. Furthermore, the applicability of the fitted model was verified in each case with diagnostic plots (residual variances, QQ plot, Cook’s distance plot). Tukey’s honest significant difference (HSD) tests were used as post hoc analyses to assess the significant differences between groups. The data were evaluated using the Kruskal–Wallis test, if the conditions for applying the chosen model were not met, with the use of the Student–Newman–Keuls (SNK) test for the comparison of the different groups at the significance level of 0.050. In addition, the observed hormetic effects were verified with the use of Brain–Cousens hormesis models available in the ‘dcr’ package of the R Statistical program 4.2.1. [110–112].

## 3. Results

### 3.1. Individual and Combined Effects on Algal Growth

During the ecotoxicological testing, significant differences were not observed in the 72 h  $EC_{50}$  values determined for the active ingredient glyphosate based on the optical density of *P. subcapitata* ( $125.2 \pm 16.5$  mg/L) and *D. subspicatus* ( $132.9 \pm 2.3$  mg/L) green algae samples ( $p = 0.467$ ). On the other hand, much higher individual toxicity of glyphosate was demonstrated on *S. obtusiusculus* ( $73.1 \pm 21.2$  mg/L) compared to the individual toxicity values determined for the two other green algae species ( $p < 0.001$ ). The individual toxicity of POEA significantly exceeded the individual toxicity of glyphosate for all three algal species ( $p < 0.001$ ). Furthermore, compared to the individual toxicity of glyphosate, the toxicity of

the formulation significantly increased in the presence of POEA during the examination of all species (*P. subcapitata*, *D. subspicatus*: ( $p < 0.001$ ); *S. obtusiusculus*  $p = 0.005$ ). In the case of the formulation, no difference can be observed between the toxicity values corrected with the nominal content of POEA and determined after the individual exposure to POEA for *P. subcapitata* ( $p = 0.146$ ) and *D. subspicatus* ( $p = 0.172$ ), but the individual toxicity of POEA was lower on *S. obtusiusculus* ( $p = 0.021$ ) (Table 4). Based on the determined 72 h EC<sub>50</sub> values, a significant difference can be observed in the sensitivity of the tested species. There is no difference between the sensitivity of *P. subcapitata* and *D. subspicatus* ( $p = 0.467$ ) for glyphosate, while the sensitivity of *S. obtusiusculus* was higher due to the toxic effects of glyphosate ( $p < 0.001$ ). In contrast, *S. obtusiusculus* was the most tolerant against the effects of the POEA-formulated herbicide and POEA, followed by *D. subspicatus*, while in the case of both tested substances, *P. subcapitata* proved to be the most sensitive green algae (Roundup Classic:  $p < 0.001$ ; POEA:  $p < 0.030$ ) (Table 4).

**Table 4.** The determined 72 h EC<sub>50</sub> values for Roundup Classic and its components based on optical density measurements during the ecotoxicological testing on various green algae species.

Algae Species	GLY	72 h EC <sub>50</sub> Values (mg/L) <sup>1</sup>		POEA
		Roundup Classic <sup>2</sup>		
		GLY cont.	POEA cont.	
<i>Pseudokirchneriella subcapitata</i>	125.2 ± 16.5	5.1 ± 1.3	12.2 ± 3.1 1.9 ± 0.5	2.6 ± 0.7
<i>Desmodesmus subspicatus</i>	132.9 ± 2.3	14.1 ± 2.9	34.0 ± 6.9 5.3 ± 1.1	4.4 ± 0.4
<i>Scenedesmus obtusiusculus</i>	73.1 ± 21.2	27.3 ± 3.7	65.8 ± 9.0 10.2 ± 1.4	6.9 ± 1.6

<sup>1</sup> The combined toxicity of the investigated active ingredient glyphosate (GLY) and formulating agent POEA (mixture of polyethoxylated tallow amines) was investigated in the form of the formulated herbicide preparation.

<sup>2</sup> The 72 h EC<sub>50</sub> values for the herbicide formulation corrected with the nominal content of GLY and POEA indicates the concentration of the given component that is present in the formulation causing a 50% effect.

Similar to the toxicity values based on the optical density measurements, the toxicity of the POEA-formulated herbicide was also higher compared to the individual toxicity of glyphosate on the investigated green algae species ( $p < 0.001$ ), according to the 72 h EC<sub>50</sub> values based on the measurements of the chlorophyll-a content. The highest individual toxicity of glyphosate (17.4 ± 6.0 mg/L) was demonstrated for the tested cyanobacterium (*A. flos-aquae*) ( $p < 0.001$ ). However, based on the chlorophyll-a content, a difference can be observed in the sensitivity of the two green algae, as *D. subspicatus* proved to be more sensitive (73.8 ± 5.3 mg/L) compared to *P. subcapitata* (105.3 ± 17.8 mg/L) ( $p = 0.004$ ). The individual toxicity of POEA also proved to be much higher compared to the individual toxicity of glyphosate based on the chlorophyll-a content ( $p < 0.001$ ), where *P. subcapitata* was more sensitive to the effect of POEA ( $p < 0.001$ ). In contrast to the active ingredient, there was no significant difference between the 72 h EC<sub>50</sub> values for the formulation corrected with the POEA content and the determined toxicity values for POEA alone on the tested green algae species ( $p \geq 0.096$ ). Moreover, differences were not indicated in the sensitivity of the green algae species exposed to the formulation ( $p = 0.838$ ) (Table 5).

According to the results of algal growth inhibition tests performed on the APG-formulated herbicide, differences were not demonstrated between the individual toxicity of glyphosate and the combined toxicity indicated by the calculated 72 h EC<sub>50</sub> values for the formulation on *P. subcapitata* ( $p = 0.856$ ). In contrast to the POEA-formulated GBH, the individual toxicity of glyphosate significantly exceeded the combined toxicity of the components determined in the form of the APG-formulated herbicide on the other two tested green algae species (*D. subspicatus*:  $p < 0.001$ , *S. obtusiusculus*:  $p = 0.002$ ). *S. obtusiusculus* proved to be more sensitive to the effects of glyphosate ( $p = 0.001$ ) (Table 6). Similar to POEA, the individual toxicity of APG was higher compared to the individual toxicity of glyphosate on *P. subcapitata* and *D. subspicatus* ( $p < 0.001$ ), where *P. subcapitata* proved

to be more sensitive ( $p < 0.001$ ). Conversely, the individual toxicity of glyphosate was higher compared to the individual effects of APG on *S. obtusiusculus* ( $p = 0.008$ ). Significant differences were not detected between the toxicity values determined for Medallion Premium corrected with the APG content and indicated after the individual exposure to the surfactant APG on *P. subcapitata* ( $p = 0.068$ ) and *S. obtusiusculus* ( $p = 0.109$ ), similarly to POEA. However, the individual toxicity of APG was higher compared to the combined effects of the components indicated by toxicity values for the APG-formulated herbicide corrected with the APG content on *D. subspicatus* ( $p = 0.001$ ). Based on the determined 72 h EC<sub>50</sub> values, significant differences can be observed in the sensitivity of the tested species. *P. subcapitata* was the most sensitive species for both the formulation and APG (Medallion Premium:  $p < 0.001$ , APG:  $p \leq 0.001$ ). In the case of the additional two green algae, the difference was not demonstrated in their sensitivity to the effects of the APG-formulated herbicide ( $p = 0.650$ ), while *S. obtusiusculus* proved to be more tolerant to the toxic effects of APG ( $p < 0.001$ ) (Table 6).

**Table 5.** The determined 72 h EC<sub>50</sub> values for Roundup Classic and its components based on the chlorophyll-a content during the ecotoxicological testing on green algae species and a cyanobacterium.

Algae Species	GLY	72 h EC <sub>50</sub> Values (mg/L) <sup>1</sup>		POEA
		Roundup Classic <sup>2</sup>		
		GLY cont.	POEA cont.	
<i>Pseudokirchneriella subcapitata</i>	105.3 ± 17.8	14.5 ± 1.36	34.9 ± 3.2 5.4 ± 0.5	1.9 ± 0.3
<i>Desmodesmus subspicatus</i>	73.8 ± 5.3	13.4 ± 3.8	32.3 ± 9.2 5.0 ± 1.4	4.9 ± 0.6
<i>Scenedesmus obtusiusculus</i>	51.1 ± 2.6	10.5 ± 3.5	25.4 ± 8.5 3.9 ± 0.6	4.4 ± 0.9
<i>Anabaena flos-aquae</i>	17.4 ± 6.0	n.m.	n.m. <sup>3</sup> n.m.	n.m.

<sup>1</sup> The combined toxicity of the investigated active ingredient glyphosate (GLY) and formulating agent POEA (mixture of polyethoxylated tallow amines) was investigated in the form of the formulated herbicide preparation.

<sup>2</sup> The 72 h EC<sub>50</sub> values for the herbicide formulation corrected with the nominal content of GLY and POEA indicates the concentration of the given component that is present in the formulation causing a 50% effect.

<sup>3</sup> not measured.

**Table 6.** The determined 72 h EC<sub>50</sub> values for Medallion Premium and its components based on the optical density measurements during the ecotoxicological testing on various green algae species.

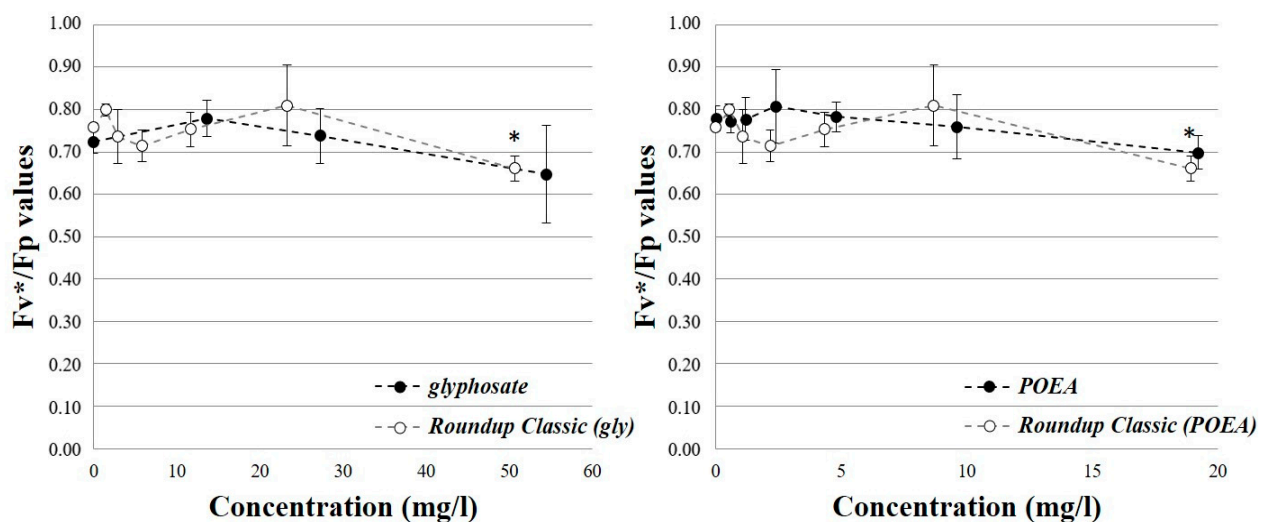
Algae Species	GLY	72 h EC <sub>50</sub> Values (mg/L) <sup>1</sup>		APG
		Medallion Premium <sup>2</sup>		
		GLY cont.	APG cont.	
<i>Pseudokirchneriella subcapitata</i>	125.2 ± 16.5	42.7 ± 4.7	125.7 ± 13.7 18.9 ± 2.1	23.0 ± 2.3
<i>Desmodesmus subspicatus</i>	132.9 ± 2.3	245.1 ± 32.8	720.9 ± 96.6 108.1 ± 14.5	64.3 ± 12.9
<i>Scenedesmus obtusiusculus</i>	73.1 ± 21.2	233.8 ± 58.4	687.5 ± 171.9 103.1 ± 25.8	137.9 ± 19.1

<sup>1</sup> The combined toxicity of the investigated active ingredient glyphosate (GLY) and formulating agent APG (alkyl polyglucosides) was investigated in the form of the formulated herbicide preparation. <sup>2</sup> The 72 h EC<sub>50</sub> values for the herbicide formulation corrected with the nominal content of GLY and APG indicates the concentration of the given component that is present in the formulation causing a 50% effect.

### 3.2. Effects on the Photosynthetic Activity of Green Algae Cells

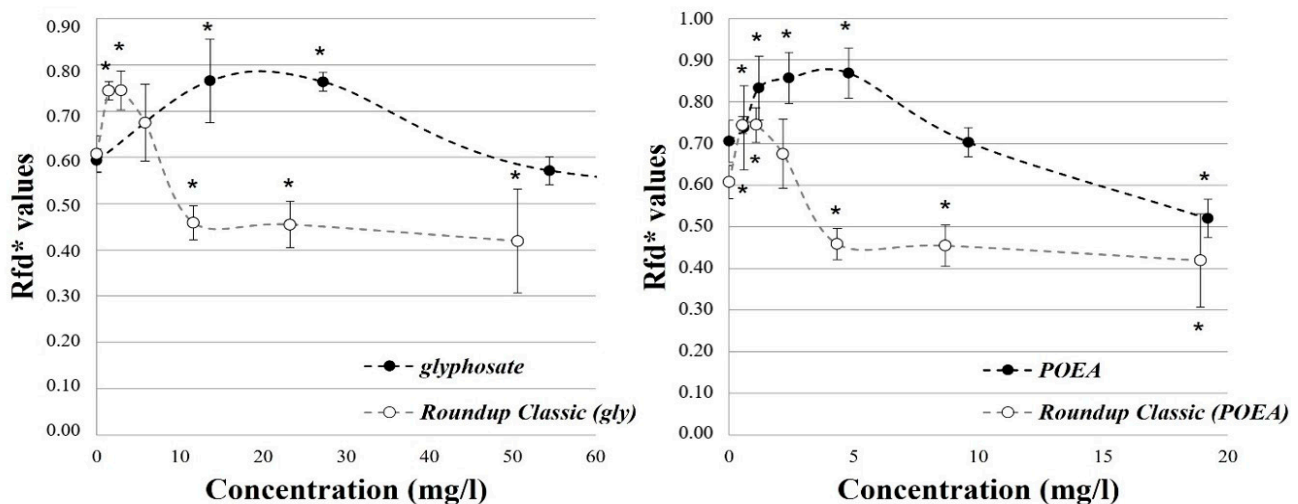
The individual and combined effects of the components presented in the tested POEA-formulated herbicide on the photosynthetic activity of *P. subcapitata* were evaluated according to the measured Fv\*/Fp values connected to the photochemical efficiency of the PS II photochemical system and Rfd\* values characterizing photosynthetic activity. During

the investigation of the effects of glyphosate on photosynthetic activity, the pure active ingredient did not result in a significant decrease in the  $F_v^*/F_p$  values compared to the control group up to a concentration of 109 mg/L ( $p = 0.034$ ). In contrast to the pure active ingredient, the formulation resulted in a significant reduction in the  $F_v^*/F_p$  value ( $p = 0.015$ ) compared to the control group, but only at the highest tested concentration (50.6 mg/L). After the individual exposure to POEA, significant changes in  $F_v^*/F_p$  values were not observed in the tested concentration range (0.6–19.2 mg/L). POEA in the presence of the active ingredient did not cause the reduction in the  $F_v^*/F_p$  value up to the highest tested concentration (18.9 mg/L) compared to the control group ( $p < 0.001$ ) (Figure 1—the  $F_v^*/F_p$  values were plotted in the common concentration range of the tested components: glyphosate: 0–54.5 mg/L; POEA: 0–19.2 mg/L).



**Figure 1.** The individual and combined effects of glyphosate and POEA on the  $F_v^*/F_p$  values characterizing the photochemical efficiency of the PS II photochemical system in the exposed algae cells.

During the investigation of glyphosate, a significant increase was observed in the  $R_{fd}^*$  values at the lower tested concentrations (13.6–27.2 mg/L) ( $p < 0.025$ ). However, above this range, no significant difference was observed compared to the control group, not even at the highest concentration (436 mg/L) ( $p = 1.000$ ). After the exposure to glyphosate in the form of herbicide formulation, increased  $R_{fd}^*$  values were also detected at the lower test concentrations (1.5–5.8 mg/L) compared to the control group. However, the difference was significant only at the two lowest concentrations ( $p < 0.018$ ). In contrast, significantly decreased  $R_{fd}^*$  values were observed at the higher concentration range (11.6–50.6 mg/L) ( $p < 0.012$ ) (Figure 2—the  $R_{fd}^*$  values were plotted in the common concentration range of the tested components: glyphosate: 0–54.5 mg/L; POEA: 0–19.2 mg/L). Similar to the effects of glyphosate, a significant increase in  $R_{fd}^*$  was observed after the individual exposure to POEA at the lower concentration range (0.6–4.8 mg/L) compared to the control group ( $p < 0.035$ ). However, a significant decrease was demonstrated in the  $R_{fd}^*$  values ( $p = 0.009$ ) at the highest tested concentration (19.2 mg/L). After the exposure to the herbicide formulation, POEA also resulted in an increase in  $R_{fd}^*$  values at the lower concentration range of POEA (0.5–2.2 mg/L) compared to the control. However, significant differences were not observed at the higher concentrations (4.8–19.2 mg/L) ( $p > 0.984$ ) (Figure 2).



**Figure 2.** The individual and combined effects of glyphosate and POEA on the Rfd\* values characterizing photosynthetic activity in the exposed algae cells.

#### 4. Discussion

According to the determined 72 h EC<sub>50</sub> values based on the measured optical density and chlorophyll-a content of the samples, the results and observed trends in toxicity correlated well between the tested endpoints. However, higher differences can be observed in some cases. Generally, lower toxicity values were determined based on the chlorophyll-a content compared to the 72 h EC<sub>50</sub> values calculated based on the optical density of the samples (Tables 4 and 5). The observed differences between the 72 h EC<sub>50</sub> values based on the two tested endpoints can presumably be explained by the fact that the determination of the optical density can be disturbed by the aggregation of cells and the presence of the remains of dead cells in the sample. On the other hand, during the analytical determination of the chlorophyll-a content, this disturbing matrix is not presented after the extraction of the samples. The determination of chlorophyll-a content proved to be a more reliable and sensitive endpoint, while in dead plant cells, chlorophyll-a begins to decompose rapidly, so the effects of inhibiting algae growth are estimated only based on living cells.

Based on the scientific literature and our results, significant differences can be observed in the sensitivity of different algal and cyanobacterial species to the effects of glyphosate and its formulated herbicides, even within the same taxa [20,70–72,113]. Therefore, the significant differences that can be observed in the available toxicity data are not surprising. Differences in the sensitivity of different algal species can presumably be explained by differences in the morphology of different algal cells (e.g., size and shape of cells, surface area to volume ratio, colony formation), the biology of cells (e.g., cell wall permeability, intracellular structure), and the physiology of different species (e.g., growth, nutrient uptake, metabolic activity) [114,115].

During the examination of the phytotoxic effects of glyphosate, the 72 h EC<sub>50</sub> values determined for *P. subcapitata* ( $125.2 \pm 16.5$  and  $105.3 \pm 17.8$  mg/L) far exceed the available literature values (24.7–41 mg/L) [15,73]. The values determined for *D. subspicatus* ( $132.9 \pm 2.3$  and  $73.8 \pm 5.3$  mg/L) fit well into the available toxicity range (72.9–166 mg/L) [74–76]. The toxicity of GBHs on algae species was investigated in several studies [115–117]. The toxicity values determined for Roundup Classic (corrected with glyphosate content) on *P. subcapitata* (72 h EC<sub>50</sub> values:  $5.1 \pm 1.3$  mg/L) correlated well with some of the published 72 h EC<sub>50</sub> values (0.7–5.8 mg/L) for Roundup formulations [15,73,118]; however, they remain well below the values published in other studies (15.6–64.7 mg/L) [52,119]. In contrast to the 72 h EC<sub>50</sub> values demonstrated on the MSDS of the tested formulations for algal test organisms (72 h EC<sub>50</sub> values = 2.1 mg/L (*P. subcapitata*, Roundup Classic) and 140 mg/L (*D. subspicatus*, Medallion Premium) [97,98]), our toxicity values were significantly higher. During the investigation of the individual and combined toxicity of the



components presented in Roundup Classic, the formulating agent POEA proved to be the most toxic component, followed by the formulation, while the toxicity of glyphosate was the lowest on the tested green algae species similar to the results of Tsui and Chu [15]. Similarly, the highest toxicity was observed for the tested surfactant APG compared to the individual toxicity of glyphosate and the combined toxic effects of the formulation on *P. subcapitata* and *D. subspicatus*. However, the highest toxicity was observed after the individual glyphosate exposure on *S. obtusiusculus*. (Tables 4–6). The increased toxicity of the formulations in the presence of formulating agents (e.g., POEA) has already been proven in several studies [15,77,120,121]. Based on our results, POEA proved to be more toxic than APG ( $p < 0.001$ ) (Tables 4 and 6). The determined 72 h EC<sub>50</sub> values of POEA ( $2.6 \pm 0.7$  mg/L and  $1.9 \pm 0.3$  mg/L) roughly correspond to the literature data for *P. subcapitata* (0.2–4.1 mg/L) [15,81,82] (Tables 4 and 5). The 72 h EC<sub>50</sub> values determined for APG on *P. subcapitata* ( $23.0 \pm 2.3$  mg/L) correspond to the toxicity range determined for long-chain APG compounds (C<sub>12–14</sub>: 11–46 mg/L) [83,84], but far exceed the value (2.7 mg/L) determined by Pavlic et al. [85] for C<sub>10</sub> carbon chain APG compounds. In contrast, the 72 h EC<sub>50</sub> values determined for APG compounds with shorter carbon chains (C<sub>8–10</sub>) on *P. subcapitata* proved to be very high (1113–1543 mg/L) [83,84]. The toxicity values determined for *D. subspicatus* ( $64.3 \pm 12.9$  mg/L) (Table 6) far exceed the average 72 h EC<sub>50</sub> values calculated for APG compounds with different chain lengths (C<sub>8–10</sub>: 21 mg/L, C<sub>12–14</sub>: 6 mg/L) [122], but mainly reflect the average value determined by Pavlic et al. [85] (C<sub>10</sub>: 0.32 mg/L). According to the results of several studies, increased toxicity on algae species has been observed with the length of the carbon chain [84,122].

In addition to herbicides that directly inhibit photosynthesis (e.g., atrazine), other active ingredients (e.g., glyphosate) can also affect photosynthesis and respiration processes through their effects on different metabolic pathways [90,91,123]. Based on our results, the pure glyphosate active ingredient did not result in a significant decrease in the photochemical efficiency of the PS II photochemical system compared to the control group up to a concentration of 109 mg/L. On the other hand, in the presence of POEA, a significant decrease was detected in the measured Fv\*/Fp values even at a lower concentration of 50.6 mg/L (Figure 1). According to the measured Rfd\* values, an increase was observed for both the pure active ingredient and the formulated herbicide preparation at low concentrations (glyphosate: 13.6–27.2 mg/L, Roundup Classic (glyphosate equivalent): 1.5–5.8 mg/L). However, the decrease in the Rfd\* values was only observed after the exposure to the herbicide formulation in the tested concentration range (Figure 2).

The adverse effects of glyphosate on the photochemical efficiency of the PS II photochemical system were observed on various green algal species (including *P. subcapitata*) from a concentration of 75 mg/L and several diatom species in the range of 15.3–37.5 mg/L [95]. Moreover, similar to our results, at a lower test concentration (0.02 mg/L), an increase in photosynthetic activity was also observed in the unicellular green algae (*Scenedesmus quadricauda*) [113]. The effects of formulating agents (e.g., POEA, APG) on the photosynthetic activity were investigated on the leaves of higher order plants (*Brassica oleracea*, *Malus domestica*), where significant effects of the tested surfactants were not demonstrated on *M. domestica*. On the other hand, a significant decrease in the photochemical efficiency of the PS II photochemical system was detected on the leaves of *B. oleracea* exposed to POEA [124]. During our measurements, POEA caused a significant decrease in the photochemical efficiency of the PS II photochemical system only in the presence of glyphosate at the highest test concentration (Roundup Classic: 18.9 mg/L POEA equivalent). POEA individually and in the presence of glyphosate induced an increase in Rfd\* values at lower test concentrations. Conversely, significantly lower Rfd\* values were demonstrated in the higher POEA concentration range compared to the control (Figure 2). After the exposure to Roundup formulations, the phytotoxic effect of glyphosate in the presence of POEA far exceeded the toxicity of the pure active ingredient on the photosynthetic activity of green and blue-green algae species (*M. aeruginosa*, *N. microcarpa* var. *wrightii*). However, at low concentrations, an increase in photosynthetic activity was also reported [80,90].

The observed stimulatory effects of toxic compounds at lower concentrations can be interpreted as hormesis effects [80,90]. Hormesis is the phenomenon when a xenobiotic has an opposite effect in low and high doses on some infra-individual level property of an organism (biochemical processes, cellular characteristics, histological and organic changes, etc.), or on some characteristic of a population/community [125]. Essentially, hormesis is a biological phenomenon where a harmful compound shows a favorable or stimulating effect in the low concentration range [126]. The phenomenon cannot be characterized by the usual sigmoid (logistic) shaped dose–effect curve [126–128]. The explanation of the phenomenon of hormesis is not completely clear, but several background mechanisms can be assumed [127,129]. One of the possible explanations is that the homeostasis of the organism is disturbed by the low concentration of the pollutant and the positive effect appears to compensate the negative effects of the xenobiotics. As a result of the disturbance, the dynamic equilibrium of the body conditions slightly exceeds the normal limits. To compensate for this imbalance, the affected organism mobilizes resources and, in the meantime, achieves a more favorable state than before (e.g., observed higher growth rates by the low concentrations of the tested compounds compared to the control) [127,130]. According to another idea, hormesis results from changes in energy allocation of the organisms [131]. The consequence of the trade-off alterations in life history traits can be indicated by changes in various population parameters (e.g., number of eggs, growth, and behavior) [129,131]. The changes of trade-off caused by pesticides can have an adaptive value, because it helps individuals maintain their fitness [132].

Based on our results, Rfd\* proved to be a more sensitive endpoint compared to the Fv\*/Fp values characterizing the photochemical efficiency of the PS II photochemical system. During the investigation of the phytotoxic effects, a higher growth rate (3.5 mg/L Roundup Classic) and increased photosynthetic activity were measured at lower test concentrations of the formulation compared to the control groups. At the lower concentrations of the pure active ingredient and POEA, the hormesis effects were only detected during the measurement of the Rfd\* values. The observed hormetic effects were also verified by the performed Brain–Cousens hormesis models ( $p < 0.048$ ). Based on the results of algal growth inhibition tests, this stimulating effect was not demonstrated after the individual exposure to the tested components. According to the measured Rfd\* values, the hormetic response of *P. subcapitata* was indicated after the individual and combined exposure to the components at the lower concentration ranges. In our study, the observed changes in photosynthetic parameters can presumably be explained primarily by the phenomenon of hormesis, as well as by the change in algal biomass resulting from toxic effects. At low concentrations, the toxic effects do not yet prevail, but on the contrary, the treated algal cells can utilize glyphosate as a source of carbon, phosphorus, and nitrogen [70,90,110,133,134]. Moreover, glyphosate can also trigger pathways for protein and metabolite synthesis [70,133], which can result in increased biomass growth. However, with the increase in concentration, the toxic effects prevail against the excess nutrient content. During the investigation of *Pseudomonas* species, the utilization of octadecyl-bis(2-hydroxyethyl) amine was demonstrated as a carbon and energy source during bacterial growth [81]. Based on certain studies, hormesis can also be interpreted as the response of the plant organism to increased stress [135].

Significant differences were demonstrated in the individual and combined toxicity of the components presented in the tested GBHs. Furthermore, our results support the scientific opinions proposing changes in the official regulations, including the strict regulation of co-formulants, the future development of standards to assess combined effects, and the environmental risks of chemical mixtures [136,137]. Generally, the active ingredients and the co-formulants almost certainly become separated relatively quickly after pesticide treatments. The mobility of the components presented in pesticide formulation highly depends on the physico-chemical properties of the chemical substances (e.g., water solubility, log  $K_{ow}$ ) and the environmental matrices (e.g., pH, level of suspended materials, dissolved oxygen content) [20–22,41,138]. The water solubility of glyphosate is 11.6 g/L (25 °C), while degradation half-life (DT<sub>50</sub>) in water varies from a few to 91 days [41]. The

water solubility of POEA increases with the increase in oxide–tallow amine ratio [139], while its persistence was demonstrated in soil by several studies [44,45]. The water solubility and biodegradability of APGs depends on the length of the alkyl chain [48]. Various co-formulants affect the solubility and stability of glyphosate, leading to variations in its bioavailability and persistence in the environment [43]. The surfactants applied in GBHs can modify the adsorption capacity of glyphosate, resulting in reduced physical adsorption of glyphosate on the surface of solid–liquid boundary phases (e.g., suspended particles in water samples) [140]. In addition, surfactants form micelles that help glyphosate stay in solution and provide protection against degradation [82,141,142]. In summary, the physico-chemical interactions between glyphosate and the additional ingredients are complex and can significantly influence the overall toxicity of GBHs. Therefore, the ecotoxicological and toxicological evaluation of the various additives is an essential condition for the proper environmental risk assessment of pesticide formulations used in agricultural practice. Currently, manufacturers are only required to indicate the exact chemical name and quantity of the active ingredient(s), synergists, and antidotes on the labels of the products in the EU. Thus, the exact composition of the formulations and information about co-formulants are not public [9,143], resulting in several uncertainties regarding the evaluation of the possible combined toxic effects [144].

However, most of the calculated toxicity values determined for the components presented in glyphosate-based formulations individually and in combination remain below the detected average environmental concentrations in surface waters [27,36], and contamination levels can rise significantly after heavy rains in the watercourses near the treated areas [34,145]. Additionally, the toxicity of glyphosate and POEA can also be significantly influenced by different environmental conditions (e.g., pH, dissolved oxygen content, temperature) [146,147]. Moreover, as a result of global climate change, increased average temperature and the modified intensity of the incident light can significantly influence the phytotoxic effects of glyphosate on phytoplankton communities [91]. Stimulatory effects were indicated at lower concentrations of glyphosate-based Roundup Classic (1.5–5.8 mg glyphosate/L and 0.5–2.2 mg POEA/L), for which concentrations approach or stay much below the measured maximums [27,34,35,37].

## 5. Conclusions

Based on the scientific evidence and our findings, significant differences can be observed in both the individual and combined toxicity of the components contained in the tested GBH formulations. According to our results, the tested co-formulants proved to be the most toxic components. Although the individual toxicity of APG is not as high as for POEA, the toxicity of the formulation is affected by the simultaneous presence of the active ingredient and the co-formulants. Therefore, the revision of GBHs formulated with APG compounds may also be necessary. In addition, significant differences were detected in the sensitivity of the tested algal species, including *D. subspicatus* and *S. obtusiusculus* species belonging to the same family (*Scenedesmaceae*). The differences in sensitivity are presumably the result of differences observed in the morphology, cell biology, and physiology of different algal cells. During the evaluation of phytotoxic effects, increased photosynthetic activity was detected on *P. subcapitata* after the exposure to the POEA-formulated GBH and its components in the lower concentration ranges. However, decreased activity was observed after exposure to POEA and the formulation at higher test concentrations. Not only the inhibitory effects but stimulating effects on the growth of algae can adversely affect the aquatic ecosystem and water quality of surface waters. Moreover, the accumulation of phytotoxins can also cause serious environmental effects on aquatic communities.

**Author Contributions:** Conceptualization, S.K.; methodology, S.K., E.T., A.B. and S.L.; software, A.B. and S.L.; validation, S.K. and E.T.; investigation, S.K., E.T. and B.D.; writing—original draft preparation, S.K. and E.T.; writing—review and editing, A.B., B.D., L.K. and A.S.; visualization, S.K.; supervision, A.S.; funding acquisition, L.K. and A.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work has been funded by the Hungarian National Research, Development and Innovation Office within the National Competitiveness and Excellence Program, project NVKP\_16-1-2016-0049, “In situ, complex water quality monitoring by using direct or immunofluorimetry and plasma spectroscopy” (Aquafluosense 2017–2021), and project 2022-2.1.1-NL-2022-00006, “Development of the Agrotechnology National Laboratory” (Grant agreement NKFIH-3524-1/2022), supported by the National Research, Development and Innovation Fund by the Hungarian Ministry of Culture and Innovation, as well as projects TKP2021-NVA-02 and TKP2021-NVA-22 within the framework of the Thematic Excellence Program 2021, National Defense, National Security Sub-Program. Our study was also supported by the Hungarian Scientific Research Fund (OTKA K109865 and OTKA K112978 projects), the Research Excellence Program 2024 of Hungarian University of Agriculture and Life Sciences, the János Bolyai Scholarship of the Hungarian Academy of Sciences, and ÚNKP-23-5-BME-448 New National Excellence Program of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy reasons.

**Acknowledgments:** The authors thank Péter Bohus for the sample of testing materials and express their sincere appreciation to Ildikó Mándics and Judit Olajos for their technical assistance during ecotoxicological testing.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## References

1. Arias-Estévez, M.; López-Periago, E.; Martínez-Carballo, E.; Simal-Gándara, J.; Mejuto, J.-C.; García-Río, L. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. *Agric. Ecosyst. Environ.* **2008**, *123*, 247–260. [CrossRef]
2. Stehle, S.; Schulz, R. Agricultural insecticides threaten surface waters at the global scale. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 5750–5755. [CrossRef]
3. Tang, F.H.M.; Lenzen, M.; McBratney, A.; Maggi, F. Risk of pesticide pollution at the global scale. *Nat. Geosci.* **2021**, *14*, 206–210. [CrossRef]
4. Solomon, K.R.; Thompson, D.G. Ecological risk assessment for aquatic organisms from over-water uses of glyphosate. *J. Toxicol. Environ. Health Part B* **2003**, *6*, 289–324. [CrossRef] [PubMed]
5. Hanke, I.; Wittmer, I.; Bischofberger, S.; Stamm, C.; Singer, H. Relevance of urban glyphosate use for surface water quality. *Chemosphere* **2010**, *81*, 422–429. [CrossRef] [PubMed]
6. Benbrook, C.M. Trends in glyphosate herbicide use in the United States and globally. *Environ. Sci. Eur.* **2016**, *28*, 3. [CrossRef]
7. Gower, S.A.; Loux, M.M.; Cardina, J.; Harrison, S.K. Effect of planting date, residual herbicide, and postemergence application timing on weed control and grain yield in glyphosate-tolerant corn (*Zea mays*). *Weed Technol.* **2002**, *16*, 488–494. [CrossRef]
8. Whigham, D.K.; Stoller, E.W. Soybean desiccation by paraquat, glyphosate, and ametryn to accelerate harvest. *Agron. J.* **1979**, *71*, 630–633. [CrossRef]
9. European Parliament and Council. Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing council directives 79/117/EEC and 91/414/EEC. *OJ EU* **2009**, *L309*, 1–50. Available online: <https://eur-lex.europa.eu/eli/reg/2009/1107/oj> (accessed on 21 March 2024).
10. European Commission. Commission Implementing Regulation (EU) 2023/2660 of 28 November 2023 renewing the approval of the active substance glyphosate in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council and amending Commission Implementing Regulation (EU) No 540/2011. *OJ EU* **2023**, *L2023/2660*. Available online: [https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=OJ:L\\_202302660&qid=1710855398897](https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=OJ:L_202302660&qid=1710855398897) (accessed on 21 March 2024).
11. Defarge, N.; Takács, E.; Lozano, V.L.; Mesnage, R.; Vendômois, J.S.; Seralini, G.E.; Székács, A. Co-formulants in glyphosate-based herbicides disrupt aromatase activity in human cells below toxic levels. *Int. J. Environ. Res. Pub. Health* **2016**, *13*, 264. [CrossRef]
12. Travlos, I.; Cheimona, N.; Bilalis, D. Glyphosate efficacy of different salt formulations and adjuvant additives on various weeds. *Agronomy* **2017**, *7*, 60. [CrossRef]
13. Foy, C. Adjuvants: Terminology, classification, and mode of action. In *Adjuvants and Agrochemicals*; Chow, P., Grant, C., Hinshalwood, A., Simundson, E., Eds.; CRC Press: Boca Raton, FL, USA, 1987; pp. 1–15.
14. Defarge, N.; Spiroux de Vendômois, J.; Seralini, G.E. Toxicity of formulants and heavy metals in glyphosate-based herbicides and other pesticides. *Toxicol. Rep.* **2018**, *5*, 156–163. [CrossRef] [PubMed]
15. Tsui, M.T.K.; Chu, L.M. Aquatic toxicity of glyphosate-based formulations: Comparison between different organisms and the effects of environmental factors. *Chemosphere* **2003**, *52*, 1189–1197. [CrossRef] [PubMed]



16. Székács, A. Mechanism-related teratogenic, hormone modulant and other toxicological effects of veterinary and agricultural surfactants. *Insights Vet. Sci.* **2017**, *1*, 24–31. [[CrossRef](#)]
17. Mesnage, R.; Benbrook, C.; Antoniou, M.N. Insight into the confusion over surfactant co-formulants in glyphosate-based herbicides. *Food Chem. Toxicol.* **2019**, *128*, 137–145. [[CrossRef](#)] [[PubMed](#)]
18. European Commission. Commission Implementing Regulation (EU) 2016/1313 of 1 August 2016 amending Implementation Regulation (EU) No 540/2011 as regards the conditions of approval of the active substance glyphosate. *OJ EU* **2016**, *L208*, 1–3. Available online: [https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L\\_.2016.208.01.0001.01.ENG](https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2016.208.01.0001.01.ENG) (accessed on 21 March 2024).
19. Székács, A.; Mörtl, M.; Darvas, B. Monitoring pesticide residues in surface and ground water in Hungary: Surveys in 1990–2015. *J. Chem.* **2015**, *2015*, 717948. [[CrossRef](#)]
20. Klátyik, S.; Simon, G.; Oláh, M.; Takács, E.; Mesnage, R.; Antoniou, M.N.; Zaller, J.G.; Székács, A. Aquatic ecotoxicity of glyphosate, its formulations, and co-formulants: Evidence from 2010 to 2023. *Environ. Sci. Eur.* **2024**, *36*, 22. [[CrossRef](#)]
21. Borggaard, O.K.; Gimsing, A.L. Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: A review. *Pest Manag. Sci.* **2008**, *64*, 441–456. [[CrossRef](#)] [[PubMed](#)]
22. Hébert, M.-P.; Fugère, V.; Gonzalez, A. The overlooked impact of rising glyphosate use on phosphorus loading in agricultural watersheds. *Front. Ecol. Environ.* **2019**, *17*, 48–56. [[CrossRef](#)]
23. Zaller, J.G.; Weber, M.; Maderthaner, M.; Gruber, E.; Takács, E.; Mörtl, M.; Klátyik, S.; Györi, J.; Römbke, J.; Leisch, F.; et al. Effects of glyphosate-based herbicides and their active ingredients on earthworms, water infiltration and glyphosate leaching are influenced by soil properties. *Environ. Sci. Eur.* **2021**, *33*, 51. [[CrossRef](#)]
24. Kjær, J.; Olsen, P.; Ullum, M.; Grant, R. Leaching of glyphosate and amino-methylphosphonic acid from Danish agricultural field sites. *J. Environ. Qual.* **2005**, *34*, 608–620. [[CrossRef](#)] [[PubMed](#)]
25. Huhn, C. More and enhanced glyphosate analysis is needed. *Anal. Bioanal. Chem.* **2018**, *410*, 3041–3045. [[CrossRef](#)] [[PubMed](#)]
26. Duke, S.O.; Powles, S.B. Glyphosate: A once-in-a-century herbicide. *Pest. Manag. Sci.* **2008**, *64*, 319–325. [[CrossRef](#)] [[PubMed](#)]
27. Székács, A.; Darvas, B. Re-registration challenges of glyphosate in the European Union. *Front. Environ. Sci.* **2018**, *6*, 78. [[CrossRef](#)]
28. Villeneuve, A.; Larroudé, S.; Humbert, J.F. Herbicide contamination of freshwater ecosystems: Impact on microbial communities. In *Pesticides—Formulations, Effects, Fate*; Stoytcheva, M., Ed.; InTech: Rijeka, Croatia, 2011; pp. 285–312.
29. Chang, F.C.; Simcik, M.F.; Capel, P.D. Occurrence and fate of the herbicide glyphosate and its degradate aminomethylphosphonic acid in the atmosphere. *Environ. Toxicol. Chem.* **2011**, *30*, 548–555. [[CrossRef](#)] [[PubMed](#)]
30. Silva, V.; Montanarella, L.; Jones, A.; Fernández-Ugalde, O.; Mol, H.G.J.; Ritsema, C.J.; Geissen, V. Distribution of glyphosate and aminomethylphosphonic acid (AMPA) in agricultural topsoils of the European Union. *Sci. Total Environ.* **2018**, *621*, 1352–1359. [[CrossRef](#)] [[PubMed](#)]
31. Lutri, V.F.; Matteoda, E.; Blarasin, M.; Aparicio, V.; Giacobone, D.; Maldonado, L.; Becher Quinodoz, F.; Cabrera, A.; Giuliano Albo, J. Hydrogeological features affecting spatial distribution of glyphosate and AMPA in groundwater and surface water in an agroecosystem. Córdoba, Argentina. *Sci. Total Environ.* **2020**, *711*, 134557. [[CrossRef](#)]
32. Grandcoin, A.; Piel, S.; Baurès, E. AminoMethylPhosphonic acid (AMPA) in natural waters: Its sources, behavior and environmental fate. *Weed Res.* **2017**, *117*, 187–197. [[CrossRef](#)]
33. U.S. Geological Survey. Common Weed Killer Is Widespread in the Environment, U.S. Geological Survey. Available online: <https://www.usgs.gov/programs/environmental-health-program/science/common-weed-killer-widespread-environment> (accessed on 21 March 2024).
34. Coupe, R.H.; Kalkhoff, S.J.; Capel, P.D.; Gregoire, C. Fate and transport of glyphosate and aminomethylphosphonic acid in surface waters of agricultural basins. *Pest Manag. Sci.* **2012**, *68*, 16–30. [[CrossRef](#)]
35. Mörtl, M.; Németh, G.; Juracsek, J.; Darvas, B.; Kamp, L.; Rubio, F.; Székács, A. Determination of glyphosate residues in Hungarian water samples by immunoassay. *Microchem. J.* **2013**, *107*, 143–151. [[CrossRef](#)]
36. Bonansea, R.I.; Filippi, I.; Wunderlin, D.A.; Marino, D.J.G.; Amé, M.V. The fate of glyphosate and AMPA in a freshwater endorheic basin: An ecotoxicological risk assessment. *Toxics* **2018**, *6*, 3. [[CrossRef](#)] [[PubMed](#)]
37. Caprile, A.C.; Aparicio, V.; Sasal, C.; Andriulo, E. Variation in glyphosate and AMPA concentrations of surface water and groundwater. *Geophys. Res. Abstr.* **2017**, *19*, EGU2017-2068. Available online: <https://meetingorganizer.copernicus.org/EGU2017/EGU2017-2068.pdf> (accessed on 21 March 2024).
38. Centrum voor Milieuwetenschappen Leiden. Atlas Bestrijdingsmiddelen in Oppervlaktewater, Universiteit Leiden, Leiden, Netherlands. Available online: <https://www.bestrijdingsmiddelenatlas.nl/atlas/1/1> (accessed on 21 March 2024).
39. Poiger, T.; Buerge, I.J.; Bächli, A.; Müller, M.D.; Balmer, M.E. Occurrence of the herbicide glyphosate and its metabolite AMPA in surface waters in Switzerland determined with on-line solid phase extraction LC-MS/MS. *Environ. Sci. Pollut. Res.* **2017**, *24*, 1588–1596. [[CrossRef](#)] [[PubMed](#)]
40. Di Guardo, A.; Finizio, A. A new methodology to identify surface water bodies at risk by using pesticide monitoring data: The glyphosate case study in Lombardy Region (Italy). *Sci. Total Environ.* **2018**, *610–611*, 421–429. [[CrossRef](#)]
41. Turner, J.A. *The Pesticide Manual*, 19th ed.; The British Crop Protection Council: Brighton, UK, 2021.
42. 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**, *15*, 7519. [[CrossRef](#)]



43. Klátyik, S.; Simon, G.; Oláh, M.; Mesnage, R.; Antoniou, M.N.; Zaller, J.G.; Székács, A. Terrestrial ecotoxicity of glyphosate, its formulations, and co-formulants: Evidence from 2010–2023. *Environ. Sci. Eur.* **2023**, *35*, 51. [\[CrossRef\]](#)
44. Tush, D.; Meyer, M.T. Polyoxyethylene tallow amine, a glyphosate formulation adjuvant: Soil adsorption characteristics, degradation profile, and occurrence on selected soils from agricultural fields in Iowa, Illinois, Indiana, Kansas, Mississippi, and Missouri. *Environ. Sci. Technol.* **2016**, *50*, 5781–5789. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Tush, D.; Maksimowicz, M.M.; Meyer, M.T. Dissipation of polyoxyethylene tallow amine (POEA) and glyphosate in an agricultural field and their co-occurrence on streambed sediments. *Sci. Total Environ.* **2018**, *636*, 212–219. [\[CrossRef\]](#)
46. Morrás, H.; Behrends Kraemer, F.; Sainz, D.; Fernández, P.; Chagas, C. Soil structure and glyphosate fate under no-till management in the Pampa region. II. Glyphosate and AMPA persistence and spatial distribution in the long-term. A conceptual model. *Soil Tillage Res.* **2022**, *223*, 105471. [\[CrossRef\]](#)
47. Green, J.M.; Beestman, G.B. Recently patented and commercialized formulation and adjuvant technology. *Crop Prot.* **2007**, *26*, 320–327. [\[CrossRef\]](#)
48. Geetha, D.; Tyagi, R. Alkyl poly glucosides (APGs) surfactants and their properties: A review. *Tenside Surfactants Deterg.* **2012**, *49*, 417–427. [\[CrossRef\]](#)
49. Rastogi, R. Fate of alkyl polyglucosides in the environment. *J. Cosmet. Sci.* **2021**, *72*, 91–98. [\[PubMed\]](#)
50. Evalen, P.S.; Barnhardt, E.N.; Ryu, J.; Stahlschmidt, Z.R. Toxicity of glyphosate to animals: A meta-analytical approach, *Environ. Pollut.* **2024**, *347*, 123669. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Dabney, B.L.; Patiño, R. Low-dose stimulation of growth of the harmful alga, *Prymnesium parvum*, by glyphosate and glyphosate-based herbicides. *Harmful Algae* **2018**, *80*, 130–139. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Fernández, C.; Asselborn, V.; Parodi, E.R. Toxic effects of chlorpyrifos, cypermethrin and glyphosate on the non-target organism *Selenastrum capricornutum* (Chlorophyta). *An. Acad. Bras. Cienc.* **2021**, *93*, e20200233. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Reno, U.; Doyle, S.R.; Momo, F.R.; Regaldo, L.; Gagneten, A.M. Effects of glyphosate formulations on the population dynamics of two freshwater cladoceran species. *Ecotoxicology* **2018**, *27*, 784–793. [\[CrossRef\]](#)
54. Iummato, M.M.; Sabatini, S.E.; Cacciatore, L.C.; Cochón, A.C.; Cataldo, D.; de Molina, M.D.C.R.; Juárez, Á.B. Biochemical responses of the golden mussel *Limnoperna fortunei* under dietary glyphosate exposure. *Ecotoxicol. Environ. Saf.* **2018**, *163*, 69–75. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Fiorino, E.; Sehonova, P.; Plhalova, L.; Blahova, J.; Svobodova, Z.; Faggio, C. Effects of glyphosate on early life stages: Comparison between *Cyprinus carpio* and *Danio rerio*. *Environ. Sci. Pollut. Res.* **2018**, *25*, 8542–8549. [\[CrossRef\]](#)
56. Bach, N.C.; Marino, D.J.G.; Natale, G.S.; Somoza, G.M. Effects of glyphosate and its commercial formulation, Roundup® Ultramax, on liver histology of tadpoles of the neotropical frog, *Leptodactylus latrans* (amphibia: Anura). *Chemosphere* **2018**, *202*, 289–297. [\[CrossRef\]](#)
57. Schaffer, J.D.; Sebetich, M.J. Effects of aquatic herbicides on primary productivity of phytoplankton in the laboratory. *Bull. Environ. Contam. Toxicol.* **2004**, *72*, 1032–1037. [\[CrossRef\]](#) [\[PubMed\]](#)
58. Jyothi, K.; Krishna Prasad, M.; Mohan Narasimha Rao, G. Algae in fresh water ecosystem. *Phykos* **2016**, *46*, 25–31.
59. Maguire, R.J.; Wong, P.T.S.; Rhamey, J.S. Accumulation and metabolism of tri-n-butyltin cation by a green alga, *Ankistrodesmus falcatus*. *Can. J. Fish. Aquat.* **1984**, *41*, 537–540. [\[CrossRef\]](#)
60. Paerl, H.W.; Otten, T.G. Harmful cyanobacterial blooms: Causes, consequences, and controls. *Microb. Ecol.* **2013**, *65*, 995–1010. [\[CrossRef\]](#) [\[PubMed\]](#)
61. Watson, S.B.; Whitton, B.A.; Higgins, S.N.; Paerl, H.W.; Brooks, B.W.; Wehr, J.D. Harmful algal blooms. In *Freshwater Algae of North America: Ecology and Classification*; Wehr, J.D., Sheath, R.G., Kociolek, P., Eds.; Academic Press: Cambridge, MA, USA, 2015; pp. 873–920.
62. Wu, N.; Dong, X.; Liu, Y.; Wang, C.; Baattrup-Pederson, A.; Riis, T. Using river microalgae as indicators for freshwater biomonitoring: Review of published research and future directions. *Ecol. Indic.* **2017**, *81*, 124–131. [\[CrossRef\]](#)
63. Vidyashankar, S.; Ravishankar, G.A. Algae-based bioremediation: Bioproducts and biofuels for biobusiness. In *Bioremediation and Bioeconomy*; Prasad, M.N.V., Ed.; Elsevier: Amsterdam, The Netherlands, 2016; pp. 457–493.
64. 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. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Hernández-García, C.I.; Martínez-Jerónimo, F. Multistressor negative effects on an experimental phytoplankton community. The case of glyphosate and one toxigenic cyanobacterium on *Chlorophycean* microalgae. *Sci. Total Environ.* **2020**, *717*, 137186. [\[CrossRef\]](#) [\[PubMed\]](#)
66. Wu, L.; Qiu, Z.; Zhou, Y.; Du, Y.; Liu, C.; Ye, J.; Hu, X. Physiological effects of the herbicide glyphosate on the cyanobacterium *Microcystis aeruginosa*. *Aquat. Toxicol.* **2016**, *178*, 72–79. [\[CrossRef\]](#) [\[PubMed\]](#)
67. Drzyzga, D.; Lipok, J. Glyphosate dose modulates the uptake of inorganic phosphate by freshwater cyanobacteria. *J. Appl. Phycol.* **2018**, *30*, 299–309. [\[CrossRef\]](#)
68. Iummato, M.M.; Fassiano, A.; Graziano, M.; Dos Santos Afonso, M.; Ríos de Molina, M.D.C.; Juárez, Á.B. Effect of glyphosate on the growth, morphology, ultrastructure and metabolism of *Scenedesmus vacuolatus*. *Ecotoxicol. Environ. Saf.* **2019**, *15*, 471–479. [\[CrossRef\]](#)

69. Smedbol, É.; Gomes, M.P.; Paquet, S.; Labrecque, M.; Lepage, L.; Lucotte, M.; Juneau, P. Effects of low concentrations of glyphosate-based herbicide Factor 540® on an agricultural stream freshwater phytoplankton community. *Chemosphere* **2018**, *192*, 133–141. [CrossRef] [PubMed]
70. Wang, C.; Lin, X.; Li, L.; Lin, S. Differential growth responses of marine phytoplankton to herbicide glyphosate. *PLoS ONE* **2016**, *11*, e0151633. [CrossRef] [PubMed]
71. Ma, J.; Qin, W.; Lu, N.; Wang, P.; Huang, C.; Xu, R. Differential sensitivity of three cyanobacteria (*Anabaena flos-aquae*, *Microcystis flos-aquae* and *Mirocystis aeruginosa* to 10 pesticide adjuvants. *Bull. Environ. Contam. Toxicol.* **2005**, *75*, 873–881. [CrossRef] [PubMed]
72. Arunakumara, K.; Walpol, B.; Yoon, M. Metabolism and degradation of glyphosate in aquatic cyanobacteria: A review. *Afr. J. Microbiol. Res.* **2013**, *7*, 4084–4090.
73. EGEIS Aquatic Ecotoxicity of Glyphosate and Formulated Products Containing Glyphosate. European Glyphosate Environmental Information Sources. Available online: <http://www.egeis.org/cd-info/Aquatic-ecotoxicity-of-glyphosate-and-formulated-products-containing-glyphosate.pdf> (accessed on 21 March 2024).
74. Giesy, J.P.; Dobson, S.; Solomon, K.R. Ecotoxicological risk assessment for Roundup herbicide. *Rev. Environ. Contam. Toxicol.* **2000**, *167*, 35–120.
75. Lewis, K.A.; Tzilivakis, J.; Warner, D.J.; Green, A. An international database for pesticide risk assessments and management. *Hum. Ecol. Risk Assess.* **2016**, *22*, 1050–1064. [CrossRef]
76. MacBean, C. *The Pesticide Manual*, 16th ed.; The British Crop Protection Council: Brighton, UK, 2012.
77. Gonzalez, D.; Juárez, A.; Krug, C.; Santos, M.; Vera, S. Freshwater periphyton response to technical-grade and two commercial formulations of glyphosate. *Ecol. Austral.* **2019**, *29*, 020–027. [CrossRef]
78. Vera, M.S.; Trinelli, M.A. First evaluation of the periphyton recovery after glyphosate exposure. *Environ. Pollut.* **2021**, *290*, 117998. [CrossRef] [PubMed]
79. Bricheux, G.; Le Moal, G.; Hennequin, C.; Coffe, G.; Donnadieu, F.; Portelli, C.; Bohatier, J.; Forestier, C. Characterization and evolution of natural aquatic biofilm communities exposed in vitro to herbicides. *Ecotoxicol. Environ. Saf.* **2013**, *88*, 126–134. [CrossRef]
80. de Campos Oliveira, R.; Boas, L.K.V.; Branco, C.C.Z. Assessment of the potential toxicity of glyphosate-based herbicides on the photosynthesis of *Nitella microcarpa* var. *wrightii* (Charophyceae). *Phycologia* **2016**, *55*, 577–584. [CrossRef]
81. van Ginkel, C.G.; Stroo, C.A.; Kroon, A.G.M. Biodegradability of ethoxylated fatty amines and amides and the non-toxicity of their biodegradation products. *Tenside Surfactant. Deterg.* **1993**, *30*, 213–216. [CrossRef]
82. Rodriguez-Gil, J.L.; Prosser, R.; Poirier, D.; Lissemore, L.; Thompson, D.; Hanson, M.; Solomon, K.R. Aquatic hazard assessment of MON 0818, a commercial mixture of alkylamine ethoxylates commonly used in glyphosate-containing herbicide formulations. Part 1: Species sensitivity distribution from laboratory acute exposures. *Environ. Toxicol. Chem.* **2017**, *36*, 512–521. [CrossRef] [PubMed]
83. Madsen, T.; Petersen, G.; Seiero, C.; Torslov, J. Biodegradability and aquatic toxicity of glycoside surfactants and a nonionic alcohol ethoxylate. *J. Am. Oil Chem. Soc.* **1996**, *73*, 929–933. [CrossRef]
84. Jurado, E.; Fernández-Serrano, M.; Núñez-Olea, J.; Lechuga, M.; Jiménez, J.L.; Ríos, F. Acute toxicity of alkylpolyglucosides to *Vibrio fischeri*, *Daphnia magna* and microalgae: A comparative study. *Bull. Environ. Contam. Toxicol.* **2012**, *88*, 290–295. [CrossRef] [PubMed]
85. Pavlic, Z.; Vidakovic-Cifrek, Z.; Puntaric, D. Toxicity of surfactants to green microalgae *Pseudokirchneriella subcapitata* and *Scenedesmus subspicatus* and to marine diatoms *Phaeodactylum tricornutum* and *Skeletonema costatum*. *Chemosphere* **2005**, *61*, 1061–1068. [CrossRef]
86. Kautsky, H.; Hirsch, A. Neue Versuche zur Kohlenstoffassimilation. *Naturwissenschaften* **1931**, *19*, 964. [CrossRef]
87. Krause, G.H.; Weis, E. Chlorophyll fluorescence as a tool in plant physiology II: Interpretation of fluorescence signal. *Photosynth. Res.* **1984**, *5*, 139–157. [CrossRef]
88. Barócsi, A.; Kocsányi, L.; Várkonyi, S.; Richter, P.; Csintalan, Z.; Szenté, K. Two-wavelength, multipurpose, truly portable chlorophyll fluorometer and its application in field monitoring of phytoremediation. *Meas. Sci. Technol.* **2000**, *11*, 717–729. [CrossRef]
89. Lenk, S.; Gáboros, P.; Kocsányi, L.; Barócsi, A. Teaching laser-induced fluorescence of plant leaves. *Eur. J. Phys.* **2016**, *37*, 064003. [CrossRef]
90. Qiu, H.; Geng, J.; Ren, H.; Xia, X.; Wang, X.; Yu, Y. Physiological and biochemical responses of *Microcystis aeruginosa* to glyphosate and its Roundup® formulation. *J. Hazard. Mater.* **2013**, *248–249*, 172–176. [CrossRef]
91. Gomes, M.P.; Juneau, P. Temperature and light modulation of herbicide toxicity on algal and cyanobacterial physiology. *Front. Environ. Sci.* **2017**, *5*, 50. [CrossRef]
92. Cobb, A.H.; Reade, J.P.H. Harmful algal blooms. In *Herbicides and Plant Physiology*; Cobb, A.H., Reade, J.P.H., Eds.; Wiley-Blackwell: Oxford, UK, 2010; pp. 176–197.
93. Gomes, M.P.; Le Manac’h, S.G.; Hénault-Ethier, L.; Labrecque, M.; Lucotte, M.; Juneau, P. Glyphosate-dependent inhibition of photosynthesis in willow. *Front. Plant Sci.* **2017**, *8*, 207. [CrossRef] [PubMed]

94. Gomes, M.P.; Le Manac'h, S.G.; Maccario, S.; Labrecque, M.; Lucotte, M.; Juneau, P. Differential effects of glyphosate and aminomethylphosphonic acid (AMPA) on photosynthesis and chlorophyll metabolism in willow plants. *Pestic. Biochem. Physiol.* **2016**, *130*, 65–70. [CrossRef] [PubMed]
95. Choi, C.J.; Berges, J.A.; Young, E.B. Rapid effects of diverse toxic water pollutants on chlorophyll a fluorescence: Variable responses among freshwater microalgae. *Weed Res.* **2012**, *46*, 2615–2626. [CrossRef] [PubMed]
96. Smedbol, É.; Lucotte, M.; Labrecque, M.; Lepage, L.; Juneau, P. Phytoplankton growth and PSII efficiency sensitivity to a glyphosate-based herbicide (Factor 540®). *Aquat. Toxicol.* **2017**, *192*, 265–273. [CrossRef] [PubMed]
97. Monsanto Europe S.A. Material Safety Data Sheet of Roundup Classic. Monsanto Europe S.A./N.V.: Antwerp, Belgium, 2015.
98. Syngenta. *Material Safety Data Sheet of Medallon Premium*; Syngenta Magyarország Kft.: Budapest, Hungary, 2018.
99. Scandinavian Culture Collection of Algae and Protozoa: Media Recipes. Available online: <https://www.sccap.dk/media/> (accessed on 21 March 2024).
100. Allen, M.M. Simple conditions for growth of unicellular blue-green algae on plates. *J. Phycol.* **1968**, *4*, 1–4. [CrossRef] [PubMed]
101. ISO 8692:2012; Water Quality-Fresh Water Algal Growth Inhibition Test with Unicellular Green Algae. International Organization for Standardization: Geneva, Switzerland, 2012.
102. Organisation for Economic Co-operation and Development Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test. OECD Publishing, Paris. Available online: [https://read.oecd-ilibrary.org/environment/test-no-201-alga-growth-inhibition-test\\_9789264069923-en#page1](https://read.oecd-ilibrary.org/environment/test-no-201-alga-growth-inhibition-test_9789264069923-en#page1) (accessed on 21 March 2024).
103. Stevenson, R.J.; Lowe, R.L. Sampling and interpretation of algal patterns for water quality assessment. In *Rationale for Sampling and Interpretation of Ecological Data in the Assessment of Freshwater Ecosystems*; Isom, R.G., Ed.; American Society for Testing and Materials: Philadelphia, PA, USA, 1986; pp. 118–149.
104. McCormick, P.V.; Cairns, J. Algae as indicators of environmental change. *J. Appl. Phycol.* **1994**, *6*, 509–526. [CrossRef]
105. ISO 10260:1992; Water Quality-Measurement of Biochemical Parameters-Spectrometric Determination of the Chlorophyll-a Concentration. International Organization for Standardization: Geneva, Switzerland, 1992.
106. Lázár, D.; Takács, E.; Mörtl, M.; Klátyik, S.; Barócsi, A.; Kocsányi, L.; Lenk, S.; Domján, L.; Szarvas, G.; Lengyel, E.; et al. Application of a fluorescence-based instrument prototype for chlorophyll measurements and its utility in an herbicide algal ecotoxicity assay. *Water* **2023**, *15*, 1866. [CrossRef]
107. Barócsi, A.; Lenk, S.; Kocsányi, L.; Buschmann, C. Excitation kinetics during induction of chlorophyll a fluorescence. *Photosynthetica* **2009**, *47*, 104–111. [CrossRef]
108. Lichtenthaler, H.K.; Buschmann, C.; Knapp, M. How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio RFd of leaves with the PAM fluorometer. *Photosynthetica* **2005**, *43*, 379–393. [CrossRef]
109. Kalaji, H.M.; Schansker, G.; Brestic, M.; Bussotti, F.; Calatayud, A.; Ferroni, L.; Goltsev, V.; Guidi, L.; Jajoo, A.; Li, P.; et al. Frequently asked questions about chlorophyll fluorescence, the sequel. *Photosynth. Res.* **2017**, *132*, 13–66. [CrossRef] [PubMed]
110. Brain, P.; Cousens, R. An equation to describe dose responses where there is stimulation of growth at low doses. *Weed Res.* **1989**, *29*, 93–96. [CrossRef]
111. Ritz, C.; Baty, F.; Streibig, J.C.; Gerhard, D. Dose-response analysis using R. *PLoS ONE* **2015**, *10*, e0146021. [CrossRef] [PubMed]
112. Ritz, C.; Jensen, S.M.; Gerhard, D.; Streibig, J.C. A hormesis effect on lettuce growth. In *Dose-Response Analysis Using R*; Ritz, C., Jensen, S.M., Gerhard, D., Streibig, J.C., Eds.; CRC Press: Boca Raton, FL, USA, 2020; pp. 23–26.
113. Wong, P.K. Effect of 2,4-D, glyphosate and paraquat on growth, photosynthesis and chlorophyll-a synthesis of *Scenedesmus quadricauda* Berb 614. *Chemosphere* **2000**, *41*, 177–182. [CrossRef] [PubMed]
114. Schönherr, J. A mechanistic analysis of penetration of glyphosate salts across stomatous cuticular membranes. *Pest Manag. Sci.* **2022**, *58*, 343–351. [CrossRef] [PubMed]
115. Ewacha, M.V.A.; Goldsborough, L.G. The response of *Scenedesmus quadricauda* and *Selenastrum capricornutum* to glyphosate toxicity (Roundup® formulation) with cellular growth and chlorophyll-a synthesis as endpoints. *Proc. Manitoba's Undergrad. Sci. Eng. Res.* **2013**, *1*, 1–19.
116. Powell, H.A.; Kerby, N.W.; Rowell, P. Natural tolerance of cyanobacteria to the herbicide glyphosate. *New Phytol.* **1991**, *119*, 421–426. [CrossRef]
117. Sáenz, M.E.; Di Marzio, W. Ecotoxicity of herbicide glyphosate to four chlorophyceae freshwater algae. *Limnetica* **2009**, *28*, 149–158. [CrossRef]
118. LISEC Alga, growth inhibition test. Effect of MON 2139 on the growth of *Selenastrum capricornutum*. In *Monsanto Unpublished Study XX-89-093*; LISEC, Study Centre for Ecology and Forestry: Bokrijk, Belgium, 1989.
119. Cedergreen, N.; Streibig, J.C. The toxicity of herbicides to non-target aquatic plants and algae: Assessment of predictive factors and hazard. *Pest Manag. Sci.* **2005**, *61*, 1152–1160. [CrossRef]
120. Pereira, J.; Antunes, S.C.; Castro, B.B.; Marques, C.R.; Goncalves, A.M.M.; Goncalves, F.; Pereira, R. Toxicity evaluation of three pesticides on non-target aquatic and soil organisms: Commercial formulation versus active ingredient. *Ecotoxicology* **2009**, *18*, 455–463. [CrossRef]
121. Lipok, J.; Studnik, H.; Gruyaert, S. The toxicity of Roundup 360 SL formulation and its main constituents: Glyphosate and isopropylamine towards non-target water photoautotrophs. *Ecotoxicol. Environ. Saf.* **2010**, *73*, 1681–1688. [CrossRef]
122. Steber, J.; Guhl, W.; Stelter, N.; Schroder, F.R. Alkyl polyglycosides -ecological evaluation of a new generation of nonionic surfactants. *Tenside Surfactants Deterg.* **1995**, *32*, 515–521. [CrossRef]

123. Gomes, M.P.; Juneau, P. Oxidative stress in duckweed (*Lemna minor* L.) induced by glyphosate: Is the mitochondrial electron transport chain a target of this herbicide? *Environ. Pollut.* **2016**, *218*, 402–409. [\[CrossRef\]](#)
124. Rasch, A.; Hunsche, M.; Mail, M.; Burkhardt, J.; Noga, G.; Pariyar, S. Agricultural adjuvants may impair leaf transpiration and photosynthetic activity. *Plant Physiol. Biochem.* **2018**, *132*, 229–237. [\[CrossRef\]](#)
125. Guedes, R.N.C.; Cutler, G.C. Insecticide induced hormesis and arthropod pest management. *Pest Manag. Sci.* **2014**, *70*, 690–697. [\[CrossRef\]](#) [\[PubMed\]](#)
126. Mattson, M.P. Hormesis defined. *Ageing Res. Rev.* **2008**, *7*, 1–7. [\[CrossRef\]](#) [\[PubMed\]](#)
127. Calabrese, E.J.; Bachmann, K.A.; Bailer, A.J.; Bolger, P.M.; Borak, J.; Cai, L.; Cedergreen, N.; Cherian, M.G.; Chiueh, C.C.; Clarkson, T.W.; et al. Biological stress response terminology: Integrating the concepts of adaptive response and preconditioning stress within a hormetic dose-response framework. *Toxicol. Appl. Pharmacol.* **2007**, *222*, 122–128. [\[CrossRef\]](#)
128. Kendig, E.L.; Le, H.H.; Belcher, S.M. Defining hormesis: Evaluation of a complex concentration response phenomenon. *Int. J. Toxicol.* **2010**, *29*, 235–246. [\[CrossRef\]](#)
129. Bakonyi, G.; Szabó, B.; Seres, A. A hormézis, mint ökotoxikológiai jelenség, különös tekintettel a növényvédelemre. *bioKontroll* **2017**, *2*, 47–53. (In Hungarian)
130. Calabrese, E.J. Overcompensation stimulation: A mechanism for hormetic effects. *Critical Rev. Toxicol.* **2001**, *31*, 425–470. [\[CrossRef\]](#)
131. Jager, T.; Barsi, A.; Ducrot, V. Hormesis on life-history traits: Is there such thing as a free lunch? *Ecotoxicology* **2013**, *22*, 263–270. [\[CrossRef\]](#) [\[PubMed\]](#)
132. Forbes, V.E. Is hormesis an evolutionary expectation? *Functional Ecol.* **2000**, *14*, 12–24. [\[CrossRef\]](#)
133. Saxton, M.A.; Morrow, E.A.; Bourbonniere, R.A.; Wilhelm, S.W. Glyphosate influence on phytoplankton community structure in Lake Erie. *J. Great Lakes Res.* **2011**, *37*, 683–690. [\[CrossRef\]](#)
134. Vera, M.S.; Lagomarsino, L.; Sylvester, M.; Pérez, G.L.; Rodriguez, P.; Mugni, H.; Sinistro, R.; Ferraro, M.; Bonetto, C.; Zagares, H.; et al. New evidences of Roundup® (glyphosate formulation) impact on the periphyton community and the water quality of freshwater ecosystems. *Ecotoxicology* **2010**, *19*, 710–721. [\[CrossRef\]](#) [\[PubMed\]](#)
135. Jalal, A.; de Oliveira Junior, J.C.; Ribeiro, J.S.; Fernandes, G.C.; Mariano, G.G.; Trindade, V.D.R.; dos Reis, A.R. Hormesis in plants: Physiological and biochemical responses. *Ecotoxicol. Environ. Saf.* **2021**, *207*, 111225. [\[CrossRef\]](#)
136. Panizzi, S.; Suci, N.A.; Trevisan, M. Combined ecotoxicological risk assessment in the frame of European authorization of pesticides. *Sci. Total Environ.* **2017**, *580*, 136–146. [\[CrossRef\]](#)
137. Lozano, V.L.; Pizarro, H.N. Glyphosate lessons: Is biodegradation of pesticides a harmless process for biodiversity? *Environ. Sci. Eur.* **2024**, *36*, 55. [\[CrossRef\]](#)
138. Aparicio, V.C.; de Gerónimo, E.; Marino, D.; Primost, J.; Carriquiriborde, P.; Costa, J.L. Environmental fate of glyphosate and aminomethylphosphonic acid in surface waters and soil of agricultural basins. *Chemosphere* **2013**, *93*, 1866–1873. [\[CrossRef\]](#)
139. Brausch, J.M.; Smith, P.N. Toxicity of three POEA surfactant formulations to the fairy shrimp *Thamnocephalus platyurus*. *Arch. Environ. Contam. Toxicol.* **2007**, *52*, 217–222. [\[CrossRef\]](#)
140. Li, M.; Du, F.; Cao, C.; Li, B.; Zhai, X. Effect of glyphosate isopropylamine on the surface tension and surface dilational rheology properties of polyoxyethylene tallow amine surfactant. *J. Dispers. Sci. Technol.* **2016**, *37*, 213–221. [\[CrossRef\]](#)
141. Mesnage, R.; Antoniou, M.N. Ignoring adjuvant toxicity falsifies the safety profile of commercial pesticide. *Front. Public Health* **2018**, *5*, 361. [\[CrossRef\]](#) [\[PubMed\]](#)
142. Klátyik, S.; Takács, E.; Mörtl, M.; Földi, A.; Trábert, Z.; Ács, É.; Darvas, B.; Székács, A. Dissipation of the herbicide active ingredient glyphosate in natural water samples in the presence of biofilms. *Int. J. Environ. Anal. Chem.* **2017**, *97*, 901–921. [\[CrossRef\]](#)
143. European Commission. Commission regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. *OJ EU* **2013**, L93, 85–151. Available online: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32013R0284&qid=1707814054061> (accessed on 21 March 2024).
144. Seralini, G.-E. Pesticides in formulations: New revolutionary findings. *Toxics* **2024**, *12*, 151. [\[CrossRef\]](#) [\[PubMed\]](#)
145. Edwards, W.M.; Triplett, G.B.; Kramer, R.M. A watershed study of glyphosate transport in runoff. *J. Environ. Qual.* **1980**, *9*, 661–665. [\[CrossRef\]](#)
146. Servizi, J.A.; Gordon, R.W.; Martens, D.W. Acute toxicity of Garlon 4 and Roundup herbicides to salmon, Daphnia, and Trout. *Bull. Environ. Contam. Toxicol.* **1987**, *39*, 15–22. [\[CrossRef\]](#)
147. Mensink, H.; Janssen, P. Glyphosate. 1994. Available online: <http://www.inchem.org/documents/ehc/ehc/ehc159.htm> (accessed on 9 February 2024).

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