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Effect of Glyphosate on Growth of Four Freshwater Species of Phytoplankton: A Microplate Bioassay

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Abstract The acute toxicity of glyphosate herbicide was tested on the four species of freshwater phytoplankton, *Scenedesmus acutus*, *Scenedesmus subspicatus*, *Chlorella vulgaris* and *Chlorella saccharophila.* Herbicide concentrations eliciting a 50% growth reduction over 72 h (EC_{50}) ranged from 24.5 to 41.7 mg L^{-1}, whilst a 10% growth inhibition is achieved by herbicide concentrations ranging from 1.6 to 3.0 mg L^{-1}, difficult to find neither in paddy fields (it is not used in rice) nor in the lake of the Albufera Natural Park. *Chorella* species are less sensitive to the herbicide than *Scenedesmus* species. It can be concluded that glyphosate has a low potential risk for the tested organisms.

Keywords Algae · Herbicide · Natural park · Toxicity test

Valencia (Spain) is one of the most important rice areas in Europe. Rice crop in Valencia is mainly cultivated within the limits of a very rich flora and fauna area, the Albufera Natural Park (ANP), which includes a freshwater lake and takes over 14,000 ha representing 12% of the total Spanish rice cultivated area.

Glyphosate, the most applied herbicide throughout the world, is (obviously) not applied in rice, but it is massively sprayed in irrigation citrus orchards that surround the ANP. Glyphosate is rapidly adsorbed on soils, practically immobile in soils, volatilization does not occur and leaching is practically negligible, but disappearance through degradation is often slow with half-lives ranging from a few days to several months or years (Sprankle et al. 1975; Rueppel et al. 1977), so that toxicology must be evaluated. Due to its high soil adsorption coefficient (2,100 mL g^{-1}) (Durkin 2003), glyphosate has a very low potential risk of leaching and ground water contamination on valencian citrus orchards (De Paz and Rubio 2006) but excess water from citrus orchards, and eventually glyphosate residues, can travel to the lake of the ANP through the rice fields due to its high water solubility (12 g L^{-1}). Chen et al. (1985) could only find in a drainage irrigation channel close to a paddy rice field and only 1 h after glyphosate spraying, residues ranging from 0.08 to 1.5 mg L^{-1}. Another similar paddy rice area in Europe (Rhone river delta in France) was investigated by Comoretto et al. (2007), and no glyphosate residues were found.

On the first level of the lake trophic chain we can find microscopic algae which are the food for the next steps in the trophic chain. Wetland contamination could result in a die-off of most algal species present, causing a severe decline of this food source. Alternatively, certain species or groups of algae could be selectively inhibited (Gómez de Barreda Ferraz et al. 2004), and although research on the effect of glyphosate on microscopic algae has increased since Eijsackers (1985), noted that few reports were available on the effect of glyphosate on microscopic algae, none of them have been conducted in the ANP ecosystem. On the other hand, low glyphosate concentrations (0.02 mg L^{-1}) can stimulate algae growth (Wong 2000). Algae are known to be comparatively sensitive to many chemicals, and the inclusion of these organisms in test

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batteries has been shown to improve the capacity to predict the most sensitive ecosystem responses (Sloof et al. 1983). Furthermore, the importance of these organisms as dominant primary producers in most aquatic ecosystems speaks for their use in test batteries for environmental hazard assessment (Källqvist and Romstad 1994).

This paper describes the effects of the herbicide glyphosate on the growth of pure cultures of four species of phytoplankton, two wild species, representatives of mediterranean wetlands, isolated from Lake Albufera (Valencia, Spain) and two laboratory strains.

Materials and Methods

The chlorophyceae *Scenedesmus acutus* (Meyens), *Scenedesmus subspicatus* CCAP 276/22, *Chlorella vulgaris* Beijerinck and *Chlorella saccharophila* (Krüger) Migula were selected for the toxicity tests. *S. acutus* and *C. saccharophila* were isolated from samples collected at Albufera lake in Valencia (Spain). *S. subspicatus* and *C. vulgaris* were kindly supplied by the Institute of Freshwater Ecology (Ambleside, UK) and by the Area of Environmental Toxicology (CISA-INIA, Spain), respectively. These four chlorophyceae were grown in a medium recommended by the ASTM (1997). The stock cultures were maintained in a liquid medium at a temperature of 22 ± 2°C and a light intensity of 1,100 lux on a 12-h light-dark cycle.

Glyphosate [*N*-(phosphonomethyl) glycine; 97.5%, analytical standard] was obtained from "Dr. Ehrenstorfer Quality" (Augsburg, Alemania).

In the microplate bioassays (Aguayo et al. 2000) the organisms were exposed to various concentrations of glyphosate analytical standard (Nominal values are shown in Table 1) for 72 h. Growth of cultures was measured at 450 nm wavelength using a microplate reader (Multiskan EX; Labsystem) at 0, 24, 48 and 72 h. After 72 h, growth rates were quantitatively determined using the exponential model $y = e^{a+\mu x}$ (where y = the population estimate, x = time, a = integration constant and μ = slope or growth rate). ANOVA and Student Newman–Keuls multiple range test were employed to determine if treatments were significantly different from each other (Reish and Oshida 1987). Results were deemed significantly different at the level $p \leq 0.05$. EC_{10} (0–72 h) and EC_{50} (0–72 h) values with 95% confidence limits were estimated by the linear regression of probit of percentage growth on log dose of glyphosate (Newman 1995). In this study, EC_{10} and EC_{50} are the concentration of herbicides, derived by the method of calculation "comparison of areas under growth curves", which results in a 10% and a 50% growth reduction relative to the control values, at 72 h, respectively (ASTM 1997). All statistical analyses were performed using the commercial software packages, STATGRAPHICS (1994) (STSC Inc., Rockville, MD) and SAS (1998) (SAS Institute, Inc., Cary).

The toxicity tests were carried out on polystyrene microplates with 12 × 8 flat bottom wells of 400 µL capacity. The microplate bioassays were conducted with eight replicates of controls (culture medium and algae), eight replicates at each test concentration of herbicide (culture medium, algae and glyphosate) and eight blanks

Table 1 Average specific growth rates (μ) of the four species of phytoplankton treated with glyphosate

C. saccharophila			*C. vulgaris*			*S. acutus*			*S. subspicatus*		
mg L^{-1}	μ	R^2	mg L^{-1}	μ	R^2	mg L^{-1}	μ	R^2	mg L^{-1}	μ	R^2
0.0	0.0282	0.80	0.0	0.0195	0.90	0.0	0.0196	0.96	0	0.0194	0.85
0.1	0.0285	0.80	0.1	0.0181	0.85	0.1	0.0161	0.93	0.1[a]	0.0191	0.87
0.39[a]	0.0279	0.79	0.20	0.0171	0.87	0.39	0.0157	0.92	0.39	0.0176	0.85
1.56	0.0275	0.80	0.39	0.0163	0.86	0.78	0.0153	0.89	1.56	0.0168	0.85
6.3	0.0258	0.79	0.78	0.0163	0.87	1.56	0.0163	0.91	6.25	0.0171	0.92
12.5	0.0255	0.80	1.56	0.0166	0.87	3.13	0.0145	0.94	12.5	0.0164	0.94
25.0	0.0228	0.83	6.3	0.0165	0.88	6.25	0.0147	0.93	25	0.0166	0.97
50.0	0.0142	0.87	12.5	0.0165	0.83	12.5	0.0164	0.94	50[b]	–	–
100.0[b]	–	–	25.0	0.0163	0.88	25.0	0.0119	0.97			
			50.0	0.0130	0.94	50.0[b]	–	–			
			100.0 [b]	–	–						

Growth rates (μ) were determined using the exponential model, $y = e^{a+\mu x}$ (y = the population estimate, x = time, a = integration constant and μ = slope or growth rate)

R^2 Correlation coefficient

[a] Concentrations tested without significant effects ($p < 0.05$) on the algal growth relative to control values

[b] Lethal concentrations

(culture medium). Each bioassay was conducted three times.

Test cultures containing the appropiate concentrations of glyphosate and the neccesary quantity of algal inoculum are prepared by diluting with sterile algal medium aliquots of stock solutions of the glyphosate and of algal suspension. Each well on the plates was filled with 200 µL of the dilute solutions. The initial algal density of *Scenedesmus acutus* and *Scenedesmus subspicatus* was 5×10^5 cel mL^{-1} and of *Chlorella saccharophila* and *Chlorella vulgaris* was 2.5×10^6 cel mL^{-1}. The inoculum was obtained from a preculture which was incubated under test conditions and used when cells were exponentially growing.

The microplates were placed in a climatic chamber at 24 ± 2°C under continuous illumination provided by daylight lamps; ligth intensity was suitable for the optimal growth of algae (8,000 lux; ASTM 1997). During the test, microplates were shaken at 100 rpm. The microplates were shaken for 10 s before reading at 0, 24, 48 and 72 h. Each microplate was read twice.

Results and Discussion

The concentrations of technical glyphosate that caused significant effects ($p < 0.05$) on the algal growth with respect to control values ranged from 1.56 to 100.0 mg L^{-1} for *C. saccharophila*, from 0.1 to 100.0 mg L^{-1} for *C. vulgaris*, from 0.1 to 50.0 mg L^{-1} for *S. acutus* and from 0.39 to 50.0 mg L^{-1} for *S. subspicatus.* Growth reduction was particularly high from 25.0 mg L^{-1} glyphosate concentration on four algal species (Table 2).

Growth of the four species of phytoplankton under different exposures of glyphosate was compared starting from average specific growth rates values. In general, reduction in growth rates was observed with an increase of glyphosate concentrations, being *Scenedesmus* species more sensitive to the herbicide than *Chlorella* species. No inhibition of the growth of *C. saccharophila* was observed at 0.39 mg L^{-1} whereas the same concentration produced an inhibition of 14.4% for *C. vulgaris*. Glyphosate concentrations of 50 mg L^{-1} inhibited *C. saccharofila* and *C. vulgaris* growth in 81.3% and 60.3%, respectively, but were lethal for both *Scenedesmus* species. However, Christy et al. (1981), found total inhibition of *C. sarakiniana* with glyphosate concentrations equal or above 4 mg L^{-1}, whilst Hernando et al. (1989), found *C. pyreinodosa* much more resistant to glyphosate than *C. vulgaris* and *C. saccharophila* tested by the authors. The chemical effect on algal growth at 0.1 mg L^{-1} differs significantly from control for *S. acutus* and *Chlorella* species, although for *C. saccharophila*, statiscal difference is due to a more growth rate than the control culture. Sáenz et al. (1993) and other authors, reported a similar effect, growth stimulation on *Chlorella* and *Scenedesmus* species.

Based on their 72-h EC_{10}, EC_{50} and EC_{90} values (Table 3), the *Chlorella* and *Scenedesmus* species responded very differently to glyphosate. *S. acutus* and *S. subspicatus* both proved to be more sensitive than *C. saccharophila* and *C. vulgaris* to the herbicide. It can be clearly observed in Table 3 with the EC_{50} values, ranging from 36.7 to 46.6 mg L^{-1} in *Chlorella* species versus 21.9 to 28.9 mg L^{-1} in *Scenedesmus* species. No differences were observed within both algal genera. Sáenz et al. (1997), reported a lower EC_{50} (10.2 mg L^{-1}, at 96-h) for *S. acutus* than the observed in this experiment (24.5 mg L^{-1}, at 72-h).

The above mentioned sensitive differences between both genera were also reported by Carrasco and Sabater (1997); Sabater and Carrasco (1996, 1997, 1998); Sabater et al. (2002) and Gómez de Barreda Ferraz et al. (2004) in

Table 2 Multiple range test of analysis of variance of cellular concentration (cel mL^{-1}) at 72 h of glyphosate exposition

C. saccharophila		*C. vulgaris*		*S. acutus*		*S. subspicatus*	
mg L^{-1}	Homogeneous groups	mg L^{-1}	Homogeneous groups	mg L^{-1}	Homogeneous groups	mg L^{-1}	Homogeneous groups
0.0	b	0.0	a	0.0	a	0.0	a
0.1	a	0.1	bc	0.1	b	0.1	ab
0.39	bc	0.20	bc	0.39	cd	0.39	b
1.56	cd	0.39	b	0.78	c	1.56	bc
6.3	d	0.78	bc	1.56	de	6.25	bc
12.5	e	1.56	cd	3.13	e	12.5	cd
25.0	f	6.3	de	6.25	e	25	d
50.0	g	12.5	e	12.5	f	50	e
100.0	h	25.0	f	25.0	g		
		50.0	g	50.0	h		
		100.0	h				

Different letters in homogeneous groups denotes a statistically significant difference

Table 3 72 h EC_{10}, EC_{50} and EC_{90} values of glyphosate for four algal species, expressed in mg L^{-1}, with confidence limits at 95%

Test species	EC_{10}	EC_{50}	EC_{90}
C. saccharophila	3.0 (−1.14 to 6.54)	40.6 (36.7–45.2)	78.1 (70.8–87.0)
C. vulgaris	–[a]	41.7 (37.5–46.6)	86.9 (78.3–97.7)
S. acutus	–[a]	24.5 (21.9–27.7)	53.6 (47.9–61.0)
S. subspicatus	1.6 (−1.26 to 4.01)	26.0 (23.5–28.9)	50.3 (45.6–56.4)

Nominal glyphosate concentrations were used to calculate EC_{10}, EC_{50} and EC_{90} values

[a] EC_{10} values and their confidence limits at 95% were negative

several studies of molinate, thiobencarb, chlorsulfuron, bensulfuron–methyl, cinosulfuron, propanil and mefenacet effect on algal growth, ranging the EC_{50} values from 0.014 to 8.36 mg L^{-1} in *Scenedesmus* species and 0.43 to 104.0 mg L^{-1} in *Chlorella* species. It can be concluded that glyphosate is less toxic for the assayed algae than the above mentioned herbicides, most of them directly applied on rice fields. Kassai and Hatakeyama (1993), also reported more toxicity of simetryn, pretilachlor and thiobencarb on *Scenedesmus capricornutum* than on *Chlorella vulgaris*. Giesy et al. (2000) and Durkin (2003) stated that the relative sentitivities to algae and aquatic vascular plants is similar and Solomon and Thompson (2003) said that the aquatic macrophytes showed intermediate toxicity between the most and the least sensitive algae.

We can conclude by saying that glyphosate is not a dangerous herbicide for the ANP ecosystem due to its low algae toxicity at low glyphosate concentrations, demonstrated in this paper, as well as the low possibility of finding high concentration episodes (point-source contamination), as this has never been reported in Albufera lake.

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References

Aguayo S, Roset J, De la Torre AI, Cuellar ML, Muñoz MJ (2000) Ensayo de ecotoxicidad miniaturizado en *Chorella vulgaris* utilizando diferentes parámetros de expresión. Rev Toxicol 17:41–45

ASTM (1997) Standard guide for conducting static 96-h toxicity tests with microalgae, E 1218-97a

Carrasco JM, Sabater C (1997) Toxicity of atrazine and chlorsulfuron to algae. Toxicol Environ Chem 59:89–99. doi:10.1080/02772249709358427

Chen Y, Chiang H, Wu L, Wang Y (1985) Effects of glyphosate on germination and seedling growth of rice. J Weed Sci Tech 30:219–223

Christy SL, Karlander EP, Parochetti JV (1981) Effects of glyphosate on the growth rate of *Chlorella*. Weed Sci 29(1):5–7

Comoretto L, Arfib B, Chiron S (2007) Pesticides in the Rhone river delta (France): basic data for a field-based exposure assessment. Sci Total Environ 380:124–132. doi:10.1016/j.scitotenv.2006.11.046

De Paz JM, Rubio JL (2006) Application of a GIS-AF/RF model to asses the risk of herbicide leaching in a citrus-growing area of the Valencia Community, Spain. Sci Total Environ 371:44–54. doi:10.1016/j.scitotenv.2006.07.018

Durkin PR (2003) Glyphosate, Human health and ecological risk assessment. USDA forest service. http://www.fs.fed.us/foresthealth/pesticide/pdfs/04a03_glyphosate.pdf. Accessed 3 Sept 2008

Eijsackers H (1985) Effects of glyphosate on the soil fauna. In: Grossbard E, Atkinson D (eds) The herbicide glyphosate. Butterworths, London, p 151

Giesy JP, Dobson S, Solomon KR (2000) Ecotoxicological risk assessment for roundup herbicide. Rev Contam Toxicol 167:35–120

Gómez de Barreda Ferraz D, Sabater C, Carrasco JM (2004) Effects of propanil, tebufenozide and mefenacet on growth of tour freshwater species of phytoplankton: a microplate bioassay. Chemosphere 56:315–320. doi:10.1016/j.chemosphere.2004.01.038

Hernando F, Royuela M, Muñoz-Rueda A, Gonzalez Murua C (1989) Effect of Glyphosate on the greening process and photosynthetic in *Chlorella pyrenoidosa*. J Plant Physiol 134:26–31

Källqvist T, Romstad R (1994) Effects of agricultural pesticides on planktonic algae and cyanobacteria, examples of interspecies sensitivity variations. Nor J Agric Sci 13:117–131

Kassai F, Hatakeyama S (1993) Herbicide susceptibility in two green algae, *Chorella vulgaris* and *Selenastrum capricornutum*. Chemosphere 27:899–904. doi:10.1016/0045-6535(93)90019-2

Newman MC (1995) Quantitative methods in aquatic ecotoxicology. Lewis Publishers, London

Reish DL, Oshida P (1987) Manual of methods in aquatic environment research. Part 10, Short-Term static bioassays. FAO Fisheries Technical Paper 247. Food and Agriculture Organization of the United Nations

Rueppel ML, Brightwell BB, Schaefer J, Marvel JT (1977) Metabolism and degradation of glyphosate in soil and water. J Agric Food Chem 25:517–528. doi:10.1021/jf60211a018

Sabater C, Carrasco JM (1996) Effects of thiobencarb on the growth of three species of phytoplankton. Bull Environ Contam Toxicol 56:977–981. doi:10.1007/s001289900141

Sabater C, Carrasco JM (1997) Effects of chlorsulfuron on growth of three freshwater species of phytoplankton. Bull Environ Contam Toxicol 58:807–813. doi:10.1007/s001289900406

Sabater C, Carrasco JM (1998) Effects of molinate on growth of five freshwater species of phytoplankton. Bull Environ Contam Toxicol 61:534–540. doi:10.1007/s001289900795

Sabater C, Cuesta A, Carrasco R (2002) Effects of bensulfuron–methyl and cinosulfuron on growth of four freshwater species of phytoplankton. Chemosphere 46:953–960. doi:10.1016/S0045-6535(01)00179-5

Sáenz ME, Accorinti J, Tortorelli MC (1993) Toxicity of paraquat to a green alga, *Scenedesmus acutus*. J Environ Sci Health B 28:193–240. doi:10.1080/03601239309372822

Sáenz ME, Alberdi JL, Dimarzio WD, Accorinti J, Tortorelli MC (1997) Paraquat toxicity to different green algae. Bull Environ Contam Toxicol 58:922–928

SAS (1988) SAS/STAT User's Guide: statistics. Version 5. SAS Institute Inc, NC

Sloof W, Canton JH, Hermens JLM (1983) Comparison of the susceptibility of 22 freshwater species to 15 chemical compounds. I. (Semi) acute toxicity tests. Aquat Toxicol 4:113–128. doi:10.1016/0166-445X(83)90049-8

Solomon KR, Thompson DG (2003) Ecological risk assessment for aquatic organisms from over-water uses of glyphosate. J Toxicol Environ Health B 6:289–324. doi:10.1080/10937400306468

Sprankle P, Meggitt WF, Penner D (1975) Adsorption, mobility and microbial degradation of glyphosate in the soil. Weed Sci 23:229–234

STATGRAPHICS (1994) Statistical Corp. Version 2.1. STSC Inc., Md

Torstensson L (1985) Behaviour of glyphosate in soils and its degradation. In: Grossbard E, Atkinson D (eds) The herbicide glyphosate. Butterworths, London, p 137

Wong P (2000) Effects of 2, 4-D, glyphosate and paraquat on growth, photosynthesis and chlorophyll—a synthesis of *Scenedesmus quadricauda*. Chemosphere 41:177–182. doi:10.1016/S0045-6535(99)00408-7