J Appl Phycol
DOI 10.1007/s10811-015-0755-6

Water polluted with glyphosate formulations: effectiveness of a decontamination process using *Chlorella vulgaris* growing as bioindicator

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Received: 28 July 2015 / Revised and accepted: 3 November 2015

Abstract The environmental pollution caused by pesticides is considered a major problem worldwide. Glyphosate is one of the herbicides most widely used, and its use has increased sharply in the last years. In this work, the toxicity of four commercial glyphosate formulations (Eskoba®, Panzer Gold®, Roundup Ultramax® and Sulfosato Touchdown®) was assessed by determining the median effective concentration at 96 h (96 h-EC_{50}) using the microalga *Chlorella vulgaris* as the biological model. Although the formulations tested are moderately to slightly toxic to *C. vulgaris* according to the World Health Organization's toxicity categories for aquatic and terrestrial organisms, this research shows that the four formulations are toxic, with Eskoba® the least toxic and Roundup Ultramax® the most toxic one. A UV/H_2O_2 remediation process for the detoxification of the samples was tested also. Its effectiveness was evaluated using a *C. vulgaris* growth inhibition test. Growth inhibition of *C. vulgaris* did not reach 18.2 %, indicating the efficacy of the UV/H_2O_2 remediation process to reduce glyphosate toxicity. In some of the samples tested within the first 48 h of the assay, *C. vulgaris* growth was even increased. The results of the present work suggest that the selected species was a good indicator to determine the toxicity level of glyphosate formulations and shows the relevance of the ecotoxicological tests to evaluate a physicochemical remediation process.

Keywords Glyphosate formulations · UV/H_2O_2 process · Ecotoxicity · *Chlorella vulgaris* · Bioindicator

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Introduction

The toxicity of agrochemicals is permanently under global debate. The environmental pollution caused by pesticides is considered a major problem worldwide. In particular, glyphosate residues are a serious hazard, as these substances can reach the aquatic environment by drifts, surface runoff, drainage and leaching, which increases significantly the risks to non-target organisms. Recent studies have shown that glyphosate can affect phytoplankton and aquatic organisms. Vera et al. (2010) reported adverse effects of glyphosate on periphyton and phytoplankton communities. Other studies have shown the effects of glyphosate on zooplankton species such as *Daphnia magna* and *Daphnia spinulata* (Domínguez-Cortinas et al. 2008; Vendrell et al. 2009), and although the herbicide glyphosate and the insecticides malathion and diazinon were recently classified as *probably carcinogenic to humans* (Group 2A) by the WHO (2015), glyphosate (*N*-phosphonomethyl glycine) is still one of the herbicides most widely used worldwide as it is broad spectrum, low cost and non-selective.

In the last decades, the implementation of genetically modified (GM) glyphosate resistant (GR) cultivars has contributed to increase the agricultural use of glyphosate. The countries with the highest soybean production are the highest consumers of glyphosate. USA, Brazil and Argentina are the major soybeans producers, according to 2013 statistics, and

Published online: 08 January 2016

commercialise 89, 81 and 49 million t of soybeans, respectively (FAOSTAT 2013). As one of the top soybean producers, the use of glyphosate in Argentina has leaped from 1 million litres in 1991 to 180 million in 2007 (Binimelis et al. 2009). In commercial glyphosate-based formulations, only the active ingredient is specified, i.e. glyphosate salt, but the formulations also contain surfactants as adjuvant or additives to facilitate the mobility of glyphosate through the waxy cuticle (WHO 1994). Although these ingredients are usually classified as "inert", it is well documented that some of them can be even more toxic than glyphosate (Székács et al. 2014).

Tsui and Chu (2003) examined the acute toxicity of technical-grade glyphosate acid, isopropylamine (IPA) salt of glyphosate, Roundup® and its surfactant polyoxyethylene amine (POEA) to a bacterium (*Vibrio fischeri*), microalgae (*Selenastrum capricornutum* and *Skeletonema costatum*), protozoa (*Tetrahymena pyriformis* and *Euplotes vannus*) and crustaceans (*Ceriodaphnia dubia* and *Acartia tonsa*). The authors found that toxicity of Roundup® to aquatic organisms could be attributed to both the IPA salt of glyphosate and its surfactant POEA, and this depended on the group of organisms considered.

Ashoka Deepananda et al. (2011) investigated the acute toxicity of a glyphosate herbicide Roundup® in two species of the most common freshwater crustaceans: the calanoid copepod (*Phyllodiaptomus annae*) and decapod shrimp (*Caridina nilotica*). The study revealed that Roundup® may cause a significant impact on native non-target organisms.

Sihtmae et al. (2013)) have studied two glyphosate-based herbicides, Roundup Max™ (containing surfactant POEA) and Roundup Quick™ (without POEA) on non-target species. The ecotoxicity testing was conducted with (i) two aquatic organisms, crustaceans *Daphnia magna* and marine bacteria *Vibrio fischeri*, (ii) five bacterial strains (*Escherichia coli* MG1655, *Pseudomonas putida* KT2440 and three bacterial isolates from the soil) and (iii) terrestrial plants *Raphanus sativus* and *Hordeum vulgare*. Roundup Quick™ (without POEA) was more toxic to the aquatic bacterium *V. fischeri* but less toxic to soil bacterial strains and terrestrial plants than Roundup Max™ containing POEA. Thus, the difference in toxicity of the two glyphosate products studied (Roundup Max™ and Roundup Quick™) depended not only on POEA addition but also on other additives used in the specific formulation. Demetrio et al. (2012) using the *Hydra attenuata* toxicity test reported that glyphosate formulation exhibited higher toxicity at low concentrations (LC_{1-10}) with respect to active ingredient, reversing this behaviour at higher concentrations (LC_{50-90}). In this context, toxicity assays using the glyphosate formulations are more appropriate from the environmental point of view because the pesticides in the cropland are used in this way.

The advanced oxidation processes (AOPs) could be an option to reduce the concentration of glyphosate to acceptable limits. AOPs usually operate at or near ambient temperature and pressure, and a highly reactive radical, such as hydroxyl radical, is used as the primary oxidant. Several papers have shown the efficacy of these processes in degrading glyphosate (Chen et al. 2007; Echavia et al. 2009). Recently, Vidal et al. (2015) showed that the combination of hydrogen peroxide and UV radiation may become a suitable and very convenient process for treating wastewater originating from glyphosate commercial formulations as equipment and container rinsates. The UV/H_2O_2 method has also some advantages regarding equipment and process, as hydrogen peroxide is a relatively inexpensive chemical reagent and the operative process is very simple. Unfortunately, the partial oxidation of organic pollutants may produce intermediates more toxic than the parent compounds, e.g. the main product of hydrolysis de 2,4-D (2,4-dichlorophenol) has been described as more toxic than the parent compound (Drzewicz et al. 2004) or the generation of chlorpyrifos-oxon, a more toxic intermediate compound produced during the oxidation process of chlorpyrifos (Bavcon Kralj et al. 2007).

Ecotoxicity tests are a useful tool to assess the decontamination by AOPs and to determine the end point for treated wastewater. In addition to this, biological assays might reduce operating costs because they could indicate that the total mineralization of the pollutant is not necessary. Several toxicity tests have been used to evaluate whether effluent detoxification is effective (Rizzo 2011; Klamerth et al. 2010). The assessment of algae sensitivity to herbicides is very important, as algae are the bases of many aquatic food webs and are the primary producers in aquatic ecosystems. Therefore, toxicity tests based on these organisms have also been developed considering their ubiquity and short life cycle (Rizzo 2011). On the other hand, algae species vary widely in their response to pesticide formulations: for example, it was found that *Chlorella pyrenoidosa* was more sensitive than *Scenedesmus obliquus* to 26 herbicides.

Vibrio fischeri and *D. magna* have been the most commonly used organisms to evaluate toxicity in pesticide solutions treated with AOPs, while the green microalga *Chlorella vulgaris* has been the least used (Essam et al. 2007; De la Cruz et al. 2013).

Taking into account the importance of developing cleaner technologies to minimise the environmental pollution in aquatic ecosystems, the aims of the present study were two: (1) to determine the effective concentration 50 at 96 h (96 h-EC_{50}) for the four formulations, considering *C. vulgaris* as the biological model, (2) to assess the remaining toxicity of the samples contaminated with glyphosate formulations after UV/H_2O_2 process and using *C. vulgaris* as the test organism. Four of the commercial glyphosate formulations most widely used in Argentina were selected for this study.

Materials and methods

The strain of *Chlorella vulgaris* (CLV2 strain) was provided by the Center for Scientific Research and Higher Education

from Ensenada, Baja California, Mexico (CICESE), and was cultured in 2000-mL Erlenmeyer flasks, under sterile conditions, using Bold's basal medium (BBM) (Bischoff and Bold 1963). The pH and conductivity of the medium were 6.5 and 1.4 mS cm^{-1}, respectively. BBM is a widely used medium for the production of biomass and ecotoxicological assays (Reno et al. 2014; Al-Shatri, et al. 2014). The culture was kept under constant conditions: aeration, agitation to 100 rpm, temperature (23 ± 1 °C), and uniform illumination (approximately 120 μmol photons m^{-2} s^{-1}).

The formulations selected—Eskoba® (Red Surcos), Panzer Gold® (Dow Agrosciences), Roundup Ultramax® (Monsanto) and Sulfosato Touchdown® (Syngenta Agro)—are among the most widely used in the eco-regions with the largest soybean production in Argentina (the pampas and Litoral region, CONICET 2009; UNL 2010), as well as in other large-scale soybean producing countries. The commercial formulations mentioned above contain the following: 48 % (*w*/*v*) isopropylamine salt, 60.8 % (*w*/*v*) dimethylamine salt, 74.7 % monoammonium salt and 62 % (*w*/*v*) potassium salt, respectively (CASAFE 2011). In these formulations, the glyphosate acid is converted to a salt in order to increase water solubility, and for this reason, glyphosate concentrations are reported as acid equivalents (a.e.) per litre (Lanctôt et al. 2014).

Algal growth inhibition test

The experimental treatments to assess the growth inhibition for *C. vulgaris* exposed to the four glyphosate formulations were conducted according to the US EPA standard protocol (US EPA 2002). Erlenmeyer flasks (250 mL) with 100 mL of BBM medium were used for the tests. Microalgae were collected at exponential growth phase, centrifuged, and finally resuspended in ultrapure, sterile water. The inoculum density for both treatment and control was 10000 cells mL^{-1}. The concentration of the algae was quantified by cell counting with a Neubauer chamber (ISO 8692 1989). Three replicates for the treatments and the control were produced for each assay (96 h), all maintained under constant illumination (approximately 120 μmol photons m^{-2} s^{-1}) and temperature (25 ± 1 °C). *C. vulgaris* in BBM culture medium without the addition of glyphosate-based formulations was the negative control.

The glyphosate concentrations used in this work were determined according to the product label information and following the works of authors who tested the toxicity of different glyphosate formulations (Wong 2000; Tsui and Chu 2003, 2004; Sáenz and Di Marzio 2009; Mensah et al. 2013; Regaldo 2013; Reno et al. 2014.

Three samples of 100 μL were collected at 24, 48, 72 and 96 h from each treatment and control; in each sample, the cell density was determined with a Neubauer chamber. For all of the cases, at least 25 squares were counted so as to ensure errors lower than 10 % (Venrick 1978).

For each assay, effective concentration at 96 h (96 h-EC_{50}) was determined by linear interpolation (US EPA 2002), and the inhibition percentage (*I*%) was assessed according to Eq. 1:

$$I\% = \frac{(\mu_c - \mu_i)}{\mu_c} \times 100 \quad (1)$$

where *I*% is the percent inhibition of the growth rate, μ_c is the mean value of the growth rate (μ) in the control group and μ_i is the mean value of the growth rate in the treatments (OECD 2011).

The growth rate in equation (1) was assessed using Eq. 2:

$$\mu_{i-j} = \frac{(InX_j - InX_i)}{t_j - t_i} \left(day^{-1}\right) \quad (2)$$

where μ_{i-j} is the growth rate from time *i* to *j*, X_i is the biomass at time *i* and X_j is the biomass at time *j* (OECD 2011).

The glyphosate concentrations tested were the following: 6.25, 12.5, 25, 50 and 100 mg L^{-1} a.e. (Eskoba®); 1.5, 3.12, 6.25, 12.5, 25, 50, 100 and 200 mg L^{-1} a.e. (Panzer Gold®); and 0.75, 1.5, 3.12, 6.25, 12.5, 25 and 50 mg L^{-1} a.e. (Roundup Ultramax® and Sulfosato Touchdown®). pH was measured at the beginning and at the end of each test.

Photoreactor: degradation of samples polluted with glyphosate

The glyphosate-based formulations were degraded in an annular reactor ($V_{reactor}$ = 870 cm^3) which contains an internal quartz tube that allows UV radiation to pass from a concentrically positioned germicidal lamp (Philips 125 TUV 15 W, low pressure Hg vapour lamp with peak emission at λ = 253.7 nm). This reactor operates in a recirculation batch system, which includes a centrifugal pump and a feed tank with continuous stirring. The total volume of the system is 2500 cm^3. Constant temperature (*T* = 20 °C) is maintained by a heat exchanger. A more detailed description of the reactor and its operation conditions can be found in Junges et al. (2013) and in Reno et al. (2015).

For the degradation assays of the four formulations tested, the initial concentration of glyphosate was 50 mg L^{-1} a.e., expressed as milligram of acid equivalents per litre (mg L^{-1} a.e); the initial concentration of H_2O_2 was 120 mg L^{-1} (30 % *w*/*v*, Ciccarelli p.a.). The total reaction time was 360 min, the operating temperature was 20 °C and the initial pH was 5.2.

For the algal growth inhibition test of the four glyphosate-base formulations, the samples tested were the following: sample M_0: untreated, corresponding to 50 mg L^{-1} a.e. of glyphosate and without H_2O_2 added; and samples M_1, M_2 and M_3, collected at different reaction times of the UV/H_2O_2

process: 120, 240 and 360 min, respectively, with the removal of the remaining H_2O_2. Bovine catalase (2197 U mg^{-1}; Fluka) (1 U decomposes 1 μmol H_2O_2 min^{-1} at pH 7.0 at 25 °C) was used to degrade the remaining H_2O_2 from the samples.

For each of the samples obtained during the process, pH was measured and salts were dissolved in the culture medium BBM. The pH in the samples (M_0 to M_3) ranged between 5.62 and 7.20 at the beginning and between 5.56 and 7.30 at the end of the test. The test was performed for 96 h and following the same methodological design as the tests previously described in relation to number of replicates, methodology for the determination of cells density, temperature, illumination, volume of the inoculum and volume of the culture medium used for each test and control.

For each sample (M_0 to M_3), the inhibition growth (I%) was determined as in the expression (1). To evaluate possible significant differences between the control and the treatments at 24, 48, 72 and 96 h (values log10 (x) transformed), ANOVA (RM) was performed ($\alpha = 0.05$) with Dunnett post-test. Pearson product–moment correlation tests were conducted by plotting cell density (cell mL^{-1}) at the different reaction times assessed (0, 120, 240, and 360 min) at 96 h.

Stock solutions of glyphosate used for the algal growth inhibition tests (1067.5, 1091, 914, 993 mg L^{-1} a.e. for Eskoba®, Sulfosato Touchdown®, Panzer Gold® and Roundup Ultramax®, respectively) and glyphosate concentrations in the samples (M_0 to M_3) collected at different reaction times of the UV/H_2O_2 process were analysed using ion exchange chromatography with conductivity detection in an analytical Ion Pac-SC AS2A-SC column (2250 mm) protected by an Ion Pac AG2A-SC guard column (250 mm). The eluent used was a mixture of 7.2 mM Na_2CO_3 and 3.2 mM NaOH at 0.6 mL min^{-1}. Glyphosate acid (AccuStandard cat. No. P-015NB-250) was used as a chromatography standard for calibration.

Results

Algal growth inhibition test to assess the toxicity of four glyphosate formulations

Table 1 shows the values of 96 h-EC_{50} obtained for the four glyphosate-based formulations tested. As can be observed, the commercial formulation Eskoba® was the least toxic and Roundup Ultramax® the most toxic. According to the World Health Organization (WHO), the toxicity categories of glyphosate formulations to aquatic and terrestrial organisms are moderate to slightly toxic for *C. vulgaris* (Schaaf 2013).

For all the formulations, cell density was higher in the lower concentrations than in the control, with values ranging from 0.9 to 33.7 %, depending on the concentrations tested. In five of the seven concentrations with Sulfosato Touchdown®, cell density was higher between 24 and 48 h, with a peak of 33.7 % at 48 h, compared to the control. This would indicate that when the toxic concentration is low, the microalgae could use some elements of the glyphosate-based formulation to grow (Fig. 1).

C. vulgaris growth inhibition test to evaluate the efficacy of the UV/H_2O_2 process

Figure 2 shows the increase of cell density as glyphosate concentration decrease during the treatment in the different samples (M_0 to M_3). The correlations between cell density (cells mL^{-1}) and glyphosate concentration of the samples collected at different times of the process (M_0, M_1, M_2, M_3) were high and significant for all the formulations after the 96-h test ($p < 0.05$). The cell densities corresponding to the control values were 32800 (±692), 40800 (±2052), 79066 (±1006) and 105000 (±4358) cells mL^{-1} at 24, 48, 72 and 96 h, respectively.

In all the formulations tested, M_0 showed significant differences in cell density in relation to the control 96-h test (ANOVA with Tukey-Kramer post-test, $p < 0.01$). The Sulfosato Touchdown® and Roundup Ultramax® showed significant differences in M_3 after 96-h assay ($p < 0.05$), inhibition of growth failed to reach 50 % (15.3 and 18.2, respectively, Table 2).

In Eskoba® assays at 24 h, the cell density in M_2 and M_3 was higher than the cell density in the control (25.7 and 34 %, respectively) and at 48 h (15 and 27 %, respectively, Table 2). In Panzer Gold® assays, the cell density in M_2 and M_3 exceeded the control in 1.12 and 9.9 %, respectively at 24 h and in 1.93 and 20.4 %, respectively at 48 h (Table 2).

In Sulfosato Touchdown® assays, treated with the UV/H_2O_2 process, the number of cells per millilitre was 3.6 and 19.8 % higher in M_3 than in the control at 24 and 48 h,

Table 1 Algal growth inhibition test

Glyphosate-based commercial formulation	96 h-EC_{50} (mg L^{-1} a.e.)	Confidence interval (95 %)
Eskoba®	29.95	25.11–36.06
Panzer Gold®	8.64	6.44–11.05
Sulfosato Touchdown®	7.37	6.08–8.87
Roundup Ultramax®	3.85	2.82–5.08

96 h-EC_{50} and confidence intervals (95 %) of the glyphosate-based formulations tested

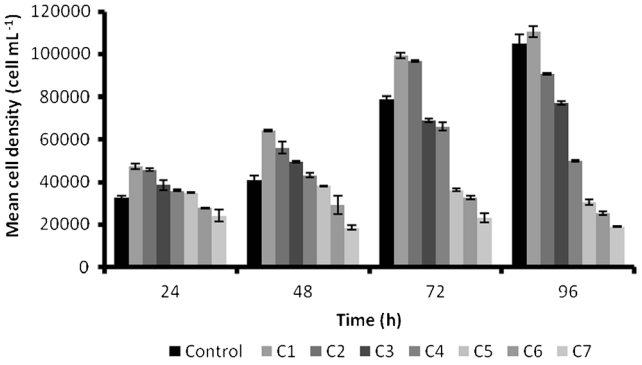

Fig. 1 Variations in cell density (cells mL^{-1}) during the algal growth inhibition test to assess the toxicity of the glyphosate formulation Sulfosato Touchdown®. *Columns* show the mean ($n=3$) cell density (cell mL^{-1}) for each concentration of glyphosate evaluated (mg L^{-1} a.e.). Control (without glyphosate): 0.75 (C1), 1.5 (C2), 3.12 (C3), 6.25 (C4), 12.5 (C5), 25 (C6), and 50 mg L^{-1} (C7) a.e.. *Error bars* represent standard error

respectively (Table 2). ANOVA with Tukey-Kramer post-test showed that at 96 h, there were no significant differences between M_1 and M_2 ($p>0.05$). The difference in glyphosate concentration between the samples ($M_1=25$ mg L^{-1} a.e. and $M_2=9$ mg L^{-1} a.e.) could be due to reaction intermediates which would increase toxicity in M_2. In Roundup Ultramax® assays, the growth of *C. vulgaris* was inhibited in all the samples during the test, except at 48 h, when M_3 exceeded the control by 6 % (Table 2).

After comparing the *I*% at 96 h (Table 3) observed in M_3 for the four glyphosate formulations exposed to the UV/H_2O_2 process with the *I*% observed for the most similar concentrations of untreated commercial formulation, it can be observed that for three formulations (Eskoba®, Panzer Gold® and Ultramax®) the *I*% was higher for the untreated formulations. This would indicate that unknown ingredients of the commercial formulations may be also toxic. The different behaviour corresponding to sample M_3 (Sulfosato Touchdown®) could be attributed to the kind of salt of glyphosate composing the commercial formulation or other "unknown compounds" that are not specified by the provider. These compounds and/or their oxidation by-products generated after treatment could affect the toxicity of the samples. This would indicate that the unknown ingredients of the commercial formulations may be also toxic.

Discussion

This work evaluated the toxicity of four glyphosate-based formulations, considering a non-target species, the microalga *C. vulgaris*, as biological model. When the toxicity of herbicides is tested, it is common to focus on the toxicity of the active ingredient rather than on the toxicity of the glyphosate formulations. However, different studies have found that glyphosate formulations are more toxic than the active ingredients (Lajmanovich et al. 2011; Piola et al. 2013). The formulations tested in this work are the most used by soybean producing countries on a large scale, with Roundup Ultramax® the most toxic one.

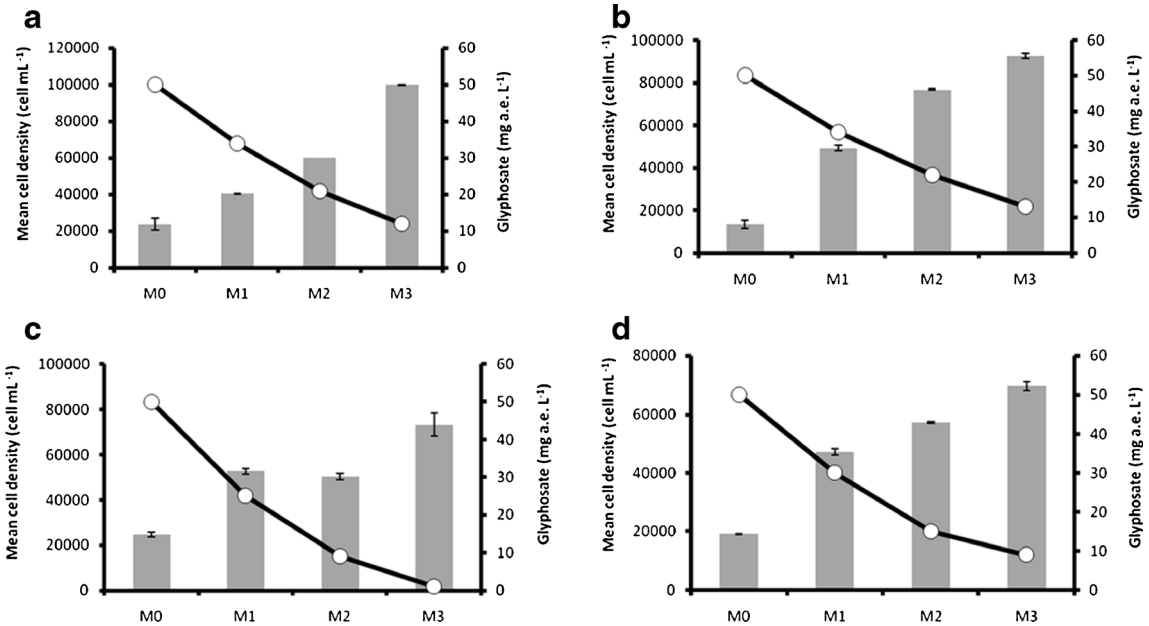

Fig. 2 Variations in cell density (cell mL^{-1}) at 96 h regarding glyphosate evolution for the different reaction times (M0 to M3, UV/H_2O_2 process). Sample M0 corresponding to 50 mg L^{-1} a.e. of glyphosate without H_2O_2; M1, M2 and M3: 120, 240 and 360 min, respectively, with the removal of the remaining H_2O_2. **a** Eskoba®, **b** Panzer Gold®, **c** Sulfosato Touchdown®, and **d** Roundup Ultramax®. Cell density (cell mL^{-1}) (*columns*). Glyphosate concentration (*white circles*). *Error bars* represent standard error

Table 2 Inhibition percentage (*I*%) of the different glyphosate-based formulations after its treatments with the UV/H_2O_2 process at different times (M_0 to M_3) (24, 48, 72, and 96 h: times of samples collection in algae growth inhibition test)

Eskoba®	M_0	M_1	M_2	M_3
24 h	39.8	4.5	25.7[a]	34[a]
48 h	47.1	1.3	15[a]	27[a]
72 h	49.2	30.6	13.6	6.8
96 h	63.9	41.6	24.5	2.2
Panzer Gold®				
24 h	99.7	17.1	1.12[a]	9.9[a]
48 h	86.5	18.4	1.93[a]	20.4[a]
72 h	71.6	20.4	19.9	15.5
96 h	88.7	31.9	13.6	5.08
Sulfosato Touchdown®				
24 h	25.7	39.5	0.8	3.6[a]
48 h	55.4	35.6	15.7	19.8[a]
72 h	59.9	28.2	21.8	8.2
96 h	60.8	28.8	30	15.3
Roundup Ultramax®				
24 h	90.4	23	38.3	5.4
48 h	84.4	20.1	17.4	6[a]
72 h	80.4	34.1	28.2	13.1
96 h	72.3	33.4	28	18.2

[a] >% of cells per millilitre in the sample M_x versus the control

After comparing the sensitivity of *C. vulgaris* with other Chlorophyceae exposed to commercial glyphosate-based formulations, it was observed that the 96 h-EC_{50} obtained in this work were lower than the EC_{50} observed by other researchers. Tsui and Chu (2003) obtained values of EC_{50} of 5.81 mg L^{-1} for *Selenastrum capricornutum* exposed to Roundup®. In this work, a lower EC_{50} was found for Ultramax® (3.85 mg L^{-1}), whereas Romero et al. (2011) found an EC_{50} of 55.62 mg L^{-1} after exposing *Chlorella kessleri* to an Atanor® formulation for 96 h. This value is higher than the EC_{50} found for the least toxic formulation of this work (Eskoba® 96 h-EC_{50} 29.95 mg L^{-1}). However, the studies carried out by Sáenz and Di Marzio (2009) showed even higher values of EC_{50} for different species of microalgae exposed to Roundup® for 96 h (120 to 154 mg L^{-1}).

The results show that the sensitivity of the microalgae to glyphosate depends on the species and that it is highly dependent on the chemical composition of the formulations tested. However, this information is not available on the label of the commercial products, with the resulting uncertainty about the potential toxicity for the non-target species (Cox 2004). In this regard, Puglis and Boone (2011) claimed that there should be more access to the information about all the ingredients used in pesticide formulations. Far from being just a formal neglect, this situation makes it difficult to standardise the correct levels for the protection of the aquatic biota (Reno et al. 2015).

After analysing the security labels of the formulations tested, it was observed that none of them communicated the value of 96 h-EC_{50} for microalgae within the potentially affected non-target species. However, many authors have claimed that phytoplankton might be the most promising early-alert indicator of changes in the ecological characteristics of wetlands caused by chemicals (Durrieu et al. 2003; Luna and Carmenate 2004). In a recent work, Mensah et al. (2013), after exposing different organisms (*Baetis harrisoni*, *Caridina nilotica*, *Chlorella protothecoides*, *Chlorella sorokiniana*, *Daphnia pulex*, *Oreochromismos sambicus* and *Tanytarsus flumineus*) to Roundup®, found that the microalgae *Chlorella protothecoides* and *C. sorokiniana* were the most sensitive species to that herbicide.

As for the assessment of UV/H_2O_2 process, for all the samples obtained at the end of the process (360 min., M_3), the *I*% of *C. vulgaris* did not reach 18.2 % in any of the

Table 3 Comparison between Inhibition percentage (*I*%) in the sample M_3 for the four glyphosate formulations exposed to UV/H_2O_2 process, with the *I*% observed for the most similar concentrations of untreated commercial formulations and in the closest concentrations used in the inhibition tests performed with the untreated formulations

Glyphosate-based commercial formulation	Glyphosate (mg L^{-1} a.e.)	*I*%
Eskoba®		
M_3	12.0	2.2
Untreated sample	12.5	14.2
Panzer Gold®		
M_3	13.0	5.08
Untreated sample	12.5	54.2
Sulfosato Touchdown®		
M_3	1.0	15.3
Untreated sample	1.5	6.2
Roundup Ultramax®		
M_3	9.0	20.0
Untreated sample	6.25	50.2

formulations tested, which would indicate the efficacy of the process to reduce the toxicity of glyphosate.

In addition, it is worth pointing out that for the four formulations tested, in M_2 and M_3 for Eskoba® and M_3 for Panzer Gold®, Sulfosato Touchdown® and Ultramax®, during the first 48 h of the test, cell density exceeded the control. It shows that with longer degradation times, more substances favouring *C. vulgaris* growth could appear. In this regard, Manassero et al. (2010), in a previous research on acid glyphosate degradation by UV/H_2O_2 process, managed to identify the following reaction intermediates: glycine, formic acid, formaldehyde, ammonium, and as terminal products, ion nitrate and phosphate. These ions could be used as nutrients for the growth of *C. vulgaris*. Afterwards, once the additional nutrients produced by the glyphosate degradation run out, an inhibitory growth effect on the microalga started to be noticed.

Regarding the stimulus to the growth of *C. vulgaris* under low concentrations of glyphosate and the M_2 or M_3 obtained in UV/H_2O_2 process observed in this work, it is worth mentioning that similar results were found by other authors such as Sáenz et al. (2012) and Regaldo (2013). Moreover, Wong (2000) and Zalizniak (2006), working with a commercial formulation of glyphosate with 35.6 % of active ingredient and Roundup Biactive®, found that at concentrations of 0.2 and 16 mg L^{-1}, respectively, the growth of *Scenedesmus quadricauda* and *Pseudokirchneriella subcapitata* was stimulated. The stimulation mentioned above could also result from the use of glyphosate as a source of carbon or nitrogen for growing, as proposed by Maršálek and Rojíčková (1996). In this regard, Qiu et al. (2013), showed that glyphosate is a suitable P source for the growth of *Microcystis aeruginosa* in the concentration of 1 mg L^{-1} P Roundup®. According to these studies, it would be important to consider the response of hormesis that generate this type of herbicides, as it could stimulate the growth of algae causing blooms.

When the 96 h-EC_{50} observed in the algal growth inhibition test with the untreated formulations were compared to the samples obtained from the UV/H_2O_2 process, in many cases, the *I*% did not correlate with the concentrations assayed. This result could be due to the unknown ingredients present in the commercial formulations.

This work contributes to the current debate on the sustainability of the grain production system on a large scale, based on the use of high volumes of glyphosate-based formulations.

In conclusion, the results presented in this study showed that the bioassay based on *C. vulgaris* is a good tool for evaluating the UV/H_2O_2 process to treat effluents with glyphosate from agricultural activities.

It was shown that the experimental conditions reached in all the samples treated with UV/H_2O_2 process in a reaction time of 360 min (M_3) are adequate to obtain decontaminated effluents when toxicity is assessed with *C. vulgaris*. Under the mentioned conditions, the maximum *I*% was 18.2, lower than the EC_{50} standard values. The result suggested a cell number stimulation when low-dose glyphosate concentration and high-dose inhibition (hormesis effect). The labels of the commercial products should provide information about all the ingredients of the pesticides and their effects on non-target organisms.

Acknowledgments This research was supported by grants from the University Universidad Nacional del Litoral, Projects CAI + D Orientado No.: 1.6 and CAI + D N°: 501 201101 00215 LI. We would like to thank to Margarita Herman for the English language editing.

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