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Microcrustaceans: biological models to evaluate a remediation process of glyphosate-based formulations

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Abstract Ecotoxicity studies using two glyphosate-based formulations (Eskoba® and Sulfosato Touchdown®) were undergone with three microcrustacean species to establish their LC_{50} values and to evaluate the efficacy of cleaning treatments with UV/H_2O_2.Samples were collected at the beginning of the process −50 mg acid equivalent per liter of glyphosate without H_2O_2 and at different treatment timepoints: 2, 4, and 6 h. Three microcrustacean species were used as biological models.The Eskoba® LC_{50} ranged between 14.49 and 95.23 acid equivalents (a.e.) mg $L^{-1}$and for Sulfosato Touchdown® between 0.31 and 1.74 a.e. mg L^{-1}. The glyphosate-based formulations registered the following order of sensitivities: *Ceriodaphnia dubia* > *Daphnia magna* > *Notodiaptomus conifer*. The treatment duration and mortality (%) were negative and significantly correlated for both formulations, indicating that the remediation process diminished the glyphosate concentration. Therefore, microcrustacean mortality decreased linearly with the remediation time. *C. dubia* and *N. conifer* were more sensitive than the holarctic *D. magna* to the remediation process, since the first two species showed greater percentage of mortality at 6 h of processes, compared with *D. magna*, for both formulations evaluated. Sulfosato Touchdown® was more toxic but showed greater degradability than Eskoba®. The results provide relevant information regarding (1) the urgency to clearly identify the additives on product labels, (2) the efficiency of UV/H_2O_2 process for reducing adverse effects of two glyphosate-based formulations, and (3) the importance of developing studies to evaluate the effectiveness of cleaner technologies with an emphasis on microcrustacean species as biological models.

Keywords UV/H_2O_2 remediation process · Glyphosate-based formulations · Microcrustaceans

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1 Introduction

Argentina is one of the three major soybean producers in the world according to current statistics, commercializing 49 million of tons per year (after USA and Brazil that produce 89 and 81 million tons, respectively) (FAOSTAT 2013).The genetically modified soy crops compose 60 % of the cultivated area all over the country (Junges et al. 2013). However, in the pampean region,

the changes in land use with the purpose of increase of this production began in the 1960s but were accelerated at the end of the 1970s. The most dramatic technological innovation occurred in 1996 with the introduction of genetically modified soybean tolerant to glyphosate and the elimination of soil tillage (no tillage). Pastures and annual forage crops were replaced by wheat–soybean relay cropping, maize, and sunflower crops. In this context, herbicides are one of the three pillars of the so-called "green revolution" the other two being new genetically modified (GM) seed varieties and high fertilizers inputs (Mc Laughlin and Mineau 1995).

Most herbicides contain the active ingredient glyphosate (*N*-phosphonomethyl) which is being aggressively used. Such use has significantly increased since 1971 (Dill et al. 2010) with 160 million liter glyphosate applications per year in 2004 (Altieri and Pengue 2006). They usually enter water bodies by washing containers, direct spray on rivers, lakes or streams, and by runoff after rainfall (Romero et al. 2011). The literature is extensive for glyphosate effects on aquatic biota (Demetrio et al. 2012; Cuhra et al. 2013; Gagneten et al. 2014). However, little is known on the active ingredient and adjuvant toxicities (Tsui and Chu 2003). While this issue has received global attention, research has focused on the effects from Roundup® (Tsui and Chu 2003, Tsui and Chu 2004; Raipulis et al. 2009; Dutra et al. 2011), but effects from the many new glyphosate-based formulations available have been poorly explored (Lajmanovich et al. 2011).

In Argentina, the use of transgenic cultivars of soy tolerant to glyphosate has been increasing from 1997 although it is been extensively demonstrated that the practice of applying glyphosate in late summer to increase forage supply during winter and spring has several negative consequences for biodiversity conservation, ecosystem functioning, and livestock management in the last semi-natural habitats in the Pampas grasslands (Rodríguez and Jacobo 2010).

Clean technologies, such as advanced oxidation processes (AOPs), can greatly reduce pollution. UV radiation combined with hydrogen peroxide has certain advantages over other AOPs; H_2O_2 is commercially available and simple to use. AOPs are based on generating highly oxidizing species, such as hydroxyl radicals (OH), which react with the pollutants and degrade them to harmless products, such as carbon dioxide, water, and mineral acids. In addition, non-selective technologies, such as oxidants, can degrade any type of chemical pollutant (MangatEchavia et al. 2009; Manassero et al. 2010). Vidal et al. (2015) proved that the combination of hydrogen peroxide and UV radiation may become a suitable and very simple process for treating wastewater originating from glyphosate commercial formulations.

However, full mineralization is not always possible at a reasonable price and time. Thus, the intermediate compounds generated during degradation might be equally or more toxic than the parent compound. Bioassays can be used to detect when the treated effluent is no longer toxic, which can reduce AOP operating costs because complete pollutant degradation is not always necessary to generate harmless effluent. Because toxicants sensitivity differs among species, organisms from different taxonomic groups must be used as biological models for the assays (Fernández-Alba et al. 2002). Microcrustaceans are commonly used in toxicological tests worldwide because—among many other practical reasons—they are fundamental to aquatic ecosystems and link the primary producers with higher trophic level consumers.

Given the need of evaluating the ecotoxicological effects of glyphosate-based formulations on key aquatic organisms as well as the effectiveness of a recently developed cleaner technology to reduce their toxicity, we proposed the following objectives: (1) to determine and compare the acute toxicity (LC_{50}) of two of the most-used glyphosate-based formulations on three microcrustacean species: *Daphnia magna*, *Ceriodaphnia dubia*, and *Notodiaptomus conifer* and (2) to evaluate the toxicity of contaminated samples with formulated glyphosate after being treated with the PAO UV/H_2O_2 at different reaction times using the same three species.

2 Materials and Methods

2.1 Glyphosate-Based Formulations

Eskoba® (Red Surcos) and Sulfosato Touchdown® (Syngenta Agro) glyphosate-based formulations were selected, which are among the most used in the pampean region and the Parana River floodplain (Argentina), the two ecoregions with the highest soybean production in the country. The glyphosate commercial formulations used herein include 48 % (w/v) as monoisopropylamine salt and 62 % of potassium salt. The chemical structures for both active principles are shown in Fig. 1.

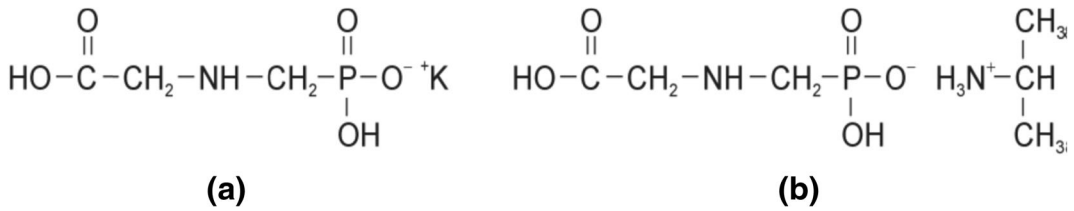

Fig. 1 Chemical structures: **a** glyphosate potassic salt in the Sulfosato Touchdown® formulation; **b** glyphosate monoisopropylamine salt in the Eskoba® formulation

2.2 Acute bioassays With Microcrustaceans

The *D. magna* specimens were generated from a monoclonal culture, which was initiated with an adult female and maintained in the laboratory for several generations under controlled temperature and photoperiod conditions. Given their abundance in the river floodplains, the *C. dubia* and *N. conifer* individuals were collected using a plankton net (100 μm) in the Parana River alluvial valley. Then, they were transferred to the laboratory for acclimation in synthetic media.

The *D. magna* and *N. conifer* samples were maintained in the same synthetic medium comprising 0.13 g K_2SO_4, 1.12 g $CaCl_2$, and 1 g $NaHCO_3$, dissolved in 5 L of distilled water, and *C. dubia* was maintained in American Public Health Association (APHA) et al. (1998) medium which includes the following: 2.4 g SO_4Mg, 3.84 g $NaHCO_3$, and 0.16 g KCl y 2.4 g $CaSO_4.2H_2O$, dissolved in 20 L of distilled water. The organisms were fed regularly with *Chlorella vulgaris* (CLV2 strain, from CISECE, Mexico) (absorbance=1.5 λ=650 nm) and maintained in a growth chamber undercontrolled and constant conditions (photoperiod 16 L, 8D and T 20±1 °C).

Prior to the experiments, stock solutions with 1000 a.e. mg L^{-1} of the two glyphosate-based formulations were prepared in sterile distilled water and maintained in the dark at −4 °C until analyzed.

The analyses were performed using a Dionex DX-100 chromatograph equipped with a Waters 430 ion conductivity detector, a Dionex ASRS300 suppressor, a Dionex Ion Pack AS2A-SC, and an Ion Pac AG2A–SC guard column. The eluent used comprised 7.2 mM Na_2CO_3 and 3.2 mM NaOH. The glyphosate concentration was 1067.5 (SD±38.48) a.e. mg L^{-1}. This stock solution was used to prepare each glyphosate-based formulations used in the assays. The assays were static of 48 h (photoperiod 16 L, 8D and T=20±1 °C). The *D. magna* and *C. dubia* neonates as well as 5th instar *N. conifer* copepodites were used. The number of replicate trials was 20 (International Organization for Standardation.ISO 6341, 1996) for cladocerans and 30 for copepods.

For the Eskoba® formulation, 6 glyphosate concentrations were tested on *D. magna*, 20 (C1), 25 (C2), 30 (C3), 35 (C4), 40 (C5), and 45 (C6) a.e.mg L^{-1} and 5 on *N. conifer* and *C. dubia*, 20 (C1), 40 (C2), 80 (C3), 160 (C4), and 320 (C5) a.e. mg L^{-1} on *N. conifer*, and 8 (C1), 12 (C2), 18 (C3), 27 (C4), and 40.5 (C5) a.e. mg L^{-1}on *C. dubia*.

For the Sulfosato Touchdown® formulation, 5 glyphosate concentrations were tested on the three studied species, 5 (C1), 2.5 (C2), 1.25 (C3), 0.5 (C4), and 0.25 (C5) a.e. mg L^{-1}on *D. magna*; 1 (C1), 0.5 (C2),0.25 (C3), 0.125 (C4), and 0.0625 (C5) mg a.e. L^{-1} on *C. dubia*, and 10 (C1), 5 (C2), 2.5 (C3), 1.25 (C4), and 0.75(C5) a.e. mg L^1 on *N. conifer*.

The results were considered acceptable when the control mortality was ≤10 %. The LC50 was determined for each species; LC_{50} is the effective concentration of a chemical that reduces the experimental population by 50 % (Rand and Petrocelli 1985).

LC50 values with a 95 % confidence interval were determined using Probit analysis (Finney, 1971). The pH and dissolved oxygen were measured throughout the experiment and maintained within a range of 7.6–8 and 8.6 mgL^{-1}, (APHA 1998).

2.3 Photoreactor and UV/H_2O_2 Process

The glyphosate-based formulations were degraded in an annular reactor with an inner quartz tube (V reactor= 870 cm^3), which allowed UV radiation to pass from a concentrically positioned germicidal lamp (Philips TUV 15 W, low pressure Hg vapor lamp with a significant emission at λ=253.7 nm). This reactor was operated in a recirculating batch system, which included a centrifugal pump and feed tank with continuous stirring. The system had a total volume of 2500 cm^3 and a constant temperature (T=20 °C) was maintained using a heat exchanger (Fig. 2).

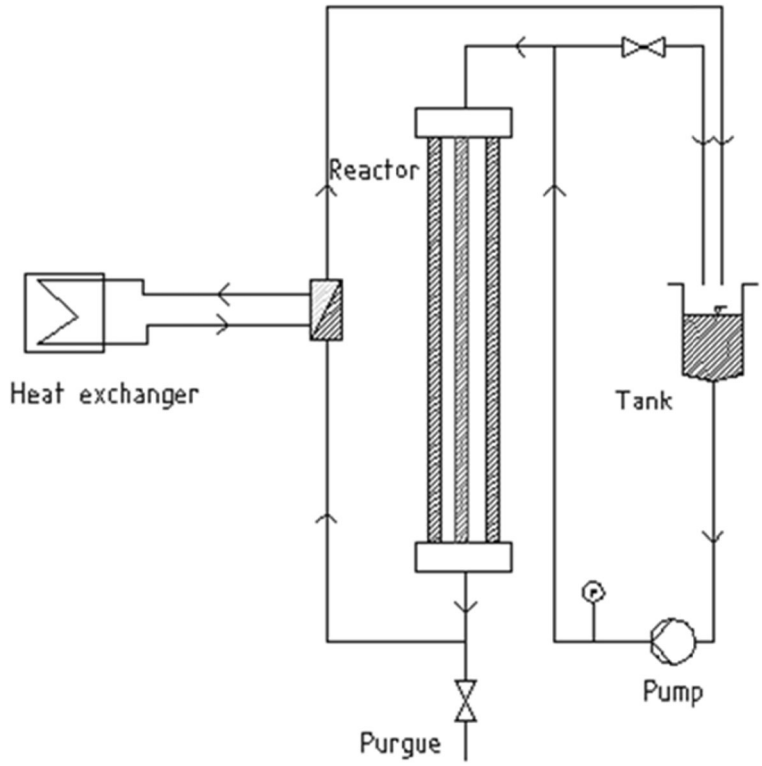

Fig. 2 Schematic representation of laboratory reactor

The reactor has a screen to block radiation from entering the reaction chamber until the system reached a steady state (uniform concentration, constant temperature, and lamp stability).

The samples were degraded in accordance with the following experimental procedure: the working solution and desired glyphosate as well as hydrogen peroxide concentrations were added to the tank. The germicidal lamp was lit with the screen in place, and the fluid was recirculated. Upon reaching steady state, the screen was removed to initiate the reaction (initial time, t=0). Samples were collected every 120 min to monitor the glyphosate concentration. The operating flow was 120 cm^3s^{-1}. The operating conditions are detailed in Table 1. 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 per liter (Lanctôta et al. 2014).

The samples tested for toxicity in the microcrustaceans were as follows. M0 (untreated sample): corresponds to 50 a.e. mg L^{-1} of glyphosate without H_2O_2. M1, M2, and M3: samples collected at different UV/H_2O_2 reaction times (2, 4, and 6 h, respectively), whereupon H_2O_2 was eliminated.

The bovine catalase (2197 units/mg Fluka; 1 unit decomposes 1 mol H2O2/min at pH 7.0 and 25 ° C) was used to decompose the remaining H_2O_2 in the samples. Glyphosate acid (AccuStandard cat. N P-015NB-250) was used as a chromatography standard for calibration.

2.4 Analytical Methods

Glyphosate was analyzed 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 comprised a mixture of 7.2 mM Na_2CO_3 and 3.2 mM NaOH at 0.6 ml/min.

Table 1 Experimental conditions

Variable	Value
Glyphosate initial concentration	0.30 (mM) (50 a.e. mg L^{-1})
H_2O_2 initial concentration	3.6 (mM) (120 mg L^{-1})
Total reaction time	6 h
Sampling	M0 (t=0); M1 (t=2 h); M2 (t=4 h); M3 (t=6 h)
Temperature	20 (°C)
Initial pH	5.2

The H_2O_2 was analyzed using colorimetry by measuring absorbance at 350 nm in a CARY spectrophotometer. The total organic carbon (TOC) was analyzed to quantify the level of mineralization in the samples, with an analyzer Shimadzu TOC-5000.

2.5 Static Assays Using Microcrustaceans to Evaluate UV/H_2O_2 Efficiency

Sample toxicity at different timepoints (M0=0 h, M1=2 h, M2=4 h, and M3=6 h) was evaluated using *D. magna*, *C. dubia*, and *N. conifer* as biological models.

Microcrustacean samples were transferred to the laboratory, where the pH was measured, and the salts in the synthetic media described above were dissolved for each species. Subsequently, each sample was aerated for 24 h, and then assays were used to assess the organisms' mortality at the different timepoints tested.

The assays were static for 48 h and followed the methodological design described in section "Acute bioassays with microcrustaceans" (T°, photoperiod, effect indicator, number of replications, and assay acceptability). The pH was measured at the beginning and end of the experiment with values ranging from 7.65 to 7.15 for Eskoba® and 7.22 to 7.68 for Sulfosato Touchdown®. Similarly, dissolved oxygen was measured, which ranged from 9 to 6.6 mg L^{-1} and 8.7 to 6.5 mg L^{-1} for Eskoba® and Sulfosato Touchdown®, respectively.

The Pearson correlation analysis was performed to assess the trend in time of the mortality (in %) of each species under study. Prior to this, data were tested for normality with the Kolmogorov-Smirnov test at a significance level of α=95 %. In addition, a Tukey test was used in order to analyze differences in mortality between species within each treatment (i.e., the pesticide at different timepoints of the remediation process: M0 to M3). This analysis was performed separately for each species, being the % of mortality the response variable. The GraphPad InStat (InfoStat 2004) statistical software was used.

3 Results

3.1 Acute Bioassays With Microcrustaceans to Assess Toxicity of Glyphosate-Based Formulations

The LC_{50} for Eskoba® was higher than the one for Sulfosato Touchdown® for the three species under study (Table 2). LC_{50} was showing greater toxicity of Sulfosato Touchdown®. The registered order of species sensitivity was the following: *C. dubia* > *D. magna* > *N. conifer*, being equal for both formulations.

3.2 Degradation of Glyphosate-Based Formulations

In the present study, glyphosate degraded more rapidly from Sulfosato Touchdown® than from Eskoba®. Glyphosate and TOC concentration were variations as a function of the reaction times for the two glyphosate-based formulations under the same experimental conditions, which generated the highest glyphosate degradation rates, as determined in previous studies (Manassero et al. 2010; Neder et al. 2011; Vidal et al., 2015) (Fig. 3).

For the same glyphosate concentrations as acid, the initial TOC concentrations were higher in Eskoba® than Sulfosato Touchdown® (23 vs. 10 mg L^{-1}). The different active ingredients that compose each formulation explain the difference. For the same initial glyphosate acid concentration (50 a.e.mg L^{-1}), the Eskoba® formulation has a 1–1 molar ratio between the anion glyphosate and the cation isopropylamine, while the Sulfosato Touchdown® active ingredient is only the potassium salt (Fig. 1). Hydroxyl radicals generated by UV/H_2O_2 (which does not react selectively) oxidized both the anion glyphosate and the cation isopropylamine. In contrast, for Sulfosato Touchdown®, the radicals exclusively oxidized the glyphosate anion.

3.3 Bioassays With Microcrustaceans to Evaluate the Efficacy of the UV/H_2O_2 Process

The Tukey test for the mortality (%) of the species showed non-significant values in the three species (p= 0.3498), indicating that they responded similarly to the remediation process. The negative correlations between the organism's mortality and timepoints (M0, M1, M2, and M3) indicate that the remediation process diminished the glyphosate concentration for Eskoba®, r^2= 0.8536, 0.9363, and 0.8526 (p<0.05) and for Sulfosato Touchdown®, r^2=0.7269, 0.8937 and 0.6914 (p<0.05) in *N. conifer*, *D. magna*, and *C. dubia*. Therefore, microcrustacean mortality decreased linearly with the remediation time. *C. dubia* and *N. conifer* were more sensitive than the holarctic *D. magna* to the remediation process, since the first two species showed greater percentage of mortality at 6 h (M3) of processes, compared with *D. magna*, for both formulations evaluated.

Table 2 LC_{50} values for the three species in acute assays with Eskoba® and Sulfosato Touchdown®

	LC_{50} glyphosate (a.e. mg L^{-1})		
	C. dubia	*D. magna*	*N. conifer*
Eskoba®	14.49 (12.40–16.77)	29.48 (27.46–31.41)	95.23 (71.82–128.2)
Sulfosato Touchdown®	0.31 (0.25–0.37)	1.62 (1.24–2.09)	1.74 (1.22–2.29)

Figure. 4 presents the mortality of the three microcrustacean species in the samples (M0=0 h, M1= 2 h, M2=4 h, and M3=6 h) and the corresponding glyphosate concentration (Eskoba® formulation).

The lowest mortality values were recorded for the longest treatment time (6 h=M3) in each species: 60 % for *C. dubia*, 47 % for *N. conifer*, and 35 % for *D. magna*.

The remaining TOC was 46 % (10.8 mg L^{-1}TOC) for sample M3. It is difficult to compare the glyphosate concentrations that correspond to the LC50 values with the mortality values at the end of the remediation process (M3). *C. dubia* showed higher percentage of mortality to 12 a.e. mg L^{-1} (M3) that LC_{50}=14.49 a.e. mg L^{-1}, while *D. magna* and *N. conifer* to 12 a.e. mg L^{-1}(M3) percentage of mortality was less than LC_{50}= 29.48 and LC_{50}=95.23 (Table 3).

Figure 5 shows the mortality percentage for the three microcrustacean species in the samples M0, M1, M2, and M3 and the corresponding glyphosate concentration (Sulfosato Touchdown® formulation). Mortality decreased with increased treatment time. Lower mortality values were observed in each species for the longest treatment time (M3), which had a 1 a.e.mg L^{-1} glyphosate concentration and 3.7 mg L^{-1} TOC concentration; the values were 25 % for *N. conifer*, 20 % for *C. dubia*, and 5 % for *D. magna* (Table 4). LC_{50} values compared with mortality at the end of the remediation process (1 a.e.mg L^{-1} glyphosate concentration) showed that the treated sample is less toxic to the three microcrustaceans.

4 Discussion

4.1 Acute toxicity of Two Glyphosate-Based Formulations and Comparison Among Species

The 48 h LC_{50} values of Eskoba® and Sulfosato Touchdown® obtained in this study for *D. magna*, *C. dubia*, and *N. conifer* showed that these species notoriously differ in their specific sensitivity. This difference can be attributed to their particular differences in life cycles, ecology, and metabolic rates and demonstrate the importance of developing toxicological test with a high range of organisms, even belonging to the same aquatic community. When compared to other ecotoxicological studies, it can also be observed that there is a high variation within species and, in particular, within the glyphosate formulations used. For example, *D. magna* recorded 48 h LC50 values of 7.9 mg

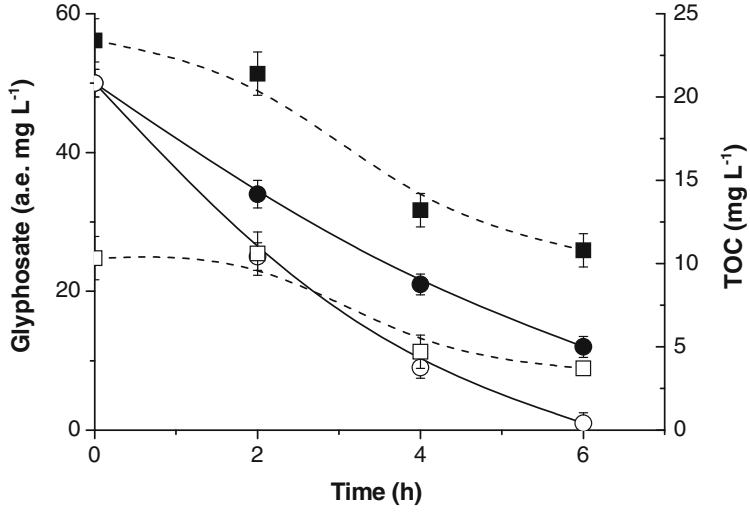

Fig. 3 Glyphosate and TOC concentrations evolution as a function of time during UV/H_2O_2 process under the best experimental conditions for both glyphosate-based formulations: $C_{gly}{}^0$=50 e.a. mg L^{-1}, $C_{H2O2}{}^0$= 120 mg L^{-1}: (*black circle*) glyphosate and (*black box*) TOC in Eskoba®, (*white circle*) glyphosate and (*white box*) TOC in Sulfosato Touchdown®. The *line* connecting the experimental values is a trend line

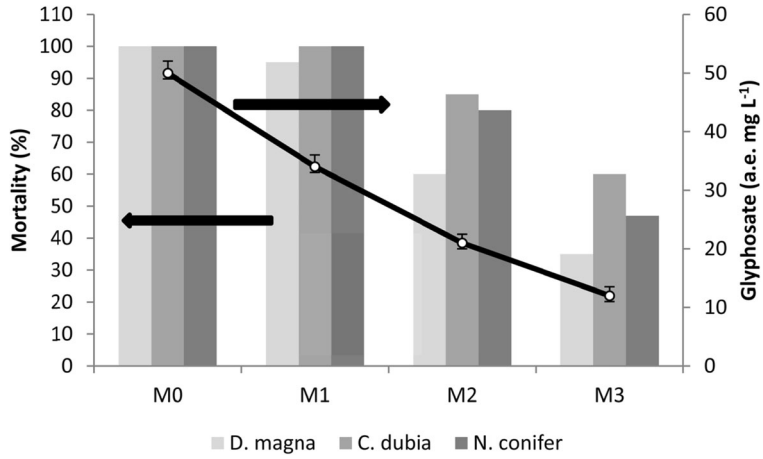

Fig. 4 Mortality (%) and glyphosate evolution at different times (M0=0 h, M1=2 h, M2=4 h, and M3=6 h) after the UV/H_2O_2 process, for Eskoba® and the three studied species. (*White circle*) glyphosate concentration (a.e. mg L^{-1}). *Rightwards arrow* indicates the axis where the concentration of glyphosate is represented determined for each reaction times of processes (UV/H_2O_2). *Leftwards arrow* indicates the axis where the% mortality represents the microcrustaceans

L^{-1} exposed to Faena®, 61.72 mg L^{-1} exposed to Ron-Do, 190 mg L^{-1} to Roundup®, and 11 mg L^{-1} for Roundup UltraMax®. The cladoceran *C. dubia* registered 48 h LC_{50} values of 5.39 mg L^{-1} exposed to Roundup®, 415 mg L^{-1} to Rodeo®, and 81.5 mg L^{-1} to Roundup Bio Active® (Alberdi et al., 1996; Tsui and Chu, 2003; Tsui and Chu 2004; Raipulis et al. 2009; Appendices to Glyphosate 2010. Other zooplancton species, such as *Simocephalus vetulus*, *Phyllodiaptomus annae*, and *Lecane quadridentata* reported 48 h LC_{50} values of 21.5, 1.6, and 13.1 a.e.mg L^{-1} when exposed to commercial formulations Eskoba®, Roundup®, and Faena®, respectively (Reno et al. 2014, AshokaDeepananda et al. 2011; Dominguez-Cortinas et al. 2008). Similar results to those found in this study were reported by Regaldo (2013) and Olvera-Ramírez et al. (2010), concerning the increased sensitivity of *C. dubia* compared with *D. magna*.

4.2 Degradation of Glyphosate-Based Formulations

The obtained results from the degradation of glyphosate-based formulations by the process UV/H_2O_2 suggest that it is more convenient to treat aqueous waste from Sulfosato Touchdown® than Eskoba® from an efficiency perspective because a higher final percentage of glyphosate degradation (98 % for Sulfosato Touchdown® compared with 76 % for Eskoba®) was generated over the same time (which is directly related to expense). Furthermore, the TOC conversions were 64 and 54 % for Sulfosato Touchdown® and Eskoba®, respectively.

Table 3 LC_{50} values for the three species in acute assays with Eskoba® and mortality (%) in M3

Mortality (%)			
Glyphosate (a.e. mg L^{-1})	*C. dubia*	*N. conifer*	*D. magna*
LC_{50}=14.49	50		
LC_{50}=29.48			50
LC_{50}=95.23		50	
M3=12	60	47	35

4.3 Bioassays With Microcrustaceans to Evaluate the Efficacy of the UV/H_2O_2 Process

The bioassays performed with the three microcrustaceans to evaluate the efficacy of the UV/H_2O_2 process indicate that it is necessary to increase the reaction time for samples with Eskoba® because in the three microcustaceans species, the percentage of mortality was below or near 50 %.

Previous studies on glyphosate acid degradation using UV/H_2O_2 identified the following reaction intermediates: glycine, formic acid, formaldehyde, ammonia, and, as final products, nitrate and phosphate ions (Manassero et al. 2010). These intermediates and other substances (i.e., additives) in such formulations at lower concentrations are not frequently showed on the product labels. Therefore, it is possible that they are affecting the toxicity of samples regardless of the glyphosate concentration.

A previous work has shown that Roundup® and its surfactant polyoxyethylene amine (POEA) were more toxic than the active ingredient, with LC_{50} values at 5.39 and 1.77 a.e. mg L^{-1} for the commercial formulation and 1.15 and 0.57 a.e. mg L^{-1} for POEA to *C. dubia* and *Acartia tonsa*, respectively (Tsui and Chu, 2003). The

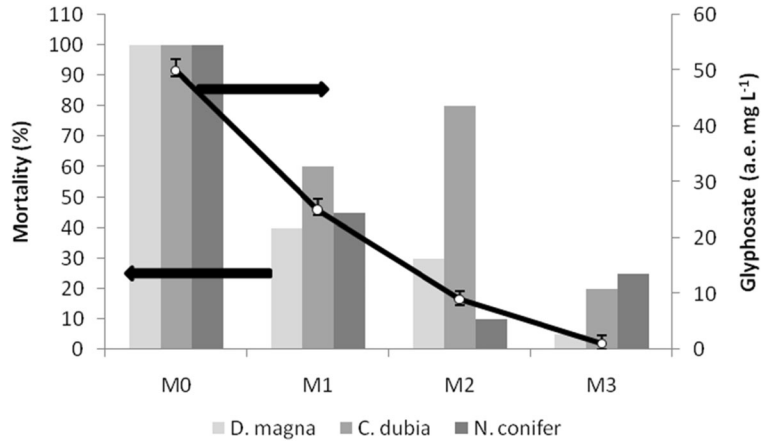

Fig. 5 Mortality (%) and glyphosate evolution at different times (M0=0 h, M1=2 h, M2=4 h, and M3=6 h) after the UV/H_2O_2 process, for Sulfosato Touchdown® and the three studied species. (*White circle*) glyphosate concentration (a.e. mg L^{-1}). *Rightwards arrow* indicates the axis where the concentration of glyphosate is represented determined for each reaction times of processes (UV/H_2O_2). *Leftwards arrow* indicates the axis where the % mortality represents the microcrustaceans

glyphosate acid LC_{50} values were 35.3 and 147 a.e.mg L^{-1} for the mentioned copepod and cladoceran, respectively. Other research has reported similar results, assuming that the Roundup® surfactant, but not glyphosate, caused the increased toxicity (Kitulagodage et al. 2008; Pereira et al. 2009). Piola et al. (2013), after comparing the toxicity of two glyphosate formulates on *Eisenia andrei*, determined that the adverse effects observed at doses close to its LC_{50} could be attributed to the effects of some of the so-called "inert ingredients" either due to a direct intrinsic toxicity or to an enhancement in the bioavailability and/or bioaccumulation of the active ingredient. Recently, Mesnage et al. (2014) informed that eight formulations out of nine were up to one thousand times more toxic to human cells than their active principles, concluding that chronic tests on pesticides may not reflect relevant environmental exposures if only one ingredient of these mixtures is tested alone. In sum, our results demonstrate the importance of specifying each compound in a pesticide on the product labels.

Table 4 LC_{50} values for the three species in acute assays with Sulfosato Touchdown® and Mortality (%) in M3

	Mortality (%)		
Glyphosate (mg a.e. L^{-1})	*N. conifer*	*C. dubia*	*D. magna*
LC_{50}=0.31		50	
LC_{50}=1.62			50
LC_{50}=1.74	50		
M_3=1	25	20	5

5 Conclusions

In this study acute tests were conducted to determine the lethal concentration 50 (LC_{50}) for both formulates. Also, the toxicity of samples collected at different timepoints after UV/H_2O_2 remediation process for two glyphosate-based formulations were evaluated. Relevant information was provided regarding the toxic effects from two glyphosate-based formulations not previously explored on a standard organism, such as *D. magna* and two microcrustaceans that are frequent and abundant in the pampas region of Argentina and the river floodplains of South America. Microcrustaceans can be used in toxicity evaluations of agricultural wastes treated with UV/H_2O_2. *C. dubia* and *N. conifer* were more sensitive to the remediation process, as they showed greater percentage of mortality during the degradation process of the glyphosate formulations (UV/H_2O_2), than the holarctic species (*D. magna*), which suggests that regionally relevant species should be used in evaluating decontamination for the pampas and other southern regions where these species exist. The results indicate the effectiveness of UV/H_2O_2 at reducing the water contamination with glyphosate, which will facilitate better pesticide waste management in agricultural activities. However, some intermediates produced in these treatments, from the active pesticide component or from some other components of the formulation, may lead to increased toxicity. Finally, commercial formulations with the same active ingredient may have different toxicity owing to different additives; therefore, they should be identified on product labels.

This paper also shows the importance of developing cleaner technologies with an emphasis on microcrustacean as suitable biological models to evaluate a remediation process.

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