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Effectiveness evaluation of glyphosate oxidation employing the H_2O_2/UVC process: Toxicity assays with *Vibrio fischeri* and *Rhinella arenarum* tadpoles

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The H_2O_2/UVC process was applied to the photodegradation of a commercial formulation of glyphosate in water. Two organisms (*Vibrio fischeri* bacteria and *Rhinella arenarum* tadpoles) were used to investigate the toxicity of glyphosate in samples M_1, M_2, and M_3 following different photodegradation reaction times (120, 240 and 360 min, respectively) that had differing amounts of residual H_2O_2. Subsamples of M_1, M_2, and M_3 were then used to create samples $M_{1,E}$, $M_{2,E}$ and $M_{3,E}$ in which the H_2O_2 had been removed. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities were measured in tadpoles to determine possible sub-lethal effects. In *V. fischeri*, $M_{1,E}$, which was collected early in the photodegradation process, caused 52% inhibition, while $M_{3,E}$, which was collected at the end of the photodegradation process, caused only 17% inhibition. Survival of tadpoles was 100% in samples M_2, M_3, and in $M_{1,E}$, $M_{2,E}$ and $M_{3,E}$. The lowest percentages of enzymatic inhibition were observed in samples without removal of H_2O_2: 13.96% (AChE) and 16% (BChE) for M_2, and 24.12% (AChE) and 13.83% (BChE) for M_3. These results show the efficiency of the H_2O_2/UVC process in reducing the toxicity of water or wastewater polluted by commercial formulations of glyphosate. According to the ecotoxicity assays, the conditions corresponding to M_2 (11 ± 1 mg a.e. L^{-1} glyphosate and 11 ± 1 mg L^{-1} H_2O_2) could be used as a final point for glyphosate treatment with the H_2O_2/UV process.

Keywords: Glyphosate, H_2O_2/UVC process, toxicity, *Vibrio fischeri*, *Rhinella arenarum*.

Introduction

Environmental pollution caused by pesticides in aquatic ecosystems has become a severe global problem. Aquatic ecosystems are also being subjected to increasing pressures from anthropogenic activities, including contamination by a variety of mineral and organic pollutants. Some organic pollutants are herbicides that are used not only in agriculture but also for many other purposes such as domestic gardens and railroad track maintenance. Herbicides can contaminate aquatic ecosystems by terrestrial runoff and, to a lesser extent, by direct application and aerial spraying.[1]

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Glyphosate, is a broad spectrum, nonselective and post-emergence herbicide that is classified as both a phosphonate and an amino acid group-containing pesticide. It has become the world's leading agrochemical, especially in recent years with the widespread use of genetically modified glyphosate-tolerant crops.[2,3] Glyphosate is highly soluble in water and, consequently, it can easily cause groundwater contamination in agricultural areas. It is used in major soybean- producing countries such as the U.S., Brazil and Argentina. In Argentina, genetically modified (GM) soybean crops account for approximately 60% of the cultivated surface and approximately 200 million L of glyphosate-based herbicides such as Roundup Ready (RR) were used to produce more than 50 million tons of soybeans per year.[4]

The potential negative effects of glyphosate-based herbicides on some sectors of the ecosystem are clear. Recent studies have shown that amphibians are one of the most sensitive vertebrate groups to the toxicological effects of glyphosate.[5,6]. The LC_{50} (lethal concentration value) for many amphibians to glyphosate is between 1 and 10 mg

a.e. L^{-1} and may be even lower, between 1 and 0.1 mg a.e. L^{-1}, in some cases.[7,8] Exposure to glyphosate induced sublethal effects such as malformations (craniofacial and mouth deformities, eye abnormalities, and bent or curved tails) in the South American species *Scinax nasicus*.[9] Another study suggested that adverse effects of glyphosate on anuran morphology could be a consequence of increasing endogenous retinoid activity during the early stages of morphogenesis in embryos[10] increasing morphological abnormalities in amphibian in east-central Argentina.[11] Lajmanovich et al.[12] demonstrated the existence of a wide range of toxic effects as well as cholinesterase inhibition causing significant reductions in both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in *Rhinella arenarum* tadpoles exposed to glyphosate formulations. Notably, in the Southern Hemisphere glyphosate application often occurs from November through March;[13] this period coincides with the reproductive period of most amphibians in the region[14] and is concurrent with the highest pluvial period; that results in intensive pesticide runoff [15] and polluted aquatic ecosystems. Thus, surface and ground water contamination by herbicides applied to soybean crops in countries with intensive production demands strategies to prevent and remediate the environmental problems caused by such agrochemicals.

The development of simple, efficient and affordable technologies is strongly urged for the on-site treatment of water contaminated by pesticides. Advanced oxidation processes (AOPs) usually operate at or near ambient temperature and pressure and are based on using a highly reactive radical such as the hydroxyl radical as the primary oxidant. Different AOPs were tested for the degradation of glyphosate in water.[16] Typical studies of degradation of glyphosate in water with AOPs include Chen et al.,[17] Mangat Echavia et al.,[18] Muneer and Boxall[19] and Shifu and Yunzhang,[20]. Manassero et al.[21] showed that the combination of hydrogen peroxide and UV radiation (H_2O_2/UVC) can be a convenient process for treating these types of pollutants.

The main problems with AOPs are frequently their cost and their usually long processing times. Because the total destruction of pollutants is not always necessary, however, one way to reduce the operating cost of these processes is to determine the toxicity of the treated water at different AOP treatment stages using ecotoxicity bioassays with various organisms.[22–24] Because toxicity is a biological response, a universal monitoring device is unlikely to be available, so to increase confidence in the evaluation it is necessary to use a battery of different organisms from different taxonomic groups.

In this work, the H_2O_2/UVC process was applied to the degradation of a commercial formulation of glyphosate in water. Two different bioassays were used for determining sample toxicity at different stages of mineralization. The bioassays used the luminescence bacterium *Vibrio fischeri* (a traditional assay for evaluating AOPs) and tadpoles of *Rhinella arenarum*, a common anuran that is frequently found in forests, wetlands, agricultural land and urban territories[25] and has an extensive Neotropical distribution.[26] *Rhinella* was used to assess acute toxicity. In addition, total AChE and BChE activities were evaluated as possible indicators of sub-lethal toxicity of both untreated wastewater and wastewater treated by the H_2O_2/UVC process.

The application of ecotoxicity tests for evaluating the effectiveness of decontamination by AOPs is a very useful tool for determining the end point for treating wastewater contaminated with herbicides. The use of these biological assays might allow for reduced operating costs because they might show that the total mineralization of the pollutant is not necessary.

Materials and methods

Chemicals

The following reactants were used: (a) glyphosate (AccuStandard) as a standard chromatographic and (b) glyphosate as the commercial herbicide Eskoba®, 35.6% (w/v) as acid or 48% as a monoisopropylamine salt (MIPA), (c) hydrogen peroxide (Ciccarelli p.a., > 99%), (d) Manganese (IV) oxide, particle size 10 micron, reagent grade > 90% (Aldrich) to eliminate residual H_2O_2 and (e) pure water (0.055 μS cm^{-1}) was used in all experiments. This water was obtained from an OSMOION™purification system. (f) Butyrylthiocholine iodide (BuSCh), (g) 5,5–dithiobis–2–nitrobenzoic acid (DTNB), (h) α- naphthyl acetate (α-NA), (i) 4–nitrophenyl valerate (4–NPV), and (j) Fast Red ITR salt were obtained from Sigma-Aldrich®. All other chemicals used in this study were obtained from Biopack® (Argentina).

Photoreactor

The degradation of glyphosate was carried out in an annular photoreactor. The experimental setup consisted of a flow-through reactor (volume equal to 870 cm^3) that was coupled to a centrifugal pump and a storage tank that had provisions for sampling and temperature measurements. A heat exchanger connected to a thermostatic water bath was inserted in the circuit to keep the system at 20°C constant temperature (Fig. 1). Experiments were performed in a batch mode by recycling the reactants in a closed loop. A germicidal lamp (Philips TUV 15 W, low pressure mercury vapor lamp) with one single significant emission wavelength at 253.7 nm (output power equal to 3.5 W = 7.4×10^{-6} Einstein s^{-1}) was used as the source of UVC radiation. The lamp was placed at the centerline of the cylindrical reactor. The total system volume was 2500 cm^3. Connections between the different components of the recycle loop were made with Teflon® tubing. The lamp was covered with an opaque mask during the times when the whole system was

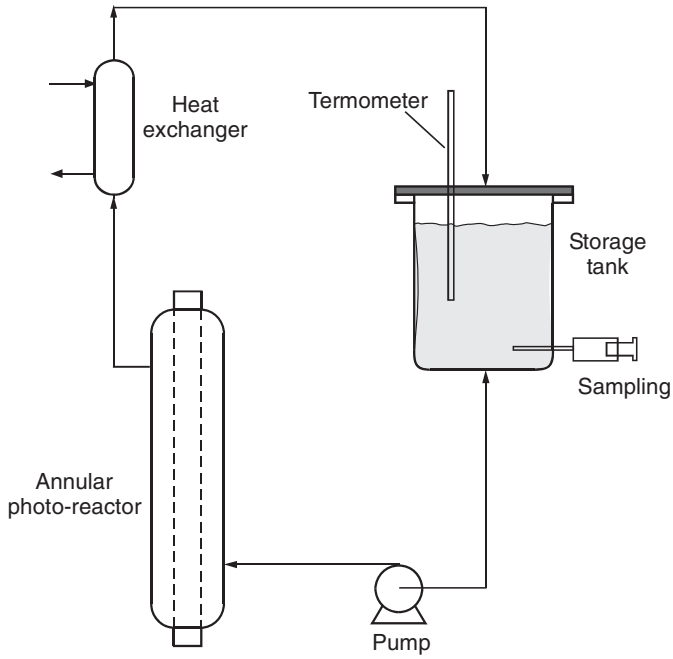

Fig. 1. Schematic of the annular photoreactor operated in a batch mode.

reaching a steady-state condition based on concentration, temperature and lamp operation.

Experimental procedures

Each experimental run was performed as follows: the working solution was added to the reactor with the desired initial concentration of glyphosate and hydrogen peroxide (H_2O_2). The germicidal lamp was turned on while the mask was on and recirculation of the fluid was set in motion. After the concentration, temperature and lamp operation all reached steady-state conditions, the cover was removed and the reaction was started at reaction time $t = 0$. Samples were collected every 120 min for monitoring the concentrations of glyphosate, H_2O_2, Total Organic Carbon (TOC) and pH. The recirculation flow rate was relatively high at 120 $cm^3\ s^{-1}$. Details of the operating conditions are described in Table 1. To enable an easier comparison of results, all concentrations of glyphosate are expressed as milligrams of acid equivalents per liter (mg a.e. L^{-1}).

For the toxicity assays the samples analyzed were:

i. M_0 or an untreated sample corresponding to 50 mg a.e. L^{-1} of glyphosate without H_2O_2.
ii. M_1, M_2 and M_3: samples collected at different times during the H_2O_2/UVC process (120, 240 and 360 min, respectively), without removal of unreacted H_2O_2.
iii. $M_{1,E}$, $M_{2,E}$ and $M_{3,E}$: samples collected at different times during the H_2O_2/UVC process (120, 240 and 360 min, respectively), with post-reaction treatment to eliminate the unreacted H_2O_2.

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	21,600 s (360 min)
Sampling	M_0 (t = 0 min); M_1 (t = 120 min); M_2 (t = 240 min); M_3 (t = 360 min)
Temperature	20 (°C)
Initial pH	5.2

Relevant samples were treated with manganese oxide powder to decompose residual H_2O_2. Those samples were filtered on 0.45 μm Millipore membranes to remove manganese oxide before further analysis.

Analytical determinations

Analyses were carried out as follows: H_2O_2 was analyzed by spectrophotometry at 350 nm with a Cary 100 Bio UV–visible instrument (Varian).[27] Glyphosate was analyzed by ion chromatography with a suppressed conductivity detector and using an Ion Pac AG2A–SC guard column, an AS2A-SC separating column, and an ion self-regenerating suppressor (Alltech DS-Plus^{TM}). A solution of Na_2CO_3 (9 mM) and NaOH (4 mM) was used as the eluent at a flow-rate of 1.5 mL min^{-1}.[28]

The mineralization of the pollutant was assessed by total TOC analysis, employing a Shimadzu TOC-5000A analyzer. The pH was determined with an Orion EA 940 pH meter.

Ecotoxicity tests

Luminescent bacteria emit light as a by-product of their cellular respiration and metabolic processes. During direct contact by the bacteria with toxic compounds, the reduction in the light emission indicates a decreased rate of respiration caused by the presence of those compounds. The inhibition of *V. fischeri* was measured by the Microtox® acute toxicity test using a Model 500 Analyzer (Strategic Diagnostics Inc.) within a short time exposure (15 min) following ASTM Standard Method D 5660–96 [29]. The samples analyzed by this assay were M_0, $M_{1,E}$, $M_{2,E}$ and $M_{3,E}$. Assays were carried out in duplicate.

Prometamorphic tadpoles (Gosner stages 36–38)[30] of *R. arenarum* (n = 84) were collected in January 2011 from temporary ponds in natural parafluvial forests of the Paraná River Boundary (31°11′31″S; 60°9′29″W, Argentina). Despite the wide distribution of soybean crops in the region, all the tadpoles were collected in non-agricultural areas and were therefore presumed to have had minimal prior exposure to pesticides. The average total length of the

tadpoles (snout-tail tip) was 0.89 ± 0.08 mm, and their average weight was 0.26 ± 0.07 g.

Short-term (24 and 48-h) static tests were performed to evaluate the toxicity of the samples collected at different times during the H_2O_2/UVC process. For these assays, we used glass tanks (12.5 cm diameter and 13.5 cm high) with 0.5 L of untreated sample M_0 plus samples M_1, M_2, M_3, $M_{1,E}$, $M_{2,E}$ and $M_{3,E}$, and seven tadpoles were housed per tank. H_2O_2 (120 mg L^{-1}) and distilled water (DW) were used as positive (PC) and negative (NC) controls, respectively. Both controls and test solutions were made and tested in duplicate. Treatments were randomly assigned to the tanks, as was the order in which the tanks were sampled. Tadpoles were not fed during the toxicity tests. Assays were conducted at 22 ± 2°C, and a 12:12 light:dark period was observed.

Enzymatic determinations

Tadpoles from control assays and those that survived sample exposure for 48 hours were euthanized in accord with American Society of Ichthyologists and Herpetologists (ASIH) et al. criteria.[31] Whole tadpoles were homogenized on ice in 0.1% *t*–octylphenoxypolyethoxy ethanol (triton X-100) in 25 mM tris (hydroxymethyl) aminomethane hydrochloride (pH 8.0) in a Polytron homogenizer. The homogenates were centrifuged at 14,000 r.p.m. for 15 min at 4°C and the supernatant was collected. Protein concentrations in the supernatants were determined according to the Biuret method.[32] After sufficient sample volumes were collected, enzyme kinetic assays were carried out in duplicate or triplicate. AChE and BChE activities were measured according to Ellman et al.[33] The reaction mixture consisted of 0.01 mL of extract, 2 mM dithio-bis-2-nitrobenzoic acid (DTNB), 20 mM acetylthiocholine and butyrylthiocholine iodide (AcSCh and BuSCh, respectively), 25 mM Tris-HCl, and 1 mM $CaCl_2$ (pH 7.6). Assays were conducted at 25°C. The variation in optical density was recorded at 410 nm for 1 min at 25°C using a JENWAY 6405 UV-VIS spectrophotometer. AChE and BChE activities were expressed as nmol min^{-1} mg^{-1} protein using a molar extinction coefficient of 14.15×10^3 M^{-1} cm^{-1} for AChE, and 13.6×10^3 M^{-1} cm^{-1} for BChE.

Data analyses

In the *R. arenarum* survival tests, the percentage of mortality expressed as the mean ± standard error of measurement (SEM) was recorded. The differences in the mortality proportions were estimated using a Chi-Squared Goodness of Fit model (with Yates correction).[34]

Enzymatic activities were expressed as the mean ± SEM. The influence of treatments on the B-esterase enzyme activities were analyzed statistically using the non-parametric Kruskal-Wallis ANOVA. Pairwise comparisons between samples from the all treatment were tested by the Dunnett's test for post-hoc multiple comparisons. Statistical significance was held at $\alpha = 0.05$. Analyses were performed with GraphPad InStats®.

Results and discussion

Glyphosate degradation

In previous work the kinetics of degradation of a commercial formulation of glyphosate in water was studied.[35] For those experiments a small bath laboratory reactor inside a recycle system was employed and a detailed description of the photoreactor was presented elsewhere.[36] Two types of prior experiments were carried out to separately investigate the effects of UVC and H_2O_2. The first experiment was performed with an initial glyphosate concentration equal to 50 mg a.e. L^{-1}, a hydrogen peroxide concentration equal to 100 mg L^{-1} and without UV radiation. After 4 h of total reaction time, no noticeable changes in glyphosate concentration were observed. A similar experiment was performed with an initial glyphosate concentration equal to 50 mg a.e. L^{-1} and using 40 W Heraeus UVC germicidal lamps turned on for a total of 4 h. No signs of direct photolysis were observed. These results confirm those previously obtained by Manassero et al.[21] with glyphosate acid alone.

It is known that there is an optimum concentration of H_2O_2 in the H_2O_2/UVC process. Because the radiation absorption coefficient of H_2O_2 at the usually employed wavelengths is rather weak, the reaction rate—more specifically, the initiation step of H_2O_2 decomposition—slows when the concentration of H_2O_2 is too low. When the concentration of H_2O_2 is too high it becomes a scavenger of hydroxyl radicals competing with the pollutant degradation reaction and decreasing the rate of the latter reaction. Moreover, excess H_2O_2 concentration might not be tolerated in the effluent discharge, such as when the outlet stream is to be post-treated biologically. In addition, excess H_2O_2 can affect the process economic feasibility.[37] Because the H_2O_2 concentration ratio is very important, unless the literature provides information concerning the most appropriate operating conditions,[37] the ratio normally has to be determined experimentally.

The results, examined for a fixed total reaction interval of 5 h were analyzed in terms of the corresponding final glyphosate conversion for the commercial formulation (Fig. 2). The hydrogen peroxide concentration range between 100–230 mg L^{-1} produced the higher conversions up to 36% for experiments conducted in the laboratory reactor at pH 5.2.

Using the annular photoreactor, an experiment was conducted under the best previously established operating conditions for glyphosate degradation. Figure 3 shows the temporal progression of the concentrations of the participating species. The initial pH was 5.2 and did not change significantly during the process. In this case, the glyphosate

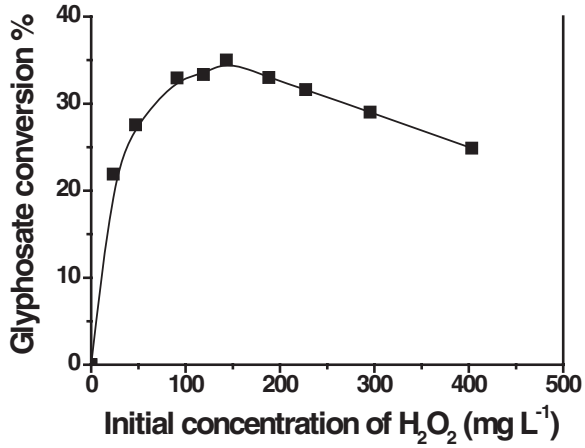

Fig. 2. Glyphosate conversion vs. initial concentration of H_2O_2. $C^0_{glyph.}$ = 50 mg a.e. L^{-1}; pH = 5.2; UV lamp: 40 W of input power; reaction time = 5 h. ■ Glyphosate monoisopropylamine salt (commercial formulation).

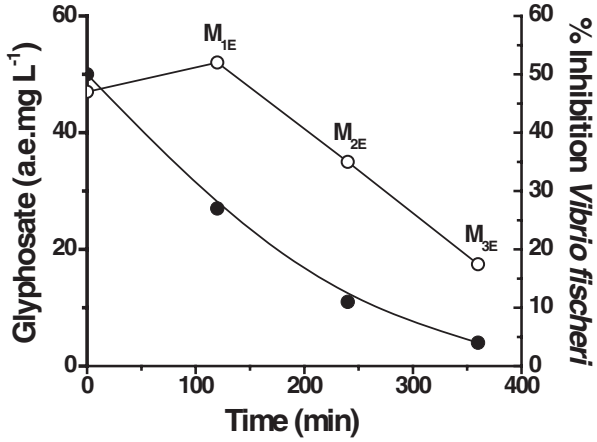

Fig. 4. Degradation of glyphosate and change in toxicity during UV/H_2O_2 process $C^0_{glyph.}$ = 50 mg a.e. L^{-1}; $C^0_{H_2O_2}$ = 120 mg L^{-1}. • Glyphosate, ○ *Vibrio fischeri*. $M_{1,E}$, $M_{2,E}$ and $M_{3,E}$ samples at different times of the H_2O_2/UVC process: 120, 240 and 360 min, respectively. Samples produced after H_2O_2 removal.

and TOC conversions in 360 minutes were 90% and 70%, respectively.

Ecotoxicity tests

Because amphibians are one of the most sensitive vertebrate groups that can be used in ecotoxicity tests and the *V. fischeri* is a rapid standard test, the correct evaluation of any AOP process applied to water and wastewater treatments must include not only the study of the disappearance of the parent compound but also the consequences for the environment under consideration. Knowledge about the toxicity of the treated water or wastewaters evaluated by different bioassays is always necessary.

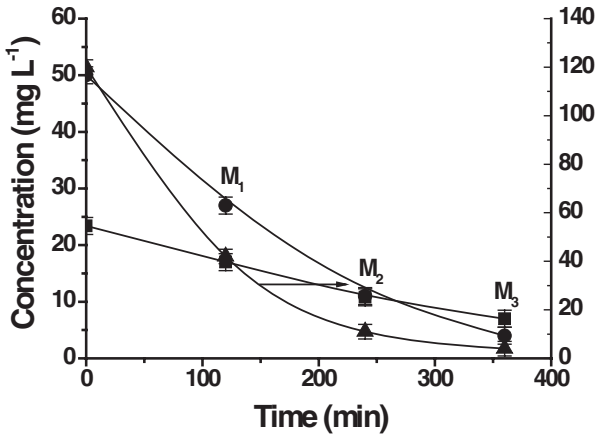

Fig. 3. Glyphosate, H_2O_2 and TOC concentration evolution as a function of time during H_2O_2/UVC process under the best experimental conditions: $C^0_{glyph.}$ = 50 mg a.e. L^{-1}, $C^0_{H_2O_2}$ = 120 mg L^{-1}. • Glyphosate, ▲ H_2O_2 and ■ TOC. M_1, M_2 and M_3: samples at different times of the H_2O_2/UVC process: 120, 240 and 360 min, respectively. H_2O_2 not yet removed.

The herbicide was tested as part of a complex commercial mixture because that is the form in which it is applied to fields and introduced into the environment.[12]

The changes in of the toxicity of the treated solution for *V. fischeri* are presented in Figure 4, along with the glyphosate concentration. The toxicity of the samples is represented by the percent inhibition of the natural bioluminescence of *V. fischeri*. The untreated sample (M_0) causes an inhibitory effect of almost 50%. A slight rise in toxicity is observed at approximately 120 min of reaction. Nevertheless, as the treatment progresses, the toxicity diminishes. At the end of the photodegradation, inhibition has been reduced to 17%.

According to the toxicity categories established in EU legislation, glyphosate is harmful to *V. fischeri* (EC_{50}, 30 min = 44.2 mg L^{-1}) where the EC_{50} is the effective concentration of a chemical that causes a 50% reduction in the bioluminescence of the bacteria.[38] A similar value is obtained for M_0, which corresponds to a concentration of 50 mg a.e. L^{-1} of glyphosate (although in this case the commercial formulation was employed).

The slight rise in the toxicity at 120 min of reaction time could be attributed to the presence of other toxic compounds. Notably, nearly 30% of the initial TOC content of the sample still remains after 360 min, which corresponds to a glyphosate concentration of 4 a.e. mg L^{-1} (Fig. 3).

Survival of tadpoles was 100% in the NC. The same result was obtained in the treated samples after 240 and 360 min without removal of H_2O_2 (M_2 and M_3), and in the all treated samples at 120, 240 and 360 min with H_2O_2 removed ($M_{1,E}$, $M_{2,E}$ and $M_{3,E}$) (Fig. 5). However, in the remaining samples (PC, M_{0-48h}, M_{1-24h} and M_{1-48h}) the larval mortality was 100% with respect to NC ($\chi 2 = 72.76\%$, df = 1, $P < 0.01$), with the exception of M_{0-24h} where the larval

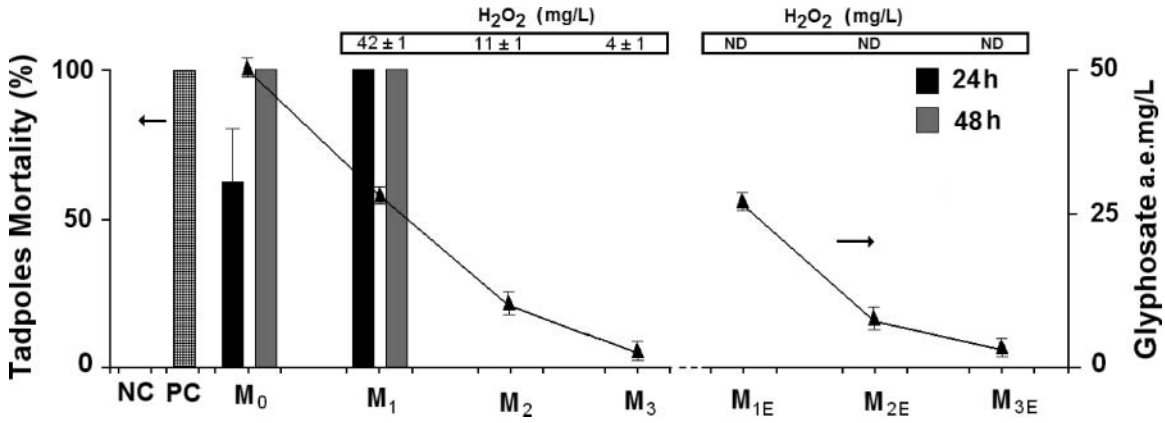

Fig. 5. Change in glyphosate concentration in water and total tadpole mortality at three different times during the H_2O_2/UVC process. NC = Negative Control; PC = Positive Control (H_2O_2 120 mg L^{-1}); M_0 = untreated sample (50 mg L^{-1}); M_1, M_2 and M_3 = samples at 120, 240 and 360 min, respectively, of the H_2O_2/UVC process (H_2O_2 not yet removed); $M_{1,E}$, $M_{2,E}$ and $M_{3,E}$ = samples at 120, 240 and 360 min, respectively, of the H_2O_2/UVC process (H_2O_2 removed). The data are expressed as the mean ± SEM.

mortality was 60% ($\chi 2$: 14.74, df = 1, $P < 0.01$) (Fig. 5). Figure 5 shows also the change in the concentration of glyphosate with the time of reaction: the initial concentration of glyphosate (50 mg a.e. L^{-1}) decreased to 27 mg a.e. L^{-1} for M_1, 11 mg a.e. L^{-1} for M_2, and 4 mg a.e. L^{-1} for M_3.

In this study, the survival of *R. arenarum* tadpoles in the presence of glyphosate at < 27 mg a.e. L-1 was similar to that observed in other studies for glyphosate commercial formulations (1.85–60 mg a.e. L^{-1}).[12] However, the combined effects of glyphosate at 27 mg L^{-1} plus H_2O_2 (42 mg L^{-1}) in the sample M_1 produced 100% mortality of tadpoles exposed to 24 and 48 h. Therefore, next, the H_2O_2 was removed, and no mortalities were observed in *R. arenarum* tadpoles in all the evaluated samples ($M_{1,E}$, $M_{2,E}$ and $M_{3,E}$).

Enzymatic determinations

The mean values of the AChE activities in the control tadpoles was 16.62 ± 1.32 nmol $min^{-1}mg^{-1}$ of protein at 48 h. The AChE activities varied significantly in $M_{2,E}$ with H_2O_2 removed ($P < 0.05$) with a percentage of AChE inhibition equal to 26.5% (Fig. 6).

Control BChE activities were 4.23 ± 0.45 nmol $min^{-1}mg^{-1}$ of protein at 48 h. BChE activities varied significantly in $M_{1,E}$ ($P < 0.01$) and $M_{2,E}$ ($P < 0.05$), where both samples had H_2O_2 removed. The percentage of inhibition varied from 25.05% for $M_{1,E}$ to 27.89% for $M_{2,E}$ (Fig. 7).

Nevertheless, sub-lethal effects (significant inhibition of cholinesterases) were detected in both $M_{1,E}$ and $M_{2,E}$ (BChE) and $M_{2,E}$ (AChE). Interestingly, the removal of H_2O_2 causes the most significant enzymatic inhibition. Regarding this point, Schallreuter el at.[39] postulated that low concentrations of H_2O_2 might increase the AChE activity. In addition, Zhang et al.[40] show that H_2O_2 increased AChE expression via transcriptional activation. Nonetheless, several aspects of the chemical interactions, such as the efficacy of H_2O_2/UVC processes in those aquatic ecosystem restorations that have undergone long-time exposure, need to be further explored.

The toxicity assays for evaluating the H_2O_2/UVC process efficiency

According to the ecotoxicity assays the conditions corresponding to M_2 (11 ± 1 mg a.e. L^{-1} of glyphosate and 11 ± 1 mg L^{-1} of H_2O_2) could be the final point of the

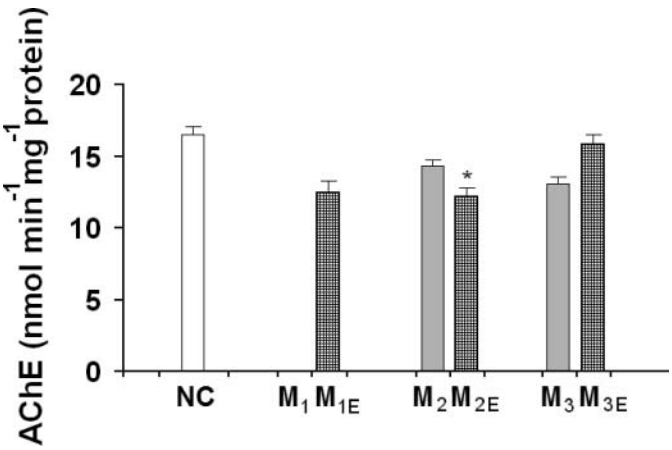

Fig. 6. Values of acetylcholinesterase (AChE) activity in surviving *R. arenarum* tadpoles exposed to samples collected at different times during the H_2O_2/UVC process. NC = Negative Control; M_1, M_2 and M_3 = samples at 120, 240 and 360 min, respectively, of the H_2O_2/UVC process (H_2O_2 not yet removed); $M_{1,E}$, $M_{2,E}$ and $M_{3,E}$ = samples at 120, 240 and 360 min, respectively, of the H_2O_2/UVC process (H_2O_2 removed). Bars represent the mean ± SEM. $^*P < 0.05$; $^{**}P < 0.01$ compared with NC by Dunnett's multiple range test.

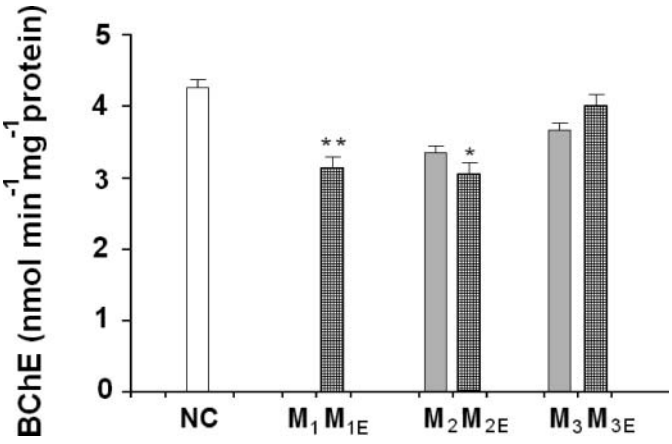

Fig. 7. Values of butyrylcholinesterase (BChE) activity in surviving *R. arenarum* tadpoles exposed to samples collected at different times during the H_2O_2/UVC process. NC = Negative Controls; M_1, M_2 and M_3 = samples at 120, 240 and 360 min, respectively, of the H_2O_2/UVC process (H_2O_2 not yet removed); $M_{1,E}$, $M_{2,E}$ and $M_{3,E}$ = samples at 120, 240 and 360 min, respectively, of the H_2O_2/UVC process (H_2O_2 removed). Bars represent the mean ± SEM. $^*P < 0.05$; $^{**}P < 0.01$ compared with NC by Dunnett's multiple range test.

treatment with the H_2O_2/UVC process. Although nearly 46% of the initial TOC content still remains in the sample, the survival of tadpoles was 100%, and the AChE and BChE activities were normal. The bacterium *V. fischeri* would be more sensitive to the reaction intermediates than tadpoles. A minor percent of inhibition of *V. fischeri* was obtained for the sample M_{3E}, but at M_{2E}, the inhibition was less than 50%.

In conclusion, the results of our study clearly show that it is not necessary to achieve a complete mineralization of the glyphosate. In addition, the conditions for M_2 as a final point of the treatment represent a minor operating cost of the process.

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