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Determination of glyphosate, AMPA and glufosinate by high performance liquid chromatography with fluorescence detection in waters of the Santarém Plateau, Brazilian Amazon

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ABSTRACT

Herbicide use, mainly glyphosate, has been intense in worldwide agriculture, including in the Brazilian Amazon region. This study aimed to validate a method for determining glyphosate and its degradation product, AMPA, and glufosinate by HPLC-FL in 58 water samples collected at the Santarém plateau region (*Planalto Santareno*), in the western of Pará state, Brazil. The method involves filtration and direct injection in the HPLC-FL for AMPA analysis, or previous concentration $(10\times)$ by lyophilization for glufosinate and glyphosate analysis. Analytes were oxidized and complexed with o-phthalaldehyde and 2-mercaptoethanol in a post-column reaction before fluorescence detection. LOQs for AMPA, glyphosate and glufosinate were established at 0.5, 0.2 and $0.3 \,\mu g L^{-1}$, respectively. A total of 58 samples were collected in 2015 (<LOQ), and AMPA was detected in any of the 30 surface water samples collected in 2015 (<LOQ), and AMPA was detected in 2017 and analyzed for glyphosate, which was detected in 11 samples (7 ground water samples), with concentrations between 1.5 and 9.7 $\mu g L^{-1}$. A continuous pesticide monitoring of the Amazonian water system is essential to guarantee the preservation of this important ecosystem.

Introduction

The increased demand for food due to the high population growth rates during the 19th and 20th centuries led to a fast development of agriculture that culminated in the Green Revolution in the mid-1960s, which incorporated new cultivation and pest management technologies.^[1] Pesticide use for pest control had a major positive impact on agricultural productivity, however, the use of these compounds can contaminate aquatic environments and their organisms.^[2]

Brazil is one of the largest agricultural producers worldwide and also one of the three largest pesticide users, following China and the United States.^[3] Glyphosate is the most commercialized pesticide in Brazil, with more than 195 thousand tons sold in 2018.^[4] The state of Pará, in Northern Brazil, has an expansion rate in soybean production above the national average, with 1.6 million tons in 2017, accounting for 30% of the regional production,^[5] with over 3 thousand tons of glyphosate commercialized in the state in 2018.^[4] The Santarém plateau, in the western region of Pará, had its agricultural area considerably expanded over the past few years, mainly with soybean.^[6] Since soybean production is directly linked to pesticide use in general, and

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specifically glyphosate, environmental monitoring in agricultural regions that use this herbicide is important. Furthermore, water contaminated by pesticides in rural areas can also pose risks to the local population, considering that it can be directly consumed by its inhabitants. Glyphosate has been classified by the International Agency for Research on Cancer as probably carcinogenic to humans.^[7]

Glyphosate can reach the water through spillage, runoff, and/or leaching, and its transport is influenced by soil composition and rainfall; it is highly water soluble and has a high soil adsorption coefficient, with a low probability of running off from fields, persist in surface waters, or leach to ground water.^[8] Half-lives of glyphosate and amino methyl phosphonic acid (AMPA), its main degradation product in the environment, range from 7 to 14 days.^[9] Several studies, however, have reported glyphosate and AMPA in surface and ground water, mainly close to agricultural areas.^[10,11]

High Performance Liquid Chromatography (HPLC) associated with fluorescence detector (HPLC-FL) has been widely used for the analysis of glyphosate and AMPA, with high sensitivity and specificity.^[12,13] Detection by fluorescence detector occurs after post-column derivatization using

CONTACT Eloisa Dutra Caldas Seloisa@unb.br E Laboratory of Toxicology, Faculty of Health Sciences, University of Brasília, Campus Darci Ribeiro, 70910-900Brasília, DF, Brazil. OPA (*o*-phtalaldehyde) and 2 -mercaptoethanol, which is a classic technique,^[14] or pre-column derivatization using FMOC-Cl (9-fluorenylmethylchloroformate).^[15] Methods using liquid chromatography coupled to mass detectors (LC-MS or LC-MS/MS) have also been reported,^[16–18] however the availability of these equipment is more restricted.

This work aimed to validate an analytical method for the determination of glyphosate, AMPA and glufosinate, an herbicide structurally similar to glyphosate also used in soybean cultivation, in water samples by HPLC-FL and post-column derivatization with OPA and 2-mercaptoethanol after concentration by lyophilization. The validated method was used to analyze water samples collected from the region of extensive soybean cultivation in the Santarém plateau, State of Pará, Brazilian Amazon region.

Material and methods

Analytical standards

AMPA was acquired from Aldrich® (99% purity), and glyphosate (99% purity) and glufosinate (98.3% purity) from Fluka[®]. Standard stock solutions were prepared in ultrapure water produced by a Milli-Q system (Millipore[®]) at a concentration of 1 mg/mL and stored in amber *vial* at -20 °C. Working solutions containing all three analytes were prepared daily from the stock solutions. Analytical standard curves were prepared with ultrapure water at levels of 0.5, 1, 7, 10, 25, 35 and 50 µg L⁻¹ for AMPA; 2, 7, 10, 25, 35 and 50 µg L⁻¹ for glyphosate; and 0.7, 2, 7, 10, 25, 35 and 50 µg L⁻¹ for glufosinate.

HPLC-FL conditions

The HPLC-FL analyses were conducted in a Shimadzu® LC20A system (Kyoto, Japan) consisting of autosampler (SIL-20SA), quaternary pump (LC20AT), column oven (CTO-20SAC), system controller (CBM-20A), post-column reaction module (CRB-6A) and fluorescence detector (10AXL). Excitation and emission wavelengths were set at 340 and 455 nm, respectively.

Chromatographic separation was obtained using an anion exchange column (PRP-X100, $10 \,\mu\text{m}$, $250 \times 4.1 \,\text{mm}$; Phenomenex[®]) and a pre-column model CTO-20SAC from Shimadzu[®]. The composition of the mobile phase was optimized after varying the concentration of methanol (HPLC grade; Merck[®]) from 4 to 20%, testing potassium phosphate (KH₂PO₄; Vetec[®]) concentration at 5 and $10 \,\mu\text{M}$, and pH from 1.9 to 2.2, adjusted with phosphoric acid (Merck[®]) in a pH meter (AJ Micronal[®], AJX-512). The isocratic mobile phase that gave the best performance in the HPLC system was 8% MeOH:92% $10 \,\mu\text{M}$ KH₂PO₄-water buffered at pH 2.1. The flow rate of the mobile phase was 0.5 mL min⁻¹.

Calcium hypochlorite oxidizing solution was prepared daily: KH_2PO_4 (0.14%), NaCl (0.12%), NaOH (0.04%) and $Ca(OCl)_2$ (0.002%). The complexing *o*-phtalaldehyde/2-mer-captoethanol (OPA/ME, 1 L) solution was prepared with 19.1 g of $Na_2B_4O_7$ ·10H₂O, 0.1 g OPA, previously dissolved in

10 mL of methanol, and $50 \,\mu$ L of ME dissolved in $50 \,\mu$ L of acetonitrile. Na₂B₄O₇·10H₂O, NaCl and NAOH were obtained from Dinâmica[®], Ca(OCl)₂, OPA, methanol and acetonitrile were acquired from Merck[®], and ME from Sigma[®]. Post-column derivatization solution flow was optimized to 0.5 mL min⁻¹, and the optimum reaction module temperature was 33 °C. The mobile phases and reagent solutions were vacuum filtered using 0.45 µm polytetrafluoro-ethylene (PTFE) microfibers (Millipore[®]) and degassed in an ultrasonic bath (Model USC – 3300 of the Unique[®]) for 15 min before use.

Sample preparation and method validation

A 5 mL aliquot of the water sample was transferred to a 15 mL falcon tube and lyophilized (K105; LIOBRAS; temperature below -70 °C and pressure below 100 μ Hg). After lyophilization, the sample was resuspended in 500 μ L of ultrapure water (10× sample concentration) and injected on the HPLC-FL.

The optimized analytical method was validated for selectivity, linearity, robustness, precision, and accuracy (recovery) according to international guidelines.^[19]

Selectively was evaluated by comparing a blank water matrix (surface water free of pesticides collected in the study area) with the sample fortified with all the analytes. Linearity (least square method), repeatability and intermediate precision of the analytical standard curves in the HPLC-FL were assessed using analytical curves prepared and analyzed on the same day or on different days by the same analyst. Repeatability (same day) and intermediate precision (7 consecutive days) were expressed as relative standard deviation (RSD, %).

Recovery of the method procedure was evaluated by fortifying an aliquot of the pesticide-free surface water sample with glyphosate, glufosinate and AMPA at three levels (0.2 up to $2 \mu \text{g L}^{-1}$), and the samples submitted to the procedure described before. Repeatability of the analytical procedure was assessed by analyzing fortified samples on the same day (n=6), and the intermediate precision was assessed by analyzing the samples on 2 different days (n=12).

Study area and sampling

The Santarém plateau is located in western region of Pará state, Brazilian Amazon, encompassing the cities of Santarém, Belterra and Mojuí dos Campos (Fig. 1). This plateau is formed by the Curuá-Una river basin, which has several streams (*igarapés*) and other tributary rivers, mainly Moju, Mojuí and Igarapé Poraquê.^[20] The local landscape is composed by a mosaic of tropical forest (Amazon Biome) cut by a dense water drainage network and occupied by soybean fields and livestock, the latter for subsistence and commercial purposes.

Samples were strategically collected in *igarapés* and streams, shallow wells and in a hydroelectric reservoir in the Santarém plateau region, close to soybean growing fields. Thirty surface water samples were collected in February



Figure 1. Water sample collecting points in the Santarém plateu, Pará state, Brazil (prepared using MapBiomas 2018).

2015, and 28 samples collected in May 2017, from which 10 ground water samples (shallow wells) and 18 surface water samples. The rural communities where the samples were collected are shown in Fig. 1, and the geographical location of each collecting point indicated in Table A1 (Appendix). At each point, a bucket was dipped to a depth of 15 to 20 cm, washed three times with the water and then filled.^[21] Approximately 100 mL of the sample was filtered through 0.45 μ m of PTFE microfiber, transferred to polyethylene bottles, kept refrigerated in a thermal box with reusable ice^[22] and sent by air for analysis at the Laboratory of Toxicology at the University of Brasilia, where they remained at -20 °C until analyzed.

Results and discussion

Method validation

Initial tests showed that lyophilization increased the response of interferents at the AMPA retention time, and this step was eliminated for the determination of this analyte. Hence, to determine the concentration of AMPA, the samples were directly injected in the HPLC-FL, while another sample was lyophilized before injecting in the system for determining glyphosate and glufosinate. Figure 2 shows the analytical procedure used in the study.

The chromatographic method proved to be selective, with no matrix interferences observed in the AMPA, glyphosate and glufosinate retention times. The system, however, is not robust as is very sensitive to small pH variations in the mobile phase. When the pH was decreased from 2.1 to 1.9, the response for glyphosate decreased considerably, and no glufosinate was detected in the chromatogram when the pH was adjusted to 2.2. As a compromise, the pH of the mobile phase was set at 2.1, which guarantees enough ionization of both glyphosate (pKa1 = 2-2.3)^[23] and glufosinate (pKa1 = <2)^[23] for interaction with the anion exchange column and optimum post-column derivatization.

The HPLC-FL response was linear for all analytes, with a coefficient of determination (\mathbb{R}^2) adjusted by the least square method greater than 0.99 in all standard curves tested. The homoscedasticity of the analytical curves was confirmed by the Cochran test. The repeatability of the analytical curve response ranged from 1.8% to 11.5% for all points (n=6) and intermediate precision ranged from 9 to 18.8% (n=7) (Table 1).

There was no handling of the sample for AMPA determination that could affect its nominal recovery, with the exception of sample filtration. Hence, the limit of quantification (LOQ) for this analyte was set at $0.5 \,\mu g \, L^{-1}$, which is the lowest level of the analytical curve that showed good repeatability and intermediate precision (Table 1). The recovery, repeatability and intermediate precision of the method for glyphosate and glufosinate, which involves lyophilization, are shown in Table 2. The recoveries ranged from 70 to 120% and repeatability an intermediate precision were below 10%, within the values considered satisfactory (recovery between 70 and 120% and precision less than



Figure 2. Method for the analysis of glyphosate, AMPA and glufosinate in water samples by HPLC-FL and pos-column derivatization with phtalaldehyde (OPA) and 2-mercaptoethanol (ME).

20%).^[19] The method's LOQs were defined as the lowest level of fortification that met the validation criteria, being established at 0.3 μ g L⁻¹ for glufosinate and 0.2 μ g L⁻¹ for glyphosate (Table 2).

The pre-concentration method commonly used in the analysis of pesticides in water, including glyphosate and AMPA, is solid-phase extraction (SPE).^[16,24,25] However, the SPE cartridge is expensive and the method uses organic solvent during preparation and elution. Water sample preconcentration by lyophilization used in this study is simple, cheap, less subject to losses and more environmentalfriendly as it does not use any organic solvent. Ramirez et al.^[15] used this procedure (20× concentration) for glyphosate and AMPA analysis by HPLC-FL after pre-column derivatization with FMOC-Cl, with LODs of 0.058 and $0.108 \,\mu g L^{-1}$, respectively. Sinha et al.^[18] analyzed various pesticides (not glyphosate) in water by LC-MS/MS after lyophilization, with LOQ of $0.1 \,\mu g \, L^{-1}$. To the best of our knowledge, this is the first study that uses lyophilization to pre-concentrate water samples for analysis of glyphosate and glufosinate after derivatization with OPA

Analysis of water samples collected in Santarém Plateau

The 30 surface water samples collected in February 2015 were lyophilized and analyzed in July 2015. Glyphosate and glufosinate were not detected in any sample (<LOQ). AMPA was detected in 6 samples, at levels between 0.65 and 1.9 μ g L⁻¹ (Table 3), indicating that, at some point, glyphosate was present in water as result of its application in

Table 1. Repeteability and intermediate precision of the HPLC-FL system using fortified ultrapure water. RSD = relative standard deviation.

	AMPA					
Concentration, μ g L ⁻¹	Repeteability $n = 6$ RSD (%)	Intermediate precision $n = 7$ RSD (%)				
0.5	7.1	18.8				
1	7.3	16.5				
10	10.9	7.1				
25	4.2	11.5				
35	2.7	15.3				
50	2.4	16				
Glufosinate						
2	11.5	16.9				
7	4.6	15.3				
10	3.0	9				
25	1.8	15.1				
35	3.1	12.5				
50	2.7	13.8				
Glyphosate						
0.7	5.9	17				
2	6.3	11.3				
10	4.4	13.9				
25	3.7	13.6				
35	4.6	12.5				
50	2.9	12.4				

the field. The non-detection of glyphosate in the samples analyzed during this period may have been due to its degradation during the storage period (5 months, at -20 °C), although the stability of glyphosate in frozen water samples has been demonstrated for up to 18 months.^[14]

In May 2017, 28 water samples were collected (10 ground water from shallow wells and 18 surface water), immediately lyophilized and stored at -20 °C for later analysis of glyphosate only, which occurred in July/August of that same year. Glyphosate was detected in 11 of the 28 samples analyzed, at levels between 1.5 and 9.7 µg L⁻¹, of which 7 were ground water samples, which also had the highest levels (Table 3). Figure 3 shows the chromatograms of two samples that contained quantified AMPA (2015 collection) and glyphosate (2017 collection) levels. The different retention times of glyphosate in the chromatographic system in the two moments are probably due to the new column (same specification, same brand) that was used in 2017, indicating that the chromatographic system is very sensitive to any condition change.

The Riacho Verde community (shown in Fig. 1) had positive samples in 2015 and 2017, which may be related to its location in the watershed. This community receives water that passes through several other communities, goes downstream and flows into the Curuá-Una hydroelectric reservoir. From the 7 samples collected in the reservoir in 2015, one contained AMPA, at the highest level found in all positive samples (1.92 µg/L; Table 3). The levels found in the samples for glyphosate and AMPA were much lower than the maximum permitted level of 500 µg/L (glyphosate alone or in combination with AMPA) established by Brazilian National Environment Council's for surface water and ground water for human consumption.^[26,27] In Europe, the upper tolerable level for all the pesticides in drinking water is administratively set to $0.1 \mu g L^{-1}$.^[28]

The Brazilian National Drinking Water Quality Surveillance Program provides data on pesticide analysis in

Table 2. Recovery (%) and precision (% RSD) of the analytical method for glyphosate and glufosinate using fortified blank surface water.

Concentration, µg L ⁻¹	Recovery $n = 6$ (%)	Repeatability $n = 6$, RSD (%)	Intermediate precision $n = 12$, RSD (%)
Glyphosate			
0.2	74	2.1	3.0
0.6	84	7.9	9.4
1.0	82	9.1	9.9
Glufosinate			
0.3	72	1.8	2.6
1.2	86	5.9	5.4
2.0	94	4.0	3.4

Table 3. Levels of glyphosate and AMPA found in the positive water samples (\geq LOQ) collected in 2015 and 2017 in the Mojuí dos Campos (MC) and Santarém (SA) counties, Pará state, Brazil.

Local (County)	inty) Water Collection, month/year		Analysis, month/year	Glyphosate, μ g L ⁻¹	AMPA, μ g L ⁻¹
Reservoir (SA)	servoir (SA) Surface 02/2015		07/2015	< LOQ	1.9
Rio Moju (MC)	Surface 02/2015		07/2015	< LOQ	1.2
Rio Moju (MC)	Surface	02/2015	07/2015	< LOQ	0.87
Riacho verde (MC)	Surface	02/2015	07/2015	< LOQ	0.86
Riacho verde (MC)	Surface	02/2015	07/2015	< LOQ	0.65
Rio Moju (MC)	Surface	02/2015	07/2015	< LOQ	0.65
Riacho Verde (MC)	Surface	05/2017	08/2017	2.3	Na
Riacho Verde (MC)	Surface	05/2017	08/2017	2.0	Na
Riacho Verde (MC)	Surface	05/2017	08/2017	1.7	Na
Riacho Verde (MC)	o Verde (MC) Surface 05/2017		08/2017	1.6	Na
Açaizal (SA)	Ground	05/2017	07/2017	9.7	Na
Guaraná (SA)	Ground	05/2017	07/2017	8.3	Na
Açaizal (SA)	Ground	05/2017	07/2017	6.0	Na
Boa Sorte (MC)	Ground	05/2017	08/2017	3.8	Na
Açaizal (SA)	Ground	05/2017	07/2017	2.2	Na
Riacho Verde (MC)	Ground	05/2017	08/2017	2.2	Na
Boa Sorte (MC)	Ground	05/2017	08/2017	1.5	Na

Na: not analyzed.

water for human consumption.^[29] Information from the period of 2014–2019 showed that about 10% of the contamination data concerns the Northern region, from which about one third from Pará state (3816 entries). Over 90% of the data from Pará showed pesticide levels < LOD/LOQ, and detected levels ranged from 0.001 to 0.005 μ g L⁻¹; 40 samples were analyzed for glyphosate/AMPA, with 80% of the samples < LOD/LOQ. The number of samples analyzed is not clearly reported in the database, neither the analytical method used; in most cases where a finite number was reported, there was no information on LOD and/or LOQ.

Few studies conducted in Brazil that analyzed glyphosate in water have been published, and none analyzed samples collected in the Amazon region. In a study conducted in São Paulo state, glyphosate was detected in 13 of the 32 surface water samples collected at 4 points on the Corumbataí river, close to sugarcane cultivation areas, but none of the samples contained quantifiable levels (LOQ of $1 \mu g L^{-1}$).^[30] In Rio Grande do Sul state, glyphosate was detected in 46.7% of the 15 water samples collected in the Passo do Pilão stream, with levels above 100 µg/L in two samples collected in an area of intense corn cultivation, in which glyphosate was used as a desiccant.^[31] In Chapecó (Santa Catarina state), glyphosate was detected in 5 of the thirteen deep tubular wells distributed in urban and rural areas, with a maximum concentration of 6.80 μ g L⁻¹^[32] close to the highest levels found in shallow well samples in the present study (6–9.7 μ g L⁻¹). More recently, Correia et al.^[33] analyzed various pesticides in 287 water samples collected from 20 farms in the Middle West region of Brazil, including surface and ground water. Glyphosate was detected in 3.4% of the samples, but only two at level greater than the LOQ of $1.2 \,\mu\text{g L}^{-1}$ (up to $11 \,\mu\text{g L}^{-1}$), and AMPA was not detected in any sample.

Glyphosate and AMPA levels in surface waters from other countries are also generally low.^[11] In a study involving 51 watercourses close to agricultural areas in the United States of America, glyphosate was detected in 36% of the 154 samples analyzed (highest level of 8.7 μ g L⁻¹), AMPA was detected in 69% of the samples (highest of $3.6 \,\mu g L^{-1}$) and glufosinate in two samples (less than $1 \mu g L^{-1}$).^[34] Higher glyphosate levels, between 100 and 700 μ g L⁻¹, were found in surface water near a transgenic soybean field in Argentina, with a direct correlation with time of pesticide application and rain events.^[35] In another study conducted in the country, stream sediments samples had the highest frequency of detections (glyphosate 95%, AMPA 100%), followed by surface water (glyphosate 28%, AMPA 50%) and groundwater (glyphosate 24%, AMPA 33%).^[36] In an agricultural area in western Yucatan peninsula, in Mexico, the highest glyphosate concentration found in 29 ground water collecting points was $1.41 \,\mu g L^{-1}$.^[37] In Malaysia, glyphosate and AMPA concentrations in surface water collected from an oil palm plantation area reached 6.23 and 3.76 μ g L⁻¹, respectively.^[38]

This study has two important limitations. The pre-concentration procedure by lyophilization cannot be used for AMPA, due to interferences that appear in the retention time of the analyte in the HPLC-FL system. Another limitation refers to the sample storage time, which reached



Figure 3. Chromatogram of a water sample collected on A: reservoir, in 2015, 1.93 μ g L⁻¹AMPA; Standards: 2 μ g L⁻¹ AMPA and glyphosate, 3 μ g L⁻¹glufosinate. B: Açaizal in 2017, 9.7 μ g L⁻¹ glyphosate; Standards: 8.0 μ g L⁻¹ glyphosate. A new column (same specification and brand) was used in 2017.

3 months in the first collecting period (2015), compromising glyphosate and glufosinate detection. Lyophilizing the sample right after collection in the second period (2017) was important for its preservation, enabling glyphosate detection.

Conclusions

This study satisfactorily optimized and validated a modified classic method of glyphosate and AMPA analysis in water samples by HPLC-FL after analyte derivatization with OPA, also including the herbicide glufosinate. Sample preconcentration using lyophilization proved to be easy to be implemented for the analysis of glyphosate and glufosinate, and when carried out right after sample collection, it preserves the integrity of analytes, which is essential when the samples cannot be analyzed right after collection.

This is the first study that investigated the presence of glyphosate, AMPA and glufosinate in the Brazilian Amazon region, where the agricultural area has been considerably expanded over the past few years. Although glyphosate levels in water were low, constant pesticide monitoring of waterbodies close to agricultural regions is important to better understand environmental processes, impacts on the watershed, and a potential risk for the human population living on the surroundings of large plantations.

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References

- Pingali, P. L. Green Revolution: Impacts, Limits, and the Path Ahead. *Proc. Natl. Acad. Sci. USA.* 2012, 109, 12302–12308. DOI: 10.1073/pnas.0912953109.
- [2] Solomon, K. R.; Stephenson, G. R.; Corrêa, C. L.; Zambrone, F. A. D. Praguicidas e o Meio Ambiente. 2010. São Paulo: International Life Sciences Institute do Brasil.
- Food and Agriculture Organization of the United Nations.
 FAOSAT Pesticide use. http://www.fao.org/faostat/en/#data/ RP (accessed Apr 13, 2020).
- [4] Instituto Brasileiro de Meio Ambiente. Relatórios de Comercialização de Agrotóxicos. http://www.ibama.gov.br/agrotoxicos/relatorios-de-comercializacao-de-agrotoxicos#sobreosrelatorios (accessed Mar 11, 2020).
- [5] Companhia Nacional de Abastecimento. Acompanhamento da Safra Brasileira - Grãos: Safra 2017/18. Monitoramento Agrícola. CONAB 2018, 1–29. https://www.conab.gov.br/info-agro/safras/ graos/monitoramento-agricola (accessed Mar 11, 2020).
- [6] Sauer, S. Soy Expansion into the Agricultural Frontiers of the Brazilian Amazon: The Agribusiness Economy and Its Social and Environmental Conflicts. *Land Use Policy* 2018, 79, 326–338. DOI: 10.1016/j.landusepol.2018.08.030.
- [7] International Agency for Cancer Research. Some Organophosphate Insecticides and Herbicides. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 112. https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Identification-Of-Carcinogenic-Hazards-To-Humans/Some-Organophosphate-Insecticides-And-Herbicides-2017. (Accessed March 11 2020)
- [8] Caldas, E. D. Toxicological Aspects of Pesticides. In Sustainable Agrochemistry, 1st ed. S. Vaz Jr. (Ed.); Switzerland AG: Springer Nature, 2019, pp 275–305.
- [9] Giesy, J.; Dobson, S.; Solomon, K. R. Ecotoxicological Risk Assessment for Roundup[®] Herbicide. In: *Reviews of Environmental Contamination and Toxicology 167*; Ware, G. W. Eds.; Springer, New York, NY, **2000**.
- [10] Battaglin, W. A.; Meyer, M. T.; Kuivila, K. M.; Dietze, J. E. Glyphosate and Its Degradation Product AMPA Occur Frequently and Widely in U.S. soils, Surface Water, Groundwater, and Precipitation. J. Am. Water Resour. Assoc. 2014, 50, 275–290. DOI: 10.1111/jawr.12159.
- [11] Van Bruggen, A. H. C.; He, M. M.; Shin, K.; Mai, V.; Jeong, K. C.; Finckh, M. R.; Morris, J. G. Jr. Environmental and Health Effects of the Herbicide Glyphosate. *Sci. Total Environ.* 2018, *616-617*, 255–268. DOI: 10.1016/j.scitotenv.2017.10.309.
- [12] Melo, K. G.; De Nucci, G.; Trape, A. Z.; Jacobucci, S. R. F.; Garlipp, C. R.; Rosa, P. C. P. Brief Review Analytical Methods for the Determination of Glyphosate. *MOJ Tox.* **2018**, *4*, 86–89. DOI: 10.15406/mojt.2018.04.00088.

- [13] Colombo, S. M.; Masini, J. C. A Sequential-Injection Reversed-Phase Chromatography Method for Fluorimetric Determination of Glyphosate and Aminomethylphosphonic Acid. Anal. Methods 2014, 6, 490–496. DOI: 10.1039/C3AY41594E.
- [14] United State Environmental Protection Agency. Method 547. Determination of glyphosate in drinking water by direct-aqueous-injection HPLC, post-column derivatization, and fluorescence detection. Environmental Monitoring Systems Laboratory Office of Research and Development U.S. Environmental Protection Agency. 1990. Cincinnati, Ohio. DOI: 10.1241/johokanri.4.12_19.
- [15] Ramirez, C. E.; Bellmund, S.; Gardinali, P. R. A Simple Method for Routine Monitoring of Glyphosate and Its Main Metabolite in Surface Waters Using Lyophilization and LC-FLD + MS/MS. Case study: canals with influence on Biscayne National Park . *Sci. Total Environ.* 2014, 496, 389–401. DOI: 10.1016/j.scitotenv.2014.06.118.
- [16] Hanke, I.; Singer, H.; Hollender, J. Ultratrace-Level Determination of Glyphosate, Aminomethylphosphonic Acid and Glufosinate in Natural Waters by Solid-Phase Extraction Followed by Liquid Chromatography-Tandem Mass Spectrometry: performance Tuning of Derivatization, Enrichment and Detection. *Anal. Bioanal. Chem.* 2008, 391, 2265–2276. DOI: 10.1007/s00216-008-2134-5.
- [17] Ibáñez, M.; Pozo, O. J.; Sancho; J, V.; López, F. J.; Hernández, F. Residue Determination of Glyphosate, Glufosinate and Aminomethylphosphonic Acid in Water and Soil Samples by Liquid Chromatography Coupled to Electrospray Tandem Mass Spectrometry. J. Chromatogr. A 2005, 1081, 145–155. DOI: 10. 1016/j.chroma.2005.05.041.
- [18] Sinha, S. N.; Vasudev, K.; Rao, M. V. V.; Odetokun, M. Quantification of Organophosphate Insecticides in Drinking Water in Urban Areas Using Lyophilization and High-Performance Liquid Chromatography-Electrospray Ionization-Mass Spectrometry Techniques. Int. J. Mass Spectrom 2011, 300, 12–20. DOI: 10.1016/j.ijms.2010.11.006.
- [19] European Commission Directorate General for Health and Food Safety; Guidance document on Analytical Quality Control and Method Validation Procedures for Pesticide Residues and Analysis in Food and Feed, SANTE/12682/2019. https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=727 (accessed Apr 13, 2020)
- [20] Azevedo, J. S.; Talgatti, D. M.; Torgan, L. C.; Pereira, A. C.; de Melo, S. The Genus Gomphonema (Bacillariophyta) in the Periphytic Community of the Curuá-Una River and Reservoir (Santarém, Pará, Brazil). *Rodriguesia* 2018, 69, 765–776. DOI: 10.1590/2175-7860201869234.
- [21] Parron, L. M.; Muniz, D. H. F.; Pereira, C. M. Manual de Procedimentos de Amostragem e Análise Físico-Química de Água. EMBRAPA - Documento 2011, 232, 69. https://doi.org/ Documentos/Embrapa. Florestas, ISSN 1980-3958 (accessed Mar 11, 2020)
- [22] Standard Methods for the Examination of Water and Wastewater. American Public Health Association, 23rd ed. E.W. Rice, R.B. Baird, A.D. Eaton, editors. 2017. Washington, USA.
- [23] PubChem. National Library of Medicine. National Center for Biotechnology Information. https://pubchem.ncbi.nlm.nih.gov/.
- [24] Mallat, E.; Barceló, D. Analysis and Degradation Study of glyphosate and of Aminomethylphosphonic Acid in Natural Waters by Means of Polymeric and Ion-Exchange Solid-Phase Extraction Columns Followed by Ion Chromatography-Post-Column Derivatization with Fluorescence Detection. J. Chromatogr. A 1998, 823, 129–136. DOI: 10.1016/S0021-9673(98)00362-8.
- [25] Corbera, M.; Hidalgo, M.; Salvadó, V. Extraction and Preconcentration of the Herbicide Glyphosate and Its Metabolite AMPA Using Anion-Exchange Solid Phases. *Microchim. Acta* 2006, 153, 203–209. DOI: 10.1007/s00604-005-0462-0.
- [26] Resolução CONAMA No. 357, March 17, 2005. Conselho Nacional do Meio Ambiente. Ministério do Meio Ambiente. Brazil, 2005.

- [27] Resolução CONAMA 396, April 3, 2008. Conselho Nacional do Meio Ambiente. Ministério do Meio Ambiente, Brazil, 2008
- [28] European Commission. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy. Official J. Europ. Union L 2000, 327, 0001–0073.
- [29] SISAGUA (Sistema de Informação de Vigilância da Qualidade da Água para Consumo humano). Amostras de Vigilância (Demais Parâmetros). Ministério da Saúde. http://www.dados. gov.br/dataset/sisagua-amostras-de-vigilancia-demais-parametros (accessed Mar 11, 2020)
- [30] de Armas, E. D.; Monteiro, R. T. R.; Antunes, P. M.; dos Santos, M. A. P. F.; de Camargo, P. B.; Abakerli, R. B. Diagnóstico Espaço-Temporal da Ocorrência de Herbicidas Nas Águas Superficiais e Sedimentos Do Rio Corumbataí e Principais Afluentes. *Quím. Nova* 2007, 30, 1119–1127. DOI: 10.1590/S0100-40422007000500013.
- [31] Da Silva, M. D.; Peralba, M. C. R.; Mattos, M. L. T. Determinação de Glifosato e Ácido Aminometilfosfônico em Águas Superficiais Do Arroio Passo Do Pilão. *Pes.* 2003, 13, 19–18. DOI: 10.5380/pes.v13i0.3161.
- [32] Delmonico, E. L.; Bertozzi, J.; de Souza, N. E.; Oliveira, C. C. Determination of Glyphosate and Aminomethylphosphonic Acid for Assessing the Quality Tap Water Using SPE and HPLC. Acta Sci. Technol. 2014, 36, 513–519. DOI: 10.4025/ actascitechnol.v36i3.22406.
- [33] Correia, N. M.; Carbonari, C. A.; Velini, E. D. Detection of Herbicides in Water Bodies of the Samambaia River Sub-Basin

in the Federal District and Eastern Goiás. [published online ahead of print, 2020 Mar 19] *J. Environ. Sci. Health B* **2020**, 1–9. DOI: 10.1080/03601234.2020.1742000.

- [34] Battaglin, W. A.; Kolpin, D. W.; Scribner, E. A.; Kuivila, K. M.; Sandstrom, M. W. Glyphosate, Other Herbicides, and Transformation Products in Midwestern Streams, 2002. J. Am. Water Resources Assoc. 2005, 41, 323–332. DOI: 10.1111/j.1752-1688.2005.tb03738.x.
- [35] Peruzzo, P. J.; Porta, A. A.; Ronco, A. E. Levels of Glyphosate in Surface Waters, Sediments and Soils Associated with Direct Sowing Soybean Cultivation in North Pampasic Region of Argentina. *Environ. Pollut.* 2008, 156, 61–66. DOI: 10.1016/j. envpol.2008.01.015.
- [36] Okada, E.; Pérez, D.; De Gerónimo, E.; Aparicio, V.; Massone, H.; Costa, J. L. Non-Point Source Pollution of Glyphosate and AMPA in a Rural Basin from the Southeast Pampas, Argentina. *Environ. Sci. Pollut. Res. Int.* **2018**, *25*, 15120–15132. DOI: 10. 1007/s11356-018-1734-7.
- [37] Rendon-von Osten, J.; Dzul-Caamal, R. Glyphosate Residues in Groundwater, Drinking Water and Urine of Subsistence Farmers from Intensive Agriculture Localities: A Survey in Hopelchén, Campeche, Mexico. *IJERPH.* 2017, 14, 595. DOI: 10.3390/ijerph14060595.
- [38] Mardiana-Jansar, K.; Ismail, B. S. Residue Determination and Levels of Glyphosate in Surface Waters, Sediments and Soils Associated with Oil Palm Plantation in Tasik Chini, Pahang, Malaysia. *AIP Conf. Proc* 2014, 1614, 795–802. DOI: 10.1063/1.4895304.

Appendix

Table A1. W	ater samples collecte	ed in the Santarém p	plateau, state of Pa	rá, Brazil and a	analvzed for o	alvphosate, AMPA a	nd alufosinate by HPLC-FL.
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Sample	Latitude	Longitude	Place	Municipality	Collected, month/year	Analyzed, month/year	Glyphosate (µg/L)	AMPA (µg/L)	Glufosinate (μg/L)
1	02°37′34.4"S	054° 30' 19.8"W	Açaizal	Santarém	02/2015	07/2015	< LOQ	< LOQ	< LOQ
2	02°37′42.6"S	054° 30′ 14.1"W	Açaizal	Santarém	02/2015	07/2015	< LOQ	< LOQ	< LOQ
3	02°38′07.0"S	054°30′26.4"W	Açaizal	Santarém	02/2015	07/2015	< LOQ	< LOQ	< LOQ
4	02°37′34.4"S	054°30′19.8"W	Açaizal	Santarém	02/2015	07/2015	< LOQ	< LOQ	< LOQ
5	02°37′35.7"S	054°30′21.9"W	Açaizal	Santarém	02/2015	07/2015	< LOQ	< LOQ	< LOQ
6	02°37′35.8"S	054°30′21.8"W	Açaizal	Santarém	02/2015	07/2015	< LOQ	< LOQ	< LOQ
7	02°37′35.7"S	054°30′21.9"W	Açaizal	Santarém	02/2015	07/2015	< LOQ	< LOQ	< LOQ
8	02°45′51.2"S	054°23′51.8"W	Guaraná	Santarém	02/2015	07/2015	< LOQ	< LOQ	< LOQ
9	02°45′53.3"S	054°23′09.6"W	Guaraná	Santarém	02/2015	07/2015	< LOQ	< LOQ	< LOQ
10	02°48′02.0"S	054°26′09.3"W	Boa Sorte	Mojuí dos Campos	02/2015	07/2015	< LOQ	< LOQ	< LOQ
11	02°53′13.5"S	054°28′40.7"W	Riacho verde	Mojuí dos Campos	02/2015	07/2015	< LOQ	< LOQ	< LOQ
12	02°52′00.5"S	054°27′23.5"W	Riacho verde	Mojuí dos Campos	02/2015	07/2015	< LOQ	0.86	< LOQ
13	02°52′48.6"S	054°28′08.7"W	Riacho verde	Moiuí dos Campos	02/2015	07/2015	< L00	0.65	< L00
14	02°40′07.6"S	054°34′44.1"W	Rio Moiu	Moiuí dos Campos	02/2015	07/2015	< L00	0.65	< L00
15	02°39'29.8"S	054°33′16.2"W	Rio Moiu	Moiuí dos Campos	02/2015	07/2015	< L00	0.87	< L00
16	02°39'30.9"S	054°33′15.5"W	Rio Moiu	Moiuí dos Campos	02/2015	07/2015	< L00	1.21	< L00
17	02°39′30.5"S	054°33′15.6"W	Rio Moiu	Moiuí dos Campos	02/2015	07/2015	< L00	< L00	< L00
18	02°41′12.6"S	054° 38' 35.2"W	Rio Moiu	Mojuí dos Campos	02/2015	07/2015	< L00	< L00	< L00
19	02°39′30.9"S	054°33′15.5"W	Rio Moiu	Mojuí dos Campos	02/2015	07/2015	< 100	< 100	< 100
20	02°40′28.6"S	054° 35′ 02.4"W	Rio Moiu	Mojuí dos Campos	02/2015	07/2015	< 100	< 100	< 100
21	02°41′04 6"S	054° 36′ 20 1"W	Rio Moju	Mojuí dos Campos	02/2015	07/2015	< 100	< 100	< 100
22	02°41′12 0"S	054° 33′ 35 6"W	Rio Moju	Mojuí dos Campos	02/2015	07/2015	< 100	< 100	< 100
23	02°41′21 0"S	054° 35′ 59 8"W	Rio Moju	Mojuí dos Campos	02/2015	07/2015	< 100	< 100	< 100
23	02°52′19 2"S	054° 23′ 24 0"W	Reservatório Curuá – Una	Santarém	02/2015	07/2015	< 100	< 100	< 100
25	02°52′54 8″S	054°24'31 5"W	Reservatório Curuá – Una	Santarém	02/2015	07/2015	< 100	< 100	< 100
25	02 52 5 4 .0 5 02°52′17 6"S	054°24'31.5 W	Reservatório Curuá – Una	Santarém	02/2015	07/2015			
20	02 52 17.0 5 02°52′42 2"S	054°24'31.5 W	Reservatório Curuá – Una	Santarém	02/2015	07/2015			
27	02 52 4 2.2 5 02°51′50 6"S	054°22′51 1"W	Reservatório Curuá – Una	Santarém	02/2015	07/2015			
20	02 51 50.0 5	054°22'00 0"\\\	Reservatorio Curuá – Una	Santaróm	02/2015	07/2015			
30	02 52 15.2 5 02°53′77 9"S	054°24'24 6"W	Reservatório Curuá – Una	Santarém	02/2015	07/2015		1 03	
21	02 JJ 22.9 J	054°24'24.0 W		Santaróm	02/2013	07/2013	200	Na	< LOQ Na
27	02 37 42.0 3 02°37′42 6"S	054°30'12 2"\\\	Açaizal	Santaróm	05/2017	07/2017	2.2	Na	Na
22	02 37 42.0 3 02°37′35 7"S	054°30'22 0"W	Açaizal	Santaróm	05/2017	07/2017	5.7	Na	Na
31	02 37 33.7 3 02°37′33 7"S	054°30′20 4"W	Açaizal	Santaróm	05/2017	07/2017	< 100	Na	Na
25	02 37 33.7 3	054°30′25 5"W	Açaizal	Santaróm	05/2017	07/2017		Na	Na
36	02 30 00.3 3 02°45′53 1"S	054°23′00 2"\\\	Açaizai	Santaróm	05/2017	07/2017		Na	Na
20 27	02 43 33.1 3 02°45′54 1"C	054 25 09.2 W	Guaraná	Santarém	05/2017*	07/2017		Na	Na
27 20	02 43 34.1 3	054 25 10.2 W	Boo Sorto	Moiuí dos Compos	05/2017*	07/2017	0.5	Na	Na
20	02 40 02.5 5 02° 40' 15 1" 5	054 20 09.9 W	Boa Sorto	Mojuí dos Campos	05/2017*	08/2017	5.0 1.5	Na	Na
10	02 40 13.1 3	054 20 09.2 W	Boa Sorto	Mojuí dos Campos	05/2017*	08/2017	< 100	Na	Na
40	02 40 01.9 3	054 20 12.0 W	Diacha Varda	Mojuí dos Campos	05/2017	08/2017		Na	Na
41	02 33 12.0 3	054 20 55.0 W	Piacho Vordo	Mojuí dos Campos	05/2017*	08/2017	2.2	Na	Na
42	02 33 14.0 3	054 20 40.4 W	Piacho Vordo	Mojuí dos Campos	05/2017*	08/2017	1.0	Na	Na
45	02 33 00.9 3	054 20 30.3 W	Riacho Verde	Mojuí dos Campos	05/2017*	00/2017		Na	Na
44	02 52 49.1 5		Riacho Verde	Mojuí dos Campos	05/2017*	00/2017	2.0	Na	Na
45	02 52 48.0 5			Mojul dos Campos	05/2017*	08/2017	2.3	Na	Na
40	02 52 00.0 5	054 Z/ Z5.6 W			05/2017*	00/2017	1./	Nd Ne	Na
4/	02°47′56.0°S	054°26'08.4"W	Boa Sorte	Mojul dos Campos	05/2017**	08/2017	< LOQ	Na	Na
48	02-41-12.1-5	054°38°35.1°W		Mojul dos Campos	05/2017**	08/2017	< LOQ	Na	Na
49	02-49.07.9.5	054°44°37.6°W	Pataua	Mojul dos Campos	05/2017**	08/2017	< LOQ	Na	Na
50	02°49′03.9″S	054°44′43.1°W	Pataua	Mojul dos Campos	05/2017*	08/2017	< LOQ	Na	Na
51	02°52′36.5"S	054°45′05.4"W		Majuí dos Campos	05/2017*	08/2017		Na	Na
52	02°52′38.1"S	054°45′01.1"W	Palhau do Una	Mojul dos Campos	05/2017*	08/2017	< LOQ	Na	Na
53	02°51′24.8″S	054°43′36.0"W	Unça	wojul dos Campos	05/201/*	08/2017	< LOQ	Na	Na
54	02°46′56.8"S	054°42′11.5"W	Brilhosa	Mojul dos Campos	05/2017*	08/2017	< LOQ	Na	Na
55	02°41′04.4"S	054°36′19.7"W	Rio Mojui	Mojui dos Campos	05/2017*	08/2017	< LOQ	Na	Na
56	02°41′20.9"	054°35′59.8"W	KIO MOJUI	Mojul dos Campos	05/2017*	08/2017	< LOQ	Na	Na
57	02°48′49.6"	054°26′19.9"W	Boa Sorte	Mojui dos Campos	05/2017*	08/2017	< LOQ	Na	Na
58	02°52′09.2"	054°44′51.5"W	Palhau do Una	Mojuí dos Campos	05/2017*	08/2017	< LOQ	Na	Na

* Samples were lyophilized 2-3 days after arriving in the Laboratory; Na: not analyzed.