



An ultrasensitive LC-MS/MS method for the determination of glyphosate, AMPA and glufosinate in water – analysis of surface and groundwater from a hydrographic basin in the Midwestern region of Brazil



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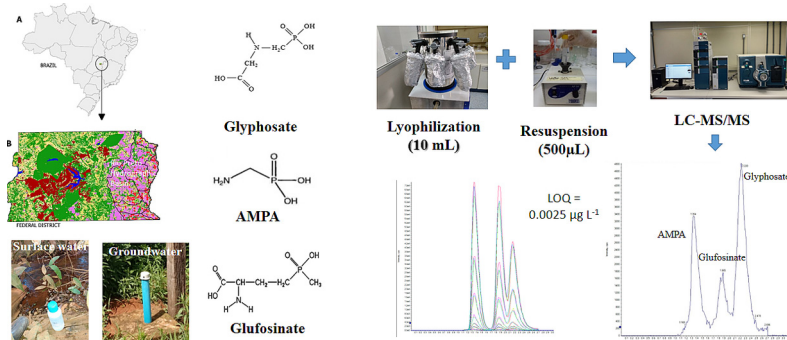
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HIGHLIGHTS

- An ultrasensitive method for glyphosate, AMPA and glufosinate
- Lyophilisation followed by LC-MS/MS, at LOQ of 0.0025 $\mu\text{g L}^{-1}$
- All 52 groundwater samples with glyphosate (up to 1.5868 $\mu\text{g L}^{-1}$) and AMPA (up to 0.2751 $\mu\text{g L}^{-1}$)
- 30% of 90 surface water samples contained glyphosate (up to 0.0236 $\mu\text{g L}^{-1}$)
- Glufosinate present in five samples

GRAPHICAL ABSTRACT



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ABSTRACT

The intensive use of glyphosate around the world in the last few decades demands constant monitoring of this compound and its metabolite in aquatic compartments. This work aimed to develop a sensitive method for the analysis of glyphosate, AMPA and glufosinate in water by liquid chromatography/tandem mass spectrometry (LC-MS/MS). The method involves analyte concentration by lyophilization ($20\times$) and direct injection on the LC-MS/MS, and was satisfactorily validated at a LOQ of 0.0025 $\mu\text{g L}^{-1}$. A total of 142 samples of surface and groundwater collected during the 2021/2022 dry and rainy seasons in the Rio Preto Hydrographic Basin were analyzed. All the 52 groundwater samples were positive for glyphosate (up to 1.5868 $\mu\text{g L}^{-1}$, dry season) and AMPA (up to 0.2751 $\mu\text{g L}^{-1}$, dry season). A total of 27 of the 90 surface water samples were positive for glyphosate (up to 0.0236 $\mu\text{g L}^{-1}$), and 31 samples for AMPA (up to 0.0086 $\mu\text{g L}^{-1}$), of which over 70 % collected during the dry season. Glufosinate was detected in only five samples, four in groundwater (up to 0.0256 $\mu\text{g L}^{-1}$). The levels found in the samples are much lower than the maximum levels established by the Brazilian legislation for glyphosate and/or AMPA and lower than the most critical toxicological endpoints for aquatic organisms. However, constant monitoring is necessary, demanding sensitive methods to allow the detection of the very low levels of these pesticides in water.

1. Introduction

Brazil is one of the main food producers worldwide (Maranhão et al., 2019), principally due to the technological advances that have taken place in the country since the second half of the 20th century, and the

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expansion of agriculture deep into the territory (Cattelan and Dall'Agno, 2018; Freitas and Landers, 2014; Silva et al., 2022). It is also among the three largest consumer of pesticides globally (FAO, 2022), with glyphosate the herbicide most used in the country and throughout the world (IBAMA, 2022; USEPA, 2017; EC, 2022; Brookes, 2019).

The Federal District, in the Midwest of Brazil, where Brasilia, the federal capital, is situated, lies within the Brazilian savannah biome, with an average altitude of about 1100 m above sea level (Paulo et al., 2013). The Brazilian savanna is considered the source of the country's waters, and it is full of springs and water bodies with small water volumes (Durigan et al., 2022). The Federal District has the highest yield of soybeans in the country (CONAB, 2022), which are planted mainly in its principal agricultural area, the Rio Preto Hydrographic Basin (RPHB), located in the eastern part of the territory (MAPBIOMAS, 2022). Crop production in this region makes significant use of irrigation by central pivot (Borges et al., 2007; CODEPLAN, 2019; Salles et al., 2018) and of pesticides in general, among them glyphosate.

According to the Brazilian Institute for the Environment and Renewable Natural Resources (IBAMA), glyphosate and its salts are the active ingredients most sold in Brazil, and sales of 219,585 tons were recorded in 2021 (IBAMA, 2022). Despite its intense use in agriculture in Brazil, there is a lack of monitoring of this compound in water. The indiscriminate use of glyphosate in agriculture can have adverse effects on the environment if it is not managed properly, with the potential to cause damage to soil microorganisms (Marques et al., 2021; Gandhi et al., 2021), to aquatic organisms (Fernandes et al., 2019; Ames et al., 2022; Rodrigues et al., 2019; Corrales et al., 2021) and to human health (Eddleston, 2020). Glyphosate can contaminate water resources, for example by run-off or leaching, and its transport is influenced by soil composition (Dotor-Robayo et al., 2022; Skeff et al., 2018; Okada et al., 2016) and rainfall (Mörtl et al., 2013). A number of studies have detected glyphosate and AMPA (aminomethylphosphonic acid), its main breakdown product, in hydric resources located close to agricultural areas in Brazil (Armas et al., 2007; Silva et al., 2003; Delmonico et al., 2014; Mendonça et al., 2020) and in other countries (Van-Bruggen et al., 2018; de Araújo et al., 2022b; Campanale et al., 2022; Geng et al., 2021).

Compared to other agricultural pesticides, however, few studies have analyzed glyphosate in water. In a systematic review published recently, the herbicide atrazine was the pesticide most analyzed in surface waters (56 % of the 146 studies), while glyphosate and AMPA were included in <7 % of the studies, and glufosinate, another herbicide, in 2 % of them (de Araújo et al., 2022b).

Analyze glyphosate in water is a challenge, because despite its large-scale usage, it is generally found in low concentrations in this compartment, demanding the use of very sensitive equipment and methods (Pires et al., 2020; Terzopoulou and Voutsas, 2016). Additionally, due to its high polarity and the fact that it is insoluble in organic solvents, glyphosate cannot be included in the multi-residue methods used for monitoring pesticides in different matrices, thus requiring a specific method, which limits its application in most laboratories, but was also used to analyze glyphosate in food samples (Chamkasem and Harmon, 2016).

Some authors used Enzyme-Linked Immunosorbent Assay (ELISA) (Osten and Dzul-Caamal, 2017; Mörtl et al., 2013) or ion chromatography (Cristofato et al., 2020) for glyphosate analysis. The analysis by high performance liquid chromatography (HPLC) with fluorescence (FLD) or UV-Vis detectors demands a derivatization step due to the absence of the fluorophore or chromophore of the molecules, including the use of 9-fluorenylmethyl chloroformate (FMOC-Cl) (Mendonça et al., 2020; Peruzzo et al., 2008), o-phthalaldehyde-mercaptoethanol (OPA-MERC) (Pires et al., 2020; Armas et al., 2007), 4-chloro-3,5-dinitrobenzotrifluoride (CNBF) (Qian et al., 2009) and 4-methoxybenzenesulfonyl fluoride (MOBD-F) (Sun et al., 2010). Most of the more recent methods use LC-MS/MS, with FMOC-Cl derivatization (e.g. Campanale et al., 2022; Cor et al., 2021; Bradley et al., 2017), or direct injection (e.g. Geng et al., 2021; Okada et al., 2020; Correia et al., 2020). However, the sensitivity of the method not always is sufficient to detect the low levels of the analytes found in water.

In this context, the objective of this work was to develop and validate a sensitive and easily executed method for the analysis of glyphosate, AMPA and glufosinate, using LC-MS/MS (Liquid Chromatography coupled with triple quadrupole mass spectrometry). The validated method was applied for the analysis of surface and groundwater samples collected in the RPHB.

2. Materials and methods

2.1. Chemicals and analytical standards

Analytical grade AMPA was acquired from Sigma-Aldrich (St Louis, USA) (98 % purity), and analytical grade glyphosate and glufosinate from AccuStandard (New Haven, USA) (98.2 and 100 % purity, respectively). Solutions of the standard solids were prepared in ultrapure water (Millipore®, Burlington, USA) with a concentration of 1 mg/mL and stored in Falcon tubes at -20 °C. Ammonium formate and formic acid were obtained from Fluka® (Buchs, Switzerland) and HPLC-grade acetonitrile (ACN) from Merck (Darmstadt, Germany).

2.2. LC-MS/MS

The analyses were performed by using a Shimadzu system (LC-20AD pumps, a SIL-20AD autosampler, and CTO-20AC column oven (Kyoto, Japan), coupled with a 6500+ QTRAP triple quadrupole mass spectrometer from AB SCIEX (Foster, USA). The software Analyst® (version 1.6) was used for control and data acquisition. Chromatographic separation was obtained by using an Acclaim Trinity Q1 (3 µm, 100 × 3 mm), tri-mode column (reversed-phase, anion-exchange, and cation-exchange), and an Acclaim Trinity Q1 (5µm, 10 × 3 mm) guard column, both from Thermo Fisher (Waltham, USA). The mass spectrometer determination was performed with negative electrospray ionization (ESI) operated in multiple reaction monitoring (MRM) mode, in which two MS/MS ion transitions (precursor/product) were monitored, the most intense being the quantification transition and the other used for confirmation. Different parameters of the source were tested: temperature (350 to 700 °C), nebulizer (GS1) and heater gas (GS2) pressures (45 to 70 psi), collisionally activated dissociation gas (CAD) (high, low and unit). Optimized ion source conditions were certain gas of 30 psi, ion spray voltage of -4500 V, CAD high, source temperature of 700 °C, GS1 of 70 psi, GS2 of 45 psi. The optimized MS/MS conditions for each analyte are shown in Table 1.

Different flows (0.4 to 0.6 mL/min), injection volumes (10 to 50 µL), pH of the mobile phase (A) (2.8, 2.9, 3.0) and its composition were tested to evaluate the chromatographic performance and sensitivity of the compounds. The best conditions selected were column temperature at 35 °C, 40 µL sample injection, and 0.5 mL/min flow. The mobile phase started with ammonium formate/formic acid (pH 2.9) for 3.1 min, changing to ACN:ammonium formate/formic acid (90:10) from 3.11 to 5.10 min, and returning to ammonium formate/formic acid up to 7.01 min. A diverter

Table 1
LC-MS/MS (6500+ QTRAP SCIEX) parameters for AMPA, glufosinate and glyphosate.

Analyte	RT (min.)	DP, volts	CE, volts	EP, volts	CXP, volts	Transition (m/z) ^a	Ion ratio ^b (RSD ^c , %)
AMPA	1.30	-15	-36	-10	-24	110 → 79	1.06 (4.92)
		-15	-26	-10	-26	110 → 63	
Glufosinate	1.80	-50	-24	-10	-11	180 → 63	0.45 (9.68)
		-50	-24	-10	-11	180 → 85	
Glyphosate	2.20	-30	-26	-10	-32	168 → 63	0.44 (5.41)
		-23	-20	-10	-15	168 → 81	

RT: retention time; DP: declustering potential; CE: collision energy CXP: collision cell exit potential

^a Quantification ion transition in bold.

^b Ratio between the qualifying and quantification ions.

^c RSD: relative standard deviation, n = 40.

valve connected between the LC column and the MS interface was turned on at 3.1 min to discard the LC eluent after the elution of glyphosate.

2.3. Study area and sample collection

The RPHB has springs in both the Federal District and in the state of Goiás. Seven hydrographic units (HUs) are located in the Federal District: Ribeirão Santa Rita HU-28; Ribeirão Jacaré HU-21; Ribeirão Extrema HU-20; Alto Rio Jardim HU-35; Alto Rio Preto HU-3; Baixo Rio Jardim HU-22 and Rio São Bernardo HU-8. The HUs localization and water sampling points in the RPHB are shown in Fig. 1. Sampling details are shown in Tables 2 and S1 (Supplementary Data). The sampling points P17, P18, P19 and PN were included as controls as they are located in areas around springs protected by natural vegetation.

The region has two well-defined seasons (a dry winter, from April to September, and a rainy summer in the other months of the year; Salles et al., 2018). In the period corresponding to sampling campaigns A and E (dry season, August 2021), there was no precipitation, and in September (campaign B), the total accumulated precipitation registered was 6.8 mm (INMET, 2022). Campaigns C, D and F (rainy season) occurred in January and February of 2022, and these had accumulated precipitation of 148.4 mm and 155.8 mm, respectively. All sampling points were close to agricultural areas (Fig. 1 and Fig. S1). In total, 142 samples of water were collected (Table 2).

The samples of groundwater were collected in Alto Rio Jardim HU-35, as this was the only hydrographic unit at which piezometer wells had been installed (Salles et al., 2018). The samples were collected using a polychloroethene bailer sampler (3 cm diameter, 100 cm high, opening at 50 cm) attached to a 20 m nylon string inserted in a 6 cm piezometric

Table 2

Information on the 142 surface and groundwater samples collected at the Rio Preto Hydrographic Basin, Federal District, Brazil.

Campaign	Sample	Season	Dates	Number
A	Surface water	Dry	August 25 and 26, 2021	19
B	Surface water	Dry	September 21 and 22, 2021	19
C	Surface water	Rainy	January 10 and 11, 2022	19
D	Surface water	Rainy	February 01 and 02, 2022	19
E	Groundwater	Dry	August 18, 2021	27
E	Surface water	Dry	August 18, 2021	7
F	Groundwater	Rainy	February 10, 2022	27
F	Surface water	Rainy	February 10, 2022	7

tube (Fig. S2). Previous studies have shown that the water table in the wells ranges between 1.8 m and 8.5 m, depending on the season (Salles et al., 2018). In this study, some wells were completely empty during the dry season and no sample could be collected.

The surface water samples were collected by manually dipping polyethylene flasks of 350 mL, about 15 to 30 cm deep, or using a van Dorn-type collector for areas that were difficult to access.

The water temperature (TEMP), electrical conductivity (EC), total dissolved solids (TDS) and salinity of the surface and groundwater samples were determined in the field using a portable multi-parameter Hq40d from Hach (Ames, USA). The samples were stored in a thermal box, under refrigeration and sheltered from light, and sent to the Toxicology Laboratory (LabTox) at the University of Brasília for analysis. The pH analyses were done in a pH meter from AJ Micronal, AJX-512 (São Paulo, Brazil) within 24 h after collection.

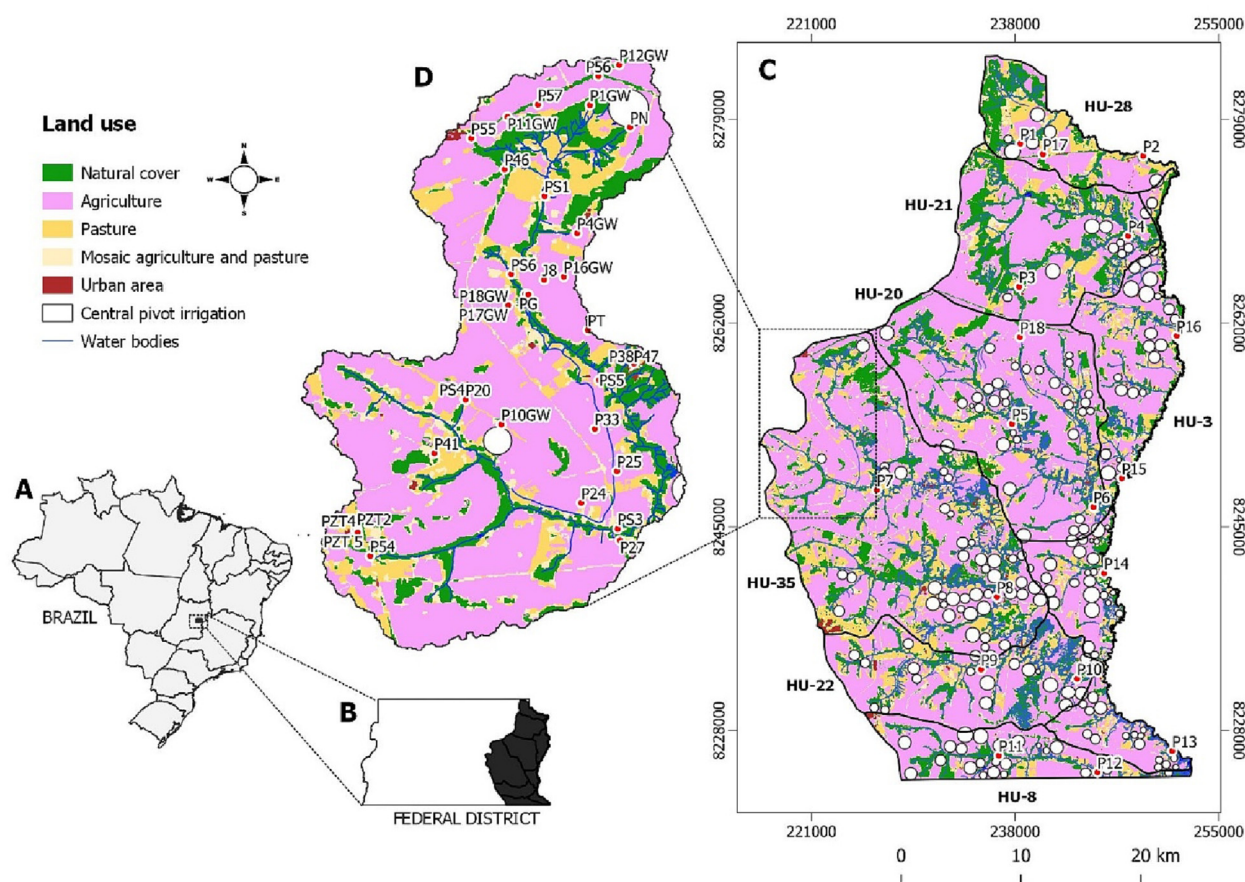


Fig. 1. Localization of the Rio Preto Hydrographic Basin in the Federal District of Brazil, indicating the hydrographic units (HU) and sampling points. Groundwater samples were only collected in HU-35 (Alto Rio Jardim). Prepared using MapBiomias (2022) and SIEG (2015).

Table 3

Recovery (%), repeatability and intermediate precision (% RSD) for AMPA, glufosinate and glyphosate analyzed by LC-MS/MS after lyophilization.

Analyte	Fortification level ^a ($\mu\text{g L}^{-1}$)	Recovery (%) (n = 5)	Repeatability (RSD, %) (n = 5)	Intermediate precision (RSD, %) (n = 10)
AMPA	0.0025	103	6.64	14.1
	0.020	79	5.42	8.99
	0.05	81	8.45	8.20
	0.1	81	7.55	7.85
	0.5	81	9.31	8.25
	1	96	8.86	8.31
Glufosinate	0.0025	91	8.47	5.86
	0.020	99	6.19	6.77
	0.05	101	6.71	5.29
	0.1	83	7.16	8.31
	0.5	94	0.88	1.96
	1	96	1.07	1.61
Glyphosate	0.0025	111	9.54	10.3
	0.020	99	6.03	6.87
	0.05	92	8.28	7.79
	0.1	93	7.14	6.20
	0.5	91	3.82	5.59
	1	102	10.1	6.93

^a Concentration in the original sample, prior to lyophilization.

2.4. Sample preparation for analysis of glyphosate, AMPA and glufosinate

At the LabTox, the samples were filtered with PTFE 0.45 μm microfibers (Millipore®) and immediately processed in order to preserve the analytes integrity (Pires et al., 2020). Aliquots of 10 mL ($n = 3$) were transferred to Falcon tubes of 15 mL and put in the freezer at $-21\text{ }^{\circ}\text{C}$ to be lyophilized ($-70\text{ }^{\circ}\text{C}$, pressure down to 50 mHg; Liobras, K105, São Paulo, Brazil). The lyophilized samples were kept in the freezer until the analysis, when they were re-suspended in 500 μL with a solution of 50 mM ammonium formate (pH 2.9), shaken in a vortex, filtered again and injected in the LC-MS/MS. The mean of three independent analysis of each sample was reported.

2.5. Method validation

The parameters for method validation were selectivity, linearity of the analytical curve, matrix effect, recovery, repeatability and intermediate precision. The validation was done with a sample of water collected from a spring in the study region (control sample), which had been tested and did not contain any of the analytes investigated in the study. Selectivity was evaluated by verifying the presence of interferences in the same retention time and monitoring transition ions. Linearity was determined in an analytical curve prepared in six concentrations (0.05, 0.5, 1.0, 5.0, 10 and 22 $\mu\text{g L}^{-1}$) with six authentic replicates prepared in 50 mM ammonium formate solution (pH 2.9). The least squares method was used to estimate the linear regression, Cochran's test was used to test the homogeneity of variance (homoscedasticity) and ANOVA to determine the coefficient of correlation (r) and regression significance (INMETRO, 2020). For the heteroscedastic calibration curves, the weightings $\ln x$, $\ln y$, $1/x$, $1/x^2$, $1/y$ and $1/y^2$ were used, in order to determine the best regression fit.

The matrix effect was investigated by comparing the equipment response of a standard curve prepared in 50 mM ammonium formate ($n = 6$) with the standard curve prepared in a lyophilized control sample dissolved in 500 μL of a 50 mM ammonium formate solution (in-matrix standard curve, $n = 6$). Matrix effect (%ME) for each calibration point was calculated by dividing the mean area of the in-matrix standard curve by the mean area of the 50 mM ammonium formate standard curve (no lyophilization), expressed in %. Matrix effect was considered relevant when higher than 20 %.

The repeatability and recovery of the analytic procedure were evaluated by analyzing fortified samples at the levels of 0.0025 to 1 $\mu\text{g L}^{-1}$ ($n = 5$ at each level), which corresponds to injected concentrations of 0.05 to 20 $\mu\text{g L}^{-1}$ (lyophilization factor of 20). The intermediate precision was evaluated by repeating the experiment on another day, and expressed as % of the relative standard deviation (RSD). Repeatability and intermediate precision were considered acceptable when % RSD was lower than or equal to 20 % and recovery within the range of 70 to 110 % (SANTE, 2019).

3. Results

3.1. Method validation

Interferents were not observed in the control matrix with the same retention time and the ions monitored, indicating the selectivity of the method. Analysis of the analytical curves in the linearity study showed heteroscedastic behavior for the three analytes. For these compounds, weighted linear regression was used with an adjustment of $1/x$ for quantification (Miller and Ambrus, 2000). Coefficients of determination (R^2) were above 0.99 for all the analytes. Fig. S3 shows the chromatogram of a standard curve with all the analytes.

Matrix effects for AMPA, glyphosate and glufosinate were within the acceptable levels (<20%), ranging from -9% (indicating ion suppression) to $+9\%$ (ion enhancement). As no significant matrix effect was observed, the analytes were quantified against a standard curve prepared in 50 mM ammonium formate (pH 2.9).

Table 3 summarizes the results of recovery, repeatability and intermediate precision. The average recovery for all analytes ($n = 5$) ranged from 79 to 111 %, with maximum RSD of 10.1 % for repeatability and 14.1 % ($n = 10$) for intermediate precision. The limit of quantification (LOQ) of the method for each analyte was defined as the lowest level that showed good recovery, repeatability and intermediate precision, defined as 0.0025 $\mu\text{g L}^{-1}$ for glyphosate, AMPA and glufosinate. The limit of detection (LOD) was 0.0012 $\mu\text{g L}^{-1}$, defined as $[\mu + 3.3 s]$, where " μ " is the average of the signal of 10 control samples and " s " the standard deviation (AAFS, 2019).

3.2. Water sample analyses

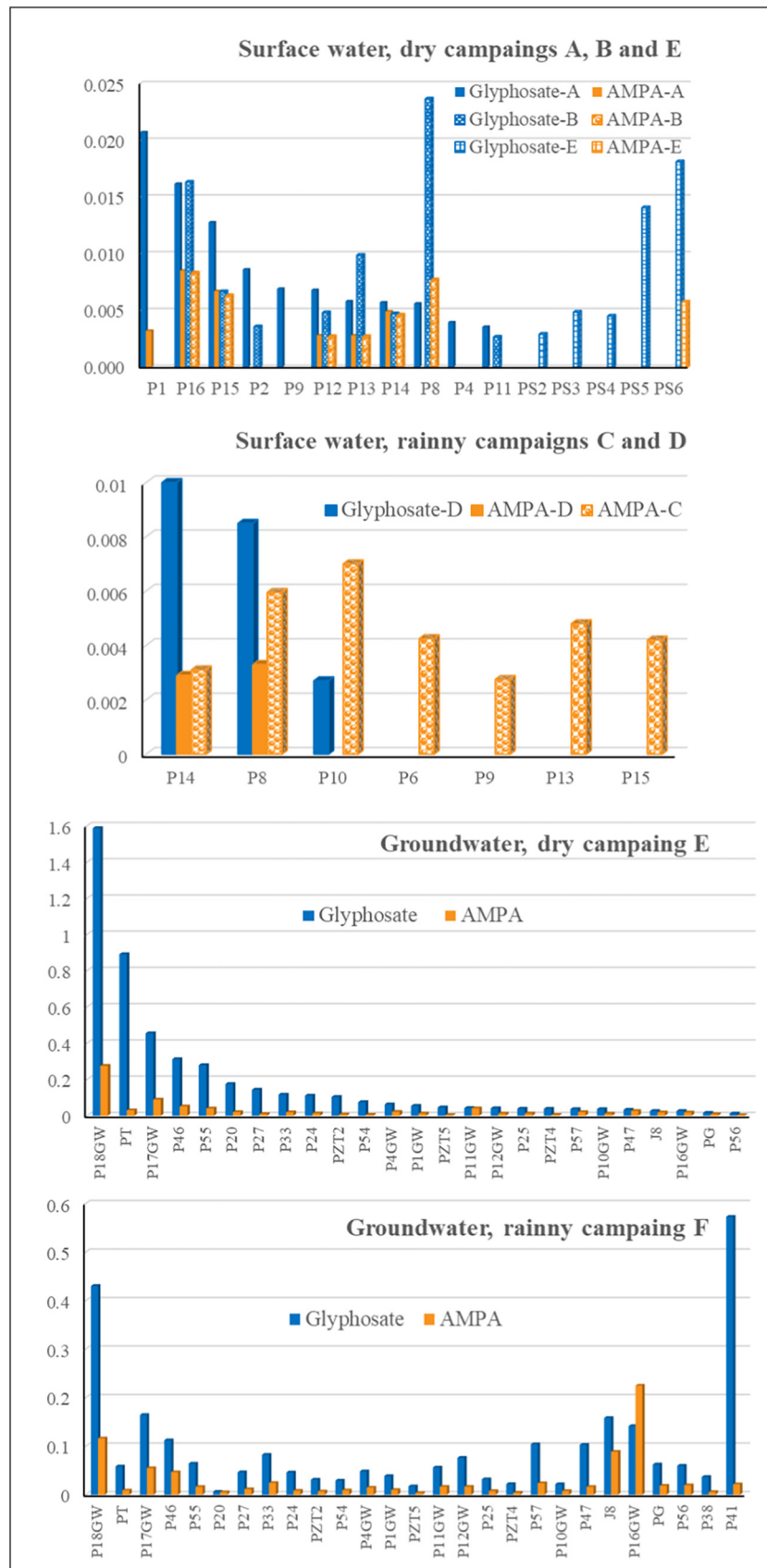
Ninety surface water samples and 52 of groundwater were analyzed, totaling 142 samples collected during the six sampling campaigns (Table 2). Of this total, 64.1 % were analyzed in the LC-MS/MS between 8 and 16 days, and 35.9 % between 17 and 33 days after collection.

Of the 90 surface water samples analyzed, 28 (31.2 %) were positive for glyphosate (>LOD), 27 of which were at levels \geq LOQ. Of the quantified samples, 24 (88.8 %) were collected in the dry season and only 3 (11.2 %) in the rainy season, in concentrations that varied between 0.0027 (P10) and 0.0236 (P8) $\mu\text{g L}^{-1}$. AMPA was detected in 31 (34.5 %) samples, and 22 samples were quantified at concentrations varying between 0.0026 (P12) and 0.0086 (P16) $\mu\text{g L}^{-1}$. Both glyphosate and AMPA were quantified in 13 dry season samples, with a concentration ratio varying from 0.7 to 3.6 (mean of 2.4 ± 1.51 , $n = 13$). Two rainy samples had both analytes, with ratios of 3.5 and 2.6.

All the 52 samples of groundwater analyzed were positive and quantified for glyphosate and AMPA. For glyphosate, the concentrations varied between 0.0062 $\mu\text{g L}^{-1}$ (P20, rainy season) and 1.5868 $\mu\text{g L}^{-1}$ (P18GW, dry season). AMPA levels ranged from 0.0026 (P56; dry season) to 0.2751 $\mu\text{g L}^{-1}$ (P18GW, dry season). The glyphosate/AMPA ratio varied from 1.0 to 29.7 (7.0 ± 6.5 , $n = 25$) during the dry season, and from 0.63 to 26.8 (4.7 ± 4.6 , $n = 27$) during the rainy season.

Fig. 2 summarizes the results found in the quantified samples (\geq LOQ) in surface and groundwater for glyphosate and AMPA, collected in the

Fig. 2. Mean ($n = 3$) concentrations of glyphosate and AMPA in groundwater and surface water ($\mu\text{g L}^{-1}$) collected during the dry and rainy seasons (2021–2022) in Rio Preto Hydrographic Basin, Federal District, Brazil. Detailed information is found in Table S1 (Supplementary Material). Surface water from campaign F ($n = 7$) had only one positive sample (AMPA < LOQ) and is not shown in the Figure.



dry and rainy seasons (mean of three independent samples). Out of the seven surface samples collected during campaign F (rainy season), only one was positive, with AMPA at levels <LOQ, and was not shown in Fig. 2. Glufosinate was detected and quantified in five samples, four of groundwater (from 0.0031 $\mu\text{g L}^{-1}$ at P25 to 0.0256 $\mu\text{g L}^{-1}$ at P11) and one of surface water (0.0062 $\mu\text{g L}^{-1}$, P13), all collected during the dry season. In all samples, glyphosate and AMPA were also detected. Detailed information for each analyzed sample can be found in Table S1.

3.3. Physical-chemical parameters

Table S2 summarizes the physical-chemical properties of the surface and groundwater samples. The temperature of surface water varied from 19.8 to 29.6 °C, with a lower mean during the dry season (22.5 °C). Groundwater temperature ranged from 21.2 to 28.5 °C, with similar means during the two seasons. The mean pH in surface water was 6.63 and 6.44 in the dry and rainy seasons, respectively, and it was more acidic in groundwater (6.12 and 5.87, respectively). The EC of surface water samples varied from 2.35 to 97.70 $\mu\text{S/cm}$, 66.6 % of them lower than 20 $\mu\text{S/cm}$. In the samples of groundwater, these values varied from 5.34 to 111.50 $\mu\text{S/cm}$, with 88.4 % of the samples showing values below 40 $\mu\text{S/cm}$. The TDS in surface water varied from 0.6 to 46.3 mg L^{-1} , with 83.3 % of the samples below 20 mg L^{-1} , and from 2.0 to 53.0 mg L^{-1} for groundwater samples, of which 88.4 % showed values below 18 mg L^{-1} . The salinity of the samples

differed from zero in only six (11.6 %) groundwater samples, three in each season (0.1 to 0.3 %).

4. Discussion

The determination of glyphosate, AMPA and glufosinate in water has limitations, due to the physical-chemical characteristics of these compounds, including their low molecular weight, high water and low organic solvent solubility and amphoteric behavior, in addition to the low concentrations normally found (Sanchís et al., 2012; Huhn, 2018). Table 4 summarizes some of these methods and the results found in water samples collected in various countries. Reported LOQs for glyphosate using ELISA or ion chromatography ranged from 0.13 to 0.85 $\mu\text{g L}^{-1}$. A HPLC-UV method using FMOC-Cl as derivatization agent had the highest LOQ among the studies shown in Table 4 (100 $\mu\text{g L}^{-1}$), while LC-MS/MS with FMOC-Cl derivatization or direct injection have a large range of reported LOQs (0.025 to 1.15 $\mu\text{g L}^{-1}$ for glyphosate and up to 3.06 for AMPA) (Table 4).

In this work, a method was satisfactorily validated to analyze glyphosate, AMPA and glufosinate in water samples by negative-mode LC-MS/MS. Separation was achieved using a tri-mode (reversed-phase, anion-exchange, and cation-exchange) column, which was initially developed for the analysis of the polar herbicides diquat and paraquat (Thermo-Scientific, 2014), but was also used to analyze glyphosate in food samples

Table 4

Analytical technique information, occurrence and concentrations of glyphosate, AMPA and glufosinate in samples of surface and groundwater in Brazil and other countries.

Ref. country	Sample preparation, analysis	LOQ ($\mu\text{g L}^{-1}$)	Samples, occurrence and concentrations
This study, Brazil	Lyophilization, LC-MS/MS	GLY/AMPA/GLU: 0.0025	Federal District. Surface water ($n = 90$): GLY, AMPA and GLU, detected in 31.2, 34.5 and 1.1 % (max. 0.0236, 0.0086, 0.006 $\mu\text{g L}^{-1}$, respectively). Groundwater ($n = 52$), 100, 100, and 9.6 % (max. 1.5868, 0.2751, 0.0256 $\mu\text{g L}^{-1}$, respectively).2
Campanale et al. (2022), Italy	UHPLC-MS/MS, FMOC-Cl	GLY/AMPA/GLU: 0.025	Puglia region ($N = 1000$). Glyphosate and AMPA: 39.5 % of the surface water and in 14 and 3 % of the groundwater samples, respectively. 36.4 % > 0.1 $\mu\text{g L}^{-1}$
Geng et al. (2021), China	UPLC-MS/MS, direct injection	GLY/AMPA/GLU: 0.1	Groundwater ($N = 694$), GLY and AMPA detected in ~1 % (max. 2.09 and 5.13 $\mu\text{g L}^{-1}$), GLU < LOQ; Surface water ($N = 196$), 14.3, 15.8, and 2.6 % (max. 32.5, 10.3 and 13.15 $\mu\text{g L}^{-1}$).
Le Cor et al. (2021), France	On line SPE, LC-MS/MS, FMOC-Cl	GLY/AMPA: 0.1/0.025	North-Eastern France, surface, continuous flow-based water sampling ($N = 237$) GLY (max. 1.7 $\mu\text{g L}^{-1}$), AMPA (max. 0.6 $\mu\text{g L}^{-1}$).
Mendonça et al. (2020), Brazil	Lyophilization, HPLC-FL, FMOC-Cl	GLY/AMPA: 0.3	Paraná state, 124 surface samples. GLY: 17.7 % \geq LOQ (0.31 to 1.65 $\mu\text{g L}^{-1}$); AMPA: 1.6 % \geq LOQ (0.50 a 1.40 $\mu\text{g L}^{-1}$).
Pires et al. (2020), Brazil	Lyophilization, HPLC-FL, OPA-MERC	GLY/AMPA/GLU: 0.2/0.5/0.07	Pará state, 58 surface samples and 7 groundwater. AMPA was detected in 6 of the 30 surface samples (0.65–1.93 $\mu\text{g L}^{-1}$), and GLY was detected in 11 samples (7 groundwater samples) (1.5 and 9.7 $\mu\text{g L}^{-1}$). GLU not detected.
Correia et al. (2020), Brazil	LC-MS/MS	GLY/AMPA: 1.15/3.06	Rio Samambaia sub-basin in the Federal District and eastern Goiás, 287 superficial and groundwater samples. GLY detected in 9 samples, only 2 > LOQ (highest at 11.3 $\mu\text{g L}^{-1}$). AMPA not detected.
Cristofato et al. (2020), Brazil	Ion chromatography, direct injection	GLY: 0.85	Southeast, six different reservoirs of Paraíba do Sul and Guandu River Basins. 43 % > LOQ (0.3–168.4 $\mu\text{g L}^{-1}$).
Okada et al. (2020), Australia	LC-MSMS, direct injection	GLY/AMPA: 0.5	Melbourne, surface, urban streams ($N = 38$), GLY 79 % (max 4.8 $\mu\text{g L}^{-1}$), AMPA 97 % (max, 4.3 $\mu\text{g L}^{-1}$). Stormwater wetlands ($N = 130$), GLY 77 % (max. 14.2 $\mu\text{g L}^{-1}$), AMPA 91 % (max, 10.0 $\mu\text{g L}^{-1}$).
Osten and Dzul-Caamal (2017), México	ELISA	GLY: 0.13	Campeche, detected in 90 % of the samples; groundwater ($N = 29$): max. 1.42 $\mu\text{g L}^{-1}$; drinking water ($N = 15$): max. 0.65 $\mu\text{g L}^{-1}$.
Bradley et al. (2017), EUA	On line SPE, LC-MS/MS, FMOC-Cl	GLY/AMPA/GLU: 0.02	Samples from 32 streams in urban and rural areas. GLY and AMPA: 79 % samples (max. 7.9 and 9.5 $\mu\text{g L}^{-1}$); GLU: 3 % (max. 0.02 $\mu\text{g L}^{-1}$).
Ronco et al. (2016), Argentina	LC-MS/MS, FMOC-Cl	GLY/AMPA: 0.3	Paraguay and Paraná rivers and tributaries. 46 surface samples, 15 % GLY, mean of 0.60 $\mu\text{g L}^{-1}$. AMPA not detected.
Ramirez et al. (2014), México	Lyophilization, LC-FLD-MS/MS, FMOC-Cl	GLY/AMPA LOD:0.058/0.108	South Florida, samples from canals. Max. of 59.9 and 9.09 $\mu\text{g L}^{-1}$, for GLY and AMPA, respectively.
Mörtl et al. (2013), Hungary	ELISA	GLY: LOD 0.12	Békés, Danube River and Lake Velencei, surface ($N = 24$): 2.1 % (max. 0.68 $\mu\text{g L}^{-1}$); groundwater ($N = 36$): 100 % GLY (max. 0.76 $\mu\text{g L}^{-1}$).
Sanchís et al. (2012), Spain	On line SPE, LC-MS/MS, FMOC-Cl	GLY: 0.0096	Catalonia, 139 groundwater samples; 47 % > LOQ (max. 2.6 $\mu\text{g L}^{-1}$).
Sun et al. (2010), China	HPLC-UV MOBS-F	GLY/AMPA: LOD: 0.1	Waste water from a pesticide factory ($N = 11$). 100 % GLY (max. 1.01 $\mu\text{g L}^{-1}$) and AMPA (max. 0.75 $\mu\text{g L}^{-1}$).
Peruzzo et al. (2008), Argentina	HPLC-UV FMOC	GLY: 100	Tributaries of the Pergamino–Arrecifes system, Buenos Aires Province. Levels from 100 to 700 $\mu\text{g L}^{-1}$.
Armas et al. (2007), Brazil	HPLC-FL, OPA-ME	GLY: 1.0	São Paulo, Corumbataí River and main tributaries, detected in 41 % of 32 samples. No quantification was performed.

ELISA: Enzyme-Linked, Immunosorbent Assay; GLY: glyphosate; GLU: glufosinate; N: total number of samples analyzed; LOQ: method limit of quantification; LOD: method limit of detection; OPA-MERC: o-phthalaldehyde-2-mercaptoethanol; FMOC-Cl: 9-fluorenylmethylchloroformate; CNBF: 4-chloro-3,5-dinitrobenzotrifluoride; MOBD-F: 4-Methoxybenzenesulfonyl fluoride.

(Chamkasem et al., 2015; Chamkasem and Harmon, 2016; Chamkasem and Vargo, 2017). Together with this specific column, the developed method includes a 20-fold concentration step by lyophilization that allowed to achieve a LOQ of $0.0025 \mu\text{g L}^{-1}$, much lower than the LOQs reported in the literature (Table 4). Lyophilization had also been used previously by our research group (Pires et al., 2020) and by Mendonça et al. (2020), with HPLC-FL (OPA-MERC or FMOC-Cl) with LOQs of 0.3 and $0.2 \mu\text{g L}^{-1}$ for glyphosate, respectively, and by Ramirez et al. (2014) using FMOC-Cl-LC-MS/MS, with a LOD of $0.058 \mu\text{g L}^{-1}$ (Table 4). In this work, degradation of glyphosate was prevented by lyophilizing the samples within a maximum of 30 days after sampling (Pires et al., 2020).

Sanchís et al. (2012) developed a sensitive method for glyphosate analysis in water using FMOC-Cl - LC-MS/MS, with a reported LOQ of $0.0096 \mu\text{g L}^{-1}$, the lowest among the studies shown in Table 4, but still almost four times higher than the present study. In addition to the derivatization step, the method includes an on-line solid-phase extraction (SPE) step, adding more complexity to the procedure. About 40 % of the 140 samples of groundwater from Catalonia (Spain) contained glyphosate at levels \geq LOQ (Sanchís et al., 2012), a lower percentage compared to the present study, where all the groundwater samples contained quantified residues of glyphosate and AMPA. Using ELISA (LOD of $0.12 \mu\text{g L}^{-1}$), Mörtl et al. (2013) also found glyphosate in all groundwater samples collected in Hungary (Table 4).

Various studies found a low incidence of glyphosate and or AMPA in the water samples, probably due to the high LOQs of the method. For example, in another study conducted in the Federal District region, only 3 % of the 287 water samples contained glyphosate equal to or higher than a LOQ of $1.12 \mu\text{g L}^{-1}$ (Correia et al., 2020; LC-MS/MS). AMPA was not detected in any sample, which is probably due to the high LOQ of the method ($3.06 \mu\text{g L}^{-1}$). The relevance of data derived from methods with low sensitivity is questionable, as it does not reflect the real environmental contamination situation.

In addition to the high incidence rate of glyphosate and AMPA in groundwater in this study, this compartment also had higher concentrations compared with surface water. While all sampling points were close to agricultural areas (Fig. 1), the sample with the highest glyphosate and AMPA concentrations (1.5868 and $0.2751 \mu\text{g L}^{-1}$, respectively) was collected at P18GW (dry season) near an empty pesticide packing room

(Fig. S2), which may have impacted the contamination. Glufosinate was detected always with glyphosate and AMPA, mostly in groundwater, and always in dry season. Fig. 3 shows a chromatogram and the extracted mass ions of sample P11GW, which contains the three analytes.

With a few exceptions, glyphosate concentration in a sample was higher than AMPA, at a ratio that could reach almost 30 for groundwater samples, much higher than in surface samples (highest ratio of 3.6). This is probably because glyphosate in groundwater is not prompt to photodegradation, an important degradation pathway in the environment (Trinelli et al., 2019).

The high incidence and levels of glyphosate found in groundwater may seem contradictory, considering the characteristics of glyphosate, which has low mobility in soil due to its high sorption coefficient (Caldas, 2019; Kaniserry et al., 2019; Vereecken, 2005). However, this is indeed confirmed by other studies (Table 4) and may mainly involve a vertical transport mechanism (Kaniserry et al., 2019). In addition to its chemical characteristics, the capacity of a molecule to bind to soil depends on soil properties, such as organic carbon and clay content, pH and texture. The soil of the groundwater sampling area (HU-35; Fig. 1) is mainly ferrasol (Salles et al., 2018), defined by a fine-textured subsurface layer of low silt-to-clay ratio, and classified as well drained soils (Reatto et al., 2000). The soil pH in the region is acidic (Muniz et al., 2020; Passos et al., 2019), which is confirmed by the acidic pH of the collected water samples. Some authors have shown that the adsorption of glyphosate into the soil diminishes with the increase in the pH at the soil-water interface, in function of the increased number of negative charges of glyphosate and of the soil surface, resulting in a greater electrostatic repulsion (Dotor-Robayo et al., 2022; Marques et al., 2021). A study carried out in Sweden showed that glyphosate did not leach in sandy soil, while it was detected at mean concentrations of $0.25 \mu\text{g L}^{-1}$ in drainage water from the clay soil on all sampling occasions (Aronsson et al., 2011). Furthermore, a study conducted in the Baltic Sea indicated that increasing the pH and or salinity significantly reduced the adsorption capacity of glyphosate, AMPA and glufosinate into sediment (Skeff et al., 2018). Indeed, the water samples in the present study showed salinity levels lower than 1 mg/L^{-1} . All these parameters together explain the high incidence and higher concentration of glyphosate, and consequently of AMPA, the glyphosate breakdown product formed mainly by microbial-mediated process, in groundwater collected in the HU-35.

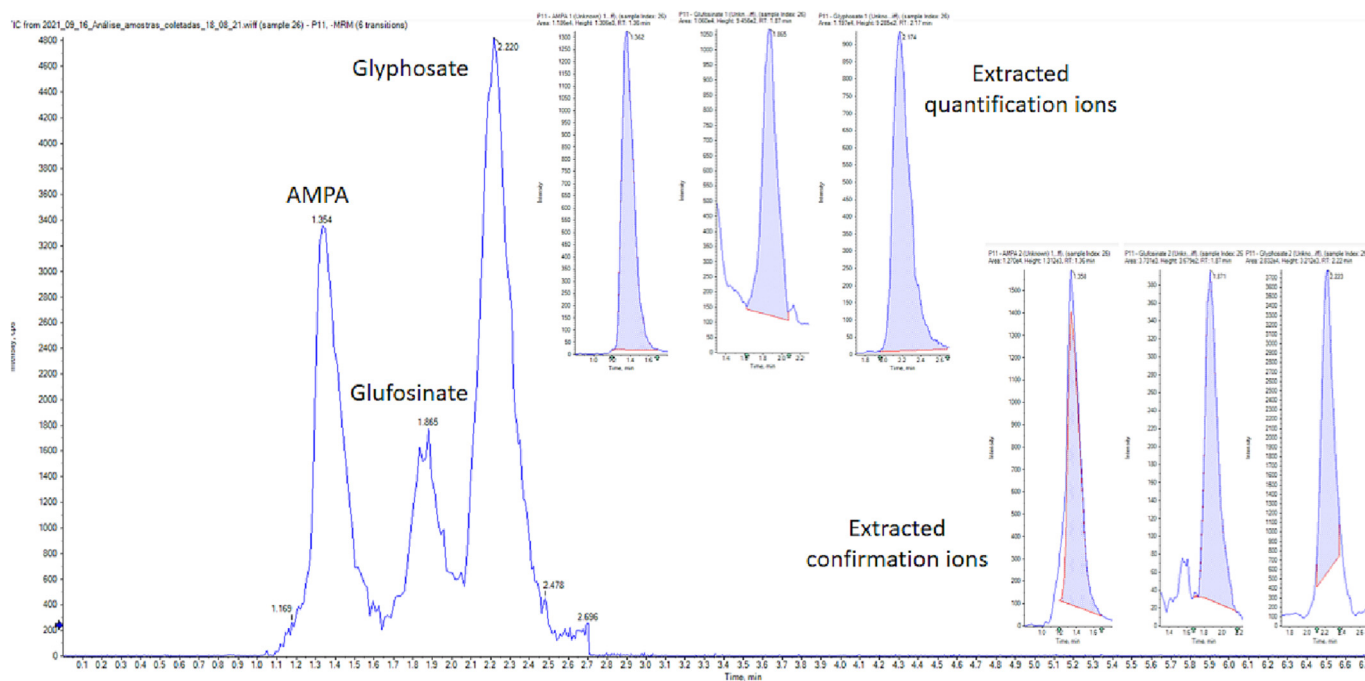


Fig. 3. Total ion chromatogram and extracted quantification and confirmation ions of groundwater sample P11GW collected during dry season (campaign E) containing 0.0421, 0.0406 and $0.0256 \mu\text{g L}^{-1}$ of glyphosate, AMPA and glufosinate, respectively.

The presence of glyphosate in surface water is generally attributed to surface water runoff and drift from the field (Kanissery et al., 2019). However, the compound was only detected in 27 of the 90 samples, which may be attributed to the lotic and dynamic regime of a surface water system compared to the lentic (static) regime of the groundwater. AMPA was detected in four additional samples, although at lower levels.

Almost 70 % of the glyphosate and/or AMPA positive surface samples were collected during the dry season, probably due to a concentration factor that allows the compound to be detected. Furthermore, as during the dry season the flow from the groundwater reservoir maintains the perennial streams in the region (Salles et al., 2018; Durigan et al., 2022), it is possible that the groundwater glyphosate may contribute to the levels found in surface water. On the other hand, a heavy rain event may facilitate the run-off and leaching of applied glyphosate into surface waters (Mörtl et al., 2013), although a dilution factor may affect the final concentration. In this study, only the samples collected at P8 and P14 (Fig. 1) had residues of glyphosate in both seasons (Fig. 2); however, the impact of the season on the analyte concentration is unclear. Close to P8 and P14, there is an important presence of center pivot irrigation (Fig. 1), which may have favored the movement of these compounds into surface water by different routes. However, further studies are needed to verify this correlation.

All the 52 samples of groundwater analyzed were positive and quantified for glyphosate and AMPA. For glyphosate, the concentrations varied between 0.0062 $\mu\text{g L}^{-1}$ (P20, rainy season) and 1.5868 $\mu\text{g L}^{-1}$ (P18GW, dry season). AMPA levels ranged from 0.0026 (P56; dry season) to 0.2751 $\mu\text{g L}^{-1}$ (P18GW, dry season).

Studies that investigated the levels of glyphosate and AMPA in water in Brazil generally report levels below the LOQ for most samples analyzed, probably due to the high LOQ of the method, as discussed previously (Pires et al., 2020; Albuquerque et al., 2016; Brovini et al., 2021; Correia et al., 2020; Mendonça et al., 2020). The low incidence of positive samples for glufosinate is a reflection of its low use in the country and in the region. In 2021, while 532.4 tons of glyphosate active ingredient were sold in the Federal District, sales of glufosinate amounted to only 14.3 tons (IBAMA, 2022).

In Brazil, there are two resolutions regarding water quality from the National Environmental Council (CONAMA). The CONAMA directive 357 (Brazil, 2005) for surface water classifies the water bodies according to the use (special, and 1 to 4 classes). The Directive establishes a maximum level (ML) for glyphosate of 65 $\mu\text{g L}^{-1}$ for water classes 1 and 2, which quality should guarantee the protection of aquatic biota, and can have various uses, including for human supply after treatment, recreation, aquaculture and fishing. In CONAMA directive 396 (Brazil, 2008) for groundwater, the ML for glyphosate and/or AMPA is 500 $\mu\text{g L}^{-1}$ for human consumption, which is the same set by the Ministry of Health for drinking water (Brazil, 2021). No Brazilian legislation has established the ML for glufosinate. Despite its high use, the levels of glyphosate and/or AMPA found in this study in surface and groundwater are much lower than any ML established by Brazilian legislation.

Furthermore, glyphosate, AMPA and glufosinate present low toxicity to aquatic organisms. The most critical toxicological parameter for glyphosate is the non-observed effect concentration (NOEC, 21 days) for the *Brachydanio rerio* fish, of 1,000 $\mu\text{g L}^{-1}$ (PPDB, 2022) and the lowest for AMPA, the non-observed effect concentration (NOEC, 21 days) for *Daphnia magna*, is 15,000 $\mu\text{g L}^{-1}$ (PPDB, 2022). As regards glufosinate, its EC_{50} for nonvascular plants is 72 $\mu\text{g L}^{-1}$ (USEPA, 2022). Thus, the concentrations detected in this study do not represent effects that are toxic to the aquatic biota. A recent study has shown that the current Brazilian legal ML levels for many pesticides in surface water represents a potential risk to the aquatic biota and should be revised (de Araújo et al., 2022a). The results of the present work indicate that this revision should consider the real environment contamination level.

5. Conclusions

This study optimized and validated an easy to implement method for the analysis of glyphosate, AMPA and glufosinate in water samples by LC-

MS/MS, with high selectivity, good accuracy and precision, and high sensitivity. The method was successfully applied in the analysis of surface and groundwater in the RPHB. To the best of our knowledge, the validated method has the lowest LOQ yet reported (0.0025 $\mu\text{g L}^{-1}$), a characteristic that is essential when analyzing environmental samples.

By developing transgenic crops that are tolerant to glyphosate over the last 25 years, agricultural productivity worldwide has certainly been raised. However, it is important to generate data and technical information about pesticide residues in the environment in regions where these products are used. Taking into consideration that agriculture has been growing in recent years in the Federal District, and that this activity is directly related to the use of pesticides, it is essential to monitor constantly the level of these contaminants in water and other environmental compartments in the region.

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CRediT authorship contribution statement

N.L. Pires: methodology, formal analysis, investigation, original draft preparation, resources, conceptualization; E.P. de Araújo: sampling, resources, conceptualization; E. Oliveira-Filho: Conceptualization, funding acquisition, supervision; E.D. Caldas: funding acquisition, supervision, data curation, project administration, writing, reviewing and editing. All authors have read and agreed to the final version of the manuscript.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.162499>.

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