Artigos

Validação de um método para análise de glifosato e AMPA em água de consumo humano usando cromatografia iônica

Validation of a method for analysis of glyphosate and AMPA in drinking water using ion chromatography

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Abstract

The analysis of pesticide residues in water is one of the requirements of drinking water legislated and related to human health. The ionic chromatography (IC) analysis is a technical alternative that contributes to the quantification of the glyphosate herbicide and its amino methyl phosphonic acid metabolite (AMPA) in drinking water because it is quick to prepare (filter and inject), high selectivity, good precision and accuracy. This research aims to develop and validate this method for direct analysis of these pesticides, without sample treatment or derivatization step. In the proposed validation, the parameters of selectivity, accuracy and precision, limits of detection and quantification, linearity and estimation of measurement uncertainty were considered. The results indicated that this technique is adequate and presents a good sensitivity for the quantification of these analytes in the water matrix, and the validation results are appropriate against the maximum concentration limits stipulated in Legislation 2914/11 of the Ministry of Health.

Resumo

A análise de resíduos de agrotóxicos em água é um dos requisitos da legislação de potabilidade ligadas à saúde humana. A análise de cromatografia iônica (CI) é uma alternativa técnica que vem contribuir para quantificação do herbicida glifosato e seu metabólito ácido amino metil fosfônico (AMPA) em água de consumo humano por ser rápida de preparo (filtrar e injetar), alta seletividade, boa precisão e exatidão. Esta pesquisa tem como objetivo desenvolver e validar este método para análise direta destes agrotóxicos, sem etapa de tratamento da amostra ou derivatização. Na validação proposta foram considerados os parâmetros de seletividade, exatidão e precisão, limites de detecção e quantificação, linearidade e estimativa de incerteza de medição. Os resultados obtidos indicaram que esta técnica é adequada e apresenta boa sensibilidade para a quantificação destes analitos na matriz água, sendo os resultados da validação apropriados frente aos limites máximos de concentração estipulados na Portaria 2914/11 do Ministério da Saúde.

1. INTRODUCTION

The intensive use of pesticides can lead to their retention in crops and in the soil and thus mainly through runoff or leaching, they can reach surface and groundwater.

In 2014, at Brazil, were sold about 360 millions ton of active ingredient (a.i.) of pesticides and the like were of commercialized of these, glyphosate and its salts correspondent to about 55%, equivalent to 195 millions tons. In the same year, the Rio Grande do Sul (RS), sold about of 10% of the such products (20 millions tons)

(IBAMA, 2016).

Pesticides have become a problem in terms of the environment and human health, especially if the application is indiscriminate. Most chemical contaminants present in surface and ground waters is related to the agricultural industry sources (HU; KIM, 1994).

In health, they can reach the applicators of products, community members and consumers of food and water contaminated with waste. At the same time, the farmer who does not know the harmful effects of pesticides on health can overestimate its benefits and use

larger doses than necessary. Glyphosate may be related to the appearance of diseases such as cancer, depression, Alzheimer's, diabetes, Parkinson's disease, autism and the effect of endocrine disruption in human liver cells, according to Technical Report No. 01/2015, produced by researchers from Santa Catarina Federal University (GASNIER et al., 2009).

In the last decade, herbicides formulated with glyphosate have gained expression and importance due to the growth in the area sown with genetically modified crops. In addition, because it is a systemic herbicide with a broad spectrum of action, it is widely used in low volume of syrup compared to conventional herbicides (RODRIGUES and ALMEIDA, 2005).

Water resources act as integrators of the biogeochemical processes of any region, characterizing itself as the final destination of some pesticides, and contamination of these sources can occur, which can cause serious damages to the aquatic ecosystem.

The persistence of glyphosate and its metabolite AMPA in aquatic environment may present half-life between 7 and 21 days depending on the levels of clays, organic matter and microbial activity (TONI et al., 2006). It is also known that its persistence in water is even shorter than in soil. Glyphosate formulations are completely soluble in water due to rapid dispersion, and do not accumulate in high concentrations in the water profile, since they are adsorbed by the sediments and degraded by the microorganisms (PATTERSON, 2016).

Glyphosate, which although it is not very mobile with regard to percolation in the soil, because it has high Koc (organic fraction), has high water solubility. Thus, the interaction of glyphosate with soil causes it not to be frequently found in groundwater, however, when applied to the soil and available on its surface, it can be carried by rainwater or irrigation and contaminate surface water (MATOS, 2014).

The characterization of glyphosate in the potential of contamination of groundwater by leaching potential considering soil types, aquifer position and precipitation can be given by the following classifications: CP (Potential Contaminant) according to USEPA, 2013 (US Environmental Protection Agency), NL (Non-leachable) by GUS method GUS <1,8 and MPCDA and MPCDA (High, medium and low Surface Water Contamination Potential Dissolved in Water or Sediment Associated) by the GOSS method (SANTOS, 2013).

Mattos et al. (2002) monitored the presence of the herbicide glyphosate and its metabolite amino methyl phosphonic acid (AMPA) in samples of water collected in orizicole area in a farm in the south coast of the RS, the lift of water ingress of Lagoa Mirim until the Arroio Bretanhas, subject to direct plantation. Levels of glyphosate were detected above the maximum permitted concentration limit. The presence of the metabolite AMPA in irrigation channel water was detected up to 120 days after application.

Armas et al.(2007) reported that 13 herbicides were detected highlighting: : glyphosate (detected in all samples but below the limit) in surface water and sediment of the Rio Corumbataí, producer of sugar cane in the São Paulo State in Brazil.

Rio Grande do Sul has areas of cultivation that extend from the Pampas to the Mountains, with great diversity of cultures, following the regional characteristics of relief and climate. The growth on the industrial scale of agricultural production and the policy of reduction of losses in each crop, lead to a relevant increase in the consumption of agrochemicals, making the crops highly dependent on these inputs (NASRALA NETO et al., 2014).

It should be noted that at the national level, Ministry of Health Ordinance No. 2914 of December 12, 2011, which provides for the quality control procedures of water for human consumption an your drinking pattern, establishes a limit of 500 $\mu g.L^{-1}$ of the sum of glyphosate and AMPA in drinking water (BRAZIL, 2011).

Several methodologies have been developed for the analysis of glyphosate and AMPA. Methods involving liquid chromatography guarantee good results due to the high polarity of these compounds, however, high cost techniques and require derivatization, which consequently makes the analysis more time consuming (WHO, 1994). Mass spectrometry or ultraviolet and fluorescence after derivatization may also be used, but ion chromatography with a conductivity detector allows the determination of glyphosate and AMPA directly in the water sample (LE FUR et al.,2000).

Ion chromatography consists of a variant of liquid chromatography that uses ion exchange resins to separate atomic or molecular ions based on their interaction with the resin. Separation of the analytes is performed either isocratically or by gradient application (AMARANTE et al., 2002).

Considering the wide-ranging discussion on the method validation, this paper aims to present the validation of the method related to the determination of glyphosate (N-phosphonomethyl glycine) and its metabolite AMPA by the ion chromatography technique. The specific objectives are: to present the criteria used in each parameter of the method validation and the measurement uncertainty of the analysis carried out, being based on the validation data. The case study presented was developed within the scope of the Official Laboratory of Rio Grande do Sul State, Brazil.

Focused on the monitoring of water for human consumption, the Program for the Quality of Human Consumption Water (VIGIÁGUA) was elaborated in the State of Rio Grande do Sul, monitoring WSS (Water Supply System), CAS (Collective Alternative Solution) and IAS (Individual Alternative Solution).

Samples (*n*=90) were collected in the following Regions: Campanha, Campos de Cima da Serra, Central, Centro Sul, Fronteira Noroeste, Fronteira Oeste, Hortênsias, Jacuí Centro, Litoral, Médio Alto Uruguai, Metropolitano Delta Jacuí, Missões, Nordeste, Noroeste Colonial, Paranhama-Encosta da Serra, Produção, Rio dos Sinos Valley, Rio Pardo Valley, Sul, Taquari Valley.

2. MATERIAL AND METHODS

The activities that integrate this validation study were carried out by two analysts, who evaluated solutions with addition of standard of glyphosate and AMPA in different concentrations.

Water from human consumption from an artesian well (Picada - São Paulo neighborhood, Morro Reuter - RS) and ultrapure water was used as "white".

As Certified Reference Material (CRMs) and traceable by ISO 9001: 2008 quality systems; ISO 17025: 2005 and ISO Guide 17034: 2016 with 99.5% purity were used: glyphosate and AMPA brand SPEXertificate.

The glassware used in the dilution step of different concentrations of the standards (pipettes and volumetric flasks) was calibrated by a laboratory belonging to the Brazilian Calibration Network (RBC).

After validation, the monitoring of VIGIAGUA program was followed.

2.1. Chromatographic Equipment and Conditions

The chromatographic separation of the two analyses (glyphosate and AMPA) was performed by conductivity on an 850 Professional IC ion chromatograph, integrated with the 858 Professional Sample Processor, and the acquisition and analysis of the data was performed through the MagIC Net 3.1 Software. A Metrosep A Supp 7-250 / 4.0 column and a RP 2 Guard 2.5 precolumn were used in flow rate of 0.7 mL.min^{-1}, temperature of 45°C and pressure of 15 MPa, with a loop of 200 μL at analysis time of 31 min.

For the chromatography the following solutions were prepared: Eluent B (1.0 mM Na_2CO_3 + 15.0 mM NaOH); Eluent C (20 mM Na_2CO_3); Suppressors (Ultrapure water and 5% H_2SO_4 in aqueous solution); wash solution (13 mL methanol + 247 mL ultrapure water).

2.2. Analytical Procedure

The aqueous sample was filtered through a 0.45 μm filtration membrane and transferred to the sample tube. The validated method was based on the procedures of Metrohm (METROHM ,2009).

2.3. Method Validation and Evaluation of Performance Criteria

The method validation was conducted based on indications quoted in scientific literature EURACHEM (2012); INMETRO(2003); Decision 2002/657 / CE(2002) and Albano and Raya-Rodrigues (2015), fulfilling the performance criteria that are expressed in terms of statistical parameters of precision, limits of detection and quantification, analytical curve, linearity, accuracy and estimation of measurement uncertainty .

2.4. Analytical Curve and Linearity

Verification of linearity reveals the ability of the method to demonstrate that the results obtained are directly proportional to the concentration of the analyte in the sample, within a specified range. The linearity of the method was evaluated by the construction of analytical curves through the external standardization in the white of the matrix.

The analytical curve was checked by analyzing standard solutions with five different concentrations (levels) in triplicate. For the construction of the curves a known amount of analytes in ultrapure water was added in the following concentration ranges: 250, 300,

500, 750 and 1000 μg.L^{-1} for AMPA and 50, 100, 500, 750 and 1000 μg.L^{-1} for glyphosate.

The criteria for approval of the curve are based on three principles: coefficient of determination (R^2) is greater than 0.99; coefficient of variation (CV) of each point on the curve is less than 10%; graph of the residuals of the calibration curve to present a random order of the points, without trends.

2.4. Limits of Detection and Quantification

The detection limit (LD) is determined as the lowest concentration of a measured and detected substance, with 95% or 99% confidence (ALBANO and RAYA-RODRIGUES, 2015). We performed seven readings of the lowest concentration of analyte (glyphosate and AMPA separately) of interest fortified in the ultrapure water sample, which can be detected by the ion chromatograph. Then, the medium values ($\overline{X}$) and the standard deviations expressed in μg .L^{-1} concentration obtained from the injected replicates were determined. The limits were calculated from Equation 1. The t value for the LD calculation was obtained from the t-Student distribution (unilateral) multiplied by the deviation of the replicates (s). The value corresponding to 99% confidence and six degrees of freedom (n-1) was 3.143 (ALBANO and RAYA-RODRIGUES, 2015).

$$LDM = \overline{X}_{analyte} + t \times s_{analyte} \qquad (1)$$

The quantification limit (LQ) represents the lowest analyte concentration value, capable of being determined with defined analytical reliability, in which the analyte is within the normal sensitivity (accuracy and precision) of the equipment or technique (ALBANO and RAYA-RODRIGUES, 2015).

Seven replicates of the ultrapure water sample were fortified with the analytes (glyphosate and AMPA separately), and the mean concentrations and standard deviations were calculated. The LQ calculation followed Equation 2. We used six standard deviations (s) plus the mean ($\overline{X}$) of the seven replicates.

$$LQ = \overline{X}_{analyte} + 6 \times s_{analyte} \qquad (2)$$

2.5. Precision

This study was based on the European Directive 657:2002. Repeatability is the degree of agreement between the results of successive measurements of the same measured carried out under the same measurement conditions. It was evaluated through the assays prepared in three distinct concentration ranges (250, 500 and 750 μg.L^{-1}) for both analytes (glyphosate and AMPA). The analysis were performed with the same test material, same analyst and even equipment, fortified with 0.5; 1.0 and 1.5 times the limit allowed by Ordinance 2914/2011, following the recommendation of Directive 657. For each level, the analysis was performed with six replicates. The mean concentration, standard deviation and CV were calculated. CV was calculated by dividing the standard deviation by the mean and multiplying this value by 100%.

The intermediate precision of the method was evaluated from the analysis by a second analyst (variation condition used), which reproduced the same amount of injections used for the repeatability parameter and with the same concentrations (250, 500 and 750 µg.L^{-1} for both analytes) as well as a number of six replicates for each of the samples. The intermediate precision took into account the deviation between the means of the different analysts, where the CV was estimated.

After the calculations were made, the maximum CVs obtained in the study were compared with the CVs estimated by Horwitz's formula, presented in Equation 3.

$$CV = 2^{(1-0,5 \times \log C)} \text{, where C (g / g)} \tag{3}$$

2.6. Accuracy

Accuracy is the difference between the actual and measured position. The determination was made by fortifying a white matrix with the analytes (glyphosate and AMPA) and calculating the recovery rate of the known analyte concentrations. In this assay quadruplicates were used at a concentration of 500 µg.L^{-1} for both analytes, a criterion established by the Laboratory and based on the reference of Albano and Raya-Rodrigues (2015).

For the evaluation of the results, the acceptance criterion was that for the pesticides studied the recovery rates are in the acceptable range of 70 to 120%. As a source of uncertainty of recovery, the relative error was used in relation to the target (theoretical) value of the sample.

2.7. Uncertainty Estimate

This parameter refers to the remaining doubt associated with the measurement result, corresponding to a range of values that can be attributed to the measurement.

The measurement uncertainty was estimated based on data from the method validation (EURACHEM, 2014). The sources of uncertainty of Type A were the deviations under conditions of repeatability and intermediate accuracy (also referred to herein as internal reproducibility), recovery error and deviation of the calibration curve. As a source of Type B, standard uncertainty (glyphosate and AMPA) was used, following the logic of the calculation based on the validation data of 350 µg.L^{-1} for AMPA and 50 µg.L^{-1} for glyphosate.

3. RESULTS AND DISCUSSION

This section presents the results obtained in the validation. From the applied methodology, it was possible to reach satisfactory detection and quantification limits: LD = 287.11 µg.L^{-1} and LQ = 311.37µg.L^{-1} for AMPA; and LD = 32.91 µg.L^{-1} and LQ = 38.94 µg.L^{-1} for glyphosate.

For a satisfactory effect, the sum of the LQs of both analytes (350.31 µg.L^{-1}) was not higher than the Maximum Residue Limit (MRL) established in Ordinance 2914/2011, which states that the sum of glyphosate and AMPA is 500 µg.L^{-1}.

The evaluation of the results for the recovery test, considering the accuracy of the method, was satisfactory (AMPA = 98.3% and glyphosate 100.46%). The analysis was based on the following criteria: for all pesticides studied, the recovery rates were in the acceptable range of 70 to 120% for the 500 µg.L^{-1} concentration level in consideration of the MRL of Ordinance 2914/2011.

From the results obtained in the repeatability and the internal reproducibility tests, the CVs were higher with the Horwitz equation, being satisfactory for both analytes (table 1). The internal variable factors were with two different analysts.

Table 1 - Coefficients of variation obtained through the repeatability and intermediate precision tests

Analyte	Result	Horwitz (CV maximum)
AMPA (Repe)	CV = 5,9%	6,97% (250 µg.L^{-1}) 6,28% (500 µg.L^{-1}) 5,91% (750 µg.L^{-1})
AMPA (intermediate precision)	CV= 5,5%	
glyphosate (Repe)	CV= 3,2%	
glyphosate (intermediate precision)	CV= 1,49%	

The maximum CV was lower than Horwitz's (maximum acceptable CV), meaning that the repeatability and the internal reproducibility of the method were satisfactory (for 250 µg.L^{-1}, 500 µg.L^{-1} and 750 µg.L^{-1}).

In the evaluation of the linearity of the method, the results obtained were considered adequate. In this assay R^2 = 0.99 was obtained for the glyphosate analytes (Figure 1) and AMPA (Figure 2).
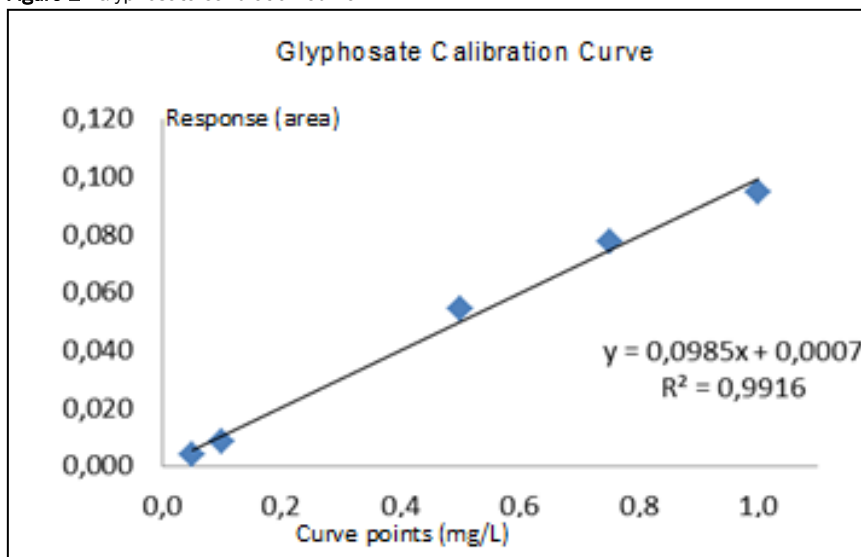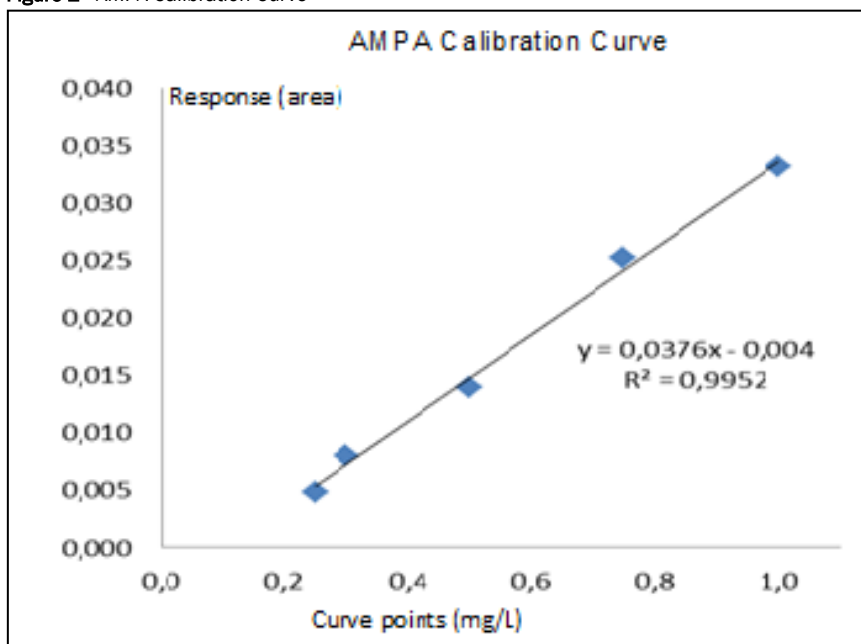
Figure 1 - Glyphosate Calibration Curve

Glyphosate Calibration Curve

$y = 0,0985x + 0,0007$
$R^2 = 0,9916$

Figure 2 - AMPA Calibration Curve

AMPA Calibration Curve

$y = 0,0376x - 0,004$
$R^2 = 0,9952$

The repeatability of the calibration curve points was also satisfactory, with CV of 2.8% (minimum) and 6.9% (maximum) for AMPA and 0.1% (minimum) and 5.8% (maximum) for glyphosate. The graph of the residuals of the calibration curve presented random results in "cloud form", meeting the criteria of randomness stipulated by Albano and Raya-Rodriguez (2015).

The uncertainty estimation worksheets are presented in Tables 2 and 3. The uncertainty estimation combined the variables that were raised in the validation study, including the standard, uncertainty, obtained in certificate (AMPA and glyphosate used were MRC produced from accordance with ISO 17034:2016).

The expanded uncertainty (U) of the analytes: glyphosate (Table 2) and AMPA (Table 3) was 21.93% and 9.3%, respectively. It was found that the main sources of uncertainty were: the calibration curve, which is expected, since chromatographic assays have this as a major contribution to method variability and repeatability. It was considered that the uncertainty obtained was adequate, since it met the internal criteria of the laboratory of maximum variation of 30%.

Table 2 - Glyphosate measurement uncertainty sheet

Result	50	ug.L⁻¹									
Result	0,05	mg L⁻¹									

Source	Measure	Entry	Converted input	Probability distribution	Splitter	Standard uncertainty	c_i	Combined uncertainty	Degrees of freedom	Effective degrees of freedom
Repeatability	CV (%)	3,20%	0,0016	t-student	2,449	0,00065	1	0,00065	5,0	
Reproducibility	CV (%)	1,49%	0,000745	t-student	2,449	0,00030	1	0,00030	5,0	
Accuracy (recovery)	Error (%)	0,5%	0,00022925	rectangular	1,732	0,00013	1	0,00013	∞	4,00
Standard	U (%)	0,97%	0,000485	t-student	2	0,00024	1	0,00024	∞	
Calibration curve	u_{curve}	0,00373	0,00373	t-student	1	0,00373	1	0,00373	4,0	

Combined uncertainty (u)		0,0038
k		2,88
Expanded uncertainty (U) ug L⁻¹		0,01
Expanded uncertainty (U) %		21,93%

Table 3 - AMPA measurement uncertainty sheet

Result	350	ug L⁻¹									
Result	0,35	mg L⁻¹									

Source	Measure	Entry	Converted input	Probability distribution	Splitter	Standard uncertainty	c_i	Combined uncertainty	Degrees of freedom	Effective degrees of freedom
Repeatability	CV (%)	5,90%	0,0206	t-student	2,45	0,0084	1	0,0084	5,0	
Reproducibility	CV (%)	5,59%	0,0196	t-student	2,45	0,0080	1	0,0080	5,0	
Accuracy (recovery)	Error (%)	-1,7%	-0,0059	rectangular	1,73	-0,0034	1	-0,0034	∞	5,08
Standard	U (%)	0,96%	0,0034	t-student	2,00	0,0017	1	0,0017	∞	
Calibration curve	u_{curve}	0,00093	0,0009	t-student	1,00	0,0009	1	0,0009	4,0	

Combined uncertainty (u)		0,012
k		2,66
Expanded uncertainty (U) em ug L⁻¹		0,03
Expanded uncertainty (U) %		9,3%

The characterization of the area where the 90 samples of drinking water were collected are litolic , oxisol ,planosol e argisol soils from the Cities: Agudo, Alegrete, Alvorada, Araricá , Bagé, Boa Vista do Cadeado, Camaquã, Candiota , Canguçu, Capão da Canoa, Capão do Cipó, Charqueadas, Coqueiro Baixo, Coxilha, Dom Pedrito, Frederico Westphalen, Giruá, Gravataí, Guaíba, Horizontina, Ivorá, Jaguarão, Júlio de Castilhos, Lajeado, Mampituba, Maquiné, Muitos Capões, Nova Palma, Nova Petrópolis, Novo Hamburgo, Palmares do Sul, Pinhal Grande, Quaraí, Relvado, Rio Grande, Rio Pardo, Rolante, Rosário do Sul, Sananduva, Santa Margarida do Sul, Santa Maria do Herval, Santa Maria, Santa Rosa, Santana do Livramento, Santo Angelo, Santo Antonio das Missões, São Borja, São Lourenço do Sul, São Sepé, Sertão, Silveira Martins, Sobradinho, Travesseiro, Três Passos, Tupanciretã, Uruguaiana, Vera Cruz, belonging to the Regions: Campanha, Campos de Cima da Serra, Central, Centro Sul, Fronteira Noroeste, Fronteira Oeste, Hortênsias, Jacuí Centro, Litoral, Médio Alto Uruguai, Metropolitano Delta Jacuí, Missões, Nordeste, Noroeste Colonial, Paranhama-Encosta da Serra ,Produção, Rio dos Sinos Valley, Rio Pardo Valley, Sul, Taquari Valley and which are bathed by the Guarani Aquifer.

From the climatological point of view, these areas are included in the subtropical climate classification. The annual temperature ranges (average) from 14 ° to 18 ° C and the annual rainfall varies from 1500 to 170 mm.

One of the main problems with the exploration of the Guarani waters is the risk of aquifer deterioration due to the increase in the volumes explored and the growth sources of pollution. Although municipalities are not water holders, they play an important role in the preservation of aquifers. They have specific competence for the disciplining of land use and occupation and for the integrated management of groundwater in their territory.

The persistence of glyphosate in water is shorter than its persistence in soil. Glyphosate formulations are completely water soluble because they disperse rapidly and do not accumulate at high concentrations in the water profile. Glyphosate dissipates in surface water rapidly by being adsorbed by sediments and degraded by microorganisms (PATERSON, 2016).

Therefore, the Precautionary Principle should continue to monitor these waters by the current legislation, while maintaining the adoption of preventive measures that determine the vulnerability to contamination of aquifers.

4. CONCLUSION

The environmental impact caused by the use of contaminating products, in the case of pesticides, can be considered as any change in the physical, chemical and biological properties of the environment caused by any matter or energy resulting from human activities that affect directly or indirectly the health, safety and well-being of the population: social, economic, biota and quality of the natural resources of the environment (FRIGHETTO, 1997; SPADOTTO, 2002). Considering these pesticides, herbicides constitute the commercial scale in the industrialized world, the main share of sales (TAROUCO, 2009), mainly formulated with glyphosate has gained expression and importance due to the growth of areas sown with genetically modified crops and also by if it is a systemic herbicide with a broad action spectrum (MORAES, 2010).

Glyphosate has very specific properties, its half-life is greater in soil than in water, which differ from most other active ingredients, as well as hinder its monitoring in environmental samples, because it depends on the physical, chemical and biological characteristics of the soil which was deposited and the number of applications influence directly or indirectly on population of the macro and micro soil fauna. Their formulations are completely soluble in water and rapidly disperse and do not accumulate in high concentrations in the water profile, and are absorbed by the sediments and degraded by micro-organisms (PATTERSON, 2016).

However, through the validation of the method it was possible to demonstrate that the technique of direct water analysis from ion chromatography is an efficient and adequate method to the scope. After this method optimization, was analyzed 90 samples of drinking water in 2016/2017 from the Surveillance Program of Water/RS (VIGIÁGUA) at the Central Laboratory RS (LACEN/RS).

All results obtained were not detected for glyphosate and its metabolite AMPA receptors in the waters for human consumption groundwater is treated and not treated researched, demonstrating that there is the possibility of the use of glyphosate for elimination of undesirable plants, since the soil characteristics of the study, do not favor the desorption of glyphosate and AMPA, thus reducing the mobility of these compounds in the environment.

Although there is a disagreement between several authors about what are the most important factors in the adsorption process of glyphosate and AMPA in soils, there is still a point of convergence that the inorganic fraction plays a major role.

In 2015, the International Agency for Research on Cancer (IARC), after evaluating the carcinogenicity of active ingredients of pesticides by a team of researchers from several countries, classified glyphosate as probable carcinogenic to humans (Group 2A). IARC assesses the potential carcinogenicity of chemicals based on long-term animal studies and epidemiological studies on occupational exposure in humans.

The analysis of mortality data in Rio Grande do Sul reveals that, out of a total of 82,294 deaths in 2015, tumors accounted for 22.21% of the causes of death, losing only to diseases of the circulatory system (27.66%), ; in third place were diseases of the respiratory system with 12.38% (RIO GRANDE DO SUL, 2016).

Finally, we considered that the objective of the work was reached, since the method was properly validated and its measurement uncertainty was estimated based on the experimental results of the validation process. The quantification limits for Glyphosate and AMPA were suitable for samples evaluation according to the Brazilian legislation. The uncertainty measurement was also fit for the laboratory purpose.

In future research it is suggested to approach the validation of methods in other pesticide tests, focusing on analytical improvements in the analysis process. It is also expected that ANVISA (National Health Surveillance Agency), IBAMA (Brazilian Institute of Environment and Renewable Natural Resources) and MAPA (Ministry of Agriculture and Food Supply) keep up with the toxicological evaluation restricting the use of agrochemicals in Brazil. And the agrotoxic name remains in Brazilian legislation so that its real danger to human health and the environment is made explicit. As well as the drinking water standard for human consumption follow the example of other countries reducing the maximum permitted amount of pesticides.

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