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Analysis of Factors Involving Drinking Water Contamination by Glyphosate and/or Nitrate in Urban Areas

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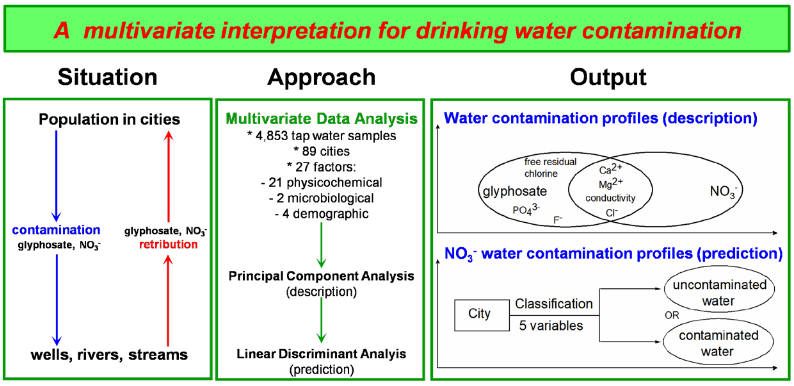
This study investigated factors that could be related to drinking water contamination in urban areas in order to obtain quality profiles that characterized presence of the glyphosate and nitrate contaminants. Thus, in a period of one year, 4,853 tap water samples from 89 cities in the northeastern region of the State of São Paulo, Brazil, were analyzed in 21 physicochemical and 2 microbiological parameters. Additionally, 4 demographic variables were also included in multivariate data analysis. Principal Component Analysis of physicochemical and microbiological data showed that glyphosate concentration is positively correlated with nitrate concentration, especially in cities that make exclusive use of groundwater, besides correlating with conductivity and with concentrations of calcium, magnesium, fluoride, chloride, phosphate and free residual chlorine. The inclusion of demographic variables in Principal Component Analysis did not significantly change waters physicochemical profiles, but in cities that exclusive use groundwater for public supply the number of hospitalizations for diarrhea correlated positively with glyphosate, nitrate and chloride concentrations, in addition to conductivity. Linear Discriminant Analysis models involving 5 variables (conductivity and concentrations of calcium, magnesium, chloride and nitrate) were able to predict the cities vulnerability to groundwater contamination by nitrate.

Graphical abstract



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1. Introduction

1.1 About glyphosate and nitrate

Broad-spectrum herbicides based on glyphosate (N-(phosphonomethyl)glycine, $C_3H_8NO_5P$) are widely used in agriculture, forestry and algae control in water, and are also

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extensively studied for various reasons such as toxicity, development of glyphosate tolerant crops, emergence of glyphosate-resistant weeds, and loss of biodiversity since tolerant crops promote monocultures that reduce diversity and, on the other hand, weeds can sustain organisms such as pollinators [1]. With regard to toxicity, in 2015 the International Agency for Research on Cancer concluded that glyphosate is a probable carcinogen in humans [2], but in 2017 the World Health Organization (WHO) considered both glyphosate and its main degradation product, aminomethylphosphonic acid (AMPA), as low toxicity substances and therefore has not established concentrations to guide actions in relation to drinking water quality [3]. In this controversial scenario [4] is important to highlight the scarcity of data investigating the exposure of human beings to glyphosate [5] and the different guidelines adopted by regulatory agencies with regard to the maximum concentrations allowed in drinking water: in the European Union, for example, 0.1 µg L^{-1} is the maximum value allowed for individual pesticides, their relevant metabolites, decomposition and reaction products [6]; in the United States, the maximum allowed level of glyphosate is 700 µg L^{-1} [7] while in Brazil the maximum allowed value for the sum of glyphosate and AMPA concentrations is 500 µg L^{-1} [8]. In addition to the contamination of water sources [9], studies have been published involving the impact of glyphosate on environment [10], its presence in grains and other foods [11] and the occurrence in biological samples such as blood and urine [12]. Thus, many analytical methods have been developed for glyphosate quantification in different matrices [13, 14]. This work is concerned with this herbicide as a contaminant in water intended for human consumption, since it can be introduced into surface water after its direct use in aquatic environments or by soil leaching; groundwater contamination is also possible but considered less likely due to low mobility of glyphosate in soil [3].

Nitrate (NO_3^-) is another water contaminant of Public Health concern. This ion is a naturally occurring substance (it is part of the nitrogen cycle) and an important nutrient for plants [15]. The main sources of human exposure are in the diet (mainly vegetables and meats) but drinking water can contribute significantly to nitrate intake in some circumstances. High concentrations may occur in surface water or groundwater as a result of agricultural activity (including excessive use of nitrogenous inorganic fertilizers and manure), disposal in the environment of untreated wastewater, oxidation of animal and human excrement (including that occurring in septic tanks) and the disposal of solid waste (landfills). Blarasin et al. used the Principal Component Analysis (PCA) of hydrochemical and isotopic data to compare the factors that control nitrate concentrations in urban and rural areas [16]: the results showed that nitrate pollution in urban area of Del Campillo city (Córdoba, Argentina) originated mainly from local sanitation systems and/or waste of domestic animals; pollution in rural areas was mainly attributed to a combination of urea-based fertilizers and manure. While nitrate concentrations can vary rapidly in surface water, in groundwater variations are usually slower. This factor is important: the relatively slow movement of water through the soil implies that groundwater residence times are generally much longer than those observed in surface water – so once polluted, an aquifer can remain in this state for decades due to the slowness of the natural recharge process with uncontaminated water [3]. The Public Health interest in the presence of nitrate in waters intended for human consumption is due to the occurrence of methaemoglobinaemia in children up to three months of age (blue-baby syndrome) and to thyroid disease: according to WHO, epidemiological studies have not reported methaemoglobinaemia or thyroid effects in areas where drinking water contained less than 50 mgNO_3^- L^{-1} [17]. In addition, the occurrence of some types of cancer and neural tube defects has also been investigated [18]. In the United States [7] and Brazil [8], the maximum allowed contaminant level expressed as nitrogen-nitrate is 10 mgN-NO_3^- L^{-1} (44.3 mgNO_3^- L^{-1}, approximately); in the European Union, the maximum allowed concentration is 50 mgNO_3^- L^{-1} [6].

1.2 About the region where this study was conducted

In the northeast region of the State of São Paulo (Brazil) approximately 3.3 million inhabitants live in 90 municipalities (Fig. 1). Agriculture contributes significantly to the economy (especially sugar cane and coffee plantations) but this region is undergoing a process of industrialization. The public water supply in the urban areas of each municipality is carried out using groundwater or surface water (or a combination of both). Groundwater abstraction can be carried out from the Bauru, Serra Geral and Guarani aquifers (see supplementary material, Fig. SM 01), while surface water can be abstracted from several rivers and streams, among which stand out the rivers Grande, Pardo, Mogi Guaçu and Sapucaí (see supplementary material, Fig. SM 02). The monitoring and evaluation of water quality are carried out through Surveillance Program of Water for Human Consumption (designated "Proagua"), which includes the analysis of eight parameters in tap water: temperature, pH, free residual chlorine (FRC), apparent color, turbidity, fluoride concentration, total coliforms and *Escherichia coli*. In this Program, the samples are collected by Municipal Sanitary Surveillance agents that perform the measurements of temperature, pH and FRC at the time of collection; the samples are then sent to laboratories where the other parameters are analyzed. In State of São Paulo there is no routine monitoring involving analyses of glyphosate and AMPA in tap water, but there is concern about nitrate presence since contamination of groundwater has been reported, such as those that occurred in urban areas of the municipalities of Marília [19], Presidente Prudente [20] and Monte Azul Paulista [21]. These studies have related high nitrate concentrations in groundwater to densely populated places and/or with older human occupation, presence of cemeteries, poorly-constructed latrine pits, older sewage collection network with higher probability of leaks, and landfills [22]. A study carried out in the region, using 4,347 samples from "Proagua" and analyzing 23 parameters, mapped tap water quality and indicated the existence of a group of 14 cities with high nitrate levels in which Monte Azul Paulista and Severínia stood out. [23, 24].

1.3 Objectives

In a complex scenario for Public Health in which cities of an agricultural region in industrialization process can perform the water abstraction from various sources (three aquifers and several rivers and streams), the objective of this work was to investigate factors that could affect the quality of water intended for human consumption in urban areas of State of São Paulo northeast, Brazil (Fig. 1) regarding nitrate and/or glyphosate/AMPA contamination. For this purpose, tap water samples were analyzed for one year in order to use PCA for pattern recognition with inclusion of 27 factors: 21 physicochemical, 2 microbiological and 4 demographic parameters. In addition, it was intended to investigate the possibility of using multivariate predictive models for nitrate tap water contamination.

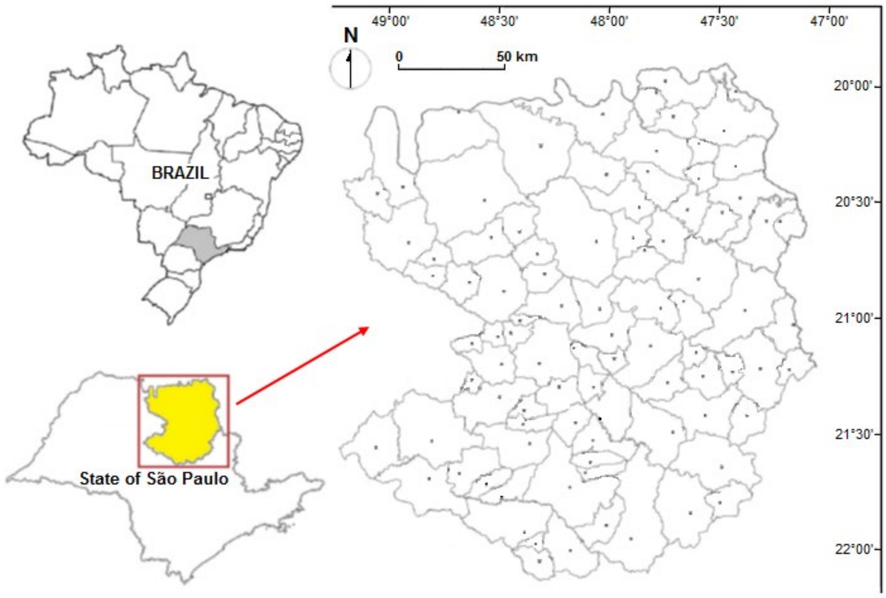

Fig. 1. Region where tap water was analyzed (on the right) and its location in the State of São Paulo, Brazil (on the left).

2. Material and Methods

2.1 Sampling of water intended for human consumption

The sampling was carried out between 03/18/2019 and 03/17/2020. Sampling frequency aimed to reach a minimum ratio of 1/10,000 between the number of samples by month and the number of inhabitants of each municipality; for municipalities where the presence of nitrate, bromate or lithium was previously known [23], the number of monthly samples was increased (the number of monthly samples from each municipality is described in Table SM 01 of the supplementary material). For physicochemical analysis, tap water samples were collected in clean plastic bottles (capacity between 250 and 1,000 mL). For microbiological analysis, 100 mL of the tap water samples were collected in plastic bottles or in *Thio Bags* (120 mL capacity)*;* these sterile and disposable containers contained sodium thiosulfate for neutralization of residual chlorine. The collections were carried out by Health Surveillance agents in each of the 89 cities; these agents also performed the measurements of temperature, pH and free residual chlorine (FRC) at the time of collection. Finally, the samples were refrigerated (4°C) and sent to laboratory.

2.2 Laboratory analysis

All reagents used in laboratory analysis were of analytical grade (Sigma-Aldrich and Merck). Aqueous standard solutions were prepared with type I water obtained from a Millipore purification system, Milli-Q Direct 8 model.

In total, 4,853 tap water samples were analyzed. Determination of the presence or absence of total coliforms and *Escherichia coli* was carried out by the chromogenic and fluorogenic method (Colilert Test Kit, *Idexx Laboratories*/USA). Conductivity was determined on Metrohm 912 meter. Apparent color was measured using a Digimed colorimeter, DM-COR model, and turbidity was determined using a Tecnopon turbidimeter, TB-1000 model. Concentrations of lithium, sodium, ammonium (as NH_3), potassium, calcium, magnesium, fluoride, chlorite, bromate, chloride, nitrate (as $N\text{-}NO_3^-$), AMPA, sulfate, phosphate (as $P\text{-}PO_4^{3-}$) and glyphosate were determined by ion chromatography using validated methods previously described [25-27].

2.3 Demographic data collection

Information for each municipality on the number of inhabitants (NINH), Gross Domestic Product per capita (GDPpc) and Municipal Human Development Index (MHDI) were obtained from the Brazilian Institute of Geography and Statistics website (https://cidades.ibge.gov.br). The number of hospitalizations due to diarrhea and gastroenteritis in the data acquisition period (03/18/2019 to 03/17/2020) was obtained for each municipality on the Ministry of Health of Brazil website (**http://www2.datasus.gov.br/DATASUS/index.php**). This number was transformed into hospitalizations per thousand inhabitants (HOSP).

2.4 Multivariate data analysis (PCA)

Microsoft Excel® 2019, *Origin*® 9.1Pro and The *Unscrambler*®X 10.3 were used in data processing. As part of data pretreatment for multivariate analysis, each city was initially represented by a set of means obtained in each of the 21 physicochemical parameters analyzed; then, the positive results of each city in the two microbiological analyses (total coliform + *Escherichia coli*) were summed and expressed as the percentage of positive microbiological results (variable %MB+). Finally, the 4 demographic data of each municipality were included. Thus, the initial matrix 4,853 x 27 (samples x variables) was transformed into an 89 x 26 matrix (cities x variables) that is presented in the supplementary material (Table SM 02). Before performing PCA, variable data were centered by the mean and scaled by the standard deviation (autoscaling).

3. Results and Discussion

3.1 Water quality: overview

Escherichia coli and/or total coliform presence was detected in 380 samples (7.8% of total). This result reveals a decrease in the disinfection process efficiency when compared to that obtained in a previous study conducted between May 2015 and April 2016 when 4,347 samples were analyzed and 6.6% were contaminated [23]. There was also a decrease in the fluoridation quality: while previously 60.2% of the samples presented a satisfactory fluoridation, in this study this index decreased to 55.0%. The quality of fluoridation was evaluated on the basis of São Paulo State legislation, which states that the fluoride concentration should be between 0.6 and 0.8 mg L^{-1} [28]. These quality drops in both disinfection and fluoridation are being investigated.

Bromate was found in 225 samples (4.6% of the total, concentrations between 3 and 199 µg L^{-1}), especially in the cities of Ribeirão Preto (121 contaminated samples) and Batatais (38 contaminated samples). These numbers are much higher than those previously obtained when only 42 samples were contaminated (1% of the total, concentrations between 5 and 30 µg L^{-1}), in which the same cities were important with 19 and 7 contaminated samples, respectively [23]. This increase in the proportion of samples contaminated with bromate is due, at least in part, to the increase in the number of samples analyzed for the cities where contamination was found according to the previous study. Bromato is considered mutagenic and a probable carcinogen; investigations are being carried out with the municipalities, but it is likely that contamination occurred due the inadequate quality of the hypochlorite solution used for the disinfection process, since no industrial sources of pollution were identified and water disinfection in the region is not performed by ozonation which could produce bromate if bromide was present [3]. In the European Union [6], United States [7] and Brazil [8] the allowed maximum bromate level in drinking water is 10 µg L^{-1}.

While in previous study [23] lithium ion was present in 34.8% of the analyzed samples (concentrations between 2 and 28 µg L^{-1}), in this work this percentage increased to 51.8% (concentrations between 1 and 23 µg L^{-1}). This increase is due, at least in part, to (i) the use of a method with lower detection limit [26] and (ii) an increase in the number of samples analyzed for the cities where lithium was determined in the previous study. Lithium salts are used in the treatment of bipolar disorder and unipolar depression, as well as in suicide prevention [29]; it has been suggested the existence of a negative correlation between low levels of lithium in waters intended for human consumption and suicide mortality [30]. In addition, studies have found evidence of a beneficial effect of lithium low concentrations in drinking water for dementia prevention [31]. Possible correlations between lithium levels found in these studies and suicide rates and/or mood disorders in the region are being investigated. In the European Union [6], United States [7] and Brazil [8] there are no reference values for lithium in drinking water.

Nitrate was detected in all cities (3,920 samples, 80.8% of the total), and the occurrence rates were higher than those previously obtained when nitrate was found in 3,119 samples (or 71.8% of the total) – see supplementary material, Table SM 03. The higher indices can be attributed, at least in part, to the increase in the number of samples analyzed for cities where there was already knowledge of important nitrate levels [23]. Seasonal fluctuations in nitrate concentrations between the dry season (March to September 2019, autumn and winter) and the rainy season (September 2019 to March 2020, spring and summer) were observed, but not all were considered significant. For example: in the city of Monte Azul Paulista, where 30 wells are used for public supply, the mean nitrate concentration increased from 3.4 (dry season) to 3.7 mg L^{-1} (rainy season), while in Severinia (16 wells) a decrease was observed, from 5.8 to 5.0 mg L^{-1} – the data were compared by a t-test ($\alpha = 0.01$) and it was found that differences were not significant in both cases (p-value 0.66 and 0.32, respectively). However, the increase from 3.8 to 4.0 mg L^{-1} observed in the case of the city of Terra Roxa (3 wells) was considered significant when the same test was applied (p value = $4x10^{-10}$). These two opposite effects on nitrate concentration depend on the terrain characteristics: an increase in concentration in the rainy season can be attributed to rainwater infiltration, while a concentration decrease can be attributed to dilution in rainfall [15].

Glyphosate was found in 151 samples (3.1%) from 42 cities (47.2%), while AMPA was not detected; concentrations ranged from 15 to 206 µg L^{-1}, and it was found most frequently in the periods from August to September (2019), and from January to April (2020) – a new stage of data acquisition is being planned to investigate this periodicity. The research of glyphosate and AMPA was not carried out in previous study, but in the present work it was initially focused on cities that use surface water for public supply since the greater vulnerability this type of water source to contamination; in fact, among 10 cities in the region that exclusively use surface water, 7 presented water contamination. Nevertheless, this study found that significant glyphosate contamination may also occur in groundwater samples. This fact will be discussed further.

3.2 Water characteristics in the region: pattern recognition by PCA

In order to perform the initial pattern recognition by PCA, involving the water quality parameters of all cities as well as their demographic characteristics (Table SM 02, supplementary material), the variables less associated with each other were eliminated (the AMPA variable was immediately discarded because the main metabolite of glyphosate was not detected in any sample). The criteria adopted for variables inclusion in this initial model were based on the analysis of the Pearson's correlations (r) matrix, indicated in Table SM 04 of the supplementary material: (i) absolute values of r greater than 0.3 ($|r| > 0.3$) were considered significant [32] and (ii) were selected the variables that presented two or more correlations with $|r| > 0.3$. According to these criteria, the following variables were eliminated: ammonium, temperature, %MB+, HOSP, fluoride, bromate, FRC, turbidity, color, NINH, GDPpc and MHDI. Finally, it was observed that although the variable chlorite presented three significant correlations (chloride, r = 0.350; phosphate, r = 0.318; glyphosate, r = 0.382), there was a large number of null values (chlorite means below the detection limit occurred in 72% of cities); in addition, scatter plots (see supplementary material, Figure SM 03) indicated a strong influence of Santa Rita do Passa Quatro city chlorite mean in the r values. In fact, when recalculating r values in the absence of Santa Rita do Passa Quatro city data, the correlations of chlorite with chloride, phosphate and glyphosate decreased to 0.032, 0.202 and -0.0095, respectively. Consequently, the chlorite variable was also eliminated.

After eliminating the variables with correlations considered non-significant, the initial PCA included the remaining 12 physicochemical variables and showed that the first three principal components (PC1, PC2 and PC3) were responsible for 66% of the explained variance (Fig. 2). The group of cities that did not present prominent variables in this descriptive model was located approximately in the center of the score plots (Fig. 2A and 2C), and was designated as "typical group". The PC1/PC2 scores plot (Fig. 2A) indicated Ibitinga as a city with tap water very different from the other tap waters in the region in terms of the physicochemical parameters analyzed. This unique profile can be attributed to higher pH and conductivity values, and to higher concentrations of sulfate, sodium, lithium and chloride, as indicated in the respective loadings plot (Fig. 2B) – in addition, some samples from Ibitinga were contaminated by glyphosate. From the center of the sores plot and in the same direction of Ibitinga, it is possible to observe a group of 5 cities in which the variable lithium was prominent ("lithium group", Fig. 2A); in fact, the same variables that defined the Ibitinga city profile (except glyphosate that was not detected in samples from these 5 cities) were also important for "lithium group" profile (Fig. 2B), but with smaller values. These characteristics in PCA ("lithium group" and unusual physicochemical parameters of Ibitinga tap water) had already been observed in a previous study [23]. In the same PC1/PC2 scores plot (Fig. 2A), it is possible to observe cities that move away from the center at 90 degrees approximately from the direction of Ibitinga clockwise – as can be seen in the respective loadings plot (Fig. 2B), these cities presented significant nitrate contamination ("nitrate group"), but simultaneous glyphosate contamination was also observed in several cities ("nitrate+glyphosate group"), and nitrate showed important correlations with calcium and magnesium variables. Still in the PC1/PC2 space (Fig. 2A), cities were observed standing out in the negative direction of PC1, influenced by potassium and phosphate concentrations ("potassium/phosphate group"), as indicated by the respective loadings plot (Fig. 2B). This small group is best visualized in the PC1/PC3 space (Fig. 2C) where it is also possible to visualize another group of 9 cities in the negative direction of PC3, characterized by glyphosate tap water contamination ("glyphosate group") as indicated in loadings plot (Fig. 2D); in this graph, glyphosate showed important correlation with chloride concentration. The "glyphosate group" included 7 cities that use only surface water, and 2 cities that use surface water and groundwater for public water supply.

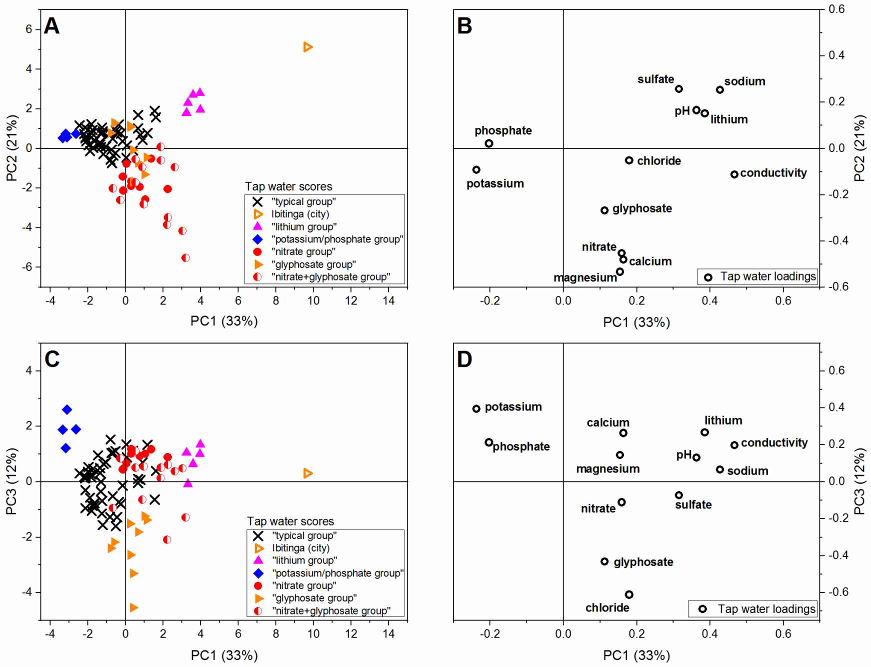

Fig. 2. PCA for initial pattern recognition: 89 samples (cities) and 12 physicochemical variables. A and B: scatter plots in PC1/PC2 space. C and D: scatter plots in PC1/PC3 space.

In three-dimensional space PC1/PC2/PC3 (Fig. 3) it was possible to clearly observe the positions of Ibitinga city and the typical, lithium and potassium/phosphate groups. However, cities with tap waters contaminated with nitrate and/or glyphosate were overlapped revealing an important correlation between these two variables. This correlation is described in detail below. The original graphs of this first PCA are in supplementary material, Fig. SM 04 to SM 06.

3.3 Nitrate/glyphosate correlation (contamination physicochemical profiles)

In order to investigate the correlation between the concentrations of nitrate and glyphosate, the cities were divided into three groups according to the source they use for public supply: 51 cities formed the group that uses groundwater, 10 cities formed the group that uses surface water, and 28 cities formed the group that use both sources. In the selection of physicochemical variables for PCA, the following procedure was followed:

(i) Three raw data matrices were constructed corresponding to the three groups of cities (supplementary material, Tables SM 05 to SM 07). The respective correlation matrices were obtained (supplementary material, Tables SM 08 to SM 10).

(ii) For the group that uses groundwater, the variables that significantly correlated with nitrate (|r| > 0.3) were initially selected due to the recognized groundwater contamination in the State of São Paulo: calcium (r = 0.422), magnesium (r = 0.665), chloride (r = 0.857), glyphosate (r = 0.531) and conductivity (r = 0.490). Next, variables were chosen that were significantly correlated with glyphosate, since it was observed that this group presented 45% of the cities with waters contaminated with the herbicide: calcium (r = 0.344), magnesium (r = 0.350), chloride (r = 0.532), nitrate (0.531) and conductivity (r = 0.329). It is important to note that they are the same variables selected in relation to nitrate. Highest mean concentrations of contaminants in groundwater raw data matrix: nitrate, 5.5 mg L^{-1}; glyphosate, 11 µg L^{-1}.

(iii) For the group of 10 cities that use surface water, the variables that correlated significantly only with glyphosate were initially chosen, considering the higher incidence of this contamination in this type of water source: calcium (r = 0.475), fluoride (r = -0.475), chloride (r = 0.819), nitrate (r = 0.356), phosphate (r = 0.828), FRC (r = -0.312) and conductivity (r = 0.436). The magnesium variable presented low correlation with glyphosate (r = 0.250), but high correlations with variables associated with nitrate contamination: calcium (r = 0.861), conductivity (r = 0.838) and nitrate (r = 0.715). Thus, magnesium was included. Although chlorite and bromate presented high r values (0.899 and 0.953, respectively), these variables were excluded due the high number of null values (below the detection limit). The pH variable (r = 0.306) was initially included, but the first exploratory PCA showed a negligible loading in the direction of glyphosate contamination (supplementary material, Fig. SM 07). Consequently, pH was also excluded. Highest mean concentrations of contaminants in surface water raw data matrix: nitrate, 1.8 mg L^{-1}; glyphosate, 14 µg L^{-1}.

(iv) The group of 28 cities that use both sources could present contamination by nitrate and glyphosate. However, nitrate correlated only with chlorite (r = 0.361), while glyphosate correlated with calcium (r = 0.458) and bromate (0.463). Considering that chlorite and bromate variables had to be excluded (many null values), PCA was not performed for this group of cities. This difficulty in developing a multivariate descriptive model by PCA can be attributed, at least in part, to the mixture of characteristics of the two water sources – the use in greater or lesser quantity of a particular source by a given city depends on several factors, such as demand and periods of the year (rainy or dry season). Thus, the water properties supplied to the population may vary significantly throughout the year for the same city, which makes it difficult to establish a characteristic physicochemical profile for water contamination. Highest mean concentrations of contaminants in groundwater/surface water raw data matrix: nitrate, 1.9 mg L^{-1}; glyphosate, 12 µg L^{-1}.

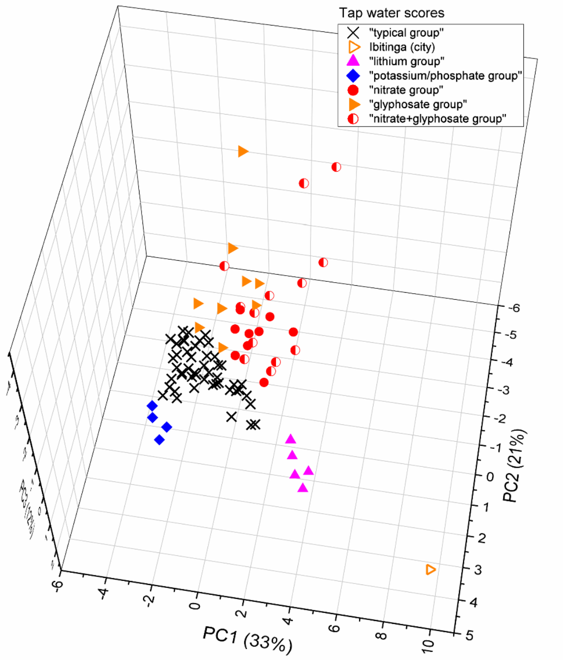

Fig. 3. Three-dimensional space (PC1/PC2/PC3) for pattern recognition score plots (initial PCA, 89 samples, 12 physicochemical variables).

The PCA including cities that use groundwater for public supply (Fig. 4A and 4B) exhibited 78% of the variance explained by the first two principal components and produced positive PC1 scores greater than 0.69 for cities with water contaminated by nitrate or by nitrate and glyphosate (original PCA graphs are in supplementary material, Fig. SM 08). Cities dispersion along the first component in the scores plot (Fig. 4A) was mainly influenced by the chloride, magnesium, nitrate, conductivity and calcium variables (Fig. 4B), with loadings 0.46, 0.44, 0.43, 0.40 and 0.38, respectively, but the contribution of glyphosate was also significant (loading 0.32). The strong influence of the chloride, magnesium, nitrate, conductivity and calcium variables on the PCA of region groundwater physicochemical parameters had already been observed in previous studies and was related to profiles of nitrate contaminated water [23, 24]. Although part of the chloride present in tap water comes from the disinfection process by chlorination, the high positive correlation between nitrate/chloride (r = 0.857) suggests contamination by anthropic action, as well as the positive nitrate/conductivity correlation (r = 0.490). On the other hand, considering that soil characteristics influence the water physicochemical variables, the positive correlations between nitrate/calcium and nitrate/magnesium (and the respective loadings in PC1, Fig. 4B) suggest the existence of soil-water equilibria involving, for example, $CaCO_3$-H_2O and $MgCO_3$-H_2O, as indicated by the hydrogeological study described for the city of Monte Azul Paulista, located in the region [33]. In this city there was a restriction on groundwater extraction established by the Department of Water and Electric Energy of the State of São Paulo due to the occurrence of high nitrate concentrations [21]. This restriction lasted six years (from 2013 to 2019) and the contamination was attributed to excessive number of wells in the urban area and inadequate drilling and maintenance of these wells; the highest levels were determined in older places of the city, where latrine pits were constructed in the past, but leaks in sewage collection network were also cited as sources of contamination [33]. In present study the water from the city of Monte Azul Paulista exhibited a nitrate mean concentration equal to 3.5 mg L^{-1}. Nitrate contamination of water intended for human consumption has also been described for the neighboring city, Severínia [24]; in present study this city presented a similar physicochemical profile to Monte Azul Paulista (Fig. 4A) and nitrate mean concentration equal to 5.5 mg L^{-1}. However, further studies are needed in the case of the city of Terra Roxa, which also presented a similar physicochemical profile (Fig.4A) and a nitrate mean concentration equal to 3.9 mg L^{-1}. In addition, the waters of these 3 cities also presented glyphosate contamination.

At first, the occurrence of glyphosate in tap waters of cities that use groundwater for public supply was not expected for two reasons: (i) this type of water source is less vulnerable to glyphosate contamination, and (ii) if glyphosate were present in the water, the chemical reaction with chlorine (used in the disinfection process) could reduce herbicide concentrations to undetectable levels [34]. Nevertheless, the significant positive correlation between nitrate and glyphosate (r = 0.531) suggests that cities vulnerability to nitrate groundwater contamination is also responsible for glyphosate contamination. In fact, it has been suggested that nitrate can be used as an inexpensive indicator to identify supply systems where other contaminants of concern may be present [35], such as agrochemicals and pharmaceutical compounds, for example. The source of groundwater glyphosate contamination is being investigated, but it is important to consider that the Health Surveillance Center of the State of São Paulo (http://www.cvs.saude.sp.gov.br) found that chemical weeding performed in urban areas is a relatively common practice for several years – this finding motivated the establishment in 2013 of a campaign entitled "Eliminating chemical weeding in the cities of São Paulo". In addition, it is necessary to consider that chemical weeding carried out in amateur gardening can also contribute to water sources contamination, given the ease of buying glyphosate in the region – currently, 1 liter of 1% solution costs the equivalent of approximately US$ 3.5 in local stores.

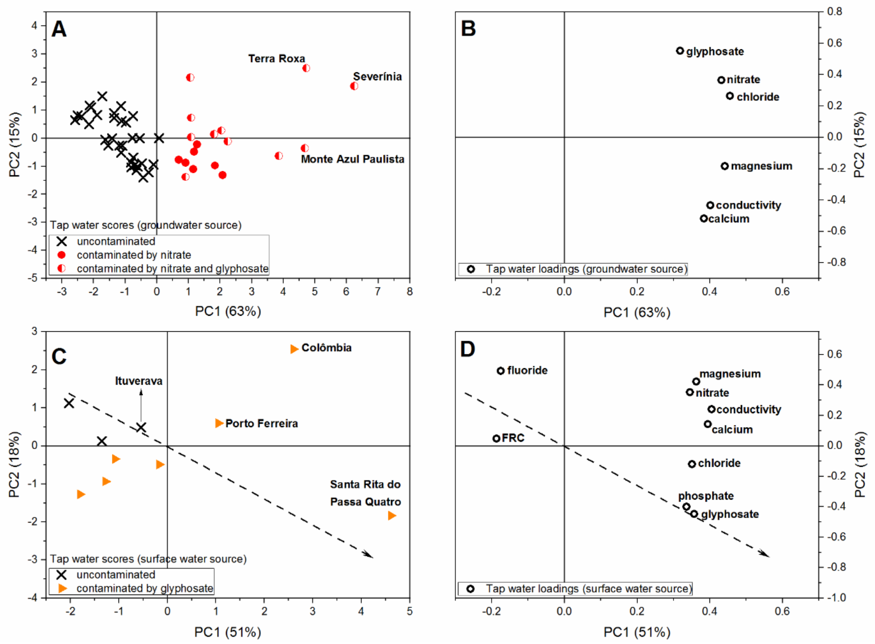

Fig. 4. A and B: PCA of groundwater samples (51 cities, 6 physicochemical variables). C and D: PCA of surface water samples (10 cities, 9 physicochemical variables).

The PCA including cities that use surface water for public supply (Fig. 4C and 4D) exhibited 69% of the variance explained by the first two principal components. In cities dispersion in scores plot (Fig. 4C) it was possible to draw a “direction of glyphosate contamination” starting from the 2nd quadrant towards the 4th quadrant (see dashed arrow in Fig. 4C), based on the same direction in the respective loadings plot (see dashed arrow in Fig. 4D). Considering PC1, there are interesting features in this loadings plot: (i) phosphate has positive loading (0.34), very close to glyphosate loading (0.36) – this proximity results, at least in part, from the high positive correlation coefficient (r = 0.828); (ii) FRC exhibits significant loading (-0.19), in a opposite position to glyphosate, as a consequence, at least in part, of its negative correlation (r = -0.312) – at first, surface waters are more susceptible to contamination by microorganisms and have a greater amount of organic material or other oxidizable substances compared to groundwater, requiring a greater amount of chlorine for disinfection [36] and, consequently, FRC acquired a greater influence in this case compared to the previous one where it was not included (no significant correlations); (iii) the relative positions between FRC and glyphosate in the loadings plot and the respective correlation coefficient (r = -0.312) indicate that the highest concentrations of FRC are associated with lower concentrations of glyphosate, a relation that can be associated with the chemical reaction between these two substances [34]; among other products, this reaction would also produce phosphoric acid [37], which would present itself in the salt form in the presence of the alkaline eluent used in the chromatographic method – thus, the high correlation between phosphate and glyphosate suggests that a significant part of phosphate was derived from the reaction between FRC and glyphosate; (iv) the relatively large distance between nitrate and glyphosate loadings indicates that the correlation observed for this group of cities (r = 0.356) was not important in determining the direction of glyphosate contamination (dashed arrows, Fig. 4C and 4D): nitrate has influenced the dispersion in PC1 and PC2, and positioning

Colombia away from other cities because it had the highest nitrate mean (1.8 mg L^{-1}) and the lowest glyphosate mean (1.6 μg L^{-1}). The cities of Santa Rita do Passa Quatro and Porto Ferreira also occupied positions far from the others (Fig. 4C), presenting the highest glyphosate means (13.9 and 4.9 μg L^{-1}, respectively) and intermediate nitrate levels (1.05 and 1.36 mg^{L-1}, respectively). In this group, glyphosate contamination may be occurring due to agricultural activities or chemical weeding. Original PCA graphs are in supplementary material (Fig. SM 09).

3.4 Influence of demographic factors on contamination physicochemical profiles

In order to verify associations between social, economic and public health indicators with the physicochemical profiles of nitrate and/or glyphosate contaminated waters, the variables NINH, GDPpc, MHDI and HOSP were added to the two matrices of raw data that originated the PCAs of the cities that use groundwater (Fig. 4A and 4B) or surface water (Fig. 4C and 4D).

In the case of 51 cities using groundwater, only the demographic variable HOSP presented significant correlations with four physicochemical variables (see Table SM 08, supplementary material): nitrate (r = 0.31), conductivity (r = 0.36), chloride (r = 0.41) and glyphosate (r = 0.42). On the other hand, among the demographic variables, only NINH and MHDI correlated (r = 0.41). Thus, the respective PCA did not promote significant changes in the distribution between the group of contaminated waters and the group of uncontaminated waters in the scores plot (Fig. 5A) compared to the respective graph obtained only with physicochemical variables (Fig. 4A). The most noticeable difference was Ribeirão Preto, which was separated from other cities – this position was mainly the result of NINH and MHDI variables (higher loadings in PC2, see Fig. 5B) for which Ribeirão Preto presented high values. In fact, the variables NINH, GDPpc and MHDI presented small weights in PC1 whose positive direction is determined by the HOSP variable and by the physicochemical variables related to contamination (Fig. 5B). On the other hand, HOSP does not present significant correlations with factors related to water disinfection quality that were not included in this PCA, such as FRC (r = 0.08), %MB+ (r = -0.16) and turbidity (r = -0.13). Thus, the data suggest that the HOSP variable was related to contamination variables in a complex context that may involve the absence of adequate and feasible public policies in cities with contaminated waters – in order to reduce the impact of acute gastroenteritis caused by viruses, for example, vaccines are necessary and a set of preventive actions that include not only improving water quality, but also adequate sanitation, nutritional interventions and hygiene, and breastfeeding incentive guidelines for the population [38].

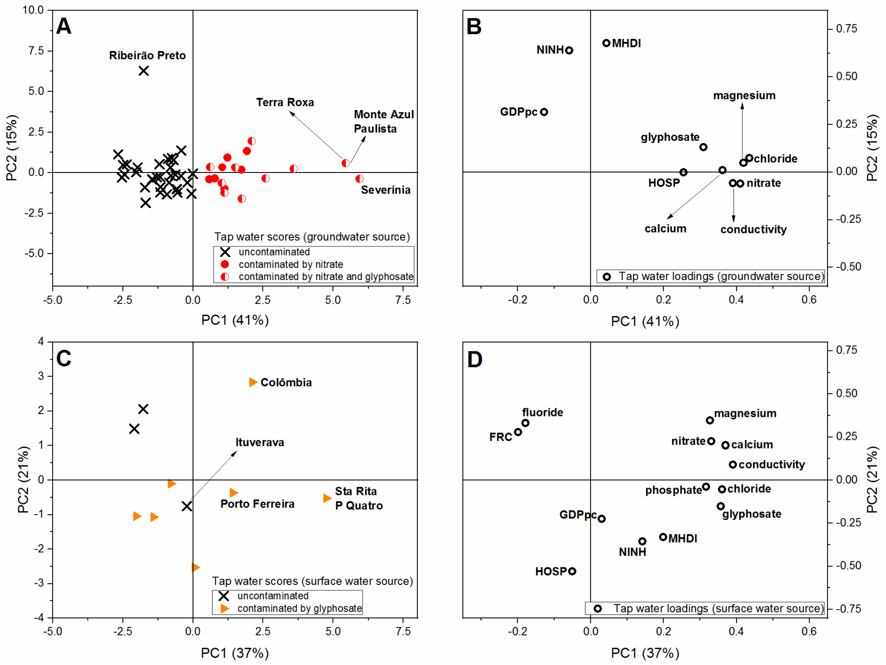

Fig. 5. A and B: PCA of groundwater samples (51 cities; 6 physicochemical and 4 demographic variables). C and D: PCA of surface water samples (10 cities; 9 physicochemical and 4 demographic variables). The original PCA graphs are in supplementary material (Fig. SM 10 and SM 11, respectively).

The demographic variables showed a higher number of significant correlations with the variables related to surface water contamination (see Table SM 09, supplementary material), but their inclusion in the respective PCA model also did not promote significant changes in the distribution between contaminated and uncontaminated waters groups in the scores plot (Fig. 5C), compared to the respective graph obtained only with physicochemical variables (Fig. 4C), with exception of Ituverava: this city (uncontaminated water) exhibited position among the cities of water contaminated by glyphosate – this position is mainly a consequence of NINH and MHDI variables for which Ituverava presented high values. On the other hand, demographic variables were relatively distant from the variables associated with glyphosate contamination (Fig. 5D); in particular, the variables HOSP and GDPpc presented loadings in PC1 close to zero (-0.05 and 0.03, respectively). These results indicate that the 4 demographic variables were not significantly related to the contamination variables. It is important to note that the proximity between FRC and fluoride variables in the loadings plot (Fig. 5D) was not a consequence of the correlation between both since it was not significant (r = 0.13); the positions were mainly due to negative correlations they presented with all other variables, except for the correlation between fluoride and magnesium (r = 0.008).

3.5 Mapping of water contamination by nitrate and/or glyphosate

Most of the nine cities with waters contaminated only with glyphosate are located in the eastern part of the region (Fig. 6) where the two highest mean concentrations were determined (13.9 μg L^{-1} in Santa Rita do Passa Quatro and 12.5 μg L^{-1} in Patrocínio Paulista). They use surface water for public supply, except Patrocínio Paulista and Jaboticabal that use surface and groundwater sources. It is important to note that Jaboticabal is neighbor of several cities that exhibited important nitrate levels (Fig. 6). In fact, when analyzing individual results of the 155 samples analyzed from Jaboticabal, it was found that 21 samples (13.5%) presented values above the general mean (1.1 mgN-NO_3 L^{-1}, calculated for 4,853 samples), including a concentration with a content equal to 12.4 mgN-NO_3 L^{-1} and three concentrations between 3 and 4 mgN-NO_3 L^{-1}. These results suggest that, alongside glyphosate contamination, Jaboticabal waters are presenting nitrate contamination at some points that were not detected by the mean.

If nitrate contamination of Jaboticabal water exists in only a few places, this is not the case for cities that presented higher concentrations and are located mainly to the west (Fig. 6). Nitrate and glyphosate contaminations were more numerous than nitrate-only contaminations, and the nitrate and groundwater relation was evident: (i) in the group of 9 cities with water contaminated only by nitrate, 8 use groundwater and 1 uses groundwater and surface water for public supply; (ii) in the group of 13 cities with water contaminated by nitrate and glyphosate, 11 use groundwater and 2 use groundwater and surface water for public supply. Considering that nitrate can be used as indicator for other possible contaminations [35], it would be important to research substances of Public Health concern in water of these 22 cities such as emerging contaminants, for example [39]. Monte Azul Paulista, Severínia and Terra Roxa are cities where such studies could be carried out primarily due to high levels of contamination and similar physicochemical profiles.

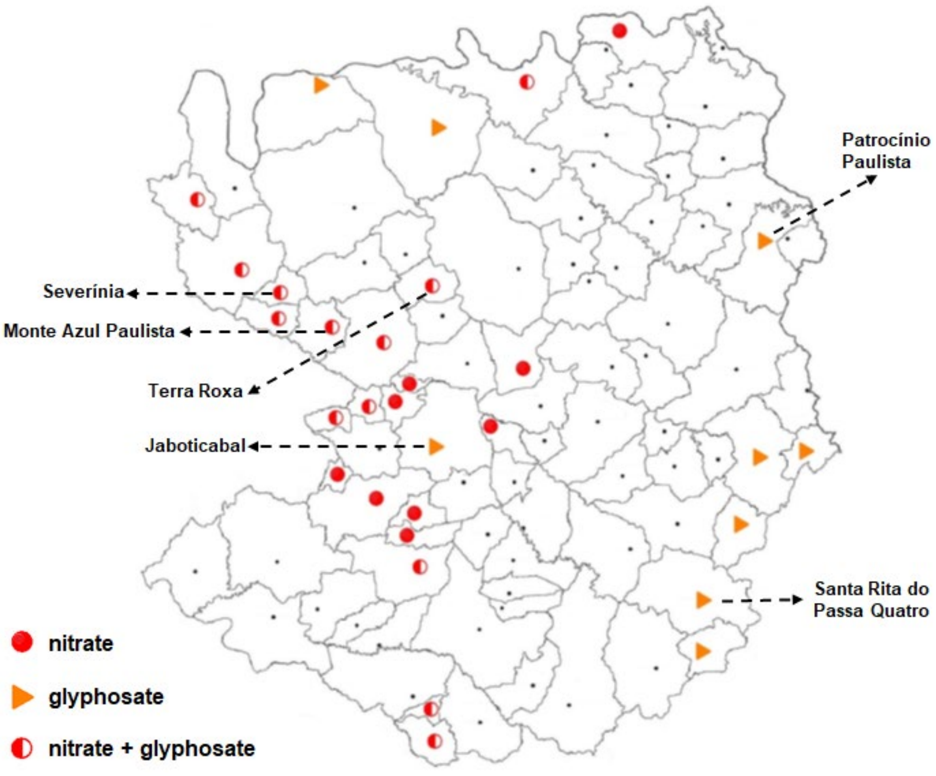

Fig. 6. Mapping of nitrate and/or glyphosate tap water contamination (northeast of State of São Paulo, Brazil).

3.6 Modelling of water contamination by nitrate: using Linear Discriminant Analysis (LDA) for classification

Considering the importance of groundwater contamination in the State of São Paulo (Brazil), two models were evaluated for cities classification regarding nitrate contamination degree of tap water. These models were developed from the physicochemical profiles of contaminated and non-contaminated water. Nitrate and the physicochemical variables that presented the most significant correlations with this contaminant (except glyphosate) were used to predict cities vulnerability degree in relation to groundwater contamination. The main objective of these models based on LDA was to use nitrate as a probe to identify cities where the search of other contaminants of interest to Public Health can be prioritized. The proposal is that models like the ones described below can be used throughout the State of São Paulo and not only in the northeast region.

3.6.1 First model: 50 cities, 5 variables

Two PCAs were initially performed for the model development. The first PCA included data obtained in a previous study conducted in the period 2015-2016, in which 4,347 tap water samples were analyzed [23]; 50 cities that exclusively use groundwater were included and, in addition to nitrate, the variables selected were those that presented significant correlations with this contaminant: calcium, magnesium, chloride and conductivity. The variance explained by the first two principal components was equal to 87% and the scores plot (Fig. 7A) indicated PC1 as the most important direction in the description of nitrate contamination according to loadings plot (Fig. 7B). This graph also showed that the 5 variables loadings are close to each other in PC1, ranging from 0.41 (calcium) to 0.48 (chloride), that is, all variables contributed significantly in PC1 construction with similar loadings. In scores plot of this first PCA was possible to draw a line separating the cities with uncontaminated waters from the cities with contaminated waters (Fig. 7A) – this separation was performed by visual inspection but the Clusters Analysis (Ward method) resulted in the formation of the same groups of cities with only two differences: the city of Matão was included in the group of contaminated waters, while Miguelópolis belonged to the group of uncontaminated waters (see dendrogram in supplementary material, Fig. SM 12). Groups were designated as “low nitrate class” or “high nitrate class” according to whether the nitrate concentrations were lower or higher, respectively.

The second PCA included data obtained in this study, and the same cities and variables were selected as the previous PCA. The variance explained by the first two principal components was equal to 85% and the scores plot was similar to the previous one with PC1 as the most important direction in the description of nitrate contamination (Fig. 7C); also similarly, the loadings plot (Fig. 7D) showed that the 5 variables loadings are close to each other in PC1, ranging from 0.41 (calcium) to 0.48 (magnesium). For comparison, in the scores plot (Fig. 7C) was traced a line approximately in the same position as the line drawn in the previous PCA – it was observed that the distribution of the cities in the two periods was similar, with small changes more noticeable in the region of the separation line. In particular, two cities were positioned on opposite sides of this separation line when comparing first with second period: Dourado (from “high nitrate class” to “low nitrate class”) and Pontal (from “low nitrate class” to “high nitrate class"); considering nitrate mean concentrations, Dourado showed a decrease (from 1.38 to 0.80 mgN-NO_3^- L^{-1}) while Pontal showed an increase (from 0.69 to 1.26 mgN-NO_3^- L^{-1}). As the 5 variables showed normal distributions (Kolmogorov-Smirnov test, α = 0.01) and the cities physicochemical profiles were similar in both periods, data from the first period (2015-2016) were used in LDA model while data from the second period (2019-2020) were used as "test samples".

For modelling, the 50 cities were separated into two groups: in the first ("high nitrate class") were included the 16 cities indicated in red in the scores plot of the period 2015-2016 (Fig 7A) and in the second group were included the 34 remaining cities of the same period ("low nitrate class"). To obtain the two discriminating linear functions were considered the 5 variables (nitrate, calcium, magnesium, chloride and conductivity). These functions were initially tested with same data used in modelling (“cross-validation”, data from period 2015-2016), and as result the confusion matrix indicated an accuracy of 98%: the city of Dourado was classified as "low nitrate class", although it belonged to the group "high nitrate class" (Fig. 7E).

To perform a second test, the model (constructed with data from 2015-2016 period) was used to classify the same 50 cities represented by the means in the 5 variables obtained in 2019-2020 period. The classification obtained in this second test was identical to that obtained in the first test. Although there was an indication for a class change to the city of Dourado in the 2019-2020 period ("high nitrate class" to "low nitrate class"), the model did not indicate a change to Pontal city classification. The raw data used in this LDA and the discriminant functions are in supplementary material (Table SM 11) as well as the original PCA graphs (Fig. SM 13 and SM 14).

3.6.2 Second model: 50 cities, 4 variables

The strategy for second LDA model development was similar to the previous one with only one difference: the variables calcium and magnesium were combined by adding the respective concentrations – the new variable was expressed as "hardness". Thus, the first PCA included samples from the period 2015-2016 of the 50 cities that use groundwater, and 4 variables: "hardness"; chloride, nitrate and conductivity. The variance explained by the first two components was 90% and nitrate contamination was defined mainly by direction of PC1 (Fig. 8A); all variables contributed significantly to the construction of this component with similar loadings (Fig. 8B), ranging from 0.48 (conductivity) to 0.53 (nitrate). By visual inspection, a line was drawn in the scores plot (Fig. 8A) indicating the separation between cities with contaminated and uncontaminated waters – the 2 groups ("high nitrate class" and "low nitrate class") presented the same cities as the groups of the previous model.

The second PCA was performed with data from this study, and the variance explained by the first two components was equal to 89%; cities distribution in the scores plot was similar to 2015-2016 period distribution with small changes in the region of the separation line (Fig. 8C): in addition to changes in Dourado and Pontal positions that had already been observed in the previous model, the cities of Taiúva ("high nitrate class", 2015-2016 data, mean nitrate concentration equal to 1.71 mg L^{-1}) and Santa Ernestina ("high nitrate class", 2015-2016 data, mean nitrate concentration equal to 2.08 mg L^{-1}) were positioned to the left of the separation line (“low nitrate class”) in the second period with decreases in nitrate mean concentrations to 1.34 and 2.01 mg L^{-1}, respectively. The variables presented similar loadings (Fig. 8D), ranging from 0.46 ("hardness”) to 0.58 (chloride). As previously, data from the first period were used in the LDA model while the data from the second period were used as "test samples".

The city groups ("high nitrate class" and "low nitrate class") and 4 variables ("hardness", chloride, nitrate and conductivity) were considered to obtain two linear discriminating functions. In the first test (cross-validation) the confusion matrix indicated an accuracy of 100%, that is, all cities were correctly classified (Fig. 8E). In the second test (cities classification using data from 2019-2020 period) the model presented sufficient sensitivity to indicate changes in Dourado and Pontal physicochemical profiles, classifying these cities as "low nitrate class" and "high nitrate class", respectively (Fig. 8F). In addition, this model was also able to indicate the change in physicochemical profile of Taiúva towards "low nitrate class" but city of Santa Ernestina remained as a “high nitrate class” – this fact can be attributed, at least in part, to a smaller decrease in nitrate mean concentration in the case of Santa Ernestina. The data used in this LDA and the discriminant functions are in supplementary material (Table SM 12) as well as the PCA original graphs (Fig. SM 15 and SM 16).

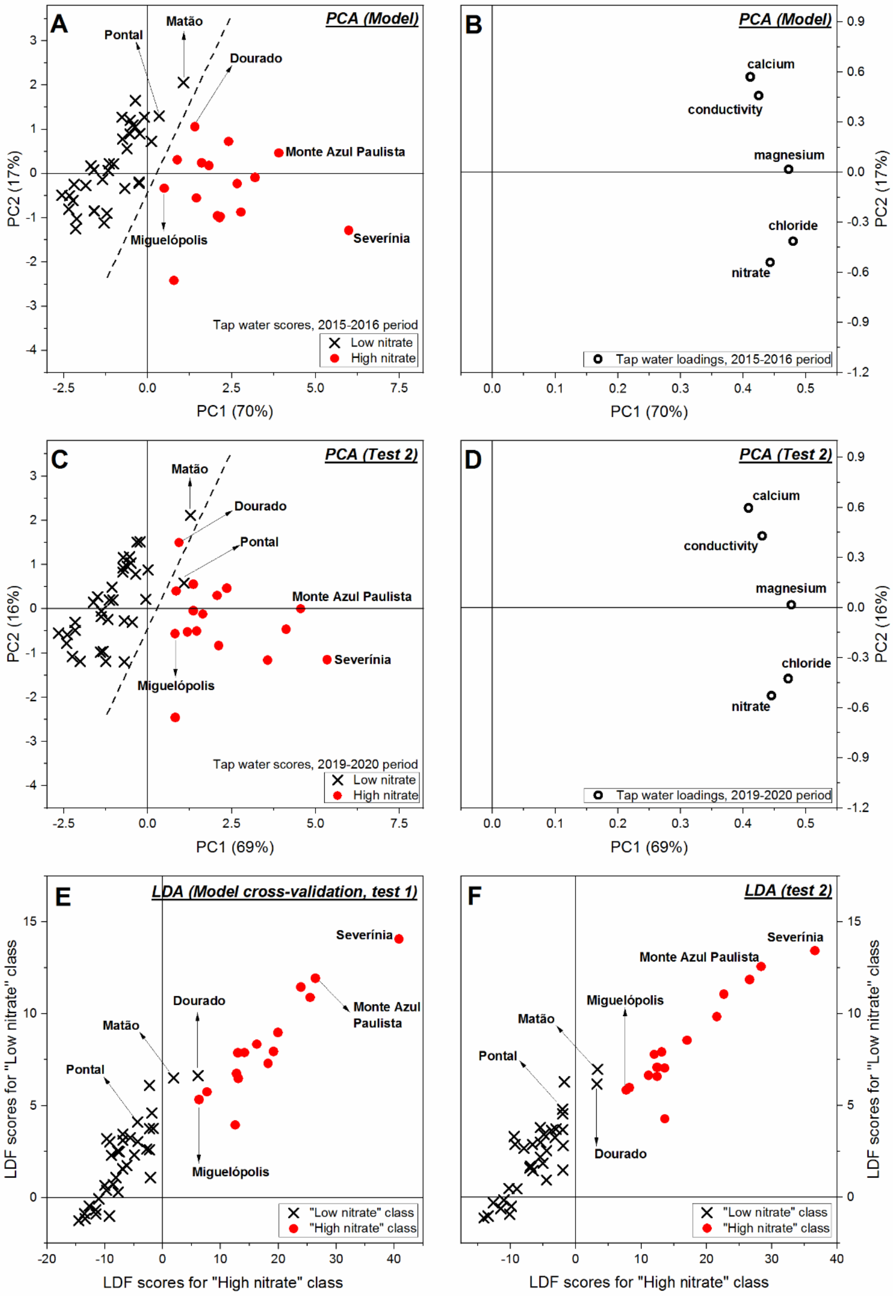

Fig. 7. A and B: PCA of groundwater samples (50 cities, 5 physicochemical variables, 2015-2016 period). C and D: PCA of groundwater samples (50 cities, 5 physicochemical variables, 2019-2020 period). E: LDA model cross-validation (test 1). F: LDA model application in samples from 2019-2020 period (test 2).

This second model was more accurate than the previous one not only in the initial validation (Test 1) but also because it was able to indicate relevant changes in the physicochemical profiles of some cities (Test 2). Nevertheless, it is important to compare this multivariate approach with that which would consider only the mean nitrate concentration (univariate approach). Table SM 13 (supplementary material) compares the LDA scores obtained for the cities in this second model (using the 2019-2020 data, Test 2), with the mean nitrate concentrations in the same period – the series were placed in descending order of values. In both approaches the city of Severínia appeared in first place, that is, it was the city with the physicochemical profile best adjusted to that of a city whose water presents significant contamination by nitrate (multivariate approach) or, alternatively, the one with the highest nitrate content among the cities investigated (univariate approach). However, in the case of Monte Azul Paulista the two approaches differed significantly: while this city occupied the second place in the "multivariate ranking", it had merely the fifth highest nitrate mean concentration in the "univariate ranking" – considering the important water contamination of this city, this fact suggests that multivariate approach may be more accurate in indicating cities that need to be prioritized in remediation planning.

Finally, it is important to point out that obtaining data for application of this predictive model is relatively simple with use of low-cost instruments: water hardness and chloride concentration can be obtained by titrimetric methods, nitrate concentration can be obtained by UV spectrophotometry and conductivity measurements can be made easily in the laboratory or at the time of sample collection [40].

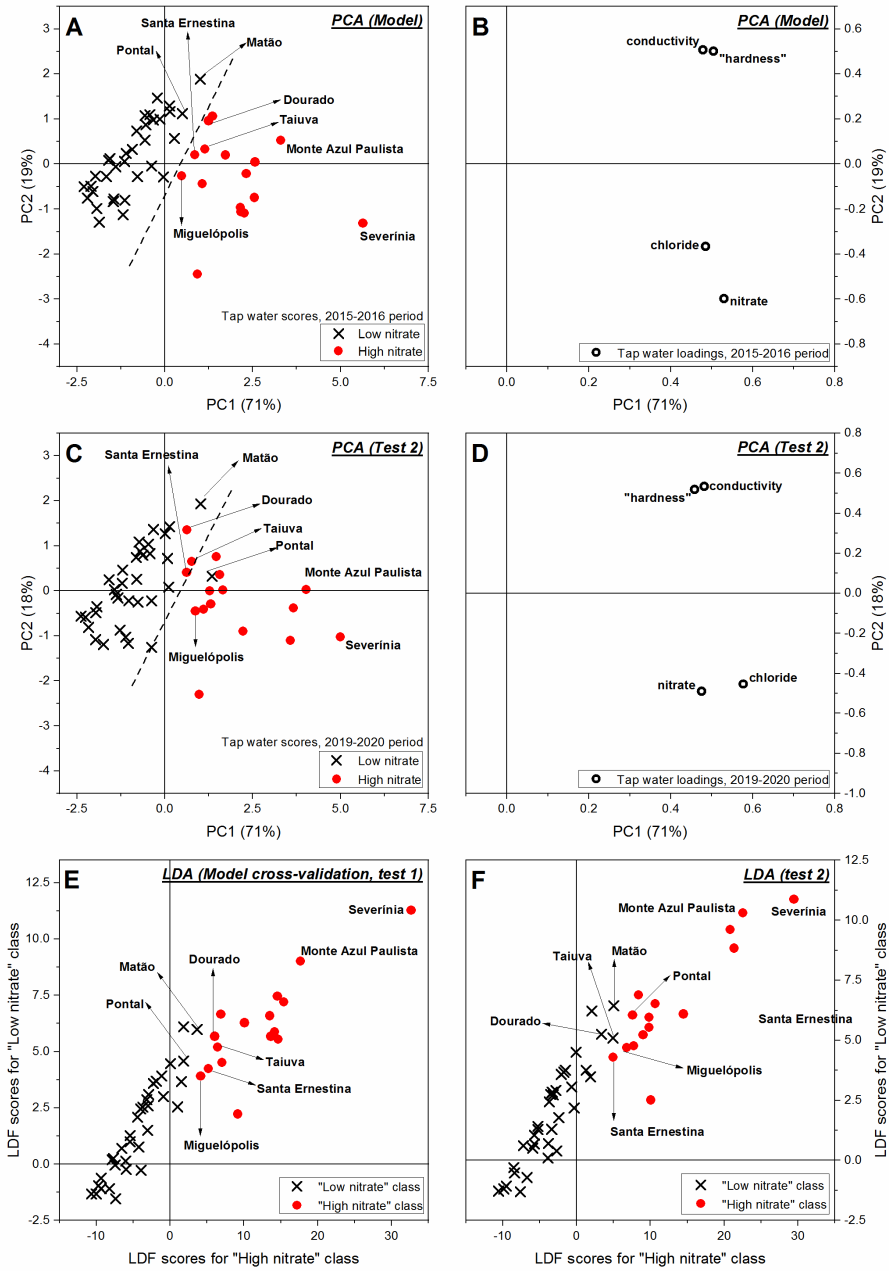

Fig. 8. A and B: PCA of groundwater samples (50 cities, 4 physicochemical variables, 2015-2016 period). C and D: PCA of groundwater samples (50 cities, 4 physicochemical variables, 2019-2020 period). E: LDA model cross-validation (test 1). F: LDA model application in samples from 2019-2020 period (test 2).

4. Conclusions

The present work used the simultaneous analysis of several factors in order to obtain a more comprehensive understanding of water contamination intended for human consumption by glyphosate and nitrate. Principal Component Analysis of variables from groundwater samples suggests that the vulnerability of cities to nitrate contamination in urban areas is also responsible for glyphosate contamination – while the nitrate contamination is likely related to urban occupation patterns including deficiencies in sanitation, the probable source of herbicide contamination is chemical weeding. With regard to surface water, Principal Component Analysis indicated that glyphosate contamination is relatively independent of nitrate contamination – both agricultural activities and chemical weeding may be contaminating the water sources with glyphosate. In general, multivariate analysis showed more complete and accurate information about risk situations with regard to contaminations compared to univariate analysis (considering only nitrate or glyphosate concentration), and inclusion of demographic variables in the analysis contributed to a more comprehensive interpretation of the dynamics of anthropogenic action on the water sources. Finally, it was possible to demonstrate that simple multivariate predictive models are able to predict vulnerability to groundwater contamination, assisting in the identification of cities whose remediation may be a priority.

Supporting Information

Supplementary material associated with this article can be found in the online version.

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Author Contributions

Sergio Dovidauskas: conceptualization, methodology, formal analysis, writing – original draft, supervision, project administration, funding acquisition. Isaura Akemi Okada: conceptualization, methodology, investigation, resources, data curation, writing – review & editing. Felipe Rodrigues dos Santos: methodology, validation, formal analysis, investigation, data curation, writing – review & editing. Marina Miyuki Okada: investigation, resources, data curation, writing – review & editing. Rita de Cássia Briganti: investigation, resources, data curation, writing – review & editing. Marco Antonio Moreira Souto: investigation, resources, data curation, writing – review & editing.

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