

Occurrence of bromate in water intended for human consumption

Ocorrência de bromato em águas destinadas ao consumo humano

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ABSTRACT

Introduction: Bromate is mutagenic and a probable carcinogen in humans. It usually does not occur in water for human consumption, but contamination can occur by industrial wastewater and in the disinfection process by ozonization (if bromide is present) or by the use of hypochlorite solution of unsatisfactory quality. **Objective:** Describe bromate concentrations in the water supply of 89 municipalities in the state of São Paulo (Brazil), the physicochemical profiles of the waters in which the contaminant occurs, and a joint action between the Public Health Laboratory, the Sanitary Surveillance Regional Group and two Sanitary Surveillance of municipalities where important levels of bromate were found. **Method:** 4,853 samples were analyzed in 21 physicochemical parameters (including bromate concentration) and 2 microbiological parameters. For multivariate analysis, 4 demographic parameters were included. **Results:** Bromate was found in 224 samples (4.6% of the total) from 17 municipalities. The concentrations ranged between 3 and 199 $\mu\text{g L}^{-1}$ and 56 samples (1.1% of the total) presented levels above the Maximum Allowed Value. Principal Component Analysis in these 17 municipalities indicated KBrO_3 as the predominant form of contamination. The contamination rates of two municipalities decreased from the availability to the Sanitary Surveillance of reports that included bromate results. **Conclusions:** The data suggest that the monitoring of bromate concentrations should be included in the routine of the Water Surveillance Program for Human Consumption in the state of São Paulo. The notifications of the Sanitary Surveillance with those responsible for the water supply of two municipalities were important to improve the quality of the water supplied to the population in relation to contamination.

KEYWORDS: Bromate; Water for Human Consumption; Water Contamination Control; Health Surveillance

RESUMO

Introdução: Bromato é mutagênico e um provável carcinogênico em seres humanos. Normalmente não ocorre em águas para consumo humano, mas a contaminação pode ocorrer por águas residuais industriais e pela desinfecção por ozonização (se brometo estiver presente) ou pelo uso de solução de hipoclorito de qualidade insatisfatória. **Objetivo:** Descrever as concentrações de bromato nas águas de abastecimento de 89 municípios do estado de São Paulo (Brasil), os perfis físico-químicos das águas nas quais o contaminante ocorre e uma ação conjunta entre Laboratório de Saúde Pública, Grupo de Vigilância Sanitária e de duas Vigilâncias Sanitárias dos municípios onde foram encontrados níveis importantes de bromato. **Método:** Foram analisadas 4.853 amostras em 21 parâmetros físico-químicos (incluindo concentração de bromato) e dois microbiológicos. Para análise multivariada foram incluídos quatro parâmetros demográficos. **Resultados:** O bromato foi encontrado em 224 amostras (4,6% do total) de 17 municípios. As concentrações variaram entre 3 e 199 $\mu\text{g L}^{-1}$ e 56 amostras (1,1% do total) apresentaram teores acima do valor máximo permitido. A Análise de Componentes

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Principais nesses 17 municípios indicou KBrO_3 como a forma predominante de contaminação. Os índices de contaminação de dois municípios diminuíram a partir da disponibilização para as Vigilâncias Sanitárias de laudos que incluíram os resultados de bromato. **Conclusões:** Os dados sugerem que o monitoramento das concentrações de bromato deveria ser incluído na rotina do Programa de Vigilância da Água para Consumo Humano do estado de São Paulo. As notificações das Vigilâncias Sanitárias junto aos responsáveis pelo abastecimento de água de dois municípios foram importantes para a melhoria da qualidade da água fornecida à população em relação à contaminação.

PALAVRAS-CHAVE: Bromato; Água para Consumo Humano; Controle da Contaminação da Água; Vigilância Sanitária

INTRODUCTION

Bromate ions (BrO_3^-) are strong oxidizing agents with standard potential E^0 equal to 1.44 V in H_2SO_4 1 mol L^{-1} , whose strength is similar to that of permanganate (MnO_4^- , $E^0 = 1,51$ V) and cerium IV (Ce^{4+} , $E^0 = 1,44$ V) oxidants¹. Most metal bromates, like sodium (NaBrO_3) or potassium (KBrO_3) bromate, are soluble in water. NaBrO_3 and KBrO_3 are widely used in cosmetic hair care products and for dyeing fabrics with dyes containing sulfur. The World Health Organization's International Agency for Research on Cancer has concluded that there is sufficient evidence to consider bromate ion to be a carcinogen, based on experiments involving high doses in animals. However, for humans the data are still insufficient to enable the same conclusion, so in this case, it is said to be potentially carcinogenic. Nonetheless, bromate is known to be mutagenic, both *in vitro* and *in vivo*².

Water intended for human consumption does not normally contain bromate ions, and Brazilian legislation establishes a maximum allowed concentration (MAC) of 0.01 mg L^{-1} (or 10 μg L^{-1})³. However, contamination can occur in three major ways²: (i) when water sources are reached by industrial wastewater containing NaBrO_3 or KBrO_3 ; (ii) during ozone disinfection, if there are bromide ions (Br^-) in the water – the chemical reaction between bromide and ozone (O_3), resulting in the conversion of bromide into bromate, depends on several factors like concentrations of Br^- and O_3 , pH and amount of organic matter in the water; (iii) when inadequate quality sodium hypochlorite (NaOCl) solution is used in the disinfection process – the NaOCl solution is produced by the electrolysis of an aqueous solution of sodium chloride (NaCl), which may naturally contain small amounts of sodium bromide (in electrolysis, bromide is transformed into bromate and the quality of the hypochlorite solution for water disinfection purposes is, in part, a function of the content of this contaminant).

In Brazil, the National Drinking Water Quality Surveillance Program (Vigiagua) consists of a set of actions adopted by public health authorities to enable control not only over bromate and other contaminants, but also to ensure the population's access to water in adequate amounts and with quality levels that fulfill the potability standard established by current legislation³. Vigiagua's actions are the responsibility of the municipal, state and Federal District health departments, in addition to the Ministry of Health, through the General Coordination of Environmental Health Surveillance.

In the Brazilian state of São Paulo, the quality of water sources is monitored by the Environmental Company of São Paulo State (CETESB), an agency under the Department of Infrastructure and Environment. CETESB periodically publishes reports on the quality of inland, coastal and groundwater^{4,5,6}. Monitoring water quality to verify its potability, in turn, is one of the actions of the Drinking Water Surveillance Program (Proágua), coordinated by the Health Surveillance Center of the Disease Control Coordination of the State Department of Health. This surveillance is done by agents of municipal health surveillance bodies and includes the collection of samples for analysis. At the time of collection, the following aspects are measured: pH, temperature and free residual chlorine (FRC). After refrigeration, the samples are sent to a laboratory for analysis of apparent color, turbidity, fluoride concentration, total coliforms and *Escherichia coli*, totalizing eight parameters in the assessment of water quality. The laboratory used for Proágua analyses of 90 municipalities in the northeast region of the state of São Paulo (Figure 1) is the Chemical and Bromatological Sciences Center of the Adolfo Lutz Institute – Ribeirão Preto Regional office (NQBRP). According to data from the 2010 census by the Brazilian Institute of Geography and Statistics (IBGE), approximately 3.3 million inhabitants live in this region, and human pressure



Source: Adapted by the authors from <http://www.igc.sp.gov.br> and <http://portaldemapas.ibge.gov.br>, 2021.

Figure 1. Region where the Laboratory of Chemical and Bromatological Sciences of the Adolfo Lutz Institute - Regional office of Ribeirão Preto (NQBRP) collaborates in monitoring the quality of water intended for human consumption (in blue).



on water sources is increasing due to growing urbanization and industrialization.

Because of the high concentration of nitrate in the water supply of a municipality in the region⁷, the Department of Water and Electricity of the state of São Paulo decided to limit the catchment of groundwater in the urban area of that municipality for six years⁸. In 2014, the NQBRP increased the number of parameters analyzed by Proágua from eight to 23, with the objective of characterizing the quality of the water supplied to the population in greater detail. Therefore, the following were included: measurement of conductivity and determination of concentrations of 14 ions (lithium, sodium, ammonium, potassium, calcium, magnesium, chlorite, bromate, chloride, bromide, chlorate, nitrate, phosphate and sulfate). The main results included a detailed map of water quality through Principal Component Analysis (PCA)⁹, with the identification of a municipality with a unique physical-chemical profile in the region¹⁰; a second municipality with significant groundwater contamination by nitrate was identified in the region¹¹; detailed description of the correlations between the concentrations of nitrate in the waters and the other parameters¹²; and assessment of the quality of water fluoridation in the municipalities through two different approaches¹³.

Motivated by the results that were obtained based on the parameters described in the previous paragraph, in 2018 the NQBRP started a second round of studies with some new objectives, namely: (i) increase the number of samples analyzed for those municipalities where relevant levels of nitrate, lithium and bromate were found in the previous study; (ii) begin to determine the concentrations of glyphosate and its main metabolite, aminomethylphosphonic acid (AMPA), since there were no comprehensive studies in Brazil on the presence of glyphosate and AMPA in water supply, like those done by the NQBRP for nitrate; and (iii) introduce demographic variables in the PCA with the objective of carrying out a preliminary investigation into the influence of social, economic and health factors on the physical-chemical profiles associated with contaminated water, obtained by multivariate data analysis.

The objective of this work was to describe the results obtained in the second phase of the research in relation to bromate. The second phase was carried out for a year without interruption, following the strategy of analyzing not only the presence of the contaminant, but also the physical-chemical profiles of the water in which the contaminant occurs. These results encouraged joint efforts between the NQBRP, the Health Surveillance Group of Ribeirão Preto and the municipal health surveillance bodies of the two cities where significant levels of bromate were found.

METHOD

In total, 4,853 water samples were analyzed. Sampling took place between March 18, 2019 and March 17, 2020, following the plan established in the previous study⁹. For physical-chemical

analysis, water samples were collected in clean plastic bottles (capacity between 250 and 1,000 mL). For microbiological analysis, 100 mL of water samples were collected in sterile and disposable plastic bottles or bags (*Thio Bag*) with a capacity of 120 mL, which contained sodium thiosulfate to neutralize FRC. Collection was made by health surveillance agents in each of the 89 municipalities of the region (the analyses of Proágua samples from the municipality of Franca were not carried out by the NQBRP). These agents also performed temperature, pH and FRC measurements at the time of collection. Finally, the samples were refrigerated (4°C) and sent to the NQBRP.

The microbiological analysis laboratory determined the presence or absence of total coliforms and *E. coli* through chromogenic and fluorogenic methods (Colilert system, Idexx Laboratories/USA).

The physical-chemical analysis laboratory used analytical-grade reagents (Sigma-Aldrich and Merck brands). In the preparation of aqueous solutions, type I water was used, obtained in a Millipore purifier, model Milli-Q Direct 8. Conductivity measurements were performed using a Metrohm conductivity meter, model 912. The apparent color was determined using a Digimed colorimeter, model DM-COR. For turbidity measurements, a Tecnonon turbidimeter, model TB-1000, was used. The concentrations of ions of lithium, sodium, ammonium (expressed as NH_3), potassium, calcium and magnesium were determined by ion chromatography using a Metrohm chromatograph, model 930 Compact IC Flex Deg, by a validated method^{10,14}. Bromate was determined by ion chromatography in a developed and validated method (limits of detection and quantification of bromate equal to 3 and 8 $\mu\text{g L}^{-1}$, respectively)¹⁵. This method also included determinations of glyphosate, AMPA, fluoride, chlorite, chloride, nitrate, phosphate and sulfate. As for the corresponding chromatographic method for anions developed and validated in the previous study¹⁰, in the present method the determinations of bromide and chlorate were not included. This is because it was not possible to obtain a satisfactory resolution of the respective chromatographic peaks without affecting the resolution and quantification of the analytes that were being introduced into the study (glyphosate and AMPA), in which the NQBRP has a particular interest.

To obtain four demographic variables, information from each municipality on the number of inhabitants (NHAB variable), gross domestic product per capita (GDPpc variable) and municipal human development index (MHDI variable) were searched on the IBGE website (<https://cidades.ibge.gov.br>), while the number of hospitalizations due to diarrhea and gastroenteritis was obtained from the website of the Brazilian Ministry of Health (<http://www2.datasus.gov.br/DATASUS/index.php>) – this number was changed into hospitalizations per thousand inhabitants (HOSP variable).

Microsoft Excel® 2013, Origin® 9.1Pro and The Unscrambler®X 10.3 software were used for data processing. To scale down the initial matrix of experimental data, consisting of 4,853 lines/samples and 23 columns/variables, initially each municipality was represented by the respective series of averages



in the 21 physicochemical variables, and the two microbiological results (presence/absence of total coliforms and *E. coli*) were combined by calculating the percentage of positive results for each municipality (%MB+ variable). Finally, the four demographic data were included, resulting in a matrix consisting of 89 rows (municipalities) and 26 columns (variables).

RESULTS AND DISCUSSION

Bromate was found in 224 samples (4.6% of the total) from 17 municipalities in the region, especially Ribeirão Preto (121 contaminated samples) and Batatais (38 contaminated samples). The concentrations varied between 3 and 199 $\mu\text{g L}^{-1}$, and 56 samples (1.1% of the total) presented levels above the MAC – samples with concentrations between the detection and quantification limits were included in the analysis because they were considered relevant¹⁶ to Public Health in relation to the presence of the mutagenic and probable carcinogenic contaminant. These numbers are much higher than those obtained in the previous study, in which 42 samples from eight municipalities

were contaminated (1% of the total, concentrations between 5 and 30 $\mu\text{g L}^{-1}$, 16 samples with levels above the MAC). In that study, Ribeirão Preto and Batatais also stood out, with 19 and seven contaminated samples, respectively. The increase in the proportion of samples contaminated with bromate (from 1% to 4.6%) can be attributed, at least in part, to the increase in the number of samples analyzed for the municipalities where contamination was initially suspected according to data from the previous study. On the other hand, the increase in the number of municipalities in which bromate was found (from eight to 17) can be attributed, at least in part, to the increase in the number of analyzed samples where significant levels of lithium and nitrate were found, which enabled the detection of bromate in municipalities where it had not been found before. In summary: the increase in the number of analyzed samples where relevant levels of bromate, lithium and nitrate had been found contributed to the increase in the rates of bromate contamination of the region's waters.

The Table shows some important details about the occurrence of bromate in the water of the 17 municipalities, arranged

Table. Occurrence of bromate in water intended for human consumption in 17 municipalities in the northeast of the state of São Paulo.

City	N. inhab.	Source	n _A	n _{Cont}	%Cont	Bromate		
						Average $\mu\text{g L}^{-1}$	c > 10 $\mu\text{g L}^{-1}$	8 ≤ c ≤ 10 $\mu\text{g L}^{-1}$
Ribeirão Preto	604,682	groundwater	294	121	41.2	3.90	40	12
Dobrada	7,939	groundwater	57	3	5.3	3.60	1	0
Brodowski	21,107	groundwater	29	1	3.4	3.30	1	0
Pradópolis	17,377	groundwater	29	7	24.1	2.90	3	1
Batatais	56,476	groundwater + surface water	152	38	25.0	2.00	5	3
Patrocínio Paulista	13,000	groundwater + surface water	33	5	15.2	1.70	2	1
Viradouro	17,297	groundwater + surface water	27	2	7.4	0.90	2	0
Sales Oliveira	10,568	groundwater	24	3	12.5	0.90	1	0
Ipuã	14,148	groundwater + surface water	29	3	10.3	0.72	0	0
Barretos	112,101	groundwater + surface water	167	15	9.0	0.50	0	0
São Carlos	221,950	groundwater + surface water	56	1	1.8	0.44	0	1
Borborema	14,529	groundwater + surface water	71	5	7.0	0.42	1	0
Orlândia	39,781	groundwater + surface water	24	1	4.2	0.41	0	1
Jaboticabal	71,662	groundwater + surface water	155	6	3.9	0.27	0	2
Santa Rita do Passa Quatro	26,478	surface water	94	9	9.6	0.21	0	0
Olímpia	39,781	groundwater + surface water	128	2	1.6	0.09	0	0
Porto Ferreira	51,400	surface water	115	2	1.7	0.08	0	0
TOTAL	1,340,276	-	1,484	224	15.1	1.31	56	21

n_A: number of samples; n_{cont}: number of contaminated samples; %Cont: percentage of contaminated samples.
Source: Prepared by the authors, 2021.



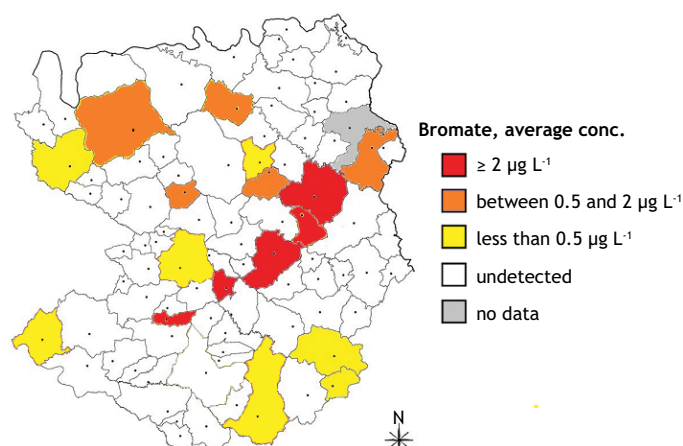
in decreasing order of average bromate concentration: (i) although they represent only 19% of the total number of municipalities, their population exceeds 1.3 million of inhabitants (40.5% of the region's total); (ii) the four highest average concentrations are from municipalities that exclusively use groundwater for supply, however, in this study we could not relate characteristics of this type of source with the observed bromate contaminations; (iii) some municipalities have high percentages of contamination (%Cont), like Ribeirão Preto (41.2%), Batatais (25.0%) and Pradópolis (24.1%), which shows that a significant part of the population of these municipalities was exposed to the contaminant (%Cont was defined as the ratio between the number of contaminated samples and the number of samples analyzed, expressed as a percentage); (iv) the number of samples with bromate concentrations close to the MAC ($8 \mu\text{g L}^{-1} \leq c \leq 10 \mu\text{g L}^{-1}$) is significant (21 samples, 0.4% of the total) – when added to the number of samples that presented levels above the MAC (1.1%), we see that 1.5% of the total samples had levels of bromate that demand special attention.

Figure 2 shows the location of the municipalities indicated in the Table. We can see that the five municipalities with the highest average concentrations of bromate are close to each other, roughly in the center of the region, and another five municipalities with intermediate average concentrations are dispersed mainly in the central-north area, whereas the others are located mainly in the central-south.

To find information on the distribution of contaminated water shown in Figure 2, we considered the possibility of performing a PCA. However, the correlation matrix, calculated from the matrix of 89 municipalities and 26 variables, has shown that the average bromate concentration in the water of the municipalities was not significantly related to any of the other 25 variables (a Pearson correlation (r) was considered significant

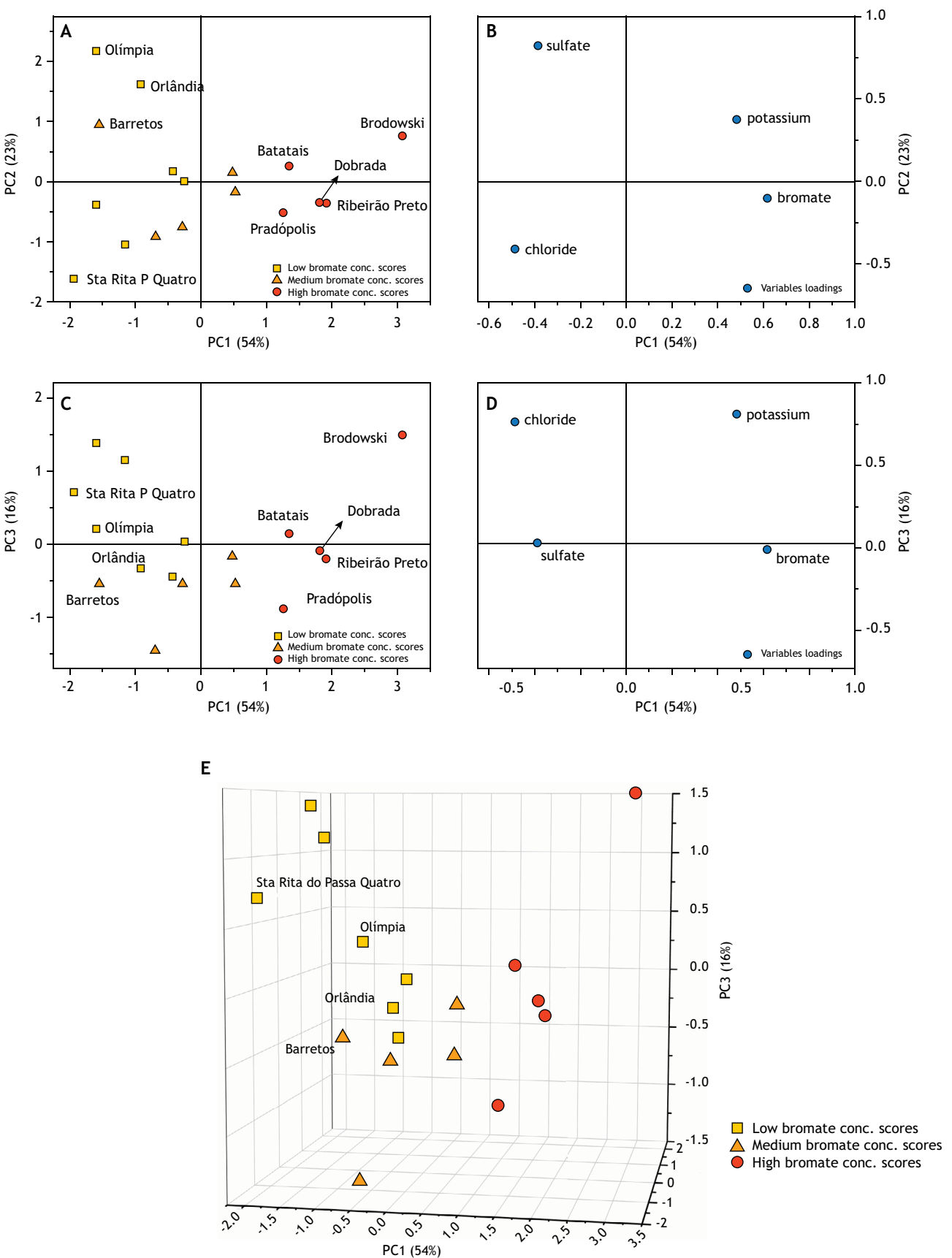
if greater than or equal to $|0.3|$, or $r \geq |0.3|$)¹⁷. Therefore, it was not possible to associate a physical-chemical profile to the water contaminated by bromate, nor to relate it to the microbiological variable (%MB+) or the demographic variables (NHAB, GDPpc, MHDI and HOSP). In particular, given that it has been suggested that nitrate can be used as an indicator for the identification of other substances of interest to Public Health¹⁸, such as emerging contaminants¹⁹, and considering that the region has municipalities with groundwater contaminated by nitrate^{7,9,11,12}, it should be noted that the extremely low correlation between bromate and nitrate concentrations ($r = -0.06$) suggests that their forms of contamination are different – in urban areas of the state of São Paulo, studies have linked high concentrations of nitrate in groundwater to densely populated areas and/or areas that have been occupied for a longer time, the presence of cemeteries, poorly constructed latrines and cesspools, and old sewage collection networks (more likely to leak)⁴.

Once the absence of variables correlated with bromate contamination in the water was verified when all municipalities were considered, only those included in the Table were investigated for common characteristics within the group. Thus, a new matrix was prepared including the 17 municipalities and the 26 variables. The respective correlation matrix indicated that the average bromate concentration correlated significantly ($r \geq |0.3|$) with 12 variables: NHAB, %MB+, conductivity, turbidity, apparent color, pH, temperature and with the average concentrations of phosphate, sulfate, chloride, potassium and sodium. However, the PCA involving the 17 municipalities in the Table and these 13 variables presented a low explained variance: 29.0% for the first principal component (PC1), 21.0% for the second principal component (PC2) and 13.0% for the third principal component (PC3), totaling 63.0% in the PC1/PC2/PC3 space and compromising more robust inferences regarding the arrangement of



Source: Adapted by the authors from <http://www.igc.sp.gov.br>, 2021.

Figure 2. Map of occurrence of bromate in water intended for human consumption in the northeast of the state of São Paulo.



Source: Prepared by the authors, 2021.

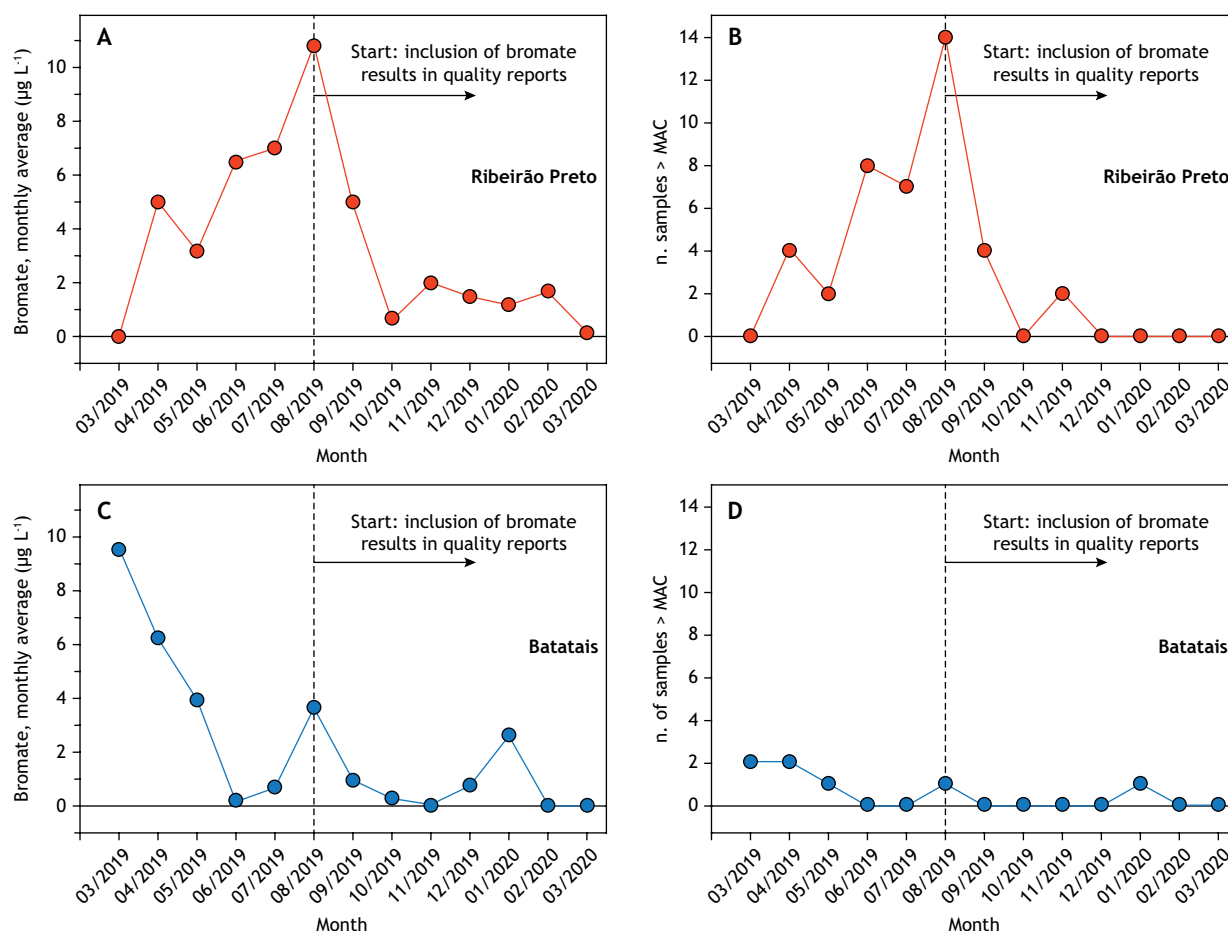
Figure 3. Principal Component Analysis (PCA) of bromate-contaminated water (17 municipalities, four variables).



municipalities in the scores plot and the arrangement of variables in the loadings plot.

In order to obtain a descriptive model with higher explained variance, the variables that had the highest correlations with the average concentrations of bromate in the group of municipalities in the table were selected: the average concentrations of chloride ($r = -0.539$), potassium ($r = 0.524$) and sulfate ($r = -0.500$). The resulting PCA presented an explained variance in three dimensions equal to 93.0%, distributed as follows: 54.0% in PC1, 23.0% in PC2 and 16.0% in PC3 (Figure 3). In the PC1/PC2 space score plot (Figure 3A), we can observe that the municipalities with the highest average bromate concentrations are located on the right, the same position of the bromate and potassium variables in the loadings plot (Figure 3B), which suggests that the predominant form of contamination in the five municipalities with the highest average concentrations is KBrO_3 . The other municipalities are distributed in Figure 3A in decreasing order of average bromate concentrations as the PC1 score decreases, but the separation between intermediate and low concentrations is not evident, although they are distributed along PC2 as they present higher sulfate (like the municipalities of Olímpia, Orlandia and

Barretos, in the positive part of PC2) or chloride (like the municipality of Santa Rita do Passa Quatro, in the negative part of PC2), as indicated in the loadings plot (Figure 3B). In particular, the position of the municipality of Barretos, apparently distant from the other municipalities that present intermediate average concentrations of bromate, is the result of its average concentration of sulfate, the 3rd highest among the 17 municipalities. Sulfate is not an important variable in PC3 (Figure 3D), and the Barretos score in this component (equal to -0.53) is compatible with the scores of municipalities with intermediate concentration, which range from -0.17 to -1.44, thus, in the PC1/PC3 space (Figure 3C) we can observe the approximation of Barretos to other municipalities with intermediate concentrations of bromate. Figure 3E shows the distribution of the 17 municipalities in the three-dimensional space of PC1/PC2/PC3, in which the preferential direction of the highest concentrations between PC1 and PC3 can be seen (defined mainly by the average concentrations of bromate and potassium), a preferential direction of smaller concentrations visualized with the addition of PC3 (influenced mainly by the average concentration of chloride) and the cities with intermediate concentrations also in intermediate positions between the two main directions.



Source: Prepared by the authors, 2021.

Figure 4. Monthly average bromate concentrations in the water of Ribeirão Preto (A) and Batatais (C), and monthly number of water samples with levels above the maximum allowed concentration (MAC) in Ribeirão Preto (B) and Batatais (D), in the period from Mar/18/2019 to Mar/17/2020.



The finding that KBrO_3 is the predominant form of contamination in the five municipalities with the highest average bromate concentrations could suggest that industrial activities are impacting the region's water sources. However, since the previous study, the NQBRP has investigated this contamination and, so far, it has not been possible to locate sources of contamination of this type. Moreover, the occurrences are random, in different regions of the same municipality. Considering that water contamination by industrial waste containing KBrO_3 is unlikely, it was decided to research other possible sources, which will be discussed below.

At the beginning of this study, it was found that, with the increase in contamination rates due to the increase in the number of samples analyzed with relevant levels of bromate, lithium and nitrate, there was a need for an intervention. For example: in the first three months of data acquisition, significant levels of bromate were found in all urban areas of the municipality of Ribeirão Preto (central, north, south, east and west). In the northeast of the state of São Paulo, water disinfection is done with hypochlorite solution, so we decided to investigate whether there was any relationship between this solution and the levels of bromate found. Initially, two municipalities were chosen, Ribeirão Preto and Batatais, whose levels were high and which had the highest percentages of contamination. Since the study did not foresee actions in the municipalities, the NQBRP met with the respective Health Surveillance Group and, as a first step, the decision was to add the results of bromate to the quality reports of the Proágua water samples of the two

municipalities, with the objective of creating instruments for municipal health surveillance bodies to notify the utilities about contamination, since a likely source of contamination could be the use of a product of unsatisfactory quality for the water disinfection process (for example: solutions of hypochlorite for industrial use may contain bromate). The inclusion of bromate results started in August 2019. As from the following month, a sharp drop was observed both in the monthly average bromate concentration and in the number of samples with concentrations above the MAC, especially for the municipality of Ribeirão Preto (Figure 4). Because of these reductions in contamination rates, other actions were not planned in the municipalities. After the notifications, the utilities were able to improve the quality of the water supplied to the population regarding the presence of the bromate contaminant.

CONCLUSIONS

Considering that bromate is mutagenic and probably carcinogenic for humans, the levels of this contaminant found in the water supply of the northeast region of the state of São Paulo, as well as the frequency with which these levels appear, suggest that the monitoring of this ion should be included in the Proágua routine. Furthermore, this work has shown that health surveillance notifications of contamination to those responsible for the water supply of the municipalities were essential to encourage measures that improved the quality of the water supplied to the population in relation to the presence of bromate.

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Authors' Contribution

Dovidauskas S - Conception, planning (study design) and writing of the manuscript. Okada IA - Conception, planning (study design), data acquisition, analysis, and interpretation, writing of the manuscript. Santos FR, Okada MM, Briganti RC, Souto MAM - Acquisition, analysis, interpretation of data and writing of the manuscript. All authors approved the final draft of the manuscript.

Disclosures

The authors report that there is no potential conflict of interest with peers and institutions, nor political or financial conflicts in this study.

Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.



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