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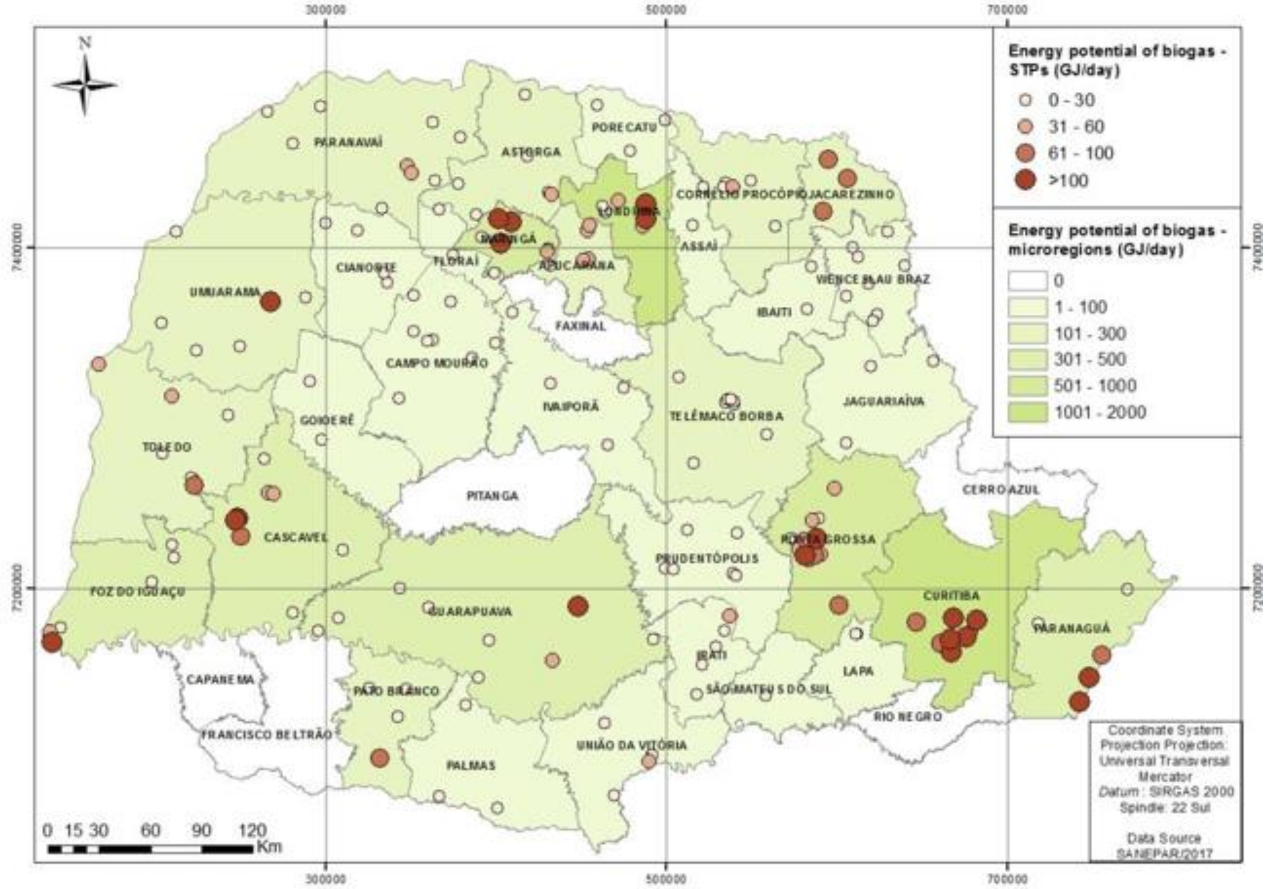

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This map shows the distribution of biogas energy potentials for sewage treatment plants (STP) in the state of Paraná in the southern region of Brazil. The results of this research showed that 19 STP units presented a biogas energy potential above 100 GJ d^{-1} and 124 units a potential below 30 GJ d^{-1}. The location of these units and associated biogas energy potential make it possible to assemble strategic plans for future investments in the energy recovery of the by-products of STPs. Source: LOPES, L. S. et al. Energy potential of biogas and sludge from UASB reactors in the State of Paraná, Brazil. **Rev. Ambient. Água,** Taubaté, vol. 15 n. 1, p. 1-15, 2020. ***doi:10.4136/ambi-agua.2398***

ARTICLES

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Intensity-duration-frequency of maximum rainfall in Mato Grosso State

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ABSTRACT

Intensive rainfall is an important meteorological variable that is of technical interest in hydraulic projects. This study therefore generated Intensity-Duration-Frequency equations (IDF) for 14 weather stations in Mato Grosso State, based on pluviograph analysis. Annual maximum rainfall data regarding 10-to-1440-minute long rainfall events were collected from digitized daily pluviographs. Data adherence to the generalized extreme value distribution (GEV) was checked through the Kolmogorov-Smirnov test at a 20% significance level. Next, the maximum probable rainfall for return periods such as 2, 5, 10, 20, 30, 50 and 100 years was calculated and the IDF equations were adjusted. The performance of the IDF equations was evaluated based on mean absolute error (MAE), root mean square error (RMSE), bias, Willmott's concordance index and Nash-Sutcliffe efficiency index (ENS). Adjusting the IDF equations was only possible for rainfall durations ranging from 10 to 360 min at each station due to the low frequency of longer rainfalls. High variation was present in parameters of the IDF equation and in maximum rainfall intensity between stations. The satisfactory performance of the models, as attested to by statistical indices, allows using IDF equations adjusted for rainfall durations from 10 to 360 min, and return periods from 2 to 100 years, in the regions of the Mato Grosso weather stations.

Keywords: IDF, intense rainfall, project rain, water resource management.

Intensidade-duração-frequência de precipitação máxima em Mato Grosso

RESUMO

A chuva intensa é uma importante variável meteorológica que apresenta interesse técnico em projetos hidráulicos. Assim, este estudo teve como objetivo obter equações intensidade-duração-frequência (IDF), obtidas por análise de pluviógrafos, para 14 estações no Estado do

Mato Grosso. As séries anuais de intensidade máximas de chuva com duração de 10 a 1440 min foram obtidas de Pluviogramas diários digitalizados. Verificada a aderência das séries à distribuição generalizada de valores extremos, pelo teste de Kolmogorov-Smirnov à 20% de significância, calculou-se as chuvas máximas prováveis para os tempos de retorno de 2, 5, 10, 20, 30, 50 e 100 anos e, ajustaram-se as equações IDF. O desempenho das equações IDF foi avaliado pelo erro absoluto médio (MAE), erro quadrático médio (RMSE), bias, índice de concordância de Willmott e índice de eficiência de Nash-Sutcliffe (ENS). Em todas as estações foi possível ajustar apenas equações IDF para chuvas com duração de 10 a 360 min, devido à baixa frequência de chuvas com duração superior. Houve grande variação na intensidade máxima da chuva e dos parâmetros da equação IDF entre as estações estudadas. O bom desempenho dos modelos, conforme atestados por índices estatísticos, permitem a utilização das equações IDF para as durações de 10 a 360 min e tempo de retorno de 2 a 100 anos, nas regiões das estações do Mato Grosso.

Palavras-chave: chuva de projeto, chuvas intensas, IDF, gestão de recursos hídricos.

1. INTRODUCTION

Intensive rainfall is one of the most important meteorological variables in climate studies, as it generates a considerable volume of water in short intervals (Pereira *et al.*, 2017). Thus, knowledge regarding the variables characterizing maximum rainfall, as well as the correlations between rainfall intensity, duration and frequency, are of technical interest to hydraulic projects such as spillways, channel terraces, agricultural, urban and road drainage systems, among others (Cheng and Amir, 2014).

The most accepted way to characterize maximum rainfall relies on the intensity-duration-frequency equation (IDF) (Campos *et al.*, 2014). The IDF curves are based on historical rainfall time-series data and are designed to capture the intensity and frequency of precipitation for different durations by fitting a theoretical probability distribution to annual extreme rainfall (Oriani *et al.*, 2017; Ouarda *et al.*, 2019). The first uses of IDF relationships date back to the 1930s (Bernard, 1932) and the first curve in Brazil was done in 1958 (Pfafstetter, 1958). Nowadays, because of its importance and ease of application, IDF curves are widely used in water management and other engineering design applications (Cheng and Amir, 2014).

Therefore, the difficulty in generating intense rainfall equations in Brazil is mainly attributed to the limited availability of data about rainfall network density and time range (Silva *et al.*, 2012). These issues are particularly concerning in the Northern and Central-Western regions of the country, as in Mato Grosso State, where pluviograph data sets comprise less than 30 years of collected data - the assessment period recommended by the World Meteorological Organization.

In addition to the lack of historical data about intense rainfall events, Mato Grosso State presents a large area that includes the Cerrado, Pantanal and Amazon Biomes, which have distinct rainfall characteristics (Marcuzzo *et al.*, 2011). Moreover, the State has been facing deep land-use and occupation changes in the last decades, mainly because of increasing urbanization, expansion of agriculture and construction of hydroelectric power plants.

However, the State has not yet defined intense rainfall equations based on the analysis of pluviograph records for most of its counties, since previous studies focused on adjusting IDF equations to Mato Grosso State were based on daily rainfall data disaggregation processes (Fietz *et al.*, 2010; Pizzato *et al.*, 2012; Mossini Junior *et al.*, 2016). Thus, the current study calibrated and evaluated the statistical performance of intensity-duration-frequency equations generated for 14 pluviograph stations located in the Cerrado, Amazon and Cerrado-Amazon-Pantanal transition biomes in Mato Grosso State.

2. MATERIALS AND METHODS

Mato Grosso State is located between geographic coordinates 06°00' S, 19°45' S and 50°06' W, 62°45' W; its territory covers 903,202,446 km^2 (IBGE, 2017). According to the Köppen classification, Aw (tropical savanna climate) and Cwa (tropical climate) are the predominant climates in the region; mean monthly temperature ranges from 23.00°C to 26.84°C, and total annual rainfall ranges from 1,200 to 2,000 mm (Souza *et al.*, 2013). The region has two well-defined seasons: the rainy season, from October to April; and the dry season, from May to September.

Rainfall data were collected from pluviographs belonging to the National Hydrometeorological Network (CPRM / ANA); these pluviographs referred to 14 counties (Table 1): 4 in the Amazon Biome (Northern mesoregion), 3 in Cerrado-Amazon-Pantanal transition biomes (Southwestern mesoregion) and 7 in the Cerrado biome (Southeastern mesoregion) (IBGE, 2012; 2013) (Figure 1). Since the stations did not have coincident data periods, no baseline study was adopted. In addition, only stations with at least 10 years of data were selected, however, and it was decided not to fill in data gaps in order to avoid bias in the estimation of the maximum annual rainfall.

Rainfall data recorded by rain gauges were obtained through the rainwater digitization system (HidroGraph 1.02) developed for the National Water Agency (ANA) by the Water Resource Research Group of the Agricultural Engineering Department of the University of Viçosa. Maximum annual precipitation heights recorded by each station for 10, 20, 30, 40, 50, 60, 120, 180, 240, 360, 720 and 1440-minute-long rainfall events were used to build the annual dataset about extreme rainfall events.

Table 1. Pluviograph rainfall stations belonging to the National Hydrometeorological Network (CPRM / ANA) used in the current study - Mato Grosso State, Brazil.

Code	Station Name	Lat.	Long.	Alt.	Dataset
	Amazon Biome (North Mesoregion)				
00956001	1 - Jusante Foz Peixoto de Azevedo	-09.64	-56.02	290	2002-2012
00956000	2 - Alta Floresta	-09.87	-56.10	400	2000-2010
01059000	3 - Humboldt	-10.18	-59.45	242	2002-2012
01157000	4 - Porto dos Gaúchos	-11.54	-57.42	260	2000-2011
	Ecotone of the Cerrado-Amazon-Pantanal Biomes (Southwest Mesoregion)				
01559006	5 - Mato Grosso	-15.01	-59.95	209	2002-2005 / 2007-2012
01559000	6 - Pontes e Lacerda	-15.22	-59.35	236	2001-2010
01558005	7 - Porto Esperidião	-15.85	-58.47	166	2001-2010
	Cerrado Biome (Southeast Mesoregion)				
01454000	8 – Paranatinga	-14.42	-54.05	484	2000-2010
01452000	9 – Xavantina	-14.67	-52.35	263	2001-2003 / 2007-2010
01654000	10 – Rondonópolis	-16.47	-54.66	220	2000-2010
01652001	11 - Ponte Branca	-16.77	-52.84	380	2000-2012
01653004	12 - Alto Garças	-16.94	-53.53	564	2000-2012
01753000	13 - Alto Araguaia	-17.30	-53.22	659	2000-2012
01853000	14 - Fazenda Taquari	-17.81	-53.29	845	2000-2012

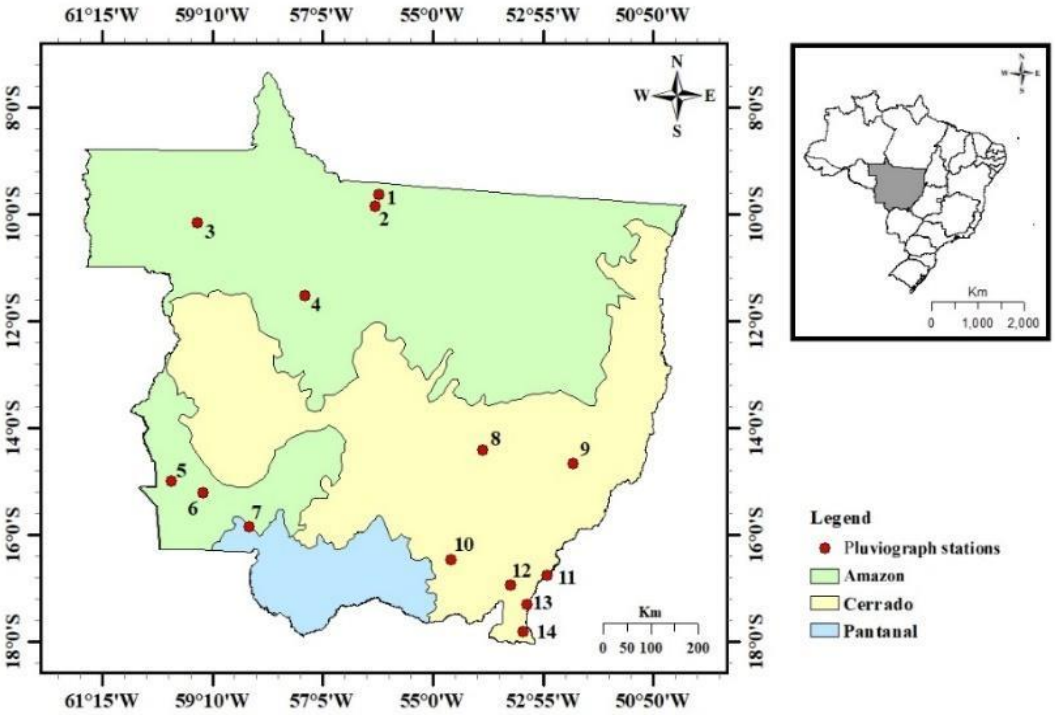

Figure 1. Location of pluviograph stations listed in Table 1.

The data set was tested for non-stationarity using the Mann-Kendall Test Modified as recommended in Cheng and Amir (2014) and Ouarda (2019). Then, the annual dataset for extreme rainfall events was adjusted to Generalized Extreme Value distribution (GEV), based on the maximum likelihood estimation (MLE) method applied to estimate probability distribution parameters. The adherence of the adjustments made in the series to the GEV distribution was investigated through the Kolmogorov Smirnov test, at a 20% probability level. This significance level was selected to limit the hypothesis test, since increased significance levels reduce the critical value of the statistical test (Naghettini and Pinto, 2007). After the GEV distribution parameters were adjusted, the probable maximum rainfall of each rainfall duration was estimated for return periods of 2, 5, 10, 20, 30, 50 and 100 years.

Parameters of the intensity-duration-frequency equation applied to each station were determined based on the Gauss-Newton non-linear adjustment technique (Chapra and Canale, 2006; Naghettini and Pinto, 2007), by using the maximum annual rainfall intensity recorded for return periods (RP) of 2, 5, 10, 20, 30, 50 and 100 years and rainfall durations (t) of 10, 20, 30, 40, 50, 60, 120, 180, 240, 360, 720 and 1440 minutes, as shown in Equation 1:

$$i = \frac{K \times RP^{a}}{(t+b)^{c}} \quad (1)$$

Where in: i is the maximum intensity (mm h^{-1}); RP is the return period (years); t is the rain duration time (min); and K, a, b and c are the adjusted local coefficients.

The performance of the IDF equations , whose models were significant at α: 0.05%, was evaluated based on the following statistical means: mean absolute error (MAE), root mean square error (RMSE), bias, Willmott's concordance index and Nash-Sutcliffe efficiency index (E_{NS}) (Willmott, 1982; Stone, 1993; Krause *et al.*, 2005; Pereira *et al.*, 2014), as shown in Equations 2, 3, 4 ,5 and 6, respectively.

$$MAE = \frac{1}{N} \sum_{i=1}^{N} |O_i - P_i| \quad (2)$$

$$RMSE = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (O_i - P_i)} \quad (3)$$

$$Bias = \frac{1}{N} \sum_{i=1}^{N} (O_i - P_i) \quad (4)$$

$$d = 1 - \left[\frac{\sum_{i=1}^{N} (P_i - O_i)^2}{\sum_{i=1}^{N} (|P_i - O| + |O_i - O|)^2}\right] \quad (5)$$

$$E_{NS} = 1 - \left[\frac{\sum_{i=1}^{N} (O_i - P_i)^2}{\sum_{i=1}^{N} (O_i - O)^2}\right] \quad (6)$$

Where in: P_i is the estimated intensity (mm h^{-1}); O_i is the observed intensity (mm h^{-1}); O is the mean of observed intensities (mm h^{-1}); and N is the number of sample values.

3. RESULTS AND DISCUSSION

Table 2 presents the means and standard deviations of the annual series of maximum rainfall intensities corresponding to rainfall durations from 10 to 1440 minutes recorded by the stations in Mato Grosso State. Although some stations presented a nonstationarity tendency, in order to avoid inconsistencies between the evaluations of different stations and due to the fact that the data series generally only had 10 years, the data series were treated in a traditional way without considering non-stationary influences.

Maximum rainfall intensities tended to be higher in the Amazonian biome (Northern region of the State), whose means varied from 102.4 mm h^{-1} (10-min-long rainfalls) to 0.7 mm h^{-1} (for 360-min-long rainfalls). Stations located in the Southwestern and Southeastern regions of the State presented similar means, which ranged from 97.8 and 98.8 mm h^{-1} (10 min) to 0.1 and 0.3 mm h^{-1} (360 min), respectively. These results match the ones found by Marcuzzo *et al.* (2011), who recorded higher rainfall indices in the Amazonian biome (Northern Mato Grosso State) than in other phyto physiognomies in Southern Mato Grosso State.

There was no record of 1440-min-long (or longer) rainfall events in Mato Grosso State; however, there were only 7 records of 720-min-long rainfall events in the State. Alves *et al.* (2013) conducted a study in Cuiabá County-MT and also found low frequency of long-term rainfall events: there were three 1440-min-long rainfall events and thirteen 720-min-long events in a 22-year dataset.

The low frequency of rainfalls longer than 360 minutes results from the predominance of convective precipitations in the State, which, according to Salio *et al.* (2007), overall last less than 9 hours. On the other hand, longer precipitations are linked to frontal rainfall caused by cold fronts brought from the Southern region of the country by polar anticyclones (Seluchi, 2009). However, the incidence of polar anticyclones strong enough to cause precipitation is rare and often results in low-intensity/volume rainfalls (Nimer, 1972; Seluchi, 2009).

Table 3 presents the IDF equations applied to the herein-investigated locations and the respective values of the adjusted parameters "K", "a", "b" and "c". Parameters "K" and "b" showed higher coefficient of variation (CV), thus, according to Silva and Oliveira (2017) indicating no spatial dependence between stations. Parameters "a" and "c" presented average variability in the State; CV values were 33.39% and 21.08%, respectively.

Table 2. Mean- and standard deviation (mm h^{-1}) of the annual dataset regarding maximum rainfall intensities with durations ranging from 10 to 1440 minutes, Mato Grosso State - Brazil.

Station	Rainfall Duration (min)											
	10	20	30	40	50	60	120	180	240	360	720	1440
Amazon Biome (North Mesoregion)												
1 – Jus. Foz Peixoto de Azevedo	105.9	79.2	65.1	59.4	43.9	34.4	11.9	4.4	2.3	1.5	0.0	0.0
	(38.5)	(23.4)	(18.6)	(15.9)	(17.1)	(20.6)	(14.4)	(10.1)	(4.2)	(3.9)	(0.1)	(0.0)
2 - Alta Floresta	106.3	84.3	67.3	56.5	53.6	41.7	16.9	9.7	4.6	0.7	0.0	0.0
	(27.1)	(19.5)	(18.1)	(12.8)	(10.8)	(19.1	(13.9)	(10.5)	(6.4)	(1.2)	(0.0)	(0.0)
3 - Humboldt	94.9	75.5	64.3	55.8	52.5	44.8	17.7	10.1	5.6	0.2	0.0	0.0
	(33.2)	(17.1)	(15.1)	(14.4)	(11.7)	(11.8	(18.0)	(13.6)	(11.8)	(0.2)	(0.0)	(0.0)
4 - Porto dos Gaúchos	102.1	80.8	67.9	58.5	48.5	43.7	17.2	6.4	0.7	0.4	0.0	0.0
	(23.3)	(19.3)	(21.2)	(21.0)	(23.9)	(25.7)	(13.6)	(10.5)	(1.3)	(1.0)	(0.1)	(0.0)
Mean	102.4	80.1	66.3	57.6	49.7	41.3	16.0	7.6	3.2	0.7	0.0	0.0
S	29.8	19.4	17.9	16.0	16.8	19.9	14.6	11.0	6.9	2.1	0.1	0.0
CV (%)	0.29	0.24	0.27	0.28	0.34	0.48	0.91	1.44	2.14	3.02	2.80	0.00
Ecotone of the Cerrado-Amazon-Pantanal Biomes (Southwest Mesoregion)												
5 - Mato Grosso	79.2	69.0	61.0	55.2	37.7	34.8	3.8	0.6	0.4	0.1	0.0	0.0
	(18.7)	(12.8)	(14.8)	(12.8)	(17.8)	(14.8	(7.1)	(0.8)	(0.7)	(0.1)	(0.0)	(0.0)
6 - Pontes e Lacerda	108.2	70.8	65.2	52.1	45.8	34.6	9.2	0.6	0.2	0.2	0.0	0.0
	(36.2)	(26.0)	(28.0)	(20.6)	(20.5)	(17.6	(15.1)	(1.0)	(0.3)	(0.3)	(0.0)	(0.0)
7 - Porto Esperidião	106.9	84.6	63.8	47.5	45.5	31.7	10.2	2.2	2.0	0.1	0.0	0.0
	(24.9)	(23.8)	(19.5)	(13.2)	(12.7)	(13.9)	(9.6)	(3.3)	(3.4)	(0.1)	(0.0)	(0.0)
Mean	97.8	74.9	63.3	51.6	42.9	33.7	7.7	1.1	0.9	0.1	0.0	0.0
S	29.6	21.9	20.5	15.5	17.0	14.9	10.9	2.2	2.2	0.2	0.0	0.0
CV (%)	0.30	0.29	0.32	0.30	0.40	0.44	1.42	1.89	2.41	1.43	0.0	0.00
Cerrado Biome (Southeast Mesoregion)												
8 - Paranatinga	94.4	74.2	66.9	53.1	43.4	39.9	12.6	4.2	0.2	0.1	0.0	0.0
	(19.7)	(13.9)	(14.2)	(14.0)	(13.9)	(13.7	(9.6)	(7.7)	(0.1)	(0.1)	(0.0)	(0.0)
9 - Xavantina	136.1	95.6	81.3	72.3	59.3	51.7	8.5	7.4	4.6	1.3	0.0	0.0
	(19.4)	(30.2)	(24.8)	(22.5)	(12.8)	(16.4	(6.0)	(6.5)	(6.0)	(3.1)	(0.0)	(0.0)
10 - Rondonópolis	90.8	71.2	61.5	49.0	42.0	35.5	4.6	0.2	0.2	0.1	0.0	0.0
	(32.8)	(26.2)	(20.8)	(19.6)	(14.9)	(15.3	(6.7)	(0.3)	(0.1)	(0.1)	(0.0)	(0.0)
11 - Ponte Branca	93.2	67.9	56.2	48.6	44.7	39.9	14.2	2.2	0.7	0.1	0.0	0.0
	(20.8)	(12.6)	(15.0)	(13.4)	(13.6)	(14.8	(11.5)	(2.8)	(1.2)	(0.1)	(0.0)	(0.0)
12 - Alto Garças	115.6	81.3	67.3	54.0	46.0	38.2	10.2	3.2	2.2	0.1	0.0	0.0
	(49.4)	(38.0)	(30.4)	(20.6)	(17.1)	(14.8	(9.6)	(5.4)	(5.3)	(0.0)	(0.0)	(0.0)
13 - Alto Araguaia	86.8	65.9	57.9	49.6	41.3	36.1	10.5	1.1	0.3	0.2	0.1	0.0
	(20.4)	(13.0)	(15.0)	(12.6)	(13.0)	(12.6	(12.5)	(2.0)	(0.3)	(0.2)	(0.2)	(0.0)
14 - Fazenda Taquari	89.1	70.4	55.8	49.7	36.7	32.5	5.2	2.8	0.6	0.5	0.0	0.0
	(35.6)	(13.8)	(8.7)	(11.8)	(10.3)	(10.4)	(9.3)	(7.4)	(1.3)	(1.3)	(0.0)	(0.0)
Mean	98.8	74.0	62.7	52.6	43.9	38.3	9.5	2.8	1.1	0.3	0.0	0.0
S	33.3	23.3	20.0	17.0	14.5	14.3	10.0	5.4	3.0	1.1	0.1	0.0
CV (%)	0.34	0.32	0.32	0.32	0.33	0.37	1.06	1.94	2.86	3.87	4.41	0.00

The number in parentheses (below the mean) corresponds to the standard deviation (S).

Table 3. Parameters “K”, “a”, “b” and “c” calibrated for Intensity-duration-frequency equations, Mato Grosso State - Brazil.

Station	K*	a*	b*	c*
Amazon Biome (North Mesoregion)				
1 – Jus. Foz Peixoto de Azevedo	3407.410	0.163	19.952	1.029
2 - Alta Floresta	4766.289	0.098	29.239	1.036
3 – Humboldt	470.837	0.112	4.347	0.580
4 - Porto dos Gaúchos	5979.295	0.131	48.214	1.022
Mean	3655.957	0.126	25.438	0.915
Standard Deviation	2369.071	0.028	18.331	0.224
****CV (%)**	64.80	22.33	72.06	24.43
Ecotone of the Cerrado-Amazon-Pantanal Biomes (Southwest Mesoregion)				
5 - Mato Grosso	987.684	0.207	13.729	0.831
6 - Pontes e Lacerda	748.555	0.205	9.945	0.700
7 - Porto Esperidião	9750.604	0.102	33.438	1.215
Mean	3828.948	0.171	19.037	0.915
Standard Deviation	5129.698	0.060	12.614	0.267
****CV (%)**	133.97	35.12	66.26	29.23
Cerrado Biome (Southeast Mesoregion)				
8 – Paranatinga	9895.483	0.124	46.840	1.174
9 – Xavantina	6246.965	0.101	23.534	1.100
10 -Rondonópolis	14615.726	0.207	39.234	1.331
11 - Ponte Branca	10828.492	0.086	47.690	1.190
12 - Alto Garças	6037.949	0.182	17.764	1.178
13 - Alto Araguaia	7339.183	0.121	37.977	1.161
14 - Fazenda Taquari	7964.394	0.228	21.612	1.351
Mean	8989.742	0.150	33.521	1.212
Standard Deviation	3051.357	0.055	12.386	0.093
****CV (%)**	33.94	36.92	36.95	7.66
State of Mato Grosso				
Mean	6359.919	0.148	28.108	1.064
Standard Deviation	4134.327	0.049	14.484	0.224
****CV (%)**	65.01	33.39	51.53	21.08

* Parameters of equations adjusted for rainfalls with duration time (t) ranging from 10 to 360 minutes. ** CV <12% - low variability; 12% < CV <60% - average variability; CV> 60% - high variability (Silva and Oliveira, 2017).

High variation in parameters of the IDF equation were also reported in Bahia (Silva *et al.*, 2002); Tocantins (Silva *et al.*, 2003), Mato Grosso do Sul (Santos *et al.*, 2009), Pernambuco (Silva *et al.*, 2012), and Piauí (Campos *et al.*, 2014) states; such variation was associated with the large number of attributes involved in the process of modeling the dynamics of environmental phenomena (Mello and Silva, 2009).

Although the parameters of the IDF equation presented high variation in most stations of Mato Grosso State, the ones located near each other in the Cerrado Biome (Southeastern Meso-region) recorded average CVs for parameters "k" (33.94%) and "A" (36.92) , as well as low variability for "b" (36.95%) and "c" (7.66%).

Table 4 presents the results of the performance analysis applied to the IDF curves generated to estimate the maximum rainfall intensity at pluviographic stations in Mato Grosso State, based on different durations and return periods. The analysis of statistical indices showed that all the equations presented satisfactory performance, as seen in R^2 values higher than 86.65%

(Humboldt), which reached maximum value of 95.96% (Jusante Foz Peixoto de Azevedo). Mean absolute error (MAE) and root mean square error (RMSE) estimates showed good fit of the equations, which recorded 15.2 mm h^{-1} (MAE) and 18.9 mm h^{-1} (RMSE) for Pontes and Lacerda stations, as well as 9.9 mm h^{-1} (MAE) and 12.7 mm h^{-1} (RMSE) when all stations were taken into consideration.

Table 4. Performance of maximum annual rainfall intensity estimates in rainfall stations in Mato Grosso State, Brazil.

Stations	R^{2*}	MAE (mm h^{-1})	RMSE (mm h^{-1})	Bias	d	Ens
Amazon Biome (North Mesoregion)						
1 – Jus. Foz Peixoto de Azevedo	0.9596	8.21	10.25	-1.87	0.9902	0.9631
2 - Alta Floresta	0.9579	6.82	8.40	-0.45	0.9899	0.9616
3 - Humboldt	0.8665	10.57	14.17	-0.76	0.9656	0.8781
4 - Porto dos Gaúchos	0.8918	12.49	14.87	-5.35	0.9707	0.9012
Ecotone of the Cerrado-Amazon-Pantanal Biomes (Southwest Mesoregion)						
5 - Mato Grosso	0.8876	12.84	14.24	-5.84	0.9710	0.8973
6 - Pontes e Lacerda	0.8912	15.18	18.91	-8.78	0.9701	0.9007
7 - Porto Esperidião	0.9275	9.31	12.34	-1.95	0.9802	0.9338
Cerrado Biome (Southeast Mesoregion)						
8 - Paranatinga	0.9447	7.46	9.58	-4.05	0.9855	0.9495
9 - Xavantina	0.9287	10.62	13.25	-1.99	0.9835	0.9349
10 -Rondonópolis	0.9536	9.49	11.77	-4.63	0.9876	0.9577
11 - Ponte Branca	0.9269	7.92	9.73	-2.78	0.9808	0.9333
12 - Alto Garças	0.9414	9.33	15.69	-2.28	0.9857	0.9465
13 - Alto Araguaia	0.9263	7.99	10.29	-2.72	0.9808	0.9327
14 - Fazenda Taquari	0.9227	9.98	13.94	0.31	0.9803	0.9295

*Significant models at α: 0.05.

The bias index analysis indicated that all models were underestimated, except for Fazenda Taquari station, where there was a model overestimation of 0.3 mm h^{-1}. The Willmott concordance (d) and the Nash-Sutcliffe efficiency (Ens) indices confirmed the good fit of the models, since their values were close to 1 and they were classified as suitable, according to the criterion set by Van Liew *et al.* (2007).

Figure 2 shows the results of the adjusted rainfall intensities at different return periods, based on IDF equations of maximum rainfall intensities in Mato Grosso State. For the probable maximum precipitation estimates to design hydro-agricultural projects, usually those obtained for rains of 10-min-duration in a 10-year return period, Alto Garça station recorded the highest intensity (183.2 mm h^{-1}), while the lowest was recorded at Ponte Branca station (106.1 mm h-1). These results are on average 9.5 mm h^{-1} lower than the ones found by Fietz *et al.* (2010) for the same stations at Mato Grosso. Although, it's important to notice that the estimates presented by Fietz *et al.* (2010) were done by means of desegregation of daily precipitation and not by pluviograph analysis, which could lead to rainfall-intensity overestimation, as can be shown when compared to the study of Castro *et al.* (2011) that found by pluviogram analysis an intensity of 165.6 mm h^{-1} at Cuiabá, results these similar to those of this work.

The lowest 60- and 360-minute rainfall estimates were recorded for Fazenda Taquari station (35.2 and 4.4 mm h^{-1}, respectively). Porto do Gaúchos and Pontes e Lacerda stations recorded the highest intensity for 60- and 360-minute-duration rainfalls (67.3 and 19.1 mm h^{-1}, respectively). The highest rainfall intensity variations happened at rainfall durations shorter than 120 minutes due to the prevalence of convective rains; the curve smoothed after 120-minute-duration rainfalls.

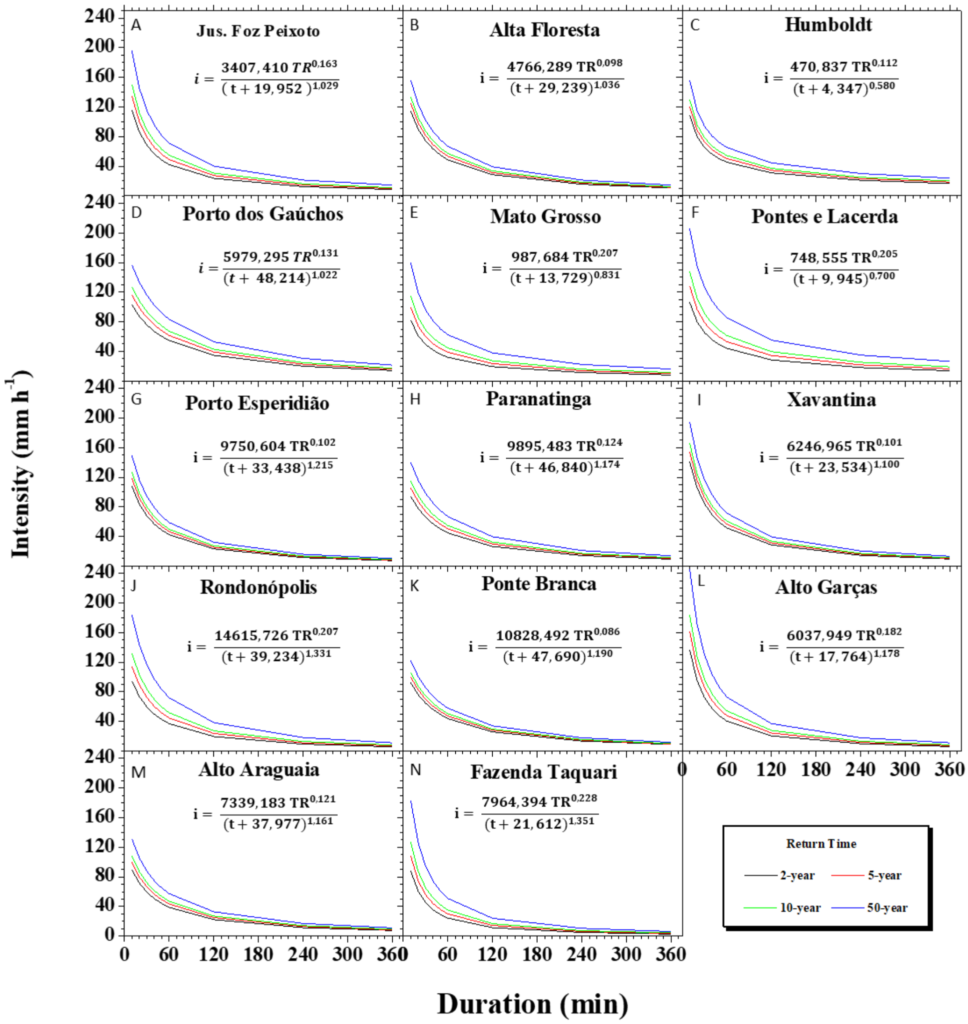

Figure 2. Maximum rainfall intensity curves recorded for different return periods and adjusted through the IDF equations - Mato Grosso State.

Topography is also a key factor for rainfall intensity in Mato Grosso State (Souza *et al.*, 2013), and as such similar rainfall estimates were found in Amazon Biome stations located at altitudes ranging from 242 to 400 m, and high variation was found in estimates for the Cerrado Biome located at Petrovina Mountain, where the altitude varied from 220 to 845 m. The orographic effect became evident when Alto Garça station was compared to Ponte Branca station. These stations were 75 km away from each other; however, they recorded rainfall-estimate differences of 77.1 mm h^{-1}, (183.2 mm h^{-1} at Alto Garça and 106.1 mm h^{-1} at Ponte Branca) for hydro-agricultural rainfall projects, corresponding to precipitation of 10-minute duration and 10 years of return time.

The small-scale variability of rainfall intensity can sensibly increase the complexity of the hydrological response, conditioned by weather and soil indicators and topography (elevation) (Oriani *et al.*, 2017). For Haiden e Pistotnik (2009), in mountainous terrain (similar to what occurs in the southern and central regions of Mato Grosso state), elevation differences strongly contribute to the small-scale spatial variability of precipitation. The effect is most pronounced for long accumulation periods such as monthly or annual; however, they may interfere with the intensity of local precipitation.

4. CONCLUSIONS

The IDF equations presented satisfactory adjustments, with determination coefficients above 87%, allowing the estimation of intense rainfall with 10 to 360 minutes duration and return periods from 2 to 100 years. The equations may therefore be used as a basis for hydrological studies in the state of Mato Grosso, and may also serve as a reference for engineering projects and prevention of extreme precipitation events.

The regionalization of the IDF equations for the state is not indicated due to the high variability of the IDF curves parameters of the current stations. The high variability is still indicative of the need for more weather stations to be distributed in Mato Grosso.

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Multivariate statistical analysis applied to the evaluation of groundwater quality in the central-southern portion of the state of Bahia - Brazil

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ABSTRACT

The objective of this study was to identify and evaluate the variables responsible for contributing to possible natural and/or human contamination in groundwater of the semiarid region of the state of Bahia, seeking to subsidize water quality monitoring and management actions in the area. To do so, multivariate analysis techniques regarding factorial analysis in principal components and cluster analysis were used. The factorial analysis allowed the grouping of variables into two principal factors that explained 93% of total accumulated variance. Variables were strongly related to concentrations of metals and salinity in the water. The cluster analysis was used to classify water sources according to the quality of waters into three clusters in each factor. The natural background of the rocks of the municipality of Boquira was shown to influence water resources. A continuous (during dry and rainy seasons) monitoring of water quality from wells and springs located upstream and downstream from contamination sources is recommended, even if these waters are not used for public supply, to determine possible contamination plumes from contaminated material.

Keywords: cluster analysis, factorial analysis, heavy metals, semiarid.

Análise estatística multivariada aplicada à avaliação da qualidade da água subterrânea na porção centro sul do estado da Bahia – Brasil

RESUMO

Esta pesquisa teve por objetivo identificar e avaliar as variáveis responsáveis pela contribuição de uma possível contaminação natural e/ou antrópica nas águas subterrâneas no semiárido baiano, visando subsidiar medidas de monitoramento e gestão da qualidade das águas na área. Para isso, foram utilizadas técnicas de análise multivariada referente à análise fatorial em componentes principais e de agrupamento. A análise fatorial permitiu agrupar as variáveis em dois fatores principais que explicaram 93% da variância total acumulada. As variáveis estão fortemente relacionadas com as concentrações de metais e salinidade na água. A análise de agrupamento classificou as fontes hídricas de acordo com a qualidade das águas, em três grupos em cada fator; onde concluiu-se que existe influência do *background* natural das rochas do município de Boquira nos recursos hídricos. Recomenda-se o monitoramento contínuo (período seco e chuvoso) da qualidade da água dos poços e nascentes situados à jusante e montante das fontes de contaminação, ainda que não sejam utilizados para abastecimento público, visando a determinação de possível pluma de contaminação do material contaminado.

Palavras-chave: análise de agrupamento, análise fatorial, metais pesados, semiárido.

1. INTRODUCTION

The municipality of Boquira is located in the central-southern portion of the state of Bahia, within the semiarid region of the state, under the geological domain of the Paramirim deformation corridor (Alkimim *et al.*, 1993). Among other lithotypes, this corridor comprises rocks of the Boquira Unit, which is widely known for lead-zinc mineralization, providing this region with a high natural background for these metals. In fact, the largest lead and zinc mine in Brazil was located in this area. It operated for over 30 years (1960 to 1992) until it was suddenly abandoned, leaving significant environmental liability in the form of particulate material composed of toxic metals, such as lead, zinc, silver, barium, copper, chromium, nickel, arsenic, cadmium, among others, deposited in the tailings pond and galleries of the underground mine (Daltro, 2017).

Although mineral extraction has been completely interrupted for over 20 years in the area, the recovery plan for degraded areas was never implemented. Even with the imminent risk of contamination, the municipal landfill was installed on the surface of the pile of tailings. In addition, the presence of urban districts, recyclable material pickers in the landfill, and rural establishments near the place where the pile of tailings is located may result in a public health issue, therefore demonstrating a situation of environmental injustice (Andrade *et al.*, 2017).

During the past years, several studies have been conducted with the objective of evaluating possible environmental impacts regarding an increase in the concentration of heavy metals in the environment. These metals originate from lithogenic processes and/or human activities, such as the use of fertilizers in agricultural zones and mining activities (Muniz and Oliveira Filho, 2006).

Studies on the evaluation of groundwater quality generally use several variables, which, in turn, are strongly correlated, thus hampering the understanding of their interrelationships. The use of multivariate analysis techniques allows a reduction in the number of variables, definition of their relationships, identification of those that are responsible for the dispersion of observations, and classification of clusters (Brito *et al*., 2006).

The multivariate analysis helps in the definition of which variables are more important for water management, assisting in the selection of variables using more objective criteria. This type of statistical analysis is an effective tool in the qualitative evaluation of waters (Vidal and Kiang, 2002; Brito *et al.*, 2006; Cloutier *et al.*, 2008; Palácio, 2009; Fernandes *et al.*, 2010; González *et al*., 2011; Finkler *et al*., 2015; Gomes and Cavalcante, 2017).

Therefore, multivariate techniques were used (factorial analysis by the principal component analysis method and hierarchical clustering analysis) with the objective of evaluating which variables (metals) are most important in the contribution to possible natural and/or human contamination of groundwater, aiming to subsidize groundwater quality monitoring and management actions in the semiarid region of the state of Bahia.

2. MATERIAL AND METHODS

In the present study, the hydrochemical database (30 variables) of the Geochemical Atlas of the Paramirim River Watershed, a project conducted in July 2013, was used (Cunha *et al.*, 2016). This database holds information on 11 samples (8 wells and 3 natural springs) distributed across the municipality of Boquira (Figure 1).

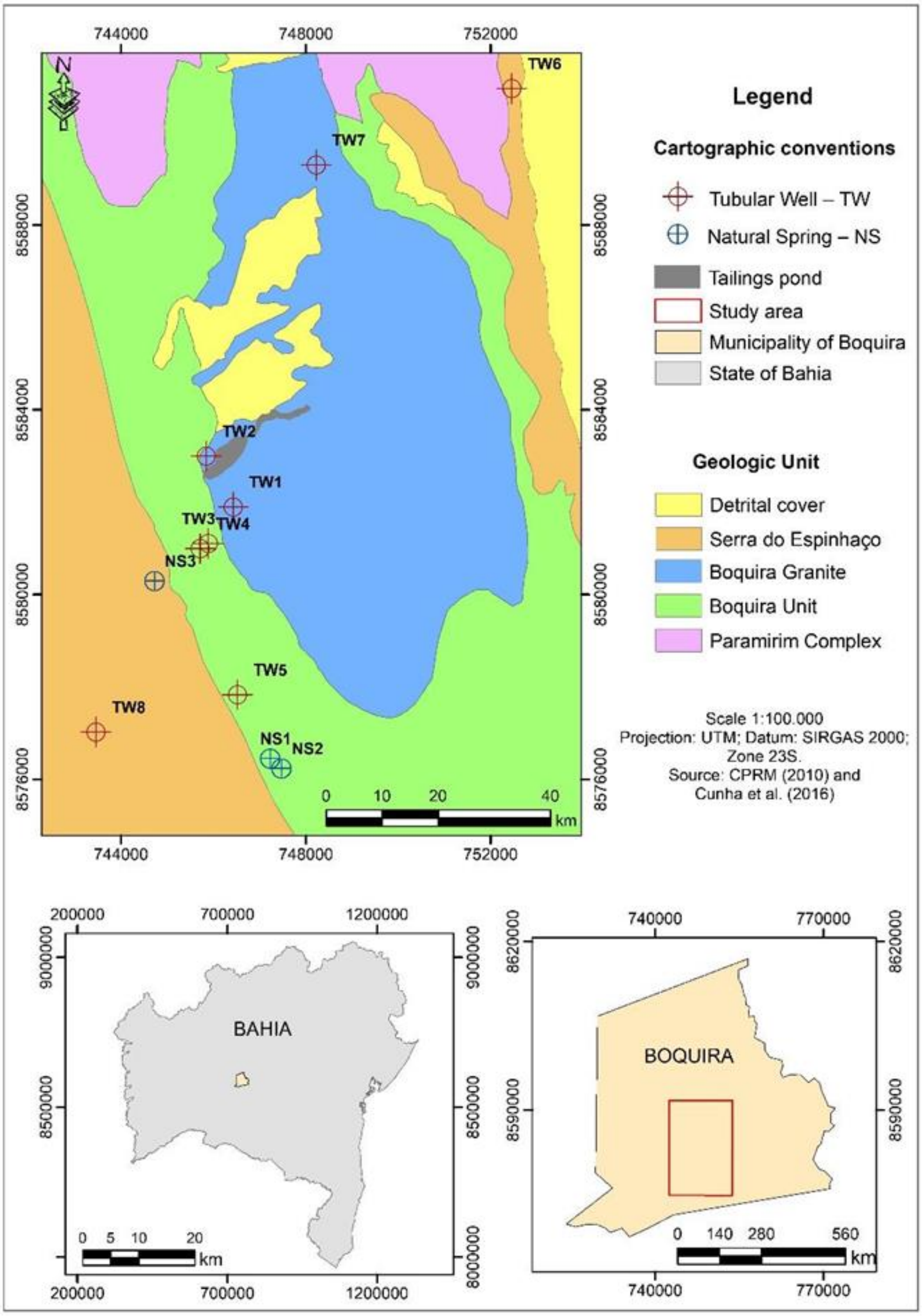

Figure 1. Location of the study area and groundwater samples analyzed.

The physicochemical variables considered in this study were: aluminium (Al), arsenic (As), boron (B), barium (Ba), beryl (Be), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), lead (Pb), antimony (Sb), silicon (Si), tin (Sn), strontium (Sr), titanium (Ti), vanadium (V), zinc (Zn), hydrogen ionic potential (pH), electrical conductivity (EC), and dissolved oxygen (DO). The 11 samples analyzed and the 30 physicochemical analyses add up to 330 results.

Two multivariate analysis techniques were used in this study: factorial and hierarchical clustering analyses, which were processed using the software *SPSS Statistics*, version 17.0.

The factorial analysis describes the correlation or covariance between a set of variables regarding a limited number of non-observable variables. These non-observable variables or factors are calculated by the linear combination of the original variables.

The factorial analysis was based on three phases: calculation of the correlation matrix between variables (R-mode factor analysis), extraction of initial factors and rotation of the matrix.

The correlation matrix was calculated based on the KMO index (Kaiser-Meyer-Olkin Measure of Sampling Adequacy), which determines the adequacy of the factor analysis data and Bartlett's test of sphericity, which tests the null hypothesis that the variables analyzed are not correlated (Hair Jr. *et al*., 1998). The method of Principal Component Analysis (PCA) was used for the extraction of the factors of the correlation matrix and the rotation of the factors was accomplished by the varimax method (orthogonal rotation was the most used and as a characteristic minimizes the number of variables with high loads in different factors, allowing the association of a variable to a single factor). This procedure aims to describe the relationships of covariance among correlated parameters, based on identified factors, and to observe through communalities how much each parameter explains each factor (Hoffmann, 1992; Manly, 1998; Landim, 2011).

Lastly, the hierarchical clustering of samples (Q-mode factor analysis) was conducted using the highest number of variables explained by a single factor in the factorial analysis. In this technique, Ward's method was used as the hierarchical clustering criterion, measuring the similarity given by the squared Euclidean distance. This clustering criterion uses the total sum of the squared values of each object's deviations in relation to the mean value of the group where it was inserted. This criterion was chosen based on its frequent application in studies on water quality (Vega *et al.*, 1998; Andrade *et al.*, 2008; Fernandes *et al.*, 2010; Salgado *et al.*, 2011; Gomes and Cavalcante, 2017).

The evaluation of groundwater adequacy for human consumption was conducted using the clusters formed primarily based on Ordinance No. 5 of 28/09/2017 of the Ministry of Health (Brasil, 2017) and the CONAMA Resolution Nº. 396 of 2008 (CONAMA, 2008) because these are the most recent norms used in Brazilian legislation. CONAMA Resolution No. 357 of 2005 (CONAMA, 2005) and the World Health Organization parameters (WHO, 2011) were used in the case of other elements that did not present maximum permissible value (MPV) parameters in the previous ordinances mentioned.

3. RESULTS AND DISCUSSION

The factorial analysis by the principal component method was initially performed with 30 physicochemical variables (Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sb, Si, Sn, Sr, Ti, V, Zn, pH, EC and DO) analyzed in July 2013. Five simulations were necessary to achieve a satisfactory result, taking into account the criteria adopted for this analysis, significantly reducing the number of variables in the last simulation. The final simulation resulted in 9 variables (B, Ba, K, Mg, Na, Si, Sr, Ca and EC) and presented two factors that adequately described the variation of the data (Tables 1 and 2), with KMO value 0.600.

The correlation analysis applied to the variables of quality of groundwater samples showed that most of them were strongly correlated (Table 1) with high statistical significance ($p \leq 0.01$).

Table 1. Correlation matrix of the physicochemical variables of groundwater samples, highlighting the strong correlations (Sampling period: July/2013).

Variables	B	Ba	K	Mg	Na	Si	Sr	EC	Ca
Boron (B)	1.000								
Barium (Ba)	0.099	1.000							
Potassium (K)	**0.643**	**0.649**	1.000						
Magnesium (Mg)	**0.750**	0.339	**0.884**	1.000					
Sodium (Na)	**0.864**	0.336	**0.872**	**0.820**	1.000				
Silicon (Si)	0.456	**0.749**	**0.940**	**0.819**	**0.683**	1.000			
Strontium (Sr)	0.18	**0.967**	**0.679**	0.423	0.371	**0.750**	1.000		
Electrical conductivity (EC)	**0.595**	**0.661**	**0.981**	**0.916**	**0.806**	**0.962**	**0.692**	1.000	
Calcium (Ca)	**0.575**	**0.696**	**0.954**	**0.901**	**0.750**	**0.944**	**0.767**	**0.979**	1.000

Concentration units: mg L^{-1}, except EC (µS/cm).

Table 2. Factorial charges, communalities, and variance explained in the factorial analysis of the variables assessed, after rotation using the varimax method (Sampling period: July/2013).

Variable	Factor 1	Factor 2	Communalities
Boron (B)	**0.919**	-0.042	0.846
Barium (Ba)	0.075	**0.971**	0.948
Potassium (K)	**0.770**	0.617	0.973
Magnesium (Mg)	**0.897**	0.336	0.917
Sodium (Na)	**0.921**	0.226	0.899
Silicon (Si)	0.597	**0.756**	0.929
Strontium (Sr)	0.146	**0.954**	0.932
Electrical conductivity (EC)	**0.741**	0.651	0.972
Calcium (Ca)	0.690	**0.700**	0.966
Variance explained by factors (%)	75.384	17.747	-
Accumulated variance (%)	93.132		

Concentration units: mg L^{-1}, except EC (µS/cm). KMO = 0.600.

The principal component factorial analysis applied in groundwater samples condensed the variables analyzed into two ordered factors, explaining 93% of total variance. Factor 1 (F1) alone was responsible for 75% of this variance. The variables with the highest factorial charges, in this factor, were B (0.919), Na (0.921), and Mg (0.897), although the remaining ones also presented a strong relationship, given the high values of factorial charges and final communalities observed. On the other hand, Factor 2 (F2) was responsible for 18% of total variance and also included variables with high factorial charges, such as Ba (0.971) and Sr (0.954) (Table 2). According to Andrade (1989, as cited by Brito *et al.*, 2006), variables whose factorial charges are high are considered representative and must always be above 0.300.

Factor 1 (75% of data variance), represented by the variables boron (B), potassium (K), magnesium (Mg), sodium (Na), and electrical conductivity (EC), was strongly related with the concentrations of metals and salinity in the water. On the other hand, Factor 2 (18% of data variance), which comprised the variables barium (Ba), silicon (Si), strontium (Sr), and calcium (Ca), was strongly correlated with concentrations of metals in the water (Table 2). Similar results were found by Celino and Rangel (2007).

Soil weathering and lixiviation are examples of natural processes that trigger the appearance of heavy metals in waters and soils. However, metal extraction and processing, industrial tailings, domestic sewage, agricultural inputs, disposal of commercial products, burning of fossil fuel, and disposal of sewage sludge are human activities associated with environmental contamination by these metals (Nriagu and Pacyna, 1988; Teixeira *et al.*, 2000;

Alleoni *et al*., 2005; Guilherme *et al*., 2005 according to Muniz and Oliveira Filho, 2006).

Alves *et al.* (2017) observed that the main lead-carrying minerals are found in advanced stages of weathering, eventually altering into lead oxides. The inadequate disposal of this type of tailing may result in favorable conditions for lead release from the structure of the carrying minerals, and consequently threatening the environment and population that lives in the surrounding areas of the tailings pond.

The hierarchical clustering analysis applied to the quality of groundwater data allowed the classification of water sources into three clusters with chemically similar characteristics in each factor (Figures 2 and 3).

The number of clusters was defined based on the first large difference among re-scaled clustering coefficients. These coefficients revealed cutoff point 2 (higher precision), where the formation of three homogeneous clusters was observed for the sampling period in each factor.

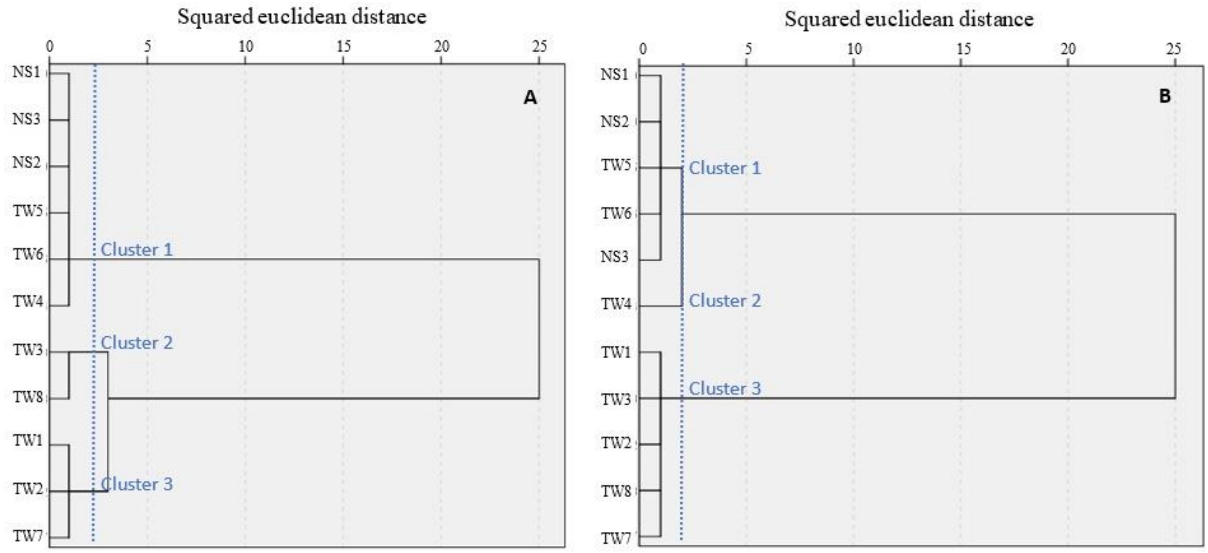

Figure 2. Dendrograms resulting from the hierarchical clustering analysis of variables explained by Factors 1 (A) and 2 (B) - (Sampling period: July/2013).

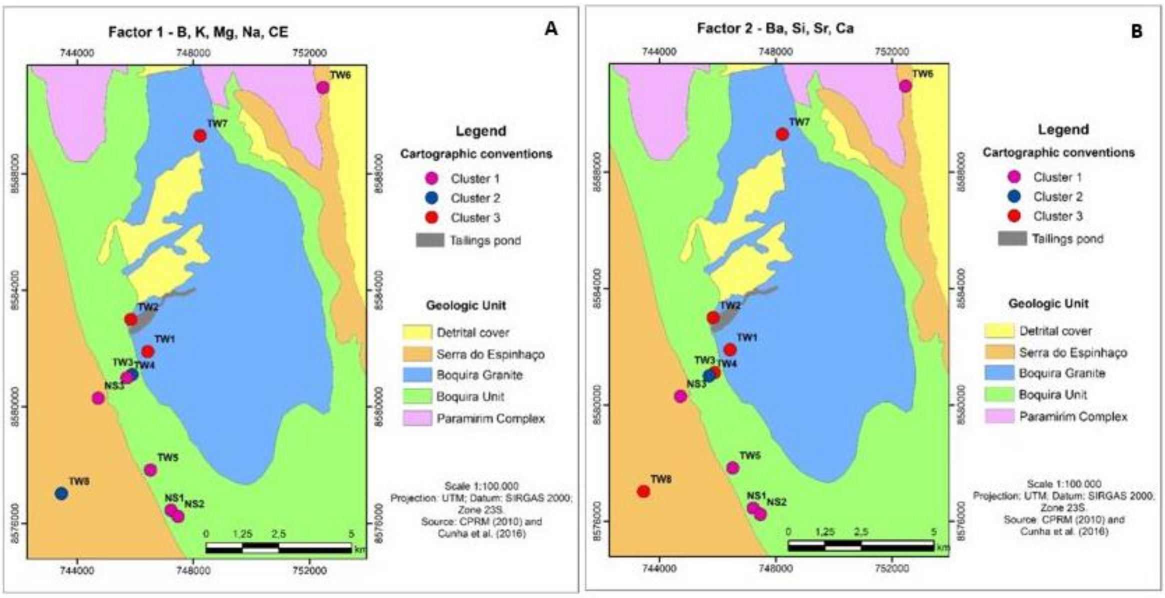

Figure 3. Distribution of tubular wells and natural springs per cluster in Factors 1 (A) and 2 (B) - (Sampling period: July/2013).

According to the variables of Factor 1 (B, K, Mg, Na, EC) (Figure 2A), three similar clusters were generated, comprising 55% (3 wells and 3 natural springs), 18% (2 wells), and 27% (3 wells) of the samples analyzed in clusters 1, 2, and 3, respectively (Figure 3A).

Cluster 1 (NS1, NS2, NS3, TW4, TW5, TW6) was characterized by waters with low concentrations of metals and salinity. In this group, boron (B) concentration ranged between 0.01 and 0.02 mg L^{-1}, which means that all samples were below the maximum permissible values according to the CONAMA Resolution No. 396/2008 (0.5 mg/L^{-1}). Ordinance No. 5 of 28/09/2017 of the Ministry of Health does not define a maximum permissible value for the concentration of boron. Magnesium (Mg) ranged between 0.53 and 34.60 mg L^{-1}, and all samples were also within the maximum permissible values according to WHO (2011) (50 mg L^{-1}). Ordinance No. 5 of 28/09/2017 of the Ministry of Health and the CONAMA Resolutions Nos. 396/2008 and 357/2005 do not define a maximum permissible value for the concentration of magnesium. Sodium varied between 1.45 and 44.10 mg L^{-1}, which is within the permissible limit according to Ordinance No. 5 of 28/09/2017 of the Ministry of Health and the CONAMA Resolution No. 396/2008 (200 mg/L^{-1}). In turn, potassium (K) varied between 0.17 and 7.47 mg L^{-1} and EC was between 13.86 and 604.10 μS/cm. Ordinance No. 5 of 28/09/2017 of the Ministry of Health, the CONAMA Resolutions Nos. 396/2008 and 357/2005, and WHO (2011) do not define maximum permissible values for the concentration of K and for EC. These results were similar to those found in the study by Daltro (2017).

Cluster 2 (TW3, TW8) was characterized by waters with intermediate concentrations of metals and salinity, presenting a concentration of boron (B) between 0.04 and 0.10 mg L^{-1}, which is below the maximum permissible value according to the CONAMA Resolution No. 396/2008 (0.5 mg/L^{-1}). Magnesium (Mg) ranged between 43.00 and 113.00 mg L^{-1}, and one sample (TW3 – Boquira Unit) was above the maximum permissible value according to WHO (2011), which is 50 mg L^{-1}. Sodium (Na) varied between 77.20 and 86.90 mg L^{-1}, therefore being within the permissible limit according to Ordinance No. 5 of 28/09/2017 of the Ministry of Health and the CONAMA Resolution No. 396/2008 (200 mg/L^{-1}). Potassium (K) varied between 9.57 and 12.50 mg L^{-1}, and EC was between 1,335 and 1,357 μS/cm. Again, ordinance No. 5 of 28/09/2017 of the Ministry of Health, the CONAMA Resolutions Nos. 396/2008 and 357/2005, and WHO (2011) do not define maximum permissible values for the concentration of K and for EC. These results also corroborate those found by Daltro (2017).

Cluster 3 (TW1, TW2, TW7) was characterized by high concentrations of metals and salinity in waters, with boron (B) concentrations that ranged between 0.01 and 0.14 mg L^{-1}. Thus, all samples were below the maximum permissible value for this element according to the CONAMA Resolution No. 396/2008 (0.5 mg/L^{-1}). Magnesium (Mg) ranged between 96.00 and 139.00 mg L^{-1}, and all samples (TW1, TW2, TW7 - Boquira Granite) were above the maximum permissible value according to WHO (2011), which is 50 mg L^{-1}. Sodium (Na) varied between 112.00 and 310.00 mg L^{-1}, and two samples (TW1 and TW2 - Boquira Granite) were above the permissible limit according to Ordinance No. 5 of 28/09/2017 of the Ministry of Health and CONAMA Resolution No. 396/2008 (200 mg/L^{-1}). Potassium (K) varied between 17.10 and 19.10 mg L^{-1}, while EC was between 1,840 and 2,399 μS/cm. As previously mentioned, Ordinance No. 5 of 28/09/2017 of the Ministry of Health, the CONAMA Resolutions Nos. 396/2008 and 357/2005, and WHO (2011) do not define maximum permissible values for the concentration of K and for EC.

According to the variables of Factor 2 (Ba, Si, Sr, Ca) (Figure 2B), three similar clusters were generated, which comprised 45% (2 wells and 3 natural springs), 10% (1 well), and 45% (5 wells) of the samples analyzed in Clusters 1, 2, and 3, respectively (Figure 3B).

Cluster 1 (NS1, NS2, NS3, TW5, TW6) of Factor 2 was represented by the same samples of Cluster 1 of Factor 1, except for sample TW4, which was characterized by good-quality waters, presenting a concentration of barium (Ba) that ranged between 0.01 and 0.05 mg L^{-1}.

All samples were within the maximum permissible value (0.7 mg/L^{-1}) for this element according to Ordinance No. 5 of 28/09/2017 of the Ministry of Health and the CONAMA Resolution No. 396/2008. The concentration of calcium (Ca) ranged between 0.98 and 2.41 mg L^{-1}, and was also within the maximum permissible limit according to WHO (2011), which is 75 mg/L^{-1}. Ordinance No. 5 of 28/09/2017 of the Ministry of Health and the CONAMA Resolutions Nos. 396/2008 and 357/2005 do not define a maximum permissible value for the concentration of calcium. Results regarding silicon (Si) and strontium (Sr) ranged between 6.88 and 11.50 mg L^{-1}, and between 0.01 and 0.07 mg L^{-1}, respectively. Ordinance No. 5 of 28/09/2017, the CONAMA Resolutions No. 396/2008 and 357/2005, and WHO (2011) do not define maximum permissible values for the concentrations of Si and Sr. Similar results were also presented in the study by Daltro (2017).

Cluster 2 (TW4) of Factor 2 was characterized by lower quality waters compared with Cluster 1, but still higher quality than in Cluster 3. In Cluster 2, barium (Ba) concentration was 0.02 mg L^{-1}, which means this sample was within the maximum permissible value (0.7 mg/L^{-1}) according to Ordinance No. 5 of 28/09/2017 of the Ministry of Health (MH) and the CONAMA Resolution No. 396/2008. In turn, the concentration of calcium (Ca) was 91.10 mg L^{-1}, therefore above the permissible limit according to WHO (2011), which is 75 mg/L^{-1}. Sample TW4 captures water from the Boquira Unit. The concentrations of silicon (Si) and strontium (Sr) reached 23.70 mg L^{-1} and 0.19 mg L^{-1}, respectively. Again, Ordinance No. 5 of 28/09/2017 of the Ministry of Health, the CONAMA Resolutions Nos. 396/2008 and 357/2005, and WHO (2011) do not define maximum permissible values for the concentrations of Si and Sr.

Cluster 3 (TW1, TW2, TW3, TW7, TW8) of Factor 2 was characterized by lower quality water compared with Clusters 1 and 2, presenting concentrations of barium (Ba) that ranged between 0.05 and 1.26 mg L^{-1}. Two samples (TW7 - Boquira Granite and TW8 - Serra do Espinhaço) were above the maximum permissible value (0.7 mg/L^{-1}) for this element according to Ordinance No. 5 of 28/09/2017 of the Ministry of Health and the CONAMA Resolution No. 396/2008. The concentration of calcium (Ca) varied between 202.00 and 303.00 mg L^{-1}, which means all samples were above the permissible limit according to WHO (2011), which is 75 mg/L^{-1}. Silicon (Si) and strontium (Sr) varied between 30.40 and 52.40 mg L^{-1}, and between 0.45 and 2.50 mg L^{-1}, respectively. As previously mentioned, Ordinance No. 5 of 28/09/2017 of the Ministry of Health, the CONAMA Resolutions Nos. 396/2008 and 357/2005, and WHO (2011) do not define maximum permissible values for the concentrations of Si and Sr.

The variables of Factor 1, which are indicators for the concentration of heavy metals and salinity in waters, suggest that the water exploited from wells and natural springs that were identified as belonging to Cluster 1 meet drinking water standards regarding the concentration of B, Mg, and Na ions. These wells and springs capture water from the Boquira Unit and Serra do Espinhaço. Only two wells (TW3 and TW8) were identified as belonging to Cluster 2. They also exploit water from the Boquira Unit and Serra do Espinhaço, but in these wells, waters are more mineralized (higher EC) than those of the previous cluster. In addition, the TW3 well presented Mg concentrations above the maximum permissible value. In turn, the wells and natural springs of Cluster 3, which exploit waters from the Boquira Granite, presented highly mineralized waters, with high concentrations of metals. These waters did not meet drinking water standards regarding the concentration of Mg and Na ions. In addition, two wells (TW1 and TW2) are located in the surroundings of the tailings pond of the mine.

The variables of Factor 2, which are indicators of concentrations of metals in waters, suggest that the wells and natural springs identified as belonging to Cluster 1 exploit better quality waters in relation to Ba, Si, Sr, and Ca ions, geologically located in the Boquira Unit and Serra do Espinhaço. The well identified as belonging to Cluster 2 (TW4), which also exploits water from the Boquira Unit, presented lower quality waters than Cluster 1, though higher than Cluster 3, with concentrations of calcium above drinking water standards. In turn, wells and natural springs of Cluster 3 presented lower quality waters (not meeting drinking water standards regarding the concentration of Ba and Ca ions) than the previous groups, where waters are captured from the Boquira Granite, Boquira Unit, and Serra do Espinhaço. These are highly mineralized waters, with high concentrations of metals.

Cunha *et al.* (2016) analyzed the mines' ramp and observed high levels of lead (Pb), zinc (Zn), cadmium (Cd), nickel (Ni), cobalt (Co), strontium (Sr), magnesium (Mg), and calcium (Ca). This may be explained due to the high amount of material from the tailings pond deposited in the galleries of the underground mine during the period it was active and after mining activities were abandoned.

According to Daltro (2017), the groundwater flow in the municipality of Boquira is preferentially oriented W-NE and, specifically in the study area, sampling points were located upstream in relation to the main human contamination sources (tailings pond, galleries of the underground mine, and open pit mine).

4. CONCLUSIONS

The factorial analysis allowed the classification of the most significant variables for water quality, prioritizing those that were strongly related with concentrations of metals and salinity in waters.

The clustering analysis classified water sources according to the quality of waters, resulting in three clusters in each factor. This allowed the conclusion that the natural background of the rocks of the municipality of Boquira influence water resources, with values found above the maximum permissible limits of metals for human consumption, such as magnesium (Clusters 2 and 3 of Factor 1), calcium (Clusters 2 and 3 of Factor 2), and barium (Cluster 3 of Factor 2).

Although the highest concentrations for metals such as lead, zinc, and cadmium were located in the waters of the underground mine galleries and tailings pond, high contents of these metals were not found in the groundwater samples analyzed.

Continuous monitoring (rainy and dry season) of the quality of water and springs located upstream in relation to contamination sources is recommended, regardless if they are not used for public supply, with the objective to determine possible contamination plumes of contaminated material.

The results of these multivariate analyses are important to support quality monitoring and management of groundwater, especially in regions that present high socio-environmental vulnerability, such as the municipality of Boquira.

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Permanent preservation areas in Mantiqueira sierra: perspectives for regularization along watercourses

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ABSTRACT

Brazilian Native Vegetation Protection Law (Law n. 12.651/2012) brought flexibility related to the sizing of Permanent Preservation Areas (PPAs) along watercourses when there was anthropogenic occupation before July 22, 2008 (consolidated rural area), which may vary according to the size of the property. To better understand the effects of this law, we analyzed land use and land occupation in PPAs along watercourses in a portion of Grande River drainage basin. Scenarios were also developed to compare the effects of the reduction of PPA limits, allowing us to discuss measures to avoid water quality deterioration. We obtained the drainage network and individualized the study area through images available in Google Earth ™, and we extracted information about land use and land occupation from a vector provided by the Laboratory of Studies and Projects in Forest Management from Federal University of Lavras. Recovery scenarios were estimated according to Article n. 61-A from the Native Vegetation Protection Law. There was a significant reduction of environmental liability compared to previous legislation. In the more restrictive scenario, there may be a 31% deficit in recovery areas, where watersheds with a strong presence of small rural properties will be the most affected. Although the reduction of recovery areas is characterized as a retrogression in the protection of native vegetation, complementary strategies could be implemented, such as economic incentive mechanisms.

Keywords: native vegetation protection law, water management, watershed management.

Áreas de preservação permanente na serra da Mantiqueira: perspectivas de regularização ao longo dos cursos d'água

RESUMO

A Lei de Proteção da Vegetação Nativa (Lei nº 12.651/2012) trouxe uma flexibilidade relacionada ao dimensionamento de Áreas de Preservação Permanente (APPs) ao longo dos cursos d'água em razão de ocupação antrópica preexistente a 22 de julho de 2008 (área rural consolidada), que pode variar de acordo com o tamanho do imóvel rural. Para melhor compreender os efeitos dessa lei, analisamos o uso e a ocupação da terra em APPs ao longo de cursos d'água em uma porção da bacia do Rio Grande. Também elaboramos cenários para comparar os efeitos da redução de limites de APPs, discutindo medidas para evitar degradação da água. Extraímos a rede de drenagem e individualizamos a área de estudo com auxílio de imagens disponíveis no Google Earth™, e extraímos informações sobre o uso e ocupação da terra a partir de um vetor fornecido pelo Laboratório de Estudos e Projetos em Manejo Florestal da Universidade Federal de Lavras. Os cenários de recuperação foram estimados de acordo com o artigo n. 61-A da Lei de Proteção da Vegetação Nativa. Houve redução significativa do passivo ambiental em comparação com a legislação anterior. No cenário mais restritivo, pode haver um déficit de 31% nas áreas de recuperação, onde as bacias com forte presença de pequenos imóveis rurais serão as mais afetadas. Embora a redução das áreas de recuperação seja caracterizada como um retrocesso na proteção da vegetação nativa, estratégias complementares podem ser implementadas, como mecanismos de incentivo econômico.

Palavras-chave: gestão da água, lei de proteção da vegetação nativa, manejo de bacias hidrográficas.

1. INTRODUCTION

Watersheds are territorial units defined by the terrain, where part of the precipitated water flows to the same point, called the “control section”. Downstream of the watershed, land use and land occupation directly influence water quality (Rodríguez-Romero *et al.*, 2018) and, in order to ensure the conservation of watercourses, the Brazilian government enacted the National Water Resources Policy (Law n. 9,433/1997). This law enshrines the watershed as a planning unit as well as for the management of water resources, requiring the adoption of conservation practices in all its extensions.

The drainage network margins play an important role in water-resource conservation by acting on slope stabilization, channel maintenance, sediment containment, siltation prevention, and soil permeability maintenance (Zaimes *et al.*, 2019). In order to ensure these functions, the Brazilian government established through the Native Vegetation Protection Law (Law n. 12,651/2012) minimum ranges that should be kept as “Permanent Preservation Areas” (PPAs).

According to the fourth article of Native Vegetation Protection Law, it is mandatory to preserve a marginal area along watercourses, which should vary according to the watercourse width. The gutter edge of the regular riverbed is taken as a reference for measuring the width of this PPA category. However, transitional provisions for vegetation recovery were established through Article n. 61-A, of this same law in areas that had been anthropized before July 22, 2008 (enactment date of Federal Decree n. 6,514/2008), called “consolidated rural areas”. According to this transitional provision, when a consolidated rural area overlaps a PPA, the marginal area will vary according to the property size - measured in Fiscal Modules (FM), rather than due to the watercourse width. This provision is known as the “ladder rule”.

In order to better comprehend the effects of the article 61-A, we analyzed land use and land occupation of PPAs along watercourses in Grande River Basin, a strategic watershed in Brazil for water supply and hydroelectric power generation (Viola *et al.*, 2014). We also elaborate scenarios to compare the effects of PPAs limit reductions, allowing us to discuss measures to avoid water degradation.

2. MATERIALS AND METHOD

2.1. Description of the study area

The area selected for analysis of recovery scenarios resulting from Law n. 12.651/2012 is located in Grande River Basin, in the cities of Itanhandu and Passa Quatro, Minas Gerais state. This region covers the springs of the Verde River, an important tributary of Grande River. We entitled the study area "Sub-Basin of Verde River Springs" (SBVRS), presented in Figure 1. The study area is geographically positioned between the coordinates 22°25'59.148" S, 44°48'12.517" W and 22°17'23.435" S, 44°55'52.356" W, with approximately 12 km². The control section of the watershed is defined by coordinates 22°17'55.246" S and 44°55'35.284" W, located in Itanhandu.

The terrain is characterized by steep hills, steep slopes, shallow soils, and embedded valleys (Minas Gerais Institute of Water Management, 2010). The climate is described as subtropical highland, with dry winter and mild summer (Cwb) (Alvares *et al.*, 2013). Regarding precipitation, the average annual rainfall in Itanhandu is approximately 1404 mm (Minas Gerais Institute of Water Management, 2010). In the study area, there is a great variation in average annual precipitation and the values vary from 1,500 mm in the northern region to 2,000 mm in the southern region. The variation of precipitation follows the altitudinal variation of the area, where the lower the altitude, the lower the precipitation (Pinto *et al.*, 2011).

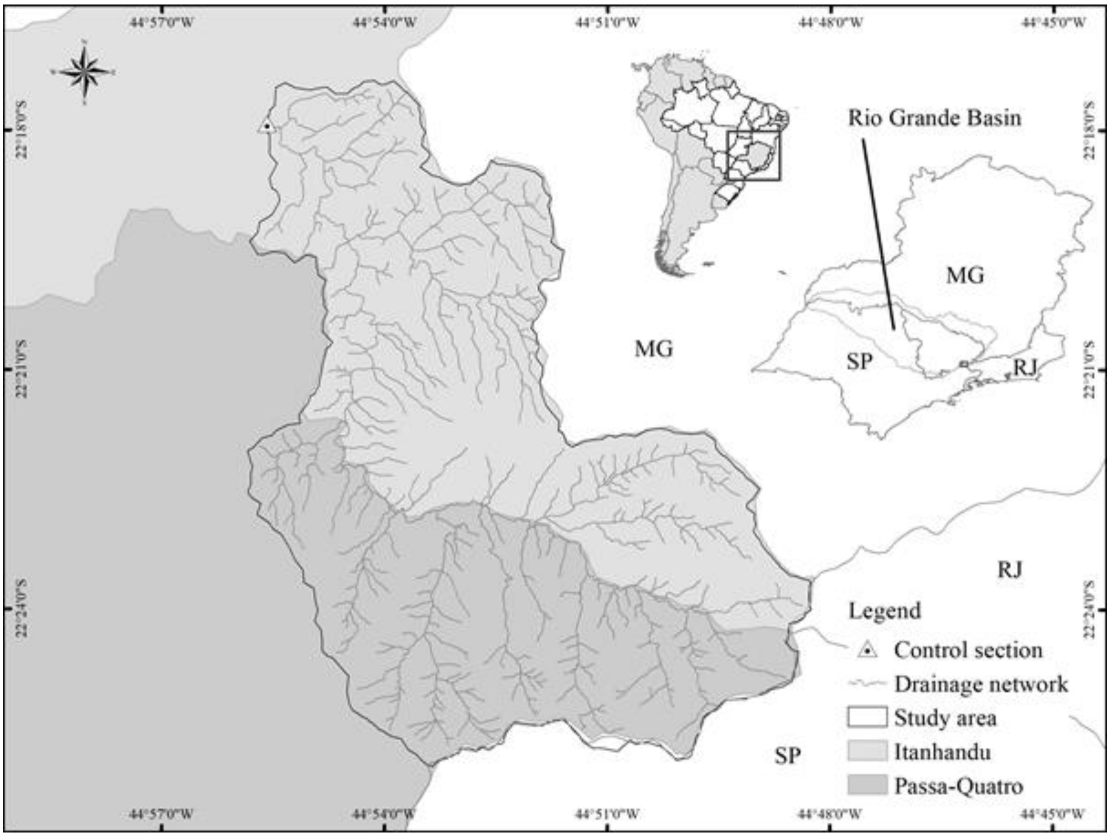

Figure 1. Study area location: the Sub-Basin of Verde River Springs.

Regarding local soil morphology, the predominant occurrence is typical dystrophic red agrisoil, both in the lower and flat portions as well as in higher steep regions. The typical dystrophic humic cambisol also occurs, but is restricted to the higher and embossed regions presenting larger declivities.

According to Brazilian morphoclimatic domains, the study area is part of the forested "Mares de Morros" domain, popularly known as "Atlantic forest". The native vegetation that occurs in the study area is distributed between grassy and forest formations. The native vegetation cover is mainly concentrated in the higher regions and associated with watercourses (Oliveira-Filho *et al.*, 2006). The study area fits into a context considered highly rich in endemic, rare and endangered species, being considered a biodiversity hotspot and one of the irreplaceable areas for world biodiversity conservation (Gonzaga, 2019).

2.2. Database and GIS procedures

In order to outline the study area, we identified the direction of surface runoff through altitude information contained in a file provided by the Earth Data project of the National Aeronautics and Space Administration (NASA), with a spatial resolution of 12.5 meters, based on the digital elevation model of the ALOS sensor. With this information, it was possible to recognize the entire contribution area of the sub-basin.

Due to watercourses in the study area being narrow and smaller than the spatial resolution of the Landsat 8 images, we obtained the drainage network manually. We've used the images available in Google Earth™ as a reference to help identify the hydrography. Google Earth™, in addition to higher spatial resolution images, features a tilt profile that guides the identification of possible water runoff channels. The manual process was based on the identification of patterns that correspond to the probable existence of watercourses, such as image texture, presence of vegetation, terrain, and color. With this information, it was possible to draw PPAs along watercourses and demarcate recovery areas with the support of a Geographic Information System (GIS).

The Laboratory of Studies and Projects in Forest Management from Federal University of Lavras (acronym in Portuguese LEMAF/UFLA) provided information about land use and land occupation. They used an image obtained from the Rapid Eye sensor from 2012, with 5 meters of spatial resolution. The raster file was generated using the object-oriented supervised classification method GEOBIA (Geographic Object-Based Image Analysis), besides the classification algorithm called 50 SVM (Support Vector Machine).

The limits of the existing properties in Itanhandu and Passa Quatro were obtained from the public consultation tab of Rural-Environmental Registry website (acronym in Portuguese CAR). To identify and quantify the properties that overlap with SBVRS, we used a GIS environment.

2.3. Analysis of Native Vegetation Protection Law

For the delimitation of the PPAs along watercourses in the study area, according to the fourth article from Native Vegetation Protection Law, the planimetric distance of 30 meters in each margin was established. We adopted this distance as a criterion assuming that all watercourses have their regular riverbed width less than 10 meters, which is consistent with the reality of the watershed terrain.

As for recovery scenarios, according to Article 61-A from Native Vegetation Protection Law, the same method of generating PPAs was used, changing only the reference widths (Table 1), where the areas of the different features of land use and occupation were quantified.

Table 1. Recovery areas according to the Article 61-A from native vegetation protection law.

Scenarios	Property Size (FM)	Property Area (ha)	Recovery Area (m)
1	0 a ≤ 1	≤ 30	5
2	>1 a ≤ 2	> 30 a ≤ 60	8
3	> 2 a ≤ 4	> 60 a ≤ 120	15
4	>4	> 120	30

For properties with an area superior to four FM, the recovery area must vary between 20 and 100 meters in planimetric projection, according to Environmental Regularization Program. In this situation, we assumed a recovery area of 30 meters, since the database from Environmental Regularization Program is not available.

3. RESULTS AND DISCUSSION

3.1. Land use and land occupation

We have identified four physiognomies related to land use and land occupation in the study area: “Native Vegetation”, “Anthropic Use”, “Urbanization”, and “Water”. Those physiognomies are represented and quantified in Figure 2.

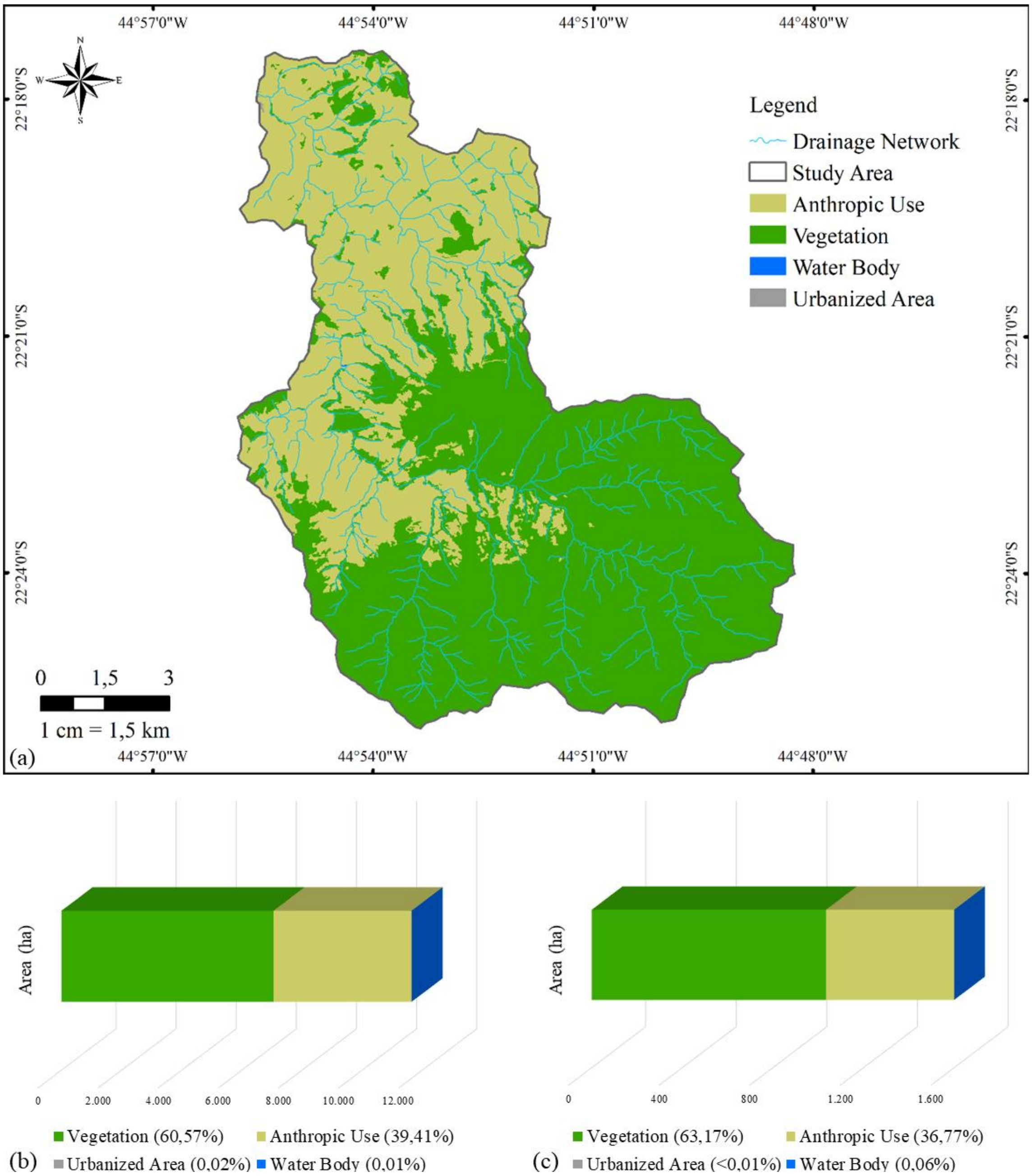

Figure 2. Land use and land occupation in (a) the study area: (b) area (ha) and percentage of each physiognomy identified in the study area, and (c) area (ha) and percentage of each physiognomy identified in PPAs along watercourses.

Nearly 60.6% of the total sub-basin area is covered by native vegetation, which occurs predominantly at altitudes above 1,100 m in Itanhandu, on the right bank of the Verde River, and above 1,400 m in Passa Quatro. We can infer that these areas were maintained due to the difficulty in managing, establishing crops or infrastructures. In both cities, native vegetation was associated with the presence of watercourses, which reflects legal impositions prior to Law n. 12.651/2012.

Regarding PPAs, almost 37% of the area is not covered by native vegetation, according to the limits fixed by the fourth article of Native Vegetation Protection Law. Most of PPAs are situated in upper portions of the study area, where the springs that supply Verde River are located. The "Anthropic Use" category occurs more expressively in the lower portions. A higher absence of native vegetation in PPAs has been located in the higher portions of Passa Quatro, near Lamins and Paiolinho communities.

3.2. The influence of properties size

Considering the self-reported data in Brazilian Rural-Environmental Registry, we have identified properties in the four proposed categories, with predominance of small rural properties (Figure 3). There is a decrease in number in the categories comprising properties with larger areas. This pattern has occurred in the distribution analysis of registered properties for cities in the study area. Among the 1,345 rural properties that were registered in Itanhandu and Passa Quatro, 202 rural properties are situated in the study area. In this sub-basin, among 97% of rural properties are smaller than four fiscal modules.

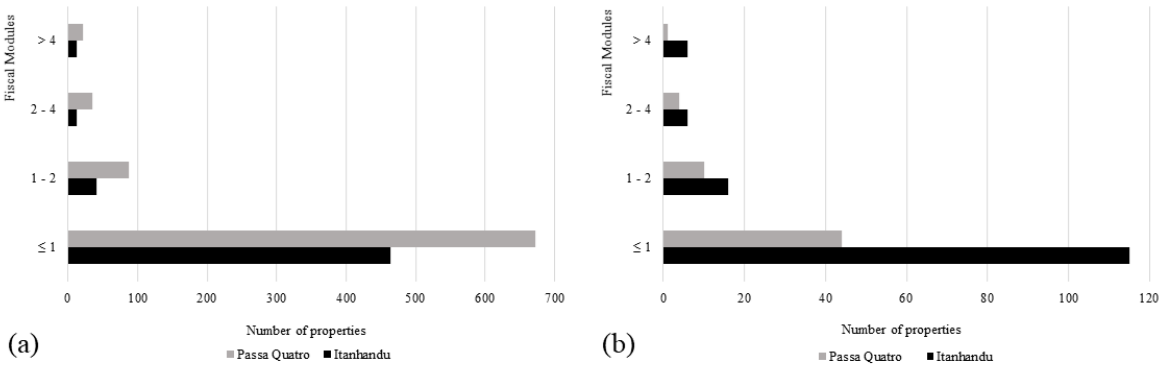

Figure 3. Number of rural properties by Fiscal Modules in (a) Itanhandu and Passa Quatro, and in (b) the study area.

In Brazilian Rural-Environmental Registry, property boundary information is registered through a self-declaration process. Therefore, owners, squatters or people who have some kind of link with the property can carry it out. However, considering that the registration platform is not intuitive for those unaware of environmental legislation, photo interpretation, and geoprocessing tools, as expected, there was a large amount of overlapping property boundaries. Consequences of this overlap does not reach land regularization scope, since information inserted in Brazilian Rural-Environmental Registry is valid only to know the environmental reality of rural properties in the country, aiming at environmental regularization. However, this process implies possible errors in property sizing and, consequently, errors in the sizing of recovery areas (Article n. 61-A from Native Vegetation Protection Law).

Although there is no reliable database containing the real properties boundary for the study area, we have identified a tendency of property distribution that corresponds to Brazilian reality. Brazilian territory presents a land profile that is comprised of a few properties with large areas, many small properties and a high number of overlaps (Sparovek *et al.*, 2019).

3.3. Perspectives for regularization

Due to spatial resolution limitation, polyline vector files have been used to outline both PPA and recovery areas, since we do not have geographic information to spatialize watercourses widths. Although unconventional, polyline vector files have been reported in literature as a suitable technique to simulate PPA limits (Alvarenga *et al.*, 2017; Rosot *et al.*, 2018; Rodríguez-Romero *et al.*, 2018; Valera *et al.*, 2019).

When comparing the proposed areas in Article 61-A of Native Vegetation Protection Law, a great variation of area to be recovered was detected (Table 2).

Table 2. Recovery scenarios in the study area.

Scenario	Property Area (FM)	Recovery Area (m)	Recovery Area (ha)	* Recovery percentage (%)	* Recovery Deficit (ha)	Recovery Deficit in PPAs (%)
1	≤ 1	5	94.97	16	508.15	31
2	> 1 and ≤ 2	8	152.25	25	450.87	28
3	> 2 and ≤ 4	15	288.66	48	314.46	19
4	> 4	30	603.12	100	0.00	0

* Related to Scenario 4 (total recovery).

In Scenario 1, only 16% of PPAs would be recovered. In Scenarios 2 and 3, we predict that, respectively, nearly 25 and 48% of PPAs would be recovered. If the limits of the fourth article from Native Vegetation Protection Law was maintained (Scenario 4), 603.12 ha of native vegetation would be recovered. PPAs recovery deficit was larger in the first scenario, which is worrying since the study area is characterized mostly by small, fragmented properties. Regions where intensive activities are developed and, consequently, have lower rates of native vegetation cover, with a strong presence of small rural properties, will be the most affected by this flexibility.

Clearly, the 2012 change in environmental legislation will lead to a reduction in areas to be recovered. This reduction is estimated to be 4.5 ± 1 million hectares (Soares-Filho, 2014; Guidotti *et al.*, 2017), an area larger than Denmark, Holland, or Switzerland, and also larger than Rio de Janeiro, Alagoas, or Sergipe state.

In a study carried out in a micro basin located inside our study area, Monteiro *et al.* (2017) presented how property size influences the recovery strings according to rticle 61-A. Nearly 90% of properties was characterized as small, being more affected by legislation changes. Environmental liability in PPAs along watercourses in this microbasin was estimated among 68% and, with this flexibilization, could be reduced to 20%. Borges *et al.* (2017) found in their studies in Pandeiros and Calindó River Basins that 95% of properties registered in Brazilian Rural-Environmental Registry are smaller than 4 fiscal modules and will be the most affected by the “ladder rule”. This value is close to the 90% identified by Soares-Filho (2014) for Brazil. Our study presents a close percentage to the above-mentioned (almost 97%).

Native vegetation around watercourses plays an important role in maintaining water quantity and quality. PPAs act as an interface between terrestrial and aquatic ecosystems and can be a source or filter of sediments available in the environment. Apparently, the width of vegetated range acts as a “thermometer” indicating the function exerted by PPA. In general, these areas act as filters when the preserved range is larger than 15 meters and as sources when the ranges are smaller. Land use and the adoption of good agricultural practices also contribute to determining the range required for PPAs to act as filters or sediment sources (Guidotti *et al.*, 2017).

PPAs along watercourses are important in terms of solar energy transfer to the aquatic environment, interception of nutrients and sediment entering rivers, and exchange of organic material between terrestrial and aquatic systems (Pusey and Arthington, 2003). They play multiple roles including “land tenure, water protection, and wildlife conservation”, and act as ecological corridors that facilitate flow of individuals into the landscape. Their benefits are related to factors such as width, topography, extent, continuity, and quality. Among these factors, the width is the most important, as it influences the relationship between interior and border, as well as microclimatic changes. The minimum width of PPAs along watercourses should be 100 meters (50 meters on each bank), regardless of biome, taxonomic group, soil class, or topography (Metzger, 2010).

Currently, vegetation recovery in PPAs along watercourses is generally established by command and control actions, such as the Native Vegetation Protection Law. However, the existence of a legislation itself does not guarantee that the vegetation deficit will be recovered, as there were liabilities concerning previous legislation (Guidotti *et al.* 2016; 2017). To support legislation compliance, it is necessary to implement complementary strategies, such as economic incentive mechanisms.

4. CONCLUSIONS

Watersheds with a strong presence of small rural properties will be the most affected by the flexibility brought by Article 61-A, which could compromise the functionality of PPAs along watercourses.

With the support of the four scenarios built in this study, we verified that there was a significant reduction of environmental liability compared to previous legislation. In the more restrictive scenario, there may be a 31% deficit in recovery areas.

Although the reduction of recovery areas is characterized as a retrogression in the protection of native vegetation, complementary strategies could be implemented, such as economic incentive mechanisms.

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Degradation of the Textile Dye Reactive Black 5 by Basidiomycetes

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ABSTRACT

Reactive Black 5 (RB5) is one of the synthetic reactive dyes most used in the textile industry, due to its solubility in water and reactive groups which form covalent bonds within the fiber. In the process of dyeing fabrics, however, it is estimated that 12-14% of dyes are released into the effluent. This work evaluated the biodegradation of RB5 dye, adsorbed in polyurethane foam, by basidiomycetes (*Phanerochaete chrysosporium* ATCC 24725, *Pleurotus ostreatus* and *Pleurotus floridae*). Results were evaluated considering the partial- or total medium discoloration, the adsorption capacity of the dye in the polyurethane foam (PUF) and the respirometric measurements. The results showed that *Phanerochaete chrysosporium* was able to partially degrade 50 mg L^{-1} of RB5 in pH 6.0, when cultivated in Petri dishes. When this microorganism was cultivated in PUF cubes saturated with RB5 solution (50 mg L^{-1}, pH 6.0), CO_2 production reached an accumulated value of 2.16 mg on the fifteenth day, revealing the growth of the microorganism and consequently the contaminant degradation, which was used as the source of nutrients.

Keywords: biodegradation, p*hanerochaete chrysosporium*, respirometric method.

Degradação do corante têxtil Preto Reativo 5 por basidiomicetos

RESUMO

O corante Preto Reativo 5 (RB5 - *reactive black* 5) é um dos corantes reativos sintéticos mais utilizados na indústria têxtil, devido à sua solubilidade em água e a grupos reativos que formam ligações covalentes com a fibra. No processo de tingimento de tecidos, no entanto, estima-se que 12-14% dos corantes sejam liberados para o efluente. O objetivo deste trabalho foi avaliar a biodegradação do corante RB5, adsorvido em espuma de poliuretano, por basidiomicetos (*Phanerochaete chrysosporium* ATCC 24725, *Pleurotus ostreatus* e *Pleurotus florida*). Os resultados foram avaliados considerando a descoloração parcial ou total do meio, a capacidade de adsorção do corante na espuma de poliuretano e a medida respirométrica. Os resultados mostraram que *Phanerochaete chrysosporium* foi o mais eficicente, sendo capaz de degradar parcialmente RB5 na concentração de 50 mg L^{-1} em pH 6.0, quando cultivado em pacas de Petri. Quando este microrganismo foi cultivado em cubos de espuma de poliuretano saturada com solução de RB5 (50 mg L^{-1}, pH 6.0) a produção de CO_2 atingiu o valor acumulado de 2.16 mg no décimo quinto dia, revelando o crescimento microbiano e a degradação do contaminante, que foi utilizado como fonte de nutrientes.

Palavras-chave: biodegradação, método respirométrico, *Phanerochaete chrysosporium*.

1. INTRODUCTION

The textile industry is one of the most important in the world. The global textile and clothing business in 2017 is estimated to be worth about US $4.395 trillion. The current global apparel market is estimated at approximately US$ 1.15 trillion, which makes up nearly 1.8% of the world Gross Domestic Product (GDP) (Textile Mates, 2018). In 2017, China was the top-ranked global textile exporter (37.2 percent of the global market share), followed by the European Union (with 23 percent of the global market share), and India (Statista, 2018).

The technology *Dye Clean* became a reference in the textile chemistry world market, consisting of dyeing cellulosic fibers with reactive dyes, such as Reactive Black 5 (RB5 - CAS Registry Number:12225-25-1/17095-24-8). Like all reactive dyes, the RB5 is highly soluble in water and difficult to degrade due to its polyaromatic (Fan *et al.*, 2009). Another troubling fact is the production of aromatic amines due to the degradation of this type of dye with azo bonds, which are highly carcinogenic (Vilar *et al.*, 2011), while also being considered highly recalcitrant, toxic and mutagenic substances for various aquatic species (Vedrenne *et al.*, 2012).

Regarding the process of dyeing fabrics, there is an estimate that 12-14% of textile dyes are released into the effluent (Safa *et al.*, 2011). These compounds cause visual pollution, changes in the micro-aquatic biota and photosynthetic processes, and bioaccumulation of toxic substances in aquatic organisms (Kunz *et al.*, 2002). In addition, most of them are classified as toxic, mutagenic and carcinogenic for aquatic organisms and humans (Ahmad *et al.*, 2011; Wang *et al.*, 2014), consisting of a serious ecological problem. The reactive dyes are highly recalcitrant to conventional effluent treatment processes, which, although removing about 80% of the dyes, leave most adsorbed in the sludge (Kunz *et al.*, 2002).

Microbial and enzyme immobilization have been extensively studied in the treatment of dye-based industrial effluents (Bilal *et al.*, 2017), being known as a solid state (SS) cultivation/fermentation process. Polyurethane foam (PUF) is one of the substrates used for fungal SSF, because it increases enzyme production and substrate oxygenation, without causing shear stress to the biomass (Sharari *et al.*, 2013).

White rot fungi (for example, *Phanerochaete chrysosporium*, *Pleurotus* sp, *Trametes* sp, *Ganoderma* sp) are among the most efficient microorganisms in the treatment of xenobiotic molecules, such as synthetic reactive dyes (Wesenberg *et al.*, 2003). Their metabolic capacity to mineralize complex polymers, like lignin, is due to the secretion of non-specific enzymes, which includes Laccases (EC 1.10.3.2), manganese peroxidases (EC 1.11.1.13) and lignin peroxidases (EC 1.11.1.14).

Biodegradation can be assessed by microbial respiration. Respirometric methodologies are capable of evaluating the degradation process of organic waste based on the quantification of CO_2 production or O_2 consumption during metabolic activities. One of the most traditional methods used to assess biodegradation of organic wastes is the Bartha's respirometric assay (Bartha and Pramer, 1965). This is a titrimetric method which measures the CO_2 trapped in a strong alkali after carbon mineralization by the microbial community present in the system (Bartha and Pramer, 1965; ABNT, 1999; Régo *et al.*, 2014). Its use includes the assessment of the complete biodegradation of petroleum wastes, general compounds and organic fertilizers in soil such as sewage sludge (ABNT, 1999; Rêgo *et al.*, 2014).

The aim of this study was to use polyurethane foam as matrix support to assess the biodegradation of the RB5 by fungi basidiomycetes *P. ostreatus, P. floridae* and *P. chrysosporium* ATCC 24725 in Bartha respirometers.

2. MATERIAL AND METHOD

2.1. Analytical curve

The RB5 dye stock solution was prepared by dissolving dye in Milli-Q water to the concentration of 100 mg L^{-1}. For analytical curve, standard solutions ranging from 1.00 to 30.0 mg L^{-1} of the dye were used, in parallel with a blank solution of water. The linear analytical calibration of the curve was furnished by a spectrophotometer UV-VIS (Varian Cary 50), in the scan range from 190 to 800 nm. The wavelength of maximum absorption of the dye (598 nm) was used to monitor the concentration of the RB5 during the experiments. The calibration curve of the RB5 described with the equation y= 0.00262 + 0.02263x and the correlation coefficient was 0.99983, indicating a good linearity.

2.2. Preliminary studies

To establish the better condition for RB5 adsorption in polyurethane foam, a full factorial design 2^2 with central point, as shown in Table 1, was used to check the effect of experimental variables (pH and dye concentration) in the adsorption of the RB5 in PUF. It was therefore determined that the dye concentrations to be used in the experiment were (mg L^{-1}): 12.5, 25 and 50.

Table 1. Full factorial design 2^2 with central point, used to evaluate the effect of pH and the concentration of Reactive Black 5 dye in its adsorption in polyurethane foam.

VARIABLE	Level (-1)	Level (0)	Level (+1)
pH	6	7	8
Dye concentration (mg L^{-1})	12.5	25	50
Experiment		**Dye concentration**	**pH**
1		-	-
2		+	-
3		-	+
4		+	+
5		0	0
6		0	0
7		0	0

The pH was adjusted using NaOH 0.1 and 0.01 mol L^{-1}, HCl 0.1 and 0.01 mol L^{-1} solutions. The polyurethane foam was ceded by the Tecnotrater Group (Federal University of Paraná State - Brazil), having the following composition: polyethylene glycerol, 4.4- diphenylmethane diisocyanate, glycerol, water and Montmorillonite 3%.

To achieve the maximum saturation of the dye in the matrix, adsorption studies were carried out introducing 100 mg of PUF cubes (1 cm x 1 cm x 1 cm) into 50 mL of RB5dye solution, being shaken (120 rpm) at room temperature (25 °C), with 3 mL samples being collected at 15 and 30 minutes. However, as the experiment progressed, magnetic agitators were also used. Samples of the supernatant (3 mL) were collected at 10-minute intervals for 2 hours, in order to verify the saturation of the adsorbent.

The amount of dye taken up and the percentage of the dye removed by the PUF were calculated by applying Equations 1 and 2, respectively.

$$q = \frac{C_o - C_f}{m} . V \quad (1)$$

$$\% \, Removal = 100. \frac{C_o - C_f}{C_o} \quad (2)$$

In which "q" is the amount of dye adsorbed by the adsorbent (mg g^{-1}), "Co" is the initial dye concentration placed in contact with the adsorbent (mg L^{-1}), "Cf" is the dye concentration (mg L^{-1}) after the batch adsorption procedure, "m" is the mass of adsorbent (g) and "V" is the volume of dye solution (L).

2.3. Microorganisms

P. ostreatus, *P. floridae* and *P. chrysosporium* ATCC 24725 were cultivated on sterile Potato Dextrose Agar (PDA), in Petri dishes, at 26°C ± 2ºC for seven days, being kept at + 4°C for up to 30 days.

Inoculum was prepared by transferring an aliquot of the mycelium to a 250 mL Erlenmeyer flask containing 50 mL of sterile PDA, and incubating it for seven days at 26°C ± 2ºC. The cell suspension was collected at the end of cultivation by adding 50 mL of sterile peptone water solution (1 g L^{-1}), 0.2% of Tween 80, 3 g of glass beads (3 mm diameter) and a magnetic bar. The suspension was subjected to agitation for 15 minutes, with sufficient rotation to promote cell dispersion. Cells were quantified using a Neubauer chamber and also by counting using Spread Plate technique, on PDA (Spier *et al.*, 2006).

2.4. Biodegradation of RB5 dye

In order to optimize RB5 biodegradation by each fungus, a full factorial design 2^2 with central point was performed in Petri dishes, as shown in Table 1 (the same conditions evaluated for adsorption of RB5 in PUF).

Biodegradation experiments were performed in 9 mm Petri dishes, containing 30 mL of the RB5 dye added with agar-agar (1.5%), and adjusted pH. The inoculation occurred in wells located in the central part of each plate, with the addition of 100 µL of the cellular suspension. The plates were then incubated at 26°C ± 2°C. Radial growth was measured when one of the fungi reached the edge of the plate, in quadruple, using a digital caliper. The growth speed was determined by the division of the growth average observed by the number of days the experiment occurred (mm day^{-1}) (Miyashira *et al.*, 2010)

After measuring the growth rate, the Petri dishes were reincubated at 26°C ± 2ºC for five days, to visually evaluate the total or partial discoloration of the media.

2.5. Respirometric Method

The respirometric assays were conducted considering the methodology described first by Bartha (1965), detailed at ABNT - NBR Method 14283/1999 (ABNT, 1999).

Each Bartha respirometer (ABNT, 1999) received 100 mg of PUF, which were saturated with dye RB5 (50 mg L^{-1}) (sole carbon and nitrogen source to the microorganisms), and the pH was adjusted to 6.0. This substrate was inoculated with 1 mL of the *P. chrysosporium* cell suspension. As the inoculum solution had 1.8 x 10^6 colony forming unity (CFU) $mL^{-1;}$ this led to an initial cell concentration of 1.8 x 10^4 CFU g^{-1} PUF.

The experiment took place for 15 days at room temperature (25ºC). The CO_2 generated during microbial respiration was measured every 24 hours, by removing KOH solution (0.2 N) of the respirometric flask and replacing it daily. The residual KOH was titrated with standard HCl (0.1 N) after addition of 1 mL of a solution of barium chloride to precipitate carbonate ions. Thus, the total amount of CO_2 produced as a function of incubation time could be calculated and plotted.

The values of CO_2 were obtained according to Equation 3 (Régo et al., 2014):

$$CO_2\ (mg) = (A - B) x\ 50\ x\ f_{HCl}\ x\ 0.044 \quad (3)$$

Where "A" is the volume of HCl (0.1 N) used to titrate the blank (mL), "B" is the volume of HCl (0.1 N) used to titrate the sample (mL), and "f_{HCl}"is the factor for HCl (0.1 N). The values 50 and 0.044 were used to transform the CO_2 from micromoles to milligrams (Régo *et al.*, 2014).

3. RESULTS AND DISCUSSION

3.1. Preliminary adsorption study

Figure 1 illustrates the decay of sample absorbance during the factorial design study of the adsorption of the RB5 dye in PUF.

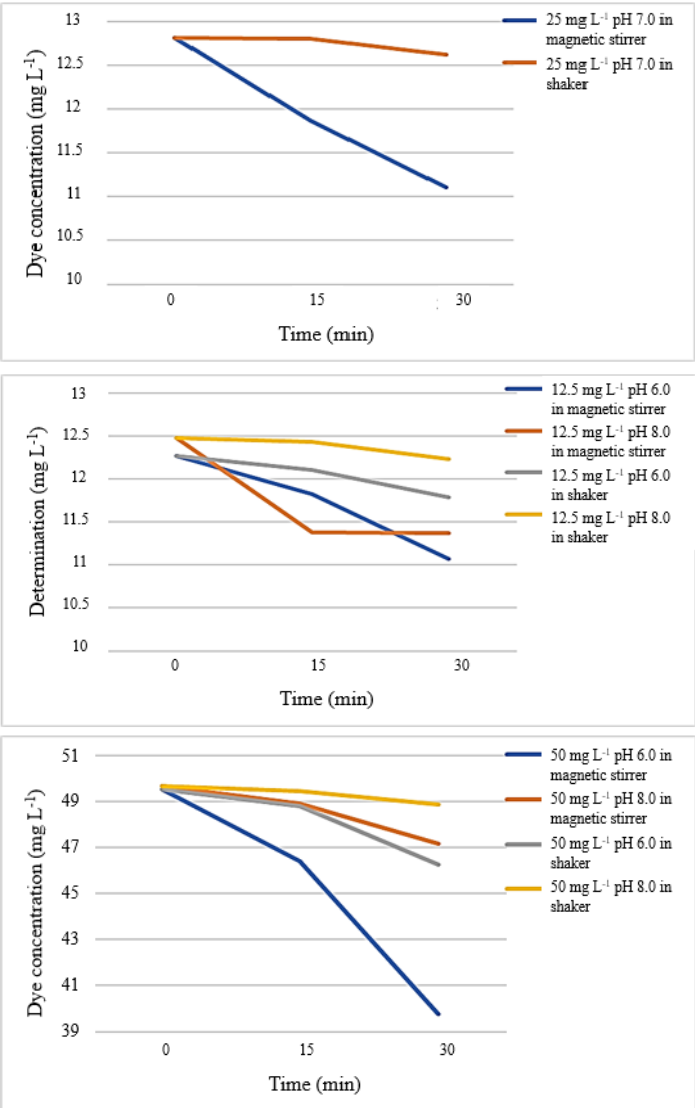

Figure 1. Absorbance variation of the samples during the factorial design study, checking the effect of pH and dye concentration in the adsorption of Reactive Black 5 in polyurethane foam.

The best adsorption result was found using the magnetic stirrer, at pH 6.0. This variation was possible because the magnetic stirrer provided better agitation than the shaker, creating a whirlwind able to plunge and completely soak all the PUF cubes in the RB5 solution, improving the retention of textile dye. In relation to the pH, it should be emphasized that pH changes the surface load of the adsorbent and the degree of ionization of different pollutants. This variable can change the adsorption process by decoupling functional groups present in the active sites of the adsorbent (Mall et al., 2014).

3.2. Adsorption study of the RB5 due on the support.

The adsorption experiment was conducted with RB5 dye at 50 mg L^{-1} in pH 6.0 and pH 7.0. The maximum adsorption of RB5 dye in PUF occurred after 110 minutes of stirring in both pH, as illustrated in Figure 2. The increase in the concentration of RB5 observed at 120 minutes indicates the desorption/adsorption equilibrium of the PUF was achieved, ending the experiment.

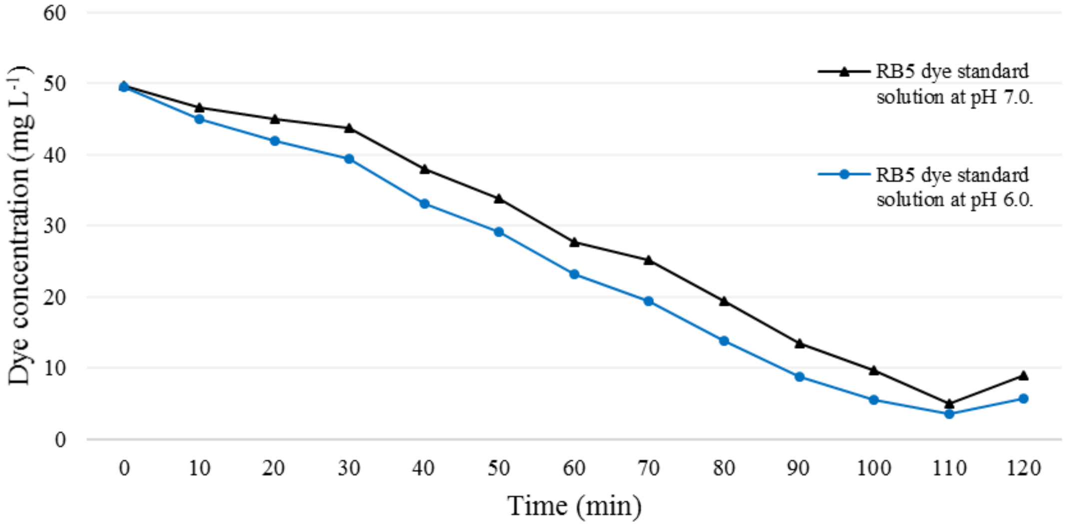

Figure 2. Adsorption efficiency of RB5 50 mg L^{-1} in polyurethane foam, at pH 6.0 and pH 7.0.

It was noted that the acidification of solution (pH 7.0 to pH 6.0) increases the adsorption of RB5 at PUF. This occurs precisely because of the dissociation of the ions present in the molecules of the textile dye and the ionization of the amino groups present in the PUF composition. However, this alternative wasn't addressed in this work, because after the adsorption of the dye in the PUF, this matrix was used as support and the textile dye as a nutrient source to the fungal culture, restricting the study to this range of work. Table 2 shows the percentage and quantification of RB5 dye (50 mg L^{-1}) adsorbed by PUF.

Table 2. Results of the adsorption experiments mentioning the percentage and the quantity of Reactive Black 5 dye (50 mg L^{-1}) adsorbed by polyurethane foam.

	Standard solution of RB 5 dye 50 mg L^{-1} without pH adjustment (pH 7.0)	Standard solution of RB 5 dye 50 mg L^{-1} at pH 6.0
Concentration in the solution after adsorption (mg L^{-1})	5.2	3.8
% of mass RB5 in the solution	10.4	7.6
Adsorbed concentration (mg L^{-1})	44.7	46.2
% of RB5 adsorbed	89.5	92.4
q (mg g^{-1})	22.35	23.1

The percentage of RB5 dye adsorption by polyurethane foam (92.4%) is close to the values mentioned by Mori and Cassella (2009), which observed 93% adsorption of violet crystal dye by PUF after 4 hours of stirring.

Góes *et al.* (2016) studied the adsorption of red dye samples by four modified polyurethane foams. PUF without cellulose adsorbed 83.8% of the dye, PUF with cellulose 75.7%, PUF with modified cellulose (1:1) 90.9%, and PUF with modified cellulose (3:1) 84.3%.

The difference in the percentage of mass adsorbed in other works, when compared to the values obtained, may be due to factors such as the presence of different ionic groups in foams, the dimensions, densities and quantities of each piece used (which directly interferes with the surface area), the presence, quantity and arrangement of the ionizable groups in each foam, favoring the adsorption of several dye molecules or rendering unusable some reactive sites due to possible steric impediments, the dye-loading used, as well as the agitation conditions (temperature, concentration , pH and agitation speed) employed in each of the adsorption tests.

In the present work, the use of PUF with nitrogenous groups was favorable for the adsorption of the RB5 textile dye, which in aqueous solution has four negatively charged sulfate groups.

3.3. Evaluation of radial growth and discoloration

Cell suspensions were counted in Neubauer chamber and PDA plates, showing similar values (Table 3), indicating that fungi had viable cells in the inoculation.

Table 3. Number of fungi cells in the microbial suspensions used as inoculums.

Fungi	Neubauer Chamber Count (cel mL^{-1})	Count on PDA Plates (CFU mL^{-1})
Pleurotus floridae	9×10^5	1.1×10^5
Pleurotus ostreatus	3×10^5	1.5×10^5
Phanerochaete chrysosporium	1.8×10^8	1.2×10^8

PDA: potato dextrose agar; CFU colony forming unity.

P. chrysosporium presented higher radial growth speed (RGS) than *Pleurotus* in all conditions studied, certainly due to the higher number of cells inoculated in the experiment (Table 3). While *Pleurotus* inoculum had 1.1 - 1.5 x 10^5 colony forming unity (CFU) (considering that 100 µL were inoculated in the wells, at the center of the Petri dishes), *P. chrysosporium* inoculum had 1.2 x 10^8 CFU.

It's well known that the performance of a biological process is significantly influenced by the size and viability of the inoculum (Pirt, 1976). In this study RGS was probably influenced by factors such as change in nutrients, presence of inhibitors, spore germination, and concentration of the inoculum. Increasing inoculum size leads to an increase of the dividing (active) cell fraction, influencing growth rate (dX/dt) which is defined as: $dX/dt = \mu X$ (where μ is the specific growth rate, and X is the inoculum size).

Although this is a difference in inoculation, Ottoni *et al.* (2013) also had better discoloration results of RB5 dye with *P. chrysosporium* (and *Trametes versicolor)* than with *P. ostreatus*. They also observed that in Petri dishes RB5 was decolorized only at a concentration equal or below 100 mg L^{-1}, being the best results obtained at 50 mg L^{-1}.

Comparing the genus *P.*, it was observed that in most of the conditions *P. ostreatus* showed higher RGS than *P. floridae*. To *P. floridae* the higher RGS occurred at pH 6.0, at the dye's lowest concentration (7.36 mm day^{-1}), while for *P. ostreatus* it occurred at 25 mg L^{-1}, at pH 7 (6.97 mm day^{-1}). Muthukumaran *et al.* (2017) in their study showed that the *P. ostreatus* was able to remove 64% of the RB5 dye (Figure 3).

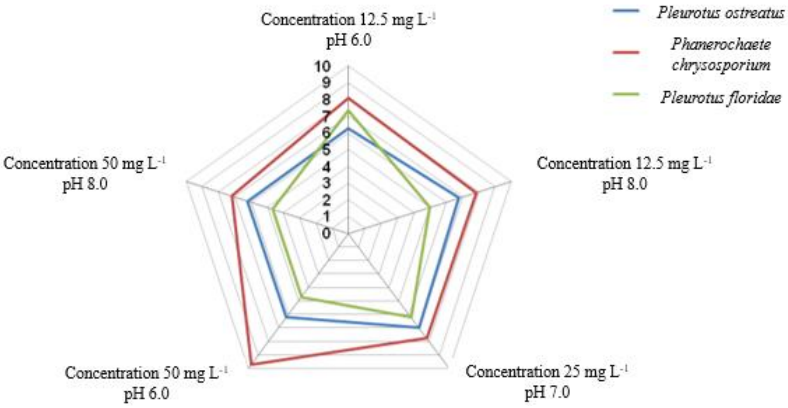

Figure 3. Radial growth speed (mm day^{-1}) of *P. ostreatus*, *P. floridae* and *P. chrysosporium* cultivated on Reactive Black 5 dye solution solidified with agar-agar (1.5 g L^{-1}) in Petri dishes.

Although it was visually observed that the color of the dye (chromophore group) in the cultivation media was not totally degraded, *P. chrysosporium* left them with a rose hue color, indicating their partial biodegradation. This color changing was also observed by Bonugli-Santos *et al.* (2013) using the fungus *Peniophora sp*.

Considering the partial degradation observed, RGS values obtained in *P. chrysosporium* cultivation were used to verify the existence of secondary interactions between the variables (dye concentration and pH). According to the response surface chart (Figure 4), *P. chrysosporium* biodegrades RB5 more efficiently when cultivated in higher dye concentration (50 mg L^{-1}) and lower pH (6.0), as shown by a red color in surface chart. Thus, the biodegradation efficiency increases from green to red color, which used lower pH and higher concentrations.

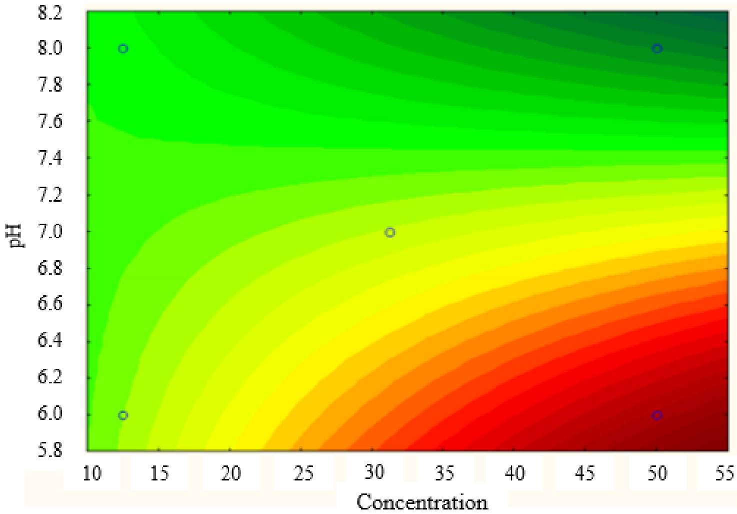

Figure 4. The response surfaces chart about *P. chrysosporium* ATCC 24725 growth when cultivated in Reactive Black 5 dye solutions in different pHs, in Petri dishes.

Radha *et al.* (2005) observed that the growth of *P. chrysosporium* and the corresponding discoloration process were essentially controlled by the pH of the medium, depending on the nature of the substrate used. Their studies with different dyes showed better biodegradation results at lower pH (4.0 – 6.0). Permpornsakul *et al.* (2016) also concluded that a higher discoloration of RB5 by *P. chrysosporium* is obtained at pH 6.0 than at pH 7.0 or 8.0, corroborating with this study.

3.4. Biodegradation Assay of Dye Reactive Black 5 Adsorbed on Polyurethane Foam

After fifteen days of *P. chrysosporium* cultivation in PUF cubes, chromophore group of RB5 dye appears to have been completely degraded, as visually observed. This degradation may have occurred by lignin or manganese peroxidase activity, the production of which was reported to be greatly improved when *P. chrysosporium* was cultivated immobilized in PUF, then comparing with the production in conventional stationary liquid culture (Bilal *et al.*, 2017). Figure 5 shows the concentration of accumulated CO_2 produced during this experiment.

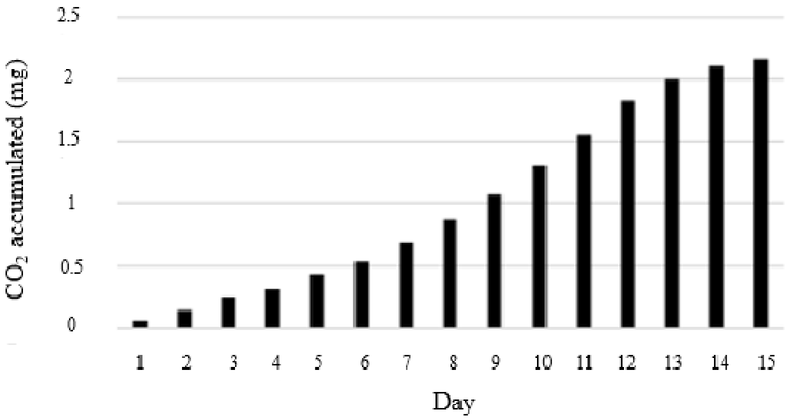

Figure 5. CO_2 accumulated (mg), produced during the biodegradation of the dye Reactive Black 5 (50 mg L^{-1}, pH 6.0) by *P. chrysosporium* cultivated in polyurethane foam cubes.

Respiration measurement directly determines the microbial activity by measuring carbon dioxide produced during microbial respiration, and, indirectly, determines the biodegradation of organic contaminants. Values shown in Figure 5 indicate that during all experiments (15 days) *P. chrysosporium* was degrading RB5. As a diazo dye, RB5 has the presence of a nitrogen double bond in the chromophore group, and respiration values combined with visual discoloration observed seem to indicate that microorganism was degrading RB5, as a carbon and/or nitrogen source.

Enayatizamir *et al.* (2011) investigated RB5 degradation by *P. chrysosporium* immobilized on cubes of nylon sponge. The SS cultivation led to the best results with a discoloration percentage of 90.3% in 72 h for an initial RB5 concentration of 100 mg L^{-1}. Their results showed that the discoloration ability of *P. chrysosporium* was greatly influenced by the support used, being the ability of *P. chrysosporium* to degrade RB5 mainly due to the secretion of the extracellular enzyme MnP.

4. CONCLUSION

It can be observed that the basidiomycete *P. chrysosporium* was the most effective for RB5 dye discoloration, besides having been the fungus studied with better radial growth speed, reaching the average value of 9.712 mm day^{-1} in Petri dish. The adsorption test has proved that polyurethane foam has great capacity in the retention of the RB5 dye, with efficiency in adsorption of 92.4%, equivalent to 46.2 mg of dye of a solution with initial concentration at 50 mg L^{-1} and pH 6.0. It was possible to ascertain that the maximum concentration condition (50 mg L^{-1}) of the dye solution RB5 and minimum pH (pH 6.0) was favorable for the development of this research, since the optimal pH value for maximum adsorption also favored the development of the *P. chrysosporium.*

With the use of the respirometric technique, it was possible to observe the increase in the amount of CO_2 produced during the inoculation, reaching the accumulated value on the last day of monitoring of 2.16 mg of CO_2. It is concluded that the *P. chrysosporium* can efficiently degrade the RB5 dye adsorbed in polyurethane foam, using it as the only source of nutrients.

5. ACKNOWLEDGMENTS

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Evaluation of ecotoxicity of contaminated water for validation of phytoremediation time

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ABSTRACT

Phytoremediation has been used as an alternative for removal of heavy metals in aquatic environments, but plant residence time and toxicity reduction need to be studied. Ecotoxicological bioassays and root anatomic studies were conducted in order to validate the phytoremediation of *Echinochloa crus-galli* L. at three different ages. The experiment was conducted using *E. crus-galli* seeds with processing factorial experimental design (2x3) and four replicates. Cadmium presence and absence (0.8 and 0 mg L^{-1}) at three times (20, 30, and 45 days after germination). Cd levels were quantified by flame atomic absorption spectroscopy on both aerial parts and roots. A bioassay was performed testing both acute and chronic effects using microcrustacean *Daphnia similis* with the purpose of evaluating phytoremediation efficiency. Regardless of biomass, *E. crus-galli* L. can be used for 16 to 19 days for 45 days after germination (DAG), tolerating the phytotoxicity of this metal. The residual solution after phytoremediation had chronic effect on *D. similis*, indicating that the time taken was not sufficient to reduce the toxicity of the solution. Thus, ecotoxicological essays are important tools in evaluating the efficiency of this type of process. While *E. crus-galli* L. is a valuable tool in Cd phytoremediation programs, exposure time must be higher than 19 days to reduce concentrations of this metal in the water to conform to the CONAMA 357/2005 e 430/2011 standards.

Keywords: acute and chronic effect, *Echinochloa crusgalli* L. macrophytes, time of exposure.

Avaliação da ecotoxicidade da água contaminada com cádmio para validação do tempo de fitorremediação

RESUMO

A fitorremediação tem sido utilizada como uma alternativa para remoção de metais pesados em ambientes aquáticos porém o tempo de permanência das plantas e redução da toxicidade precisam ser estudados. Neste sentido, bioensaios ecotoxicológicos e estudos da anatomia radicular foram realizados com o objetivo de validar a fitorremediação de *Echinochloa crusgalli* L. em três tempos após período de germinação. O experimento foi conduzido utilizando sementes de *E. crusgalli* L. com esquema fatorial (2x3) e quatro repetições. Presença e ausência de cádmio (0,8 e 0 mg L^{-1}) em três idades (20, 30 e 45 dias após a germinação). Os níveis de Cd foram quantificados por espectroscopia de absorção atômica com chama nas partes aéreas e raízes das plantas. Bioensaios para avaliar os efeitos agudos e crônicos frente ao microcrustáceo *Daphnia similis* foi utilizado com intuito de avaliar a eficiência do processo de fitorremediação. Independentemente da biomassa, *E. crusgalli* L. até 45 dias após germinação (DAG) pode ser usado por 16 a 19 dias para remediação tolerando a fitotoxicidade deste metal. A solução residual após a fitorremediação teve efeito crônico sobre *D. similis* indicando que o tempo utilizando não foi suficiente para reduzir o efeito tóxico da solução. Conclui-se que os ensaios ecotoxicológicos são ferramentas importantes na avaliação da eficiência deste tipo de processo. Assim, *E. crusgalli* L. pode ser usado em programas de fitorremediação de Cd em baixas concentrações, porém o tempo de exposição deverá ser maior que 19 dias visando reduzir as concentrações deste metal na água de forma a atender os padrões CONAMA 357/2005 e 430/2011.

Palavras-chave: *Echinochloa crusgalli* L. macrófitas, efeito crônico e agudo, tempo de exposição.

1. INTRODUCTION

The removal of heavy metals from aquatic ecosystems has attracted attention, all around the world because of their pronounced or drastic effects as biosphere contaminants (Ullah *et al.,* 2015). Among heavy metals, cadmium (Cd) is widely found in phosphates and zinc mining. It is also commonly used in rechargeable batteries (Ko *et al.*, 2012) and eventually can reach aquatic bodies. Inhalation by occupational exposure has been historically registered, but the carcinogenic risk of cadmium-contaminated food and water have been ignored by regulatory agencies. The negative impact of Cd comes not only from its toxic effect but also from different accumulative capacities in different aquatic organisms (Ullah *et al.,* 2015). In this context, Qiu and Wang (2016) observed in Daya Bay, a typical subtropical bay in southern China, that higher cadmium concentrations (5.11 $\mu g.g^{-1}$ dw) were found in mollusks (*Veremolpa scabra*) than in shrimp and crab. In addition, according to these authors, higher Cu, Zn and Cd concentrations have been recorded in wild fish than in animals kept in tanks. For example, Ekweozor *et al.* (2017) and Shovon *et al.* (2017) found heavy metals, including Cd in fish tissues above the limits allowed for human consumption according to FAO/WHO, which implies problems for human health. Remediation solutions, such as phytoremediation, need to be evaluated. Fawzy *et al.* (2012) indicate that plants might accumulate Cd in all tissues. In contrast to chemical analyses that identify and quantify the concentrations of toxic substances, the bioassays toxicity test evaluates the effect of substances on the organisms (Costa *et al.,* 2008).

In the exhaustive search for alternatives to clean-up aquatic environments polluted with cadmium, two major requirements stand out, the simplicity of method efficiency and lower costs (Sharma and Pandey, 2014). According to De Souza and Silva (2019), the phytoremediation process is an excellent biotechnological technique in terms of expense and sustainability, since it aims at improvements with minimum impact to nature. Knowledge of the ideal plant age for higher absorption/accumulation in relation to the period of exposure is a key aspect for better implementation and usage of this technique. Gomes (2012) and De Souza and Silva (2019) emphasize that longer periods of exposure to contaminants are a negative point of this process; however, information about exposure time is largely unknown. Like any other remediation process, the use of plants is designed to reduce contaminant levels to levels that are safe and compatible with human health (Andrade *et al.*, 2007). Singha *et al.,* (2019) elucidate the importance of aquatic macrophytes in facilitating phytoremediation for global sustainability and environmental pollution. Aquatic macrophytes have been used in phytoremediation programs with metals in tropical regions. Their use has shown high rates of heavy metal removal of 85% - 95% for iron, copper, zinc, cadmium, arsenic and chrome with plants of tested species such as *Eichhornia crassipes*, *Pistia stratiotes*, *Lemna minor*, *Echinochloa crusgalli,* among other macrophytes (Pereira *et al.*, 2011; Martelo and Lara Borrero, 2012; Silva *et al.*, 2013). Peng *et al.* (2017) cite that *E. crusgalli* has potential for phytoremediation of Cd in soils, but there is no data for water or how much time is required to perform the phytoremediation process.

This work evaluated the cadmium removal capacity of *Echinochloa crusgalli* L. at three different ages (20, 30 and 45 days) to validate the duration of the phytoremediation by chemical analysis and ecotoxicological essays with both acute and chronic exposure by *Daphnia similis* as test-organism. Anatomical modifications will also be evaluated in the presence of the metal.

2. MATERIAL AND METHODS

The experiments were carried out in a greenhouse and *E. crus-galli L. Beauv.* seeds were used. Plants at 20, 35 and 45 days after germination (DAG) were transferred to 2-L trays with modified Hoagland and Arnon 1950 nutritive solution (ionic force 40%) supplemented with 0.8 mg L^{-1} of Cd or, in the negative control, without Cd and incubated for 10 days. During this period, plants were sorted by size and shape to keep the population uniform as the test organism. Figure 1 shows a general design of the methodology used.

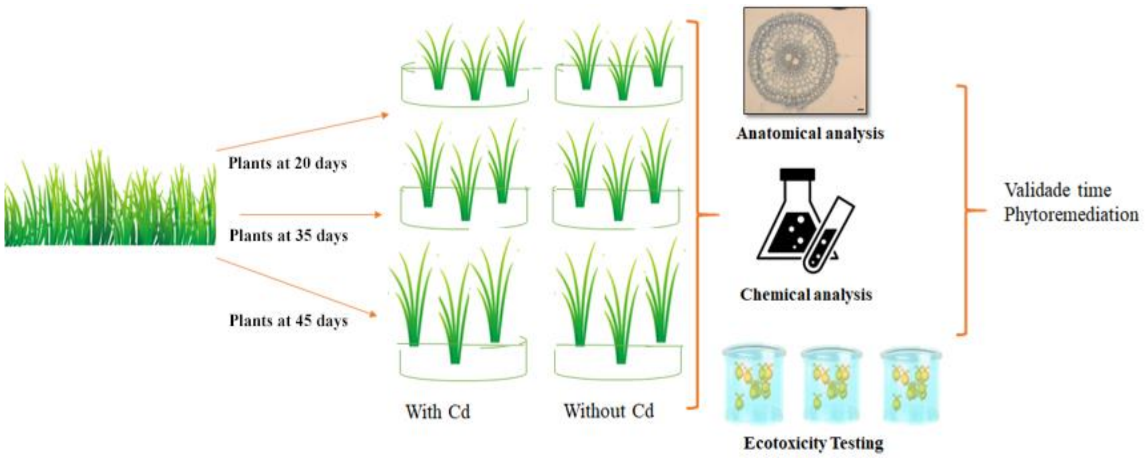

Figure 1. Design of the methodology using *E. crus-galli L. Beauv.*

The cadmium concentration used here (0.8 mg L^{-1}) was four times higher than the maximum allowed according to Brazilian legislation CONAMA No. 430/2011 (Conama, 2011) for discharges in water bodies and nearly the maximum tolerated by *E. crus-galli L. Beauv* (unpublished data).

The experimental design was completely randomized (CRD) with 2x3 factorial (2 concentrations x 3 Days After Germination – DAG) and four replicates. Each replicate contained 15 plants. Initially, the normality of data was tested using Shapiro Wilk normality test ($p > 0.05$). In cases of non-normal data, the (x + 1)0.5 transformation were applied. After this, variance analyses were performed using the F test ($p < 0.05$) and the Scott-Knott test was implemented in SISVAR software (Ferreira 2011).

To evaluate the capacity of *Echinochloa crusgalli* L. in removing cadmium at three different DAG, after 10 days of exposure the plants were removed and root samples were selected for anatomy analyses. Their roots were collected and fixed in FAA (formaldehyde, acetic acid and 70% alcohol) and later conserved in alcohol 70° GL. They were cross-sectioned with manual microtome. The sections were stained with a 0.5% toluidine blue mixture, following methods described by Kraus and Arduin (1997). The sections were placed on slides with glycerinated gelatin and covered with coverslips. Three sections of blade were analyzed, one blade was mounted for each experimental replicate, and three measurements per section. The sessions were photomicrographed using an Olympus U-TV1X-2 digital camera coupled to the Olympus CX41 microscope and assisted by Carl Zeiss AxioVision Viewer 4.8 software.

Measurements were performed without software for image analysis.

For each root section, an endoderm index, a count and a sequence of metaxylem calculations, and the Carlquist-IVC Vulnerability Index calculation (Carlquist, 1975) were measured according to Equation 1:

$$\text{IVC} = \text{Tracheal Element Diameter } (\mu\text{M}) \;/\; \text{Number of Tracheal Elements} \quad (1)$$

The remainder, aerial parts and root system were separated for oven drying at 60°C for 72 hours. For Cd quantification (in μg g^{-1}), about 0.5g of dry material was digested in 10 mL of HNO_3, filtered in a rapid-flow quantitative filter and washed with 10 mL of MILLI Q water, according to USEPA (1998). The Cd accumulation and concentration in roots and in aerial parts were evaluated according to Equations 2 and 3:

$$\text{Aerial part} = \text{Cd content in aerial part x dry} - \text{matter weight} \quad (2)$$

$$\text{Roots} = \text{Cd content in roots x dry} - \text{matter weight} \quad (3)$$

Quantification of Cd in plants and in the aqueous solutions after phytoremediation in the three phases of development and in the control were performed by flame atomic absorption spectrometry (Agilent Technologies 200 Series).

The Phytoremediation Index (PI) can be understood as the plant's ability to remove a certain amount of toxic element over time. This index is dependent on the relative growth rate (TCR), which represents the dry masses in grams (g), the time in days and biomass accumulation of Cd in milligrams (mg) divided by the amount of dry mass of the plant in grams (g) for each age and proportional to the exposure time, according to Equation 4 (Pereira, 2010):

$$\text{PI} = (\text{TCR x metal concentration}) \quad (4)$$

To determine how many days plants should be kept in the system so that phytoremediation could reach the desired levels, Equation 5 was used - Phytoremediation in Days (PD).

$$\text{PD} = (\text{Concentration} \;/\; \text{PI}) \;/\; \text{Plant's number} \quad (5)$$

An acute and chronic bioassay was performed with the microcrustacean *Daphnia similis* Claus (Cladocera, Crustacea) according to Brazilian law Nbr 12713/2016 and Nbr 13.373/2017 (ABNT 2016; 2017). The test consisted of the exposure of 10 females of *D. similis* to the nutrient solution with and without Cd after phytoremediation with *E. crus-galli L*. within the 3 stages of growth: 20, 35 and 45 days after germination (DAG). As control 1, water was used to culture these organisms. The dead / living organisms were counted after 24 h to 120 h of exposure in the acute bioassay and for 10 days in the chronic bioassay. The following variables were quantified: Temperature (T °C), Conductivity (μS.cm^{-1}), Dissolved Oxygen (mg L^{-1}) and pH at the beginning and end of tests.

3. RESULTS AND DISCUSSION

3.1. Anatomical analysis

Cd and other heavy metals removal through phytoremediation emerges as a sustainable technology for contaminated wastewater (Mahajan and Kausha, 2018). In this experiment, no association was observed between the ages of *E. crus-galli* and Cd concentration in different anatomical parameters. These variables behaved independently the presence of Cd, as in Table 1:

Table 1. Percentage of aerenchyma in cortex, Carlquist vulnerability index (CVI) and endoderm thickness (µm) of *Echinochloa crusgalli* roots in relation to DAG.

Time DAG	Aerenchyma (%)	CVI	Endoderm thickness (µm)
20	24.8 a	14.1 a	10.7 b
35	14.4 b	9.6 a	12.2 a
45	8.4 b	17.6 a	12.2 a
CV (%)	48	51	7.35

* Averages followed by the same letter do not differ statistically from each other (Skott-knott, 5%).

E. crus-galli is considered a weed to several crops from flooded and/or irrigated areas, particularly rice crops. As a strategy for anaerobic conditions, rice developed aerenchyma, which allows the transport of O_2 from the air to the roots. Aerenchyma formation can be constitutive or induced by an environmental factor (Castro *et al.*, 2009). In the present study, there was higher aerenchyma formation in roots of plants at 20 DAG, which might be associated with a strategy for establishment in a flooded environment (as the trays used in our assays). The same occurred at a lower intensity in older plants (Table 1). Starting at 35 DAG, there was a lower number of aerenchyma, which may favor the adaptation of these plants to environments contaminated with heavy metals, since with more cells, more Cd probably could fixate within their walls (Tian *et al.*, 2011; Küpper and Andresen, 2016). The result of CVI of *E. crus-galli* plants for all ages analyzed indicate that the transport of assimilates and nutrients were not affected by the presence of Cd in the solution.

Plants cannot escape fluctuations in nutrient availability and / or toxicity in their environment, so they create endodermal barriers. They are modified in response to a multitude of abiotic stresses (Doblas *et al.*, 2017). Endoderm thickening, as observed in Table 1, may have protective roles for the root system because of lignin impregnation within the cell walls. These barriers can be formed when roots are exposed to high concentrations of potentially toxic elements such as Cd (Lux *et al.*, 2011). According to Silva *et al.*, (2013) and Castro *et al.*, (2009), apoplastic barriers can act as biological filters, avoiding the excessive absorption of Cd following the obligatory symplast path, which reduces metal translocation to the aerial part, thus protecting the photosynthetic system from its toxic effects. The plant`s root is the organ in charge of nutrient acquisition, but at the same time it must provide an efficient boundary against external stresses in order to maintain plant fitness.

3.2. Chemical analysis

The results of the present study indicated that the cadmium in plants showed higher concentration and accumulation in roots than in the aerial parts during 10 days of exposure for all plant ages (20, 30 and 45 DAG) (Table 2).

Table 2. Cd concentration and accumulation in root and aerial part of *Echinochloa crus-galli* L. grown in solution with Cd and phytoremediation in days. Phytorem: Phytoremediation.

Time DAG	[Cd] (mg L^{-1})	Weight of root dry mass (g)	[Cd] in root (µg g)	Cd Accumulation in root (µg g)	Weight of aerial part dry mass (g)	[Cd] in aerial part (µg g)	Cd accumulation in aerial part (µg g)	Phytorem. in days (PD)
20	0	0,413 ±0,04 b	3,3 ±0,3 d	0,01 ±0,003 d	0,520 ±0,04 c	2,3 ±1,7 c	0,01 ±0,001 d	0
35	0	0,416 ±0,04 b	6,2 ±0,3 d	0,02 ±0,003 d	0,850 ±0,04 b	3,1 ±1,7 c	0,02 ±0,001 d	0
45	0	0,503 ±0,04 b	6,4 ±0,3 d	0,03 ±0,003 d	0,916 ±0,04 a	5,4 ±1,7 c	0,04 ±0,001 d	0
20	0,8	0,346 ±0,04 b	313,7 ±9,1 a	0,55 ±0,03 c	0,516 ±0,04 c	51,7 ±1,7 a	0,27 ±0,01 c	19 ±1a
35	0,8	0,466 ±0,04 b	270,3 ±9,1 b	0,83 ±0,03 b	0,796 ±0,04 b	38,9 ±1,7 b	0,35 ±0,01 b	16 ±1a
45	0,8	0,896 ±0,04 a	231,7 ±9,1 c	1,36 ±0,03 a	0,906 ±0,04 a	54,0 ±1,7 a	0,40 ±0,01 a	16 ±1a

* Averages followed by the same letter in the column do not differ statistically from each other (Skott-knott, 5%). Transformed data.

Plants at 45 days when grown in cadmium solution presented higher weight of dry root matter compared to plants without metal solution. This result demonstrates the tolerance of these plants at low concentrations of Cd. Ezaki *et al.* (2008) also observed that at concentrations below 0.1mM of Cd and Cu, *Echinochloa crus-galli* showed tolerance, not changing the relative length of the roots. The higher dry-mass weight of the 45-day Cd treatment contributed to the higher accumulation of Cd in the root. The weight of aerial part dry mass increased over time, contributing to the accumulation of Cd in aerial parts over time. This root accumulation can also be explained by endoderm thickness on the 35- to 45-day-old plants which act as apoplastic barriers in the roots protecting the aerial part. Similar results were also observed by De Maria *et al.,* (2013) with sunflowers, concluding that in the initial growth phase, the first most probable response of the plant was metal accumulation (with the production of phytochelatins that immobilize Cd in cells), whereas later, plants reduce and/or block the absorption and translocation of the toxic metal. Lux *et al.,* (2011) confirmed that cadmium-concentrations are often higher in roots than in aerial parts. Regarding time, Gomes *et al.,* (2014) observed that efficiency in reducing mercury (Hg) using young *Typha domingensis* (10 cm of the water column) is dependent on the time of exposure. The authors found that at less than 5 days, 90% of Hg had been removed from the water column. This result can be explained by the biomass difference between *T. domingensis* and *E. crus-galli* macrophytes. It was observed that at all ages of *E. crus-galli*, there was 25% absorption, which indicates inadequate exposure time, since it did not reach the maximum limit allowed for the release of effluent by Brazilian Legislation (0.2 mg L^{-1} of Cd). This fact may be due to the occurrence of saturation of the binding sites of this metal in *E. crus-galli* roots (Table 2). This same result was found by Oliveira *et al.,* (2001) when exposing *Eichhornia crassipes* to solution with different Cd concentrations. The authors observed that above 1 g L^{-1}, saturation of the binding sites of this metal in the roots plants was observed, since the increase in the Cd content in roots was only 7.3%, even with a two-fold increase in the concentration of this element in the nutrient solution. Bindu *et al.,* (2010) evaluated the phytoremediation capacity of *Colocasia esculenta* at different Cd concentrations and observed that at the same concentration over time there was an increase in the Cd removal percentage. Plants were evaluated at 5, 10, 15 and 20 days of exposure, obtaining 30.5%, 34.5%, 40.5% and 41% of removal, respectively, at a Cd concentration of 2 mg L^{-1}. In the present study, since *E. crus-galli L* had smaller biomass compared to *C. esculenta* and no rhizome, the presented removal percentage was around 25% at 10 days of exposure.

The calculation in phytoremediation days was performed because of the lack of this information in the literature. This information is useful for companies that wish to employ phytoremediation as an alternative to remove Cd from their contaminated wastewaters. It was found that values between 16 and 19 days (estimated by Phytoremediation in Days (PD), regardless of plant age, would be sufficient time for remediation of the solution containing Cd at the test concentration (Table 1). *E. crus-galli* L. has potential for Cd phytoremediation at low concentrations of Cd in solution; however, in our experiment, Cd concentrations in the solution remained higher than that allowed by Brazilian law. It is possible to conclude that *E. crus-galli* L with higher biomass, that is, with greater DAG, would be more likely to survive for 16 to 19 days, tolerating the phytotoxicity of this metal (Table 1). According to Fernandes (2014), at Cd concentrations of up to 1 mg L^{-1} in solution, this plant did not present morphological and physiological damage (non-published data). However, in order to achieve this result, it is necessary to increase root biomass, which is responsible for assimilation.

Within the analyzed the ages of *E. crusgalli* (20, 35 or 45 DAG), the exposure time of 10 days was not sufficient to verify differences in the cadmium removal from the solution. For DAG times of 20, 35 and 45, Cd concentration in the solution (mg L^{-1}) was 0.60 ±0.02; 0.57 ±0.02; 0.63 ±0.02, respectively. The control was 0.09 ±0.02 mg L^{-1}.

3.3. Ecotoxicity testing

In relation to the ecotoxicological assays, no acute effect after 48 hours of exposure of *D. similis* was observed in the solution after phytoremediation. However, at this residual concentration (0.6 mg L^{-1} of Cd), exposed for longer time (10 days), chronic effects were observed (Figure 2). Although the addition of the nutrient solution has caused a considerable increase in the conductivity values, this alteration was not enough to provoke an osmotic imbalance in *D. similis*, but the effect observed was due to the presence of residual cadmium.

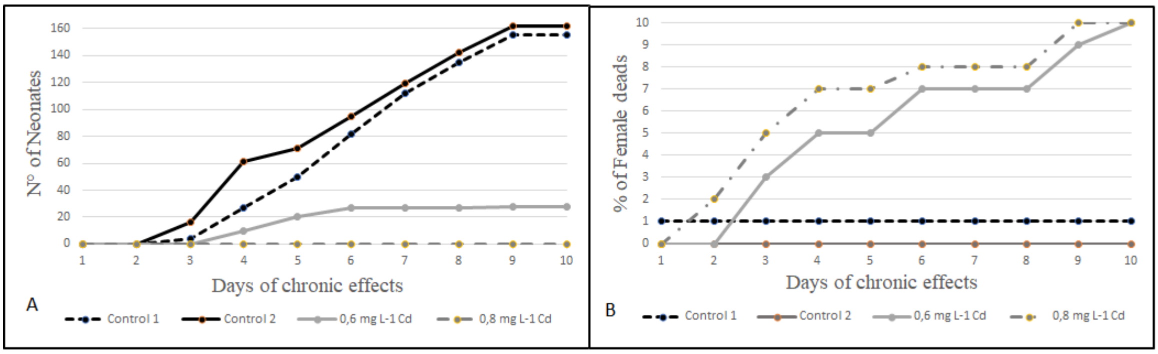

Figure 2. Results of ecotoxicological assays. *Daphnia similis* survival at different exposure times in solution with and without Cd after phytoremediation with *Echinochloa crusgalli* L. for 10 days. Legend: Control 1 - Cultivation water; Control 2 - Hoagland and Arnon nutritive solution (1950) without Cd; [0.6 mg L^{-1} Cd] - Nutritive Solution with Cd after 10 days (Onset); [0.8 mg L^{-1}Cd] - Hoagland and Arnon Nutritive Solution (1950) with Cd (Ending).

For females of *D. similis* exposed for 10 days at 0.6 mg L^{-1} Cd concentration (after phytoremediation), there was an effect on reproduction, with low production of newborns and non-reproduction in those exposed to 0.8 mg L^{-1} Cd (initial concentration) (Figure 2a). These results are corroborated by the low survival rate of females (Figure 2b). Phytoremediation using *E. crus-galli* reduced the toxicity by only 25%. Penha *et al.* (2014) observed similar data when exposing *Girarda* sp. to 0.8 mg L^{-1} $CdSO_4$ for 96 hours, with only 35% survival. The results of ecotoxicological tests corroborate those obtained in the present study, in which this effluent cannot be released into aquatic ecosystem according to Brazilian Resolution 430/2011, since toxicity has not been fully removed, remaining above the legal threshold.

4. CONCLUSIONS

Our data indicate that exposures of *E. crus-galli* for periods longer than 19 days may be required to reduce the concentrations of Cd in water to levels that comply with Brazilian legislation. Under these conditions, ecotoxicological tests were important tools for evaluation of the efficiency of the process. However, this kind of evaluation is not common to phytoremediation studies.

Independent of DAG, *E. crus-galli* reduces around or up to 25% Cd toxicity in the contaminated solution. For this, this species can be used in phytoremediation programs of Cd with other plants.

As a characteristic of aquatic macrophytes, the species used adapts to different environmental conditions, a fact that was observed after root anatomy analysis.

5. ACKNOWLEDGMENTS

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Sustainability analysis of new household connections to the municipal sewage collection network in Paraná

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ABSTRACT

The implementation of sewage collection systems alone does not guarantee public health, since households must be correctly connected to the network in order to derive any benefit from these public works. In order to ensure an environmental and social return on investments in sanitation, a) population must be instructed concerning the role of each citizen, and b) companies responsible for the provision of water and sewage services must be managed in a coordinated and participative manner. This study sought to understand why many households do not respond promptly to the "Connect to the Network" program in the state of Parana and how much the further efforts cost to the sanitation company to regularize this problem. The methodology consisted of analyzing the program's socio-environmental intervention strategies, interviews and service reports for 17 projects (13,286 household connections). The results: no projects achieved 100% of household connections; total connections were less than 80% in 40% of the projects; the additional cost of re-implementing social and environmental initiatives was R$ 680,000.00. We recommend that user representatives be allowed to participate in the network-deployment process, that social awareness and mobilization strategies be expanded and diversified, and that free home connections be completed for low-income families. This study may aid development agencies to arrive at a standardized methodology for the objective evaluation of socio-environmental initiatives that are to be carried out within the community; it may also aid in the fulfillment of Sustainable Development Goals (SDG), particularly Goal 6 (sanitation services for the entire population).

Keywords: sanitary sewer, socio-environmental intervention, wastewater collection.

Análise da sustentabilidade de novas ligações domiciliares à rede municipal coletora de esgotos no Paraná

RESUMO

A implantação de redes coletoras de esgoto, por si só, não é garantia de saúde pública, pois os benefícios decorrentes dessas obras dependem também da correta ligação dos domicílios à rede. Esse processo demanda a conscientização da população, sobre o papel de cada cidadão, e a gestão coordenada e participativa das empresas responsáveis pela prestação de serviços de água e esgoto do município, para garantir o retorno ambiental e social dos investimentos em saneamento. O objetivo deste estudo foi compreender porque muitas famílias não respondem prontamente ao programa “Se ligue na Rede” no estado do Paraná e quanto custam os esforços para a companhia de saneamento regularizar esse problema. A metodologia consistiu na análise das estratégias de intervenção socioambiental do programa, de entrevistas e relatórios de serviços em 17 empreendimentos (13.286 ligações domiciliares). Como resultado: nenhum dos empreendimentos alcançou 100% de moradias regularizadas; em 40% dos empreendimentos as ligações foram inferiores a 80%; o custo adicional para recontratações de ações socioambientais foi R$ 680.000,00. Como conclusão, recomenda-se: garantir a participação de representantes dos usuários no processo de implantação de redes; promover a ampliação e diversificação das estratégias de sensibilização e mobilização social; oferecer serviço gratuito de ligação domiciliar à rede para famílias de baixa renda. Este estudo pode auxiliar agências de fomento na consolidação de uma metodologia padronizada de avaliação objetiva das ações socioambientais praticadas junto à comunidade, assim como o cumprimento dos ODS, em especial o Objetivo 6 (saneamento para toda população).

Palavras-chave: coleta de esgoto sanitário, intervenção socioambiental, ligação predial.

1. INTRODUCTION

The process of urbanization that began in Brazil in the 1950s has contributed to the degradation of the quality of its urban rivers (Gorski, 2010). One of the main contributing factors associated with this process is the lack of adequate sanitary sewage services (Liliamtis and Mancuso, 2003). In 2015, the United Nations established a blueprint for the purpose of transforming the world, expressed in the form of 17 Sustainable Development Goals (SDG). Goal no. 6, the subject of this article, is to ensure the availability and sustainable management of water and wastewater by 2030. This involves providing access to adequate and equitable sanitation and hygiene for all (UN, 2015). In the *Safer water, better health* report (WHO, 2009), the World Health Organization (WHO) presents cases of illnesses caused by the absence or inefficiency of a basic sanitation infrastructure, illnesses that could be prevented through the distribution of treated water in conjunction with the collection, transport and treatment of domestic sewage (Pruss-Ustun *et al.*, 2008). Thus, the issues of health and sanitation are directly related, since investments in sanitation reduce the number of deaths and illnesses due to exposure to contaminated water (Souza and Freitas, 2010; Souza *et al.*, 2014).

Moreover, houses that are connected to a sanitary sewer network but improperly discharge their waste into storm drains also contribute to the contamination of water bodies, the generation of foul odors and the persistence of public-health problems (Liliamtis and Mancuso, 2003; Shamsollahi *et al.*, 2019). Thus, the implementation of a sewage collection system alone does not guarantee public health, since households must be correctly connected to the network in order to derive any benefit from these public works. In order to ensure an environmental- and social return on investments in sanitation, the population must be instructed concerning the role of each citizen, and companies responsible for the provision of water and sewage services must be managed in a coordinated and participative manner. Therefore, mobilization initiatives and social participation are essential elements of this process (Lins *et al.*, 2003; Toro and Werneck, 2007). According to Sheely (2015), mobilization is based on the participatory inclusion of the population of a community as the center of action. Both mobilization and participation are seen as means by which certain economic or social demands of the population may be met (Boland and Zhu, 2012; Broeder *et al.*, 2017), gaining necessary support for environmental policies. Therefore, a continuous and structured educational process can promote individual and collective awareness in the community of its role in building and maintaining environmental quality through the correct use of sanitation infrastructures (Dias, 2011; Santos, 2011).

The State Ministry of Cities in Ordinance 21/14 establishes guidelines for the execution of socio-educational initiatives involving sewage works; these initiatives make use of environmental education concepts and tools to stimulate social participation and improve the living conditions of the population, the effectiveness of social rights and intervention sustainability (the term used to refer collectively to public engineering projects paired with socio-environmental work). These guidelines for social and environmental work are further regulated by Law 11455/07 and Normative Instruction 39/12, which focus on social and environmental sustainability, factors which in turn must be compatible with the basic sanitation plan for river basin planning and management (Brasil, 2007a; 2012, 2014).

In view of these legal provisions, the Brazilian Federal Bank (Caixa Econômica Federal - CEF), one of the main sanitation agencies in the country, through the "Sanitation for All" Program, has established that interventions in new land connections must include socio-environmental community initiatives. However, these guidelines provide no details regarding which are the most appropriate practices, leaving to the sanitation companies the responsibility to adopt the measures considered the most appropriate in view of the specific characteristics of each location where an intervention is to be performed, as well as its environment. In addition, the CEF determines an adhesion target of at least 80% of households correctly connected to the sewage collection system for the project to be considered complete (CEF, 2015).

In response to Normative Instructions 46/07 and 39/12 and Ordinance 21/14 of the federal government (Brasil, 2007b; 2012; 2014), the water and sewage concessionaire of Paraná in 2008 created its own methodology for social and environmental work called the "Connect to the Network" program, in order to establish guidelines for social and environmental intervention in sewage works. This program establishes guidelines for work involving the Technical Project on Socio-Environmental (PTTS), whose objective is to guide the population regarding the theory and practice of environmental sanitation, by means of a socio-educational process that makes use of mobilization and community participation in order to enable social control of new works involving the implementation of a sewage collection network (SANEPAR, 2015). The institutional strategies under the “Connect to the Network” program will be presented in detail in the “Results” section.

It is imperative that the environmental and social return of public investments in sanitary sewage infrastructure be ensured, and social mobilization is an important tool for strengthening shared management through community participation in decisions concerning initiatives that affect its quality of life (Eugenio *et al.*, 2015). Sensitizing a community involves making individuals aware of new possibilities that can impact their lives and the space they occupy, since such sensitization presupposes a work that affects the emotions and foments satisfaction in the care and preservation of the place where one lives (Pereira *et al.*, 2005). In order to sensitize a community regarding basic sanitation issues, educational campaigns must be carried out so that the population understands the importance of individual participation in tasks such as the proper disposal of garbage and human waste (Toro and Werneck, 2007). In addition, an environmental technical survey must be carried out among the households in order to ensure that the residences have been properly connected to the collection network, that is, that the waste generated in the homes is being properly discharged into the sewage collection network, while rainwater is being channeled into the storm drainage network (Bracht, 2007).

In the state of Paraná, whose cities were the object of this study, State Law 13.331/2001 (Article 194) establishes that "all households are required to establish a connection to the sewage collection network as soon as such an opportunity arises" (Paraná, 2001, p.76). In practice, however, this does not occur, and many municipalities in the state of Paraná (such as Castro) show connection rates of approximately 50%, whereas others (such as Cianorte) achieve a connection rate of 90%, according to information provided by the Paraná sanitation company. Moreover, among the 15 municipalities analyzed in this study, only 2 have universalized sewage collection services (100% connection rate), 4 have connection rates inferior to 50%, and 4 others have connection rates that vary between 50% and 70% (Brasil, 2019a).

The aim of this study was to understand why many households do not respond promptly to the “Connect to the Network” program in the state of Parana and how much the further efforts cost the sanitation company to regularize this problem. To achieve this goal, the authors analyzed 15 municipalities in the state of Paraná, which encompass 17 sewage collection projects that have been carried out since the "Connect to the Network" methodology was created. This study may aid development agencies to arrive at a standardized methodology for the objective evaluation of socio-environmental initiatives carried out by sanitation companies within the community.

2. METHOD

The research conducted for this work is characterized as a descriptive, quantitative and qualitative study, based on documental analysis using data extracted from a questionnaire applied to the target population. It is also a case study that allows one to understand and explain the relationship and impact of the institutional strategies on the actions of the users, as well as to identify costs involved in raising the awareness of users who did not promptly respond to the program (Yin, 2010). The study was designed to include all municipalities in the state of Paraná (Brazil) that have concluded work on a sewage collection system since the “Connect to the Network” program was created (between 2009 and 2014) and that have household connection rates inferior to 100% (a total of 13,286 connections). The scope of the study included 15 municipalities: Almirante Tamandaré, Cascavel, Campina Grande do Sul, Campo Mourão, Castro, Cianorte, Colombo, Foz do Iguaçu, Paranavaí, Pinhais, Piraí do Sul, Pitanga, Quatro Barras, Teixeira Soares, and Ubiratã. This study was divided into three steps in order to reach the proposed goal: 1) analysis of the socio-environmental actions under the “Connect to the Network” program; 2) analysis of the socio-environmental items in the questionnaire applied to the target population (see annex); 3) calculation of the additional cost generated by further efforts necessary to raise awareness among users who did not respond promptly to the “Connect to the Network” program. Each step is described in detail below.

2.1. Socio-environmental strategies under the “Connect to the Network” program

The first step consisted of evaluating the socio-environmental intervention strategies established by the "Connect to the Network" program toward the community that will benefit from the sewage collection and treatment works. For this analysis, the following documents were consulted: the institutional “Reference Term” for contracting socio-environmental actions (SANEPAR, 2015); the financing contracts for the implementation of the sewage systems in the 15 selected municipalities; the “Technical Projects of Socio-Environmental Works”, which describes the planning of initiatives carried out within the communities and includes their respective opinions and follow-up reports; the guidelines of the “Manual for the Promotion of the Federal Savings Bank” (Sanitation for All Program) (CEF, 2015); the requirements regarding the composition of the technical team assigned to carry out the socio-environmental initiatives, as defined in Administrative Rule no. 21/14 (Brasil, 2014).

2.2. Socio-environmental analysis of the target population

The objective of the second stage of this study was the identification of the socio-environmental elements that influence the effectiveness of the program at each of the intervention sites, that is, for each new project involving the implementation of a sewage collection network. For this purpose, the socio-environmental data of the target population were collected from a structured questionnaire (annex) applied during the implementation of the sewage collection system in all 15 municipalities studied (13,286 households). The information collected was as follows: 1) socio-economic profile (average family income and any social program in which the family is enrolled); 2) general knowledge of the local residents about the sewage system (regarding the importance of the collection and treatment of sewage); 3) the cost of installing a connection to the sewage collection system; 4) the proper way in which the connection of the home network to the sewage system must be carried out; 5) the procedure used by the water and sewage company when inspecting the property to verify the existence of a proper connection to the network; 6) the existence of Law 13.331/2001, Art. 179/194, which requires all properties to have a connection to the sewage system, provided such a network is available; and 7) the procedures in place regarding the properties that have not been connected to the system after the surveys, including potential notifications sent to the resident and sanctions imposed by the sanitary surveillance of the municipality or local green police should the resident fail to connect his residence to the sewage collection system).

2.3. Additional Costs

In order to determine additional costs incurred by the sanitation company due to potential reactivation of social and environmental initiatives in order to raise awareness among users who did not respond promptly to the "Connect to the Network" program, the costs of technical surveys and programs for raising environmental awareness were used. From this survey, the additional financial cost was calculated per project for the period studied.

3. RESULTS AND DISCUSSION

Table 1 presents the data for the 17 projects in the 15 municipalities studied regarding the status of the 13,286 household connections (projects) carried out.

Table 1. Status of the projects analyzed.

MUNICIPALITY	NUMBER OF HOUSEHOLD SEWAGE CONNECTIONS		
	AVAILABLE	REGULARIZED	
Almirante Tamandaré	200	131	65.5%
Cascavel	2,671	2,276	85.2%
Campina Grande do Sul	253	156	61.7%
Campo Mourão	915	785	85.8%
*Castro (I)	683	377	55.2%
*Castro (II)	289	175	60.5%
Cianorte	610	553	90.7%
Colombo	583	480	82.3%
Foz do Iguacu	2,990	2,623	87.7%
Paranavaí	706	570	80.7%
Pinhais	708	687	97.0%
Pirai do Sul	591	473	80.0%
Pitanga	1,013	889	87.8%
*Quatro Barras (I)	155	82	80.4%
*Quatro Barras (II)	127	95	74.8%
Teixeiras Soares	304	247	74.8%
Ubiratã	488	404	82.8%
TOTAL	13,286	11,003	78.4 (±11.2)%

*Municipality with more than one financing agreement with funding agency.

The results showed a variation between 55.2% and 97.0% regularization of household connections, with none of the projects having achieved 100% household connection to the sewage network. Moreover, 40% of the 17 projects analyzed did not reach the goal of 80% established by the development agency (Caixa Econômica Federal) for sanitary sewage infrastructure. The overall average of regularized connections was 78.4%.

3.1. Analysis of the socio-environmental intervention strategies

The institutional actions under the "Connect to the Network" program are summarized in Table 2.

Table 2. Strategies under the "Connect to the Network" program.

TYPE OF INITIATIVE	DESCRIPTION
Community meeting	An initiative carried out among the residents of the project site in order to initiate a dialogue with the population, including a presentation of the engineering project and social technical work, a description of its benefits for the population and the environment and an explanation regarding the role of the citizens in this process.
Socio-environmental agents	The training of community leaders, health surveillance inspectors, community health agents, educators, etc., in order to train information and knowledge multipliers for work with the population.
Environmental awareness	A visit made to each residence involved in the project after the community meeting. In this phase, a socio-environmental agent visits the dwellings in order to underscore the need for sustainable environmental behaviors on the part of the population and to highlight the role of the user regarding the deployment of the sewage collection network, as well as to conduct a survey of the residents.
Accession to the project	A visit to the houses among the population to authorize the household connection to the sewage collection network, accompanied by the delivery of an instructional kit containing information explaining how to carry out this procedure.
Environmental technical survey	An evaluation of the conditions of home connections to the sewage network, conducted by applying dyes in each compartment (toilets, sinks, drains, rainwater). The residence is considered to be properly connected to the system when its effluents are discharged into the collection network, rainwater is directed to the storm drains, septic tanks and cesspits are deactivated, and the house has been equipped with a grease trap that conforms to the standards adopted by the local prefecture and meets the requirements set forth in ABNT 8160/1999.
Technical orientation for the user	Follow-up socio-educational work conducted by the socio-environmental agent in homes that did not regularize their situation. In this phase, the agent reiterates the importance of commitment to the population.
Course	Training for plumbers and stonemasons residing in the area of the project to work with the community in servicing home connections.
Socio-environmental survey	The application of a structured survey developed by a team of social workers, sociologists, statisticians and socio-environmental managers for the purpose of evaluating the community's understanding of the actions taken.
Educational kit	Distribution of didactic material to the population regarding important issues related to environmental sanitation (water, sewage, garbage, urban drainage and health).

Although the company took an active stance in the elaboration of strategies for a socio-environmental approach toward the population serviced by the new sewage collection networks, no effective participation of the population in the planning process for the implantation of the collection network was observed in the analyzed projects (Struecker *et al.*, 2017). This fact may have contributed to the lack of 100% regularization of home connections to the collection network seen in Table 2. The purpose of community involvement in the planning of a new project is to guarantee the effectiveness of the initiative for connecting households to the collection network. In this regard, social and environmental work is expected to achieve remarkable results, provided there exists an active (not passive) understanding and participation of the community in the process (Struecker *et al.*, 2017). This is in line with the recommendations of the Environmental Education and Social Mobilization in Sanitation Program of the State Ministry of Cities, which addresses socio-environmental intervention in sanitation conducted before, during and after the works have been completed (Brasil, 2009). In addition, Ordinance no. 21/14 of the Ministry of Cities reinforces the importance of joint formulation of social solutions, with an emphasis on participatory activities such as community meetings, workshops, etc., in order to promote the participation of the population in the planning and execution of social and environmental initiatives (Brasil, 2014).

According to the methodology used by the "Connect to the Network" program, one of the first actions taken during the intervention should be the formation of a management group during the period prior to the beginning of the project and the community meetings. This group is to be formed soon after the signing of the service order. However, it was observed that after 2011 the formation of management groups was no longer carried out, despite the fact that there is no record of a thoughtful analysis on the part of the process managers concerning the impact of removing this step from the scope of the social and environmental works. This should have been a key consideration since the role of this group is based on community participation during the execution of the projects, communicating the relevant information to all those involved (Struecker *et al.*, 2017).

It is imperative that training and meetings with management groups be resumed, since these groups leverage social mobilization as a result of the effective participation of their members in the society. Mobilization must be seen as a collective action in which the participation of responsible and conscious agents and the use of strategies and decisive decisions work together to consolidate a particular project (Pacheco, 2011; Hassan *et al.*, 2010; Struecker *et al.*, 2017). The management group acts as a link between the project and the community, providing up-to-date information regarding what is happening during the execution of the project, in addition to promoting public policies and explaining their benefits, especially for the low-income population (Eugenio *et al.*, 2015, Broeder *et al.*, 2017; Hassan *et al.*, 2010).

The first contact of socio-environmental agents with the community occurs in the community meeting. At this stage, a dialogue is initiated in order to inform the users regarding the benefits of installing a sewage collection system and land connections, with the aim of socializing information of common interest, developing the community and clarifying its role in promoting the environmental sustainability and social impact of the enterprise. Krug (1984) and Schmitt *et al.* (1999) point out that a community meeting is one of the means used to raise public awareness and strengthen its link with the environment, health and quality of life after the establishment of the collection network. According to the strategies under the “Connect to the Network” program (Table 2), the community meetings must take place 30 days before implementation of the sewage collection system begins. However, the reports analyzed revealed not only the lack of any community meetings in 5 of the municipalities, but also that the number of meetings that were held was lower than the minimum laid out in the "Connect to the Network" methodology (1 for every 250 households) Hassan *et al.* (2010). This fact may have contributed to the non-compliance. In view of these findings, it is suggested that community meetings be held in all new ventures and that the criteria used to define the number of meetings necessary to reach its goal toward the population should be reevaluated. Moreover, community meetings must be held prior to the commencement of the civil works in order to inform residents regarding the benefits of network deployment, as well as regarding the inconvenient impacts that may also result from civil works, such as sidewalk cracking, asphalt removal, etc. (Leff, 2000). It is also important that broader and more effective communication channels (radio stations, local newspapers, social networks) be employed in order to encourage as many families as possible to participate in the meetings (Nehme *et al.*, 2014). Therefore, new techniques such as recreational workshops to reinforce the community awareness process must be implemented in addition to those already known (community meeting), in order to sensitize the community regarding the importance of the participation of the population in the implementation of the sewage collection network, with the aim of making them collaborators in the preservation of the environment, assuming their co-responsibility, so that their children and grandchildren enjoy a quality of life (Paviani and Fontana, 2009). It is also important to create an evaluation process after the meetings in order to compare the planned initiatives with those actually executed and identify any points that may be improved.

The second contact of socio-environmental agents with the community occurs in the home visit for the purpose of raising environmental awareness. In this step, the families are visited individually for a conversation about the implementation of the public work and the procedure for connecting their home to the collection network (Kamaruddin *et al.*, 2016). It is during this meeting that a socio-environmental questionnaire is filled out by the resident. This meeting was in fact carried out in all instances studied; but it was observed that in each case the visits were carried out outside the term stipulated in the Term of Reference. According to the Term of Reference, this step should occur soon after the community meeting, preferably before the beginning of the civil work, since it is at this point that the community will be the most engaged and looking forward to the benefits brought by the public work, thus potentially yielding more promising results (Faya and Paganini, 2013). According to Santos and Ota (2002), the procedure for tabulating questionnaire responses must also be created, enabling this information to be used for defining new socio-environmental intervention policies and identifying which families fall within the social tariff program (and thus are exempt from paying for water and sewage services).

The commercialization of the sewage connection to the user occurs by means of a home visit whose goal is adherence to the project, this visit being conducted by the sanitation company's own staff. Employees involved in this activity should be trained and educated concerning the impact of inappropriate sensitization (Hassan *et al.*, 2010). The environmental technical survey consists of verifying the connection of the household to the sewage system. This test is conducted using dyes (green, yellow, blue and red) applied to the kitchen sink and toilet in the residences in order to determine the destination of the sewage. The methodology stipulates that this service should be carried out as soon as the adhesion is complete; however, the analysis of the reports and questionnaires revealed that for 86.84% of the residences visited, these services occurred months or even years later. A change in methodology in 2012 introduced a new step before the surveys, calling for a home visit for the purpose of technical customer orientation, whose aim was to create a more personalized experience for the community while providing guidance regarding a proper house connection to the sewage collection system. However, such a technical orientation for the client would be wholly unnecessary if the sensitization initiatives were actually being carried out within the deadlines stipulated in the Term of Reference. The authors therefore suggest that an evaluation be conducted to determine whether this service is truly necessary, since the work that should precede it would render it unnecessary (Uzun and Keles, 2012).

In order to mobilize leaders such as school teachers, health workers, community leaders, etc., at the intervention site, the "Connect to the Network" methodology establishes a training course for the socio-environmental agents, having a workload of up to 20 hours and a maximum of 30 students per class. This course aims to train socio-environmental agents to act as multipliers of knowledge concerning the relationship between human health and a change in the attitude of the population toward the environment; however, only one municipality, Foz do Iguaçu, actually held the course (Uzun and Keles, 2012). When planning the training of socio-environmental agents, it is important to encourage the participation of as many people as possible from the community, in order to identify entities that can participate in the socio-environmental education processes regarding sanitation. Another way to make the agent feel that he is part of the process that he will later replicate is to stimulate the use of workshops during the courses (Bernardes, 2007; Uzun and Keles, 2012).). In view of these observations, it is suggested that these courses actually be provided (1 for every 2,500 houses), considering that only 53% of the enterprises studied make provision for such an initiative in their financing agreement.

Regarding training for plumbers and masons in the community, the analysis revealed that 72% of the projects did not anticipate such courses in the financing contract, 1% of the projects actually held the courses, and the remaining 27% of the financing contracts did not actually execute this step, although it was provided for in the contract. Figure 1 presents an overview of the socio-environmental initiatives carried out in the projects analyzed. This chart clearly shows a decline in the number of meetings with both the community and the management group, as well as in the number of courses offered for the training of socio-environmental agents and plumbers. Technical guidance to users and sporadic surveys (Figure 2) were included in 2013 and 2014, respectively, but these actions were not enough to guarantee compliance, since connections did not achieve 100%.

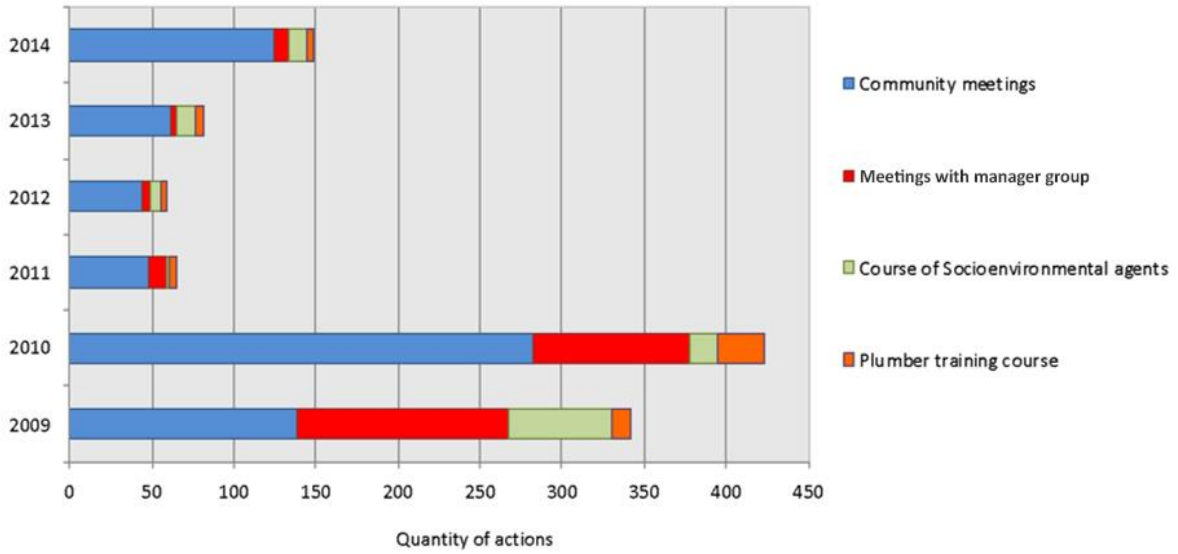

Figure 1. Socio-environmental initiatives carried out in the projects analyzed.

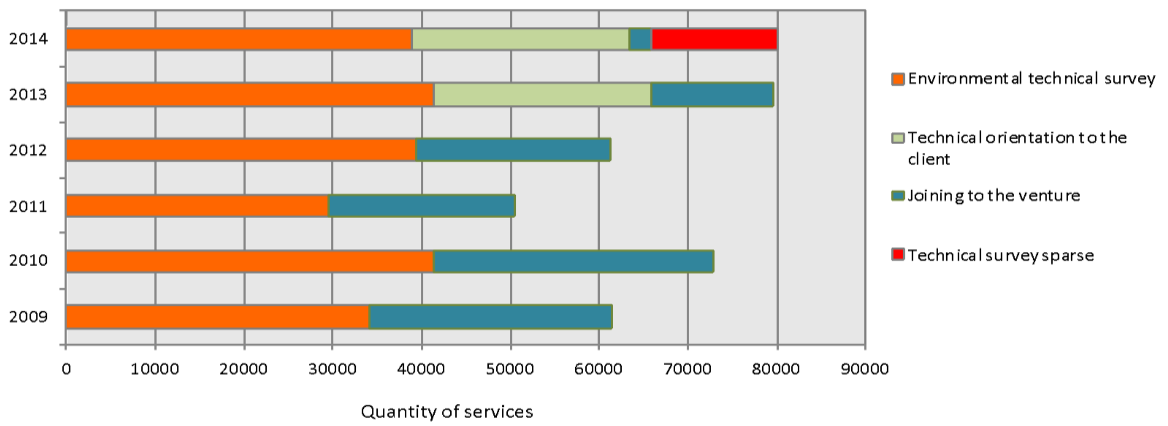

Figure 2. Technical services performed in the projects analyzed.

In order to improve training courses (for plumbers and stonemasons), partnerships should be established with local institutions to absorb skilled labor, especially professionals working in their profession. It is of utmost importance that the course be carried out before the environmental technical surveys. It is therefore suggested that an assertive timetable be created, so that the course can be carried out in the month preceding the surveys, thus allowing the plumber to work with the community in a timely manner to provide the service of connecting the dwelling to the collection system in a manner that is cost-appropriate to the local economic reality (Uzun and Keles, 2012).

The pedagogical support material should seek to present in a didactic manner the details regarding sanitary sewage works, their advantages and the initial impacts they may cause. However, in practice, the didactic material used by the sanitation company in the socio-environmental actions carried out between 2009 and 2014 was summarized in an orientation folder describing the residential connection to the network, distributed at the time of the residence accession to the project and redistributed during the orientation to the customer. It is suggested that the teaching material be revised and reprinted to produce a kit composed of booklets having the following themes: water, sewage, solid waste, health, urban drainage, environmental awareness, adherence to the project and technical surveys (Uzun and Keles, 2012).

The strategies used in the monitoring of the socio-environmental services should be reviewed. From the Term of Reference and Technical Project of Socio-Environmental Work, it is recommended that indicators be created that are organized by type of service and monthly goals. It is imperative that dynamic and interactive methods be implemented in order to evaluate the true extent of the information actually transmitted to the population during the social and environmental services. Promoting educational projects that go beyond formal educational spaces may contribute to the learning and awareness of the population, in addition to strengthening social participation, resulting in a positive impact on the number of connections made to the sewage collection system (Silva and Naval, 2015).

3.2. Analysis of Socio-Environmental Factors

Table 3 presents a summary of the socioeconomic status of the households in the 17 enterprises studied. The results from this analysis revealed that 71.10% of the households interviewed lived on incomes of less than 2 minimum wages; thus, these are families who fit a low-income profile. Moreover, 27.71% of the households received benefits from the Federal Government's *Bolsa Família* Program to guarantee their subsistence, 26.11% were enrolled in the "*Luz Fraterna*" program (Paraná, 2013) and 17.97% participated in the "*Leite das Crianças*" program (Paraná, 2010), the last two being programs provided by the government of the state of Paraná.

The analysis of the questionnaires further revealed that 20.9% of the families interviewed were beneficiaries of the social tariff for water and sewage services (Paraná, 2004) and 28% were registered in the Bolsa Família, government food program. Therefore, family income may be a limiting factor that contributes to the non-regularization of land connections. The granting of an exemption for low-income families and those registered in social programs from fees for connecting the dwelling to the sewage collection network (that is, fully subsidizing the costs involved) is a strategy that could be used to encourage socially vulnerable users to comply with the request to regularize their sewage connections. Table 4 shows the results of the interviews, whose goal was to evaluate the understanding of the network user regarding the importance of sewage collection in his community and regarding his legal obligation to properly connect to the sewage network.

Table 3. Analysis of the socioeconomic status of the families interviewed.

MUNICIPALITY	HOUSEHOLD INCOME		SOCIAL PROGRAM
	*< 0.5 Minimum wage (%)	*1 to 2 minimum wages (%)	Receive household social assistance (%)
Almirante Tamandaré	8.0	0.0	6.5
Campina Grande do Sul	48.7	63.7	26.5
Campo Mourão	2.4	59.1	17.0
Cascavel	0.8	59.0	-
**Castro I	11.2	43.8	2.4
**Castro II	4.8	48.5	14.5
Cianorte	0.2	45.6	4.0
Colombo	10.5	0.0	9.9
Foz do Iguaçu	7.5	61.5	40.0
Paranavaí	1,3	62,4	8,8
Pinhais	8,3	0,0	7,3
Piraí do Sul	3,0	61,8	15,8
Pitanga	6,0	87,5	32,5
**Quatro Barras (I)	10,4	27,2	38,5
**Quatro Barras (II)	1,0	51,9	25,0
Teixeira Soares	74,4	70,0	32,5
Ubiratã	5,1	0,0	2,1

*Current Brazilian Minimum Salary: R$ 998.00 (U$ 236.76 in November 25, 2019) (Brasil, 2019b).
**Municipality with more than one financing agreement with funding agency.

Table 4. Results of the questionnaire applied to the target population.

MUNICIPALITY	QUESTIONS ON THE QUESTIONNAIRE					
	1. Collected and treated sewage (%)	2. Cost of sewage connection (%)	3. Proper home connection to the network (%)	4. Method used for inspecting home connection (%)	5. Law requiring connection to the network (%)	6. Penalties for irregular connections (%)
Almirante Tamandaré	80.0	84.0	80.0	81.5	94.0	79.0
Campina Grande Sul	75.6	77.9	77.9	76.7	76.7	76.7
Campo Mourão	76.6	73.5	72.9	69.1	70.7	69.1
Cascavel	4.1	15.7	18.1	17.0	15.7	16.5
*Castro (I)	48.0	19.4	31.6	31.8	47.2	30.2
*Castro (II)	37.0	10.3	20.4	27.0	48.4	33.2
Cianorte	0.0	0.0	0.0	0.0	0.0	0.0
Colombo	64.9	67.2	67.2	64.3	64.3	63.7
Foz do Iguaçu	15.6	10.6	11.8	14.7	14.5	13.5
Paranavaí	63.9	22.0	53.5	68.5	76.1	60.0
Pinhais	81.5	1.5	86.4	82.5	84.5	83.5
Piraí do Sul	60.3	35.7	55.2	60.8	54.5	52.8
Pitanga	13.7	4.8	6.3	14.8	15.1	15.1
*Quatro Barras (I)	14.4	14.2	14.3	14.3	14.2	14.3
*Quatro Barras (II)	14.2	14.3	14.1	14.1	14.4	4.4
Teixeira Soares	10.0	98.8	100.0	98.8	97.6	96.4
Ubiratã	81.4	77.4	45.1	2.7	22.1	20.0

*Municipality with more than one financing agreement with the funding agency.

According to the results from the questionnaires, in 6 of the 17 ventures less than 50% of respondents were knowledgeable about the information asked. This reveals a limitation in the effectiveness of the socio-environmental intervention steps of the "Connect to the Network" program with regard to the transfer of knowledge concerning the importance and the proper manner of home connection to the sewage collection network for the resident population in the place of implantation of the enterprise. However, despite the fact that the interviewees did not exhibit adequate knowledge regarding the sewage collection enterprise, in each of these cases (with the exception of Castro I and II and Quatro Barras II) the percentage of regularized connections was higher than 80%. In the municipality of Cianorte, the interviews were not conducted. The projects showing the best results (above 80% regularized households) were Almirante Tamandaré, Pinhais and Teixeira Soares. However, when the results from the questionnaires (Table 4) were crossed with the results from the analysis of the number of regularized land connections (Table 2), it was observed that only the municipality of Pinhais presented a percentage of sewage connections above 80%. This reveals a discrepancy between the knowledge that the respondents claimed to have regarding the subject and their effective action in response to the requirement of having a proper home connection to the sewage collection network (Kamaruddin *et al.*, 2016).

It is suggested that a field survey be carried out among the users after the completion of the network deployment in order to identify their perception of the services performed and to establish measures for improvement in future projects. However, it is recommended that such a study be carried out by a company other than the one responsible for the social and environmental actions and services, in order to guarantee impartiality in the evaluation of the activities performed.

In addition to the aspects mentioned above, it is important that social workers or sociologists be included on the technical team responsible for the socio-environmental actions of new project for the purpose of improving the quality of the services provided to the sewage collection network users.

Finally, stronger measures should be developed and applied to users who, even after orientation and awareness efforts, do not adhere to the request to regularize their household connection to the sewage system. It is considered necessary to establish a procedure for prosecuting cases of non-compliance at the Public Prosecutor's Office and Sanitary Surveillance, institutions that have the power to fine the non-compliant homes.

3.3. Quantification of Additional Financial Costs

The analysis of the additional costs incurred by the "Connect to the Network" program in order to achieve an index of 100% households properly connected to the sewage collection system, that is, full user membership, revealed an increase in cost due to re-implementation of the awareness and technical environmental connection survey of between 3.8% and 200.4% (depending on the number of initiatives that need to be re-contracted), in relation to the value initially contracted for the 17 projects (a total additional cost of R$ 680,000.00). Although in some cases a single re-contracting was sufficient, others required two or even three repetitions to bring about the regularization presented in Table 2. The following projects exhibited the highest additional costs: Pitanga (200.4%), Almirante Tamandaré (183.2%), Campo Mourão (130.7%), Piraí do Sul (129.2%) and Quatro Barros I (123.6%). However, none of them achieved a regularization of more than 90% (Pitanga: 87.8%), Almirante Tamandaré: 65.5%, Campo Mourão: 85.5%, Piraí do Sul: 80.0%, and Quatro Barras I: 80.4% %). Even with the additional cost, none of the 17 projects analyzed achieved 100% regularization of sewage connections (Dominick and Diulio, 1981).

Ensuring a totality of regularized connections (100%) is a matter of great importance, since it is essential for promoting public health and environmental quality (irregular connections may be associated with the contamination of soil, rivers and groundwater), as already discussed (Shamsollahi *et al.*, 2019). The Federal Brazilian Bank (Caixa Econômica Federal) currently considers 80% to be the minimum goal for regularization of pre-existing connections to sanitary sewage infrastructure (CEF, 2015). Based on the results obtained, failure to meet this goal may be related to the following three main factors: insufficient assimilation of the information transmitted to the population; a lack of understanding of the information passed on by the socio-environmental agents during the sensitization phase; and the socioeconomic conditions of network users.

4. CONCLUSION

In order for the benefits of the sewage system to be perceived by the population, it is imperative that water and sewage service companies promote socio-environmental actions among the community (Mei *et al.*, 2016). The implantation projects for sewage collecting networks must go through a process of participatory planning in conjunction with the population, beginning with the stage of identifying the needs of the local community, through the execution and subsequent deployment stages of the enterprise to the users (UN, 2019). None of the 17 development projects analyzed (13,286 land links) obtained 100% regularized home connections. In 40% of the projects, regularization was lower than the 80% goal established by the development agency, and the additional cost paid by the water and sewage company of Paraná to re-implement social and environmental initiatives toward the population that did not adhere promptly to the requirement of a home connection to the sewage collection network was R$ 680,000.00.

The socio-environmental actions contracted in the projects analyzed were not executed as planned. Moreover, the "Connect to the Network" program does not fit the framework of an effective socio-environmental program, as may be concluded from the predominance of technical services (surveys, guidelines and accession) as compared to educational and informative socio-environmental initiatives (community meetings, meetings with the managing body, courses for socio-environmental agents, plumber training courses). It is understood that this factor, together with the low household income of the users in the analyzed projects, may have contributed to the non-compliance regarding connections to the sewage collection network (Kamaruddin *et al.*, 2016).

It is recommended that the participation of representatives of the users in the process of network deployment be ensured in order to promote the expansion and diversification of the social awareness and mobilization strategies, and that a free home connection to the network be provided for low income families (Kamaruddin *et al.*, 2016). Since no specific guidelines exist to direct the companies that provide sanitary sewage services with regard to the users of the sewage collection systems, this study can help aid development agencies to arrive at a standardized methodology for the objective evaluation of socio-environmental initiatives to be carried out within the community. For future research, it is recommended that the socio-environmental initiatives carried out within the framework of the "Connect to the Network" program be compared with similar initiatives that are planned and executed with sanitation companies in other Brazilian states.

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ABSTRACT

This article presents the first detailed geochemical data of the water from *Camaquã das Lavras* and *Hilário* Streams from Lavras do Sul-RS. Geochemical and statistical analyses were used in this study to establish the anthropogenic or natural influence on the region. The results classified the waters as soft and intermediate with acidic and neutral pH. The electrical conductivity varies from 37.2 to 62.9 µs cm^{-1} and the total alkalinity ranges from 4 to 30 mgL^{-1}. The distribution patterns and ratios indicate that clay mineral weathering is dominant on the investigated samples. It is followed by feldspar weathering in an environment under temporary hardness conditions. Cu, Rh, and Cd enrichment is associated with the many mineral deposits of the region, lithological diversity and human activities. All the measured values conform with the standards set by Brasil-Ordinance nos. 2914/2011, 36-GM/1990 and 1469/2000.

Keywords: geostatistics, hydrochemistry, water resources.

Geoquímica da água dos arroios Camaquã das Lavras e Hilário, Lavras do Sul-RS: Antropogênico ou natural?

RESUMO

Este artigo apresenta os primeiros dados geoquímicos de detalhe da água dos arroios Camaquã das Lavras e Hilário, Lavras do Sul-RS. Neste estudo foram utilizadas análises geoquímicas e geoestatísticas para estabelecer as influências antropogênicas ou naturais na região. Os resultados permitiram classificar as águas como mole e intermediária com pH ácido e neutro. A condutividade elétrica variou de 37.2 a 62.9 µs cm^{-1} e a alcalinidade total entre 4 e 30 mg L^{-1}. Razões e padrões de distribuição indicam que o intemperismo de argilominerais é dominante nas amostras investigadas. Seguido pelo intemperismo do feldspato em ambiente sob condições de dureza temporária. O enriquecimento de Cu, Rh e Cd é associado à depósitos minerais da região, à diversidade litológica e às atividades humanas. Todos os valores medidos atendem aos padrões estabelecidos pelas Portarias-Brasil n^{os} 2914/2011, 36-GM/1990 e 1469/2000.

Palavras-chave: estatística, hidroquímica, recursos hídricos.

1. INTRODUCTION

Concern about possible forms of environmental contamination is a constant theme in global debates. Among the forms of environmental pollution, anthropic pollution stands out. Anthropic pollution has become one of the main aggravating polluters and this reflects the illogical behavior of humans. It generates irreversible effects on the environment and on human health. Thus, one of the points of view that concerns most of the researchers is water contamination. In Brazil, the quality of water is protected by specific environmental legislation, based on principles of the Brazilian Federal Constitution of 1988, which declares that "[...] Everyone has the right to an ecologically balanced environment, good for the common use of the people and essential to the quality of healthy life [...]" (Brasil, 1990, p. 127).

Water's chemical composition is intimately related to its quality standards, allowing the parameters of classification and use, such as human consumption, agriculture, livestock, among others (Steffens *et al.,* 2015). Several factors can influence water composition, such as climate, anthropogenic contamination (progressive urban occupation, increased industrial processes, mining activities and the distribution of urban waste) and lithologies related to each region.

On the other hand, water quality also depends on the geological environment. The chemical elements available in the primary geochemical environment (chemical elements on the mineralogical structure of the minerals that compose the rocks) spread into secondary environments, soil, water, and sediments (Batista, 2003). The decomposition and desegregation of the rocks by weathering processes occur on the continental crust surface. Weathering is the interaction between the distinct terrestrial layers (lithosphere, atmosphere, hydrosphere, and biosphere) changing the rocks to transportable materials.

The weathering process disaggregates and turns the rocks into fragments, modifying their composition, decomposing the most fragile minerals and forming new ones. Weathering may cause chemical or physical transformations, or a combination of both, with or without anthropic influence (Cheremisinoff, 1997).

The contact of water with rocks promotes chemical reactions, carrying dissolved substances along its way. The result of these reactions will be a collection of secondary minerals. According to Cheremisinoff (1997), the main reactions that occur during chemical weathering are hydration, hydrolysis, oxidation, and complexation.

Hydration is the insertion of water into the chemical structure of a mineral, causing its weakness and forming another mineral. Oxidation occurs with all minerals that have elements that can suffer oxidation, such as iron, for example. The complexation is a reaction that involves organic compounds dissolved in water, which poorly retain soluble chemical elements in their structure, mobilizing them. Hydrolysis is the most important reaction of chemical weathering. It destroys the mineral structure and makes cations and anions available in the water. According to Bakalowicz (1994), the most important soluble salts occurring in relatively substantial amounts in the rocks are carbonates and chlorides, and the most relevant ions found in the waters are Ca^{2+}, Mg^{2+}, Na^{+}, Cl^{-}, SO_4^{2-} and HCO_3. Alkaline and alkaline-earth metals are dissolved by water and removed. The less soluble Ca and Mg carbonates are dissolved in the presence of CO_2 in the water.

As water penetrates the soil and the rocks, it leaches components and becomes enriched in mineral salts originated by the dissolution and oxidation of the rocks. The dissolution process is influenced by the pH, temperature and saturation degree of each element (Steffens *et al.,* 2015). The chemical characteristics of water reflect the water circulation zone, showing a close relationship with the percolated rocks types and the products of anthropic activities added in the water along this circulation path.

Therefore, environmental monitoring is a primary need in the context of maintaining a population's quality of life, paying attention to the urbanization growth rate and consequently, environmental degradation. When evaluating the quality of an urban environment, water reveals the integration of biological, physical and chemical processes that occur in an aquatic ecosystem. Thus, the study of these individual or grouped processes may be used to detect the presence of contaminants in the environment.

This work involved the geochemical and statistics analysis of the water samples from *Camaquã das Lavras* and *Hilário* Streams, in the rural area of Lavras do Sul/ RS. This work characterizes the water quality of streams and determines whether the geochemical influence in the water is anthropic or natural. To detect and evaluate metals in the investigated waters in a simple, fast and inexpensive way, the Energy Dispersive X-ray Fluorescence (EDXRF) was used (Wastowski *et al.*, 2010). Principal component analysis was also used to help identify the close relationship between sample/variables (Lawrence and Upchurch, 1982). Multivariate statistical analysis has been successfully applied in numerous hydrochemical studies, in which it has helped to simplify and organize a large amount of data and show the anthropic impact and/or investigate water contamination (Gomes *et al.*, 2018a; 2018b).

1.1. Studied Area

The *Camaquã das Lavras* and *Hilário* Streams are located in the Rio Grande do Sul State of Brazil, in *Lavras do Sul, at* the coordinates 30°48'46 "S and 53°53'42 "W (Figure 1). Access to the area is by highway Br-392 and then by Rs-357, 2.5 km from the secondary access, to the west towards *Caçapava do Sul-Lavras do Sul*. The studied area was strategically chosen due to its susceptibility mainly to urban waste contamination and its proximity to mining areas.

The *Lavras do Sul* District has been known since the nineteenth century for exhibiting great lithological diversity and significant metallogenic importance (Pestana and Formoso, 2003). The region is characterized by the occurrence of base metal mineralization associated with sulfides, gold, copper, lead, zinc and silver (Lopes, 2013). The district concentrates ~ 70% of the mining companies that are active in the area of *Camaquã* watershed, which includes the investigated *Camaquã das Lavras* and *Hilário* Streams (High Camaquã Streams watershed).

Camaquã das Lavras Stream and its tributaries flow near the urban center, being classified as Class 4 (Brasil-Ordinance N^{os}. 206/2016). In the sample collecting points, the transported sediments show a wide variety, including iron hydroxide, aluminum hydroxide, clay, silt and sand. The *Hilário* Stream, is located east of the *Lavras do Sul* urban center and was classified as Class 1 (Brasil-Ordinance N^{os}. 206/2016). The sampled stretch the stream presents the highest flow speed. There is almost no alluvial plain (flood) as the stream extends for approximately the entire valley.

Regarding the composition and formation of the vegetative cover, it is essentially composed of shrubs and small- to medium-sized spaced trees. Hilly grasslands geomorphologically characterize the relief, also demarcated by a soft, slightly steep low slope. Granitic and acidic volcanic rocks characterize the bedrock of the *Lavras do Sul* Intrusive Complex and the *Hilário* Formation. The soil originated from the degradation of these rocks shows certain variations. In the uplifted topography portions, the soil exhibits a sandy-silt texture and orange coloring, marked by iron oxides and hydroxides (hematite and limonite). In the lower topography portions, the ground has more sandy textures and dark brown colors. Higher organic matter content in the soil, as well as the concentration of leached chemical elements of the higher portions, may explain the color change.

The Lavras do Sul Intrusive Complex is a circular body with 216 km2 (Figure 1) formed by Monzodiorite, Monzogranite, Quartz Monzonite and Petite Granite facies (Gastal et al., 2015). This body has intrusive behavior in orthogneiss and pre-and non-tectonic granitoid in W-SW and N-NW portions. S-SE is delimited by the São Rafael Formation, whose contact is controlled by the fault zone with direction NE-SW, named São Domingos, and to the East by the Hilário Formation (Ribeiro et al., 1966). These two formations are overlapped in an angular disagreement, which represents the basal units of the Camaquã Basin (Porcher and Lopes, 2000). The deposit configuration of the area is known to fit into the copper porphyrin system, in which porphyrins igneous rocks subjected to intense hydrothermal processes end up enriched in sulfides.

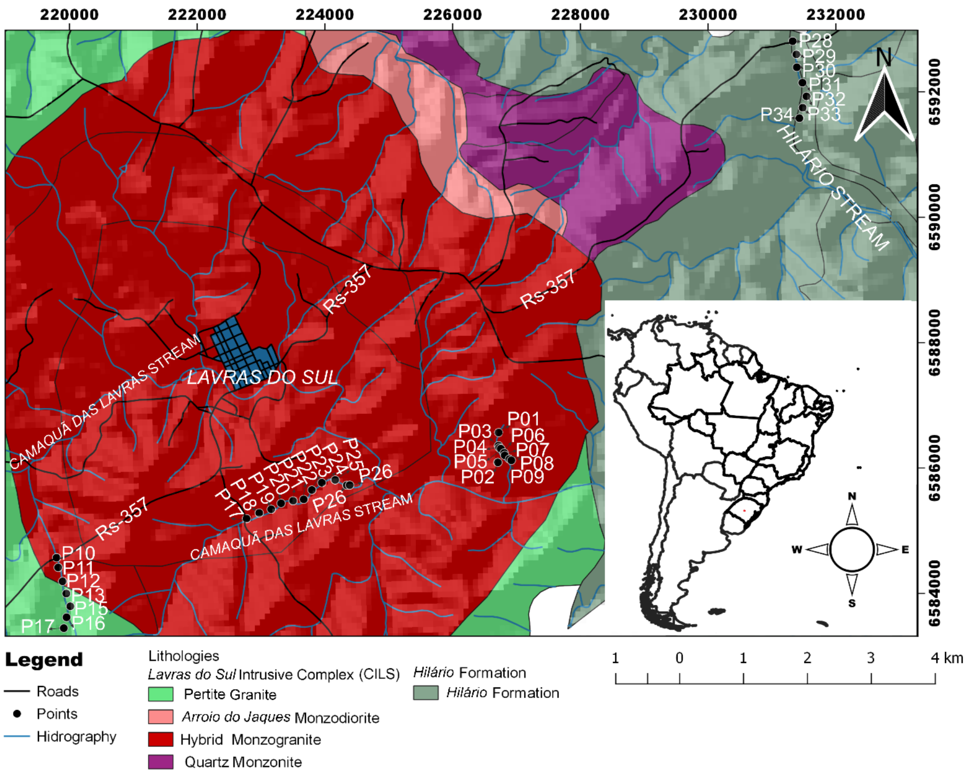

Figure 1. Geological map of the Lavras do Sul region with the location of the points. On the right, the graphical representation of South America, with *Lavras do Sul* highlighted (red point).

The mineralogy of this granitoids is composed of alkali feldspar ($KNaAlSi_3O_8$) with alteration to white mica and iron oxides (hematite), plagioclase (Ca, Na)Al(Al, Si)Si_2O_8) with alteration to kaolinite ($Al_2Si_2O_5$ (OH_4)) and/or gibbsite ($Al(OH)_3$), biotite ($K_2(Mg,Fe^{2+})_{6-4}(Fe^{3+},Al,Ti)_{0-2}Si_{6-5}Al_{2-3}O_{20}(OH,F)_4$ with alteration for reddish and black metallic oxides, quartz (SiO_2) and oxides. The accessory minerals are hornblende ($Ca_2Na(Mg,\ Fe)_4\ (Al,\ Fe,\ Ti)\ AlSi_8AlO_{22}\ (OH,\ O)_2$), pyroxene (Ca, Na)(Mg,Fe,Al,Ti)(Si_2O_6), zircon and apatite. Over the outcrops an occurrence of differential alterations in the rocks is observed. A sign of these changes is the filling of rock fractures and the occurrence of euhedral quartz of 0.5 cm long. Beside this are pervasive iron oxide films (hematite) and generalized alterations to sericite and white mica. This describes the change processes responsible for claylization, chloritization and carbonation in the rocks.

Around the *Lavras do Sul* Intrusive Complex two formations may be observed: *São Rafael* and *Hilário* (Figure 1). The *São Rafael* Formation includes arcosean to subarcoseans sandstones, intercalated with conglomerate sandstones (Porcher and Lopes, 2000). The *Hilário* Formation is composed of effusive facies with porphyritic andesites and volcaniclastic facies and auto breccias composed of porphyritic andesite fragments (de Liz *et al.*, 2009). In the studied area, porphyritic andesites of dark-gray to reddish staining were found, due to the strong alteration. The andesites show a porphyritic texture, characterized by euhedral plagioclase phenocrystals up to 2.0 cm (around 30%), and rare (<5%) augite phenocrystals immersed in an aphanitic matrix composed mainly of plagioclase. In some specimens, opaque minerals are present. The accessory minerals are zircon and apatite. They show a strong alteration in almost all samples, evidenced by the presence of alteration minerals such as chlorite $(Mg,Al,Fe)_{12}(Si,Al)_8O_{20}$, carbonates ($CaCO_3$ – calcite; $MgCO_3$ – dolomite) and white mica (like muscovite – $KAl_2Si_3AlO_{10}(OH,F)_2$). Resorption features such as corrosion are common in most crystals, in addition to change processes responsible for the claylization, chloritization and carbonation at the edges and cores of the minerals. A moderate fracture degree may be observed in the grains that contributes to the alteration and oxidation processes, besides a filling of post-magmatic silica.

2. MATERIALS AND METHODS

A total of 33 samples were collected at the end of April 2018 (Figure 1), twenty-seven along the *Camaquã das Lavras* Stream (samples P1 a P27) and six along the *Hilário* Stream (samples P28 a P33). The sampling area was strategically selected due to the concentration of mining areas and because it reflects the conditions of the environment under anthropic and lithological influence. One half liter of water samples was collected in polyethylene bottles. The bottles were previously decontaminated with nitric acid (10%) for 48 hours and washed with distilled water and taken to an oven to dry (25°C), according to FUNASA (2006). They were then sealed and brought to the Chemistry and Mineralogy/Petrography Laboratories (LMP – UNIPAMPA University). All samples were preserved and analyzed within seven days of the date of collection, according to ABNT (1992) and FUNASA (2006). Immediately after the samples had been collected, the pH was checked by using a pHmeter at the laboratory. The electric conductivity of the samples was measured by a GEHAKA CG1800 apparatus. To determine the hardness of the samples, the EDTA served as titrant and the eriochrome-T as an indicator (APHA *et al.*, 2005; ABNT, 1992). The blank reagent titration was done simultaneously using distilled/deionized water.

Approximately 50 ml of water was isolated from each sample for the elemental analysis by Energy Dispersive X-ray Fluorescence (EDXRF), Model S1 Turbo SD. The chemical elements and quantities in the water were analyzed at the Laboratory of Mineralogy and Petrography (LMP-Unipampa). The following equipment conditions were selected: tube voltage of 15 keV (Na to Sc) and 50 keV (Ti to U), with a current in the tube of 184 and 25 μA, respectively; 10 mm collimator; 120 s of real-time integration. The equipment uses an Ag anode, which allows the measuring of 25 elements, and a detector of 10 mm^2 with thermoelectric cooling and resolution of ~145 eV to MnKα that maintains a speed of 100.000 counts per second (Bona *et al.*, 2007; Teixeira *et al.*, 2017).

In this study, the Principal Component Analysis (PCA) method was used as a multivariate statistical technique, commonly used to investigate the variability in large geochemical data sets (Linhai Jing and Panahi, 2006; Scheib *et al.*, 2011). The analysis of correlations and main components were carried out using Statistical Package Statsoft Version 10. PCA is a variable reduction method that produces a smaller number of artificial variables, known as main components (PCs). Each PC represents a certain amount of variability in the data, and the first two PCs usually show the most variations within the entire data group (Reimann *et al.*, 2008).

3. RESULTS AND DISCUSSION

The hydrochemical characteristics of the studied water samples reflect the reactions involving sulfates, carbons, chlorides, alkali metals, alkaline-earth and CO_2, O_2 and S. The water samples from *Camaquã das Lavras* Stream were separated into different geographical groups: Group 1, composed of samples P10 to P16; Group 2, formed by samples P17 to P27; and Group 3, samples P1 to P9 (Figure 1). The *Hilário* Stream samples were treated as a single and comparative group to the *Camaquã das Lavras* Stream samples. The results of the analyses are presented in Tables 1, 2, 3, 4 and 5.

All pH samples were between 5.47 and 6.72 (Table 1), classified as acidic water or close to neutral. In the *Camaquã das Lavras* Stream, the pH samples varied between 5.71 and 6.72, and in the *Hilário* Stream, the pH varies from 6.12 to 6.35. The pH value is also a relevant result to the composition of the water quality indices, being a potability pattern. Thus, the majority of the measured values of pH for investigated samples are within the standards established by Ordinance No. 206 (Conselho de Recursos Hídricos, 2016). The recommended pH values should be 6.0 and 9.5. Exception samples showed pH <6.0 (Table 1), for example, in Group 3, the samples P1 (5.71) and P2 (5.94), and in Group 1 the samples P10 (5.89), P11 (5.96), P14 (5.94), P15 (5.61) e P16 (5.47). In these cases, the presence of free CO^2, and acids derived from the mineral alterations (e.g. boric acid, hydrochloric, nitric or sulfuric) may be present. They release hydrogen ions to the system and/or organic compounds as a result of synthetic activities of plants and animals (Steffens *et al*., 2015).

The alkalinity variation was between 4 and 30 mlL^{-1} of $CaCO_3$ (Table 1), which is equivalent to water resources of freshwater (FUNASA, 2006). The *Camaquã das Lavras* Stream presented the highest alkalinity values, ranging from 4 to 28 mg L-1. In Group 1, the alkalinity ranged from 22 to 28 mg L-1; in Group 2, the measured values were from 22 to 30 mg L^{-1}; and in Group 3 (samples P1 to P9), the alkalinity varied between 4 to 8 mg L-1. In the *Hilário* Stream, the alkalinity oscillated between 12 to 24 mg L^{-1} (Table 1). The total alkalinity is directly associated with the processes of chemical weathering (partial or total hydrolysis) and the capacity of water or components tamponade of an effluent (Piveli and Kato, 2006). Alkalinity can indicate three causes: 1) hydroxide and bicarbonate alkalinity; 2) alkalinity of hydride, and 3) carbonate alkalinity. The variation of the values obtained in the analyzed waters suggests possibly varying degrees of carbonates or salts of Na, K and Mg dissolved in water by passage through soil or rock substrate.

The electrical conductivity (EC) of the analyzed samples ranged from 48.2 to 66 $\mu S.cm^{-1}$ (Table 1), with this variation being an indirect measure of the pollutant concentration. Levels above 100 $\mu S /cm^3$ indicate environmental impacts and corrosive water characteristics, but do not indicate the relative quantity of the various components (CETESB, 2016). Higher EC values were found in waters of the *Hilário* Stream samples, ranging from 60.4 to 62.9 $\mu S/cm^3$. The lowest values were obtained for the waters of the *Camaquã das Lavras* Stream. In Group 1, the EC values ranged from 37.2 to 53.5 $\mu S/cm^3$; in Group 2 ranged from 48.2 to 66 $\mu S/cm^3$; and in Group 3 is between 41.6 and 50.9 $\mu S/cm^3$. The EC can be classified as Type I if the enrichment of salts is < 1.500 $\mu S/cm^3$; Type II, if the enrichment of salts is between 1.500 and 3.000 $\mu S/cm^3$; and Type III, if salts enrichment is > 1.500 $\mu S/cm^3$ (SubbaRao *et al.*, 2012). Thus, the waters of both studied streams can be classified as Type I, showing an increasing trend along Group 3 < 1 < 2 and < *Hilário* Stream. Coincidentally, the most acidic pH is noticed in the water samples of Group 1, < *Hilário* Stream, < 3 and < 2.

The total hardness results of the samples showed elevated variation, with values between 14.29 and 88.77 mg L^{-1} of $CaCO_3$ (Table 1), allowing the classification of these waters between soft and intermediary (Sawyer *et al.*, 2000). The data presented corroborate the hardness ratings for urban and rural waters of *Rio Grande do Sul* State and Brazil (Steffens *et al.*, 2015; Gomes *et al.*, 2017; 2018a; 2018b), and are within the standards of Ordinance N°.1469 (Anvisa, 2001).

The main origin associated with total hardness in water bodies is the dissolution of rocks with minerals rich in Ca and Na, K and Mg elements due to reactions with CO_2 present in water. This property can be quantified when water is in contact with HCO^{3-}, SO_4, NO_3 and Cl (Pivelli, 2014), being classified as temporary or permanent (Pereira *et al.*, 2010). The temporary hardness is due to the presence of $CaCO_3$ and Mg that, by heat action, break down to CO_2, and precipitate insoluble carbonates. The permanent hardness reflects the presence of SO_4, Cl e Ca $(NO_3)_2$ e Mg $(NO_3)_2$. This hardness also resists the action of soaps, but does not produce encrustations since its salts are highly soluble in water and do not decompose in heat.

Table 1. Results obtained in tests for the total hardness, pH, conductivity, total alkalinity, calcium, magnesium and temperature determinations.

		Samples	Total alkalinity (mg L^{-1})	EC (µS cm^{-1})	Temperature (°C)	Calcium (mg L^{-1})	Total hardness (mg L^{-1} $CaCO_3$)	Classification of hardness	pH	Magnesium (mg L^{-1})
Camaquã das Lavra Streams	Group 3	P1	6	50.9	13.3	40.90	70.41	Soft	5.71	24.45
		P2	4	49	15.1	40.90	76.53	Medium hard	5.94	24.48
		P3	6	48	17.4	40.90	58.16	Soft	6.66	24.37
		P4	6	44.3	16	44.99	42.86	Soft	6.72	24.16
		P5	4	43.5	20.02	44.99	56.12	Soft	6.52	24.31
		P6	6	45.4	19.8	40.90	50.00	Soft	6.45	24.30
		P7	8	45.6	17	49.08	37.75	Soft	6.55	24.00
		P8	4	41.6	19.1	40.90	66.33	Soft	6.61	24.42
		P9	4	41.6	19.9	44.99	60.20	Soft	6.65	24.34
	Group 1	P 10	22	52.7	8.9	61.35	81.63	Medium hard	5.89	24.34
		P 11	28	53.5	10	53.17	86.73	Medium hard	5.96	24.43
		P 12	24	49.7	10.7	65.44	81.63	Medium hard	6.1	24.31
		P 13	28	46.2	10.7	61.35	85.71	Medium hard	6.12	24.36
		P 14	26	43	10.2	61.35	71.43	Soft	5.94	24.27
		P 15	28	40.2	9.3	89.97	82.65	Medium hard	5.61	24.13
		P 16	26	37.2	10.6	77.71	82.65	Medium hard	5.47	24.22
	Group 2	P 17	28	53.8	11	53.17	80.61	Medium hard	6.19	24.40
		P 18	28	48.2	11	73.62	14.29	Soft	6.32	21.62
		P 19	24	52.8	11.5	69.53	27.55	Soft	6.38	23.24
		P 20	30	53.4	11.4	77.71	32.65	Soft	6.42	23.33
		P 21	28	53.2	10.6	65.44	38.78	Soft	6.48	23.76
		P 22	26	53	10.4	69.53	40.82	Soft	6.47	23.75
		P 23	26	53.1	10.1	73.62	51.02	Soft	6.58	23.91
		P 24	28	49.3	10.5	85.89	48.98	Soft	6.56	23.72
		P 25	28	51.5	11.2	89.97	61.22	Soft	6.6	23.90
		P 26	28	49.6	12	3.68	47.96	Soft	6.31	20.06
Hilário Stream		P27	22	66	9.2	2.45	36.73	Soft	6.4	20.67
		P28	20	62.4	7.4	2.86	61.22	Soft	6.12	21.91
		P29	12	61.8	10.1	2.86	61.22	Soft	6.17	21.91
		P30	10	62.9	8.4	2.45	52.04	Soft	6.45	21.89
		P31	24	60.4	10.5	2.86	64.29	Soft	6.35	22.05
		P32	16	62.1	10	2.86	45.92	Soft	6.33	20.95
		P33	14	61.9	8.8	2.45	88.77	Medium hard	6.26	23.10

The Ca quantity oscillated from a minimum of 2.45 mg L^{-1} (sample P27) to a maximum of 89.97 mg L^{-1} (samples P 15 and P 25; Table 1). The Mg presented low-amplitude variation with values between 20.06 and 24.48 mg L^{-1}.

Figure 2 shows the value variation between Ca and Mg in relation to the total hardness measured in the investigated water samples. The variation of the Ca value in relation to the three sampling groups of the *Camaquã das Lavras* and the *Hilário* Streams is clear. The samples are randomly distributed and it is not possible to suggest positive or negative correlations.

The equations in Figure 2 show that the total hardness coefficient in Ca of Groups 1, 2 and 3 from *Camaquã das Lavras* Stream is 0.1129, 0.0391 and 0.1707 mg L^{-1}, respectively. In the *Hilário* Stream, the total hardness coefficient in Ca tends to zero. The most significant correlation was found between the total hardness and the Ca ions of the water samples in Group 3 (r = 52%; Figure 2); however, the correlation obtained between the total hardness and the Mg ions for all water samples was r = 20% (Figure 2). This suggests that the water hardness of Group 3, probably, is a direct influence of the mineralogical composition of granitoids (*Lavras do Sul* Intrusive Complex) and volcanic rocks. The Mg is associated with iron-magnesium minerals of granitoids and volcanic rocks, such as pyroxene and hornblende, and with secondary minerals such as chlorite. The same way, $CaCO_3$ is associated with plagioclase and pyroxene minerals. Therefore, the dissolution of these minerals during the fluid/rock interaction process is responsible for the presence of elements in the water surface.

Among the three sampling groups of the *Camaquã das Lavras* Stream, approximately 86% of the samples from Group 1 were classified as intermediate hardness and only one sample (P14) was classified as soft. In the other two groups, the opposite occurs: 88% of the samples were classified as soft and only two samples show intermediate hardness (P17-Group 2; P2-Group 3). In the *Hilário* Stream, the scenario repeats itself, only one sample (P33) shows intermediate hardness, while the other five samples of this stream were classified as soft.

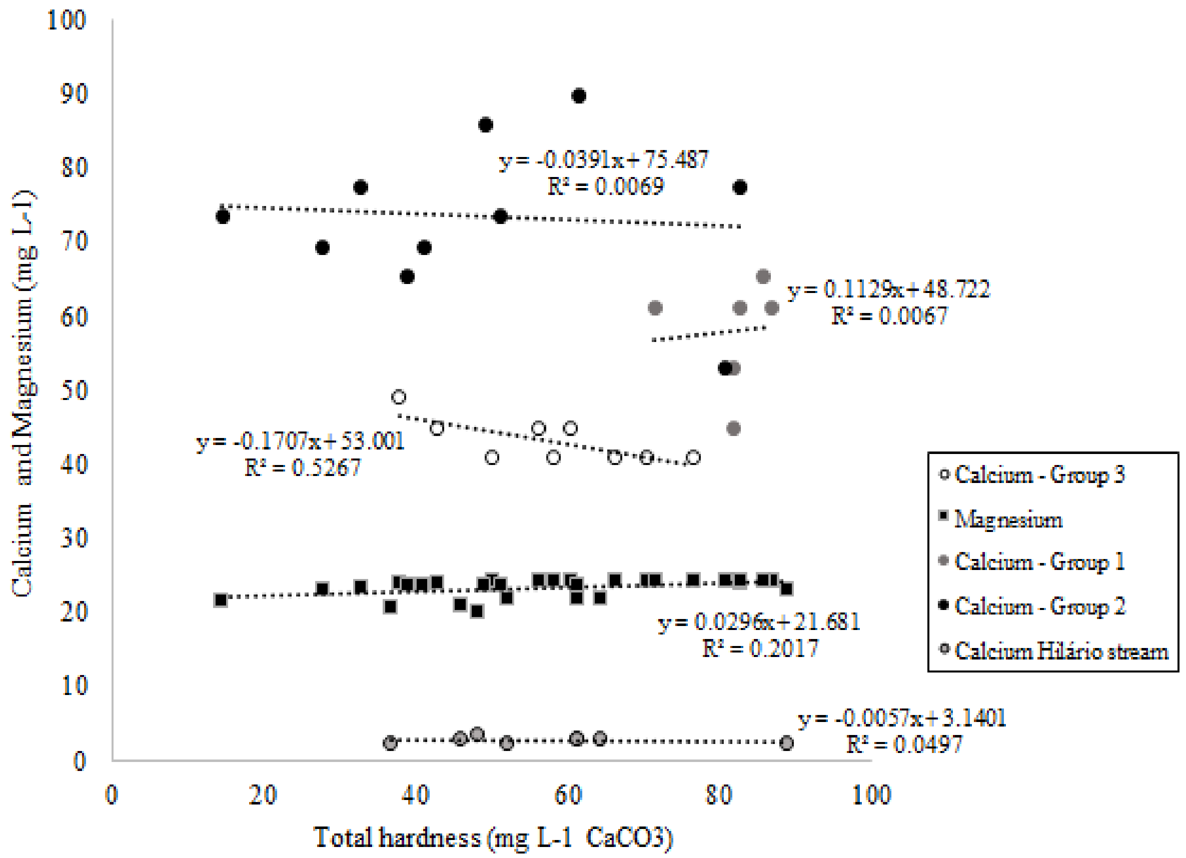

Figure 2. Relationship of calcium and magnesium with the total hardness.

3.1. EDRXF

The EDXRF analysis showed that the concentrations of major and minor elements obtained (Table 2) are among the maximum values allowed for the elements contained in water as predicted by the Brasil-Ordinance N°. 357/2005.

In terms of major elements, the silicon values obtained range from 0.13 (sample P25) to 0.65% (sample P31). In the *Camaquã das Lavras* Stream, there were varying amounts of silicon in the three groups. SiO_2 (0.19 and 0.13%) was detected in one sample of each group (samples P14 and P25). In Group 3, SiO_2 content ranged from 0.17% (sample P1) to 0.26% (sample P4). In the *Hilário* Stream samples, the highest SiO_2 content was observed, and values varied from 0.21 to 0.65%. The SiO_2 concentrations are associated with a filling of post-magmatic silica of andesites from the *Hilário* Formation.

The Al_2O_3 content detected varies according to the geographic layout of the samples. For example, the highest values are compatible with the water samples from the *Hilário* Stream, varying from 3.45 to 4.53%. In the *Camaquã das Lavras* Stream, in Group 1, only two samples presented Al_2O_3 (samples P10 and P11; Table 2). In Group 2, Al_2O_3 was detected in nine samples from a total of ten samples, ranging from 0.43 to 0.84%. In Group 3, only one sample (P6) presented Al_2O_3 (0.5%). The variation of Al_2O_3 values in the investigated samples was attributed to the presence of weathered clay minerals such as kaolinite.

To complement the chemical weathering ratio, the K_2O/Al_2O_3 elemental ratio was calculated using an indicator of the source rock composition (Cox *et al.,* 1995). Cox *et al.* (1995) suggest a differentiated K_2O/Al_2O_3 ratio for clay minerals that varies from 0.0 to 0.3 and for feldspars from 0.3 to 0.9. The $K_2O/$ Al_2O_3 ratio ranged from 0.08 to 0.38 in the *Camaquã das Lavras* Stream samples and from 0.01 to 0.02 in the *Hilário* stream samples. These values suggest that clay minerals are the dominant weathered mineral in the investigated samples, with the exception of three water samples from the *Camaquã das Lavras* Stream, Group 2, samples P17, P20 and P21, the K_2O/Al_2O_3 ratios were 0.38, 0.36 and 0.37, respectively. In this case, it is suggested that the dominant weathered mineral was feldspar.

The K_2O was detected in all investigated water samples and the highest contents were obtained in the samples from Group 2 of the *Camaquã das Lavras* Stream, where they ranged from 0.14 to 0.20% (Table 2). In Groups 1 and 3, also from *Camaquã das Lavras*, the values ranged from 0.13 to 0.19% and from 0.01 to 0.06%, respectively. Group 3 presents the lowest K_2O values of the *Camaquã das Lavras* Stream samples as the water samples from the *Hilário* Stream. In this last one, the K_2O values obtained ranged from 0.05 to 0.09%. The K_2O concentrations were attributed to the dissolution in a greater or lesser degree of white mica and alkali feldspar (Monzogranite facies and Perthite Granite), and clay minerals (andesite porphyritic from the *Hilário* Formation). These data are supported by petrography.

The SiO_2 concentration ranged from 0.21 to 0.65% in the samples from the *Hilário* Stream. Groups 1 and 2 of the *Camaquã das Lavras* Stream show only two samples with SiO_2 (P14- 0.19%; P25- 0.13%, respectively). In Group 3, also from *Camaquã das Lavras*, the values ranged from 0.17 to 0.26%. The SiO_2 concentrations in the water samples are associated with a filling of post-magmatic silica of andesites (*Hilário* Formation).

Cl was detected in the water samples of the *Hilário* Stream, varying from 0.07 to 0.09%. In water samples from Group 3 of the *Camaquã das Lavras* Stream, the SiO_2 oscillates between 0.04 and 0.05%. The Cl variation in *Hilário* and *Camaquã das Lavras* Streams is associated, probably, to two processes such as the weathering of white mica and chlorite (*Lavras do Sul* Intrusive Complex) and domestic waste.

Table 2. Percentage of major and trace elements from the analyzed water.

		Sample	Al_2O_3 (%)	SiO_2 (%)	Cl (%)	K_2O (%)	Rh (mg L^{-1})	Cd (mg L^{-1})	Cu (mg L^{-1})	K_2O/Al_2O_3
Camaquã das Lavras Stream	Group 3	P1	0	0.17	0.05	0.02	21.3	0	0	0
		P2	0	0.26	0.04	0.01	22.4	4.6	0	0
		P3	0	0	0.04	0.02	21.6	0	0	0
		P4	0	0.26	0.05	0.03	21.5	0	0	0
		P5	0	0	0.04	0.03	21.6	0	0	0
		P6	0.5	0.25	0.05	0.04	20.3	0	0	0.08
		P7	0	0	0.04	0.06	22.1	4.8	0	0
		P8	0	0	0.05	0.05	21.7	0	0	0
		P9	0	0	0.05	0.02	21.1	4.4	0	0
	Group 1	P 10	0.51	0	0	0.14	10.2	0	100	0.27
		P 11	0.57	0	0	0.16	10.4	9.3	100	0.28
		P 12	0	0	0	0.15	12	0	100	0
		P 13	0	0	0	0.13	13.7	6.9	0	0
		P 14	0	0.19	0	0.14	14.3	0	100	0
		P 15	0	0	0	0.15	14.3	7.3	100	0
		P 16	0	0	0	0.19	13.3	0	100	0
	Group 2	P 17	0.45	0	0	0.17	13.8	7.4	100	0.38
		P 18	0.61	0	0	0.17	13.5	7.4	100	0.28
		P 19	0.58	0	0	0.14	12.8	6.9	100	0.24
		P 20	0.45	0	0	0.16	13.1	0	100	0.36
		P 21	0.43	0	0	0.16	12.4	7.6	100	0.37
		P 22	0.5	0	0	0.19	12.2	8.4	100	0.38
		P 23	0.84	0	0	0.19	10.8	0	0	0.23
		P 24	0.48	0	0	0.17	11.1	7.4	100	0.35
		P 25	0.71	0.13	0	0.19	11	8.3	100	0.27
		P 26	0	0	0	0.2	11.4	0	100	0
Hilário Stream		P 27	3.45	0.21	0.07	0.05	0	0	100	0.01
		P 28	4.53	0.29	0.07	0.05	0	0	0	0.01
		P 29	3.45	0.57	0.07	0.07	0	0	0	0.02
		P 30	4.37	0.34	0.07	0.09	0	0	0	0.02
		P 31	3.45	0.65	0.07	0.08	0	0	0	0.02
		P 32	3.45	0.54	0.09	0.08	0	0	0	0.02
		P 33	3.45	0.25	0.09	0.07	4.3	0	0	0.02

Note: SiO_2=0.10; Al_2O_3=0.1; K_2O=0.01; Cl= 0.01; Rh= 0.01 mg L^{-1}; Cd=0.01 mg L^{-1}.

Attention is called to the Rh and Cd contents detected in the samples of the *Camaquã das Lavras* Stream that are above that allowed by Ordinance N°. 357 (Conama, 2005). In Group 1, the Rh content of the samples ranged from 10.2 to 14.3 mg L^{-1} (Table 2; Figure 3). In Group 2, the variation of the values was 10.8 to 13.8 g L^{-1} (Table 2; Figure 4). In Group 3 the highest Rh values for *Camaquã das Lavras* Stream are observed, which ranged from 20.3 to 22.4 mg L^{-1} (Table 2; Figure 3). Rh is an element of the platinum group, considerably resistant to weathering. Thus, its detection may be related to the weathering of soluble components from secondary minerals (like sericite and kaolinite) from the *Lavras do Sul* Intrusive Complex.

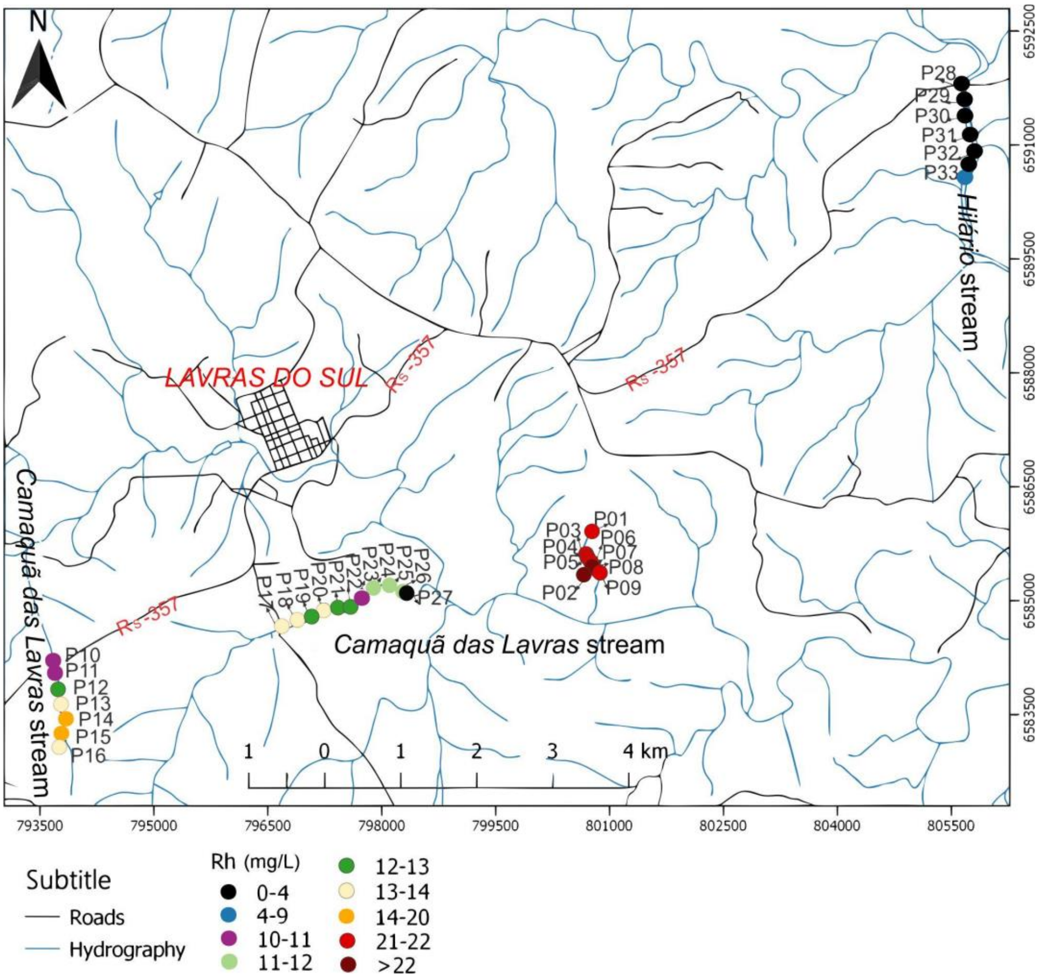

Figure 3. Rh concentrations in the studied area.

Cd was detected in most samples of *Camaquã das Lavras* Stream and in Group 1 and 3 only three samples presented Cd (P11, P13, and P15; P2, P7, and P9) ranging from 6.9 to 9.3 mg L^{-1} and 4.4 to 4.8 mg L^{-1} (Table 2; Figure 4), respectively. In Group 2 of *Camaquã das Lavras* Stream, Cd contents ranged from 6.9 to 8.4 mg L^{-1} (Table 2; Figure 4) and P20, P23 and P26 samples was not detected Cd element. In the *Hilário* Stream, Rh was only detected in the sample P33 (4.3 mg L^{-1}; Table 2; Figure 4) and Cd element was not detected.

Some factors are related to Cd detection, for example, the use of phosphate fertilizers in the soil (1-170 mg.kg^{-1}) (Tack, 2010) Cd is leached by rainwater and deposited in near water resources (Steffens *et al.*, 2015). An anthropogenic source for Cd concentrations in the water streams is the improper disposal of equipment such as batteries (Martin and Griswold, 2009). In order to identify a mineral source for the Cd and Rh concentrations in the water streams, it can be added that both Cd and Rh behave similar to Al, K, Ca, and Mg. Cd and Rh can replace the elements in the aluminosilicates, even though they have a larger atomic weight they can be adsorbed on clay minerals, kaolinite and smectite type (Caldarone *et al.*, 1994; Guerra *et al.*, 2008). However, in Figure 5 it may be observed that the highest Cd concentrations are close to or in the direction of the flow of the stream from the *Lavras do Sul* urban center, which suggests an anthropic influence.

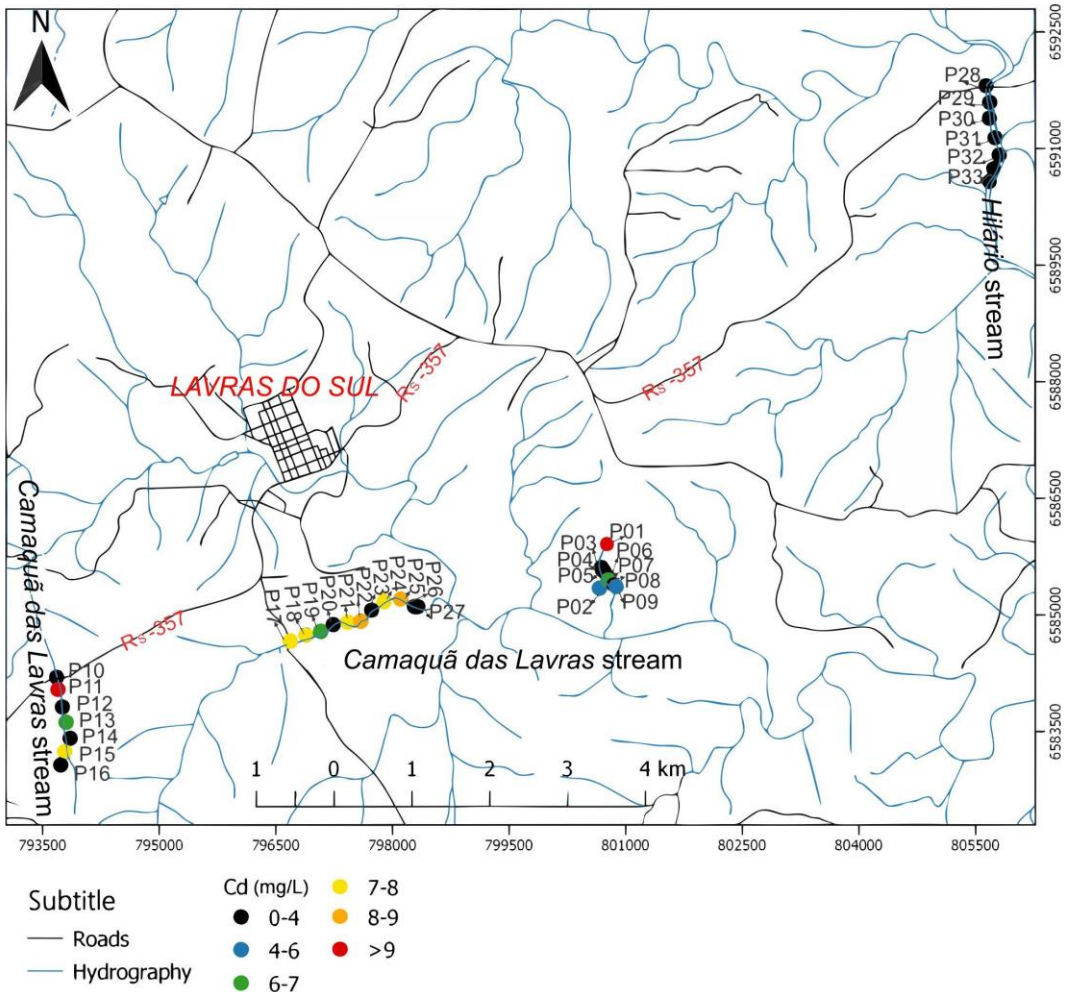

Figure 4. Cd concentrations in the studied area.

In terms of trace elements, Cu was notably detected in the samples of Groups 1 and 2 of the *Camaquã das Lavras* Stream, possibly associated with the mineralization of weathered sulfides (Pestana and Formoso, 2003; Rieuwerts *et al.*, 2006).

3.2. Statistical Analysis

In order to investigate the positive or negative correlations between the traces and larger elements of the water samples of the investigated streams, in terms of descriptive statistical correlation (Table 3), the Pearson's correlation matrix was applied (Mukaka, 2012). In this way, it was possible to relate the elements to the lithology of the studied area, enabling the knowledge of which elements are in fact, the source material and which elements are present due to human intervention.

Using the Pearson correlation matrix, it is possible to summarize the relationship between the two variables. The most common way to present and analyze bivariate data sets is through the X and Y axes. This correlation measures the similarity between two different variables. The coefficient varies between -1 to + 1. The variables that present $r > 0.7$ are considered strongly correlated, while $r > 0.5$-0.7 shows moderate correlation at a significant level ($p \leq 0.05$ with a confidence level of 95% (Table 3 and 4; Mukaka, 2012).

The Pearson correlation matrix was constructed for the water samples of the *Camaquã das Lavras* Stream (Table 3), where it shows significant positive correlations between Cl-Rh; and K_2O-alkalinity, Cu. Moderate positive correlations were observed among the following parameters: Al_2O_3-K_2O, alkalinity, EC; K_2O-Ca; alkalinity-Ca; and Hardness-Mg. Significant negative correlations are observed between Cl-K_2O, alkalinity, Cu; and Rh-K_2O, alkalinity, Cu; and moderate negative correlations on Al_2O_3-Rh; Hardness-pH; Ca-Cl, Rh. In the *Camaquã das Lavras* samples, the Cd shows weak positive correlations among Al_2O_3, alkalinity, EC, K_2O, Ca, and Rh, and the negative correlations are also weak between Cl and Rh.

The main ion exchanges can be related to the correlation coefficients found within the same order. Thus, it is possible that the simultaneous increase or decrease in cations is the result, mainly, of ion-exchange effects in the mineral assemblage of the geological substrate in the investigated waters. Cd is usually associated with zinc ores, such as sphalerite (ZnS; Rimstidt *et al.*, 1994). It is a chemical element extensively used in the industry to build materials such as cement and phosphate fertilizers. Thus, the Cd presence in the water samples can be associated to the fact that *Lavras do Sul* region has a strong mining activity of copper and lead, and agricultural activities, that make use of large amounts of phosphate fertilizers for soil correction.

Table 3. Pearson correlation matrix among the data obtained for the water samples from the *Camaquã das Lavras* Stream.

Variable	Mg	Al_2O_3	SiO_2	Cl	K_2O	Alkalinity	EC	Ca	Hardness	Ph	Rh	Cd	Cu
Mg	**1**	-0.15	0.23	0.34	-0.43	-0.37	-0.19	0.22	**0.55**	-0.14	0.34	-0.04	-0.37
Al_2O_3	-0.15	**1**	-0.15	-0.49	**0.55**	**0.50**	**0.68**	0.48	-0.36	0.24	**-0.61**	*0.40*	0.40
SiO_2	0.23	-0.15	**1**	0.47	-0.47	-0.43	-0.13	-0.24	0.06	-0.06	0.44	-0.24	-0.35
Cl	0.34	-0.49	0.47	**1**	**-0.95**	**-0.98**	-0.40	**-0.58**	-0.05	0.31	**0.95**	*-0.40*	**-0.84**
K_2O	-0.43	**0.55**	-0.47	**-0.95**	**1**	**0.96**	0.37	**0.53**	-0.04	-0.20	**-0.95**	*0.36*	**0.82**
Alkalinity	-0.37	**0.50**	-0.43	**-0.98**	**0.96**	**1**	0.39	**0.58**	0.00	-0.27	**-0.94**	*0.43*	**0.83**
EC	-0.19	**0.68**	-0.13	-0.40	0.37	0.39	**1**	0.08	-0.22	0.18	-0.48	*0.31*	0.33
Ca	0.22	0.48	-0.24	**-0.58**	**0.53**	**0.5**8	0.08	**1**	-0.03	-0.15	**-0.53**	*0.43*	0.49
Hardness	**0.55**	-0.36	0.06	-0.05	-0.04	0.00	-0.22	-0.03	**1**	**-0.63**	-0.05	-0.08	-0.02
pH	-0.14	0.24	-0.06	0.31	-0.20	-0.27	0.18	-0.15	**-0.63**	**1**	0.23	0.02	-0.33
Rh	0.34	**-0.61**	0.44	**0.95**	**-0.95**	**-0.94**	-0.48	**-0.53**	-0.05	0.23	**1**	*-0.36*	**-0.82**
Cd	-0.04	0.40	-0.24	-0.40	0.36	0.43	0.31	0.43	-0.08	0.02	-0.36	**1**	0.38
Cu	-0.37	0.40	-0.35	**-0.84**	**0.82**	**0.83**	0.33	0.49	-0.02	-0.33	**-0.82**	*0.38*	**1**

The Pearson correlation matrix was also built for the water samples of the *Hilário* Stream (Table 4). It shows that there is a significant positive correlation among the following parameters: Hardness-Mg, Rh; SiO_2-Ca; EC-Cu; and Rh-Mg. There is a moderate positive correlation with SiO_2-K_2O; and Cl-Rh. The correlation analysis for the *Hilário* Stream samples also reveals a significant negative correlation between only SiO_2-Hardness. Moderate negative correlations are observed with Mg-Hardness, Cu; SiO_2-Cu; K_2O-alkalinity, Hardness, Cu; EC-Mg, K_2O, Ca, Hardness; pH-Ca; and Hardness-Cu.

Table 4. Pearson correlation matrix among the data obtained for the water samples from the *Hilário* Stream.

Variable	Mg	Al_2O_3	SiO_2	Cl	K_2O	Alkalinity	EC	Ca	Hardness	pH	Rh	Cu
Mg	**1**	0.10	-0.04	0.21	0.22	-0.31	**-0.61**	-0.12	**0.97**	-0.35	**0.73**	**-0.62**
Al_2O_3	0.1	**1**	-0.36	-0.40	-0.07	-0.19	0.05	-0.04	-0.07	-0.14	-0.26	-0.26
SiO_2	-0.04	-0.36	**1**	-0.05	**0.54**	0.06	**-0.71**	**0.75**	-0.01	-0.06	-0.39	**-0.50**
Cl	0.21	-0.40	-0.05	**1**	0.22	-0.24	-0.20	-0.09	0.36	-0.01	**0.65**	-0.26
K_2O	0.22	-0.07	**0.54**	0.22	**1**	**-0.50**	**-0.54**	0.00	0.12	0.49	0.00	**-0.58**
Alkalinity	-0.31	-0.19	0.06	-0.24	**-0.50**	**1**	0.10	0.27	-0.20	0.00	-0.24	0.43
Conductivity	**-0.6**	0.05	**-0.71**	-0.20	**-0.54**	0.10	**1**	**-0.60**	**-0.62**	0.34	-0.15	**0.90**
Ca	-0.12	-0.04	**0.75**	-0.09	0.00	0.27	**-0.60**	**1**	-0.03	**-0.57**	-0.47	-0.47
Hardness	**0.97**	-0.07	-0.01	0.36	0.12	-0.20	**-0.62**	-0.03	**1**	-0.44	**0.81**	**-0.58**
pH	-0.35	-0.14	-0.06	-0.01	0.49	0.00	0.34	**-0.57**	-0.44	**1**	-0.14	0.38
Rh	**0.73**	-0.26	-0.39	**0.65**	0.00	-0.24	-0.15	-0.47	**0.81**	-0.14	**1**	-0.17
Cu	**-0.62**	-0.26	**-0.50**	-0.26	**-0.58**	0.43	**0.90**	-0.47	**-0.58**	0.38	-0.17	**1**

For the chemical analysis of water properties, Principal Component Analysis (PCA) was applied, based on the correlation matrix between the components and the standardized variables. In the same way, as in Pearson correlation matrix, the data of water samples investigated for each stream were interpreted separately.

Therefore, in the *Camaquã das Lavras* Stream, 10 PCs represent 100% of the variance of the obtained results (Table 5). The first three PCs are > 1, representing 77.45% of the variance (Table 5; Figure 5). For the water samples of the *Hilário* Stream, 6 PCs represent 100% of the variance in the results obtained (Table 5). The first four PCs presented values higher than 1, representing 87.57% of the variance (Table 5; Figure 5). The first and second principal components (PC1 and PC2) of the samples investigated from *Camaquã das Lavras* Stream are the result of the linear combination of 15 variables studied, and both PCs explained 43.23% and 21.62% of the variance, respectively (Table 5). On the other hand, PC1 and PC2 of the samples investigated from *Hilário* Stream are the results of the linear combination of 12 variables studied, and both PCs explained 32.35% and 22.43% of the variance, respectively (Table 5).

Table 5. Eigenvalues of correlation matrix and related statistics of the water from *Camaquã das Lavras* and *Hilário* Stream.

PCs (*Camaquã das Lavras* Stream)	Eigenvalue	Total variance %	Cumulative Eigenvalue	Cumulative %	PCs (*Hilário* Stream)	Eigenvalue	% Total Variance	Cumulative Eigenvalue	Cumulative %
1	4.323426	43.23426	4.32343	43.2343	1	3.234724	32.34724	3.23472	32.3472
2	2.161721	21.61721	6.48515	64.8515	2	2.242775	22.42775	5.47750	54.7750
3	1.260404	12.60404	7.74555	77.4555	3	1.947951	19.47951	7.42545	74.2545
4	0.914707	9.14707	8.66026	86.6026	4	1.331926	13.31926	8.75738	87.5738
5	0.732895	7.32895	9.39315	93.9315	5	0.788816	7.88816	9.54619	95.4619
6	0.288557	2.88557	9.68171	96.8171	6	0.453809	4.53809	10.00000	100.0000
7	0.204632	2.04632	9.88634	98.8634					
8	0.062680	0.62680	9.94902	99.4902					
9	0.032276	0.32276	9.98130	99.8130					
10	0.018701	0.18701	10.00000	100.0000					

The PCA also produces eigenvectors, known as coefficients or charges of principal components (Figure 5). It describes the relative importance of a component, for example, a chemical element and its variability between a data group. Eigenvectors automatically calculate the score for each PC. The values of the element loads determine the score of a sampling point. Thus, the grouping of high-load elements provides high scores, and the grouping of low-payload elements provides low scores. The number of eigenvectors calculated will be equal to the number of variables used, which in this study is the number of chemical elements and parameters analyzed (Reimann *et al.*, 2008).

For PC1 (variation of 43.23%) of the elements and parameters of *Camaquã das Lavras* Stream, high scores are commensurate to high load elements (> 0.2), for example, Mg, SiO_2, Cl and Rh (Figure 5A). The lowest scores are equivalent to the lowest rates (< 0.19), such as pH and Hardness. High scores for PC2 are related to strong positive loads (> 0.2) for Ca, alkalinity and Cu, with low rates related to Cd and K_2O (Fig. 5A).

In the *Hilário* Stream, the highest scores of PC1 with a variation of 32.35% are represented by pH, EC, Cu and alkalinity (Figure 5B), being the lowest rate equivalent to Al_2O_3. For PC2, the highest scores are equivalent hardness, Mg, Cl and Rh, in that only K_2O shows numbers lower than 0.19.

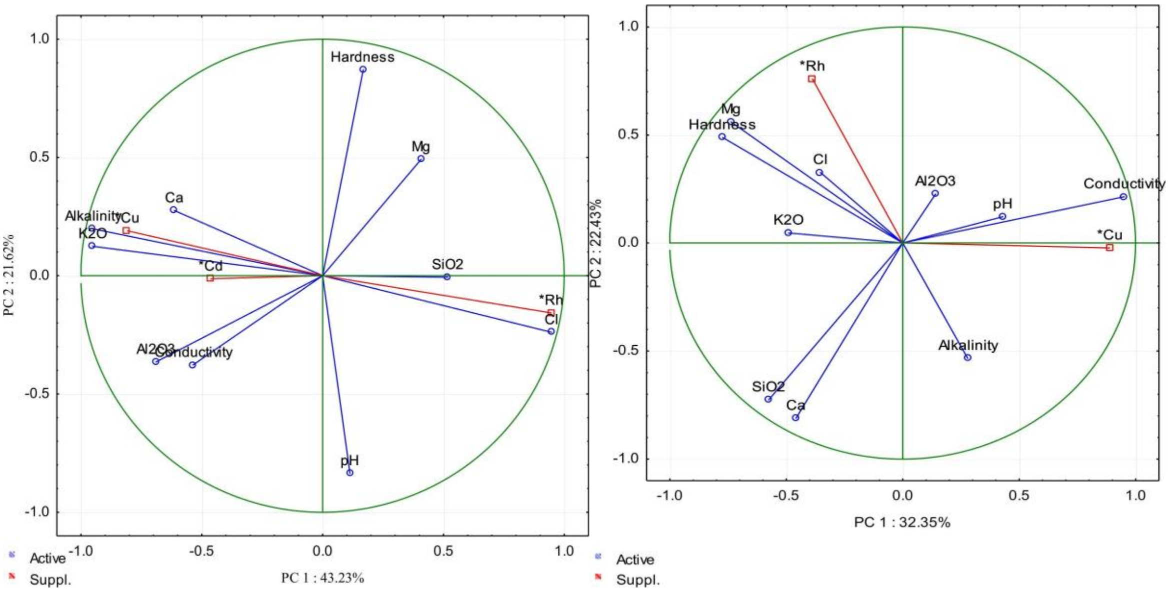

Figure 5. (A) Projection of the variables on the factor-plane (PC1 and PC2) from eigenvectors in *Camaquã das Lavras* Stream. (B) Projection of the variables on the factor-plane (PC1 and PC2) from eigenvectors in *Hilário* Stream.

4. CONCLUSIONS

The data provide new understanding of the chemical composition of the *Camaquã das Lavras* and *Hilário* streamS, in *Lavras do Sul* municipality. Thus, it is observed that:

(1) The pH values indicate acidic to near neutral waters, with indices ranging from 5.47 to 6.72;

(2) The measured the total alkalinity values varied geographically, with enrichment of hydroxides and increasing bicarbonates in the samples from Group 3 (*Camaquã das Lavras* Stream) towards the samples of the Hilário and from Group 1 to the samples from Group 2 samples of the *Camaquã das Lavras* Stream.

(3) The plagioclase, chlorite, amphibole and pyroxene weathering suggest a direct influence of the granitoids from *Lavras do Sul* Intrusive Complex and the volcanic rocks from *Hilário* Formation (andesite). After weathering, Ca and Mg ions are released into nearby water resources. These data corroborate with the soft, intermediate and temporary hardness of the water samples in the studied area;

(4) The EDXRF data of the K_2O/Al_2O_3 ratio show that clay minerals are the dominant weathered mineral in *Camaquã das Lavras* and *Hilário* Streams, resulting from the alkali feldspar weathering;

(5) The SiO_2 quantities found in water samples are associated with a filling of post-magmatic silica in andesites fromthe *Hilário* Formation. The Al_2O_3 values were attributed to the weathering of clay, such as kaolinite, with the highest values obtained in the *Hilário* Stream. The K_2O suggests the dissolution of white mica, alkali feldspar (Monzogranite and Perthite Granite), and clay minerals (*Hilário* Formation). The greatest K_2O contributions were obtained in the water samples of the *Camaquã das Lavras* Stream;

(6) Cd and Rh elements were detected in the *Camaquã das Lavras* Stream, above the values permitted by the Ordinance N$^{o.}$ 2914/2011 (Brasil, 2011).

All the geochemical and statistical analyses carried out the *Camaquã das Lavras* and *Hilário* Streams conform with a natural influence of the rock substrate type in these waters, except for the Cd that may also be related to anthropic influence. The results point to the need for further studies to better characterize the possible Cd sources in the local waters and an association with cases of infectious and other diseases. Removal of heavy metals is an important step towards safe potable water. We suggest some methods for removing Cd, such as adsorption, chemical precipitation, physical separation, ion exchange, membrane filtration, membrane distillation and hybrid methods.

5. ACKNOWLEDGEMENTS

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Biodegradation of dairy wastes using crude enzymatic extract of *Yarrowia lipolytica ATCC 9773*

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ABSTRACT

Effluents generated by the food industry have become a serious environmental concern. Bioremediation is a biological process developed as an alternative for the treatment of contaminated areas. In current research, the biodegradation of fat, Biochemical Oxygen Demand (BOD_5), Chemical Oxygen Demand (COD) and total solids were evaluated in dairy waste employing enzymatic extract of *Yarrowia lipolytica* ATCC 9773 as biological agents. All the variables were determined following the specifications of the Standard Methods of the American Water Works Association. Enzymatic extract of *Y. lipolytica* at different concentrations (8, 12 and 16.0%) was used in a fermentative medium at two pHs (5.0 and 6.5) for 32 h. The highest percentages (%) of fat (82.88), BOD (43.32), COD (44.3) and total solids (13.58) removal were obtained using an inoculum concentration of 16% at pH 5.0 for 32 h of fermentation. These results may have industrial relevance for the reduction of contamination of industrial effluents with high levels of fat and other contaminants.

Keywords: biological treatment, fatty effluents, removal, *Y lipolytica*.

Biodegradação de um residuo leiteiro usando *Yarrowia lipolytica* ATCC 9773

RESUMO

Os efluentes gerados pela indústria de alimentos tornaram-se uma séria preocupação ambiental. A biorremediação é um processo biológico desenvolvido como alternativa para o tratamento de áreas contaminadas. Na pesquisa atual, a biodegradação de gordura, a demanda bioquímica de oxigênio (DBO_5), a demanda química de oxigênio (DQO) e os sólidos totais foram avaliados como rejeitos lácteos empregando extrato enzimático de *Yarrowia lipolytica* ATCC 9773 como agentes biológicos. Todas as variáveis foram determinadas seguindo as especificações dos Métodos Padrão da American Water Works Association. O extrato enzimático de Y. lipolytica em diferentes concentrações (8, 12 e 16,0%) foi utilizado em meio fermentativo a dois pH (5,0 e 6,5) durante 32 h. As maiores porcentagens (%) de remoções de gordura (82,88), DBO (43,32), DQO (44,3) e sólidos totais (13,58) foram obtidas utilizando uma concentração de inóculo de 16% a pH 5,0 durante 32 h de fermentação. Esses resultados podem ter relevância industrial para a redução da contaminação de efluentes industriais com altos níveis de gordura e outros contaminantes.

Palavras-chave: efluentes gordurosos, remoção, tratamento biológico, *Y lipolytica*.

1. INTRODUCTION

The food industry is a sector with a high incidence of environmental pollution. Different industries such as dairy, meat and vegetable oil refining produce large amounts of wastewater. The high oil content of these increase the spoilage of some ecosystems (Porwal *et al.*, 2015).

Dairy wastes are pollutants that affect the environment when they are discarded without adequate treatment (Liu *et al.*, 2015b). In the last decades, fatty effluents have been released to the environment without previous treatment (Kumari *et al.*, 2017; Tarón-Dunoyer *et al.*, 2017), which affects public health and environmental sustainability. Considering the aforementioned, the treatment of fatty effluents is an economic and hygienic requirement.

Many of these effluents require pretreatment in order to remove incompatible substances before they are discharged into sewer systems (Kumari *et al.*, 2017; Pilusa *et al.*, 2013). The main components present in wastewater are: oils, fats and long-chain fatty acids, which represent a great problem in the pretreatment due to the fact that they are contaminants of aquatic ecosystems (Becerra-Gutiérrez *et al.*, 2015; Fachin *et al.*, 2013; Abass *et al.*, 2011)

Currently, there has been an emphasis on finding new biotechnological alternatives for the treatment of wastewater that also minimize the adverse effects previously mentioned. Biological treatment is one alternative used to decontaminate wastewater, where lipolytic properties of living organisms are employed to eliminate high-fat waste from the aquatic ecosystem (González *et al.*, 2012; Kushwaha *et al.*, 2011). Various microorganisms such as filamentous fungi, bacteria and yeast are well known as lipolytic microorganisms. Within this group of microorganisms, the yeast *Yarrowia lipolytica* is highlighted due to its extracellular and intracellular activity (Darvishi *et al.*, 2017). Its lipase production depends on the medium composition and environmental conditions (Deive *et al.*, 2010).

Yarrowia lipolytica is an adequate biological agent for biodegradation of substrates with a high fat content (Lopes *et al.*, 2018). *Y. lipolytica* has been approved as GRAS (Generally Recognized As Safe) in several industrial processes. *Yarrowia sp. have* been isolated from lipid-rich foods such as cheese and olive oil as well as from wastewater (Aloulou *et al.*, 2007). The preference of *Y. lipolytica* for these substrates has been attributed to the efficient production and secretion of proteolytic enzymes (Fickers *et al.*, 2005; Liu *et al.*, 2015a; Brigida *et al.*, 2014). In this sense, there are no references in the literature regarding the use of crude enzymatic extract for the biodegradation of dairy waste. Hence, this research focused on the utilization and evaluation of the biodegradation capacity of the crude enzymatic extract of *Yarrowia lipolytica* ATCC 9773 in dairy waste.

2. MATERIALS AND METHODS

2.1. Biological material

Yarrowia lipolytica strain (ATCC 9773) was purchased from Medimark © Europe, 38033 Grenoble Cedex 2. France.

2.2. Industrial dairy waste

The dairy waste sample was obtained from a dairy industry located in Valledupar (Colombia). The sample was collected in an 8 L plastic container. The container used for sample collection was pre-treated by washing with alcohol and later rinsed four times with distilled water. The sample was stored at a temperature below 4°C to avoid any physico-chemical changes in the effluent. Finally, the sample volume was divided in order for the pH values to be adjusted to 5.0 and 6.5 by employing HCl solution 0.5 N.

2.3. Activation and conservation of *Yarrowia lipolytica* ATCC 9773

The strain was inoculated at 25°C for three days in petri dishes containing PDA agar (Figure 1a) and olive oil as a lipid source. A microscopic morphology (Figure 1b) was then carried out employing lactophenol blue as colorant. Lastly, 0.5 mL of the inoculum was adjusted by turbidimetry at MacFarlan scale (3) and stored at 4°C until use.

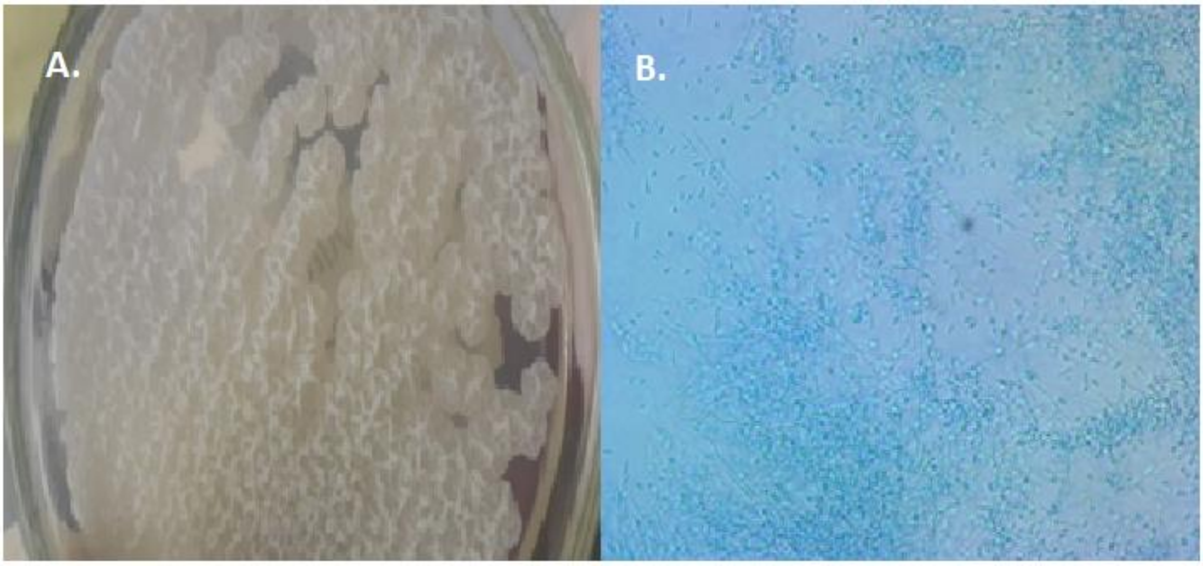

Figure 1. Morphology of *Yarrowia lipolytica* ATCC 9773: A) Macroscopic., B) Microscopic morphology.

2.4. Preparation of the inoculum and obtaining the crude enzymatic extract (CEE)

The inoculum was obtained from a suspension of mature spores of *Y. lipolytica* cultivated for five days at 25°C in PDA agar supplemented with olive oil. The biomass obtained was suspended in a solution of 0.9% (w/v) sodium chloride (NaCl). Subsequently, 200 mL of culture medium containing salt water (30% SW), sodium chloride (5.0%), yeast extract (0.5%), olive oil (1.0%) and Triton X-100 (0.1%) were inoculated with a suspension of *Y. lipolytica* for an incubation time of 8 hours. The fungal biomass was then separated from the supernatant by centrifugation at 5000 rpm for 10 min. Finally, the supernatant [enzymatic extract (EE)], was filtered through cellulose acetate membranes (0.22 to 0.45 mm) and the suspension cell viability was determined by spectrophotometry (Spectronic 20D) at 600 nm (Wu *et al.*, 2009).

2.5. Physicochemical effluent characterization

The physicochemical analyses were performed using the Standard Methods protocols established for raw water and wastewater. The fat and oil content was determined by the Soxhlet method according to the Standard Methods of the APHA *et al.* (2012). The hardness was measured by titration using EDTA solution as titrating agent, the results were expressed as mg of $CaCO_3$/L (Method 2340 C). Biological oxygen demand (BOD) was estimated by preparing the required volume of dilution water with the addition of nutrients and incubating for a period of five days at 20°C, while chemical oxygen demand (COD) was determined based on the rapid dichromate oxidation method (APHA *et al.*, 2012). The phosphorus content was determined by acid digestion, using the ascorbic acid method expressed in mg of P/L. Protein content was determined by the Kjeldahl method. Hydrogen potential was determined potentiometrically using a digital potentiometer (Bench pH-Conductivity meter PC 510).

2.6. Evaluation of biodegradation

This test was performed during the effluent's discontinuous fermentation using different concentrations (8%, 12% and 16%) of CEE of *Y. lipolytica*, considering the volume of the effluent's residual fat (pH 5 and 6.5 at 25°C). Fat content was determined each 8 hours until reaching 32 hours of fermentation. It is noteworthy to mention that BOD_5 and COD were calculated only for that time where the best fat removal was reached (inoculum concentration: 16%, incubation time: 32h and pH: 5.0).

2.7. Statistical analysis

The percentage of fat removal was used as the response variable. These data were analyzed by means of analysis of variance (ANOVA one way) in order to determine statistically significant differences ($P < 0.05$) between the samples. SPSS software (Version 17.0 for Windows) and the multiple comparison test of Tukey were used. All tests were completed in triplicate.

3. RESULTS AND DISCUSSION

Growth of *Y. lipolytica*

The growth curve of *Y. lipolytica ATCC 9773* under experimental conditions is illustrated in Figure 2a y 2b. These conditions were chosen from previous analyses of the research group. The figures show that there were mainly three phases: (1) lag phase, which lasted from 0 to 8 h, and the absorbance was 0.332 (pH 5) and 0.044 (pH 6.5); (2) logarithmic phase, which lasted from 8 to 36 h, and the absorbance increased from 0.332 to 1.319 (pH 5) and 0.044 to 0.412 (pH 6.5); and (3) death phase, the absorbance decreased after 42 h. It is interesting to mention that no stationary phase was observed, since the fermentation process was stopped at 44 h, which was before the appearance of the stationary phase.

This experimental result showed that *Y. lipolytica* was capable of using oil salts as the sole source of carbon, nitrogen and energy, and *Y. lipolytica* presented remarkable growth in oil and salt wastewater.

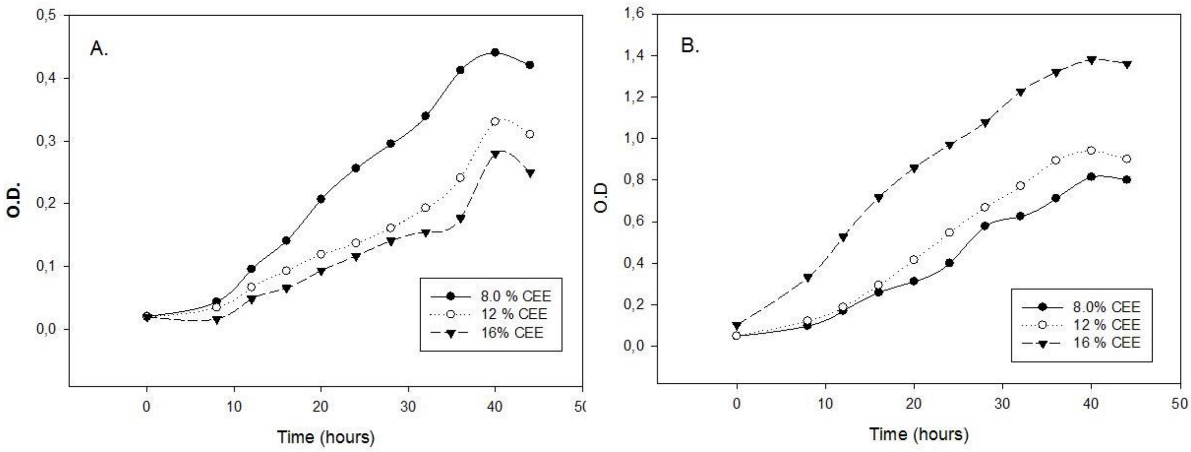

Figure 2. Growth curve of *Yarrowia lipolytica* ATCC 9773 at different pH values. A: pH 6.5; B: pH 5.0.

Table 1 shows the physical-chemical characterization of the effluent over the course of the experiment:

Table 1. Physical-chemical characterization of the residual fat effluent before and after the biodegradation process using Y. lipolytica ATCC 9773 under different conditions.

Parameters	C_i	C_f *(a)*	C_f *(b)*	*Unit*
BOD_5	17299±14.8	9805±21.2	10680±75.5	mg de 0_2/L
COD	53118±27.5	29576±15.2	32316±15.2	mg de 0_2/L
Fat	3260±20.80	558±5.290	1083±8.540	mg/L
pH	8.080±0.00	5.0±0.000	6.5±0.000	U de pH
Total solids	21308±14.4	18415±29.7	19676±92.9	mg/L
Phosphorus	<0.07	<0.075	<0.075	mg de P/L
Hardness	486.6±5.77	487.3±7.50	503.3±5.7	mg $CaCO_3$/L
Protein	2.26±0.057	1.94±0.051	1.81±0.028	%

C_i: Initial conditions of the effluent. C_f (a): Final conditions; pH 5,0 inoculum concentration of 16%, 32 hours of fermentation.
C_f (b): Final conditions; pH 6,5 inoculum concentration of 8%, 32 hours of fermentation.

According to the results of the BOD_5 and COD analyses, the dairy residue can be considered highly biodegradable. *Y lipolytica* ATCC 9773 is able to reduce BOD_5 43.32% and COD 44.30%. The best result in fat removal were obtained when a pH of 5 and an inoculum concentration of 16% were used. It seems that a pH increase in the effluent has effects on *Y. lipolytica* which influence its growth and therefore the lipases production.

One-way ANOVA of BOD_5 and COD values revealed significant differences ($p<0.05$) among the studied samples when the effluents' pH increased. Regarding to the inoculum concentration, also significant differences ($p<0.05$) were found at pH 5.0 when inoculum increased; however, the same was not observed at pH 6.5. Similar results have been reported by other researchers (Wu *et al.*, 2009; Wu and Wan, 2008) due to the fact that a high inoculum concentration can encourage microbial growth. Studies involving considerable reduction in COD and BOD in wastewater by using bacterial isolates have been published by Das and Santra (2010), Gaikwad *et al.* (2014). As with most other agro-industries, the dairy industry produces strong wastewaters characterized by COD and high BOD absorptions signifying their elevated organic content (Orhon *et al.*, 1993). The drop in COD values may be due to the concentration of nutrients, which microbial cultures could use for growing. Current results are in accordance with the reduction in COD reported by Guillen-Jimenez *et al.* (2000), where maximum COD fall was found up to 65–70%. Similar decrease in COD of the dairy wastewater (99.9%) was noted by Cosa and Okoh (2014) with a consortium of two marine species.

When the pH is 5.0 and the inoculum concentration 16%, the total solids removal reached values close to 13.58%. These results are opposite those reported by Stefańsk *et al.* (2018) who reported similar removal values employing a pH of 6.5 and an inoculum concentration of 8%. On the other hand, the hardness values are slightly higher than those initial values; this increase could be caused by the production of solid materials generated by the microbial growth.

Figure 3 shows the values of fat removal at different concentrations of inoculum (8%, 12% and 16%) in the effluent, adjusted at two pH values (5.0 and 6.5). To pH 5.0 the highest fat removal values (88. 82%) were achieved using an inoculum concentration of 16% and 32 h fermentation time. No significant differences were found ($p>0.05$) among different inoculum concentrations at the same fermentation times. It should be mentioned that significant differences ($p<0.05$) were found in fat biodegradation for all fermentation times.

The fermentative process at a pH of 6.5 using different inoculum concentrations is illustrated in Figure 3, where a removal percentage of 49.63% after 8 hours of fermentation may be observed. When the inoculum amount was increased, no significant differences ($P>0.05$) were appreciated in the fat removal percentage for different samples in the same fermentation time. After 8 hours of fermentation, the percentage of fat removal remained constant, although Y. lipolytica ATCC 9773 continues to present lipase activity, which results in a decreasing of fat percentage until 32 hours of fermentation time is reached.

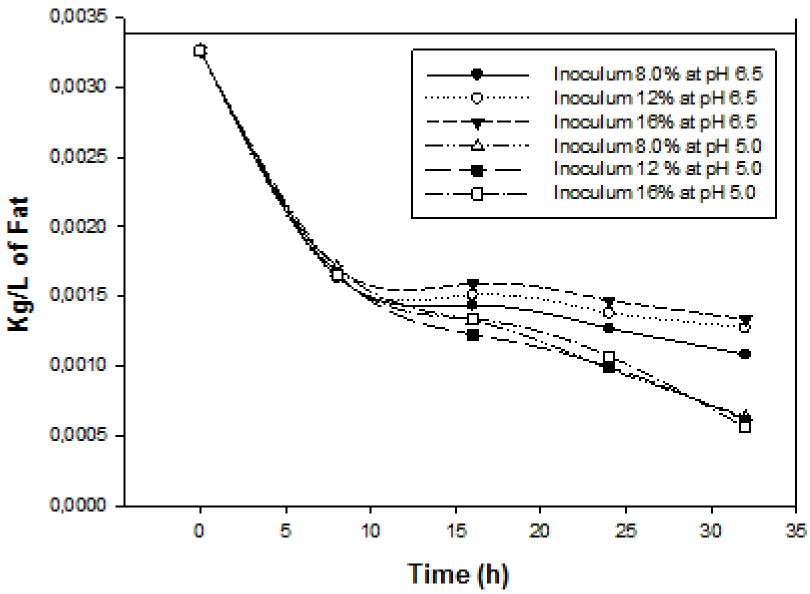

Figure 3. Fat removal from industrial effluent at different concentrations of CEE and a pH of 5.0 and 6.5.

It must be highlighted that significant differences are found ($p < 0.05$) in the percentages of biodegradation obtained at higher fermentation times. At 8% inoculum concentration, more than 16% fat biodegradation was obtained during the same fermentation time (32 hours). It seems that an increase in cell concentration causes cyanic changes in the microorganism, which result in a low metabolic activity, resulting in a reduction of lipase activity. On the other hand, no significant differences were found ($p > 0.05$) between inoculum concentrations of 12% and 16%.

The values of fat removal at different pH concentrations and fermentation times are shown in Table 2. The highest fat removal value (82.88) was obtained at pH 5 using an inoculum concentration of 16%, while the lowest value (47.50) was reached at pH 5 and an inoculum concentration of 8%. It should be highlighted that significant differences ($p < 0.05$) were found for all the fermentation times.

Table 2. Percentage of fat biodegradation using CEE at two pH values over 32 days.

Time (hours)	*Fat removal (%)*					
	pH 5			*pH 6.5*		
	Concentration of CEE Y. lipolytica			*Concentration of CEE Y. lipolytica*		
	8%	12%	16%	8%	12%	16%
8	47.50[a]	48.89[a]	50.18[a]	49.63[a]	49.81[a]	47.79[a]
16	59.29[b]	62.39[b]	58.98[b]	55.88[b]	53.65[b]	51.16[b]
24	70.00[c]	69.60[c]	67.23[c]	61.07[c]	57.66[c]	54.93[c]
32	80.52[d]	81.22[d]	82.88[d]	66.77[d]	60.85[d]	59.01[d]

Rows with no common letter showed statistically significant difference (significance level\0.05).

4. CONCLUSIONS

The study showed that fatty effluent from the dairy industry contains high levels of BOD_5, COD, total solids and fat. The results show that CEE from strain culture (*Yarrowia lipolytica*) reduced the levels of BOD_5 and COD until reaching the values of 43.32 and 44.3%, respectively. Likewise, the *Y. lipolytica* ATCC 9773 strain is able to biodegrade the fat content of the effluent to values close to 82.88%. These results may have relevant implications in the industry for the reduction of the contamination of effluents with large amounts of contaminating material, specifically fat.

5. ACKNOWLEDGMENT

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Best practice production to reduce the water footprint of dairy milk

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ABSTRACT

This study evaluated the impact of diet as a mitigation action to improve the water efficiency of lactating cows. An intensive pasture dairy system was considered to calculate direct and indirect water use. Group 1 was fed with a diet containing 20% crude protein content. The crude protein content of Group 2 was adjusted according to milk production, ranging from 23% to 14.5%. The total water footprints had a value of 502.4 L kg^{-1} fat protein corrected milk for Group 1 and 451.2 L kg^{-1} fat protein corrected milk for Group 2. The diet with the adjusted protein provided a reduction of 10% in the footprint value. The green water footprint was the most representative of consumption in the total value of the water footprint, 86.4% and 85.5% for Groups 1 and 2, respectively. The animals in Group 1 had a mean total drinking water consumption of 83.3 L $animal^{-1}$ day^{-1} and those of Group 2, 80.4 L $animal^{-1}$ day^{-1}. This study demonstrated that high crude protein content in the diet provided a greater water footprint, therefore lower water efficiency. The proposed nutritional practice proved viable as a water-mitigating action, making the ratio of liters of water per liter of milk more advantageous. The results of this study could be considered a validation of a nutritional mitigation practice to improve water efficiency and could be used as best management for the dairy supply chain.

Keywords: blue, crude protein, green, grey, nitrogen.

Boa prática de produção para reduzir a pegada hídrica do leite bovino

RESUMO

O objetivo do estudo foi avaliar o impacto do teor de proteína bruta da dieta de vacas em lactação como ação mitigadora para dar maior eficiência hídrica. Um sistema intensivo de produção de leite a pasto foi considerado para calcular os usos indiretos e diretos da água. Os animais do Grupo 1 receberam dieta contendo 20% de proteína bruta. A proteína bruta do Grupo 2 foi ajustado de acordo com a produção de leite, variando de 23% a 14.5%. A pegada hídrica total teve um valor de 502,4 L kg^{-1} de leite corrigido para gordura e proteína para o Grupo 1 e 451,2 L kg^{-1} de leite corrigido para gordura e proteína para o Grupo 2. A dieta com a proteína ajustada apresentou redução de 10% no valor da pegada hídrica. A pegada hídrica verde foi a mais representativa no valor total da pegada hídrica, 86,4% e 85,5% para os Grupos 1 e 2, respectivamente. Os animais do Grupo 1 tiveram um consumo médio de água de 83,3 L $animal^{-1}$ dia^{-1} e os do Grupo 2, 80,4 L $animal^{-1}$ dia^{-1}. O estudo demonstrou que o elevado teor de proteína bruta na dieta resulta em uma maior pegada hídrica e, portanto, menor eficiência hídrica. A prática nutricional proposta mostrou-se viável como ação mitigadora, tornando mais eficiente a relação de litros de água por litro de leite. Os resultados podem ser considerados como uma validação de uma prática de mitigação nutricional para melhorar a eficiência de uso da água e para o melhor manejo hídrico do complexo agroindustrial do leite.

Palavras-chave: azul, cinza, nitrogênio, teor de proteína, verde.

1. INTRODUCTION

One of the greatest present and future challenges for livestock is to remain a provider of quality food and conserve natural resources. This activity demands large volumes of water and has a high polluting potential as a point and no-point source. Therefore, a detailed understanding of livestock water uses and the impact of production practices on water efficiency can help the internalization of water management by the sector, reduce conflicts with society, and show how management practices promote water conservation.

Water is one of the most important factors on a dairy farm because it is essential for livestock consumption to support milk production. This dependence may lead to more active regulation and monitoring of water use, which puts a great amount of pressure, especially financially, on farmers (Robinson *et al.*, 2016). Nowadays studies are estimating water consumption by ruminants, but the efforts are only based on direct water use (Murphy *et al.*, 2014; Fischer *et al.*, 2017; Legesse *et al.*, 2017).

The physiological importance of water to dairy is well established. It is important when it comes to the health and well-being of the animal, but is a reductionist vision if the goal is the efficient use of water in the production system and the management of the natural resources. Investigations by Fischer *et al.* (2017) emphasize that the discussion about water consumption in livestock is from the perspective of production. However, this aspect must also be seen in the context of water demand by people, industry and services.

The water footprint approach provides information about water consumed and the impact of the product in the quantity and quality of water. Two internationally accepted concepts of water footprint have been developed; the water footprint concept (Hoekstra *et al.* (2017) and the Life Cycle Assessment (LCA). Very few studies have been performed to evaluate the impact of mitigation practices on the footprint value. The majority of the studies explored the impact of dairy production on freshwater resources of countries, basins, or production systems (Murphy *et al.*, 2014; Palhares and Pezzopane, 2015). To evaluate the relationship between dairy production systems and water use, it is necessary to know the consumptive water used in the “feed production-dairy-manure management” chain and to identify the major factors affecting the water consumption for milk production (Lu *et al.*, 2018). Legesse *et al.* (2017) reported that feed production/utilization, best water management practices, and animal production efficiency are strategies to increase the water efficiency of ruminant production.

Nutritional management is one of these strategies. By adopting better precision nutrition, it can improve water efficiency and reduce environmental impact. This will promote green, blue, and grey water efficiency. Palhares *et al.* (2018) showed that animal nutrition is a mitigation aspect to reduce the cost of water, natural resource consumption, and livestock polluting potential.

This study evaluated the impact of diet as a mitigation action to improve the water efficiency of lactating cows. Diet formulation for lactating cows considering the ideal crude protein content is not new to the science of animal nutrition. This investigation proposes a holistic view, considering the various aspects of dairy production systems, in this case animal nutrition, water consumption, water efficiency, and polluting potential. The innovation of this study is to give a water view to nutritional management, demonstrating that a nutritional practice routine in dairy production, besides all the productive and economic advantages, also has environmental advantages.

2. MATERIALS AND METHODS

The concept of the Water Footprint Standard (Hoekstra *et al.*, 2011) was used to evaluate the water footprint of dairy milk in a cradle-to-farm gate perspective. This method was chosen considering the experience of the authors in its use and because it distinguishes between green, blue and grey water. Water footprint is a comprehensive indicator of freshwater resource appropriation, which goes beyond traditional restrictive measures of water withdrawal. The water footprint is defined as the total volume of fresh water that is consumed to produce a product. The consumptive water footprint is split into three categories: green, measuring consumption of rainwater; blue, measuring consumption of groundwater or surface water; and grey, measuring how much water is needed to assimilate polluting substances.

Water footprints were calculated from primary data related to direct and indirect water consumption, effluent nitrogen load, and feed and milk production.

2.1. Systems data

An intensive pasture dairy production system was considered in order to produce the raw milk. The lactating cows were milked twice daily at 0800 h and 1600 h. The lactation period was 305 days. Raw milk production yield for Group 1 and Group 2 was 52,267 kg and 56,622 kg, respectively. The average daily production per cow was 23.1 L day$^{-1 \text{ for}}$ Group 1 and 25.2 L day$^{-1 \text{ for}}$ Group 2.

The diets of both experimental groups are presented in Table 1. Group 1 was fed with a diet containing 20% crude protein content in the diet of dry matter throughout the lactation. The crude protein content of Group 2 was determined according to milk production during the lactation period. Diets (roughage + concentrate) were given twice daily for each group.

Pasture and corn silage was cultivated on-farm and soya and maize were cultivated off-farm. Protein and fat content of milk varies depending on the type and age of animal, feed, and production management. International standards were determined for Fat Protein - Corrected Milk (FPCM) (FAO, 2013).

Table 1. Nutritional management and diets for the two experimental groups (average dry matter intake animal^{-1} day^{-1}).

Group 1												
	Lactating Period (month)											
Intake (kg)	1	2	3	4	5	6	7	8	9	10	11	12
Total Dry matter	25.3	21.1	24.5	21.6	21.7	22.9	20.2	20.1	20.1	20.1	20.1	20.1
Pasture	7.8	2.5	4.5	4.5	4.5	4.5	4.8	9.5	9.5	9.5	9.5	9.5
Maize Silage	5.9	7.0	8.4	5.6	6.6	7.8	4.8					
Concentrate*	11.6	11.6	11.6	11.5	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6
Soybean Meal	3.5	3.5	3.5	3.5	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
Maize	7.5	7.5	7.5	7.4	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Crude Protein (%)	20	20	20	20	20	20	20	20	20	20	20	20
Group 2												
	Lactating Period (month)											
Intake (kg)	1	2	3	4	5	6	7	8	9	10	11	12
Total Dry matter	26.0	21.3	24.6	21.7	20.5	21.7	19.0	20.2	20.2	20.2	20.2	20.2
Pasture	7.8	2.5	4.5	4.5	4.5	4.5	4.8	9.5	9.5	9.5	9.5	9.5
Maize Silage	5.9	7.0	8.4	5.6	6.6	7.8	4.8					
Concentrate*	12.3	11.8	11.7	11.6	9.4	9.4	9.4	10.7	10.7	10.7	10.7	10.7
Soybean Meal	2.4	4.3	4.3	4.2	2.4	2.4	2.4	2.0	2.0	2.0	2.0	2.0
Maize	9.2	6.8	6.7	6.7	6.5	6.5	6.5	8.2	8.2	8.2	8.2	8.2
Crude Protein (%)	23	23	23	17	17	17	17	17	17	14.5	14,5	14.5

*Concentrate- is the sum of soybean meal, maize, and minerals.

2.2. Water footprint calculations

The green water footprint is the sum of evapotranspiration water plus the water in the feed product. Each feedstuff produced or imported was considered to calculate the green water footprint. Feed produced at the farm considered field data and yields. Feed imported was taken from farm documents. The water volume of each feedstuff was reported from the dry matter content.

Green water footprint was calculated as Equation 1:

$$GreenW = \frac{\sum_{p=1}^{n}(ETc+Product)}{Production\ of\ Milkcorrected} \quad (1)$$

Green water footprint (GreenW) (L kg^{-1}); ETc [a,s] is the potential crop evapotranspiration of each feed ingredient p consumed by each experimental group (L kg^{-1}); and Product is water content in feed ingredient p in each experimental group (L kg^{-1}).

The potential crop evapotranspiration for pasture, corn silage, maize, and soya was calculated multiplying reference evapotranspiration (ETo) by based crop coefficient (Kc) (Allen *et al.*, 1998). Reference evapotranspiration was derived from a climatic station installed at the farm using the Food and Agriculture Organization Penman–Monteith equation (Allen *et al.*, 1998). Climatic parameters values were obtained monthly from the Agritempo database (AGRITEMPO, 2017).

The following productive conditions were used to calculate ETc: maize produced in Parana State, (Prudentopolis,) and soybean produced in Mato Grosso State (Rondonopolis,). It was considered that these crops were produced in a rainfed manner. To calculate Tanzania grass amounts, daily values of crop coefficient were used. Silage and grass were produced on the farm.

Soybean meal is the form that cows feed on. Technical Conversion Factors for Agricultural Commodities (FAO, 2013) were used to calculate the water quantity from evapotranspiration; considering the Brazilian condition, it is 77% meal and 23% oil.

Pasture irrigation, animal drinking, and water in the product were considered in the blue water calculation. Blue water footprint was calculated as Equation 2:

$$BW = \frac{(Wirr)+(Wani)+(Wprod)}{Production\ of\ Milkcorrected} \quad (2)$$

Blue water footprint (BW) (L kg^{-1}); Wirr is consumption of water to irrigate (L kg^{-1}), Wani is water consumed by animals (L kg^{-1}), and Wprod is water in milk, considering an average of 87% water per kg of milk (NEPA, 2011).

The drinking water intake of each lactating cow was recorded daily and measured by automatic water bin (Oliveira *et al.*, 2018).

The irrigation water was evaluated considering the type of equipment. Irrigation characteristics were: 32 paddocks divided into seven irrigation sectors, each sector with twelve sprinklers with a flow rate of 0.45 m^{-3} h^{-1}; nocturnal irrigation with two hours of irrigation per sector; irrigation efficiency of 85%. Irrigation started on 06/09/2015 and ended on 10/25/2015 (97 days).

The milking parlor comprised of a one-stage dairy shed effluent stabilization system. Effluent was considered water for cleaning and disinfection. Water meters were installed on the milking parlor to record effluent volume. To calculate the grey water footprint, nitrate was set as the pollutant under consideration. Grey water footprint was calculated as Equation 3:

$$GreyW = \frac{[(\alpha\ x\ (Efu\ x\ Cefu)]/(Cmax-Cnat)}{Production\ of\ Milkcorrected} \quad (3)$$

Grey water (GreyW) (L kg^{-1}); α dimensionless factor, defined as the fraction of applied chemical substance reaching freshwater bodies; Eflu is volume of effluent (L); Ceflu is the nitrate concentration of the effluent (23.6 mg L^{-1} N-NO_3 – Group 1 and 22.8 mg L^{-1} N-NO_3 – Group 2) (Palhares and Pezzopane, 2015); Cmax is maximum acceptable concentration (mg L^{-1}) with reference to CONAMA Resolution 357/2005 (10 mg L^{-1} N-NO_3); and Cnat is natural concentration in a receiving water body (mg L^{-1}), and was considered zero (CONAMA, 2005).

The average volume of effluent produced by each lactating cow during the study period was 39.8 L $animal^{-1}$ day^{-1}. This value was multiplied by the number of cows in each group to get the volume of effluent.

3. RESULTS AND DISCUSSION

The total water footprints had a value of 502.4 L kg^{-1} FPCM for Group 1 and 451.2 L kg^{-1} FPCM for Group 2 (Table 2). This study demonstrated that high crude protein content in the diet, besides not producing more milk, caused a greater water footprint, therefore lower water efficiency of the product. It is emphasized that a higher protein concentration has a higher cost, which will also negatively impact the cost of production of the milk.

The manipulation of the crude protein content of the concentrate resulted in better water efficiency. Ran *et al.* (2016) indicate that feed quality, digestibility, and feed conversion efficiency impact livestock water productivity. Bosire *et al.* (2015) found diet composition can determine the magnitude of the water footprint of milk, because diet translates into better feed conversion and more efficient use of freshwater. White (2016) showed that management to reduce water consumption of dairy cattle improves the use of protein and energy. Palhares *et al.* (2017) demonstrated that diet formulation is a tool to improve the water efficiency of animal products.

Table 2. Water footprints by experimental group.

Waters	Group 1		Group 2	
Green (L kg^{-1} FPCM)	434.0	(86,4%)	386.0	(85,5%)
Blue (L kg^{-1} FPCM)	67.7	(13,5%)	64.6	(14,4%)
Gray (L kg^{-1} FPCM)	0.7	(0,14%)	0.6	(0,13%)
Total Footprint (L kg^{-1} FPCM)	502.4		451.2	

The green water footprint was the most representative, 86.4% and 85.5% for Groups 1 and 2, respectively, in the water footprint value. Bai *et al.* (2018) found that indirect water footprint accounting for more than 92% of dairy farm water footprint. Noya *et al.* (2018) showed that feed/fodder production contributed 99% of the water footprint of milk while the dairy farm stage has a minor influence. These results showed the importance of the indirect water consumption instead of the minor influence of the direct water requirements. The water footprint of animal and plant products involves biological systems that have different phases of development, have specific nutrient requirements and are influenced by different climatic and management aspects. Therefore, there is a need for all these aspects to be evaluated together, as well as their interrelations, in order to achieve results of significant impact.

The diet with the adjusted protein provided a reduction of 11% in the green footprint value. Owusu-Sekyere *et al.* (2017) verified that dairy diets with high protein concentrates had the highest water footprint. According to Mekonnen and Hoekstra (2014), green water footprint for milk in pasture systems is 1.087 L kg^{-1} of milk. This represents more than double the values found in this study. The difference can be the result of the type of diet considered in the studies, not as a comparable functional unit, the geographical and temporal location of crop cultivation, and milk production. De Léis *et al.* (2015) point that the productive factors that most impact the value of the green water consumption and footprint are the animals' diet, dry-matter intake, and agricultural productivity. Zhuo *et al.* (2016) propose choosing crops that have more nutritional value and consume less water.

The water footprint of soybean was 4,304 m^3 ha^{-1} and for maize it was 3,246 m^3 ha^{-1}. As the adjusted diet presented a total lower demand of soybean meal, the green water consumption in Group 2 was lower. This is characteristic of the tropical regions and the major Brazilian grain-producing regions due to the fact that the rainfall satisfies the crops' water demands. The maize WF quantified by other authors was 1,222 m3 ton-1 (Mekonnen and Hoekstra, 2010), 910 m3 ton-1 (Wang *et al.*, 2014), 900 m3 ton-1 (Chapagain and Hoekstra, 2004), 868 m3 ton-1 (Huang *et al.*, 2014), and 750 m3 ton-1 (Williams and Al-Hmoud, 2015).

To calculate the green water consumption of the pasture, only the portion of pasture ingested daily by the animals was recorded. Thus, the total green water consumed was 403 m^3. The quantification of green water volume in cattle production systems that include grazing basically can have two interpretations: considering only the portion of grass that was ingested by the animal or the total evapotranspiration of the grazing area. In this study we chose to calculate considering only the amount of grass ingested by the animals. If the total area of the rotational grazing system (1.7 ha) were considered, the green water consumption from pasture would be 95% higher. There is still no methodological standardization for the calculation of the green water consumed from grazing systems. It is known that in a rotational grazing system the entire area contributes to the production of the products and that this system provides reduced environmental services.

The silage consumed by Group 1 represented the volume of 1,448 m^3 of green water and, for Group 2, was 1,478 m^3. If pasture irrigation had not been used in the dry period, the animals would need to be fully supplemented with maize silage. This situation means that green water consumption of Group 1 would increase by 6.8% and that of Group 2 by 7.7%. Consequently, blue water consumption would reduce by 72.8% because no irrigation would be used.

The blue water footprint of Group 1 was 67.7 L kg^{-1} FPCM, and for Group 2 64.6 L kg^{-1} FPCM. Mekonnen and Hoekstra (2014) estimated 56 L kg-1 FPCM and Huang *et al.* (2014) estimated 69 L kg^{-1} FPCM. Palhares and Pezzopane (2015) calculated the same production system evaluated in this study as 75 L kg^{-1} FPCM, but without nutritional management intervention.

In Group 1, the composition of the blue water footprint was 93.3% (63.2 L kg^{-1} FPCM) irrigation water, 5.4% (3.6 L kg^{-1} FPCM) drinking water, and 1.3% (0.87 L kg^{-1} FPCM) water in the product. For Group 2, these percentages were 93.3% (60.3 L kg^{-1} FPCM) irrigation water, 5.3% (3.4 L kg^{-1} FPCM) drinking water, and 1.3% (0.87 L kg^{-1} FPCM) water in the product. Irrigation represented the highest consumption of blue water, totaling 6,297 m^3. If irrigation were not used, the value of the blue water footprint would be reduced by 15 times. Henderson *et al.* (2017) calculated the national average blue water consumption for the US associated with milk production. It was 210 L kg $milk^{-1}$ and is dominated by feed irrigation. Studies that calculated the dairy blue water footprint without considering irrigation found values between 1.2 to 9.7 L kg^{-1} FPCM (Murphy *et al.*, 2014).

Irrigation is a practice used to maintain pasture production at the beginning of the dry season in tropical regions because it means reduced feed costs due to the lower need to buy concentrated feed. But the practice should be based on best practices and scheduling does not mean inefficient water use. As the cost of water is zero or insignificant compared to the cost of concentrated feed, farmers use irrigation without worrying about the volume of water consumed if they are in a region without water scarcity problems.

The animals in Group 1 had a mean total drinking water consumption of 83.3 L $animal^{-1}$ day^{-1} and those of Group 2, 80.4 L $animal^{-1}$ day^{-1}. The average daily water consumption per Holstein lactating cows was 114 L $animal^{-1}$ day^{-1} (Le Riche *et al.*, 2017) and 78.4 L $animal^{-1}$

day^{-1} (Appuhamy *et al.*, 2016). Considering a lactation period, the animals of Group 1 drank 165.9 m^3 and those of Group 2,165 m^3. The difference is 900 liters. Chouchane *et al.* (2015) and Owusu-Sekyere (2016) noted that blue water use is associated with production costs.

In Group 1, the drinking water intake per kg of milk produced was 3.6 L kg^{-1} FPCM and for Group 2, 3.4 L kg^{-1} FPCM. The adjustment of the protein content of the diet contributed to a reduced consumption of drinking water and positively impacted the relation between water consumption by the quantity of product. The lack of routine measuring of water consumption in dairy farms is a great challenge to have more precise calculations of blue water consumption and footprint. Considering the various consumptions of blue water in the dairy system, drinking water consumption by lactating cows is one of the largest. Therefore, the use of water meters is essential for advancements in the water management of dairy production.

Group 1 presented a higher value of grey water footprint. The results demonstrate that excessive crude protein content in the diet results in a higher consumption of water from the environment to assimilate the pollutant loads generated by the production system. By integrating nutrient-use efficiency (adjustable protein) with the calculation of the grey water footprint, it was possible to identify the relationship between the type of diet, the polluting potential, and the value of the footprint. The evaluation by White (2016) of the environmental benefits of animal-nutrition research demonstrated that improved protein efficiency greatly reduced the cost of achieving targeted reductions in water use in dairy production. Animals from the groups presented different nitrogen-use efficiencies, and consequently had different concentrations of it in their effluents. Therefore, the diet with adjustable protein produced an effluent with a lower nitrogen load, requiring less water to dilute the effluent. In this way, the study promoted the advantages of the relationships between the grey water footprint from diffuse sources and the nutritional management of the animal systems. The connection between the livestock productive aspects is essential. For example, diets with low green and/or blue footprints may result in high grey footprint values, because if the digestibility of the nutrients of the diet is poor, they will be eliminated as wastes, which will affect the volume of greywater.

4. CONCLUSIONS

The results demonstrated that the manipulation of the crude protein content of the lactating cow diet improved milk water efficiency in all water footprint dimensions. Footprint values showed that the nutritional approach can be a general recommendation across all regions and production systems to increase water efficiency and reduce the conflicts of resource availability. Therefore, the proposed nutritional practice proved viable as a water-mitigating action, making the ratio of liters of water per liter of milk more advantageous. The results reinforce the fact that the use of best practices in animal nutrition can also have positive impacts on several aspects of the dairy system.

The results of this study could be considered a validation of a nutritional mitigation practice to improve water efficiency and could be used as best management by farmers, the dairy industry and governments as a water-saving strategy from an environment and economic cost–benefit point of view. The study also contributes to the provision of benchmarks regarding mitigation actions that can be used to improve dairy water-use efficiency. Future studies should continue to explore the relationship between practices and technologies in animal nutrition and water efficiency, as well as other productive aspects such as genetics, the type of production system and the use and reuse of water and effluents in productive management.

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Evaluation of the cytotoxic and genotoxic effect of *Allium cepa L.* (Amaryllidaceae) root cells after exposure in water samples of five lakes of Alta Floresta, State of Mato Grosso

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ABSTRACT

This study evaluated genotoxic and cytotoxic potential of water samples through the analysis of *Allium cepa* root cells exposed to samples from five urban lakes of Alta Floresta, state of Mato Grosso. The samples were obtained in the dry and rainy periods, October 2014 and April 2015, respectively, at five distinct points. The collection points were as follows: Lake 01, located in MT 206; Lake 02, in Avenida Perimetral Rogério Silva; Lake 03, in Sector C; Lake 04 on Teles Pires Avenue; and Lake 05, in the Cidade Bela District. Bioassays using *Allium cepa* bulbs were taken from the water at each point, and comparisons were made with the negative and positive controls. The results found the presence of genotoxic and cytotoxic activities on the roots of *A. cepa*, indicating high potential in cell cycle inhibition. This result may also be caused by the influences of seasonal periods, taking into account that in the rainy season there is a greater transport of cytotoxic substances by rainwater, and in the dry season, a higher concentration of pollutants due to the reduction of water volume, resulting in a greater interaction of the concentrators as well as a greater concentration of the elements found in the water of these lagoons.

Keywords: bioassay, genotoxic and cytotoxic potential, seasonal periods.

Avaliação do efeito citotóxico e genotóxico das células radiculares de *Allium cepa* L. (Amaryllidaceae) após exposição em águas de cinco lagoas de Alta Floresta, Estado do Mato Grosso

RESUMO

O presente estudo teve como objetivo avaliar o potencial mutagênico e citotóxico por meio da análise das células da raiz de *Allium cepa* expostas a amostras de águas de cinco lagoas urbanas de Alta Floresta - MT. As amostras foram obtidas nos períodos da seca, outubro de 2014 e chuvoso, abril de 2015, em 5 pontos distintos, sendo os pontos de coletas, a Lagoa 01, situada na MT 206, Lagoa 02, na Avenida Perimetral Rogério Silva, Lagoa 03, no Setor C, Lagoa 04, na Avenida Teles Pires e Lagoa 05, no Bairro Cidade Bela. Bioensaio com bulbos de *Allium cepa* foram expostos à água de cada ponto, comparando-se com o controle negativo e positivo. Diante dos resultados obtidos foi possível verificar a presença de atividades mutagênico e citotóxico sobre as raízes de *A. cepa*, indicando elevado potencial na inibição do ciclo celular. Esse resultado também pode vir a ser decorrente das influências dos períodos sazonais, levando em consideração, que na estação chuvosa, há maior carreamento de substâncias citotóxicas pela água da chuva e na estação seca, uma maior concentração dos poluentes pela redução do volume de água, conduzindo maior interação dos concentrantes como também, maior concentração dos elementos presente na água dessas lagoas.

Palavras-chave: bioensaio, períodos sazonais, potencial mutagênico e citotóxico.

1. INTRODUCTION

Degradation of water resources is a matter of concern, with increasing diagnosis and monitoring of pollution of aquatic environments (Christofoletti, 2008). According to Odum (1998), freshwater ecosystems and estuaries provide the systems that are considered to be the cheapest for effluent disposal. The largest cities in various regions are located near rivers, lakes, lagoons and estuaries, which eventually serve as garbage dumps and sewers; therefore, aquatic contamination by industrial and domestic discharges are of great concern each day for public health (Maria *et al.*, 2003).

Water is an essential resource for sustaining life. However, due to the different pressures applied to water resources, the issue of water availability and quality is one of the problems that is considered fundamental (Gaffney *et al.*, 2015). Household and industrial effluents contain numerous toxic substances that can cause serious damage to genetic material. Aquatic contaminants from landfills in the riparian areas, use of pesticides and fertilizers from agriculture, population growth and the lack of public policies all fuel environmental impacts (Ronconi, 2013).

Allium cepa is considered suitable for analysis of chromosomal damage and disorders in the mitotic cycle induced by toxic agents, a short-term assay with several advantages, such as its low cost, easy handling, chromosomes at adequate conditions for studies on damage or disorder in cell divisions and risk assessments of aneuploidy (Belcavello *et al.*, 2012). *A. cepa* can detect several classes of contaminants, such as heavy metals, domestic and industrial sewage, landfill extracts and water samples from rivers and lakes, whose solutions include a complex mixture of substances of different compositions. It is efficient and confirms sensitivity and effectiveness (Barbério, 2013).

Biological toxicity and genotoxicity tests are therefore indispensable and recommended for the development of monitoring studies and their purpose is to evaluate the extent of environmental pollution and the reactions of living organisms to the facts proposed (Caritá, 2010). This study evaluated genotoxic and cytotoxic potential of water samples through the analysis of *Allium cepa* root cells exposed to samples from five urban lakes in Alta Floresta, state of Mato Grosso.

2. MATERIAL AND METHODS

The experiment was carried out at the Cytogenetic and Plant Tissue Culture Laboratory of

UNEMAT, Campus Alta Floresta, state of Mato Grosso, located in Jardim Flamboyant, Alta Floresta, state of Mato Grosso.

The collection sites were named “Lake 01” - located at MT 206; “Lake 02” – on Rogério Silva Avenue; “Lake 03” - at Sector C; “Lake 04” – on Teles Pires Avenue; and “Lake 05” - Cidade Bela Neighborhood (Table 1). All lakes belong to the urban area of Alta Floresta, state of Mato Grosso (Table 1 and Figure 1).

In Lake 01, the local population disposes of sewage contaminant materials, remains of dead animals, among other things. From this same lake, the city's water sanitation company collects water to supply the region's population.

In Lake 02, the local population bathes and fishes. The municipal works secretariat draws water from this site during dry periods to wet the dirt streets to mitigate the effects of dust. In Lake 03, some people fish; however, it is used for sewage disposal by the local population.

Lake 04 is located in the central area of the city; just like 03, the local population uses it to dispose of sewage and household waste, while at the same time some people fish there for food. Lake 05 is located in a more-peripheral area of the city. The local population uses this water for bathing, fishing and garden irrigation. However, the same population disposes of contaminants in this same lake, such as sewage and household waste.

Table 1. Identification of the water collection site in the lakes and GPS data, Alta Floresta - MT.

Treatments	Collection site	Coordinates
Lake 1	Alta Floresta – MT	"South: 09°53'1.23" "West: 56°8'46.25"
Lake 2	Alta Floresta – MT	"South: 09°53'42.95" " West: 56°4'44.69"
Lake 3	Alta Floresta – MT	"South: 09°52'28.94" " West: 56°5'43.20"
Lake 4	Alta Floresta – MT	"South: 09°52'12.52" " West: 56°5'38.16"
Lake 5	Alta Floresta - MT	"South: 09°51'12,13" "West: 56°4'50.29"

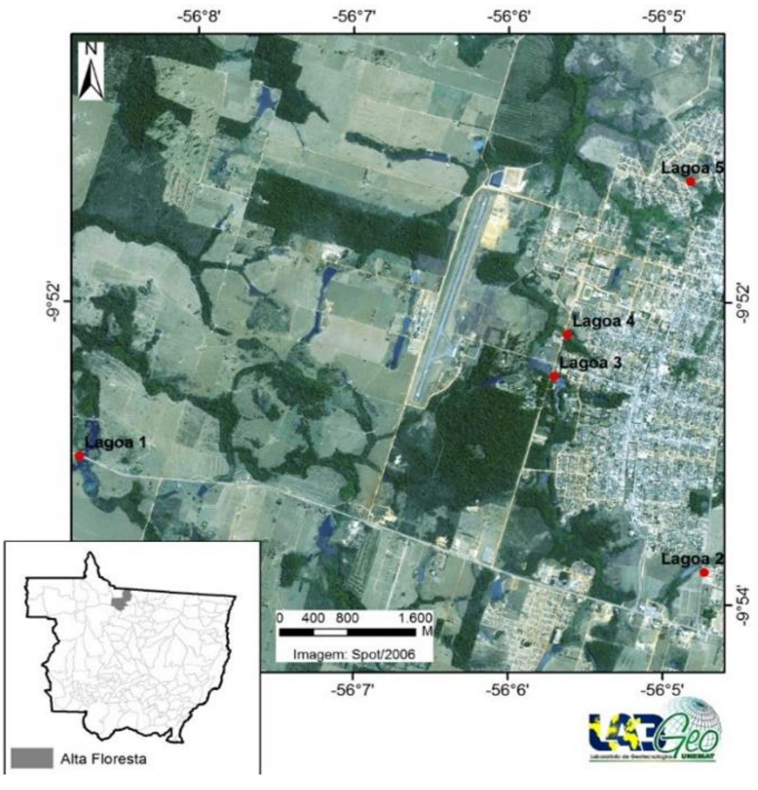

Figure 1. Location of the lakes, environments of water sample collection for the study, Alta Floresta MT.

Two water sample collections were carried out, in the dry season, October 2014, and in the rainy season, April 2015. Samples were collected from each lake, bank-inland direction, with a maximum distance of 50 cm from the bank in the first 30 cm of depth, using a 5-L polyethylene container for water storage.

The experiment (*A. cepa* test system) was done soon after the water samples were collected from the lakes. For the tests, seven treatments were used, out of which five were placed in the water sample from the lakes and one in distilled water with dipyrone for the positive control and another only in distilled water for the negative control. The bulbs, 5 repetitions per treatment, were placed in disposable plastic cups (50 mL) for rooting of the onions. Water was changed every 24 hours. After 72 hours, rootlets of approximately 5-10 mm length were fixed in ethanol-acetic acid (3:1) and kept in a refrigerator at 4^{o}C until use.

For the material digestion process, the root meristems of each sample were washed in distilled water using a plastic pipette on the Petri dish with three water changes, one after the other. Subsequently, the rootlets were dried on absorbent paper and then placed in 5N HCL solution for a period of 15 minutes. At the end of this process, the roots were washed again according to the previous procedure.

For the preparation of the slides, the roots were placed on the slide with the aid of a pair of tweezers and a scalpel, and then the meristematic region was cut, identified by the bleached stains. The rest of the material was discarded, leaving the material with cellular activity on the blade. Following the selection of the material, two drops of 2% acetic orcein dyes were added, following the maceration and dissociation of the material with the glass rod. The excess of the dye was removed with filter paper for optical microscope visualization and image selection. Ten slides were made per treatment.

Cells were randomly analyzed using the scanning method. A total of 15,000 cells were analyzed, including positive and negative control. The slides were evaluated by observing the cells (interphase, prophase, metaphase, anaphase and telophase) with the aid of an optical microscope at 40 X magnification.

The calculated index values followed the formulas below: MI = (normal cell number / 250 * 100); AP = (abnormal cell number / 250 * 100); PI = (number of interphase cells / 250 * 100); CTV = Cytotoxicity limit value = (positive mitotic index / negative control mitotic index * 100)

After counting *A. cepa* cells, the analysis of variance at 5% probability was performed and the means were compared by the test of Scott-knott, with the aid of the R software, Version 3.3.2 (R Core Team, 2016).

3. RESULTS AND DISCUSSION

Table 2 shows the data on the Mitotic Index (MI) and Anomaly Percentage (AP) of *A. cepa* obtained from the analysis of the water samples of five lakes collected in Alta Floresta, state of Mato Grosso. The analysis of the respective data showed that when the seasonal periods were compared, L2 followed by L5 and L1 obtained the highest percentages of Mitotic Index (MI) in the dry period, while for the rainy season, L1 had the highest Mitotic Index values of *A. cepa* cells. The negative control was significant in comparison to the other treatments.

In relation to the Anomaly Percentage (AP=) of A. cepa (Table 2), when comparing the seasonal periods, the dry period obtained the highest percentage of abnormal cells. The L1, L5 followed by L3 showed the highest abnormal cell indices. In the rainy season, the lakes did not differ statistically and the positive control showed significant statistical data in relation to the other treatments.

According to Tedesco and Laughinghouse IV (2012), Mitotic Index data are used as indicators of adequate cell proliferation. When analyzing the waters of two streams in the state of Paraná, Ferreira et al. (2012), using the bioindicator A. cepa did not find cytotoxicity through mitotic index (MI) analysis.

Table 2. Mitotic Index (MI), Anomaly Percentage (AP) of *Allium cepa* obtained in water samples of five lakes in the municipality of Alta Floresta, state of Mato Grosso.

TREATMENT/PERIOD	MI		AP	
	Dry	Rainy	Dry	Rainy
LAKE 1	13.24 bA	13.4 bA	7.48 aA	4.92 aB
LAKE 2	16.36 b A	8.64 cB	5.32 bA	3.16 aB
LAKE 3	11.72 cA	8.44 cB	7.00 aA	4.04 aB
LAKE 4	8.48 dA	8.76 cA	5.76 bA	3.68 aB
LAKE 5	15.08 bA	9.04 cB	7.48 aA	4.04 aB
POSITIVE CONTROL	11.8 c	11.8 b	3.93 c	3.93 a
NEGATIVE CONTROL	23.08 a	23.08 a	0 d	0 b
CV (%)	27,09		33,47	

Means followed by the same lower-case letter in the column and upper-case letter in the line are not different from each other by the test of Scott-knott at 5% probability.

In a study developed in the Paraíba do Sul River (SP), Oliveira *et al.* (2011) found that the mitotic index (MI) did not differ significantly from the control group.

Differences in toxicity between collection points may be related to water volume and may influence between seasonal periods and concentrations of toxic substances (Scalon, 2009; Oliveira *et al.*, 2012). This was observed in this study for all lakes in the dry season, where the values of the mitotic index and percentage of anomalies were higher than the rainy season.

In a study using *A. cepa* bulbs in Rio dos Sinos (Rio Grande do Sul - RS), Oliveira *et al.* (2012) obtained results with occurrence of cytotoxicity in the dry season. On the other hand, for the rainy season period, they did not find any significant results; these results corroborate those found in this paper. Galvão *et al.* (2015) reported in one of their works that *A. cepa* is considered an efficient bioindicator in this kind of analysis

Cuchiara *et al.* (2012), in an analysis of the waters of Arroio Padre Doutor (RS) in the rainy season using bioindicator *A. cepa*, did not obtain any results of interference with root growth. These results may be caused by the greater fluviometric volume in this period.

For the data obtained in the rainy season analysis, Bianchi *et al.* (2011), in tests carried out with water samples from the Monjolinho River, were not statistically different when compared to the negative control data; however, it differed from the dry period.

Faria *et al.* (2017), in a study of the toxicity potential in water samples of the Jaru River in the State of Rondônia during the dry season, August 2011, and rainy season, February 2012, using *A. cepa* cells, obtained results where the *A. cepa* test showed cytotoxicity in relation to root growth only for the dry season. Nevertheless, this fact was not observed for the Mitotic Index with significance in any of the analyzed periods.

Table 3 shows the Interphase Percentage (IP) numbers and the *A. cepa* Cytotoxicity Limit Value (VLC). The analysis of these data shows that L4 obtained the highest interphase cell indices as the positive control obtained percentages that are considered significant when compared to the other treatments of the experiment. For the cytotoxicity threshold values (CTV), the treatments of the dry period reduced the Mitotic Index, impairing cell division. Therefore, it is possible to observe by the cytotoxicity threshold value that the treatments of the dry period have greater cytotoxic effect than the treatments of the rainy season. This may have occurred due to the dissociation of some substances in the lake water, and in relation to the water volume of each lake in each period. Statistically, the negative control showed significant results in relation to the positive control and the other treatments.

Mitosis takes only a small proportion of the cell cycle, approximately 5 to 10%. The remaining time is for the interphase, composed of stages G1, S and G2. DNA is replicated during the S phase. Chromosomes cannot be seen during interphase because they are in a distended and intertwined state (Griffiths *et al.*, 2011).

Table 3. Percentage of Interphase (PI) and Cytotoxicity Threshold Value (CTV) of *Allium cepa* obtained from the water samples of five lakes in the municipality of Alta Floresta, state of Mato Grosso.

TREATMENTS/PERIOD	PI		CTV	
	Dry	Rainy	Dry	Rainy
LAKE 1	79.28 bA	81.68 bA	57.36 bA	58.05 bA
LAKE 2	78.32 bB	88.20 aA	70.88 bA	37.43 cB
LAKE 3	81.28 bB	87.52 aA	50.78 cA	36.56 cB
LAKE 4	85.76 aA	88.16 aA	36.74 dA	37.95 cA
LAKE 5	77.44 cB	87.32 aA	65.33 bA	39.16 cB
POSITIVE CONTROL	84.26 a	84.26 a	51.12 c	51.12 b
NEGATIVE CONTROL	76.92 b	76.92 c	99.99 a	99.99 a
CV (%)	4.98		27,09	

Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ statistically by the test of Scott-knott at 5% probability.

According to Fachinetto *et al.* (2007), the occurrence of an interphase cell index act may be associated with the antiproliferative consequence of mitotic cells. Souza *et al.* (2013) report that the antiproliferative effect can be observed through the inhibition of mitosis, or if there is a high number of interphase cells.

Oliveira *et al.* (2013) report that a reduction in the CTV of less than 22% of the negative control value can cause lethal effects, and reductions below 50% usually cause sublethal effects.

While studying the antiproliferative effect of *Ilex paráguariensis* on the cell cycle of *A. cepa* bioindicator, Conceição (2010) found that the treatments using infusions (*chimarrão*) had VLC lower than 50%, and sublethal effects; however, for treatments using cold water (*tereré*), the Mitotic Index was not reduced.

Based on the cells observed in the study, different types of chromosomal anomalies were found: micronucleus binucleate interphase, three-block metaphase, isolated chromosome metaphase, isolated chromosome anaphase, delayed displacement chromatin, delayed telophase in organization of the chromatids in its block, final telophase with presence of micronucleus, metaphases with chromosomal adhesion, metaphases and anaphases with chromosomal bridges. Based on the analyses, it is found that genotoxic action occurred, as abnormal cells were found during cell division processes, with genotoxic effects. The results obtained for the mitotic index from the study using the *A. cepa* test system were considered satisfactory and indicative of genotoxic potential on *A. cepa* cells (Figure 2).

Souza *et al.* (2012) report in one of their works that the genotoxic potential is evident because of the presence of chromosomal anomalies analyzed at all collection points, in which alteration in cells in metaphase with C-metaphase, in anaphases with bridges, loss and multipolarity, and binucleated cells were the most observed anomalies in the trials, besides chromosome breaks and losses. Therefore, water treatment with concentrating agents causes genotoxicity in the meristematic cells of *A. cepa*.

Leme *et al.* (2009), report that chromosomal abnormalities in *A. cepa* can be found in the stages of mitosis (prophase, metaphase, anaphase and telophase). The micronuclei originate from acentric chromosomal fragments resulting from mitotic spindle dysfunctions and may appear more than once in each cell (Balieiro *et al.*, 2010).

Marcano *et al.* (2004), point out that chromosomal bridges may have occurrences with consequences of a junction and then a failure in the separation of chromosomes in anaphase, and it can also be attributable to an unequal translocation or inversion of chromosome segments.

According to Esteves (1998), in flooding environments, lakes or very shallow lakes, there may be a greater interaction of the elements found in the sediment with the water column, and this may occur seasonally.

However, it is observed that a higher rate of abnormal cells occurred over the dry season, because in the dry season, there may be a greater interaction of concentrates in the waters of these lagoons, resulting in a concentration of residues in the water.

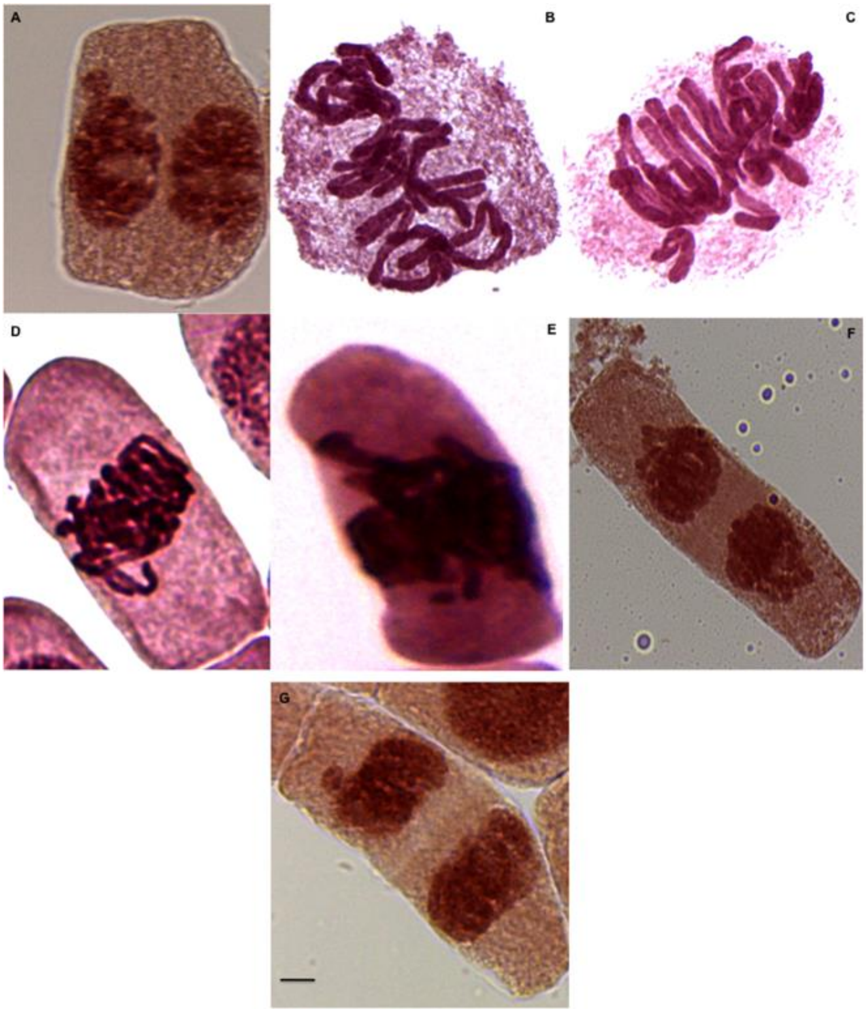

Figure 2. *Allium cepa* cell from cell division, obtained from different exposure treatments in water samples from lakes located in Alta Floresta - MT. (a) micronucleus binucleate interphase; b) Metaphase with formation of three blocks; c) Metaphase with isolated chromosome; d) Anaphase with isolated chromosome; e) Anaphase with isolated chromosome and delayed displacement chromatin; f) Telophase with delayed organization of the chromatids in each block; g) Final telophase with presence of micronucleus, Bar = 10 µm.

4. CONCLUSIONS

Based on the results of the study, it was possible to observe the presence of genotoxic and cytotoxic activities on the roots of *Allium cepa*, indicating high potential in cell cycle inhibition. This result may also be caused by the influences of seasonal periods, taking into account that a higher transport of cytotoxic substances by rainwater occurs in the rainy season, and in the dry season, there is a higher concentration of pollutants due to the reduction of water volume, leading to greater interaction of the concentrators as well as greater concentration of the elements found in the waters of these lakes.

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Energy potential of biogas and sludge from UASB reactors in the State of Paraná, Brazil

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ABSTRACT

Upflow Anaerobic Sludge Blanket (UASB) reactors are widely used for domestic sewage treatment in Brazil, and generate sludge and biogas as by-products. Typically, the sludge is sent to sanitary landfills, and the biogas is burned in flares. This study assessed the energy potential of these by-products in sewage treatment plants (STPs) located in the state of Paraná, Brazil. First, an overview of biogas energy recovery potential in a full-scale STP based on UASB reactors was carried out. Afterwards, a total of 239 STPs in the state of Paraná were assessed, and the energy potential of the by-products was estimated by a mathematical model. Data were spatially classified using ArcGIS®, taking into account the different effluent characteristics (worst, typical and best scenario), sludge dehydration systems (drying bed and centrifuge) and sizes (small, medium and large). Among the states in Brazil, Paraná presented the highest biogas potential, although nowadays it exploits only 0.4% of its capacity. Biogas was the main by-product generated by UASB reactors in Paraná, and if used in the entire state, it could supply the energy demands of a city with 111,000 inhabitants. Biogas corresponded to 65, 64 and 74% of the total potential (sludge and biogas) for small, medium and large STPs, respectively. The study of the energy potential of the by-products associated with GIS tools positively contributes to the decision-making process in sanitation management and to improve energy sustainability.

Keywords: energy recovery, sewage treatment plant, UASB reactor.

Potencial energético do biogás e lodo de reatores UASB no Estado do Paraná, Brasil

RESUMO

Os reatores anaeróbios de fluxo ascendente e manta de lodo (UASB) são amplamente utilizados para tratamento de esgoto doméstico no Brasil e geram como subprodutos lodo e biogás. Normalmente o lodo é enviado para aterros sanitários e o biogás é queimado em *flare*.

O objetivo desse estudo foi avaliar o potencial energético desses subprodutos em estações de tratamento de esgoto (ETEs) localizadas no estado do Paraná, Brasil. Inicialmente foi elaborada uma visão geral do potencial de recuperação de energia do biogás em ETEs, em escala plena, que utilizam reatores UASB. Posteriormente, 239 ETEs no estado do Paraná foram avaliadas, sendo o potencial energético dos subprodutos estimado por um modelo matemático. Os dados foram espacialmente classificados utilizando o ArcGIS®, levando-se em consideração as diferentes características do efluente (pior cenário, cenário típico e melhor cenário), sistemas de desidratação do lodo (leito de secagem e centrífuga) e tamanhos (pequeno, médio e grande). Entre os estados do Brasil, o Paraná apresentou o maior potencial energético de biogás, embora explore apenas 0,4% de sua capacidade. O biogás é o principal subproduto gerado pelos reatores UASB no Paraná de modo que, caso utilizado em todo o estado, poderia suprir a demanda de energia de uma cidade com 111.000 habitantes. O biogás correspondeu a 65, 64 e 74% do potencial energético total (lodo e biogás) para as pequenas, médias e grandes ETEs, respectivamente. O estudo do potencial energético dos subprodutos avaliados associado à utilização de ferramentas de SIG contribui positivamente para o processo de tomada de decisão na gestão de saneamento e para melhorar a sustentabilidade energética.

Palavras-chave: estação de tratamento de esgotos, reator UASB, recuperação de energia.

1. INTRODUCTION

Among the technological alternatives for sewage treatment in Brazil, the anaerobic systems can be highlighted (Santos *et al.*, 2016), especially with the wide use of upflow anaerobic sludge blanket reactors (UASB). Chernicharo *et al.* (2018) have recently accounted for more than 650 full-scale UASB based STPs with treatment capacity of 43 m^3 s^{-1}, corresponding to nearly 23 million inhabitants. The interest in such systems is particularly associated with their low costs for installation and operation, as well as the low demand for area and energy consumption (Khan *et al.*, 2011).

The expansion of STPs that use anaerobic treatment systems in Brazil has increased the generation of by-products of anaerobic digestion, such as biogas and sludge (ROSA *et al.*, 2018). These by-products can be used for energy recovery purposes, which has raised interest in their management, due to the possibility of diversifying and expanding the Brazilian energy matrix and reducing the consumption of fossil fuels and the emission of greenhouse gases (Bernal *et al.*, 2017).

Several studies have reported the experience of developed countries in recovering biogas and using the produced energy to add energy, economic and financial benefits to the sewage treatment (Larson *et al.*, 2016; Lindkvist and Karlsson, 2018). However, a great part of the biogas produced in STPs in developing countries is not used to produce energy, as observed in Brazil, where such use is still incipient and limited to a small number of treatment plants (Santos *et al*., 2016).

The sludge produced in STPs is also regarded as a feasible energy source by contributing self-sufficiency energy in STPs and reducing residue for the final destination (Yang *et al.*, 2016). Sludge in Brazil is typically sent to landfills, disregarding among other issues its energy potential. In addition, the management of this by-product corresponds up to 60% of total operational expenses in an STP (Valente, 2015). For many countries of the EU, the use of thermal processes for the management of sludge produced in STPs, for energy purposes, is estimated to double by 2020, with 2012 as a base year, corresponding to around 37% of sludge management options (Kelessidis and Stasinakis, 2012). Therefore, it is expected that the sludge, usually characterized as the type of residue disposed of in landfills, will become an energy source, a paradigm change regarding its final destination (Gu *et al.*, 2017).

For Bernal *et al.* (2017) and Rosa *et al.* (2016), studying the energy recovery from by-products generated in UASB reactors is important and can contribute to enhance the Brazilian energy matrix by using renewable sources from the sanitation sector.

Paraná State has the greatest number of UASB reactors in Brazil: 89% (235) of the STPs use this technology, and treatment flows range from 3 L s^{-1} to 4032 L s^{-1}, but only 0.4% of this potential is actually used to produce electricity (Sanepar, 2016). These numbers show the energy potential of the biogas produced as a result of anaerobic sewage treatment.

In order to enable and encourage energy recovery from by-products, mathematical models and Geographical Information Systems (GIS) can be useful tools to estimate and assess the energy potential of UASB reactors, as well as to improve studies on energy self-sufficiency in STPs and contribute to the decision-making process in sanitation management. GIS have been considered a powerful tool that allows, among other resources, the location of potential areas for biogas use, as recently carried out by Sahoo *et al.* (2018).

In Brazil, studies that evaluate the potential energy of by-products (biogas and sludge) in UASB reactors do not have geo-referenced information. When the energy potential is combined with operational and local issues, this could be essential for rapid assessment and diagnosis of its potential in different regions.

In this context, to provide a basis for the decision-making process in sanitation management in Brazil, and to promote the use of sewage treatment by-products to produce energy, this study assessed sludge and biogas energy potentials from UASB reactors in the state of Paraná, Brazil, and also verified spatial distribution using GIS based tools.

2. MATERIALS AND METHODS

2.1. An overview of biogas energy recovery potential in STP based on UASB reactors

First, an inventory of full-scale STPs that actually implemented the biogas energy recovery in Brazil was carried out. This survey was conducted using direct contact with Sanitation Companies that already promote electricity generation by the use of biogas as an energy source in 2018. The information requested includes the following: an installed wastewater treatment system, the actual population that is served and the average influent flow.

Afterwards, the STPs operated by UASB reactors with energy recovery of biogas was evaluated. It was then compared with the UASB installed capacity to treat domestic sewage in Brazil.

The current situation of biogas energy potential in STPs operated by UASB reactors was estimated using the actual population served. An estimate was also made of the full potential of biogas of STPs operated by UASB reactors associated with an installed capacity that would promote the energy recovery of this by-product. The unitary relationship of 133.8 MJ $inhab^{-1}$ y^{-1} proposed by Lobato (2011) was used to determine the current and full energy potential of biogas.

After the overview that highlighted the biogas energy recovery at STPs in Brazil, the potential of renewable energy sources (biogas and sludge) was assessed in Paraná, the state with the highest number of full-scale UASB-based treatment plants. The evaluation was carried out using secondary data provided by the Water and Sanitation Company of Paraná State for 2016. The database consisted of sewage flow, dehydration units and STPs localization. The Geographic Information System (GIS) was used as a tool for the decision-making process in sanitation management.

2.2. Energy potential of biogas and sludge from UASB reactors in Paraná State

The study was carried out in several STPs in Paraná State, Brazil. Out of the 239 currently operating STPs, 182 treat sewage using UASB reactors, of which only the Ouro Verde STP, which is able to treat up to 70 L s^{-1} of sewage, produces electricity from biogas.

Out of the 182 STPs assessed, 158 operate with drying beds and 8 operate with centrifuges; 16 units do not have dehydration systems. The sludge is sent to landfills or disposed in agricultural areas. The STPs were classified by size as “small” (lower than 56 L s^{-1}), “medium” (from 57 up to 434 L s^{-1}) and “large” (greater than 435 L s^{-1}), according to the Paraná Environmental Institute (IAP) and CONAMA Resolution no. 377/2006.

Figure 1 shows spatially the STPs operated by UASB reactors in Paraná State according to their sizes.

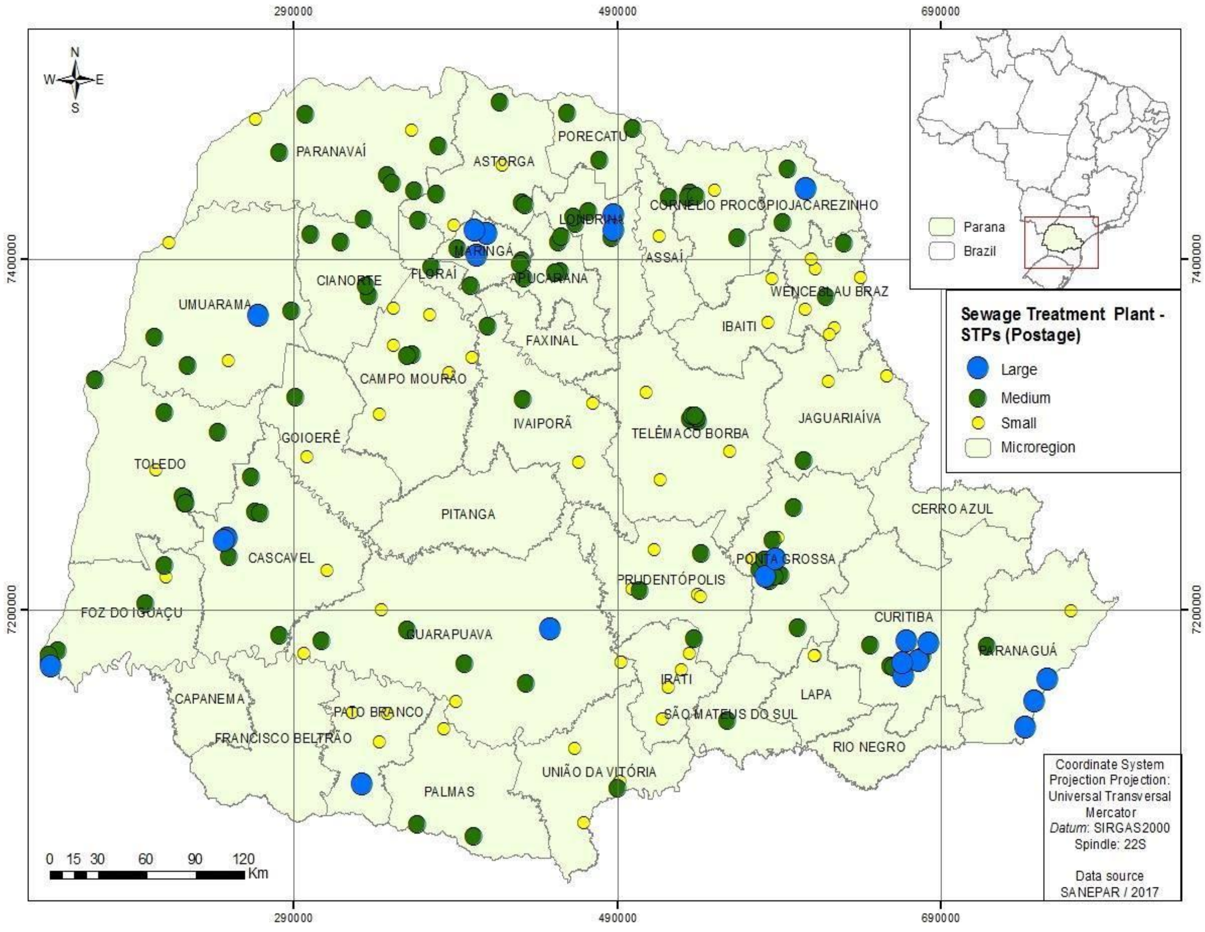

Figure 1. Location of STPs based on UASB reactors in Paraná State, according to their size.

2.3. Energy potential of by-products (biogas and sludge)

A mathematical model was structured in an electronic spreadsheet using the premises of simplicity and a reduced number of input data. The mathematical model was developed to estimate the energy potential of biogas and sludge in three scenarios that correspond to different sewage and reactor characteristics, namely: (i) worst scenario; (ii) typical scenario; and (iii) best scenario, as reported by Rosa *et al.* (2013).

2.4. The input data of the model

The variability of input data presented by value range (Table 1) was incorporated into the model through uncertainty analysis, which is based on the execution of a high number of simulations (in this case 250 simulations for each situation), called a "Monte Carlo" simulation. In each model execution, a different group of input values is selected randomly according to the uniform distribution within pre-established ranges.

Table 1. Input data of biogas and sludge used in the model.

	Parameter	Unit	Scenario		
			Worst	Typical	Best
Biogas	Population equivalent (Pop)	inhab.	1,000 - 1,000.000		
	Contribution per capita of sewage (PSC)	m^3 $inhab^{-1}$ d^{-1}	0.12 - 0.22		
	Contribution per capita of COD (QPC_{COD})	m^3 $inhab^{-1}$ d^{-1}	0.09 - 0.11		
	Removal efficiency of COD (E_{COD})	%	60	65	70
	Sulphur concentration in sewage (CSO_4)	$kgSO_4$ m^{-3}	0.08	0.06	0.04
	Sulphur efficiency reduction (ESO_4)	%	80	75	70
	Operational temperature in the reactor (T)	°C	20 - 30		
	Percentage of CH_4 in the biogas (CCH_4)	%	70	75	80
	COD_{CH4} lost as waste gas (p_W)	%	75	5.0	2.5
	Other COD_{CH4} losses (p_o)	%	75	5.0	2.5
	Dissolved COD_{CH4} lost with the effluent (p_L)	kg m^{-3}	0.025	0.020	0.015
	Inside the UASB reactor				
Sludge	Sludge density (ρs)	kg m^{-3}	1,020 - 1,030		
	Solids concentration (C)	%	3 - 6		
	Sludge yield (Y_{ss})	kgSS $kgCOD_{app}^{-1}$	0.18		
	Dehydrated sludge				
	Solids capture – Centrifuge (Sc)	%	90 - 91	92 - 93	94 - 95
	Solids capture – Drying bed (Sc)	%	90 - 92	93 - 95	96 - 98
	Solids concentration – Centrifuge (C’)	%	18 - 21	22 - 24	25 - 27
	Solids concentration – Drying bed (C’)	%	30 - 34	35 - 39	40 - 44
	Sludge density (ρs’)	kg m^{-3}	1,050 – 1,080		
	Carbon (C)[1]	%	18	30	38
	Hydrogen (H)[1]	%	3.6	4.4	6.1
	Nitrogen (N)[1]	%	1.6	1.9	2.5
	Sulphur (S)[1]	%	0.8	1.2	1.4
	Oxygen (O)[1]	%	21	22	24
	Ashes[1] (A)[1]	%	55	40.5	28

The model developed to estimate the energy potential of biogas was conceptually structured based on the COD conversion routes and methane flows in UASB reactors. The input data needed for calculating the energy potential of biogas were: population equivalent (Pop), per capita contribution of sewage (PSC), per capita contribution of COD (QPC_{COD}), removal efficiency of COD (E_{COD}), sulfate concentration in sewage (C_{SO4}), sulfate reduction efficiency (E_{SO4}), reactor operational temperature (T), percentage of CH_4 in the biogas (C_{CH4}) and losses in the system (p_W, p_o, p_L). The value ranges for the input data of the model were derived from a review of the literature as presented in Lobato *et al.* (2011). The calculation of mass energy balance parcels for COD and the biogas energy potential are presented by Lobato *et al.* (2012) (Table 2).

The following variables were considered for estimating the energy potential of sludge: density (ρs), solids concentration (C); solids yield coefficient (Y); solids capture in the dehydration unit (Sc) after dehydration; solids concentration (C'); density (ρs') and elemental composition. The value ranges for the elemental composition of the sludge were derived from a review of the literature as presented in Rosa *et al.* (2016). The calculation of energy potential of sludge are presented by Rosa *et al.* (2013) and transcribed in Table 3. The mathematical models for sludge and biogas were developed by Rosa *et al.* (2013) and Lobato *et al.* (2012), respectively. Since the models were validated by those authors, the simulated data regarding the energy potential of biogas and sludge are compatible with monitored data for STPs of different sizes.

The production of solids in UASB reactors (Ps) and the production of solids in dehydration (Ps') for mechanical dehydration alternatives (drying beds and centrifuges) were determined according to Von Sperling and Chernicharo (2005). The lower Calorific Values (LCV) were determined according to Cortez *et al.* (2008). The sludge energy potential takes into account the sludge production and the LCV, as presented in Table 3.

2.5. The energy potential estimation of by-products in UASB reactors in Paraná State

After running the mathematical model, 250 theoretical STPs were simulated and then **(i)** linear regressions were obtained for the simulated data to estimate the by-products energy potential considering the sewage flow; **(ii)** following these equations, the sewage flows of STPs in Paraná State were used to determine the biogas and sludge energy potential; and finally, **(iii)** the energy potential of the by-products was assessed for different sizes, scenarios and dehydration units. Thematic maps were used to represent the energy potential of the by-products in full-scale STPs in Paraná/Brazil by using the software ArcGIS® 10.2, where the STPs' geographic locations, flows and energy potentials were converted into point-vector shapefiles (*shp). Using the *Analysis Tools (Spatial Join)*, the spatial elements of the spreadsheet with the shapefile's attribute table were associated with the microregions of Paraná State and the classification of the potentials was performed. Thematic maps were designed considering STPs based on UASB reactors in Paraná State and the microregions were delimited according to the base system presented by the Brazilian Institute of Geography and Statistics (IBGE).

3. RESULTS AND DISCUSSIONS

3.1. An overview of biogas energy recovery potential in STP based on UASB reactors

In accordance with the evaluation done in Brazil, there are only ten full-scale STPs where biogas energy recovery is in process, as shown in Table 4. Out of the ten STPs, only four treat sewage using full-scale UASB-based treatment plants. These units are concentrated in the States of Minas Gerais, São Paulo and Paraná. Table 4 indicates the current- and full energy potential of biogas for the states. The state of Paraná presents the highest biogas energy potential, albeit only 0.4% of its potential is exploited.

Table 2. Equations for calculating the portions of the mass balance of COD and energy recovery potential.

Parcels	Equations	Notes
Estimate of mean influent flow rate	$F_{mean} = Pop \times PSC$	F_{mean} = Mean influent flow rate ($m^3 d^{-1}$) Pop = population (inhab.) PSC = *per-capita* sewage contribution (m^3 $inhab^{-1}$ d^{-1})
Estimate of daily COD mass removed from the system	$COD_{remov} = Pop \times PSC \times COD_{inf}$ $COD_{remov} = F_{mean} \times COD_{inf} \times E_{COD}$	COD_{remov} = daily COD mass removed from the system (kgCOD d^{-1}) COD_{inf} = COD concentration influent (kgCOD m^{-3}) E_{COD} = efficiency of COD removal (%) F_{mean} = mean influent flow rate ($m^3 d^{-1}$)
Estimate of daily COD mass used by the biomass	$COD_{sludge} = COD_{remov} \times Y_{COD}$ $Y_{COD} = YTVS \times K_{TVS-COD}$	COD_{sludge} = daily COD mass converted into biomass ($kgCOD_{sludge}$ d^{-1}) Y_{COD} = sludge yield, as COD ($kgCOD_{sludge}$ $kgCOD_{rem}^{-1}$) Y_{TVS} = sludge yield, as total volatile solids (TVS) (kgTVS $kgCOD_{rem}^{-1}$) $K_{TVS-COD}$ = conversion factor (1kgTVS = 1.42 $kgCOD_{sludge}$)
Estimate of sulfate load converted into sulfide	$CO_{SO_4 converted} = F_{mean} \times C_{SO_4} \times E_{SO_4}$	$CO_{SO4converted}$ = load of SO_4 converted into sulfide ($kgSO_4$ d^{-1}) C_{SO4} = average influent SO_4 concentration ($kgSO_4$ m^{-3}) E_{SO4} = efficiency of sulfate reduction (%)
Estimate of daily COD mass used in sulfate reduction	$COD_{SO_4} = CO_{SO_4 converted} \times K_{COD-SO_4}$	COD_{SO4}= COD used by the sulfate reducing bacteria for sulfate reduction ($kgCOD_{SO4}$ d^{-1}) $K_{COD-SO4}$ = COD consumed in sulfate reduction (0.667 $kgCOD_{SO4}$ $kgSO_4^{-2}$)
Estimate of daily COD mass converted into methane	$COD_{CH_4} = COD_{remov} - COD_{sludge} - COD_{SO_4}$ $Q_{CH_4} = \frac{COD_{CH_4} \times R \times (273+T)}{P \times K_{COD} \times 1{,}000}$	COD_{CH4} = daily COD mass converted into methane ($kgCOD_{CH4}$ d^{-1}) Q_{CH4} = theoretical volumetric production of methane ($m^3.d^{-1}$ R = gas constant (0.08206 atm L mol^{-1} K^{-1}) T = operational temperature of the reactor (ºC) P = atmospheric pressure (1 atm) K_{COD} = COD of one mole of CH_4 (0.064 $kgCOD_{CH4}$ mol^{-1})
Estimate of methane loss	$Q_{O-CH_4} = Q_{CH_4} \times p_O$ $Q_{W-CH_4} = Q_{CH_4} \times p_w$ $Q_{L-CH_4} = Fmean \times p_L \times f_{CH_4}\left(\frac{R \times (273+T)}{P \times K_{COD}}\right)$ $p_L = \frac{\%CH_4}{100} \times k_h \times F_s$	Q_{W-CH4} = methane loss as waste gas ($m^3 d^{-1}$) p_w = percentage of methane in the gaseous phase lost as waste gas (%) Q_{O-CH4} = other methane losses in the gaseous phase ($m^3 d^{-1}$) p_O = percentage of methane in the gaseous phase considered as other losses (%) Q_{L-CH4} = loss of dissolved methane in the liquid effluent ($m^3 d^{-1}$) p_L = concentration of dissolved methane in the liquid effluent (kg m^{-3}) f_{CH4} = conversion factor of methane mass into COD mass (4 kgCOD $kgCH_4^{-1}$) K_h = constant of Henry ($mg.L^{-1}$ atm^{-1}) F_s = supersaturation factor of CH_4 in the liquid phase % CH_4 = percentage of CH_4 in the biogas
Estimate of actual methane production	$Q_{actual-CH_4} = Q_{CH_4} - Q_{W-CH_4} - Q_{O-CH_4} - Q_{L-CH_4}$	$Q_{actual-CH4}$ = actual production of methane available for energy recovery ($m^3 d^{-1}$)
Energy recovery potential	$PE_{actual-CH_4} = Q_{N-actual-CH_4} \times E_{CH_4}$	$PE_{actual-CH4}$ = energy potential of biogas (MJ d^{-1}) $Q_{N-actual-CH4}$ = normalized methane production ($Nm^3 d^{-1}$) E_{CH4} = calorific energy of methane (35.9 MJ Nm^{-3})

Table 3. Equations for calculating the energy potential of sludge.

Parcels	Equations	Notes
	UASB reactor	
Influent COD load	$COD_{app} = Pop \times PCL_{COD}$	COD_{app} = COD load applied to the system (kgCOD d^{-1}) Pop = population (inhab.) PCL_{COD} = *per capita* contribution of COD (kg.inhab^{-1} d^{-1})
Sludge production	$P_s = COD_{app} \times Y_{ss}$	P_s = sludge production in the system (kg SS d^{-1}) Y_{SS} = sludge yield (0.18 kgSS kg CODapp^{-1})
Volumetric sludge production	$V_s = \frac{P_s}{\left[\frac{C}{100}\right] \times \rho_s}$	V_s = volumetric sludge production (m^3 d^{-1}) C = solids concentration in the sludge (%) ρ_s = sludge density (kg m^{-3})
	Dewatering processes	
Dry sludge production	$P_s' = \frac{S_c}{100} \times P_s$	P'_s = sludge production in the dehydration unit (kgSS d^{-1}) S_c = solids capture in the dehydration unit (%)
	Energy potential of sludge	
Theoretical HCV	$HCV = \frac{\left[337{,}3 \times C + 1.418{,}9 \times \left(H - \frac{O}{8}\right) + 93{,}1 \times S + 23{,}3 \times N\right]}{1.000}$	HCV = higher calorific value, (MJ kg^{-1}), d.b. *Elemental composition* *C; H; O; S; N* = % of carbon, hydrogen, oxygen, sulfur, nitrogen in the material, d.b.
Theoretical LCV	$LCV = [(HCV - \lambda \times (r + 0{,}09 \times H))(100W_t)/100]$ $r = \frac{W_t}{(100 - W_t)}$	LCV = lower calorific value (MJ kg^{-1}), m.b. λ = latent heat of water (2,31 MJ kg^{-1} in 25 °C); r = solids content and dehydrated sludge moisture ratio H_s = hydrogen content (%), d.b. W_t = solids content in the dehydrated sludge (%), m.b.
Sludge energy potential	$PE_{sludge} = LCV \times P_s'$	PE_{sludge} = energy potential of sludge (MJ d^{-1})

d.b: dry basis, m.b: moist basis (real moisture of the sample after collection).

Table 4. The summary of full-scale STPs in Brazil with energy recovery of biogas.

Location	Sanitation company	STP	Treatment system	Population served (inhab.)	Installed capacity (inhab.)[1]	CEP (GJ d^{-1})[2]	FEP (GJ d^{-1})[3]
Minas Gerais	COPASA	Arrudas	Activated sludge	1,343,867	4,049,193	218	1,484
		Betim	UASB	266,495			
		Ibirité	Activated sludge	115,604			
		Vieira/ Montes Claros	UASB	326,931			
São Paulo	SABESP	Barueri	Activated sludge	4,400,000	5,520,818	161	2,024
		Franca	Activated sludge	4,100.000			
	SEMAE	São José do Rio Preto	UASB	438,000			
	AMBIENT	Ribeirão Preto	Activated sludge	620,000			
Paraná	SANEPAR	Belém	Activated sludge	600,000	7,300,603	11	2,676
		Ouro Verde	UASB	29,500			

[1]Installed capacity in STPs based on UASB reactors for the states (Chernicharo *et al.*, 2018). [2]Current energy potential (CEP = Population served by UASB reactors * 133.8 * 0.001/365). [3]Full energy potential (FEP = Installed capacity of UASB reactors * 133.8 * 0.001/365).

3.2. Potential of renewable energy sources in Paraná, Brazil

3.2.1. Overall energy potential of the STP

Table 5 presents a summary of the linear regression relationships obtained for the simulated data to estimate the by-products' energy potential considering sewage flow. According to the characteristics described for the worst scenario that indicates no free energy after sludge combustion, there are not any applicable regression equations. This could be due to high level of ash, low concentration of hydrogen, low level of fixed solids and low solids concentration.

The use of the model allowed the simulation of the energy potential of biogas and dehydrated sludge in units with drying beds and centrifuges for different sizes of STPs, for the three scenarios considered for the sewage flow of STPs in Paraná State.

Table 5. Summary of linear regressions obtained for various proposed relations for biogas and sludge.

By-product	**System**	**Relation (x,y)**	Scenario: Worst		Typical		Best	
			Equation	(R^2)	Equation	(R^2)	Equation	(R^2)
Biogas	-	L s^{-1} x MJ d^{-1}	y = 129.46x	0.98	y = 180.91x	0.73	y = 244.09x	0.82
Sludge	DB	L s^{-1} x MJ d^{-1}	y = 4.96x	0.78	y = 108.82x	0.88	y = 186.23x	0.89
	C	L s^{-1} x MJ d^{-1}	Not applicable		y = 7,50x	0.84	y = 21,25x	0.86

DB: drying bed; C: centrifuge.

3.2.2. Biogas energy potential

Among the 182 STPs based on UASB reactors in Paraná State, 61 were classified as “small”, 99 as “medium” and 22 as “large”. The energy potential of small STPs for the typical scenario was 380 GJ d^{-1}. Medium- and large STPs presented biogas energy potentials of 2,869 GJ d^{-1} and 4,871 GJ d^{-1}, respectively, for the typical scenario. Despite the lower number of units, the large STPs (22 units) presented the highest biogas energy potential in Paraná State, 13 times higher than that of small STPs and 1.7 times higher than that of medium STPs. Considering a conversion yield of 30% by the power generator and the typical scenario, Paraná State could produce 677 MWh d^{-1} of electricity, enough to supply the energy demand around 111,000 inhabitants on the basis of a per capita energy consumption of 0.0061 MWh d^{-1} (Balanço Energético Nacional, 2017). Figure 2 presents the accumulated biogas energy potential of STPs, considering the different scenarios and sizes.

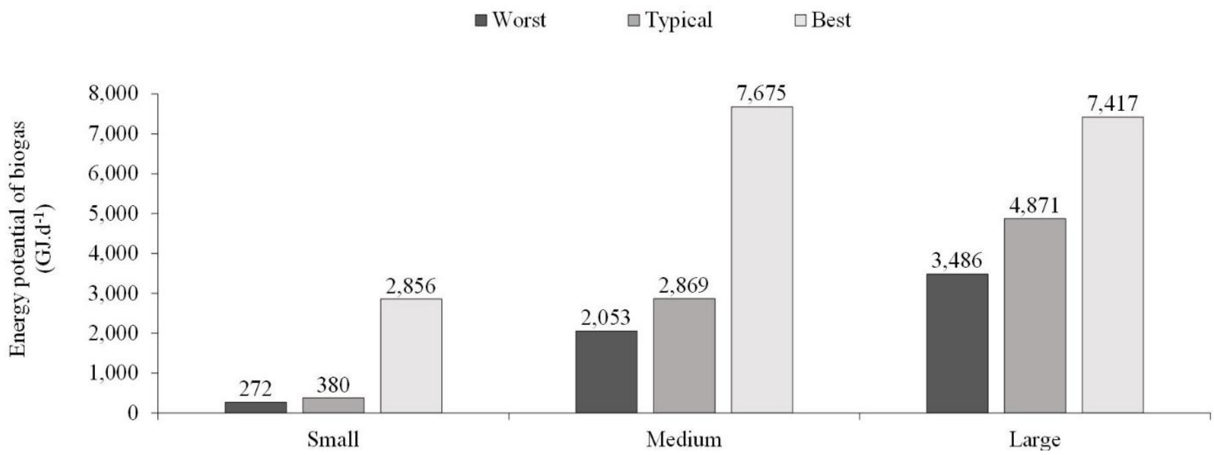

Figure 2. Biogas energy potential for the different scenarios and STP sizes in Paraná State.

Ouro Verde STP generates electricity from biogas in Paraná, but it produces only 0.4% of the maximum capacity to be achieved by a medium-size STP. For small- and large STPs, there are no records of biogas recovery for energy purposes, which indicates the need for investments in energy recovery at STPs, regardless of their size.

The analysis of the 99 medium-sized STPs resulted in mean energy potentials of 19, 29 and 39 GJ d^{-1} for the worst, typical and best scenarios, respectively. For the typical scenario, 74% of the STPs presented biogas energy potential of up to 38 GJ d^{-1}.

As previously mentioned, despite the lower number of STPs classified as “large” (22), all of them presented higher biogas potential in comparison to small- and medium STPs. The mean energy values obtained from the biogas energy recovery in large STPs corresponded to 142, 221 and 299 GJ d^{-1} for the worst, typical and best scenarios, respectively.

Figure 3 presents the biogas energy potential for the STPs in each microregion of Paraná for the typical scenario. By estimating the energy potential of the biogas produced by UASB reactors, it is possible to outline strategic plans for future investments in the energy recovery of the by-products. Most of the STPs (124 units) presented biogas energy potentials below 30 GJ d^{-1} and 19 STPs presented potentials higher than 100 GJ d^{-1}. The microregions of Curitiba and Londrina presented the highest biogas-energy potentials, more than 1,000 GJ d^{-1}, and the microregion of Curitiba accounted for 30% of the entire state's potential.

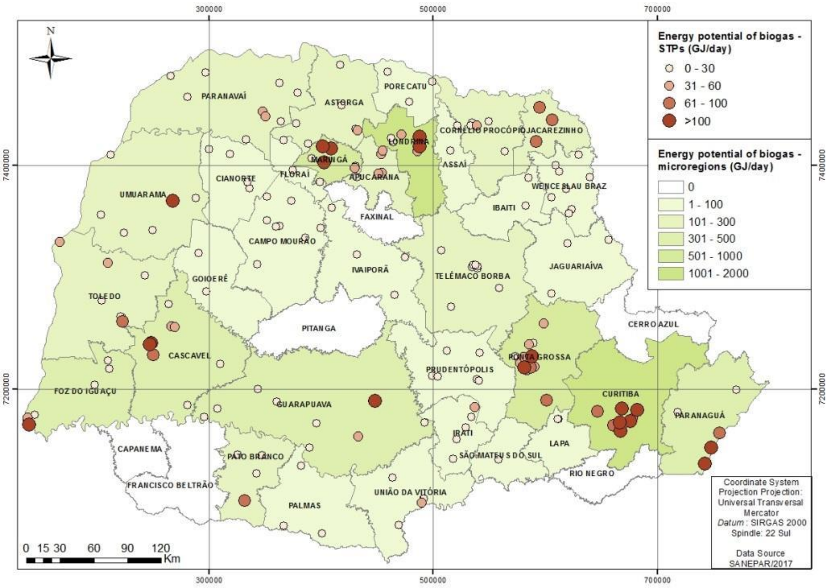

Figure 3. Biogas energy potentials for STPs in each microregion of Paraná State.

3.2.3. Sludge energy potential

For sludge management, 87% of the STPs use drying beds for the dehydration process, of which 33% (52 STPs) are small, 58% (91 STPs) are medium and 9% (15 STPs) are large. According to the typical scenario, the accumulated sludge energy potential for small, medium and large STPs corresponded to 205 GJ d^{-1}, 1,613 GJ d^{-1} and 1,582 GJ d^{-1}, respectively, indicating that the medium- and large STPs presented the highest potentials for energy recovery from this by-product. Figure 4 presents the accumulated energy potential of the dehydrated sludge removed from the drying beds, considering the different STP sizes and scenarios assessed.

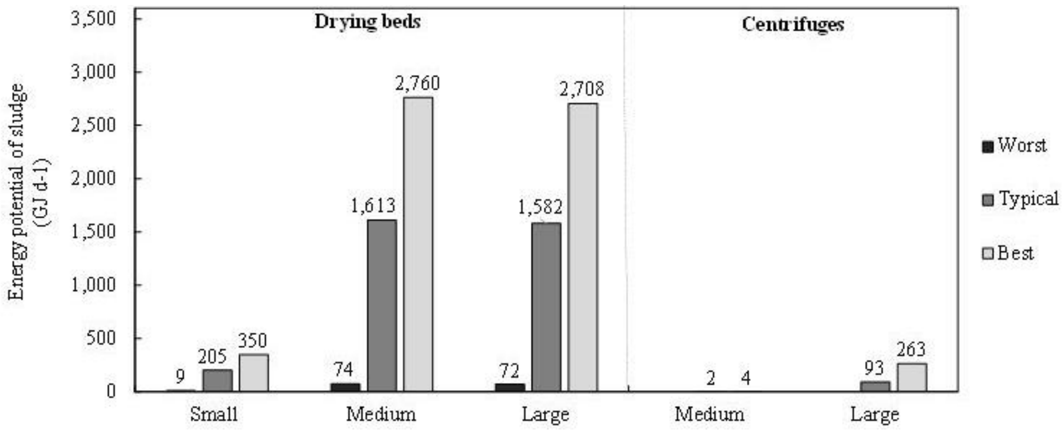

Figure 4. Sludge energy potential for the different scenarios and sizes of STPs that use drying beds and centrifuges for dehydration, in Paraná.

Also, small STPs presented mean values of 0.2, 3.9 and 6.7 GJ d^{-1} for the worst, typical and best scenarios, respectively. Overall, the use of drying beds instead of centrifuges points to a greater interest in sludge recovery from the energy aspect, because of the lower levels of moisture in the sludge cake.

Figure 4 shows the full energy potential of sludge from 8 STPs operated with centrifuges in Paraná State for the different scenarios. An absence of small STPs with centrifuges as the dehydration system is seen.

Considering STPs where the dehydration is performed by centrifuges, the large size presented the highest potential. The 7 STPs would produce for the typical and best scenarios around 263 to 93 GJ day^{-1}. In the worst scenario the dehydrated sludge does not contribute useful energy after its combustion due to the higher moisture of the material.

The medium STPs presented a low contribution in terms of energy potential owing to the presence of only one unit operated by centrifuge. Finally, the sludge energy potential for typical- and best scenarios were around 2 and 4 GJ day^{-1}, respectively.

Figure 5 presents the sludge potential for the STPs based on UASB reactors in each microregion of Paraná State, considering the energy of this by-product in the different dehydration units (drying beds and centrifuge) for the typical scenario.

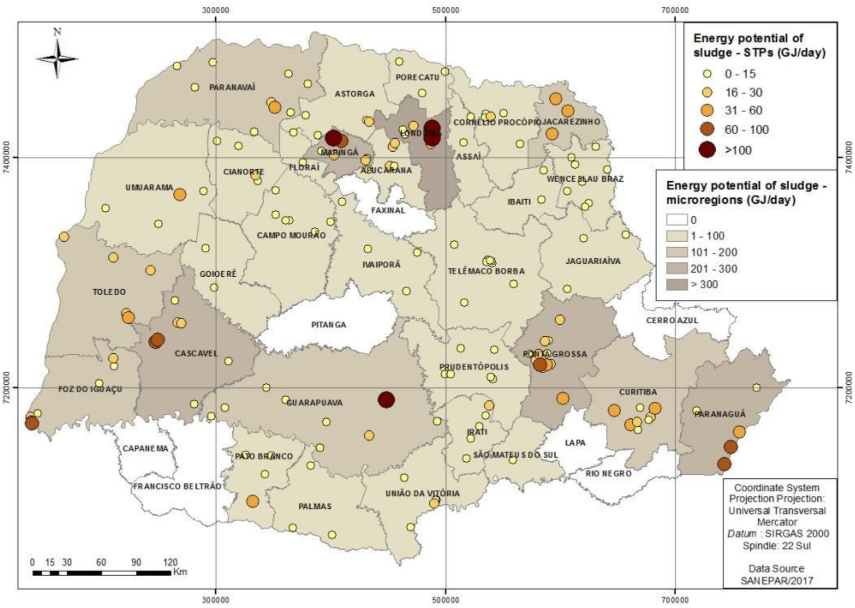

Figure 5. Spatial distribution of the energy potential of the sludge produced in UASB reactors in Paraná.

Most of the STPs in Paraná State presented sludge energy potentials below 15 GJ d^{-1}. However, 4 STPs presented potentials higher than 100 GJ d^{-1}. This analysis enables the diagnosis of the sludge energy potential and the encouragement of its recovery, which leads to better strategic plans for investment in sludge final destination. The microregions of Curitiba and Londrina presented the highest energy potentials, of more than 300 GJ d^{-1}.

3.2.4. By-products energy potential

For all STP sizes, there was a predominance of biogas over sludge in terms of energy potential. Biogas corresponded to 65, 64 and 74% of the energy contained in the by-products of small-, medium- and large STPs, respectively. For the 158 STPs that use drying beds for dehydration, the mean biogas potential corresponded to 64% of the by-products potential, whereas for the 8 STPs that use centrifuges, biogas accounted for 95% of the total potential. Valente (2015) also reports that biogas is the most representative by-product in terms of energy. Rosa *et al.* (2016) observed a similar tendency at the Laboreaux STP (medium-sized), where the energy potentials of sludge and biogas were 7,518 MJ d^{-1} and 10,962 MJ d^{-1}, respectively, with 60% of the energy associated with biogas.

3.3. Perspectives on the energy recovery in UASB reactors in Brazil

Considering the growing use of UASB reactors in Brazil, and the expansion of basic sanitation, the relevance of energy recovery from sludge and biogas is evident. Despite the proven energetic characteristics of the sludge and biogas from UASB, energy self-sufficiency in STPs and the evident financial viability (Valente, 2015), there are several limiting conditions for such practices in Brazil.

Moreira *et al.* (2017), after verifying the strategic demands in terms of biogas recovery in Brazil, observed the need for qualified training for operators, sanitation managers and designers. Furthermore, there is an evident need for qualified professionals who can operate STPs that employ biogas recovery, given the complex operation and monitoring of such systems. Moreover, the technological alternatives (measurement and monitoring equipment, among others) are not national, thus professional qualification not aligned with the demands of the sanitation sector and the limitations in technical assistance compromise the expansion of resource recovery in Brazilian STPs.

Possetti *et al.* (2018) highlight the use of some engineering practices that would increase biogas recovery by maximizing the sustainable and clean energy in Brazil. For these authors, some improvements in the design, construction and operation of UASB reactors could be pointed out, such as: construction of three-phase separator, sealing and water-tightness of biogas pipe, methane recovery from the liquid phase, scum removal, installation of efficient biogas burners, measuring and monitoring of biogas.

Despite the discussed factors, a change of paradigm is expected with the increase of research on resource recovery from effluent treatment, as well as the greater visibility of the subject in the sanitation sector by sanitation and engineering companies and by the society. The progressive updating, although modest, of environmental legislation, norms and guidelines for the recovery of by-products and residues from the sanitation sector, as well as the creation of the National Institute of Science and Technology in Sustainable Sewage Treatment Plants is also evident.

Lastly, the use of GIS to evaluate the energy potential proved to be an easy tool to identify sites with high potential for energy recovery. The application of this tool for this purpose was carried out in other studies, as pointed by Zoreei (2018), who indicated the advantages of its use. The mapping could be used to diagnose the energy potential of by-products in UASB reactors based on geographic location and is also useful in strategic plans and decision-making in sanitation companies.

4. CONCLUSIONS

In spite of the advanced level of sewage treatment in the Paraná, there is considerable energy potential for by-products (sludge and biogas) from UASB reactors to be exploited.

The States of São Paulo, Minas Gerais and Paraná include all of the STPs that exploit energy recovery of biogas, though their potential is not sufficiently explored. Paraná State presents the highest biogas potential; however, it recovers only 0.4% of its capacity for 2018.

There are few large STPs in Paraná State, but they correspond to the highest potentials of energy recovery from sludge and biogas, and biogas was the main energy by-product.

GIS is a relevant tool for sanitation companies and its use can enable studies for the implementation of energy self-sufficiency projects by allowing analyses of energy recovery by-products that take spatial distribution into consideration

The studies of energy recovery from biogas and sludge provide the sanitation companies with support for the decision-making process, and show the energetic advantages of UASB reactors that are currently not exploited.

Energy recovery from the main sewage treatment technology in Brazil (UASB reactors) is incipient. Paraná State is at the forefront of energy recovery from biogas and sludge, but it still presents low levels of STP energy self-sufficiency.

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Furosemide in water matrix: HPLC-UV method development and degradation studies

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ABSTRACT

This study developed a method for furosemide quantification through high performance liquid chromatographic technique. Special attention was given to solute loss and storage stability due to furosemide's low solubility and photosensitivity, respectively. The performance of Nylon and PVDF filters was tested in a 2 mg.L^{-1} furosemide solution. PVDF filters showed better recovery capacity and therefore are more suitable for furosemide filtration. Over eight days, three different storage conditions were studied to access furosemide degradation susceptibility: (i) exposure to light at room temperature, (ii) storage at room temperature without exposure to light, and (iii) storage at 4ºC without exposure to light. The study demonstrated that after 48 h under natural light exposure furosemide was completely degraded. Furosemide solution stored in the dark was stable. Storage temperature did not seem to affect furosemide concentration. The study shows that the selection of more suitable filter and storage conditions for furosemide determination is crucial to avoid underestimation errors.

Keywords: filter retention, pharmaceutical, photodegradation.

Furosemida em matriz de água: desenvolvimento de um método HPLC-UV e estudos de degradação

RESUMO

O presente estudo desenvolve um método para a quantificação de furosemida através de cromatografia de alta resolução. Devido à baixa solubilidade do composto e à sua fotossensibilidade, neste estudo avaliou-se as possíveis perdas do composto ao longo do método de quantificação e a sua estabilidade durante o armazenamento ao longo de oito dias. Para tal, foram testados dois filtros (Nylon e PVDF) com o mesmo diâmetro e tamanho de poro para uma concentração de furosemida de 2 mg.L^{-1}. A degradação da furosemida foi estudada para três condições diferentes de armazenamento: com exposição solar à temperatura ambiente, sem exposição solar à temperatura ambiente e sem exposição solar a 4°C. O estudo demonstrou que o composto furosemida atinge a degradação completa com exposição solar após 48h. A solução de furosemida permaneceu estável ao longo da experiência quando protegida da luz. A temperatura de armazenamento não parece afetar a concentração da solução de furosemida. O presente estudo mostrou que para análises da furosemida, a escolha do filtro adequado e as condições de armazenamento devem ser consideradas para evitar erros de subestimação.

Palavras-chave: fármaco, foto degradação, retenção do filtro.

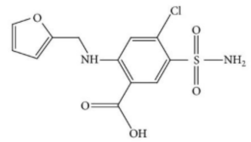
1. INTRODUCTION

The presence of pharmaceutical active compounds (PhACs) in different water bodies is well documented in several studies (Böger *et al.*, 2018; Kuster *et al.*, 2008; Paíga and Delerue-Matos, 2016; Pereira *et al.,* 2017). After ingestion, pharmaceuticals are only partially absorbed and the remainder is excreted as the parent compound or their metabolites via urine or feces. Conventional wastewater treatment processes are not completely efficient in removing PhACs (Ternes, 1998; Martín *et al.,* 2012). Therefore, the development of methods to correctly quantify these compounds in specific matrices are required, and several options already exist. Environmental studies commonly determine the presence of PhACs in samples using methods for the determination of a broad variety of compounds (Cruz-Morató *et al.,* 2014). Although this approach brings benefits such as reductions in time and cost, some inherent restrictions to the accuracy of the determination of each compound is associated, such as analyte loss due to membrane filter adsorption. HPLC-UV analyses requires samples to be filtered to avoid clogging the equipment and capillaries and to extend the column's lifespan (Carlson and Thompson, 2000). Moreover, compound stability studies are also necessary to prevent associated quantification errors, particularly along analyses procedures. For some pollutants, the knowledge of all interferences in the quantification process are still not fully known and require further investigation.

In Europe, anti-hypertensive substances, recently included in the cardiovascular system pharmacotherapeutic group, have the highest consumption rates (OECD, 2016). Included in this group, furosemide (FUR) is a loop diuretic pharmaceutical prescribed to treat cases of hypertension and edema (Bosch *et al.*, 2008). In Portugal in 2014, FUR occupied the 15th place in the top 100 active substances ranking with the highest number of packages sold in the National Health System (INFARMED, 2014). According to Prandota and Witkowska (1976), after human consumption up to 70% of furosemide is absorbed. However, 90% of the non-absorbed drug is excreted as the parent compound (Zuccato *et al.*, 2005). Therefore, in countries such as Portugal, FUR is expected to be found in wastewater treatment plants (WWTPs) (Santos *et al.*, 2013). According to the literature, the presence of FUR in WWTP influents and effluents can widely fluctuate from $mg.L^{-1}$ to $ng.L^{-1}$ (Jelic *et al.*, 2011; Salgado *et al.*, 2010; Santos *et al.*, 2013), while in surface water bodies it usually occurs in low concentrations of $\mu g.L^{-1}$ to $ng.L^{-1}$ (Silva *et al.*, 2011; Valcárcel, 2011; Matamoros *et al.*, 2012).

Although several studies show the presence of FUR in different environmental samples, its concentration varies widely, inducing uncertainty in the PhAC relevance in terms of environment contamination and particularly in the accurate efficiency assessment of the WWTPs on removing this contaminant. Therefore, developing a quantification method for FUR that accounts for the different interferences that can exist along the analyses is vital. Moreover, the use of simple matrices such as ultrapure water for preliminary tests can provide more clear results, especially for compounds such as FUR that are hydrophobic and have low solubilities (Table 1), and thus are more prone to be lost during simple sample preparation procedures, such as filtration. Saturation of the filter adsorption sites generally can minimize these losses (Pillai *et al.*, 2016). However, sometimes the sample volume is not enough for pre-wetting the filters, and thus the selection of the most suitable type of filter can be a key aspect.

Table 1. Structure, physical and chemical properties of furosemide.

Common Name	Furosemide
Chemical structure	
CAS-Number	54-31-9
Molecular formula	$C_{12}H_{11}ClN_2O_5S$
Molecular weight (g.mol^{-1})	330.75[a]
Melting Point (˚C)	206[a]
Ionisation constant, pKa	pKa= 3.8[b]
Octanol/Water Partition Coefficient, log Kow	2.03[c]
Water solubility, at 30˚C (mg.L^{-1})	73.1[d]

Source: [a]O'Neil (2001). [b]Berthod *et al.* (1999). [c]Sangster (2001). [d]Yalkowsky and Dannenfelser (1992).

Another relevant factor is that the literature on methodologies for FUR quantification as a single target is only common in the scope of human or animal health, or for pharmaceutical dosage control (Bosch *et al.*, 2008). Hence, most of the studies on FUR determination were performed in matrices of human and animal urine or plasma. The complexity of such matrices can mask other interferences along the analytical procedure.

This study sought to contribute to the definition of an appropriate methodology for FUR determination in a water matrix by HPLC-UV and to better understand the stability of the compound along the analysis-preparation procedure.

2. MATERIALS AND METHODS

2.1. Chemicals and materials

Analytical grade furosemide (99.8% purity) was purchased from Sigma-Aldrich (Lisbon, Portugal); see Table 1 for its main characteristics. Phosphoric acid (>85% purity), HPLC-grade solvents acetonitrile and methanol were obtained from Enzymatic, S.A. (Loures, Portugal). Ultra-pure water was prepared from a Millipore Milli Q system. Both Whatman 13 Puradisc syringe filters (nylon and PVDF - Polyvinylidene fluoride) were supplied by Enzymatic, S.A. (Loures, Portugal).

2.2. Standards solutions

Furosemide stock standard solution containing 10 mg.L^{-1} was prepared. Furosemide was dissolved in 1 ml of methanol and filled to the 1 L mark with ultra-pure water. An ultrasonic bath was used to help the dissolution of furosemide. For furosemide quantification, standard solutions were prepared within the range of 0.1-4 mg.L^{-1}. All solutions were stored in the dark and covered with aluminium foil to avoid photodegradation. The 10 mg.L^{-1} FUR solution was scanned between the 190 and 350 nm UV region. The wavelength of 233 nm corresponding to the maximum absorption peak has been therefore selected to carry on the experiment. Electric conductivity and pH were also assessed.

2.3. Quantification and analytical method validation

Furosemide quantification was carried out in a HPLC - Beckman Coulter System Gold, with a Solvent Module 126 and a Diode Array Detector 168, using 32 Karat Software, version

8.0, with a variable wavelength detector (190-800 nm) at 233 nm and with a 20 µL volume injector loop. Samples were determined with a flow rate of 1 $mL.min^{-1}$. A reversed-phase analytical column Zorbax Eclipse XDB-C8 (4.6x150 mm; 5 µm) was used. The composition of the mobile phase used in furosemide analyses was previously selected for an isocratic elution mode using trial mixtures of acetonitrile and ultra-pure water acidified with phosphoric acid (0.1% v/v) in different proportions (50:50, 55:45, 60:40, 65:35 and 70:30). The mixture composed of 60% acetonitrile and 40% ultra-pure water (acidified with phosphoric acid) was selected, as the corresponding retention time for furosemide was below 4 min., thereby allowing the quantification of the pharmaceutical in a reasonably short time. Samples were injected (three replicates) with an automatic injector manufactured by Spark Holland BV – MIDAS, at room temperature (16-20˚C).

This analytical method was validated according to the International Conference on Harmonization (ICH) guidelines for validation of analytical procedures (United States, 1996), based on the following parameters: linearity, sensitivity, precision (intra-day) and accuracy.

A calibration curve was constructed using a set of furosemide standard solutions with concentrations of 0.1-4 $mg.L^{-1}$. Three replicate injections were made for each standard solution. The regression equation and the value of the correlation coefficient were calculated using linear regression analysis. The sensitivity of the analytical method was estimated in terms of the limits of detection (LOD) and of quantification (LOQ). LOD and LOQ were defined based on signal-to-noise ratios of 3:1 and 10:1, respectively. Moreover, for the assessment of the analytical method's accuracy and precision (intra-day), standard solutions at seven different concentrations (from 0.25 $mg.L^{-1}$ to 4 $mg.L^{-1}$) were injected at three different times in the same day. The precision of the proposed method was obtained by calculating the relative standard deviation (RSD) values of the peak areas for the three different injections with acceptance criteria of not more than 2% (United States, 1996). The accuracy of the quantification method was assessed through the percentage ratio between the measured concentrations of the furosemide standards and their nominal concentrations.

2.4. Procedure

Different syringe filters were tested to study the FUR recovery percentage throughout the process. Nylon and PVDF (Polyvinylidene fluoride), both with 0.45 µm pore size as recommended for HPLC analysis, were selected. The filter size (13 mm) was chosen in accordance with the low sample volume required for further analysis. Through HPLC analysis, filter recovery capacity was tested for 2 $mg.L^{-1}$ of FUR solution, filtering a volume of 2 mL. Additionally, the recovery capacity was further validated comparing a standard curve, between 0.25 and 2 $mg.L^{-1}$ of FUR, with and without filtration.

For FUR degradation studies, an experiment over 8 days was performed under three different storage conditions. At room temperature (12-18˚C), three replicates of a FUR solution with approximately 2 $mg.L^{-1}$ were exposed to daylight (L), and the other three were kept in the dark (D) to test photodegradation sensitivity. The effect of storage temperature was tested with another three replicates that were kept at 4˚C. Samples were taken from all the standard solutions after 0, 24, 48, 72 and 192 hours of exposure and analysed through HPLC.

3. RESULTS AND DISCUSSION

3.1. Furosemide quantification

3.1.1. Furosemide recovery

Figure 1 compares the extent of FUR retention by two different filters, Nylon and PVDF, for the same solution. Nylon filters presented 75% more FUR adsorption than PVDF filters.

Hence, the PVDF filter seems more efficient for FUR solution filtration. Several factors can affect adsorption onto membranes, from characteristics of the compounds to the properties of the filters. Both filters have hydrophilic membranes, the same pore size (0.45µm) and the same effective filter area (1.3 cm^2). Nylon filters, compared with PVDF, bind proteins and are more suitable for filtering samples with a high pH. In turn, PVDF filters are highly inert and have low protein binding (GE Healthcare, 2018). Since the standard samples have a pH of 5.5±0.17, both filters were appropriate. Hence, the results can be an outcome of the inert feature of the PVDF filters. To further validate the PVDF filters' suitability, a comparison of a standard curve with and without filtration was performed (see Figure 2). No significant losses were observed. Therefore, the PVDF filter was considered appropriate to carry on experiments with FUR.

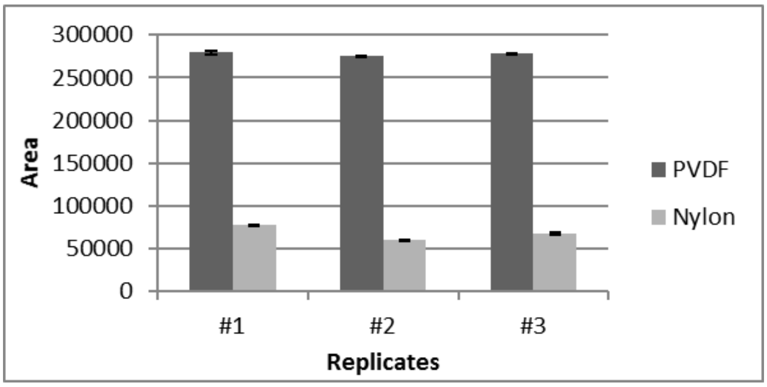


Figure 1. FUR retention (2 $mg.L^{-1}$) in Nylon and PVDF filters.

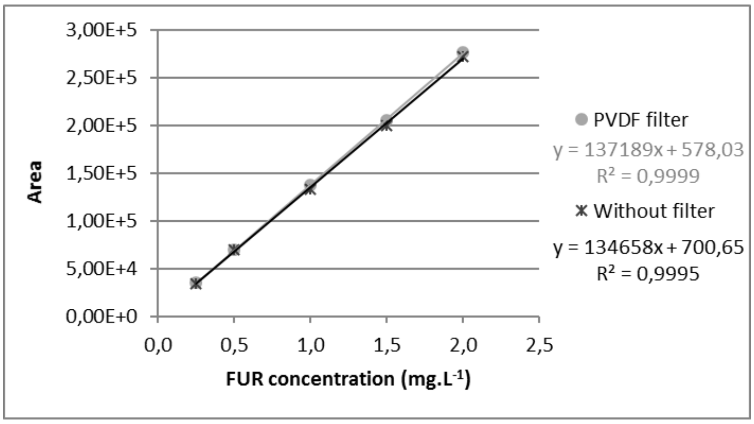


Figure 2. Standard curve of FUR for samples without filtration and filtered through PVDF filters.

3.1.2. Method validation

An optimal peak shape and retention time of FUR was obtained for the selected chromatographic conditions (Figure 3). The method employed also showed a linear pattern for the tested concentration range (0.1-4.0 $mg.L^{-1}$) with a correlation r^2=0.9999 (Figure 4). Limit of quantification (LOQ) was 0.144 $mg.L^{-1}$ and limit of detection (LOD) was 0.048 $mg.L^{-1}$ (Equations 1 and 2, respectively). The accuracy of the method was estimated to be within 93.7-101.8%, whereas in regard to the intra-day precision, RSD of measurements were below 2%. Therefore, the method can be considered to provide a good sensitivity, precision and accuracy.

$$LOQ = \frac{10 \text{ x } \sigma}{slope\ of\ calibration\ curve} \quad (1)$$

$$LOD = \frac{3.3 \text{ x } \sigma}{slope\ of\ calibration\ curve} \quad (2)$$

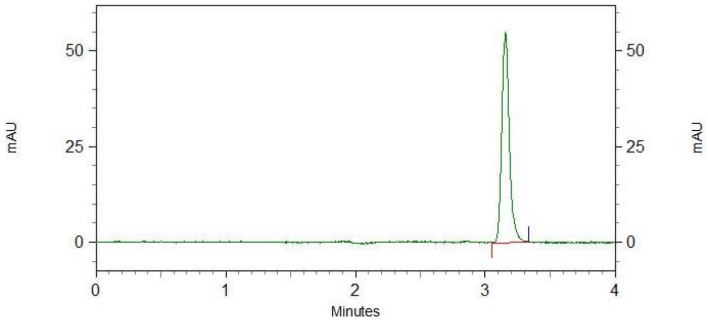


Figure 3. Chromatogram of FUR in ultrapure water matrix.

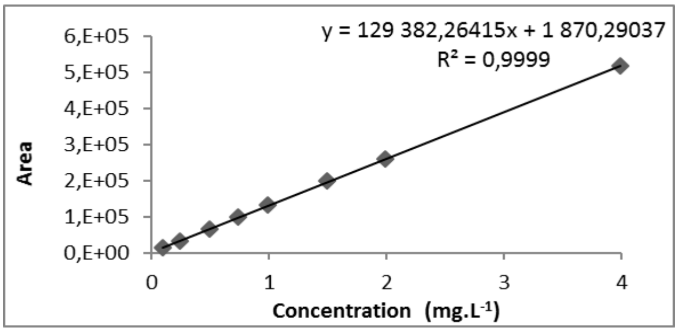


Figure 4. Linearity of FUR in an ultrapure water matrix.

3.2. Degradation studies

Figure 5 shows FUR stability over 8 days under different storage conditions. The FUR solutions kept from light exposure maintained their concentration throughout the experiment. Storage temperature does not seem to affect FUR concentration. Moreover, since FUR quantification is performed at room temperature, FUR-solution storage should benefit if similar temperatures are maintained to avoid potential precipitation. Figure 5 shows that during the first 24h of exposure there is a slight increase in concentration in 4D solutions. The minor increase is due to the initial lower values of one of the replicates. Poor initial solubility of the replicate could explain the lower value at 0 hours of exposure, compared to the 24-hour sample.

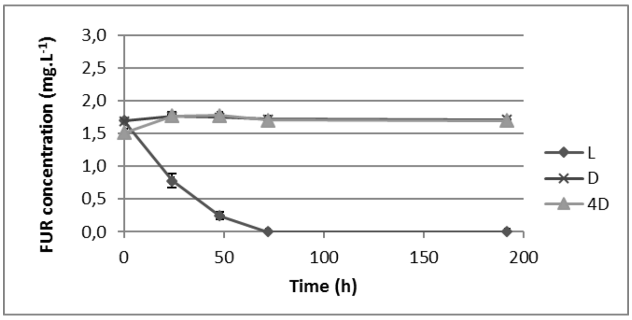


Figure 5. FUR conservation over 8 days for three different conditions: L – Light exposure at room temperature; D – Kept in the dark at room temperature; and, 4D – Kept in the dark at 4˚C.

FUR's sensitivity to light is well-documented, and the appearance of photodegradation products as a result of the exposure to light is to be expected (Greca *et al.*, 2004). In fact, Figure 6a shows the presence of two new peaks (2 and 3) along with the peak of FUR (1). Substance (3) appears in all samples after 24h and disappears before 48h of exposure; therefore, light exposure and temperature are not the key factors to explain this phenomenon. Moreover, substance (3) seems to suffer further degradation since it disappears after 48h.

In the first 72h of exposure, the decrease of FUR is followed by an increase of the concentration of substance (2). Afterwards, substance (2) decreases to concentrations below detection levels. Greca *et al.*, (2004) tested the photodegradation of furosemide in different water matrices through a 150-W solar simulator over 36h. In all water matrices, including a simple distilled water medium, only one new photoproduct was found. The same result occurred when the distilled water solution was exposed to natural solar radiation. Therefore, the main photoproduct mentioned above, substance (2), which is visible in the solution exposed to light, can be the same as the one found in the previously referred to work. FUR, in aqueous solutions exposed to UV light, hydrolyses into 2-amino-4-chloro-5 sulfamoylanthranilic acid (CSA) and furfuryl alcohol that will be further degraded into levulinic acid (Ruiz-Angel *et al.*, 2006). Therefore, substance (2) could be the FUR photoproduct CSA, but further liquid chromatography mass spectrometry analysis should be undertaken for substance identification (Figure 7).

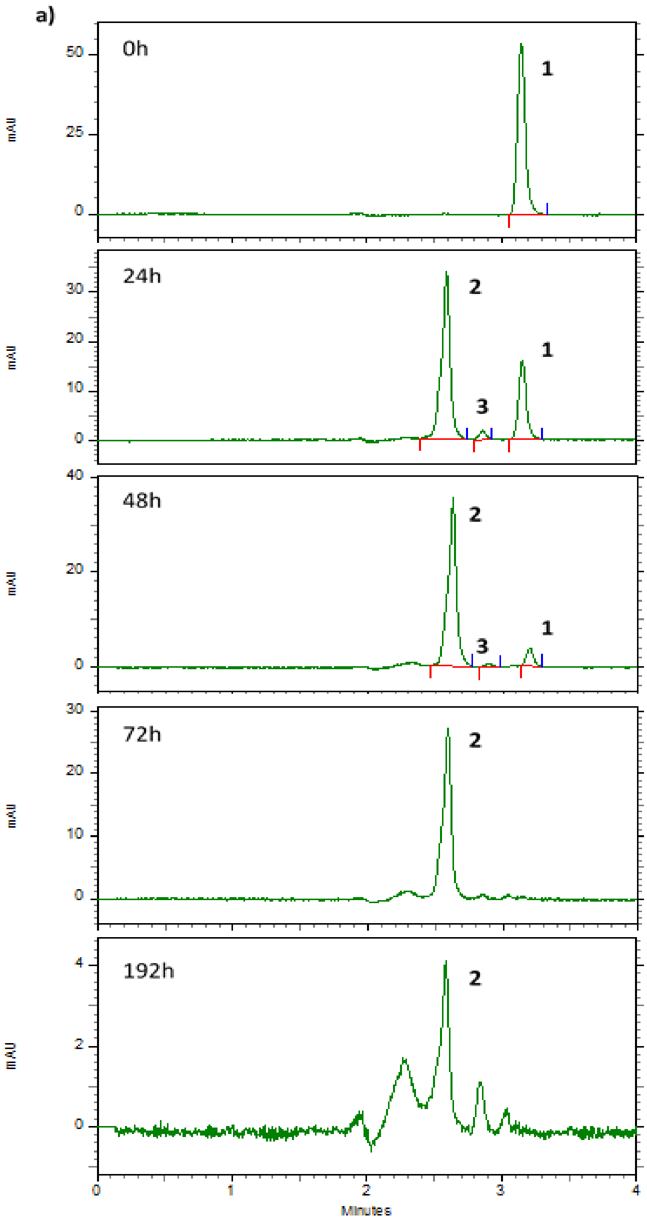


Figure 6. Chromatogram of furosemide in ultrapure water under: **a)** light exposure/room temperature; **b)** darkness/room temperature; **c)** darkness/4˚C. Continue.

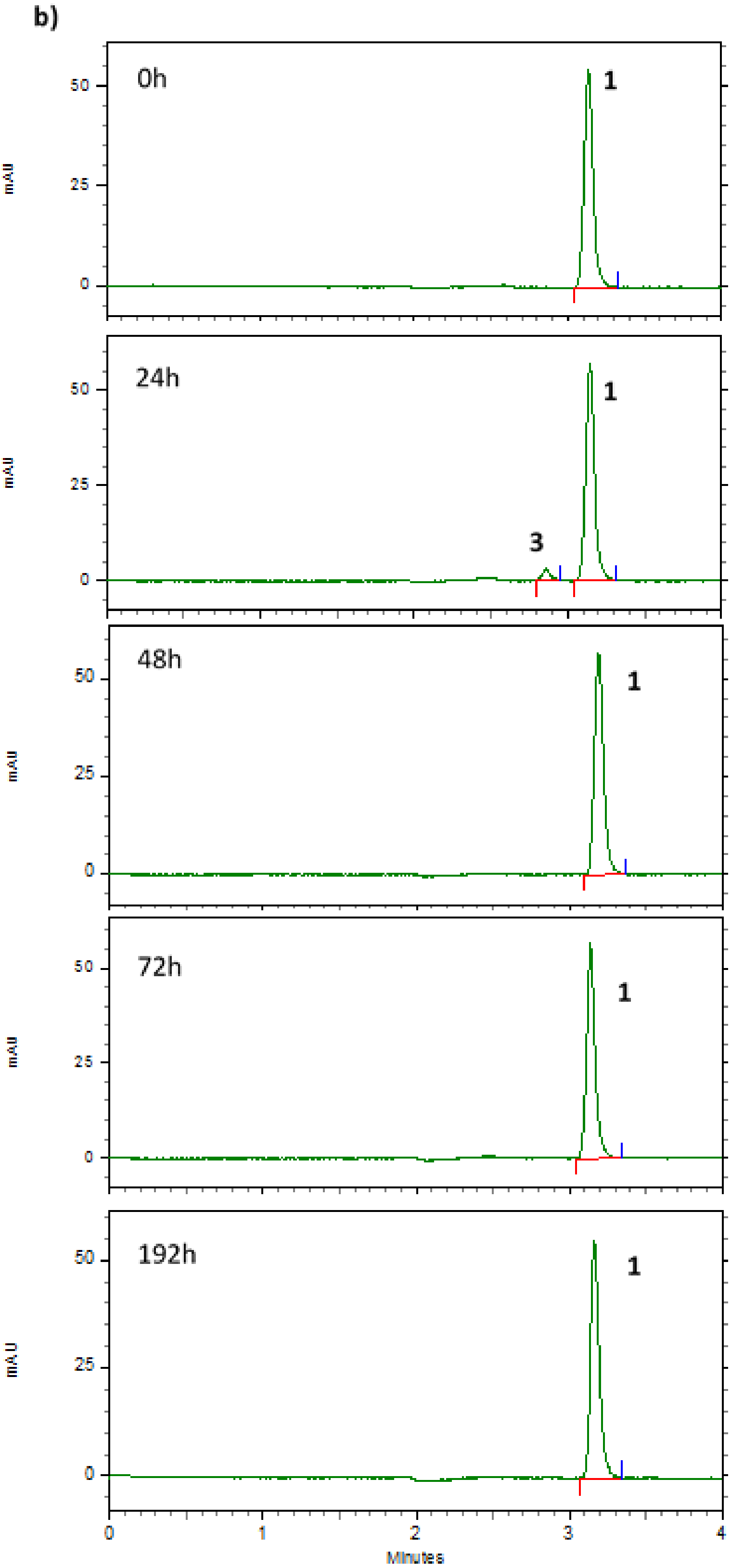


Figure 6. Continue.

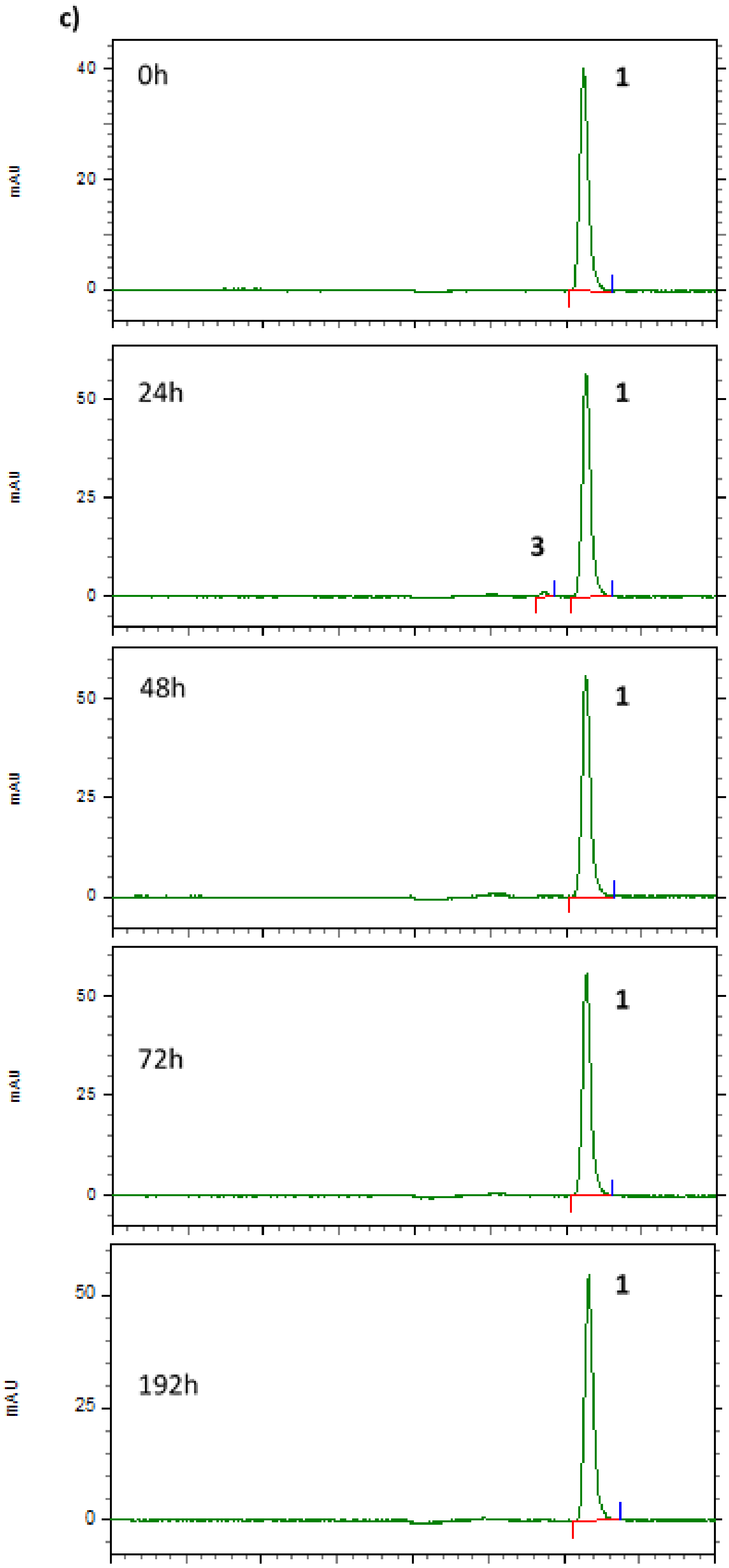


Figure 6. Continue.

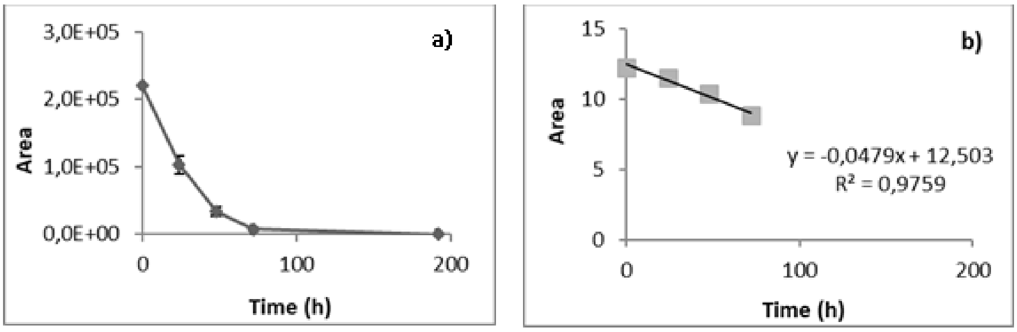


Figure 7. a) Furosemide photodegradation over eight days; **b)** First-order kinetic of furosemide photodegradation.

4. CONCLUSIONS

The developed method allows fast and accurate determination of FUR in water solution by HPLC-UV. In the 0.1-4 mg.L^{-1} concentration range, the quantification of FUR follows a linear regression. Due to its low solubility and sorption susceptibility, FUR standard solutions should be filtered through PVDF filters or others with a similar inert feature. Stability studies validated the well-known photo sensitivity characteristic of FUR. Total photodegradation of a FUR solution was achieved after 48h of exposure to natural light. Therefore, it is recommended that FUR standards solutions should be stored in the dark at room temperature to guarantee concentration conservation. The study emphasizes that when targeting FUR in low volume environmental samples, special attention should be given to the chosen filter, as well as to storage conditions to avoid underestimation errors.

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