ISSN = 1980-993X (Online)
<http://www.ambi-agua.net>

50th Edition of Revista Ambiente & Água - An Interdisciplinary Journal of Applied Science,
Taubaté, V. 14, N. 2, p. 1-27 Mar/Apr. 2019. ([doi:10.4136/ambi-agua.v14.n2](https://doi.org/10.4136/ambi-agua.v14.n2))

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Revista Ambiente & Água - An Interdisciplinary Journal of Applied Science / Instituto de Pesquisas Ambientais em Bacias Hidrográficas. Taubaté. v. 14, n.2 (2006) - Taubaté: IPABHi, 2019. Quadrimestral (2006 – 2013), Trimestral (2014 – 2016), Bimestral (2017), Publicação Contínua a partir de Janeiro de 2018.

Resumo em português e inglês.
ISSN 1980-993X

1. Ciências ambientais. 2. Recursos hídricos. I. Instituto de Pesquisas Ambientais em Bacias Hidrográficas.

CDD - 333.705

CDU - (03)556.18

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Acute ecotoxicity on *Daphnia magna* to evaluate effluent samples of Kraft pulp mill treated by UV/H₂O₂ process

ARTICLES doi:10.4136/ambi-agua.2208

Received: 03 Nov. 2017; Accepted: 11 Jan. 2019

Joicy Micheletto^{1*} ; Naiara Mariana Fiori Monteiro Sampaio²
Henrique Zavattieri Ruiz³ ; Lucia Regina Rocha Martins¹
Marcus Vinicius de Liz¹ ; Adriane Martins de Freitas¹

¹Universidade Tecnológica Federal do Paraná (UTFPR), Curitiba, PR, Brasil
Departamento de Química e Biologia (DAQBi). E-mail: joicymicheletto@hotmail.com,
luciaregi@utfpr.edu.br, marcusliz@utfpr.edu.br, adrianefreitas@utfpr.edu.br

²Universidade Federal do Paraná (UFPR), Curitiba, PR, Brasil
Departamento de Química (DQUI). E-mail: naiaramfms@gmail.com

³Universidade Federal do Paraná (UFPR), Curitiba, PR, Brasil
Centro Mesorregional de Excelência em Tecnologia do Leite (CMETL).

E-mail: henriquezr@hotmail.com

*Corresponding author

ABSTRACT

The pulp and paper industry is one of world's largest water consumers, generating high volumes of effluents. The Kraft process produces effluents with high BOD, COD, suspended solids, lignin and a myriad of potentially toxic compounds, which require treatment before discharge into the aquatic environment. Advanced oxidation processes, such as UV/H₂O₂, have been applied as treatment alternatives because they can destroy many compounds before they mineralize. However, when the oxidation process is incomplete, occurs could be produced by products with high toxicity. This study evaluated the acute toxicity on *Daphnia magna* of two effluent samples of Kraft pulp mill (KE1 and KE2) treated by UV/H₂O₂ process. The effects of the pH variation and oxidant concentration on the removal of DOC, total UV-vis spectral area and apparent color were considered to adjust the experiments' conditions with diluted effluent KE1. Both samples were treated at pH 4.0 and 70 mg L⁻¹ of H₂O₂ for 40 min, achieving removals of up to 69.4% in apparent color, 73.7% of phenolic compounds and 68.9% of lignin compounds. When the reaction was applied in undiluted effluent samples, the acute toxicity for *Daphnia magna* decreased for KE1 after 780 min of treatment, whereas KE2 became four times more toxic. The data showed that although the treatment had been efficient considering physics and chemicals parameters, it is necessary follow the oxidative processes with ecotoxicological bioassays to guarantee their safety, since different effluents of the Kraft pulp mill could present different levels of organic compound mineralization.

Keywords: advanced oxidation process, ecotoxicology bioassays, industrial effluent.

Ecotoxicidade aguda em *Daphnia magna* para avaliação de amostras de efluente de celulose Kraft tratadas por processo UV/H₂O₂

RESUMO

A indústria de papel e celulose está entre as que mais consome água no mundo, gerando grandes volumes de efluentes. O processo Kraft produz efluentes com elevada DBO, DQO, sólidos suspensos, teor de lignina e uma mistura complexa de compostos potencialmente tóxicos, os quais necessitam de tratamento para posterior descarte no meio ambiente. Os processos oxidativos avançados, dentre eles o processo UV/H₂O₂, têm sido aplicado como alternativa de tratamento pois é capaz de degradar os mais diversos compostos até sua mineralização. No entanto, quando o processo oxidativo é incompleto, podem ser formados produtos intermediários com elevada toxicidade. Este estudo avaliou a toxicidade aguda em *Daphnia magna* de duas amostras de efluente Kraft (KE1 e KE2) tratadas por processo UV/H₂O₂. Os efeitos da variação de pH e da concentração do oxidante sobre a redução de COT, área espectral UV-vis e cor aparente foram utilizados para ajustar as condições experimentais com a amostra KE1 diluída. Ambas as amostras foram tratadas em pH 4.0 e com 70 mg L⁻¹ de H₂O₂ por 40 min, alcançando remoções de até 69,4% na cor aparente, 73,7% de compostos fenólicos e 68,9% de compostos lignínicos. Quando a reação foi aplicada nas amostras não diluídas de efluentes, a toxicidade aguda para *Daphnia magna* diminuiu para KE1 após 780 min de tratamento, enquanto KE2 tornou-se três vezes mais tóxica. Os dados mostraram que embora o tratamento tenha sido eficiente, considerando os parâmetros físicos e químicos, é necessário acompanhar os processos oxidativos por ensaios ecotoxicológicos para garantir sua segurança, uma vez que cada efluente kraft pode apresentar diferentes níveis de mineralização de seus compostos orgânicos.

Palavras-chave: bioensaio ecotoxicológico, efluente industrial, processos oxidativos avançados.

1. INTRODUCTION

The pulp and paper industry consumes a large amount of water (Ashrafi *et al.*, 2015) and is one of the largest polluters due the variety of chemicals that are released into the environment (Savant *et al.*, 2006). One of common pulping technologies is the Kraft process, which is applied for wood delignification with alkaline solutions at high pressures and temperatures (Kamali and Khodaparast, 2015). Kraft effluent has high biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids, lignin and its derivatives and a variety of toxic compounds (Ali and Sreekrishnan, 2001). If these effluents are poorly treated, toxic substances are released into the aquatic environment, affecting many ecosystems (Oller *et al.*, 2011).

In order to mitigate these negative effects, studies have evaluated options of treatments; the application of advanced oxidation processes (AOPs) is a promising alternative (Rueda-Márques *et al.*, 2015; Merayo *et al.*, 2013). In wastewater treatment of the pulp and paper industry, the AOPs most used are those wherein a source of radiation is applied (UV, visible or solar light), such as UV/H₂O₂, photo-Fenton and TiO₂ photocatalysis (Catalkaya and Kargi, 2008). These processes are based on the generation of hydroxyl radicals *in situ* that exhibit high oxidizing power, low selectivity and in many cases result in complete degradation of organic matter (Del Moro *et al.*, 2013). In the UV/H₂O₂ process, the hydroxyl radicals are formed by the homolitic break of H₂O₂ according to Equation 1:

The hydroxyl radicals ($\bullet\text{OH}$) are capable of mineralizing organic contaminants by three different routes of oxidation: hydrogen abstraction, electron transfer and radical addition (Malato *et al.*, 2009). The efficiency of these processes have been applied to different matrices such as urban wastewater (Silva *et al.*, 2018), petroleum refinery effluent (Moser *et al.*, 2018) and pharmaceutical substances (Alharbi *et al.*, 2017). There are many advantages in the application of AOPs compared with other treatment systems, such as non-phase transfer, high solubility of H_2O_2 in water, no sludge formation and relatively low operational cost; but despite these, its application in the treatment of pulp and paper mill effluent is still incipient (Rizzo, 2011).

Some studies show that if the amount of hydroxyl radicals generated in AOPs was insufficient to the complete mineralization the partial oxidation of organic contaminants may result in formation of toxic byproducts (Rizzo, 2011; Rueda-Márquez *et al.*, 2015). In order to avoid this issue, toxicity tests could be used as important tools for the monitoring of the effectiveness of the treatment process (Oller *et al.*, 2011). The main objective of this study was to evaluate the acute ecotoxicity of two effluent samples of a Kraft pulp mill submitted to treatment with the UV/ H_2O_2 process, using the *Daphnia magna* as a test organism.

2. MATERIALS AND METHODS

2.1. Kraft effluent samples

Two effluent samples (named KE1 and KE2) were collected in different days in a kraft pulp mill of *Pinus taeda* and *Pinus elliottii*. These samples were collected before primary treatment (raw effluent) in plastic vessels (5 L) and stored at 4°C. After filtration (7-11 μm , Quantity) the samples were characterized by standardized methods for pH, Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD_5), Dissolved Organic Carbon (DOC), turbidity, apparent color (440 nm), alkalinity, total phenolics and UV-Vis spectral area (200-800 nm) (APHA *et al.*, 2012). Lignin compounds were analyzed by absorbance measured at 280 nm, according to Çeçen (1999). All analyses were performed in triplicate.

2.2. Experimental design

The effects of two variables (pH and H_2O_2 concentration) were evaluated by 2^2 factorial design with triplicate of the central point. The levels for each variable were pH 4.0 and 8.0 and for the H_2O_2 concentrations 50 and 70 mg L^{-1} . The performance of experiments was measured by color, UV-Vis spectral area and DOC and the results were expressed as percentage of reduction. The end of the reaction was determined as the time when consumption of hydrogen peroxide was above 95%. Residual H_2O_2 was determined by the spectrophotometric method based on reaction with ammonium metavanadate (Nogueira *et al.*, 2005).

The effluent samples (500 mL) were filtered (7-11 μm , Quantity), diluted with demineralized water (fifteen times) and the pH was adjusted before treatment by the UV/ H_2O_2 process. The experiments were conducted in a borosilicate bench photoreactor equipped with a water-cooler and a magnetic stirrer. Artificial radiation was provided by a high-pressure mercury vapor lamp (125W, $\lambda_{\text{max}} = 254 \text{ nm}$, Philips) immersed in the solution through a quartz bulb. Before the addition of oxidant and at the end of the treatment, an aliquot of each sample was collected for the measurements indicated in Item 2.1.

The significance of the variables effect was evaluated by the Pareto graph (95% confidence level, Statistica 7.0). The treatment condition chosen was applied to the other sample.

2.3. Bioassays with *Daphnia magna*

Acute toxicity tests were conducted with effluent samples (KE1 and KE2) before (initial time) and after the UV/ H_2O_2 treatment, which was performed with filtered and undiluted

effluent samples, after adjusting the pH (4.0). The hydrogen peroxide added was fifteen times more (1050 mg L^{-1}) than in the factorial design because the samples were not diluted. For this reason, the reaction time was 780 min (hydrogen peroxide consumption above 95%). Before the tests the samples had the pH adjusted (~ 6.5) and catalase bovine solution (1%, Sigma) added before and after the treatment for remotion of residual H_2O_2 . The bioassays with *D. magna* were performed in triplicate with 20 neonates organisms (6-24h) that were exposed to effluent samples at concentrations of 100, 50, 25, 12.5 and 6.25% (v/v) diluted in standard medium, that was used also for the control experiments. All tests were maintained at 20°C by 48h of exposure and after that the number of immobile organisms was recorded (ABNT, 2016). The results were expressed by 50% effective concentration (EC_{50}) that was calculated through the Probit method and by toxic units (TU), defined as $100/\text{EC}_{50}$. The sensitivity of the organism batches was evaluated monthly with KCl solutions (Panreac; purity 99%) in concentrations between $570\text{--}840 \text{ mg L}^{-1}$. In this work, the organisms used for the bioassays presented $\text{EC}_{50, \text{KCl}} = 710 \pm 15 \text{ mg L}^{-1}$.

3. RESULTS AND DISCUSSIONS

3.1. Characterization of the effluent samples

The physical and chemical properties of the effluent samples (KE1 and KE2) reveal that although they originated from the same pulping process there were differences among them (Table 1). In both samples, the COD were quite similar; but there was a wide variation in the BOD_5 values. The most adopted method used to quantify biodegradability is the assessment of the BOD_5/COD ratio (Sarria *et al.*, 2002). According to this, the KE2 sample ($\text{BOD}_5/\text{COD} = 0.905$) was more biodegradable than the KE1 sample ($\text{BOD}_5/\text{COD} = 0.395$) which may be related to the low phenolic content and the turbidity of KE2.

Table 1. Chemical and physical properties of Kraft effluent samples.

Parameter	KE1	KE2
pH	10.5 ± 0.1	9.7 ± 0.1
Turbidity (FTU)	129 ± 0	73.4 ± 1.2
True color (440 nm)	0.4144 ± 0.0005	0.4560 ± 0.0007
Apparent color (440 nm)	0.7261 ± 0.0001	0.6070 ± 0.0002
BOD_5 ($\text{mg L}^{-1} \text{O}_2$)	414 ± 39	971 ± 93
COD ($\text{mg L}^{-1} \text{O}_2$)	1047 ± 21	1073 ± 1
DOC ($\text{mg L}^{-1} \text{C}$)	316 ± 7	308 ± 15
Total phenolics (mg L^{-1} gallic acid)	83.6 ± 2.8	75.8 ± 1.7
Lignin compounds (280 nm) *	0.4944 ± 0.0001	0.4811 ± 0.0003
Spectral area (200-800 nm) *	106.40 ± 3.56	108.89 ± 5.45
Alkalinity ($\text{mg L}^{-1} \text{CaCO}_3$)	40.1 ± 0.9	46.4 ± 1.1

* values obtained from diluted sample (factor = 15).

3.2. Experimental design

The effect of pH variation and hydrogen peroxide concentration on the performance of the UV/ H_2O_2 process was initially evaluated on the KE1 sample (Table 2). The central point (exp. 5) was assayed in triplicate and demonstrated a satisfactory repeatability among experiments (mean SD = 2.67). The results indicated low reduction of the apparent color in all conditions tested, except for the DOC and UV-vis spectral area best results were reached in acidic media and with high peroxide concentration.

Table 2. Factorial design data for UV/H₂O₂ process for the KE1 sample (reaction time = 40 min).

Exp	Variable		Reduction (%)		
	H ₂ O ₂ / (mg L ⁻¹)	pH	Apparent Color	DOC	UV-vis Spectral area
1	50	4	23.12	62.85	56.76
2	70	4	41.33	77.45	71.04
3	50	8	21.43	28.44	45.44
4	70	8	22.49	45.58	45.88
5*	60	6	24.06 ± 3.72	48.70 ± 2.17	52.14 ± 2.12

*triplicate of central point, mean ± standard deviation estimative (SD).

The Pareto chart (Figure 1) confirmed that an increase in the dosage of oxidant contributes significantly ($p = 0.95$) to the decrease of DOC. The pH variation had a significant effect over the reduction of DOC and UV-vis spectral area; its increase interfered negatively in all studied parameters. This effect was not expected, since H₂O₂ have a higher absorptivity at 254 nm in basic pH (HO₂⁻), generating more hydroxyl radicals.

Figure 1. Main effect Pareto charts for apparent color, DOC and spectral area reductions obtained from 2² factorial design (Standardized Effect Estimate for absolute values - 95% confidence interval).

For color removal, the low efficiency of the process can be explained by the presence of some recalcitrant compounds, such as condensed tannins which contribute to the coloration of the sample (Kamali and Khodaparast, 2015). During the oxidative process, these recalcitrant compounds are broken down into smaller chromophore fractions, reaching less-expressive results in the removal of color (Kemeny and Benerjee, 1997). No linear response explain the low correlation ($R^2 = 0.59032$) and adjustment (0.1864) for this parameter in comparison to DOC ($R^2 = 0.96415$ and adjustment = 0.9283) and UV-vis spectral area ($R^2 = 0.95391$ and

adjustment = 0.90782). Based on these data, the best treatment condition for KE1 was an oxidant concentration of 70 mg L⁻¹ and pH 4.0, which was then applied to the treatment of the two samples (KE1 and KE2).

3.3. Effluent treatment by UV/H₂O₂ process

The adjusted conditions of the UV/H₂O₂ reactions were applied in both effluent samples, and the process performance was evaluated through analytical parameters (Table 3). The variations in the results are probably correlated with the different chemical properties of the samples. Although the treatment was conducted under the same pH and oxidant concentration conditions, it is not possible to predict how the hydroxyl radicals formed will interact with the organic matter in the effluent. Such interactions are critical for color reduction as a consequence of the breakdown of conjugated double bonds, but the non-selectivity of the hydroxyl radical could affect the samples in different ways (Lee and Von Gunten, 2010). Besides that, the difference in DOC removal between the samples can be explain by the higher biodegradability of KE2 (Table 1). This indicates that this sample had a great amount of labile compounds.

Table 3. Percentual reduction of the parameters after treatment of Kraft effluent samples ([H₂O₂] = 70 mg L⁻¹, pH = 4.0, reaction time = 40 min).

Parameter	Removal (%)	
	KE1	KE2
Turbidity	-41.8	0.91
True color (440 nm)	19.4	69.4
Apparent color (440 nm)	35.0	34.2
DOC	18.8	50.7
Total phenolics	79.3	73.7
Lignin compounds (280 nm)	40.5	68.9
UV-vis spectral area (200-800 nm)	49.4	69.9

The best result was expected in the DOC removal in the KE1 sample (18.8%), since in the literature COD reductions between 45% and 74% have been reported with AOPs applied for the treatment of pulp and paper effluents, although the largest reduction was described by combining a coagulation-flocculation process followed by heterogeneous photocatalysis (Catalkaya and Kargi, 2008; Rodrigues *et al.*, 2008).

Satisfactory reductions of lignin compounds and total phenolics can be attributed to aromatic content in the start of the process; above those occur the electrophilic addition of hydroxyl radicals, generating new phenols in the media. Once the oxidative process continues, these formed compounds undergo the breakdown of the aromatic rings, lose their chromophoric groups and afterwards would mineralize (Tiburtius *et al.*, 2009; Kamali and Khodaparast, 2015). Theses results indicate that higher H₂O₂ doses should be implemented to mineralize of the organic compounds.

3.4. Acute ecotoxicity on *Daphnia magna*

The bioassay with *Daphnia magna* is one of the most broadly used in the laboratory routine of industries to evaluate the ecotoxicity of effluent samples. The bioassays data for non-treated samples showed acute toxicity to *Daphnia magna*. However, for treated samples the results were quite variable for KE1 and KE2 (Table 4), once the toxicity of KE2 increased after UV/H₂O₂ treatment.

Table 4. Acute toxicity of Kraft effluent samples on *D. magna* before and after UV/H₂O₂ process (pH = 4.0; [H₂O₂] = 1050 mg L⁻¹; reaction time = 780 min).

Sample	KE1		KE2	
	Before UV/H ₂ O ₂	After UV/H ₂ O ₂	Before UV/H ₂ O ₂	After UV/H ₂ O ₂
48h -EC ₅₀ ^a (% v/v)	132.90	Non-toxic ^b	183.51	50.05
TU	0.752	Non-toxic ^b	0.545	1.998

^aconcentration that cause immobilization in 50% of the test organisms; for results before treatment the values correspond to extrapolation because the effluent did not reach the EC₅₀ index. ^bno organism presented immobility at the concentrations tested.

The increase of KE2 acute toxicity after treatment could be at least partially explained by the formation of byproducts once complete mineralization has not been achieved. Previous studies suggested that the degradation of high molecular weight compounds can generate highly toxic compounds (Fernandez-Alba *et al.*, 2002; Pereira *et al.*, 2009). The literature suggests that lignins, tannins and resin acids are some of the major compounds in this complex kind of effluent that are responsible for the toxicity in untreated samples (Peitz and Xavier, 2017). Carvalho *et al.* (2009) described the production of some compounds such as p-hydroxy-phenyl, guaiacyl and syringyl when oxidation process are carried out in degradation of wood. The electrophilic addition of the hydroxyl radical to the aromatic rings of these subunits can lead to the formation of quinone and catechol derivatives (Azevedo *et al.*, 2006). Some of similar molecules such as phenol and hydroquinone have been demonstrated high toxicity to *D. magna* (48 h-EC₅₀ = 6.6 mg L⁻¹ and 0.15 mg L⁻¹, respectively) (Guerra, 2001). Considering that almost same removal of total phenolics were achieved for both samples, the higher toxicity may have been caused by compounds in a more advanced stage of oxidation. Once the KE2 was a more biodegradable sample, the byproducts formed in the oxidation process grew abundant enough to increase the toxicity after treatment.

4. CONCLUSIONS

This study demonstrates that the physical and chemical measures are not sufficient to evaluate the effectiveness of the oxidative treatment of effluent samples of a Kraft pulp mill. Moreover, it was possible to verify that each batch of these complex effluents should be considered a particular case and require additional performance criteria, such as toxicity monitoring. In the same way as in this study, the application of reactional parameters obtained by factorial planning in a preliminary sample on other similar effluent can result in an increase of toxicity due to the partial oxidation of organic compounds.

5. ACKNOWLEDGEMENTS

The authors would like to thank CNPq for financial support.

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The importance of climate scenarios in setting the productivity indexes in the Pampa Arenosa sub-region of the province of Buenos Aires, Argentina

ARTICLES doi:10.4136/ambi-agua.2286

Received: 04 Jun. 2018; Accepted: 18 Dec. 2018

**Silvia Patricia Pérez^{1*} ; Julieta Irigoin² ;
Mariano Tomás Cassani³ ; Marcelo Juan Massobrio³ **

¹Universidad de Buenos Aires (UBA), Buenos Aires, Argentina
Facultad de Agronomía (FAUBA). Departamento de Recursos Naturales y Ambiente.
E-mail: perezgrima@gmail.com

²Instituto Nacional de Tecnología Agropecuaria (INTA), Hurlingham, Buenos Aires, Argentina
Instituto de Suelos (IS). E-mail: irigoin.julieta@inta.gob.ar

³Universidad de Buenos Aires (UBA), Buenos Aires, Argentina
Facultad de Agronomía (FAUBA). Departamento de Ingeniería Agrícola y Uso de la Tierra.
E-mail: mcassani@agro.uba.ar, massobrio@agro.uba.ar

*Corresponding author

ABSTRACT

Starting in the 1970's, the Pampa Arenosa sub-region experienced an increasing water regime that generated an increased area for dryland farming. With increasing agricultural activity, examining the land is an essential strategic tool for its planning. The objective of this work was to highlight the importance of considering several climate scenarios when setting the productivity index in the sector of longitudinal dunes in the Pampa Arenosa sub-region in the province of Buenos Aires, Argentina. The climate scenarios were set in relation to the shifts in average rain values, according to the Pettitt Test. The land were classified according to their productivity index. It was found that the productivity index of the land increased with the rain, reaching its highest value in the period right after the abrupt shift. Land of moderate productive capacity whose productivity index values are between 65 and 51 comprised the majority of the area of our study.

Keywords: agro climatology, land evaluation, Pettitt test, rainfall.

Importância dos cenários climáticos na determinação de índices de produtividade na sub-região Pampa Arenosa da província de Buenos Aires, Argentina

RESUMO

A partir da década de 70, a sub-região Pampa Arenosa experimentou um aumento no seu regime de água, o que permitiu o crescimento da área dedicada à agricultura de secas. Em vista da intensificação da atividade agrícola, uma avaliação da terra é uma ferramenta estratégica essencial para o planejamento. O objetivo do trabalho foi destacar a importância de considerar diferentes cenários climáticos, na determinação do Índice de Produtividade, no setor de dunas longitudinais da sub-região Pampa Arenosa na Província de Buenos Aires, Argentina. Os

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cenários climáticos foram estabelecidos de acordo com as mudanças nos valores médios de precipitação, pelo teste Pettitt. As terras foram classificadas pelo índice de produtividade. Verificou-se que o índice de produtividade do solo aumentou com o aumento da precipitação, atingindo sua máxima expressão climática no período após a mudança abrupta. As terras de capacidade produtiva moderada com valores de Índice de Produtividade entre 65-51 ocuparam a maior área da área de estudo.

Palavras-chave: agroclimatologia, avaliação da terra, precipitação, teste de Pettitt.

1. INTRODUCTION

The Pampa region, located in the east-central part of the country (30°S - 40°S y 56°O - 65°O), is Argentina's main agricultural area. The Pampa Region has a humid temperate climate (Cf in the Köppen-Geiger classification, as revised by Kottek *et al.*, 2006). East winds predominate, driven by a semi-permanent anticyclone from the coast of Brazil. After being drawn across the Brazilian coastline, maritime subtropical air moves southeast, reaching up to 40° latitude in summer and about 30° latitude in winter. In this way, the Pampa Region receives sea winds throughout the year, with a moisture gradient decreasing from east to west (Pérez *et al.*, 2015).

Several studies indicate that the westward advance of the agricultural frontier in the Pampas during the last quarter of the twentieth century (Viglizzo *et al.*, 1995) was partly a consequence of increased rainfall (Castañeda and Barros 1994; Pérez *et al.*, 2011; 2015; Pérez and Sierra 2012). This increase in precipitation acted synergistically with an increasing demand from international markets (Trigo, 2005) and technological innovations (Satorre, 2005).

Some authors believe that the above-mentioned increase in rainfall is permanent. They attribute it to increased energy in the climate system caused by global warming. In their view, this has led to an increased thermal regime throughout the country, affecting the whole of its climate (Carril *et al.*, 1997; Minetti *et al.*, 2003; Barros, 2004). In contrast, others (Suriano and Ferpozzi, 1993; Roberto *et al.*, 1994; Pérez *et al.*, 2003; 2011; Sierra and Pérez, 2006, Pérez *et al.*, 2015) suggest that these changes are reversible. In their view, the Pampas have a long-term water cycle with wet and dry phases separated by transition periods during which the agricultural frontier either advances or retreats.

Starting in the 1970's, the Pampa Arenosa sub-region experienced an increasing water regime that generated an increasing area for dryland farming.

With increasing agricultural activity, examining the land becomes an essential strategic tool for its planning.

Several methods are used when creating land evaluation systems in terms of adaptability and/or vulnerability (De la Rosa *et al.*, 2004). In Argentina, the most frequent quantitative indirect classification is the productivity index (PI). This system was developed initially by FAO (Riquier *et al.*, 1970) and adopted by the National Institute of Agricultural Technology (in Spanish, Instituto Nacional de Tecnología Agropecuaria) according to the local agro-ecological conditions. The productivity index relates the property values or levels that have some influence on land productivity (Irigoin, 2011).

The objective of this work was to highlight the importance of considering several climate scenarios when setting the productivity index in the sector of longitudinal dunes in the Pampa Arenosa sub-region in the province of Buenos Aires, Argentina.

2. MATERIAL AND METHODS

The area of study comprises the second-level administrative subdivisions of Nueve de Julio, Carlos Casares, Pehuajó and Trenque Lauquen, which are located in the province of Buenos Aires, Argentina, in the sector of longitudinal dunes of the Pampa Arenosa sub-region. Annual rainfall data from four towns in the Pampa Arenosa sub-region for the period 1918-2011 was used (Table 1). Data was provided by the National Weather Service and the National Institute of Agricultural Technology.

The homogeneity of the precipitation series was tested using Alexandersson and Moberg's (1997) Standard Normal Homogeneity Test (SNHT) on AnClim software (Štěpánek, 2006). The test was applied to series of annual values, using the average annual rainfall of each sub-region as a reference series.

Table 1. Locations of towns in the Pampa Arenosa sub-region.

Town/City	Latitude (S)	Longitude (W)	Altitude (msl)
Nueve de Julio	35° 27'	60° 53'	76
Carlos Casares	35° 55'	61° 20'	88
Pehuajó	35° 48'	61° 54'	85
Trenque Lauquen	35° 58'	62° 43'	80

With the homogeneous precipitation series of each town, shifts in the average values were found using the Pettitt test. The climate scenarios were defined using the temporal series of rainfall for the periods before and after the shifts in the average values, identified as the dry period (DP) and the humid period (HP), respectively.

The productivity index (PI) was obtained by using the changes introduced by the National Institute of Agricultural Technology (INTA) for the Pampa region (Sobral and Nakama 1988; Maccarini, 1990; Sobral *et al.*, 2010). This parametric index comprises the following factors: Macro-climate condition (H); drain (D); effective depth (Pe); surface texture (Ta); sub-surface texture (Tb); salinity (Sa); sodicity (Na); organic matter (Mo); cation-exchange capacity (T), current and potential water and wind erosion (E).

The quantitative expression of the productivity index is calculated by multiplying the factors listed above. This multiplication intends to highlight the influence of each factor on the final value of the index (Equation 1):

$$PI = H * D * Pe * Ta * Tb * Sa * Na * Mo * T * E, \quad (1)$$

The results of the PI calculation are positive values, the highest of which is equal to 100, where the highest value represents the greater productive capacity of the land.

2.1. Climate factor of the PI

The climate factor H of the PI was calculated following the INTA methodology (Sobral and Nakama 1988; Maccarini, 1990).

The climate factor was calculated using Thornthwaite's global humidity index (Im), the soil moisture regimes (Moscatelli, 1991; Van Wambeke and Scoppa, 1980) and the 16°C isotherm (Table 2).

El Im (Thornthwaite, 1948; 1955) was obtained from the difference between the annual water excess (humidity index, Ih) and the deficiencies (aridity index, Ia) as the result of the water balance for maximum water storage capacity in soil of 100 mm and its relation with the potential evapotranspiration (ETP) estimated by Thornthwaite, where $Im = (Ih - Ia) / ETP$

(Castellví Sentí and Castillo, 2001). The value for Im for the area as a whole was obtained from the average of the value for Im for each area.

Table 2. Conversion of climate characteristics into coefficient H values (Macroclimate condition) (Maccarini 1990).

Global humidity index (Im)	Average annual temperature (°C)	Macro-climate condition (H)
> 10	≥16	100
10 to 0	≥16	95
0 to -10	≥16	90
-10 at ustic/aridic	≥16	75

2.2. Edaphics factors of the PI

The edaphic information for the different factors that make up the PI is the following: drain, alkalinity, salinity, texture, cation-exchange capacity, content of organic matter, current and potential water and wind erosion. All this data was obtained from soil charts (INTA, 1990).

The PI value was obtained for each taxonomic unit defined at a series level, from the use of conversion tables (Irigoin, 2011), where each edaphic factor participating in the parametric equation is classified into categories or ranges with their respective coefficients with values between 0 and 1 (Sobral *et al.*, 2010, Sobral and Nakama, 1988).

The PI value of the cartographic unit was calculated by weighing the PI values of each taxonomic unit and in relation to the percentage of area covered by each of them.

The PI values of the cartographic units were divided into six productive categories: very high (>80), high (80-66), moderate (65-51), moderately low (50-36), low (35-20) and very low (<20) (Irigoin, 2011).

3. RESULTS AND DISCUSSION

3.1. Homogeneity Test

According to the Homogeneity Test (SNHT), two of the annual precipitation series available (Table 3) showed a T value lower than the critical value (Khaliq and Quarda, 2007), and can be considered homogeneous at the level of significance $\alpha = 0.05$. The other two series showed T values higher than the critical value and were thus considered non-homogeneous. For the analysis, the non-homogeneous series were adjusted according to the method proposed by Alexandersson (1986).

Table 3. Test results of the SNHT applied to annual precipitation series for the Pampa Arenosa sub-region.

Town/City	Shift year	T Value	Adjusted shift year	Adjusted T value
Nueve de Julio	1984	7.107		
Carlos Casares	2010	4.157		
Pehuajo	1935	10.747 *	1954	2.191
Trenque Lauquen	1972	14.080 *	2010	4.647

(* indicates that the T value exceeds 95%).

3.2. Pettitt Test

The shifts in the values of the annual precipitation average according to the Pettitt Test (Pettitt, 1979) (Table 4) were produced in 1962 and 1965, thus defining the dry scenarios, before the shift (1918-1962/65) and wet, after the shift (1962/65-2005).

Table 4. Results after the Pettitt Test for the annual precipitation of the Pampa Arenosa sub-region.

Town/City	Shift year	Average annual rainfall (mm)	
		Before the shift	After the shift
Nueve de Julio	1962	893	16.8
Carlos Casares	1965	787	22.5
Pehuajo	1965	820	19.3
Trenque Lauquen	1962	708	38.3

The average of Nueve de Julio (Figure 1) showed a positive abrupt change, with the average annual precipitation rising from 893 mm during the 1918-1962 sub-period, to 1043 mm during the 1963-2011 sub-period.

Figure 1. Annual precipitation and means for sub-periods in the Nueve de Julio by Pettitt's method.

The average of Carlos Casares (Figure 2) showed a positive abrupt change, with the average annual precipitation rising from 787 mm during the 1918-1965 sub-period, to 964 mm during the 1966-2011 sub-period.

Figure 2. Annual precipitation and means for sub-periods in the Carlos Casares by Pettitt's method.

The average of Pehuajo (Figure 3) showed a positive abrupt change, with the average annual precipitation rising from 820 mm during the 1918-1965 sub-period, to 978.2 mm during the 1966-2011 sub-period.

Figure 3. Annual precipitation and means for sub-periods in the Pehuajo by Pettitt's method

The average of Trenque Lauquen (Figure 4) showed a positive abrupt change, with the average annual precipitation rising from 708 mm during the 1918-1962 sub-period, to 979.2 mm during the 1963-2011 sub-period.

Figure 4. Annual precipitation and means for sub-periods in the Trenque Lauquen by Pettitt's method.

3.3. Productivity Index

Table 5 shows the productivity indices in the sector of longitudinal dunes in the Pampa Arenosa sub-region in the province of Buenos Aires, Argentina. In the study area,

approximately 458,700 hectares have values of productivity index between 65 and 51, indicating that these lands have some type of permanent limitations for the production of common crops. On the other hand, these IP values would indicate that the feasible yield to obtain will correspond between 65 and 50% of the optimum yield for the region (Sobral and Nakama, 1988). A similar area (460,720 hectares) corresponds to lands of moderately low productive capacity with IP values of 50-36. These two IP categories represent more than half of the study area, approximately 54.7%. At the same time, lands with severe and very severe limitations for agriculture occupy 668,320 hectares with IP values of less than 35.

Table 5. Classification of the lands according to the Productivity Index (PI) for the study area and for each Town/City, expressed in % of occupation and km².

		Productivity Index (PI)					
Town/City	Occupation	100-81	80-66	65-51	50-36	35-21	20-0
Nueve de Julio	km ²	88.5	623.1	1717.8	215.2	1026.2	614.9
	%	2.1	14.5	40.1	5.0	23.9	14.4
Carlos Casares	km ²	0.0	200.0	830.4	28.3	943.5	516.5
	%	0.0	7.9	33.0	1.1	37.5	20.5
Pehuajó	km ²	0.0	3.4	1312.6	1381.7	740.6	1081.8
	%	0.0	0.1	29.0	30.6	16.4	23.9
Trenque Lauquen	km ²	0.0	0.0	726.2	2982.0	1340.1	419.6
	%	0.0	0.0	13.3	54.5	24.5	7.7
Study area	km ²	88.5	826.5	4587.0	4607.2	4050.4	2632.8
	%	0.5	4.8	27.3	27.4	24.1	15.7

According to the PI values for both climate scenarios and their location, we can observe a shift in the productivity of the land as the consequence of shifts in the climate factor.

The PI weighted average obtained for the period right after the abrupt shift for the Nueve de Julio area was 48.5, for Carlos Casares was 43.7, for Pehuajó was 39.9 and for Trenque Lauquen was 46.1 (Figure 5). On the other hand, the PI values for the period before were 39.4, 36.1, 36.9 and 34.4, respectively, according to the transect of these areas, from East to West (Figure 6).

Figure 5. Distribution pattern of the classes of productivity index of the land (PI) for the period after the abrupt shift in the area of Nueve de Julio (A), Carlos Casares (B), Pehuajó (C) and Trenque Lauquen (D).

Figure 6. Distribution pattern of the classes of productivity index of the land (PI) for the period before the abrupt shift in the area of Nueve de Julio (A), Carlos Casares (B), Pehuajó (C) and Trenque Lauquen (D).

In this way, the land resulted of moderately low productive capacity (50-36), both for the period after the abrupt shift and for the period before, thus showing that such land has permanent edaphic limitations for the production of conventional crops. The productivity index is interpreted as a proportion of the potential maximum yield of the most common crops in the region, eco-typically adapted, under a determined level of management.

4. CONCLUSION

The Pettitt Test (Pettitt, 1979), applied to the annual precipitation series of the Pampa Arenosa sub-region, showed that the sub-region had abrupt positive shifts during the second half of the 20th century. This sub-region was considerably affected by such shifts. Indeed, they changed from a semi-arid steppe climate (Bs in the Köppen Climate Classification System) (Köppen, 1948) during dry phases to a dry-summer subtropical climate (Cw in the Köppen classification) during wet phases, which significantly affects agricultural capacity.

It was found that the PI of the lands increased with the rain, reaching its highest value in the period right after the abrupt shift for all the studied areas. The Trenque Lauquen area was the area most positively affected by increasing precipitation.

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Adaptation of domestic effluent for agricultural reuse by biological, physical treatment and disinfection by ultraviolet radiation

ARTICLES doi:10.4136/ambi-agua.2292

Received: 12 Jun. 2018; Accepted: 18 Dec. 2018

Andressa de Almeida Soares Oliveira^{1*}; **Reinaldo Gaspar Bastos²**; **Claudinei Fonseca Souza¹**

¹Universidade Federal de São Carlos (UFSCar), Araras, SP, Brasil
Departamento de Recursos Naturais e Proteção Ambiental (DRNPA).
E-mail: dressasoares89@gmail.com, cfsouza@ufscar.br

²Universidade Federal de São Carlos (UFSCar), Araras, SP, Brasil
Departamento de Tecnologia Agroindustrial e Socioeconomia Rural (DTAISer).
E-mail: reinaldo@cca.ufscar.br

*Corresponding author

ABSTRACT

Domestic effluent reuse is an alternative for irrigated agriculture in situations of reduced water availability. However, as there is the presence of pathogens in wastewater, the disinfection process is necessary before use. This research evaluated the sanitary and agricultural viability of treated wastewater at a pilot-scale station composed of a septic tank, *Wetlands* and an ultraviolet radiation (UV) disinfection system. The Sewage Treatment Station (STS) is installed in the Agricultural Sciences Center, UFSCar, in Araras city, SP, and receives 2000 L of sewage daily, which was monitored in terms of pH, turbidity, electrical conductivity (EC), dissolved oxygen (DO), sodium (Na), potassium (K), calcium (Ca), total nitrogen (TN), total phosphorus (TP), magnesium (Mg), total organic carbon (TOC), total coliforms (TC), *Escherichia coli* (*E. coli*) and Sodium Adsorption Ratio (SAR). The removal efficiency of the parameters was calculated based on the input and output means of each treatment unit. Results indicated removal efficiency for K (68.2%), TN (54.1%) and TP (36.1%). The levels of Na (26.7%) and Ca (22.9%) demonstrated the contribution of these salts to the wastewater; however, SAR did not present any risks for soil sodification. Microbiologically, the STS presented removal efficiency of 4 and 5 log-cycles for TC and *E. coli*, respectively. Therefore, results of monitoring indicate that the treated wastewater presents sanitary and agricultural viability, and conforms with the current legislation.

Keywords: nutrients, sewage, ultraviolet radiation.

Adaptação de efluente doméstico para uso agrícola por tratamento biológico, físico e desinfecção por ultravioleta

RESUMO

O reúso de efluente doméstico é uma alternativa para a agricultura irrigada em situações de reduzida disponibilidade de água. No entanto, como é comum a presença de patógenos em águas residuárias, torna-se necessário um processo de desinfecção previamente à sua utilização. Com o desenvolvimento da pesquisa, avaliou-se a viabilidade sanitária e agrícola do efluente

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doméstico tratado em Estação Piloto composta por Tanque Séptico, *Wetlands* e sistema de desinfecção por radiação ultravioleta (UV). A Estação de Tratamento de Esgoto (ETE) se encontra instalada no Centro de Ciências Agrárias, UFSCar, em Araras-SP, e recebe diariamente 2000 L de esgoto, o qual foi monitorado em termos de pH, turbidez, condutividade elétrica (CE), oxigênio dissolvido (OD), sódio (Na), potássio (K), cálcio (Ca), nitrogênio total (NT), fósforo total (PT), magnésio (Mg), carbono orgânico total (COT), coliformes totais (CT), *Escherichia coli* (*E. coli*) e Razão de Adsorção de Sódio (RAS). A eficiência de remoção dos parâmetros foi calculada com base nas médias de entrada e saída de cada unidade de tratamento. Os resultados apontaram remoção para K (68,2%), NT (54,1%) e PT (36,1%). Os teores de Na (26,7%) e Ca (22,9%) demonstraram aporte desses sais ao efluente, porém, a RAS não apresentou riscos quanto à sodificação do solo. Com relação aos microbiológicos, a ETE apresentou uma remoção de 4 e 5 ciclos log para CT e *E. coli*, respectivamente. Sendo assim, os resultados do monitoramento indicam que o efluente tratado da ETE Piloto apresenta viabilidade sanitária e agrícola, estando de acordo com a legislação vigente.

Palavras-chave: esgoto, nutrientes, radiação ultravioleta.

1. INTRODUCTION

Population growth, climatic instability and increased demand for food culminated in the scarcity of water of adequate quality to irrigate agriculture (Ahmadi and Merkley, 2017). One of the strategies to ameliorate the water crisis is to invest in the reuse of effluents, which can intensify agricultural production even during off-season periods (Hespanhol, 2008).

Recycling nutrients in agriculture is a sustainable strategy since it minimizes the pollution of aquatic environments and decreases the dependence on mineral fertilizers (Pantano *et al.*, 2016).

However, the practice of reuse with effluents requires treatment steps that are allied with its purpose and application. The most commonly used systems employed for this are septic tanks and *Wetlands*.

New techniques are being studied in order to improve/increase the efficiency of removal of organic matter, salts and pathogens from Septic Tanks, employing the use of anaerobic reactors filled with zeolites modified with copper (Anil and Neera, 2016) or with the installation of electrodes of stainless steel (Lin *et al.*, 2017).

Increasingly, *Wetlands* are been implemented for the treatment of effluents from cleaning trucks, septic tanks, chemical toilets and even grease residues. A simple method, but one that requires caution as to its required efficiency, in order not to cause imbalances, such as low disinfection efficiency due to the inhibition of ultraviolet rays (Andrade *et al.*, 2017).

Treatment systems are designed according to removal efficiency, thus enabling the disposal of the effluent. Souza *et al.* (2015) developed a pilot-scale station consisting of a septic tank, anaerobic filter, microalgae tank and *Wetlands*, and noted that these associated units removed part of the essential nutrients for agriculture. In addition, there was an impediment to such application: the expressive presence of bacterial from the Coliform class.

In this sense, a disinfection as tertiary treatment becomes essential, considering the high burden of pathogens usually present in domestic effluents, in order to ensure the integrity of human health (Zhou *et al.*, 2015).

One of the options used for sewage disinfection is the application of the ultraviolet system (UV), which is based on electromagnetic radiation and wavelengths less than violet light, without the generation of by-products harmful to health or the release of residuals to the wastewater.

Fluorescent lamps responsible for the emission of UV radiation have in their interior a tube coated with phosphorus powder containing gas (argon type) and mercury. From an electrical potential difference, the electrons fluxes collide with the mercury atoms providing the UV light, which is absorbed by the phosphorus becoming visible (Salvetti, 2008).

Microorganisms are inactivated by the action of UV radiation, which causes damage to nucleic acids, macromolecules responsible for carrying genetic information (deoxyribonucleic acid - DNA) and protein synthesis (ribonucleic acid - RNA) (USEPA, 2003).

According to Regulatory Brazilian Standard NBR 13.969 (ABNT, 1997), the irrigation technique for some crops with the reuse of treated wastewaters is classified as Class 4 and may be destined to orchards, cereals, fodder, cattle pastures and other crops through surface runoff or point irrigation. Considering the World Health Organization (WHO, 2006) the types of irrigation can be:

- Restricted: cultures cannot be ingested raw, or unprepared, with the maximum of 10^5 allowed of *Escherichia coli* in 100 mL in the treated wastewater and;
- Unrestricted: can be consumed without pre-preparation with a total of 10^3 of *Escherichia Coli* in 100 mL of treated wastewater.

The perspective of sustainable development proposes the use of treated wastewaters in irrigated agriculture. However, the pathogenic load of sewage is high and may interfere with the advancement of this technique, causing sanitary problems to an extensive population through food and contaminated soil. The incidence of UV radiation in the treated wastewater is mainly aimed at microbiological reduction, especially that of the group of total coliforms and *Escherichia coli*.

Thus, the objective of the research was to evaluate the sanitary and agricultural viability of a treated domestic effluent, from a Sewage Treatment Station composed of the septic tank units, *Wetlands*, and UV disinfection.

2. MATERIAL AND METHODS

2.1. Pilot system of treatment of domestic sewage

Pilot Sewage Treatment Station (STS) is installed and operating at the CCA (Center of Agricultural Sciences - Federal University of São Carlos) in Araras city, State of São Paulo, latitude 22° 18'53.23" South, and longitude 47° 23'00.91" West; to 701 m of altitude. With Cwa type-subtropical climate and average annual precipitation of 1300 mm (Souza *et al.*, 2015). The STS was designed to receive daily volumes of 2000 liters of wastewater from the refectory and bathrooms of the University. Figure 1 shows a schematic of the arrangement of its treatment steps.

Figure 1. Schematic of the sewage treatment units of the pilot-scale station.

Source: Adapted from Souza *et al.* (2015).

In this way, the wastewater treatment system consisted of:

- Fat box: with a capacity of 24 L and constructed of PVC material, its function is to prevent clogging by coarse materials;
- Septic Tank: constructed according to NBR 7.229/93 (ABNT, 1993) of volume 5.0 m³ (1.723 m in diameter and 2.57 m in height), with a hydraulic retention time of 22 hours;
- Wetlands: 2.0 m³ tanks, divided in two (1.64 m in diameter x 0.44 m in height for each tank), in which was cultivated macrophyte Papyrus (*Cyperus*);
- Ultraviolet system: made in stainless steel, brand SODRAMAR (2016)¹ model SUV 7. Consisting of a lamp, model TUV 4P S/E of 16W, tubular format, length of 32.8 cm and life of approximately 7000 hours (Philips¹). The light is emitted in the UV-C band, which corresponds to about 260 nm.

The ultraviolet radiation dose of the lamp in question is 0.43 mWscm⁻², being calculated by the intensity of the flow (mWcm⁻²) and multiplied by the time of exposure of the effluent to the UV (seconds) (Philips, 2006).

All units have appropriate seals so that there is no contamination in the water table.

2.2. Monitoring of Station

Sewage samples were collected between October 2016 and September 2017, from 09:30 am to 11:30 am, and were placed in sterilized containers and immediately sent to the Water and Soil Laboratory of the Department of Natural Resources and Environmental Protection (DRNPA/CCA/UFSCar).

Wastewater monitoring was carried out according to the methodology indicated by the Standard Methods for the Examination of Water and Wastewater (APHA, 2012), in triplicate, corresponding to following parameters:

- Biweekly analyses: pH, dissolved oxygen (DO), turbidity and electrical conductivity (EC);
- Monthly analyses: total phosphorus (TP), total nitrogen (TN), calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), total coliforms (TC), *Escherichia coli* and Sodium Adsorption Ratio (SAR).

For bacteriological analysis, the enzymatic method Colilert¹ was used according to IDEXX (2016).

Sodium Adsorption Ratio (SAR) is calculated by sodium (Na), calcium (Ca) and magnesium (Mg) according to Equation 1 (Lesch and Suarez, 2009):

$$SAR = \frac{Na}{\sqrt{Ca+Mg}} \quad (1)$$

On what:

SAR = Sodium Adsorption Ratio (mmol_c L⁻¹)^{1/2};

Na = concentration of sodium (mmol_c L⁻¹);

Ca = concentration of calcium (mmol_c L⁻¹);

Mg = concentration of magnesium (mmol_c L⁻¹).

The efficiency of each parameter was calculated from a balance between the input and output means of each treatment unit (Equation 2) (Von Sperling, 2005):

$$E = \frac{C_e - C_s}{C_e} \times 100 \quad (2)$$

On what:

E = removal efficiency (%);

C_e = concentration of the parameter in the raw wastewater (input);

C_s = concentration of the parameter in the treated wastewater (output).

¹Trademark references do not constitute an endorsement by the authors.

3. RESULTS AND DISCUSSION

Table 1 presents the results of wastewater characterization from 24 samples during the one-year monitoring.

Table 1. Average values of raw wastewater (input) and treated wastewater (output) of monitoring parameters and removal efficiency.

Parameters	Input	Output	Overall efficiency (%)
pH	6.8	6.4	---
Turbidity (NTU)	87.3	2.7	96.9
EC (μS cm ⁻¹)	639.5	444	30.6
DO (mg L ⁻¹)	3.3	6.5	98.5*
Na (mg L ⁻¹)	8.2	10.3	26.7*
K (mg L ⁻¹)	49.4	15.7	68.2
Ca (mg L ⁻¹)	13.5	16.6	22.9*
TN (mg L ⁻¹)	23.4	10.7	54.1
TP (mg L ⁻¹)	5.6	3.6	36.1
Mg (mg L ⁻¹)	10.3	8.8	14.6
TOC (mg L ⁻¹)	60	23.6	60.6
TC (MPN 100mL ⁻¹)	4.7x10 ⁷	8.8x10 ³	99.98
<i>Escherichia coli</i> (MPN 100mL ⁻¹)	2.6x10 ⁷	6.1x10 ²	>99.99

EC = electrical conductivity; DO = dissolved oxygen; Na = sodium; Ca = calcium; TN = total nitrogen; TP = total phosphorus; K = potassium; TOC = total organic carbon; Mg = magnesium; TC = total coliforms in Most Probable Number (MPN).

* percentage increase of the parameter in the units of the Station.

Reuse in agriculture requires wastewaters to have a pH that does not negatively influence the development of agricultural crops. Martínez *et al.* (2015) describe that the pH for reuse in agriculture should be between 5 and 6.5, to facilitate the absorption of nutrients by plants.

In this sense, the crude wastewater presented averages of 6.8 at the entrance and 6.4 at the exit. Colares and Sandri (2013) also observed a decrease in pH in their cultivated bed surveys, concluding that the bacteria produce organic acids due to the anaerobic degradation of the organic matter, leading to discrete acidification of the wastewater.

The station showed a reduction of 96.9% of turbidity, with input and output averages of 87.3 and 2.7 NTU, respectively. At the exit of the septic tank, the turbidity presented an index of 80.9% and, after *Wetlands*, reached 83%. Colares and Sandri (2013) found lower values in relation to the septic tank of 37.8%; for the flooded systems, the averages found were close to those observed in the Station, with rates from 66.9 to 82.5%, which demonstrates excellent removal of suspended solids from the Pilot Station septic tank and *Wetlands*.

Electric Conductivity had a reduction efficiency of 30.6%, from 639.5 μS cm⁻¹ to 444 μS cm⁻¹, and the Na, Ca and K parameters showed variations in their concentrations. Souza

et al. (2015) found an increase in EC of 36.3% during the wastewaters treatment and stated that the main reason would be to raise the same parameters mentioned above in units containing brittle.

The content of DO had a mean input of 3.3 mg L⁻¹ and the output with 6.5 mg L⁻¹, totaling an increase in oxygenation of wastewater of 98.5%. In the *Wetlands*, there is an occurrence of photosynthesis, leading to an increase of dissolved oxygen at the outlet of the flooded system.

Sodium showed an increase of 26.7% in the final wastewater, the highest increase was observed at the exit of the septic tank, with rates of 15.8%. The final wastewater of the STS has 0.4 mmol L⁻¹ of sodium and, according to Ayers and Westcot (1999), does not present risk in the case of surface irrigation to the soil and the culture.

The overall efficiency of removal of K of the STS Pilot, since the entry, with 49.4 mg L⁻¹ until the exit with 15.7 mg L⁻¹, was 68.2%. The limit established for irrigation water is up to 2 mg L⁻¹ (Ayers e Westcot, 1999). Therefore, the value of the final wastewater of the STS presents restrictions on K, since they can present problems of loss of water to the plants, reducing transpiration, damaging growth and the absorption of other essential nutrients to the plant, such as calcium, magnesium, boron, zinc, manganese, and ammonium (Fia *et al.*, 2015).

Calcium had an increase of 22.9% from input to output (0.41 mmol L⁻¹) and its greatest contribution to this elevation was after the wastewater entered the system of *Wetlands*. According to Ayers and Westcot (1999), the threshold value found in irrigation water would be 10 mmol L⁻¹.

Results of NT demonstrated a contribution of 30.6% after the septic tank; however, the wastewater entering the *Wetlands* had a reduction efficiency of 74.6%. In the final wastewater, the concentration of NT detected was 10.7 mg L⁻¹. The total efficiency of removal was 54.1%. According to the recommended literature, the appropriate range for irrigation with wastewater would be 10 mg L⁻¹ of total nitrogen (Ayers and Westcot, 1999).

Total phosphorus showed an average input of 5.6 mg L⁻¹. The Pilot STS presented total removal efficiency of 36.1% TP. The highest reduction rate recorded in the Pilot Station was in the *Wetlands*, with 82.7%.

However, after the incidence of UV radiation on the wastewater, TP increased the rate from 2.4 to 3.6 mg L⁻¹, representing 50%. This elevation in TP contents may be associated with phosphate accumulating organisms present in wastewater under anaerobic and aerobic conditions.

These microorganisms submitted to ultraviolet radiation showed a breakdown of genetic material, where phosphorus would be absorbed intracellularly. After undergoing UV cell lysis, the nutrient becomes available and detectable in the final analysis of the effluent. By classification of Ayers and Westcot (1999), the maximum found in irrigation water for TP would be 0-2 mg L⁻¹.

A concentration of Mg had a total removal efficiency of 14.6%, the final wastewater was observed with 8.8 mg L⁻¹. The recommendation for application of water in irrigation is not to exceed the margin of 100 mg L⁻¹ (Ayers and Westcot, 1999).

The overall efficiency of removal of TOC from input to output was 60.6%. Only at the exit of the septic tank was the removal 59.8%, due to the biodegradation of the organic matter. After this process, the wastewater practically remained with the same average until the exit of the pilot STS.

Total coliforms reached a full efficiency of removal, that is to say, 99.98%, from 4.7x10⁷ to 8.8x10³ MPN per 100 mL in the crude wastewater. Moreover, removal was greater than 99.99% for *E. coli* from 2.6x10⁷ to 6.1x10² MPN per 100 mL in the crude wastewater, which demonstrates the high disinfection potential of ultraviolet radiation, reducing more than 4 log-

cycles from an initial load of TC and 5 log-cycles from an initial load of *E. coli*, suggesting microbiological safety.

The NBR 13.969/97 allows the application of water for reuse to include maximum values of 5.000 NMP 100 mL of *E. coli*. However, the World Health Organization (WHO, 2006) recommends that the values for *E. coli* do not exceed 10^3 in 100 mL for unrestricted irrigation, that is, crops that can be ingested raw.

As with the efficiency of the UV treatment unit, removals were 96.6 and 91% for TC and *E. coli*, respectively, reducing 1log-cycle with a radiation dose of $0.43 \text{ mW.s.cm}^{-2}$.

For this result, the difference between the wastewater values of the *Wetlands* outlet and the UV outlet was analyzed (Table 2).

Table 2. Monitoring of total coliforms (TC) and *E. coli* in the input and output of the treatment unit by UV radiation.

	Wetlands output	UV output
TC	$8.2 \times 10^4 \text{ MPN } 100 \text{ mL}^{-1}$	$8.8 \times 10^3 \text{ MPN } 100 \text{ mL}^{-1}$
<i>E. coli</i>	$6.8 \times 10^3 \text{ MPN } 100 \text{ mL}^{-1}$	$6.1 \times 10^2 \text{ MPN } 100 \text{ mL}^{-1}$

Bilotta and Daniel (2012) tested the effect of effective UV doses (123.0 and 247.0 mWscm^{-2}) on effluent from secondary treatment with exposure times of 60/120 seconds and noted the inactivation of 1.6 to 5.2 log-cycles of *E. coli*.

Souza *et al.* (2012) in one of their UV assays observed that 317.1 mWscm^{-2} were required to inactivate 3.7 and 2.0 log-cycles of *E. coli* and CT, respectively. However, a dose of 42.28 mWscm^{-2} was able to disinfect approximately 5 log of the same microorganisms.

Therefore, it is understood that the effluent quality interferes directly with the dose applied by UV radiation.

Sodium Adsorption Ratio (SAR) of the final wastewater presented an average of $0.5 (\text{mmolcL}^{-1})^{1/2}$, where the minimum observed was $0.2 (\text{mmolcL}^{-1})^{1/2}$ and the maximum $2.2 (\text{mmolcL}^{-1})^{1/2}$. This discrepancy in SAR may be associated with a disproportionate increase in Na, Ca and Mg. In order to avoid impacts by the application of the wastewater, the classification according to Ayers and Westcot (1999) for SAR should be between 0-15 $(\text{mmolcL}^{-1})^{1/2}$.

The septic tank, *Wetlands* and UV showed distinct behaviors, in which the parameters suffered inflow or removal efficiency (Table 3).

Table 3. Removal efficiency of parameters for treatment units.

	Removal efficiency %		
	Septic Tank	Wetlands	UV
Turbidity	80.9	83	4.3
EC	NR	38.5	NR
DO	NR	NR	NR
Na	NR	NR	NR
K	11.8	71.4	NR
Ca	NR	NR	9.9
TN	NR	74.6	NR
TP	NR	82.7	NR
Mg	14.3	NR	3.7
TOC	59.8	NR	2.19
TC	96.3	95.2	89.3
<i>E. coli</i>	99.3	96.1	91

NR: not removed.

The septic tank, considered a unit of primary treatment, was responsible for the largest removals of TOC, Mg, TC and *E. coli*.

The first two parameters are associated with the degradation and stabilization of organic matter by the anaerobic bacteria present in this treatment phase, and the release of methane gas. For the bacteriological indicators, the average of efficient removal may range from 60 to 90% (Von Sperling, 2005).

In the *Wetlands*, due to filtration and the polishing of wastewater, there was greater removal turbidity and a decreased EC, due to the consumption of salts by the macrophytes (Santos *et al.*, 2016).

The nutrients NT (74.6%), PT (82.7%) and K (71.4%), the main ones for the development of agricultural crops, demonstrated the removal efficiency of the percentages found in the literature (Fia *et al.*, 2017).

According to the presented results, the viability of the agricultural use of the treated wastewater of the Pilot STS is verified, mainly due to the contribution of nutrients. Therefore, this proposal is in line with sustainable agricultural production, since it represents an economy in terms of nutrients/water resources, combining agriculture with the environment.

4. CONCLUSIONS

In experimental conditions, it is possible to state that the final wastewater from the pilot scale Sewage Treatment Station has sanitary and agricultural viability for irrigation application.

5. ACKNOWLEDGMENTS

The authors gratefully acknowledge Coordination for the Improvement of Higher Education Personnel (CAPES) for the scholarship granted to A. A.S. Oliveira. Financial support was provided by FAPESP (2010/20081-7 and 2013/14893-7) and National Council for Scientific and Technological Development (CNPq) (303583/2012-6).

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Evaluation of water resource preservation areas in the Hydrographical Basin of Andreas Stream, RS, Brazil, using environmental monitoring programs

ARTICLES doi:10.4136/ambi-agua.2307

Received: 26 Jul. 2018; Accepted: 11 Jan. 2019

Rodrigo Augusto Klamt^{1*}; **Eduardo Alexis Lobo²**;
Adilson Ben da Costa²; **Dionei Minuzzi Delevati³**

¹Universidade de Santa Cruz do Sul (UNISC), Santa Cruz do Sul, RS, Brasil
Programa de Pós-Graduação em Tecnologia Ambiental (PPGTA). E-mail: rodrigoklamt@hotmail.com

²Universidade de Santa Cruz do Sul (UNISC), Santa Cruz do Sul, RS, Brasil
Departamento de Biologia e Farmácia. Programa de Pós-Graduação em Tecnologia Ambiental (PPGTA).
E-mail: lobo@unisc.br, adilson@unisc.br

³Universidade de Santa Cruz do Sul (UNISC), Santa Cruz do Sul, RS, Brasil
Departamento de Engenharia e Ciência Agrárias.
Programa de Pós-Graduação em Tecnologia Ambiental (PPGTA). E-mail: dionei@unisc.br

*Corresponding author

ABSTRACT

This research evaluated the effectiveness of the implementation of water resource preservation areas in the Hydrographical Basin of Andreas Stream, county of Vera Cruz, RS, Brazil, established through Payment for Environmental Services (PES), using an environmental monitoring program to assess physical, chemical and microbiological variables. The PES is linked to the "Water Guardian" project, which aims to ensure the preservation of water resources by paying farmers for providing the environmental services to protect the springs and riparian areas that lie within their properties. Twenty sampling stations were selected for collecting water samples monthly during the period July 2012 to June 2014 (460 samples) to evaluate the following variables: water temperature, pH, turbidity, dissolved oxygen, biochemical oxygen demand, nitrate, ammonia nitrogen, phosphate, total dissolved solids and thermotolerant coliforms. The evaluation was made based on decree 357/2005 of the National Environmental Council (CONAMA) and by applying the Water Quality Index (WQI). The results indicated a significant improvement in the water quality when comparisons were made between the periods before and after the installation of the preservation areas. The sampling points classified as "good" (CONAMA and WQI), which indicates water of good quality that is appropriate for multiple uses, increased from 52.0% to 72.0%. In this sense, promoting the preservation of headwaters and riparian zones becomes of fundamental importance and highlights the role of PES as an operational tool for water resource preservation.

Keywords: hydrographical Basin of Andreas Stream, payment for environmental services (PES), water quality.

Avaliação de áreas de preservação de recursos hídricos na Bacia Hidrográfica do Arroio Andreas, RS, utilizando programas de monitoramento ambiental

RESUMO

A presente pesquisa objetivou avaliar a efetividade da implantação de áreas de preservação de recursos hídricos na Bacia Hidrográfica do Arroio Andreas, município de Vera Cruz, RS, estabelecida através do Pagamento por Serviços Ambientais (PSA), utilizando um programa de monitoramento ambiental para avaliar variáveis físicas, químicas e microbiológicas. O PES está vinculado ao projeto "Guardião da Água", que visa garantir a preservação dos recursos hídricos, pagando aos agricultores a prestação de serviços ambientais para proteger as nascentes e áreas ribeirinhas que se encontram dentro de suas propriedades. Vinte estações de amostragem foram selecionadas para coleta mensal de amostras de água no período de julho de 2012 a junho de 2014 (460 amostras) para avaliar as seguintes variáveis: temperatura da água, pH, turbidez, oxigênio dissolvido, demanda bioquímica de oxigênio, nitrato, nitrogênio amoniacal, fosfato total sólidos dissolvidos e coliformes termotolerantes. A avaliação foi feita com base no decreto 357/2005 do Conselho Nacional do Meio Ambiente (CONAMA) e na aplicação do Índice de Qualidade da Água (IQA). Os resultados indicaram uma melhora significativa na qualidade da água quando comparações foram feitas entre os períodos antes e depois da instalação das áreas de preservação. Os pontos de amostragem classificados como "bons" (CONAMA e WQI), que indicam água de boa qualidade e adequada para usos múltiplos, aumentaram de 52,0% para 72,0%. Nesse sentido, promover a preservação de cabeceiras e zonas ribeirinhas passa a ser de fundamental importância e destaca o papel do PSA como ferramenta operacional para a preservação dos recursos hídricos.

Palavras-chave: Bacia Hidrográfica do Arroio Andreas, pagamento por serviços ambientais (PSA), qualidade da água.

1. INTRODUCTION

In Brazil, water is considered an abundant natural resource compared to the current demand, although there are dry areas where water is used only for human needs. Although Brazil holds 8% of all freshwater in the world, the situation in the country is delicate. Most of the available volume (80%) is in the Amazon region, and the remaining 20% is unevenly divided among the other regions in which 95% of the population lives (UNEP; IETEC, 2001). In the South, there are water shortages despite the large rivers and flow. These shortages are due to the cumulative effects of environmental processes and a broad spectrum of multiple water use in agriculture, industry, fisheries, navigation and recreation (Tundisi, 2000). Additionally, the quality of the small amount of available freshwater is degraded, mainly due to the rapid population growth observed in recent decades. This growth has generated a growing number of polluting processes that significantly alter the ecological balance of ecosystems, highlighting cultural eutrophication as one of the biggest problems for surface water and one of the most visible examples of the changes caused by humans to the biosphere (Smith and Schindler, 2009).

According to Esteves (2011), eutrophication is the concentration of nutrients, particularly nitrogen and phosphorus species or derivatives, in aquatic ecosystems; one result of eutrophication is increased productivity of biomass and decreasing of dissolved oxygen content, and thus the death of living biomass and biodegradation intensification. Eutrophication is a global problem that produces a marked deterioration of rivers, reservoirs, lakes and coastal

waters and causes direct and indirect effects on biodiversity. Additionally, eutrophication jeopardizes the multiple uses of surface and groundwater resources, causing substantial losses to the local and regional economy. Eutrophication is a threat to public health and the general functioning of aquatic systems and is an important economic factor that can prevent or delay sustainable development (Tundisi, 2000; 2003; 2006 and 2007).

Among the complementary alternatives to local-sustainable development, payment for the provision of environmental services (PES) has been highlighted as a recent and innovative policy for environmental conservation. PES is defined as the voluntary transfer of funds from beneficiaries of such environmental services to those who perform them for the proper management of the ecosystem where these environmental services are being provided (Wunder *et al.*, 2008). Because PES is a relatively new concept and practice, governments have only recently begun to recognize the role that farmers and users in rural areas can have on the improvement of environmental management. Tools for payment or compensation suggest that through incentives there will be a change in the behavior of economic agents in relation to the environment. According to the United Nations (ONU, 2011), the following environmental services are included in PES: the conservation and natural treatment of water resources, the preservation and conservation of biodiversity, soil erosion protection, carbon sequestration in the growth stage of the plant, regulating the local climate, besides the landscape formation and composition.

Based on these antecedents, the University of Santa Cruz do Sul (UNISC), in partnership with the Universal Leaf Tobacco Company and Fundación Altadis (non-profit organization, part of the Imperial Tobacco Group) signed a contract in 2011 for the execution of the project "Payment for Environmental Services" (PES) in the "Hydrographical Basin of Andreas Stream, county of Vera Cruz, RS, Brazil." The project, "Water Guardian," was to be developed over a period of five years (2011-2015) with the goal to protect the headwaters and the riparian areas of this basin and ensure the preservation of water resources by paying smallholder farmers for providing the environmental service to protect the headwaters located in their properties.

In this context, this study evaluated the effectiveness of the implementation of these conservation areas in the basin of Andreas Stream, RS, from July 2012 to June 2014, using environmental monitoring programs to assess physical, chemical, and microbiological variables.

2. MATERIALS AND METHODS

2.1. Field of study

The Hydrographical Basin of Andreas Stream, RS, is of fundamental importance as the source of water for the city of Vera Cruz. The soil relief map shows variations and terrain altitudes ranging between 500 and 100 m. Land use is characterized by agriculture, including the cultivation of rice. It has an area of 80.2 km² with a 21 km extension, with a population of 11,183 inhabitants in the urban area and 2,964 inhabitants in the rural area (IBGE, 2010). Along this basin, 20 sampling points have been selected (Figure 1) to carry out environmental monitoring studies in the rural properties that have joined the "Water Guardian" project.

2.2. Collection/analysis of samples

Monthly scientific expeditions were carried out from July 2012 to June 2014 in the 20 sampling points distributed along the Andreas Basin stream to measure physical, chemical and microbiological variables; a monthly sample was collected at each point during the period. The study period was divided into two stages: from July 2012 to June 2013, during which the conservation areas around the sampling points were being installed; and from July 2013 to June 2014, during which the areas of preservation around the sampling points were installed.

Figure 1. Map of the study area showing the location of Hydrographical Basin of Andreas Stream in the state of Rio Grande do Sul, highlighting the selected sampling points.

The following variables were considered as environmental evaluation parameters: temperature, pH, turbidity, dissolved oxygen, biochemical oxygen demand after five days, nitrate, nitrite, total ammonia nitrogen, phosphate, total dissolved solids and thermotolerant coliforms. The samples were collected on the water surface using 300-1000 ml glass bottles packed in a Styrofoam box containing ice and the samples were analyzed the day after collection. The techniques used in sample collection and analytical determination of the physical, chemical and microbiological variables are described in APHA (2005).

2.3. Water Quality Assessment

Based on the environmental analysis, the assessment of water quality was determined by Resolution Number 357 of the National Environmental Council (CONAMA), 2005 (CONAMA, 2005), which classifies fresh water into five different classes depending on the uses for which it is intended. The assessment of water quality was also made using the Water Quality Index (WQI) originally developed by the National Sanitation Foundation (NSF, 2007) of the United States and adapted for Brazilian waters by COMITESINOS (1993). To use the WQI in southern Brazilian river systems, Moretto *et al.* (2012) conducted a calibration of the weights of the variables used in the WQI formulation, checking for a significant correlation between the new calibrated weights and resolution 357/2005 of CONAMA (2005). The WQI calculations were made using the IQADData program developed by Posselt and Costa (2010). This software allows for both the application of the original NSF model or the development of new quality models, such as the calibrated WQI proposed by Moretto *et al.* (2012).

To operationally compare the water-use classes of CONAMA related to the quality requirements set by the IQA, a relative scale of agreement was used (Table 1). The scale reflects the main objective of the "Water Guardian" project, which is to achieve good water quality in the preserved areas, providing water that is suitable for multiple uses (human consumption after simplified treatment, protection of aquatic communities and primary contact recreation), a condition that characterizes the water-use Classes 1 and 2 of CONAMA (2005).

Table 1. Relative scale of agreement between the water use classes of CONAMA and the quality levels of the IQA.

Classes of use of CONAMA 357/2005	Quality levels
Special	Excellent
Class 1	Good
Class 2	Good
Class 3	Regular
Class 4	Poor/very poor

3. RESULTS AND DISCUSSION

The results of water quality evaluation in the 20 sampling points distributed along the Andreas stream from July 2012 to June 2013, (Table 2), (before installation of the protected areas), using Resolution 357/2005 of CONAMA (2005), are shown in Figure 2. Among the 220 sampling points, 32.0% were evaluated as "Class 1," 29.0% as "Class 2," 22.0% as "Class 3," and 17.0% as "Class 4."

Table 2. Results of physico-chemical and biological analyses 2012-2013.

Quality indicator	Average	Standard deviation
Dissolved oxygen, mg O ₂ / L	6,887	0,437
pH	6,876	0,903
Temperature water, °C	18,496	2,163
Turbidity, FTU	23,592	13,938
Nitrate, mg / L NO ₃ -	1,351	0,723
Thermotolerant coliforms, NMP/100 mL	2273,926	7740,408
BOD-biochemical oxygen demand, mg O ₂ / L	4,181	0,846
Phosphate, mg/L (PO ₄) ₃ -	0,119	0,108
Total ammonia nitrogen, mg / L NH ₃	163,452	0,565
Nitrite, mg/L NO ₂ -	174,425	0,017
Total dissolved solids, mg/L	114,512	28,007

Figure 2. Classification of the 220 sampling points according to the decree 357/2005 of CONAMA (points P1 to P20), from July 2012 to June 2013.

The sampling points classified as Class 1 of CONAMA (32.0%) are characterized as having good water quality that is appropriate for human consumption after simple treatment, the protection of aquatic communities and primary contact recreation (bathing), as its main features (CONAMA, 2005). The sampling points classified as Class 2 of CONAMA (29.0%) also correspond to good quality water; the main difference compared to Class 1 is that Class 2 water can be used for human consumption only after conventional treatment (CONAMA, 2005).

The sampling points classified as Class 3 of CONAMA (22.0%) have much more restrictive uses for their water than Class 2; for example, human consumption after conventional or advanced treatment, secondary contact for recreation and for cattle. Finally, the sample points classified as belonging to Class 4 of CONAMA (17.0%) correspond to a class of lower quality water intended only for navigation and landscape harmony (CONAMA, 2005).

The results of evaluation using the Water Quality Index (WQI) adapted to the conditions in southern Brazilian by Moretto *et al.* (2012) in the 20 sampling points distributed along the basin of Andreas stream, RS, from July 2012 to June 2013 (before installation of the conservation areas), are shown in Figure 3. Among the 220 sampling points, 52.0% were classified as "good," 46.0% as "Regular" and 2.0% as "Poor."

Overall, during the period prior to the installation of the conservation areas, the results of the application of WQI indicated that 52.0% of the 220 samples collected were classified as "good," which corresponds to the 61% of Class 1 and 2 samples of CONAMA, indicating that these sampling points are characterized by good water quality that is appropriate for multiple uses. For the critical sampling points that showed water quality lower than the desired standards, the results of the application of WQI indicated that 48.0% of the 220 samples collected were classified as "Fair" or "Poor," values that correspond to the 39.0% of samples classified as Classes 3 and 4 of CONAMA, indicating that these sampling points have far more restrictive uses.

Figure 3. Rating of sampling points according to the WQI (points P1 to P20), from July 2012 to June 2013.

From July 2013, the installation of conservation areas in the 20 sampling points distributed along the Andreas stream was completed, and then a new sampling campaign started, according to results of the (Table 3). The results of the water quality evaluation, using the Resolution 357/2005 of CONAMA (2005), from July 2013 to June 2014 are shown in Figure 4. Among the 240 sampling points, 49.0% were classified as "Class 1," 24.0% as "Class 2," 15.0% as "Class 3," and 12.0% as "Class 4". The results of the WQI are shown in Figure 5. Among the 240 sampling points, 72.0% were classified as "Good" and 28.0% as "Regular."

Table 3. Results of physico-chemical and biological analyses 2013-2014.

Quality indicator	Average	Standard deviation
Dissolved oxygen, mg O ₂ / L	8,695	0,952
pH	6,919	0,036
Temperature water, °C	19,561	0,531
Turbidity, FTU	13,638	2,628
Nitrate, mg / L NO ₃ -	1,023	0,235
Thermotolerant coliforms, NMP/100 mL	744,891	851,480
BOD-biochemical oxygen demand, mg O ₂ / L	2,334	1,455
Phosphate, mg/L (PO ₄) ₃ -	0,069	0,126
Total ammonia nitrogen, mg / L NH ₃	0,510	0,258
Nitrite, mg/L NO ₂ -	0,033	0,005
Total dissolved solids, mg/L	100,489	11,286

Figure 4. Classification of the 240 sampling points according to the decree 357/2005 of CONAMA (points P1 to P20), from July 2013 to June 2014.

Figure 5. Rating of sampling points according to the WQI (points P1 to P20), from July 2013 to June 2014.

The results of the application of WQI after the installation of the conservation areas indicated that 72.0% of the 240 samples collected were classified as "good," which corresponds to the 73.0% of samples belonging to Classes 1 and 2 of CONAMA. The results indicated that 28.0% of the critical sampling points were classified "Regular," which corresponds to the 27.0% of samples belonging to classes 3 and 4 of CONAMA.

Comparing the results of the WQI with those of Resolution CONAMA 357/2005, small differences, 9.0% and 2.6%, respectively, were found in the percentages separating sampling points appropriate for the noble uses of water (level "good" or Classes 1 and 2) from the critical sampling points (levels "Regular or Bad" or Classes 3 and 4) in the period before the installation of the conservation areas, whereas 1% differences were found in both cases in the period after the installation of the conservation areas. These differences could be explained by the differences in evaluation methods; when applying the CONAMA resolution in any point of collection, the value of the variable with the worst performance will be responsible for the final framing of this sampling point, while the WQI is calculated by the weighted multiplier of the

corresponding water quality for each parameter evaluated, from the weights of importance of the nine variables used to set the index. The WQI is, therefore, a holistic index that characterizes the overall condition of a sampling point from the interaction of the nine variables that comprise the index. The WQI used was modified by Moretto *et al.* (2012) for their application in the Brazilian southern river systems. Thus, this WQI constitutes an effective technological tool to be used in environmental monitoring programs in Brazilian southern river systems, and it also provides significant information about the general environmental condition of a sampling point, complementing the results from the implementation of Resolution 357/2005 of CONAMA.

Overall, the results indicated that there was a significant improvement of water quality from the physical, chemical and microbiological points of view, comparing the periods before and after the installation of the preservation areas. We can actually see the results comparing Table 2 with Table 3, which demonstrates that the parameters obtained a significant improvement. There was a 38.5% increase of sampling points classified as having "good" water quality (CONAMA Classes 1 and 2), represented by increases from 52.0% to 72.0%, indicating that these sampling points are characterized as having good water quality suitable for multiple uses. Concomitantly, it was found that there was a 39.1% reduction of sampling points classified as "Regular" (CONAMA Class 3), represented by decreases from 46.0% to 28%. Additionally, in the period after installation of the conservation areas, there was no sampling point ranked as "Poor" (CONAMA Class 4) compared to the previous period, when 2% of the sampling points were determined to be in this condition.

Although the reductions of the number of sampling points classified as "Regular" was evident, these points are critical, because the uses for the water in these points is more restrictive than for the water in sampling points classified as Class 1 and 2. These results could be explained by considering that these areas, even though protected, are still subject to the impact of a series of local human activities, such as the supply of nutrients and organic load from domestic sewage, cattle raising and the excess of fertilizers and agricultural inputs used in crops.

These environmental impacts can be evaluated from the variables responsible for the classification of the 28.0% of the samples as "Regular" (CONAMA Class 3), namely, the biochemical oxygen demand, dissolved oxygen, phosphorus, turbidity, nitrate and coliform. Thus, according to these variables, it is concluded that organic contamination and eutrophication of the water are the main environmental problems that characterized these water bodies. These results corroborate the environmental monitoring work in the regional water systems performed by UNISC, which has demonstrated that these water systems already have advanced stages of eutrophication (Bes *et al.*, 2012; Böhm *et al.*, 2013; Düpont *et al.*, 2007; Hermany *et al.*, 2006; Lobo *et al.*, 2004a, 2004b, 2004c, 2004d, 2010, 2014; Oliveira *et al.*, 2001; Salomoni *et al.*, 2006; 2011; Schuch *et al.*, 2012; 2014; Wetzel *et al.*, 2002). According to Tundisi (2006), this condition characterizes in a general way all the waterways throughout the southern region of Brazil, according to the results obtained by the project "Brazil Waters."

4. CONCLUSION

The results clearly show that the preservation of water resources in headwaters and riparian zones plays an important role in water quality because the forests develop naturally and act as a protection zone in addition to being a source of food and protection for wildlife (Felfili *et al.*, 2000). It is essential to recognize the importance of the environmental services provided by riparian forests and their influence on soil conditions, biodiversity, hydrological cycle and climate (Franco, 2010). Thus, promoting the recovery of degraded areas, especially those that are part of the permanent protection areas along rivers and springs, is of fundamental importance and highlights the role of the Payment for Environmental Services (PES) as an operational tool in the preservation of water resources (Tundisi *et al.*, 2007).

Currently in Brazil, there is a number of projects aimed at water resource preservation that utilize PES, such as the project "Production of Water" in the basin of Pipiripau stream, DF. This project verified a 74% reduction of the costs of water treatment and a reduction of approximately 70% in water pollution by mercury and pesticides (Melo, 2013). There are other ongoing projects, such as the River Basin Benavente, ES, which is expected to expand to the entire state (ANA, 2014). In the basin of the Cantareira system, there are also PES experiences in Ribeirões do Moinho in Nazaré Paulista, SP, and Cancã in Joanópolis, SP (Melo, 2013). Finally, the Secretary of State for the Environment in Rio de Janeiro launched a River Parks Program aimed at the preservation of water resources by planting millions of trees on the banks of the Guandu and Macacu Rivers (INEA, 2014).

5. ACKNOWLEDGEMENTS

The authors thank CAPES/FAPERGS (Higher Education Personnel Training Coordination/Rio Grande do Sul Research Foundation) for the scholarship grant to the first author to pursue graduate studies. We like to thank CNPq for financial support through the Edict MCT/CNPq/Universal nº 14/2011. Also thanks are due to Universal Leaf Tobacco Company and Fundación Altadis, for financial support to the project "Water Guardian" in the period 2011/2015.

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A proposal to integrate the legal definition and official delineation of watersheds in Mexico: eight model case studies

ARTICLES doi:10.4136/ambi-agua.2198

Received: 18 Oct. 2017; Accepted: 18 Jan. 2019

**Sergio Arturo Rentería-Guevara^{1,2} ; Antonio Sanhouse-García¹ ;
Yaneth Bustos-Terrones³ ; Abraham E. Rodriguez-Mata³ ;
Jesus Gabriel Rangel-Peraza^{1*} **

¹Instituto Tecnológico de Culiacán, Culiacán, Sinaloa, Mexico

E-mail: sergiorenteria@uas.edu.mx, asanhouse@itculiacan.edu.mx, jesus.rangel@itculiacan.edu.mx

²Universidad Autónoma de Sinaloa, Culiacán, Sinaloa, Mexico

E-mail: sergiorenteria@uas.edu.mx

³Consejo Nacional en Ciencia y Tecnología (Conacyt), Mexico City, Mexico

Cátedras Conacyt. E-mail: yabustoste@conacyt.mx, arodriguez@itculiacan.edu.mx

*Corresponding author

ABSTRACT

A hydrological basin is established as a geographic frame to officially determine surface water availability and is basic to confer or deny water rights. In Mexico, the legal definition of a hydrological basin is confusing and inconsistent with the official basin delineation published by the authority. This situation is demonstrated through the analysis of eight study cases located in Hydrological Region 10 Sinaloa. A proposal to define the legal term of hydrological basin, ensuring its consistency with official delineation of hydrological basins is presented. Geographic analysis was performed using QGIS software, by using official sources of Mexico and worldwide data bases. By law, in Mexico a water parting is defined as a borderline of maximum elevation. Nevertheless, the legal definition fails to explain hydrologic basin official boundaries where the basin limit is the location of a hydrometric station. Besides, the presence of this equipment does not mean that a waterbody exists in a hydrological basin, or that the delimited basin is an autonomous differentiated unit, as it is required by the legal definition. Other inconsistencies are presented dealing with the presence of inland water bodies as watershed limits and with groups of independent watersheds incongruously considered as a single basin. An amendment is proposed to harmonize this situation, with a minimum of changes in policy documents. The method proposed can be useful to analyze similar situations in other countries where discrepancies between the legal and official definitions of watershed delineation are also observed, or in countries where no official watershed definition has been established.

Keywords: hydrological basin delineation, surface water availability, water parting.

Uma proposta para harmonizar a definição legal e delimitação oficial das bacias hidrográficas no México: oito casos estudados como exemplo

RESUMO

Uma bacia hidrológica é estabelecida como um quadro geográfico para determinar a disponibilidade oficial de água superficial. Portanto, a delimitação da bacia hidrológica é básica para conferir ou negar os direitos da água. Apesar disso, em certas regiões, como o México, a definição legal da bacia hidrológica é confusa e não é consistente com a delimitação oficial da bacia publicada pelas autoridades. Isso é demonstrado neste trabalho por meio de oito estudos de caso. É apresentado um esquema para definir o termo legal da bacia hidrológica, garantindo sua consistência com a delimitação oficial das bacias hidrológicas. A análise geográfica foi realizada utilizando o software QGIS, utilizando fontes oficiais do México e bases de dados internacionais. Por lei, no México, uma separação de água é definida como uma fronteira de elevação máxima. Porém, a definição legal não explica os limites oficiais da bacia hidrológica, onde o limite da bacia é a localização de uma estação hidrométrica. Além disso, a presença deste equipamento não significa que existe um corpo de água em uma bacia hidrológica, ou que a bacia delimitada é uma unidade autônoma diferenciada, como é exigido pela definição legal. São apresentadas outras inconsistências que tratam da presença de canos de água do interior como limites de bacia hidrográfica e com grupos de bacias hidrográficas independentes consideradas incongruentes com uma bacia única. Uma alteração é proposta para harmonizar esta situação com mudanças mínimas nos documentos oficiais. O esquema proposto neste trabalho pode ser útil para analisar situações semelhantes em outros países onde também são observadas discrepâncias entre a definição legal da bacia hidrográfica e a delimitação oficial das bacias hidrográficas ou em países onde nenhuma bacia hidrográfica oficial esteja estabelecida.

Palavras-chave: delimitação da bacia hidrológica, disponibilidade de água superficial, linha divisória de águas.

1. INTRODUCTION

A hydrological basin is an area delineated by nature itself, fundamentally by the borders of surface runoff zones that converge towards a single watercourse (Reis *et al.*, 2015). It is considered the suitable territorial unit to manage water resources, even to manage the whole environment. Watershed management is an essential component of adequate environmental administration (Imperial, 2005). The watershed concept is also considered a risk management unit for hydrometeorological events due to the fact that climate change has made it necessary to develop adaptation programs to maintain and increase surface and ground water availability, with a focus on integrated hydrographic watershed management (Tomer and Schilling, 2009).

Thus, this natural territorial division tends to acquire an administrative connotation: “*The current National Water Bank (NWB) watersheds is basically an administrative application*” (Chile, 2013). Such connotation can even become official as in Chile and Mexico, respectively: “[...] *this watershed delineation is the administrative official delineation of General Water Administration (GWA)*” (Chile, 2013); “*Therefore, it is necessary to specify and publish the watershed limits to provide water users with a higher certitude about the exact localization of the geographic sites where water use occurs and to improve water management*[...]” (CONAGUA, 2016a).

This watershed delineation is an input for governmental instruments for river basin

development planning and management (RBDPM), by which water is distributed among users in a watershed-based territory division. However, *“sometimes political influence is observed in RBDPM boundaries, where the political territorial limits match rather than natural drainage basin delineation”* (Barrow, 1998). In Mexico, the National Water Commission (CONAGUA) recognizes 37 Hydrological Regions whose basin-based frontiers are adjusted to municipal political limits to form the borders among hydrological administrative regions (CONAGUA, 2010). The distribution of municipalities among Mexican watersheds was carried out depending on their socioeconomic, demographic and territorial dynamics (Espejel *et al.*, 2005; Sotelo *et al.*, 2010). In this sense, territorial divisions in natural hydrological basins and administrative hydrological basins do not match: *“The first evident problem, and the most important of all, is the differences between NWB watersheds and real watersheds”* (Chile, 2013).

When natural and administrative boundaries do not match, problems arise in calculating water balances in hydrological basins. Further problems generated by mismatch between natural and official limits affect underground water recharge calculation, hydrogeological modeling, surveillance plan design for water quality norms and aquifer sectorization (Chile, 2013). Therefore, the official delineation of a hydrological basin, as an administrative and a legal instrument, must be consistent with the legal definition of this territorial entity.

Countries like Argentina (2003), Bolivia (1992), Chile (2018), Colombia (2004), Ecuador (2014), México (CONAGUA, 2017a) and Peru (1969), among others, have their respective legal definitions of a hydrological basin named according to local terminology: *“As a consequence of this lack of conceptual consensus, due to the Spanish terminology, there have been interagency problems about hydrological basin management actions and law formulation deficiencies”* (CEPAL, 1994).

The aim of this paper is to contrast the definition of a hydrological river basin established in the Mexican Water Law with officially delineated basins to determine discrepancies and to present a proposal to overcome them. This is achieved by analyzing eight official river basins in Sinaloa, Mexico. Based on the differences found, a new definition of hydrological basin is proposed with the purpose of harmonizing this situation.

2. MATERIALS AND METHODS

2.1. Study area

The Mexican territory is divided in 37 hydrological regions. Eight basins were taken as study cases for this paper. All of these basins are in Hydrological Region 10 of Sinaloa (Figure 1).

Figure 1. Hydrological regions in Mexico, Hydrological Region 10 Sinaloa and basins under study.

Sources: Prepared by authors with shape files from CONABIO (2009) and basin vertices published in CONAGUA (2016a).

2.1.1. Humaya and Tamazula basins

These basins are part of the Culiacan River Basin that is located between parallels 26°03'12.98" N and 24°27'09.78" N and between meridians 107°45'38.81" W and 105°48'34.16" W. Culiacan River watershed occupies the second-most important terrain portion of Hydrological Region 10. The Culiacan River is 305 km long; it is a sixth-order stream and has an angular drainage pattern. This basin is exorheic, draining to the California Gulf and has a maximum altitude of 3,207 meters above sea level. Culiacan River Basin occupies territories of the Mexican states of Chihuahua, Durango and Sinaloa. The Culiacan River is the main stream which is formed by the junction of Humaya and Tamazula rivers in the city of Culiacan (Sanhouse-Garcia *et al.*, 2017).

The Humaya River is the largest affluent of the Culiacan River. This river has its origin in Durango state, located at 3,100 m.a.s.l. The Humaya River Basin has a surface area of 11,104.0 km², where its runoff is estimated in 1,907.0 Mm³ per year (CONAGUA, 2016b). The Adolfo Lopez Mateos dam is situated on the Humaya River upstream from its confluence with the Tamazula River located in Culiacan City. The Tamazula River Basin has a catchment area of 3,307.0 km² and a natural runoff annual volume of 755.4 Mm³ (CONAGUA, 2016b). The Sanalona Dam is located on this river before its confluence with the Humaya River.

2.1.2. Group of streams Altata and Pabellones basins

Group of Streams Altata Basin is in an area situated between parallels 24°45'45.54" N and 24°32'27.20" N and between meridians 107°59'37.21" W and 107°46'10.81" W. Its topography is mainly flat, with a maximum altitude of 9 m.a.s.l. This watershed has a surface area of 299.1 km² and a natural surface runoff of 17.6 Mm³ per year (CONAGUA, 2016b). The Group of Streams Altata area has no main stream. Its territory is drained by diverse small watercourses to the sea through Altata cove

Group of Streams Pabellones is an area placed between parallels 24°47'28.51" N and 24°16'17.63" N and between meridians 24°16'17.63" W and 107°05'09.77" W. Its surface area is of 1 818 km² and its natural surface runoff is 92.1 Mm³ per year (CONAGUA, 2016b). The Group of Stream Pabellones area has a mean altitude of 330 m.a.s.l. Because this watershed has no main stream, it is drained by multiple small streams that flow to Pabellones cove that is connected to the Pacific Ocean.

2.1.3. Piaxtla River watershed

The Piaxtla River Watershed is located between parallels 23°40' N and 26°30' N and between meridians 105°20' W and 106°50' W. The catchment area is about 7,447.1 km² and the natural annual surface water runoff is 1,397 Mm³ (CONAGUA, 2016b). This watershed is exorheic with a very elongated ellipse shape having a maximum altitude of 3,206 m above sea level (de la Lanza Espino *et al.*, 2015). Its hydrographic scheme is simple. Its main collector follows an approximate southwest direction towards the sea receiving streams on both sides. The Piaxtla River springs in Sierra Madre Occidental ridge above 2,800 meters above the sea.

The Piaxtla River has a longitude of 220 km and an angular drainage pattern. Based on CONAGUA criterion, the Piaxtla River Watershed is divided in two basins: the Rio Piaxtla 1 and the Rio Piaxtla 2 that are described below. Rio Piaxtla 1 basin drains an area of 4,842.7 km² and is bordered to the north by the San Lorenzo River watershed, to the south by the watersheds of the rivers Presidio and Quelite, to the east by Hydrological Region 36 Nazas-Aguanaval, and to the west by the Elota River Watershed. Rio Piaxtla 1 basin extreme altitude sites are the source of the Piaxtla River and the Piaxtla hydrometric station. Rio Piaxtla 2 basin drains an area of 2,604.4 km² and it is limited at north by the Elota River watershed, to the south by the Quelite River watershed, to the east by the Presidio River watershed, and to the west by the Pacific Ocean.

2.1.4. Quelite River Watershed

The Quelite River Watershed is situated between 23°48'43.85" N and 23°18'16.30" N and between 106°36'57.09" W and 106°10'04.35" W. This watershed has a surface area of 1,194.5 km² and its main water course has a longitude of 67 km. It has a natural surface runoff of 154.0 Mm³ (CONAGUA, 2016b).

The Quelite River watershed has the shape of an irregular four-sided polygon having a very simple hydrographic structure with a single central collector flowing to the southwest. It receives unnamed small streams on both sides. The Quelite River originates in the Sierra del Espinazo del Diablo ridge around 1,050 m.a.s.l. running through Los Naranjos village and six kilometers downstream through El Quelite village, from which this river takes its name (Ramos and Gracia, 2012).

The Quelite River watershed is divided into two basins, Rio Quelite 1 and Rio Quelite 2, as described below. Rio Quelite 1 basin includes an area from the Quelite River origins to the El Quelite hydrometric station. It drains an area of 829.9 km² and it is limited to the north and to the west by the Piaxtla River watershed, to the south by the Rio Quelite 2 Basin, and to the west by the Presidio River Watershed. Rio Quelite 2 Basin extends from the El Quelite hydrometric station to the sea. It drains an area of 364.6 km² and limits to the north by Quelite 1 Basin, to the east by the Presidio River Watershed, to the south by a marsh zone and by the Presidio River Watershed, and to the west by the Piaxtla 2 Basin and the Pacific Ocean.

2.2. Software and databases

The dataset used in this study was obtained from Mexican government and worldwide databases. Mexican official information includes hydrography, official watershed delineation, and localization of discharge gauging stations. In turn, worldwide databases provided terrain elevations and satellite images.

Maps were generated using QGIS 2.18.3 Las Palmas software. GIS software selection was based on the criterion that this software is an open-source tool. Also, this software is useful and simple and shows a friendly interface that allows for the processing of many file formats (both input and output) and the exchange of information through the publication of cartography. QGIS was executed from the Windows platform. Georeferencing of the basic cartography set was carried out using the WGS84 reference system (World Geodetic System, 1984) and UTM projection (Universal Transverse Mercator), zone 13.

Maps were generated using vector files containing information such as the delineation of basins, sub-basins, watercourses and waterbodies. This information is available from National Institute of Geography and Statistics (INEGI, 2017a). Layers corresponding to Hydrological Region 10 Sinaloa (scale 1:50 000) were downloaded. They are vector files in shape format (*.shp).

Mapping of the official delineation of river basins in Mexico was performed based on vertex coordinates. Official delineation in Mexico is published in the Official Journal of the Federation (CONAGUA, 2016c) in PDF text format. These coordinates were converted from Adobe Acrobat to Excel files. They were then saved in comma delimited text format and added to the GIS canvas using option "Add comma delimited text layer". Terrain elevation layers were generated from elevation data using GIS tool "Level curves".

Watercourses and water bodies were incorporated into maps by loading vector layers from Hydrographic Network 1:50 000. The Hydrographic Network used in this paper is a drainage model of river basins (INEGI, 2010). This information is stored in a database in geographic coordinates Datum ITRF92 epoch 1988.0 with eight decimal digits of decimal degrees. In some maps, terrain satellite images were used as background to enhance visual perception of terrain. Hydrometric station coordinates were consulted in the Surface Water National Data Bank (CONAGUA, 2017b). In addition, ASTER GDEM v2 Worldwide

Elevation Data with one arcsec resolution (NASA, 2011) and satellite images from Google Earth Pro were used to identify elevations of official river basin boundaries and to display orography as a natural terrain background, respectively. Both data sources were selected as cartographic sources.

It should be noted that neither river basin boundaries nor other hydromorphological features were generated by using digital elevation model processing. This is because the purpose of this paper is to compare official information issued from different governmental sources: official river delineation from the water authority (CONAGUA), hydrography from INEGI and the legal definition of “river basin” from the Federal Official Diary.

2.3. Analysis of the legal definition of watershed and watershed official delineation

The definition of watershed established in Mexican Water Law was examined and compared with officially delineated basins. The characteristics that distinguish the official watershed definition were separated and examined in eight basins, which constitute the study cases. They were selected from a representative hydrological region with diverse hydrographic features, like basins with and without water reservoirs, borders established by the presence of hydrometric stations and territories drained by no main streams.

The GIS capabilities were used to prepare maps having official basin delineation and hydrography coming from different data sources. This allowed for the examination of specific characteristics of selected official basins and for the comparison of these with their corresponding concept established in the legal definition of watershed.

Finally, a new watershed definition is proposed, which tries to keep the essence of the original one proposed by the Mexican Federal Government. No changes in official watershed delineation are mandatory for this definition proposal. The eight study cases were also used to probe the suitability of the new definition.

3. RESULTS AND DISCUSSION

3.1. Legal definition of watershed in Mexico

In Mexico, a legal definition of hydrological basin is established in the National Water Law (NWL) (CONAGUA, 2017a):

Hydrological basin: is a territory unit, differentiated from other units, usually delimited by a water parting or water divide, which is a polygonal line formed by the highest elevation points in that unit, where water courses in different ways and this resource is stored or flows to an outlet point that can be the sea or other inland waterbody, through a hydrographic network of streams that converges in a main one, or it is the territory in which the waters form an autonomous unit or differentiated from others, even without draining water towards the sea. In this space, delimited by a topographic diversity, water, soil, flora, fauna, and other natural resources coexist. The hydrological basin in conjunction with the aquifers constitutes the management unit of water resources. The hydrological basin is in turn composed of sub-basins and they are integrated by micro-basins.

This definition shows inconsistencies with respect to the official delineation of hydrological basins in Mexico, which are published in the Official Journal of the Federation. The territory division in official hydrological basins is used as a geographic framework to determine water availability “*Natural runoff is among the elements considered to update mean annual national water availability in the 731 hydrological basins[...]*” (CONAGUA, 2016d). In turn, official water availability is the base to confer or deny water use rights (CONAGUA, 2017a).

According to official information, 26 new small basins or watersheds were aggregated. Hence, there are 757 official hydrological basins in Mexico (CONAGUA, 2016a; 2016b; 2016c; 2016d; 2016e; 2016f). However, these delimited basins do not meet the characteristics given in the legal definition, as it is discussed in this article through the respective study cases.

3.2. Water parting: a line of the highest elevation points

In the hydrological basin definition, the NWL establishes that *"Hydrological basin: is a territory unit, differentiated from other units, usually delimited by a water parting or water divide, which is a polygonal line formed by the highest elevation points in that unit[...]"*

Based on this definition, the word "usually" gives the idea that there are unusual situations. Commonly a hydrological basin is defined as a polygonal line formed by the highest elevation points. However, there are some official hydrological basins in Mexico which are delimited, and they do not meet with this requirement. Official delineation of Piaxtla River 1 and Piaxtla River 2 Basins are shown in Figure 2 (CONAGUA, 2016a).

Figure 2. Contour lines in the frontier of Piaxtla River 1 and Piaxtla River 2 Basins.

Source: Prepared by the authors using Google Earth and CONAGUA (2016a).

Boundary details of both basins demonstrate that delineation boundary dramatically decreased from an elevation of 2100 m.a.s.l. to 400 m.a.s.l. This situation indicates that an important part of the basins' border, i.e., the common frontier, does not meet the hydrological basin definition of the NWL. An arbitrary unnatural delineation was carried out for dividing both basins. A similar situation is presented for Quelite River 1 and Quelite River 2 Basins, as shown in Figure 3. Based on the level curves of the study area, elevations of 20 m.a.s.l were registered as part of the delineation boundaries of these basins.

Figure 3. Contour lines in the frontier of Quelite River 1 and Quelite River 2 Basins.

Source: Prepared by the authors using Google Earth and CONAGUA (2016a).

3.3. Location of hydrometric stations as a criterion for basin delineation

A common practice for hydrographic basin delineation in Mexico is to define its boundaries through the presence of hydrometric stations located on the main stream. In the previous section (Figures 2 and 3), two study cases were present: Piaxtla River Basin and Quelite River Basin. Based on the description of Piaxtla River 1 Basin boundaries, this hydrological basin "[...]starts from the beginning of the Piaxtla River until where the Piaxtla hydrometric station is located." (CONAGUA, 2013a). This hydrometric station is located in the coordinates 106°25'30.0" and 23°56'00.0" (CONAGUA, 2017b). In accordance with official information, the division between these basins was determined by the locations of the hydrometric stations registered in the National Data Bank of Surface Waters (NDBSW). This situation could explain the fact that none of the basins is formed by the highest elevation points. A similar situation occurs for the Quelite River Basin. This hydrological basin: "[...]starts from the beginning of the Quelite River until where the hydrometric station El Quelite is located" (CONAGUA, 2013b). The hydrometric station mentioned above is located in the coordinates 106°29'00" N and 23°30'45" S (CONAGUA, 2017b).

On the other hand, Figures 2 and 3 show that the sea or other interior water bodies are not located at the outlet of the upstream basins (Piaxtla and Quelite). In this sense, these study cases do not fulfill with the definition of hydrological basin of the NWL, which specifies that the territory of a hydrological basin is: "[...]where water courses in different ways and this resource is stored or flows to an outlet point that can be the sea or other inland waterbody". The basins demonstrated that the legal definition of hydrological basin in Mexico is not linked to the official delineation of hydrological basin. Consequently, it is not consistent with the characteristics that legal definition establishes. Furthermore, according to legal definition, a hydrological basin is "[...]the territory in which the waters form an autonomous unit or differentiated from others, even without draining water towards the sea." Piaxtla River 1 and Piaxtla River 2 Basins do not form autonomous units, nor are differentiated from one another.

The only thing that distinguishes them is the existence of a hydrometric station located on a site, which is considered appropriate for measurement purposes. A similar situation is presented in the Quelite River 1 and Quelite River 2 Basins. Thus, the legal definition of hydrological basin should be flexible and should include cases such as those described here.

3.4. Water bodies as boundaries of a hydrological basin

The National Water Law establishes that surface water in a hydrological basin: "[...]is stored or flows to an outlet point that can be the sea or other inland waterbody, through a hydrographic network of streams that converges in a main one". In this statement, there is a parallelism between the sea and other waterbody as destinations of the water that drains out the basin. This means that neither the sea, nor the "other waterbody" are part of the basin. According to this wording, this receptor waterbody should be located outside the basin, downstream from it.

In Mexico, waterbodies' boundaries are used to define hydrological basins. The presence of water bodies inside a hydrological basin demonstrates that the official delineation of hydrological basin does not meet with the characteristics established by the legal definition. Such a discrepancy is observed specially in hydrological basins with reservoirs. The Humaya River and Tamazula River Basins could be considered as examples. The official description of Humaya River Basin indicates that: *"The available volume of Humaya River basin goes from the beginning of the Humaya River up to the Adolfo López Mateos reservoir"*, while the official description of Tamazula River basin is: *"The volume available of Tamazula River basin goes from the beginning of Tamazula River until where the Sanalona reservoir is located."* (CONAGUA, 2010).

CONAGUA (2016a) provides the vertices of the polygon lines that define these hydrological basins boundaries. This polygon includes Adolfo López Mateos reservoir inside Humaya River Basin and Sanalona reservoir inside Tamazula River Basin, as shown in Figure 4.

Figure 4. Humaya River and Tamazula River Basins and their reservoirs.

Source: Prepared by the authors using INEGI (2017a) and CONAGUA (2016a).

In both cases, the water does not drain towards a waterbody located outside the basin, because the waterbody (reservoir) is inside of it. In addition, some streams belonging to the hydrographic network of the basin do not converge towards a main course but converge towards the reservoir. An inspection of the surrounding areas reveals that there are multiple inputs to each reservoir (Figures 5 and 6). Since each waterbody located inside the basin has multiple inlets, they could be also considered as hydrological basin outlets, including the corresponding to the main stream.

Figure 5. Streams draining to the reservoir of Adolfo López Mateos Dam.

Source: Prepared by the authors using INEGI (2017a) y CONAGUA (2016a).

Figure 6. Streams draining to the reservoir of Sanalona Dam.

Source: Prepared by the authors using (INEGI, 2017b) y (CONAGUA, 2016a).

Therefore, if the whole drained area is characterized, maintaining the principle that a basin should have a single outlet point, the first alternative would be to indicate that in the case of a waterbody presence in a hydrological basin: 1) the drained area is constituted by the main stream basin and a set of minor basins; 2) the convergence entity is the waterbody located inside of the basin; and, 3) the boundary of this basin is the output point of the reservoir or where its outflows merge. A second alternative would be to modify the definition of hydrological basin in such a way that this definition could be consistent with the existing official delineations.

3.5. Groups of minor coastal basins

In the Federation Official Diary (CONAGUA, 2016a), it is also noted that:

"[...]within the 37 hydrological regions of the country, there are areas where no perennial streams exist, which are not within the 731 basins identified previously. Hence the identification of 26 new hydrological basins is required to cover the whole national territory."

In the area under study there are two of these types of areas. This type of terrain is characterized by multiple small watercourses draining to the sea, each of them having multiple drainage points, as shown in Figure 7. These officially-delimited basins do not fulfill with the established in the legal definition, where an only drainage point is required (CONAGUA, 2017a).

Figure 7. Group of streams Altata and Pabellones.

Source: Prepared by the authors using CONAGUA (2016b).

In order to look for consistency between the legal definition of hydrological basin and these types of drainage entities, which are already defined and officially referred as "new hydrological basins", it is necessary to consider the configuration variability of these territories. Cotler (2007) suggests that:

"Some hydrological basin boundaries might vary due to the time of the year, an anthropogenic modification of the drainage network and even because of the presence of some special meteorological phenomenon like hurricanes, which could generate the displacement of basin boundaries downstream".

According to this, the establishment of individual boundaries for each of these basins is not a viable option, given the permanent nature of the official precepts. Gathering them in a single drainage official entity, as a "group of minor basins", could make consistent the natural configuration variability of these basins with official denomination. In addition, this boundary would be at the same time a limit of adjacent basins with more stability in their configuration. "Group of minor basins" is a concept that can be extended to a set of small hydrological basins, which drain into a waterbody located inside a basin.

3.6. Complexity of the legal definition

According to the Oxford Dictionary, a definition is: "[...]an exact statement or description of the nature, scope, or meaning of something" (Oxford, 2018). The Dictionary of the Royal Spanish Academy adds "clarity" to the characteristics that a definition must have (Real Academia Española, 2017). The definition of "hydrological basin" in Mexico is provided by the National Water Law. Neither references nor technical fundamentals are specified. However, the wording of this definition is inconsistent with the concept of definition itself. The definition of hydrological basin established in the NWL has a generic character, as being "[...]a territory unit". This could be a physiographic region, a municipality or other natural or artificial zoning.

In the CONAGUA definition of hydrological basin, it is also established that the basin is *usually* delimited by a water parting. The word *usually* refers to the existence of different cases. However, it does not indicate how these cases would be delimited. This wording fails to fulfill the exactitude and clarity that a definition should have. On the other hand, the wording of the hydrological basin definition is comprised of several watershed definitions, as shown in Table 1.

Table 1. Definitions contained in the definition of watershed of Mexican National Water Law.

National Water Law	Other sources
"[...]is a territory unit, differentiated from other units, usually delimited by a water parting or water divide[...]"	Watershed: Surface bordered by a water parting so that its runoff flows towards a main water course, or waterbody. It is a subdivision of a hydrological region (Omernik and Bailey, 1997). basin syn. drainage basin, catchment, river basin, watershed (USU): Area having a common outlet for its surface runoff (OMM, 2012).
"[...]a polygonal line formed by the highest elevation points in that unit[...]"	Watershed divide (USU) syn. drainage divide, water parting: Summit or boundary line separating adjacent drainage basins (OMM, 2012).
"[...]where water courses in different ways and this resource is stored or flows to an outlet point that can be the sea or other inland waterbody, through a hydrographic network of streams that converges in a main one[...]"	Exorheic basin: A basin draining to the sea (García-Tortosa <i>et al.</i> , 2008). Endorheic basin: A basin having no outlet to the sea but receiving temporary or permanent streams. That is the reason why a permanent or temporal aquifer exists (CONANP, 2016).
"[...]or it is the territory in which the waters form an autonomous unit or differentiated from others, even without draining water towards the sea."	Endorheic basin: A basin having no outlet to the sea but receiving temporary or permanent streams. That is the reason why a permanent or temporary aquifer exists (CONANP, 2016).

Source: Prepared by the authors.

In addition, this definition includes differential characters (CONAGUA, 2017a):

- *"In this space, delimited by a topographic diversity, water, soil, flora, fauna, and other natural resources coexist.*
- *The hydrological basin in conjunction with the aquifers constitutes the management unit of water resources.*
- *The hydrological basin is in turn composed of sub-basins and they are integrated by micro-basins".*

The elevated number of definitions and differential characters in the same paragraph which constitutes a single definition complicates its interpretation. In this sense, hydrological basin definition is not presented with clarity. These differential characters are excluded from the hydrological watershed definition in a new Mexican water law project proposed since 2015.

On the other hand, the Mexican Modern Government Program for 2013-2018 states that the initiatives must be communicated in plain language (*line of action 1.5.2 Diffusion of the progress and results of the programs derived from the National Development Program (NDP) must be in plain language*) (Mexico, 2013). Plain language is: "[...]a simple expression, clear and direct information that readers (public servants and citizens) need to know" (Valdovinos Chávez *et al.*, 2004). This initiative comes from an international effort of several countries, including some Spanish-speaking countries like Spain, Mexico and Chile.

As can be seen, the hydrological basin definition is not expressed in plain language; however, it is "[...]information that readers (public servants and citizens) need to know". This is by virtue of the fact that the definition of basin contained in the NWL is an essential element in determining the average annual availability of surface water (SEMARNAT, 2015). The availability of surface water is one of the central themes of the Water National Program 2014 - 2018 (CONAGUA, 2014), a document that is derived from the NDP.

3.7. Proposal of a hydrological basin definition

A proposal for a new hydrological basin definition is presented to harmonize this definition with the hydrological basin delineation published in the Federation Official Diary (FOD). This modification proposal represents minor consequences in terms of: 1) technical work to review the boundaries of the 757 hydrological basins already defined; 2) editing the vertices that do not meet the legal definition; 3) administrative work to review and modify the publications of the FOD that establish the official delineation (CONAGUA, 2016a; 2016b; 2016c; 2016d; 2016e; 2016f); and 4) dealing with technical, administrative and even legal implications on calculation of average annual availability of surface water and consequently with the already given or denied water rights (CONAGUA, 2017).

The proposal has the following characteristics:

- Defines the concept of hydrological basin in a simple way, in a single paragraph and in accordance with the examples of basins analyzed in this manuscript and based on a definition supported by a recognized publication in the hydrology field.
- Considers the concept of "new hydrological basins" and the rest of the concepts that the current definition includes.
- When an area is drained by various minor currents, their basins are congregated in groups and are considered a special unit for the management of water resources (Group of minor basins or sub-basins).

Derived from the above, a proposal for hydrological basin definition is presented in a plain language, followed by six definitions, corresponding to the characteristics that the Mexican authority establishes for resource management purposes:

Hydrological Basin

Area that has a single outlet point for its surface runoff (adapted from OMM, 2012).

In addition, the following definitions are proposed to complement this definition; they include the aspects considered in the original definition, as well as the concept that defines published "additional basins".

Sub-basin

Next-level subdivision of a hydrological basin.

Micro-basin

Next-level subdivision of a sub-basin.

Group of minor basins

A land area where surface flow originates entirely within the hydrologic unit and drains to multiple points along a large waterbody such as the ocean, or large reservoir or lake (adapted from SSWD, 2003).

Water Parting

Boundary line that separates adjacent hydrological basins (OMM, 2012).

Exorheic basin

Drainage basin whose surface water runs into the ocean or into another waterbody having a connection to the ocean (CONANP, 2016).

Endorheic basin

Basin whose surface waters do not drain towards the sea but receives temporary or permanent flows. For that reason, a permanent or temporary aquifer is present (CONANP, 2016).

3.8. Discussion

The aim of this paper is not to propose criteria to delineate new hydrologic units. Discussion deals with the suitability of the proposed definition for a watershed and its applicability to the current official watershed delineations in Mexico. Countries mentioned in this paper also must deal with current official delineations that are used for administrative and legal purposes. Thus, congruence between legal definitions of watershed and official delineations is an issue that this manuscript addresses.

Strong interagency efforts are needed to establish watershed delineations. *"The delineation shall be as simple as it is practical, and will keep hydrologic units from benefiting a particular agency, program, administrative area or special project."* (SSWD, 2003). Cotler (2007) suggests that this delineation should be determined solely upon science-based hydrological principles to assure a homogeneous national seamless digital data layer.

In this sense, several basin delineations have been carried out by government entities in Mexico. CONABIO (2012) reported 3115 sub-basins in 2001. This entity has also reported different numbers of natural basins for the Mexican territory: 160, 142 and 1,474 (CONABIO, 2008; 2012 respectively). National Institute of Statistics and Geography (INEGI) considered 150 basins and 1,001 sub-basins (INEGI, 2001). National Water Commission (CONAGUA) has also stated a number of basins of 314 (CONAGUA, 1996). In 2007, based upon the *"[...]join effort of INEGI, National Institute of Ecology and CONAGUA to establish concerted and common criteria to delimitate the basins of Mexico and toponymy definition"*,

the Mexican territory was divided into 1,471 basins (Cotler, 2007). This delineation was performed with a rigorous and documented process based on natural criteria such as topography and hydrography. Despite these efforts, CONAGUA published different numbers of basins for Mexico: 718, 728, 722 (CONAGUA, 2009; 2008; 2010, respectively). In 2012, the number of basin was defined in 731 (CONAGUA, 2012). This number of basins and their respective vertex coordinates were formalized in the Federation Official Diary (CONAGUA, 2016d). Later, 26 new additional hydrological basins were set up. Therefore, a total of 757 basins is the currently official number of watersheds in Mexico.

Despite having access to a delineation of 1,471 basins obtained by a rigorous process based on natural criteria (Cotler, 2007), the water authority decided to make a different basin delineation and give it an official status. This is evidence that the working criteria were also different. The 757-basin delineation was configured progressively, probably by technical and administrative departments of CONAGUA. This delineation aims “[...]to give higher certainty to water users with respect to the exact localization of the site where the exploitation, usage or harnessing of water takes place as well as to improve the administration and management of water resources” (CONAGUA, 2016a). In other words, this watershed delineation has a clear administrative purpose with intrinsic legal and technical implications that prevents its drastic modification (Cuevas *et al.*, 2010). This kind of inertia is recognized even in systematic science-based efforts to delineate watersheds: “the diversity of hydrological conditions nationwide, complexity of surface hydrology and the number of factors involved in the delineation process preclude an all-inclusive guideline. Variations will generally be limited to previously delineated units with a long history of use” (SSWD, 2003).

In Mexico, a modification of official basin delineation can motivate important issues to deal with in water administration. Water-right title is a legal document which specifies the basin where water usage occurs (CONAGUA, 2017a). Thus, if a change of basin delineation or denomination is carried out, then a proper legally-valid procedure must be followed. Similar situations can occur with other legal documents, such as discharge of wastewater permits, water quality certificates, among others (CONAGUA, 2017c). Even legal processes against water authorities’ resolutions about surface water availability should be considered before such changes.

The difficulty of modifying the official delineation of hydrological basins was also considered unfeasible in Chile:

[...]it was concluded that new water delineation could be useful in some cases... but being a new delineation, it is very likely to have a different number of watersheds and database with respect to the current NWB System, which means that this new watershed division will be separated from NWB codification. This disconnection would represent that the new delineation will not be used (Chile, 2013).

The proposal for legal definition of hydrological basin in Mexico made in this paper is based on the definition of the World Meteorological Organization (OMM, 2012). The purpose of defining terms separately is to consider the concepts included in the original definition and the results obtained in this study. The proposed definition is expressed in a simple language, which is clear and direct. On the one hand, the hydrological basin definition proposed in this manuscript fulfills with the initiative to express concepts of public interest in plain language. On the other hand, this definition does not establish the maximum elevation points as a criterion to define the hydrological basin boundaries, which offsets the deficiencies identified in the official definition.

Based on the inconsistencies found in this study, the proposed definition establishes that the surface water of a basin should not flow towards the sea or a waterbody, nor should the basin be autonomous from others. Therefore, this definition proposal could adapt to the official delineation of hydrological basins defined by the criterion of hydrometric station

presence. The proposed definition could be also adapted to official delineations where the hydrological basin boundaries are determined by the presence of a waterbody. The area referred to in the proposed definition covers a surface area without including water stored in a waterbody, and the single outlet is constituted by the structures built with the purpose of extracting the water stored in the inland waterbody. The introduction of "group of minor basis" concept refers to an aggregate of basins with reduced dimensions which serves as a management unit of water resources. These are the 26 cases that were recognized by federal authority and called "new hydrological basins". This concept could also be applied to groups of small hydrological basins defined by the presence of a waterbody.

It is also noted that official hydrological basins were defined based on superficial terrain criteria. This situation can be deduced by the fact that its configuration is used to calculate and disclose the average annual surface water availability. The average annual groundwater availability is computed separately from surface water availability (SEMANAT, 2015). Groundwater availability is based on the aquifers' delineation and then it is calculated with an independent method (SEMANAT, 2015). Hence, official definition of hydrological basin in Mexico (CONAGUA, 2017a) is, in fact, the definition of hydrographical basin. According to Moura and Henry-Silva (2015), hydrographic basins are part of larger systems, involving aspects of geology, vegetation, climate and land use and occupation, consisting of a mosaic of functional subsystems interconnected by biotic and abiotic processes. In contrast, the concept of hydrological basin: *"Unlike the hydrographic basins, hydrological basins, besides including the first ones, they also include underground hydrogeological systems. Therefore, hydrological basins could vary in their purely hydrographic limits"* (INECOL, 2005).

In accordance with Campos Aranda (1992), the difference between the territorial delineation of a hydrographic basin (the topographic basin) and a hydrological basin (the groundwater table basin) is reduced to special cases, such as karst terrain or regions with coarser sediment on rock beds. Except in the cases mentioned above, both boundaries should match. Therefore, if discrepancy cases are sought, a detailed analysis is required to determine and compare the aquifer-recharge areas in conjunction with the surface topography. At most, when the water balance in the basins and aquifers is carried out, these differences would be clear and a distinction between the boundaries of the hydrological and hydrographic basins would be required.

Hydrological basin definition is used in the totality of the publications in the FOD dealing with surface water availability, as well as all derived documents of a normative character. Therefore, the use of hydrological basin definition is generalized in this area. Changing this term to hydrographic basin would imply substantial modifications to official, legal, and regulatory documents. A duly substantiated and instrumented superior law would be required to make this change. Hence, if both types of basins are equivalent from the point of view of topographic layout maps, the official delineation can be considered as hydrological basins.

4. CONCLUSIONS

Official delineation of hydrological basin is fundamental to determine official water surface availability, which is in turn essential to confer or deny water rights. This delineation must be consistent with the legal definition of hydrological basin, a term that is persistently defined in water laws of Latin America. This manuscript presents a discussion about inconsistencies between the legal definition and the official delineation of eight study cases constituted by hydrological basins in Sinaloa, Mexico.

Based on the analysis of the current hydrological basin definition included in the National Water Law, it is concluded that the legal definition should be amended to establish

consistency with the basins delineated in the Official Federation Diary. This is because significantly less technical and administrative effort is required to modify one definition than to modify the official watershed delineation, as it is also recognized by Chilean experts.

In this article, an amendment is proposed to harmonize this situation, with a minimum of changes in policy documents. This definition preserves the essence of the current legal definition, which highlights the hydrological basin as a management unit of water resources in Mexico. However, it is recognized that it is necessary to confront progressively the proposed definition with study cases of basins located in other parts of Mexico for its further improvement. Besides, for future analyses, it is convenient to use digital elevation models of higher resolution to perform more precise basin delineation.

This paper also proposes a scheme to establish a legal definition of watershed and issues directly associated with it, which can be useful to analyze similar situations in other countries where there is a watershed legal definition and official watershed delineation. In Latin American countries where there is no official watershed delineation to confer or deny water rights, this definition should be used from the beginning to assure consistency between legal definitions and such delineation. This could prevent technical, administrative and even legal problems to manage water resources derived from a lack of consistency between the basin's legal definition and the official watershed delineation.

5. ACKNOWLEDGMENTS

The authors would like to thank the Associate Editor and all the reviewers for their constructive suggestions. They also gratefully acknowledge the support of Ulysses Zaldívar Higuera, José Emerio Parra Flores, Rafael Sanz Ramos. This work was supported by Cátedras CONACYT 2014 project ("*Diseño e instrumentación de una red de monitoreo y de alerta para la gestión del agua en Sinaloa*" Ref. 2572).

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Simultaneous removal of nitrogen and organic carbon from swine wastewater using the pre-denitrification/nitrification process

ARTICLES doi:10.4136/ambi-agua.2241

Received: 12 Feb. 2018; Accepted: 29 Jan. 2019

Marcelo Bortoli^{1*}; **Airton Kunz^{2,3}**; **Marina Celant De Prá⁴**;
Marcio Luis Busi Da Silva²; **Ana Cé⁵**; **Hugo Moreira Soares⁶**

¹Universidade Tecnológica Federal do Paraná (UTFPR), Francisco Beltrão, PR, Brasil
Programa de Pós-graduação em Engenharia Ambiental (PPGEA).

Departamento Acadêmico de Engenharia Ambiental (DAEAM). E-mail: marcelobortoli@utfpr.edu.br

²Embrapa Suínos e Aves, Concórdia, SC, Brasil

E-mail: airton.kunz@embrapa.br, marcio.busi@embrapa.br

³Universidade Estadual do Oeste do Paraná (UNIOESTE), Cascavel, PR, Brasil

Programa de Pós-graduação em Engenharia Agrícola (PGEAGRI). E-mail: airton.kunz@embrapa.br

⁴Universidade Tecnológica Federal do Paraná (UTFPR), Dois Vizinhos, PR, Brasil

Coordenação do Curso de Engenharia de Bioprocessos e Biotecnologia. E-mail: marinapra@utfpr.edu.br

⁵Universidade Tecnológica Federal do Paraná (UTFPR), Francisco Beltrão, PR, Brasil

Programa de Pós-graduação em Engenharia Ambiental (PPGEA). E-mail: aninhace_@hotmail.com

⁶Universidade Federal de Santa Catarina (UFSC), Florianópolis, SC, Brasil

Departamento de Engenharia Química e de Alimentos. E-mail: soares@enq.ufsc.br

*Corresponding author

ABSTRACT

Wastewater from confined animal feeding operations (CAFOs) can interfere significantly with the natural nitrogen and phosphorus balance in the environment if not treated adequately prior to disposal. In this work, a modified Lutzak-Ettinger (MLE) consisting of sequential pre-denitrification/ nitrification was used to determine the effects of nutrient loading rates and hydraulic retention times (HRT) on total nitrogen (TN) and organic carbon (TOC) removal from swine wastewater. MLE reactor was continuously fed swine wastewater for over 205 days using different recycle ratios (Rr) and HRT. Higher TN and TOC removal efficiencies (90.7% and 96.1%, respectively) were obtained when recycling effluent from the nitrification tank (4.5:1) combined with effluent from the final clarifier (1:1). Removal efficiencies of 96% for TOC and 90% for TN were obtained for the maximum loading rates of 0.56 gTN L⁻¹ d⁻¹ and 2.15 g TOC L⁻¹ d⁻¹, respectively. TN and TOC removal rates were achieved with HRT in as little as 3.5 days. Overall, MLE was a robust bioprocess withstanding variations in wastewater physical-chemical composition and/or changes in operational conditions without significant impairment of N and TOC removal efficiencies.

Keywords: ammonia, high organic rate, nitrate.

Remoção simultânea de nitrogênio e carbono orgânico em efluentes da suinocultura utilizando processo pré-desnitrificação/ nitrificação

RESUMO

Os efluentes dos sistemas de produção de animais confinados (SPACs), se não tratados adequadamente antes do descarte, podem interferir significativamente no balanço natural do nitrogênio no meio ambiente. Nesse trabalho, um sistema Lutzak-Ettinger modificado (MLE), consistindo de dois reatores sequenciais, um pré-desnitrificante (RD) e um nitrificante (RN), foi utilizado para determinar os efeitos da carga de alimentação e o tempo de retenção hidráulico (TRH), na remoção simultânea de nitrogênio total (NT) e carbono orgânico total (COT) de efluentes da suinocultura. O sistema MLE foi alimentado com efluente da suinocultura por 205 dias, usando diferentes razões de recirculação (Rr) e TRH. Foram obtidas eficiências de remoção de NT e COT (90,7% e 96,1%, respectivamente) com a recirculação do meio do RN (4.5:1) combinado com o lodo do decantador final (1:1). Essas eficiências foram obtidas com TRH de 3,5 dias e cargas de alimentação de 0,56 gNT L⁻¹ d⁻¹ e 2,15 g TOC L⁻¹ d⁻¹. Concluiu-se que o processo MLE se demonstrou robusto, resistindo a grandes variações na composição físico-química do efluente de alimentação e nas condições de operação, sem sofrer impacto importante nas eficiências de remoção de NT e COT.

Palavras-chave: alta carga orgânica, amônia, nitrato.

1. INTRODUCTION

Concentrated animal feeding operations (CAFOs) are increasingly being used for the enhancement of swine production. Consequently, much higher wastewater volumes are expected which, if not treated properly, could contaminate soils and water resources (Kunz *et al.*, 2012). For instance, the presence of high organic content and nutrients (e.g., nitrogen and phosphorus) in these effluents can exacerbate biological oxygen demand (BOD) and favor eutrophication (Techio *et al.*, 2011).

Different system configurations are considered including intermittent aeration, the use of selective membranes, sequencing batch reactors (Lee and Han, 2012), and/or the modified Ludzack-Ettinger (MLE). The MLE, also known as anoxic pre-denitrification process (Rajagopal *et al.*, 2011; Hafez *et al.*, 2010), was initially proposed by Ludzack and Ettinger for treatment of municipal sewage (Ludzack and Ettinger, 1962).

In the MLE, the oxidized nitrogen species ($\text{NO}_x = \text{NO}_2^- + \text{NO}_3^-$) are recycled into an anoxic reactor to serve as electron acceptors for heterotrophic denitrification. MLE has been used to remove TOC and nitrogen from a wide variety of effluents, including landfill leachate (Hafez *et al.*, 2010) and wastewaters from animal farming (Castrillón *et al.*, 2009; Vanotti *et al.*, 2009).

In the MLE system, the NO_x recycle ratio (Rr) to the anoxic reactor can limit nitrogen removal efficiency (Tchobanoglous *et al.* 2014). A high Rr can increase the oxidation-reduction potential (ORP) in the anaerobic reactor, inhibiting denitrification. Conversely, a low Rr can lead to insufficient concentrations of NO_x as electron acceptors, thus hindering complete denitrification. Thus, whereas the use of the MLE to remove N and TOC from swine wastewater effluents has already been demonstrated (Hafez *et al.*, 2010; Vanotti *et al.*, 2009), uncertainties still remain on how different Rr affects MLE system performance, especially treating CAFOs effluents.

Therefore, this study investigated the effects of different recycling ratios and nitrogen and the increase of organic loading rates (by decreasing HRT) on N and TOC removal efficiencies, using an MLE system to treat swine wastewater effluent.

2. MATERIALS AND METHODS

2.1. Experimental setup

A schematic representation of the lab scale MLE experimental setup is shown in Figure 1. Two 5 L completely stirred bioreactors connected in series were continuously fed swine wastewater. The first bioreactor was designed to promote anaerobic denitrification. The second was aerated to stimulate nitrification. Aerobic conditions were maintained with the use of a pneumatic air pump (PowerAIR 2000) and two cylindrical air diffusers (bubble stones) placed at the bottom of the bioreactor. The nitrified mixed liquor (NML) from the nitrification bioreactor as well as the settled solid (SS) from the clarifier were both recycled into denitrification bioreactor. Peristaltic pumps (Masterflex® L/S® Standard Digital Pump) were utilized to control different R_r flow rates (Q) in the MLE system (Table 1).

Figure 1. Schematic representation of the modified Ludzak-Etinger (MLE) experimental setup utilized in this work.

Table 1. MLE experimental configurations.

Period	Time (d)	Q (flow) (mL min ⁻¹)	R _r	Nitrogen Loading Rate (NLR) (gN L ⁻¹ d ⁻¹)	Hydraulic retention time (HRT) (d)
I	1 - 126	1.0	4-5.5-6.5	0.15	6.9
II	127-140	1.5	5.5	0.20	4.6
III	141-152	1.8	5.5	0.24	3.9
IV	153-164	2.0	5.5	0.30	3.5
V	165-185	2.0	5.5	0.41	3.5
VI	186-196	2.7	5.5	0.60	2.6
VII	197-205	2.0	5.5	0.41	3.5

The initial HRT was calculated using Equation 1 in order to obtain the initial nitrogen-loading rate (NLR) of 0.15 gN L⁻¹ d⁻¹ achieved by Vanotti *et al.* (2009) and based on the initial concentration of N-NH₃ from the first bath.

$$HRT = \frac{[NH_3 - N]}{NLR} \quad (1)$$

Where:

$[NH_3 - N]$ = NH₃ – N concentratio (g L⁻¹)

NLR = Nitrogen loading rate (g L⁻¹ d⁻¹)

The initial calculated HRT is close to the HRTs used by other studies, mainly as a function of NLR, such as Vanotti *et al.* (2009) and Loughrin *et al.* (2009), who studied MLE systems

for the swine wastewater treatment and used HRT between 10 and 17 d and 4.1 and 6 d, respectively.

2.2. MLE Inoculation

Sludge from a pilot scale activated sludge bioreactor treating swine wastewater effluents at Embrapa Swine and Poultry [total suspended solids (TSS) of 7.1 g L^{-1}] was used as inoculum (Viancelli *et al.*, 2013). For the startup, the nitrification and denitrification reactors were inoculated with 0.25 L of inoculum and the reactor was filled with 4.75 L of diluted swine manure effluent (1:10 swine manure/tap water; v/v) to minimize likely inhibition by substrate restriction.

2.3. Operational conditions

Liquid swine wastewater effluent collected from a solid-liquid separation tank at wastewater treatment plant was utilized as feeding solution. 250 L of the same wastewater batch was kept under refrigeration (-5°) and utilized throughout the experimental time frame as feeding solution. Therefore, confounding effects due to variations in influent wastewater physical-chemical characteristics were avoided. The first batch ($\text{NH}_3\text{-N} = 1.05 \text{ g L}^{-1}$) was used for 157 days. The second batch ($\text{NH}_3\text{-N} = 1.36 \text{ g L}^{-1}$) was used from day 158 to day 205. Different flow rates (Q), R_r , NLR , and HRT were evaluated (Table 1). It was not necessary to discard sludge from the MLE system, and consequently, the Solid Retention Time (SRT) does not need to be controlled, which reduces disposition and treatment costs.

2.4. Analytical methods and calculation of recycle ratio and removal efficiencies

TOC was measured in a TOC analyzer (Multi Elemental Analytic® Multi C/N 2100 Analytik Jena). The $\text{NH}_3\text{-N}$ concentration was determined electrochemically by the ion-selective electrode following the methodology described by APHA *et al.* (2012). Free ammonia (FA) and free nitrous acid (FNA) concentrations were estimated using the standard Equations proposed by Anthonisen *et al.* (1976).

Dissolved oxygen and pH were determined using an oximeter (YSI Model 55) and a pH meter (HANNA - HI8424), respectively. $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ were quantified by the colorimetric method using a Multi-Channel Flow Injection (FIALab – 2500).

The recycle ratio was defined by the ratio of the recirculation flow sum (mixer liquor + sludge) by the system feed rate (Equation 2). The sludge recycle pump flow was fixed at 1 mL L^{-1} during the experiment.

Nitrogen removal efficiencies (NRE) were obtained after the establishment of steady-conditions in the MLE reactor according to Equation 3.

$$R_r = \frac{Q_{RML} + Q_{RS}}{Q_{in}} \quad (2)$$

Where:

Q_{RML} is nitrified mixed liquor recycle flow;

Q_{RS} is clarified sludge recycle flow;

Q_{in} is feeding flow.

$$\text{NRE}(\%) = R_r \cdot (1 + R_r)^{-1} \quad (3)$$

3. RESULTS AND DISCUSSION

3.1. MLE reactors start up

$\text{NH}_3\text{-N}$ concentrations in the batches were continuously monitored over time to account for significant losses. $\text{NH}_3\text{-N}$ concentrations in the first and second batches were 10.6% (after 165 days of storage) and 0.8% (after 40 days of storage), respectively (data not shown). The observed decrease in $\text{NH}_3\text{-N}$ influent concentrations observed in period I (Figure 2) was primarily due to dilution effects as results of the increasing recirculation ratios. Measured $\text{NH}_3\text{-N}$ concentrations at the MLE effluent were higher at the beginning of the experiments, probably due to the lack of significant nitrifying-specific biomass (Figure 2). After 32 days of continuous operation, nitrification acclimation time was reached as observed by the reduction in ammonia and the concomitant increase in effluent $\text{NO}_3^-\text{-N}$ concentrations.

Figure 2. Nitrogen concentration profile over time in the nitrifying reactor under different reactor operational conditions (Table 1).

The average temperature ($23 \pm 1.6^\circ\text{C}$), pH (7.8 ± 0.3) and dissolved oxygen concentration ($> 3 \text{ mg L}^{-1}$) obtained during this experimental time frame were within the physiological requirements for nitrifying microorganisms (Ordaz et al., 2008). Interestingly, the observed acclimation period was much shorter than the 100 to 175 days (Jubany *et al.*, 2008) reported for other nitrification systems treating swine wastewater. The rapid acclimation time obtained in this study was most likely due to the use of a diluted raw effluent (1:10 v/v), which minimizes the inhibitory effects of high influent NH_3 concentrations on microorganisms (De Prá *et al.*, 2012). Therefore, the use of a diluted wastewater influent during the initial MLE start up process could minimize time required to establish steady-state conditions at field-scale operations.

To stimulate denitrification processes, the reactor was continuously fed at an influent organic loading rate (L_{TOC}) of $1.26 \pm 0.32 \text{ g TOC L}^{-1} \text{ d}^{-1}$ and a nitrogen loading rate (L_{TN}) of $0.15 \text{ g TN L}^{-1} \text{ d}^{-1}$. Temperature and pH were kept at $28 \pm 1.2^\circ\text{C}$ and 8.4 ± 0.2 , respectively. After 11 days of continuous operation, effluent $\text{NO}_x\text{-N}$ concentrations decreased from 52.1 mg L^{-1} to 0.21 mg L^{-1} , indicating the establishment of denitrifying processes.

3.2. MLE - Nitrification reactor performance

After 45 days, $\text{NH}_3\text{-N}$ and TN removal efficiencies of 97.7% and 84.4%, respectively, were obtained for an influent $L_{\text{NH}_3\text{-N}}$ of $0.21 \pm 0.1 \text{ g L}^{-1} \text{ d}^{-1}$ (Period I, Figure 2). During this

experimental time frame, dissolved oxygen (DO) ($3.2 \pm 0.4 \text{ mg L}^{-1}$) was unlikely limiting to nitrification because DO concentration $> 2 \text{ mg L}^{-1}$ is sufficient to maintain the growth and activity of ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) populations (Ordaz et al., 2008).

Changes in Rr from 5.5 to 6.5 decreased $\text{NH}_3\text{-N}$ removal efficiency from 96.1 to 87.5% (Figure 2). Following 86 days of experiment (Period I), however, NH_3 removal efficiency reestablished from the initial 87.5 to 96.1% as result of microorganism adaptation to the newer imposed operational conditions. A further decrease in HRT (periods II, III, and IV) did not affect nitrification efficiencies, which remained within 96.4 to 98.4%.

Increasing $\text{NH}_3\text{-N}$ influent loading rate by 50% (Period V, $\text{LNH}_3\text{-N}$ of $0.39 \pm 0.1 \text{ g L}^{-1} \text{ d}^{-1}$) did not show significant changes in ammonia removal efficiencies ($93.5 \pm 4.3\%$), demonstrating the robustness of the MLE process to withstand variations in operational feeding rates. A decrease in HRT from 3.5 d to 2.7 d (period VI), however, led to a decrease in NH_3 removal efficiency from $93.5 \pm 4.3\%$ to $57.8 \pm 51.1\%$. In order to re-establishment NH_3 removal efficiencies, HRT was adjusted to the previous 3.5 d.

Free ammonia (FA) concentrations in the reactor were below the inhibitory threshold reported for NOB (0.1 and 10.0 mg L^{-1}) and AOB ($>150 \text{ mg L}^{-1}$) (Rongsayamanont *et al.*, 2010) (Figure 3). Additionally, the absence of detectable free nitrous acid (FNA) suggested that nitrification was unlikely inhibited by the formation of FNA. Efficient NH_3 oxidation ($>99\%$) was observed throughout the remaining experimental time frame (Figure 3).

Figure 3. FA and FNA concentrations profile over time in the nitrifying reactor. Solid and dashed lines represent inhibitory thresholds for AOB and NOB, respectively (Rongsayamanont *et al.*, 2010).

Nitrification efficiency ($>95\%$) was in agreement with previously reported data obtained for reactors treating cattle manure (Castrillón *et al.*, 2009) or digested sow manure (Rajagopal *et al.*, 2011). Total nitrogen removal as low as $19.1 \pm 13.2\%$ was achieved in the nitrification reactor. This low removal value was somewhat unexpected, considering the favorable nitrification conditions in the reactor (i.e., $>2 \text{ mgO}_2 \text{ L}^{-1}$ and low FA concentrations). Therefore, circumstantial evidence supports the notion that other biological nitrogen removal processes were occurring concomitantly with nitrification. Whereas the investigation of such biological mechanisms was beyond the scope of this study, it is worth mentioning that biomass agglutination in MLE systems could aid the formation of anoxic micro niches, thus favoring autotrophic denitrification and/or anammox (Park *et al.*, 2009).

3.3. MLE – Denitrification reactor performance

Changes in R_r can play an important role in denitrification efficiency. Denitrification and TOC removal efficiencies of 88.9 and 83.5%, respectively, were achieved when R_r was set at 4.0:1 (Period I) (Figure 4). During this experimental time frame, the reactor was fed with a L_{TOC} of $0.41 \pm 0.1 \text{ g L}^{-1} \text{ d}^{-1}$ and L_{NOx} of $0.26 \pm 0.1 \text{ gN L}^{-1} \text{ d}^{-1}$. Denitrification and TOC removal efficiencies were increased with higher influent organic and nitrogen loading rates (Figure 4). Negligible denitrification and TOC removal efficiencies were observed when raising R_r from 5.5 to 6.5:1. Therefore, we opted to maintain the R_r at 5.5:1 throughout the experiment.

Figure 4. Correlation between Organic loading rate (OLR) and Nitrogen loading rate (NLR) on nitrogen removal efficiency by denitrification bioprocesses.

A reduction in HRT from 6.9 to 3.5 (from day 127 to 164) did not affect denitrification and TOC removal, which remained at 95.4% and 99.4%, respectively. These results served to demonstrate the effectiveness of MLE to operate at relatively low HRT.

In experimental period V, a new batch of wastewater effluent sample was used as substrate to feed the MLE system. Denitrification efficiency was kept at 99.1% with a TOC removal efficiency of 96%. Interesting to note, changes in wastewater physical-chemical characteristics, such as the higher TOC, did not affect reactor efficiency. From the operational point of view, this is relevant considering the inherent variability in swine wastewater effluent composition commonly observed at field scale. After reaching steady-state conditions, the HRT was intentionally adjusted from 3.5 to 2.6 d, resulting in an influent L_{TOC} of $3.68 \pm 0.79 \text{ g L}^{-1} \text{ d}^{-1}$ and L_{N-NOx} of $0.43 \pm 0.02 \text{ g L}^{-1} \text{ d}^{-1}$ (0.01 NO_2^-/NO_3^- ratio). Even at this low HRT, the average denitrification and TOC removal efficiencies were notably high (93.7% and 94.4%, respectively).

3.4. MLE efficiency

Overall, the MLE system was not sensitive to major changes in nitrogen loading conditions maintaining satisfactory TOC and TN removal efficiencies of 96.1% and 90.7%, respectively (for a L_{TOC} of $2.15 \pm 0.41 \text{ g L}^{-1} \text{ d}^{-1}$ and L_{TN} of $0.56 \pm 0.03 \text{ g L}^{-1} \text{ d}^{-1}$; Figure 5). These results were comparatively higher than the removal efficiencies reported for similar nitrification-denitrification systems treating swine wastewater effluent. For instance, Park et al. (2009), utilizing an anoxic-aerobic-anoxic sequence reactors, obtained TN removal of 90% for a L_{TN} of $0.18 \text{ g L}^{-1} \text{ d}^{-1}$. Removal efficiency below 80% was reported by Vanotti et al. (2009) for a nitrification-denitrification system using a L_{TN} of $0.31 \text{ g L}^{-1} \text{ d}^{-1}$. Castrillón et al. (2009) reported 68% removal efficiency for influent L_{TOC} of $4.5 \text{ gCOD L}^{-1} \text{ d}^{-1}$ and L_{TN} of $0.40 \text{ gTN L}^{-1} \text{ d}^{-1}$.

Figure 5. TOC and TN removal efficiencies in a MLE system under different carbon and nitrogen influent concentrations.

The final effluent obtained at the exit of the MLE system was relatively transparent, oxidized, and odorless, with 1.9 mg L^{-1} of $\text{NH}_3\text{-N}$, 161.2 mg L^{-1} of $\text{NO}_3\text{-N} + \text{NO}_2\text{-N}$, and 144.7 mg L^{-1} of TOC. These are physical-chemical parameters that meet water quality standards that permit the reuse of water to wash swine facilities (Kunz *et al.*, 2012).

4. CONCLUSIONS

The lab scale MLE reactor was continuously fed swine wastewater effluent for over 210 days under different N and TOC loading rates and HRT operational conditions. Dilution of swine wastewater effluent at the reactor's startup minimized acclimation time required to reach steady state conditions. Denitrification and nitrification conditions were established after 12 and 32 days, respectively. NH_3 oxidation above 95% was achieved. Denitrification efficiencies $> 90\%$ were observed under different N and TOC influent loading rates and HRT. The highest total nitrogen removal of 89% was accomplished by recycling effluent from the nitrification reactor (4.5:1) together with the effluent from the clarifier (1:1). HRT as low as 3.5 days (L_{TN} of $0.56 \text{ g L}^{-1} \text{ d}^{-1}$ and L_{TOC} of $2.15 \text{ g L}^{-1} \text{ d}^{-1}$) led to nitrogen and TOC removal efficiencies $> 90\%$.

This study demonstrated that nitrogen-rich swine wastewater effluent can be efficiently treated by the MLE process. Changes in raw wastewater physical-chemical composition and/or operational conditions should not significantly affect N and TOC removal efficiencies. This denotes the robustness of the approach and its potential application to field-scale operations.

5. ACKNOWLEDGMENTS

The authors thank the CNPq for its financial support.

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Background and the use of isoscapes in the Brazilian context: essential tool for isotope data interpretation and natural resource management

ARTICLES [doi:10.4136/ambi-agua.2282](https://doi.org/10.4136/ambi-agua.2282)

Received: 23 May 2018; Accepted: 01 Feb. 2019

João Paulo Sena-Souza^{1*} ; **Fábio José Viana Costa²** ; **Gabriela Bielefeld Nardoto¹**

¹Universidade de Brasília (UnB), Brasília, DF, Brasil
Instituto de Ciências Biológicas (IB). Departamento de Ecologia. E-mail: jpsenasouza@gmail.com,
gbnardoto@unb.br

²Instituto Nacional de Criminalística (INC), Brasília, DF, Brasil
Departamento de Polícia Federal. E-mail: mr.f.bio@gmail.com

*Corresponding author

ABSTRACT

Spatial patterns of stable isotope ratios can be represented in spatial models called isoscapes, and have been widely used to track biogeochemical processes in natural and anthropic systems. Isoscapes have the potential to improve isotope dissemination and interpretation of spatial patterns, increase scientific results appropriation by non-specialists and improve natural resource management. However, the isoscape approach has not commonly been used in studies performed in the Brazilian context. Isoscapes with oxygen, hydrogen, nitrogen and carbon stable isotopes contribute in areas such as animal migration, forensics, hydrology, and studies on population, community and ecosystem level, among others. Here, we show the well-known global use and applications of isoscapes in different studies worldwide as a background to point out the potential for more Brazilian researchers to employ this approach in their studies, taking advantage of existing methods and filling spatial and methodological gaps. The incorporation of isoscapes may broaden the understanding of mechanisms and processes of major biogeochemical cycles in Brazil, assist in solving crimes, track illicit drug origins, help to detect wild animal trafficking, and increase Brazilian knowledge about the hydrological cycle and animal migration patterns in the Neotropics.

Keywords: isotopic landscape, spatial patterns, stable isotope.

Isoscapes e seu uso no contexto brasileiro: ferramenta essencial para a interpretação dos dados isotópicos e a gestão dos recursos naturais

RESUMO

Padrões de distribuição espacial de isótopos estáveis podem ser apresentados em mapas chamados de isoscapes e têm sido amplamente utilizados para rastrear processos biogeoquímicos em sistemas naturais e antrópicos. Isoscapes têm o potencial de melhorar a disseminação e interpretação dos padrões espaciais de isótopos estáveis, aumentar a apropriação de resultados científicos por não-especialistas e melhorar a gestão de recursos naturais. No entanto, a abordagem de isoscape não tem sido comumente usada em estudos realizados no Brasil. Isoscapes de isótopos estáveis de oxigênio, hidrogênio, nitrogênio e carbono contribuem em áreas como migração animal, ciência forense, estudos hidrológicos e

ecológicos a nível de população, comunidade e ecossistema, entre outros. Aqui nós mostramos uma revisão sobre o uso e as aplicações de isoscapes em diferentes estudos em todo o mundo. Também apontamos uma forma de mais pesquisadores brasileiros empregarem essa abordagem em seus estudos, aproveitando os métodos existentes e preenchendo lacunas espaciais e metodológicas. A incorporação de isoscapes pode ampliar o entendimento dos mecanismos e processos dos ciclos biogeoquímicos no Brasil, auxiliar na resolução de crimes, tráfico de drogas ilícitas e de animais silvestres e aumentar o conhecimento brasileiro sobre o ciclo hidrológico e os padrões de migração animal nos neotrópicos.

Palavras-chave: isótopos estáveis, padrões espaciais, paisagem isotópica.

1. INTRODUCTION

Stable isotope ratio distribution is related to geographic space. This is a fundamental feature that has led to the emergence of many studies in this growing field of knowledge with a series of new applications in recent years. This spatial variation is related to how spatially explicit variables influence isotopic fractionation and discrimination. For example, altitude and latitude highly influence $\delta^2\text{H}$ and $\delta^{18}\text{O}$, differences in regional nitrogen cycles influence $\delta^{15}\text{N}$, and differences in the plant photosynthetic pathways (C_3 or C_4) influence $\delta^{13}\text{C}$.

Spatial patterns of stable isotope ratios have been represented in spatial models, called isoscapes (isoscapes = isotopic + landscapes), which allow visualization and help both data interpretation and decision making when managing natural resources (Bowen, 2010a). Isoscapes enable the statistical filling of spatial gaps where isotope ratio information has not been represented (Bowen, 2010a). Isoscapes can be based on any of both abiotic elements, such as water (Terzer *et al.*, 2013) and soil (Weintraub *et al.*, 2016), as well as biotic elements, such as plant (Powell *et al.*, 2012), animal (Vander Zanden *et al.*, 2018) and human tissues (Valenzuela *et al.*, 2011). For this reason, isoscapes have been used in a wide range of scientific areas, such as ecology, climate change, biogeochemistry, hydrology, forensic sciences, anthropology, and commercial regulation, among others (Bowen *et al.*, 2009).

The effort to map stable isotope ratios in the landscape precedes the term "isoscape", which was idealized in 2006 (West *et al.*, 2006). Before that, mathematical models were developed to map global $\delta^{15}\text{N}$ patterns in soil (Amundson *et al.*, 2003), global $\delta^{13}\text{C}$ patterns in biosphere focusing on carbon isotope discrimination during photosynthesis (Lloyd and Farquhar, 1994), and of $\delta^{18}\text{O}$ in atmospheric CO_2 (Farquhar *et al.*, 1993). The $\delta^{18}\text{O}$ and $\delta^2\text{H}$ global maps were also developed, mainly based on a database with precipitation isotopic ratios provided by the Global Network for Isotopes in Precipitation (GNIP) (Bowen and Revenaugh, 2003). With improvements in mapping technologies, interpolation techniques, and transfer functions, the isoscape approach has become a fertile scientific application field (West *et al.*, 2010).

Reviews of isoscapes (Bowen *et al.*, 2009; Bowen, 2010a) and a book focused on isoscape concepts and techniques (West *et al.*, 2010) have already been published. However, the present compilation is the first that presents an extensive update on isoscape application followed by indications of perspectives and future applicability in Brazil, a region recognized worldwide for its high biological diversity and landscape heterogeneity. As a country of continental size, Brazilian scientific works that use stable isotopes can adapt global isoscapes from literature to solve national scientific problems. In addition, creating new isoscapes in different spatial and temporal scales to Brazil may fill existent scientific gaps on stable isotope distribution, improving the knowledge on spatial patterns of carbon and nitrogen biogeochemical cycles, hydrological cycles, trophic niches and animal movements, and food authentication and forensics.

In this context, we explore here the key concepts and methods used in the design of isoscapes presenting a brief explanation about stable isotope spatial distribution mechanisms, the major problems related to scale definition, and the already used methodologies to generate isoscapes. With that in mind, associated to a summary of the main applications of isoscapes in the areas of water cycle, ecosystem ecology, animal movement and forensic studies, we present how the use of isoscapes could be an essential tool for isotope data interpretation and natural resource managing in Brazil.

2. METHODOLOGY

We searched for publications in the Web of Science (<http://thomsonreuters.com/web-of-science>) databases using the word "isoscape" or "isoscapes" as a topic (present in the abstract, keyword, or title). In order to expand the search, we explored the reference lists of the retrieved articles. We classified the articles according to the topics defined in this work: water cycle studies, ecosystem approach studies, animal movement, and forensic applications. Although there are isoscapes of several elements, we restricted the search to $\delta^2\text{H}$, $\delta^{18}\text{O}$, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isoscapes. To present papers in which isoscapes could be used in order to show the potential use of this approach in the Brazilian context, we searched for articles using the word "stable isotopes". This search was performed on the Web of Science and Scielo databases.

To illustrate some existent isoscapes that can be used in the Brazilian context, we used isoscapes available in literature. World precipitation $\delta^2\text{H}$ and $\delta^{18}\text{O}$ isoscapes are accessible to download on <http://www.waterisotopes.org> in raster format, with 10 km x 10 km spatial resolution. We used climatic data from <http://www.worldclim.org> to elaborate soil $\delta^{15}\text{N}$ isoscape to Brazil based in Amundson *et al.* (2003) equation for 0 – 10 cm depth ($\delta^{15}\text{N}_{\text{soil}(0-10)} = 0.134 * \text{MAT} - 0.0005 * \text{MAP} + 3.1985$). For $\delta^{13}\text{C}$ isoscape, we used the model elaborated by Powell *et al.* (2012) to South America under their previous authorization.

3. KEY CONCEPTS AND METHODS USED IN THE DESIGN OF ISOSCAPES

3.1. Describing stable isotopes and their natural variations

Isotopes are atopic species of the same element. Therefore, they contain the same number of protons in the atomic nucleus, but different numbers of neutrons. The stable isotopes are those that do not emit any kind of radiation. Carbon stable isotopes, for example, are ^{12}C and ^{13}C . The first one contains 6 protons and 6 neutrons in the nucleus, the second has the same number of protons, but 7 neutrons. The elements of major biogeochemical cycles that have more than one isotope (hydrogen, oxygen, carbon and nitrogen) are so called the light stable isotopes.

The natural abundance of heavier isotope atoms is significantly smaller compared to the lighter atoms. Therefore, the isotope ratio (R) of the sample is compared to the R of a preset international standard to define the value of delta: $\delta = (R_{\text{sample}}/R_{\text{standard}} - 1) * 1000$, where R_{sample} is the ratio between heavier and lighter atoms of the sample, and R_{standard} is the ratio between heavier and lighter atoms of a standard. The R value is dimensionless and via criteria of provenance is less than zero. Thus, its value is multiplied by a thousand, creating a notation called δ per mil (‰).

Biogeochemical and physical processes lead to differences in reaction between light-heavy isotopes generating different proportion of them on materials or environment. The main process that leads the carbon isotope discrimination ($\Delta^{13}\text{C}$) is photosynthesis. There are two main metabolic pathways of CO_2 absorption by plants: C_3 (Calvin Cycle) and C_4 (Hatch-Slack Cycle). C_3 plants discriminate more ^{13}C from CO_2 , thus have lower $\delta^{13}\text{C}$, varying between -35

‰ and -20 ‰. In contrast, C₄ plants have higher $\delta^{13}\text{C}$ values, with variation pattern between -15 ‰ and -11 ‰ (Powell *et al.*, 2012). In general, C₃ plants are tree and shrub species, while C₄ plants are represented by tropical grasses (e.g. corn and sugarcane). The $\delta^{13}\text{C}$ isoscapes are generally elaborated through methods that differentiate plants with C₃ or C₄ metabolisms (Powell *et al.*, 2012).

However, differences in carbon isotope ratio can be found in the same photosynthetic group (C₃ or C₄) or even in multiple individuals in the same species. Those differences occur due to environmental control or genotype-specific physiological influences in carbon isotopic discrimination among plants (Cernusak *et al.*, 2013). In C₃ plants, leaf $\delta^{13}\text{C}$ might decrease with increasing the ratio of intercellular to ambient CO₂ concentrations (c_i/c_a), which is a function of CO₂ supply from the atmosphere to the intercellular air spaces through stomata (Orchard *et al.*, 2010). In addition to allowing the diffusion of CO₂ in the leaf, the stoma prevents water loss. Therefore, soil water availability and atmospheric pressure also influence $\Delta^{13}\text{C}$. Plant $\delta^{13}\text{C}$ has a negative correlation with the transpiration efficiency and has been used to access water-use efficiency, which can change following environmental gradients. Generally, $\Delta^{13}\text{C}$ decreases with the decrease of MAP (Cernusak *et al.*, 2013).

There is a general pattern of increasing $\delta^{13}\text{C}$ value with the increase of elevation among C₃ plants. In higher altitudes, the c_i/c_a ratio decreases with the decrease of atmospheric pressure. The lower temperature in high altitudes can increase the $\Delta^{13}\text{C}$ as well. The availability of nutrients in the soil is another factor that influences the isotopic discrimination of carbon by the C₃ plant due to the direct influence on photosynthetic capacity. Discrimination of the carbon isotope decreases with increasing N concentration in the leaf (Cernusak *et al.*, 2013). Light availability is the main environmental condition that causes $\Delta^{13}\text{C}$ variation among C₄ plants. Generally, $\Delta^{13}\text{C}$ increases with irradiance in C₄ plants. Water use efficiency also influences C₄ plants $\Delta^{13}\text{C}$; however, with an opposite trend compared with C₃ plants. Also, there may be a trend for increased isotope carbon discrimination with increased drought stress in C₄ plants (Murphy and Bowman, 2009).

Global atmospheric circulation of water is the most important process that influences global distribution of $\delta^{18}\text{O}$ and $\delta^2\text{H}$. Oxygen and hydrogen isotope ratios of water change primarily from the isotopic discrimination by sea water evaporation and precipitation. The standard mean ocean water (SMOW) is the international standard for $\delta^{18}\text{O}$ and $\delta^2\text{H}$, which is 0‰, by convention. Furthermore, $\delta^{18}\text{O}$ and $\delta^2\text{H}$ ratios become negative due to differential evaporation between heavier and lighter molecules, where lighter molecules evaporate more easily. In the process of rain formation and precipitation, clouds turn more negative as they advance toward the continent. In this way, $\delta^{18}\text{O}$ and $\delta^2\text{H}$ ratios present latitudinal, longitudinal and/or altitude variation, undergoing continental effects.

At smaller scales, water isotopes differ according to the scale and processes that drive the hydrological cycle. In a watershed, regardless of its extent, the water isotopic variation goes beyond the influence of precipitation. The O and H isotope ratios of surface water depend mainly on the water source (Bowen and Good, 2015; Liu *et al.* 2010; Birkel *et al.*, 2018) and downstream transport-related processes during the terrestrial water fluxes in a watershed (Jasechko *et al.*, 2013). Differences in soil texture or hillslope angle can drive differential variation in the evaporation leading to variation in water isotope values at local scales (Mueller *et al.*, 2014). Differences in environmental conditions (temperature and air humidity) influence leaf water evaporation leading to possible variation of tissue O and H isotope ratios and it can vary according to the plant species (West *et al.*, 2008).

The spatial variation of $\delta^{15}\text{N}$ depends on a complex set of processes. Each process of oxidation and reduction of nitrogen causes isotope fractionation. Nitrogen makes up about 78% of the atmosphere in the form of N₂, a little-reactive gas. This gas is the international standard for $\delta^{15}\text{N}$ which is 0‰, by convention. Processes such as dry and/or wet deposition of N, and

decomposition of organic matter transform N into more reactive forms. When nitrogen is transformed from N_2 to NH_3 and NH_4^+ , assimilated by the organisms and transformed into organic molecules, $\delta^{15}N$ values of the substrate increase. Therefore, soil $\delta^{15}N$ is generally high, especially in tropical ecosystems (Martinelli *et al.*, 1999).

Climatic influences on soil $\delta^{15}N$ values occur mainly due to the higher gaseous N losses in hot/dry places than in wet/cold places (Craine *et al.*, 2015a). High values of $\delta^{15}N$ in the soil can indicate high relative losses of N for the atmosphere while N losses due to leaching and erosion do not cause significant fractionation. Soil $\delta^{15}N$ varies as a function of clay content and soil organic carbon concentration at global scale (Craine *et al.*, 2015a) while at local and regional scales, soil $\delta^{15}N$ values are a function of organic matter decomposition (Craine *et al.*, 2015b). Generally, the higher the level of organic matter decomposition, the greater the soil $\delta^{15}N$ (Craine *et al.*, 2015b). Moreover, topographic position influences soil particle size by erosion and deposition processes and can be an important predictive variable of the nitrogen cycle, which consequently influences soil $\delta^{15}N$ spatial variation (Berhe *et al.*, 2018).

3.2. Scaling isoscapes

Scale is a fundamental aspect of studying all phenomena and processes that vary in space and/or time (Goodchild, 2011). In general, a scale reflects the limit of a given phenomenon representation. Several studies use categoric terminology from geographical scale approach in the application or elaboration of isoscapes or in works on processes that lead to a spatial variation of isotopic fractionation and discrimination, which can be used as predictive variables for isoscape elaboration (eg.: landscape-scale - Bai *et al.*, 2009; continental-scale - Powell *et al.*, 2012; ecosystem-scale Wang *et al.*, 2013; community-scale - Rascher *et al.*, 2012).

Large-scale isoscapes often need to be simplified and generalized due to the necessary match with secondary data sources available at these scales (Bowen, 2010a). In this sense, global or continental-scale isoscapes may not be suitable for landscape-scale applications without going through a downscaling process considering more variables. Likewise, isoscapes elaborated on very detailed scales may not address issues at larger scales (Bowen, 2010a).

The isotope ratio of a given substrate varies with time, location, and spatially. Therefore, depending on the chosen scale, the predictive variables of a given isotopic ratio may change. The spatial variation of water stable isotopes of precipitation ($\delta^{18}O$ and δ^2H), for example, is clearly influenced mainly by climatic patterns, with the main variables predicting the annual mean precipitation and temperature (Terzer *et al.*, 2013). However, in smaller scales, the influence of other variables such as topography may be more evident (Baisden *et al.*, 2016).

3.3. Mapping isoscapes

One of the main principles of isoscapes is to determine isotopic composition in places where there is no sampling, extrapolating a limited number of sampled sites. Therefore, we mainly use methods and algorithms from the geostatistics. The choice of the appropriate method for the mapping depends on the purpose of the work, the number of spatially explicit dependent variables and predictors available, and the scale. At the local and regional scale, it is feasible to collect in the field to feed isoscapes models. Usually, these studies have used ordinary kriging after semivariogram fit to construct the isoscapes (Oliver and Webster, 2014).

Isoscapes with larger scales are usually built by the compilation of data from literature. Another way is setting up global networks of isotopic monitoring. As an example, $\delta^{18}O$ and δ^2H isoscapes in water were used to compile the GNIP database that has been applied in hydrological and ecological approaches in many spatial scales (IAEA/WMO, 2015).

Spatial autocorrelation is considered as a fundamental condition for the application of geostatistical methods. That is, values from nearby places tend to be more similar than values from places more distant in space. In this sense, the independent or predictive variable must influence the values of the target variable, which are the stable isotope values. Geostatistical

models usually bring an error that must be measured and considered in the studies. The model settings seek to reduce the error, increasing accuracy.

When the spatial process influencing the variable is well-known, the search for a methodological standardization is more feasible. That is the case of the influence of atmospheric water circulation on precipitation $\delta^{18}\text{O}$ and $\delta^2\text{H}$, for example, which allows the application of special regression in most mappings (Bowen, 2010b).

Most of the well-known global isoscapes are for $\delta^{18}\text{O}$ and $\delta^2\text{H}$, and they are basically based on equations of spatial regressions, with variations and adaptations between studies. Since there are global isoscapes of environmental elements such as precipitation of water, they can be adjusted for global isoscapes of biological tissues, as was done for leaf water (West *et al.*, 2010) and bird feathers (Bowen *et al.*, 2005; Hobson *et al.*, 2012a). It is important to highlight here the work of Terzer *et al.* (2013) that proposed a new approach to raise accuracy of $\delta^{18}\text{O}_p$ and $\delta^2\text{H}_p$ isotope global models, called Regionalized Cluster-based Water Isotope Precipitation (RCWIP). The method differs from previous ones because the authors made a more accurate model from a set of regionalized multivariate regression equations. The predictive variables were gridded climatic and geographic variables (elevation, latitude / longitude). Terzer *et al.* (2013) compared the results with the model of Bowen and Wilkinson (2002), showing that uncertainty generally decreased. These results are available for download on the internet in raster format with $10' \times 10'$ spatial resolution on <http://www.iaea.org/water>.

The only existing global nitrogen isotope was proposed by Amundson *et al.* (2003), based on a regression using global patterns of precipitation and temperature as predictive variables. They used values of foliar $\delta^{15}\text{N}$, soil $\delta^{15}\text{N}$, and enrichment factor available in the literature at that time, showing that, on a global scale, soil and plant $\delta^{15}\text{N}$ have a negative relation with MAP and positive correlation with MAT. It served as a first proxy of $\delta^{15}\text{N}$ global distribution. However, it presented considerable limitations due to the complexity of factors that affect $\delta^{15}\text{N}$ fractionation, many gaps in spatial distribution data, which results in augmented error in the final model (Pardo and Nadelhoffer, 2010).

For carbon isoscapes, Still and Powell (2010) developed a methodology to estimate the percentage of C_3 and C_4 plants by area unit, allowing $\delta^{13}\text{C}$ plant spatial distribution pattern estimation in a large mapping scale. Powell *et al.* (2012) used similar methodology to map $\delta^{13}\text{C}$ in South America in 2000, using empirical measurements of plants and soils. They estimated $\delta^{13}\text{C}$ values for plants and soil organic matter from vegetation type mapping (grasses, shrubs and trees) by classifying each pixel of an image as potentially favorable for C_3 plant or C_4 plant. The authors used temperature and precipitation dataset incorporated to previous classifications of agricultural cover and type of crop. First, they considered vegetation pattern and visual interpretation of satellite image and incorporated information of managed agro-ecosystems. Second, they partitioned the area considered herbaceous in the previous step in natural grasses percentage and crop percentage based on global data of agricultural area distribution, separating in C_3 and C_4 plant culture from a global database of types of agriculture. This approach may be applied at a regional scale by incorporating new datasets that represent regional relationships between ^{13}C discrimination and abiotic factors.

4. MAIN APPLICATIONS OF THE ISOSCAPES

4.1. Water isoscapes

Global and regional $\delta^{18}\text{O}$ and $\delta^2\text{H}$ isoscapes provide information that integrate a number of water cycle mechanisms and have strong spatial correlation (Bowen and Good, 2015). This approach serves as base to models that assign samples to their regions of origin, such as animal migration and forensic models. Due to the importance and utility of maps for water $\delta^{18}\text{O}$ and $\delta^2\text{H}$, many works adapted methodologies by downscaling precipitation, surface water and

groundwater isoscapes for different locations. Water isoscapes have been used to measure contributions of surface water and groundwater to rivers, water loss to atmosphere through evaporation or transpiration, and rain-type contribution to total precipitation (Aggarwal *et al.*, 2016). Precipitation $\delta^{18}\text{O}$ isoscapes also contribute to the understanding of climatic variations and anomalies (Kern *et al.*, 2014) and the determination of seasonal variation in water sources (Brooks *et al.*, 2012). In the latter, during the dry season, the major contribution was found to be from snow accumulated at higher altitudes (60-80%), helping to understand the vulnerability of the Willamette River hydrographical system in the climate change scenario (Brooks *et al.*, 2012).

4.2. Isoscapes on ecosystem ecology approach

Isoscapes have been used in the ecosystem approach mainly to provide a basis for studies of carbon and nitrogen biogeochemical cycles from local to global scales.

At the global scale, atmospheric and plant $\delta^{13}\text{C}$ isoscapes provide fundamental information to the comprehension of CO_2 exchanges between the biosphere and atmosphere by calculating net assimilation of carbon by plants based on carbon discrimination models (Lloyd and Farquhar, 1994; Suits *et al.*, 2005); global plant $\delta^{13}\text{C}$ isoscape models have great potential to improve estimates of the carbon sinks and sources spatial distribution, knowledge about plant physiology, and their interaction with climatic and edaphic factors (Powell *et al.*, 2012); global isoscapes of soil $\delta^{15}\text{N}$ integrate important information about N global input and output tendencies of ecosystems (Amundson *et al.*, 2003; Houlton *et al.*, 2015).

At smaller scales, isoscapes have been used for both aquatic and terrestrial environments with an ecosystem approach to understand regional and local patterns of carbon and nitrogen cycles as well as human activities and local environmental changes are affecting the dynamics of these elements. In aquatic ecosystems, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isoscapes have been coupled with various elemental ratios to analyze nutrient dynamics (N and P) (Fourqurean *et al.*, 2015). In terrestrial ecosystems, soil $\delta^{15}\text{N}$ spatial variation may be shaped considering elevation, which has a direct relationship to climatic patterns (e.g.: MAT and MAP) (Arnold *et al.*, 2009; Weintraub *et al.*, 2016), but may also help to compose future predictive models about nutrient cycling and CO_2 emission in a climate change scenario (Houlton *et al.*, 2015). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isoscapes have also been applied to measure spatial correlation of soil $\delta^{13}\text{C}$ in natural environments and pastures (Powers, 2006), to evaluate invasive plant effects in local ecosystem processes (Bai *et al.*, 2009; 2012a; 2013; Rascher *et al.*, 2012; Hellmann *et al.*, 2016a; 2016b; 2017; Nielsen *et al.*, 2016) and to assess the effects of land use change to nutrient cycle in rural areas (Nitzsche *et al.*, 2016) as well as in urban areas (Boeckx *et al.*, 2006). Local $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isoscapes have been also used to track soil organic matter dynamics. For instance, variations in tropical forest soil $\delta^{15}\text{N}$ may also be explained by topographic variations, mainly by slope (Hilton *et al.*, 2013; Weintraub *et al.*, 2015) and elevation (Arnold *et al.*, 2009; Weintraub *et al.*, 2016).

4.3. Isoscapes on animal movement and biological conservation studies

There are two major approaches to use isoscapes in animal migration studies: nominal assignment and continuous surface assignment (Wunder, 2012). Nominal assignment approach divides the continuous surface with isotopic ratios (predictive variables) in smaller named blocks, easier to manipulate (categorical variables). The most-used methods in the nominal assignment approach are decision trees or discriminatory functions that create attributions and clusters (Hobson *et al.*, 2012b; Vander Zanden *et al.*, 2015). Continuous surface assignment approach is based on continuous surface models with isotopic ratios attributed to each pixel on a model. In these cases, the continuous surface approach requires a small set of sample data adjusted to inorganic variable models (such as precipitation, for instance) (Wunder, 2012).

Assignments have usually been made by applying Bayesian analysis in order to estimate the likelihood that each isoscape pixel represents the origin of a given sample. The same approach is used in forensic studies (see section below). Some prior information may lead to greater likelihood that an unknown sample may have originated from an area. In the absence of isotope data, given the greater natural abundance of individuals from a same species in a region versus in another region, it is reasonable to think that the target individual would have a greater chance to have come from the former region. This way of thinking may be traduced to mathematical terms using Bayesian rules: $P(B/A) = P(A/B) * P(B)/P(A)$, where, $P(B/A)$ is the posterior probability of an event B given event A , $P(A/B)$ is the probability of the observed data given the model parameters (for example, the normal probability function cited below), $P(B)$ is the prior probability for B and $P(A)$ is a marginal probability that serves as a normalizing constant.

There are several algorithms and computational platforms that support the Bayesian analysis specific for sample assignment to isoscapes. One of the most-used platforms is the IsoMAP, cyber-GIS system that supports basic isoscape modeling and sample assignment based on Bayesian analysis (<http://isomap.org>; Bowen *et al.*, 2014). Recently, many packages have emerged on the R statistical computing environment (R Core Team, 2018) that permit geostatistical analyses, isoscape modelling, and sample assignment. Some of them combined different GIS packages in one specific for isoscape assignment, such as IsoriX package, for example (Courtiol *et al.*, 2019).

Assignment models use tissue isoscape as a probability surface, where an unknown origin sample value may be assigned to a region as a function of a normal distribution, given its isotopic value and the expected standard deviation (Wunder, 2012; Reed *et al.*, 2018; Hobson *et al.*, 2018). For that reason, the construction of tissue isoscapes is a priority for studies on animal migration. Studies with this objective should consider the characteristics of each species and generate isoscape models with appropriate spatial resolution. Also, the majority of existing models are static, not taking into account temporal variation that may be incorporated to animal tissue. Isoscapes based on global databases, for example, should be associated with field-validated samplings and other, more refined, local climatic models (Hobson *et al.*, 2010). The mechanisms of tissue renewal and how they affect isotopic fractionation in the animal must be considered in the analysis (Wunder and Norris 2008). Studies about migratory species using isoscapes in countries with few available data should elaborate ground-validated models for the species in question (Gutiérrez-Expósito *et al.*, 2015). The tissue-calibrated isoscape can be made by using the residual from a regression analysis between the environment isoscape and the target tissue isotopic value (Hobson *et al.*, 2018). The tissues to calibrate the isoscapes must have known origins.

Scientists must be aware of the challenges of using organic tissue for stable isotope analysis, mainly for $\delta^2\text{H}$ values (Hobson *et al.*, 2012a; Soto *et al.*, 2017). Samples for this type of analysis are very sensitive to contamination by any water from the natural ambient humidity. The analysis of the same sample at different sites may also affect the final result, which can be solved by more intensive drying of the sample (Soto *et al.*, 2017). Even more complex is the exchangeable nature of some hydrogen molecules, which may affect the final bulk tissue $\delta^2\text{H}$ value. That is due to the characteristics of some chemical structures of the materials, such as amino (NH_2), carboxyl (COOH), hydroxyl (OH), or thiol (SH), which can exchange hydrogen atoms with ambient water (Meier-Augenstein *et al.*, 2013). Therefore, exchangeable hydrogen must be considered in the analysis to maintain the accuracy of assignment models with precipitation isoscapes (Meier-Augenstein *et al.*, 2013; Soto *et al.*, 2017).

Building an organic tissue $\delta^2\text{H}$ dataset must follow controlled methods to determine non-exchangeable ^2H abundance in animal tissue, so the analysis can be validated and applied in spatial models (Meier-Augenstein *et al.*, 2013). Some methods have recently been accessed in

an attempt to standardize $\delta^2\text{H}$ analyzes on tissues in different laboratories and should be considered in future analyses (Soto *et al.*, 2017). These challenges must be considered on any analysis that uses organic tissue $\delta^2\text{H}$ to track animal movement, as well as any forensic application involving tracking humans, food provenance, and illegal trade of wildlife, timber and drugs.

Most papers on the use of isoscapes in animal migration studies are focused on birds, due to the large number of migratory species (eg Vander Zanden *et al.*, 2015; Reed *et al.*, 2018). However, the isoscape approach has been used in studies of the movement of any species or group of animals that exhibit some geographic variation during the life cycle. In terms of wild fauna conservation studies, regional isoscapes have been used with great potential to assist decision makers (Hénaux *et al.*, 2011). For example, Hénaux *et al.* (2011) observed the dispersion routes of pumas (*Puma concolor*) using $\delta^2\text{H}$ and $\delta^{13}\text{C}$ isoscapes to approach prey with sedentary behavior, an important work in identifying critical conservation areas for big carnivores, and Rodríguez-Pérez *et al.* (2018) identified critical habitats of vaquitas (*Phocoena sinus*) using sediment and zooplankton $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isoscapes in marine ecosystems.

4.4. Isoscapes on forensic applications

The success of prior uses of animal-tissue isoscapes has led the increased use of isoscape approach in human forensic studies. Stable isotopes serve as geographical indications of illegal material seized, such as drugs or trafficked wild animals and have the potential to link these materials to geographic positions (Bowen *et al.*, 2007; Ehleringer *et al.*, 2008; Warner *et al.*, 2018; Chesson *et al.*, 2018).

The success of using isoscapes to help solve forensic questions depends on a series of factors. First, is important to know how mechanisms and chemical materials used on fixation and storage affect the material, component or organism isotope ratio. Second, is necessary to know the predictive power of the model, which may be recognized from a regression, to describe relations between a specimen isotopic ratio with environmental variables (Ehleringer *et al.*, 2010).

Ehleringer *et al.* (2008) mapped $\delta^2\text{H}$ and $\delta^{18}\text{O}$ human hair for the United States, while Valenzuela *et al.* (2011) elaborated human hair isoscapes for the United States, using $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{34}\text{S}$. Both papers discussed the possible applications of this model to identify the region of unidentified human origin, movement reconstruction, and studies on human diet. Isoscape from tap water is also a solid base to forensic studies (Bowen *et al.*, 2007) and has been useful in identifying human geographical origin (Warner *et al.*, 2018). Hydrogen and oxygen isoscapes have been also employed for the identification of the geographic region of food production, such as wine, olive oil, coffee, meat (Ehleringer *et al.*, 2000; Carter *et al.*, 2015; Chiocchiani *et al.*, 2016), beer, bottled water, soda, and milk (Chesson *et al.*, 2010a; 2010b).

Although the use of isoscapes are efficient in numerous forensic studies, generally the models are not capable of predicting the exact specimen origin place due to climatic conditions that shape stable isotopes spatial patterns in similar ways in different regions (Ehleringer *et al.*, 2010). If there is some previous knowledge of the specimens' probable origin areas or assistance from other complementary tools, it is possible to increase the predictive capacity of coupled models. As an example, Mallette *et al.* (2016) used O and H isoscapes coupled to alkaloid studies to detect the possible origin of seized cocaine in the United States among 19 crop areas in South America; but also, they could compare cocaine isotopic ratios to a geographically validated database and that allowed the authors to discover new cocaine cultivation areas formerly unknown.

5. POTENTIAL USE OF ISOSCAPES IN BRAZIL

In Brazil, the number of studies using light stable isotope ratios to track local and regional carbon and nitrogen dynamics in the soil-plant-atmosphere system from different Brazilian regions has grown considerably in the last decades: in the North region - Amazonia (Ometto *et al.*, 2002; 2005; 2006; Pérez *et al.*, 2006; Nardoto *et al.*, 2008; 2014); northeastern region - Caatinga (Teixeira *et al.*, 2006; Freitas *et al.*, 2010), Central region - Cerrado (Bustamante *et al.*, 2004; Coletta *et al.*, 2009; Viani *et al.*, 2011), southeastern region – Atlantic forest (Lins *et al.*, 2016; Vitoria *et al.*, 2018).

The combination of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ has been the basis for studies about diet of different animals such as aquatic macrofauna (Hardt *et al.*, 2013; Castro *et al.*, 2016), fishes and dolphins (Di Benedetto *et al.*, 2013), arthropods (Salgado *et al.*, 2014) and small mammals (Galetti *et al.*, 2016), and human diet in different regions of Brazil (Nardoto *et al.*, 2006, 2011; Gragnani *et al.*, 2014; Rodrigues *et al.*, 2016).

The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ have been applied to hydrological cycle studies in Brazil. These isotopes have been applied in the understanding of local and regional hydrological cycles (Martinelli *et al.*, 2004; Soler i Gil and Bonotto, 2014). Mixture models of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ indicated the role of plant transpiration and soil evaporation on local hydrological cycle in Amazonia (Moreira *et al.*, 1997), Cerrado (Jackson *et al.*, 1999), and Atlantic forest ecosystems (Cassana *et al.*, 2015). An isotope map has been made to verify chemical and isotopic characteristics at the west portion of the Guarani Aquifer System (among the states of Mato Grosso do Sul, Mato Grosso and Goiás) for a paleoclimate study (Gastmans *et al.*, 2010) while Martinelli *et al.* (2004) showed the potential of using $\delta^2\text{H}$ and $\delta^{18}\text{O}$ isotopes from precipitation and river water in the Piracicaba's River Basin's hydrological monitoring station, in São Paulo State.

The applications of stable isotopes to detect food authentication and adulteration have also been used in Brazil to evaluate the quality and origin of Brazilian wines (Martinelli *et al.*, 2003) and beers (Mardegan *et al.*, 2013), soy sauce (Moraes *et al.*, 2018), but also to determine differences in diet of barn versus free-range chickens for studying animal nutrition with food authentication implications (Coletta *et al.*, 2012).

Stable-isotope forensic studies have sporadically been used in Brazil. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ helped to identify the origin of Brazilian marijuana seized (Shibuya *et al.*, 2007), to trace origin and effect of illegal discharge of residues in streams and sediment (Barbieri *et al.*, 2014; Roth *et al.*, 2016), but also for an isotopic assessment to help understand the effects of the 2012 Brazilian Forest Act on tropical riparian zones (Salemi *et al.*, 2016).

Very few studies using $\delta^{13}\text{C}$ spatial patterns have been employed to measure land-use effects in aquatic (Sanaïotti *et al.*, 2002; Augusto *et al.*, 2015) and terrestrial ecosystems (Assad *et al.*, 2013; Salemi *et al.*, 2016; Figueira *et al.*, 2016), which, therefore, might closely fit what has been applied in Brazil in terms of the isoscape approach in any published study led by Brazilian research groups.

5.1. Perspectives of using isoscapes in Brazil

Published global and continental isoscape models can be downloaded for the Brazilian context. These isoscapes may serve as a first approximation to perform larger scales mapping in Brazilian biomes. $\delta^{13}\text{C}$ isoscape elaborated by Powell *et al.* (2012) for South America shows carbon stable isotope spatial distribution patterns along Brazilian biomes (Figure 1 A). The equations from Amundson *et al.* (2003) for soil and vegetation $\delta^{15}\text{N}$ may be applied to Brazil from records of spatial climatic variables (Figura 1 B).

Global precipitation $\delta^2\text{H}$ and $\delta^{18}\text{O}$ isoscapes can also be downloaded and easily applied in Brazilian scale (Figure 2). However, elaboration of local and regional isoscapes in the Brazilian context is essential in order to reduce associated model errors. Developing precipitation

isotope models specific for Brazil is imperative, since current global models mask part of the natural variations of isotopic ratios. For instance, average rates presented in Figure 2, show a significantly lower spatial variation in comparison to temporal and spatial fluctuation found by Soler i Gil and Bonotto (2014) only in the area of São Paulo State.

Figure 1. Plant $\delta^{13}\text{C}$ isoscape from Powell *et al.* (2012) with author's consent (A); and soil $\delta^{15}\text{N}$ isoscape based in Amundson *et al.* (2003) equation for 0 – 10 cm depth ($\delta^{15}\text{N}_{\text{soil}(0-10)} = 0.134 * \text{MAT} - 0.0005 * \text{MAP} + 3.1985$) applied using climatic data from <http://www.worldclim.org> to elaborated (B).

Figure 2. Mean annual precipitation $\delta^{18}\text{O}$ (A) and $\delta^2\text{H}$ (B) isoscapes. Data were downloaded from <http://www.waterisotopes.org>.

Taking into account the number of studies that have been using the stable isotope approach in Brazil together with the facility to use the equations and methods already tested and applied worldwide, the potential to develop carbon, nitrogen, oxygen and hydrogen and isoscapes with improved scale will definitely support both basic and applied studies in different areas of knowledge as pointed out in Section 4 above.

In terms of developing $\delta^2\text{H}$ and $\delta^{18}\text{O}$, local to regional isoscapes to study hydrological cycles in different regions of Brazil could be easily applied to groundwater management in Brazil and should be complemented by spatial modelling for isoscape creation that would

facilitate data interpretation. There are 26 geographic locations with precipitation stable isotope historic data from GNIP stations in Brazil, which are available for download in shapefile format (<http://wateriso.utah.edu/>). Currently, 10 GNIP monitoring stations are operating in Brazil (Peeva, 2018). This amount of GNIP stations cannot represent the climatic and hydrographic diversity for the Brazilian territory. Some alternatives are suggested in the literature, such as the Ehleringer *et al.* (2008) work collecting tap water and human hair, or using specific resident species (Hobson *et al.*, 2009) to construct indirectly water based isoscapes in a continental level. These approaches can be used to fill-in gaps on water based isoscapes for the continental territory of Brazil.

Future $\delta^{13}\text{C}$ isoscapes for Brazil should be made using samples with large spatial representation, such as the one performed by Assad *et al.* (2013) to study changes in carbon stock caused by land-use changes. It may help to decrease model errors. For the Cerrado region, for example, Powell *et al.* (2012) indicated considerable uncertainty due to huge land cover heterogeneity in Cerrado biome. Brazilian researchers can integrate soil and vegetation samples from collections of previous field work and publications to increase the number of sampling sites for biogeochemical studies, both for carbon and nitrogen stable isotopes.

Local and regional biogeochemical changes result in problems related to N_r in Latin America (Austin *et al.*, 2013), especially in Brazil. $\delta^{15}\text{N}$ isoscapes for vegetation and, mainly, for soil are important tools to study N input and output mechanisms from ecosystems (Bai *et al.*, 2012b, Houlton *et al.*, 2015). Nitzsche *et al.* (2016) discussed the importance of $\delta^{15}\text{N}$ mapping in complex agricultural areas to disclose results to farmers and decision makers. It may be applied in Brazil, considering the large extension of agricultural areas. Another possibility would be to map $\delta^{15}\text{N}$ in agricultural areas considering landforms, potential ways in which the land can influence soil characteristics and agricultural productivity (Siqueira *et al.*, 2010). In this case, a regional and local soil $\delta^{15}\text{N}$ isoscape could point to the topographic position where N losses to the atmosphere or leaching area are happening, aiding agricultural management.

Some plants used in agriculture and livestock were introduced in natural ecosystems in Brazil. There are at least 54 potentially invasive plants in Brazil and their effects on local biogeochemical cycles are little known (Zenni and Ziller 2011; Dias *et al.*, 2013). Similar approaches as above described (Bai *et al.*, 2013) should be used to help in the understanding of invasion vectors and the biogeochemical cycle changing (Rascher *et al.*, 2012, Bai *et al.*, 2012a, Hellmann *et al.*, 2016a, 2017).

Considering the extensive biodiversity and animal migration in Brazil, existing global isoscape models may be used for first approximations of animal wintering and reproduction localities. Geographic assignment models of migratory birds to geographic origins have already been made for South America (García-Pérez and Hobson, 2014; Hobson and Kardynal, 2016). Despite the use of water isoscapes applicability to infer origin or wintering places of animals at regional and continental scales, the highest potential of this approach lies at medium to high latitude regions due to the strong spatial gradient of isotopic ratios in precipitation. However, this limitation does not narrow the application of isoscapes in issues of this nature in regions of low latitude, such as South America and Africa. In these cases, a multi-isotope approach coupling $\delta^2\text{H}$ isoscapes with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isoscapes may raise the inference efficiency (García-Pérez and Hobson, 2014; Hobson and Kardynal, 2016). Another way to raise the isoscape efficiency is to perform an integrated use with other tracing methods, such as satellite tracking or combined with genetic markers (Rundel *et al.*, 2013).

Isoscape approach used in animal migration studies could be adapted to determine the origin of seized animals and trafficking routes. There is still a huge number of live animals and animal products being seized every year in illegal activities (Alves *et al.*, 2012; Regueira and Bernard, 2012). Illegal animal trade usually results in the withdrawal of animals from those

natural origins and transporting them to other regions where illegal markets, intermediate sellers or final consumers are present (Alves *et al.*, 2012; Destro *et al.*, 2012). Transportation caused by illegal wildlife traffic may be compared to natural bird migration, as both imply animal displacement at a continental scale (Destro *et al.*, 2012).

In the forensic context, many applications should be accomplished in numerous investigation cases, since it is usually necessary to link crime traces to their geographical origin (Cerling *et al.*, 2016). In addition to wildlife illegal trade, other common crimes, such as illicit drug trafficking, wood trafficking, food frauds cases, and individual human crime investigations would benefit from isoscape approaches in the Brazilian context. All materials or components seized that go through isotopic analysis should have their isotopic ratios compared to spatial databases which may indicate the origin or traveled path (see Cerling *et al.*, 2016). For example, the US Drug Enforcement Agency (DEA) has a drug signature program, with the aim to construct specific drugs isoscapes (Chesson *et al.*, 2018). This initiative should be replicated here, for marijuana and cocaine drugs. Shibuya *et al.* (2007) studied different marijuana-producing regions in Brazil and could assign seized samples. This knowledge can be updated and used for drug enforcement.

Illegal logging in Brazil, specially in the Amazon region, is still a big issue, with selected species being collected using fraud in mechanisms of control (Brancalion *et al.*, 2018). Considering the Brazilian potential for wood commercialization, certification of commercialized wood would benefit from the isoscape approach, considering the well-known relation between local water and wood isotopic ratios (Gori *et al.*, 2018).

Provenance of food production for human consumption is also a forensic issue. Regional certified products have specific organoleptic and culinary qualities, such as wine, cheese, and coffee, which sometimes are subject to mislabeling or other fraud (Camim *et al.*, 2017). Camim *et al.* (2017) reviewed methods and necessary legal aspects to state geographic origins of food products. Besides basic element isoscape knowledge, they state the importance of complementing isotopic information with standard values of authentic products in available databases.

6. SYNTHESIS AND FUTURE

The isoscape approach has been applied in different scales and its use as a basis for many scientific subjects has increased in the last decade. Its main advantage is deriving isotopic ratios with a statistical consistency in real sample points gap. Since spatial and temporal isotopic continuous maps integrate chemical processes occurring in ecosystems, the use of grid-based isoscape models has facilitated and simplified the isotope interpretation in different contexts, from terrestrial to aquatic ecosystems, either in natural or anthropic conditions.

Both global and regional $\delta^2\text{H}$ and $\delta^{18}\text{O}$ isoscapes indicate elements of water cycle, such as inputs and outputs in water pools, and have helped in water management. However, these isoscapes are also used as tracers to identify origin of animals, products and chemical elements and may amplify their utility when coupled with $\delta^{15}\text{N}$ and/or $\delta^{13}\text{C}$ isoscapes.

Existent global and continental isoscapes can already be framed into the Brazilian boundaries and applied in many studies, although they are general models with relatively low spatial resolution, not being able to represent regional or local variations of stable isotope values. However, by improving technologies and possibilities of isoscape applications, studies tend to seek more-refined isoscapes, with better spatial and temporal resolution. There is a tendency pointed out by Bowen (2010a), a transition from descriptive models to comprehensive models, where isoscapes help explain ecosystem mechanisms and processes, and it must be incorporated into future studies in Brazil. The Brazilian challenge will be to develop regional and local isoscapes, upscaling existing isoscapes and/or elaborating new ones from new systematic sampling.

There is a global trend for the development of an integrated and centralized isotopic database. An example is an initiative from scientists, museum curators, data analysts, and educators to build the IsoBank, which they say would be a dynamic and sustainable repository that would accelerate the resolution of urgent issues in all disciplines involving stable isotopes (Pauli *et al.*, 2017).

Another example is the GNIP for precipitation data, which has currently 10 stations in operation in Brazil. The integration of laboratories and the establishment of a national network could increase the number of monitoring stations. However, to build a national network to collect water samples regularly and to have them analyzed to $\delta^2\text{H}$ and $\delta^{18}\text{O}$ is far from being an easy task considering the immense Brazilian territory and especially the logistics and infrastructure involved. It should take into account the need to measure the years on a regular basis (rainfall event or least monthly). Most important is to be aware of the global networks and to be integrated with them.

Brazil has a basic structure of laboratories that carry out isotopic analyses in the northeastern, central-western and southeastern regions; however, the challenge is to increase the amount of specific funding involving stable isotope research. The establishment of museum networks and integrated access to animal and plant collections has great potential to increase the spatial distribution of samples with isotopic values in Brazil. The same is true for an integrated network for accessing soil samples from collections in Brazilian laboratories. Both networks would improve the access to data and the construction of isoscapes to answer current issues in biogeochemistry, ecology, conservation and forensics.

7. ACKNOWLEDGMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. We would like to thank the Graduate Program of Environmental Sciences of University of Brasília for the financial support to English review. We would like to thank to the Graduate Program of Environmental Sciences of University of Brasília for the financial support to English review.

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ARTICLES doi:10.4136/ambi-agua.2296

Received: 27 Jun. 2018; Accepted: 11 Jan. 2019

Douglas Leite Figueira^{ID}; Ricardo Valcarcel^{ID}; Marcelle Nardelli Baptista^{*ID}

Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica, RJ, Brasil

Instituto Florestas (IF). Departamento de Ciências Ambientais (DCA).

E-mail: douglas_nf@hotmail.com, ricardo.valcarcel@gmail.com, marcellenardelli@gmail.com

^{*}Corresponding author

ABSTRACT

The catchment basin of the Dois Rios River (R2R) (3159 km²) unites the climatic and environmental effects typical of areas located on the leeward side of mountain chains. It has a deficient water balance, with sparse and very specific wet areas. This study identifies delimitation areas, and characterizes and groups those basins and floodplains which show the greatest capacity for producing ecosystemic services related to maintaining the continuity of water resources in the R2R basin. Locations with higher pluviometric indices (1500 to 2800 mm/year) were selected and grouped into micro basins with the highest potential for capturing rainfall due to their being on the leeward side of the mountains in the region (with their slopes oriented to permit them to capture humidity and concave plane curvature). The 42 basins formed 4 groups, from which the 17 micro basins with the highest probability of providing ecosystemic services were chosen, belonging to Groups III and IV. Within these groups, the 498 floodplains with the highest potential for offering ecosystemic services (Group D) present the greatest capacity for water retention and sustainable effects (high average circularity index, higher altitudes and low occupation rates). The correct use of 0.0006% of the basin can guarantee the direct flow of the drainage for the entire basin. This strategy only requires renaturation measures, discipline with regard to the land uses and strict observance of the processes which generate ecosystemic services in the floodplains of these basins, resulting in relevant socio-environmental benefits for all socioeconomic segments within the R2R basin.

Keywords: floodplain, planning, water regulation.

Subsídio para ordenamento territorial e aproveitamento de serviços ecossistêmicos em bacia hidrográfica com déficit hídrico na Mata Atlântica

RESUMO

A bacia do rio Dois Rios (R2R) (3.159 km²) recolhe os efeitos climáticos e ambientais típicos de zonas situadas a sotavento de cadeias montanhosas. Tem balanço hídrico deficitário, havendo poucos e específicos locais úmidos. Este estudo identifica, espacializa, caracteriza e agrupa as planícies de inundação com maior habilidade em produzir serviços ecossistêmicos

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relacionados à perenidade de recursos hídricos na bacia R2R. Foram segregados locais com maiores índices pluviométrico (1500 a 2800 mm/ano), agrupados em microbacias com maior potencial de captação em função de estarem a sota-vento na região (slopes oriented to permit them to capture humidity and concave plane curvature). As 42 bacias formaram 4 grupos, onde levantou-se 17 microbacias com alto potencial de fornecer serviços ecossistêmicos pertencentes aos Grupos III e IV. Nestes grupos, 498 planícies de inundação com maior potencial de oferta de serviços ecossistêmicos (grupo D) apresentam habilidades para retenção hídrica e efeitos de perenização (high average circularity index, higher altitudes and low occupation rates). O uso correto de 0,0006% pode garantir o escoamento direto das drenagens de toda a bacia. Esta estratégia implicaria em medidas de renaturalização, disciplinamento dos usos e estrita observação dos processos que geram serviços ecossistêmicos nas planícies das bacias, trazendo relevantes benefícios socioeconômicos para todos os segmentos da bacia do R2R.

Palavras-chave: planejamento, planície de inundação, regulação hídrica.

1. INTRODUCTION

Floodplains are the first areas to be saturated after intense precipitation and the overflow of river channels in hydrographic basins (Baptista *et al.*, 2014). They accumulate water and gradually release it into channels, filling up with a backflow (Baptista *et al.*, 2017). The ecosystemic services related to water production permit the survival of these ecosystems, in which humans are an integral and inseparable part and are directly benefited. Among the relevant hydrological ecosystemic services are water regularization, lamination of flooding and amplification of the minimum flows during dry periods (Baptista *et al.*, 2017). The conservation of wet areas in the floodplains is considered to be more efficient from the hydrological point of view, and more economical than the construction of large reservoirs (Baptista and Valcarcel, 2018) when the principles of sustainable development prevail.

Steeply sloping regions which are blocked from the direct influence of humid winds by mountain chains present specific environmental constraints and require objective planning strategies in order to achieve sustainability, especially those areas with a potential for water storage, such as floodplains. Headwaters present characteristics such as precipitation, sizes and forms of floodplains which are distinct from the rest of the catchment basin, affecting the hydric performance of lower areas. In this upper section of the basin, drainage patterns are particularly rectilinear, with fewer contributions from upstream and lower retention capacity, with fast flows and less lateral connection between the channel and the floodplains.

Floodplains are distinguished by their capacity to store water in “flood buffering basins”, which reduce the discharge from the headwaters, thus affecting the potential for flooding during rainfall and the contribution from the basic flow afterwards (Reinhardt *et al.*, 2011). The drainage coefficient can be variable in small basins which contribute to the direct runoff (both surface and sub-surface) in catchment basins with low storage capacity (Oswald *et al.*, 2011), as well as the basic flow in catchment basins with high storage capacity.

Decentralized measures for administering the hydrographic basins of rivers may be efficient strategies for managing floods, water retention and recharging of the aquifers (Reinhardt *et al.*, 2011). Being open systems, they join the combined effects of characteristics such as the surface area, the circularity index, the plane of the curve of the slope and the orientation of the slopes in relation to the humid winds (windward slopes) which interfere in the micro basins (Cardoso *et al.*, 2006) and extrapolate the products to the larger catchment basins, influencing the dynamics of water capture and transmission from the slopes to the floodplains, where that water will be stored.

The Dois Rios River Basin (3159 km²) is located on the right bank (14,165 km²) of the Paraíba do Sul River (total catchment area 55,400 km²), has a gradient of 2348 m and receives the climatic and environmental effects typical of sheltered areas, situated on the leeward slopes of the Serra do Mar Mountains. Its water balance is predominantly deficient, with few and specific wet areas, determined by the aerial basins, vertical precipitation and horizontal interception (dew, mist and other hidden precipitation). The region has a strong agricultural presence with high production, which leads to high water consumption. This study identifies, delimitation areas, characterizes and groups the floodplains with the highest capacity for producing ecosystemic services related to maintaining constant water resources in the R2R catchment basin for the purpose of the sustainable management of those resources.

2. MATERIALS AND METHODS

2.1. Study Area

The slopes and channels of the Paraíba do Sul River catchment basin (55,400 km²) have their orientation and shape conditioned by the geological limits of the Serra da Mantiqueira Mountains on the left side and the Serra do Mar Mountains on the right side of the main channel. Its headwaters in the region of Guararema, in the Brazilian state of São Paulo, present a reduction in declivity, which decreases from 4.9 m/km to 0.19 m/km. This configuration of the relief conditions the drainage pattern and the formation of floodplains.

The left bank features a high plateau on top of the mountains and gentler slopes which, associated with the topographic divisions far from the channels, form the relief with reduced steepness, similar to the regions known as “oceans of hills”. The extensive rivers are nestled in the valleys that separate these hills from the high plateaus of the southern portion of Minas Gerais state, where they receive the climatic influence of the sea, in spite of the distance and the interference of the Serra do Mar Mountains.

The right bank has a few low plateaus and presents a sharper relief, with steep cliffs and small valleys. Even though it is closer to the sea, it receives less influence from the moist winds because it is sheltered, which justifies the predominance of a generalized water deficit, most accentuated in the middle and lower stretches of the Paraíba do Sul catchment basin. In this region, the lack of water and the low capacity for supporting ecosystems confer less resilience, with an increase in erosive processes. In these areas, 50% of the territory presents high to very high vulnerability to erosion (AGEVAP, 2006).

The R2R catchment basin (3159 km²) is located on the right bank of the Paraíba do Sul River (Figure 1). It is formed by the Negro River and the Grande River, and has a predominantly ENE orientation. The entrance of moist air masses is descending due to the orientation and orography. The circulation of humid air masses immediately downstream of the interfluvial areas alters the saturation of atmospheric humidity and creates zones with different levels of humidity, conditioning the ecological diversity and offer of environmental attributes (Barboza *et al.*, 2015).

The high-altitude tropical climate (Cf) and subtropical climate (Cw), according to Köppen's classification, have an average annual rainfall of 1500 mm and average annual temperature of 17.8°C (average in winter of 9°C; average in summer of 28°C). The vegetation in this catchment basin was originally composed of Dense Ombrophilous Mountain Forest and Semi Deciduous Broadleaved Forest of the Atlantic Rainforest biome (IBGE, 2012).

The Serra do Mar mountain chain affects the water balance of the catchment basin, with the discharge from the moist winds occurring on the slopes of Cachoeiras de Macacu, on the windward side of these mountains. The majority of the hidden precipitation in the zones located on the leeward side occurs at points near gorges, where the passage of humid air masses is concentrated (Cortines *et al.*, 2011) and where the vegetation is able to reinforce the maximum

horizontal interception. These points and the neighboring steep slopes, more exposed to these humid winds, are the most important locations for recharging the soil with water (Barboza et al., 2015). In these mountainous cliffs, the average annual rainfall is 2700 mm, due to the orography which contrasts with the high plateau beyond the Serra do Mar Mountains, where the average humidity is less than 1100 mm per year (CPRM, 2001). The altitude of the catchment basin reaches 2366 meters at the peaks and drops to 12 meters at the mouth. The direction in which the drainage networks are oriented is influenced by the geology of the reactivated faults of the Mesozoic separation and the consequent subdivision of the Gondwana supercontinent (AGEVAP, 2006).

Figure 1. Dois Rios River Basin (R2R) – Rio de Janeiro State, Brazil.

2.2. Hydrologically Consistent Digital Elevation Model

The Hydrologically Consistent Digital Elevation Model (HCDEM) was generated from the continuous vectorial cartographic base of the state of Rio de Janeiro, on a scale of 1:25,000 (IBGE, 2012), using the procedures performed by ArcGIS 10.2 software (Figure 2).

Figure 2. Procedures employed for determining the Hydrologically Consistent Digital Elevation Model (HCDEM).

2.3. Geospatial Attributes and Ecosystemic Services

Ecosystemic services related to water resources are factors of the physical environment itself combined with the biotic environment, which, when functioning in an integrated fashion,

can improve the water supply in hydrographic basins. In order to delimit, characterize, group and select the floodplains with similar propensities for producing ecosystemic services which maximize the perennialization of water in the R2R catchment basin, the following indicators were established: *i)* Regions with the highest pluviometric indexes; *ii)* Identification of their micro basins (capture zones) based on morphometric attributes (area, circularity index, orientation and plane of the curve); and *iii)* Selection of floodplains with the greatest capacity for retaining water based on the parameters of area, circularity index, altitude and land use and occupation.

The minimum precipitation which was considered was 1500 mm/year. This was spatialized with help from the Clip tool in the QGIS 2.18 software, with an isohyetal map as database (CPRM, 2001). This part of the catchment basin was sectorized according to its micro basins of up to 3rd order, utilizing the Basin tool of the ArcGIS 10.2 software, available in Spatial Analyst Tools → Hydrology, as well as the circularity index (CI) (Equation 1) (Cardoso *et al.*, 2006).

$$CI = \frac{(12.57 \times A)}{P^2} \quad (1)$$

In which:

CI = Circularity Index (adimensional);

A = Drainage Area (m²);

P = Perimeter (m).

In the ArcGIS 10.2 software, the slope areas were calculated according to their orientation (Aspect, in Spatial Analyst Tools → Surface), then the results were vectorized (from Raster to Polygon, in Conversion Tools). After this procedure, the area of each stretch of the slope was calculated according to its orientation (Calculate Geometry → Area). The plane of the curve was obtained using the Curvature command in Spatial Analyst Tools → Surface, followed by vectorization of the results (from Raster to Polygon, in Conversion Tools). Using the results of this vectorization, the area of each stretch of the slope was calculated according to its orientation (Calculate Geometry → Area). Based on these parameters, the micro basins were grouped according to the hydrographic attributes they offer and their similarities. The Cluster analysis of the SPSS 15.0 software was used to define the group with the best hydrological attributes.

The hydrological model for identifying areas saturated with water – “Topographic Wetness Index” (TWI) – was extracted from the SAGA GIS 2.0.8 HCDem software (Olaya and Conrad, 2009), using the tool Terrain Analysis > Hydrology > Topographic Indices. The division of these areas was established by the cut-off line in the TWI by the adimensional value 5.85. These data were later validated during *in situ* field visits in 10% of the areas studied. They were then verified using Landsat images with a resolution of 15 meters.

The hydrological attributes of water retention analyzed in the floodplains were: *i)* Area; *ii)* Circularity Index; *iii)* Altitude; and *iv)* Land Use and Coverage. To obtain the area and circularity index of these floodplains, the commands described previously were used. Altitude was obtained by using Elevation – Obtain Elevation, in the QGIS 2.18 software, clicking on the highest point, where the floodplain ends.

Soil use and coverage for the floodplains were obtained by using the cutout of the vector provided by the IBGE, on the scale of 1:25,000, which, superimposed on the areas of the floodplains, identify land use, plant coverage and the existence of stone outcroppings in the areas surrounding the floodplains. Subsequently, these floodplains were grouped by similarity of hydrological attributes, using the Cluster analysis of the SPSS 15.0 software and selecting the group of floodplains with the best hydrological attributes for water retention.

3. RESULTS AND DISCUSSION

The R2R basin (3159 km²) is influenced by the leeward side of the Serra do Mar mountains, and contains 220.000 inhabitants, 09 municipalities, 42 micro basins and 7467 floodplains. The distribution of rainfall varies between 1000 and 2800 mm/year, with the management of the part with rainfall higher than 1500 mm/year being of strategic priority, where the use of the catchment basins is an important form of managing their ecosystemic services, both for capturing horizontal precipitation which is a hidden component of the total humidity on the slopes during the rainy seasons, and for storing and maintaining the flow during the dry seasons through the action of the floodplains in the smaller areas which belong to the higher parts of the basin. Thus, the strategic areas which generate water benefits for the entire catchment basin can be defined.

3.1. Areas with High Rainfall

The orographic and environmental condition of the catchment basin shows that the greatest volumes of rainfall (between 1500 and 2800 mm/year) occur in only 13.1% of its area (Figure 3), and that these locations must be treated with the highest priority. Below these locations, an increase in temperature and a decrease in the volume of precipitation occur, increasing loss due to evapotranspiration.

Figure 3. Floodplains located in the area with annual rainfall greater than 1.500 mm.

3.2. Grouping micro basins

Excessive rainfall can also be a source of degradation, if the landscape is not environmentally adjusted, as can be seen by the frequent landslides and floods and prolonged droughts in the low areas (CPRM, 2001; Corrêa and Costa, 2016). The combination of the water

entering the catchment basins and the storage of that water in the floodplains is fundamental for sustainable water supply projects, for both agriculture and the population.

During this study, 42 micro basins of up to the third order were encountered which presented a potential for producing relevant ecosystemic services related with water resources. Area is a morphometric characteristic which can be related to the maximum discharge (Horton, 1945), depending on the types and characteristics of the soils. The average size of the micro basins (320-1835 ha) determines the prioritization of the smaller basins, both because these show greater hydrological sensitivity to the uses to which they are subject, and because they contain fewer users, thus facilitating regional agreements with the objective of adopting conservationist practices. The smaller basins offer greater options for the management of their slopes and other areas (Tonello *et al.*, 2006). Another fact which attests to the greater interactivity between characteristics of their physical environment and the management of these basins is the influence of a greater number of factors, such as the effects of water storage in floodplains in large catchment basins, with less pronounced flood peaks (Borsato and Martone, 2004).

The circularity index of these 42 micro basins was an average of 0.48, ranging from 0.43 to 0.53, similar to the results encountered by Oliveira *et al.* (2010). CI values greater than 0.51 indicate a tendency for the basin to be circular (Schumm, 1956), thus favoring flooding processes; lower values indicate elongated forms, suggesting shorter water paths from the slopes, reducing the concentration time and draining the water more rapidly. Cardoso *et al.* (2006) state that the greater the circularity of a catchment basin, the greater the tendency for intense rain to occur simultaneously throughout its entire extension, which concentrates a large volume of water in the principle tributary. Tonello *et al.* (2006) add that the elongated form of the catchment basin – defined by the index number farthest from unity – causes a lower concentration of runoff and less risk of flooding. Therefore, the micro basins with the highest CI were selected.

The predominance of convex slopes (an average of 50.2%) was detected in relation to concave plane curvature (39.8%), which demonstrates the character of these slopes for dispersing water (Dragut and Blaschke, 2006). The micro basins selected present a higher average of slopes with a concave plane curvature (40.4%) than the others (39.2%). The concave plane curvature is related to a concentration of water, increasing the potential for mass movements (flow of debris and landslides) (Silveira *et al.*, 2013), and thus being a factor associated with erosive processes (Sartori *et al.*, 2005). The concentration of water indicates a delay in the runoff of that water.

The predominance of slopes oriented to leeward (an average of 44.3%) was apparent, with only 7 micro basins showing a predominance of windward orientation. Micro basins with a higher percentage of windward slopes (34.4%) in comparison to the others (29.6%) were selected, because due to the steep terrain, there is a difference in the precipitation received between windward slopes and leeward slopes, considering the masses of moist air to which they are exposed (Dragut and Blaschke, 2016).

After the identification and selection of the micro basins with the best environmental attributes for water production (larger areas, higher CIs, concave plane curvature and slopes oriented to windward), it was possible to identify, group and select the floodplains situated within these micro basins which present geospatial environmental attributes which favor the supply of hydrological ecosystemic services. Grouping them according to similar capacities offers classifications to the hydrological processes in 4 groups (Table 1).

Groups III (12 micro basins) and IV (5 micro basins) include areas with slopes oriented to permit them to capture humidity (35.33% e 33.42% of their areas, respectively) and a concave plane curvature (40.5% and 40.2%, respectively), as well as possessing larger surface areas, conditioning factors which increase the rainfall they receive and induce a higher concentration

of humidity per area, physical attributes capable of conferring a higher probability of offering ecosystemic services.

Table 1. Groups of micro basins, units per group and average values of morphometric variables.

Micro- Basin Group	n	Average Area (hectares)	Southern Orientation (SO/SE/S) ¹ (%)	Concave Plane Curvatura (%)	CI ²
Group I	16	635.1	28.9	40.0	0.50
Group II	09	320.0	30.4	38.4	0.43
Group III	12	1061.6	35.3	40.5	0.47
Group IV	05	1835.3	33.4	40.2	0.53
Total	42	963.0	32.0	39.8	0.48

n – Number of Units (micro basins).

¹ - Oriented to Southwest, Southeast or South.

² - Circularity Index.

Groups III (12 micro basins) and IV (5 micro basins) include areas with slopes oriented to permit them to capture humidity (35.33% e 33.42% of their areas, respectively) and a concave plane curvature (40.5% and 40.2%, respectively), as well as possessing larger surface areas, conditioning factors which increase the rainfall they receive and induce a higher concentration of humidity per area, physical attributes capable of conferring a higher probability of offering ecosystemic services.

3.3. Grouping of Floodplains

In the R2R Hydrographic Basin, 7467 floodplains cover 241.91 km², which represents 6.58% of the basin's total area. This value is similar to that observed in other literature (Hamilton, 2002). In the basin of the main river (Paraíba do Sul), the floodplains are larger and fewer in number, totaling 1.16% of the surface area (Baptista *et al.*, 2014). This information shows their high potential for producing ecosystemic services related to water regularization. Of these floodplains, 1071 are located in the region with precipitation greater than 1500 mm/year and within the 42 micro basins with the highest priority in terms of territorial management, based on the differentiated supply of environmental attributes which can offer distinct environmental services, corresponding to 20.37 km² or 8.42% of the area of all the floodplains in the basin (Figure 3).

Of the floodplains located in the region with rainfall of more than 1500 mm/year, 729 (68.06%) are located in the 17 micro basins whose morphometric attributes confer the highest probability of producing water through their ecosystemic services (Groups III and IV). These micro basins are steep, with a high number of floodplains and heterogeneous geospatial attributes, which, to a certain degree, justify the integration and interdependence of their effects for guaranteeing the continuity of water production.

The average area of 1.5 hectares is not significant, because of the high variability (ranging from 0.008 to 119.6 ha). This high variability was also observed in relation to the circularity index (0.07 to 0.98). The larger floodplains present low circularity indices, affecting the rate at which water is liberated and contributing to its regularization. It can be observed that floodplains along the channel of the Paraíba do Sul River follow this trend, with CIs ranging from 0.001 to 0.52 (Baptista *et al.*, 2014), with no significant difference being noted between this characteristic of the main river and that of the tributaries of the Paraíba do Sul, confirming the replicability of the study.

Of these 729 floodplains, 42.1% present forest coverage. Agriculture and pastures compose another 42.8% of that coverage. Even though nearly half of the floodplains are

anthropized by farming activities, these areas are still subject to alterations of their land use which can be reversed through renaturation activities. Only 14.9% of the areas are occupied by residences or infrastructure (consolidated uses). This value is near to that encountered in the floodplains of the Paraíba do Sul River (18.94%), showing that, in general, the floodplains present a low level of urbanization, with part of their hydrological functions still being preserved. This fact, associated with adequate planning for the orderly advance of urbanization in the floodplains, may enhance services for the renaturation of their hydrological functions (Baptista *et al.*, 2014). Urbanization impairs the hydrological functions and ecosystemic services provided by floodplains (Baptista *et al.*, 2017), as it leaves the soil compacted and impermeable, and also impedes the connection with the river channel. The low proportion of occupied areas demonstrates that it is still possible to adequate the land uses demanded by society with the offer of ecosystemic services, permitting the implantation of renaturation measures to improve the performance of areas with high water production (Baptista e Valcarcel, 2018).

As mentioned earlier, by using the Cluster function, 4 groups of floodplains with similar environmental attributes were formed (Table 2). The fourth group stands out from the rest because of its capacity for retaining water, with a total of 498 floodplains with high potential for water reservation, if the necessary conservational measures are utilized.

Table 2. Groups of floodplains, units per group and averages of the variables analyzed.

	N	AA (ha)	ACI	UAP (%)	AAI (m)
Group A	213	0.9	0.63	92.2	1014.9
Group B	3	91.2	0.12	86.5	981.6
Group C	15	30.9	0.21	49.0	984.1
Group D	498	0.4	0.60	1.9	1112.1
Total	729	30.8	0.40	57.4	1023.2

N – Number of Units (Floodplains); AA – Average Area in hectares; ACI – Average Circularity Index; UAP – Percentage of the area occupied by Urbanization, Agriculture and Pasture; AAI – Average Altitude in meters.

Considering the high percentage of the surface area occupied by Urbanization, Agriculture and Pasture, Groups A and B do not possess the ability to administer water resources, because the anthropic uses of the floodplains have diminished their capacity for water storage and their ability to offer ecosystemic services (Baptista *et al.*, 2017). In these areas, the elevated levels of anthropization have prioritized strategic solutions, although actions for water renaturation should be reinforced, with a view toward increasing the delay time for filling those floodplains which show a high potential for flooding and water regularization within Group A (a high ACI).

While half of the floodplains in Group C are not anthropized, this group has a low ACI. According to Schumm (1956), CI values greater than 0.51 indicate that the basin tends to be circular, favoring flooding processes (higher flood peaks); values lower than 0.51 suggest that the basin tends to be elongated, contributing to the drainage process and the escape of water resources from the hydrographic basin. Considering the analogous reasoning between hydrological units and floodplains, this means that the floodplains of Group C have a tendency to disperse water, similar to the findings of Oliveira *et al.* (2010).

Group D has a high ACI, which indicates a lower capacity for superficial runoff, and consequently an increase in water retention. The higher altitude guarantees that precipitation normally exceeds evapotranspiration, providing enough water to maintain a constant supply to the aquifers which are responsible for the springs that feed the watercourses (Cardoso *et al.*, 2006). Low occupation rates increase the infiltration and water storage capacity (Vanzela *et al.*,

2010) and contribute to low-cost management. These factors permit this group of floodplains, which correspond to 0.01% of the total area of all the floodplains and 0.0006% of the total area of the catchment basin, to be selected as the best areas in the catchment basin in terms of potential for offering ecosystemic services for water retention.

3.4. The Supply of Ecosystemic Services

The 13.1% of the basin with rainfall between 1500 and 2800 mm/year has a greater horizontal interception, which may add up to 44% to the pluviometric precipitation (Barboza *et al.*, 2015) in its 6.93% of the territory total of the basin in Groups III and IV, which has an environmental advantage related to a greater propensity for receiving both pluviometric rainfall and occult precipitation. This volume of water entering the subsystems of the slopes and the floodplains, if managed according to conservationist techniques and/or reinforced through measures for the renaturation of water functions, can produce benefits for water regulation in the areas located downriver which present a water deficit. This effect also reduces flooding and its consequences in both urban and rural spaces (Baptista and Valcarcel, 2018). The ecosystemic services for water regularization observed in the 7467 floodplains (241.91 km²), which represents 6.58% of the basin's total area, affect the recharge and discharge. These are the main ecosystemic services related with water regularization (Schindler *et al.*, 2016).

A variety of renaturing actions have been utilized around the world, including the hydromorphological restoration in the Danube River (Riquier *et al.*, 2015) and the revitalization of wetlands in the Anacostia (Baldwin, 2004), Isar (Binder, 2010) and Rhine rivers (Lamers, 2006). These measures require the integration of technical, socio-economic and legal approaches (González *et al.*, 2017). The mapping of floodplains with high potential for the renaturation of water functions is fundamental for increasing the supply of water in terms of both quantity and quality for the population located downriver. This mapping and the identification of the peculiar characteristics of each location are essential to the success of such measures.

Physical and biological measures are important for potentializing ecosystemic services. An example of a physical measure is the opening of recharge areas at sites far from the river channel (Hein *et al.*, 2016), providing great benefits at a low cost because they increase the water storage capacity of the floodplains. Biological measures such as selective cutting of trees (instead of the clear cutting of forests), maintenance of woody vegetation and prioritization in planting species with appropriate root systems are efficient in maintaining the hydrological functions of floodplains, integrated with social demands (González *et al.*, 2017). As a renaturalization proposal for the middle stretch of the Ebro River, the creation of a "river territory" was suggested, aimed at creating conditions for the river to resume its conditions of navigability (Ollero, 2010). Other measures based on a combination of physical, physical-biological and biological works could also be implemented (Schindler *et al.*, 2014).

Even by selecting strategic floodplains, these do not function in a homogeneous way throughout their entire extension. Their main processes must be studied in order to separate them into sectors with distinct hydrological capabilities, based on their specific geomorphological characteristics, aimed at achieving functional synergy and increasing their water capture and storage functions, thus permitting the development of techniques which can be directed towards the better administration of water resources.

4. CONCLUSIONS

In order to contribute to territorial planning and the optimal utilization of the ecosystemic services of floodplains, groups of micro basins (I, II, III and IV) were first formed based on favorable environmental attributes (slopes oriented to permit them to capture humidity and concave plane curvature to retain that humidity) so that the best locations for receiving

pluviometric precipitation (rainfall between 1500 and 2800 mm/ano) and occult precipitation could be defined, according to the aerial corridors which transport humidity and the locations with frontal exposure to the geographical entrances of those corridors into the region, determined by the lowest passes in the high Serra do Mar mountain range. In the groups with adequate attributes and the highest rate of capture of rainfall (Groups III and IV), the floodplains were identified and grouped according to the highest propensity for storing water and contributing to water regularization, forming 4 groups (A, B, C and D). Group D presented the greatest potential for water retention, with the highest average characteristics of surface area, circularity index and altitude, and the lowest occupation rates, for the implantation of measures for renaturizing the hydrological functions, with the objective of increasing the offer of ecosystemic services downriver and promoting water regularization in the catchment basin.

The precipitation concentrated in the headwaters of hydrographic basins (13.1% of the total area, in the case of the R2R region) is an important element to be considered in planning land use focused on the management of the ecosystemic services capable of interfering in the production and administration of water. Of the 7467 floodplains in the R2R catchment basin, only 498 (corresponding to 0.0006% of the total surface area) present optimum conditions for promoting water regulation through the management of their distinctive supply of ecosystemic services, which can benefit the entire catchment basin. These criteria for safeguarding their hydrological functions may affect the collection of water in the micro basins and the water regulation in the floodplains. These results will be materialized in the form of water regulation and continuity of flow, notably in the dry periods, improving the quality and quantity of water in the catchment basin. These focal actions offer greater responses with less investment. Any planning activities which pursue the sustainability of the water system should take these areas into consideration, because they are strategic for guaranteeing perennial flow in the springs and providing water for all the different activities being developed within the catchment basin.

Considering the repressed demands for an adequate water supply, the potential ecosystemic services offered by floodplains and the scarcity of information about these environments, the development of studies which permit deeper knowledge of the sectors within those floodplains, which offer different ecosystemic services and different functional relationships with the physical surroundings, is of extreme importance.

5. ACKNOWLEDGEMENTS

We wish to thank the Municipal Government of Nova Friburgo for having permitted time off from other functions to perform these studies. Also, the UFRRJ/PPGCAF (the Post-Graduate Program for Environmental and Forestry Sciences of the Federal Rural University of the State of Rio de Janeiro) for permitting this opportunity for learning, and the CAPES (the Coordination for Perfection of University-Level Personnel, of the Brazilian Ministry of Education) for providing the necessary support.

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Analytical solution for the stationary model of pollutant propagation in an aquatic medium

ARTICLES doi:10.4136/ambi-agua.2298

Received: 13 Jul. 2018; Accepted: 11 Jan. 2019

Cíntia Ourique Monticelli^{1*} ; Jorge Rodolfo Zabadal²
Daniela Muller Quevedo¹ ; Carlos Augusto Nascimento¹

¹Universidade FEEVALE (FEEVALE), Novo Hamburgo, RS, Brasil
Instituto de Ciências Exatas e Tecnológicas (ICET). E-mail: cintiam@feevale.br,
danielamq@feevale.br, nascimento@feevale.br

²Universidade Federal do Rio Grande do Sul (UFRGS), Tramandaí, RS, Brasil
Departamento Interdisciplinar. E-mail: jorge.zabadal@ufrgs.br

*Corresponding author

ABSTRACT

This work presents a new analytical approach for solving pollutant dispersion problems along irregular-shaped water bodies. In this approach, the advection-diffusion equation is expressed in terms of orthogonal curvilinear coordinates, defined by the velocity potential and the corresponding stream function for inviscid flows. The boundary condition rewritten in terms of these new coordinates is reduced to a classical third kind one, i.e., the derivative of the concentration distribution with respect to the stream function is proportional to the numerical value of the local pollutant concentration. The solution obtained from the proposed formulation was employed to simulate pollutant dispersion (thermotolerant coliforms) along the Pampa Creek, a tributary of the Sinos River at the outskirts of Novo Hamburgo city, South Region of Brazil. The results obtained reproduce the qualitative behavior of the expected concentration distribution.

Keywords: advection-diffusion equation, analytical solutions, mass transport, orthogonal curvilinear coordinates system, pollutant dispersion.

Solução analítica do modelo estacionário de propagação de poluentes em meio aquático

RESUMO

Este trabalho apresenta uma nova abordagem analítica para a solução de problemas de dispersão de poluentes ao longo de corpos hídricos com contornos irregulares. Nesta abordagem, a equação advectivo-difusiva é expressa em termos de coordenadas curvilíneas ortogonais que são definidas pelo potencial de velocidade e pela função de corrente correspondente para fluidos invíscidos. A condição de contorno reescrita em termos destas novas coordenadas é reduzida a uma de terceira espécie clássica, isto é, a derivada da distribuição de concentração em relação à função de corrente é proporcional ao valor numérico da concentração de poluente local. A solução obtida a partir da formulação proposta foi empregada para simular dispersão de poluentes (coliformes termotolerantes) ao longo do Arroio Pampa, afluente do rio dos Sinos, na periferia de Novo Hamburgo, cidade do sul do Brasil. Os

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resultados obtidos reproduzem o comportamento qualitativo da distribuição de concentração esperada.

Palavras-chave: dispersão de poluentes, equação advectivo-difusiva, sistema de coordenadas curvilíneas ortogonais, soluções analíticas, transporte mássico.

1. INTRODUCTION

The conservation of surface water quality is of great importance for society, since this water is collected in order to supply public distribution systems. However, due to their power of dilution and self-purification, several rivers also serve as the final destination of sanitary sewage. Hence, the technical tools to aid in the planning, monitoring and management of water resources, among which mathematical modeling stands out as a fundamental instrument, are very important. Mathematical models allow the generation of properly organized data, spatially and temporally integrating dispersed data, as well as promoting a better understanding of the dynamics of the processes and a prediction of future conditions of the system regarding relevant parameters.

Rivers are the main source of water for human consumption. Thus, controlling the water quality of the rivers is very important because water is directly related to human health (Qishlaqi *et al.*, 2017). In environmental impact studies of sewage transport network projects, part of the data to be produced is the determination of a safe distance from the vicinal area or points of raw water collection for water treatment plants, where effluents can be discharged through an emissary so that the dispersion plume does not reach these areas. Surely, one can also estimate the contamination level that would be produced if the pollutant inevitably reached the regions of interest.

To obtain reliable results regarding the distance of the sewage outfall in relation to the source and to the point of water collection, and thus minimize the total costs of an enterprise, it is necessary to carry out several computational simulations. These simulations would be able to predict the approximate spatial distribution of pollutant concentrations considering a large quantity of scenarios by using mathematical models for mass transport. However, the time required by computational programs capable of implementing such models can become a critical point for certain applications that require a high level of detail, since the large number of mathematical calculations in a commonly used numerical model (Rosman, 2001), (Gomes *et al.*, 2018) causes delays in the generation of the results.

The governing equation of pollutant transmission in rivers is the advection diffusion equation (ADE). This is a partial equation that is very important in environmental engineering, and several hydraulic phenomena such pollution transmission, suspended sediment transport modeling, etc. are involved (Parsaie and Haghiabi, 2017). The ADE includes two differential parts, advection and diffusion; the advection part characterizes the velocity's contribution in the pollutant transmission, and the diffusion or dispersion part characterizes the molecular transmission from highest concentration region to lowest concentration region. In addition to the differential terms present in ADE, there are the physical parameters which will be described throughout this work.

Three possible solutions could be proposed to solve the differential equation that describe the problem in question: the numerical, analytical and hybrid methods. Numerical methods, usually employed by the scientific community, often provide sufficiently realistic results for particle transport problems (Jobim, 2012). In the application of numerical solutions to computational fluid dynamics, either by applying the finite difference, finite element or finite volume methods and artificial neural network - ANN (Parsaie and Haghiabi, 2017), it is common to have to discretize the domain into a mesh with thousands or even millions of points.

This may produce algebraic systems of a very high order (Fernandez, 2007); thus, in most cases, the numerical solutions demand a high computational effort. Consequently, they require a high processing time, which may make it impossible in some cases to generate a sufficient number of scenarios in a timely manner.

In order to avoid these problems, the use of analytical solutions is more advantageous. As they are expressed in closed form, it is possible to write small source codes that are executed in a shorter processing time, which is due to the decrease in the number of operations to be performed and, consequently, in the memory required for all the necessary routines. The advection-diffusion equation, which describes the transport of particles, has several proposed analytical solutions, such as the integral transform method (Mikhailov and Ozisik, 1984), the generalized integral transform method (Cotta, 1993), the change of variables combined with the integral transform method (Guerrero *et al.*, 2009), using the Green's function (Sanskritayn and Kumar, 2016), among others. However, changes in the geometry of the domain are not considered, whereas this would be required in the problem of transporting particles in a water body.

This study presents a new analytical approach to solve the problem of pollutant dispersion in the aquatic environment, in which the advection-diffusion equation is expressed in terms of new orthogonal curvilinear coordinates, defined by the velocity potential and by the stream function. Such reformulation aims to standardize the boundary conditions to be prescribed, in addition to considerably increasing the size of the elements of the corresponding mesh. The use of this technique produces a closed solution for the propagation model of conservative or non-conservative pollutants and its implementation generates very compact symbolic codes and high computational performance.

In addition, the that way the physical parameters, present in the ADE, are obtained has fundamental importance. The determination of the longitudinal dispersion coefficients that properly describes the stream behavior is one of the main objectives of research works, like those of Paisaie *et al.* (2018) Haghiabi (2016; 2017), that use different techniques to obtain them. In the solution proposed by this study, the dispersion coefficients used are the ones obtained by Garcia in 2009 and the velocity coefficients obtained by Lersh *et al.* in 2013.

In order to prove the feasibility and validity of the proposed method, we present the results of a one-dimensional model for the simulation of dispersion of organic pollutants (thermotolerant coliforms) in a 7 km-stretch of the Pampa Creek until its mouth in the Sinos River – located in an urban area of the city of Novo Hamburgo, Rio Grande do Sul, Brazil.

2. MATERIAL AND METHODS

2.1. Equations Model

In this study, in order to obtain an approximation for estimating the concentration of thermotolerant coliforms downstream from a point source, the flow was considered to be: homogeneous, isotropic, uniform, incompressible and irrotational. That is, respectively: absence of regions with “superconcentrations” or even actions of fields that interact with the substance of interest, absence of the characteristic that causes the molecule to migrate from one point to another that differ in each direction of pollutant propagation, and the pressure and turbulence temperature conditions are approximately the same at all points. The velocity field has divergent zero, i.e., there are no diffusive terms nor generation or decay. And finally, considering the irrotational flow to be the viscosity effects forming the hydrodynamic boundary layer on the banks of the river, they can be neglected and the fluid considered with viscosity equal to zero, which does not constitute vorticity.

Yet, many rivers and lakes can be reduced to a two-dimensional problem by behaving like water slides, since the depth is much smaller than the distance between the boundaries, so the

dispersion would have enough time to homogenize the vertical concentration profile at the moment the plume has spread horizontally on a geographic scale. Consequently, the dispersion in the z -direction will be neglected.

With the changes and simplifications applied to the general equation of the particle balance (Cranck, 1975; Kambe, 2007) based on an Eulerian view of the process, and assuming that the kinetics of reactions could be represented by a first-order decay, the two-dimensional advection-diffusion equation for non-conservative pollutants is given as Equation 1:

$$\frac{\partial C}{\partial t} + u \frac{\partial C}{\partial x} + v \frac{\partial C}{\partial y} = D \left(\frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2} \right) - kC \quad (1)$$

In Equation 1, C is the pollutant concentration, x and y are the directions of motion, u and v are the velocities of the water body in the directions x and y respectively, D is the surface oscillation diffusion coefficient (Garcia *et al.*, 2009) and k is the coefficient of velocity.

When one intends to estimate the level of pollution caused by the discharge of sewage systems in different regions of a watercourse, whether for classification and framing according to current regulations or for the projection of bathing conditions, it is desirable to generate results that express the system in its steady state. Since the time scale in which the dispersion occurs is much higher than the characteristic period of the thermal and hydrodynamic fluctuations of the flow, the time evolution pattern of the diffusion, kinetic and velocity field coefficients present stationarity, that is, low amplitude stochastic oscillations around a fixed mean value. In this study, the coefficients of diffusion and velocity shall be considered constant, representing temporal arithmetic means independent of time.

Since the only transient effect on the concentration distribution to be considered is due to the effects of degradation kinetics, which is a phenomenon independent of mass transport, Equation 1 can be decoupled through the split process (Zabada and Ribeiro, 2012):

In the kinetic Equation 2:

$$\frac{\partial C}{\partial t} = -kC \quad (2)$$

And in the transport Equation 3:

$$u \frac{\partial C}{\partial x} + v \frac{\partial C}{\partial y} = D \left(\frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2} \right) \quad (3)$$

2.2. Analytical Solution

With the target equation decoupled in a system of two equations, Equation 2 and Equation 3, they can be solved in parallel.

2.2.1. Solution of the kinetic equation for first-order decay

The asymptotic behavior of the kinetic model for most pollutants is exponential, thus solving Equation 2 by separation of variables we obtain Equation 4:

$$C(x, y, t) = f(x, y)e^{-kt} \quad (4)$$

The function $f(x, y)$ is precisely the exact solution for the transport equation, Equation 3. Thus, substituting Equation 5 into Equation 3 restored the transport equation:

$$\left(u \frac{\partial f}{\partial x} + v \frac{\partial f}{\partial y} \right) = D \left(\frac{\partial^2 f}{\partial x^2} + \frac{\partial^2 f}{\partial y^2} \right) \quad (5)$$

2.3. Solution of transport equation for point sources

It is possible to produce the solution of the transport equation by writing it in terms of a new curvilinear coordinate system (ϕ, ψ) and, only then, solve it. To perform this task, initially, it is necessary to redefine the first and second order partial derivatives of the function $f(x, y)$ in terms of the velocity potential $\phi(x, y)$ and the stream function $\psi(x, y)$. By using the chain rule (Equations 6 and 7):

$$\frac{\partial f}{\partial x} = \frac{\partial f}{\partial \phi} \frac{\partial \phi}{\partial x} + \frac{\partial f}{\partial \psi} \frac{\partial \psi}{\partial x} \quad (6)$$

$$\frac{\partial f}{\partial y} = \frac{\partial f}{\partial \phi} \frac{\partial \phi}{\partial y} + \frac{\partial f}{\partial \psi} \frac{\partial \psi}{\partial y} \quad (7)$$

And the identities and conditions of Cauchy-Riemann Equation 8:

$$u = \frac{\partial \phi}{\partial x} = \frac{\partial \psi}{\partial y}, \quad v = \frac{\partial \phi}{\partial y} = -\frac{\partial \psi}{\partial x} \quad (8)$$

Writing the second-order derivative, regrouping the terms and making the necessary simplifications, the Laplacian of $f(x, y)$ is yielded in terms of the new coordinates ϕ and ψ Equation 9:

$$\nabla^2 f(x, y) = (u^2 + v^2) \left(\frac{\partial^2 f}{\partial \phi^2} + \frac{\partial^2 f}{\partial \psi^2} \right) + \frac{\partial f}{\partial \phi} \left(\left(u \frac{\partial u}{\partial \phi} - v \frac{\partial u}{\partial \psi} \right) + \left(v \frac{\partial v}{\partial \phi} + u \frac{\partial v}{\partial \psi} \right) \right) + \frac{\partial f}{\partial \psi} \left(\left(v \frac{\partial u}{\partial \phi} + u \frac{\partial u}{\partial \psi} \right) - \left(u \frac{\partial v}{\partial \phi} - v \frac{\partial v}{\partial \psi} \right) \right) \quad (9)$$

The partial derivatives of the velocity field components u and v can be defined with the help of the chain rule, thus Equations 10 and 11:

$$\frac{\partial u}{\partial x} = u \frac{\partial u}{\partial \phi} - v \frac{\partial u}{\partial \psi} \quad \text{and} \quad \frac{\partial u}{\partial y} = v \frac{\partial u}{\partial \phi} + u \frac{\partial u}{\partial \psi} \quad (10)$$

$$\frac{\partial v}{\partial x} = u \frac{\partial v}{\partial \phi} - v \frac{\partial v}{\partial \psi} \quad \text{and} \quad \frac{\partial v}{\partial y} = v \frac{\partial v}{\partial \phi} + u \frac{\partial v}{\partial \psi} \quad (11)$$

Once the relations Equation 10 and Equation 11 had replaced into Equation 9, the divergent and rotational of the velocity field will be cancelled, since incompressible and irrotational flows are being considered. This allows us to rewrite the diffusion terms of the target Equation 12 as:

$$\nabla^2 f(x, y) = (u^2 + v^2) \left(\frac{\partial^2 f}{\partial \phi^2} + \frac{\partial^2 f}{\partial \psi^2} \right) \quad (12)$$

In order to complete the procedure, it is also necessary to rewrite the advection terms of Equation 5 as a function of the new coordinates Equation 13:

$$u \frac{\partial f}{\partial x} + v \frac{\partial f}{\partial y} = u \left(u \frac{\partial f}{\partial \phi} - v \frac{\partial f}{\partial \psi} \right) + v \left(v \frac{\partial f}{\partial \phi} + u \frac{\partial f}{\partial \psi} \right) = (u^2 + v^2) \frac{\partial f}{\partial \phi} + (uv - vu) \frac{\partial f}{\partial \psi} \quad (13)$$

Finally, the advection-diffusion equation for the steady state can be explained as a function of the new coordinate system. Equating the two sides in Equation 12 and Equation 13 and dividing by $(u^2 + v^2)$, Equation 5 is thus rewritten as a function of curvilinear coordinates Equation 14:

$$\frac{\partial f}{\partial \phi} = D \left(\frac{\partial^2 f}{\partial \phi^2} + \frac{\partial^2 f}{\partial \psi^2} \right) \quad (14)$$

The Equation 14, that describes the bi-dimensional model, can be solved by using Bäcklund (Polyanin and Zaitzev, 2004) and Fourier transformations (Spiegel, 1976). However, in this study, due to the restricted number of experimental data, we will consider the one-dimensional problem.

2.4. One-dimensional Model

In the case of narrow or slow-flow rivers, as soon as the concentration distribution reaches the steady state the cross profile will be homogenized. Therefore, the diffusion term in this direction, $(\frac{\partial^2 f}{\partial \psi^2})$, can be neglected and the model will become one-dimensional, described through the ordinary differential Equation 15:

$$\frac{df}{d\phi} = D \frac{d^2 f}{d\phi^2} \quad (15)$$

Solving Equation 16 by the method of separation of variables and a second integration, the result is:

$$f(\phi) = b_0 + c_0 e^{\frac{\phi}{D}} \quad (16)$$

In this solution b_0 represents a constant buffer, namely, the minimum value of concentration that the substance of interest will reach, which can be considered null for non-conservative pollutants, since for these the concentration C will fall to zero when $\phi \rightarrow -\infty$ in the absence of new sources. The constant c_0 represents the initial concentration of the contaminant at the point of discharge.

Reacting the solution obtained by Equation 17 to the solution of the kinetic equation given by Equation 4, we obtain:

$$C = c_0 e^{\left(\frac{\phi}{D} - kt\right)} \quad (17)$$

Expression Equation 17 results in the concentration of the pollutant downstream from the point source given that the initial concentration of the pollutant and its velocity profile in the stretch are known.

3. RESULTS AND DISCUSSION

3.1. Experimental data

In order to validate the proposed solution, experimental data were used (Nascimento and Naime, 2009). The data were collected at the source, main body and mouth of the Pampa Creek, an affluent of the Sinos River, shown in Figure 1. The Sinos River supplies approximately 97% of the urban population of the city of Novo Hamburgo in Rio Grande do Sul, Brazil (COMUSA, 2015). This city has an estimated population of 249,113 inhabitants (IBGE, 2017). Pampa Creek receives a domestic load from a region with approximately 40% of the total population of the city (Novo Hamburgo, 2017). Their effluents are discharged in the stream without any treatment, subsequently flowing into the Sinos River for about 1.5 km to the water collection point for treatment and subsequent public distribution for the city.

Point P1, located at the source of the Pampa Creek, has been taken as the starting point of the particles. Point P2, located midstream the course of Pampa is 3919 m away from P1, as well as P3, the mouth of the creek, which is located at 7669 m away from P1. Point P4, on the Sinos

River, located next to the raw water pumping station of the water treatment plant is 1668m downstream of P3.

Although the model described here has the ability to change the geometry under study, once the coordinate system has been modified, we intend to validate the results based on the one-dimensional approach given by Equation 17. For validation of the two-dimensional solution, Equation 14, it is necessary to obtain a more robust amount of data, such as pollutant concentration and velocity in more than one line along the water body.

Figure 1. Pampa creek's location and data collection points.

The next figures show that the expression found as a problem solution (Equation 17) is experimental data adherent. The mean values for the thermotolerant coliforms measured from May 2006 to August 2006, and from January 2007 to March 2007, are presented, respectively, in Figures 2 and 3. We choose these periods because they had a similar precipitation profile. The samples were collected at the source of the Pampa Creek (P1), midstream along the course (P2), at the mouth of the brook (P3) and at the raw water collection point for water treatment in the Sinos River (P4), on eight different dates, at the four points of interest. The mean values show that the profile of coliform concentration from the data collected experimentally presents exponential decay, as suggested by the solution.

Figure 2. Average coliform concentration profile, from May to August 2006 at the 4 sampling points.

Figure 3. Average coliform concentration profile, from January to March 2007 at the 4 sampling points.

3.2. Velocity Potentials

The hydraulic variables are directly related to the watercourse flow. Thus, during rainy periods, and more frequently throughout autumn and winter, in this region of the country the flow velocity increases, as well as the level of the watercourse. Consequently, the depth and width of the water resource, along with the drainage area, increase.

The velocities at the four points selected for this study were obtained with the Flo-Mate™ portable speed meter Model 2000. Monthly measurements were performed at each of the points and are described in Tables 1, 2 and 3. The mean velocity was used in each point and period of interest.

At the raw water collection point by the Municipal Company of Sanitation of Novo Hamburgo (COMUSA), P4, the mean velocity of the watercourse was used for the spring/summer period, which was measured at 0.37m/s, and for the fall/winter period, 0.69m/s.

The $\phi(x)$ coordinate values were calculated at each point and date considering the mean value of flow velocity, $\bar{u}$, by the definition of the velocity potential (Equation 18):

$$\phi(x)_i = \alpha \int_0^{-x_i} \bar{u} dx \quad (18)$$

In Equation 18, $\alpha = 10^{-4}$ represents a dimensionless correction value at the order of magnitude of the velocity to contemplate the effect that the stirring of the liquid mass exerts on the velocity measurement float, since it moves in all directions in very short intervals of time. This effect causes considerable amplifications on the diffusion coefficient as it promotes an essentially isotropic mixture when the shells formed on the surface of the water rise and fall alternately. Thus, it is not necessary to calculate the effective distance traveled over time accumulating the projections of the velocity vector on the main direction of the flow. The diffusion coefficient for the stagnant water (Brownian motion), for which the order of magnitude is 0.000001 (Bird *et al.*, 2002), is then divided by the diffusion coefficient – estimated by means of an oscillatory model derived from Navier-Stokes equations (model Korteweg-de Vries), the value of which is approximately 0.01 (Garcia *et al.*, 2009). Thus, this quotient is on the order of 10^{-4} . Note that this quotient equals the product between the instantaneous velocity and half of the mean free course.

Table 1. Measured velocity and velocity potentials at P1.

Period	May/06	Jul/06	Aug/06	Oct/06	Nov/06	Jan/07	Mar/07	May/07
$\bar{u}$ (m/s)	0.081	0.10	0.175	0.149	0.134	0.062	0.154	0.12
$\phi(x)$ (m ² /s)	0	0	0	0	0	0	0	0

Table 2. Measured velocity and velocity potentials at P2.

Period	May/06	Jul/06	Aug/06	Oct/06	Nov/06	Jan/07	Mar/07	May/07
$\underline{u}(\text{m/s})$	0.23	0.102	0.376	0.326	0.392	0.367	0.394	0.39
$\phi(x)(\text{m}^2/\text{s})$	-0.0966	-0.03997	-0.1435	-0.1279	-0.15391	-0.14393	-0.15441	-0.15284

Table 3. Measured velocity and velocity potentials at P3.

Period	May/06	Jul/06	Aug/06	Oct/06	Nov/06	Jan/07	Mar/07	May/07
$\underline{u}(\text{m/s})$	0.03	0.456	0.28	0.4518	0.063	0.221	0.242	0.18
$\phi(x)(\text{m}^2/\text{s})$	-0.02301	-0.34971	-0.21473	-0.3465	-0.04875	-0.16948	-0.18559	-0.13804

3.3. Coefficient of diffusion

In the absence of any other characteristic that might make the probability of a molecule to migrate from one point to another be different for each direction of propagation of the pollutant, it is safe to assume that the diffusion coefficient is a constant value. Thus, the coefficient of diffusion by surface oscillation in microscale, D , was given by the quotient between the mean free courses and double the period between successive molecular collisions, in which $D = 0.25\text{m}^2/\text{s}$ (Garcia *et al.*, 2009).

3.4. Coefficient of velocity

The coefficient of velocity, k , presented in the model referring to the kinetics of bacterial and chemical pollutants decay, is indirectly dependent on the position and on the time variable. This dependency is due to the velocity constant being influenced by factors that can vary both in space and time, such as temperature, turbulence and wind incidence, which cause oscillations on the surface of the water body, in addition to the pollutant concentration itself.

For the determination of k , the data for the calculation of another coefficient, T90, was used. This coefficient was obtained through laboratory assays and measures the time required to reduce the level of bacteria present in water by 90%, i.e., to reduce the concentration to 10% of the original value at the point of emission. In the experiment (Lersch *et al.*, 2013), this reduction occurred in 48h, which, based on the solution of the first order differential equation that describes the degradation kinetics, allowed us to estimate that the rate constant in $k = -0.0479$.

3.5. Time

The time has been calculated based on the distance that the particle traveled in each section and the mean velocity obtained experimentally. For each section, on each different date, the mean travel time was calculated. Given the ease of calculation by the reader and the great amount of information, no further explanations on this specific topic will be given, since there are eight different dates and four different points of the course evaluated.

3.6. Simulations

By using all the described data, it was possible to simulate the dispersion of thermotolerant coliforms based on the hypothesis that they are discharged at a specific location in the Pampa Stream and dispersed along its course.

The results of the simulations are presented on the different dates in which the samples were collected. Point P1 was taken as a source of pollutant discharge near the source of the stream and the amount of coliforms remaining at point P2 (midstream) was then simulated. Figure 4 presents the results obtained in the simulations and the measured values distributed in a scatter plot. In this graph, the complete agreement of the results with the experimental data is not shown, since this area is a highly urbanized zone, so point P1 is certainly not the only source of load in this stretch.

Figure 4. Experimental and simulated data. Discharge influence of P1 in P2.

Considering P2 as a point source of discharge, the amount of thermotolerant coliforms remaining at point P3, that is, at the mouth of the stream, was simulated. This result was presented in Figure 5, which shows a better agreement of the data obtained through the simulation and the values measured experimentally, due to the order of magnitude of the parameter involved.

Figure 5. Experimental and simulated data. Discharge influence of P2 in P3.

The stretch from the mouth of the Pampa Creek (P3) to the point of water collection for treatment in the Sinos River (P4) is not urbanized, so there is no discharge point in between these places. Thus, all coliforms arriving at P3 were considered as input data and the remaining value at P4 was simulated, as shown in Figure 6. However, although the results obtained in the simulations were expected to agree almost totally with experimental data, the expected results were not verified on three of the dates. This is due to the input of water from the Sinos River near the mouth of the Pampa Creek, which impacts the poor quality of the experimental data obtained and, consequently, on the simulations. Given all hypothesis constraints imposed to the model performed, the reality hasn't reflected, at all.

Figure 6. Experimental and simulated data. Discharge Influence of P3 in P4.

The points P1, P2 and P3 are in the same watercourse, the Pampa Stream, whereas P4 is located in the course of the Sinos River, where the water flow and velocity change considerably. Thus, the difference between the calculated values and the respective experimental data is basically due to two causes of error: flow inversion and considerable fluctuations in the velocity field. Both factors are observed, frequently in periods of high flow rates, as was the case when experimental data were obtained. Since the hydrodynamic model employed considers the flow to be potential, implicitly the hypothesis of steady state is assumed. Therefore, any perturbation that produces transients in the velocity field had been neglected in the proposed model. Moreover, even the most refined hydrodynamic formulations based on the Navier-Stokes and Helmholtz equations (Bird *et al.*, 2002; Mott, 2006) produce equally disparate results, since it is not possible to reproduce such fluctuations with fidelity, but only to estimate their amplitudes and frequencies in a relatively coarse manner. Haghiabi *et al.* (2018) examining models of artificial neural networks (ANN) and support vector machine (SVM), showed that all of them had some over-estimation properties. In short, the simplified hydrodynamic model is, in practice, as realistic as the finer formulations in fluid mechanics, even though they contemplate turbulence models.

In order to enable the analysis of the agreement of the results with the experimental data, both data were contrasted in dot cloud graphs, shown in Figure 7 and Figure 8. The bisecting line $y = x$, was plotted, in which the correlation coefficient was above 80%, thus evidencing that the data are correlated.

4. CONCLUSIONS

The results obtained in this work are qualitatively consistent with the behavior of the phenomenon and relatively concordant from the quantitative point of view, proving the feasibility of the proposed solution. Even if some divergence is present, at least two main reasons could be listed. First, there may be regions where the cross stratification of the concentration profile is not negligible, whereas sampling may have been done in different flow lines, and such precision is not attainable experimentally. Second, the adoption of a constant mean velocity in each modeled portion could have caused a insignificant error in the calculation.

Figure 7. Distribution of the results around the bisector. Influence of P2 in P3.

Figure 8. Distribution of the results around the bisector. Influence of P3 in P4.

However, despite the limitations of this preliminary analysis, the overall trend displayed by the complete set of numerical results agrees well with the experimental data given the proportion of order of magnitude involved. Considering that a deterministic model was proposed, while the studied phenomenon is strongly influenced by stochastic factors, a certain difference is not only expected but also perfectly understood due to the different characteristics of the data.

Once results had been obtained for the one-dimensional model, it seems reasonable to assume that the use of a two-dimensional model will refine the estimates. This assumption has been based on the possibility of reducing, for example, the potential error generated by not considering the different flow lines of each section. Thus, the authors intend to implement simulations of the two-dimensional model based on the solution of Equation 14 proposed in this study as soon as new experimental data of concentration and velocity are obtained in different flow lines along the water body.

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Potential for biogas generation from sweet potato genotypes

ARTICLES doi:10.4136/ambi-agua.2317

Received: 22 Aug. 2018; Accepted: 18 Dec. 2018

Samantha de Paula Batista^{1*}; **Edson Perez Guerra²**;
Juliano Tadeu Vilela de Resende²; **Matheus Vitor Diniz Gueri³**;
Guilherme Campos Carvalho⁴; **Jessica Naiara dos Santos Crestani²**;
Israel Felipe Lustosa da Silva²

¹Universidade Estadual do Centro-Oeste (UNICENTRO), Guarapuava, PR, Brasil
Programa de Pós-Graduação em Bioenergia. E-mail: sasah_batista@hotmail.com

²Universidade Estadual do Centro-Oeste (UNICENTRO), Guarapuava, PR, Brasil
Departamento de Agronomia. E-mail: epguerra@unicentro.br, jvresende@uol.com.br,
jessicacrestani@hotmail.com, israel.felipe30@gmail.com

³Universidade Estadual do Oeste do Paraná (UNIOESTE), Cascavel, PR, Brasil
Programa de Pós-Graduação em Bioenergia. E-mail: mgueri@hotmail.com

⁴Universidade Tecnológica Federal do Paraná (UTFPR), Guarapuava, PR, Brasil
Departamento de Engenharia Mecânica. E-mail: guicampos96@gmail.com

*Corresponding author

ABSTRACT

Biogas is a technology that enables the generation of energy through the process of anaerobic biodigestion, and sweet potato is an option as an energy production source. This research evaluated biogas production in anaerobic biodigesters and characterized the physico-chemical composition of two commercial sweet potato cultivars, BRS Cuia and BRS Rubissol, and two genotypes, Bela Vista and Laranjeiras. Variance analysis, the Tukey test and the physical-chemical characterization were completed, and the results were correlated using principal component analysis (PCA). In the analyses of tuberous root composition, significant differences between the genotypes were observed because of the influence of the absorption of moisture, decomposition, and variations in the yield and quality of roots. For the biochemical methane potential (BMP) test, the estimate of biogas production was 2,906.5 liters ha⁻¹ for the cultivar BRS Cuia, 2,712.4 liters ha⁻¹ for Laranjeiras, 2,906.5 liters ha⁻¹ for BRS Rubissol, and 398,2 liters ha⁻¹ for Bela Vista. The PCA analysis of sweet potato genotypes, physical-chemical and agronomic parameters showed all genotypes have a direct correlation with the amount of reducing sugars and humidity, with 97.77% of the data variability explained. The Laranjeiras genotype has the most significant potential to produce biogas, followed by the BRS Cuia cultivar. The results emphasize that the sweet potato crop is a good source of biomass for energy generation.

Keywords: biodigester, bioenergy, *Ipomoea batatas* L.

Potencial de geração de biogás de genótipos de batata-doce

RESUMO

O biogás é uma tecnologia que possibilita a geração de energia através do processo de biodigestão anaeróbia, sendo uma opção a produção a partir de batata-doce como fonte

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energética. A presente pesquisa teve por objetivo avaliar a produção de biogás em biodigestores anaeróbios de bancada, caracterizar os parâmetros físico-química das cultivares de batata-doce BRS Cuia e BRS Rubissol e dos genótipos Bela Vista e Laranjeiras. Foi feita análise de variância, teste Tukey e correlação com a caracterização físico-química dos genótipos por análise de componentes principais (ACP). A partir das análises da composição das raízes tuberosas, foram observadas diferenças significativas entre os genótipos, devido a influência na absorção da umidade, decomposição, ocorrendo variações na produtividade e qualidade das raízes. Para os ensaios do potencial bioquímico de metano (BMP – *biochemical methane potential*) a estimativa de produção de biogás foi de 2.906,5 L ha⁻¹ para a cultivar BRS Cuia e de 2.712,4 L ha⁻¹ para Laranjeiras, de 2906,5 L ha⁻¹ para BRS Rubissol e de 398,2 L ha⁻¹ para Bela Vista. A partir da ACP com a produção de biogás dos genótipos de batata-doce, dos parâmetros físico-químicos e agrônômicos, foi possível identificar que todos os genótipos têm correlação direta com a quantidade de açúcares redutores e umidade, com explicação de 97,77% da variabilidade dos dados, e que o genótipo Laranjeiras foi o que apresentou maior potencial significativo em produzir biogás, seguido da BRS Cuia. Diante dos resultados obtidos enfatiza-se a cultura da batata-doce como fonte de biomassa para a geração de energia.

Palavras-chave: biodigestor, bioenergia, *Ipomoea batatas* L.

1. INTRODUCTION

The sweet potato (*Ipomoea batatas* L.) is a herbaceous plant native to Central and South America. It is adapted to the tropical and subtropical climate, presenting favorable characteristics such as low production cost, resistance to pests, wide adaptation in degraded soils, prolonged harvest, being considered a rustic crop (Miranda *et al.*, 1995; Silva *et al.*, 2008; Stathers *et al.*, 2013).

Biomass is a renewable source of energy that can generate heat, electricity and biofuels (McKendry, 2002). With the expansion of clean and renewable energy, the production of biofuel from the biomass of several crops has been used, since they contain high content of organic matter from the carbohydrates (sugars and starches) stored (Brasil, 2015). Among the biomass used are: sugarcane, corn, oats, manioc, castor bean, pine, sugar beet, sunflower, potato (Silveira, 2008).

The sweet potato genotypes differ in many aspects, such as root structure, root shape, reserve root bark color, pulp color, flavor, texture, resistance to pests and yields (Borém, 2005). These characteristics are important in deciding which variety to use for trade and for other processing (Stathers *et al.*, 2013).

In the harvest period of the sweet potato, losses of tuberous root can occur in the field, in the process of washing, grading, packaging and transport, or even in market wholesale, retail and with the consumer. When the tuberous root is considerably depreciated and does not meet the aesthetic standards of market quality, such as showing small or very large deformities, part of the harvest is destined to animal feed or become waste and is sent to landfills or even left in the field (Chitarra; Chitarra, 2005). One way to take advantage of these sweet potato tailings would be to use their biomass energetically through the anaerobic process to obtain biogas (Brasil, 2015).

Sweet potatoes have high potential as an energy source in the production of ethanol and biogas. In an experiment conducted at a small industrial plant in Palmas, Tocantins, Brazil, Silva *et al.* (2018) obtained a high yield of sweet potato bioethanol of 161.4 L t⁻¹ corresponding to 10,598 L ha⁻¹, based on the productivity of 65.5 t ha⁻¹ of the evaluated cultivar. They considered the result 46% superior to the ethanol yield of sugarcane, 34% superior to that of

sugar beet and 149% superior to that of corn ethanol. For biogas production, however, research work is needed to determine the potential for energy production from the sweet potato.

The process of anaerobic bio-digestion is the biological conversion of organic matter in the absence of oxygen. There are four phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis transforming into biogas, consisting mainly of methane and carbon dioxide. This process occurs in biodigesters, structures designed and constructed from a closed chamber where the organic material is deposited and undergoes decomposition, generating the biogas (Yu; Schanbacher, 2010; Zhang *et al.*, 2018).

In Brazil the most-used models of biodigesters are the Indian, tubular and Chinese, considered the oldest of those for agricultural use. With the use of biodigesters in expansion, several models have been proposed and the technology is being adapted to the needs of the field and for sanitary treatment (Deganutti *et al.*, 2002).

In this context, biogas is a technology that enables solutions to problems such as the production of alternative energy, the safe management of human, animal, municipal and industrial waste and the control of environmental contamination (Xumeng *et al.*, 2014).

2. MATERIALS AND METHODS

The BRS Cuia and BRS Rubissol sweet potato cultivars developed by Embrapa Clima Temperado (Pelotas, RS) and the Bela Vista and Laranjeiras genotypes were selected for the experiment and come from the germplasm bank of the Horticulture Research Center.

Samples of the four sweet potato genotypes were placed in a drying oven at 60°C for approximately eight hours. Flour was obtained by grinding and sieving, and stored in polyethylene bags packed in a vacuum to avoid contamination. The characterization parameters were moisture, acidity, pH, starch, and reducing sugars for sweet potato genotypes (Cecchi, 2003; IAL, 2008).

The presence of inoculum benefits the biodigestion process of the raw material, improving the methane content in the biogas composition (Xavier *et al.*, 2010; Hassan *et al.*, 2017). Cattle manure inoculum was collected and transported in borosilicate vials and kept under refrigeration conditions at 10°C. This material presented 72.68% humidity and pH of 5.93, characterized according to AOAC (2007).

According to Azevedo (2010), it is necessary to dilute the inoculum in a solution of mineral salts in a 1:1 ratio. The mineral salt solution was prepared using 1.0 L of distilled water with 2.0 g of potassium dibasic phosphate (K_2HPO_4) 20 g of potassium phosphate monobasic (KH_2PO_4) and 3.5 g of urea (NH_2CO). After the addition of the salts, the solution was homogenized with the cattle manure inoculum and samples of the sweet potato genotypes were added. Subsequently, they were transferred to the biodigesters until the volume of 150 mL was completed for 250 mL glass vials.

2.1. Laboratory tests to obtain biogas

The anaerobic biodigesters was monitored by means of the biochemical methane potential (BMP) tests, which measure the biodegradability of organic matter through total methane production (Gueri, 2018). Nine lab bench biodigesters were used (Figure 1).

Figure 1. Bench scale anaerobic biodigesters (Personal Archive, 2016).

2.2. Preparation of biodigesters

The bench anaerobic biodigesters were assembled using BMP assays. Each biodigester was composed of 250 mL borosilicate vials, with a nylon screw cap, containing a gas outlet valve and a valve with manometer for the internal pressure monitoring (Figure 1).

Two treatments were used for the tests:

Treatment 1: Raw material + Inoculum, composed of 50 g of sweet potato flour inoculated with 20 g of bovine manure diluted in 20 g of salt solution;

Treatment 2: only inoculum in biodigester as control (white) composed of 20 g of bovine manure.

After inoculation, the moisture content obtained from the substrate with the genotypes was 83.3% for Bela Vista, 79.18% for Laranjeiras, 76.08% for BRS Cuia and 83.28% BRS Rubissol. The screw caps were fixed in the vials and a stream of nitrogen gas was circulated into the headspace of each biodigester for about four minutes to ensure anaerobiosis of the medium.

2.3. Test Monitoring BMP

The biogas volume generated was measured by the U-liquid column type manometric method, by reading of the liquid column coupled in the biodigesters.

The experiment was installed into a BOD Illumination Incubator Automatic, with lights turned off, to have constant temperature of 36°C, monitored daily; the local atmospheric pressure (National Institute of Meteorology - INMET) was monitored every six hours.

The internal pressure of the flasks was converted into volume of the generated biogas by Equations 1 and 2, for the different sweet potato genotypes evaluated and transformed to the normal conditions of temperature and pressure (CNTP) (Harries *et al.*, 2001). This procedure was performed by Alves (2008), Gueri (2018), recommended by Angelidaki *et al.* (2009) and reported by Alzate *et al.* (2012).

$$\text{Volume of biogas generated between } T + (T + 1) = \frac{[PF(\text{mbar}) \cdot VUF(L) \cdot 22,42] \cdot 1000}{[83,14 \cdot TF(K)]} \quad (1)$$

Where: T = time (days); PF (mbar) = bottle pressure in millibar; VUF (L) = useful volume of the bottle in liters; TF (K) = bottle temperature in Kelvin.

$$\text{Volume of accumulated biogas}(mL) = [\text{Generated between } T + (T + 1)] + VGA (mL) \quad (2)$$

Where: T = time (days); VGA (mL) = volume of accumulated biogas from the previous day in milliliters.

2.4. Statistical analysis

The physico-chemical analyzes and the BMP tests of the biogas production process were submitted to Analysis of Variance (ANOVA), to the Tukey test ($p < 0.05$) of the means significant differences, and the correlation with the physicochemical characterization of the genotypes by the principal components analysis (PCA). The statistical program Minitab17 was used.

3. RESULTS AND DISCUSSION

3.1. Physico-chemical characterization

The flour of sweet potato genotypes was characterized for physicochemical composition (Tables 1 and 2).

Table 1. Summary of physical-chemical variance analyzes of flour of sweet potato genotypes.

Variation of source	GL	Means Square					
		Moisture	Acidity	Starch	R.S.	pH	Ashes
Block	2	0.03331	0.00212	0.02083	0.12	0.00033	0.19341
Genotypes	3	36.8768**	0.0498**	66.6244**	2.996389**	0.0123**	0.7120*
Residue	6	0.04181	0.00203	0.1492	0.101667	0.00075	0.1149
Total	11	-	-	-	-	-	-
CV (%)		2.63	4.69	1.89	5.99	0.45	10.90

*, ** significant at the level of 5% and 1% of probability, ($p < 0.05$) and ($p < 0.01$), respectively; R.S. (Reducing Sugars); CV (coefficient of variation).

Table 2. Means of physical-chemical parameters in sweet potato genotypes.

Parameters ¹	Bela Vista	BRS Cuia	BRS Rubissol	Laranjeiras
Moisture (%)	10.62 a	3.40 c	10.60 a	6.50 b
Acidity (%)	1.09 a	1.04 a	0.84 b	0.85 b
pH	5.99 b	6.12 a	6.10 a	6.00 b
Starch (%)	53.04 b	67.60 a	37.69 c	30.30 c
Reducing sugars (%)	3.70 c	4.30 b	2.92 d	4.49 a
Total sugar	56.73 b	71.90 a	40.61 c	34.79 c
Ashes (%)	2.78 b	3.17 ab	2.70 b	3.77 a

Means followed by the same letter in row do not differ statistically from each other by the Tukey test ($p < 0.05$); ¹ n = three replicates.

According to the Technical Regulation for Setting Identity and Quality Standards for Tubers, the sweet potatoes samples were within the maximum range of 2.0% of titratable total acidity desirable (Table 2). So, when acidity is decreased, the fermentation intensity decreased and the processing time increased (Brasil, 1978).

The flour purity index is measured by the ash percentage. A high ash content will indicate more minerals present. The values obtained (Table 2) were similar to those found by Lima *et al.* (2001), with an averaged of 2.0%.

According to Alves (2008), the ideal pH for anaerobic digestion should be in the 6.3 to 7.8 range, which demonstrates greater efficiency of the methanogenic phase. Considering the pH values obtained (Table 2), it was lower than ideal, and the process could be more efficient.

The moisture obtained for the sweet potato flour (Table 2) was similar to that found by Borba *et al.* (2005), 8.7%, considering an average value of the cultivars.

Sugar levels are variable due to cultivar, growing season, climate and storage time, and because the starch over time is converted to simple sugars. The values obtained in sweet potato flour (Table 2) were similar to those of Silva *et al.* (2008), who established values of 4.8 to 7.8% without specifying the cultivar.

The starch is the main component of dry matter of the sweet potato tuberous roots (66.8 - 78.5%) followed by soluble sugars (8.2 - 15.3%) which represent 6.17 to 7.69% of the total dry matter (Kohyama; Nishinari, 1992). The values obtained in sweet potato flour (Table 2) were similar to those of Borba *et al.* (2005), who established values of 51.8% starch without specifying the cultivar.

3.2. Assay of the biochemical potential of methane (BMP)

After calculations (Equations 1 and 2), a biogas production profile graph was generated for each genotype over time. It is observed that they were very different from the inoculum used as control (Figure 2). The daily production of biogas varied for days of incubation and for sweet potato genotypes, making it necessary to check the production at each hour of the day, for five consecutive days.

Figure 2. Profile of biogas production of sweet potato genotypes every hour from first day to fifth day (2A to 2E).

From the inoculums incubation, there were different production peaks in the process of anaerobic biodigestion, as observed between the 16h-18h (Figure 2A), due to physicochemical factors such as pH, sugars and atmospheric pressure. The temperature was kept constant at $\pm 36^{\circ}\text{C}$.

However, it was necessary to monitor the biogas production test every hour as observed in Figures 2A to 2E, as the sweet potato undergoes the biodigestion process rapidly, consuming its simple sugars, since no hydrolysis was performed to test the carbohydrate reserve of the starch. This justifies the different displacement reading peaks that occurred each day. Schirmer *et al.* (2014) obtained similar results, with peak generation of biogas reached in the first five days of the experiment.

3.3. Biogas generation

The generated and accumulated average volume of biogas produced during the incubation period is shown in Table 3. The atmospheric pressure was measured every six hours during the test period. The values of the internal pressure of the bottles, by displacing the bubble in the hose column, were converted into the volume of biogas generated by means of Equations 1 and 2 and transformed to the normal conditions of temperature and pressure (CNTP).

The maximum daily rate of biogas production was reached on the first monitoring day (Figure 3A); this chart shows the daily accumulated biogas volume of the four sweet potato genotypes and for the inoculum (control).

Table 3. Biogas production of sweet potato genotypes with water column displacement measurement every hour for five days, with calculation of CNTP.

Biogas production						
Day	SDC (cm H ₂ O)	CNTP Ger. (mL)	CNTP Ac. (mL)	SDC (cm H ₂ O)	CNTP Ger. (mL)	CNTP Ac. (mL)
BRS Cuia				BRS Rubissol		
1	135.85	9.80	9.80	125.60	9.06	9.06
2	102.15	7.65	17.83	104.15	7.80	17.21
3	83.00	6.02	23.29	72.50	5.26	21.93
4	29.50	2.13	25.29	35.50	2.56	24.37
5	8.50	0.64	27.05	10.25	0.77	26.22
Bela Vista				Laranjeiras		
1	132.65	9.57	9.57	145.60	10.50	10.50
2	98.70	7.39	17.33	113.60	8.51	19.41
3	82.00	5.95	22.73	72.00	5.22	24.03
4	32.00	2.31	24.92	33.75	2.44	26.34
5	7.25	0.55	26.57	9.75	0.73	28.23
Inoculum						
1	63.50	4.58	4.58			
2	35.70	2.67	7.43			
3	23.50	1.71	8.90			
4	14.00	1.01	9.87			
5	5.30	0.40	10.70			

SDC - Sum of the displacement of the water column; CNTP Ger. - normal conditions of temperature and pressure generated; CNTP Ac.- accumulated.

Figure 3. Profile of the medium (A) and accumulated (B) biogas production of sweet potato genotypes.

The biogas generation peak of the sweet potato genotypes can also be explained by the absence of oxygen, as the circulation of the gaseous nitrogen stream in the headspace of the biodigesters provides anaerobiosis of the medium. The high generation observed and accumulated in the five days is due to the easily biodegradable substances of its composition, the simple sugars (Figure 3B).

According to Alves (2008), in the BMP assays it is justified that this rapid generation may be due to the small amount of substrate used, since 50 g of sample was used in the present experiment. It is observed that there was a correlation of the internal pressure of the flask with the atmospheric pressure, thus having different peaks of displacement in the biogas production generated. Figure 4 shows, in the first and fifth days, as an example, the values of atmospheric pressure and the normal conditions of temperature and pressure (CNTP) every six hours of evaluation for the different evaluated sweet potato genotypes.

Figure 4. Correlation of atmospheric pressure with the volume of biogas generated from sweet potato genotypes under CNTP conditions from the first day (A) to the fifth day (E).

The internal pressure of the bottles was monitored daily and converted into daily biogas generation. On the first day (Figure 4A), when the peak atm pressure was maximum, the gas production decreased. When the pressure drops, the gas increases. All cultivars and inoculum obtained the same behavior, thus observing the correlation with the volume of biogas generated.

In the second and third days (Figure 4B, 4C), this correlation was not completely observed, because the pH was reduced in the anaerobic biodigestion process, using simple sugars from the beginning of the process. However, the pH can not be monitored due to strict anaerobiosis of the flask.

On the fourth day (Figure 4D) the correlation was inverted due to the behavior of the biodigestion caused by the biochemical factors of the genotypes composition. Biodigestion and pH may have influenced. Then, on the fifth day (Figure 4E), biogas production decreased, but the highest peak volume occurred in the morning, although less than in previous days. Consequently, because the process was in the final stage, as the reaction rate was high, the experiment was shorter in time. If the starch reserve were available, the experimental time would be longer.

The atmospheric pressure played an important role in the interaction of the flask internal pressure with the volume of biogas generated. It was similar for the genotypes, except for the inoculum that practically obtained the production generated on the fifth day due to the difference in the proportion of the biodegradability.

Hansen *et al.* (2017) indicate that the determination of methane generation potential is a biological method dependent on several factors, since the inoculum used are not standardized and the matter has heterogeneous composition, which denotes the need for procedures that allow repeatability and reproducibility of the experiments.

To verify the influenced the biogas production, principal components statistical analysis (PCA) was applied, in order to determine the spatial distribution of the physicochemical analyses and of the genotypes, decomposing the original data matrix in two other matrices: the Loading with the physical-chemical analyses and the Scores with the genotypes (Table 4). For the analysis, a preliminary selection was made for the variables that showed the highest correlation with biogas generation: pH, humidity, biogas generated and reducing sugars.

Table 4. Correlation of the variables (*Loading*) and genotypes (*Scores*) with the principal components (PCA) of the physicochemical analysis of sweet potato genotypes.

Physicochemical analysis	Principal Components		
	PCA1	PCA2	PCA3
Moisture	-0.52695	0.84984	0.0096883
pH	-0.56791	-0.80817	0.15598
Reducing sugars	0.93057	-0.34601	-0.11962
Biogas	0.91307	0.34043	0.22452
Genotypes	PCA1	PCA2	PCA3
Bela Vista	-0.042424	1.2052	-0.3466
BRS Cuia	0.13833	-1.7876	-0.1517
BRS Rubissol	-1.9019	0.2032	0.24101
Laranjeiras	1.806	0.37922	0.25729

Figure 5 shows the distribution of the variables with PCA1 presenting 57.50% explanation of the variations and, in order of importance, the reducing sugars, pH and humidity. In PCA2 of 40.27%, the variables with higher loadings were moisture, pH and reducing sugars. The sum of components 1 and 2 is equivalent to 97.77%; that is, how much of the variability of the data can be explained by the PCA model.

Figure 5. Loadings (A) and Scores (B) of the principal components (PCA) of the physical-chemical variables of sweet potato.

The pH is related to the first two principal components, and has influence on the biogas production, but did not present a significant correlation, shown by the obtuse angle formed in relation to the biogas (Figure 5A). In the case of reducing sugars and moisture, there was a correlation with the biogas production, evidenced by the acute angle.

The positive signals denote a direct correlation with the component and the negative signals mean an inverse correlation. Each line corresponds to a principal component that

explains a percentage of data variation, and the selected variables relate to these principal components (Crovador, 2014).

In relation to biogas production with the variables, the Laranjeiras genotype presented most significant potential to produce biogas, followed by BRS Cuia correlated with the three variables, in an intermediate region of the graph, (Figure 5B). The Bela Vista genotype and BRS Rubissol are more correlated with moisture than sugar, and have not been efficient in producing biogas. All genotypes have direct correlation with the amount of reducing sugars and moisture.

With the set of scores and loadings, it was possible to estimate the domain of each variable in each sample. The variables that caused the most biogas generation were the reducing sugars of each genotype, followed by humidity and pH.

The BMP assays has shown the lack of compatibility of the experiments results, highlighted by Angelidaki *et al.* (2009). According to Elbeshbishy *et al.* (2012), there is no standard protocol to perform the experiment, or standardize parameters such as: equipment used, inoculum, bottle volume, pH, headspace, among other factors. Another point that makes comparisons difficult is how the results are expressed, often in different units and incubation times.

The volume of biogas produced from the four genotypes was verified in the present test. The sweet potato genotype that stood out for the agronomic, physical-chemical characteristics and the greatest biogas generation potential were BRS Cuia and Laranjeiras. The information collected, such as the agronomic characterization of the new genotypes, viability and performance in the field, were important for possible release as a new cultivar. It was also possible to demonstrate the quality of the genotypes for energy or food destination through their physicochemical characterization.

The knowledge of biogas generation potential is a key factor in the study of the technical and economic viability of the energy obtained by biodigestion. This enables exploration of an alternative energy source, aimed at producing bioenergy with low environmental impact.

4. CONCLUSION

Among the evaluated sweet potato genotypes, the cultivars BRS Cuia and genotype Laranjeiras demonstrated bioenergy potential for biogas production, with 2,906.5 liters ha⁻¹ and 2,712.4 liters ha⁻¹, respectively.

The cultivation of the genotypes in field experiments, morphological characterization and the physico-chemical composition characteristics, mainly the amount of sugars available and the phenotypic aspects of the size and shape of their tuberous roots, demonstrate the quality of the genotypes.

The proposed procedure for the production of biogas in anaerobic biodigesters, carried out with the Biochemical Potential of Methane (BMP) test, was easy to perform and an efficient analytical method to analyze the biodegradability of organic materials.

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Shifting habitat mosaic: identification and mapping

ARTICLES doi:10.4136/ambi-agua.2242

Received: 13 Mar. 2018; Accepted: 18 Dec. 2018

João Paulo Simioni^{1*} ; **Laurindo Antonio Guasselli²** ; **Tatiana Silva da Silva²**

¹Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brasil
Centro Estadual de Pesquisas em Sensoriamento Remoto e Meteorologia (CEPSRM).
E-mail: joao.delapasse@ufrgs.br

²Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brasil
Instituto de Geociências (IGeo/UFRGS).
Centro Estadual de Pesquisas em Sensoriamento Remoto e Meteorologia (CEPSRM).
E-mail: laurindo.guasselli@ufrgs.br, tatiana.silva@ufrgs.br

*Corresponding author

ABSTRACT

The Shifting Habitat Mosaic refers to temporal changes in the spatial pattern of habitats, through the flood and dry pulses. This work mapped the dynamics of a Shifting Mosaic associated with the flood and dry pulses in the Environmental Protection Area of the Banhado Grande (EPABG). The research was divided into four stages: i) acquisition of satellite images; ii) obtaining rainfall river basin data from the Gravataí River; iii) identification of the flood and dry pulses; and iv) mapping of the Shifting Mosaic in the wetlands of the EPABG. In large swaths of flooding, a shifting connectivity is established between the AUs of EPABG, providing several interactions among these wetlands, such as nutrient exchange, sediments and living organisms. It was found that in periods where the accumulation of precipitation in the last 30 days is greater than 200 mm, we have the presence of predominantly aquatic mosaic with the presence of predominantly aquatic animals and vegetation; In periods where the cumulative precipitation in the 30 days is less than 200 mm, a terrestrial mosaic predominates, with increase of ciliary vegetation.

Keywords: floodplain, flood pulses, shifting mosaic, wetlands.

Mosaico de habitat móvel: identificação e mapeamento

RESUMO

O Mosaico de Habitat Móvel (SHM) refere-se às mudanças temporais no padrão espacial dos habitats, através dos pulsos de inundação e seca. Este trabalho teve por objetivo mapear a dinâmica de um Mosaico Móvel associado aos pulsos de inundação e seca, na Área de Proteção Ambiental do Banhado Grande (APABG). A realização desta pesquisa dividiu-se em quatro etapas: i) aquisição das imagens de satélite; ii) obtenção dos dados de precipitação pluviométrica e de cota do rio Gravataí; iii) identificação dos pulsos de inundação e seca; e iv) mapeamento do Mosaico Móvel nas Áreas Úmidas da APABG. Em grandes pulsos de inundação se estabelece, no mosaico móvel, uma conectividade entre as AUs da APABG, proporcionando diversas interações entre essas AUs, tais como, troca de nutrientes, sedimentos e organismos vivos. Identificou-se que em períodos onde o acumulado de precipitação nos

últimos 30 dias é maior que 200 mm, tem-se a presença de um mosaico predominantemente aquático com a presença de peixes e macrófitas; já em períodos onde o acumulado de precipitação nos 30 dias é menor que 200 mm, predomina-se um mosaico terrestre, com aumento da vegetação ciliar.

Palavras-chave: áreas úmidas, mosaico móvel, planície de inundação, pulsos de inundação.

1. INTRODUCTION

Considered environments of high productivity and ecological value, flood plains are areas flooded by the lateral overflow of rivers or lakes, by the rainwater or groundwater (Schindler *et al.*, 2014). Due to phytosociological features and substrates, they constitute a variety of ecosystems (Haines-Young *et al.*, 2012).

The flood plains can be called the Aquatic/Terrestrial Transition Zone - ATTZ. This alternation between aquatic and terrestrial increases the decomposition of organic matter and the nutrient cycling, in addition to reducing the periods of stagnation in comparison with the conditions in permanently flooded habitats (Tracy-Smith *et al.*, 2012).

Neiff (2003) points out that the transition concepts, ecotone and wetlands have been used without considering their conceptual differences. For floodplains to be ecotones (intermediate areas of tension between two communities) the physicochemical environment should have a flotation system (climate, hydro-sedimentological) that is difficult to predict. Also, populations of narrow niches should predominate (estenotípicos) as ecotone components.

However, the floodplain areas are constant in time and their operation is predictable (Schindler *et al.*, 2014) due to the regularity of flood pulses (Junk, 2015). Therefore, environments commonly with flood pulses have no ecotone features, being characterized as macro-ecosystems that have no transitional features between terrestrial and aquatic ecosystems (Neiff, 2003).

Nevertheless, there may exist locally and continuous ecotones in some flood plains, depending on the degree of environmental interactions and the hydrological regime associated with flood pulses (Piazza, *et al.*, 2014). As a result this highly complex structure of floodplains, there is the formation of functional landscape mosaics as from the drastic seasonal changes to which the system is periodically subject (Milne and Bennett, 2007).

Stanford *et al.* (2005) deal with these mosaics as "Shifting Habitat Mosaic" (SHM) that change seasonally, determining an important spatiotemporal variability, characterized by the presence of habitats that interrelate in different degrees (Lim *et al.*, 2016).

The SHM refer to temporal changes in landscape fragments, which are presented sometimes dry, sometimes flooded, with exposed vegetation or soil. These changes seasonally create a mosaic of landscapes by natural processes such as floods, mass movements, fluvial deposits, defrosting, etc., but also by human activities such as burnings, deforestation and dams (Engle *et al.*, 2008).

In floodplains, the SHM approach recognizes that the interactions between physical and biotic processes produce constant changes of its spatial pattern through flood pulses (Wang and Finley, 2011).

The concept of flood pulse (Junk, 2005) considers the lateral exchanges between the river and its floodplain, as well as the change and exchanges between the terrestrial (dry) and aquatic (flood) phases on the same plain. Therefore, the system can incorporate and efficiently take advantage of the organic matter, which explains the richness and diversity of rivers with floodplains (Sanches *et al.*, 2012).

Studies involving SHM are characterized by a multidisciplinary approach: Wimberly (2006) deals with the dynamics of species in degraded landscapes, to understand if the

connectivity (Tischendorf and Fahrig, 2000; Luque *et al.* 2012) between landscapes is influenced by SHM; Engle *et al.* (2008) analyze the sensitivity of the invertebrate community; Armstrong and Schindler (2013) analyzed the relationship between the water temperature and the spatial distribution of the silver-Salmon (*Oncorhynchus kisutch*), address the restoration of successional dynamics of a SHM in a floodplain.

Thus, this study maps the dynamics of a SHM associated with flood pulse and drought in the connectivity area between the Wetlands of Environmental Protection Area of Banhado Grande (EPABG).

2. MATERIAL AND METHODS

2.1. Study area

The Environmental Protection Area of Banhado Grande was established by State Decree N. 38,971/1998, and its basic objectives are to protect biological diversity, to discipline the occupation process and to ensure the sustainable use of natural resources (Brenner, 2016).

Located in the Metropolitan Region of Porto Alegre (MRPA), the EPABG has an area of 136,935 ha, covering the municipalities of Santo Antonio da Patrulha, Gravataí, Viamão and Glorinha (Figure 1) (Brenner, 2016; Simioni *et al.*, 2017).

The EPABG comprises three areas of marsh, the Banhado Grande, the Banhado Chico Lomã and the Banhado dos Pachecos, which form a large composition of wetlands in the basin of the Gravataí River, defined as Banhado Grande System (BGS) (Scherer, 2014). The BGS is delimited by an altimetry quota of 20 m, and according to Accordi and Hartz (2006) it consists of a continuous area of swamps or marshes, flooded lands and rice fields.

Figure 1. Lithology of the Environmental Protection Area of Banhado Grande, RS.

According to the geological map (Figure 1), the BGS area consists of four geological compartments: lagoon plains deposits, peat deposits, alluvial deposits and floodplain deposits. The areas of marshes associated with turf deposits are found in areas where the marshes of Banhado Grande, Chico-Lomã and Pachecos are located (Scherer, 2014). In large flood pulses, a polygon associated with 11 m quota floods connects (Simioni *et al.*, 2017) the areas of Banhado Grande and Pachecos and the floodplain of the Gravataí River.

EPABG is part of the Subtropical Climate III (Rossato, 2011), with its average annual temperature ranging between 17 and 20°C and pluviometric regime between 1,700-1,800 mm per year (Alvares *et al.*, 2013). The monthly average rainfall between 1981-2010 in the INMET Meteorological Station in Porto Alegre is 112.6 mm. The months with the lowest average rainfall (dry) are April, May and December, with 86 mm, 95 mm and 100 mm, respectively (Inmet, 2016). On the other hand, the months with the highest average rainfall (floods) are June, August and September, with 135 mm, 140 mm and 139 mm, respectively.

2.2. Methodological procedures

The work was divided into four stages: i) the acquisition of satellite images; ii) obtaining the rainfall and the Gravataí River quota data compatible with the dates of the images; iii) identification of the flood pulse and drought in the polygon area concerning the quota of 11 meters; and iv) mapping of the SHM in the wetlands of APABG.

Unlike Stanford *et al.* (2005) which defined the term SHM, this work will only address the spatialization of a SHM, associated with a flood pulse (Junk, 2005) and drought, in the polygon area associated with elevation of 11 m.

2.3. Acquisition of Satellite Images

The satellite images were obtained from the USGS website (United States Geological Survey). We chose to work with images in periods of large flood pulses and drought. Six Landsat images were selected from 1983 to 2014, Table 1.

Table 1. Landsat TM images utilized.

Image Date	Satellite	Cumulative rainfall in 30 days (mm)	Quota (m)
11/24/1983	Landsat 4	186.5	2.3
09/21/1992	Landsat 4	111.1	2.8
10/21/2000	Landsat 7	250.8	4.5
04/29/2009	Landsat 5	31.0	0.9
08/31/2013	Landsat 8	247.5	5.5
10/21/2014	Landsat 8	204.3	4.6

2.4. Rainfall and River level quota

The rainfall data were obtained from the website of the Brazilian Institute of Meteorology-INMET, daily rainfall data for the 30 days prior to obtaining the satellite images.

On the Weather Forecasting and Climate Studies Center - CPTEC/INPE website, event data were obtained from El Niño and La Niña, to relate to the flood pulses and drought in the wetlands of EPABG.

For the data analysis of variations in the level of the river quota, the measurement gauge of Passo das Canoas was used as the basis, administered by the Mineral Resources Research Company (CPRM), installed in Gravataí, available in the system of the Hidroweb of the National Water Agency (ANA).

2.5. Identification of pulses flood and drought

To identify and map the flood pulses and drought (Junk *et al.*, 2012) in EPABG, the Normalized Difference Water Index - NDWI was used (Gao, 1996), using satellite images from Landsat 4, 5 TM and 7 ETM+, Equation 1:

$$NDWI = \frac{(B2-B4)}{(B2+B4)} \quad (1)$$

Where: B2 - green wavelength; and B4 - near infrared.

And to calculate the NDWI using Landsat 8 OLI images, Equation 2:

$$NDWI = \frac{(B3-B5)}{(B3+B5)} \quad (2)$$

Where: B3 - green wavelength; and B5 - near infrared.

The NDWI images (Gao, 1996) have identified the flood pulses and drought. The six images used were added through the Raster Calculator tool. The NDWI images were overlapped on the polygon referring to the limits of EPABG quantified floodplains located in the EPA. The calculation was performed using the ArcGIS Calculate Geometry tool.

2.6. Characterization of the Shifting Habitat Mosaic

The methodology of Kleindl *et al.* (2015) was adapted to characterize the SHM in the wetlands of the EPABG. The focus of this work is the river-floodplain system and wetlands dynamics in the corridor between two major wetland areas, Banhado Grande and Pachecos, considering an 11-meter quota. Differently from Kleindl *et al.* (2015), this study analyzed these areas using only data derived from remote sensing, measured precipitation data, and quota level data of Gravataí River, to understand and characterize the dynamics of an SHM in the EPABG.

The study of Kleindl *et al.* (2015) consisted in analyzing the expansion of a floodplain due the forest fires, using the concept of SHM. The authors worked with remote sensing images, rainfall data, outflow data (empirical and shaped), and data on the extension and frequency of forest fires of the past, to examine the relations between hydrology, fire, land use, as well as geomorphological and dynamic position of the SHM. In this way, the authors had concluded that the current composition of the SHM in the area of the floodplain of the River Flathead resulted from diverse interactions between flooding events, erosive processes, and forest fires, influenced by the geomorphic composition of the floodplain.

3. RESULTS

The average annual growth (1973-2014) of the river-level maximum quota, as registered by the measurement gauge of Passo das Canoas, in the Gravataí River (Figure 2), shows a range between 1.25 m to 2.70 m over the 41 years of analysis.

Figure 2. Monthly variation (1980-2014) of the maximum quota as measured by the measurement gauge of Passo das Canoas, Gravataí River.

According to Brazil (Inmet, 2016), the years of occurrence of the El Niño classified as “strong” were 1983, 1986-1988, 1991-1992, 1997-1998, 2002 and 2009-2010. In these years, water levels were higher when compared to other years analysed. In all the years of occurrence of El Niño considered strong by the CPTEC/INPE, the average fluctuation of water levels was higher than 2.00 m.

On the other hand, the La Niña events, considered strong by the CPTEC/INPE, occurred in the years of 1988-1990 and 2006-2008. These years were those which had the lowest maximum monthly values of water level elevation in the Gravataí River (1.25 to 1.50m) (Inmet, 2016).

Figure 3 shows the rainfall and the Gravataí River quota for the 30 days preceding the date of satellite images. The years 1983 (Figure 3a) and 2009 (Figure 3d) were characterized by the strong influence of the La Niña (Inmet, 2016). Rainfall totals in the official INMET Meteorological Station in Porto Alegre were 186.5 mm and 31.0 mm, respectively, and the quotas were 2.3 m and 0.9 m. In Figs. 3b, 3c, 3e and 3f, the events of the El Niño are highlighted. In Figure 3b, it rained 101.1 mm, and the Gravataí River quota reached its maximum value for the period (4.25 m).

Figure 3. Relationship between the accumulated rain and the Gravataí River quota.

Figure 3c shows the highest rainfall, detected of 250.8mm, distributed in 14 days. However, since the maximum rainfall peak occurred 20 days before obtaining the image, this period did not present the highest Gravataí River quota, which marked 4.5m.

According to Colombo and Pessoa (2013), the year of 2009 was characterized by a severe drought in Rio Grande do Sul, causing several impacts, especially on the economy of the state. The year of 2009, Figure 3d, was the one with the lowest rainfall, when it rained a total of 31.0 mm. As a result, this period also had the lowest quota in the level of the Gravataí River (0.9 m).

In Figure 3e, the rainfall value recorded between August 25th and 26th, 2013 is highlighted, 72 mm. This amount resulted in the highest Gravataí River quota among the analyzed dates (5.5 m).

Similar to the date of 2013, Figure 3f also presents only one maximum rainfall peak, verified on 17 October 2014, when it rained 80 mm in 24 hours. This peak increased the Gravataí River quota in more than 2 m. The quota value was 2.5 m, and at the time of image acquisition the registered quota was 4.6 m.

Figure 4 refers to the flooded area for each Landsat image. The accumulated precipitation was 186.5 mm, and flooded area in the polygon referring to the quota of 11 meters was 367.4 ha, corresponding to 3.8% polygonal area (Figure 4a). This area is located mostly in the floodplain of the Gravataí River, in the area popularly known as Anastacia Lagoon. On that date, the two dams did not exist, located within and south of the polygon related to the 11-meter quota. The Gravataí River quota level on the date of the image was 2.3m.

In Figure 4b, the two dams already existed. The accumulated rainfall was 111.1 mm and the flooded area was 322.5 ha. The quota of the Gravataí River on the date of the image was 2.8 m. Figure 4c presents, along with Figure 4e, larger amounts of accumulated precipitation, 250.8 mm and 247.5 mm, respectively. Consequently, these figures show the biggest flooded areas among the analyzed images, 7577.5 ha, and in Figure 4c and 7853.9 ha in Figure 4e. The quota of the Gravataí River on the dates of the images was 4.5m and 5.5m, respectively.

Figure 4d shows a period of drought, the flooded area was 67.5 ha. The quota of the Gravataí River in Figure 4d was 0.9 m. In Figure 4f, the flooded area is 3,796 ha and the accumulated rainfall 204.4 mm. The quota of the Gravataí River on this date was 4.6 meters.

It is understood, therefore, that when the accumulated precipitations are greater than 200 mm, during 30 days, SHM are formed in the EPABG wetlands. Figure 5 shows a SHM in the EPABG wetlands. The rectified stretch of the Gravataí River is highlighted, near the area known as Anastacia Lagoon. In this area, the river flood pulses create a mosaic that seasonally alternates between aquatic and land, being responsible for the habitat heterogeneity (Junk *et al.*, 1989). In Figure 5a, the drought period, there is a predominance of a terrestrial environment. The flooded area is restricted to the main channel (rectified) and the intricacies that, although abandoned, still remain active in Anastacia Lagoon. This lagoon is erroneously named as such, since this environment is just an old meander of the Gravataí River, which was closed by rice farmers to stop the water so that it could be used for crop irrigation.

Figure 5b shows a flood period in the Gravataí River. It is noticeable that the meanders are completely flooded, forming a predominantly aquatic SHM that remains until the recession of the waters, which lasts from 15 to 30 days.

Figure 4. Flood area for the studied images.

Figure 5. Shifting Mosaic in a rectified section of the Gravataí River: (a) dry phase, 04/30/2009; and (b) flooded phase, 08/11/2011.

Source: Google Earth Pro (2016).

4. DISCUSSION

The quota of the Gravataí River is considered normal between 2.70 to 3.00 m, on the measurement gauge of Passo das Canoas (Relf, 2015). From the quota of 4.5 m, the Gravataí River begins to overflow, and riverine families need to be relocated (Relf, 2015). Still, in the 1970s, part of the Gravataí River was rerouted with the intention of draining the wetlands, seeking to expand the areas intended for rice cultivation (Brenner, 2016).

However, despite the correction, the area near the Anastacia Lagoon remains with its old meanders, being horizontally connected to the river, in large flood pulses. In this area, Brenner (2016) has proposed measures to return the meanders to a more-natural state, using bioengineering techniques. This process would reduce the speed of the water runoff, since the channel would go from rectilinear to meandering, reducing erosion problems and drainage of wetlands.

Regarding the discharge, according to the Institute of Hydraulic Research (IPH, 2002), the minimum discharge of 7 days to 10 years of TR ($Q_{7.10}$) in the post of Passo das Canoas (located at the boundary between the low and medium Gravataí) is $6.02 \text{ m}^3/\text{s}$, which corresponds to 25% of the long-period average. For the same post, the discharge full of TR of 10 years and 100 years are respectively $206 \text{ m}^3/\text{s}$ and $311 \text{ m}^3/\text{s}$.

Simioni *et al.* (2017) point out that the DNOS report in 1985, analysing of the number of flow rates from 1940 to 1981, states that the course correction of the channel did not cause changes in the water regime, nor an increase in flow of the Gravataí River.

However, according to the authors, through the analysis of the flow in the period after 1981, the increase of flow in the post-correction period is evident. The maximum flow rates (Rio Grande do Sul, 2011) increased due to the ease of flow provided by the correction. The river course, when meandering, favored the decrease in the flow speed and, with the correction, the rectilinear course favored the increased flow speed (Brenner, 2016). In Figure 5, the images allow easy identification of the SHM providing a concise overview of rivers, even for non-scientists (Stanford *et al.*, 2005).

Thus, when analyzing the maps of variation of the SHM, it is possible to realize that the fluvial action of floods and temporal migration of the channel, especially in Anastacia Lagoon, create different SHM through the riverside landscape (Stanford *et al.* 2005).

Thus, ecotones (present in the floodplain) (Neiff, 2003), connectivity (between Banhados Grande, Pachecos and the floodplain of the Gravataí River) (Vanderhoof *et al.*, 2016; Simioni *et al.*, 2017) and succession play important roles in the structuring of spatiotemporal heterogeneity that leads to high biodiversity that characterizes the Gravataí River (Rio Grande do Sul, 2011; Cardoso, 2016).

In periods of great flood pulses, this connectivity established between the wetlands of EPABG, especially in the corridor that connects the Banhado Grande, Pachecos and floodplain of the Gravataí River, provides several interactions between wetlands, such as the exchange of nutrients, sediments and organisms (Vanderhoof *et al.* 2016).

Given this ecological dynamism, Wang and Finley (2011) pointed out how critical this is to managers of Conservation Units (CU's), because they often choose to preserve the shape of the landscape in its present condition, or lead to landscape back to some historical appearance based on studies of changes in the landscape. The interference in natural patterns alters or disturbs the ecological flows. So, if the goal is to maintain the natural ecosystem processes, it should be accepted that the configuration of the landscape is changing over time (Wang and Finley, 2011).

Temporal shifts in the mosaic associated with temporal changes in the main channel and in the floodplains create fragments with different environmental conditions (Lima *et al.*, 2013), the SHM. These conditions are directly related to the dynamics of the EPABG wetlands, which are presented sometimes dry, sometimes flooded, forming the SHM, with the growth of the vegetation and the presence of land animals in dry periods, and the presence of fish and aquatic plants in flood periods (Cardoso, 2016).

Furthermore, Tittensor (2013) states that the result of SHM in flood plains are considered productivity hotspots because they provide the proliferation of algae during the flood and enrichment of nutrients in the soil after drought.

Additionally, according to Junk *et al.* (2012), the wetlands along small rivers, small depressions, such as wetlands of the EPABG, have an unpredictability in the occurrence of flood pulses (Neiff, 2003), and its amplitude is variable. Given this, the characterization of the SHM in the wetlands of EPABG is essential, as it allows us to understand the dynamics and compartments subject to the formation of this mosaic.

5. CONCLUSION

The satellite images allowed concise mapping of the SHM in EPABG. It was found that in periods where the cumulative rainfall in the past 30 days is greater than 200 mm, there is the presence of a predominantly aquatic mosaic on the polygon referring to the 11 m quota, with the presence of amphibians, reptiles, fish and birds that depend on them for feeding.

On the other hand, in periods where the cumulative rainfall is less than 200 mm, a terrestrial mosaic predominates, with the increase of riparian vegetation and animals, especially amphibians and mammals. Regarding the methodology, its adaptation allowed the analysis and spatial distribution of the SHM throughout the polygon area concerning the 11 m quota, not restricting it only to the temporal displacement of the meanders.

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Rainfall trends for the State of Paraná: present and future climate

ARTICLES doi:10.4136/ambi-agua.2258

Received: 21 Mar. 2018; Accepted: 18 Dec. 2018

Luciana Espindula de Quadros^{1*} ; **Eloy Lemos de Mello²** ;
Benedito Martins Gomes² ; **Fernanda Cristina Araujo²**

¹Instituto Federal do Paraná (IFPR), Foz do Iguaçu, PR, Brasil

E-mail: luciana.quadros@ifpr.edu.br

²Universidade Estadual do Oeste do Paraná (UNIOESTE), Cascavel, PR, Brasil

E-mail: eloymello@gmail.com, benedito.gomes@unioeste.br, fer.crisaraujo@yahoo.com.br

*Corresponding author

ABSTRACT

This paper analyzes the variability and the precipitation trend of the State of Paraná, in Brazil. For that, monthly precipitation data belonging to 24 precipitation stations in a 30-year period (1980-2010) were analyzed and they were compared with projections of precipitation for the years 2016-2050. These data were simulated by Eta/Miroc5 for RCP 4.5 (Representative Concentration Pathways) from the Center for Weather Forecasting and Climate Studies CPTEC/INPE and the historical data of precipitation were taken from National Water Agency (ANA). The Mann-Kendall non-parametric test and the Sen's slope estimator were applied to detect trends and magnitudes, respectively. The Mann-Whitney test was used to compare the median of the historical series (1980-2010) with the simulated series (2016-2050) and the comparison of the means between the two series was performed by Test *t*. The results draw attention to the great variability and significant changes in the monthly average rainfall that may occur, if the climate change scenarios that were considered become a reality in the near future.

Keywords: climate changes, climate models, precipitation variability.

Tendência da precipitação no Estado do Paraná: clima presente e futuro

RESUMO

O objetivo deste artigo é analisar a variabilidade e a tendência de precipitação do Estado do Paraná, Brasil. Para isso, foram analisados os dados de precipitação mensal pertencentes a 24 estações de precipitação pluviométrica em um período de 30 anos (1980-2010) e foi comparado com as projeções futuras sobre a precipitação para os anos de 2016-2050. Estes dados foram simulados por Eta/Miroc5 para RCP 4.5 (Caminhos Representativos de Concentrações) proveniente do Centro de Previsão de Tempo e Estudos Climáticos CPTEC/INPE e os dados históricos de precipitação utilizados foram da Agência Nacional de Águas (ANA). O teste não-paramétrico de Mann-Kendall e o estimador de Sen foram aplicados para detectar tendências e sua magnitude, respectivamente. O teste de Mann-Whitney foi usado para comparar a mediana da série histórica (1980-2010) com a mediana da série simulada (2016-2050) e a comparação das médias entre as duas séries foram realizadas pelo Test *t*. Os resultados chamam atenção para a grande variabilidade e mudanças significativas na

precipitação média mensal que poderá ocorrer, se os cenários de mudanças climáticas que foram consideradas se tornarem uma realidade no futuro próximo.

Palavras-chave: modelos climáticos, mudanças climáticas, variabilidade de precipitação.

1. INTRODUCTION

Among the many effects that climate variability could be responsible for, a major concern is the imminence of a possible increase in the occurrence of extreme events around the globe, which could directly affect the human population and other living organisms. Several studies and investigations have been conducted in order to identify which elements are causing such impacts and what are their intensities and frequencies of occurrence (Zandonadi and Acquattro, 2016).

According to the Brazil Panel on Climate Change (PBMC, 2013), some regions of Brazil may experience changes in temperature and rainfall with global warming. Intensifications of severe events should occur, causing severe impacts in cities and areas vulnerable to climate change. The possible impacts of these changes will occur at different scales, according to the specific characteristics of each region of Brazil. It is necessary to know and map the vulnerabilities of Brazilian regions to identify, propose and implement adaptation measures.

Among all the variables, the precipitation is one of the most important meteorological variables which can impact the occurrence of drought or flood. Analysis of precipitation data yields relevant information which can be used to improve water management strategies, protect the environment, agricultural production planning or impact economic development of a certain region (Gocic and Trajkovic, 2013).

In recent years, scientists worldwide have compared and analyzed precipitation trends, but precipitation isn't the only focus; other meteorological parameters have also been studied for adaptation and mitigation strategies for potential future changes. The Global Climate Models (GCMs) and dynamical downscaling using Regional Climate Models (RCMs) are the main tools of the analyses aimed at assessing what climate we are likely to have in the near- and not-so-near future (Marengo *et al.*, 2012). According to Adam and Collischonn (2013), the GCMs take into account the behavior of climate compartments (atmosphere, oceans, vegetation, soils, etc.) and their interactions in a quantitative model (numerical), allowing the simulation of probable climate evolution projections for various scenarios of emissions of greenhouse gases. These simulations have resulted in four pathways being adopted by the IPCC, denominated "Representative Concentration Pathways" (RCPs). The four RCPs included one mitigation scenario leading to a very high baseline emission scenarios (RCP 8.5), two medium stabilization scenarios (RCP 4.5/RCP 6.0) and one very low forcing level (RCP 2.6) (Vuuren *et al.*, 2011).

The use of RCMs also contributed to information enhancement, because on a regional scale the information is more accurate and the computational advancement allowed the inclusion of more components and processes in the simulations and spatial resolution of the models, thus increasing their complexity (Marengo *et al.*, 2009). In South America, the Eta Model has been used to provide time forecasts, because this model is able to produce satisfactory results in regions that contain sharp orography, such as the Andean Mountains (Chou *et al.*, 2011).

Singh and Goyal (2016) analyzed the spatial and temporal variability of precipitation lapse rate and of precipitation extreme indices of the Teesta River catchment, which corresponds to north Sikkim eastern Himalayas. For this, they used series durations observed (1980–2005) and simulated precipitation data sets (2006–2100) simulated by CMIP5 ESM-2 M Model (Coupled Model Intercomparison Project Phase 5 Earth System Model 2), employing three different radiative forcing scenarios Representative Concentration Pathways (RCP).

In Korea, Kim *et al.* (2016) researched heat waves associated with climate change using a series of observational data from 1994 to 2012 to deduce the causal factors that affect the number of deaths from heat disorders, because the greater concern is that the steady increase in death rate is expected to be intercepted by more severe events in the future compared to the present period. To simulate future changes in heat wave incidences in Korea for the period from 2013 to 2060, they used data simulated using the Hadley Centre Global Environmental for two RCP scenarios: RCP 4.5 and RCP 8.5.

In the southeast Brazil, a study was done on possible changes in air temperature and the effect of this on coffee beverage quality. Based on climate projections using the Eta/HadCM3 for the period of 2011 to 2100, the simulation of future changes was possible. The effects of this process on coffee beverage quality were simulated and the results indicated that, in the case of an occurrence of A1B emission scenario, the coffee beverage quality could be affected in this region due to the fact that the flavor may become stronger and unpleasant caused by rising air temperatures (Giarolla *et al.*, 2012).

Therefore, this study sought to use simulations to understand changing trends in different contexts. This paper analyzes the variability and the precipitation trend of the observed precipitation data (1980-2010) and simulated data by Eta/Miroc5 (2016-2050) for RCP 4.5 of the State of Paraná, in Brazil.

2. MATERIAL AND METHODS

Monthly precipitation series from 1980 to 2010 from the National Water Agency (ANA) database from 24 precipitation stations in the State of Paraná were used. Figures 1 and 2 represent the spatial distribution of the stations. Data gaps were filled using values from the nearest neighboring observatories. To apply this method, two criteria were established: 1) the correlation (Pearson's r) between the monthly precipitation series from both observatories was required to be higher than 0.7 (Barbosa *et al.*, 2005; Pruski *et al.*, 2004); and 2) the nearest stations were considered, with a maximum distance of 100 km, taking into consideration that Baba *et al.* (2014) states that the nearest stations are more likely to have similar behaviors with respect to the treated variables (Table 1).

Figure 1. Spatial distribution of the 24 precipitation stations and grid of Eta/MIROC5 model with 824 pixels covering the State of Paraná.

Figure 2. Spatial distribution of the 24 precipitation stations in the State of Paraná and the simulated data set simulated with Eta/MIROC.

In order to detect trends, the Mann-Kendall non-parametric statistical was used in the precipitation series. The series of 1980 to 2010 were put together with the series 2016 to 2050, considering a single series at each point studied. For this, the test statistic Z was used (Equations 1, 2, 3 and 4):

$$Z = \begin{cases} \frac{(S-1)}{\sqrt{\text{var}(S)}} & \text{if } S > 0 \\ 0 & \text{if } S = 0 \\ \frac{(S+1)}{\sqrt{\text{var}(S)}} & \text{if } S < 0 \end{cases} \quad (1)$$

where:

$$S = \sum_{i=1}^{n-1} \sum_{j=i+1}^n \text{sgn}(x_j - x_i) \quad (2)$$

$$\text{sgn}(x_j - x_i) = \begin{cases} +1, & \text{if } x_j > x_i \\ 0, & \text{if } x_j = x_i \\ -1, & \text{if } x_j < x_i \end{cases} \quad (3)$$

$$\text{var}(S) = \frac{n(n-1)(2n+5) - \sum_{i=1}^m t_i(t_i-1)(2t_i+5)}{18} \quad (4)$$

Where $\text{sgn}(x_j - x_i)$ is the sign function, n is the number of data points, m is the number of tied groups and t_i denotes the number of observations of the i -th tied group. A tied group is a set of sample data having the same value.

Positive values of Z show increasing trends while negative Z values indicate decreasing trends. For this study a 5% significance level was considered.

Table 1. The information of precipitation stations and simulated data set by RCP scenario 4.5 Miroc5.

Nº	Stations	Historical data			Simulated data set		Distance between them (km)
		Latitude	Longitude	Elevation	Latitude	Longitude	
1	2454018	-24.9061	-54.2014	243	-25.000	-54.2	10.41
2	2653016	-26.0644	-53.3622	557	-26.000	-53.4	8.08
3	2453056	-24.9628	-53.2439	697	-25.000	-53.2	6.05
4	2453008	-24.0147	-53.4397	427	-23.999	-53.4	4.39
5	2353010	-23.0817	-53.4811	349	-22.999	-53.4	12.37
6	2552044	-25.9833	-52.5667	700	-26.000	-52.6	3.81
7	2452019	-24.8858	-52.4739	741	-24.800	-52.4	12.06
8	2452029	-24.0906	-52.6214	582	-23.999	-52.6	10.37
9	2352052	-23.0831	-52.6667	450	-22.999	-52.6	11.55
10	2551019	-25.85	-51.7667	1245	-25.800	-51.8	6.46
11	2451010	-24.9333	-51.8833	900	-25.000	-51.8	11.19
12	2451049	-24.1144	-51.7342	618	-24.199	-51.8	11.5
13	2351065	-23.0403	-51.8056	485	-22.999	-51.8	4.6
14	2651005	-26.0306	-51.1419	840	-26.000	-51.199	6.64
15	2450049	-24.9575	-50.8917	743	-25.000	-50.799	10.47
16	2351041	-23.9831	-51.0831	1011	-23.999	-50.999	8.73
17	2251039	-22.8517	-51.0319	370	-22.799	-50.999	6.47
18	2650005	-26.0833	-50.3167	770	-26.000	-50.399	12.36
19	2450021	-24.9831	-50.2667	950	-25.000	-50.199	7.08
20	2350041	-23.9167	-50.25	600	-23.999	-50.199	10.48
21	2250033	-22.9653	-50.2661	423	-22.999	-50.199	7.82
22	2549003	-25.95	-49.3931	810	-26.000	-49.399	5.57
23	2449006	-24.8	-49.2833	270	-24.800	-49.199	8.52
24	2548040	-25.0833	-48.8333	670	-25.000	-48.799	9.86

The Mann-Kendall test allows us to detect statistically significant trends, but does not provide estimates of the steepness of the trend slope. For this reason, the application was complemented by Sen's slope estimator. It is defined as (Equation 5):

$$B = Md\left(\frac{x_i - x_j}{t_i - t_j}\right) \quad (5)$$

Where Md is the value of median and x_i and x_j are data values at times t_i and t_j ($i > j$), respectively.

The Mann-Kendall statistical test and Sen's slope estimator have often been used to detect trend in hydro-meteorological time series. (Gocic and Trajkovic, 2013; Santos and Frago, 2013; Yürekli, 2015).

To determine if there are any statistically significant differences between the medians, the Mann-Whitney test was applied; this the series of 1980 to 2010 was compared with the series 2016 to 2050. The Mann-Whitney test statistics are described as (Equation. 6):

$$Z = \frac{w - \frac{n \cdot m}{2}}{\sqrt{\frac{n \cdot m (n + m + 1)}{12}}} \quad (6)$$

Where n is the historic size series, m is the simulated size series, w is defined as the smaller value of w_1 and w_2 , where (Equations 7 and 8):

$$w_1 = r_1 - \frac{n(n+1)}{2} \quad (7)$$

$$w_2 = r_2 - \frac{m(m+1)}{2} \quad (8)$$

To calculate r_1 and r_2 , both groups (historic series and simulated series) were put in a single set. Afterwards, numeric ranks were assigned to all the observations. In case of equal numbers, an average of the ranking was made to find out their position. Then, the historical and simulated series were separated again, r_1 is the sum of the ranks of the historic series and r_2 is the sum of the ranks of the simulated series.

The null hypothesis is stated as the median of the historic series equals the median of the simulated series. If the p-value were less than or equal to the significance level of 0.05, the decision will be to reject the null hypothesis. It must be concluded that the difference between the population medians is statistically significant.

The 2-Sample Test t was used to compare the precipitation averages between two independent groups, the historical data and simulated data, and to determine if there is a significant difference between them. The null hypothesis is the difference between the population means ($\mu_1 - \mu_2$) equals zero. Rejecting the null hypothesis, it can be concluded that the difference between the population means that it is not equal to, or greater than, or less than the reference value chosen in case zero. The Test t (Equation 9):

$$t = \frac{\mu_A - \mu_B}{\sqrt{S^2 \left(\frac{1}{n_A} + \frac{1}{n_B} \right)}} \text{ where } S^2 = \frac{(n_A - 1)S_A^2 + (n_B - 1)S_B^2}{n_A + n_B - 2} \quad (9)$$

In which: S_A^2 and S_B^2 are the variances of precipitation values of historical and simulated data; μ_A and μ_B sample means of precipitation values of historical and simulated data, n_A and n_B is the length of the series, respectively. If the variances are equal, S^2 will correspond to the variance of both samples.

3. RESULTS AND DISCUSSION

For the period 1980 to 2010 and 2016 to 2050, monthly trends of precipitation were obtained by the Mann-Kendall test, the Sen's slope estimator, the Mann-Whitney Test and the Test t and the results are given in Table 2.

According the results of the Mann Kendall test, there is a significant trend in monthly precipitation series and this was detected on seventeen points of the twenty-four points at the 5% significance level, while other points had no significant trends.

The monthly precipitation at fifteen points over the State of Paraná tends to increase until 2050 and two points tend to decrease. The points with increased trend are 6, 7, 9, 10, 12-15, 17-21, 23 and 24. The points with decreased trend are point 2 and point 11 (Table 2).

From 1980 to 2050, the monthly rainfall has been declining at the rate of 0.0605 mm month⁻¹ at point 2 and 0.0369 mm month⁻¹ at point 11. The points that have an increasing trend, the rates of raising are 0.0967, 0.1307, 0.0479, 0.1273, 0.1642, 0.0515, 0.1186, 0.1641, 0.0018, 0.1612, 0.1372, 0.0443, 0.0319, 0.0428, 0.0192, 0.1896 and 0.0794 mm month⁻¹, respectively in points 6, 7, 9, 10, 12, 13, 14, 15, 17, 18, 19, 20, 21, 23 and 24.

Still in Table 2, note the results of the Mann-Whitney test (nonparametric) and t tests (parametric). The test results relate to the use of the statistics at each point comparing the period of 1980 to 2010 and 2016 to 2050. It is observed that, even as with the Mann-Kendall test, seventeen points of the twenty-four points show a trend with a significance level of 5%, meaning that there is a change in the trend of monthly precipitation in the State of Paraná. Only at point 20 does the Mann-Whitney test differ from the other tests, but at the other points, the Mann-Whitney test and Test t confirm the result of the Mann-Kendall test.

Table 2. Results of statistical tests trend of rainfall in the State of Paraná.

Mann-Kendall			Sen's slope		Mann-Whitney		Test <i>t</i>		
	<i>p-value</i> increasing	<i>p-value</i> decreasing	<i>mm</i> <i>month</i> ⁻¹		Median	<i>p-value</i>	Mean	Increment	<i>p-value</i>
1	0.501	0.498	0.0000	Hist1	130.20		142.8		
				Fut 1	121.90	0.992	143.4	+0.42%	0.930
2	0.999	0.000*	-0.0605	Hist2	160.80		177		
				Fut 2	120.77	0.000*	142.2	-19.66%	0.000*
3	0.083	0.917	0.0218	Hist3	153.10		168		
				Fut 3	154.92	0.204	178	+5.95%	0.195
4	0.243	0.757	0.0089	Hist4	122.65		136.6		
				Fut 4	121.91	0.584	140.1	+2.56%	0.590
5	0.153	0.846	0.0137	Hist5	115.00		136.8		
				Fut 5	121.00	0.256	143.9	+5.19%	0.295
6	0.000*	1.000	0.0967	Hist6	153.15		169		
				Fut 6	207.08	0.000*	227	+34.32%	0.000*
7	0.000*	1.000	0.1307	Hist7	138.70		157		
				Fut 7	204.38	0.000*	230	+46.49%	0.000*
8	0.399	0.600	0.0031	Hist8	122.30		138.9		
				Fut 8	121.37	0.963	140.3	+1.01%	0.821
9	0.000*	0.999	0.0479	Hist9	100.55		116.1		
				Fut 9	121.77	0.000*	141.3	+21.7%	0.000*
10	0.000*	1.000	0.1273	Hist10	167.35		180		
				Fut 10	229.32	0.000*	251	+39.44%	0.000*
11	0.995	0.004*	-0.0369	Hist11	144.70		164.7		
				Fut 11	122.00	0.001*	144.1	-12.51%	0.003*
12	0.000*	1.000	0.1642	Hist12	119.75		131.5		
				Fut 12	197.46	0.000*	226	+71.86%	0.000*
13	0.000*	0.999	0.0515	Hist13	103.10		120.4		
				Fut 13	128.94	0.000*	148.6	+23.42%	0.000*
14	0.000*	1.000	0.1186	Hist14	134.15		148.8		
				Fut 14	201.88	0.000*	218	+46.5%	0.000*
15	0.000*	1.000	0.1641	Hist15	122.10		139.5		
				Fut 15	212.52	0.000*	235	+68.46%	0.000*
16	0.451	0.549	0.0018	Hist16	133.20		151		
				Fut 16	121.85	0.508	147	-2.65%	0.551
17	0.000*	1.000	0.1612	Hist17	93.55		114.5		
				Fut 17	183.20	0.000*	206	+79.91%	0.000*
18	0.000*	1.000	0.1372	Hist18	112.65		128.4		
				Fut 18	193.35	0.000*	202	+57.32%	0.000*
19	0.000*	0.999	0.0443	Hist19	110.65		123.5		
				Fut 19	121.61	0.011*	142.6	+15.46%	0.003*
20	0.009*	0.991	0.0319	Hist20	112.70		129.1		
				Fut 20	119.89	0.063	142.8	+10.61%	0.043*
21	0.000*	0.999	0.0428	Hist21	100.80		116.9		
				Fut 21	121.83	0.001*	139.3	+19.16%	0.000*
22	0.064	0.935	0.0192	Hist22	120.50		129.4		
				Fut 22	121.38	0.514	137.9	+6.57%	0.164
23	0.000*	1.000	0.1896	Hist23	101.15		113.8		
				Fut 23	197.23	0.000*	216	+89.81%	0.000*
24	0.000*	1.000	0.0794	Hist24	115.70		129.0		
				Fut 24	152.82	0.000*	164.3	+27.36%	0.000*

* Statistically significant trends at the 5% significance level.

A study by Cera and Ferraz (2015) analyzed precipitation trends in Rio Grande do Sul for three sets of data, with a comparison of the observed precipitation data (1982-2006) and simulated data by RegCM3 Regional Model (2070-2086) also showed an increasing trend for that State. They applied the Run test and Pettit test to certify the homogeneity of data for the series 1982 to 2006; but the tests gave different results and the Mann-Kendall test was used and this confirmed the Pettit test, indicating an increased trend of precipitation at a significant level of 99% in extreme north and central areas of the State of Rio Grande do Sul. The same study also analyzed the trend for future climate and the increase in precipitation trend at a 90% level of significance in the western, southern and central state was observed.

Other research similar to the study above was that of Doyle *et al.* (2012) in the La Plata Basin (LPB), where the northern part of this basin includes the State of Paraná. They employed the PRECIS regional climate modeling system for the annual rainfall and used precipitation data in the recent past (1961–1990) and in a future (2071–2100) climate at 5 and 10% confidence levels and all stations showed positive trends with the values exceeding 8 mm yr⁻¹ over southern Brazil. For that same location, Marengo *et al.* (2012) made use of the Eta/HadCM3 Model configured with a 40-km grid size and was run over 1961 to 1990 to represent baseline climate, and 2011 to 2100 to simulate possible future changes. They detected a rainfall increase in the South East of South America (by about 30–50%).

According to PBMC (2013), in southern Brazil and northern Argentina, trends for increased rainfall and river flows have been observed since the mid-twentieth century, with Paraná and La Plata Rivers showing a downward trend from 1901 to 1970 and a systematic increase in flows from the early 1970s to the present. It is also claimed that Southern region disasters such as landslides, death by drowning and building collapses may be more frequent. Agriculture and livestock in the region are vulnerable to climate change. There are numerous records of the El Niño Southern Oscillation (ENSO) phenomenon, which accentuates the conditioning/adversity features of the climate on agricultural production, determining production records or widespread losses. It should be added to the weather condition already affected by the ENSO, records of air temperature increase in various municipalities of Rio Grande do Sul, Santa Catarina and Paraná, which inevitably influence not only the agriculture and food security, but environmental conditions such as the hydrologic cycle and population health. Increases in rainfall and river flow, despite the uncertainties, are likely to intensify, as projected by the IPCC scenarios (IPCC, 2014). Temperatures will follow the pattern of increase in average values, with reduced episodes of frosts and cold days.

The location of points and the direction of trends for monthly precipitation data series during the period 1980 to 2050 are shown in Figure 3. Significant increasing trends were found in almost all the Mesoregions for the State of Paraná, except in West and West Central, where there wasn't any trend. In the South West and South Central, two points were detected for decreasing trend, but increasing trends also were found. This result is similar to that obtained by Zandonadi and Acquaotta (2016) for Paraná State: both results exhibited an increasing trend in the region of Curitiba (located in Metropolitan Curitiba), Castro (located in East Central), Irati (located in South East), Maringá and Londrina (both located in North Central), but differ in Campo Mourão region (located in West Central), where this study didn't show a trend and the other showed decreased trend.

Figure 3. Spatial distribution of precipitation stations with increasing, decreasing and no trends during the period 1980 to 2050.

The Figure 4 shows the monthly precipitation of seventeen points where there is trend increasing and decreasing the values. A large variability in monthly precipitation accumulation in graphics is observed, for instance, in Figure 4j, where the maximum value of monthly accumulation reaches approximately 1000 mm, whereas the graphic of Figure 4o) reaches 500 mm/month⁻¹. The red line confirms the trend displayed in Table 2 and adrees with Grimm *et al.* (2000), that say that in South Brazil there are seasonal characteristics of rainfall that can change when there is the El Niño/La Niña, causing extreme precipitation events as excess or deficit.

Observe the high variability between each month in Figure 4. In Figure 4b), the average of the historical series is 169 mm month⁻¹ and the mean in the simulated series is 227 mm month⁻¹. This represents an increase of 34.32%. In the time series of graphic Figure 4k), the means are 114.5 and 206 mm month⁻¹, an increase of 79.91%. The same happens in the graphic of Figure 4l), a rain increase of 57.32%. According to Table 2, the maximum increment (89.81%) is in Figure 4p), and the maximum reduction (19.66%) in Figure 4a). Thus, if materializing expectations of these increases, there may be positive and negative impacts. An example of a positive impact is the replenishment of groundwater and an example of a negative impact is the increase of floods.

4. CONCLUSIONS

Considering the historical series (1980-2010) with the simulated series (2016-2050) that was simulated by the Eta/MIROC5 at twenty-four points, monthly precipitation showed increasing trends at fifteen locations in the State of Paraná and two sites showed a decrease in the monthly rainfall trend.

In some places, there is a big difference between the average monthly precipitation of 1980 to 2010 compared with the average expected precipitation of 2016 to 2050.

The results also exhibited great variability in monthly rainfall for both the historical series and the simulated series.

Figure 4. Temporal variation at points where show changes in precipitation trends during the period 1980-2050, along with the mean of historical and simulated precipitation. Continue.

Figure 4. Continued.

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Relationships between land use and water quality obtained for the evaluation of genotoxic effects in plant bioindicators

ARTICLES doi:10.4136/ambi-agua.2299

Received: 16 Jul. 2018; Accepted: 18 Dec. 2018

**Jéssica Sieklicki^{id}; Nilton Cesar Pires Bione^{id}; Paulo Costa de Oliveira-Filho^{id};
Viviane Fernandes de Souza^{id}; Kelly Geronazzo Martins*^{id}**

Universidade Estadual do Centro-Oeste (UNICENTRO), Irati, PR, Brasil

Departamento de Engenharia Ambiental (DENAM). E-mail: jessica.sieklicki@gmail.com,
bione@irati.unicentro.br, paulocostafh@gmail.com, vfsouza@irati.unicentro.br, kellygm77@gmail.com

*Corresponding author

ABSTRACT

Anthropic activities as well as land use and occupation are closely linked to water and soil quality. An alternative to assess their influence on water quality in water bodies is through biomonitoring using *Allium cepa* as a bioindicator organism. This technique allows the detection of cytogenetic abnormalities in *Allium cepa* root meristematic cells after contact with analyzed water samples. Thus, we evaluated the genotoxic potential of water samples from the Rio das Antas (Antas River) along the urban perimeter of the city of Irati, Paraná, Brazil. With the aid of GeoEye Satellite high-resolution fused orbital images, we searched for possible relationships between the use and occupation of land around this river. Biomonitoring was performed at three different sampling points. Statistical equality between the negative control and Point 1 and between Points 2 and 3 was obtained using Fisher's test and a Principal Coordinate Analysis (PCoA). The former presented a low frequency of abnormalities chromosomes and the latter presented larger averages. In addition, Point 1 had strong influence of tree vegetation. Points 2 and 3, demonstrated a strong influence of urbanized area, with significant degradation of permanent preservation areas (áreas de preservação permanente – APP). The results showed remarkable anthropogenic interference to the ecosystem. Furthermore, this indicates importance of an APP watercourses functioning to preserve the quality of the water resources.

Keywords: alternative method of water quality analysis, chromosomal abnormalities, remote sensing.

Relações pontuais entre o uso e ocupação da terra e a qualidade da água obtida pela avaliação dos efeitos genotóxicos em bioindicadores vegetais

RESUMO

As atividades antrópicas bem como o uso e ocupação da terra estão intimamente ligadas à qualidade da água e do solo. Uma alternativa para avaliar suas influências na qualidade da água em corpos hídricos é através do biomonitoramento utilizando *Allium cepa* como organismo bioindicador. Esta técnica permite detectar anormalidades citogenéticas nas células meristemáticas radiculares de *Allium cepa* após o contato com a amostra de água analisada.

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Assim, realizou-se a avaliação do potencial genotóxico de águas do Rio da Antas no perímetro urbano do município de Irati-PR e com o auxílio de imagens orbitais fusionadas de alta resolução do satélite *Geoeye*, buscou-se possíveis relações com o uso e ocupação da terra do entorno deste rio. O biomonitoramento foi realizado em três pontos amostrais distintos. Integrando-se os dados, obteve-se, com o teste de Fisher e mediante uma análise de coordenadas principais (PCoA), uma igualdade estatística entre o controle negativo e o ponto 1 e entre o ponto 2 e 3, sendo que os primeiros apresentaram baixa frequência de anormalidades cromossômicas e os últimos, médias maiores. Além disso, o ponto 1 apresentou forte influência de vegetação arbórea e os pontos 2 e 3, forte influência de área urbanizada, com expressiva degradação das áreas de preservação permanente (APPs). Os resultados confirmaram a notável interferência antrópica ao ecossistema, bem como, a importância da APP na preservação e qualidade do recurso hídrico.

Palavras-chave: anormalidades cromossômicas, metodologia alternativa de análise da qualidade da água, sensoriamento remoto.

1. INTRODUCTION

The urbanization process of many Brazilian cities occurred along riverbanks and brought detrimental consequences for bodies of water, the quality of the air, soil, fauna and flora of the region (Fendrich and Oliynik, 2002).

Anthropogenic activities such as land use and occupation are factors that interfere with water and soil quality. Another factor of significant relevance is the advancement of technologies and products in the industrial and agricultural sectors, which have helped to bring about the introduction of new chemical agents in the environment, thereby impacting the ecosystem equilibrium (FUNASA, 2002). The destruction of the riparian forests contributes to the silting of water bodies. As a result, this caused increases of turbidity, erosion, disequilibrium of the flood regime and the involvement of wild fauna (Oliveira-Filho *et al.*, 1994).

Environmental quality monitoring can be performed through conventional physico-chemical and microbiological assessments combined with specific assessments to investigate changes in the cellular metabolism of bioindicators. Moreover, they can be applied to evaluate differences in some genetic structures due to the presence of substances with varying degrees of toxicity. In addition to representing a low cost, according to Grant (1994), plant species such as *Allium cepa* and *Tradescantia ssp.* are effective in environmental monitoring to assess the mutagenic potential of air, water and soil pollutants. Because of the similarities in the morphology of the chromosomes of eukaryotic organisms (plants and animals), they may also respond in an action mode of mutagenic agents (Constantin and Owens, 1982).

Experiments using *Allium cepa* as a test organism reveal and classify parameters such as cytotoxicity with mitotic index, mutagenicity with frequency of micronuclei, and genotoxicity with the frequency of chromosomal abnormalities during meristematic cell division of roots exposed to the analyzed samples (Fiskesjö, 1993). Thus, the genotoxic action of possible pollutants in the samples can be determined (Leme and Morales, 2009). The genotoxicity test using *Allium cepa* was validated by the United Nations Environment Program and the International Chemical Safety Program (Grover and Satwinderjeet, 1999).

It is a fact that the use and occupation of the land interfere with the quality of a water body, and this interference does not occur only in a localized way, but reaches the whole region that surrounds it (Oliveira *et al.*, 2012; Costa, *et al.*, 2015). Thus, proximity analyses are valuable to detect the use and occupation of the land around the sampling points and to assist in the evaluation of water quality.

The use of remote sensing as a tool for analysis and control of environmental issues has become essential. The evolution in the technological area increasingly favors the development of numerous terrestrial-environmental monitoring satellites allowing for the collection of qualitative and quantitative data on a global, regional or local scale, as well as determining the degree of environmental degradation among other measurements (Mascarenhas *et al.*, 2009).

The purpose of this study was to evaluate the influence of urban and peri-urban soil use and occupation through the monitoring of the Rio das Antas' (Antas River) water quality and its possible genotoxic effects at predefined points. By means of high-resolution orbital images the classification of land use and cover was carried out by comparing them with the results obtained in the cytogenetic evaluations of the bioindicator vegetation.

2. MATERIALS AND METHODS

2.1. Study area

The study was carried out in the urban area of the municipality of Irati, Paraná, Brazil. (Figure 1) located 150.34 km from the state capital, Curitiba (IPARDES, 2017). The municipality was founded in 1907, and since then it has experienced increases in population along with economic and industrial growth. These factors interfere directly and indirectly with the preservation of the water quality of the municipality. It has a 999,517 km² extension, an estimated population of 60,425 inhabitants with 73.6% of households having access to adequate sanitary sewage (IBGE, 2017). The rivers that surround the municipality are the Rio dos Patos, Rio Caratuva, Rio das Antas, Rio Preto, Rio Riozinho, Rio Cachoeira, Rio Ponte Alta, Río Quente and Rio Taquari, with their courses being directed to the Tibagi, Iguaçu and Ivaí river basins. These tributaries all belong to the Paraná River Basin (Andrade and Felchak, 2009).

Figure 1. Urban area of the municipality of Irati, Paraná showing the sampling points P1, P2 and P3.

Source: research data.

The Rio das Antas is considered one of the main water bodies of the municipality of Irati. Its origin is in the Serra do Nhapindazal (Nhapindazal mountain range), an area of urban expansion of the municipality. Additionally, its greater extension crosses the urbanized area,

crosses several neighborhoods, receiving several tributaries before leaving this perimeter when passing under the bridge in BR 277, between the coordinates 25°28'02" South and 50°39'04" West. It flows into the river Imbituvão, belonging to the Tibagi River Basin (Andrade and Felchak, 2009) and according to the SUREHMA Ordinance nº003/91 it is classified as a second order river (SUREHMA, 1991).

Through orbital images, an accurate visual analysis of the surroundings of the Antas River along its urban route was carried out at three different collection points (P1, P2 and P3) in relation to the use and occupation of the land in its surroundings. Since Point 1 is located near the springs; Point 2 and Point 3 are located in areas of urban consolidation.

2.2. Mapping of study areas

With the aid of multispectral orthoimagery fused with spatial resolution of 0.50 m, from the GeoEye satellite from 2013, obtained from the Municipality of Irati, the mapping of the study area was carried out, and for each collection point, a buffer zone, that is, a distance band that surrounds each of these points, with a radius of 500 m, covering a total area of 79 ha (encompassing the proximity around each point).

After the buffers were created, they were mapped. Classes were defined for the use and occupation of the land with the greatest influence in the analyzed areas. Then, through cognitive interpretation and utilizing the technique of vectorization on canvas, polygons were created and associated with the defined classes.

2.3. Assays with *Allium cepa*

Three water collections (campaigns) were carried out, in the months of September and December of 2016 and March of 2017, at the Antas River at predefined collection points. Bioassays with *Allium cepa* (onion) bulbs were placed in separate aquariums. Three assays were performed by collecting, one for each sample and leaving a fourth assay for positive control; following the protocols for plant cell mitosis (Jordão, 1998) along with adaptations of the assay as validated by the United Nations Environment Program (UNEP) as a standard efficient for the monitoring of genotoxicity and mutagenicity (Kumari *et al.*, 2009).

The bioassays were placed randomly by distributing twenty onion bulbs into four aquariums (five bulbs each) containing distilled water (negative control) and left for a period of 96 hours for root growth. Then, five roots of each bulb were randomly collected in each aquarium to perform cytogenetic analyses. The distilled water was replaced by water samples from each collection point of the river. And in the aquarium room, the distilled water was replaced by potassium dichromate solution 1.4 mg.L⁻¹ (positive control).

After 48 hours, the same root harvesting procedure was performed and the roots collected were fixed in ethanol and acetic acid solution (3: 1 v / v) and stored in 70% alcohol under refrigerated conditions until they were analyzed by light microscopy. For the cytogenetic analyses, the roots were hydrolyzed in 1N HCl solution at 60°C for 10 minutes and the cytological slides were made by a crushing technique and were stained with 1% carmin propionic.

Three cytological slides were prepared for the mitotic analyses. Each slide containing one root per bulb, being evaluated 500 cells per phase of mitosis totalizing 2000 cells in a total of 15 slides per treatment. Cells with the most representative abnormalities were photographed using the Olympus CX31 Optical Microscope / SC30 camera.

2.4. Statistical analyses

In order to determine whether there were statistical differences in the percentage of abnormalities (dependent variable) as a function of the 3 study points and the controls (factor), a one-way ANOVA analysis was performed after checking for gaussianity assumptions

(Shapiro-Wilk Normality test) and homogeneity of variances (Bartlett). Peer-to-peer comparisons were also performed by using Fisher's test.

The relationships between the percentage of chromosomal abnormalities and the classes of land use and occupation were investigated through a Principal Coordinate Analysis (PCoA). The analyses were carried out with the aid of software R (specifically, the *vegan* and *MASS* packages). For all statistical procedures, values of $p < 0.05$ were considered significant.

3. RESULTS AND DISCUSSION

After completing the mapping of the study areas, eight classes were defined for land use and occupation, which through cognitive interpretation were more evident. They are:

- Urbanized areas: according to Magalhães *et al.* (2013): "[...]the result of anthropic occupation over the territory; are areas that have continuous occupancy of buildings, or that have suffered anthropic impact for urban occupation in some way";
- Reforestation: Area used for the cultivation of species of commercial interest;
- Tree vegetation: can be defined as a group of species constituted by large trees, present mainly in forests and woodlands.
- Underground vegetation: mainly grasses;
- Bodies of water: rivers, lakes and lagoons;
- Exposed soil: soil devoid of any vegetation;
- Capoeira: area of secondary vegetation (Linhares *et al.*, 2003);
- Agricultural cultivation: occupied area by any stage of agricultural culture.

The classification of the image allowed the calculation of the areas equivalent to each class previously described. The results of area and respective occupation by class can be verified in Figure 2. Point 1 area near the springs of Rio das Antas showed about 77% of green areas, tree vegetation (48.21%), undergrowth (9.84%), reforestation (10.69%) and capoeira (8.07%). Along the way the population density increases and in Points 2 and 3 the predominant class was the urbanized area with 84.06% and 56.84% respectively.

Cytogenetic analyses in meristematic cells of *Allium cepa* roots showed some type of chromosomal abnormality in all phases of mitosis, which demonstrates the genotoxic potential of water samples at all collection points. Chromosome abnormalities visualized as chromosomal bridges and adhesions, inconclusive chromosomes in the equatorial plate of the cell, chromosomal fragments, early rise of chromosomes or retardant chromosomes culminated in the formation of micronuclei (Figure 3). The micronuclei are formed by cytoplasmic portions of chromatin, have a round or oval shape, are located near the nucleus and are formed by lysis in the DNA molecule during mitotic division, days or weeks after this undergoes mutagenic agent interference (Stich and Rosin, 1983; Stich *et al.*, 1984). The other chromosomal alterations, according to Vidaković-Cifrek *et al.* (2002) can be visualized at any stage of cell division as evidence of mutagenic effects arising from agents that cause DNA breaks (clastogenic agents) or that induce abnormal chromosomal segregation or aneuploidy (aneugenic agents), classified by the type of alteration it produces.

Genotoxic effects of different chemical agents have been described in the literature as being responsible for varied chromosomal alterations in different exposed organisms, among them man (Natarajan, 2002). According to Grant (1994), the evaluation of the genotoxicity caused by environmental pollutants in tests using *Allium cepa* as a bioindicator has been shown to be very efficient, as well as for the quality evaluation of waters affected by anthropic influences (Fiskesjö, 1993; Fatima and Ahmad, 2006).

Figure 2. Classification of the use and occupation of the land to the surroundings of the sampling points.

Source: research data.

Figure 3. Major chromosomal abnormalities found in *Allium cepa* cells. a) Prophase with micronucleus (arrow); b) Metaphase with non-conjoined chromosome fragments (arrows); c) Anaphase with chromosome fragment (arrow); d) Telophase with chromosomes not assembled to the nucleus (arrows); e) Prophase with stickiness chromosomes (arrow); f) Metaphase with early ascending chromosomes (arrows); g) Anaphase with early ascending chromosomes (arrow) and stickiness chromosomes (arrowhead); h) Non-conjoined chromosome telophase (arrow) and chromosome bridge (arrowhead).

Source: research data.

In relation to the cytogenetic analyses in the three sample points and controls, three statistically distinct groups ($F = 33,10$; $p < 0,01$) were identified, the first one referring to Points 2 and 3 (statistically equal averages) by Point 1 and the negative control and finally a third

group formed by the positive control, (Table 1, Figure 4). These results are consistent with what is expected since Point 1, near the source (origin) of the study river, is characterized as a region with higher vegetation index and less urbanized when compared to Points 2 and 3, which are characterized as regions with less vegetation, more urbanized and consequently, more eutrophic. Moraes and Jordão (2002) report that plants collected in eutrophic rivers present a higher index of chromosomal alterations when compared to plants that develop in uncontaminated waters. Results similar to those found in this work are described by Oliveira *et al.* (2011; 2012), and Taniwaki *et al.* (2013).

Table 1. Number of cells analyzed and percentage of abnormalities in controls and by collection at each sampling point.

	Collection 1	Collection 2	Collection 3	NTCA	(%) TCA
	(%) CAA	(%) CAA	(%) CAA		
CN	-	-	-	6000	12.90c
CP	-	-	-	6000	24.80a
Point 1	2000 – 13.25	2000 – 13.50	2000 – 12.15	6000	12.97c
Point 2	2000 – 22.70	2000 – 19.45	2000 – 16.95	6000	19.70b
Point 3	2000 – 18.55	2000 – 19.35	2000 – 16.30	6000	18.07b

CAA= Number of Abnormal Cells Analyzed; NTCA= Total Number of Cells Analyzed; MCA= Average Cells Assayed; CN= Negative Control; CP= Positive Control. Averages followed by the same letter do not differ statistically from each other, by the Fisher test at 5% significance. Source: research data.

Figure 4. Principal Coordinates Analysis (PCoA).

Source: research data.

In relation to the results already presented, it is possible to discriminate the statistically significant interference of land use and occupation on water quality via genotoxic effect. The Principal Coordinates Analysis (PCoA) (Figure 4), showed that Point 1 is heavily influenced by tree vegetation, especially by areas of natural regeneration. Points 2 and 3, are strongly influenced by the urbanized area in part due to the percentage of chromosomal abnormalities in *Allium cepa* test.

The sampling Points 2 and 3 were the ones that presented the worst results referring to the water quality of the Antas River as evidenced by the presence of chromosomal abnormalities in the *A. cepa* toxicity test. Therefore, it is possible to say that the use and occupation of the land directly influence the quality of these waters, being that the anthropic occupation near the water course negatively impacts its quality.

According to a study by Andrade and Felchak (2009), the Antas River presents several problems resulting from disorderly occupation, such as the absence of riparian forest due to the occupation (accumulation) of the valley bottom, silting due to the creek-free banks and the rectilinearization (straightening) process of some stretches of the river, accumulation of organic matter due to lack of sanitation and disposal of industrial chemical wastes not treated correctly. The main problem is the clandestine (possibly illegal) disposal of sewage and the extravasation of it in areas where the sewage network intersects with the rainwater network, and the pluvial network with the sewage network.

The riparian forests are areas around bodies of water, established as APPs. According to Law No. 12,651 / 2012, which established the New Forest Code, APP is: "[...]protected area, covered or not by native vegetation, with the environmental function of preserving water resources, landscape, geological stability and biodiversity, facilitate the genetic flow of fauna and flora, protect the soil and ensure the well-being of human populations." Also, in accordance with this law, it is considered as APP, in rivers with a width of less than 10m, a marginal range of 30m, as is the case in Antas River. The law also provides for the urban land regularization of specific interest of areas that are occupying APP and for environmental regularization purposes that a non-buildable strip with a minimum width of 15m on each side must be maintained. Nonetheless, it should be pointed out that in most of the areas analyzed in this study, it was not widely observed in Points 2 and 3, located in areas of urban consolidation.

Works such as Marmontel and Rodrigues (2015), Cândido *et al.* (2015) and Silva *et al.* (2016), have already verified that anthropic actions, together with the devastation of the ciliary forest, negatively influence the water quality of the water bodies. Furthermore, in places where there is greater preservation of the vegetation the quality water is also better.

4. CONCLUSIONS

The results were obtained in the bioassays using *Allium cepa* as test organisms. Combined with orbital satellite imagery, statistical analyses were effective as alternative validation tools to monitor the toxic effects of possible pollutants present in water bodies with compromised water quality. Accompanied by physical-chemical analyses to prove their results, these can become even more significant.

The Antas River (Rio das Antas) is experiencing significant degradation of PPAs, especially in urban consolidation areas. The results indicated that water quality is intrinsically related to land use and occupation. The sampling area near the sources of the Rio das Antas (P1) has a significant portion of arboreal vegetation, a fact that contributes to a better quality of the water of the locale (region). However, the remaining sample areas (P2 and P3), suggest a greater deterioration in water quality as evidenced by the greater presence of chromosomal abnormalities in the *Allium cepa* test. These areas suffer from the detrimental impact on the anthropic occupation and a consequent lack of PPAs; a fact that supports the observed results.

One can then verify the remarkable interference that man can bring to the ecosystem. Also, the importance of PPAs for a watercourse, can assist in greater preservation and better quality of the water resources.

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Impacts of land-use change on southeast Amazonia basin streamflow

ARTICLES doi:10.4136/ambi-agua.2303

Received: 22 Jul. 2018; Accepted: 24 Jan. 2019

Vinicius Marques Louzada* ; **Celso Bandeira de Melo Ribeiro**

Universidade Federal de Juiz de Fora (UFJF), Juiz de Fora, MG, Brasil
Faculdade de Engenharia. Departamento de Engenharia Sanitária e Ambiental (ESA).
E-mail: vinicius.marques@engenharia.ufjf.br, celso.bandeira@ufjf.edu.br

*Corresponding author

ABSTRACT

The Amazon region is the subject of growing interest in the international scientific community due to the environmental services provided by its dense forests in climate regulation and freshwater. Despite many efforts from environmental management agencies and research centers, this region can still be considered poorly monitored, especially given its large regional and global importance. Rainfall-runoff models are presented as a good alternative to the estimated flow rates in inaccessible or difficult to watch places. This study simulated stream flows for a representative part of the Amazon basin using the Soil and Water Assessment Tool (SWAT) redistributed hydrological model, adopting as reference the streamflow observed in Altamira gauge station. The authors emphasize that a database was specially prepared with physical parameters such as land use, topography and soil types, and weather data encompassing the years from 1985 to 2012, at a daily time step. The chosen approach allowed carrying out hydrologic simulations that were consistent with the flow values observed at the Altamira gauge station, providing better understanding of hydraulic-hydrological processes in the Xingu subbasin and information for planning and decision-making on the management of water resources in this important river basin.

Keywords: Amazon basin, hydrologic modeling, land use change.

Impacto na mudança de uso do solo na vazão de uma bacia do sudeste da Amazônia

RESUMO

A Região Amazônica vem despertando interesse crescente da comunidade científica internacional devido às amplas possibilidades ambientais que suas densas florestas têm a oferecer no que diz respeito à regulação climática e recursos hídricos. Apesar dos muitos esforços de agências reguladoras de meio ambiente e centros de pesquisa, esta região ainda pode ser considerada pouco monitorada, especialmente dada sua grande importância regional e global. Modelos de vazão são apresentados como uma boa alternativa para taxas de fluxos estimadas em lugares inacessíveis ou de difícil monitoração. O objetivo deste trabalho é simular vazões para uma porção significativa da Bacia Amazônica usando o modelo hidrológico semidistribuído SWAT (Soil and Water Assessment Tool), adotando como referência as vazões

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observadas na estação de monitoramento de Altamira. Ressaltamos que um banco de dados foi especialmente preparado com parâmetros físicos, como uso do solo, topografia e tipos de solo, e informações do clima compreendidas entre os anos de 1985 e 2012, numa escala de tempo diária. A abordagem metodológica permitiu que fossem realizadas simulações hidrológicas muito condizentes com os valores de fluxos observados na estação de monitoramento de Altamira, permitindo um melhor entendimento dos processos hidráulico-hidrológicos na sub-bacia do Xingu e fornecendo informação para planejamento e tomada de decisões no que concerne ao gerenciamento de recursos hídricos nesta importante bacia.

Palavras-chave: bacia Amazônica, modelagem hidrológica, mudança de uso do solo.

1. INTRODUCTION

The Amazon is one of the most complex ecosystems in the world; it is a place where it is possible to find researchers from all over the Earth trying to find new species or new medicine from plants (Gutbelert, 2002). Non-governmental and governmental organizations from all over the world also pursue the preservation of animals, plants and water resources (Goodwin, 2014).

Although it is known that natural resources must be preserved, the Amazon is facing issues with deforestation, especially for wood, commerce and plantations (Ferreira *et al.*, 2005). According to Brazil's Ministry of the Environment, deforestation has decreased since 2004. However, the region still loses about 6000 km² of natural forest every year. Another significant issue is the construction of the Belo Monte hydroelectric power plant, which is going to generate 11.233 MW of electricity and cause the inundation of 668 km² (Berman, 2012).

Analyzing all that has been said, it was possible to conclude that studies should be done on that area to guarantee a sustainable exploitation of all natural resources. One type of study that is increasing worldwide is the use of mathematical models.

To plan the use of a basin and predict how this basin will respond to changes in soil use, deforestation and occupation, a mathematical model is used that simulates reality and provides an important tool to make the decisions. With a long data series, it is possible to estimate the impact of an action on a basin (Lenhart *et al.*, 2002). Models are mathematical equations that intend to describe and simulate what will occur in the basin, and there are different models that can be used for a variety of purposes, according to the author's goal (Souza, 2015).

The model being used in this article is the Soil and Water Assessment Tool (SWAT) model. The SWAT model was developed by USDA Agricultural Research Service (USDA-ARS) and Texas A&M AgriLife Research (Arnold *et al.*, 1998). This model has recently been used in different hydrological studies in Brazil (Bressiani *et al.*, 2015; Pereira *et al.*, 2016). This research simulates different scenarios and how the streamflow responds to changes in land use.

2. MATERIALS AND METHODS

2.1. Study Area

The hydrological modeling was made in the Xingu River's subbasin (Figure 1), in the Amazon, between the coordinates UTM 8330743.862 m and 9851708.200 m; 1319966.175 m and 1915083.081 m. The basin has an approximate area of 509 000 km² that is distributed along the states of Mato Grosso (MT) and Pará (PA).

According to Koppen's classification model, the basin has two climates; the north part is classified as Am – Tropical Monsoon, with most of the rain occurring during the 7 to 9 hottest months. The rest of the basin the classification is Aw – Tropical Wet and Dry, where the precipitation occurs during the summer season and has a dry winter (Sampaio et al., 2011).

Figure 1. Xingú sub-basin, located in Amazon basin.

2.2. Meteorological Data

The meteorological data (relative humidity, solar radiation, wind speed and temperature) were obtained from 40 weather stations from INMET - National Meteorology Institute. Using this data, it was possible to calculate the necessary statistics for the model to run the simulation. It is noteworthy that rainfall data from 204 ANA – National Water Agency rainfall stations were used too.

2.3. Types of Soil, Land Use Map, and Types of Soil and Land Use Map

The main types of soils found in the Amazon basin are: Acrisol (38.47%), Ferrasol (37.64%) and Leptosol (11.78%). The soil map (Figure 2a), with 5 km resolution was obtained in the ISRIC global database. The soil characteristics were obtained from the Pedo-Transfer Function (Saxton and Rawls, 2006). The land-use map (Figure 2b) was obtained from the MODIS sensor with a 1 km resolution (Friedl *et al.*, 2010). The digital elevation models (Figure 3a) and slope maps (Figure 3b) were obtained from the TOPODATA global database (Hydrosheds), with a 1 km resolution.

The model was previously prepared in ArcGis with the following data: soil use, soils type, climatological data and the digital elevation model (DEM). The database comprehends the years between 1985 and 2012.

The model was used to simulate streamflow in the basin cited above, during a period of 28 years, between 1985 and 2012, of which the first two years were used to warm-up the model.

The simulation results were imported to the Soil & Water Assessment Tool – Calibration and Uncertainty Procedures (SWAT-CUP), where it is possible to do a static analysis and compare the data simulated by the program with the observed data. It is also possible to verify which parameters are more sensible, which allowed the calibration and validation of the model, making it possible to predict the stream flow in a more accurate way.

Figure 2. a) Types of soil; b) Land-use map.

Figure 3. a) Digital Elevation Model; b) Slope map.

For the calibration process, data from two monitoring points were used, belonging to the ANA – National Agency of Water. The first one at the beginning of the basin and the second at another point located in the course of the same river. The first monitoring point is named Boa Sorte (code 18460000). On this point, there is data available from January 1976 to February 2009. However, the model's database only starts on 1985; therefore, data from before that year was not taken into consideration.

After calibrating and validating the model, the values in all subbasins that contribute to the point where the first monitoring point is located were replaced. After changing the values, the model was run one more time. Next, the calibration and validation process for the second point, called Altamira (code 18850000), was initiated. Once again, the database available was larger than the model, so the authors only considered data from after 1985.

In the calibration and validation process for the second point, the values were only replaced on the subbasins between the first and the second points; the authors did not change any values on the subbasins that contribute for the first calibration point or the subbasins after the second point.

The second point was of interest because it is situated where the government of Brazil is building a hydroelectricity plant. Once the model is calibrated and validated, it will be possible to simulate and verify the impact of land use change in streamflow and power generation.

SWAT-CUP makes some statistical analyses and gives a few parameters to evaluate the model's efficiency. In this paper, the authors are going to use three parameters to check if the model is corresponding in a good way to reality.

2.4. Statistical Evaluation Criteria

Streamflow simulations were evaluated using different statistical criteria. According to Moriasi *et al.* (2007), the parameters that can be used are:

Coefficient of determination (R^2): describe the proportion of the variance in measured data. R^2 ranges from 0 to 1 and values greater than 0.5 are acceptable.

Nash-Sutcliffe efficiency (NSE): determine the relative magnitude of data variance compared to the measured data variance.

Percent bias (PBIAS): indicates the tendency of the simulated data to be larger or smaller than the observed values (Equations 1 and 2).

$$NSE = 1 - \frac{\sum_{i=1}^n (Y_{obs} - Y_{sim})^2}{\sum_{i=1}^n (Y_{obs} - Y_{mean})^2} \quad (1)$$

$$PBIAS = \frac{\sum_{i=1}^n (Y_{obs} - Y_{sim}) * 100}{\sum_{i=1}^n (Y_{obs})} \quad (2)$$

Model evaluations used in this study were based on performance ratings suggested by Moriasi *et al.* (2007) for a monthly time step, as shown in Tables 1 and 2.

Table 1. Reported performance ratings for PBIAS.

Model	Value	Performance rating	Modeling Phase	Reference
SWAT	<10%	Very Good	Calibration and Validation	Van Liew <i>et al.</i> (2007)
SWAT	<10% to <15%	Good	Calibration and Validation	Van Liew <i>et al.</i> (2007)
SWAT	<15% to <25%	Satisfactory	Calibration and Validation	Van Liew <i>et al.</i> (2007)
SWAT	>25%	Unsatisfactory	Calibration and Validation	Van Liew <i>et al.</i> (2007)

Adapted from Moriasi *et al.* (2007).

Table 2. Reported performance ratings for NSE.

Model	Value	Performance rating	Modeling Phase	Reference
SWAT	>0.65	Very Good	Calibration and Validation	Saleh <i>et al.</i> (2000)
SWAT	0.54 to 0.65	Adequate	Calibration and Validation	Saleh <i>et al.</i> (2000)
SWAT	>0.50	Satisfactory	Calibration and Validation	Santhi <i>et al.</i> (2001); adapted by Bracmort <i>et al.</i> (2006)

Adapted from Moriasi et al. (2007).

2.5. Land Use Change

Images with the different types of land use were obtained from the MODIS Land Cover Product Type, (MCD12Q1). These products have annual basis and its historical series is available from 2001. These images were produced by supervised classification algorithms, with a spatial resolution of 500 m, covering the soil surface of the planet (Friedl *et al.*, 2010).

3. RESULTS AND DISCUSSION

3.1. Calibration Process

Lelis *et al.* (2012), Paim and Menezes (2009) and Andrade *et al.* (2013) present the most common parameter used to calibrate the SWAT model, especially for hydrology variables. Based on the parameter they presented, a few tests were made to check which one would be more sensitive for the specific model. After the simulations, the best parameters were determined and are shown in Table 3.

Table 3. Most sensitive parameters.

Parameter	Description	Range of parameter	Best Value	Units
CN2	Surface runoff	35 to 98	75.163	-
ESCO	Compensation of soil evaporation	0 to 1	0.2958	-
ALPHA_BF	Base flow	0 to 1	0.40416	l/day
RCHRG_DP	Deep aquifer percolation	0 to 1	0.5458	-
SLSUBBSN	Average length of lateral ramp	10 to 150	32.75	m
EPCO	Compensation for plant grown	0 to 1	0.85416	-
SURLAG	Surface runoff retardation coefficient	0.05 to 24	20.3078	-
CH_W2	Average width of main channel at top of bank	0 to 1000	287.5	m
CH_L2	Length of main channel	-0.05 to 500	160.383	km
CH_N2	Manning's roughness coefficient value for the main channel	-0.01 to 0.3	0.200	-

After determining which parameters should be changed, a simulation including all ten variables was made as to define the best value for each one. The statistical analysis is shown in Table 4. The data shown in the table is for the second point used.

Table 4. Calibration results.

R²	NSE	PBIAS
0.63	0.59	17.3

As stated by Rocha *et al.* (2012), the SWAT model is highly sensitive to the input data and R^2 represents the correlation between observed and simulated data. As cited by the author and by Moriasi *et al.* (2007), values of R^2 greater than 0.5 are usually acceptable. This shows a good correspondence between the values estimated by the model and the ones simulated by the program.

For the NSE parameter, Moriasi *et al.* (2007) defines the range between 0.54 and 0.64 as adequate. This corroborates what Rocha *et al.* (2012) present values higher than 0.5 are not discarded, and other authors have used this efficiency. The value of 0.59 is classified as adequate for the specific model.

The last parameter used to evaluate the model was PBIAS; according to Moriasi *et al.* (2007), PBIAS can indicate poor model performance. The range of 15% to 25% is considered adequate by Van Liew *et al.* (2007). The value of 17.3% found after calibration was considered satisfactory.

All three parameters used to evaluate calibration process are sensitive to input data quality and indicate how well the model is representing the observed values in the basin. Analyzing the results shown in Tables 1 and 2, based on the literature, it was found that the model satisfactorily corresponds to the observed data.

3.2. Land Use Change

The land-use spatial-temporal analysis for the *Xingu* watershed was performed using MODIS images with 500 m spatial resolution along the 2001-2012 period, with sixteen different classes of land cover, as follows: evergreen needleleaf forest, evergreen broadleaf forest, deciduous needleleaf forest, deciduous broadleaf forest, savannas, grasslands, woody savannas, permanent wetlands, cropland/natural vegetation mosaic – CNVM, cropland, mixed forest, closed shrublands, open shrublands, urban and built-up and barren or sparsely vegetated.

The greatest identified coverage loss in *Xingu* watershed between 2001 and 2012 refers to the evergreen broadleaf forest class (Figure 4a), while the largest expansions occurred in the areas of savannas (Figure 4b) and croplands (Figure 4c), respectively.

Figure 4. Land use change in *Xingu* watershed: a) Evergreen Broadleaf Forest; b) Savannas, c) Cropland/Natural Vegetation Mosaic.

3.3. SWAT Simulation

After the calibration, two simulations were made using different land use maps: one for 2002, and another to 2012. The simulation dataset project present 307 watersheds with average area of around 1769.84 km² (Figure 5).

Figure 5. Discretization of representation watershed in SWAT model project for the *Xingu*: a) Watersheds; b) Stream.

Simulated and observed monthly flows at Altamira gauge station are presented for 01/1985 to 12/2012 in Figure 6, to visually evaluate the performance.

Figure 6. Observed and simulated monthly flows from 01/1985 to 12/2012.

Figure 6 shows that the simulated stream flows for 2002 and 2012 are higher than the observed data from Altamira station. It is also possible to observe on the graphic that simulated flow for 2002 is almost the same as for 2012, with a few higher peaks for the year of 2002. This fact goes against prediction, due to changes in land use, once the Evergreen Broadleaf forest had decreased as the Savanna had increased, less evapotranspiration and rainfall is expected, and an increase of water percolation, resulting in the decrease of streamflow in the *Xingu* River.

4. CONCLUSION

The model has been calibrated for the subbasin of *Xingu* River, presenting satisfactory results for the years between 1997 and 1999. However, it is still possible to improve the calibration of the model, achieving a model that represents the reality in a more accurate way.

The streamflow results demonstrate that the land use change from 2002 to 2012 did not cause a significant difference at the Altamira gauge station. There little difference in power plant energy generation in Belo Monte during this period.

When properly calibrated and validated, the SWAT model is a very efficient tool to plan interventions and changes in basins. It saves time and can predict results for any modifications that may occur at the site.

5. ACKNOWLEDGEMENTS

We would like to acknowledge the financial support provided by CNPq, Brazilian National Council for Scientific and Technological Development for conducting this research work.

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Biomonitors to evaluate the toxic potential of urban solid waste landfill leachate

ARTICLES doi:10.4136/ambi-agua.2326

Received: 06 Sep. 2018; Accepted: 18 Dec. 2018

Catiele Vieira¹ ; Annette Droste^{2*}

¹Universidade FEEVALE (FEEVALE), Novo Hamburgo, RS, Brasil
Instituto de Ciências da Saúde (ICS). E-mail: catiele_2003@hotmail.com

²Universidade FEEVALE (FEEVALE), Novo Hamburgo, RS, Brasil
Programa de Pós-graduação em Qualidade Ambiental. E-mail: annette@feevale.br

*Corresponding author

ABSTRACT

The accentuated increase in the production of solid urban waste (SUW) and the consequent accumulation of leachate in landfills increase the risk of environmental contamination. Biomonitoring is used to assess the toxicity of pollutants on living organisms. In this study, the pollutant potential of leachate from SUW from a deactivated landfill was evaluated by bioassays with *Lactuca sativa* L. and *Lycopersicon esculentum* Mill., and the relationship between chemical characteristics of the effluent and biological parameters was analyzed. The effluent was tested in its raw form and diluted in distilled water at concentrations of 75 and 50%. The percentage of germination, root growth (RG), mitotic index (MI), chromosomal abnormalities index (CAI), and micronuclei frequency (MCN) were analyzed. In the presence of effluent, germination and MI decreased, while RG, CAI and MCN frequencies increased in relation to the negative control (distilled water) for both species. Lead, iron and zinc presented negative relation with seed germination for both species, with RG of *L. sativa* and MI of *L. esculentum*, as well as a positive relation with MCN frequency in the studied species. Because of its larger chromosomes, *L. sativa* is a more suitable biomonitor of SUW leachate toxicity than *L. esculentum*. Even though the landfill is deactivated, it is necessary to treat this effluent, in order to minimize environmental impacts.

Keywords: bioassay, effluent, toxicity.

Biomonitores para avaliação do potencial tóxico do lixiviado de aterro de resíduos sólidos urbanos

RESUMO

O aumento acentuado da produção de resíduos sólidos urbanos (RSU) e o consequente acúmulo de lixiviado em aterros potencializam o risco de contaminação do meio ambiente. Biomonitoring são utilizados para avaliação da toxicidade dos poluentes sobre os organismos vivos. Neste estudo, foram avaliados o potencial poluidor do lixiviado de RSU de um aterro desativado por bioensaios com *Lactuca sativa* L. e *Lycopersicon esculentum* Mill. e a relação entre características químicas do efluente e os parâmetros biológicos analisados. O efluente foi testado na sua forma bruta e diluído em água destilada, nas concentrações de 75 e 50%. Foram

analisados o percentual de germinação, o crescimento radicular (CR), os índices mitótico (IM) e de anomalias cromossômicas (IAC), e a frequência de micronúcleos (MCN). Na presença do efluente, a germinação e o IM diminuíram, enquanto que o CR, IAC e a frequência de MCN aumentaram em relação ao controle negativo (água destilada) para ambas as espécies. Chumbo, ferro e zinco apresentaram relação negativa com a germinação das sementes de ambas as espécies, com o CR de *L. sativa* e com o IM de *L. esculentum*, além de uma relação positiva com a frequência de MCN nas espécies estudadas. *L. sativa*, por apresentar cromossomos maiores, é uma biomonitora de toxicidade de lixiviado de RSU mais adequada do que *L. esculentum*. Mesmo que o aterro esteja desativado, é necessário o tratamento deste efluente, com vistas a minimizar os impactos ambientais.

Palavras-chave: bioensaio, efluente, toxicidade.

1. INTRODUCTION

Population growth, together with technological development and increased consumption, lead to a rise in solid urban waste (SUW). In Brazil, the generation of SUW in 2016 totaled approximately 78.3 million tons (ABRELPE, 2016). The need for large expanses of land for final waste disposal and the possibility of environmental contamination make SUW management a major challenge for city administration (Rosa *et al.*, 2017).

Landfills remain the most common SUW disposal practices, due to simple execution and relatively low cost (Yang *et al.*, 2014). Landfills can be classified as sanitary, controlled and dumps. Sanitary landfills employ technology to minimize the environmental impact and possible human health risks, using things like composite liners that act as a low-permeable barrier, preventing liquid from leaching. At controlled landfills, the waste is simply covered with earth, with no leachate or biogas collection or processing. At dumps, the waste is left exposed on the ground (IBAM, 2001).

According to the most recent outlook on solid waste in Brazil, from 2016, conducted by the Brazilian Association of Public Cleaning and Special Waste Companies (ABRELPE), of all the waste collected in Brazilian municipalities, 58.4% is sent to sanitary landfills and 41.6% to controlled landfills and dumps (ABRELPE, 2016). This is despite Law 12,305/2010, which instituted the National Policy on Solid Waste, determining an end to controlled landfill and dump activities, as these means of disposal are considered incompatible with environmental protection. A maximum period of four years from the date of sanctioning was established for replacement with sanitary landfills.

Leachate, the byproduct resulting from the breakdown of solid waste, calls for closer attention due to its variability and the complexity in treating it, not to mention the myriad environmental impacts it can cause. Leachate can contain high levels of metals, suspended particles and organic matter. Leaching occurs in operating landfills, but also once they have been deactivated, as the organic matter continues to degrade and breakdown (Rosa *et al.*, 2017).

Characterizing the leachate is an important factor in establishing a strategy to effectively manage it (El-Fadel *et al.*, 2002), as the chemical composition tends to vary greatly (Budi *et al.*, 2016). The characteristics depend on the type of deposited waste, the means of disposal, management and length of time the landfill has existed. They are also greatly influenced by meteorological factors, among which rainfall volumes and air temperature are important (El-Fadel *et al.*, 2002). Due to the highly soluble substances and toxic composites, beside soil contamination, leaching may lead to the contamination of groundwater and aquifers in areas surrounding landfills, negatively impacting the environment and public health (Rosa *et al.*, 2017). CONAMA Resolution 397/2008 of Brazil's National Council on the Environment (CONAMA, 2008) establishes the conditions and standards for disposing of this class of effluents.

Leachate toxicity tests can be conducted by means of bioassays using plant species sensitive to toxicity of complex composites, although studies are scarce (Silva *et al.*, 2015; Budi *et al.*, 2016). Among the leading species for conducting toxicity bioassays evaluating seed germination, root growth, cell division and chromosome behavior and structure organization are *Lactuca sativa* L. (lettuce) and *Lycopersicon esculentum* Mill. (tomato), as they constitute species sensitive to toxic agents and germinate rapidly, while are also easily found in stores throughout the year at a low cost (Silva *et al.*, 2015).

Within this context, the study proposed the following objectives: (a) to assess the toxic potential of SUW leachate in a deactivated controlled landfill by means of bioassays using *L. sativa* and *L. esculentum*; (b) to identify the biomonitoring organism and biological parameter most suited to detecting effluent toxicity; (c) to verify the relation between the physical and chemical properties of the leachate and the data obtained for biological parameters.

2. MATERIALS AND METHODS

2.1. Deactivated controlled landfill

The SUW landfill, occupying an area of 4,750 m², is located in a municipality with a population of 22,514 inhabitants (IBGE, 2017), located in the Metropolitan Region of Porto Alegre, the capital of Rio Grande do Sul state, in the south of Brazil. The landfill remained activated for 13 years until 2005.

2.2. Collection and analysis of leachate physical and chemical parameters

A 5-liter sample of leachate was collected at the landfill in January 2017. Collection, storage, preservation and transport of the leachate sample were in full accord with criteria established by the Standard Methods for the Examination of Water and Wastewater (APHA *et al.*, 2012). The following parameters were noted: pH, temperature, electric conductivity (EC) and dissolved oxygen (DO) with the aid of the AK88-ASKO[®] multi-parameter meter; biochemical oxygen demand (BOD₅), by manometry (SM 5210); chemical oxygen demand (COD), by titration (SM 5220); ammoniacal nitrogen (NH₃-N), by titration (4500 NH₃); nitrite (NO₂), through ultraviolet–visible spectroscopy (SM 4500 NO₂-); nitrate (NO₃), through ultraviolet–visible spectroscopy (SM 4500 NO₃-) and concentrations of iron (Fe), zinc (Zn), lead (Pb), cadmium (Cd), nickel (Ni) and copper (Cu), by flame atomic absorption spectroscopy (SM 3111).

2.3. Bioassays

Bioassays were prepared in a laminar flow chamber in the laboratory. Leachate was used in its raw form (100% original concentration) and diluted in distilled water, at concentrations of 75% and 50%. Distilled water was used as the negative control, and for positive control, the minimum inhibitory concentration of copper (3 mg L⁻¹ of CuSO₄) was used, equal to the lowest concentration of the metal able to inhibit root growth (Di Salvatore *et al.*, 2008). *Lactuca sativa* and *L. esculentum* seeds were germinated in Petri dishes with a diameter of 9 cm, containing a sheet of quantitative filter paper (GE Healthcare[™]) sterilized and moistened with 5 mL solution of the specified solutions (leachate concentrations and controls). Three dishes were prepared for each treatment and biomonitor, each containing fifteen seeds and sealed with plastic film. The dishes were kept at a temperature of 25±1 °C, for a 16-hour photoperiod.

2.4. Germination

Germinated seeds in each dish were counted after two days of exposure to the treatments, according to Aguiar *et al.* (2016). Seeds showing evidence of root protrusion, visible without the aid of instruments, were considered germinated. The data were used to calculate the average of germinated seeds for each biomonitor in each treatment.

2.5. Root growth

Root growth was assessed after seven days of exposure to the treatments (Carvalho *et al.*, 2014) with the aid of a millimeter rule. Root lengths of five random seedlings per dish were measured, totaling 15 seedlings per treatment and biomonitor. Root length was measured from the hypocotyl of the seedling to the apical meristem of the root system, according to Gatti *et al.* (2004).

2.6. Mitotic index, chromosomal abnormality index and micronuclei frequency

Root tips were randomly removed from five seedlings from each Petri dish after two days of exposure, totaling 15 roots per treatment and organism. The tips were fixed in an ethanol: acetic acid (3:1 v/v) solution for 24 hours at room temperature and then transferred to 70% ethanol at 4°C. Of the 15 roots removed per treatment, the meristem region of only 10 random roots were used. The remaining material was kept in reserve. To prepare the microscopic slides, the root tips were sequentially treated for 2 minutes in distilled water, then hydrolyzed for 6 minutes in HC11N and washed again for 2 minutes in distilled water. Microscopic slides were prepared according to Cuchiara *et al.* (2012). The hydrolyzed root tips were squashed on microscopic slides and stained with 2% aceto-orcein. The number of cells in mitosis and cells with chromosomal abnormalities, and the number of micronuclei (MCN) were counted at 500 cells per root through the scanning technique, using an optic microscope (Nikon Eclipse E200) at 400x magnification. The mitotic index (MI) was calculated using the formula $MI = [(number\ of\ mitotic\ cells / total\ cells) 100]$. The chromosomal abnormalities index (CAI) was calculated by $CAI = [(number\ of\ cells\ with\ abnormality / total\ cells\ in\ division) 100]$. Micronuclei frequency was expressed as MCN/100 cells.

2.7. Statistical analysis

Data were subjected to the Shapiro-Wilk normality test and as they complied with assumption of normality, differences between the averages were analyzed by ANOVA, followed by the Duncan test. The relation between biological parameters and the metals Pb, Fe and Zn was verified using the Pearson correlation coefficient. All statistical analysis was conducted using the SPSS program, version 20.0, at 5% probability.

3. RESULTS AND DISCUSSION

The COD value was three times greater than the BOD₅ in the raw leachate sample (Table 1). BOD₅ refers to the biodegradable organic mass, while COD refers to all the oxidizable matter in the effluent (CETESB, 2016). The BOD₅/COD calculated for the raw leachate was 0.29. Due to the BOD₅/COD ratio, the landfill in the present study would be classified as moderately stable, according to the Solid Waste Association of North America (SWANA, 1997) classification. This ratio could be considered an indicator of the level of organic matter biodegradability, which diminishes over time (El-Fadel *et al.*, 2002).

The leachate presented pH levels higher than 7 (Table 1). The pH variation over the years is linked to the COD and BOD₅ values, with the pH starting at around 5.5 and increasing to around 8 within two years of the landfill initiating operations. An alkaline pH is usually found in landfills after depositing waste for 10 years (Farquhar, 1989), which would corroborate the findings of this study.

The electric conductivity (EC) value of the raw leachate sample was 1,562 $\mu S\ cm^{-1}$ (Table 1), double the average found by Rigueti *et al.* (2015), which, when analyzing the toxicity of leachate from a sanitary landfill located in the state of Minas Gerais, Brazil, recorded an EC average of approximately 630 $\mu S\ cm^{-1}$ in a period of one year. High EC indicates a large volume of ions present in the effluent. In general, levels above 100 $\mu S\ cm^{-1}$ indicate environmental impacts (CETESB, 2016).

Table 1. Physical and chemical characteristics of different leachate concentrations, with their respective detection limit.

Parameters	Leachate			Detection limit	CONAMA 397/2008
	100%	75%	50%		
pH	7.22	7.26	7.35	2.00	n.i.
Temperature (°C)	17.8	16.8	16.5	0.0	n.i.
BOD ₅ (mg O ₂ L ⁻¹)	39	40	44	5.0	n.i.
COD (mg O ₂ L ⁻¹)	131	92	62	3.10	n.i.
EC (μS cm ⁻¹)	1,562	1,200	852	200	n.i.
DO (mg L ⁻¹)	3.2	5.9	7.4	0.0	n.i.
Ammoniacal nitrogen (mg L ⁻¹)	36.3	28.2	18.1	5.0	20.0
Nitrite (mg L ⁻¹ N in NO ₂)	0.289	0.206	0.225	0.002	n.i.
Nitrate (mg L ⁻¹ N in NO ₃)	0.2516	0.2418	3.1063	0.0785	n.i.
Fe (mg L ⁻¹)	1.181	0.541	0.277	0.1414	15.0
Zn (mg L ⁻¹)	0.017	0.020	0.018	0.0095	5.0
Pb (mg L ⁻¹)	0.056	0.041	0.037	0.0112	0.5
Cd (mg L ⁻¹)	n.d.	n.d.	n.d.	0.0075	0.2
Ni (mg L ⁻¹)	n.d.	n.d.	n.d.	0.0643	2.0
Cu (mg L ⁻¹)	n.d.	n.d.	n.d.	0.0316	1.0

n.i. = not informed by CONAMA Resolution 397/2008.

The concentration of ammoniacal nitrogen was 36.3 mg L⁻¹, considered high, according to CONAMA Resolution 397/2008 (20.0 mg L⁻¹), with this constituting the only parameter found over the limit permitted in legislation (CONAMA, 2008). To the contrary, the concentrations of DO and metals detected were low (Table 1). In the early years, young landfills produce a leachate with greater pollutant potential, due to the presence of metals in higher concentrations. The older the landfill, the higher the index of ammoniacal nitrogen due to hydrolysis and fermentation, and the lower the concentrations of organic matter and metal ions (Renou *et al.*, 2008). In the raw leachate, these ions are associated with organic and inorganic colloidal particles and in the form of complexes. The reductive conditions of the effluent and the alkaline pH lead to metal ions being complexed in the form of sulfides, sulfates, carbonates and oxyhydroxides and also precipitated in insoluble composites considerably reducing bioavailability of the metals (Riguetti *et al.*, 2015).

At its maximum concentration, leachate reduced the germination capacity of *L. sativa*, with 86.6% of the seeds germinating. Comparably, the raw leachate from a deactivated sanitary landfill located in the region of Vale dos Sinos, in the state of Rio Grande do Sul, Brazil, also presented toxicity for this species, with 90% of the seeds germinating (Klauck *et al.*, 2015). *Lycopersicon esculentum* seeds appeared to be more sensitive than the *L. sativa* seeds, with the leachate reducing the number of germinated seeds with an increase in concentration and completely inhibited germination at a 100% concentration (Table 2).

This study recorded a significant increase in root length in the presence of leachate when compared to the controls in both plant species (Table 2). The lower concentration of leachate (50%) led to a higher average in root growth in *L. sativa* (5.70 cm) and *L. esculentum* (8.28 cm). Rodrigues *et al.* (2013) also noted root growth stimulation for *L. sativa* when analyzing the water from an urban stream located in the municipality of Alfenas, in the state of Minas Gerais, Brazil, and attributed this result to a greater offer of nutrients provided by the organic load in water.

Table 2. Values (average $\pm$ standard deviation) of germination percentage (GM), root growth (RG in cm), mitotic index (MI), chromosomal abnormality index (CAI) and micronuclei frequency (MCN) in *Lactuca sativa* and *Lycopersicon esculentum* exposed to leachate and control treatments.

Parameters		Treatments				
		Leachate			Controls	
		100%	75%	50%	H ₂ O	CuSO ₄
<i>L. sativa</i>	GM	86.66 $\pm$ 6.66 c	93.33 $\pm$ 0.00 abc	95.55 $\pm$ 3.84 ab	100.00 $\pm$ 0.00 a	88.88 $\pm$ 3.84 bc
	RG	4.24 $\pm$ 0.68 c	4.99 $\pm$ 0.85 b	5.70 $\pm$ 0.92 a	4.11 $\pm$ 1.08 c	2.00 $\pm$ 0.98 d
	MI	4.98 $\pm$ 1.13 b	5.36 $\pm$ 1.41 b	5.14 $\pm$ 1.29 b	9.58 $\pm$ 1.79 a	4.58 $\pm$ 0.52 b
	CAI	28.04 $\pm$ 11.48 b	25.68 $\pm$ 7.69 b	13.94 $\pm$ 5.72 b	13.94 $\pm$ 5.72 a	30.93 $\pm$ 8.05 b
	MCN	4.64 $\pm$ 1.23 c	3.56 $\pm$ 1.35 b	3.06 $\pm$ 0.83 ab	2.38 $\pm$ 0.51 a	4.70 $\pm$ 1.40 c
<i>L. esculentum</i>	GM	0	24.44 $\pm$ 16.77 c	51.11 $\pm$ 13.87 b	88.88 $\pm$ 10.18 a	66.66 $\pm$ 6.66 ab
	RG	n.a.	7.94 $\pm$ 1.44 ab	8.28 $\pm$ 1.15 a	6.83 $\pm$ 1.41 b	5.67 $\pm$ 1.95 c
	MI	n.a.	2.74 $\pm$ 0.66 c	4.24 $\pm$ 0.54 b	4.82 $\pm$ 0.45 a	2.10 $\pm$ 0.31 d
	CAI	n.a.	12.96 $\pm$ 5.95 a	7.29 $\pm$ 5.16 a	7.04 $\pm$ 2.82 a	21.63 $\pm$ 13.77 b
	MCN	n.a.	0.14 $\pm$ 0.16 b	0.04 $\pm$ 0.08 a	0.00 $\pm$ 0.00	0.06 $\pm$ 0.09 ab

Averages followed by the same letter on the line do not differ significantly in accord with the Duncan test at 5% probability / n.a. = not analyzed.

For both species studied, the MI was significantly lower in the different leachate concentrations and in the positive control than in the negative control (Table 2). In *Allium cepa*, besides recording negative effects from the leachate on the MI in five of the six municipal solid waste landfill sites analyzed in Southern Poland, a negative relation was noted between the physical and chemical quality of each leachate and their genotoxicity (Kwasniewska *et al.*, 2012), which corroborates with the results obtained in this study with *L. sativa* and *L. esculentum*. Alterations of the mitotic index are considered good indicators of environmental pollution, especially in the assessment of substances with cytotoxic potential (Leme and Marin-Morales, 2009).

In *L. esculentum*, CAI values in the leachate concentrations and in the negative control did not differ and were lower than those of the positive control. *Lycopersicon esculentum* chromosomes are smaller, as such, this characteristic complicated the analysis of chromosomal abnormalities for this plant species. Further, in the raw leachate treatment (100%), the *L. esculentum* seeds did not germinate, as such, the comparison with other plant species is jeopardized. *Lactuca sativa*, on the other hand, showed significantly higher CAI values in leachate concentrations and in the positive control, when compared to the negative control (Table 2). Chromosomal abnormalities (CA) are changes that occur in the chromosome structure through the action of chemical and physical agents or can even occur spontaneously (Leme and Marin-Morales, 2009).

The quantified CA were constituted by C-metaphase, isolated chromosome, or anaphase and telophase with bridges (Figure 1). A break down in the spindle apparatus is responsible for aneugenic alterations resulting from disturbances in spindle activity. The chromosomes are not correctly linked to the spindle and do not take on the correct position in the cell and, thus, they are spread throughout it internally instead. The chromosomal bridges are clastogenic alterations observed in anaphase and telophase and occur due to a loss of telomeres, the region responsible for guaranteeing chromosome protection and stability, preventing erroneous fusions and pairings (Leme and Marin-Morales, 2009).

The occurrence of MCN in *L. esculentum* was low, with the negative control not presenting them. In *L. sativa*, greater MCN frequency was noted, even registering MCN in the negative control (2.38 MCN). The highest frequencies were found when plants were exposed to raw leachate (4.64 MCN) and the positive control (4.70 MCN). We may infer that the MCNs found

in the negative control form spontaneously. For other treatments, the higher MCN frequency in relation to the control shows the toxic effect of leachate and CuSO_4 . The MCNs are tiny cell structures formed through chromosomal loss or chromosomal fragmentation caused by CA and also due to the structural alteration in the spindle apparatus fibers, which can be induced by genotoxic agents or even may be a result of spontaneous mutation (Kieling-Rubio *et al.*, 2015).

The CuSO_4 was shown to be a good positive control, as it was able to damage both plant species, observed by means of reduced germination and root growth, by the reduced number of cells in mitosis and also by the stimulation and increase of CA and MCN frequency (Table 2). Despite copper figuring as an essential micronutrient in plant development, in elevated concentrations it becomes toxic, inhibiting several plant metabolism processes (Kabata-Pendias, 2011).

Figure 1. Chromosomes with and without abnormalities observed in *Lycopersicon esculentum* (A - H) and *Lactuca sativa* (I – P) root meristematic cells exposed to different leachate concentrations. (A and I) prophase; (B and J) metaphase; (C and K) anaphase; (D and L) telophase; (E and M) C-metaphase; (F and N) metaphase with isolated chromosome; (G and O) anaphase with bridge; (H and P) telophase with bridge and MCN. Bar: 10 μm

L. esculentum germination and MI related negatively with Pb, Fe and Zn ($r=-0.728$; $p<0.001$), ($r=-0.757$; $p<0.001$), respectively, while the frequency of MCN was positively related to the same metals ($r=0.449$; $p=0.013$). In *L. sativa*, germination was also negatively related to Pb and Fe ($r=-0.720$; $p<0.001$). Root growth showed a negative relation with Pb ($r=-0.571$; $p<0.001$) and Fe ($r=-0.585$; $p<0.001$), while the frequency of MCN related positively to Pb ($r=0.570$; $p<0.001$) and Fe ($r=0.582$; $p<0.001$). Despite being considered essential micronutrients in plant metabolism (Kabata-Pendias, 2011), when Fe and Zn are present in elevated concentration in the environment, they may induce the formation of micronuclei (Kieling-Rubio *et al.*, 2015) and reduce germination and root growth (Wang *et al.*, 2011). Lead does not play an essential role in plant metabolism and it is also considered one of the most toxic metals to them. Its toxic effects are chiefly related to fundamental biological process damage, such as photosynthesis, growth and mitosis (Kabata-Pendias, 2011). This metal may delay or inhibit plant germination, as described for wheat (Lamhamdi *et al.*, 2011), while also interfering in root growth (Moraes *et al.*, 2014). The relation found between the formation of MCN and Pb was also observed in *Vicia faba* root cells, with MCN frequency used as one of the parameters to determine the toxic potential of Pb (Pourrut *et al.*, 2011). The reduction in cell division induced by Pb may be associated to this metal's link to the cell wall and membranes, increasing rigidity and defects in microtubule organization. As a result, malformation of the microtubules can lead to an increase in the number of chromosomal abnormalities (Jiang *et al.*, 2014).

4. CONCLUSIONS

The physical and chemical results indicate that the controlled landfill is at the methanogenic stage. Based on the analysis of biological parameters in different leachate concentrations, one may conclude that the effluent presents potential toxicity for *L. sativa* and *L. esculentum*, as it reduced the germination percentage and the number of mitotic divisions in both plant species, while also promoting the formation of chromosomal abnormalities and micronuclei, especially in *L. sativa*. The rise in root growth may be associated with the volume of organic matter present in the effluent. Biological parameters that best responded to the effluent toxicity were seed germination and the number of mitotic divisions, along with MCN frequency for *L. sativa*. The reduced index of CA and MCN in *L. esculentum* may be associated with the small size of chromosomes in comparison with *L. sativa*, thus jeopardizing visibility. It is also possible to infer that both plant species demonstrated being efficient biomonitors of environmental quality by showing evidence of sensitivity to the analyzed variables. However, *L. sativa* showed a more significant treatment x response relation in all analyzed biological parameters, which allowed for a unique interpretation of the results from specific leachate treatments. The relation noted between germination, root growth, MI and MCN frequency indicated a negative influence on the species studied from Pb, Fe and Zn present in the effluent. This relation reiterates the importance of associating physical and chemical analysis with an assessment of biological parameters, by means of assays using environmental quality biomonitors. Based on these findings, one may suggest that, even with a controlled SUW landfill deactivated for over a decade, there is a need to treat this effluent, with the goal of minimizing possible impacts on the environment.

5. ACKNOWLEDGEMENTS

The authors thank Universidade Feevale for the infrastructure and the Scientific Initiation Grant awarded to the first author.

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Ozonation improves physical attributes in domestic sewage effluent

ARTICLES doi:10.4136/ambi-agua.2328

Received: 13 Sep. 2018; Accepted: 24 Jan. 2019

**Osli Barreto Camilo Júnior^{1*} ; Delvio Sandri² ;
Ernandes Rodrigues de Alencar² ; Lucas Ferraz Hebling² **

¹Universidade de Brasília (UnB), Brasília, DF, Brasil

Faculdade de Agronomia e Medicina Veterinária (FAV/UnB).

Programa de Pós-Graduação em Agronomia. E-mail: oslijr@gmail.com

²Universidade de Brasília (UnB), Brasília, DF, Brasil

Faculdade de Agronomia e Medicina Veterinária (FAV/UnB). E-mail: sandri@unb.br,

ernandesalencar@unb.br, hebling123@gmail.com

*Corresponding author

ABSTRACT

Root Zone Wastewater Treatment (RZWT) is an option of natural wastewater treatment widely studied throughout the world, being a technology based on physical, chemical and biological processes. However, there are some attributes in effluent that occasionally are not eliminated by RZWT. In this context, the use of ozone may contribute to mineralization, disinfection, detoxification, and, especially, color and turbidity removal. Ozone is an excellent oxidant, with great potential to reduce color, turbidity and other attributes in liquids, such as in wastewater treatment. This paper evaluated the effect of different ozone exposure times (0, 30, 60, 90, 120 and 150 minutes) and the application of different dosages of ozone (0, 4, 10, 17, 25 and 35 mg L⁻¹ of O₃) on Hydrogeonic Potential (pH), Electrical Conductivity (EC), Dissolved Oxygen (OD), Total Alkalinity (Alk.), Sodium (Na) and Potassium (K) in domestic sewage effluent treated by the RZWT. Different exposure times and ozone dosages did not influence the attributes Potassium, Sodium, Ammonia and Total Solids. Color and turbidity decreased, while pH, Alkalinity and Dissolved Oxygen increased at all exposure times and O₃ dosages in relation to the non-ozonized effluent.

Keywords: color, ozone, root zone wastewater treatment, turbidity.

Ozonização promove a melhoria de atributos físicos em efluente de esgoto doméstico

RESUMO

O Sistema de Zona de Raízes (SRZ) é uma opção natural de tratamento de efluentes amplamente estudado em todo o mundo, sendo uma tecnologia baseada em processos físicos, químicos e biológicos. No entanto, existem alguns atributos no efluente que ocasionalmente não são eliminados pelo SZR. Nesse contexto, o uso do ozônio pode contribuir para a mineralização, desinfecção, desintoxicação e, principalmente, remoção de cor e turbidez. O ozônio é um excelente oxidante, com grande potencial para reduzir a cor, a turbidez e outros atributos em líquidos, assim como no tratamento de efluentes. O objetivo deste trabalho foi avaliar o efeito de diferentes tempos de exposição ao ozônio (0, 30, 60, 90, 120 e 150 minutos) e a aplicação de diferentes dosagens de ozônio (0, 4, 10, 17, 25 e 35 mg L⁻¹ de O₃) sobre

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Potencial Hidrogeônico (pH), Condutividade Elétrica (CE), Oxigênio Dissolvido (OD), Alcalinidade Total (Alk.), Sódio (Na) e Potássio (K) no efluente doméstico tratado pelo SZR. Diferentes tempos de exposição e dosagens de ozônio não influenciaram os atributos Potássio, Sódio, Amônia e Sólidos Totais. A cor e a turbidez diminuíram, enquanto o pH, a alcalinidade e o oxigênio dissolvido aumentaram em todos os momentos de exposição e as dosagens de O₃ em relação ao efluente não-ozonizado.

Palavras-chave: cor, ozônio, sistema de zona de raízes, turbidez.

1. INTRODUCTION

The use of septic tanks, followed by Root Zone Wastewater Treatment (RZWT), is an option of natural wastewater treatment system widely studied throughout the world, being a technology based on physical, chemical and biological processes which normally occur naturally in ecosystems, such as in natural floodplains (Kadlec and Wallace, 2009).

The main function of a wastewater treatment system is to remove the pollutant load, whether colloidal or soluble compounds. However, some attributes in the effluent occasionally are not eliminated. In this context, the use of ozone may contribute to mineralization, disinfection, detoxification, and, especially, color and turbidity removal (Bhatta *et al.*, 2015).

Today, ozone is still used for disinfection, but due to its superior oxidizing ability it is also used for a several other purposes including taste-and-odor control, color removal, iron and manganese oxidation, turbidity removal, algal removal, increasing the biodegradability of natural organic matter (NOM), and oxidation of specific micropollutants (Araújo *et al.*, 2016; Bhatta *et al.*, 2015; Bukhari *et al.*, 2019; Freitas-Silva *et al.*, 2013; Lage Filho, 2010).

Once dissolved in water, the ozone reacts with a large number of organic compounds in two different possible ways: direct oxidation, such as molecular ozone, or indirect reaction through the formation of secondary oxidants, such as free radicals, particularly the hydroxyl radical (Subha and Muthukumar, 2012; Wajahat *et al.*, 2019).

Despite the importance of ozone, it is noteworthy that the use of innovative technologies such as biogenic nanoparticles (BNPs), magnetotactic bacteria (MTB), and Phytogenic Magnetic Nanoparticles (PMNPs) is increasing in wastewater treatment, especially in the removal of heavy metals and specific compounds. In this way, studies related to ozonation should take into account all drawbacks including high cost, complex operation, and toxic and chemical reducing agents required to synthesize and control system operation (Ali *et al.*, 2017; 2018).

Considering the importance of ozonation in wastewater treatment, whether domestic or industrial, the objective of this paper was to evaluate the application of different dosages and exposure times to ozone on physical and chemical attributes in domestic sewage effluent, obtained after Root Zone Wastewater Treatment (RZWT).

2. MATERIAL AND METHODS

The Root Zone Wastewater Treatment (RZWT) is located at the Água Limpa Farm (ALF) of the University of Brasília (UnB) (15°57'16" S, 47°55'89" W and altitude of 1.103 m). The Laboratory of Water Analysis and the Laboratory of Storage and Preprocessing of Agricultural Products of the Faculty of Agronomy and Veterinary Medicine (UnB) performed the analyses of the effluent before and after applying ozonation.

The effluent generated at the Água Limpa Farm is mostly from sanitary discharges and a refectory, which, for its composition, is characterized as domestic effluent. The daily volume

generated is quite variable due to its frequency of use and oscillation of amount of people who attend the ALF, and it is not equal among the days of the week.

The RZWT consists of a set of four equal units built in rectangular fiberglass structures, with dimensions of 2.5 m (width), 6.5 (length) and 0.5 m (height). The first unit was cultivated with taboa (*Typha spp*); the second with Brazilian Papyrus (*Cyperus Giganteus*); the third with umbrella papyrus (*Cyperus alternifolius*), and a fourth system without a plant (witness).

An ozone generator O&LM, Ozone & Life® brand generated the ozone gas. In the process of gas generation, oxygen 90% pure and humidity free was used as input, obtained from the Mark Plus Oxygen Concentrator.

In order to produce the ozone gas (O_3), the oxygen went through a refrigerated reactor, in which the Dielectric Barrier Discharge (DBD) occurs. This type of discharge consists in applying a high voltage between two parallel electrodes, having between them a dielectric (glass) and a free space. In this free space, a discharge is produced in the form of filaments, where electrons are generated with sufficient energy to produce the breakdown of the oxygen molecules, forming the ozone (O_3) (Lage Filho *et al.*, 2008).

To evaluate the effect of exposure times of ozonation (0, 30, 60, 90, 120 and 150 minutes) and to optimize the analyses, two glass containers were used in each stage, where O_3 was equally divided. The containers were hermetically sealed and had 10 cm of internal diameter and 20 cm of useful height, having in its plastic cover, a porous diffuser through which the gas was introduced in a flow of 1.0 L min^{-1} .

The first two treatments were the exposure times of 30 and 90 min (one in each container). As soon as the time of 30 min finished, it was replaced by the 60 min treatment, thus, at the end of the 90 min, four treatments had already been completed (0 - witness, 30, 60 and 90 min). The last two treatments (120 and 150 min) followed the same sequence. At the end of the 120 min treatment, this container was replaced with distilled water until the completion of the experiment, i.e., for another 30 min until the treatment of 150 min was completed.

For the treatment with different dosages of ozone (0, 4, 10, 17, 25 and 3 mg L^{-1} of O_3), only one container was used. The treatments performed consecutively from the lowest to the highest dosage. The first effluent sample was exposed to a dosage of 4 mg L^{-1} of O_3 for 30 min, the second sample was added and the dosage was increased from 4 to 10 mg L^{-1} which remained for more 30 min. The dosages of 17, 25 and 35 mg L^{-1} of O_3 followed the same sequence.

In order to carry out the laboratory analyses, the methodologies of analysis of the Standard Methods for the Examination of Water and Wastewater (APHA *et al.*, 2005) were used or adapted. APHA Method no. 4500-0 (for Dissolved Oxygen); no. 2320 (for Total Alkalinity); no. 2130 (for Turbidity); no. 2120 (for Color); no. 2540 (for Solids); no. 4500 – NO_3 (for Ammonia); no. 3500 – Na (for Sodium); and no. 3500 – K (for Potassium). To apply different exposure times to O_3 , four collection campaigns were carried out in November 2017, and to evaluate the different dosages of O_3 , three collection campaigns were carried out in December 2017. In each campaign, 6 L of effluent in sterilized containers were collected, always at 1 p.m. due to higher volume of sewage at this time. The containers were packed in ice packs, which were transported to the laboratory and refrigerated at 4°C (APHA *et al.*, 2005).

The results of the experiments were analyzed using the Software Assistant (Silva and Azevedo, 2016), applying the Duncan test at 1 and 5% of probability, considering the collection dates as the replicates.

3. RESULTS AND DISCUSSION

3.1. Potential Hydrogen (pH)

There was an increase in pH values in the effluent samples, either as a function of exposure times due to the variation of the ozone dosages (Figure 1).

The pH values of the effluent exposed to the times of ozonation were higher than the effluent without ozonation, being the largest of 8.38 and the lowest of 7.46 (without ozonation). The time of 30 min differed from the times of 90, 120 and 150 min. A variation of 8.7% might be observed for the time of 30 min, up to 12.3% for the times of 120 and 150 min, demonstrating that the greatest variation occurred up to 30 min, after the beginning of O₃ application (Figure 1).

Figure 1. pH values of the domestic sewage effluent as a function of the exposure times 0, 30, 60, 90, 120 and 150 min at the dosage of 12 mg L⁻¹ of O₃ and to the dosages of 0, 4, 10, 17, 25 and 35 mg L⁻¹ of O₃ for 30 min and variation, in %, in relation to the effluent without ozonation. Different letters between the times of ozonation or O₃ dosages differ from each other by the Duncan test at 1% probability.

As the O₃ dosages varied, the pH values increased, reaching 8.14 (10 mg L⁻¹ of O₃), differing from the effluent without ozonation, but equal to the other dosages. After the dosage of 10 mg L⁻¹ of O₃, a reduction in the pH values was observed until the highest dosage evaluated (35 mg L⁻¹ of O₃), but not significantly (Figure 1). The pH drop from the 25 mg L⁻¹ O₃ dosage can be attributed to the formation of short chain acid compounds (acetic and oxalic acid, for example), resulting from the partial oxidation of more complex substances promoted by ozone as observed by Aquino and Pires (2016).

As ozone acts on the effluent, there is the release of hydroxide ions (OH⁻) making these predominant over hydrogen H⁺ ions. When the pH reaches 8.0, practically half of the O₃ introduced is decomposed into several intermediate forms of oxygen, over a period of 10 min (Lage Filho *et al.*, 2008; Coelho *et al.*, 2015).

This fact may explain the increase in pH values in the effluent samples with variation of the exposure time. When the pH value of the effluent is above 8.0, most of the ozone is decomposed into OH⁻, which induces an increasingly alkaline condition, interfering with the attributes of the effluent sample.

Bhatta *et al.* (2015) observed a slight increase in pH values in their study on the treatment of wastewater by ozone produced in dielectric barrier discharge, in agreement with the results found here. Likewise, Silva and Daniel (2015) observed an increase in pH values ($p = 0.05$) for the ozonized effluents compared to the UASB reactor effluent.

3.2. Electrical Conductivity (EC) and Total Alkalinity

Ozonation promoted a significant reduction in EC values as a function of time of ozonation and a reduction of Alkalinity as a function of O₃ dosages (Figure 2).

As the time of exposure of the effluent to the O₃ increased, there was a reduction of 7.5% in the EC values at 120 min compared to the effluent without exposure to O₃. The grand mean

of the treatment for different ozone dosages was $1281 \mu\text{S cm}^{-1}$, with values ranging from 1295 (0 mg L^{-1} of O_3) to 1272 (17 mg L^{-1} of O_3), but not differing between dosages.

Regarding total alkalinity, the grand mean obtained by the different exposure times was 56 mg L^{-1} of CaCO_3 , with values ranging from 53 to 59 mg L^{-1} of CaCO_3 , showing no significant difference by the 1% Duncan test.

Figure 2. Electrical Conductivity of the domestic sewage effluent as a function of the exposure times of 0, 30, 60, 90, 120 and 150 min at the dosage of 12 mg L^{-1} of O_3 , and total alkalinity as a function of the time of exposure of the effluent at the dosages of 0, 4, 10, 17, 25 and 35 mg L^{-1} of O_3 for 30 min and variation, in %, in relation to the effluent without ozonation. Different letters between times of ozonation or O_3 dosages differ from each other by the Duncan test at 1% probability.

In the treatment as a function of the O_3 dosages, there was a reduction in the alkalinity values with the increase in the ozone dosages up to 17 mg L^{-1} , tending to stabilize from this dosage. The grand mean in the treatment was 52.33 mg L^{-1} of CaCO_3 , with values ranging from 46.67 mg L^{-1} (17 mg L^{-1} of O_3 dosage) to 60 mg L^{-1} (without ozonation), an average reduction of 22.2% (Figure 2).

Considering the basic pH values in both treatments, the buffering power (alkalinity) of the effluent treated with O_3 is adequate. It is worth highlighting that alkalinity is not the standard classification for natural waters or emission of sewage, showing the importance of this parameter in the control of certain unit processes used in water treatment plants (Von Sperling, 2014).

3.3. Dissolved Oxygen (DO)

The Dissolved Oxygen (DO) values were higher at all times of ozonation and at all O_3 dosages compared with the effluent without ozonation (Figure 3).

The grand mean in the treatment as a function of time of exposure to O_3 was $15.16 \text{ mg O}_2 \text{ L}^{-1}$, with values ranging from 5.5 to $19.3 \text{ mg O}_2 \text{ L}^{-1}$ for 0 and 90 min, respectively, representing elevation of 351%. While at the exposure times of 30, 60, 120 and 150 min the increases were 253, 282, 349 and 318%, respectively (Figure 3).

The grand mean when varying the O_3 dosages was $21.28 \text{ mg O}_2 \text{ L}^{-1}$, with values ranging from 6.2 to $26.2 \text{ mg O}_2 \text{ L}^{-1}$. There was an increase in DO values of 424% (17 mg L^{-1} O_3) and 413% (25 mg L^{-1} O_3) in relation to the effluent without ozonation.

Figure 3. Dissolved Oxygen (mg O₂ L⁻¹) of the domestic sewage effluent as a function of times of the exposure of 0, 30, 60, 90, 120 and 150 min at the dosage of 12 mg L⁻¹ of O₃ and as a function of the dosages of 0, 4, 10, 17, 25 and 35 mg L⁻¹ of O₃ for 30 min and variation, in %, in relation to the effluent without ozonation. Different letters between times of ozonation and O₃ dosage differ from each other by the Duncan test at 1% probability.

The significant increase in DO as a function of the time of exposure and to the O₃ dosages is explained by the fact that the O₃ applied to the effluent is rapidly converted into O₂, raising its concentration in the environment. This phenomenon was also observed by Bhatta *et al.* (2015) where the DO values of the effluent, after the treatment with ozone, increased significantly, reaching the value of 9.55 mg O₂ L⁻¹ in that study.

3.4. Turbidity

There was a decrease in the turbidity values in the effluent samples both in relation to the exposure times and in relation to O₃ dosages (Figure 4).

Figure 4. Turbidity in NTU of domestic sewage effluent treated as a function of the exposure times of 0, 30, 60, 90, 120 and 150 min at the dosage of 12 mg L⁻¹ of O₃ and as a function of the dosages of 0, 4, 10, 17, 25 and 35 mg L⁻¹ of O₃ for 30 min and variation, in %, in relation to the effluent without ozonation. Different letters between times of ozonation and O₃ dosages differ from each other by the Duncan test at 1% probability.

The grand mean in treatment as a function of time of exposure was 6.2 NTU, being the highest value 8.63 and the lowest 5.1 NTU (0 and 120 min of exposure), representing a reduction of 40.0%.

As a function of the O_3 dosages, the grand mean of turbidity was 5.8 NTU, representing a reduction of 23.42% in the dosage of $mg\ L^{-1}$ of O_3 , and of 52.43% and 58.86% in the dosages 25 and 35 $mg\ L^{-1}$ of O_3 , respectively (Figure 4).

In this study, the greatest reductions in turbidity values were 40.0% and 58.9% at 120 min of exposure and 17 $mg\ L^{-1}$ of O_3 , respectively. Cabrera-Díaz *et al.* (2016) observed higher turbidity removal values, reaching 99.2% in combined treatment of vinasse (anaerobic filter-reactor and ozonation process); however, using a concentration of 100 $mg\ L^{-1}$ of O_3 and a contact period of 3 hours.

The reason for the decreasing values of turbidity while increasing ozone dosages and exposures times are that some particles and high weight organic compounds would be destroyed by ozone and converted into dissolved compounds like observed by Xu *et al.* (2002).

The reduction in turbidity values were positive, considering there was a reduction close to 60.0% in the dosage of 17 $mg\ L^{-1}$ of O_3 for 30 min. On the other hand, Li *et al.* (2015), in a study on biofiltration and ozonation for effluent treatment, observed a turbidity reduction of 90.0% using a dosage of 5 $mg\ L^{-1}$ of O_3 and a flow of $1.5\ m^3\ h^{-1}$, respecting local limits for the discharge of urban effluents into water bodies.

3.5. Color

There was a reduction in color values at all exposure times and at all ozone dosages (Figure 5).

The grand mean of the color as a function of exposure times to O_3 was 96.0 of standard APHA color with progressive reduction, reaching 49.7 at 120 min of exposure. In percentage, the reduction was 47.0% at 30 min and 76.2% at 120 min

Regarding the treatment with different dosages of ozone, there was a reduction of 67.7% at the dosage of 4 $mg\ L^{-1}$ of O_3 and of 81.5% at the dosage of 17 $mg\ L^{-1}$ of O_3 (Figure 5).

Figure 5. Standard APHA color (dimensionless) values of treated domestic sewage effluent as a function of the exposure times of 0, 30, 60, 90, 120 and 150 min at the dosage of 12 $mg\ L^{-1}$ of O_3 and as a function of the dosages of 0, 4, 10, 17, 25 and 35 $mg\ L^{-1}$ of O_3 for 30 min and variation, in %, in relation to the effluent without ozonation. Different letters between times of ozonation and dosages of O_3 differ from each other by the Duncan test at 1% probability.

The greatest color reductions (76.2% and 81.5%), are corroborated by Cabrera-Díaz *et al.* (2016) and Li *et al.* (2015), in which Cabrera-Díaz *et al.* (2016) observed color removals of 93.7% (UPt-Co) in a combined treatment of vinasse (anaerobic filter-reactor and ozonation process) emphasizing the effectiveness of the ozonation process in the color and turbidity

removal of this residue. Li *et al.* (2015), on the other hand, using biofiltration and ozonation for effluent treatment, observed a general efficiency for color removal of 70.0%, similar to the one obtained in the present study. Bhatta *et al.* (2015) observed visually, in their study on the treatment of wastewater by ozone, that the ozonized samples went from a dark gray color to a light color, close to the standard color of the water.

3.6. Ammonia, Sodium, Potassium and Total Solids

The average values of ammonia, sodium, potassium and total solids were not influenced as a function of exposure times and O₃ dosages.

Considering the average values, there was a tendency of ammonia elevation in absolute values, and there was no statistical difference, attributed, in part, to the great variability of the results, i.e., there was no consistent evidence that the time of ozonation and the O₃ dosages have influence on the ammonia contents.

Regarding sodium and potassium, there was great homogeneity of the values, both as a function of time and of O₃ dosages, i.e., parameters that were not influenced by ozonation.

The total solids, although showing some variations between the exposure times and O₃ dosages, did not present significant differences, leading to the conclusion, as well as in the parameters ammonia, sodium and potassium, that ozonation does not influence their values.

4. CONCLUSIONS

Ozonation reduced color and turbidity values both as a function of exposure times and as a function of O₃ dosages. There was a maximum reduction of 76.2% in color at 120 min of exposure and of 81.5% in turbidity at the concentration of 17 mg L⁻¹ of O₃.

There was an increase in pH, Total Alkalinity and Dissolved Oxygen (OD) values. At 120 min of exposure, there was a 12.30% increase in pH and at the dose of 4 mg L⁻¹ of O₃ the increase was 9.4%. Alkalinity increased 22.2% at the dose of 17 mg L⁻¹ of O₃, whereas OD values increased 351% at 90 min of exposure and 424% at the dose of 17 mg L⁻¹ of O₃.

Potassium, sodium, ammonia and total solids were not influenced by exposure time and ozone dosages.

5. ACKNOWLEDGMENTS

To CNPq for the financial assistance. Universal Call - MCTI / CNPq N° 14/2013. Process: 480332 / 2013-4.

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Frequency of mentum deformity in *Chironomus sancticaroli* (Diptera: Chironomidae) in a laboratory culture

ARTICLES doi:10.4136/ambi-agua.2337

Received: 01 Oct. 2018; Accepted: 01 Feb. 2019

Mariana Silveira Guerra Moura e Silva^{1*} ; Ana Lúcia Silva Marigo²
William Viveiros³ ; Mônica Luisa Kuhlmann³

¹Embrapa Meio Ambiente (EMBRAPA), Jaguariúna, SP, Brasil

Laboratório de Ecossistemas Aquáticos. E-mail: mariana.silveira@embrapa.br

²Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brasil

Instituto de Geociências (IGE). E-mail: analuciamarigo@yahoo.com.br

³Companhia Ambiental do Estado de São Paulo (CETESB), São Paulo, SP, Brasil

Divisão de Análises Hidrobiológicas. E-mail: wviveiros@sp.gov.br, mkuhlmann@sp.gov.br

*Corresponding author

ABSTRACT

The midge *Chironomus sancticaroli* (Diptera: Chironomidae) has been used in ecotoxicological tests because it is sensitive to a variety of inorganic pollutants. Among the parameters used to evaluate the toxicity of a substance is the frequency of mentum deformity, which is part of the oral system of this organism. However, there is still no consensus on the baseline level (percentage) of acceptable deformities in laboratory cultures not exposed to pollutants. The determination of this variable is important to ensure the validity of bioassays and to compare cultures from different research and teaching institutions. Once this value is established, it will also be used to monitor the quality of organisms cultured, since factors such as inbreeding could increase the frequency of mentum deformity. Thus, the objective of this study was to quantify the percentage of mentum deformity in the fourth instar of *C. sancticaroli* larvae from the culture of the Laboratory of Aquatic Ecosystems, at Embrapa Meio Ambiente. The average frequency of mentum deformity obtained was 6,63%. It is believed that factors such as the renewal of the culture with the inclusion of spawns from the laboratories of other institutions, as well as the control of the quality of the dilution water and the sediment of the breeding may have contributed to a low frequency of mentum deformity of the culture observed in this study.

Keywords: bioindicator, ecotoxicology, inbreeding.

Estudo para determinação da frequência de deformidade do mento em *Chironomus sancticaroli* (Diptera: Chironomidae) em cultura de laboratório

RESUMO

A espécie *Chironomus sancticaroli* (Diptera: Chironomidae) tem sido utilizada em testes ecotoxicológicos por ser sensível a uma variedade de poluentes inorgânicos. Um dos parâmetros utilizados para avaliar a toxicidade de uma substância é a frequência de deformidade

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do mento, que faz parte do sistema oral deste organismo. Entretanto, ainda não há consenso a respeito do nível basal (porcentagem) de deformidade aceitável em culturas de laboratório não expostas a poluentes. A determinação desta variável é importante para assegurar a validade de bioensaios e comparar culturas de diferentes instituições de pesquisa e ensino. Uma vez estabelecido, este número também será usado para o controle da qualidade dos organismos criados, já que fatores como o endocruzamento poderiam aumentar a frequência de deformidade do mento. Assim, o objetivo deste estudo foi quantificar a porcentagem de deformidade do mento em larvas de quarto instar de *C. sancticaroli* de cultura do Laboratório de Ecossistemas Aquáticos da Embrapa Meio Ambiente. A média de frequência de deformidade obtida para a cultura foi de 6,63%. Acredita-se que fatores como a renovação da cultura com a inclusão de desovas de laboratórios de outras instituições, assim como o controle da qualidade da água de diluição e do sedimento da criação, podem ter contribuído para uma baixa frequência de deformidade do mento da cultura.

Palavras-chave: bioindicadores, ecotoxicologia, endocruzamento.

1. INTRODUCTION

Aquatic insects of the family Chironomidae (Diptera) are widely used as bioindicators of environmental impacts, because their entire larval cycle occurs in sediment, where they interact with contaminants that tend to accumulate in this compartment of the aquatic ecosystem. Furthermore, they are ubiquitous in distribution, sensitive to various pollutants and present a relatively short life cycle, being ideal candidates for ecotoxicological studies (Park and Choi, 2009). They are also indicated for ecotoxicological studies because they present rapid responses and easy manipulation of the cultures (USEPA, 2000; OECD, 2007). They present varieties of feeding habits, from the filtration of particles in the water column to the predation of small organisms.

The genus *Chironomus* (Diptera, Chironomidae) is an organism of great importance in the group of benthic bioindicators. It plays a relevant role in the food chain and in sediment nutrient cycling. Further, it is easily cultured in the laboratory. Its larval cycle is aquatic, in direct contact with the sediment, where it remains buried. The life cycle of the individuals of the genus *Chironomus* comprises four phases: egg, larval period (4 stages), pupa, and adult. Soon after the egg hatches, in the first stage, the larva remains between the sediment particles and feeds on detritus (*Chironomus* is classified as a gathering collector), but can also be planktonic. From the second stage, the larvae construct cocoons that shelter them and increase in size, until reaching the stage of pupa and soon after, adult, which is terrestrial (Bonani, 2010). They are found in most aquatic environments and can be very tolerant to extreme environmental conditions such as variations in pH, temperature, dissolved oxygen, salinity, depth, and speed of currents. In order to survive in habitats with low concentrations of dissolved oxygen, the larvae present hemoglobin, which gives it a reddish coloration, long abdominal gills and undulating movement (Viveiros, 2012).

Sanseverino and Nessimian (2008) describe that several genera of the family Chironomidae are reported in studies of deformities, and the genus *Chironomus* (Meigen 1803) is the most-cited. Comparing the incidence of deformities in many genera of Chironomidae in Canadian lakes, Hudson and Ciborowski (1996b) suggested *Chironomus*, *Phaenopsectra* and *Procladius* because of their wide distribution and sensitivity to the formation of deformities. In the cephalic capsules of the larvae of *Chironomus* several sensorial structures and mouthparts are inserted; one of them is the mentum. They are composed of chitin which is a biological component of high strength.

Chironomus may present deformities in mentum when exposed to radiation-contaminated

sediments (Williams *et al.*, 2001), heavy metals (Callisto *et al.*, 2000; Deliberalli *et al.*, 2018), pesticides (Hamilton and Saether, 1971), among others. These same authors report high frequencies of deformity in the chironomids in their different larval instars, evidencing such structure as one of the most sensitive and important for use in biomonitoring. Such deformities can be of different types and intensities, including lack or excess of teeth, gap, bifurcation of the central tooth, asymmetry, wear and break. To apply the metric as a quality criterion, CETESB (2017) standardized the data acquisition in order to minimize subjectivity considering as deformities missing and excessive teeth, Kohn gap and bifurcation of the central tooth. In these field studies, deformity is observed as an effect of toxic substances, however, there is no national or international standard protocol for its use as an endpoint in ecotoxicological bioassay (Viveiros, 2012).

At the individual level, deformities do not impair emergency, but they decrease body size and retard development. Further bioaccumulation may occur in deformed individuals. Individual differences reflect microhabitat conditions and genetic differences linked to resistance mechanisms (Janssens de Bisthoven *et al.*, 1998). Studies suggest that some types of deformity are related to the inbreeding and age of laboratory-maintained creations (Vogt *et al.*, 2013). Besides that, there are few published studies with *C. sancticaroli* for deformity in culture. However, there is no consensus on the rate of acceptable baseline deformity in laboratory for *Chironomus* cultures. These data are important for the assessment of test organism fitness before their use in ecotoxicological tests in order to determine the level of deformity.

This study evaluated the incidence of mentum deformities in individuals of *Chironomus sancticaroli* in cultures maintained in the Laboratory of Aquatic Ecosystems at Embrapa Meio Ambiente.

2. MATERIAL AND METHODS

2.1. Culture conditions:

C. sancticaroli egg masses from Laboratory of Aquatic Ecosystems (LEA) – Embrapa Meio Ambiente were placed into plastic trays of approximately 30 x 37 x 8 cm containing ~ 1 cm of sand washed and calcinated in muffle for total elimination of organic matter (Dornfeld, 2006). The water used was sterilized and reconstituted in the laboratory (Jonsson and Maia, 1999), with controlled aeration and temperature ($25^{\circ}\text{C} \pm 1^{\circ}\text{C}$) and photoperiod 12h light / 8h dark. The water column was aerated through porous stone and air mini compressor. The newly hatched larvae were fed once daily with Tetramin[®], according to the concentration indicated in Fonseca and Rocha (2004) until reaching the last larval stage (4th instar).

2.2. Bioassay procedures:

After reaching the 4th instar, 454 larvae were randomly separated, on different dates, from the same culture to prepare microscope slides with the cephalic capsules, with an average size of 0.32 ± 0.02 mm, corresponding to this larval stage, according to Strixino and Strixino (1982). The cephalic capsule was separated from the larvae body using a fine-pointed forceps and mounted in slides with ventral part upward in the Hoyer medium. That medium is composed of gum arabic, chloral hydrate, glycerol and water and is used to make semi-permanent slide mounts for examination of the cephalic capsule under microscope. For the calculation of deformity frequencies, mentum was considered deformed when presenting: exceeding or missing teeth, central teeth bifurcation or Kohn gap. The last one is a type of deformity where a cavity is formed in the tooth. It differs from a break by having smooth edges. The frequency of deformities was expressed as a percentage of the total of analyzed larvae. Four batches of larvae were analyzed under the same laboratory conditions at different dates: 10/04/17; 11/06/17 e 12/07/17 and 12/12/18.

3. RESULTS AND DISCUSSION

A total of 454 fourth instar larvae of *Chironomus sancticaroli* from the Laboratory of Aquatic Ecosystems of Embrapa Meio Ambiente were analyzed, and an average frequency of 6.63% of mentum deformity was found (Figure 1). The types of deformity found were: “missing teeth”, “excess teeth”, “gap” and “central tooth bifurcation” (Figure 1).

Figure 1. Example of mentum deformity found at *Chironomus sancticaroli* culture from Laboratory of Aquatic Ecosystems, at Embrapa Meio Ambiente. Normal tooth (A) and mentum with absence of lateral teeth deformity type (B). Microscope amplification of 100x.

For field data (larvae in the natural environment), Burt *et al.* (2003) defined the frequency of deformities on *Chironomus* population collected in reference sites of the Great Lakes as $2.1 \pm 0.2\%$, but the authors considered that for rivers, streams and bogs the basal value could be less than 1%. Hudson and Ciborowski (1996b) also observed smaller percentages of mentum deformity at sites considered as "reference" ones (not impacted). Considering the quality criteria stated by CETESB (CETESB, 2017), frequencies above 6% mean that the site has bad quality and probably receives chemical contaminants. In laboratory assays, larger percentages than the present study have been observed in control condition. In test control, Hudson and Ciborowski (1996a) observed until 7.4% of deformity for *C. salinarius*; a very similar result (until 7.3%) was obtained by Arambourou and coworkers (2015) for *C. riparius*; Kwak and Lee (2005) found 18.2% for *C. plumosus*, Bonani (2010), when exposing organisms of *C. sancticaroli* to the test control sediment obtained a mean deformity of 20.2% and 13% at Viveiros (2012). On the other hand, Dornfeld (2006); evaluating the toxicity of cadmium and copper in *C. xanthus* assays, observed only 2.0% deformity in control larvae. In laboratory culturing, Rebechi and Silva (2012) observed 9% of mentum deformity in *C. sancticaroli* (instar II) reared in standard conditions and 48% in III instar, while Viveiros (2012) observed an average of 13% for the same specie (IV instar). Despite these data, the criteria used for field studies should not be applied directly to laboratories tests, because the environmental conditions are different.

Long time chironomid cultures generally exhibit high levels of inbreeding, and this could impact the reliability of ecotoxicological tests using this strains. The introduction of new cultures from other laboratories, and also the use of field-collected strains are alternatives to reduce inbreeding (Nowak *et al.*, 2007).

Vogt *et al.* (2013) and Bird *et al.* (1995) have highlighted the importance of inbreeding in the level of mouthpart deformities in *C. riparius* larvae. According to them, unpredictable environmental variables may have a greater influence on the approach to field studies, whereas population effects (e.g. inbreeding) may be more pronounced in laboratory studies. These same authors observed a direct relation between the increase of deformities of the mentum in *C.*

riparius with the increase of inbreeding. Sanseverino and Nessimian (2008) mentioned increased central tooth deformities in the 4th and 6th generations of their cultures, associating the fact with inbreeding. Thus, field populations of *C. riparius* have greater genetic diversity than the laboratory populations, being less subject to the deformity of the mentum induced by the stressors. Vermeulen *et al.* (2000) also found a high incidence of deformities in their cultures and in the controls of their tests (from 34 to 56%) and concluded that the crossing between relatives in the cultures is the main thing responsible for the high indexes found. The same condition was observed by Bird (1997) and Janssens de Bisthoven (2001) including the organisms age and no renovation of the cultures. Culture condition can also promote deformities, as observed by Madden *et al.* (1992). These researchers obtained high deformity frequencies (20 – 46%) in larvae maintained in artificial substrate (Martin's solution) but diminished frequency (19%) when natural water was used in the culture. On the other hand, Dickman *et al.* (1992) observed a reduction of mentum deformity in organisms maintained in a clean sediment after collecting them in contaminated sites with HPA's. According to Jeyasingham and Ling (1997), the origin of the organisms and the substrate that is used in the cultures can influence the frequencies of mentum deformity, but it is possible to reduce them until F2 generation.

Further study still has to be performed in order to define if the level of deformity found is in this experiment can be considered low, and if it may be related to the fact that the culture of Embrapa Meio Ambiente received spawnings from other institutions, reducing inbreeding within the population. A further aspect in this context concerns inheritance. The insertion of new spawns, preferably from other laboratories, also brings the advantage of renewing culture from the point of view of its age. Sanseverino and Nessimian (2008) observed that the presence of some mentum deformities (split of median tooth, multiple teeth) was related to the age of the culture for *Chironomus tentans*, because of inbreeding.

In addition, the control of water and sediment quality variables (using reconstituted water and calcinated sand) may contribute to the low percentage of deformity found. Nevertheless, it is important to evaluate and monitor the fluctuation of the deformity in this culture in order to be able to use this parameter (frequency of mentum deformity) in assessment of the health of organisms and for detecting the need for culture renewal.

4. CONCLUSIONS

The cultures of *Chironomus sancticaroli* of Embrapa Meio Ambiente showed low frequency of deformities when compared to other studies, suggesting that the procedures developed have been effective in prevent inbreeding effects.

Evaluating and monitoring the fluctuation of mentum deformity in laboratory culture is important in order to be able to use this parameter in the evaluation of the health of the organisms and to detect the need for culture renewal.

Further studies are required in order to define the acceptable level of deformity in *Chironomus sancticaroli* raised in laboratory which can ensure the reliability of the culture.

It is interesting to perform genetic tests to quantify inbreeding among insect populations cultured in laboratory.

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