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Decision support system for selecting sectoral data-bases in studies of the water–energy–agricultural–environmental nexus

Sistema de apoio à decisão para seleção de bases de dados setoriais em estudos Nexus água–energia–agricultura–meio ambiente

Cássia Juliana Fernandes Torres[1], *Rodrigo Saldanha Xavier da Silva*[1], *Andrea Souza Fontes*[2], *Daniel Veras Ribeiro*[1], *Yvonilde Dantas Pinto Medeiros*[1]

ABSTRACT

Obtaining databases to develop multidisciplinary studies in complex intersectoral network systems presents great challenges. Databases often lack compatibility or data standardization because they are organized differently by sector. Therefore, this article aims to propose a Decision Support System (DSS) to assist in the identification, analysis, and selection of sectoral databases to support the development of quantitative studies. The concept of the "Nexus of water, energy, agriculture, and the environment" is used to illustrate the development of the DSS. To this end, a conceptual structure defined in six stages was presented: institutional analysis, definition of alternatives, definition of criteria, analysis of databases, classification matrix, and organization and selection of alternatives. Validation of the proposed DSS was carried out using national-scale databases for the Brazilian context. From the application of DSS in the databases surveyed, it appears that: Brazil does not have interconnected databases, nor does it share databases between sectors; the information is dispersed across a large number of institutions, and includes a multiplicity of spatial and temporal scales, hindering their integration; the adoption of macro-scales, both spatially and temporally, facilitates the integration of the collected information, and the country's sectoral organizational structures tend to hamper the development of systems integrated into complex networks. The proposed DSS allows a better understanding and visualization of possible simplifications and limitations inherent in integrated studies of quantitative scope, minimizes uncertainties, and directs systemic planning and management strategies.

Keywords: intersectoriality; natural resources; integrated management.

RESUMO

A obtenção de bases de dados para auxiliar o desenvolvimento de estudos multidisciplinares em sistemas de redes complexas intersetoriais apresenta grandes desafios devido à falta de compatibilização e nivelamento entre as informações, uma vez que estão segmentadas de diferentes formas nos setores correlacionados. Diante disso, o presente artigo tem por objetivo propor um Sistema de Apoio à Decisão (SAD) para auxiliar na identificação, análise e seleção de bases de dados setoriais, visando subsidiar o desenvolvimento de estudos quantitativos mediante o conceito "Nexus água, energia, agricultura e meio ambiente". Para tanto, foi apresentada uma estrutura conceitual definida em seis etapas: análise institucional, definição das alternativas, definição dos critérios, análise das bases de dados, matriz de classificação, e organização e seleção das alternativas. A validação do SAD proposto foi realizada por meio das bases de dados de âmbito federal presentes no Brasil. Após a aplicação do SAD nas bases de dados levantadas, verifica-se que: até o momento, o Brasil não possui bases de dados interconectados e compartilhados entre diferentes setores; as informações concentram-se em uma grande quantidade de instituições e contemplam uma multiplicidade de escalas espaciais e temporais, dificultando suas interações; a adoção de macroescalas, espacial e temporal, facilita a integração das informações levantadas; e as estruturas organizacionais setoriais do país tendem a inviabilizar o desenvolvimento de sistemas integrados em redes complexas. O SAD proposto permite uma melhor compreensão e visualização de possíveis simplificações e limitações inerentes em estudos integrados, de âmbito quantitativo, minimizando incertezas e direcionando estratégias sistêmicas de planejamento e gestão.

Palavras-chave: intersetorialidade; recursos naturais; gerenciamento integrado.

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Introduction

The nexus concept refers to the interconnected and interdependent management between the water, energy, agricultural, and environmental sectors. It is based on the good governance of each of these sectors, and provides for the design and implementation of more efficient and sustainable policies and actions (Hoff, 2011; WEF, 2011; Bamwesigye et al., 2019).

The nexus concept was initially promoted in 2008, at the World Economic Forum (2011). It was developed based on the need to better understand the interrelations and interdependencies that exist between the sectors in question so as to better manage them. The initiative came about through the efforts of the "2030 Water Resources Group (WRG)," a group of multinational companies in the food and beverage sector which were concerned about the impacts of the water crisis on their global operations (Leese and Meisch, 2015). The WRG prepared the report, "Water security: the water-food-energy-climate nexus," which directed global attention toward these four main pillars of the world economy and their interrelationships (Bizikova et al., 2013; Leese and Meisch, 2015).

Water is necessary for each stage of energy production, and energy is essential for the functioning of the sanitation sector (Maas et al., 2017; Meldrum et al. 2013; Walker et al., 2014; Chang et al., 2016; Sanders and Masri, 2016). The energy sector is highly vulnerable to changes in water resources, especially those that may result from climate change (Meldrum et al., 2013). Both water and energy are inputs for the agricultural sector (Lawford et al., 2013; Chang et al., 2016; Urbaniec et al., 2017); agricultural products provide bioenergy (Wu and Chiu, 2011; Moioli et al., 2018), and the consumption of all these resources has a negative impact on the environment (Wicaksono et al., 2017).

Through the recognition of these interrelationships and their relevance for the structuring of shared intersectoral management, the "nexus" theme has gained increasing interest in academic literature, as evidenced by the growing body of technical and scientific research (Baleta et al., 2019; Meng et al., 2019; Zhang et al., 2019). The greater level of global interest and visibility that surrounds this theme may be attributable to a lack of natural resource security, increasing climatic imbalances, and the recession of the global economy (Allouche et al., 2015; Leese and Meisch, 2015; Al-Saidi and Elagib, 2017). These factors have prompted the nexus concept to emerge as a new paradigm for the public management of natural resources (Torres, 2020).

Since its emergence, research on the nexus concept can be observed across three thematic areas (Torres et al., 2019):

- qualitative studies that recognize and understand the interactions between the different nexus elements (water, energy, agriculture, climate, land, etc.) in different contexts and across scales;
- quantitative studies that evaluate the interconnections and interdependencies between correlated nexus elements;
- socio-political and economic research that analyzes governance models and public policies to outline the nexus concept in terms of practical issues related to management and planning.

As the authors pointed out, the major challenges for conducting quantitative research are the lack of available data on the water, energy, agricultural, and environmental sectors, and the lack of capable models, methods, and instruments to represent the multiple interactions that exist between them (King and Carbajales-Dale, 2016; Albrecht et al., 2018; Embid and Martín, 2018; Nhamo et al., 2018; Shannak et al., 2018; Mercure et al., 2019).

An ideal model or method to represent the nexus concept must be flexible, dynamic, and interactive; it needs to represent and evaluate the relationships between the systems (water, energy, agriculture, and environment), and it must (Miralles-Wilhelm, 2016):

- consider the management and regulation of these resources;
- assist decision makers in defining planning strategies and integrated sectoral policies;
- allow the development of socioeconomic scenarios;
- subsidize evaluations that consider the trade-offs and synergies between the multiple sectors involved;
- allow analyses that consider the effects of variability in the spatial and temporal scales in the systems considered.

Therefore, integrated databases are required. However, obtaining sufficient data to support integrated research on the nexus concept is complex (Embid and Martin, 2018); information on water, energy, agriculture, and the environment is spread across different institutions, information systems, and documents, and there is no shared and accessible database. Additionally, even when this information exists, it often presents divergences in terms of spatial and temporal scales, and a variety of metrics, thereby making it difficult or prohibitive to conduct analyses due to the lack of compatibility (Eftelioglu et al., 2016; Huckleberry and Potts, 2019).

Dispersed databases that do not integrate information are often a reflection of the independent organizational format that has historically been implemented in public sectors, wherein each entity follows its own strategies, policies, plans, data collection, and actions (Eftelioglu et al., 2016; Embid and Martín, 2018). Due to the multidisciplinary nature of these problems, over time, this independent organizational format has been confronted with the need for studies integrated into complex network systems (WEF, 2011; Hanlon et al., 2013).

According to Lawford (2019), an information service must be shared between the multiple correlated sectors to support a well-structured dialogue that fosters joint planning; the leveling of databases based on the identification of common information needs between them is also essential. This service must be developed through an open access platform supported by joint governance; such a service would improve management, planning, and decision-making that involves both the public and private sectors.

In view of this, it is observed that there is a need for research on the existing weaknesses in sectoral databases to analyze the possibilities of carrying out integrated studies that include quantitative assessments of natural resources. The results of this investigation vary according to the reality of each location, and the progress in this area, especially of a quantitative nature, did not occur without the support of databases for its development.

Given the above, this article aimed to propose a Decision Support System (DSS) to assist in the identification, analysis, and selection of sectoral databases. The development of a DSS will support the development of quantitative studies on the "Nexus of water, energy, agriculture, and the environment."

Materials and Methods

The methodology developed comprises the proposal of a DSS, which is an information management system that analyzes different variables to assist in the decision-making process. In this study, a conceptual structure of the DSS is presented. This is an innovative methodology that has been developed to address the gaps identified in the literature regarding the complexity in obtaining compatible information between the involved sectors proposed in the nexus concept.

The proposed conceptual model follows the organizational logic of multicriteria analysis, where different alternatives are evaluated using multiple criteria. The multicriteria analysis is composed of alternatives, criteria, a classification matrix (checklist), and objectives (goals) of the process (Hajkowicz and Collins, 2007). The structural basis of the model used in the present study builds upon that used by Torres et al. (2021).

In this research, the alternatives represent the sectoral databases and the matrix functions as a checklist (Checklist) that verifies which criteria are present in the alternatives. The methodology was divided into six stages, as shown in Figure 1:

- sectoral analysis;
- identification of the databases;

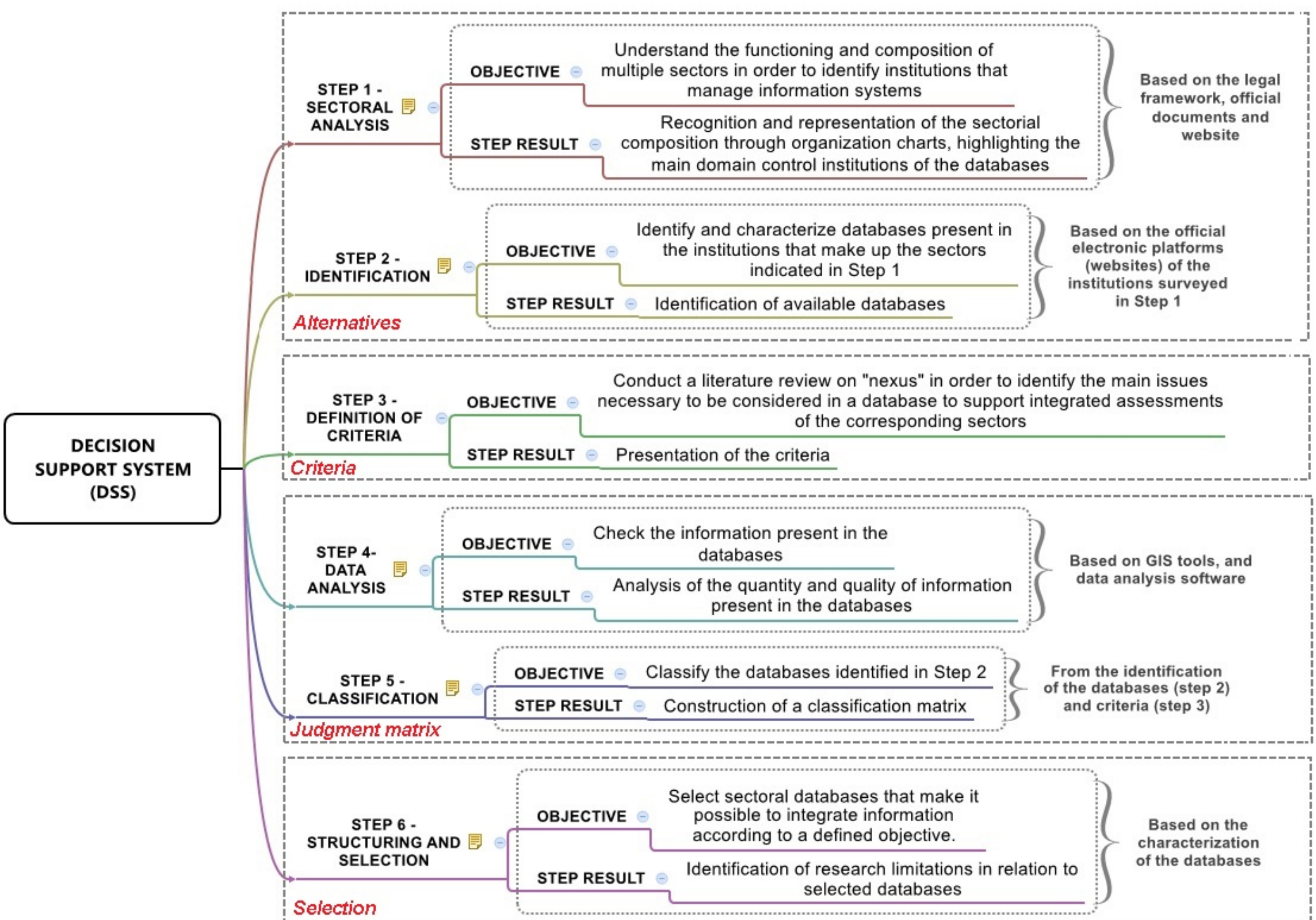

Figure 1 – Conceptual structure of DSS for analysis and selection of databases.

- definition of the criteria;
- analysis of the databases;
- construction of the classification matrix;
- structuring and selecting databases according to the defined objective.

Step 1: identification of the institutions that make up each sector

The first stage of the DSS consists of an institutional analysis to understand the functioning and composition of the sectors, and to identify and access their databases. For this purpose, the institutional arrangement of the sectors were initially verified through the elaboration of an organization table (hierarchical systematization of the institutions and bodies involved). Once the organizational composition of each sector was identified, the databases were identified, as described in the next section.

The application of the methodology in Federal scale databases was chosen based on two aspects:

- achieving an overview of the information systems of the four sectors in the Brazilian context;
- establishing a hierarchical priority according to the Federal Constitution of 1988: Union, States, and municipalities.

Step 2: identification of alternatives

This step comprises the identification of the main national databases in the institutions surveyed in the previous step. For this, online consultations were carried out on the official websites of the "National Water Agency (*Agência Nacional de Águas* – ANA)", "Ministry of Mines and Energy (*Ministério de Minas e Energia* – MME)" "Ministry of Agriculture, Livestock and Supply (*Ministério da Agricultura, Pecuária e Abastecimento* – MAPA)," and "Ministry of the Environment (*Ministério do Meio Ambiente* – MMA)."

In addition to consulting the aforementioned websites, the webpages of the institutions that make up the ministries were also searched, including those of the National Petroleum, Natural Gas and Biofuels Agency (*Agência Nacional do Petróleo, Gás Natural e Biocombustíveis* – ANP), Petrobrás, National Supply Company (*Companhia Nacional de Abastecimento* – CONAB), Brazilian Institute of the Environment and Renewable Natural Resources (*Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis* – IBAMA).

As a complementary research, the Brazilian Open Data Portal was verified, which functions as a data centralizer in accordance with Decree No. 8.777/2016. According to this decree, government ministries must "promote the publication of data contained in databases of organs and entities of the direct, autarchic and foundational federal public administration in the form of open data" (Brasil, 2016).

In view of this, the federal level databases of the sectors relevant to the nexus concept were selected and investigated. The selection considered the scope of the information to represent the sector in the Brazilian context. In other words, databases that presented information regarding the regional or local scale were excluded from this study. Following this step, the set of criteria for evaluation were defined.

Step 3: defining the criteria

Aspects for the database analysis were defined to ascertain the possibility of integrating information. The criteria were defined from two points:

- systematic review of the literature, based on Torres et al. (2019), contemplating publications involving the theme "nexus," in the research platforms aiming to identify authors who deal with information systems for integrated evaluations. The main authors who guided these definitions were: WEF (2011), Eftelioglu et al. (2016), Endo et al. (2017), Albrecht et al. (2018), Embid and Martín (2018), Dai et al. (2018), Shannak et al. (2018), and McGrane et al. (2019);
- survey of the main aspects to be considered in a database to support integrated assessments of the corresponding sectors.

The highlighted aspects outlined the construction of seven criteria and 27 categories, as shown in Table 1: Criterion 1 (C1) with three categories; Criterion 2 (C2), seven categories; Criterion 3 (C3), three categories; Criterion 4 (C4), six categories; Criterion 5 to Criterion 7, three categories. To facilitate the evaluation of alternatives against the criteria, in the classification matrix, a color palette was defined by a family of criteria categories. It is important to highlight that, from the completion of new bibliographic reviews, other criteria can be included in the list in Table 1, as long as they are characterized and justified.

After defining the criteria listed in Table 1, analyses of some characteristics present in the databases were carried out for a better evaluation of the classification matrix, as shown in the following step.

Step 4: analysis of databases

In this stage, a careful check is carried out on the quality of the available information. Four main issues are investigated:

- quantity and type of information present;
- divergences from the same information presented in different databases;
- presence of databases that include the monitoring of data from multiple sectors in an interconnected manner (for example, water-energy or energy-agriculture);
- survey of databases from other sectors that include information of interest for the development of integrated studies.

The investigations cited above were defined based on the bibliographic review mentioned in Step 3, as based on the systematic review by Torres et al. (2019).

Geographic information system (GIS) tools and other data analysis tools are essential to support the development of this phase. As a result of this analysis, the construction of the classification matrix for the evaluation of cataloged databases was elaborated.

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Table 1 – Database classification criteria.

Criterion	Definition	Category	Color scale
C1: Access to Information	Represents access to information present in a database.	(I) Available - Free access for download	
		(II) Conditioned - Can be accessed through authorization from the segment that represents them	
		(III) Not available - Not accessible for download, however, can be viewed	
		(IV) Mixed - Database classified in more than one category of this criterion	
C2: Data monitoring period	Corresponds to the duration of the data monitoring.	(I) Historical series - Sequence of observations of data collected continuously over a period of time, at regular intervals	
		(II) Restricted - There is no sequence of observation of the data, the information is presented for irregular intervals	
		(III) Not applicable - Does not have a monitoring period, includes only characterization data bases	
		(IV) Mixed - Database classified in more than one category of this criterion	
C3: Time Scale	Time scale for data presentation.	(I) daily	
		(II) weekly	
		(III) monthly	
		(IV) Yearly	
		(V) Others	
		(VI) Multiple - Use of more than one time scale	
		(VII) Not applicable - does not have a time scale	
C4: Geographic Scale	Refers to the spatial scale of data presentation.	(I) National	
		(II) Regional	
		(III) State	
		(IV) Hydrographic basin	
		(V) Location (cities, companies, reservoirs, etc.)	
		(VI) Multiple - Presentation of more than one geographical scale.	
C5: Mathematical quantities	It depicts the measurement of a phenomenon. For example, volume, flow, production, etc.	(I) Single mathematical quantity - Representation of only a single mathematical quantity	
		(II) Multiple mathematical quantities - Representation of more than one mathematical quantity	
		(III) Not applicable - These are documentary and spatial databases	
C6: Type of databases	It represents the presentation format of the information present in the databases.	(I) Characterization - Databases aimed at describing some characteristics of a sector, without monitoring the information presented (for example, the description of the profile of a power plant without presenting the monitoring of its production)	
		(II) Productivity - Data presented in a format that allows its manipulation, containing monitoring of its data (for example, history of the production of a plant exposed in Excel spreadsheets)	
		(III) Documentary - Data presented in technical and scientific documents, published in journals, theses, reports, among others. In this case, the information has already been manipulated by the authors.	
		(IV) Mixed - Database classified into more than one type.	
C7: Spatialization of data	Data georeferencing	(I) Georeferenced	
		(II) Not georeferenced	
		(III) Mixed - Databases that include both georeferenced and non-georeferenced information.	

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Step 5: classification of alternatives

The classification of alternatives was carried out by means of matrix analysis. The matrix is composed of rows and columns that vary according to the number of defined criteria and alternatives. In the present study, the lines represent the alternatives and columns, and the categories of the criteria are listed in Table 1. The matrix functions as a checklist that indicates the categories of criteria present in the database framework. All criteria have the same level of priority, and should be used equally in the evaluation of all alternatives. Thus, filling out the matrix was done using two numeric codes: "1" indicates the presence of the category according to each criterion, and "0" indicates its absence.

Step 6: structuring and selecting alternatives

Structure refers to the elaboration of a selection system complementary to the classification matrix. In this system, the databases are organized by a criterion category session within each sector under study. Two points were considered when completing the proposed structure:

- identification of the criterion category that obtained the least number of databases inserted;
- analysis of the level of relevance of the criterion category for the development of the research.

In the case of a relevant category, which cannot be modified, the databases entered are those selected. However, in the event that this category is not relevant to the study it will be disregarded as a selection parameter, and another category must be analyzed successively until the selection process is closed. Once the DSS was structured, it was validated using federal databases present in Brazil.

Case study: Brazilian context

In recent years, Brazil has experienced novel uncertainties due to water crises in various regions, and the simultaneous political and economic conflicts that have taken place at the national level. The water crisis that has affected the energy sector in recent years has also impacted other sectors in some parts of the country. This situation has made managers question the real causes of these problems in hopes of determining actions to stabilize this scenario.

Thus, it is pertinent to investigate the country's databases belonging to the main sectors that guide its economy and sustain the well-being of its nation. These investigations tend to corroborate the identification of weaknesses and advances in sectoral databases.

The Federal Constitution of 1988 organizes political and administrative Brazil in entities of the Federation formed by the Union, States (26), Federal District (*Distrito Federal* – DF), and municipalities (5,570); all are endowed with autonomy in legislative, governmental, tax, and administrative powers (Brasil, 1988). In other words, according to the constitution, all entities of the Federation can legislate, organize, and manage their territory, and the Union should intervene only in situations of disorder that interfere with national integrity. Despite the autonomy of the federated entities, their competencies follow a hierarchy of power, from the greatest to the least powerful: federation, state, DF, and municipality.

The organizational model approved in 1988 is based on the autonomy of federated entities according to their respective competencies. The development of the public sectors in these spheres took place in a segmented and disaggregated way. Their plans, programs, and projects were elaborated largely with no prospect of integration. Today, this course of development reflects the difficulty in planning the articulation between the databases of different sectors due to the diffusion of the integrated management model.

Regarding the water sector, it is the domain of the Union "Lakes, rivers and any water resource in its territory, or that cross more than one State, that serve as limits with other countries or that extend over foreign territory, as well as marginal lands and river beaches" (Brasil, 1988). Water management in Brazil took place in a fragmented way, as each sector carried out its planning in isolation, without participation from municipal governments, water users, and civil society (Abers and Jorge, 2005).

Like the water sector, the energy sector has undergone several institutional changes during the 1970s, 1980s, 1990s, and to the present day. The sector left a monopoly system for free competition in energy generation, commercialization, transmission, and operation distributed between public and private companies. As federal assets, the constitution mentions the potential of hydraulic energy and mineral resources; therefore, the exploitation or use of these resources can only be carried out with authorization or concession from the federal government (Brasil, 1988).

With regard to the agricultural sector, the constitution played a fundamental role in its development by promoting the country's agricultural policies, including rural insurance, agricultural planning, technical assistance, and rural extension (Brasil, 1988).

For the environmental sector, Law No. 6.938/1981 (Brasil, 1981) organized the management of environmental resources at the federal level, and instituted the National Environment System. Complementary Law No. 140/2011 (Brasil, 2011) establishes the instruments and actions of cooperation between the Union, States, DF, and municipalities in administrative actions resulting from the exercise of the common powers established in the constitution. In view of the above, it is observed that despite the great advances that the country has achieved since the adoption of the Federal Constitution, historically, the organizational structures of the public sector have several obstacles and challenges within the scope of integrated intersectoral management.

Results and Discussion

In Brazil, information on water resources, energy, agriculture, and the environment are segmented into independent databases, managed by a large number of institutions at the federal, state, and municipal levels. For the present study, only the main databases in the federal sphere were considered. This section presents the results obtained with the development of the steps that make up the DSS.

Step 1: institutions that make up the sectors under analysis

For the development of the first stage of the DSS, public policies and legislation relevant to the sectors under analysis in Brazil were used, highlighting federal decisions, provisional measures, and national policies (Brasil, 1981, 1991, 1997a, 1997b, 2019a, 2019b, 2019c, 2019d, 2020). The main institutions, bodies, and collegiate bodies identified in each sector were structured in an organization table (Figure 2), corresponding to the governmental structure in force in the country. The institutions surveyed in the organization table hold the databases of the sectors mentioned at the federal level.

In general, it can be seen that each sector is inserted in a government ministry, and in line with each ministry are the specific secretariats, departments, councils, and bodies.

For the water resources sector, the data generated by the bodies that are part of the National Water Resources Management System (*Sistema Nacional de Gerenciamento de Recursos Hídricos* – SINGREH) are incorporated into the Water Resources Information System (*Sistema Nacional de Informações sobre Recursos Hídricos* – SNIRH). The National Water Agency (*Agência Nacional de Águas* – ANA) is responsible for managing this system in its area of operation (Brasil, 1997a).

In the energy sector, the Energy Research Company (*Empresa de Pesquisa Energética* – EPE) is responsible for "promoting studies and producing information to support energy development plans and programs" (Brasil, 2004). As for the agriculture sector, it is the responsibility of the MAPA to aggregate "agricultural, meteorological, and climatological information for use in agriculture" (Brasil, 2019a).

In the environmental sector, it is important to mention two aspects:

- according to the National Environment Policy (*Política Nacional do Meio Ambiente* – PNMA), energy, water, agriculture, other natural resources, and economic activities make up the environmental sector (Brasil, 1981);
- although multiple sectors include monitoring data from the environmental sector, this does not imply that there is integration between these different institutions and between the data monitored by them.

Once the institutional composition of each sector under analysis was identified, the main databases belonging to each sector were identified, as shown in the next section.

Step 2: identification of the databases

Based on the organizational system present in each sector, 35 national databases were identified (Table 2); they are distributed among their bodies and institutions. Of the databases surveyed, seven comprised the water resources sector (20%), eight comprised the energy sector (22.8%), seven constituted the agricultural sector (20%), and 13 made up the environmental sector (37.14%).

In the water resources sector, different databases contain different information; of note are the: HIDROWEB, which can be highlighted for its pluviometric and fluviometric information; the reservoir monitoring system (*Sistema de Acompanhamento de Reservatórios* – SAR) for monitoring reservoirs; the grant registration for water volumes captured by different users distributed by hydrographic basin; the National Sanitation Information System (*Sistema Nacional de Informações sobre Saneamento* – SNIS) for monitoring the sanitation sector; the groundwater information system (*Sistema de Informações de Água Subterrânea* – SIAGAS) for groundwater; the National Dam Safety Information System (*Sistema Nacional de Informações sobre Seguranca de Barragens* – SNISB), which involves the registration of dams in the context of their safety; and the Metadata Portal for geographic information.

In the energy sector, the main databases include that of the National Electric System Operator (*Operador Nacional do Sistema Elétrico* – ONS), which contains the operation history of the National Interconnected System (*Sistema Interligado Nacional* – SIN); the National Electric Energy Agency (*Agência Nacional de Energia Elétrica* – ANEEL) developed the ANEEL Generation Information System (*Sistema de Informações de Geração da ANEEL* – SIGA) to provide data on installed electricity generation capacity from its multiple sources in Brazil; the Union of Sugarcane Industries (*União da Indústria de Cana-de-Açúcar* – UNICA), which is not directly linked to the ministry of agriculture and energy, but which has a very rich database on the production and commercialization of sugarcane, ethanol production, and bioelectricity; open data from the ANP, which brings a large amount of historical information on the production of oil, natural gas, and biofuels, among many others; and the geographic information bases involving the SIN Cadastral Geographic Information System (*Sistema de Informações Geográficas Cadastrais do SIN* – SINDAT), the Geographic Information System for the Electricity Sector (*Sistema de Informações Georreferenciadas do Setor Elétrico* – SIGEL), the geographic information system for the energy sector (WEB MAP EPE/EPE), and the Information System Mining Geographic Areas (*Sistema de Informações Geográficas da Mineração* – SIGMINE).

In the agricultural sector, open data from the MAPA predominate; the National Institute of Meteorology (*Instituto Nacional de Meteorologia* – INMET), which provides a history of meteorological data; CONAB, which provides the historical data on the Brazilian harvest, agricultural prices, production costs, and other information; documentary databases including the Agricultural Research Database (*Base de Dados da Pesquisa Agropecuária* – BDPA) of Brazilian Agricultural Research Corporation (*Empresa Brasileira de Pesquisa Agropecuária* – EMBRAPA), and the Bibliographic Base of Brazilian Agriculture (*Base Bibliográfica da Agricultura Brasileira* – AGROBASE); the EMBRAPA Spatial Data Infrastructure (GEOINFO), which comprises a set of spa-

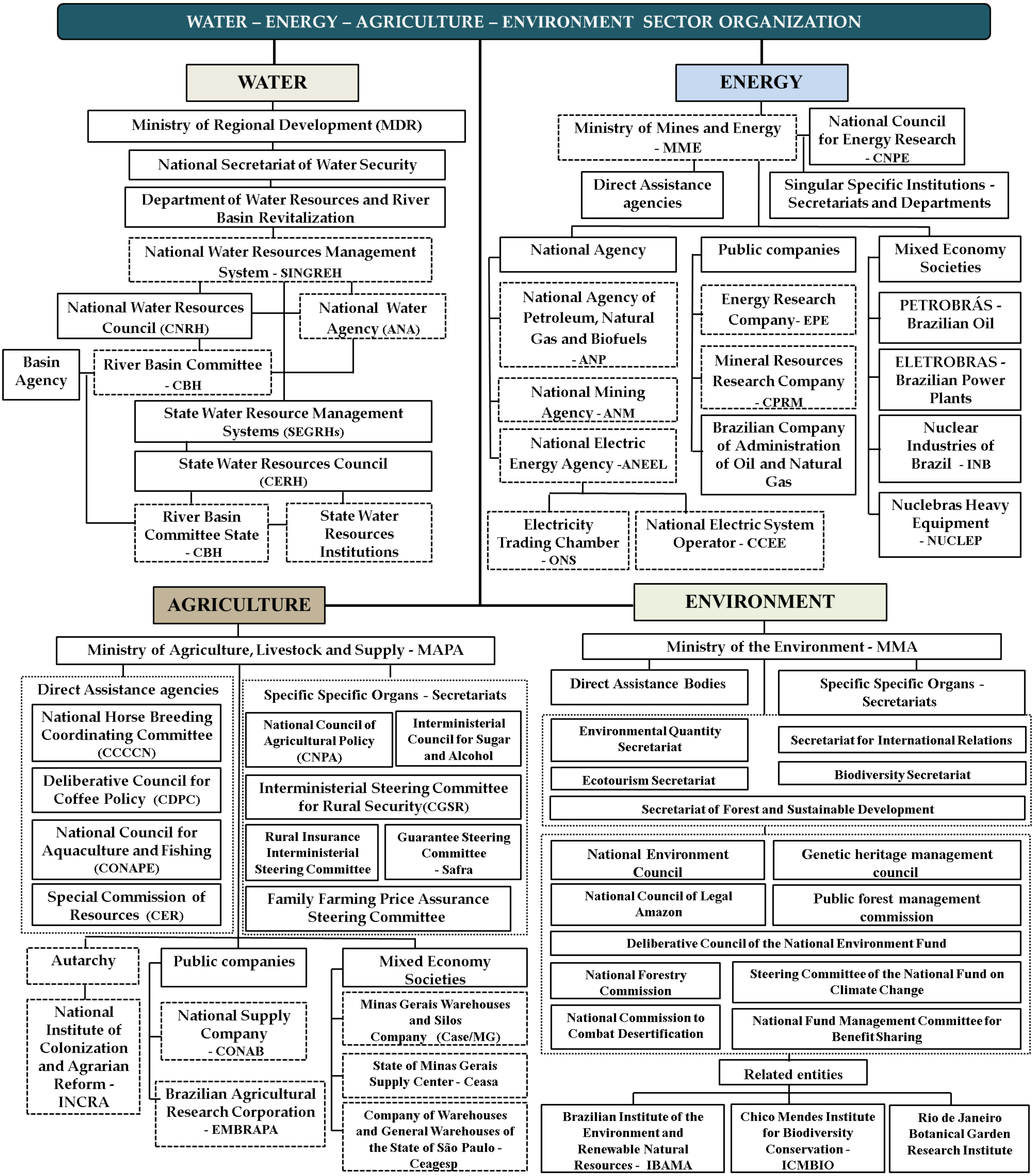

Figure 2 – Organization table of the Water - Energy - Agriculture - Environment sectors in Brazil.
Source: adapted from Torres et al. (2021).

Table 2 – Main national databases of the Water–Energy–Agricultural–Environmental sectors.

Sector	ID	Initials	Name	Entity
Water	A	HIDROWEB	Hydrological Information System	ANA
	B	METADADOS	Metadata Portal of the National Water Agency	ANA
	C	SAR	Reservoir Monitoring System	ANA
	D	Grant registration for water volumes	National Water Agency Grant Registry	ANA
	E	SIAGAS	Groundwater Information System	ANA
	F	SNISB	National Dam Safety Information System	ANA
	G	SNIS	National Sanitation Information System	MDR
Energy	A	ONS database	ONS Electricity Operation History	ONS
	B	SINDAT	Cadastral Geographic Information System of the National Interconnected System (SIN)	ONS
	C	SIGEL	Geographic Information System for the electricity sector	ANEEL
	D	WEB MAP EPE	Geographic Information System for the Energy sector	EPE
	E	SIGMINE/ANM	Mining Geographic Information System	ANM
	F	Open Data / ANP	Open data from the National Petroleum Agency	ANP
	G	SIGA	ANEEL's Generation Information System	ANEEL
	H	UNICA	Union of the sugarcane industries	UNICA
Agriculture	A	Open Data /MAPA	Open data from the Ministry of Agriculture, Livestock and Supply	MAPA
	B	INMET	National Institute of Meteorology	MAPA
	C	CONAB	National Supply Company	MAPA
	D	BDPA	Agricultural research database	EMBRAPA
	E	GEOINFO	Embrapa Spatial Data Infrastructure	EMBRAPA
	F	AGROBASE	Bibliographic Basis of Brazilian Agriculture	MAPA
	G	Agricultural Census	IBGE Agricultural Census	IBGE
Environment	A	Open Data /MMA	Open data from the Ministry of the Environment	MMA
	B	Open Data /IBAMA	Open data from the Brazilian Institute of the Environment and Renewable Natural Resources	IBAMA
	C	Open Data /ICMBIO	Open data from the Chico Mendes Institute for Biodiversity Conservation	ICMBIO
	D	Open Data /INPE	Open data from the National Institute for Space Research	INPE
	E	SFB	Database of the Brazilian Forest Service	MAPA
	F	BDiA/IBGE	Environmental Information Database	IBGE
	G	SIBBR	Information Systems on Brazilian Biodiversity	MCTI
	H	UC/ICMBIO	Database of Conservation Units	ICMBIO
	I	SISolos/ EMBRAPA	Soil Information System	EMBRAPA
	J	GeoSGB	System of geosciences of the Geological Survey of Brazil	CPRM
	K	CPTEC/INPE	Weather Provision Center and Climate Studies	INPE
	L	AdaptaCLIMA	Knowledge Platform on Adaptation to Climate Change	MMA
	M	BDA	Environmental Databases	ANP-IBAMA

tial information; and the IBGE agricultural census. Although IBGE is not directly part of the agricultural sector, it has significant relevance in the collection of data through the Agricultural Census.

In the environmental sector, a large amount of information distributed in different institutions were highlighted, including: open data from the MMA; IBAMA; Instituto Chico Mendes for Biodiversity Conservation (ICMBIO) which includes information from Conservation Units, the National Institute for Space Research (*Instituto Nacional de Pesquisas Espaciais* – INPE), and the Weather Forecast and Climate Studies Center (*Centro de Previsão de Tempo e Estudos Climáticos* – CPTEC); the Brazilian Forest Service (*Serviço Florestal Brasileiro* – SFB), which comprises the National Forest Information System (*Sistema Nacional de Informações Florestais* – SNIF); the IBGE's Environmental Information Database (*Banco de Dados de Informações Ambientais* – BDiA), which addresses information on geology, geomorphology, pedology, and vegetation; the Brazilian Biodiversity Information System (*Sistemas de Informações sobre a Biodiversidade Brasileira* – SIBBR), a platform that brings together information about ecosystems; the Brazilian Soil Information System (*Sistema de Informação de Solos Brasileiros* – SISolos) of EMBRAPA, which has soil samples and profiles from all over Brazil; the System of Geosciences of the Geological Survey of Brazil (*Sistema Geociências do Serviço Geológico do Brasil* – GeoSGB) that deals with geological information from the national territory; and the Environmental Database (*Sistema de Dados Ambientais* – BDA) created by MMA Ordinance No. 422 of 10/26/11, which represents an integrated database between ANP and IBAMA.

Lawford (2019) states that social, economic, political, and biophysical data are essential for any integrated assessment of the water, energy, and agricultural sectors. The author also highlights some essential variables to enable studies contemplating the nexus approach: precipitation (pluviometric stations), air temperature, evapotranspiration, soil moisture (meteorological stations), water quality, operational and physical data of the reservoirs, flow (fluviometric stations), underground water, energy, land use, and occupation. Most of this information is present in the databases listed in Table 2; however, many of them do not cover all regions of the country, much information is out of date, and there is a disparity in the monitoring of data, both in terms of time and spatial scales. Torres (2020) adds that Brazil has a large number of inactive pluviometric and fluviometric monitoring posts, especially when it comes to small state hydrographic basins.

When inserting the environment into the context of integrated assessment, it is observed that, despite the significant number of databases illustrated in Table 2, most of the information available in the environmental databases is not integrated and does not interrelate, such as the Environmental Information Database with the Environmental Database.

Step 4 was taken from the identified databases, which conceives the analysis of its information. It should be mentioned that it was not necessary to present Step 3, since it involves the definition of the analysis criteria, that is, this phase has already been structured in the methodology of the study.

Step 4: analysis of the databases

The analysis of the databases was carried out through the investigation of five points, as listed in the methodology. For analysis of the first point, it was found that 74.3% of the databases surveyed in Table 2 were georeferenced, and were mostly distributed in the environmental, water, and energy sectors.

Spatial data greatly contributes to quantitative studies of integrated assessment of nexus elements. It enables research on multiple geographic scales if at least one of the following aspects are met:

- The presence of national databases that are spatialized and, at the same time, productive. They present the monitoring of their information and not just their characterization, and are able to mention HIDROWEB among the systems identified in Figure 2;
- Presence of georeferencing databases. In this case, it is necessary to integrate this information into a productivity database, such as the association between SIGEL and ONS.

As for the variability of the type of geographic scale to be worked on, cutouts of information present in a macro- to micro-scale can be made through the use of GIS tools. This is possible as long as the information is georeferenced and positioned within the geographical limits.

Several authors in the literature have pointed out significant benefits from the use of spatial data in different sectors of the economy. Gonçalves et al. (2009) developed a method based on a GIS to integrate physical data with the type and use of the soil to assist in the management and granting of the right to use groundwater applied in the DF. Souza and Farias (2010) provide in their study that the systematic organization in a GIS of the water resources sector and other related sectors facilitate the assessment and management of water in conservation units (UC). According to the authors, the integration between databases associated with the GIS allows a greater approximation of different public and private institutions that share specific themes in decision-making processes, which allows interoperability and cooperation between them (Souza and Farias, 2010). Pereira et al. (2019) addressed the relevance of spatial data and geological instruments to increase productivity and public capacity to define strategies to solve complex problems related to local geography.

Another point considered was the relationship between the organizational structure present in each sector and the geographical scale of the presentation of its data. In this case, it was observed that databases of the water resources sector normally present their data for the hydrographic basin scale, since the National Water Resources Policy (*Política Nacional de Recursos Hídricos* – PNRH) defines the hydrographic basin as a planning and management unit (Brasil, 1997a).

For the electricity sector, the main databases are for the regional scale (sub-systems), and depend on the functioning of the SIN. For the agricultural sector, CONAB presents information on the state and regional scales. The main information for the environment is presented on regional (biome), state, and national scales.

The application of DSS for databases of other spatial scales allows the identification of the level of integration between them. For example, this can be used to verify the level of integration of the State grant registry with the ANA grant registration; integration of surface water availability with groundwater and its interrelationships with water quality; and demands for water and energy in the agricultural projects of the different regions of the country.

In addition to analyzing these issues, the databases were checked for the presence of divergences in the presented information . It should be considered that the investigation of this aspect is possible as long as more than one institution presents the same type of information for a given sector. This was conceivable only for the energy sector, where the WEB MAP EPE, SIGEL, SIGA, and ONS power generation history databases present information on the number of power plants present in the country.

The research revealed the presence of divergence between the quantities presented in these databases. For example, analyzing the number of wind farms, a total of 455 plants were found in the WEB MAP EPE database, 877 plants in SIGEL, 606 plants in SIGA, and 601 plants in ONS (considering the same time period for all). With regard to solar plants, 67 plants were identified on the WEB MAP EPE, 2,524 plants in SIGEL, 2,469 plants in SIGA, and 81 in ONS. The same is true for other power-generation plants.

In the water resources sector, Torres (2020) observed a disparity between the number of pluviometric and fluviometric stations reported in HIDROWEB for the number of active stations. For example, in the São Francisco River Basin (BHSF), the author identified that 32% (567 of the total of 1,748) of the river stations, and 53% (1,009 of 1,878) of the rain stations were deactivated.

After analyzing these aspects, an investigation was carried out on the presence of databases that integrate information from different sectors. In this case, the only databases (Table 2) that meet these requirements are:

- the grant registry/ANA, which lists the elements of water-irrigated agriculture, and water - energy (water demand for some types of energy, such as thermoelectric and hydroelectric);
- UNICA, which relates energy and agriculture (production of bioenergy from sugar cane);
- IBAMA, which brings multiple variables, such as biome-energy sources-solid waste;
- the Bank of Environmental Data shared between ANA–IBAMA (aimed at sharing different projects between ANP and IBAMA aiming at the optimization of the environmental licensing processes of the oil and gas industry in Brazil);
- the SNIS that relates the elements energy–water (consumption in the sanitation segment).

Specifically, for the registry of grant/ANA, it was observed that this database holds the geographic coordinates of the points of water collection for irrigation, and may represent the irrigated agriculture sector in terms of the location and number of the cultures practiced by the hydrographic basin.

As a last point of data analysis, nine national and international platforms were identified, which have information of interest for the sectors in question:

- National Spatial Data Infrastructure (*Infraestrutura Nacional de Dados Espaciais* – INDE);
- Information and Knowledge Management System for the Brazilian Semiarid Region (*Sistema de Gestão da Informação e do Conhecimento do Semiárido Brasileiro* – SIGSAB);
- Comex Stat: Database linked to the Ministry of Industry, Foreign Trade and Services;
- SIDRAS - IBGE database;
- PROCEL INFO: Energy efficiency information center;
- SISPPI - Information System on Public Irrigation Projects;
- DATAHIDRO–PETROBRÁS Corporate System on Water Resources and Effluents;
- UN Comtrade Database: UN database;
- FAOSTAT - Food and Agriculture Organization (FAO) database;
- WaterStat–Water footprint statistics.

All of these information systems, despite not being directly linked to the institutions listed in Figure 1, should be considered in the investigations contemplating the nexus concept as it contains a large amount of data measured for multiple spatial scales and contain open access data. For example, the information platform of the Brazilian semiarid region presents data on a regional scale, and the international database (WaterStat) displays information on the water footprint and virtual water of a wide variety of products and services.

In general, some aspects of interest in research related to the nexus concept have been discussed. However, other points of analysis deserve to be deepened, depending on the need for investigation for a given study.

Step 5: classification of databases

Table 3 lists the classification matrices of the databases listed in Table 2. It can be seen that more than 80% of the databases express information that is available for download; approximately 31.4% have a historical data monitoring period; the time scales most used as a data output format are annual (14.3%) and multiple (37%), which includes, annual, regional, and state data; more than 80% have multiple spatial scales, mainly national and state; more than 70% have multiple quantities; 37% represent more than one type of database (mainly characterization and productivity); and more than 50% are geo-referenced.

From the classification matrix, the databases were structured by sets of similarities in the information according to the categories presented in the criteria presented in Table 1 of the methodology.

Table 3 – Matrix for classifying the databases raised in Table 2.

Sector	Database	Access to Information				Data monitoring period				Time Scale							Geographic Scale						Mathematical quantities			Type of databases				Spatialization of data		
		Available	Conditioned	Not available	Mixed	Historical series	Restricted	Not applicable	Mixed	Daily	Weekly	Monthly	Yearly	Others	Multiple	Not applicable	National	Regional	State	Hydrographic basin	Location	Multiple	Single mathematical quantity	Multiple	Not applicable	Characterization	Productivity	Documentary	Mixed	Georeferenced	Not georeferenced	Mixed
Water	A	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0
	B	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0
	C	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0
	D	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0
	E	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0
	F	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	1	0	0
	G	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	1	0
Energy	A	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	1	0
	B	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	1	0	0
	C	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0
	D	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	1	0	0	0	1	0	0
	E	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	1	0	0	0	1	0	0
	F	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	1	0
	G	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	1	1	0	0
	H	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	1	0
Agricultural	A	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	1	0
	B	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0
	C	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0
	D	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0
	E	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	0	0	0	1	0	0
	F	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0
	G	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	1	0
Environment	A	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1
Environment	B	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1
	C	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1
	D	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1
	E	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1
	F	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	0	0	0	1	0	0
	G	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1
	H	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0
	I	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	1	1	0	0
	J	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0
	K	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0
	L	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0
	M	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0
Total		31	2	0	2	11	2	15	7	1	0	0	0	0	0	16	1	0	1	0	0	0	0	26	9	8	10	4	13	20	9	6
Total (%)		88.6	5.7	0	5.7	31	5.7	42.8	20	2.8	0	0	14.3	0	37	45.7	2.6	2.6	2.6	2.6	2.6	85.7	0	74.3	25,7	23	29	11	37	57	26	17

WATER SECTOR: A: HIDROWEB; B: METADADOS; C: SAR; D: Grant registration for water volumes; E: SIAGAS; F: SNISB; G: SNIS; ENERGY SECTOR: A: ONS database; B: SINDAT; C: SIGEL; D: WEB MAP EPE; E: SIGMINE/ANM; F: Open Data /ANP; G: SIGA; H: UNICA; AGRICULTURE SECTOR: A: Open Data/MAPA; B: INMET; C: CONAB; D: BDPA; E: GEOINFO; F: AGROBASE; G: Agricultural Census; ENVIRONMENT SECTOR: A: Open Data/MMA; B: Open Data/IBAMA; C: Open Data /ICMBIO; D: Open Data /INPE; E: SFB; F: BDiA/IBGE; G: SIBBR; H: UC/ICMBIO; I: SISolos/ EMBRAPA; J: GeoSGB; K: CPTEC/INPE; L: AdaptaCLIMA; M: BDA/ANP-IBAMA.

Step 6: structuring and selecting databases

This step represents the last phase of the DSS, in which the databases are structured and selected. For this process, a structure was elaborated that facilitated the visualization and selection of the databases (Figure 3). In this structure the databases are grouped by category according to the color scale presented in the methodology (Table 1). Database selection can be manual, or automated in Microsoft Excel. Figure 3 shows an example of filling in the structure according to the selection of certain criteria categories.

From the DSS application example (Figure 3), it is possible to analyze two aspects:

- the INMET, despite being present in practically all categories of the criteria reported in Figure 3. This database represents meteorological-climatological information and does not provide specific information related to agricultural productivity;
- despite the lack of databases in the agriculture sector to represent the hydrographic basin category, it can be seen that the registry of grant/ANA the water sector offers valuable information associated with the agricultural sector and can supplement this lack of data.

As for the energy and environmental sectors, the greatest lack of databases also consists of the geographical scale criterion referring to the hydrographic basin category. In the case of information from the environmental sector, this deficiency can be addressed as long as the databases are georeferenced, allowing integration with multiple spatial scales through GIS tools. When viewing the results in the DSS structure, two directions tend to be considered: the first corresponds to the possibility of changing from a more restrictive category to a less restrictive one to serve all sectors under analysis; the second involves choosing to remain in restrictive categories even though they do not include all the databases of the different sectors. This assumes the necessary simplifications and limitations in the development of an integrated study.

In view of the structure presented in Figure 3 the development of the steps presented in the methodology was conducted for the databases indicated in Table 2. The discussions exposed in the development of the steps do not consider other data systems. Therefore, in the case of insertion of new databases or application to other territorial limits the methodological, procedure must be adjusted or implemented again and new investigations must be considered.

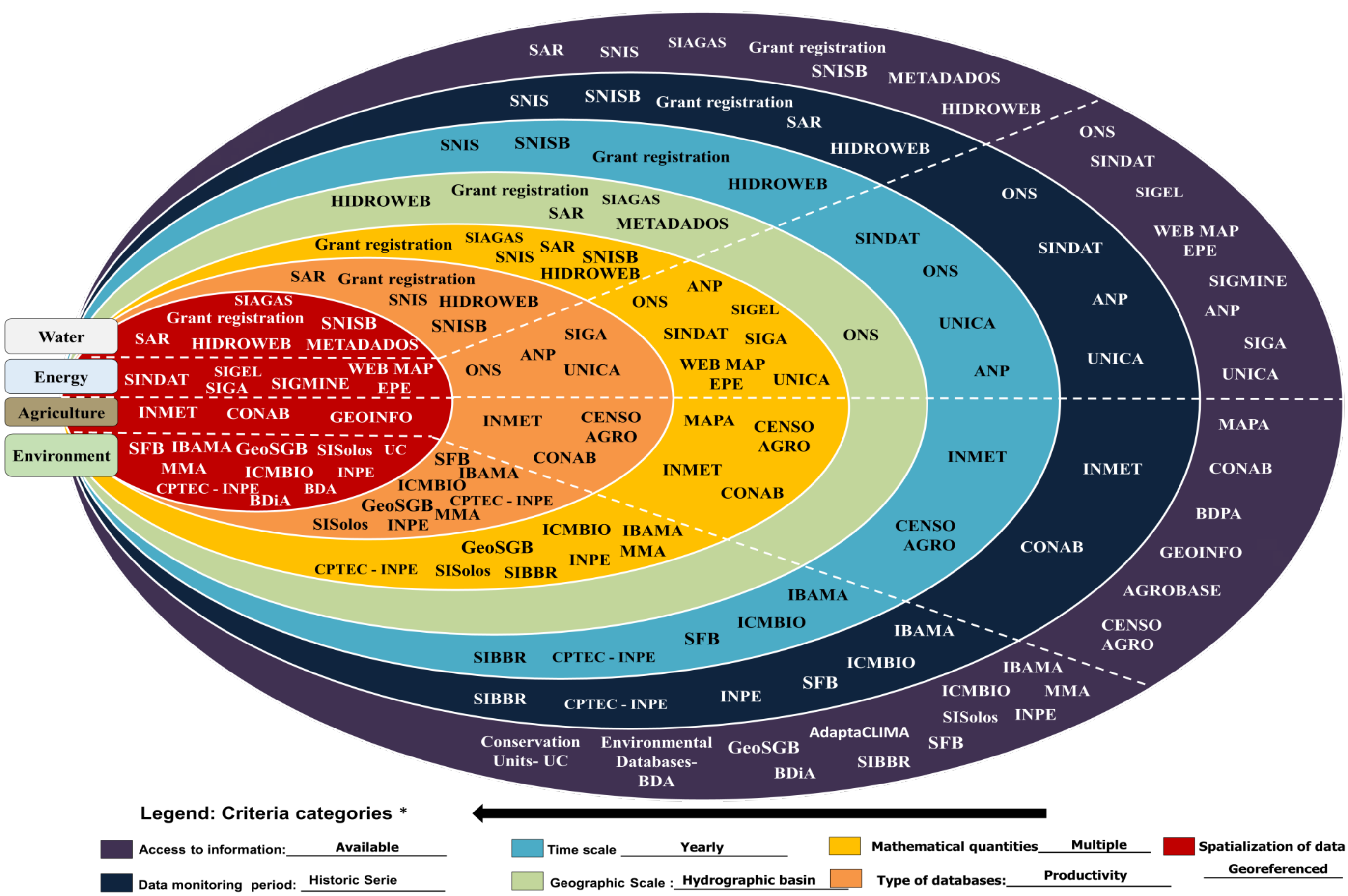

Figure 3 – Structure to assist in the selection of databases in a DSS - Example of filling.
*As the categories are modified. the structure's filling colors and databases are also changed.

Applicability of DSS in public management

Complex and multidimensional problems involving different sectors of the government require systematic databases that enable the integration of information. The lack of an information system or the lack of integration between the data generated by different sectors, often cause overlapping of projects (Cisne, 2012), misaligned or weak decision-making processes, and wastage of time and money.

According to Rosales-Asensio et al. (2020), access and integration of databases represent a major challenge for the management of natural resources. The compilation of standardized data can help to deal with problems related to consistency, comparability and scale, in addition to the lack of information from certain sectors (Rosales-Asensio et al., 2020).

In this sense, the DSS proposed in the present study, based on the "Nexus of water–energy–agriculture–environment" approach is a new tool for public managers to assist in investigating the level of compatibility and data leveling between information present in different databases. The DSS represents a powerful tool for identifying and organizing problems, analyzing different alternatives, and determining the best course of action (Porto et al., 2003; Ahmadi et al., 2020). For this, it is essential to automate the DSS for time optimization and its application to databases covering other sectors and spheres of government, whether they are from the union, state, or municipal.

Therefore, any plan, program or project that involves multiple sectors can use the DSS. This system aims to optimize planning and contribute to a better decision-making process and definition of integrated strategies, which tend to be reflected in greater efficiency and effectiveness.

Conclusion

The DSS proposed in the present study has the main purpose of assisting the development of integrated and interdependent studies (nexus concept) of a quantitative character. From the application of the DSS to the databases of the water, energy, agricultural, and environmental sectors present in the Brazilian context, it was possible to point out some limitations of this approach as well as future directions.

Limitations

- In general, the information corresponding to the sectors analyzed is dispersed in different institutions and does not have data standardization or uniformity; that is, most databases were structured to answer the demands of their sectors, without the prospect of integration with data from other sectors;
- The uniformity of some information is difficult to achieve, especially when it involves different spatial and time scales. In the case of spatial scales, standardization becomes easier when the data are georeferenced;
- To date, there are no national databases in the country that make it possible to investigate the impacts of water use by power plants (thermoelectric, solar, oil, coal, and natural gas) on the water availability of the hydrographic basins where they are located (systems water allocation);
- In the energy to water (sanitation) relationship, the database that stands out is the SNIS; however, it does not present the energy consumption in the different stages of the process;
- In the environmental sector, a large number of elements are at play, and consequently, it has the largest number of databases. However, in most cases, the information is not related to data from other sectors;
- Some interactions between water, energy, agriculture, and the environment cannot be developed by considering the analyzed databases; for example, energy consumption in irrigated agriculture and consumption of water and energy for all types of energy in the Brazilian matrix.

Directions and recommendations

- The adoption of macro-scales, spatial (national), and temporal (annual) uniformity facilitates the integration of the information collected. In general, working with micro- scales (such as small hydrographic basins) makes it difficult to develop integrated studies because of the low quantity of monitoring data; however, the micro-scale does facilitate database development in terms of reducing the number of variables accounted for in the process;
- It is necessary to link the georeferenced databases with the productivity databases;
- The databases of the energy sector, which may mention SIGA/ANEEL and ANP, should expand their information in the plant's register. For example, for thermoelectric plants and oil refineries, the type of cooling system, type of thermodynamic cycle, fuel used, and water situation in the region where they are located should be recorded;
- The agricultural and energy sectors should structure an integrated database combining different energy crops for the production of biofuels. The UNICA database for sugarcane is a fine example; however, it could be expanded to contain other information such as energy consumption, and water and energy use in production;
- It is recommended to apply the proposed procedure to databases present in other entities of the Federation, and to expand analyses to global databases, mainly related to the environmental and agricultural sectors. It is also recommended to structure integrated databases between the sectors involved, aiming at greater leveling and uniformity in the presentation of information. Doing so will reduce uncertainties and direct systemic planning and management strategies.

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Forest fragmentation and its potential implications for the management of the Tarumã-Açu River basin, Central Amazon, Brazil

Fragmentação florestal e suas potenciais implicações para a gestão da bacia do Tarumã-Açu, Amazônia Central, Brasil

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ABSTRACT

The intensification of deforestation and the consequent fragmentation of the natural landscape in urban and periurban watersheds affect the entire eco-hydrological system, increasing the need to understand how these changes can affect their sustainability. In this sense, the present study evaluated the potential implications of forest fragmentation for the management of the Tarumã-Açu basin, based on the characterization of the structural and functional patterns of the landscape. For this, we mapped and categorized the basin's forest fragments, based on the supervised classification (Bhattacharyya Method) of Landsat/OLI image, and, subsequently, we calculated the landscape metrics (area, density and size, edge, form, core, isolation and connectivity). The metrics showed a very fragmented landscape, especially in the region of the basin's low course, which concentrates the smallest, most dispersed, and vulnerable fragments even in conservation units. The headwater region, on the other hand, has the largest patches, with a large amount of central area and high structural and functional connectivity, which are fundamental for the sustainability of the basin and, therefore, deserve attention and prioritization by managers. The results offer important subsidies and unpublished data that can contribute to elaboration of the basin's management plan and for the definition of conservation and restoration strategies of the forest remnants, indicating priority areas for the implementation of these actions.

Keywords: connectivity; forest; landscape metrics; water resources.

RESUMO

A intensificação do desmatamento e a consequente fragmentação da paisagem natural em bacias hidrográficas urbanas e periurbanas afetam todo o sistema eco-hidrológico, aumentando a necessidade de entendimento de como essas mudanças podem impactar sua sustentabilidade. Nesse sentido, o presente estudo avaliou as potenciais implicações da fragmentação florestal para a gestão da bacia do Tarumã-Açu, a partir da caracterização dos padrões estruturais e funcionais da paisagem. Para tanto, realizou-se o mapeamento e a categorização dos fragmentos florestais da bacia, a partir da classificação supervisionada (Método Bhattacharyya) de imagem Landsat/OLI, e, posteriormente, o cálculo de métricas da paisagem (área, densidade e tamanho, bordas, forma, área central, isolamento e conectividade). As métricas mostraram uma paisagem bastante fragmentada, especialmente na região do baixo curso da bacia, que concentra os menores, mais dispersos e vulneráveis fragmentos, mesmo em unidades de conservação. Já a região da cabeceira possui os maiores fragmentos, com grande quantidade de área central e alta conectividade estrutural e funcional, fundamentais para a sustentabilidade da bacia e que, portanto, merecem atenção e priorização dos gestores. Os resultados oferecem subsídios importantes e dados inéditos que podem contribuir para a elaboração do plano de gestão da bacia e para a definição de estratégias de conservação e restauração dos remanescentes florestais, indicando áreas prioritárias para implementação dessas ações.

Palavras-chave: conectividade; floresta; métricas de paisagem; recursos hídricos.

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Introduction

The Amazon basin accommodates 10–15% of terrestrial biodiversity and most of the remnants of tropical forests in the world, still constituting an important source of atmospheric moisture, freshwater entering the oceans (approximately 15%) and carbon storage — 150–200 billion tons (Fearnside, 2016; Nobre et al., 2016). Also, despite the strong dependence of Amazonian populations on regional ecosystems (forests, rivers, wetlands and others), 20% of forest cover has already been lost, in the last 60 years, due to extensive anthropogenic changes (Nobre et al., 2016; Ruiz-Agudelo et al., 2020), with the highest rates of pantropical deforestation (Numata and Cochrane, 2012).

According to Fearnside (2016) and Ruiz-Agudelo et al. (2020), in the Amazon, deforestation begins with the opening of roads, and from there occurs the occupation (urban or not) and the expansion of the anthropic land uses (extensive agriculture, logging, mining, hydroelectric, exploration of oil and gas, and increased production of biofuels), fragmenting the natural landscape and exerting a serious influence on forest remnants and biodiversity and associated environmental services. According to Nobre et al. (2016), the conversion of forests to anthropogenic uses in this region affects hydrology, climate and biogeochemical flows at different spatial scales.

These changes in the Amazonian land use and land cover are driven by variables and complex political and socioeconomic interactions, which have reduced contiguous forests to smaller fragments (< 400 ha), irregular and increasingly isolated (Ruiz-Agudelo et al., 2020). This induces changes in the eco-hydrological dynamics of the landscapes that form the watersheds and puts their sustainability at risk, especially at the local scale (Numata and Cochrane, 2012; Laurance et al., 2018).

Laurance et al. (2011, 2018) explain that changes in the structural and functional patterns of the natural landscape of the watershed, with a decrease in the size (area) of forest fragments, have significant deleterious effects on its biophysical dynamics, making it more vulnerable to internal and compromising their resilience. In the case of the Amazon watersheds, due to the distance from the nearest ocean, and, therefore, the greater dependence on the forest evapotranspiration rates themselves, much of this susceptibility derives from the loss of leaf area and the effects to which the fragments edges are subjected (Nobre et al., 2016; Wang et al., 2017). Thus, as fragmentation increases, the impacts on the hydrological regime of the basin are also intensified (Laurance et al., 2018).

According to the studies by Laurance et al. (2011, 2018), desiccation conditions caused by the opening of clearings can penetrate up to 100–200 m inside the fragments, and lead to great spatiotemporal variations in the flow of fragmented watersheds in the Central Amazon. In fragments of the Eastern Amazon, these desiccant effects can be even more aggressive, and penetrate 1.0–2.7 km into the forests (Briant et al., 2010), as a result of the advanced state of fragmentation in that region. For Aragón et al. (2015) and Cabral and Costa (2017), a new eco-hydrological cycle begins in each new fragment, increasing the risks of erosion and silting of streams; and all these changes affect not only the local biodiversity, but also the populations dependent on that biota (indigenous and riverine, especially), forcing them to change their livelihoods and traditions.

In general, the fragmentation of the watershed landscape can affect the formation of clouds and lead to an increase in local precipitation in the short term, but a decrease in rainfall rates over time, according to changes in the size and pattern of the patches remaining (Nobre et al., 2016). As pointed out by Laurance et al. (2011, 2018), Maeda et al. (2015) and Nobre et al. (2016), the increase in soil surface temperature in watersheds with forest cover loss superior to 20% alters low-level atmospheric circulation, forming zones of low pressure on clearings, which attract moisture from forested areas and lead to formation of convection clouds. This effect redistributes moisture over deforested areas and affects the recycling of water into the atmosphere, which can lead to the collapse of the hydrological system in the long term (Laurance et al., 2018).

As if the effects of fragmentation were not enough, the occurrence of forest fires (common in the region) hypersatures the atmospheric condensation cores, absorbs solar radiation, promotes atmospheric heating, and also prevents the formation of rain clouds (Sheil, 2018). In periods of drought, when vegetation is already subjected to a certain degree of water stress, forest fragments become more vulnerable. It is precisely in this season that fires happen most frequently (Laurance et al., 2018). Jiménez-Muñoz et al. (2016) suggest that forest fires are the main agents of transition to scenarios of frequent droughts, which should be repeated in the coming decades, as recorded in 2015–2016.

Although many studies propose to assess the potential consequences of forest fragmentation and its relationship with hydrological, structural and functional changes in the largest and most important river basins on the planet, the potential impacts on small Amazon watersheds are still little explored (Maeda et al., 2015), especially in the State of Amazonas. In addition to the complexity of the relationships between the hydrological system and the structural and functional changes of tropical forests, the scarcity of historical data and in part the natural variability of precipitation in these watersheds make this understanding an even greater challenge, besides causing divergences between future possible scenarios (Marengo et al., 2011; Trenberth, 2011; Gloor et al., 2013).

The difficulty of fully understanding this complex and integrated set of natural and anthroponatural formations that characterizes the landscape of urban and periruban watersheds extends to the planning and sustainable management of its natural resources (Rodríguez et al., 2017, 2019). According to Trombeta and Leal (2016), the hydrographic basin as a management unit established by Law nº 9,433/97 (Brasil, 1997) requires the elaboration of a water resources plan based on the concept of landscape (landscape planning), which allows the proposition of rational strategies for the use and conservation of natural resources, aiming at sustainable development (Trombeta and Leal, 2016; Scholten et al., 2020).

In this sense, Pereira and Cestaro (2016) consider that even the creation of conservation units (CUs) — considered one of the best preservation strategies — tend to suffer the consequences of isolation over time, becoming more susceptible to the effects of edge and adjacent anthropic changes. Thus, the planning and management of watersheds and their resilience capacity must consider network structures (CUs, ecological trampolines and corridors, enrichment of buffer zones, land use planning, and others) and not just the definition of areas unique and isolated (Pereira and Cestaro, 2016).

Considering the variety of eco-hydrological distortions that can permeate fragmented basins, one of the challenges for managers is to identify those aspects of greatest importance and generality (Laurance et al., 2018). This identification can be done through structural (related to physical characteristics) and functional (possibilities of gene flow, individuals and populations) analysis of landscape fragmentation, supported by landscape metrics, increasing the possibilities of efficiency and effectiveness, as they reduce subjectivity in considering conflicts and restrictions (existing and probable) and, consequently, in making decisions (Rudnick et al., 2012).

However, Ruiz-Agudelo et al. (2020) indicate that the management of Amazon watersheds presents yet another obstacle — the institutional and management instruments fragmentation, which have been partially dissociated in the region. The State of Amazonas, for example, reformulated its State Water Resources Policy in 2007, also instituting its management instruments (Amazonas, 2020). However, only two of the nine predicted instruments were implemented: in 2016, the granting of the right to use; and more recently the State Water Resources Plan (Amazonas, 2020).

The State of Amazonas was the first in the North Region of Brazil to create, in 2009, a watershed committee, the Tarumã-Açu River Basin Committee (Amazonas, 2020). However, more than ten years after its creation, the respective basin plan has not yet been implemented. According to Scholten et al. (2020) and Trindade and Scheibe (2019), in addition to the lack of technical, physical and financial support from the States and the difficulty in articulating the various stakeholders, the inefficiency and limitation of the decision-making capacity of committees is due to the lack of basins plans, the main management tool.

This whole context shows that the watershed management plan must be based on the dynamics of occupation, use and anthropogenic alteration of the pre-existing matrix, balancing the prospects for the conservation of water resources and socioeconomic development, increasing the chances of success and reducing negative risks. This requires understanding and anticipating structural and/or functional changes, as well as managing the system's ability to reorganize and recover (Scheffer et al., 2018).

A multifactorial analysis of impacts on the watershed's systemic resilience potential, combining structural and functional connectivity metrics in a Geographic Information System (GIS) environment constitutes, according to Pereira and Cestaro (2016) and Rocha et al. (2018), an important tool to assist managers in decision making, endowing the choices with scientific and methodological rigor. Thus, studies in this sense do not necessarily seek the indication of better and/or worse alternatives for the resilience of socio-ecological systems, but illustrate the potential and/or weaknesses of possible choices (Biggs et al., 2015; Pereira and Cestaro, 2016; Ruiz-Agudelo et al., 2020).

Magnuszewski et al. (2015) ratify that management instruments, such as river basin plans and/or land use planning in traditional ways, do not apply to Amazonian landscapes. It is necessary to understand, with the support of landscape metrics, the changes in structural and functional patterns of forest fragmentation promoted by the complex local biophysical, political and social context, and, with this holistic perspective, proceed to elaborate and implement the management and monitoring plan (Santos et al., 2019).

The construction of basin plans based on the recognition of the spatial-functional interdependence between biophysical components and social well-being makes it possible to design new management arrangements for natural resources, which guarantee the provision of environmental services and which increase the capacity for adaptability and resilience of the remaining natural ecosystems (García-Márquez et al., 2017; Stimson, 2017). In this sense, the present study aimed to evaluate the potential implications of forest fragmentation for the management of the Tarumã-Açu River basin (TARB), based on the characterization of the structural and functional patterns of the landscape.

Materials and Methods

Study area

The TARB is located in the central region of the Amazon, on the left bank of the Negro River, upstream of Manaus city; and it constitutes one of the four large watersheds on which the capital of Amazonas was built (Siqueira, 2019). With a total area of 137,273 hectares, it is a fifth-order watershed, morphometrically wide, elongated and few dissected and sinuous, which reduces the risk of flooding, but favors the carrying of sediments and the appearance of erosion processes, mainly in areas without vegetation cover (Costa et al., 2013) (see Figure 1).

According to Antonio (2017), the climate is of the super humid type, according to the De Martonne Index, with three months of mild to moderate drought (July to September) and six months (December to May) of higher humidity and rainfall; June and October are transition months between periods. The mean annual temperature is 27°C, the mean annual relative humidity is around 80%, and the annual rainfall varies from 1,900 to 3,500 mm (Costa et al., 2012; Laurance et al.,

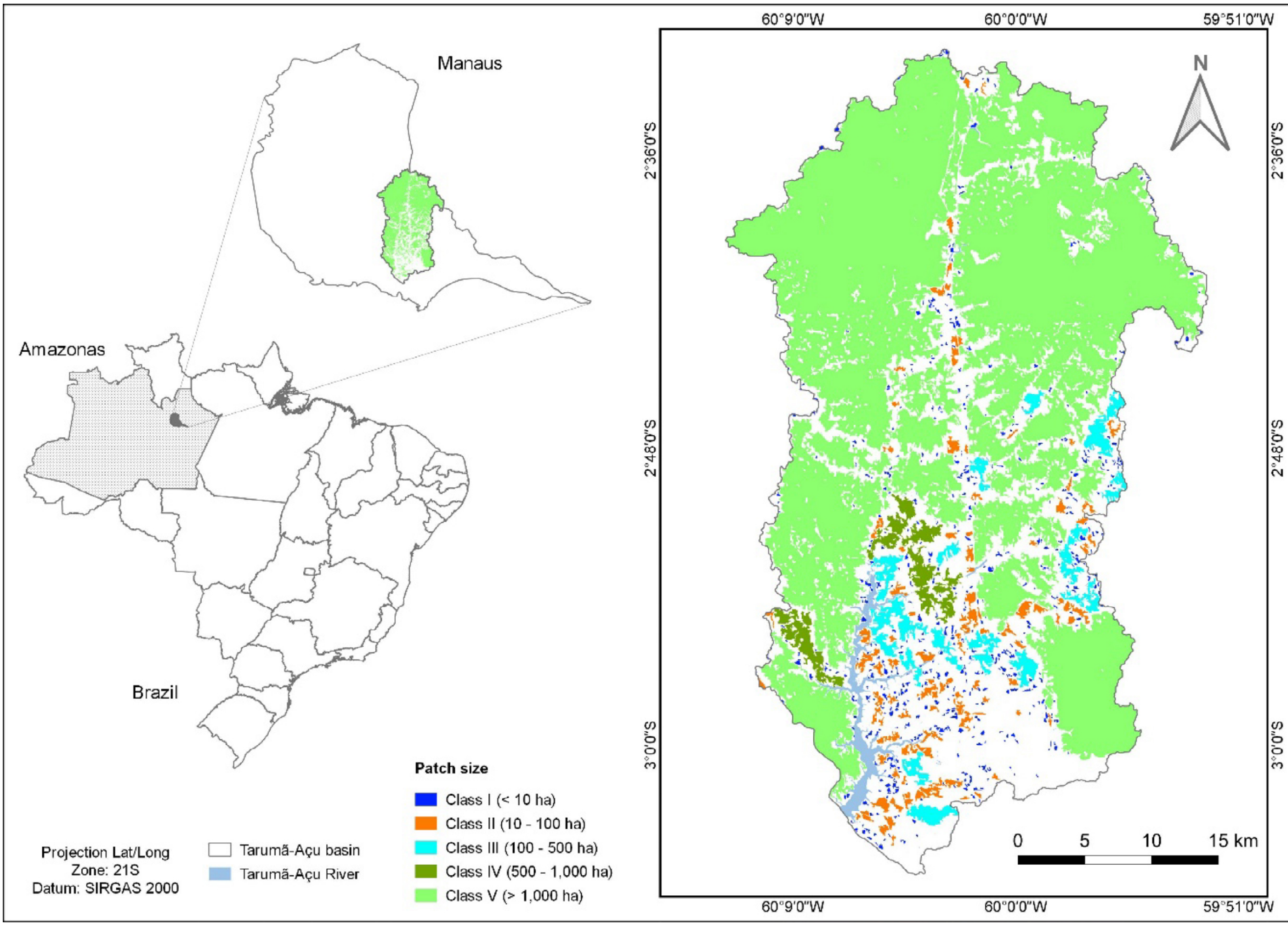


Figure 1 – Location of the Tarumã-Açu River basin with distribution of forest remnants by size class.

2018), which are characteristic of tropical forests. According to Embrapa (2003) and Laurance et al. (2018), the vegetation in the TARB region is predominantly Dense Rainforest, with canopy 30–37 m high and emerging reaching 55 m. There is also an Open Rainforest, Periodically Flooded Alluvial Forest (*Igapó* Forest), Campinarana and areas of ecological tension (secondary vegetation and agriculture).

Derived from the Alter do Chão Formation, the basin soils are classified as: Yellow Latosol in the plateaus; Red-yellow Podzolic on the slopes; and Hydromorphic soils in the shallows (EMBRAPA, 2003). The sandy-clay textures of the slopes, close to the shallows, and sandy in the shallows also facilitate the occurrence of erosion in areas without vegetation (PROAMBIENTE, 2002). Regarding land use, according to Costa et al. (2012), it is an area of urban and agro-industrial expansion that also has conservation units, agrarian settlement (Tarumã-Mirim) and traditional communities (riverine and *Saterê-Mawé Inhambé* and *Caniço-Rouxinol* indigenous peoples).

Structural and functional analysis of the landscape

To generate the forest fragmentation map (primary and secondary forest formations), we used the Landsat 8 OLI sensor image (30 m resolution) of July 27, 2016, made available by the National Institute for Space Research (in Portuguese: *Instituto Nacional de Pesquisas Espaciais* – INPE). The image selection took into account the absence of cloud and/or shadow coverage over the study area. For image processing, we used the SPRING 5.5.6 software (IBGE, 2013).

In the pre-processing stage, we generated the compositions of the scenes from the spectral bands 4 (red), 5 (near infrared) and 6 (medium infrared), widely used in this type of mapping due to the different spectral responses of the vegetation (Ribeiro et al., 2017). We used the QGIS 3.4.5 software for stacking, clipping and false color composition of the 456/RGB bands. Then, we proceeded to the forest area supervised classification, using the SPRING 5.5.6 software, according to the segmentation and classification regions of Bhattacharyya Method, 95% acceptance (Ribeiro et al., 2017).

According to Oliveira et al. (2015), the planning and management of territorial units based on landscape indexes/metrics requires the interpretation of geostatistical maps, whose quality/accuracy is fundamental for the correct analysis of the spatial pattern and fragmentation indexes. In this sense, as adopted by Rusca et al. (2017), in post-processing, we first verify the accuracy of the classification through the Kappa coefficient (K = 0.9861, calculated by SPRING), categorized by Landis and Koch (1977) as perfect. Subsequently, for classification validation, we resampled the data from georeferenced points distributed along the TARB according to the conditions of accessibility (Jesus et al., 2015). Finally, we vectored and quantified the thematic classes and set the final layout, following the color chart of the Technical Manual for Land Use (IBGE, 2013).

Considering the specificities of fragmentation in the Amazon (Briant et al., 2010; Numata and Cochrane, 2012; Haddad et al., 2015; Sonter et al., 2017; Laurance et al., 2018; Lisboa et al., 2019; Santos et al., 2019; Hansen et al., 2020; Ruiz-Agudelo et al., 2020), we distributed forest patches in five size classes, which were used in landscape analysis: < 10 ha (Class I), 10–100 ha (Class II), 100–500 ha (Class III), 500–1,000 (Class IV) ha and > 1,000 ha (Class V), as shown in Figure 1.

Following fragmentation studies in Amazonian environments (*e.g.* Rusca et al., 2017; Lisboa et al., 2019; Santos et al., 2019; Andrade et al., 2020; Castro et al., 2020; Duarte et al., 2020), we apply the metrics described in Table 1 to characterize the structural and functional patterns of the TARB landscape. The selection included metrics that, according to Baranyi et al. (2011), Saura et al. (2011) and Ziólkowska et al. (2014),

Table 1 – Landscape metrics selected for analysis of forest fragmentation in the Tarumã-Açu River basin.

Metrics	Abbreviation	Unit	Feature
Area			
Class Area	CA	ha	Total area of fragment patches by class.
Occupancy percentage	ZLAND	%	Percentage of occupation of each class in relation to the landscape.
Density and size			
Number of Patches	NUMP	-	Number of patches in the landscape by class.
Mean Patch Size	MPS	ha	Mean size of forest patches by class.
Patches Size Standard Deviation	PSSD	ha	Variation in the size of forest patches in relation to the mean of each class.
Patches Size Variance Coefficient	PSCoV	%	PSSD/MPS ratio, in percentage, for each class.
Edge			
Total Edge	TE	m	Total length of the edges by class.
Shape			
Mean Shape Index	MSI	-	Circularity of the patches. The closer to 1 the value is, more circular is the fragment.
Area-Weighted Mean Shape Index	AWMSI	-	Area-weighted MSI measure.
Core			
Total Core Area	TCA	ha	Total core area by class – reference edge for this study is 300 m (Laurance et al., 2011, 2018).
Total Core Area Index	TCAI	%	Relative measure of core area.
Isolation			
Mean Neighbor Near	MNN	m	Mean distances from the nearest neighbor at class level (between patches) and in the landscape (between classes).
Connectivity			
Integral Connectivity Index	IIC	-	Degree of connectivity based on determined dispersion radius.
Probability of Connectivity	PC	-	Probability dispersion of organisms among the fragments that make up the landscape.

Source: adapted from Jesus et al. (2015) and Thiago et al. (2020).

present better performance and higher frequency of use in the evaluation of structural and functional aspects of watersheds.

The isolation metric (Mean Neighbor Near (MNN)) measures the inaccessibility of patches to migration from other fragments. Functional indexes (Integral Connectivity Index (IIC) and Probability of Connectivity (PC)), on the other hand, take distances from all possible origins (patches) into account, being good predictors of colonization events, especially in very fragmented landscapes (Ziólkowska et al., 2014). According to Saura et al. (2011), when analyzed together, these indices provide a more complete view of the potential for promoting and/or maintaining connectivity, as they are based on spatial graphs (Graph Theory) and on the concept of measuring habitat availability (accessibility) on the landscape scale.

The Graph Theory considers the fragments as "nodes" and the connections between them as borders, and allows evaluating a network of patches (Gross et al., 2019). For this reason, it is a powerful way of representing complex landscape patterns with advanced performance in the analysis of connectivity (Saura et al., 2011; Ziólkowska et al., 2014). Accessibility measurement integrates the connection resources existing inside the large fragments (intrapatches) with those reachable through strong connections with other fragments in the landscape (interpatches), or the combination of both (Saura and Torné, 2012).

For the structural analysis of forest fragmentation in the TARB, we used the free extensions Patch Grid (raster), for the metrics MNN and Occupancy percentage (ZLAND), and Patch Analyst (shapefile) for the others, both from ArcGIS 10.8 (Andrade et al., 2020). Functional metrics were calculated using the free software Conefor 2.6, designed as a tool to support landscape planning and management through the identification and prioritization of critical locations for ecological connectivity (Saura and Torné, 2012).

Considering the results presented in the studies by Laurance et al. (2011, 2018), which indicate limitations of 100–250 m for the dispersive process of some resident species (Euglossini bees, Scarabaeidae beetles and some birds), we defined 100 m threshold for quantification of functional connectivity indexes (Rusca et al., 2017). For the PC calculation, we used the 0.05 probability that corresponds to the maximum possibility of dispersion between the nodes, following the one applied by Ziólkowska et al. (2014). The statistical analyzes were processed using the StatSoft Statistica 12.5 software.

Results and Discussion

The results of the structural metrics of the landscape of the TARB are shown in Table 2. The mapping identified 683 forest fragments distributed in 5 size classes (as shown in Figure 1), which correspond to 66.50% of the total area of TARB. In the landscape context, the mean patches size (MPS) was 133.66 ha, with the smallest fragment measuring around 0.02 ha and the largest 28.092 ha, resulting in high standard deviation values (Patches Size Standard Deviation (PSSD)) and variation coefficient (Patches Size Variance Coefficient (PSCoV)). This information suggests that despite being quite fragmented, the basin still has good forest conservation potential, which, according to Oliveira et al. (2015), facilitates the planning and management of conservation and restoration actions.

Most of the remaining forest patches (NUMP = 553, or 80.97%) belong to Class I, whose area is less than 10 ha (Table 2 and Figure 2A). The second most representative class in number of fragments is Class II (10–100 ha), which covers 102 fragments or 14.93%

Table 2 – Landscape metrics by size class of forest fragments in the Tarumã-Açu River basin.

Metrics	Unit	Size classes				
		I	II	III	IV	V
CA	ha	1,683.16	2,938.76	3,930.49	2,264.69	80,474.86
ZLAND	%	1.84	3.22	4.31	2.48	88.15
NUMP	-	553	102	16	3	9
MPS	ha	3.04	28.81	245.66	754.90	8,941.65
PSSD	ha	2.17	19.36	112.53	15.74	9,853.15
PSCoV	%	71.24	67.20	45.81	2.08	110.19
TE	m 10^3	452.82	387.01	318.65	169.06	2,016.72
MSI	-	1.37	2.00	3.58	5.78	6.64
AWMSI	-	1.44	2.19	3.77	5.79	8.73
MNN	m	482.33	502.56	399.03	872.70	161.63

CA: class area; ZLAND: occupancy percentage; NUMP: number of patches; MPS: mean patch size; PSSD: patches size standard deviation; PSCoV: patches size variance coefficient; TE: total edge; MSI: mean shape index; AWMSI: area-weighted mean shape index; MNN: mean neighbour near.

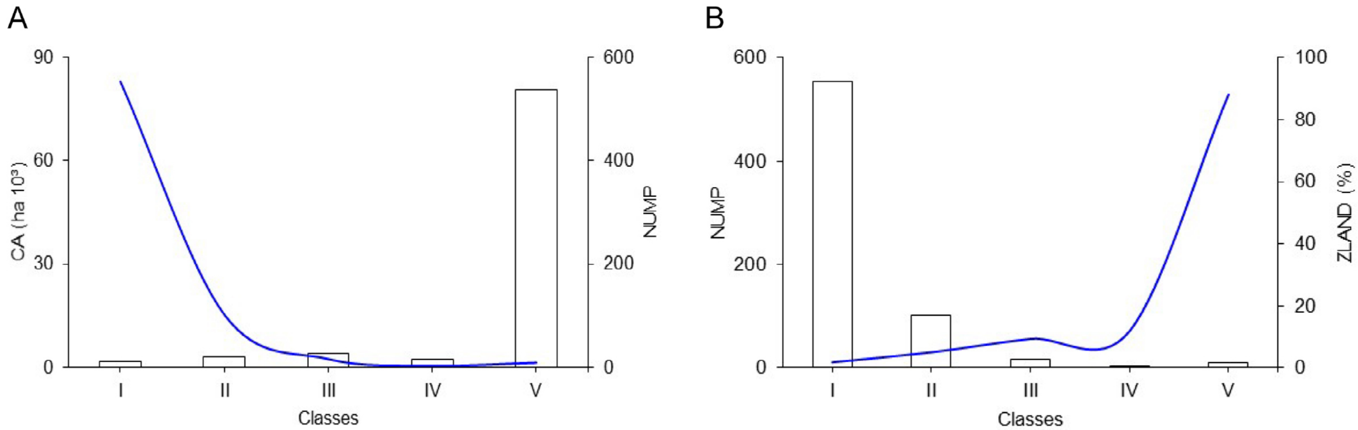


Figure 2 – Number of fragments (NUMP) by size class and total area of forest patches in: (A) hectares – CA; and (B) percentage – ZLAND.
NUMP: number of patches; CA: class area; ZLAND: occupancy percentage.

of the total. However, these classes together account for only 5.06% of all forest vegetation in the TARB (Figure 2B). This diagnosis converges with the studies by Numata and Cochrane (2012), Haddad et al. (2015) and Hansen et al. (2020), whose data showed an increase in the number of fragments < 10 ha in Amazonian forests, where there is a numerical predominance of patches smaller than 100 ha. Concerning hydrographic basins as a territorial unit, several studies have found similar results, both in the Amazon and in other biomes (Jesus et al., 2015; Rusca et al., 2017; Rex et al., 2018; Lisboa et al., 2019; Andrade et al., 2020; Cavalcante et al., 2020; Castro et al., 2020; Thiago et al., 2020).

Although small fragments generally function as habitat sinks (Odum and Barret, 2008) in very anthropized matrices — is the case of the TARB low basin region — these scattered patches can function as ecological "stepping stones" or connection points, facilitating the flow of some species in the landscape (Saura et al., 2014; Andrade et al., 2020). Managing strategies for these fragments should be considered in watersheds management plans (Castro et al., 2020; Cavalcante et al., 2020; Thiago et al., 2020), aiming at maintaining and/or increasing connectivity with larger fragments. These, in turn, although in smaller numbers (Class V: NUMP = 9), represent 88.15% of the forest areas (Figure 2B), and function as sources for eco-hydrological processes, standing out in importance for the resilience and sustainability capacity of the watershed landscape (Jesus et al., 2015).

Approximately 70% of the fragmentation is concentrated in the low basin, the region most affected by anthropogenic uses (urbanization, mining, agribusiness and tourism), even within the limits of conservation units (*e.g.* Tarumã/Ponta Negra Environmental Protection Area). Duarte et al. (2020) also recorded high rates of deforestation in CUs in the lower Acre River, Western Amazon. These changes in land use and coverage, plus the hyperdinamism of urban and periurban watersheds, increase the vulnerability to sediment transport, and consequently to the occurrence of erosion and silting of stretches of the drainage network (Serrão et al., 2019; Rocha and Lima, 2020).

As Jesus et al. (2015), the reduction of natural landscapes to small patches is related to the economic land use. According to Farias et al. (2018), Laurance et al. (2018), Cavalcante et al. (2020) and Duarte et al. (2020), in Amazonian landscapes, this use is mainly agricultural and often associated with rural settlements, even when overlapping with CUs. This pattern is observed on left bank of the Tarumã-Açu River and close to basin's headwater, where fragmentation is notable into areas corresponding to the Tarumã-Mirim and Santo Antônio settlements, respectively. In the low basin, since it is a periurban watershed, the main factor in transforming the natural landscape was disordered urbanization.

The constructions of highways and opening of roads induces anthropic occupation and transforms the landscape (Gomes et al., 2019). This is very common at TARB, for access to the settled communities and the streams used for non-consumptive purposes of primary contact (balneary). According to Khanna et al. (2017) and Spera et al. (2016), such changes in forest cover impact the variability of precipitation and discharges from river channels, especially in small watersheds. If fragmentation occurs in *Permanent Protection Areas* (PPAs), susceptibility to erosion and silting of water bodies increases, compromising water quality (Silva et al., 2020).

Regarding the shape, the landscape mean index (Mean Shape Index (MSI)) was 1.61, and, in the division by classes, while Class I presented more circular shapes, the Class V patches showed more irregular shapes (Figure 3). In the weighted analysis (Area-Weighted Mean Shape Index (AWMSI)) the pattern remained. This concentration of fragments in less complex shapes is evident in the dispersion analysis (Figure 3B). Jesus et al. (2015), Rusca et al. (2017), Rex et al. (2018) and Thiago et al. (2020) found similar results in the shape/area relationship of the fragments when studying urban and periurban basins.

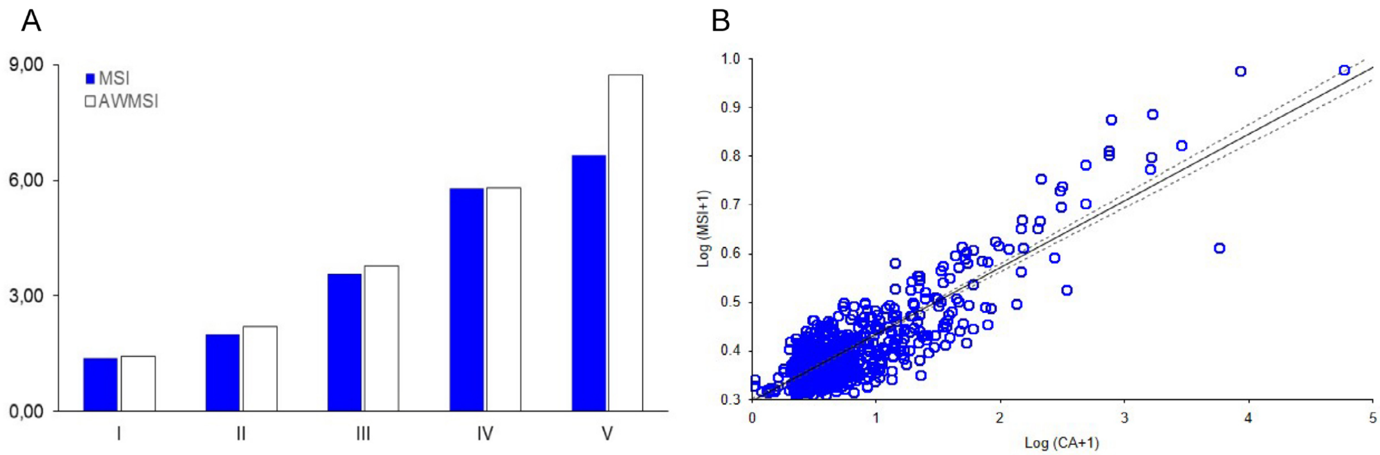


Figure 3 – (A) Circularity indexes (MSI e AWMSI), and (B) dispersion of forest fragments in relation to area (CA) and shape (MSI) values – confidence interval 0.95.
MSI: mean shape index; AWMSI: area-weighted mean shape index; CA: class area.

Fragments with more irregular shapes (MSI > 2) indicate greater border area and smaller core. Thus, in addition to size, shape is another aspect that influences the vulnerability of the patch to different edge effects and other external instabilities (Numata and Cochrane, 2012; Laurance et al., 2018). In this sense, there was a higher total value of edge length (Total Edge (TE)) for the class of the largest fragments. Class IV had the lowest TE, which is justified by the low class area (CA) and ZLAND. The mean edge length at TARB landscape was 668.85 10^3 m.

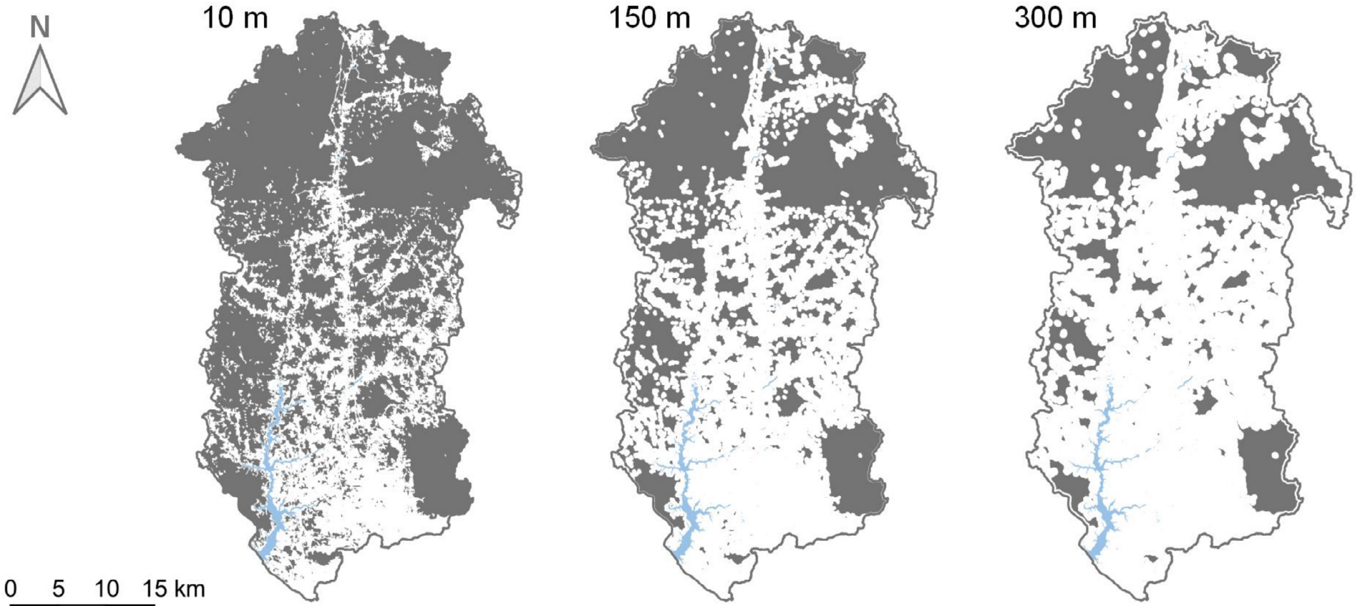
According to Thiago et al. (2020), the large amount of edge area favors the generalist, predators and invasive parasites species development, which act mainly on the edges of the fragments. Laurance et al. (2011, 2018) emphasize that the edge effects are among the most important vectors of temporal-flow changes in fragmented watersheds in the Central Amazon, due to the desiccant effects of clearings on low-level atmospheric circulation, impacting the clouds formation and local precipitation, raising its susceptibility to the vicissitudes of anthropic transformations and fires.

The studies by Laurance et al. (2011, 2018) about fragments of Central Amazonia suggest that the edge effects can reach from 10 to 300 m towards the interior of the patches, and the wider the edge (> 60 m), the greater susceptibility to hydroclimatic alteration effects. For this reason, Lisboa et al. (2019), Cavalcante et al. (2020) and Thiago et al. (2020) are unanimous in considering the core metrics (Total Core Area (TCA) and Total Core Area Index (TCAI)) as measures of habitat quality, since they indicate the effective habitat availability into fragments, that is, where there is no subjection to edge effect.

Thus, Figure 4 expresses the influence of three distinct ranges of the edge over the Tarumã-Açu basin core patches, by size class, weighing the thresholds suggested by Laurance et al. (2011, 2018): minimum width of 10 m and maximum of 300 m, and an intermediate edge of 150 m. It is observed that even the smallest border width (10 m) already represents, approximately, 25% of the Class I fragments total area. However, in this scenario, the TCAI of the basin landscape is still 96.76%. Considering the wider edges (150 and 300 m), it is possible to verify that the smallest class does not have core area availability, and only 69.74% and 49.99% of Class V is equivalent to the core, respectively. From this perspective, 31.03% and 50.25% of TARB's forest areas correspond to edge.

These results are in agreement with the work of Haddad et al. (2015) and Numata and Cochrane (2012), whose analysis of fragmentation in the Amazon found that the forest areas subject to the edge effects were already larger than the total deforested areas, and this proportion has been increasing due to anthropic transformations. Lisboa et al. (2019) and Cavalcante et al. (2020) recorded similar patterns in São Félix do Xingu, Pará, and at the Belém Endemism Center, between the States of Pará and Maranhão, suggesting the worsening of the quality of the remaining patches and the greater fragility of the landscape.

As for the degree of isolation (MNN), the fragments with more than 1,000 ha (Class V) had the shortest average distance from the nearest neighbor (161.63 m), reinforcing their potential function as maintainers of the watershed's eco-hydrological dynamics. Patches with area 500–1,000 ha (Class IV) have the highest intraclass insulation (872.70 m). In the TARB landscape assessment, the mean distancing of the fragments was 470.40 m. The registered MNN values suggest, based on Lisboa et al. (2019) and Cavalcante et al. (2020), positive aggregation from the perspective of environmental restoration, and, according to Thiago et al. (2020), confirms the importance of small fragments as connecting elements.



Classes	TCA	TCAI	NUMP	TCA	TCAI	NUMP	TCA	TCAI	NUMP
I	1.250,94	74,33	539	-	-	-	-	-	-
II	2.556,05	86,98	102	130,52	6,01	46	0,98	0,44	3
III	3.612,87	91,88	16	824,71	16,27	16	137,35	5,50	9
IV	2.095,56	92,53	3	528,76	23,55	3	67,19	3,25	3
V	78.443,32	97,63	9	55.300,54	69,74	9	39.828,00	49,99	9

Figure 4 – Comparative simulation between available habitat (core) in hectares (TCA) and percentage (TCAI), and the number of fragments (NUMP) by size class for edges of 10, 150 and 300 m.

TCA: total core area; TCAI: total core area index; NUMP: number of patches.

According to Leite et al. (2013), this positive aggregation derives from the high density of forest fragments, which can facilitate biological flows across the landscape, especially for groups with less mobility (Crouzeilles and Curran, 2016). Radford and Bennett (2007) add that this would increase the structural landscape connectivity, supporting larger populations, but it does not compensate for the reduced extension of available habitats. In other words, it is necessary to think about management strategies that use this aggregation of forest fragments to improve the connectivity of the basin's landscape, increasing matrix permeability. Especially in the low basin, the creation of habitat networks, amalgamation of discrete patches, trampolines, for example, would facilitate mobility and increase recolonization rates, making the fragments smaller nuclei of regeneration (Crouzeilles and Curran, 2016), and potentiating the effects of restoration actions in time and space (Antongiovanni and Metzger, 2005). The benefits can still involve minimizing costs, since the natural process of plant succession could be in charge of reestablishing ecosystem processes (Chazdon et al., 2017).

As indicated by Silva et al. (2020) for the Apeú River basin (Pará), the isolation of TARB fragments was greater in the region where urbanization was consolidated (low basin). It should be noted that an anthropic matrix added to the isolation enhances the fragmentation effects and reduces the resilience capacity of natural patches (Jesus et al., 2015). Thus, watershed management strategies should consider the possibility of increasing size of urban fragments and/or enriching the buffer zones, using fast-growing native forest species, helping to support connectivity (Jesus et al., 2015; Brasil, 2017).

In general, larger fragments were more important for the connectivity of the Tarumã-Açu basin landscape, according to both indexes evaluated — IIC and PC, especially the large patches located in the headwater (Figures 5 and 6). The connectivity indexes were grouped according to the Jenks Optimization Method, also known as "natural breaks", whose principle is to minimize the differences between the values arranged in the same class and to maximize the differences between the classes; in addition to being suitable for application in choropleth maps, its use is standard in GIS (Ramos et al., 2016).

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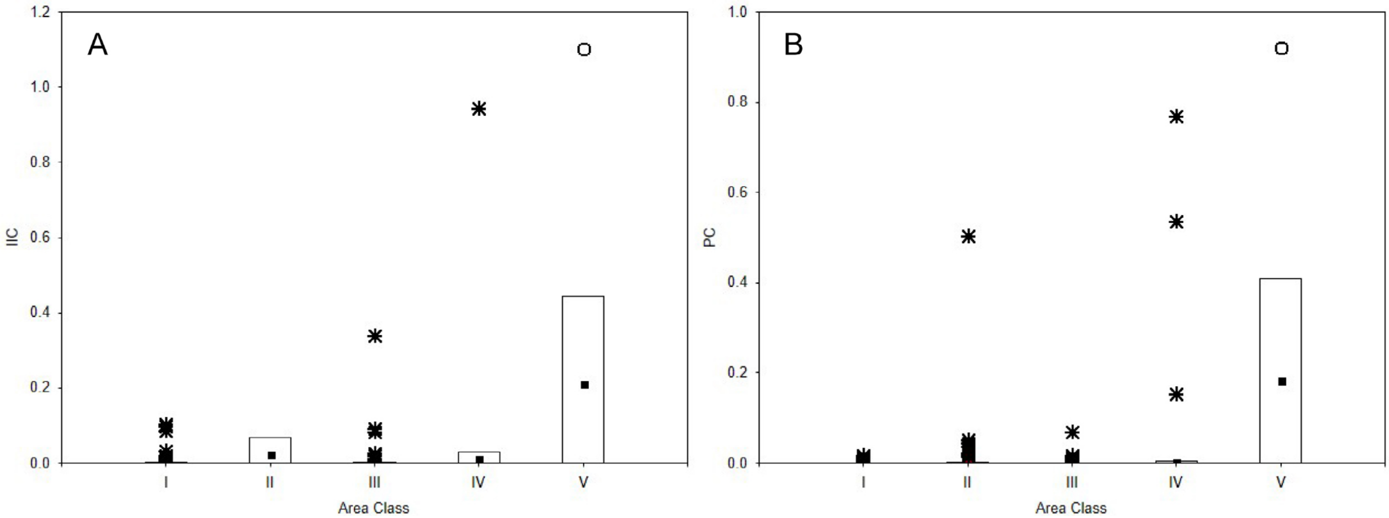

Figure 5 – Variation values of connectivity of the Tarumã-Açu basin fragments (based on 100 m dispersion threshold) by size class, according to: (A) Integral Connectivity Index; (B) Probability of Connectivity. The columns show the mean and the variation of the indexes, while the asterisks and circles indicate outliers and very discrepant values, respectively.

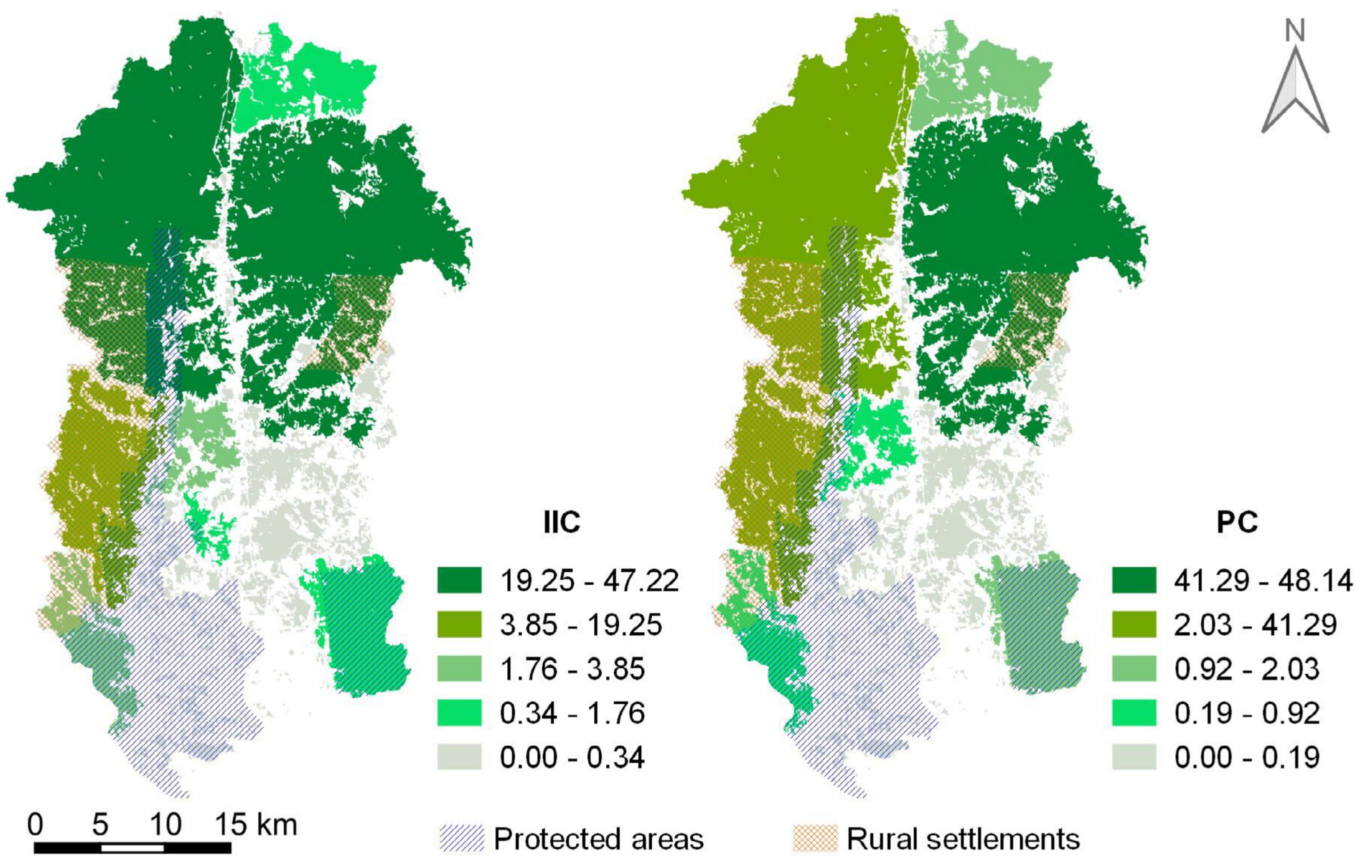

Figure 6 – Classification of remaining forest patches in the Tarumã-Açu River basin under 100 m dispersion threshold, based on the importance for connectivity determined by the Integral Connectivity Index (IIC) and the Probability of Connectivity (PC).

The results make it evident that most areas with high potential for functional connectivity are outside the limits of the CUs, while those patches with low values of IIC and PC are within protected areas. It is also clear the importance of the Adolpho Ducke Forest Reserve for maintaining connectivity, especially in the low basin. Barber et al. (2014), Castro et al. (2020) and Duarte et al. (2020) warn that protected areas play a fundamental role in the sustainability of the Amazon fragmented landscapes. However, the authors attribute good results to the existence of the respective management plans, and even more to the effectiveness of the inspection actions.

Andrade et al. (2020) and Silva et al. (2020) underscore the importance of connectivity as a way of promoting key ecosystem services for water and soil conservation in watersheds. Therefore, the planning and management of conservation and recovery strategies for connectivity in small basins, such as TARB, where the impacts of fragmentation on environmental services (crop pollination, pest control, erosion and silting of water bodies, etc.) are felt more directly should be a priority (Haddad et al., 2015).

As recorded by Castro et al. (2020) in the Xingu Endemism Area, the conflict of interests and the potential for exploiting varied natural resources (water, forests, soils, non-metallic minerals) shaped the occupation and transformation of the Tarumã-Açu basin landscape. The impacts of forest cover loss are possibly aggravated by reducing fragment size, creating edges and reducing connectivity (structural and functional). In addition, there is a lack of governance, integrated policies and management instruments, such as the watershed plan and the CUs management plans.

It was seen that the creation of individual conservation units did not bring the expected collective benefit to TARB. Thus, the planning and use of network strategies can present better results for Tarumã-Açu basin landscape conservation/restoration. As proposed by Haddad et al. (2015) and Castro et al. (2020), these strategies can initially focus on prioritizing the protection of those fragments with high connectivity potential (IIC and PC), which are not protected; the enrichment of buffer zones of small fragments with fast growing native species in the low course, due to the urban use already consolidated in this region; and effective land use planning.

Whatever the plan, its implementation and effectiveness will depend, above all, on the articulation capacity of the basin committee, responsible for promoting dialogue between the various stakeholders, trying to balance management, socioeconomic development and natural biophysical aspects of the basin's landscape, as stated Lima and Garcez (2017) and Trindade and Scheibe (2019). These aspects are interdependent and mutually influential when it comes to the planning and management of urban and periurban watersheds, and, although complex, this integration must start from the assumption of the forest-water relationship transversality (Diniz et al., 2020).

Conclusions

The TARB is very fragmented with a higher degree of forest degradation in the low basin, including protected areas except the Adolpho Ducke Forest Reserve, although it still has good vegetation conservation potential. The headwater region, in turn, has the largest size and core area amount fragments, in addition to high structural and functional connectivity, which are fundamental for the natural resources and basin's landscape sustainability, however it is not under protection.

The small fragments of the low basin, despite the greater vulnerability to edge effects and anthropogenic pressures, and reduced potential for functional connectivity, present physical isolation values close to landscape mean value, indicating their potential role as a link between larger patches, functioning as connection elements and requiring equal attention from watershed and UCs managers. In the urban context, well-planned and executed landscape measures, using fast-growing native species, can help in the process of maintaining TARB's ecosystem services.

The present characterization of structural and functional patterns of the Tarumã-Açu basin landscape offers important subsidies to elaboration of the management plan and to definition of the forest remnants conservation and restoration strategies, indicating priority areas for the implementation of these actions from production of unprecedented data on forest fragmentation in the TARB's context. The management of the basin's natural landscape needs to envision mitigating the impacts of human transformations, which, in this context, are numerous (and inevitable, from the perspective of socioeconomic development). This should help to support the eco-hydrological dynamics responsible for water resources, soils, biodiversity sustainability and, therefore, for the very economic and social activities that occur into the TARB.

Contribution of authors:

Costa, J.S.: Conceptualization, Methodology, Formal analysis, Data curation, Writing — original draft, Writing — review and editing; Rodrigues, L.S.: Formal analysis, Data curation, Writing — review; Silva, A.G.C.: Formal analysis, Data curation, Writing — review; Reis, T.C.: Formal analysis, Data curation, Writing — review; Melo, M.G.G.: Supervision, Validation, Writing — review; Liberato, M.A.R.: Supervision, Validation, Writing — review.

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Metals bioremediation potential using *Pseudokirchneriella subcapitata*

Potencial de biorremediação de metais pela microalga *Pseudokirchneriella subcapitata*

Mônica Ansilago[1], Franciéli Ottonelli[1], Emerson Machado de Carvalho[2]

ABSTRACT

Microalgae are unicellular organisms, photosynthesizers that present cell duplication exponentially and biosorption capacity of nutrients dissolved in water. The objective of this work was to evaluate the capacity of the microalga *Pseudokirchneriella subcapitata* for bioremediation of metals and salts. In this aspect, the reduction of the metals and salts in the synthetic effluents by the microalga *P. subcapitata* was evaluated: (T1) culture medium (control); (T2) culture medium contaminated with aluminum chloride; (T3) culture medium contaminated with ferrous sulfate; (T4) culture medium contaminated with zinc sulfate; (T5) culture medium contaminated with the combination of aluminum chloride, ferrous sulfate and zinc sulfate. The bioremediation process was evaluated by comparing culture media with suspended microalgae to a filtrate version of the same medium. Iron and zinc metals, as well as nitrogen and phosphorus salts, showed depleted values in the filtered medium, indicating efficiency in the treatment of water by microalgae. Aluminum content was below the limit of detection in all treatments. The cumulative values in the microalgae biomass were, in descending order: nitrogen, zinc, iron and phosphorus, thus indicating the assimilation of the contaminants in the algal biomass. In addition, high biomass production of the microalgae was observed. The highest production rate was verified in the synthetic effluent with the association of metals, indicating a synergy between contaminants, which was probably responsible for reducing the toxic effect on the microalgae. These results indicated high potential for bioremediation by microalga *P. subcapitata*, besides the possibility of using algal biomass for biotechnological applications.

Keywords: adsorption; biosorption; Chlorophyceae; contaminants.

RESUMO

As microalgas são organismos unicelulares, fotossintetizadores, que apresentam duplicação celular exponencial e capacidade de biossorção de nutrientes dissolvidos na água. O objetivo deste trabalho foi avaliar a capacidade de biorremediação de metais e sais pela microalga *Pseudokirchneriella subcapitata.* Nesse aspecto, avaliou-se a redução de metais e sais nos efluentes sintéticos pelas microalgas *P. subcapitata*: (T1) meio de cultura (controle); (T2) meio de cultura contaminado com cloreto de alumínio; (T3) meio de cultura contaminado com sulfato ferroso; (T4) meio de cultura contaminado com sulfato de zinco; (T5) meio de cultura contaminado com a combinação de cloreto de alumínio, sulfato ferroso e sulfato de zinco. O processo de biorremediação foi avaliado comparando o meio de cultura com microalgas em suspensão e o mesmo meio filtrado. Metais de ferro e zinco, assim como sais de nitrogênio e fósforo, apresentaram valores esgotados no meio filtrado, indicando eficiência no tratamento da água por microalgas. O teor de alumínio ficou abaixo do limite de detecção em todos os tratamentos. Os valores acumulados na biomassa de microalgas, em ordem decrescente, foram nitrogênio, zinco, ferro e fósforo, indicando assim a assimilação dos contaminantes na biomassa de algas. Além disso, foi observada alta produção de biomassa das microalgas. A maior taxa de produção foi verificada no efluente sintético com a associação de metais, indicando sinergia entre contaminantes, provavelmente responsável pela redução do efeito tóxico nas microalgas. Esses resultados indicaram alto potencial de biorremediação pela microalga *P. subcapitata*, além da possibilidade de utilização de biomassa de microalgas para aplicações biotecnológicas.

Palavras-chave: adsorção; biossorção; Chlorophyceae; contaminantes.

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Introduction

Population growth and the development of new production technologies generate continuous environmental impacts. In this scenario, there is a possibility of increasing concentration of contaminants in wastewater, which would require adequate treatment for these effluents (Mahapatra et al., 2014), with the goal of reducing environmental impacts. These contaminants may present a wide variety of organic and inorganic materials, including toxic metals that pose high risks to aquatic environments (Carvalho et al., 2012; Zhang et al., 2016).

In order to reduce the amount of contaminants in wastewater, conventional measures have been adopted, but these are often unsustainable from an environmental, economic and energetic point of view. Thus, there is great interest in the use of microorganisms/biological materials in effluent treatment processes, due to economic feasibility and low risk to the environment. The use of microorganisms in bioremediation processes is considered a clean and sustainable technology. In addition, this technique makes it possible to recycle nutrients efficiently and add value to the biomass produced (Ramachandra et al., 2013).

Microalgae have high applicability in the field of biotechnology, due to the high rate of biomass production and higher growth tendency (two to ten times) in relation to terrestrial plants. Consequently, the ability of these organisms to absorb solar energy increases the fixation of CO_2 by their metabolism (Priyadarshani and Rath, 2012; Sathasivam et al., 2019), and it can increase the production of carbohydrates, proteins, amino acids, lipids and other compounds of interest in algal biomass. Data from the literature suggests high efficiency in the use of microalgae for the reduction of contaminants resulting to effluent treatment. It is possible to observe representative values in the removal of mineral salts (Leong et al., 2018; Mohammadi et al., 2018; Ridley et al., 2018; Saavedra et al., 2018), metals (Peng et al., 2017; Saavedra et al., 2018; Shen et al., 2018), pesticides (González et al., 2012), pharmaceutical compounds (Escapa et al., 2017), oils (Ammar et al., 2018), among others.

Microalgae have been noted for producing lipids (Abdelaziz et al., 2014), proteins, carbohydrates, pigments and carotenoids (β-carotene, lutein, chlorophyll, etc.), vitamins (A, B1, B6, folic acid, etc.), antioxidants (catalases, polyphenols, etc.) and other interesting molecules. These bioactive compounds are essential inputs for the food, pharmaceutical and cosmetic industries. In addition, they are an energy source (Priyadarshani and Rath, 2012) and present high market value due to the low costs of the production process. In this strategy, it is possible to use the organism for production of bioactive compounds and surfactants, for different uses in biofuels, biofertilizers, biopolymers, biofilms, among many others (Carvalho et al., 2012; Schmitz et al., 2012; Gouveia et al., 2016).

Some factors are important to regulate the kinetics growth of microalgae and contribute with high production of algal biomass; for instance, the concentration of nutrients present in the medium, the luminosity and the temperature (Carvalho et al., 2012; Wang et al., 2014; Ansilago et al., 2016). Currently, one of the major obstacles in the production of industrial scale microalgae lies in the high cost of culture medium. An alternative culture medium that has been widely used is chemical fertilizer based in nitrogen, phosphorus and potassium (NPK), due to the high concentrations of micro and macronutrients. Besides, it presents low cost, availability in the market and facility in the preparation of culture medium (Sipaúba-Tavares et al., 2009; Carvalho et al., 2012; Ansilago et al., 2016). In order for it to become even more economically attractive, it is necessary to add value to the cultivation process.

Different species of microalgae were used in wastewater treatment processes. Leong et al. (2018) obtained results of removal of up to 98% of nitrogen in domestic effluent using the microalga *Chlorella vulgaris*. Saavedra et al. (2018) tested the removal of metals in effluent, obtaining efficiency in manganese (99.4%), arsenic (40.7%), barium (38.6%), zinc (91.90) and copper (88%) removal, using the microalgae *C. vulgaris*, *Scendesmus almeriensis* and *Chlorophyceae* spp. The microalga *C. vulgaris* was also used for mercury bioremediation, obtaining removal values of 62.85 and 94.74% (Peng et al., 2017).

The species *Pseudokirchneriella subcapitata* has been reported in nutrient removal studies concerning elements such as nitrogen and phosphorus (Gonçalves et al., 2016), as well as in toxicity studies on media containing toxic metals (Gao et al., 2016; Sousa et al., 2018). It is a half moon-shaped, single-celled green algae, with a single chloroplast containing chlorophyll *a* and *b* (Granados et al., 2008). This microalga has been used in biotechnological trials to evaluate its tolerance and production under conditions of contamination by toxic metals (Lima, 2010; Carvalho et al., 2012). The data obtained is indicative of the potential of *P. subcapitata* in the complementary treatment of domestic or industrial wastewater.

Although the microalga *P. subcapitata* presents desirable characteristics for wastewater bioremediation, experiments in controlled environments are still needed to evaluate its ability to remove nutrients of water, whether salts and/or metals, as well as to evaluate the production of algal biomass in liquid media substrates with the presence of contaminants. For this purpose, the objective of this study was to evaluate the bioremediation capacity of metals and salts by *P. subcapitata* microalgae in laboratory culture.

Materials and Methods

The *P. subcapitata* inoculum was obtained from the Laboratory of Algae Physiology at Universidade Federal de São Carlos (UFSCar), isolated from the Broa Dam (São Carlos, SP, Brazil). The microalga was cultured and maintained in standard medium CHU_{12} (Chu, 1942) in the laboratory of the Center for Biodiversity Research (CPBio) at Universidade Estadual de Mato Grosso do Sul

(UEMS/Dourados, MS, Brazil). The cultivation system was static nonaxenic, with constant aeration and room temperature (22 ± 2.0°C). The tests were maintained in a BOD incubator with photoperiod control of 2,500 LUX provided by white fluorescent lamps (12 h light/ 12 h dark).

The culture medium of the microalgae was prepared by adding 1 mL of NPK stock solution to 1 liter of distilled water that was autoclaved at 120°C for 20 minutes (*e.g.*, Ansilago et al., 2016). The NPK stock solution was prepared with 0.70 gL^{-1} of chemical fertilizer N:P:K (20-5-20 gL^{-1}), according to Sipaúba-Tavares and Rocha (1993) and Carvalho et al. (2012). The rates of daily growth were obtained by Equation 1:

$$(N_n - N_1)/T \tag{1}$$

Where:

N_n = the algal density value at the desired sample time (number of microalgae cells);

N_1 = the algal density value at the initial time of the experiment (number of microalgae cells);

T = the desired sample time (days).

The elaboration of each treatment is described in Table 1. Treatments were performed in triplicate. The value used from each contaminant was established based on the limit allowed by CONAMA's Resolution nº 357/2005 (Brasil, 2005), which was doubled.

For the metal analysis, 100 mL was collected from each Erlenmeyer flask at the beginning of the test (day 1) and at the end of the test (day 21). On day 1, the sample was collected before insertion of *P. subcapitata*. On the last day of the test, the samples were divided into two equal fractions: one filtered (Whatman microfiber filter, chemically inert, with porosity of 0.45 μM) and another unfiltered (Figure 1), in order to get the Percentage of nutrient removal (% R) between suspension medium and filtered medium on day 21.

The analysis of metals (zinc, aluminum and iron) was determined by flame atomic absorption spectrometry techniques, EAA-flame, according to Welz and Sperling (1999). The total phosphorus analyzes were read by visible ultraviolet spectroscopy, UV-VIS, according to Soares et al. (2001). For the total nitrogen analyses, the Kjeldhal micro-distillation technique was used, as described in Mantovani et al. (2005), and then titrated by sodium hydroxide (NaOH); subsequently, the value consumed was converted to mg L^{-1} of mineral nitrogen, according to Tedesco et al. (1995).

To evaluate the potential bioremediation in each treatment, the concentration values of each nutrient (mg L^{-1}) were compared in the microalgae suspension samples and in the filtered samples. For this, the percentage of removal was used through Equation 2:

$$\%R = \frac{C_0 - C_e}{C_0}(100) \tag{2}$$

Where:

C_0 and C_e = the concentrations of nutrients in the liquid phase (mg L^{-1}) on the 21st day, when microalgae reach the stationary phase of growth, in the suspended material and in the filtrate one, respectively. The data was evaluated via analysis of variance (ANOVA) and Tukey's test, in the statistical program GENES, version DOS and Visual Basic 5.0.

The chemical soil analysis manual of the Paraná Agronomic Institute (Pavan et al., 1992) was used as methodology for the analyses. The laboratory analyses were performed in the Laboratory of Environmental and Instrumental Chemistry at Universidade Estadual do Oeste do Paraná (Unioeste), Marechal Cândido Rondon, PR, Brazil.

Results and Discussion

To evaluate the bioremediation potential of the *P. subcapitata* microalgae, the capacities of production in the contaminated medium and removal of contaminants were considered.

The daily growth rate of *P. subcapitata* indicated that the treatment contaminated with aluminum, iron and zinc simultaneously (T5) showed the best algal biomass doubling rate. In addition to the control, this treatment was significantly superior to the other treat-

Table 1 – Composition of treatments used to evaluate microalgae bioremediation by *P. subcapitata*.

Treatments	Adw	NPK	MPs	Contaminants		
				$AlCl_3$	$FeSO_4$	$ZnSO_4$
T1	400	50	50	-	-	-
T2	400	50	50	0.32	-	-
T3	400	50	50	-	0.32	-
T4	400	50	50	-	-	0.6
T5	400	50	50	0.32	0.32	0.6

Adw: autoclaved distilled water (mL); NPK: cultivation medium with nitrogen, phosphorus and potassium 20-5-20 g L^{-1}, respectively (mL); M*Ps*: inoculum with the microalgae *P. subcapitata* (mL); contaminants: synthetic effluent based on aluminum chloride ($AlCl_3$), iron sulphate ($FeSO_4$) and zinc sulphate ($ZnSO_4$) (g L^{-1}).

ments in the final period of the experiment (21st day of experiment). Treatments contaminated only with aluminum (T2), iron (T3) or zinc (T5) showed significantly lower growth rates than control over almost the entire experimental period (Table 2).

Table 2 also shows the analyses resulting from growth curves of *P. subcapitata*. The analysis of the algal growth curves by the covariance analysis indicated that there was no significant difference between the control and the treatment contaminated with aluminum, iron and zinc simultaneously (T5), corroborating the observations mentioned above. The exponential growth rate (K) indicated better productivity for treatment contaminated simultaneously with the metals and for control, while treatment with zinc contamination presented poor performance. The specific growth rate (μmax) also indicated satisfactory production values for control and treatment contaminated with metals simultaneously, as well as treatment contaminated only with iron (T3).

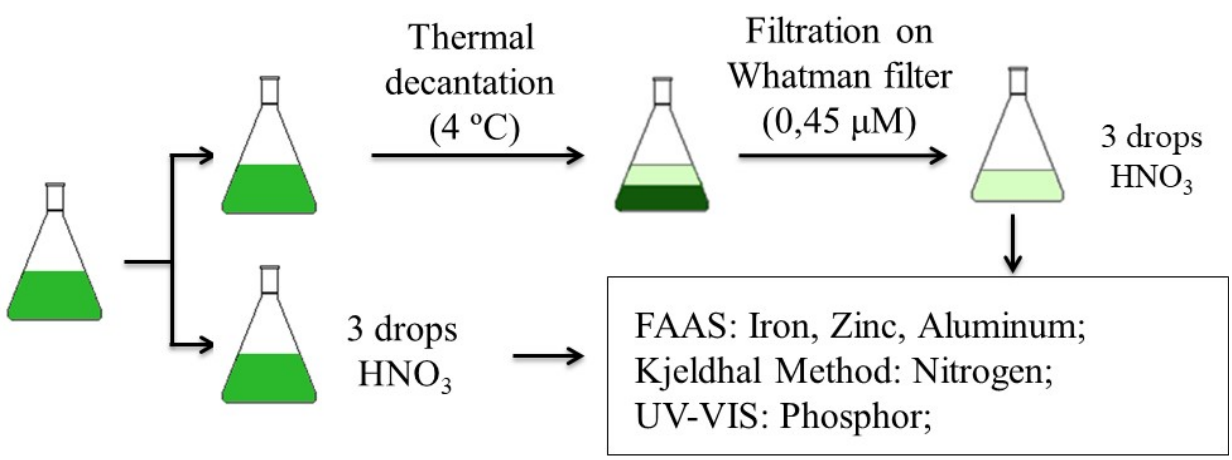

Figure 1 – Schematization of the methodology used for analysis of metals and salts present in microalgae in suspension and filtered culture medium. FAAS: Flame Atomic Absorption Spectrometry; UV-VIS: ultraviolet-visible spectrophotometry.

Table 2 – Analysis of variance (ANOVA) of the daily growth rate of *P. subcapitata* microalgae (mean ± standard error) of (T1) culture medium (control); (T2) culture medium contaminated with aluminum chloride; (T3) culture medium contaminated with ferrous sulfate; (T4) culture medium contaminated with zinc sulfate; (T5) culture medium contaminated with the combination of aluminum chloride, ferrous sulfate and zinc sulfate, in each sampling period, followed by analysis of covariance (Ancova), exponential growth rate (k) and specific maximum growth (μmax) of the 21 days of experiment*.

	Anova		Treatments				
	F	p	T1	T2	T3	T4	T5
3rd	11.1	*< 0.05*	4.8 a ± 0.16	-3.4 c ± 0.83	-2.1 c ± 0.61	0.1 b ± 0.55	-2.2 c ± 0.52
6th	53.4	*< 0.05*	2.2 b ± 0.37	2.3 b ± 0.19	0.1 c ± 0.43	0.4 c ± 0.33	4.8 a ± 0.23
9th	13.5	*< 0.05*	6.0 a ± 0.16	1.9 b ± 0.1	1.1 b ± 0.54	-2.0 c ± 0.16	2.2 b ± 0.25
12th	43.4	*< 0.05*	3.2 ab ± 0.06	2.4 b ± 0.45	0.2 c ± 0.22	0.3 c ± 0.12	4.0 a ± 0.31
15th	48.0	*< 0.05*	3.0 a ± 0.47	0.3 c ± 0.38	1.2 bc ± 0.13	0.5 c ± 0.10	2.3 ab ± 0.09
18th	71.5	*< 0.05*	2.2 ab ± 0.35	2.3 ab ± 0.25	1.6 b ± 0.17	0.0 ± 0.19	3.3 a ± 0.20
21st	99.3	*< 0.05*	4.6 a ± 0.28	2.9 b ±0.009	1.4 c ± 0.25	1.0 c ± 0.04	5.3 a ± 0.19
Ancova	14.19	*< 0.001*	AB	BC	BC	C	A
k			0,19	0,13	0,10	0,051	0,24
μmax			0,91	0,69	0,89	0,50	0,90

*Analysis of variance at 95% confidence followed by Tukey's test, represented by lowercase letters in comparison in the lines, where equal letters indicate statistically equal means and different letters have statistically different means between them. Analysis of covariance in the algal growth curve at 99% reliability, where upper case letters indicate statistically equal means, and different letters have statistically different means between them.

It was verified, however, that the growth curves of the algal biomass have an exponential behavior only for the medium without contaminant (control) and for the medium contaminated with all the metals simultaneously. It has been observed that all trace elements, even those with biological function, such as zinc, when in higher concentrations, can cause toxicity to organisms. On the other hand, the treatment contaminated with aluminum, iron and zinc (T5) presented the best production, superior even to the control one. In this case, it is possible to observe an antagonistic dynamic, in which the effect of zinc on the exposure of other chemicals, such as iron and aluminum, resulted in the reduction of its toxic effect to microalgae, bringing about effects different from those expected for the action of contaminants alone, which result from synergistic, potentiation, antagonistic and additive interactions (Mozeto and Zagato, 2008).

Another important factor to be analysed in the production of microalgae and in the process of contaminant bioremediation is the monitoring of the pH of the culture medium. It is observed in Figure 2 that zinc and iron contamination raised the pH of the culture medium. However, a pH buffering in the culture medium is observed on the 3rd day of experiment. It is possible to verify the extent to which microalgae absorbed nutrients from the medium, carried out chemical reactions and excreted residues, tending to alter its acid-base balance, which can be verified by pH fluctuation. Carvalho et al. (2012) observed that the microalga *P. subcapitata* had considerable growth in an acidic medium, even playing a role in capping the medium.

The increase in density of the algal biomass increases the fixation of CO_2 through photosynthesis, providing greater dissociation of carbonate (CO_2^{-2}) and bicarbonates (HCO_2^{-}) ions, which induces the removal of carbonic acid, and may even precipitate metals in the form of carbonates, followed by the release of OH^- ions for the neutralization of the medium (Mota and Von Sperling, 2009; Gardner et al., 2011). All these chemical reactions may explain the increase and subsequent stabilization of pH in all treatments.

Table 3 presents the percentage of removal of metals and salts during the process of biomass production of the microalgae *P.*

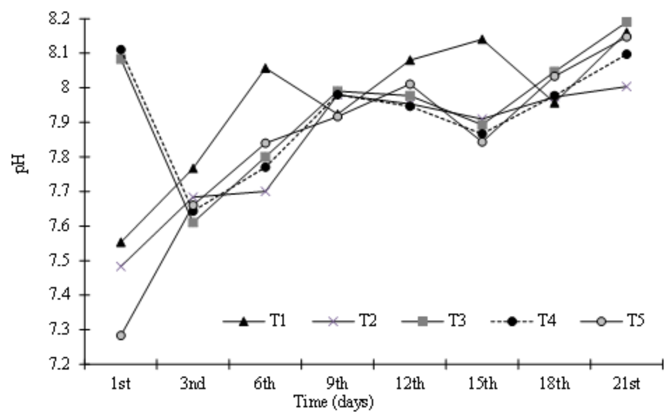

Figure 2 – Ionic potential of hydrogen in culture media used for the production of *P. subcapitata*.

Table 3 – Percentage of nutrient removal (% R) between suspension medium and filtered medium on day 21 of experiment (mean ± standard error).

	Fe	Al	Zn	N	P
T1	$39.7^{B} \pm 1,1$	< LD	$-600.1^{C} \pm 98,1$	$78.9^{A} \pm 1.6$	$19.6^{ns} \pm 0.9$
T2	$-8.7^{C} \pm 18,7$	< LD	$-433.3^{C} \pm 38,5$	$21.9^{C} \pm 3,4$	$9.2^{ns} \pm 1,1$
T3	$75.3^{A} \pm 3,4$	< LD	$-183.3^{BC} \pm 67.3$	$6.9^{D} \pm 4.0$	$17.5^{ns} \pm 3.7$
T4	$-16.9^{C} \pm 18,7$	< LD	$-46.6^{B} \pm 81.3$	$46.0^{B} \pm 1.6$	$12.8^{ns} \pm 4.3$
T5	$27.3^{B} \pm 6.9$	< LD	$88,9^{A} \pm 1,8$	$57.2^{B} \pm 0.9$	$13.4^{ns} \pm 3.8$
P	< 0.05	-	< 0.05	< 0.05	0.25
F	*84.10*	-	*11.41*	*121.50*	*1.59*

T1: control – no contaminants; T2: treatment contaminated with aluminum; T3: treatment contaminated with iron; T4: treatment contaminated with zinc; T5: treatment contaminated with aluminum + iron + zinc; < LD: elements that have an absorbance value below the detection limit. The analysis of variance ($p < 0.05$) was performed, followed by Tukey's test in comparison with the lines, where the same letters indicate statistically equal means and different letters present statistically different means.

subcapitata adsorbed or (bio)sorbed by microalgae. In the analysis of the iron content in the culture medium, values of removal were significantly higher in treatment contaminated with iron (T3), followed by control (T1) and treatment contaminated by all metals simultaneously (T5). Thus, it is possible to infer that the microalgae is able to adsorb or (bio)sorb iron from the medium, even in high concentrations. The capture of metal ions involves some biosorption mechanisms, which are based on ion exchange, coordination, complexation, adsorption and chemical precipitation (Silva et al., 2013).

The results of the aluminum analysis in the medium were below the limit of detection in all treatments, rendering the biore-

Table 4 – Compilation of studies in bioremediation using different microalgae.

Main microalgae used	Objective	Growing medium	Main results	Reference
Oocystis sp.	Biomass production and sulfate removal	Effluent from power plant	Removal of up to 32% sulfate	Mohammadi et al. (2018)
Chorella sp.			Biomass production of 50 mg L^{-1}	
Scenedesmus intermedius	Adaptation and removal of lindane	BG11	Resistance by rare mutations after exposure period up to 40 mg L^{-1}; subsequent removal by up to 99% of lindane	González et al. (2012)
Chlorella vulgaris	Simultaneous cultivation with activated sludge for bioremediation and lipid production	Domestic effluent	Removal of up to 98% of nitrogen; lipid yield up to 130 mg L^{-1}	Leong et al. (2018)
Chlorella vulgaris	Absorption of arsenic, boron, copper, manganese and zinc from water by different green microalgae	Synthetic medium based on the water composition of the Loa river (Chile).	Removal of 99.4% for manganese	Saavedra et al. (2018)
Scenedesmus almeriensis			40.7% for arsenic 38.6% for boron	
Chlorophyceae spp.			91.9% for zinc 88% for copper	
Chlorella vulgaris	Bioremediation of mercury by mineralized microalgae	Blue green medium (BG11) contaminating with mercury	Removal of mercury from 62.85% to 94.74%	Peng et al. (2017)
Nannochloropsis oculata	Oil removal and Chemical oxygen demand (COD)	Oil effluent	Removal up to 89% of oil and up to 90% of COD	Ammar et al. (2018)
Isochrysis galbana			Removal of up to 82% of oil and up to 83% of COD	
Phaeodactylum tricornutum	Growth capacity in nitrate-containing medium	Modified F/2 medium	Removal of up to 1700 mg L^{-1} of nitrate in 100 L bioreactor	Ridley et al. (2018)
Chlorella sp.	Efficiency in removal of cadmium	Immobilization in pellets derived from a complex of the botanical genus *Eichhornia*	Removal up to 92.45% cadmium	Shen et al. (2018)
Chlorella sorokiniana	Response of microalgae to high concentrations of paracetamol (PC) and salicylic acid (AS)	Mann and Myers Medium	Removal up to 69% for PC and up to 98% for AS	Escapa et al. (2017)

mediation process inconclusive. However, it is possible that aluminum is able to associate with other elements present, making the reading by the method adopted inefficient.

The percentage of zinc removal was effective only in the treatment contaminated with all metals (T5), which showed a reduction of almost 90%. Other treatments, including treatment contaminated with only zinc (T4), had negative removal values. Gao et al. (2016) observed, in their study with *P. subcaptata*, an elevation of zinc, without toxic potential, in the microalgae with greater exposure to phosphorus, due to potentially induced by Zn–P complexation or precipitation inside the cell. Saavedra et al. (2018) also observed removal of up to 91.9% for zinc in a study carried out with microalgae of the class *Chlorophyceae*. Other studies indicate that dead algae biomass may be even more efficient at retaining and accumulating metal elements than living cells and tissues (e.g., Cossich, 2000). This process occurs due to the changes in the nature of the cell surface because of the absence of active transport of the dead microalgae, causing better adsorption of metals efficiency by the algal biomass.

The removal of nitrogen in the culture medium was significantly higher in the control (T1). The value of phosphorus removal was also higher in the control, but it did not differ significantly from the previous treatments. It was possible to observe that, in treatments contaminated with metals, the biosorption or adsorption of salts of nitrogen and phosphorus was low. The maximum reduction value recorded was 78.9% for nitrogen and 19.6% for phosphorus, all in the control treatment. This fact may have resulted from the Redfield ratio ($C_{106}H_{118}O_{45}N_{16}P$), which means that algae, on average, demand 16 times more nitrogen than phosphorus (Redfield, 1958; Sperling, 2001). In a study by Wang et al. (2014), a reduction of up to 95% and 95.7% for phosphorus and nitrogen, respectively, was observed in a wastewater treatment system using the microalgae genera *Chlorella* and *Micractinium*.

Based on the results, it is possible to observe a complex relationship between microalgae bioremediation processes and the antagonistic and synergistic effects of the contaminants, with each other and with the microalgae itself. However, it is important to establish standards of control and monitoring for these contaminants in the effluent, so that it is possible to understand the metabolism and the efficiency of this system. In Table 4, we present a compilation of studies using different species of microalgae for the specific purpose of culturing, adaptation and bioremediation in media containing metals and other organic and inorganic compounds that can be biosorbed. Through these studies, it can be established that wherever there was insertion and/or presence of stressors in the culture medium of the microalgae, positive results were obtained for the removal of these contaminants and consequent increase in the production of algal biomass. This corroborates the results obtained in the present study, since the treatment contaminated with all metals (iron, aluminum and zinc) (T5) obtained a higher rate of algal biomass duplication, which can indicate synergy between the contaminants, thus reducing the toxic effects.

Conclusion

The experimental results showed that the microalga *P. subcapitata* presented bioremediation capacity (either by adsorption and/or biosorption) and algal biomass production. The contaminants with higher concentration indices in the culture medium obtained a higher percentage of removal (N > Zn > Fe > P > Al). It was also possible to observe that, on a bench scale, the microalgae were able to develop in the presence of the toxic metals inserted in the medium.

In addition, the highest production rate was verified in the synthetic effluent with the association of metals, indicating possible synergy between aluminum, iron and zinc, which was probably responsible for reducing the toxic effect on the microalgae, meaning biomass gain for further biotechnological applications.

However, more detailed studies are needed to improve the technique and the methodology used, requiring shorter test times and the use of other contaminating metals for influence and toxicity analysis.

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Contribution of authors:

Ansilago, M.: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration, Funding acquisition. Ottonelli, F.: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft. Carvalho, E.M.: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration.

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Model of integrated territorial assessment for environmental justice applied to sanitation

Modelo de avaliação territorial integrada da justiça ambiental aplicada ao saneamento

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ABSTRACT

Equitable access to water and sanitation is still a challenge worldwide and in Brazil. In this sense, the concept of environmental justice was used in this paper as a basis for establishing an Integrated Territorial Assessment Model for Environmental Justice Applied to Sanitation. This research aims to give scientific support for the State Government to improve public policies and promote the universalization of water and sanitation services as established by the Sustainable Development Goals (SDGs). This study was based on a quali-quantitative methodology. Secondary data were selected as key information to analyze environmental justice in sanitation, including the following: hydric vulnerability (IV), water supply (WS); untreated sewage collection (SC); sewage collection with treatment (ST); water supply investments (WSI); sewage system investments (SSI); municipal per capita income (MPI); and municipal human development index (MHDI). The data were presented in maps by overlapping the State official regional division and the discussion was carried out based on regional differences and similarities. The repetition of a pattern was noted, in which unfavorable rates were concentrated in the North and Jequitinhonha-Mucuri regions: water vulnerability, sewage system with collection and without treatment, total investment, average investment, per capita income and municipal human development index. Both also have low rates of the sewage system and water supply when compared to others. On the other hand, Zona da Mata and Triângulo regions have favorable rates for hydric vulnerability, sewage system with collection and without treatment and water supply. The Triângulo Mineiro region also presented favorable

RESUMO

O acesso equitativo ao saneamento básico ainda é um desafio no mundo e no Brasil. É nesse sentido que o conceito de justiça ambiental foi utilizado no presente trabalho como base para estabelecer um Modelo de Avaliação Territorial Integrada da Justiça Ambiental Aplicada ao Saneamento. Pretende-se apoiar os estados no aprimoramento de políticas públicas de promoção à universalização dos serviços de saneamento, conforme estabelecido pelos Objetivos do Desenvolvimento Sustentável (ODS). Para o desenvolvimento do trabalho, foi elaborada metodologia quali-quantitativa em duas etapas, com base nos seguintes dados secundários selecionados: vulnerabilidade hídrica (VH); os seguintes índices: abastecimento de água (AA), coleta de esgoto sem tratamento (CE), coleta de esgoto com tratamento (TE), investimento em abastecimento de água (IAA), investimento em esgotamento sanitário (IES), renda per capita municipal (RPM); e desenvolvimento humano municipal (IDHM). O modelo proposto foi aplicado no estado de Minas Gerais. Os dados foram apresentados em mapas com sobreposição da divisão regional oficial do estado, e a discussão foi realizada com base nas divergências e semelhanças regionais. Notou-se a repetição de um padrão, em que índices desfavoráveis concentraram-se nas regiões Norte e Jequitinhonha-Mucuri para vulnerabilidade hídrica, atendimento com coleta e sem tratamento de esgoto, investimento total, investimento médio, renda per capita e IDHM. Ambas também apresentam valores baixos de índice de atendimento com coleta e com tratamento de esgoto, e abastecimento de água quando comparadas às demais. Por outro lado, as regiões Zona da Mata e Triângulo apresentam índices favoráveis para vulnerabilidade hídrica, atendimento com coleta e

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rates of total investment, average investment, per capita income, and municipal human development index. It is concluded that the inequality between the regions is, initially, of natural origin, and reinforced by the social context and inequality in sanitation investments in the different regions.

Keywords: sanitation access; socioeconomic conditions; human right; investment; water vulnerability.

sem tratamento de esgoto e abastecimento de água. A região do Triângulo Mineiro apresentou ainda índices favoráveis de investimento total, investimento médio, renda per capita e IDHM. Dessa forma, a desigualdade entre as regiões mineiras no tema estudado é claramente influenciada por condições naturais, assim como pelo contexto social.

Palavras-chave: acesso ao saneamento; condições socioeconômicas; direito humano; investimento; vulnerabilidade hídrica.

Conceptualization

Brazil concentrates 12% of all freshwater on the planet. However, this scenario does not mean the absence of conflicts or broad access to water for all (ANA, 2019). It is contradictory, but there are populations in a situation of serious water vulnerability in the country that concentrates 30% of the water in the American continent; there are almost 35 million people who are not served by water supply in Brazil (Trata Brasil, 2019). In the different Brazilian regions, there is a great difference in water supply between population groups. In increasing order, these are the following percentage rates per region: North, 57.49%; Northeast, 73.25%; South, 89.68%; Midwest, 90.13%; and Southeast with 91.25% of the population supplied by treated water (Trata Brasil, 2019).

In the State of Minas Gerais the situation is not different, since 13.9% of the households do not have access to the general water network, and 24.7% to the general sewage network (Rodrigues et al., 2019b). After evaluating the historical trend, no significant advances were observed (Costa et al., 2016). When analyzing the Economic Ecological Zoning of the State of Minas Gerais (Minas Gerais, 2008), it is possible to notice that the regions with lower water availability are also the ones with greater social vulnerability, where the human component is in a precarious or very precarious state (SISEMA, 2019).

Rodrigues et al. (2019a), when analyzing the evolution of the spatial distribution of access to basic sanitation services (water supply, sanitary sewage and garbage collection) in Brazilian micro-regions, concluded that the growth in sanitation rates was uneven among the studied areas, showing that the lack of access to these services was predominant in the less populated and lower-income regions in the North and Northeast of the country. The authors also identified spatial concentration in sanitation access rates in regions with large urban agglomeration and higher per capita income.

The National Basic Sanitation Policy — NBSP (Act nº 11.445/2007) includes universal access to sanitation (the concept embraces water supply, sewage collection and treatment) as one of its principles (Brasil, 2007). The new regulation mark for sanitation, Act No. 14.026/2020, reinforces the principle of universalization, establishing the year of 2033 as a limit for achieving universalization. It also presents the need to prioritize plans, programs and projects that aim at the implementation and expansion of services and basic sanitation actions in areas occupied by low-income populations, including informal consolidated urban centers as a guideline (Brasil, 2020). In fact, access to drinking water and basic sanitation is an essential human right, recognized by the United Nations (UN, 2010) and by the Brazilian constitutional system, in which it is intrinsically related to citizenship (art. 1, II), to the dignity of the human person (art. 1, III), to the rights to life (art. 5), health, food, housing (art. 6) and an ecologically balanced environment (art. 225), whose guarantee is part of the primacy of the prevalence of human rights (art. 4, II, all of the Federal Constitution).

In Minas Gerais, State Law no. 11.720/1994 introduced the State Policy on Basic Sanitation, providing the right to sanitation for all and establishing the State System on Basic Sanitation, responsible for the creation of policies, definition of strategies and execution of sanitation actions (Minas Gerais, 1994).

The main sanitation policy planning instrument in the country is the Sanitation Plan, applied to the three existing federal spheres (municipalities, State governments and federal government). Marchi (2015) explains that this Plan should gradually seek for equitable and sustainable progress, so that sanitation services be inserted in the criteria of welfare and social equity, as well as environmental risk reduction. However, according to Ventura and Albuquerque (2020), a lack of social participation and content unrelated to the local reality is observed in these plans.

Among the 853 municipalities in Minas Gerais, only 28.5% had this Plan ready by 2014, and another 48.1% were preparing their respective plans (FJP, 2017). The regions with the highest rates of municipalities that do not have a Municipal Basic Sanitation Plan are Jequitinhonha, South, Southeast, North and Northeast (FJP, 2017), which means that they cannot receive transfers of funds from the Union and the State to execute sanitation actions, since the Plan is a *sine qua non* condition for the investment of these resources.

Heller and Castro (2007) state that it is necessary to understand the access to sanitation as a human right, rather than a market asset, i.e., observe the premises of environmental justice, understood as a set of principles and practices as defined in the Manifesto for the Launching of the Brazilian Environmental Justice Network. These are about the isonomy of social groups to absorb the environmental liabilities inherent to the political and socio-economic system in which we are inserted; fair and equitable access to the country's environmental resources; principles of publicity and social participation in environmental matters of relevant interest; and the encouragement of social protagonism in the construction of alternative models for development (Brasil, 2001).

Environmental justice is defined as the fair treatment and significant involvement of all people, regardless of race, color, national origin or income, concerning the development and enforcement of environmental laws, regulations and policies (USEPA, 2016). In a section about the specific concept of water, Perreault et al. (2018) advocate that water justice should integrate the economic, cultural, political and socio-ecological aspects of justice.

Other authors reinforce the relationship between environmental justice and sanitation. Menton et al. (2020) and Hurlbert (2020) establish a correlation between environmental justice and the Sustainable Development Goals (SDG), including universalization of sanitation. Corroborating this thesis, Ezbakhe et al. (2019) state that the SDGs help overcome inequality in access to sanitation among social classes and vulnerable groups, especially SGDs 6 and 10. Ataide and Borja (2017) further reinforce that sanitation is an inducer of social justice. Finally, it is important to note that the U.S. Environmental Justice Action Agenda defines the provision of water as a structural axis (USEPA, 2016).

Correlation between access to sanitation and social and economic conditions has been the object of several analyses. Jongh et al. (2019), when evaluating the correlation between access to water and sanitation and social and economic variables in the Sedibeng region (South Africa), concluded that both access to water and sanitation seem to play a significant role in the region's economic and social well-being. Another study in Africa relates access to sanitation to social and demographic variables (Mosimane and Kamwi, 2020). Luh and Bartram (2016) evaluated the correlation of sanitation progress with socioeconomic indicators in 73 countries, and concluded that national socioeconomic characteristics may not be primary determinants of progress in access to water and sanitation. Therefore, it is expected that isonomy and equity, two concepts related to environmental justice, should be the basis for the guidelines of sanitation provision policies.

Thus, the present work aims at proposing a Model for Integrated Territorial Assessment of Environmental Justice Applied to Sanitation. The intention is to support the State Government to improve public policies that promote universalization of sanitation services as established by the SDGs, especially SDG 6 (UN, 2015). The proposed model was applied in the State of Minas Gerais, Brazil.

Methodology

The research was based on a qualitative and quantitative methodological approach, using secondary data. It was divided in two methodological moments. In the first stage, an Integrated Territorial Assessment Model for Environmental Justice Applied to Sanitation was proposed; and in the second, the proposed model was applied for the State of Minas Gerais.

An explanatory perspective on water supply and sanitary exhaustion distribution over a State territory was presented, seeking to evaluate compliance with the principle of isonomy in the delivery of these services, and evaluating reasons for the distribution pattern found; and finally, whether there is environmental justice in access to services.

The integrated territorial assessment model for environmental justice applied to sanitation

The proposed model considered two main dimensions: "access to sanitation" and "socioeconomic conditions", involved in the concept of environmental justice and its relationship with the integrated variables "water vulnerability" and "investments in environmental sanitation" (for water and sewage). For the first dimension, the primary variables were: water supply and sanitation; for the second: per capita income and human development index.

The dimensions, with their respective primary variables and the integrated variables, are described in Table 1. The integrated territorial analysis model, which involves all of them, is presented in the flowchart of Figure 1.

The integrated variable "water vulnerability", which represents a natural condition, is an intervening factor in the provision of water services and was adopted in the study to identify its influence on the model of environmental justice. The integrated variable "investments in environmental sanitation" is in line with the concept of environmental justice and human rights, since it allows the evaluation of whether there is equality in the priority of investments regardless of the social and economic condition of the region.

Treatment of variables and integrated analysis

The primary variables that characterize the access to sanitation and to social and economic conditions are systematized by municipality and by State administrative regions. Therefore, for each variable, a layer was elaborated with the ArcGIS® software, considering both divisions.

To evaluate the territorial correlation in the administrative regions, the local Moran's I coefficient was used. According to Neves et al. (2000), the local Moran's I coefficient is considered a Local Spatial Association Index (LISA) and hence produces a specific value for each object, allowing the identification of groups with similar attribute values (clusters), anomalous objects (outliers) and more than one spatial regime.

Table 1 – Dimensions and variables of the Integrated Territorial Analysis Model of Environmental Justice in Sanitation.

Variables		Abbreviation	Variable classes	Description
Sanitation access	Water supply coverage	WS	-	It considers the number of buildings served by the system and the number of people supplied with water. It allows assessing the degree of supply systems coverage and the service deficit.
	Sewage without treatment	NT	-	Index of people served with collecting network.
	Sewage with treatment	TR	-	Index of collected wastewater that is sent for treatment.
Socioeconomic conditions	*Renda* per capita (*Per capita* income)	PCI	Classes: * 1 – from 0 to R$ 461.40; 2 – from R$ 461.41 to R$ 690.80; 3 – from R$ 690.81 to R$ 920.20; 4 – from R$ 920.21 to R$ 1149.60; 5 – from R$ 1149.61 to R$ 1379.00.	Index that measures the income of each individual component of the municipal population.
	Municipal human development index	MHDI	Classes: ** Very low – 0 a 0.499; low – from 0.500 to 0.599; average – from 0.600 to 0.699; high – from 0.700 to 0.799; very high – from 0.800 to 1.000.	Numerical index that varies from 0 (zero) to 1 (one), based on the Global HDI, considering the same three dimensions - longevity, education and income.
Integrated variables	Water vulnerability	WV	-	Factor composed by three indicators: availability of surface water, groundwater and potential contamination of aquifers. Each with a weight: 50% for the natural availability of surface water and 25% for each of the others (IGAM, 2018).
	Water supply investment	WSI	-	Investments: i) made by service providers; ii) made by municipalities; and iii) made by States.
	Sanitary sewage investment	SSI	-	

*Income classes were obtained by subtracting the lowest value from the biggest one and then dividing the result by five (number of desired groups); **IDHM varies from zero (0) to one (1).

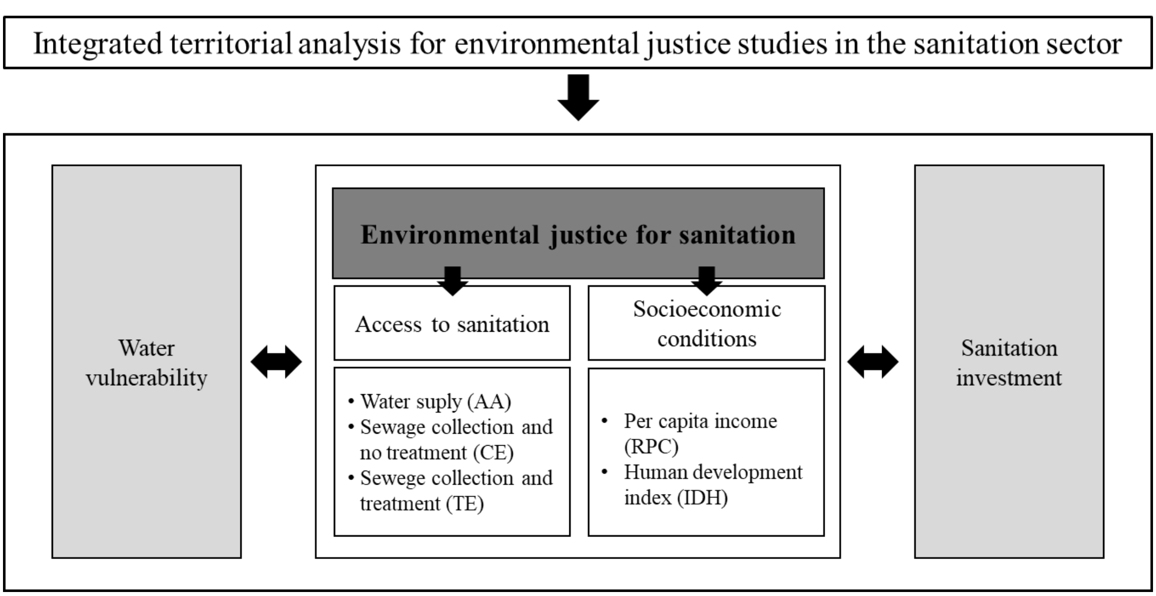

Figure 1 – Flowchart of integrated territorial analysis for environmental justice in the sanitation sector.

The local index was chosen because it allows to understand the spatial correlations considering a more detailed scale (Krempi, 2004). With the application of this index, it is possible to observe the relationship between municipalities and to understand the local and regional dynamics of the entire State in relation to the evaluated parameters. The results are presented as maps and must be interpreted according to Table 2.

Different procedures were adopted to work with the integrated variables. Water vulnerability was analyzed through a map which overlapped this variable and the Minas Gerais planning regions, allowing the visualization of inter-regional similarities and divergences. For investments in sanitation, the following were considered:

- the sum of the investments made by the different entities (sanitation service providers, municipality and State government) in all municipalities of each region in each year – called total investment (TI);
- the average amount invested in the region, obtained by dividing the total investments by the number of municipalities of each region – called average investment (AI).

The relationship between invested values, water supply and sanitary indexes was evaluated by the Pearson's correlation coefficient (r), which allowed us to understand if the increase or decrease of a unit in one of the variables generates the same impact on the other one.

Pearson's coefficient (r) is a measure of variance shared between two variables, whose distributions are linear, and indicates the association between them. Pearson's (r) values vary from -1 to 1, in which the closer to 1 (one), regardless of the sign, the higher the level of linear statistical dependence between the variables. On the other hand, the closer to 0 (zero), the weaker the strength of this relationship (Figueiredo Filho and Silva Júnior, 2009). According to those same authors, a perfect Pearson's correlation, which means values equal to -1 or 1, indicates that the variable score can be predicted by knowing the correlated variable score. If r equals 0 (zero), there is no linear relation between variables.

Finally, an integrated analysis was established among all of the analyzed variables to find the answer to the question presented: is there environmental justice in sanitation?

Case study: Minas Gerais State

At this stage, the model was applied to the State of Minas Gerais. Table 3 shows the sources and respective evaluation period for the considered variables.

The primary variables that characterize the access to sanitation and socioeconomic conditions were systematized by municipality, with the overlapping of the official regional division of Minas Gerais (Minas Gerais, 2019), in a layer elaborated with the ArcGIS® software. This allowed an evaluation of the regional spatial correlation, based on the divergences and similarities, as presented in the previous methodological items.

In conclusion, an argumentative discussion was carried out based on all the data collected to seek answers to the presented question: is there environmental justice for the Minas Gerais State's population regarding access to sanitation?

Validation and formal analysis

Water vulnerability evaluation

Figure 2 shows a map created from the overlapping of water vulnerability and the planning regions of the State of Minas Gerais.

The Northwest, North and Jequitinhonha-Mucuri regions concentrate the areas of high and very high vulnerability, whereas the other regions contain the less vulnerable zones (Figure 2). Thus, it is possible to say there is naturally lower surface and underground water availability, as well as greater potential for aquifer contamination (or a combination of the three factors) in the Northwest, North and Jequitinhonha-Mucuri regions.

The northern part of the central region presents an area with high water vulnerability, which includes all or part of the 110 municipalities, with 85 of them inserted in the basin of São Francisco River (Upper São Francisco), and the others in Rio Doce Basin. Of this total, Belo Horizonte and the municipalities of its metropolitan region, Diamantina and Três Marias, stand out. The same is true for a portion of the Midwest region and other areas in the São Francisco River Basin, but in a more fragmented way.

Table 2 – Spatial analysis results interpretation – local Moran coefficient.

Result	Interpretation
Not significant	No spatial association between the municipality and its neighbors
High-High cluster	Positive spatial association between the municipality and its neighbors
High-low outlier	Atypical situations in which the municipality has the variable value higher than the average while its neighbors have values lower than the average
Low-high outlier	Atypical situations in which the municipality has the variable lower than the average while its neighbors have values higher than the average
Low-Low cluster	Negative spatial association between the municipality and its neighbors

Source: adapted from Neves et al. (2000).

Table 3 – Variables considered for the case study in the Minas Gerais State.

Variable	Type	Abbreviation	Evaluation period/Year	Source
Water vulnerability	Integrated	WV	-	Zoneamento Ecológico e Econômico – ZEE (SISEMA, 2019)
Water supply coverage	Primary	WS	2014	SEIS (FJP, 2017)
Sewage without treatment	Primary	NT	2013	SNIRH Atlas Esgoto: Agência Nacional de Águas – ANA (ANA, 2017)
Sewage with treatment	Primary	TR	2013	SNIRH Atlas Esgoto: Agência Nacional de Águas – ANA (ANA, 2017)
Water Supply Investment	Integrated	WSI	1995 e 2017	SNIS: série histórica (SNIS, 2018)
Sanitary Sewage Investment	Integrated	SSI	1995 e 2017	SNIS: série histórica (SNIS, 2018)
Per capita income	Primary	PCI	2010	Censo 2010 (IBGE, 2010)
Municipal human development index	Primary	MHDI	2010	Atlas do Desenvolvimento Humano no Brasil 2013 (Brasil, 2013).

SEIS: *Sistema Estadual de Informações sobre Saneamento*. The FJP work was selected for this study due to the scope and accuracy of the data and since it is not self-declaratory data; SNIS: *Sistema Nacional de Informações sobre Saneamento*; IBGE: *Instituto Brasileiro de Geografia e Estatística*.

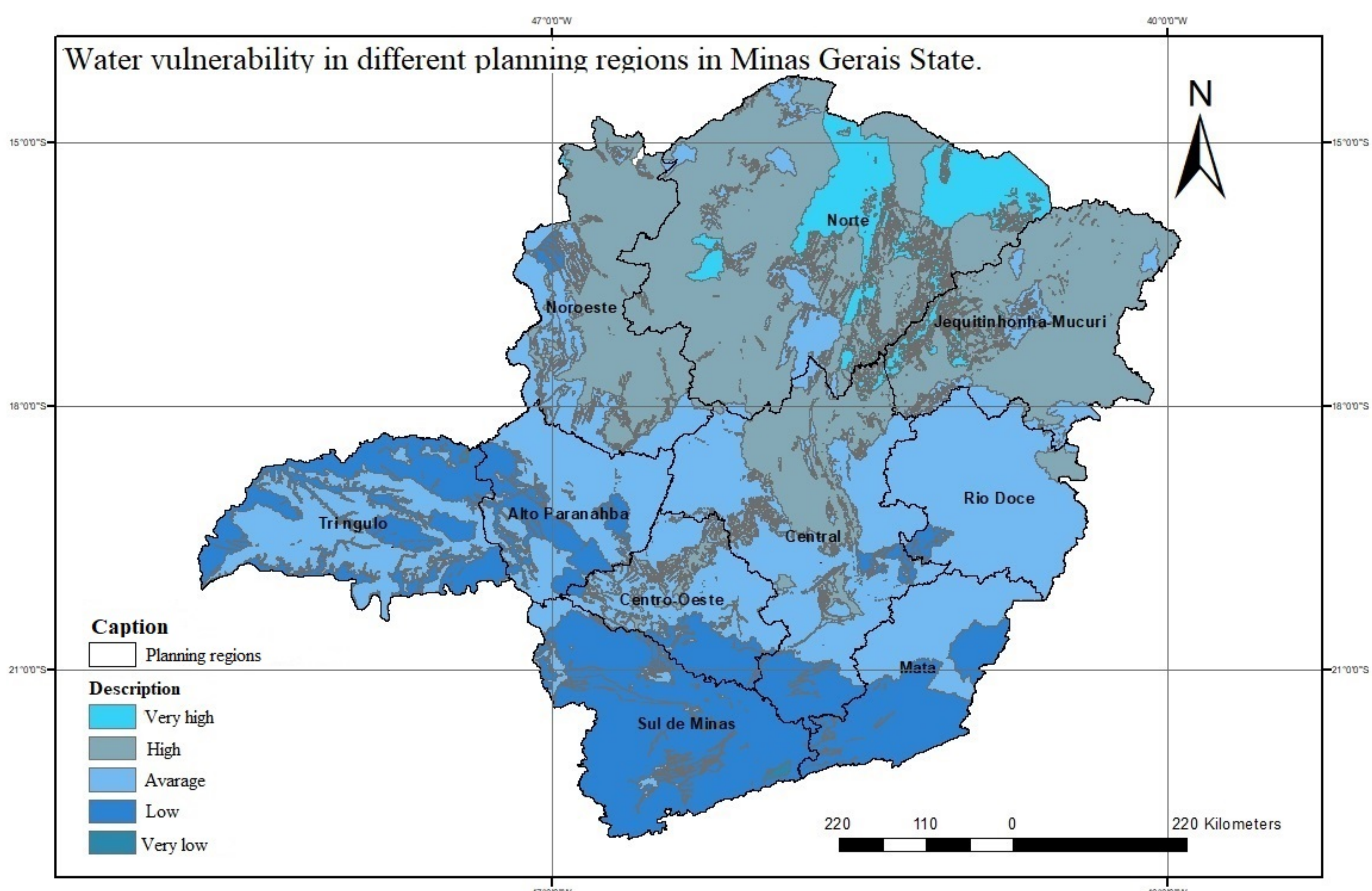

Figure 2 – Water vulnerability in the different planning regions.

Assessment of water supply coverage and sewage disposal

Based on data provided by Fundação João Pinheiro (FJP) and by the Brazilian National Water and Sanitation Agency (ANA), the rates of water and sewage coverage of the urban population in the planning regions of Minas Gerais for 2013 were surveyed, as shown in Table 4.

Regarding water supply, the regions present very close numbers, all of them above 95%, which means there is no evidence of a difference in distribution. Triângulo Mineiro and Alto Paranaíba regions present the highest rates of water supply, very close to 100%. The limitation of the available analyzed data is owed to the fact that they refer only to the urban population, that live a reality known to be different from that of rural areas, thus not providing a more comprehensive analysis of the inequality related to access to water (Silveira, 2013; Agra Filho et al., 2010; Metha et al., 2014).

With these results, it is also possible to observe that the regions of Jequitinhonha-Mucuri, Northwest and North have low rates of sewage without treatment in common, lower than 60%, highlighting the lowest value of 29.04% in the North region.

For the water supply indexes per municipality, the local Moran coefficient was analyzed and presented in Figure 3.

Although regional water supply average rates are homogeneous, the spatial correlation analysis shows some inter and intraregional discrepancies. Especially in the Triângulo Mineiro, Alto Paranaíba and Zona da Mata regions, it is possible to see groups of municipalities that show water supply rates above the general average (high-high relation).

The low-low relationships, which are groups of cities with below-average rates, are prevalent in the North and Northwest regions, as do the high-low outliers. In these regions there are cities with above-average rates surrounded by cities with below-average rates.

Regarding the sewage without treatment index in the municipalities of Minas Gerais, a map showing the local Moran coefficient was also prepared, as shown in Figure 4.

It is possible to identify high-high clusters concentrated in the South, Rio Doce and, in a smaller area, Zona da Mata regions, indicating there is a group of cities within the same region with similar sewage without treatment rates and above the average value for the State.

At the same time, the low-low associations are concentrated in the North, Central (especially in the São Francisco River Basin) and, to a lesser extent, Jequitinhonha-Mucuri region. The Central region has intraregional discrepancies in relation to sewage collection.

The North and Central regions are the ones with the most high-low outliers, i.e., cities with discrepant values from their neighbors. The South, Zona da Mata and Rio Doce regions concentrate the opposite condition.

Finally, the local Moran coefficient for sewage with treatment in the municipalities of Minas Gerais is presented in Figure 5.

The Zona da Mata, Sul de Minas and Rio Doce regions concentrate the low-low clusters, i.e., they present groups of municipalities with below-average sewage with treatment rates. The North and Jequitinhonha-Mucuri regions concentrate the high-high clusters, followed by the Midwest and Triângulo Mineiro regions.

The low-high outliers are dispersed in the South, Triângulo Mineiro, Northwest, Central, Midwest, Rio Doce, Jequitinhonha-Mucuri and North regions. The high-high outliers prevail in the Central and Zona da Mata regions and in smaller groups, in Alto Paranaíba, South and Northwest.

The spatial correlation analyses for the three evaluated indexes (water supply, sewage without treatment and sewage with treatment) demonstrate, considering the concentration of clusters and outliers, there are differences between the North and Jequit-

Table 4 – Distribution of water and sewage service rates among the planning regions of Minas Gerais.

Region	Sewage (urban population)		Water (urban population)
	Average coverage index with collection and without treatment	Average coverage index with collection and treatment	Average service index of water supply network
North	29.04%	24.76%	96.85
Northwest	30.27%	34.88%	96.53
Jequitinhonha-Mucuri	54.21%	18.08%	96.70
Midwest	56.73%	27.62%	96.91
Central	60.30%	14.54%	96.74
Rio Doce	62.49%	16.53%	97.36
Zona da Mata	66.02%	11.28%	97.54
South	66.63%	14.99%	95.68
Alto Paranaíba	67.45%	20.22%	98.10
Triângulo Mineiro	67.67%	18.09%	99.38

Source: Adapted from *Agência Nacional de Águas* (2017) and IBGE (2013) *apud* FJP (2017).

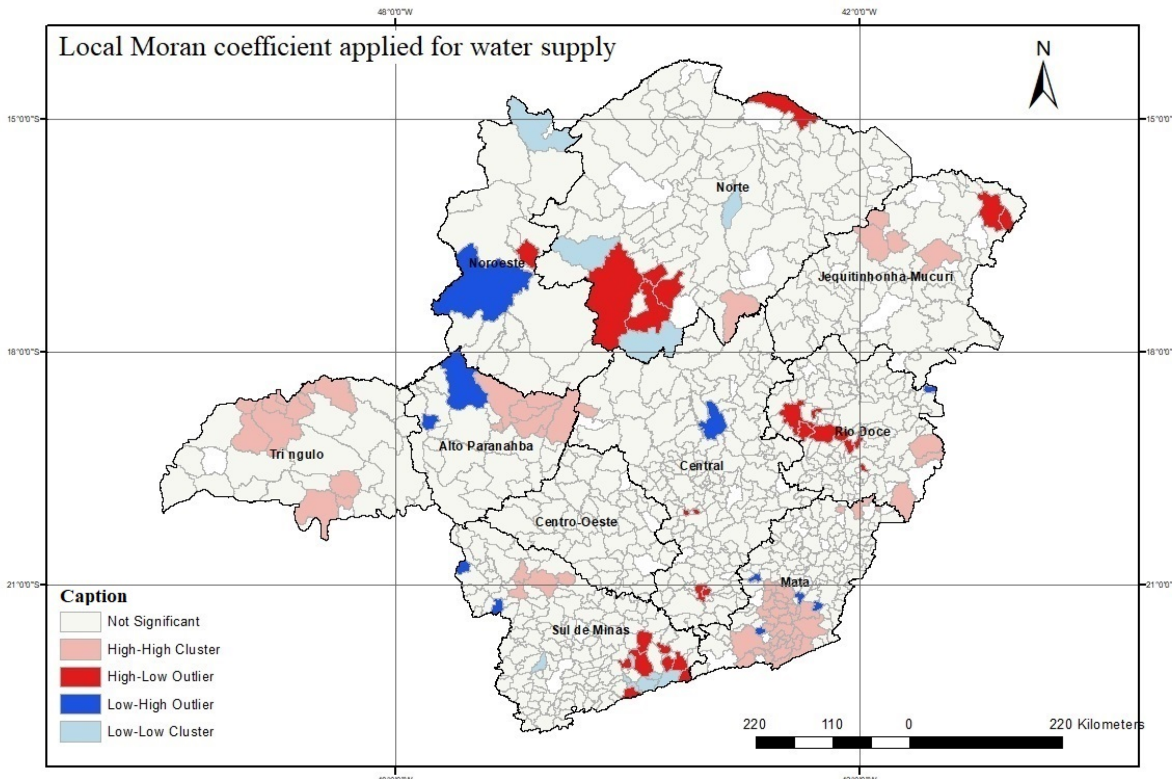

Figure 3 – Spatial correlation map for water supply index in the State planning regions of Minas Gerais.

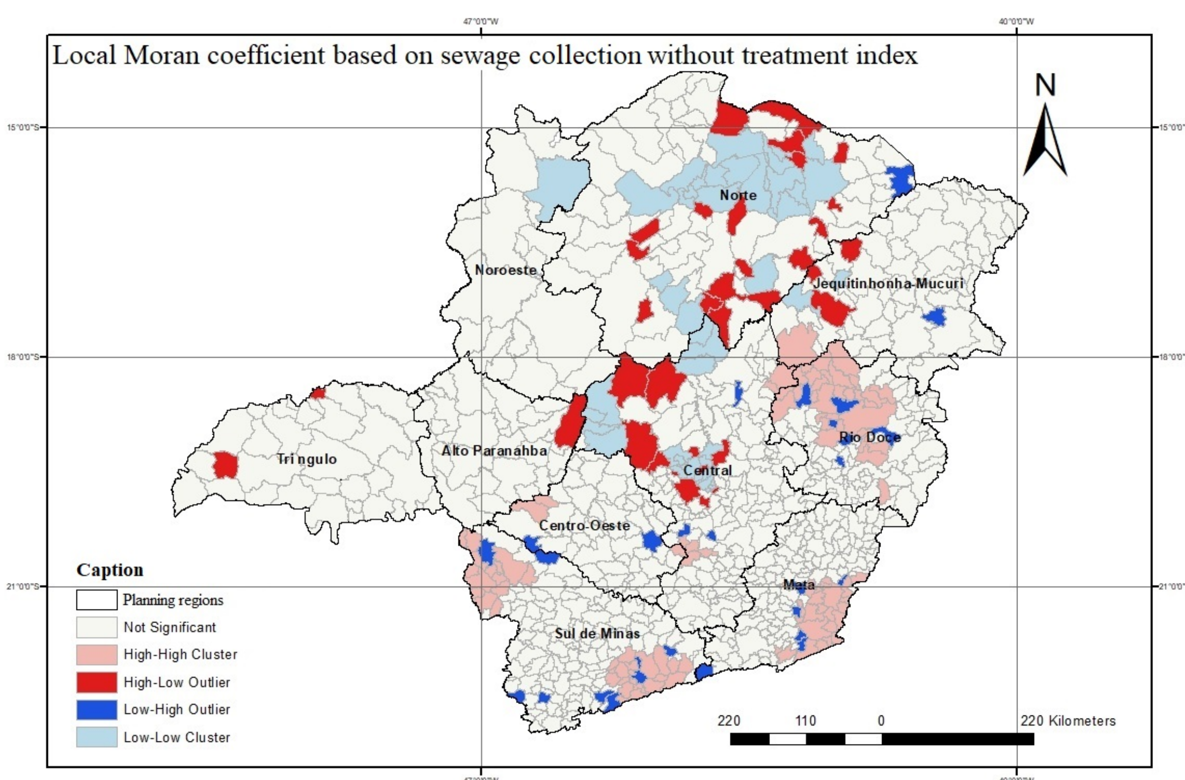

Figure 4 – Spatial correlation map for sewage without treatment index in Minas Gerais.

inhonha-Mucuri regions and the other ones. In the North and Jequitinhonha-Mucuri, low-low clusters are prevalent, indicating a concentration of municipalities with below-average water supply and sewage without treatment rates, while in the others, high-high clusters are prevalent.

The biggest difference occurs between the North and Jequitinhonha-Mucuri *versus* the Rio Doce, Zona da Mata and Sul de Minas regions. The Central region represents a mixture of high and low rates, with highs predominating in its southern portion, and lows in its northern portion.

This analysis also allowed us to understand that even regions with high-high clusters concentration and with high mean indexes in comparison to the others, according to Table 1, present intraregional discrepancies, with low-high outliers. The results show that access to water supply and sewage is unequal among the municipalities and between the planning regions of Minas Gerais.

Correlation with social indicators - per capita income distribution and IDHM

At this stage, per capita income and IDHM data were evaluated in order to identify a possible correlation between water and sewage rates and regional social conditions.

The North region presents a little more than 75% of its cities with average IDHM and approximately 19% with low IDHM; Jequitinhonha-Mucuri has 42% of the cities with low IDHM, and 52% average; and in the Rio Doce region, 70% of the cities have average IDHM, with only 13% presenting low index. The Alto Paranaíba and Triângulo Mineiro regions have 62% of their cities with high IDHM, followed by the Midwest region, where 50% of the cities have high index and Sul de Minas, with 41%. In Zona da Mata, the average indexes appear in almost 78% of the municipalities.

The North and Jequitinhonha-Mucuri regions present a higher concentration of municipalities with the lowest per capita income

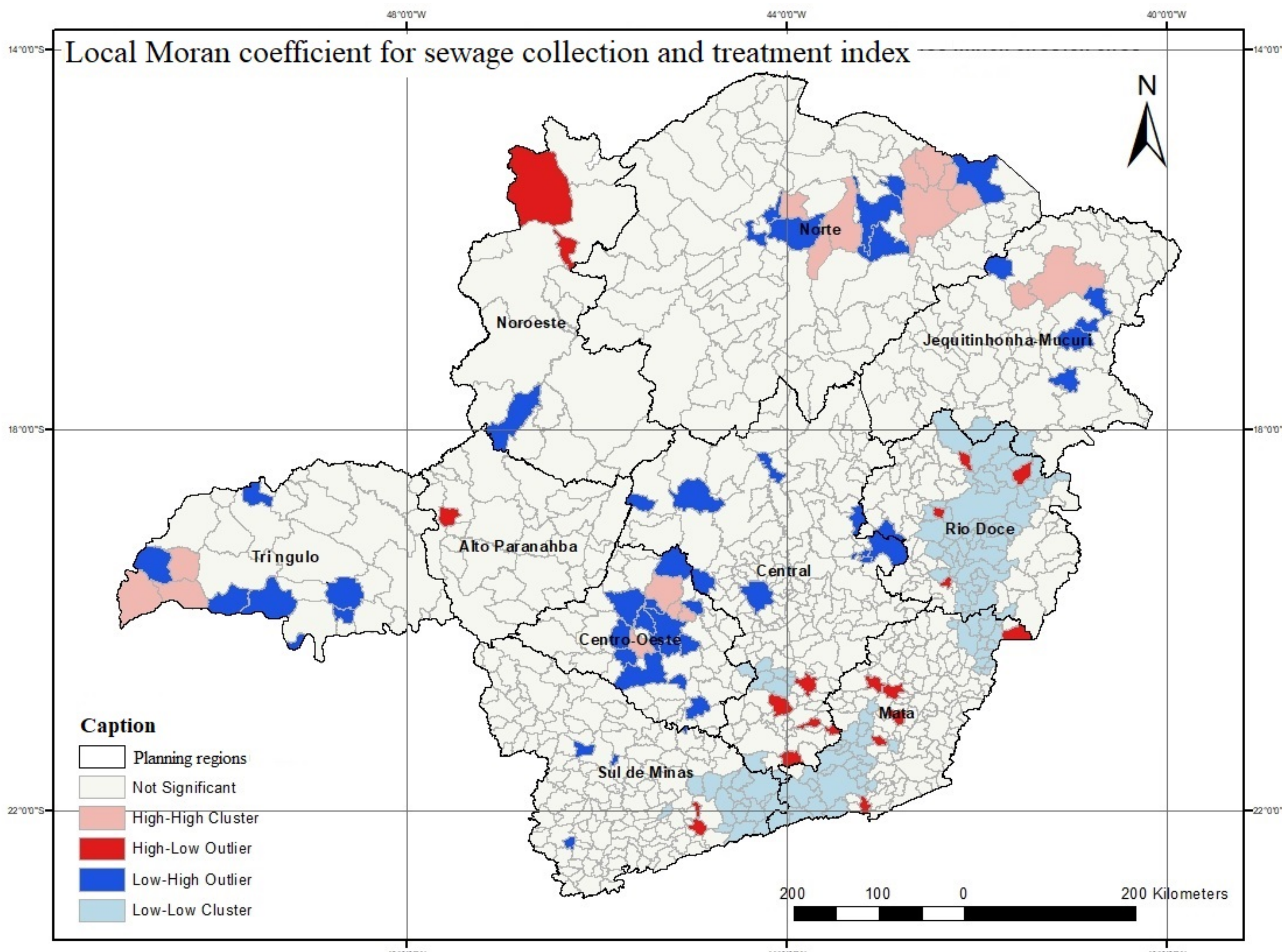

Figure 5 – Spatial correlation map for sewage with treatment index in the planning regions of the State of Minas Gerais.

and IDHM values in the State, reinforcing a possible divergence of standards between these and the other regions of Minas.

The result presented in Figure 6 is similar to that observed in Figure 7, both showing that municipalities of the North, Jequitinhonha-Mucuri, Rio Doce regions, and the northern portion of the Central region, are predominantly in the low and average IDHM ranges, whereas most of the cities in Sul de Minas, Centro-Oeste and especially Alto Paranaíba and Triângulo Mineiro have high IDHM. Only Belo Horizonte and Nova Lima, in the Central region, are in the very high range.

The local Moran correlation coefficient was calculated for the per capita income and IDHM data, whose results are in Figures 8 and 9, and show the existing heterogeneity between the different planning regions of the state of Minas Gerais.

We observed a higher concentration of low-low clusters of IDHM data in the North of the State of Minas Gerais. Part of the regions of Zona da Mata and the North of Rio Doce displays the positive spatial association between a city and its neighbors (high-high cluster). High-low outliers are also observed in the central region.

Regarding income distribution, in the North and Jequitinhonha-Mucuri regions, besides the Northern extremities of the Rio Doce and Central regions, there is a greater concentration of low-low clusters. In these, there is a predominance of municipalities with income values below-average. In the same regions, the high-low outliers are concentrated, demonstrating that some municipalities have above-average per capita income values, with neighbors having below-average values. The opposite happens with the Sul de Minas, Midwest, Alto Paranaíba, Northwest, Triângulo Mineiro and Central regions, where there is concentration of high-high clusters, but occurrence of low-high outliers.

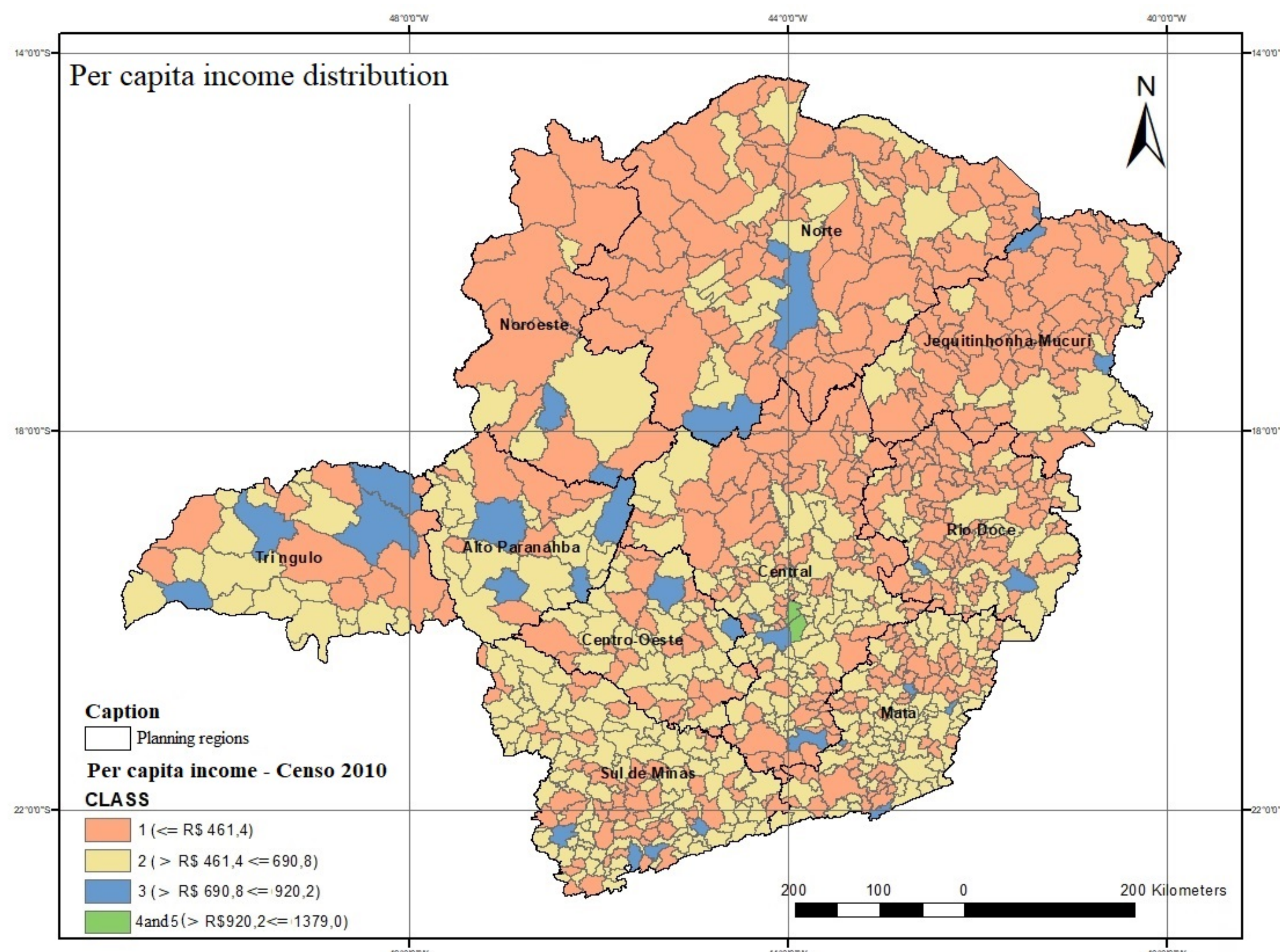

Figure 6 – *Per capita* income data in the municipalities and planning regions of Minas Gerais.

Investments' evaluation in water and sewage

At this stage, data regarding regional investments were evaluated by measuring the total amount of investments made between 1995 and 2017 in Minas Gerais, in water supply and sewage per region, whose results are presented in Table 5.

The total amount invested in water supply and sewage in the cities of Minas Gerais by the municipalities' administrations, providers and the State in the last 20 years was R$24.76 billion. Analyzing the data presented in Table 5, Zona da Mata, Rio Doce, Triângulo, and Central are the regions with the highest total and where the average investment amounts stand out. The Central region concentrated almost 80% of the total investment in Minas Gerais, of which more than 95% went to the city of Belo Horizonte.

Jequitinhonha-Mucuri has the lowest average investment value, followed by the North, both of which are in the semiarid climate region — according to SUDENE (2017a), this region is characterized by an annual average rainfall of 800 mm or less; SUDENE (2017b) also says this region covers 1,262 Brazilian municipalities (and 91 from Minas Gerais). These same regions present 54.21% and 29.04% of sewage without treatment, and 18.08% and 24.76% of sewage with treatment, respectively.

It is important to point out that some of these regions present a concentration of investment in only one municipality. In the Jequitinhonha-Mucuri region, Teófilo Otoni received more than 97% of the investments; and in the Northwest, Unaí received 76.7% of them. One factor that may have contributed to the concentration of investments is the centralization of regional populations in these municipalities. Forty percent of the population of the Central region and 14% of the entire population of the State lives in Belo Horizonte; in Teófilo Otoni, this number drops to 13.5% in relation to Jequitinhonha-Mucuri, and to 0.65% concerning to the State; and in Unaí, to 21.19% of the inhabitants of the Northwest region and 0.37% of the State.

The correlation between the average investment variables and sewage index per region was evaluated by the Pearson's correlation index (r), which generated a value of approximately 0.21, indicating a weak positive relationship (as one variable grows, the other also grows) among them. This can be observed in real life in Central region data, which despite having received almost 80% of the investments in the State, presents a water supply index smaller than the Rio Doce, Zona da Mata, Alto Paranaíba and Triângulo Mineiro regions. About sewage index, this region is still behind Sul de Minas (Table 4).

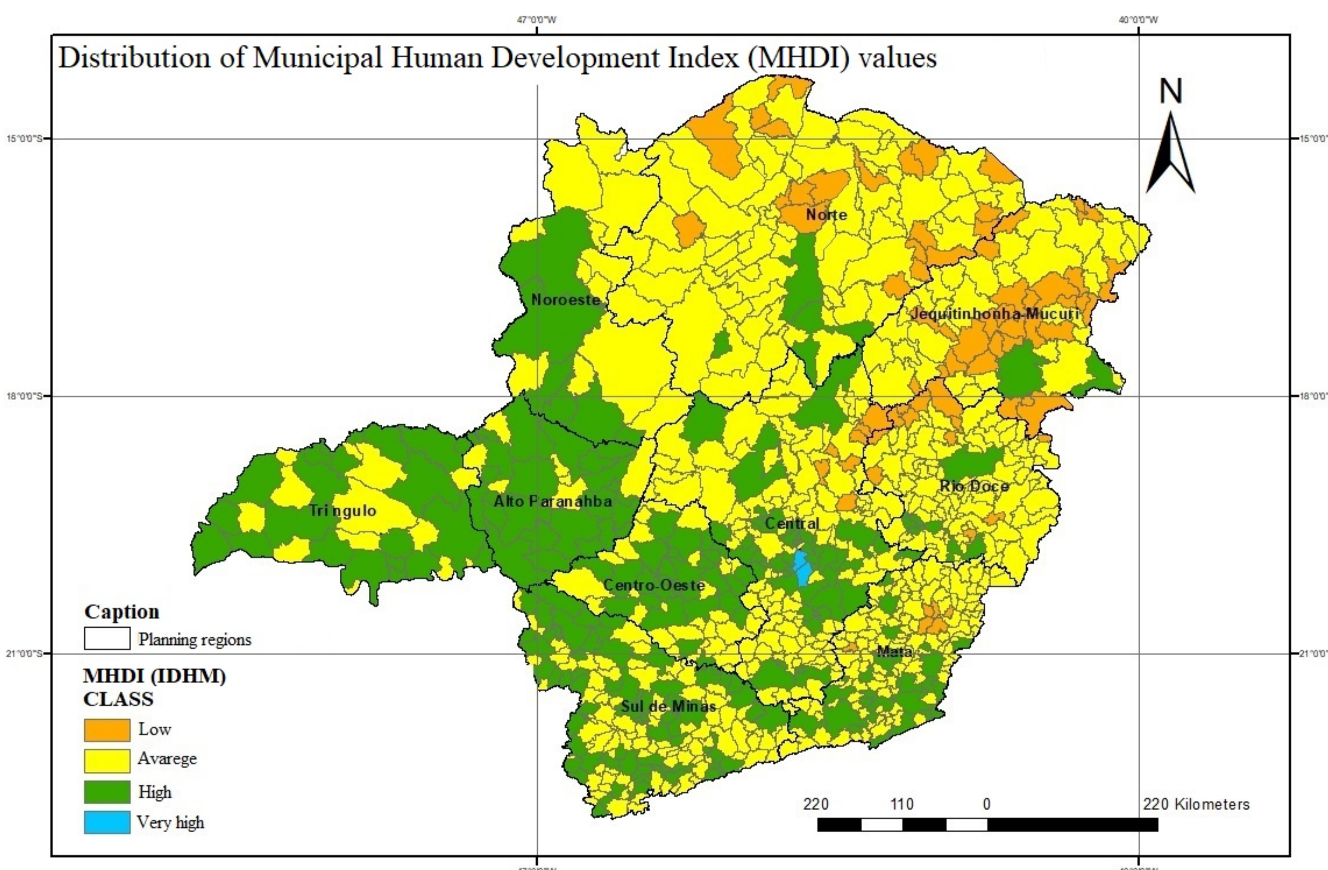

Figure 7 – MHDI data map by municipalities and planning regions of Minas Gerais.

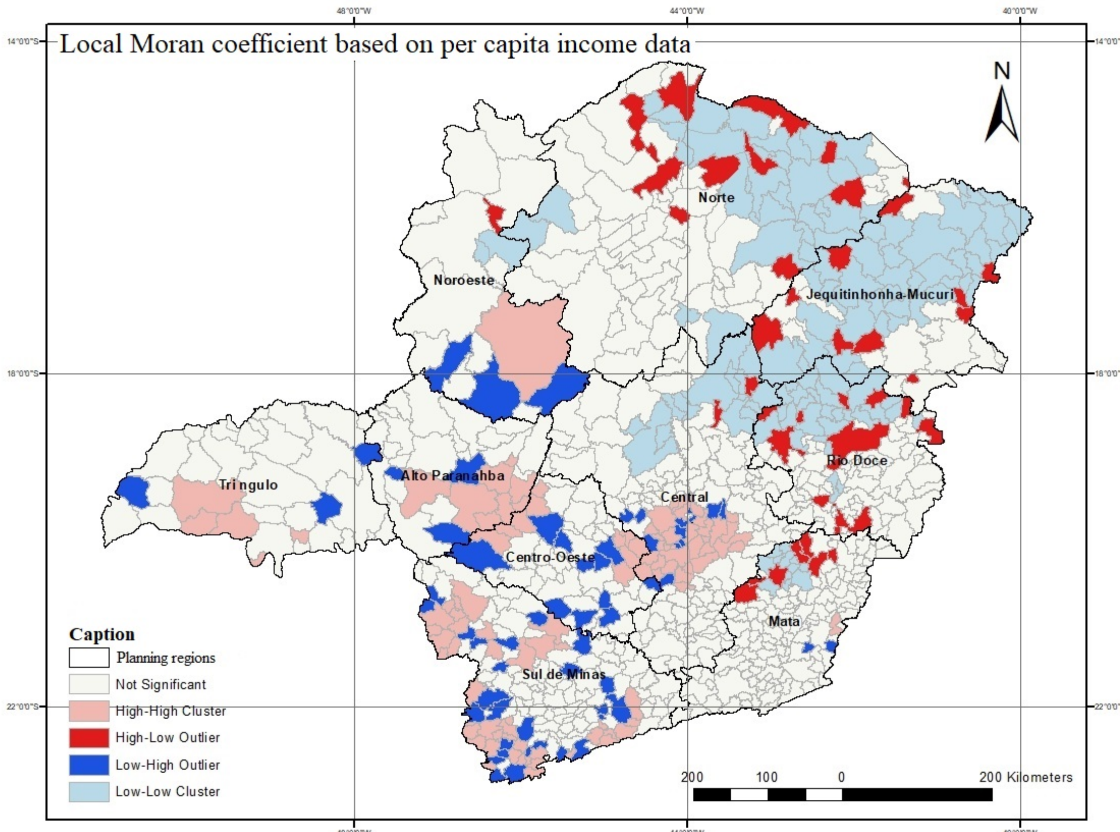

Figure 8 – Spatial correlation map for *per capita* income in the planning regions of Minas Gerais.

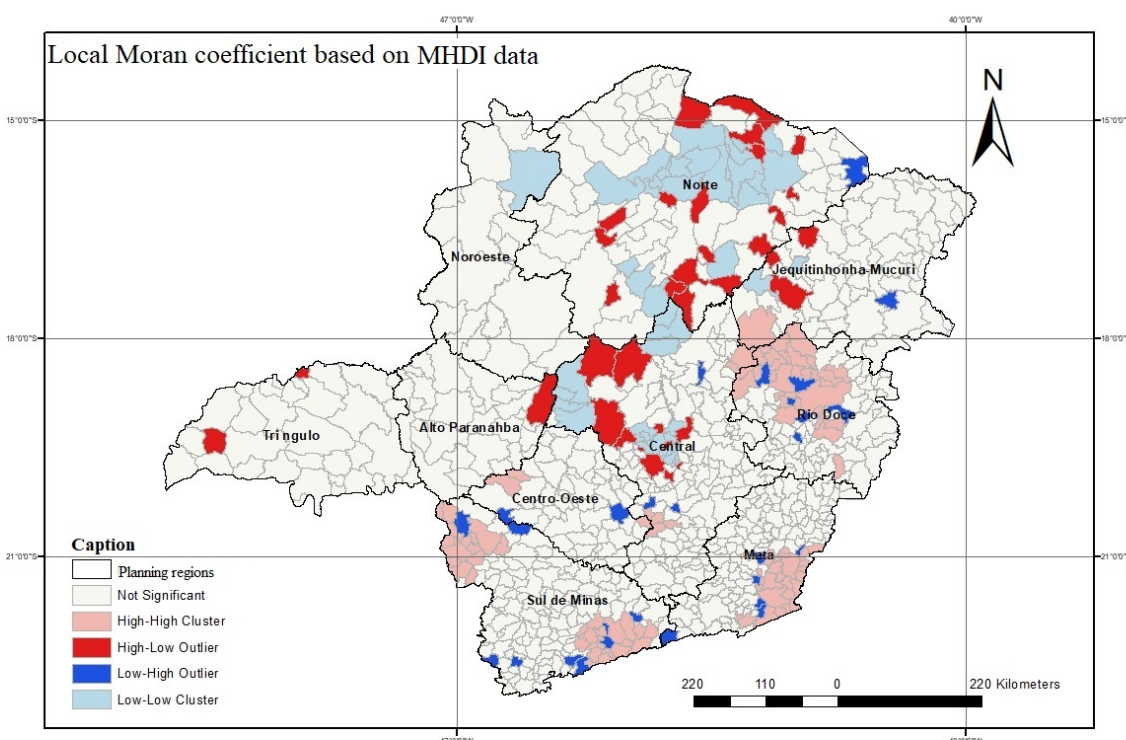

Figure 9 – Spatial correlation map for MHDI through the planning regions of Minas Gerais.

Table 5 – Investments in water supply and sewage by region in Minas Gerais.

Region	Total investment (R$)*	Average investment (R$)**
Jequitinhonha/Mucuri	523,356,236.76	1,466,942.41
North	31,479,926.26	3,497,769.58
Alto Paranaíba	91,788,358.78	4,830,966.25
South	448,449,454.14	5,214,528.54
Midwest	276,314,253.68	7,467,952.80
Northwest	62,041,045.33	7,755,130.67
Zona da Mata	897,057,729.03	10,679,258.68
Rio Doce	1,177,616,442.70	21,807,711.90
Triângulo	1,633.916,404.21	85,995,600.22
Central	19,619,064,989.45	248,342,594.80
MINAS GERAIS	R$24,761,084,840.34	R$397,058,455.86

*Sum of the investments made by different entities (providers, municipality and State) in all the municipalities of each region in each year; **average amount invested in the region, obtained by dividing the total investments and the number of municipalities of each region.

Integrated analysis

An integrated analysis of the data compiled in Table 6 was conducted, resulting in a repetition pattern that shows unfavorable results concentrated in the North and Jequitinhonha-Mucuri regions for water vulnerability, sewage index with and without treatment, total investment, average investment, per capita income and MHDI. On the other hand, the Zona da Mata and Triângulo regions present the best indexes in relation to water vulnerability, sewage index with and without sewage treatment and water supply. The Triângulo Mineiro region also presents high rates of total investment, average investment, per capita income and MHDI.

It is also important to stress that the Sul de Minas and Alto Paranaíba regions present better conditions in the evaluated indexes. An exception of the correlation model was observed in Sul de Minas. Despite the high MHDI values, sanitation indexes do not have the same behavior. A similar conclusion was presented by Souza et al. (2016) for the State of Goiás.

In a study on access to water supply and sewage in the mesoregions of Minas Gerais, Rodrigues et al. (2019b) noticed a relationship between the greatest access deficits and the lowest rates of urbanization and income levels, especially in the north of the State. Oliveira and Ervilha (2019)

Table 6 – Compilation of the values and attributes related to the variables analyzed for the planning regions of Minas Gerais.

Region	WV	WE (%)	TR (%)	WS (%)	AI (R$)	PCI (class)	MHDI
North	Very high, high and average	29,04	24,76	96,85	3,497,769.6	1, 2 and 3	Low, average and high
Northwest	High and average	30.27	34.88	96.53	7,755,130.7	1, 2 and 3	Average and high
Jequitinhonha-Mucuri	Very high, high and average	54.21	18.08	96.70	1,466,942.4	1, 2 and 3	Low, average and high
Midwest	High, average and low	56.73	27.62	96.91	7,467,952.8	1, 2 and 3	Average and high
Central	High, average and low	60.30	14.54	96.74	248,342,594.8	1, 2, 3, 4 and 5	Low, average, high and very high
Rio Doce	Average and low	62.49	16.53	97.36	21,807,711.9	1, 2 and 3	Low, average and high
Mata	Average and low	66.02	11.28	97.54	10,679,258.7	1, 2 and 3	Low, average and high
South	Average and low	66.63	14.99	95.68	5,214,528.5	1, 2 and 3	Average and high
Alto Paranaíba	Average and low	67.45	20.22	98.10	4,830,966.25	1, 2 and 3	Average and high
Triângulo	Average and low	67.67	18.09	99.38	85,995,600.2	1, 2 and 3	Average and high

WV: water vulnerability; NT: sewage without treatment; TR: sewage with treatment; WS: water supply coverage; AI: average investment; PCI: *per capita* incom); MHDI: municipal human development index.

concluded there are significant inequalities between the municipalities of Minas Gerais concerning access to basic sanitation, with the worst rates in the North, Jequitinhonha and Mucuri valleys. Assessing the inequity in access to water, Aleixo et al. (2016) concluded there is an obvious influence of regional (country, state, type of area — urban or rural), socioeconomic (income and schooling), demographic (gender and race) and cultural factors (religion) in access or lack of access to water.

Conclusion

Based on the aforementioned results, the Model of Integrated Territorial Assessment for Environmental Justice Applied to Sanitation proposed in this paper and applied for the State of Minas Gerais allows a systemic and integrated analysis of environmental justice in the sanitation sector.

Principles and practices of environmental justice in access to sanitation were not considered when allocating financial resources in Minas Gerais. It is worth noting that in only 43 – out of the 674 municipalities in Minas Gerais studied by Siqueira et al. (2018) – the quality of investment in sanitation showed high level of efficiency in the allocation, which, in fact, may be related to political determinants (Kresch and Schneider, 2020).

The comparison between the Central and North regions, representing extremes, shows that the Central region received 79.23% of the investment in water supply and sewage in the last 20 years, whereas the North received 0.13%. The Central region has about 2/3 of its territory with average and low water vulnerability, while the North has almost all cities with high and very high vulnerability. In the Central region, 60.3% of the sewage is collected, but not treated, against 29.04% in the North. The lowest income and MHDI rates occur in the municipalities of the North region.

The North and Jequitinhonha-Mucuri planning regions have the highest water vulnerability, as well as the lowest levels of water supply, sewage without treatment and investment. This, associated with the unfavorable socioeconomic results (low income and MHDI), points to a critical situation of the mentioned regions in relation to environmental justice in sanitation, coherent with what is verified in the global scenario (Local Burden of Disease WaSH Collaborators, 2020).

The results show there is inequality between the Minas Gerais regions regarding water supply and sewage, not only because of the natural context, marked by water vulnerability, but also by the socioeconomic status and different concentrations of investments.

Authors' contributions:

Elias, L.G.R.: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing — Original Draft, Writing — Review & Editing; Melo, M.C.: Validation, Formal Analysis, Writing — Review & Editing, Supervision; Santos, A. S. P.: Writing — Review & Editing; Maia, L. C.: Writing — Review & Editing.

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Study on Brazilian agribusiness wastewaters: composition, physical-chemical characterization, volumetric production and resource recovery

Estudo sobre águas residuárias do agronegócio brasileiro: composição, caracterização físico-química, produção volumétrica e recuperação de recursos

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ABSTRACT

Brazil is a significant producer of agricultural and agro-industrial waste, which can be used to recover valuable resources, such as struvite, hydroxyapatite, methane gas, hydrogen gas, and carboxylic acids, to mitigate the environmental impacts of the agro-industrial sector, add economic value to organic waste, and promote the sustainability of natural resources. Thus, this work's objective was to compile and analyze data on the composition, physical-chemical characterization, and volumetric production of six agricultural and agro-industrial wastewaters (AWWs) from activities of paramount importance in Brazilian agribusiness and to report studies on resource recovery from those liquid wastes. The literature review was carried out by analyzing scientific works obtained by searching for keywords in different databases. It was concluded that swine wastewaters (SWs), slaughterhouse wastewaters (SHWs), and dairy wastewaters (DWs) are the most promising for struvite recovery. DWs also stand out for the recovery of hydroxyapatite. SWs and brewery wastewaters (BWs) are commonly used for prospecting for algae or bacterial biomass and their derivative products. All AWWs analyzed are considered promising for biogas, methane and hydrogen, while the most soluble AWWs are more valuable for carboxylic acid production.

Keywords: nexus concept; organic liquid waste; environmental sustainability; agro-industrial wastewater; wastewater treatment.

RESUMO

O Brasil é um grande produtor de resíduos agrícolas e agroindustriais, os quais podem ser utilizados para a recuperação de recursos valiosos, como a estruvita, a hidroxiapatita, o gás metano, o gás hidrogênio e os ácidos carboxílicos, visando mitigar os impactos ambientais do setor agroindustrial, agregar valor econômico aos resíduos orgânicos e promover a sustentabilidade dos recursos naturais. Assim, o objetivo deste trabalho foi compilar e analisar dados de composição, de caracterização físico-química e de produção volumétrica de seis águas residuárias agrícolas e agroindustriais (ARA) provenientes de atividades de suma importância ao agronegócio brasileiro e reportar estudos sobre recuperação de recursos a partir desses resíduos líquidos. A revisão de literatura foi elaborada por meio da análise de trabalhos científicos obtidos mediante à busca de palavras-chave em diferentes bancos de dados. Concluiu-se que as águas residuárias da criação de suínos (ARCS), as águas residuárias de abate bovino (ARAB) e as águas residuárias do beneficiamento de leite (ARBL) são as mais promissoras para a recuperação de estruvita. As ARBL também se destacam para a recuperação de hidroxiapatita. As ARCS e as águas residuárias da produção de cerveja (ARPC) são comumente utilizadas para a prospecção de biomassa algácea ou bacteriana e seus produtos derivados. Todas as ARA analisadas são adequadas para a prospecção de biogás, metano e hidrogênio, enquanto as ARA mais solúveis são as mais promissoras para a produção de ácidos carboxílicos.

Palavras-chave: conceito *nexus*; resíduos líquidos orgânicos; sustentabilidade ambiental; águas residuárias agroindustriais; tratamento de águas residuárias.

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Introduction

According to the Center for Advanced Studies in Applied Economics at the University of São Paulo (Centro de Estudos Avançados em Economia Aplicada da Universidade de São Paulo — CEPEA/USP) and the National Confederation of Agriculture and Livestock of Brazil (Confederação Nacional da Agricultura e Pecuária do Brasil — CNA), in the first half of 2020, Brazil was the fourth largest exporter of agricultural products in the world, second only to the European Union, the United States of America (USA) and China. It was also a world leader in coffee production and a leader in the export of sugar, coffee, orange juice, soybeans, beef and chicken, showing agribusiness as one of the main activities that sustain the Brazilian economy (CNA, 2020).

In 2019, agribusiness was responsible for 43% of national exports, 21.4% of the Brazilian gross domestic product (GDP), and generating more than 18.2 million jobs, corresponding to 19.54% of the employed population with and without formalization (CEPEA and CNA, 2020). The share of agribusiness in the national GDP increased from 20.8% to 21.4% between 2018 and 2019 and showed a growth of 1.9% of GDP in the first quarter of 2020 compared to the same period of 2019 (Barros et al., 2020).

Due to agro-industry growth, there is an increasing generation of liquid organic waste, such as agricultural and agro-industrial wastewaters (AWWs), in production processes and related activities. These residues cause environmental impacts when they are not treated and disposed of properly, which contributes to soil, air and water pollution, contributing to the eutrophication of water bodies, in addition to causing possible harm to human and animal health (Dornelles et al., 2017; Morais et al., 2020b). Only dairy wastewaters generated worldwide, for example, have a polluting potential equivalent to 60% of the world population (Silva et al., 2020a).

In addition to the high generation of effluents from agribusiness activities, it is estimated that the global demand for water, energy and food will increase by more than 50% by 2050 compared to 2015 (Zhang et al., 2018). Such demand, driven by rapid population growth, urbanization, climate change and the depletion of fossil fuels, requires the adoption of solutions or alternatives that allow global resources management in a comprehensive, interconnected and efficient manner. Thus, the nexus water-energy-food concept was designed to study how these three systems are related and propose integrated planning strategies to optimize the use of natural resources in a sustainable way (Zhang et al., 2018).

In this context, aiming not only to mitigate the environmental impacts caused by its inadequate disposal but also to add economic value to organic liquid waste, the development of new treatment technologies and the study of the characteristics and potential of production and resource recovery from AWWs and other wastes have become essential to the progress of society and the maintenance of environmental resources (Bustillo-Lecompte and Mehrvar, 2015).

Thus, wastewater treatment can be directed towards the production and recovery of resources of commercial and industrial interest, such as struvite, hydroxyapatite, methane (CH_4), hydrogen (H_2), and carboxylic acids (Song et al., 2018). In this scenario, due to the high agricultural and agro-industrial activity in Brazil, it is essential to analyze the potential for prospecting these resources from different residues, stimulating the design and implementation of treatment plants with resource recovery.

Accordingly, scientific studies on resource recovery from AWWs can be facilitated by reviewing the technical literature that provides scientists with fundamental knowledge for developing their research. Despite several scientific studies on the treatment of AWWs, data on the composition and physical-chemical properties are dispersed in the literature and, generally, are reported superficially in applied research.

Thus, this work's objective was to compile and analyze data on the composition, physical-chemical characterization, and volumetric production of six agricultural and agro-industrial wastewaters from activities of paramount importance in Brazilian agribusiness and to report studies on resource recovery from those liquid wastes.

Methodology

Agricultural and agro-industrial wastewaters

The AWWs analyzed in this work were: slaughterhouse wastewaters (SHWs), swine wastewaters (SWs), brewery wastewaters (BWs), dairy wastewaters (DWs), fruit processing wastewaters (FPWs) from the production of ice cream and biodiesel production wastewaters (residual glycerol – RG).

It is already consolidated in the technical literature that these AWWs can be used to recover bioenergy from anaerobic methane production (Silva et al., 2020a), demonstrating the potential of these liquid residues for the recovery of other bioproducts. Thus, the possibility of recovering resources from low-cost substrates prompted us to investigate these AWWs and to build a literature review compiling data on their composition, physical-chemical characterization and volumetric production, aiming to provide researchers, in an only material, fundamental knowledge about each wastewater evaluated and to stimulate the development of new scientific research.

Resource recovery studies

The technical literature reports several chemical, physical and biological processes in which AWWs can be submitted aimed at the production and prospecting of resources. Due to the impossibility of deepening and discussing all the strategies reported in the literature, the most common ones were adopted in the context of wastewater treatment, such as anaerobic and aerobic biological treatment. In addition to these, in the context of nutrient removal, the chemical precipitation process was selected (Chernicharo, 2007; Metcalf and Eddy, 2016).

On the basis of scientific studies reporting the use of AWWs for resource recovery, a significant amount of research has been observed associated with the removal of nutrients from the recovery of minerals (such as struvite and hydroxyapatite), the production of biomass for the prospecting of compounds of industrial interest (such as algal biomass) and the production and extraction of compounds commonly exploited in the anaerobic digestion of organic waste (methane, hydrogen and carboxylic acids, for example). Therefore, these resources were chosen for detail in this review. Thus, on the basis of the composition and physical-chemical characterization of the AWWs selected for evaluation, one can analyze which ones are most promising for obtaining these resources.

Data collection

The literature review was carried out through the analysis of scientific articles and other academic works, such as monographs, master's dissertations, and doctoral theses, obtained by searching keywords in different databases, such as SciVerse Scopus, Google Scholar, SciELO, Science Direct and CAPES Journals (Higher Education Personnel Improvement Coordination Journals – Periódicos da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES). The data survey took place between January and July 2020.

The main keywords used (in English and in Portuguese) in the databases to search for scientific works were: slaughterhouse wastewaters (*águas residuárias de abate bovino*), swine wastewaters (*águas residuárias da criação de suínos*), brewery wastewaters (*águas residuárias da produção de cerveja*), dairy wastewaters (*águas residuárias do beneficiamento de leite*), fruit processing wastewaters (*águas residuárias do beneficiamento de frutas*), residual glycerol (*glicerol residual*), methane (*metano*), hydrogen (*hidrogênio*), carboxylic acids or volatile fatty acids (*ácidos carboxílicos*), algae biomass (*biomassa algácea*), struvite (*estruvita*) and hydroxyapatite (*hidroxiapatita*). Context-related words were also used, such as slaughterhouse (*abatedouro*), swine farming (*suinocultura*), brewery (*cervejaria*), dairy (*laticínios*), fruit processing (*beneficiamento de frutas*), ice cream (*sorvetes*), and glycerol (*glicerol*). These words were combined in different ways to carry out the research.

For the selection of scientific articles, the summary and objectives of each one were read. Subsequently, articles that contained data on the composition, physical-chemical characterization, volumetric production of raw wastewaters and those that addressed resource recovery using these AWWs were selected. The publication date was another selection criterion used, considering only articles published between 1990 and 2020. However, preference was given to collecting data reported in papers published since 2010 in international journals with scientific relevance. Articles with cross-cutting themes were also selected, such as environmental pollution, the nexus concept, biological treatment of wastewater and bioproducts, to support the introduction and discussion. The choice of articles published in recent years (2015 to 2020) was prioritized.

Through consultation with electronic portals (websites), technical and economic reports were also obtained and analyzed from different relevant institutes or organizations, such as the Brazilian Institute of Geography and Statistics (Instituto Brasileiro de Geografia e Estatística — IBGE), the Food and Agriculture Organization of the United Nations (FAO), the Ministry of Agriculture, Livestock and Supply (Ministério da Agricultura, Pecuária e Abastecimento — MAPA), the Center for Advanced Studies in Applied Economics (Centro de Estudos Avançados em Economia Aplicada — CEPEA) and the National Confederation of Agriculture and Livestock (Confederação Nacional da Agricultura e Pecuária — CNA). In this case, only works published since 2016 were considered to provide more recent information on the evaluated agribusiness activities' economic aspects.

The Statista portal (https://www.statista.com/), which consists of a statistics and infographics platform for consultation, was also used in data collection in some cases. After selecting scientific papers, the data of interest were compiled, and the review article was constructed.

Composition, Physical-Chemical Characterization and Volumetric Production of Agricultural and Agro-Industrial Wastewaters

Table 1 summarizes the importance of some Brazilian agribusiness activities in a national and international panorama based on 2017 and adjacent years. This table also presents a range of volumetric production for each AWW. Figure 1 shows the leading Brazilian states that produce these wastes. It is noticed that these agribusiness sectors are concentrated in the South, Southeast and Midwest regions.

The constant generation of wastewaters from cattle slaughter (slaughterhouse wastewater — SHWs) and swine farming (swine wastewaters — SWs) is the result of the growing demand for livestock products due to the high nutritional value of meat (proteins, minerals, and bioavailable vitamins), which is a crucial part of the diet of the population of several countries, including Brazil (Moukazis et al., 2018).

SHWs are generally composed of blood, manure and viscera and contain a high proportion of proteins and lipids, thus having a high amount of biodegradable organic matter (Fia et al., 2015). Furthermore, SHWs have a high concentration of pathogenic and non-pathogenic microorganisms and can promote ecotoxicity at acute and chronic levels, which makes the treatment of this effluent indispensable before it is disposed of in water bodies (Pereira et al., 2016). SHWs may also contain heavy metals and residues of cleaning agents and veterinary medical products, which is why they are considered highly polluting worldwide due to their complex composition and high volumetric production (Souza and Orrico, 2016).

In the same sense, SWs are complex liquid wastes that can be defined as a mixture of wasted feed, water spilled from drinking fountains, animal excrement (feces and urine) and water used for cleaning and hygiene purposes in swine farming.

Table 1 – National and international relevance and range of volumetric production of wastewaters generated by Brazilian agribusiness activities, based on 2017 and adjacent years.

Agribusiness activities	National relevance	International relevance	Volumetric production	References
Cattle slaughter	It is estimated that in 2017, 30.8 million heads were slaughtered. The actual bovine data in 2018 indicate that the country had, in that year, 222.0 million head. In 2019, Brazil was the largest beef exporter, with around 2.00 million tons, and the second largest producer, with 9.90 million tons.	In 2017, world beef production totaled 70 million tons. Meat production is dominated by Brazil, China, the European Union, the Russian Federation and the United States.	During the bovine slaughter process, about 3 m^3 of wastewater are generated, on average, by slaughtered cattle. A processing facility can consume between 2.5 and 40 m^3 of water per ton of meat produced.	Bustillo-Lecompte and Mehrvar (2015) Pereira et al. (2016) Brasil (2018) CNA (2020)
Swine farming	Around 3.75 million tons of pork were produced in 2017, making Brazil fourth in world production. In 2019, Brazil was the fourth largest pork producer and exporter, making 3.70 million tons available on the national market and exporting 0.7 million tons.	World net pork production totaled around 109.85 million tons of carcass weight in 2016, with production dominated by China, the European Union, the United States and Brazil.	It is estimated that 4 - 9 L of wastewater are generated daily by swine on a farm.	García et al. (2017) USDA (2018) Nagarajan et al. (2019) CNA (2020)
Beer production	Brazil produces about 13 billion L of beer per year. In 2016, 140 million hL of beer were produced in the country, giving Brazil third place worldwide.	In 2017, global beer production reached around 1.95 billion hL, compared to 1.3 billion hL in 1998. The main countries in beer production are China, the United States and Brazil.	It is estimated that for each liter of beer produced, 4.5 - 10 L of wastewater are generated.	Marcusso and Müller (2017) Arantes et al. (2017) Pachiega et al. (2019) Statista (2019a)
Milk processing and production of derivatives	In 2017, Brazil produced around 35.1 billion liters of milk. Over four decades, national production has quadrupled. Sales of dairy products abroad were mostly powdered milk (62.2%), UHT milk (18.7%) and different types of cheese (9.1%).	World milk production reached 811 million tons in 2017. The world's top five milk producers are the European Union (20%), India (20%), the United States (12%), Pakistan (6%) and China (5%).	The dairy and milk processing industry produces 0.2 - 10 L of wastewater per liter of milk processed.	Fernandes and Naval (2017) EMBRAPA (2018) FAO (2018) OECD and FAO (2018) Silva, A.N. et al. (2019)
Fruit processing for ice cream production	In 2017, about 1,129 million L of ice cream were produced, with a per capita consumption of 5.44 L per year, making Brazil the sixth leading country worldwide in consumption (3.1%).	In 2017, the global ice cream market had an estimated value of 56.91 million USD. By 2024, it is expected to be worth 74.96 billion USD.	The ice cream industry generates 3 - 7 L of wastewater per 0.45 kg of ice cream produced.	Enteshari and Martínez-Monteagudo (2018) ABIS (2019) Statista (2019b)
Biodiesel production	In 2017, biodiesel production was 4.3 million m^3, which corresponded to 56.2% of the total national production capacity (21.2 thousand m^3 per day). That same year, 374,500 m^3 of glycerol were generated as a by-product of biodiesel production.	The global production of biodiesel was just over 34 million tons in 2016. The most important producer of biodiesel is the European Union, followed by the United States and Brazil.	For every 90 m^3 of biodiesel produced by transesterification, 10 m^3 of glycerol are generated.	Mota et al. (2009) Yazdani and Gonzalez (2007) ANP (2018) UFOP (2017)

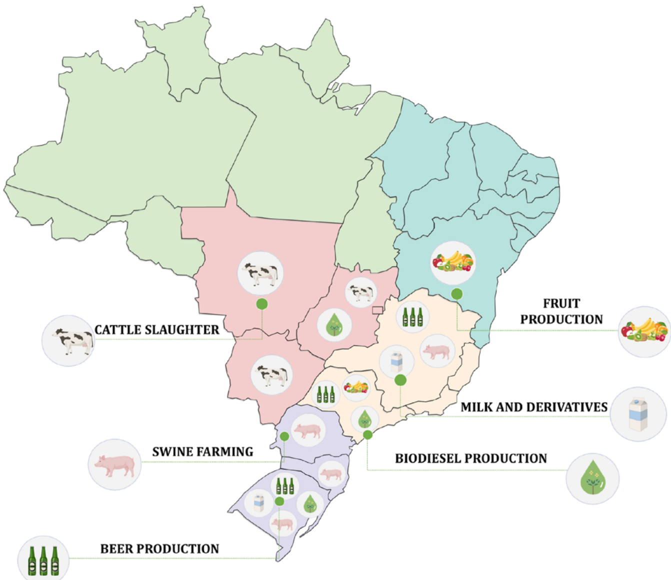

Figure 1 – Leading Brazilian states producing agricultural and agro-industrial wastewaters.
Source: adapted from Marcusso and Müller (2017), EMBRAPA (2018), ANP (2018), ABIS (2019) and CNA (2020).

For this reason, SWs are characterized by having high levels of organic matter, suspended solids, nutrients and high microbial load (Pereira et al., 2009). SWs can also contain considerable concentrations of antibiotics (tetracyclines, sulfonamides and macrolides) and hormones (estrogens, androgens, glucocorticoids and progestogens), extensively used to treat infections and even used as promoters of animal growth. For this reason, this type of AWW can introduce micropollutants, such as antibiotics and hormones, indiscriminately into the environment (Cheng et al., 2018).

In another perspective, the beverage sector makes up a fundamental part of the agribusiness in the national and international panorama, emphasizing the production of soft drinks, coffee, beer and milk (Cervieri Júnior et al., 2014). In this type of industry, a large amount of water is used to produce beverages, washing bottles and cleaning equipment and machines, with an estimated 50% of the total wastewater generated coming from washing bottles. Thus, the beverage sector contributes to the generation of polluting liquid effluents due to the high concentrations of sugars and other biodegradable compounds derived from its production process (Abdel-Fatah et al., 2017).

In the beverage sector, beer is the fifth most consumed beverage globally, behind tea, soda, milk and coffee (Olajire, 2012). The beer industry generates residues called wastewaters from beer production (brewery wastewaters – BWs), containing residual amounts of raw materials for the drink, including suspended solids, sugars and yeasts. BWs come from the beer production chain's unitary operations, such as filtration, equipment discharges, container washing and cleaning of tanks, vats, pipes, and floors (Arantes et al., 2017).

On the other hand, the dairy industry is one of the largest sectors of food processing and, for this reason, consumes large amounts of water for cleaning and sanitizing machines and equipment, exchanging heat and washing its locations. Wastewaters from milk processing (dairy wastewaters – DWs) and derived products are composed of wasted milk residues, lactose, fats, nutrients and residues of detergents and disinfectants. Depending on the season and the production system, the characterization of DWs varies considerably (Chandra et al., 2018; Daneshvar et al., 2019).

Still in the context of the food industry, ice cream is one of the most popular luxury items in the world and the sector is overgrowing (Konstantas et al., 2019). During the washing and pulping of fruits for ice cream production, liquid residues referred to as wastewaters from the processing of fruits for ice cream production (fruit processing wastewaters – FPWs) are generated. These consist of a complex colloidal mixture of suspended solids (flavoring compounds), soluble molecules (carbohydrates, milk proteins and other sources, lipids and minerals) and detergent and disinfectant residues (Demirel et al., 2013).

Biodiesel is increasingly considered an environmentally viable substitute for diesel in the biofuels business due to global energy needs. Residual glycerol from biodiesel plants (RG) is a by-product of the manufacture of this biofuel from a transesterification reaction of oils (vegetables or animals) with alcohol (Pereira et al., 2019). Besides having a high organic load, the generated RG includes many impurities and chemicals, such as methanol, organic and inorganic salts, vegetable dyes, traces of mono- and diglycerides and soap, making it a polluting by-product (Anitha et al., 2016).

Due to the importance of the agro-industrial activities shown in Table 1 and the environmental impacts potentially caused by them, it is essential to know the physical-chemical characteristics of raw AWWs generated in their production processes. Table 2 summarizes the main physical-chemical characteristics of raw AWWs reported in the technical literature.

According to data expressed in Table 2, there is significant variability in the physical and chemical characteristics of AWWs due to the production processes, the routine inherent to each agribusiness and the sustainability practices adopted (Ranade and Bhandari, 2014). Despite this variability, all of them have high levels of organic matter and nutrients, not meeting the effluent release standards established by the federal legislation in force in Brazil (Brasil, 2011), showing that they must be treated before being disposed of in the environment (Morais and Santos, 2019).

Resource Recovery from Agricultural and Agro-Industrial Wastewaters

The following topics will briefly present an overview of resource recovery from the AWWs evaluated in this work, mainly discussing these resources' industrial applications.

Table 2 – Range of values of physical-chemical characteristics of raw agricultural and agro-industrial wastewaters evaluated.

AWWs	Parameter	Value range	Unit	References
SHWs	pH	4.9–8.1	-	Bustillo-Lecompte and Mehrvar (2015) Pereira et al. (2016) Plácido and Zhang (2018) Morais (2019) Morais et al. (2020b) Morais et al. (2021)
	Al	83–1,500	mg L^{-1} $CaCO_3$	
	COD	1,018–13,800	mg L^{-1} O_2	
	BOD	420–5,770	mg L^{-1} O_2	
	TN	50–840	mg L^{-1}	
	$N\text{-}NH_4^+$	20–340	mg L^{-1}	
	TP	20–2,260	mg L^{-1}	
SWs	pH	7.0–8.5	-	Suto et al. (2017) Ding et al. (2017) Cheng et al. (2018) Xiao et al. (2018) Morais et al. (2020a)
	Al	560–4,780	mg L^{-1} $CaCO_3$	
	COD	3,000–30,000	mg L^{-1} O_2	
	BOD	1,500–8,700	mg L^{-1} O_2	
	TN	800–6,000	mg L^{-1}	
	$N\text{-}NH_4^+$	400–2,000	mg L^{-1}	
	TP	100–1,400	mg L^{-1}	
BWs	pH	5.0–11.0	-	Shi et al. (2010) Bakare et al. (2017) Arantes et al. (2017) Enitan et al. (2018) Silva, A.S. et al. (2019)
	Al	190–3,170	mg L^{-1} $CaCO_3$	
	COD	2,000–32,500	mg L^{-1} O_2	
	BOD	1,200–3,600	mg L^{-1} O_2	
	TN	25–450	mg L^{-1}	
	$N\text{-}NH_4^+$	5–22	mg L^{-1}	
	TP	0.5–20	mg L^{-1}	
DWs	pH	4.7–1.0	-	Lu et al. (2016) Justina et al. (2018) Murcia et al. (2018) Daneshvar et al. (2019) Coelho et al. (2020b)
	Al	140–620	mg L^{-1} $CaCO_3$	
	COD	80–95,000	mg L^{-1} O_2	
	BOD	40–48,000	mg L^{-1} O_2	
	TN	14–380	mg L^{-1}	
	$N\text{-}NH_4^+$	1–48	mg L^{-1}	
	TP	9–280	mg L^{-1}	
FPWs	pH	3.2–7.7	-	Borja and Banks (1994) Borja e Banks (1995) Hu et al. (2002) Demirel et al. (2013) Morais et al. (2020b)
	Al	220–1,500	mg L^{-1} $CaCO_3$	
	COD	4,500–10,480	mg L^{-1} O_2	
	BOD	1,620–2,450	mg L^{-1} O_2	
	TN	145–165	mg L^{-1}	
	$N\text{-}NH_4^+$	32–43	mg L^{-1}	
RG	pH	3.7–10.3	-	Sittijunda and Reungsang (2012) Oliveira et al. (2015) Silva et al. (2017) Ren et al. (2017) Pan et al. (2019) Coelho et al. (2020a)
	COD	1,023–1,260	g L^{-1} O_2	
	TN	0–500	mg L^{-1}	
	$N\text{-}NH_4^+$	5–50	mg L^{-1}	
	TP	53–90	mg L^{-1}	

pH: hydrogenionic potential; Al: total alkalinity; COD: chemical oxygen demand; BOD: biological oxygen demand; TN: total nitrogen; $N\text{-}NH_4^+$: ammonium nitrogen; TP: total phosphorus.

The constructive and operational aspects of treatment technologies and resource prospecting will not be analyzed. Table 3 presents a compilation of recent studies that aim to treat AWWs with production and resource recovery.

Production of struvite and hydroxyapatite by chemical precipitation

AWWs have considerable concentrations of nutrients, such as phosphorus (P) and nitrogen (N). These nutrients can be recovered in the forms of orthophosphates (PO_4^{3-}, HPO_4^{2-}, H_2PO^{4-}, H_3PO_4) and ammonium nitrogen ($N\text{-}NH_4^+$), respectively, through solid precipitates, such as struvite ($MgNH_4PO_4{\cdot}6H_2O$) and hydroxyapatite ($Ca_5(PO_4)_3OH$). The great interest in the biorefinery of these nutrients is since they can be applied in the production of fertilizers for the agricultural industry and as building block chemicals in the chemical industry for the production of nylon, plastic, explosives, rocket fuels and animal feed supplements (Carey et al., 2016).

Struvite is considered a slow-release fertilizer, being less soluble in water, and it contains low levels of heavy metals compared to conventional fertilizers, causing less severe environmental impacts on groundwater and water bodies (Wang et al. 2005). The recovery of struvite from AWWs provides the advantage of simultaneously removing phosphorus and nitrogen, depending on the composition of the AWWs, and has been extensively studied for a variety of liquid effluents, especially wastewaters from animal husbandry, particularly swine farming (in the case of struvite), and for effluents from the dairy and soft drink industry as well (Muhmood et al., 2019).

Recently, Kwon et al. (2018) investigated nitrogen and phosphorus removal via struvite formation by treating SWs (initial concentrations of ammonia and phosphate of 3.141 and 60.8 mg L^{-1},

Table 3 – Research on resource recovery from agricultural and agro-industrial wastewaters evaluated in this study.

AWWs	Technology	Recovered resource	Efficiency of resource recovery process	References
SHWs	Anaerobic membrane bioreactor (AnMBR)	CH_4	95% COD removal. Yield of CH_4: 365 L per kg COD applied.	Jensen et al. (2015)
	Tubular anaerobic digestors	Biogas	COD removal: more than 70%. Maximum biogas production: 0.2 $m^3\ m^{-3}\ d^{-1}$.	Martí-Herrero et al. (2018)
	Pilot scale two-stage anaerobic digesters	CH_4	68.5% COD removal. Maximum CH_4 yield: 384 mL per g COD applied.	Wang et al. (2018)
	Microbial fuel cell with aerobic and anaerobic bioreactors	Bioelectricity and nitrogen	99% COD removal efficiency. Generation of 162.55 mW m^{-2} of bioelectricity and 100% nitrogen recovery.	Mohammed and Ismail (2018)
	Batch anaerobic reactor Fed-batch anaerobic reactors Semi-continuous anaerobic reactors	CAs	Maximum concentration obtained of 100 g L^{-1} CAs. The predominant acids were C2, C4 and iso-C5.	Plácido and Zhang (2018)
	Batch anaerobic reactor	CAs	On average, 76% of the applied COD was converted to CAs. Yield 0.76 mg COD per mg COD applied.	Morais (2019)
SWs	Microbial electrolysis cell (MEC)	H_2	69-75% COD removal. Productivity: 0.9 - 1.0 $m^3\ m^{-3}\ d^{-1}$ H_2.	Wagner et al. (2009)
	Photobioreactor	Biomass and carbohydrates	60-70% COD removal. 40-90% removal of NH_4^+-N. 3.96 g L^{-1} of maximum biomass concentration and 58% dry weight in carbohydrates.	Wang et al. (2015)
	Anaerobic sequential batch reactor (ASBR)	CH_4	Maximum CH_4 production rate: 1.952 L $L^{-1}\ d^{-1}$.	Yang et al. (2016)
	Constructed wetlands (CWs)	Nitrogen	87.7–97.9% removal for $N\text{-}NH_4^+$ and 85.4–96.1% for TN. Nitrogen recovery: of 120–222 g m^{-2} $year^{-1}$.	Luo et al. (2018)
	Anaerobic digester	Struvite	Removal of ammonia and phosphorus of 91.95 and 99.65%, respectively, with struvite formation in the molar ratio of 1.2 (Mg^{2+}): 1.0 ($P\text{-}PO_4^{3-}$): 1.0 ($N\text{-}NH_3$).	Kwon et al. (2018)
	Batch anaerobic reactor	CAs	On average, 40% of the applied COD was converted to CAs. Yield 0.40 mg COD per mg COD applied.	Morais et al. (2020a)

Continue...

Table 3 – Continuation.

AWWs	Technology	Recovered resource	Efficiency of resource recovery process	References
BWs	Batch anaerobic reactor	H_2	Maximum H_2 yield: 149.6 mL per g COD applied.	Shi et al. (2010)
	Upflow anaerobic sludge blanket (UASB) reactor	CH_4	The highest rate of CH_4 production occurred at 29 °C. Methane production rate increased from 0.29 to 1.46 L g COD^{-1}, when the loading rate was increased from 2.0 to 8.26 g COD L^{-1} d^{-1}.	Enitan et al. (2015)
	Immobilized-cells continuously stirred tank reactors (immobilized-cells CSTR)	H_2 and energy	The maximum rate of H_2 production of 55 L L^{-1} d^{-1} was obtained in hydraulic detention time of 1.5 h. High energy efficiency with an energy production rate of 641 kJ L^{-1} d^{-1}.	Sivagurunathan et al. (2015)
	Anaerobic membrane bioreactor (AnMBR)	CH_4	Removal efficiency of 98% COD with 0.53 L of biogas per g COD removed. Biogas composition: 59% CH_4, 31% CO_2 and 10% N_2.	Chen et al. (2016)
	Upflow anaerobic sludge blanket (UASB) reactor	CH_4	78.97% COD removal; 60.21% BOD removal; CH_4 corresponded to about 60-69% of the biogas.	Enitan et al. (2018)
	Photosynthetic bacterial-membrane bioreactor (PS-MBR)	Algal biomass, bacterial biomass and derived products	COD removal above 97%. Yields of biomass, proteins, polysaccharides, bacteriochlorophyll and coenzyme Q10 were respectively 0.51, 0.21, 0.089, 0.0013, 0.0054 and 0.019 g per g COD removed.	Lu et al. (2019)
	Batch anaerobic reactor	CAs	On average, 76% of the applied COD was converted. Yield of 0.60 g acids per g COD applied.	Silva, A.S. et al. (2019)
DWs	Fed-batch anaerobic Reactors	Struvite	Recovery of 89% ammonia and 93% phosphate in various compounds, such as struvite.	Krishan and Srivastava (2015)
	Photobioreactor	Biodiesel and CAs	Maximum COD, TN, and TP removal rates obtained in indoor conditions were 88.38, 38.34, and 2.03 mg L^{-1} d^{-1}.	Lu et al. (2015)
	Two-stage anaerobic reactors	H_2 and CH_4	64% COD removal. Yield of H_2: 105 mL per g COD applied. Yield of CH_4: 190 mL per g COD applied.	Kothari et al. (2017)
	Combined system: continuously stirred tank reactor (CSTR) and compartmented anaerobic reactor	Biogas	82% COD removal. Yield of Biogas: 0.26 m^3 per kg COD removed.	Jürgensen et al. (2018)
	Anaerobic fluidized-bed reactor (AFBR)	H_2	32.2% COD removal. Maximum H_2 productivity: 0.80 L h^{-1} L^{-1}.	Silva, A.N. et al. (2019)
	Batch anaerobic reactor	CAs	On average, 83% of the COD applied was converted to CAs. Yield of 0.83 mg COD per mg COD applied.	Coelho et al. (2020b)
FPWs	Upflow anaerobic sludge blanket (UASB) reactor	CH_4	87% TOC removed. Yield of CH_4: 0.93 m^3 per kg TOC removed.	Goodwin et al. (1990)
	Upflow anaerobic sludge blanket (UASB) reactor	Biogas	Biogas yield: 0.31 - 0.52 L g^{-1} COD. 48.2% - 71.0% of CH_4 in biogas.	Borja and Banks (1994)
	Anaerobic filter	CH_4	70% COD removal. Yield of CH_4: 0.36 m^3 per kg COD removed.	Hawkes et al. (1995)
	Batch anaerobic reactor	CH_4	Yield of CH_4: 0.338 L per g COD removed. Percentage of methane in biogas: 70%.	Demirel et al. (2013)
	Batch anaerobic reactor	CH_4	Yield of CH_4: 0.283 L per g COD removed and 0.228 L per g COD applied. Biogas rich in CH_4 (83.7%).	Morais (2019)

Continue...

Table 3 – Continuation.

AWWs	Technology	Recovered resource	Efficiency of resource recovery process	References
RG	Upflow anaerobic sludge blanket (UASB) reactor	H_2	Total energy conversion efficiency of 44.8%. Maximum H_2 productivity: 6.2 mmol L^{-1} h^{-1}.	Sittijunda and Reungsang (2012)
	Batch anaerobic reactor	CAs	Yield 0.51 g COD per g COD applied. Maximum CAs concentration: 38.5 g COD L^{-1}.	Yin et al. (2016)
	Photobioreactor	Biomass and lipids	Average biomass production of 16.7 g m^{-2} d^{-1}, lipid content of 23.6%, and the removal of 2.4 g m^{-2} d^{-1} $N\text{-}NH_4^+$, 2.7 g m^{-2} d^{-1} TN, 3.0 g m^{-2} d^{-1} TP, and 103.0 g m^{-2} d^{-1} of COD.	Ren et al. (2017)
	Upflow anaerobic sludge blanket (UASB) reactor	H_2	Total energy conversion efficiency of 63.63%. Yields of 368 mmol H_2 per mol glycerol, 55 mmol H_2 per L of 1,3-PDO, and 71 mmol H_2 per L of ethanol.	Sittijunda and Reungsang (2017)
	Batch anaerobic reactor	CAs and 1,3-PDO	Production of C2, C3, C4, C5, C6 and 1,3-PDO at concentrations of 0.21, 0.50, 0.50, 2.31, 3.84 and 1.62 g L^{-1}, respectively. Conversion of 100% glycerol.	Dams et al. (2018)
	Batch anaerobic reactor	CAs	On average, 82% of the COD applied was converted to CAs. Yield 0.82 mg COD per mg COD applied.	Coelho (2019)

COD: chemical oxygen demand; BOD: biological oxygen demand; CAs: carboxylic acids; TOC: total organic carbon; TN: total nitrogen; $N\text{-}NH_4^+$: ammonium nitrogen; TP: total phosphorous; CH_4: methane; H_2: hydrogen; C2: acetic acid; C3: propionic acid; C4: butyric acid; iso-C5: isovaleric acid; C5: valeric acid; C6: caproic acid; 1,3-PDO: 1,3-propanediol.

respectively) in an anaerobic digester. The authors obtained ammonia and phosphorus removal efficiency of 91.95 and 99.65%, respectively, with the formation of struvite in the molar ratio of 1.2 (Mg^{2+}):1.0 ($P\text{-}PO_4^{3-}$):1.0 ($N\text{-}NH_3$). Krishan and Srivastava (2015) established the same objective as the aforementioned authors, but treating DWs (initial concentrations of ammonia and phosphate of 69.96 and 45.05 mg L^{-1}, respectively) in an anaerobic batch reactor and achieved recovery of 89% ammonia and 93% phosphate in the form of various compounds together with struvite.

For the efficient recovery of struvite from AWWs that have low concentrations of magnesium and phosphorus about ammonium nitrogen concentrations, it is necessary to supplement magnesium and phosphorus salts, since, theoretically, the molar ratio 1:1:1 of Mg:N:P is a requirement for its precipitation (Muhmood et al., 2019).

Among the AWWs presented in this work, the SWs would be the most promising for the recovery of struvite due to the high levels of total nitrogen (800–6,000 mg L^{-1} N) and total phosphorus (100–1,400 mg L^{-1} P) commonly found in its composition (Kumar and Pal, 2015; Ding et al., 2017), which confirms the struvite recovery potential widely reported in the literature for this type of AWW (Suzuki et al., 2007; Wang et al., 2019). However, it is expected that it will be necessary to supplement the reaction medium with magnesium, since studies show that, in general, SWs are rich in ammonium and phosphate, while their magnesium content is low (Muhmood et al., 2019).

Despite this, the recovery of phosphorus as struvite is not always possible or appropriate since the precipitation of this compound competes with the precipitation of hydroxyapatite when the Ca^{2+}/P molar ratio is greater than 1, which can be a problem in the recovery of struvite in AWWs that include calcium in their composition (Monballiu et al., 2018). Ca^{2+} ions block struvite's growth surface, thus impairing its formation (Pastor et al., 2008). Tao et al. (2016) reported that a high Ca^{2+}/Mg^{2+} molar ratio (greater than 0.5) causes a competitive environment between the formation of struvite and calcium-based phosphate products, causing adverse impacts on the formation of struvite.

Accordingly, Hakimi et al. (2020) evaluated the recovery of struvite from SHWs using an anaerobic membrane bioreactor (AnMBR). They reported that the molar ratio Mg^{2+}/Ca^{2+} of 0.8 (high concentration of calcium ions) has a significant negative impact on the production and quality of struvite. The authors also observed that when SHWs were treated with a negligible concentration of Ca^{2+} (molar ratio of $Mg^{2+}/Ca^{2+} > 20$), 80% of total phosphorus was removed via struvite precipitation. Also, higher rates of nitrogen and phosphorus removal were obtained at pH 9.5 with a 2:1 Mg^{2+}/PO_4^{3-} molar ratio. Sreyvich et al. (2020) also evaluated SHWs for nutrient recovery and concluded that this residue is promising for struvite recovery by obtaining 99.3% phosphate and 98.1% ammonium removal using a PO_4^{3-}/Mg^{2+} molar ratio of 1:3 at pH 9.0.

In addition to SWs and SHWs, DWs are also commonly investigated for struvite recovery due to their high concentrations of nitrogen and phosphorus (Rabinovich et al., 2018; Lavanya and Thanga, 2020). However, DWs have high concentrations of Ca^{2+} ions, which prompts many researchers to study techniques and operational strategies aimed at optimizing struvite recovery through the use of this AWW as a substrate (Numviyimana et al., 2020).

Thus, another possibility is the recovery of phosphorus from hydroxyapatite precipitation. Hydroxyapatite is used in the medical and dental fields as a coating material for metal implants and for filling bone cavities (Valente, 1999). Ease of handling and low cost of inputs are considered the main advantages of recovering this mineral from AWWs (De-Bashan and Bashan, 2004). However, compared to struvite, hydroxyapatite has no potential as a fertilizer for agriculture due to its low solubility and sufficient Ca^{2+}/P specific binding strength to hinder P availability in the soil (Hao et al., 2013; Shashvatt et al., 2018; Li et al., 2020).

DWs are promising for the recovery of phosphorus via hydroxyapatite precipitation since they commonly have high concentrations of phosphate, ammonium and calcium (up to 950 mg L^{-1} Ca^{2+}) (Demirel et al., 2005; Kharbanda and Prasanna, 2016). Generally, calcium salts are used during the production of dairy products, which could decrease costs by supplementing this compound in hydroxyapatite recovery (Karadag et al., 2015).

Production of algal biomass, bacterial biomass and products derived from biological treatment of agricultural and agro-industrial wastewaters

Besides prospecting for nutrients, a diversity of bioproducts can be recovered through biological treatments in aerobic processes. Among these products, algal biomass, bacterial biomass and products derived from aerobic treatment stand out (Wang et al., 2015; Lu et al., 2019). Aerobic treatment is an oxidation process by which microorganisms degrade organic matter and other pollutants in the presence of oxygen (O_2). In this type of treatment, the main objective is to achieve a high degree of substrate conversion. One of the fundamental advantages is that the oxidative degradation of carbon present in wastewater provides the necessary energy to propagate microorganisms that act as catalysts. (Ranade and Bhandari, 2014). For this reason, biological treatment in aerobic AWW processes has been studied to obtain biomass to extract cellular components that may be useful industrially, such as carbohydrates, proteins, lipids and enzymes.

Wang et al. (2015), for example, studied the treatment of SWs at different dilutions (without dilution and dilutions of 5, 10 and 20 times), using the microalga *Chlorella vulgaris* JSC-6 in a photobioreactor to obtain biomass rich in carbohydrates, which can be used as a raw material for the fermentative production of biofuels. The authors achieved about 60–70% removal of COD and 40–90% removal of N-NH_3 depending on the dilution rate adopted, with the highest removal rate obtained at 20 times dilution. Mixotrophic cultivation using wastewater diluted five times resulted in the highest biomass concentration (3.96 g L^{-1}). Besides, the carbohydrate content in microalgae produced from the SWs can reach 58% (dry weight).

Lu et al. (2019) investigated photosynthetic bacteria's use to treat BWs in a photosynthetic bacterial-membrane bioreactor (PS-MBR) on a pilot scale, aimed at the production of bacterial biomass rich in compounds of industrial interest. The authors obtained satisfactory results, reaching COD removal above 97% and yields of biomass, proteins, polysaccharides, carotenoids, bacteriochlorophyll and coenzyme Q10 of respectively 0.51, 0.21, 0.089, 0.0013, 0.0054 and 0.019 g g^{-1} COD removed. These bioproducts are added value compounds that can be used in agriculture and the cosmetic and medical industries.

Methane and hydrogen production from anaerobic treatment of agricultural and agro-industrial wastewaters

Another biological treatment option for AWWs is anaerobic treatment, which is more complex than aerobic treatment due to the different metabolic pathways that microorganisms can use to degrade organic matter present in waste. In recent years, this type of treatment's relevance has increased due to higher energy costs and the operational and managerial problems of aerobic processes, such as excessive sludge disposal (Show and Lee, 2017).

Conventionally, anaerobic digestion has been adopted for the production of bioenergy from different organic residues, mainly for CH_4 prospecting, which can be used for the generation of electricity, heat, steam, as a vehicle fuel and for injection into the natural gas network for domestic use, as well as for the production of H_2, which can be used as fuel in vehicles and for electricity generation (Silva et al., 2020a). CH_4 is the biogas purification product, that is, the removal of impurities present, such as CO_2, water, H_2S and siloxanes. The main technologies used for this purpose are pressure swing adsorption, high-pressure water washing, organic solvent washing, amine clearance, membrane separation and cryogenic separation (Khan et al., 2017). This stage is considered the most expensive in the CH_4 production and recovery process. Therefore, it is crucial to adopt the most effective and efficient technology to obtain the required degree of purity for a specific application (Muñoz et al., 2015).

When considering the viability of waste as a raw material for a biogas generation plant, it is necessary to understand how the production of CH_4 or H_2 occurs from this substrate (Alzate et al., 2012). For this, the biogas potential production (BPP) test is widely used, and as the experimental conditions of this test are not yet fully standardized, it is possible to adapt it to evaluate different substrates, inoculants and gases, mainly CH_4 and H_2 (Strömberg et al., 2014). The BPP test results mostly in stoichiometric coefficients of biogas production and cell growth, which can be used to estimate the gas of interest and manage the sludge produced in anaerobic treatment units (Sun et al., 2015).

The technical literature presents research on the BPP for each of the AWWs analyzed in this work, showing that these substrates have potential for prospecting this bioproduct (Table 3). As an example, Yang et al. (2016) studied the production of CH_4 in an anaerobic sequential batch reactor (ASBR) using SWs as substrate. They reported that the highest volumetric methane production rate (1.95 L L^{-1} d^{-1} CH_4) was obtained when the system was operated at 35 °C with an organic load of 7.2 g L^{-1} d^{-1} VS. Enitan et al. (2018) reported that 80% of the organic matter used from the BWs was converted to CH_4 in an upflow anaerobic sludge blanket — UASB reactor.

Martí-Herrero et al. (2018) investigated the production of biogas treating SHWs in tubular anaerobic digesters, aiming to identify the best operational conditions for its production. The organic loading rate (OLR) varied from 0.04 to 1.13 kg m^{-3} d^{-1} VS and the hydraulic detention time (HDT) from 3.2 to 87.4 days. The authors observed a peak of biogas production of 0.2 m^3 m^{-3} d^{-1} for OLR of 0.37 kg m^{-3} d^{-1} SV with 9.7 days HDT. COD removal above 70% was achieved with HDT greater than 19 days.

Regarding the production of H_2, Silva, A.N. et al. (2019) evaluated the effect of OLR on H_2 production from the use of DWs as a substrate in an anaerobic fluidized-bed reactor (AFBR). Different OLRs of 28.7, 53.2 and 101.7 kg m^{-3} d^{-1} COD and different HDT corresponding to 8, 6 and 4 h, respectively, were applied. The researchers concluded that the increase in OLR negatively affects the production of H_2 since the gas yield fell from 2.56 ± 0.62 to 0.95 ± 0.28 mol H_2 per mol of carbohydrate as OLR increased from 28.65 to 95.76 kg m^{-3} d^{-1} COD. The content of H_2 in biogas and the highest volumetric production of H_2 were respectively 35.72 ± 9.43% and 0.80 ± 0.21 L h^{-1} L^{-1} H_2 when the OLR was 53.25 ± 7.81 kg m^{-3} d^{-1} COD. Sittijunda and Reungsang (2017) evaluated the production of H_2 from RG and found a maximum productivity rate of 3.2 L L^{-1} d^{-1} H_2, but the COD removal efficiency was not presented.

Despite extensive research on the anaerobic digestion of AWWs and on the prospecting and purification of biogas, the use of CH_4 in Brazil from landfills and sewage treatment plants (STPs) was only regulated in 2017 by the National Petroleum Agency, Natural Gas and Biofuels (Agência Nacional do Petróleo, Gás Natural e Biocombustíveis — ANP) with ANP Resolution No. 685/2017, which promoted an increase in the share of this resource in the Brazilian energy matrix (ANP, 2017). According to the International Center for Renewable Energies-Biogas (Centro Internacional de Energias Renováveis-Biogás — CIBiogás) between 2010 and 2018, the share of biogas in the Brazilian energy matrix increased from 0.01%, (14 thousand toes, a ton of oil equivalent) to 0.07% (CIBiogás, 2020). In 2018, the primary sources of biogas production were landfills (72%), the food/beverage industries (14%), swine farming (7%) and sewage sludge (5%) (EPE, 2019). Thus, the importance of STPs and agribusiness for consolidating a market for biogas and CH_4 is perceived. Nonetheless, Brazil still has an underutilized potential, having only 275 biogas production plants in operation, mostly located in Brazil's South and Southeast regions (CIBiogás, 2020).

Production of carboxylic acids from anaerobic treatment of agricultural and agro-industrial wastewaters

There has been a trend in the search for by-products from the acidogenic fermentation stage of anaerobic digestion, such as short-chain carboxylic acids (SCCAs) (acetic acid – C2, propionic – C3, butyric – C4, and valeric – C5). These compounds are building block chemicals widely applied in the industry in producing varnishes, paints, perfumes, disinfectants, surfactants, textile auxiliaries, medicines and food products (Lee et al., 2014). The organic production of carboxylic acids (CAs) has been advocated as an effective way to generate sustainable fuels and chemicals from biomass and organic waste (Silva et al., 2020b). Conventionally, CAs are produced by petrochemical routes, and for this reason, their production by biological means from substrates rich in organic matter, as is the case of AWWs, is interesting both from an environmental and economic point of view (Sittijunda and Reungsang, 2017).

The biological production of CAs occurs during the hydrolytic-acidogenic process of organic matter, requiring the inhibition of the methanogenesis and sulfate reduction steps to block the conversion of CAs to CH_4 and H_2S (Kleerebezem et al., 2015). The main strategies for inhibiting the activity of methanogenic archaea and sulfate-reducing bacteria are the addition of chloroform, which acts by inhibiting the coenzyme M reductase necessary for the metabolism of archaea (Viana et al., 2019), the addition of 2-bromoethanesulfonic acid (2-BES), which acts in the inhibition of the acetyl-CoA route, and the combined addition of chloroform and molybdate, which works directly in the enzymatic inactivation. However, the use of chemical agents in this inhibition increases the production of CAs, which encourages the development of studies related to other inhibition strategies, such as pH reduction and nutritional restriction (Siriwongrungson et al., 2007; Ge et al., 2015).

SCCAs are the main products of acidogenic fermentation of organic residues, while medium-chain carboxylic acids (MCCAs), which have six to twelve carbon atoms, are formed in lower concentrations (Steinbusch et al., 2011). MCCCs are formed during the carboxylic chain elongation process (CCEP), in which an SCCA such as C2 reacts with reduced organic material, usually ethanol or other alcohol, forming an MCCA such as caproic acid (C6) and caprylic acid (C8) (Cavalcante et al., 2017).

MCCAs are more economically attractive than SCCAs and can be used as food additives, antimicrobial agents, precursors for biodiesel production, and bioplastics production (Strazzera et al., 2018). In addition to greater industrial applicability, MCCAs are easier to extract from the reaction medium compared to SCCAs because of their greater hydrophobicity, reducing costs with downstream processing of these bioproducts (Grootscholten et al., 2013). It is estimated that the aggregate value of MCCAs is 2,000-2,500 USD ton^{-1}, thus being higher than the market price of SCCAs (400–2,500 USD ton^{-1}), CH_4 (200–600 USD ton^{-1}) and H_2 (800–1,600 USD ton^{-1}) (Bastidas-Oyanedel et al., 2015; Moscoviz et al., 2018; Silva et al., 2020a).

In this sense, studies indicate that the recovery of CAs from the acidogenic fermentation of agricultural and agro-industrial waste is possibly more advantageous in economic terms than CH_4 and H_2 (Bastidas-Oyanedel and Schmidt, 2018). Silva et al. (2020a) reported that the gross added value of CAs is higher than that of CH_4 and H_2. However, it is necessary to carry out further economic studies, mainly involving the analysis of downstream processing costs (e.g., costs involved in extracting CAs from the fermentation medium).

Morais et al. (2020a) and Coelho et al. (2020b) assessed the CA production potential of SWs and DWs, respectively. The experiments were carried out in four batch reactors made of borosilicate glass with a reaction volume of 250 and 50 mL of headspace, inoculated with the brewery sludge at a substrate/inoculum (S/I) of 0.60 ± 0.04 g COD per g SSV. To inhibit methanogenic activity, the authors added chloroform 0.05% (v/v) to the basal medium. Morais et al. (2020a) reported that 40% of the applied organic matter from SWs was bioconverted to CAs, with a yield of 0.33 g acids per g COD. Coelho et al. (2020b) observed that DWs have a high potential for acidification under acidogenic conditions and obtained the conversion of 83% of the COD applied to CAs, representing a yield of 0.66 g acids per g COD. The leading CAs formed were C2 and C4; however, the authors pointed out that SWs and DWs have the potential for the production of MCCAs because even without the addition of electron donors or the application of other methods to enhance CCEP, C6 was formed in low concentrations.

In further research, Morais et al. (2021) and Coelho et al. (2020a) also evaluated the prospecting of CAs from SHWs and RG, respectively, using the same operational conditions as their previous studies with SWs and DWs. For SHWs, the authors reported the conversion of 76% of COD to CAs (yield of 0.55 g acids per g COD), while for RG a yield of 0.62 g acids per g COD was obtained, corresponding to 82% conversion of applied organic matter.

On the basis of these studies, the authors concluded that more soluble AWWs generally have a greater potential for CA production due to the reduction of the hydrolysis step and the fact that organic matter is readily available for acidogenic microorganisms, which promotes rapid use and bioconversion of the substrate into CAs. In this sense, SHWs, DWs, BWs and RG because of their high levels of soluble organic matter are more promising substrates to be investigated for the production of CAs than substrates that have a higher concentration of organic matter in the particulate fraction, such as SWs. In this perspective, showing the high potential of soluble substrates for the organic production of CAs, Yin et al. (2016) investigated the acidogenic fermentation of glucose, peptone and RG in batch reactors inoculated with mixed biomass from a brewery wastewater treatment. They reported average yields of 0.66, 0.60 and 0.51 g COD per g COD for these substrates, respectively.

To increase the production of MCCAs in acidogenic processes and to enhance, several strategies have been investigated, such as the establishment of optimized operational parameters (pH, temperature, volumetric organic load, hydraulic detention time) and the external addition of donors of electrons to the fermentation process (Steinbusch et al., 2011). Approximately 80% of studies aimed at producing C6 use ethanol as an electron donor to favor CCEP. However, this external addition can cause environmental impacts and increase the costs of the fermentation process. Therefore, one of the focuses of current research is to optimize ethanol's dose required by CCEP (Moscoviz et al., 2018).

Another technique to favor CCEP is bio-augmentation (addition of microorganisms specialized in chain extension to mixed microbial cultures). According to Hung et al. (2011), bio-augmentation can improve the rate of degradation of complex compounds by combining microorganisms' metabolic pathways and assisting in producing precursors (C2 and C4) for CCEP. Dams et al. (2018) conducted a C6 production experiment in batch reactors by the RG's acidogenic fermentation using bio-increased brewing sludge with *Clostridium acetobutylicum* ATCC 824 as an inoculum and adding 100 mM ethanol to stimulate CCEP. The authors reported that the C6 concentration increased from 1.61 to 3.82 g L^{-1}.

Another resource studied to increase the production of MCCAs in anaerobic reactors is the extraction concomitant with its output. This process reduces problems of inhibition of microbial activity due to the toxicity of the CAs formed. The production of CAs in anaerobic reactors requires extraction techniques specific to the type of acid produced. Usually, a selective extraction module for only one MCCA is the preferable choice to relieve toxicity to microorganisms of this product and recirculate to the reactor the remaining SCCAs not yet elongated to be reused in the process (Cavalcante et al., 2017).

Hybrid membrane modules with extracting liquids have been among the leading technologies for prospecting CAs (Moraes et al., 2015). In this process, the more hydrophobic CA in greater concentration has removal priority (Ge et al., 2015). Although there are several techniques capable of extracting CAs from anaerobic fermentation processes, including ion exchange resin, electrodialysis and liquid-liquid extraction membrane, the recovery of these bioproducts from the reaction (fermented) liquid is still one of the main barriers of the biological process production of CAs, requiring further studies on the optimization of operational parameters (López-Garzón and Straathof, 2014).

Conclusion

On the basis of the technical literature review carried out on the composition and physical-chemical characterization of agricultural and agro-industrial wastewaters (AWWs), it was possible to conclude that swine wastewaters (SWs), slaughterhouse wastewaters (SHWs) and wastewaters from milk processing (DWs) are the most promising for the recovery of struvite since they generally have high levels of phosphorus and nitrogen. DWs also stand out for the recovery of hydroxyapatite due to high concentrations of phosphorus and calcium. SWs and wastewaters from beer production (BWs) are commonly used in research to recover biomass rich in carbohydrates, proteins and polysaccharides. All the AWWs analyzed are suitable for prospecting for biogas, methane and hydrogen. The more soluble AWWs (e.g., SHWs, DWs, BWs and residual glycerol — RG) are more promising for research in producing carboxylic acids compared to more particulate substrates, such as SWs. The scarcity of studies with fruit processing wastewaters (FPWs) from ice cream production is noteworthy. Thus, due to the high output of AWWs, Brazil is a promising country for the implementation of treatment plants that aim to recover resources from these liquid wastes.

Contribution of authors:

Morais, N.W.S.: Conceptualization, methodology, validation, formal analysis, investigation, data curation, writing - original draft, and writing — review and editing. Coelho, M.M.H.: Conceptualization, methodology, validation, formal analysis, investigation, data curation, writing — original draft, and writing — review and editing. Sousa e Silva, A.: Methodology and writing — review and editing. Pereira, E.L.: Conceptualization, methodology, supervision, validation and acquisition of funding.

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Isotopic variations of carbon and nitrogen and their implications on the conversion of Cerrado vegetation into pasture

Variações isotópicas de carbono e nitrogênio e suas implicações na conversão da vegetação do cerrado em pastagens

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ABSTRACT

Conversions of natural vegetation into pasture can, in a short time, change the carbon stock and the natural abundance of $\delta^{13}C$ in the soil. The objective of this study was to evaluate changes in carbon (C) and nitrogen (N) stocks, as well as in the natural abundance of $\delta^{13}C$ and $\delta^{15}N$ of Argissolo Vermelho distrófico (Acrisol), in an area of natural vegetation and planted pasture in the Cerrado region of Aquidauana (MS), Brazil. In order to do this, an area of pasture (PA), cultivated for 25 years with *Urochloa brizantha*, and an area of natural vegetation (NV) were evaluated. Soil samples were collected at intervals of 0.05 m up to 0.60 m depth, and physical attributes, C and N stocks (CSt and NSt) and isotopic variations of $\delta^{13}C$ and $\delta^{15}N$ of soil were determined. In the 0–0.05 m layer, the highest C and N stocks occurred in NV, 21.99 and 1.9 Mg ha^{-1}, respectively. In the conversion to PA, 14.62 Mg ha^{-1} of CSt and 1.36 Mg ha^{-1} of NSt were lost in the 0–0.05 m layer. The area with PA had greater isotopic enrichment of $\delta^{13}C$ in the layers of 0–0.05 and 0.05–0.10 m, with values of -18.3 and -17.4‰, respectively, while in the other layers the isotopic values decreased with the mixture between C of C_3 and C_4 plants. NV showed enrichment in the isotopic signals, in the layers from 0.25–0.30 m up to 0.40–0.45 m, with values between -21.74 and -21.54‰, respectively, which is characteristic of mixed vegetation of C_3 and C_4 plants. The values of δ^{15} N showed isotopic enrichment as depth increased, indicating greater mineralization of soil organic matter in both areas. The conversion of Cerrado into pasture and its consequent fragmentation causes negative impacts on the C and N sequestration and storage capacity, both in pasture and in natural vegetation.

Keywords: acrisol; isotopic composition; vegetation conversion; organic matter.

RESUMO

As conversões de vegetação natural em pastagem podem, em um curto intervalo de tempo, alterar o estoque de carbono e a abundância natural de $\delta^{13}C$ no solo. O objetivo deste trabalho foi avaliar alterações nos estoques de carbono (C) e nitrogênio (N) e na abundância natural de $\delta^{13}C$ e $\delta^{15}N$ no Argissolo Vermelho distrófico (Acrisolo) em uma área de vegetação natural e pastagem plantada no Cerrado em Aquidauana (MS). Para isso, foram avaliadas uma área de pastagem (PA), cultivada durante 25 anos com *Urochloa brizantha*, e uma área de vegetação natural (VN). Foram coletadas amostras de solo em intervalos de profundidade de 0,05 m até 0,60 m, e a partir delas foram determinados os atributos físicos, os estoques de C e N (EstC e EstN) e as variações isotópicas de $\delta^{13}C$ e $\delta^{15}N$ do solo. Na camada de 0–0,05 m, os maiores estoques de C e N ocorreram na VN; 21,99 e 1,9 Mg ha^{-1}, respectivamente. Na conversão para PA, 14,62 Mg ha^{-1} do EstC e 1,36 Mg ha^{-1} do EstN foram perdidos na camada de 0–0,05 m. A área com PA apresentou maior enriquecimento isotópico do $\delta^{13}C$ nas camadas de 0–0,05 e 0,05–0,10 m, cujos valores foram de -18,3 e -17,4‰, respectivamente, enquanto, nas demais camadas, os valores isotópicos diminuíram com a mistura entre C de plantas C_3 e C_4. A VN apresentou enriquecimento nos sinais isotópicos nas camadas 0,25–0,30 até 0,40–0,45 m, com valores entre -21,74 e -21,54‰, respectivamente, o que é característico de vegetação mista de plantas C_3 e C_4. Os valores de $\delta^{15}N$ apresentaram enriquecimento isotópico de acordo com o aumento da profundidade, indicando maior mineralização da matéria orgânica do solo em ambas as áreas. A conversão do Cerrado em pastagem e, consequentemente, sua fragmentação, provoca impactos negativos na capacidade de sequestro e armazenamento do C e N, tanto na pastagem quanto na vegetação natural.

Palavras-chave: argissolo vermelho; composição isotópica; conversões da vegetação; matéria orgânica.

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Introduction

Brazilian Cerrado is an ecological hotspot with great diversity of species that are endemic, but vulnerable to anthropic modifications, which cause several environmental impacts and the disorderly conversion of original vegetation into land used by agriculture and livestock, one of the main factors of degradation of this ecosystem (Rocha et al., 2011; Silva and Bacani, 2017; Ozório et al., 2019). According to deforestation data gathered up to the year 2013 by TerraClass Cerrado — a project implemented by Instituto Nacional de Pesquisas Espaciais (Inpe) and Empresa Brasileira de Pesquisa Agropecuária (Embrapa) —, the remaining natural vegetation represents 54.5% of the total Cerrado area. Regarding the classes of anthropic use, planted pasture was the one with the highest predominance (29.5%), which leads to the conclusion that this activity has great impact in Cerrado (Brasil, 2015).

Recent estimates predict an increase in deforestation in the Cerrado biome at an average annual rate of 0.34% to 0.5% (772 ha year^{-1} on average), particularly affecting forests and savannas (Sano et al., 2019; Alencar et al., 2020). Considering that Cerrado is a vegetation complex consisting of forest vegetation (riparian forest, gallery forest, dry forest and Cerradão), grassland vegetation (dirty grassland, clean grassland and rupestrian grassland) and savanna vegetation (Cerrado *sensu stricto*, Cerrado park, palm grove and vereda), the environmental impact of its deforestation will be even greater (Ribeiro and Walter 2008). Specifically, the state of Mato Grosso do Sul has lost an average 17% of its native vegetation in the last 33 years, and the greatest losses occurred between the years 1985 and 1995, a period of expansion of livestock farming and agriculture in the state (Alencar et al., 2020).

The conversion of native vegetation for anthropic use, if poorly managed, can have negative effects on the carbon (C) and nitrogen (N) cycle. In Cerrado, the imbalance in C and N stocks is largely due to the replacement of the original vegetation with pasture, the main anthropic class of land use in the biome, which leads to changes in the physical and chemical attributes of soil organic matter (SOM), both in degree of oxidation and lability, and may result in the simultaneous release of large amounts of C and N accumulated in the vegetation, increasing the release of greenhouse gases (Carvalho et al., 2010; Dortzbach et al., 2015).

Changes in land use forms also alter the nature of the soil's C and N sources. C_3 plants (typical in tree vegetation) and C_4 plants (typical in Poaceae species) leave different isotopic values of C and N in organic matter, an indicator of the type of existing vegetation and the modifications to which an area has been subjected, which can be determined using isotopic techniques based on the natural abundance of ^{13}C ($\delta\delta^{13}C$) and ^{15}N ($\delta^{15}N$). These isotopic techniques have been widely used in studies on landscape transformations, since they presuppose that organic matter reflects the plant material from which it was derived, constituting an efficient method to identify anthropic effects on the structure of ecosystems (Costa et al., 2009; Loss et al., 2014).

The objective of this work was to evaluate changes in C and N stocks, as well as in the natural abundance of $\delta^{13}C$ and $\delta^{15}N$ in Argissolo Vermelho distrófico (Acrisol), in areas of natural vegetation and planted pasture (*Urochloa brizantha*) of the Cerrado region of Aquidauana (MS), Brazil.

Materials and Methods

The study was carried out at Universidade Estadual do Mato Grosso do Sul, Aquidauana unit, located in the municipality of Aquidauana, Mato Grosso do Sul, at 20°27'20" S latitude and 55°40'17" W longitude, with an altitude of approximately 180 m (Figure 1). The predominant soil class in the region is Argissolo Vermelho distrófico (Acrisol), with sandy loam textural class (Schiavo et al., 2010; Santos et al., 2018). According to Köppen's classification, the climate of the region is Aw, defined as sub-humid hot tropical, with an average annual rainfall of 1,200 mm, rainy season in summer and dry season in winter.

For the study, two areas were selected under two conditions of land use. The first was an area of planted pasture (PA) for cattle grazing in the extensive system, implemented in 1973 and reformed in 2005, by removing *Urochloa brizantha* and planting *Panicum maximum* (Guinea grass), with no fertilizer application. In 2011, the pasture was reformed for the second time, removing *Panicum maximum* and replanting *Urochloa brizantha* cv. BRS Piatã, which remained in the sampled picket in 2015. The second, used as reference, was an area of natural Cerrado vegetation, with gallery forest, on the Fundo stream (NV) (Figure 2).

In each studied area, a representative plot of 10,000 m^2 was demarcated, and one soil pit with dimensions of approximately 1 × 1 m surface and 0.6 m depth was opened in a random position. In each of the soil pits, undisturbed samples were collected using a volumetric ring, taking one sample every 5 cm deep. Leaf samples were also collected from the main plant species of the NV area — *Anadenanthera colubrina*, *Anadenanthera peregrina*, *Bauhinia forficata*, *Cecropia sp.*, *Xylopia sp.* and *Tabebuia sp.* — and from *Urochloa brizantha* in the PA area. After collection, the soil samples were air dried, pounded to break up clods and passed through a 2-mm-mesh sieve, in order to obtain air-dried fine earth (ADFE), which was subjected to the physical analyses (Teixeira et al., 2017).

Bulk density (BD) was determined using the volumetric ring method (Teixeira et al., 2017). Particle density (PD) was determined by the volumetric flask method, and this data, together with BD data, was used to calculate the percentage of total porosity (TP).

C and N contents were determined by dry combustion in a CHNS analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Data of C and N contents and BD were then used to calculate carbon

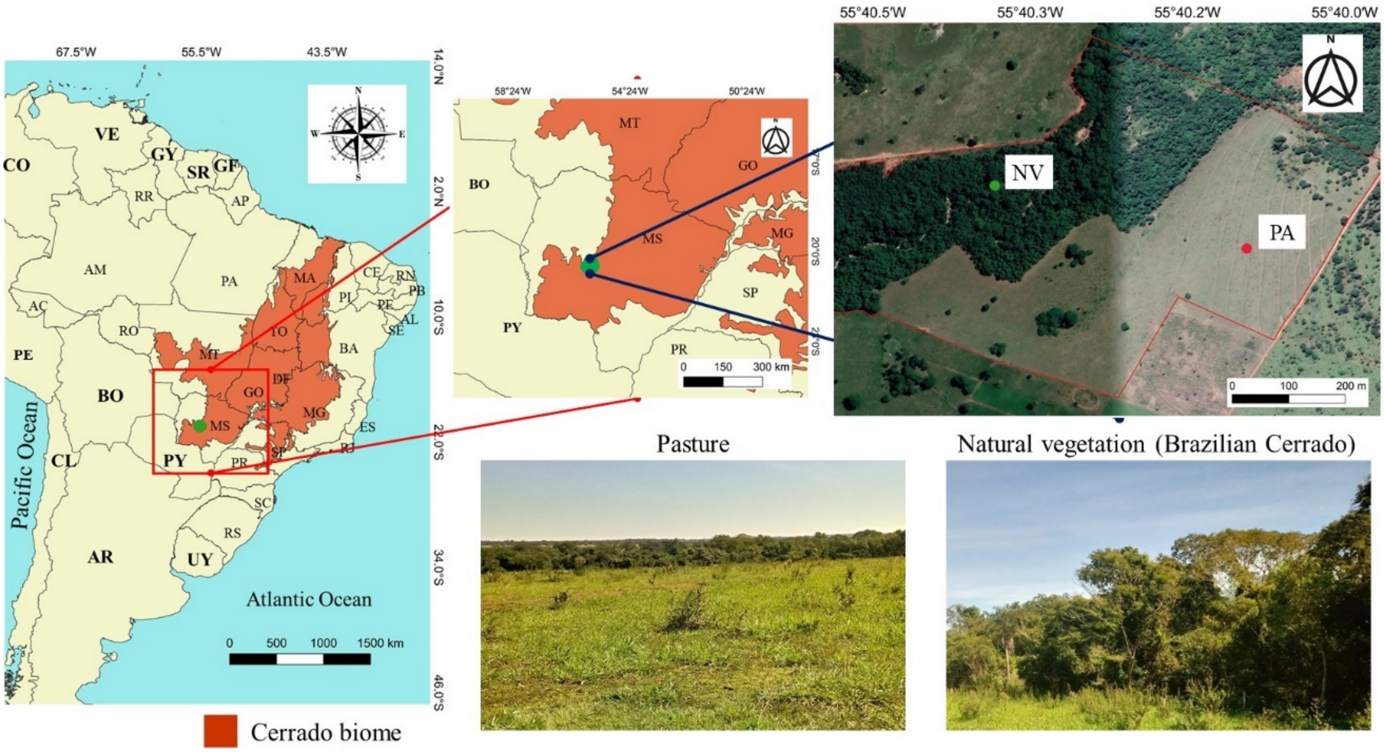

Figure 1 – Location of collection points in the natural vegetation (NV) of Cerrado and in the pasture area (PA), Mato Grosso do Sul, Brazil.

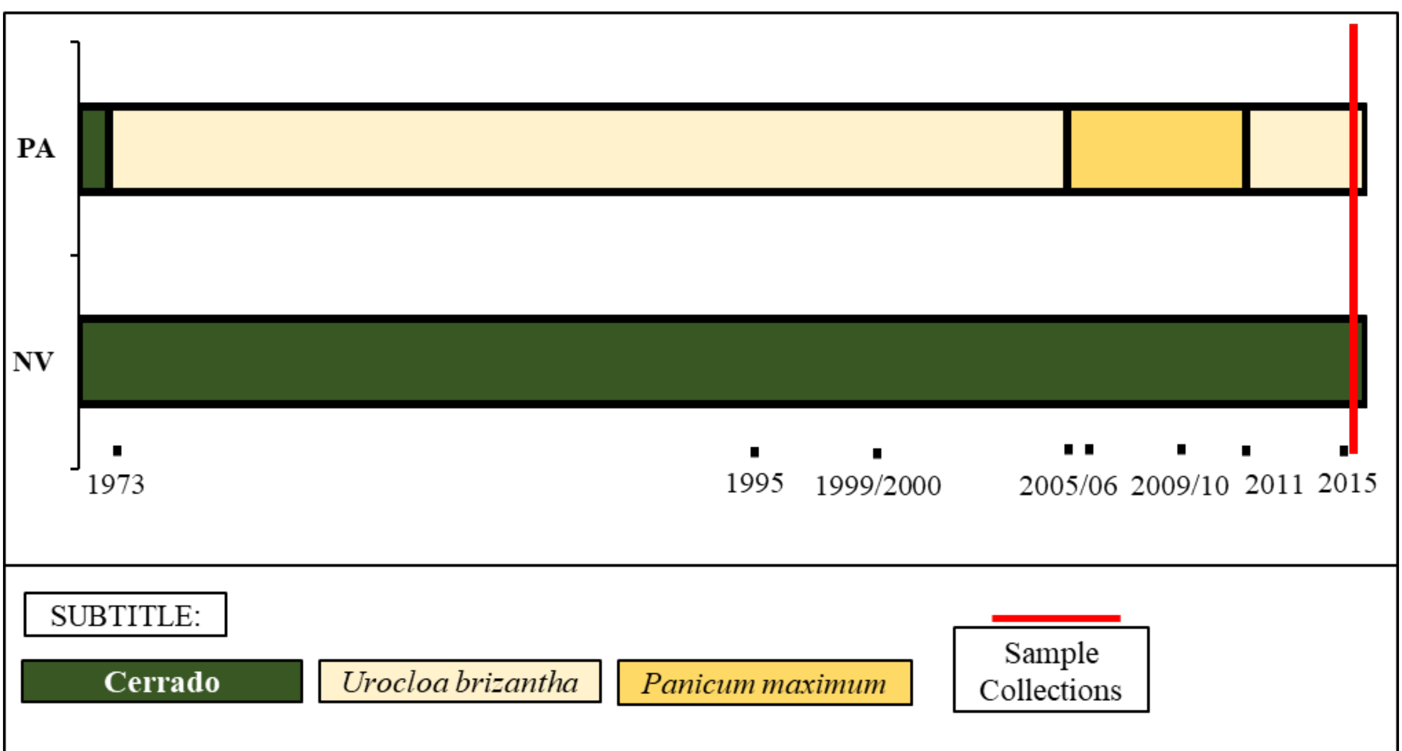

Figure 2 – History of land use change processes, with the respective dates for implementing of the areas: planted pasture (PA) and natural vegetation (NV), in the Cerrado biome in Mato Grosso do Sul, Brazil.

(CSt) and nitrogen (NSt) stocks through the mathematical expression proposed by Veldkamp (1994) (Equation 1).

$$St = \frac{(E \ x \ BD \ x \ e)}{10} \quad (1)$$

Where:

St = the stock of C or N in a given layer (Mg ha^{-1});

E = the total content of organic C or N in the sampled layer (g kg^{-1});

BD = the bulk density of the layer (Mg m^{-3});

e = the thickness of the layer considered (m).

The natural abundance of ^{13}C and ^{15}N was determined with the Finnigan Delta Plus mass spectrometer at the Isotopic Ecology Laboratory of CENA–USP, in Piracicaba–SP. The results of ^{13}C were expressed in the form of delta δ^{13}C (‰), in relation to the international standard PDB (*Belemnitella americana* from the Pee Dee Formation). The results of ^{15}N were expressed in the form of delta δ^{15}N (‰), in relation to the δ^{15}N of air (0.3663%).

Isotopic dilution was calculated according to Equation 2, with the objective of identifying the percentage of carbon from C_4 plants (%C_4):

$$\%C_4 = \frac{\delta^{13}C_{Amostra} - \delta^{13}C_{C3}}{\delta^{13}C_{C4} - \delta^{13}C_{C3}} \ x \ 100 \quad (2)$$

Where:

$\delta^{13}C_{C4}$ = the value of δ^{13}C of the pasture C_4 plant, *Urochloa brizantha* (-13.33‰);

$\delta^{13}C_{C3}$ = δ^{13}C of C_3 plants, averages of species of natural vegetation (-31.77‰).

The percentages of remaining carbon from native vegetation (C_f) were obtained through Equations 3 and 4:

$$C_p = \frac{\delta^{13}C_{PA} - \delta^{13}C_{NV}}{\delta^{13}C_{C4} - \delta^{13}C_{NV}} \ x \ 100 \quad (3)$$

$$C_f = 100 - C_p \quad (4)$$

Where:

$\delta^{13}C_{PA}$ = δ^{13}C of the soil sample analyzed;

$\delta^{13}C_{C4}$ = the value of δ^{13}C of the pasture C_4 plant, *Urochloa brizantha* (-13.33‰);

$\delta^{13}C_{NV}$ = the value of δ^{13}C of the soil underforest (Balbinot, 2009).

Pearson's correlation and multiple linear regression analyses were performed. Statistical analyses were carried out using Microsoft Excel.

Results and Discussion

Physical attributes

Bulk density (BD) showed an increase in subsurface trend, with higher values in the PA area, ranging from 1.72 to 1.83 Mg m^{-3} in the layers from 0–0.05 to 0.45–0.50 m, respectively, whereas, in the NV area, values ranged from 1.45 to 1.72 Mg m^{-3} in the layers of 0.05–0.10 and 0.50–0.55 m, respectively (Figure 3). The total porosity of the soil (TP) was higher in the NV area, ranging from 41.4 to 30% in the layers of 0.05–0.10 and 0.40–0.45 m, respectively, when compared to the values of PA, which ranged from 30.4 to 28.4% in the layers of 0.05–0.10 and 0.10–0.15 m, respectively. The higher BD values and lower TP values in the PA area when compared to NV, both in surface and in subsurface, are probably attributed to the intense trampling of animals, often exceeding the adequate stocking rate, triggering the process of soil compaction (Ozório et al., 2019).

The increase of BD in subsurface is directly related to the reduction of organic matter contents, lower aggregation, lower root penetration, reduction of soil fauna activity, greater compaction caused by the weight of overlying layers, reduction of total porosity due to clay eluviation, among other processes (Reichert et al., 2007; Silva et al., 2011). These relationships are confirmed by the correlation analysis, where the BD values of the NV area showed negative correlations with TP (r = -0.95), CSt (r = -0.66) and NSt (r = -0.70), and TP showed a positive correlation with CSt (r = 0.76) and NSt (r = 0.80) (Table 1).

The values of BD in PA only showed negative correlation with porosity (r = -0.81). The nonsignificant correlation with the attributes CSt and NSt can be explained by the intensification of compaction, resulting from the irregular management of the extensive livestock system, hampering the development of the pasture root system in subsurface, thus drastically reducing the contents of organic matter in subsurface (Ferreira et al., 2010).

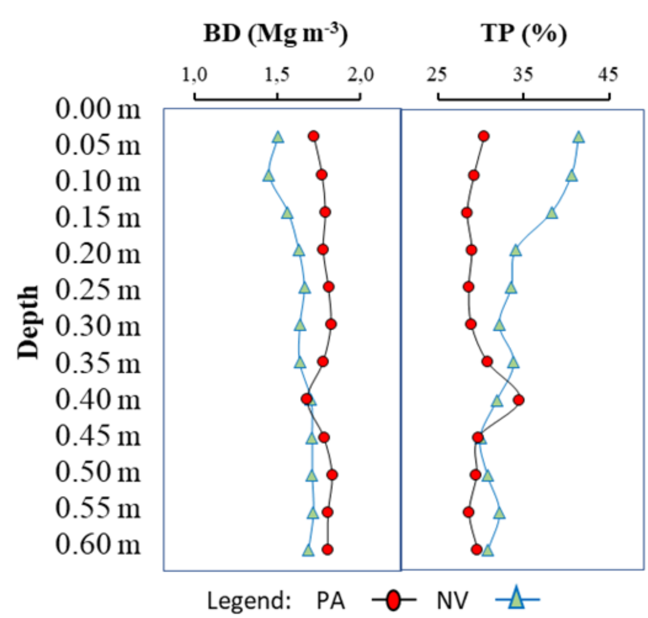

Figure 3 – Bulk density (BD) and total porosity (TP) of the soil in areas with planted pasture (PA) and natural vegetation (NV), in the Cerrado biome in Mato Grosso do Sul, Brazil.

Soil carbon and nitrogen stocks

CSt and NSt in NV in the 0–0.05 m layer were 21.99 and 1.9 Mg ha^{-1}, respectively, higher than those of the PA area in the same layer, which were 7.37 and 0.54 Mg ha^{-1}, respectively. These differences between the stocks of NV and PA in this layer are equivalent to 14.62 Mg ha^{-1} of C and 1.36 Mg ha^{-1} of N. No differences between the evaluated areas were observed in the other layers, and there was only a reduction in both contents in subsurface (Figure 4). The higher stocks of these elements in the NV area (0–0.05 m) can be attributed to the greater supply of litter causing greater entry of C in the surface layers (Rosset et al., 2016). The density of tree species present in the NV area promotes higher quality of residues in the soil, which contributes to the results of stocks in the surface layer, mainly for NSt (Carvalho et al., 2017).

Many studies in the literature have shown that soils under well-managed pastures with good fertility conditions have C contents equal to or higher than those found in forest environments, due to the greater supply of organic matter provided by the roots, which explains similar C and N contents between the areas in subsurface (Carvalho et al., 2010; Rosset et al., 2016; Assunção et al., 2019; Falcão et al., 2020). However, the PA area is still under intensive grazing system, a common practice in the sandy soils of Cerrado, which results in the restriction of root system distribution and reduction in the accumulation of residues, consequently restricting the increment of C in subsurface, as observed in the PA area (Carvalho et al., 2010; Macedo et al., 2013).

The PA area had a higher C/N ratio than the NV area in the layers from 0.05–0.10 m to 0.40–0.45 m, with values from 14.10 to 14.63, respectively, and a maximum of 17.13 in the 0.35–0.40 m layer. Because it has a higher content of lignin, a carbon-rich organic polymer, the pasture has organic matter with high C/N ratio and difficult degradation, which contributes to the C values in subsurface being similar to or even higher than that of NV (Costa et al., 2009; Braz et al., 2013). However, the higher C contents in the layers from 0.45–0.50 m up to 0.55–0.60 m of the NV area may be associated with the presence of coal, due to the history of regular fires in the Cerrado biome and the preservation of SOM in structures, or complexed with oxides and clay minerals, resulting in increased C/N ratio at these depths (Costa et al., 2009; Sant-Anna et al., 2017).

Natural abundance of ^{13}C and ^{15}N

The values of $\delta^{13}C$ (‰) reflect the current vegetation in each area (Figure 5). The NV area had the lowest values of $\delta^{13}C$ (‰), with variation from -26.9‰ (0–0.05 m) to -21.5‰ (0.30–0.35 m). The isotopic values of the first layers of NV discriminate the predominance of C_3 plants, resulting in the intensity of ^{13}C, which is described in the literature with values of $\delta^{13}C$ between -33‰ and -22‰ (Tarré et al., 2001; Carvalho et al., 2017), confirmed by the percentages of %C_4 below 50% in the 0–0.05 m and 0.05–0.10 m layers.

From the layers of 0.25–0.30 m extending up to 0.40–0.45 m, there was isotopic enrichment, with a difference greater than 4‰ from the surface and %C_4 greater than 50%. Isotopic variations greater than 4‰ are associated with changes in plant communities (Saia et al., 2008). This result indicates that, in some past period, the existing vegetation was mixed and more open than the current one, or that it underwent anthropic interferences, such as wood extraction and introduction of exotic species of grass and cattle, resulting in the mixture of C_3 plants (arboreal vegetation) and C_4 plants (pasture) (Assad et al., 2013; Sant-Anna et al., 2017).

In the deeper layers, below 0.45–0.50 and 0.55–0.60 m, the enrichment remained below 4‰, with values of $\delta^{13}C$ (‰) below -22‰ and %C_4 less than 50%. This enrichment in subsurface is due to the processes of decomposition and humification of organic matter, where ^{12}C is released in greater amount, which leads to an increase in the concentration of the enriched forms in ^{13}C compared to the recently incorporated organic matter (Boutton et al., 1998; Dortzbach et al., 2015).

Table 1 – Pearson's correlation between the variables bulk density (BD), total porosity (TP) and carbon and nitrogen stocks (CSt and NSt) of the soil in areas in the Cerrado biome, Mato Grosso do Sul, Brazil.

Natural vegetation (NV)				
	BD	TP	CSt	NSt
BD	1			
TP	-0.95**	1		
CSt	-0.66*	0.76**	1	
NSt	-0.70**	0.80**	0.99**	1
Pasture (PA)				
	BD	TP	CSt	NSt
BD	1			
TP	-0.81**	1		
CSt	-0.30	-0.20	1	
NSt	-0.26	-0.26	0.96**	1

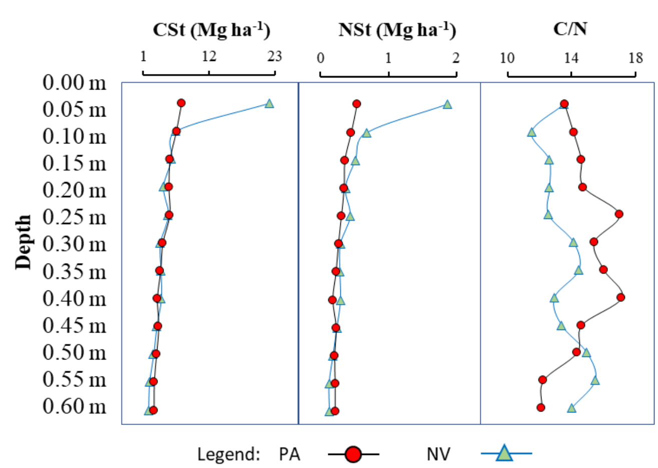

Figure 4 – Carbon stocks (CSt), nitrogen (NSt) and carbon/nitrogen ratio (C/N) of the soil in areas with planted pasture (PA) and natural vegetation (NV), in the Cerrado biome, Mato Grosso do Sul, Brazil.

The PA area had the highest values of natural abundance of $\delta^{13}C$ (‰) in the 0–0.05 and 0.05–0.10 m layers, with isotopic values from -17.4‰ to -18.3‰, respectively, and $\%C_4$ higher than 70% at these depths, from the organic matter of C_4 plants. These results indicate that there was considerable deposition of C_4 plants derived from grass residues up to a depth of 0.10 m (Sant-Anna et al., 2017). In the other layers, the isotopic values decrease between -19.0‰ and -21.8‰, typical of vegetation mixed between C_3 and C_4 plants. Thus, it is possible to observe the evolution of a C_3 photosynthetic cycle vegetation to C_4, but it still indicates that the organic matter in subsurface has remnants of the characteristics of transition from native vegetation to native pasture and of natural vegetation (Carvalho et al., 2010; Strey et al., 2016; Menezes et al., 2017).

The percentage of remaining C from native vegetation in the PA area (%Cf) is below 40% in the first two layers, but these values increase dramatically in subsurface, exceeding 70% in most layers and reachg 91.7% in the 0.30–0.35 m layer. Some studies report similar results, with an average of 70% of soil organic carbon derived from the original forest in soils conducted from low-productivity pastures (Costa et al., 2009; Dortzbach et al., 2015).

The values of %Cf in subsurface suggest the preservation of the remaining organic matter from NV in these layers, corroborating the results of the C/N ratio at these same depths. The poorly formed pasture with high animal stocking rate in Argissolo with sandy texture resulted in a low carbon increment to the soil, associated with the rapid cycling of this material by microorganisms (Dortzbach et al., 2015). Argissolos or Acrisols (IUSS Working Group WRB, 2015) tend to lose less C derived from the forest when compared to Ferralsols, due to their physical characteristics in the subsurface layers (Strey et al., 2016). The addition of fertilizer can lead to higher rates of decomposition of the remaining organic matter from natural vegetation and an increase in the release of C from grasses (Sant-Anna et al., 2017). However, the area with PA has no fertilization management in its history, which restricts the development of the pasture root system and the decomposition of the remaining SOM in subsurface.

The values of $\delta^{15}N$ showed an isotopic enrichment as depth increased, and this pattern was more pronounced in NV. In the NV area, the values of $\delta^{15}N$ ranged from 3.99 to 16.83‰, whereas, in the PA area, there were values between 7.93 and 15.15‰ (Figure 5). The lower values of $\delta^{15}N$ in the surface layers, mainly in NV, are attributed to the constant addition of organic matter in the surface layers of the soil, as well as to the diversity of sources present in NV, with some species of the Fabaceae family, which promotes higher contents of readily available nitrogen (^{14}N, lighter isotope) (Loss et al., 2014).

The enrichment of $\delta^{15}N$ values in subsurface can also be attributed to transformations from organic N to mineral N. Thus, as the reactions of mineralization, nitrification, denitrification and volatilization occur associated with N assimilations by plants, there is greater decomposition of the isotope ^{14}N, leaving the remaining organic matter enriched in ^{15}N atoms (Couto et al., 2017). Therefore, isotropic values of $\delta^{15}N$ serve as an indication of the decomposition of organic matter, as the highest values of $\delta^{15}N$ are found in areas with low contents of organic carbon.

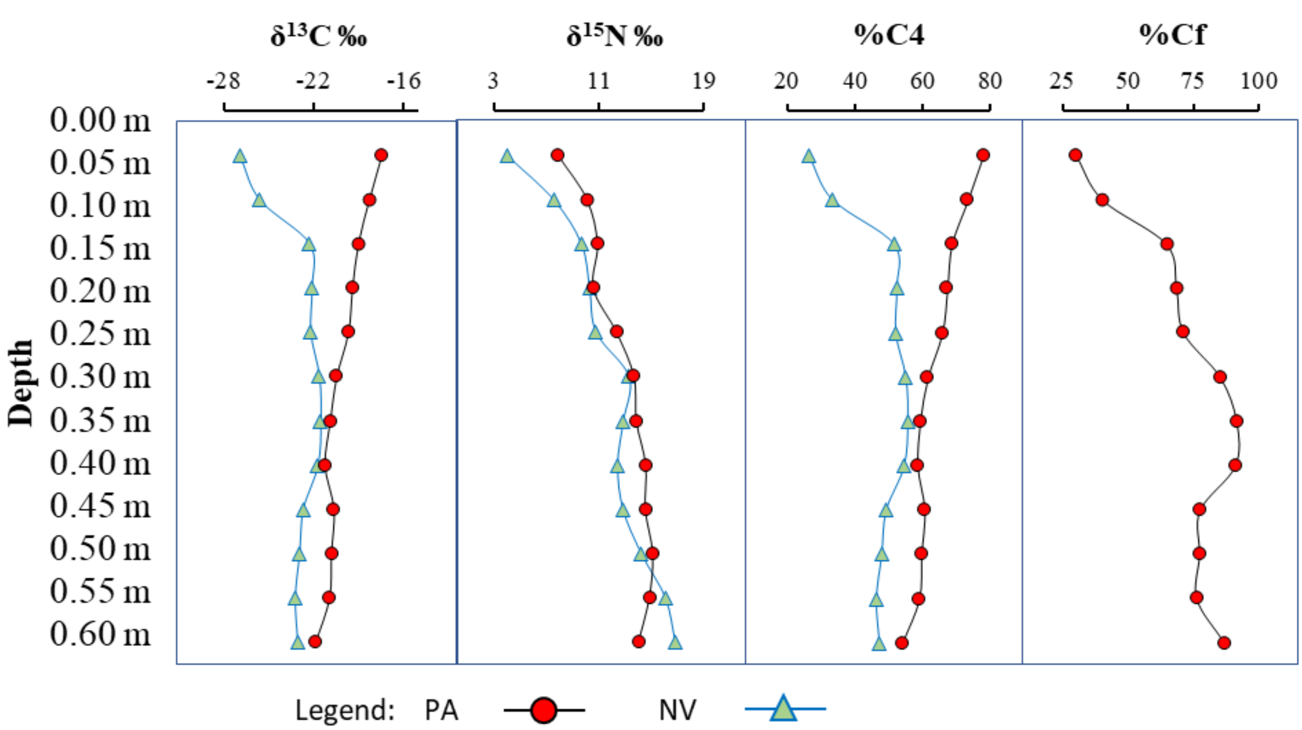

Figure 5 – Natural abundance of $\delta^{13}C$ and $\delta^{15}N$ (‰), percentage of carbon from C_4 plant origin (%C4) and carbon remaining from native vegetation (%Cf) in the soil of areas with planted pasture (PA) and Natural vegetation (NV), in the Cerrado biome, Mato Grosso do Sul, Brazil.

Effects of Cerrado conversion and fragmentation

The conversion of native vegetation into cultivated pasture for extensive livestock farming in the 1970s, 1980s and 1990s, in the Cerrado area of Aquidauana, resulted in a fragmentation of this biome in the region, as it was shown in the NV area evaluated, with the fragmentation of natural vegetation in favor of the expansion of livestock farming and agriculture, a common practice in Cerrado, an ecological hotspot in Brazil (Silva and Bacani, 2017; Ozório et al., 2019; Alencar et al., 2020).

The NV area (fragmented area) possibly suffered or is still suffering from anthropic actions (introduction of animals, wood extraction and advance of pasture) and edge effect, factors that were not evaluated in the present study, as the collection was performed at the center of the NV area. However, the drastic reductions of CSt and NSt in subsurface and the isotopic enrichment in the layers of 0.25–0.30 m up to 0.40–0.45 m of NV may be associated with these factors (Nascimento and Laurance, 2004; Barros and Fearnside, 2016).

The fragmentation of NV may have resulted in a change in the composition and structure of the vegetation from edge to center, with death of climax trees and the development of pioneer species, affecting the distribution and dynamics of aboveground biomass, enabling the increase in the rate of decomposition and shifting the C flow to the soil (Barros and Fearnside, 2016; Ma et al., 2017). Thus, fragmentation contributed with negative impacts within biogeochemical cycles, such as reduction of the capacity of sequestration and storage of C and N in the vegetation, rapid mineralization of these elements in the soil, reduction of C and N stocks in surface and subsurface and, consequently, increase in greenhouse gas (GHG) emissions (Nascimento and Laurance, 2004).

Conclusions

The highest carbon and nitrogen stocks occur in the 0–0.05 m layer of natural vegetation, and conversion to pasture leads to significant losses in the carbon and nitrogen stocks of the 0–0.05 m layer. In the subsurface layers, the area of natural vegetation has similar contents to those of planted pasture.

The conversion of natural vegetation into pasture causes changes in the signal of $\delta^{13}C$, with the highest isotopic values in the first two layers of pasture; however, in subsurface, the signals of $\delta^{13}C$ decrease, indicating the presence of the mixture between C_3 and C_4 plants, and the percentage of remaining carbon from the native vegetation in the pasture area increases.

The enriched values of $\delta^{13}C$ in a subsurface layer of natural vegetation suggest change in vegetation community in this area during past periods, with a mixed vegetation of C_3 and C_4 plants.

The values of $\delta^{15}N$ showed an isotopic enrichment as depth increased, indicating greater mineralization of soil organic matter. Because it was composed of a C_4 species, the area with pasture had the highest values of $\delta^{15}N$, with low enrichment in subsurface.

Contribution of authors:

Oliveira, N.S.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. Schiavo, J.A.: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. Lima, M.F.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Visualization, Writing – original draft. Laranjeira, L.T.: Data curation, Formal analysis, Investigation, Methodology, Resources, Visualization, Writing – original draft. Nunes, G.P.: Data curation, Formal analysis, Investigation, Methodology, Resources, Visualization. Cruz, S.C.: Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Visualization.

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ABES

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Optimization of palm oil biodiesel production using response surface methodology

Otimização na produção de biodiesel de óleo de palma utilizando a metodologia de superfície de resposta

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ABSTRACT

The purpose of this paper was to analyze palm oil biodiesel production under different conditions and to verify the relationships between production variables in order to optimize biofuel production using response surface methodology (RSM). Biodiesel was produced through transesterification process by methyl route and alkali catalyst (NaOH) 1% (m/m). The analyzed variables were: four molar ratios (3:1, 4:1, 6:1 and 8:1); three temperature reactions (45°, 52° and 60°C); and three time reactions (40, 60 and 80 minutes). For the palm oil biodiesel production, the highest yield was 93%, obtained via a molar rate of 3:1, 52°C and 60 minutes. This result differs from previous studies that found a higher yield with molar ratio increases, implying greater expenses of methanol. Kinetic viscosity and specific mass were also analyzed, and the values are within the Brazilian, American, and European standards. The results showed that the most influent factor in biodiesel production was the molar rate. In relation to the biodiesel characterization, using the RMN H1 technique, it was possible to obtain the transesterification reaction yield of 79.50% for the 3:1 palm oil biodiesel. Through gas chromatography, it can be verified that the predominant fatty acids in the samples were palmitic and oleic acids. .

Keywords: methyl esters; biofuel viscosity; biofuel specific mass; production efficiency.

RESUMO

O objetivo deste trabalho foi analisar a produção de biodiesel de óleo de palma em diferentes condições e verificar as relações entre variáveis de produção para otimizar a produção de biocombustíveis usando a metodologia da superfície de resposta (*response surface methodology* – RSM). O biodiesel foi produzido através do processo de transesterificação via rota metílica e com catalisador alcalino (NaOH) a 1% (m/m). As variáveis analisadas foram: quatro razões molares (3:1, 4:1, 6:1 e 8:1); três temperaturas de reação (45°, 52° e 60°C) e três tempos de reação (40, 60 e 80 minutos). Para a produção de biodiesel de óleo de palma, o maior rendimento foi de 93%, obtido na razão molar de 3:1, 52°C e 60 minutos. Esse resultado difere de outros estudos que encontraram maior rendimento com o aumento da razão molar, implicando em maiores gastos com metanol. A viscosidade cinética e a massa específica também foram analisadas, e os valores estão dentro dos padrões brasileiro, americano e europeu. Os resultados mostraram que o fator mais influente na produção de biodiesel foi a razão molar. Em relação à caracterização do biodiesel, pela técnica de RMN 1H, foi possível obter o rendimento da reação de transesterificação de 79,50% para o biodiesel 3:1 de óleo de palma. Por meio da cromatografia gasosa, pode-se verificar que os ácidos graxos predominantes nas amostras foram os ácidos palmíticos e oleico.

Palavras-chave: ésteres metílicos; viscosidade de biocombustível; massa específica de biocombustível; eficiência de produção.

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Introduction

Alternative fuel sources have been widely studied due to the non-renewable character of the current and most used energy source, oil. For Razack and Duraiarasan (2016), biodiesel has emerged as the main substitute for petroleum diesel. Biodiesel promotes sustainable development through energy savings, in addition to presenting characteristics, such as low toxicity and low emission of polluting gases (Ambat et al., 2018).

Biodiesel is a renewable energy source, biodegradable and derivates from renewable sources, such as vegetable oils and animal fat. There are several technologies for biodiesel production, such as cracking, esterification and transesterification, which involve the management of variables, such as the molar ratio of alcohol:oil, temperature, time, and catalyst amount, determinants for the efficiency of biodiesel production. The transesterification process of fatty acids present in oils and fats is the most common and can be carried out using ethanol (ethyl route) or methanol (methyl route), which generally present better yields in the presence of an acid or basic catalyst (Rodrigues et al., 2011; Victorino et al., 2016; Abdullah et al., 2017; Gonçalves et al., 2019).

In both processes the production of glycerol is obtained as a by-product, which contributes in the increasing biodiesel competitiveness, since this substance can be used as raw material in the production of paints, pharmaceuticals, and textiles.

Biodiesel or fatty acid methyl ester (FAME), when using methanol, or fatty acid ethyl ester (FAEE), when using ethanol, is a fuel that can be applied pure or mixed with petroleum derived diesel in various proportions in internal combustion engines without the need for mechanical modifications in the engine (Rincón et al., 2014).

Brazilian national law no. 12,490 of September 16, 2011 (in item XXIV of Article 6) defines biofuel as a "substance derived from renewable biomass, such as biodiesel, ethanol and other substances established in Brazilian National Agency of Petroleum, Natural Gas and Biofuels (ANP) regulations, which can be used directly or through changes in internal combustion engines or for other types of power generation, being able to replace fossil fuels partially or totally" (Brasil, 2011).

Due to its origin, biodiesel provides lower environmental impacts when compared to diesel fuel, emitting less particulate material, carbon dioxide (CO_2) and nitrogen oxides (NO_X), which are gases that contribute to the greenhouse effect. When this biofuel shows similar characteristics to diesel fuel, it is able to replace it as an energy source (Kumar et al., 2016).

However, as stated by Ge et al. (2020), a possible disadvantage is that viscosity and density tend to be slightly higher in biodiesel than in diesel due to the presence of saturated and unsaturated long chain fatty acids.

The USA is the country with the highest biofuel production, followed by Brazil (Sawin et al., 2017). According to Sawin et al. (2017), the total production of biofuels in Brazil was 30.8 billion liters in 2016, 27 billion of which were ethanol and 3.8 billion were biodiesel. In Brazil, biodiesel has soy oil and pork fat as its main feedstocks, with a participation of 72.51 and 14.93%, respectively, while palm oil represents only 0.16%. These facts show the minor participation on a wide variety of potential raw materials for biodiesel production, so it is necessary to increase the participation of raw materials in matrix, especially of products with highly productive yield such as the palm oil crop.

According to Feroldi et al. (2014), palm is considered the raw material with the highest oil productivity among the oilseeds for consumption around the world, with 20 to 22% of oil and a yield of four to six tons per hectare each year. Besides, palm oil is also a significant profitable oil for biodiesel production due to its compositional characteristics, high productivity with low cost, whole year well distributed production and no competition with other crops for feeding purposes. Nowadays in Brazil, most palm oil plantations are concentrated in the states of Pará, Amazonas, Amapá, and Bahia (D'Agosto et al., 2015). Moreover, palm oil biodiesel production is encouraging a new segment for the productive chain that is strengthening, generating, and multiplying employment and increasing income in the agricultural phase, input market and services and transport, storage, blending and biodiesel marketing activities (Lebid and Henkes, 2015).

The cultivation of the African palm (*Elaeis guineenses*), originating from the Gulf of Guinea on the West coast of Africa, is an oleaginous plant species from which oil is extracted out of its fruit mesocarp. This palm has a life cycle of 20 to 30 years and begins to produce clusters at the age of 3.5 years after planting, reaching its peak between 7 and 15 years, after which it begins to decrease slowly until the 25th year. The favourable development conditions are moderate air temperature, solar radiation associated with a good distribution of precipitation with 2,000 mm year^{-1} and deep soils without compaction, with a maximum slope of 5% and an altitude up to 600 meters (Kuss et al., 2015).

Palm oil is the main raw stock for biodiesel production in Malaysia (Mekhilef et al., 2011), which is one of the leading palm oil producers in the world. There are many advantages and disadvantages from the economic, social, and environmental features of the Malaysian biodiesel palm production.

Apart from the economic aspect, the environmental issue is a major fact. According to Kong et al. (2014), the producing biochar from palm oil biomass provides promising co-benefits, including the generation of renewable electricity, liquid and gas biofuels, large amounts of heat or low-pressure steam and the potential of a net withdrawal of carbon dioxide from the atmosphere. In the future, biochar alone is not going to be enough to reduce Greenhouse Gas Protocol (GHG) to manageable safety levels. However, it can be implemented and integrated to the palm oil producing countries using many different approaches to create a substantial positive impact on the challenges of climate change and crop productivity.

Different studies carried out by Ali and Tay (2013), Sukjit and Punsuvon (2013), Feroldi et al. (2014), Wong et al. (2015), Anguebes-Franseschi et al. (2016), among others, analysed the variables associated with the biodiesel production from palm oil, and found that the molar ratio ethanol:oil, temperature and stirring time are the most important factors involved in the reaction. Some authors, such as Ali and

Tay (2013) and Feroldi et al. (2014), found the optimal conditions for palm oil biodiesel production using methanol in the transesterification process: the methanol:oil molar ratio of 6:1, reaction time of 60 minutes and temperature of 60°C with 1% of KOH as a catalyst, resulting in 88% yield. Likewise, Anguebes-Franseschi et al. (2016) obtained a yield of 90% in palm oil biodiesel production with a reaction temperature of 56°C, 135 minutes, and a NaOH catalyst proportion of 0.65%.

It is important to monitor the process from the formation of the main product, the fatty esters, in the transesterification. Thus, high-performance liquid chromatography, gas chromatography, proton nuclear magnetic resonance (1H NMR) and thin layer chromatography methods are used. Among these techniques, gas chromatography is considered the most effective to determine the amount of fatty acid esters present in the biodiesel composition (Marques et al., 2010).

Using response surface methodology (RSM), Sukjit and Punsuvon (2013) determined that the best conditions were a molar ratio of 7:1 methanol:palm oil, temperature of 60°C with 70 minutes of reaction time and a proportion of 1.2% of KOH catalyst, resulting in a 96.24% yield. Using RSM, the highest yield obtained in the reaction with palm oil was 97.67% with a molar ratio of 13.04:1 methanol:oil, time of 2.67 hours and a catalyst proportion of 3.60% (Wong et al., 2015).

Thus, taking into account the different studies about molar ratio, temperature and reaction time for biodiesel production, as well as the importance of determining a more efficient process for the production of renewable energies, this paper aimed to establish the process with the highest yield using RSM in the production of palm oil biodiesel, testing the values of factors, such as molar ratio, temperature and time, as well as analyzing the kinetic viscosity and specific mass.

Materials and Methods

Biodiesel production

The structure of this paper comprised two steps: the first involved the biodiesel produced experimentally and the second consisted in biodiesel characterization and elaboration of the response surface. The palm oil biodiesel production and characterization were performed at the Universidade Federal Fluminense (UFF, Brazil). For biodiesel production, palm oil and methanol were used and NaOH was the catalyst.

The different characteristics of molar ratio, temperature and time were considered for the experimental analyses of biodiesel production. The data were obtained for three temperatures (45, 52 and 60°C), three production times (40, 60 and 80 minutes), and four molar ratios of methanol:oil (3:1, 4:1, 6:1, 8:1), as shown in Table 1.

Table 1 – Analysis of variation sources.

Molar ratio (methanol:oil)	3:1, 4:1, 6:1, 8:1
Temperature (°C)	45, 52, 60
Time (min)	40, 60, 80

Palm oil is red due to the presence of carotenoids and is rich in vitamins, coenzymes, and sterols (Porcayo-Calderon et al., 2017). In addition, it is more saturated than soybean oil and rapeseed due to its higher amount of fatty acids, such as palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids (Issariyakul and Dalai, 2014). High-quality palm oil is mainly used in the food industry, and low-quality (non-edible) oil is used to produce soap, waxes, cosmetics, biofuels, and other types of goods (Porcayo-Calderon et al., 2017).

Table 2 shows the fatty acid composition of palm oil. It is possible to observe 50% of saturated fatty acids (SFA), mainly palmitic acid (44%), and lower amounts of stearic acid (5%), 40% of monounsaturated fatty acids (AGMI), mainly oleic acid and 10% of polyunsaturated fatty acids (PUFA), mainly linoleic acid.

Biodiesel was obtained via alkali transesterification reaction. Initially, the quantities of palm oil, methanol and NaOH based on the treatment were weighed. Consequently, the oil was heated in the corresponding treatment temperature (45, 52 and 60°C) and then mixed with the methanol and NaOH mixture in the magnetic stirrer at the temperature and reaction time of the treatment. After the reaction time, the biodiesel was separated from the glycerol phase and washed with distilled water and hydrochloric acid, heated at 105°C and finally filtered. The mass yield production of each treatment was determined from the biodiesel mass divided by oil mass, as shown in Equation 1.

$$\text{Yield (\%)} = (\text{biodiesel mass (g)})/(\text{oil mass (g)}) \quad (1)$$

Biodiesel characterization

The biodiesel characterization was carried out according to the Brazilian (ABNT), American (ASTM) and European (EN ISO) standards (Table 3). A specific mass at 20°C was determined, as well as biodiesel kinetic viscosity, separated according to molar ratio (3:1, 4:1, 6:1 and 8:1). The Brazilian National Agency of Petroleum, Natural Gas and Biofuels (ANP) defines biodiesel as a fuel composed of alkyl esters of long-chain carboxylic acids, produced through the transesterifica-

Table 2 – Fatty acid composition of palm oil.

Fatty Acid Name		Composition (%)
Lauric	(12:0)	0.2
Myristic	(14:0)	1.1
Palmitic	(16:0)	44
Stearic	(18:0)	4.5
Oleic	(18:1)	39.2
Linoleic	(18:2)	10.1
Linolenic	(18:3)	0.4
Arachidic	(20:0)	0.1

Source: adapted from Mancini et al. (2015).

tion and/or esterification of fatty substances, of fats from vegetable or animal origins (Brasil, 2014).

The specific mass was determined using a pycnometer with biodiesel at 20°C (Lima et al., 2010). The known volume using a pycnometer was 50 mL, and the specific mass was calculated applying Equation 2, considering the mass difference between empty and full pycnometers.

$$\rho = (fpm-epm)/pv*1000 \tag{2}$$

In which:
ρ = specific mass of biodiesel (kg m^{-3});
fpm = full pycnometer mass (g);
epm = empty pycnometer mass (g);
pv = pycnometer volume (mL).

Kinetic viscosity was calculated from the dynamic viscosity divided by specific mass. Dynamic viscosity was determined using rheometer Haake RS50, and specific mass was calculated. Biodiesel samples at 40°C (ASTM, 2012) were used for these analyses.

Biodiesel characterization by hydrogen nuclear magnetic resonance

1H NMR spectrometry characterization of palm oil and biodiesel samples with higher mass yield was carried out at the Multi-Nuclear Magnetic Resonance Laboratory (LaReMN) at UFF (Brazil). The samples were diluted in deuterated chloroform (CDCl3) and analyzed on a Varian spectrometer — VNMRS 300MHz. Tetramethylsilane (TMS) was used as reference, according to the methodology proposed by Fagundes (2011).

Gas chromatography

Samples with the highest mass yield biodiesel were analyzed qualitatively in the gas chromatograph coupled to mass spectrometry (GC-MS) GCMS-QP2010 (Shimadzu, Tokyo, JP) using the following conditions: injection with flow division in the ratio of 1:20; DB5-MS column (30 m × 0.25 mm D.I. and 1 μm of 5% phenyl-polydimethylsiloxane); the carrier gas used was He (99.999% pure) under a constant flow of 3 mL min^{-1}; oven temperature setting was 50–180°C with heating rate of 8°C min^{-1}, 180–230°C with heating rate of 5°C min^{-1}, 230–310°C with heating rate of 20°C min^{-1}, followed by isotherm for 15 minutes. The chromatographic profiles were made in comparison with the Nist 147 library (US National Institute of Standards and Technology 147), indicating the presence of some methyl esters in the samples. The distribution of the observed substances was determined by standardizing the area of each present peak, that is, in percentage of relative chromatographic area.

Degree of transesterification conversion

The biodiesel sample with the highest mass yield had its transesterification reaction characterized by the spectrum of nuclear magnetic resonance. The conversion of oil into biodiesel was calculated according to Equation 3, following the methodology proposed by Ruschel et al. (2016). Based on this equation, the conversion is determined by the integration of the biodiesel sample 1H NMR signals. The integration of each spectrum was generated using the MestreNova software v. 12.0.

$$C_T = ((I_{CH3}/3)/(I_{CH2}/2))*100 \tag{3}$$

In which:
C_T = the conversion rate of the transesterification process;
I_{CH2} and I_{CH3} = obtained integrating the signals attributed to hydrogen of methylene group adjacent to carbonyl and hydrogen of methyl ester group, respectively.

As mentioned, the signal from methylene group adjacent to carbonyl (2.1–2.4 ppm) is used in the conversion parameter, as it is present in all triglyceride derivatives.

Response surface

In order to elaborate the response surface, the Matlab Software® (academic version) was used. The response surface was obtained for the variables molar ratio (x), reaction time (y), temperature (z), and yield (response), shown in Equation 4, using the linear interpolation method. Maximum yield was then obtained from the maximum value of the surface in Equation 4.

$$\text{Yield} = F(xq, yq, zq) \tag{4}$$

Table 3 – Biodiesel specifications established by Brazilian, American, and European regulations.

Characteristic	Limit	Method		
		ABNT	ASTM D	EN ISO
Specific mass at 20°C	850 to 900 kg.m^{-3}	7148 14065	1298 4052	EN ISO 3675 EN ISO 12185
Kinematic viscosity at 40°C	3 to 6 $mm^2.s^{-1}$	10441	445	EN ISO 3104

Source: adapted from Brasil (2014).

Results and Discussion

Specific mass

The specific mass results of biodiesel samples at 20°C are presented in Table 4. The results obtained are according to the Brazilian (ABNT), American (ASTM) and European (EN ISO) standards (Table 3), which demand a specific mass value from 850 to 900 kg m^{-3}. It is also possible to observe that the molar ratio did not significantly alter the specific mass values of the produced biodiesel.

According to Lôbo et al. (2009), biodiesel specific mass is directly linked to its molecular structure. The higher the length of the alkyl ester carbon chain, the greater the density. However, density can decrease with a greater number of unsaturation present in the molecule. Density can also be affected by the presence of impurities, such as alcohol or adulterants. Compared to diesel, biodiesel is less compressible and denser, causing a decrease in the calorific value and increasing consumption.

The specific mass is a determinant parameter of maximum biodiesel percentage in the mixture with diesel, since mixtures with a high proportion of biodiesel or those which diesel density is close to the upper limit allowed could exceed the limits established by the standards (Avellaneda, 2010).

Based on Ali et al. (2014), the raw material used in the production process has a direct influence on the specific biodiesel mass. The results presented for specific mass are according to the ones obtained by Ali et al. (2012), Vargas (2010), Sarkar and Bhattacharyya (2012). These authors indicated values for specific mass from palm oil biodiesel as 867, 871.6 and 874 kg m^{-3}, according to Ali et al. (2012), Vargas (2010), Sarkar and Bhattacharyya (2012)".

Table 4 – Specific mass of biodiesel at 20°C according to molar ratio*.

Molar ratio	Specific mass (kg m^{-3})
3:1	871.90 a
4:1	872.46 a
6:1	872.26 a
8:1	871.58 a

*Average values followed by the same letter do not differ in the 5% probability level by Tukey test.

Table 5 – Biodiesel kinetic viscosity according to molar ratio*.

Molar ratio	Kinetic viscosity (mm^2 s^{-1})
3:1	4.89 a
4:1	5.45 d
6:1	5.39 c
8:1	5.09 b

*Average values followed by different letter differ in the 5% probability level by Tukey test.

Kinetic viscosity

The kinetic viscosity results obtained are presented in Table 5 and are according to the Brazilian (ABNT), American (ASTM) and European (EN ISO) standards (Table 3), which require values of 3 to 6 mm^2 s^{-1}.

Several authors, such as Ali et al. (2012), Yusop et al. (2018), Kim et al. (2019), Tziourtzioumis and Stamatelos (2019), Yoon et al. (2019), and Ong et al. (2020), who produced biodiesel from palm oil, obtained kinematic viscosity values between 4.56 and 4.74 $mm^2.s^{-1}$.

According to Zahan and Kano (2018), biodiesel produced from palm oil has better properties compared to biodiesel produced from other raw materials, including kinematic viscosity. As explained by Yusop et al. (2018), fuel viscosity plays an important role in engine injection systems, as lower viscosities cause injectors and pumps to leak, interfering with the engine's power production. In addition, viscosity also interferes with atomization and spraying within the combustion chamber.

Hydrogen nuclear magnetic resonance

Using the hydrogen nuclear magnetic resonance (1H NMR) technique, it is possible to determine whether the transesterification reaction of triglycerides in monoesters occurred directly. The results from RMN1 H were similar for both palm oil and 3:1 molar ratio biodiesel. Figure 1 shows the 1H NMR spectrum of palm oil, in which the presence of a multiplet signal in the area of 4–4.4 ppm can be observed. This area shows the presence of hydrogen characteristic of triglycerides.

After the transesterification reaction, the 1H NMR spectrum in the produced biodiesel (Figure 1B) has no sign of the characteristic area of triglycerides 4–4.4 ppm. In addition, it is also possible to observe that in this spectrum, unlike the oil sample, a singlet is observed in the 3.4–3.7 ppm area, and this signal is attributed to the hydrogen of the methoxy group -OCH3 present in the produced methyl esters.

Furthermore, it appears that the signal from the 2.1–2.4 ppm area was highlighted, as it can be observed both in the source material (vegetable oil) and in the biodiesel samples. This area is attributed to the hydrogen in the carbonyl (α-CH2) adjacent group. In addition to the chemical information that this signal provides, it is also used to determine the degree of the transesterification conversion process.

The main chemical shifts (ppm) observed in the spectra of the produced biodiesel are shown in Table 6.

Analyzing the 1H NMR spectrum of 3:1 palm biodiesel, a triplet with an integration of approximately 3 H is observed in the displacement range of 0.92–0.84 ppm in relation to TMS. In the displacement range of 1.27–1.21 ppm, a singlet with an integration of approximately 13 H is observed. A singlet is observed in the displacement range of 1.56–1.59 ppm with an integration of approximately 2 H. In the displacement range of 2.32–2.28 ppm, a triplet with an integration of approximately 2 H is observed. In the displacement range of 3.69–3.65 ppm, a singlet with an integration of approximately 3 H is observed and in the displacement range of 5.42–5.29 ppm a multiplet with an integration of approximately 1 H is observed.

Analysis of the data from the 1H NMR spectrum of the produced biodiesel proved that the transesterification reaction occurred observing the disappearance of the signals from the area of 4–4.4 ppm, indicating the disappearance of the hydrogen attributed to the triglycerides and the appearance of the singlet in the 3.5–3.7 ppm area, which is attributed to the hydrogen of the formed ester. The obtained results for chemical displacement and yield were compared to data already described in previous studies.

Transesterification conversion degree

As shown in Table 7, the 1H NMR analysis suggested that the yield of the transesterification reaction is mainly associated with the presence or absence of signal in the 4–4.4 ppm area attributed to the triglyceride H. As a result, a 79.50% conversion rate was observed.

It is important to note that the greater the conversion of biodiesel, the lower the amount of glycerin produced, suggesting that the cotton-coconut mixture has a lower amount of glycerin compared to other oils.

Gas chromatography analysis coupled to mass spectrometry

In the chromatogram corresponding to the palm oil methyl biodiesel sample (Figure 2), it is evident that the 16–22-minute interval shows signs related to the fatty esters that contribute the most to the composition of palm oil biodiesel. Comparing the experimental data to the data from ANVISA (Brasil, 1999) and Mancini et al. (2015), there is an agreement in relation to the largest compositions, which were oleic and palmitic acids, such as those of a greater area present in the sample (Table 8).

Statistical analysis

The software used for statistical analysis was the SISVAR v. 5.6 (Ferreira, 2014) with the Tukey test at a 5% significance level ($p < 0.05$). 108 samples were also analyzed in order to estimate the effects in biodiesel production yield of the studied variables and their interactions (Table 9). All three studied variables and the interactions between molar ratio versus temperature and temperature versus time had a significant effect on biodiesel production yield. However, the interaction between molar ratio versus time did not present significant effects.

It can be observed in Figure 3 that the highest yield in biodiesel production (93%) was obtained in the 3:1 molar ratio with a temperature of 52°C and stirring time of 60 minutes. The curves are quadratic regressions over average yield and temperature (or time) for all sets of molar ratios experiments. For the other molar ratios, the yield was lower and decreasing as the molar ratio increased. It can also be observed that the yield variation (between temperatures and stirring times) was lower at the 3:1 molar ratio.

Table 6 – Chemical shift (ppm) of biodiesel samples.

Biodiesel	Chemical shift (δ)
Palm 3:1	1H NMR (CDCl3, 300 Hz): 5.42-5.29 (m, 1H); 3.69-3.65 (s, 3H); 2.32-2.28 (t, 2H); 1.59-1.56 (s, 2H); 1.27-1.21 (s, 13H); 0.92-0.84 (t, 3H)

Table 7 – Conversion rate values for higher mass yield biodiesel.

Biodiesel	Conversion (%)
Palm 3:1	79.50

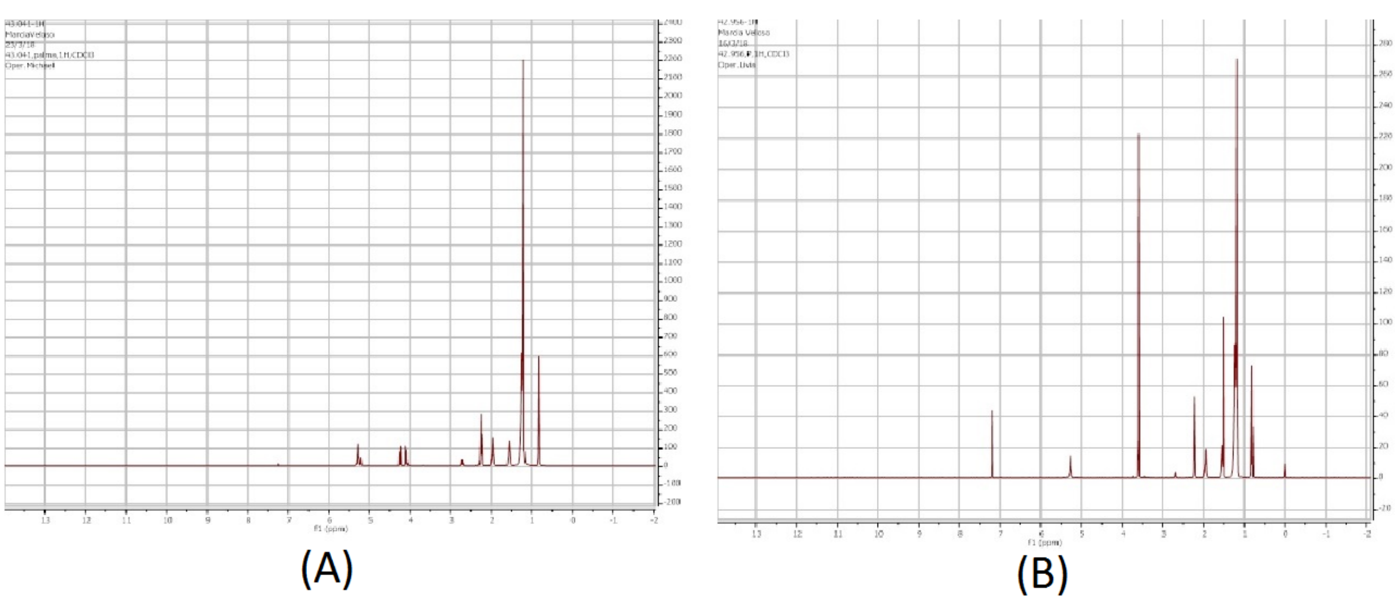

Figure 1 – 1H NMR spectrum of the source of triglycerides in (A) palm oil and (B) biodiesel formed.

Figure 3A shows the temperature performance in the production of biodiesel from palm. It was expected that the yield would be increased at higher temperatures, since, according to Feng et al. (2017), the molecular interaction between the reagents benefits the increase in the reaction temperature. However, the increase in temperature causes loss of reaction yield. According to Ramos et al. (2011), a possible ex-

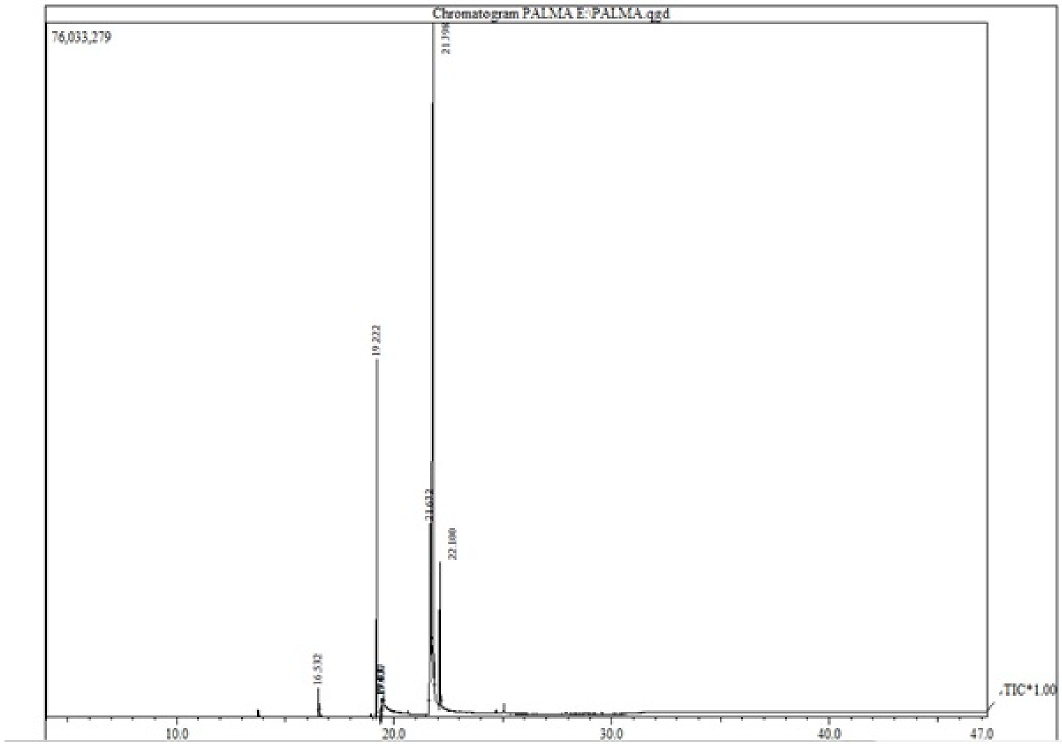

Figure 2 – Palm oil methyl biodiesel chromatogram.

Table 8 – Palm oil biodiesel fatty acid.

Fatty acid	Nomenclature	Retention time (min)	Area (%)	Mancini et al. (2015)	Brasil (1999)
C14:0	Myristic	16.53	0.82	1.1	0.5–2
C16:0	Palmitic	19.22	16.72	44	35–47
C18:0	Stearic	21.10	8.23	4.5	3.5–6.5
C18:1	Oleic	21.79	64.39	39.2	36–47
C18:2	Linoleic	21.67	9.34	10.1	6.5–15

Table 9 – Variance analysis of the effect on biodiesel production yield of the studied variables and their interactions.

Source	Degree of freedom	Sum of squares	Mean of square	F-value	Pr > Fc
Molar ratio	3	0.2213	0.0738	313.528	0.0000
Temperature	2	0.0508	0.0254	107.849	0.0000
Time	2	0.0050	0.0025	10.592	0.0000
Molar ratio*Temperature	6	0.0233	0.0039	16.530	0.0000
Molar ratio*Time	6	0.0023	0.0004	1.653	0.1429
Temperature*Time	4	0.0051	0.0013	5.458	0.0000
Error	84	0.0198	0.0002		
Corrected Total	107	0.3277			
CV (%)	1.78				
Mean	0.8606				

planation is that the increase in temperature not only favors the desired kinetics, but also the competing reactions such as hydrolysis. Ahiekpor and Kuwornoo also (2010) concluded that low temperatures in the reaction decrease the saponification degree.

Figure 3B shows the reaction time effect on the transesterification of palm oil. The yield showed a slight increase when the reaction time went from 40 to 60 minutes in the molar ratio of 3:1. However, the increase in time from 60 to 80 minutes led to a reduction in the reaction yield. For the other molar ratios, the increase in reaction time resulted in decreased yield. These results differ from those of Roschat et al. (2018), which obtained higher yields with increased reaction times.

Figure 4 shows the standardized distribution of production yields, and it is possible to verify that the lower molar ratios (3:1 and 4:1) present a higher density of values in the area of 85 to 90% yield, with emphasis on the molar ratio of 3:1, which showed greater density in the area of 90 to 95% of yield. At the highest molar ratios (6:1 and 8:1), the value density was greater in the area of 85 to 90% yield for the 6:1 molar ratio (with a density lower than the 4:1 ratio), and in the area of 80 to 85% yield for the 8:1 molar ratio. These values are evidenced by the better performance of the 3:1 molar ratio in the different reaction times studied.

Response surface

Figure 5 shows the effects of molar ratio, temperature, and time over a response surface of palm oil biodiesel production yield.

A maximum yield response, indicated by the dark red color, corresponds to a molar ratio of 3:1, temperature of 52°C and 60 minutes time, whose yield is 93%. The response surface indicates that, as molar ratio increases, yield decreases. This is different from the results observed by Feroldi et al. (2014), which suggested that in order to obtain a higher yield it is necessary to increase molar ratio in palm oil biodiesel production. Off the surface maximum, the yield decreases, especially for a molar ratio of 8:1 and 80 minutes, illustrated by the dark blue color on surface, resulting in a yield of 70%. According to Guerrero-Peña et al. (2013), a possible cause of this effect is linked to the reversibility reaction, due to long reaction time, resulting in a decrease of methyl esters production.

Sukjit and Punsuvon (2013), while also using the RSM determined optimum conditions for palm oil biodiesel production at a molar ratio of 7:1, temperature of 60°C and 70 minutes reaction time with a proportion of 1.2% KOH catalyst, resulting in a yield of 96.24%. Wong et al. (2015), using RSM, observed that the highest yield of 97.67% was obtained by molar ratio of 13.04:1, reaction time of 2.67 hours, and a proportion of 3.60% of catalyst.

Using the RSM and the Taguchi method, Tan et al. (2017) found a biodiesel yield around 95% under optimal reaction conditions at a temperature of 65°C, 1.9 at 2 hours' time and a molar ratio of 10 at 11:1, indicating that the integration of both methods is practically effective. However, RSM is more reliable in predicting the nonlinear relationship between the processing variables and response.

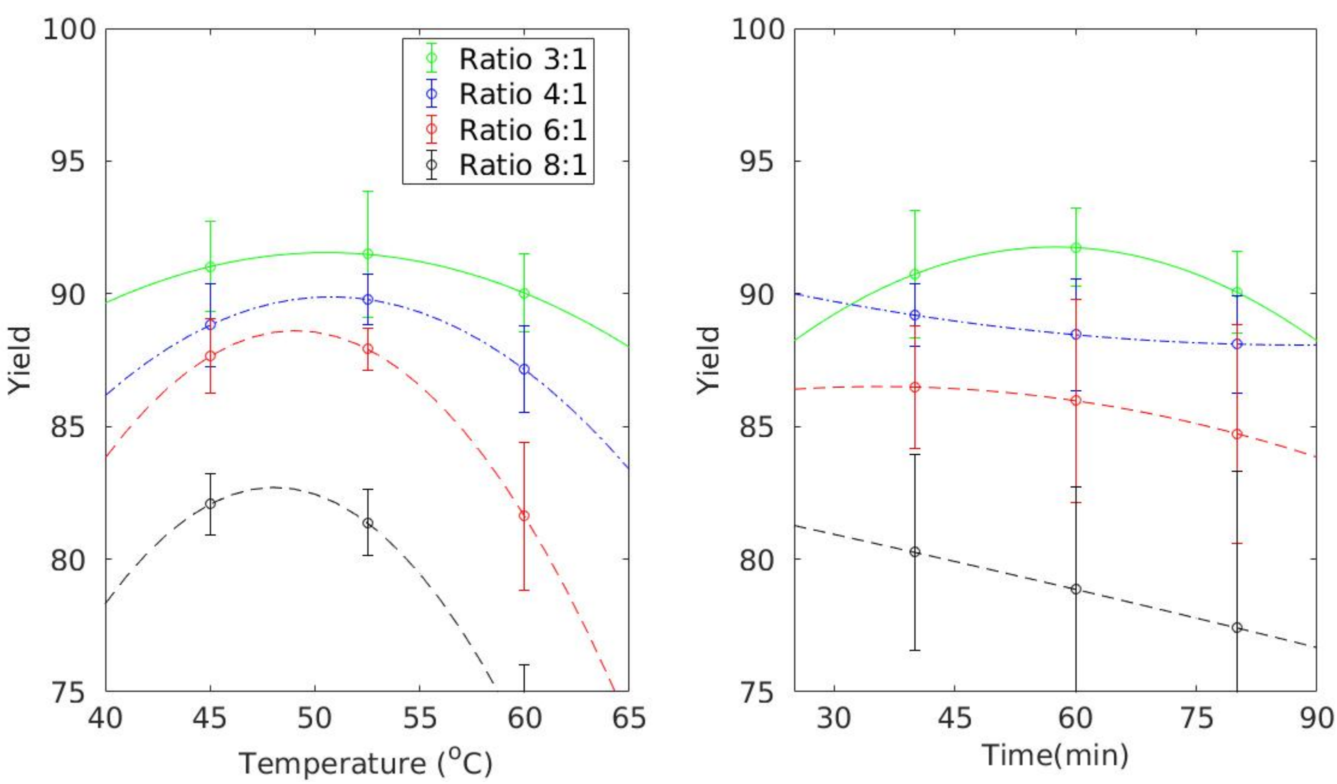

Figure 3 – Average and standard deviation of the experimental set (molar ratio) for (A) temperature (°C) and (B) time (minutes).

According to the results of Alkabbashi et al. (2009), the optimal conditions for biodiesel production with palm oil using methanol in the transesterification process were a reaction time of 60 minutes, a temperature of 60°C, a molar ratio of 10:1 ($m.m^{-1}$), and a proportion catalyst of 1.4% based on the oil mass, thus obtaining a yield of 93.6%.

These results corroborate those obtained by Paula et al. (2017), which state that the characteristic molar ratio of the reaction is 3:1, meaning that for every 3 moles of alcohol, 1 mol of triglycerides is required for the stoichiometric balance of the complete transesterification reaction to ensure that all oil is consumed in the process and transformed into methyl ester and glycerin.

The results found in the current study also corroborate with Uribe et al. (2014), as the authors state that methanol has high toxicity, although its advantages refer to the greater use in biofuel plants for biodiesel production, once it is more reactive and relatively cheaper than ethanol, requiring a shorter reaction time and lower molar ratios.

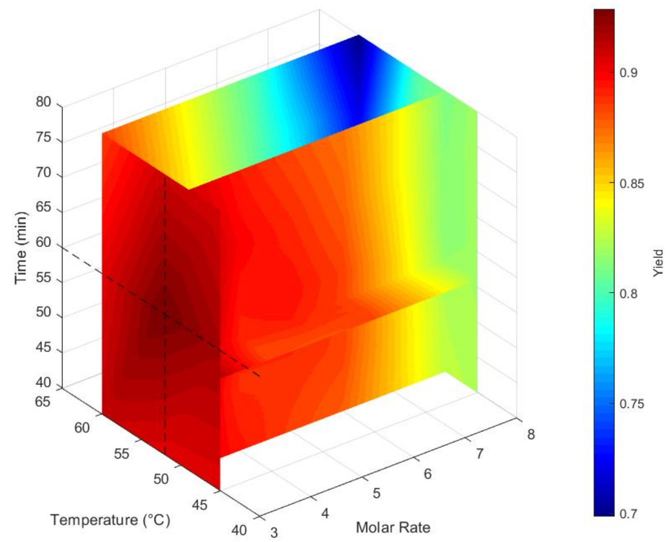

Figure 5 – Response surface of biodiesel production yield based on molar ratio, temperature, and time.

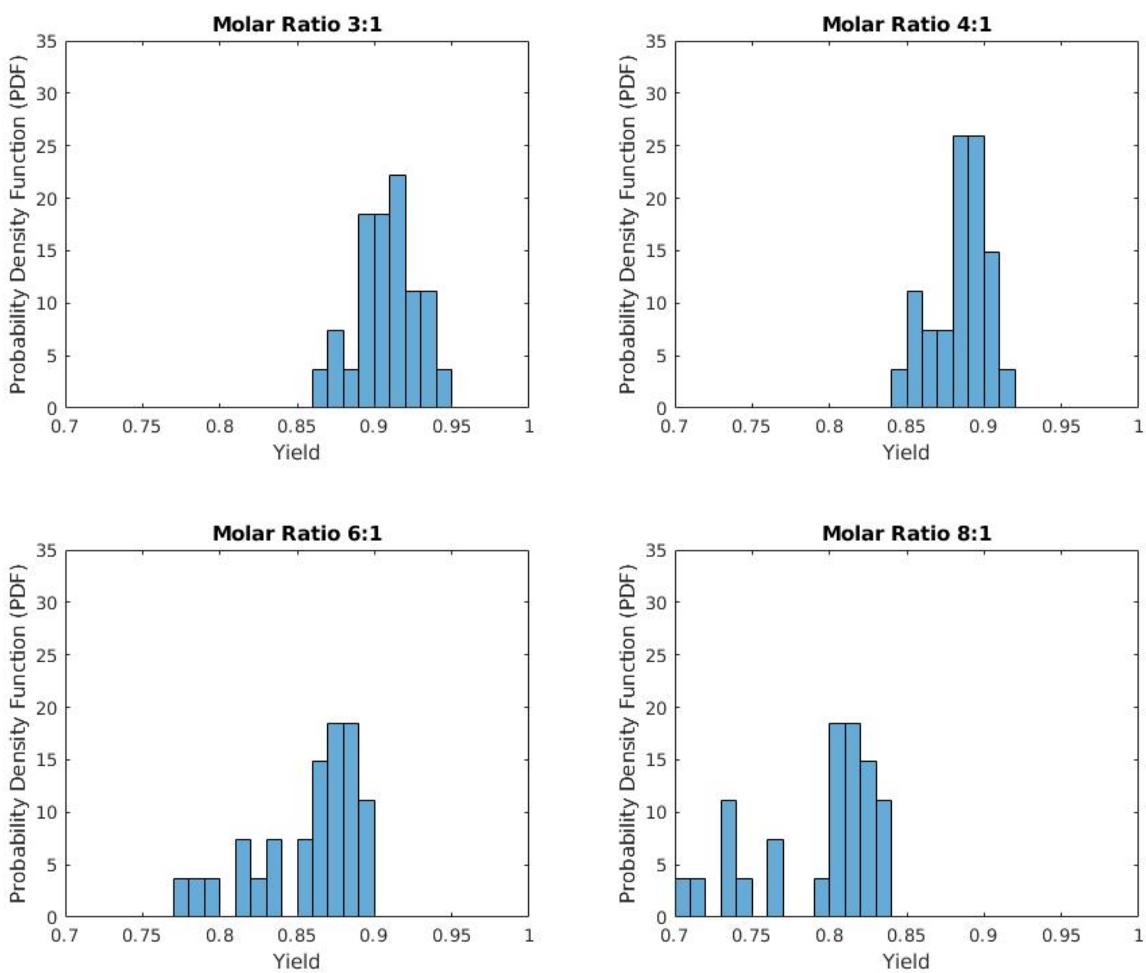

Figure 4 – Normalized probability distribution of production yields for 3:1, 4:1, 6:1 and 8:1 molar ratios.

The optimization method results from the importance of improving the performance of the systems and processes without increasing the costs. The RSM is a set of statistical and mathematical techniques used for the development, improvement and optimization of processes, in which the response is influenced by several variables, with the goal of optimizing the response (Khuri and Mukhopadhyay, 2010).

Conclusions

Based on the methodology and the results, the molar ratio was the most influential variable in the production yield of palm oil biodiesel using alkali catalyst and methyl route. Through response methodology and variation of the production factors (molar ratio, temperature, and time), the highest yield was for the molar ratio of 3:1, 52°C and 60 minutes. This result varies from those previously investigated in other studies, which generally had increased yields for higher molar ratios and indicated that when the molar ratio increases, the yield increases as well, opposing the results in the current study.

The produced biodiesel was characterized according to specific mass and kinetic viscosity properties, and the found values are included in Brazilian, American, and European standards. Therefore, the palm oil biodiesel has may be used as fuel for igniting compression engines.

In relation to the biodiesel characterization, using the H1 NMR technique, the transesterification reaction yield for the 3:1 palm oil biodiesel reached 79.50%. Using gas chromatography, the fatty acids present in the 3:1 palm oil biodiesel showed predominance of palmitic and oleic acids.

Contribution of authors:

Silva, F.C.: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing — original draft, Writing — review & editing, Visualization, Supervision, Project administration. Guardiola, J.F.H.: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing — original draft, Writing — review & editing, Visualization, Project administration. Teixeira, L.P.: Methodology, Validation, Formal analysis, Investigation, Data curation. Maria, A.C.L.: Formal analysis, Data curation, Writing — original draft, Writing — review & editing. Souza, L.: Validation, Formal analysis, Data curation, Writing — original draft. Belém, A.L.: Methodology, Validation, Formal analysis, Investigation, Data curation, Visualization.

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Stock and indices of carbon management under different soil use systems

Estoque e índices de manejo de carbono sob diferentes sistemas de uso do solo

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ABSTRACT

The aim of this study was to evaluate the stock of total organic carbon (TOC) and to perform the physical-granulometric fractionation of soil organic matter (SOM) in different management systems (MS). Three MS and one reference area of Native Forest (NF) were studied, and the three systems were sugarcane (SC), permanent pasture (PP) and no-tillage system (NTS). Soil samples were collected in the 0–0.05, 0.05–0.10, 0.10–0.20-m layers. Soil density (Sd), TOC, stratification index (SI), carbon stock (StockC), variation in StockC (ΔStockC), carbon content of particulate organic matter (C-POM) and mineral organic matter (C-MOM), carbon stock index (CSI), lability (L), lability index (LI), and carbon management index (CMI) were determined. The MS presented higher Sd than the NF area. The NF area had higher TOC contents in the first layers, reaching 25.40 g kg^{-1} in the 0–0.05-m layer, with the PP area having higher values than the NF in the 0.10–0.20-m layer. The NF area showed the highest levels of C-POM (15.25 g kg^{-1}) and C-MOM (10.15 g kg^{-1}) in the first layer. In the 0.10–0.20-m layer, the PP and NTS systems were superior to the others. Regarding the C-MOM content, SC and PP showed higher levels in the 0.10–0.20-m layer. The highest CMI values were observed in the NTS and PP areas in the 0.10–0.20-m layer. The MS increased the Sd and reduced the TOC levels. The different MS modified the POM fraction, and the MOM fraction was most impacted by the SC area. The lability of the SOM was altered by the MS in the most superficial layers.

Keywords: soil quality; labile carbon; environmental assessment.

RESUMO

O objetivo do presente trabalho foi avaliar os estoques de carbono orgânico total (COT) e realizar o fracionamento físico-granulométrico da matéria orgânica do solo (MOS) em diferentes sistemas de manejo (SM). Foram estudados três SM e uma área de referência de Mata Nativa (MN), sendo os três sistemas: cana-de-açúcar (CA); pastagem permanente (PP) e sistema plantio direto (SPD). Foram coletadas amostras de solos nas camadas 0–0,05, 0,05–0,10 e 0,10–0,20 m. Foram determinados a densidade do solo (Ds), o COT, o índice de estratificação (IE), o estoque de carbono (EstC), a variação do EstC (ΔEstC), os teores de carbono da matéria orgânica particulada (C-MOP) e mineral (C-MOM), o índice de estoque de carbono (IEC), a labilidade (L), o índice de labilidade (IL) e o índice de manejo de carbono (IMC). Os SM apresentaram Ds superior à área de MN. A área de MN apresentou maiores teores de COT nas primeiras camadas, chegando a 25,40 g kg^{-1} na camada 0–0,05 m, sendo a área de PP superior à MN na camada de 0,10–0,20 m. A área de MN apresentou os maiores teores de C-MOP (15,25 g kg^{-1}) e C-MOM (10,15 g kg^{-1}) na primeira camada. Para a camada de 0,10–0,20 m, os sistemas de PP e SPD foram superiores aos demais. Para os teores de C-MOM, a CA e PP apresentaram maiores teores na camada 0,10–0,20 m. Os maiores valores de IMC foram observados nas áreas de SPD e PP na camada de 0,10–0,20 m. Os SM aumentaram a Ds e reduziram os teores de COT. Os diferentes SM modificaram a fração MOP, sendo a fração MOM mais impactada pela área de CA. A labilidade da MOS foi alterada pelos SM nas camadas mais superficiais.

Palavras-chave: qualidade do solo; carbono lábil; avaliação ambiental.

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Introduction

The conversion of natural areas into production systems can, in addition to modifying the landscape, change the edaphic quality when not properly handled (Freitas et al., 2018). The different uses and managements directly influence soil attributes, such as carbon (C) (Lal, 2018; Ozório et al., 2019), besides causing changes in physical (Sales et al., 2018; Falcão et al., 2020), chemical (Souza et al., 2018; Assunção et al., 2019), and biological attributes of the soil (Borges et al., 2015; Barbosa et al., 2018).

Among the many attributes analyzed to evaluate the effects of the management systems and soil quality (SQ), soil organic matter (SOM) stands out (Nanzer et al., 2019; Lavallee et al., 2020; Poffenbarger et al., 2020). Thus, one of the methods for evaluating SQ is the analysis of C compartments of the physical fractions of the SOM (Cambardella and Elliott, 1992). Those are divided into two fractions, the particulate organic matter (POM), a fraction with high potential to indicate SQ in a short period of time (Bayer et al., 2002; Bongiorno et al., 2019); and the mineral organic matter (MOM), which is the most stable fraction of the SOM, being less sensitive to changes in a short period of time (Cambardella and Elliott, 1992).

With the data of physical fractionation, it is possible to obtain the carbon management index (CMI), developed by Blair et al. (1995), which is a useful tool to analyze the effects of different management systems, as it analyzes the effects of the systems on the quality and quantity of SOM in the same index (Ghosh et al., 2019).

The implementation of conservation production systems, such as well-managed pastures and the no-tillage system, can maintain or even increase soil carbon stocks (Salton et al., 2008; Rosset et al., 2019; Falcão et al., 2020), maintaining productive capacity and mitigating the emission of C dioxide (CO_2) into the atmosphere (Borges et al., 2015; Besen et al., 2018). In conventional soil tillage systems, the yearly plowing hinders the formation of stable soil aggregates, with consequent damage to the storage of C (Salton et al., 2008), as reported in studies analyzing soil quality in systems with sugarcane cultivation (Bordonal et al., 2018; Gomes et al., 2019).

Thus, the evaluation of SQ by quantifying the total organic carbon (TOC) contents and their respective fractions in areas with a known history of cultivation can produce accurate and conclusive results on the edaphic quality of the area. Therefore, the present study aimed to evaluate soil TOC contents and stocks, and the physical fractions of SOM in different management systems.

Materials and Methods

Soil collections were carried out at Vezozzo Farm, located in the municipality of Eldorado (Figure 1), Southern Cone region of the state of Mato Grosso do Sul, Brazil. The climate of the region is subtropical (Cfa), according to Köppen's classification and native vegetation of Atlantic Forest – Semideciduous Seasonal Forest (SEMADE, 2015), with soils classified as *Argissolo Vermelho Amarelo distrofico típico* (Santos et al., 2018), equivalent Acrisols (IUSS WORKING GROUP WRB, 2015) and Ultisols (SOIL SURVEY STAFF, 2014), of sandy texture (Santos et al., 2018).

Four different areas were evaluated, three management systems in addition to a reference area of native forest, namely: sugarcane crop area (SC) – with 350 hectares, cultivated with sugarcane since 2006; permanent pasture area (PP) –implanted in 2003, with 2.5 hectares, with *Brachiaria brizantha* species subjected to grazing pressure by goats, with approximately 12 AU ha^{-1}; no-tillage system area (NTS) – 240 hectares, where a succession of corn/soybean and cassava has been cultivated since 2002; and a native forest area (NF) of legal reserve with 160 hectares.

Disturbed and undisturbed soil samples were collected in the 0–0.05, 0.05–0.10 and 0.10–0.20-m layers, with four replicates per management system and layer. Each composite sample of deformed soil was represented by ten simple samples within the four evaluated areas. In the NTS and SC, collection was carried out between the cultivation rows. In the areas of PP and NF, samples were randomly collected. The undisturbed samples for soil density analysis (Sd) were collected with the aid of a volumetric ring with a volume of 48.86 cm^3, with four replicates in the areas and layers. In order to characterize the study areas, soil samples from the 0–0.20-m layer were collected and then sent to the laboratory for chemical and physical characterization (Table 1).

Sd analyses were performed according to Claessen (1997), using the volumetric ring method. TOC was determined according to the methodology adapted from Yeomans and Bremner (1988). Based on the TOC results, the total organic carbon stocks (StockC) were calculated according to the equivalent mass method (Ellert and Bettany, 1995; Sisti et al., 2004).

To assess trends of accumulation or loss of TOC in relation to the NF (reference system of the original soil condition in this study), the variation in the StockC (ΔStockC) was calculated by the difference between the mean values of StockC of the NF and each of the management systems. The obtained value was divided by the thickness (cm) of each layer and in the profile of 0–0.20 m. With the results of the TOC contents, the carbon stratification index (SI) (Franzluebbers, 2002) was calculated using the relation between the TOC contents of the 0–0.05-m and the 0.10–0.20-m layers.

The physical-granulometric fractionation of the SOM was performed according to the methodology of Cambardella and Elliott (1992), in which 20 g of air-dried fine earth (ADFE), together with 60 mL of sodium hexametaphosphate (5 g L^{-1}) were placed in Erlenmeyer flasks of 250 mL, being stirred for 16 hours in stirring table at a speed of 150 rpm. After the stirring period, samples were washed in a 53-μm sieve, and the material retained in the sieve comprised the POM. Subsequently, the carbon content of particulate organic matter (C-POM) was obtained by the methodology of Yeomans and Bremner (1988), and the carbon content of mineral organic matter (C-MOM) was obtained from the difference between TOC and C-POM. For the calculations of carbon stock of particulate organic matter (StockC-POM) and carbon stock of mineral organic matter (StockC-MOM), the methodology of the equivalent mass was used (Ellert and Bettany, 1995; Sisti et al., 2004).

After the determination of C fractions, the following indices were calculated to evaluate the quality of the SOM: carbon stock index (CSI), lability of SOM (L), lability index (LI), and carbon management index (CMI), according to Blair et al. (1995).

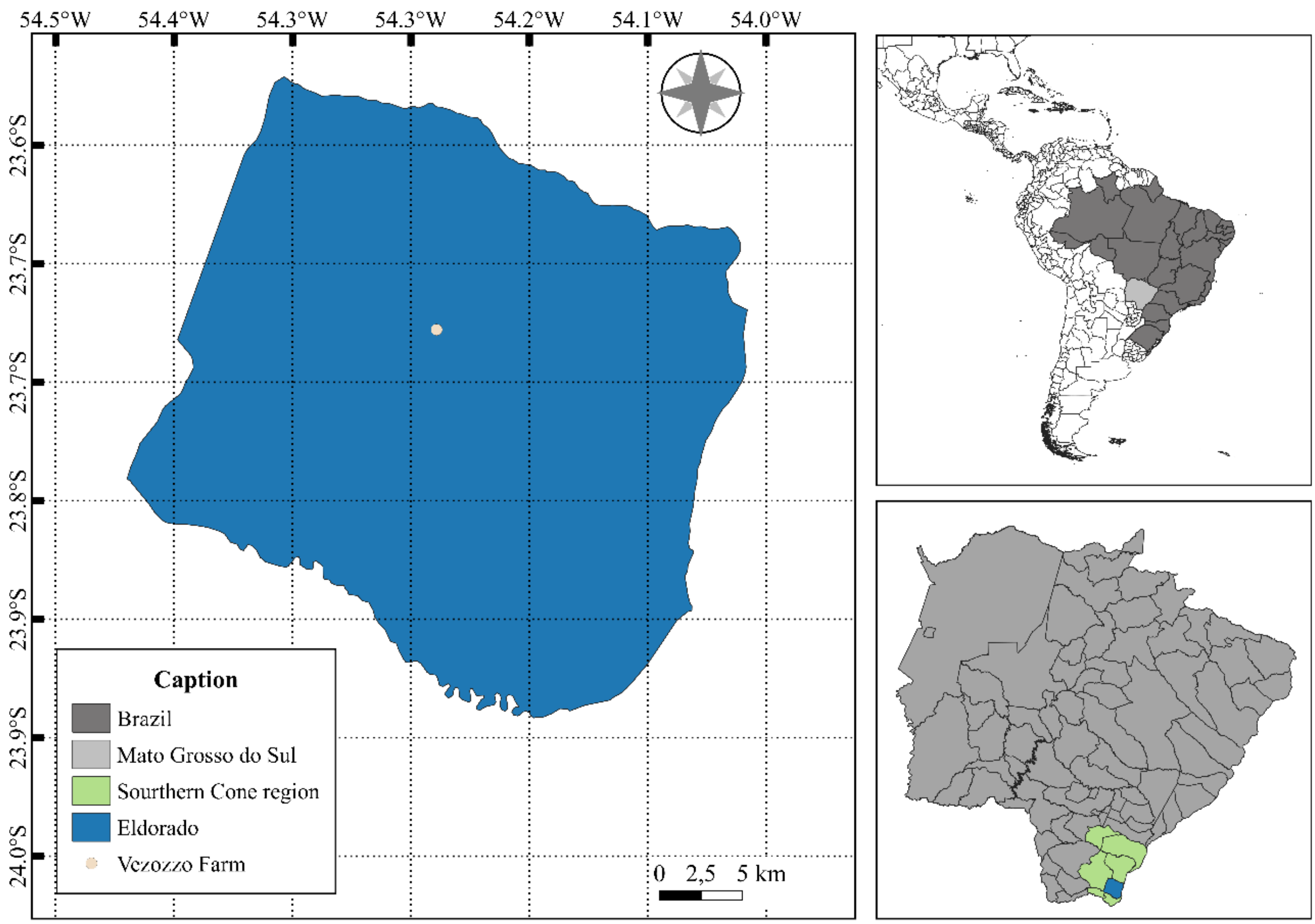

Figure 1 – Location of the municipality of Eldorado, state of Mato Grosso do Sul (MS), indicating the location of Vezozzo Farm, where the study collections were carried out. Cartography software: QGIS 3.12 Bucuresti.

Table 1 – Physical and chemical characterization of the soil (0-0.20-m layer) in the study areas.

SA	Sand	Silt	Clay	pH	OM	P	K	Ca	Mg	Al	H+Al	SB	CEC	V
	--------g kg^{-1}--------			$CaCl_2$	g dm^{-3}	mg dm^{-3}	-------------------- cmol$_c$dm^{-3}--------------------							%
SC	779	100	121	5.13	10.11	3.52	0.20	2.1	1.1	0.02	1.4	3.40	4.80	70.8
PP	831	84	85	5.53	12.85	26.10	0.17	1.6	1.2	0	1.2	2.97	4.17	71.2
NTS	815	83	102	4.53	16.94	35.58	0.14	0.8	0.5	0.13	1.8	1.44	3.24	44.4
NF	831	50	119	4.16	14.76	3.03	0.03	0.5	0.3	0.49	2.8	0.87	3.67	23.7

SA: Study area; SC: sugarcane crop area; PP: permanent pasture; NTS: no-tillage system; NF: native forest. Physical characterization – Granulometry: pipette method. Chemical characterization – Calcium Chloride (pH); Mehlich (P and K); KCl 1N (Ca, Mg and Al); Calcium Acetate pH 7 (H + Al); OM: Organic matter; CEC: Cationic exchange capacity; V: Base Saturation; SB: Sum of bases.

The results were subjected to variance analysis with F-test application, and the mean values were compared with each other by the Tukey test at 5% probability with the aid of the GENES software (Cruz, 2006).

Results and Discussion

Regarding the Sd, it can be observed that the three managed areas had higher values than the area of NF in the 0-0.05-m layer, being similar to each other, ranging from 1.35 to 1.52 Mg m^{-3}, whereas the area of NF presented a value of 1.08 Mg m^{-3} (Table 2). In the 0.05–0.10-m layer, the highest Sd values were observed in the SC and PP areas, with values of 1.63 and 1.74 Mg m^{-3}, respectively. In this same layer, the area of NTS (1.35 Mg m^{-3}) and NF (1.20 Mg m^{-3}) were similar to each other. In the 0.10–0.20-m layer, the areas of SC and PP had the highest values of Sd, and the area of NTS was similar to the NF (Table 2).

The highest values of Sd in the areas of SC and NTS are associated with the use of agricultural machinery during crop management procedures, which increase the pressure under the soil surface, promoting soil compaction (Sales et al., 2018). Awe et al. (2020), studying changes in soil physical attributes in sugarcane areas, reported an increase in Sd up to the 0.40-m layer, due to machine traffic and soil revolving, corroborating the results of Rosset et al. (2014b) in sugarcane crop areas in the state of Mato Grosso do Sul. The highest values of Sd presented by the PP area are due to the absence of pasture maintenance, which favors degradation processes, such as surface disaggregation and particle rearrangement, thus increasing the Sd (Falcão et al., 2020). Vasques et al. (2019) in a pasture management study in Brazil, concluded that inadequate pasture management results in direct impacts on soil physical attributes, directly on Sd and soil porosity, which are extremely important in soil water regulation.

Table 2 – Soil density (Sd), total organic carbon (TOC), and carbon stock (StockC) in the different management systems in the municipality of Eldorado, MS.

MS	Sd	TOC	StockC
	Mg m^{-3}	g kg^{-1}	Mg ha^{-1}
		0–0.05 m	
SC	1.52a	12.68c	6.84b
PP	1.50a	15.79b	8.53ab
NTS	1.35a	15.01b	8.05ab
NF	1.08b	25.40a	10.18a
CV (%)	9.0	4.8	15.1
		0.05–0.10 m	
SC	1.63a	11.63c	7.00a
PP	1.74a	13.71b	8.25a
NTS	1.35b	12.57bc	7.56a
NF	1.20b	16.60a	7.63a
CV (%)	5.2	5.5	8.4
		0.10–0.20 m	
SC	1.62a	9.21b	11.42bc
PP	1.70a	13.00a	16.11a
NTS	1.49ab	11.13ab	13.82ab
NF	1.24b	9.72b	10.46c
CV (%)	7.9	8.8	9.3

MS: management systems; SC: sugarcane; PP: permanent pasture; NTS: no-tillage system; NF: native forest; CV (%): coefficient of variation. Means followed by equal letters in the column, in each layer, do not differ from each other according to the Tukey test ($p \leq 0.05$).

In the area of NF, the lowest values of Sd are associated with the intense litter deposition in these areas – which, together with the fact that there is no revolving, favors the activity of organisms (Borges et al., 2015), especially the edaphic macrofauna, that directly contribute to the decrease in Sd through their movement of the soil profile (Menandro et al., 2019; Velasquez and Lavelle, 2019). In all evaluated areas and layers, Sd values did not exceed 1.75 Mg m^{-3}, considered an impediment for root development of crops in this sandy soil condition (Reinert et al., 2008; Sales et al., 2016).

It is noteworthy that in the 0–0.05 and 0.05–0.10-m layers, the TOC contents of the managed areas are lower than the NF. In the 0.05–0.10-m layer, the SC, PP, and NTS presented contents of 11.63, 13.71, and 12.57 g kg^{-1}, respectively. These higher contents in the NF area are due to the higher litter deposition of different forest extracts (Ozório et al., 2019), which increases the TOC contents in the most superficial layers of the soil (Assunção et al., 2019). Several authors have reported higher TOC contents in higher native areas compared with production systems in different regions of Brazil, soil types, and management systems (Borges et al., 2019; Maia et al., 2019; Santos et al., 2019; Ferreira et al., 2020; Medeiros et al., 2020).

Regarding the 0.10-0.20-m layer, the highest levels of TOC were found in the PP area, with 13 g kg^{-1} (Table 2). These higher levels of TOC in more subsurface layers of areas cultivated with PP are explained because the root system of grasses deposits significant amounts of TOC in subsurface (Nanzer et al., 2019). Another explanation for the higher levels of TOC in the 0.10–0.20-m layer of PP, even with lower levels of TOC in surface layers, might be related to the process of pasture degradation by excessive grazing, with increased production of exudates in the roots, which increase the intake of C in more subsurface layers, as reported by Shen et al. (2020).

The highest StockC value in the 0–0.05-m layer was observed in the NF area, with 10.18 Mg ha^{-1}, being higher than the SC with 6.84 Mg ha^{-1}. In the 0.05–0.10-m layer, all the evaluated areas were similar to each other, ranging from 7.00 to 8.25 Mg ha^{-1}. In the 0.10–0.20-m layer, the highest values of StockC were observed in the areas of PP (16.11 Mg ha^{-1}) and NTS (13.82 Mg ha^{-1}), being higher than the area of NF (Table 2).

These results show that the management adopted in NTS and PP have contributed to the maintenance of the StockC both in surface and subsurface soil layers, a behavior previously observed by other authors (Rosset 2014a; 2014b; Rosset et al., 2016; Sales et al., 2018; Assunção et al., 2019; Rosset et al., 2019). This increase in soil StockC in the PP and NTS areas is important for improving soil quality, considering that C directly acts in the reduction of Sd (Velasquez and Lavelle, 2019; Falcão et al., 2020), porosity maintenance (Bertollo and Levien, 2019) as well as in the regulation of water infiltration (Silva et al., 2019), improving microorganism activity (Souza et al., 2018) and providing greater soil aggregation (Ozório et al., 2019; Udom and Omovbude, 2019).

The results of the TOC stratification index (SI) assessed in all studied areas, presented values above 1 (Figure 2). The managed areas did not present differences between them, with values ranging from 1.22 to 1.38, which were different from the NF area, which presented an SI value of 2.63.

According to Franzluebbers (2002) and Sá and Lal (2009), SI values greater than 1 indicate a high stratification ratio of soil C, which contributes to storing C in more subsurface soil layers. The highest SI value in the NF area is due to the constant entry of SOM into the soil surface, which causes the TOC content of the first layer to be higher in relation to deeper layers, as observed in Table 2. Salton et al. (2014) found SI of 1.70 for NF area in the *Cerrado*. Rosset et al. (2014a) reported a value of 3.43 SI in an Atlantic Forest area in western Paraná, Brazil.

Regarding the variation in carbon stock (ΔStockC), all managed areas showed negative variation, especially in the 0–0.05-m layer (Figure 3). This negative variation in StockC in the 0–0.05-m layer is more evident in the SC area. This is mainly due to the intense soil management in the SC area, where gradation is used for crop renewal (Lopes et al., 2017). This process breaks the soil aggregates, leaving the SOM exposed to microorganisms that consume this organic matter, releasing CO_2 (Bertollo and Levien, 2019; Gonçalves et al., 2019). This fact

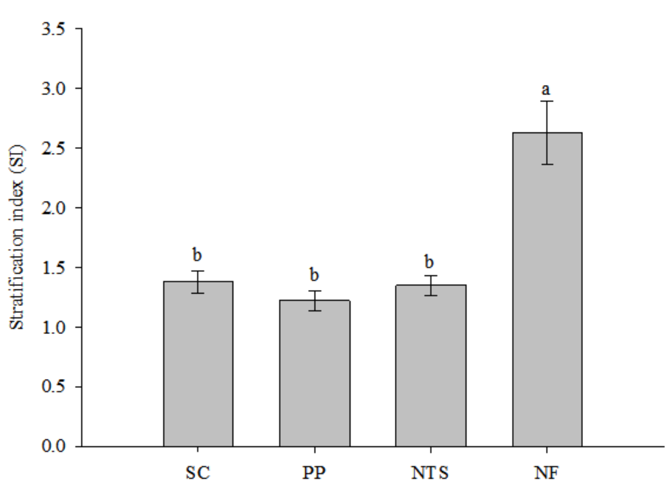

Figure 2 – Stratification index (SI) of total organic carbon as a function of different management systems in the municipality of Eldorado, MS. SC: sugarcane crop area; PP: permanent pasture; NTS: no-tillage system; NF: native forest.

was also observed by Rosset et al. (2014b) in areas with different forms of sugarcane cultivation in the municipality of Maracajú, MS, Brazil.

In the PP area, especially in the 0.10–0.20-m layer, but also in the 0–0.2-m profile, the positive variation in the StockC was higher than the other managed areas. Nanzer et al. (2019) found higher StockC in a *Brachiaria brizantha* PP area, mainly due to the continuous renewal of the root system. Shen et al. (2020) highlights the importance and contribution of pastures to increase the StockC in deeper soil layers, even in pastures that show signs of degradation.

Considering the ΔStockC in the three stratified layers evaluated together (profile of 0–0.2 m), it is possible to observe negative variation in the StockC only in the area of sugarcane cultivation (Figure 2). Some authors studying the effect of sugarcane production on soil C also observed StockC reduction in the layer of 0–0.2 m in SC areas in Brazil (Rosset et al., 2014b; Oliveira et al., 2016; Borges et al., 2019).

In the 0-0.05-m layer, the highest C-POM contents were observed in the NF area with 15.25 g kg^{-1}, and the lowest content was observed in the SC area (4.93 g kg^{-1}), whereas the PP and NTS areas presented intermediate levels. This same pattern was observed in the 0.05–0.10-m layer, where the SC area presented 43.68% of the C-POM content compared with the reference area (Table 3). Gomes et al. (2019), in a study in southeastern Brazil, concluded that areas of SC are prone to losses of C-POM, both by soil revolving during crop renewal, and by losses in erosive processes that occur between the rows of these areas.

In the NF area, the highest levels of C-POM are attributed to the continuous deposition of soil litter (Gazolla et al., 2015; Rosset et al., 2019), with different plant extracts and different carbon-to-nitrogen (C/N) ratios between them (Ozório et al., 2019). The C-POM is extremely important for soil quality due to the strong relationship with the formation of soil macroaggregates (Tisdall and Oades, 1982; Six et al., 2000).

Regarding the C-MOM in the 0–0.05-m layer, the highest content was observed in the NF area, 10.15 g kg^{-1}, and the areas of SC, PP and NTS were similar to each other, with contents from 6.75 g kg^{-1} to 7.75 g kg^{-1}, respectively (Table 3). The absence of soil disturbance in the NF area favors the humification of SOM in the most superficial layer of the soil (Rosset et al., 2019). Similar results were found by Bueno et al. (2017), according to which a secondary forest area presented C-MOM values higher than managed areas, in the 0-0.10-m layer, and by Rosset et al. (2019) in Atlantic Forest vegetation.

In the deepest layer evaluated (of 0.10–0.20 m), there is an increase in the C-MOM content in the PP area (Table 3). This increase corroborates the pattern of increase in the TOC content of this area (Table 2). The increase in TOC content in subsurface layers and soil stability in PP areas favor the SOM humification process, becoming more recalcitrant (Rosset et al., 2019; Shen et al., 2020).

The representativeness of POM was higher in the most superficial layer of the soil, while the MOM percentage (%MOM) increased according to increased depth in all areas (Table 3). The SC area presented lower POM percentage (%POM) than the other areas, with 38.75%, 35.14%, and 31.80% in relation to TOC, in layers of 0–0.05 m; 0.05–0.10 m; and 0.10–0.20 m, respectively. On the other hand, the SC area presented %MOM higher than 60% in all evaluated layers. This characterizes the absence of constant deposition of SOM (Bordonal et al., 2018), which hinders the balance between these two fractions of the SOM (Lal, 2018).

The areas of NF, PP, and NTS in all layers showed variations between 39% and 60% for %POM and %MOM, respectively, and they were influenced by the evaluated layer (Table 3). Higher %POM in surface soil layers is common due to the entry of organic matter deposited on the soil surface (Cotrufo et al., 2019; Rosset et al., 2019; Lavallee et al., 2020).

The NF area had the highest value of StockPOM in the first layer evaluated, 6.12 Mg ha^{-1}, with PP and NTS areas having similar values, and the SC area had the lowest StockPOM, 2.68 Mg ha^{-1}. In the 0.05–0.10-m layer, the areas of PP, NTS, and NF were similar to each other, ranging from 4.23 Mg ha^{-1} to 4.30 Mg ha^{-1}, higher than the SC area. Finally, in the 0.10–0.20-m layer, the PP and NTS areas showed the highest StockPOM, 7.17 Mg ha^{-1} and 7.22 Mg ha^{-1}, respectively (Table 3). C accumulation in the particulate fraction is important to keep the flow of biological activities in the soil (Batista et al., 2013), mainly

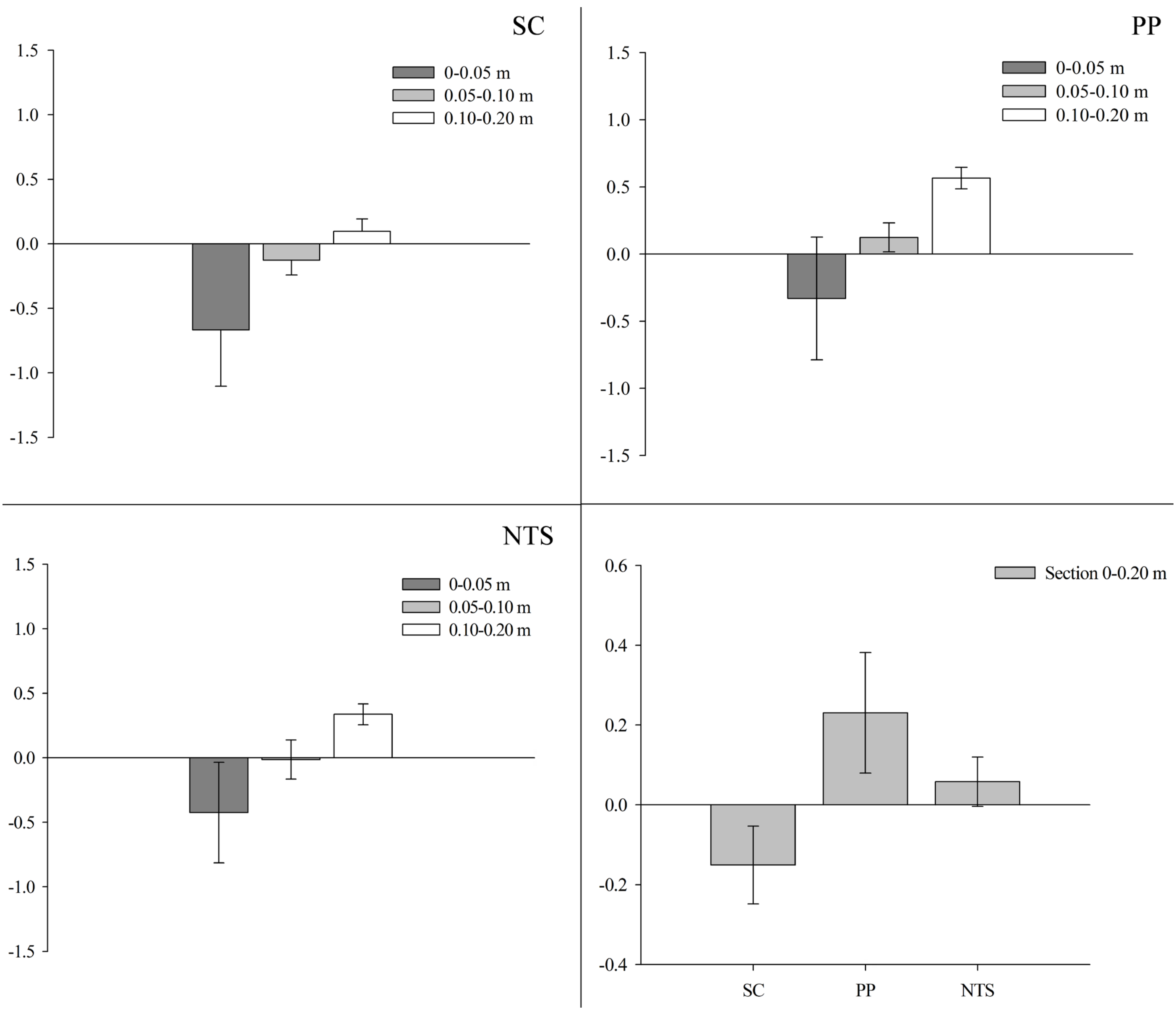

Figure 3 – Variation in TOC stock (ΔStockC) in the managed areas in the 0–0.05-m, 0.05–0.10-m, and 0.10–0.20-m layers in relation to the native forest area, and in the 0–0.20-m layer.

by microorganisms that consume this SOM, turning it into more stable fractions (Borges et al., 2015; Rosset et al., 2019).

When observing the carbon stocks associated with minerals (StockMOM), in the first two layers evaluated, no difference was observed between the studied areas, ranging from 3.62 Mg ha^{-1} to 4.16 Mg ha^{-1} in the 0–0.05-m layer, and from 3.32 Mg ha^{-1} to 4.53 Mg ha^{-1} in the 0.05–0.10-m layer. In the 0.10–0.20-m layer, the highest values were observed in the SC and PP areas differing from the NF area (Table 3). This fraction presents advanced humification stage, being highly stable due to the interaction with the soil colloids and being located inside stable microaggregates (Assunção et al., 2019; Rosset et al., 2019; Ferreira et al., 2020).

The NF area had higher values of CSI for the first two layers studied (Table 3). This difference between the managed areas and the NF demonstrates the potential for C accumulation in the first layers of soil of areas under native vegetation (Ozório et al., 2019). When only assessing the managed areas, the NTS and PP areas differed from the SC area in the 0–0.05-m layer. It is worth highlighting, mainly in the 0–0.05-m layer, the low value of CSI (0.50) in the SC area, indicating that this area is not efficient in stocking C (Bordonal et al., 2018). In the 0.10–0.20-m layer, the highest CSI was found in the PP area, being even higher than in the NF and SC areas (Table 3). The higher CSI in areas cultivated with PP are due to their volume of root system, which is efficient in the accumulation of C (Nanzer et al., 2019; Ozório et al., 2019; Shen et al., 2020).

In general, in all evaluated layers, the lability values (L) of the SOM ranged from 0.47 to 1.50. In the three layers evaluated, the SC area had values lower than 0.64, different from the other managed areas, PP and NTS, and the NF area (Table 3). Regarding the LI, the same L pattern was found in the 0–0.05-m and 0.05–0.10-m layers, with the SC presenting the lowest values and the other treatments being similar to each other.

In this study, the L and LI values showed differences in C quality between the managed areas, especially comparing the SC area with the others (Table 3), demonstrating sensitivity in detecting changes in soil organic fraction of the evaluated areas. This assessment is essential to evaluate how different management systems alter soil attributes, thus allowing to develop strategies to minimize the negative impacts of agricultural production over the years of cultivation (Lal, 2018).

Table 3 – Carbon of particulate organic matter (C-POM), carbon of mineral organic matter (C-MOM), POM percentage (%POM), MOM percentage (%MOM), carbon stock of particulate organic matter (StockPOM), carbon stock of mineral organic matter (StockMOM), carbon stock index (CSI), lability of SOM (L), lability index (LI), and carbon management index (CMI) in the different management systems in the municipality of Eldorado, MS*.

MS	C-POM	C-MOM	POM	MOM	StockPOM	StockMOM	CSI	L	LI	CMI
	--------g kg^{-1}--------		-------%-------		--------Mg ha^{-1}--------		----------------------------			
	0–0.05 m									
SC	4.93c	7.75b	38.75b	61.25a	2.68c	4.16a	0.50c	0.64b	0.42b	21.30c
PP	8.54b	7.25b	54.44a	45.56b	4.58b	3.94a	0.62b	1.22a	0.81a	49.80b
NTS	8.26b	6.75b	54.92a	45.08b	4.42b	3.62a	0.59b	1.22a	0.82a	48.37b
NF	15.25a	10.15a	60.05a	39.95b	6.12a	4.06a	1.00a	1.50a	1.00a	100.00a
CV (%)	6.1	10.0	7.6	8.2	14.6	18.4	4.7	15.9	15.8	10.6
	0.05–0.10 m									
SC	4.08c	7.55a	35.14b	64.86a	2.45b	4.53a	0.70c	0.55b	0.42b	29.73b
PP	7.30b	6.41ab	53.66a	46.34a	4.38a	3.86a	0.83b	1.20a	0.96a	78.04a
NTS	7.05b	5.52b	56.25a	43.75b	4.23a	3.32a	0.75c	1.30a	1.03a	77.46a
NF	9.34a	7.26ab	56.24a	43.76b	4.30a	3.32a	1.00a	1.29a	1.00a	100.00a
CV (%)	7.2	14.2	10.5	10.6	9.6	16.0	4.0	23.7	29.9	24.6
	0.10–0.20 m									
SC	2.92c	6.29ab	31.80c	68.20a	3.61b	7.80ab	0.95b	0.47c	0.59c	56.57d
PP	5.79a	7.21a	44.61b	55.39b	7.17a	8.93a	1.34a	0.81b	1.03b	137.39b
NTS	5.82a	5.31b	52.31a	47.69c	7.22a	6.59bc	1.15ab	1.10a	1.40a	160.36a
NF	4.26b	5.46b	44.00b	56.00b	4.59b	5.86c	1.00b	0.79b	1.00b	100.00c
CV (%)	8.5	10.7	4.6	3.5	8.9	11.2	8.9	6.8	7.3	8.1

*Means followed by equal letters in the column, in each layer, do not differ from each other according to the Tukey test ($p \leq 0.05$); CV: coefficient of variation.

It was observed that in the 0–0.05-m layer, the NF area presented higher CMI in relation to the managed areas, and the cultivation areas presented values between 21.30 and 49.80 in the 0–0.05-m layer, and 29.73 and 78.04 in the 0.05–0.10-m layer (Table 3). This shows the impact that the areas of SC, PP, and NTS caused on the quantity and quality of C in the most superficial layers of the soil, compared with the NF area. Similar results were observed by other authors in several study areas (Rosset et al., 2019; Lavallee et al., 2020; Poffenbarger et al., 2020).

Considering the CMI results in the 0.10–0.20-m layer, the PP and NTS areas exceed the reference value of the NF, which indicates that even compromising the quantity and quality of C on the surface, the systems have been contributing to the improvement of SOM in the most subsurface layer of the soil. This may benefit the edaphic quality in these areas, favoring other chemical, physical, and biological attributes of the soil (Lal, 2018; Assunção et al., 2019; Ozório et al., 2019; Rosset et al., 2019; Ferreira et al., 2020).

Conclusions

The managed areas modify the density, total organic carbon content, and soil carbon stock when compared with the reference area.

The particulate and mineral fractions of soil organic matter are altered in the different management systems, and the sugarcane area compromised the presence of particulate organic matter.

The managed areas, through the evaluation of the carbon management index, compromise organic matter in the most superficial layers.

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Contribution of authors:

Morais, D.H.O.: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing — original draft. Silva, C.A.: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing — original draft. Rosset, J.S.: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Visualization, Supervision, Project administration. Farias, P.G.S.: Methodology, Validation, Formal analysis, Investigation, Resources. Souza, C.B.S.: Formal analysis, Investigation, Resources, Data curation, Writing — review & editing. Ozório, J.M.B.: Resources, Software, Project administration, Formal analysis, Funding. Castilho, S.C.P.: Methodology, Validation, Formal analysis, Investigation, Visualization. Marra, L.M.: Methodology, Validation, Formal analysis, Investigation, Supervision, Visualization.

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ABES

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Medicinal plants of the Unified Health System (Sistema Único de Saúde) with antifungal potential

Plantas medicinais do Sistema Único de Saúde com potencial antifúngico

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ABSTRACT

Medicinal plants synthesize various secondary metabolites that can be used for therapeutic and antimicrobial purposes. In Brazil, the Unified Health System (SUS) offers several herbal medicines as an alternative in the treatment of various diseases. Considering the importance of these plants in the production of chemicals that expand therapeutic options and improve the health of SUS users, this review was carried out to quantitatively determine the antifungal activity of plants used as phytotherapeutics at RENAME. The selection of papers was performed at three distinct stages: examining and choosing titles related to antifungal action, reading the abstracts, and reading the whole selected articles. This review selected 22 studies of interest; 12 of them were conducted in Brazil and 10 were carried out in other countries. The papers chosen tested the growth inhibitory effect of plants against fungal species of agricultural and health importance, ranging from filamentous to yeast-like fungi, and *Candida albicans* was the most tested species. The growth of 39 fungal species were inhibited by some concentration of the extract used, with either an increase or decrease in antifungal activity depending on the extract used. The most frequently analyzed plant was the species *Schinus terebinthifolius* Raddi., studied in seven papers. The results found demonstrate the importance of analyzing medicinal plants and incorporating plant-based medicines in healthcare as an alternative source of treatment, highlighting the need for studies that evaluate the mechanisms action of their cytotoxicity and therapeutic effects in the human body.

Keywords: herbal medicines; fungi; secondary metabolites.

RESUMO

As plantas medicinais produzem uma série de metabólitos secundários que podem ser usados para fins terapêuticos e antimicrobianos. No Brasil, o SUS disponibiliza uma série de medicamentos fitoterápicos como alternativa ao tratamento de diversas enfermidades. Considerando a importância da utilização dessas plantas na produção de medicamentos que ampliem as opções terapêuticas e melhorem a atenção à saúde de usuários do sistema, elaborou-se este estudo de revisão com o objetivo de estimar quantitativamente a atividade antifúngica das plantas utilizadas como fitoterápicos contidas na RENAME. A seleção de artigos deu-se por meio de três etapas distintas: leitura e escolha de títulos relacionados à ação antifúngica, leitura dos resumos e leitura na íntegra dos artigos selecionados. Esta revisão selecionou 22 estudos de interesse, sendo 12 elaborados no Brasil e 10 em outros países. Os artigos escolhidos testaram a ação inibitória das plantas contra espécies de fungos de importância agrícola e sanitária, entre filamentosos e leveduriformes, sendo *Candida albicans* a espécie mais testada. Trinta e nove espécies foram inibidas por alguma concentração do extrato utilizado, havendo aumento ou diminuição da atividade antifúngica conforme substância extratora utilizada. A planta mais analisada foi a espécie *Schinus terebinthifolius* Raddi., estudada em sete artigos. Os resultados encontrados demonstram a importância da análise de plantas medicinais e da incorporação de medicamentos à base de plantas como fonte alternativa de tratamento, salientando a necessidade de estudos que demonstrem sua citotoxicidade e mecanismos de ação terapêutica no organismo humano.

Palavras-chave: medicamentos fitoterápicos; fungos; metabólitos secundários.

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Introduction

the set of chemical reactions performed by plants produces a series of substances called secondary metabolites, which include flavonoids, tannins, alkaloids, saponins, coumarins and quinones, also encompassing the so-called essential oils or essences. These metabolites have various functions, which are related to the defense against animals, insects and other plants, protection from physical factors and attraction of animals for reproductive purposes (Simões et al., 2017; Tamariz-Angeles et al., 2018). In addition, many of these substances are important for characterizing and confirming the identity and quality of the plant in question, also manifesting antimicrobial activities, especially against bacteria, protozoa and fungi (Maciel et al., 2017).

Several studies have shown the beneficial use of extracts and active principles extracted from plants against pathogens that can infect and cause harm to animals, humans and plant crops (Chansue, 2007; Itako et al., 2009; Marmitt et al., 2015a; 2015b; Pinho et al., 2012; Rodrigues et al., 2007). New antimicrobial assets derived from natural sources can decrease the amount of pesticides and other chemical products for environmental control applied on farms, reducing the risks to our health and to the environment. Hence, they become a safer — and often more effective — alternative than pesticides (Mauli et al., 2009; Schwan-Estrada et al., 2000). they can also be an alternative to prevent many microorganisms from developing resistance to drugs used for their control, which increases the incidence of diseases caused by these pathogens and hampers the treatment of people affected (Cavalcanti et al., 2012; França et al., 2009; Maciel et al., 2017; Nogueira et al., 2008).

In 2009, the National List of Medicinal Plants of Interest to SUS (RENISUS) was established. Consisting of 71 plant species with therapeutic potential according to traditional medicine knowledge, with the purpose of promoting the study of herbal medicines and their production in Brazil (Brazil, 2009a; 2009b). The provision of plant-derived medicines by the Brazilian public healthcare system began in 2007 through the National List of Essential Medicines (RENAME) (Brazil, 2017b), and currently comprises 12 plants: *Aloe vera* (L.) Burm.f., *Cynara scolymus* L., *Glycine max* (L.) Merr., *Harpagophytum procumbens* (Burch.) DC. ex Meisn., *Maytenus ilicifolia* Mart. ex Reissek, *Mentha piperita* L., *Mikania glomerata* Spreng., *Plantago ovata* Forssk, *Rhamnus purshiana* DC., *Salix alba* L., *Schinus terebinthifolius* Raddi, and *Uncaria tomentosa* (Willd. ex Schult.) DC.; offered in the form of syrups, tablets, capsules, gels, creams and dyes (Brazil, 2012; 2017a; 2017b).

Considering the purpose for which RENISUS has been created, the aim of this systematic review was to show the amount of studies published on medicinal plants with antifungal activity as described in the national list of medicinal plants provided by SUS, using the CAPES (Coordination for the Improvement of Higher Education Personnel) Journals Portal as database, which encompasses publications from several other platforms, such as SciELo, PubMed and Springer, among others.

Materials and methods

The present study used a systematic review of the literature as a technical procedure for gathering scientific information encompassing different cases, locations and perspectives from different researchers, summarizing their objectives and results in a simplified manner (Greenhalgh, 1997; Carneiro; Takayanagui, 2009). The studies addressed here were those concerning the antifungal potential of medicinal plants provided as phytotherapeutics by SUS through RENISUS, and those with the full text available at the Capes Journals Portal in English, Portuguese or Spanish, were analyzed with no restrictions as to their publication year. Descriptors used were the scientific names of the plants (or phytotherapeutics) at RENAME and the term "antimicrobial", followed by the Boolean operator "and", with no restrictions of language or publication year.

The access link used was the one made available in the database. The titles of all the papers found when searching each species were read and the repeated ones were excluded, thus being counted only once. Review studies, interviews, reports, and studies on the practical use of plants or referring to their chemical constituents without either confirming or proving their antifungal effect were also excluded.

A total of 10,763 articles were found in the initial search; 382 were reports and 267 were repeated scientific papers, and they were thus excluded from the study. Of all articles found in the initial search, 417 studies were selected to comprise the further stages of this review. The publications chosen were analyzed in three stages: first, by reading the title of all articles found in the database, and selecting those that included terms related to antifungals, such as "fungos", "fungi", "levedura", "yeast", "*Aspergillus*", "*Candida*", "*Penicillium*", "*Fusarium*" and "antifúngico", among others. A total of 67 studies were selected. Afterwards, the next stage consisted of reading the abstracts of the papers selected in the first stage, which resulted in 32 papers selected. Those that somehow mentioned the method used and proved the antifungal effect of the studied plant were chosen. In the third and last stage, the papers selected in the second phase were completely read, to prove the antifungal effect of the plants of interest, and 22 studies were found to be compatible with the criteria established here, and were thus chosen to be included in the present review. The scientific names of the plants mentioned were written according to updated references for native plants in Brazil (Reflora, 2010) and introduced plants (Taxonomic Name Resolution Service, 2020).

During the search, a methodological adjustment was required to include the plant *Glycine max*. While using the same procedure adopted for the other plants, it was not possible to read the papers available starting on page 281 due to problems in the platform used for the search. To complete the search for this plant, restricting the publication year to 2009, the year in which RENISUS was created, was required. Only with this adjustment, was it possible to access all the studies published as of this date and the search for this descriptor was not complete.

Results and Discussion

After reading the 32 selected papers completely, 22 studies of interest were selected for inclusion in the present review, which corresponds to 5.3% of the total relevant papers initially found in the database. Chart 1 shows the total number of papers selected for each study of interest of each plant analyzed, followed by methodology and main results.

Of the 12 plants that comprised this study, no studies on *Maytenus ilicifolia*, *Salix alba* and *Rhamnus purshiana* were selected, due to the fact that none of the papers found met the selection criteria. The papers were organized in a distribution chart by antifungal potential and therapeutic action (Chart 1). There were three publications in 2011, which was the year with the highest number of publications. The most recent research among the papers selected was from 2017, and the oldest was from 2002.

Of the 22 selected studies, 12 (54.5% of the total) were conducted by Brazilian researchers (Biasi-Garbin et al., 2016; Braga et al., 2007; Duarte et al., 2005; Freire et al., 2012; Holetz et al., 2002; Johann et al., 2008; Martinelli et al., 2017; Moraes et al., 2015; Moura-Costa et al., 2012; Schmourlo et al., 2005; Santos et al., 2010; Souza Júnior et al., 2011), which indicates that the creation of RENISUS did promote medicinal plant studies in Brazil.

Reports indicate that medicinal plants were already used in Europe in 460 B.C. in the form of hot and iced teas, moist compresses and dried herbs. They are still used today by many European countries as complementary medicines in the treatment of many diseases. Several other cultures have also relied on the properties of medicinal plants for thousands of years, with several species still in use to date (Van Wyk; Wink, 2018). Of the 22 selected articles in this study, nine (41%) were carried out in other countries, especially India and Iran, with two studies each. The most tested plants in these studies were *Mentha piperita*, with 6 studies, and *Cynara scolymus*, with 2 studies.

In the international scenario, studies on medicinal plants, especially those focused on public health systems, have been disseminated since the First International Conference on Primary Health Care, held in 1978 in Russia (Gonçalves et al., 2017). Heinrich (2010), studying medicinal plants worldwide, indicated an increase in the search for phytotherapeutics for the treatment of AIDS/HIV and viral diseases. In the same study, he points out that research has focused on the innovation of phytochemical studies for the isolation and identification of active ingredients. Since 1979, the World Health Organization has suggested the inclusion of complementary alternative medicine therapies in public health policies, with their use reaching 75% in France, 70% in Canada, and 42% in the USA (Zeni et al., 2017).

Estimates indicate that 70–80% of drug development depends exclusively on plants, and much of the research in this area is focused on the isolation of plant active ingredients (Aslam; Ahmad, 2016). According to Rajeswara Rao and Rajput (2010), the major research areas on medicinal plants in the world are: development of technologies for the cultivation and isolation of chemical compounds, implementation of quality control protocols, scientific validation of the traditional use of these plants, development of products using them as a production base, selection of markers for genotypes, creation of a DNA profile of the species studied, identification and isolation of enzymes and bioactive molecules, and screening of phytochemical compounds with antimicrobial properties.

The nine international studies selected for this review were *in vitro* and 66.7% (6) also analyzed the chemical compounds present in the study plant, using chromatographic methods. None of them, however, identified the specific compound accounting for the antifungal activity observed.

Of the international studies selected, seven (77.8%) were carried out after 2010, one of which was published in 2019. Compared to the Brazilian studies, where 58.3% were published in or after 2010, there was an increase in studies in this area at the global level after this period. In a comparative study, Assis et al. (2015) showed a strong growth trend in research on medicinal plants in analyzing 111 research groups between 1997 and 2010, a trend that has continued to intensify in later years.

It is known that most medicinal plants are available in tropical countries (Dar et al., 2017). India and Iran stood out as the countries with the highest number of papers of interest, with two studies each (Desam et al., 2019; Tyagi; Malik, 2011; Saharkhiz et al., 2012; Mahboubi; Kazempour, 2014), having an important diversity of plants relevant to the healthcare area (Panda et al., 2018; Tandon; Yadav, 2017; Sadat-Hosseini et al., 2017; Parsaei et al., 2016).

The pharmacology of natural products allows determination of the bioactive compounds present in plants, that are responsible for growth-inhibitory effects. These bioactive compounds, also called secondary metabolites, have antimicrobial effects due to their cytotoxicity, and because of their neuroactive effects, they can be used as analgesics, anesthetics and antidepressants, among others (Vizzotto et al., 2010). As indicated by Piriz et al. (2014), medicinal plants have several beneficial properties that can be used for the benefit of humans. In their study, Piriz et al. (2014) found relevant results of studies on anti-inflammatory activity, especially in Brazil, which was the country with the highest number of papers published. The same number of papers produced in Brazil (12) was found in the present study, showing the importance and competence of Brazilian research demonstrated in this area.

Marmitt et al. (2015b), in a review, searched for plants with antibacterial effect in RENISUS, and found that 42% of the 19 selected published studies of interest were conducted in Brazil, thus indicating an increase in studies in the country after the creation of the National Policy on Medicinal and Herbal Plants (PNPMF). Similar results were found in the present study: of the 13 studies carried out in Brazil, 69% (9) were published after the creation of PNPMF. PNPMF (Brazil, 2006) has contributed to the increase in research aimed at the development of new plant-derived drugs, which are already used popularly in conventional medicine.

Chart 1 – Antifungal potential and therapeutic action of medicinal plants evaluated in the studies of interest selected.

Plant species (Family)	Part of the plant / compound / concentration used / treatment regimen (dose)	Therapeutic activity	Type of study	Year of publication/ Journal/ Country
Aloe Vera (Xanthorrhoeaceae)	Gel from the mucilaginous part of the leaf plus potato dextrose agar (PDA) / Concentrations of 1, 5, 25, 50, and 100 mL/L	Growth-inhibitory activity was detected at the lowest concentrations of the gel, which was effective against the fungi *Penicillium digitatum* and *Botrytis cinerea*	In vitro	2010/ *Postharvest Biology and Technology*/ Spain
Cynara scolymus (Asteraceae)	Chloroform, ethyl acetate and butanol extract of dry leaves / Doses of 2.5, 5 and 10 mg/mL	The butanol extract exhibited higher growth-inhibitory activity and was the only one capable of preventing the growth of *Candida lusitaniae* and *Mucor mucedo*	In vitro	2004/ *Journal of Agricultural and Food Chemistry*/ China
Cynara scolymus (Asteraceae)	Extract of powder from dry leaves with ethanol at the concentrations 25, 50, 75 and 97% v/v, 50% methanol, and pure water / Concentrations from 2.5 to 20 mg/mL	The 75 and 97% ethanol extracts proved to have the highest activity against strains of *Candida albicans Candida* sp.	In vitro	2011/ *Tropical Journal of Pharmaceutical Research*/ Romania
Mentha piperita (Lamiaceae)	40 g of fresh plant parts to obtain the essential oil, using 50 mL of dichloromethane for extraction / Dose of 2–0.03 mg/mL	It showed moderate activity (MIC of 0.6 mg/mL) in the control of *Candida albicans* strains	In vitro	2005/ *Journal of Ethnopharmacology*/ Brazil
Mentha piperita (Lamiaceae)	Dried seed extract obtained using 95% ethanol / Concentrations of 50, 25, 12.5, and 6.25 mg/mL / Dose 1 mg/mL	The extract could inhibit *Aspergillus niger* and *Candida albicans* with MIC of 5 mg/mL	In vitro	2006/ *Biology*/ Turkey
Mentha piperita (Lamiaceae)	Hydrodistilled extract of leaves (1:7 w / v), extracted using dichloromethane (5:1 v/v) / Concentration 0.1-0.2% (v/v)	The essential oil inhibited the growth of *Aspergillus flavus, Aspergillus glaucus, Aspergillus niger, Aspergillus ochraceous, Colletotrichum gloeosporioides, Colletotrichum musae, Fusarium oxysporum*, and *Fusarium semitectum*	In vitro	2011/ *Journal of Food Safety*/ Brazil
Mentha piperita (Lamiaceae)	Essential oil extracted using ethanol and diethyl ether / Concentrations of 0.14–18 mg/mL / Doses of 10–40 µL	The extract was effective against *Aspergillus flavus, Aspergillus niger, Mucor spp., Fusarium oxysporum, Candida albicans*, and *Saccharomyces cerevisiae*, and its highest efficacy was against *Penicillium digitatum*	In vitro	2011/ *Food Control*/ India
Mentha piperita (Lamiaceae)	Hydrodistillation of aerial parts during flowering / at 1:50 and 1:1,000 dilutions	The oil could inhibit *Cryptococcus neoformans, Aspergillus flavus, Aspergillus fumigatus, Aspergillus oryzae, Aspergillus clavatus*. It also completely inhibited biofilm formation by *Candida albicans* and *Candida dubliniensis*	In vitro	2012/ *International Scholarly Research Network*/ Iran
Mentha piperita (Lamiaceae)	Extraction of essential oil by hydrodistillation of the aerial parts of the plant at the beginning of flowering / Concentrations of 16–0.25 µg/mL	The oil exhibited antimicrobial activity against strains of *Candida albicans, Candida glabrata*, and *Aspergillus niger*	In vitro	2014/ *Songklanakarin Journal of Science and Technology*/ Iran

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Chart 1 – Continuation.

Plant species (Family)	Part of the plant / compound / concentration used / treatment regimen (dose)	Therapeutic activity	Type of study	Year of publication/ Journal/ Country
Mentha piperita (Lamiaceae)	Hydrodistillation of dry leaves to obtain the extract dissolved in 95% ethanol / Concentrations of 4, 2, 1, 0.5, 0.25, 0.125 µL/mL	It prevented the growth of *Microsporum canis, Epidermophyton floccosum, Trichophyton rubrum*, and *Trichophyton mentagrophytes* at concentrations of 2.0 and 4.0 µL/mL	In vitro	2015/ *Environmental Health and Preventive Medicine*/ Egypt
Mentha piperita (Lamiaceae)	Hydrodistillation at 1:5 concentration / dose of 1 µL	The essential oil exhibited strong antifungal activity against *Alternaria, Penicillium* spp., *Fusarium oxysporum, Fusarium tabacinum, Aspergillus fumigatus, Candida albicans, Cladosporium herbarum*, and *Rhizoctonia solani*	In vitro	2017/ *Journal of King Saud University*/ India
Mikania glomerata (Asteraceae)	Lower parts of the plant were macerated with water and ethanol (90–10%) / Dose of 2 mg/mL	It exhibited moderate activity (MIC of 100–500 µg/mL) against *Candida krusei* and *Candida parapsilosis*	In vitro	2002/ *Memórias do Instituto Oswaldo Cruz*/ Brazil
Mikania glomerata (Asteraceae)	40 g of fresh parts of the plant were used to obtain the essential oil, using 50 mL of dichloromethane for extraction / Dose of 2-0.03 mg/mL	It exhibited weak inhibitory activity (MIC > 2.0 mg/mL) compared to *Candida albicans*	In vitro	2005/ *Journal of Ethnopharmacology*/ Brazil
Schinus terebinthifolius (Anacardiaceae)	Aqueous-ethanol extract (20:3 mg/mL) from the aerial parts / Concentration of 1 mg/mL	The aqueous extract was able to prevent the growth of *Candida albicans*	In vitro	2005/ *Journal of Ethnopharmacology*/ Brazil
Schinus terebinthifolius (Anacardiaceae)	Methanol extract (3:2,000) / Dose of 100 mg/mL	It exhibited antifungal activity against *Candida albicans* and *Cryptococcus neoformans*	In vitro	2007/ *Journal of Ethnopharmacology*/ Brazil
Schinus terebinthifolius (Anacardiaceae)	Oil extraction from leaves with 80% ethanol and addition of water, hexane, dichloromethane and ethyl acetate / Concentrations from 1,000 to 7.8 µg/mL	The ethyl acetate extract portion exhibited higher antifungal activity against strains of *Candida albicans*	In vitro	2008/ *World Journal of Microbiology and Biotechnology*/ Brazil
Schinus terebinthifolius (Anacardiaceae)	Extract obtained by hydrodistillation / Concentrations of 25, 50, 75 and 100% / Dose of 100 µL/mL	The 25% dilution was able to inhibit the growth of *Colletotrichum* sp., *Alternaria* spp. and *Botrytis* spp. *Fusarium* spp. was only inhibited by the 50% dilution	In vitro	2010/*Revista Brasileira de Farmacognosia*/ Brazil
Schinus terebinthifolius (Anacardiaceae)	Hydroalcoholic extract of the bark (1:10) / Dose of 2 mg/mL	It exhibited inhibitory activity against strains of *Candida tropicalis* and *Candida parapsilosis*	In vitro	2012/ *Journal of Ethnopharmacology*/ Brazil
Schinus terebinthifolius (Anacardiaceae)	Aqueous, ethanol and acetone extracts / Concentrations from 1,000 to 1.95 µg/mL / Dose of 2,000 µg/mL	Ethanol extract exhibited strong activity against *Trichophyton rubrum* and *Trichophyton mentagrophytes*	In vitro	2016/*Revista do Instituto de Medicina Tropical de São Paulo*/ Brazil

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Chart 1 – Continuation.

Plant species (Family)	Part of the plant / compound / concentration used / treatment regimen (dose)	Therapeutic activity	Type of study	Year of publication/ Journal/ Country
Schinus terebinthifolius (Anacardiaceae)	Dichloromethane and oleoresin extract / Concentrations of 1–0.05%	Oleoresin and the extract exhibited moderate activity against strains of *Candida albicans* and *Penicillium* sp.	In vitro	2017/ *Ciência Rural*/ Brazil
Uncaria tomentosa (Rubiaceae)	Hydroalcoholic extract 70% of the stem in a 1:1 ratio / Concentrations at 1:512 dilution	It exhibited strong activity against the strains of *Candida albicans*, *Candida krusei*, *Candida tropicalis*, *Candida guilliermondii* up to 1:16 dilution	In vitro	2011/ *Pesquisa Brasileira em Odontopediatria e Clínica Integrada*/ Brazil
Uncaria tomentosa (Rubiaceae)	Hydroethanolic extract (50% v/v) / Concentration of 1 mg/mL	It exhibited strong activity against the resistant species *Candida krusei* and *Candida glabrata*	In vitro	2015/ *Industrial Crops and Products*/ Brazil

Source: Castillo et al. (2010), Ertürk (2006), Ibrahim and El-Salam (2015), Vamanu et al. (2011) and Zhu et al. (2004).

Different numbers of papers were found for each plant species selected, indicating a variety and effectiveness of different mechanisms of action in each species. The therapeutic activity shown is related to the extraction method and dose used. Extraction using aqueous-ethanol solvents afforded higher growth-inhibitory activity (27 different strains growth-inhibited), followed by extraction of essential oils using hydrodistillation (22 different strains growth-inhibited) and extraction using dichloromethane (12 different strains growth-inhibited). Different solvents extract different active principles from the plant, which are responsible for the effects caused by the use of medicines containing these substances (e.g., anti-inflammatory, hypoglycemic, anticoagulant and antiplatelet activities) (Sixel; Pecinalli, 2005).

Studies show that the use of the crude extract, shows a considerably higher bioactivity than using each active principle separately (Sixel; Pecinalli, 2005). The studies analyzed in this review, therefore, showed higher antimicrobial activity, since all the assays were performed using crude extracts. Among all the plants analyzed, approximately 54% (12) showed growth-inhibition in all of the fungal species tested, and 41% (9) at least prevented the growth of 50% of the species analyzed. The butanol extract of *Schinus terebinthifolius* was tested against *Candida neoformans*, *Candida albicans*, and *Trychophyton rubrum*, and inhibited only *Candida albicans* strains.

Analyzing the data obtained, *Candida albicans* was the most tested microorganism in all papers selected, totaling 16 studies analyzing extracts and oils against strains of this species. Overall, 18 studies (78.3% of the total) analyzed seven *Candida* species regarding their sensitivity to medicinal plant extracts and oils. Filamentous fungi were also part of the selected studies. Nine species of *Aspergillus* were used in seven articles (39.2% of the total), and *Aspergillus niger* was most studied, appearing in six papers.

According to Correia et al. (2006), the family Anacardiaceae is rich in bioactive compounds that may have antimicrobial effects, with the genus *Schinus* being among the 11 most studied in this family. Correia et al. (2006) demonstrated the presence of phenolic lipids in this plant. These compounds have amphipathic characteristics, which facilitate their penetration into the plasma membrane, causing changes in their structure and properties. Therefore, seven of the 22 studies selected for this review analyzed the bioactive and antimicrobial activity of *Schinus terebinthifolius*, which prevented the growth of 78% (14) of the 18 strains tested.

Another important factor, pointed out by Gobbo-Neto and Lopes (2007), indicates that changes in the concentrations of active compounds may occur due to circadian cycle (day/night), seasonality, plant age, development period, temperature, attack by pathogens, pollution, and the hormonal development process in the plant. Evans (2009) showed significant differences in the presence of chemical compounds during winter and summer. Vasconcelos Silva et al. (1999) demonstrated how the circadian cycle affects the production and concentration of bioactive substances, showing high quantitative contrasts at different times of the day.

Stress caused by temperature variations can also affect the concentrations of these compounds, as indicated by Christie et al. (1994). Plant age is also related to these changes. Flowering period leads to increase or decrease in certain substances, as well as the period of leaf emergence or loss in deciduous plants. This indicates why the same plant species had different results in this paper. Two studies carried out with *Mentha piperita* (Mahboubi; Kazempour, 2014) (Saharkhiz et al., 2012) used *Aspergillus flavus* samples to test the antimicrobial action of essential oils extracted using hydrodistillation. The first study used the aerial parts at the beginning of flowering to obtain essential oils and observed excellent growth-inhibitory activity. However, the second study, which also used aerial parts to extract essential oils, although after the beginning of flowering, found no growth-inhibitory action at any concentration in *Aspergillus flavus* strains.

The same occurred in studies carried out by Schmourlo et al. (2005) and Biasi-Garbin et al. (2016), who tested the action of the ethanol extract of the species *Schinus terebinthifolius*. The extract was prepared using different parts of the plant in each study, differing only in the concentration used (Chart 1). At the end of the study, Biasi-Garbin et al. (2016) were able to inhibit the growth of the pathogenic fungus *Trichophyton rubrum*, while Schmourlo et al. (2005) showed no effect on this fungus. Schmourlo et al. (2005) and Braga et al. (2007) conducted a study with the same plant species using extracts against *Candida neoformans* strains. Alcoholic extracts were obtained for both studies; Schmourlo et al. (2005) used ethanol as extraction solvent and Braga et al. (2007) used methanol. In the first study, the extract did not prevent the growth of *Candida neoformans*, while it was inhibited in the second study, thus indicating that different solvents affect the composition of bioactive substances, either intensifying or preventing the effect of the extract, as already observed by Silva (2010). Silva (2010) compared different extracts of essential oils, classifying the different activities exhibited by each extract. Thus, he identified significant differences in the action of different solvents, related to solubility and affinity with the plasma membrane of the strains he compared.

Conclusion

Of the 417 papers initially read, 22 that demonstrated an antifungal effect were selected for the present review. The two plants that exhibited the highest antifungal activity were *Mentha piperita*, with eight studies, and *Schinus terebinthifolius*, with seven studies. *Candida albicans* was the most frequently tested fungal species, as it was present in 11 different studies. Other species of the genus *Candida* were tested in 10 different studies. The genera *Fusarium* and *Aspergillus* also stood out, with five and 13 studies, respectively.

The incorporation of integrative and complementary practices within the scope of SUS through PNPMF helps to understand and perceive the importance of studies on this topic. RENAME has also played an important role in the increase and constant evolution of the research on herbal medicines in Brazil, especially after their inclusion in SUS.

There is a therapeutic equivalence between essential oils and extracts used to obtain active ingredients. Thus, the importance of evaluating these compounds for cytotoxicity, bioavailability and therapeutic action in humans is evident to ensure that they can be used as drugs, having proven their extensive activity against microorganisms.

Contribution of authors:

Maciel, M. J.: Supervision, Funding Acquisition, Formal Analysis, Writing, Review & Editing. Rempel, C.: Supervision, Funding Acquisition, Formal Analysis, Writing, Review & Editing. Stroher, A. L.: Methodology, Investigation, Data Curation, Writing. Bergmann, P. C.: Methodology; Investigation, Data Curation, Writing. Marmitt, D. J.: Supervision, Formal Analysis, Writing, Review & Editing.

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ABES

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Water loss associated with food loss and waste in Brazil

Perda de água associada a perda e desperdício de alimentos no Brasil

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ABSTRACT

This article aimed to estimate the loss of water associated with food loss and waste in Brazil in 2013. Data from the Food and Agriculture Organization (FAO) of the United Nations (UN) on food balance and waste, as well as the Water Footprint (WF) of agricultural products available at Water Footprint Network (WFN) were used. Results show that food waste reaches 49 million metric tons per year, compromising a total of 87 billion cubic meters of water, which is higher than the average annual flow of the river São Francisco. Major water loss is associated with the agricultural production stage (32%), followed by consumption (19%). Amongst food groups, major water loss is associated with meat (49%), followed by cereals (19%). Roughly 96% of water loss is attributed to the green water component, which highlights that attention must be paid to rainfed agriculture to ensure food and water for everyone. The loss of blue water was more than half of the volume consumed in the urban sector, and the grey component (polluted water) was equivalent to 80% of this consumption. Measures such as improving agricultural practices, logistics, irrigation, expanding and improving rainfed agriculture, developing campaigns and policies to reduce exportation of primary products, as well as consumption of products from animal origin, can contribute to managing the food supply chain more sustainably when the focus is water. Reducing food loss and waste means preserving water.

Keywords: agriculture; water-energy-food nexus; water footprint; virtual water; green water.

RESUMO

Neste artigo estimou-se a perda de água associada aos alimentos desperdiçados no Brasil no ano de 2013. Tomou-se por base estudo da Organização das Nações Unidas para Agricultura e Alimentação (FAO) sobre desperdício de alimentos, o banco de dados FAOStat com o balanço de alimentos e o banco de dados de Pegada Hídrica (PH) de produtos agrícolas disponíveis na Water Footprint Network (WFN). Os resultados mostram que as perdas e desperdícios de alimentos atingem 49 milhões de toneladas por ano, comprometendo um volume anual de água de 87 bilhões de metros cúbicos, superior à vazão média anual do Rio São Francisco. A principal parcela das perdas de água está associada às perdas de alimento na etapa de produção agrícola (32%), seguida da de consumo (19%). Dentre os grupos de alimentos, as maiores perdas de água estão associadas às carnes (49%), seguida pelo grupo de cereais (19%). Cerca de 96% das perdas de água referem-se à água verde, o que evidencia a necessidade de uma maior atenção à agricultura de sequeiro para assegurar alimento e água para todos. A perda de água azul foi superior à metade do volume consumido no setor urbano; e a parcela cinza (água poluída) equivaleu a 80% desse consumo. Medidas como melhoria das práticas agrícolas, logística, irrigação, expansão da agricultura de sequeiro, desenvolvimento de campanhas e políticas para redução da exportação de produtos primários, bem como do consumo de produtos de origem animal, podem contribuir para uma gestão mais sustentável da cadeia de suprimento de alimentos quando o foco é a água. Reduzir a perda e o desperdício de alimentos significa preservar água.

Palavras-chave: agricultura; nexo água-energia-alimento; pegada hídrica; água virtual; água verde.

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Introduction

Data from the Food and Agriculture Organization (FAO) of the United Nations (UN) show that, every year, about a third of the food produced worldwide is not consumed by the population, being lost throughout the production chain, or wasted in the endpoint (restaurants and households) instead. This represents about 1.3 billion metric tons of food that is not used or, in monetary value, approximately US$ 1 trillion (FAO, 2014).

This situation tends to be even more aggravated with the growth of the world population. According to the United Nations Department of Economic and Social Affairs (2019), by the middle of this century, the world population will reach about 10 billion people. Concerning Brazil, the population is estimated to be around 238 million inhabitants by 2050, according to the intermediate projection (United Nations, 2019). In order to feed this additional population and include the current 870 million hungry people and two billion people who suffer from moderate to severe food insecurity in the country (FAO, 2019), it would be necessary to increase food supply by 70 to 100% (Alexandratos and Bruinsma, 2012; Reddy, 2016). In this scenario, the agricultural sector in Brazil would be pressured to increase production.

Nevertheless, agriculture is one of the greatest drivers for the transgression of planetary boundaries, including the use of freshwater (Campbell et al., 2017). Overall, this sector accounts for 70% of the total water withdrawal, with regional variations of 44% in the countries of the Organisation for Economic Cooperation and Development (OECD), 87% in Africa, and more than 90% in some Middle Eastern countries (Campbell et al., 2017). In Brazil, the consumptive water use for agricultural production in 2017 was 78.3% of the total consumed of 1,109 m^3/s, an increase of 21% compared to 2006, *versus* 6% for the industrial sector (ANA, 2019). In a broader view, considering the rainwater stored in the soil (green water), agricultural and livestock production accounts for 92% of all water used by humanity (Hogeboom, 2020).

This scenario already points to the threat of exceeding the limit of freshwater use. The Millennium Ecosystem Assessment Report (MEA) warned of this when it estimated, with low to medium certainty, that this limit was already exceeded by 5 to 25% (Millennium Ecosystem Assessment, 2005). More recently, Jaramillo and Destouni (2015) estimated the total freshwater use at $4.664 \cdot 10^{12}$ m^3 per year. The Stockholm Resilience Centre proposed a freshwater use limit of $4.0 \cdot 10^{12}$ m^3 per year (Rockström et al., 2009). This number, however, has been criticized for not considering regional specificities and/or the flow of green water (Bogardi et al., 2013; Gerten et al., 2013; Steffen et al., 2015). This limit had a revision suggested to be $2.8 \cdot 10^{12}$ m^3 per year, which is the mean value within an uncertainty range from $1.1 \cdot 10^{12}$ m^3 to $4.5 \cdot 10^{12}$ m^3 per year (Gerten et al., 2013). Assuming the consumption suggested by Jaramillo and Destouni (2015), the most conservative value of $2.8 \cdot 10^{12}$ m^3 per year and the most liberal of $4.0 \cdot 10^{12}$ m^3 per year would already be exceeded, by 67 or 17%, respectively. Such criticism led to the revision of the safe limit for the use of freshwater on Earth, considering the diverse flows, and climatic and ecosystem particularities of the various regions, aiming at a more robust definition of this limit (Gleeson et al., 2020), but the indications are of a water scarcity situation.

Meanwhile, tonnes of food are lost and/or wasted daily (FAO, 2013). Food Loss and Waste (FLW) occur both in developed and developing countries, although at different stages of the food chain for each case (Gustavsson et al., 2011). In the case of developing countries, such loss occur mainly due to the lack of infrastructure and investment in storage structures, whereas in developed countries, the origin is in the stages of distribution and consumption (Godfray et al., 2010).

The FLW occur throughout the supply chain, which involves the production, storage, transportation, processing, distribution, and consumption of food; and reflects significant equivalent water loss, revealing the inefficiency in the use of this critical resource and imposing an additional difficulty to fully meet future demands.

In Brazil, water security is considered a regional issue, given that the spatial distribution of the large water resources in the country is extremely unequal. The major availability, in which the worrying, critical or very critical level has not yet been reached, is found in areas of high ecological interest: the Cerrado region, the Amazon Forest, and the Pantanal (ANA, 2019).

The order of magnitude of FLW and, consequently, of water wasted, is large enough to deserve greater attention from the managers and users of this resource. Strategies that focus on reducing loss along the food production chain, and on the efficient and sustainable use of water, are crucial to achieving the Sustainable Development Goal No. 2 (Lundqvist et al., 2008). Doubling production simply implies that water damage will also double.

Thus, knowing the volumes of water wasted due to FLW will enable the managers of this resource to define priorities to tackle the problem. The appropriate indicator for this is the Water Footprint (WF), used to evaluate the volume of water needed for each agricultural product and its portions of blue water, the most cited and the one which corresponds to what is extracted from rivers, lakes, and aquifers; green water, stored in the soil and used by plants; and grey water, the volume of water polluted as a result of the activity (Hogeboom, 2020).

The Water Footprint Network (WFN) defines the WF of a product as the total volume of freshwater used directly or indirectly to produce that product and can be decomposed into the blue, green, and grey components (Hoekstra et al., 2011). The WF can be considered as a comprehensive indicator of the appropriation of water resources, *vis-à-vis* the traditional and restricted concept of water withdrawal. The WF of a product is the volume of water used to produce it, measured throughout the entire production chain (Hoekstra et al., 2011). It is, therefore, an indicator of the appropriation of the freshwater resource as opposed to the traditional and restricted measurement of water withdrawal.

The concept of WF has been widely used in agricultural and livestock production, with a large number of studies evaluating the impact of agricultural products on the water system (Ding et al., 2018; Xinchun et al., 2018; Fulton et al., 2019), livestock (Barden et al., 2017; Asevedo et al., 2018), forestry (Schyns et al., 2017), agroindustry (Bleninger and Kotsuka, 2015; Munoz Castillo et al., 2017), besides being used to support water management (Empinotti and Jacobi, 2013; Silva et al., 2016; Nouri et al., 2019).

However, relatively few studies assess the water loss associated with FLW. Except for the study of the water footprint of the FLW in the European Union (Vanham et al., 2015) and more recently research by Sun et al. (2018), the literature is limited to the blue water component, which is present in rivers and aquifers (Kummu et al., 2012; FAO, 2013; Le Roux et al., 2018; Spang and Stevens, 2018; Read et al., 2020) or does not identify the various components of water (Liu et al., 2013). A recent report evaluated the WF of bovine meat in Brazil, although without considering the loss (Pavão et al., 2020).

Nevertheless, given the importance of water in agricultural production, especially food, many authors have recommended the inclusion of the green water component in integrated water management (Rockström et al., 2014; Rodrigues et al., 2014; Schyns et al., 2015; Porkka et al., 2016; Falkenmark, 2018). This is particularly relevant for Brazil, whose culture is of an abundance of water, at a time when there is a growing trend of export of primary products.

Therefore, the objective of the present article is to assess the volume of water compromised due to food loss and waste in Brazil for the year 2013, which may constitute a subsidy for planning water allocation in the Brazilian agricultural production.

Material and Methods

The scope of this study is the food portion intended to meet domestic demand in Brazil for the year 2013. The most recent data are available on the FAO's statistics division website and Food Balance Sheet, FBS (FAOSTAT, 2015).

First, the loss throughout the Food Supply Chain (FSC) was estimated in terms of mass, then the volume of water needed to produce these foods was calculated (Figure 1).

Differently from the related and global scope literature, which has accounted only for the volume of water withdrawn for irrigation (blue water), this study also accounts for the components associated with the rainfed production (green water) and water for diluting the residues generated (grey water).

Food loss and waste accounting

Data concerning food production and use were obtained from the FAOSTAT's and FBS's websites (FAOSTAT, 2015) for the year 2013, Brazil. The food groups were organized as shown in Table 1.

The elements contained in the FBS have been divided into production and utilization elements. For each product group, the Quantity intended for Domestic Supply (QDS) is equal to the sum of production, import quantity, stock variation, and export quantity. The food available for human consumption is QDS minus other utilization elements, such as feed, seeds, processing, and others (Figure 2).

The calculations to estimate loss and waste were carried out for each food group separately, to take into account their specificities. The method was based on the FAO report, entitled Global Food Losses and Food Waste (Gustavsson et al., 2011), which was later detailed in the publication entitled The Methodology of the FAO Study: " Global Food Losses and Food Waste-extent, causes and prevention" - FAO, 2011 (Gustavsson et al., 2013).

Allocation factors (af) were used to estimate the fraction of the production intended for human consumption. Conversion factors (cf) were applied to determine the edible portion of primary products. The values provided by Gustavsson et al. (2013) for Latin America were adopted, as shown in Table 2. Based on the same method, the evaluation was made considering five stages of the supply chain whose percentages of loss are shown in Table 3.

Water footprint

This study included the component associated with the agricultural production stage alone, which is the most important, although water could be considered to be also used in the stages of processing and consumption. In this sense, factors such as climate, soil and crop management, crop varieties, among others, directly affect their accounting.

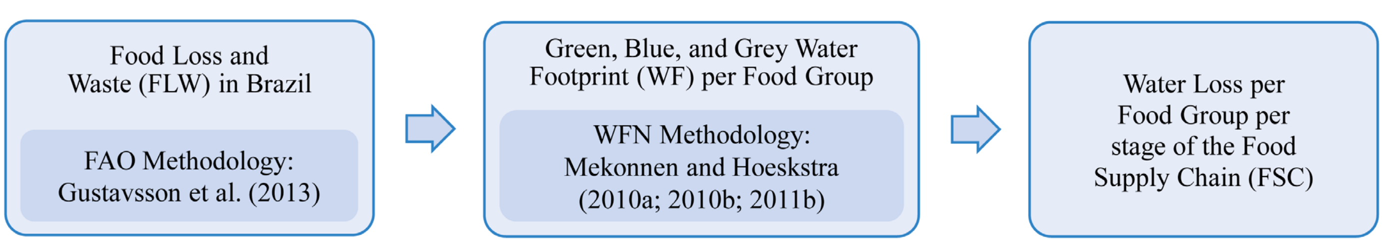

Figure 1 – Stages of the study.

To estimate the green, blue, and grey WF of agricultural and animal products, the studies by Mekonnen and Hoekstra (2010a; 2010b; 2011b) were used, whose annexes present the values of these indicators referring to the food produced in several countries, including Brazil. This study considered the value of the global average for the country.

The WF calculation for each group of food was performed according to Equation 1.

$$\sum WF_{\text{Group n}} = \frac{P_1 \cdot WF_1 + P_2 \cdot WF_2 + P_n \cdot WF_n}{P_1 + P_2 + P_n} \quad (1)$$

In which:

WF = water footprint ($m^3 \cdot t^{-1}$);

P = production of each food (t).

Results and Discussion

Based on the methods used, it is estimated that Brazil lost, in the FSC, about 49 million metric tonnes of food in 2013, which represents 39% of the QDS that includes the portion intended for human consumption (Table 4).

These losses and wastes occur throughout the FSC, from cultivation to final consumers, with emphasis on the initial stages in emerging countries such as Brazil. About 38% of the FLW occur in the production stage, followed by 22% in post-harvest and storage, 15% in processing and packaging, 13% in distribution, and 12% in the consumption stage.

Even though the consumption stage has the lowest contribution to the total FLW, it is still surprising that about 6 million tonnes are wasted in Brazilian households. This value may be underestimated in view of a study by Porpino et al. (2015) that points to high waste generation during the consumption stage in the lower-middle-class population, which can be associated with cultural traits, which differentiates Brazil from countries with equivalent *per capita* income in which the loss in this stage is small.

This situation is aggravated considering that about 39.4% of households in the country live in food insecurity (Araújo et al., 2020). Furthermore, 5% of all disease burdens in Brazil are associated with food shortages (GBD 2016 Brazil Collaborators, 2018).

In terms of mass, the food group with the highest percentage of loss was fruit and vegetables. According to the Paraná Institute of Technical Assistance and Rural Extension (*Instituto Paranaense de Assistência Técnica e Extensão Rural*), EMATER (Trento et al., 2011) the agribusiness of fruits and vegetables faces several problems such as low productivity, low quality, and high production costs; environmental and sanitary problems in production, processing, and marketing; deficiency in storage, transport, and marketing logistics; low consumption and restricted eating habits; lack of more advanced technology and market knowledge. Due to the fragility of these products, the greatest loss occur in the agricultural production system and in transportation, in which the distance between production and consumption sites is a determining factor.

The cereals group was a major agricultural production with 97 million tonnes, of which 79 million were allocated to the domestic market. Compared to another group of high gross production, oilseeds and legumes, whose total production was 90 million tonnes, only 47 mil-

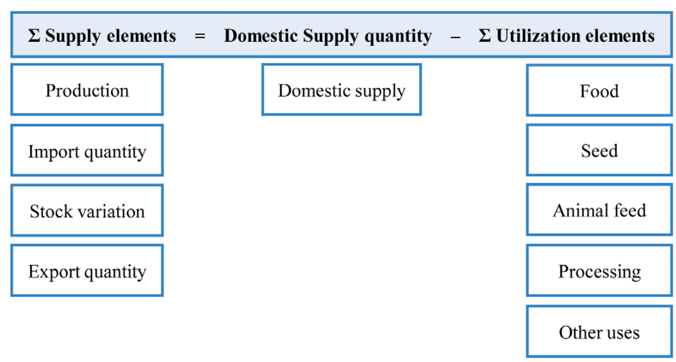

Figure 2 – Food mass balance.
Source: Gustavsson et al. (2011).

Table 1 – Food groups.

Group	Food
Cereals	Wheat, rice, barley, maize, rye, oats, millet, sorghum, and other cereals
Roots and tubers	Cassava, yam, potatoes, and sweet potatoes
Oilseeds and legumes	Soybeans, peanuts, sunflower, grape pomace and mustard seed, cotton seed, coconuts, sesame seed, palm seed, olives, and other oilseeds
Fruits and vegetables	Orange and mandarin, lemon and lime, grapefruit, other citrus fruits, banana, apple, pineapple, date, grape, other fruits, tomato, onion, and other vegetables
Meat	Bovine meat, mutton/goat meat, pork meat, and bird meat
Milk and eggs	Milk (not including derivatives) and eggs

Table 2 – Allocation factors and conversion factors for food groups.

Food group	Stage of the chain				
	Agricultural production	Post-harvest and storage	Processing and packaging	Distribution	Consumption
Cereals	0.40 (af)	0.40 (af)	-	-	-
Roots and tubers	0.82 (cf)	0.82 (cf)	0.90 (cf)	0.74 (cf_m) 0.90 (cf_i)	0.74 (cf_m) 0.90 (cf_i)
Oilseeds and legumes	0.12 (af)	0.12 (af)	-	-	-
Fruits and vegetables	0.77 (cf)	0.77 (cf)	0.75 (cf)	0.80 (cf_m) 0.75 (cf_i)	0.80 (cf_m) 0.75 (cf_i)
Meat	-	-	-	-	-
Milk	-	-	-	-	-

af: allocation factor; cf: conversion factor; i: industrial; m: manual.
Source: Gustavsson et al. (2013).

Table 3 – Values of the percentage of food loss per group for Latin America.

Food group	Stage of the chain				
	Agricultural production	Post-harvest and storage	Processing and packaging	Distribution	Consumption
Cereals	6%	4%	2.0% (g) 7% (p)	4%	10%
Roots and tubers	14%	14%	12%	3%(f)	4% (f) 2% (p)
Oilseeds and legumes	6%	3%	8%	2%	2%
Fruits and vegetables	20%	10%	20%	12% (f) 2% (p)	10% (f) 1% (p)
Meat	5.6%	1.1%	5%	5%	6%
Milk	3.5%	6%	2%	8%	4%

m: grinded; f: fresh; p: processed.
Source: Gustavsson et al. (2013).

Table 4 – Food Loss in Brazil in 2013.

Stage	Food loss (10^3·t)						
	Cereals	Roots and tubers	Oilseeds and legumes	Fruits and vegetables	Meat	Milk	Total
Agricultural production	2,480.6	3,406.6	857.3	9,174.9	1,543.0	1,330.2	18,792.7
Post-harvest and storage	1,554.5	2,929.7	402.9	3,670.0	286.1	1,959.2	10,802.4
Processing and packaging	1,869.0	965.3	711.2	2,235.0	977.6	607.2	7,365.3
Distribution	745.7	264.9	284.3	1,581.8	928.7	2,416.5	6,222.0
Consumption	2,147.6	201.8	278.6	1,064.2	1,058.7	1,147.4	5,898.4
Total	**8,797.4**	**7,768.4**	**2,534.4**	**17,725.9**	**4,794.2**	**7,460.6**	**49,080.7**

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lion were allocated to the domestic market.The difference between these proportions has implications for losses in the processing, distribution, and consumption stages.

The relative loss in the FSC reached 70% in the roots and tubers group, and 61% in the fruit and vegetable group. The more animal products, and fruits and vegetables in the diet, the less is the durability of food, thus increasing the loss. This is driven by the growth of urbanization rates that increases the distance between the production and consumption sites, demanding more transportation.

These losses also imply substantial financial losses. The Brazilian Institute of Applied Economic Research (*Instituto de Pesquisa Econômica Aplicada* - IPEA) (Carvalho, 2009) cites a study by the Department of Supply and Agriculture of São Paulo State, which estimated the value of food loss at 1.4% of Brazil's Gross Domestic Product, worth R$ 17.25 billion at the time.

In addition to other economic, social, and environmental impacts, FLW should be associated with the loss of water used in food production, which is usually ignored in scientific work focused on this subject and virtually absent in the official documents that deal with water management. The WF and its components for each food group are shown in Table 5.

Table 6 shows the WF associated with FLW by stage of the chain per food group. The greatest impact occurs on agricultural production. The total value of water loss reached 87.3 billion m^3 in 2013. This is equivalent to 2,768 m^3/s, which is in the same order of magnitude of the average long-term flow at the source of the river São Francisco Basin, equal to 2,914 m^3/s or three times the offerable flow of 875 m^3/s (ANA, 2019). It is almost an entire river São Francisco wasted.

According to Falkenmark and Rockström (2008), the water used in the production of the amount of food to meet a person's needs is 1,300 L/day, considering 20% of calories being of animal origin. The virtual water from FLW would therefore be sufficient to produce food for a population of about 180 million people, which corresponds to roughly 90% of the Brazilian population in the study year (IBGE, 2013).

The value of this loss in 2013 was 435.0 m^3 per capita, corresponding to about 20% of the average WF of consumption of each Brazilian, which is equivalent to 2,027 m^3/year (Mekonnen; Hoekstra, 2011a).

The major share of water loss is associated with loss in the agricultural production stage (32.1%), followed by consumption (18.7%), processing and packaging (18.5%), distribution (16.8%), and, finally, post-harvest and storage (13.9%).

Moreover, according to the results in Table 6, the analysis of the total WF per food group shows a greater contribution of meat, which is associated with 49.2% of water losses, followed by the group of cereals (19.0%), milk and eggs (12.1%), fruits and vegetables (9.5%), oilseeds and legumes (6.3%), and roots and tubers (3.8%). The FLW of animal products account for more than half of the total water loss.

Considering the three components of WF (green, blue, and grey), the largest contribution to the total WF comes from the green component, 96.0% (Figure 3), which has no direct impact on watercourses. However, several authors (Rockström et al., 2014; Rodrigues et al., 2014; Schyns et al., 2015; Porkka et al., 2016; Falkenmark, 2018) warn both of the mistake of seeing only blue water as a productive resource and of the strong dependence of food production by green water. The green component can account for up to 97% of the water used in this activity and support this concern realizing that the appropriation of this resource towards society compromises the maintenance of ecosystem services.

Blue water and green water work as "communicating vessels", and their availability depends on precipitation, which is, ultimately, the allocated resource. The main distinction between one and the other is that green water is allocated according to decisions on land use, whereas blue water can be captured at distant points from where precipitation fell (Schyns et al., 2019). Due to this "invisibility" of green water, the limits of its use and the use of associated scarcity indicators have only recently been debated and researched, such as the study in the agricultural basin of the Cantareira system (Rodrigues et al., 2014), and the review and classification article of indicators of availability and scarcity of green water (Schyns et al., 2015). Schyns et al. (2019) propose a green water Scarcity Index, given by the relation between the total green WF of the area and the maximum sustainable green WF, the latter being equal to total green WF minus that of the reserve of 17% to ensure the biodiversity target and areas without aptitude for agriculture. This method was applied on a planetary scale with a cell mesh of

Table 5 – Water footprint by component per food group.

WF component	Water footprint (m^3/t)						
	Cereals	Roots and tubers	Oilseeds and legumes	Fruits and vegetables	Meat	Milk	Total
Green	1,712.8	407.8	2,153.4	431.8	8,669.1	1,350.8	14,725.6
Blue	46.2	3.8	2.9	15.7	141.9	38.4	249.0
Grey	131.1	17.0	20.9	20.0	152.7	25.3	367.0
Total	1,890.1	428.6	2,177.2	467.5	8,963.8	1,414.5	15,341.6

Source: Mekonnen and Hoekstra (2010a; 2010b; 2011b).

5 × 5-minute arc; and the results show that 56% of the availability of green water in the world is already used, 51% in Brazil (Schyns et al., 2019). These are aggregated values that do not include cases of use of the green WF flow in protected areas, whose overall value is 18% and, for Brazil, 14% (Schyns et al., 2019).

On the other hand, green WF is the main resource for agricultural production, since the urban and industrial supply depends exclusively on the blue water flow. Thus, aiming at meeting an increasing demand for food, the reduction of FLW should be associated with what is called sustainable intensification of rainfed agriculture by adopting techniques that increase and retain moisture in the soil for longer, in addition to the application of water according to the concept of supplementary or deficit irrigation (Reddy, 2016; Schyns et al., 2019).

The blue and grey WF, which are the components that have an impact on water bodies, represent approximately 136 m^3/s, or about 50% of the entire availability of the East Atlantic Hydrographic Region, where 15 million people live (ANA, 2019). This number is also equivalent to 70% of all water consumed by the Brazilian industrial sector. Another way to look at this number is to compare it with the sum of all the flows for urban supply in Brazil in 2018, whose value was 501 m^3/s (ANA, 2019).

Even though the absolute numbers of food waste are already alarming *per se*, there are a set of built-in wastes that further cloud the global scenario. The production and distribution stages in the FSC need land, mineral fertilizers, pesticides, electricity, fossil fuels, and, above all, water. Wasted food buries all these resources with it (Rodrigues, 2017).

Table 6 – Water footprint of food loss by stage of the chain per food group.

Stage of the chain	Water footprint by food group (10^9 m^3/year)						
	Cereals	Roots / tubers	Oilseeds / legumes	Fruits / vegetables	Meat	Milk	Total
Agricultural production	4.69	1.46	1.87	4.29	13.83	1.68	28.0
Post-harvest and storage	2.94	1.26	0.88	1.72	2.56	2.77	12.1
Processing and packaging	3.53	0.41	1.55	1.04	8.76	0.85	16.2
Distribution	1.41	0.11	0.62	0.74	8.32	3.32	14.6
Consumption	4.06	0.09	0.61	0.50	9.49	1.53	16.4
Total	**16.63**	**3.33**	**5.52**	**8.29**	**42.97**	**10.13**	**87.3**

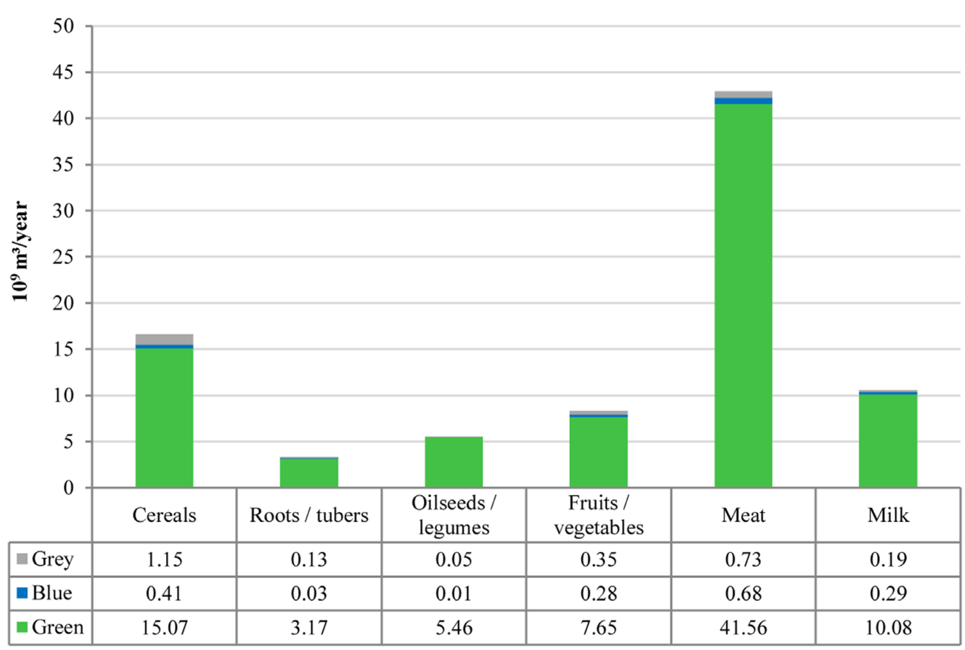

Figure 3 – Water footprint by component per food group.

Reducing FLW throughout the FSC may be the best strategy towards sustainability within food security. At the same time, this reduction can represent a relief in the pressure on water bodies, saving water for other uses, including environmental demands.

Food production is the activity with the largest water use in Brazil, accounting for 79.2% of the total consumption in 2017, or a flow of 917.1 m^3/s (ANA, 2019), which is in the same order of magnitude as the world's average. Meeting the 12.3 goal of the Sustainable Development Goals of halving the FLW by 2030 would mean, alone, the availability of 458.6 m^3/s, equivalent to about 90% of the total withdrawal for urban supply.

The current situation of water use already demands attention. Vörösmarty et al. (2010) analyzed the world's water resources and found that the incidence of a threat to water security was high in many regions, including Brazil. Roughly 11,500 km of rivers have withdrawals above 20% of their availability, of which 4,900 km have withdrawals over 70% (ANA, 2019). Not coincidentally, the concentration of watersheds in critical situations occurs where the largest populations are also concentrated. Despite the claimed abundance of water, extensive areas are under threat.

The food production system, therefore, urges for critical advances in water use efficiency. On the one hand, both reducing losses before considering increasing production capacity (Freire Junior and Soares, 2014) and minimizing their implications for water resources must be key concerns; and, on the other hand, an increase in the productivity of water use in agriculture is needed.

The first approach consists of goal 12.3 of the Sustainable Development Goals (SDGs): by 2030, halve the global food waste *per capita* at retail and consumer levels, and reduce food loss along the production and supply chain, including post-harvest loss.

In less developed countries, which includes Brazil, FLW can be associated with factors that mainly penalize the initial stages of the FSC. The main factors pointed out are improper management, inadequate harvesting techniques, inappropriate post-harvest management, lack of logistics infrastructure, irregular processing and packaging, and poor-quality marketing information (Gustavsson et al., 2011; Lipinski et al., 2013; Dung et al., 2014).

Nevertheless, attention must also be focused on the consumption stage, which represents 12% of the FLW. Various reasons for loss in this stage can be pointed out: lack of awareness of the amount of food wasted by consumers or the impact it causes; income high enough to afford wasting; high-quality standard and sensitivity to food security; lack of planning for the acquisition of food, which results in overbuying; lack of ability in the kitchen to size the portions in each meal; and changes in daily planning due to busy routine (Kibler et al., 2018).

The World Resources Institute (WRI), linked to the United Nations Environment Programme (UNEP), suggests several measures, listed in Table 7, without, however, claiming to exhaust the possibilities (Lipinski et al., 2013).

It is worth adding, among other measures, food production in the concept of urban and peri-urban agriculture of perishable products, such as some horticulture producers (Kibler et al., 2018). This could have a strong impact on the loss observed in these food groups, which is mainly influenced by transportation.

Concerning the increasing water efficiency in food production, the relevance of green water cannot be ignored: rainwater stored in soil and which sustains rainfed agriculture. Conservation practices such as terracing, land leveling, soil fertility management, tillage, sediment, and moisture containment dams, etc.

Table 7 – Potential approaches to reduce food loss and waste per stage.

Production	Post-harvest and storage	Processing and packaging	Distribution and marketing	Consumption
Facilitate the donation of inadequate crops to the market	Improve access to low-cost storage technologies	Reengineer manufacturing processes	Facilitate the donation of unsold products	Facilitate the donation of unsold products in restaurants
Improve the availability of extension services	Improve the management of the ethylene and microorganisms in the storage stage	Improve supply chain management	Change practices on food date labels	Run consumer education campaigns
Improve market access	Use low-carbon cooling techniques	Improve packaging to keep products fresh longer	Modify promotions in the market	Reduce the size of the served portions
Improve harvesting techniques	Improve transport infrastructure		Provide consumer guidance on food storage and preparation	Ensure home economics education in schools, universities, and communities

Source: Lipinski et al. (2013).

can increase and retain soil moisture for longer, reducing water loss by evaporation given the same amount of rain (Springer and Duchin, 2014). Based on Falkenmark and Rockström (2006), the use of such techniques could potentially increase productivity by up to 50% in Latin America.

This claim is shared by de Fraiture and Wichelns (2010) when they state that, for a scenario with high productivity of rainfed agriculture, the demand for food in 2050 could be met by increasing 7% of the cultivated rainfed area, without the expansion of the irrigated area.

In the opposite direction, the Brazilian Government, through the Ministry of Agriculture, Livestock, and Supply (MAPA), has been adopting measures aimed at expanding the irrigated area. Between 1960 and 2015, the irrigated area grew from 455,000 to 6.95 million hectares, equivalent to an average growth rate of 6% per year. Furthermore, adding 2.8 million hectares by 2020 was also planned, and another 7.0 million from 2020 to 2030 (Rocha and Christofidis, 2015).

From the point of view of water management, there are two trends that should foster demand over the next few years. One is the consolidation of the economic model based on the export of primary products, as shown in Figure 4. Brazilian exports in the last fifty years of bovine meat, chicken meat, soybean, and maize have grown 27, 1,000, 450, and 2,000 times, respectively (FAOSTAT, 2015).

Godfray et al. (2010) draw attention to the need of better understanding the effects of globalization on the food production system and its externalities. According to Mekonnen and Hoekstra (2011b), Brazil's exports in primary products are equivalent to 110 billion cubic meters of virtual water, *versus* an import of 33 billion. This situation characterizes the country not only as an exporter of water, but also of land and soil fertility.

In a pandemic situation, as occurred in 2020 with COVID-19, a new geopolitical context is observed. Given the scarcity of water, it is natural for developed economies to seek to avoid wasting it for production. As such, they opt for importing from countries capable of providing them with this resource at competitive prices. However, international trade in agricultural products may be affected by the rise of neo-nationalism after the COVID-19 pandemic (Brasil, 2020). Virtual water export management, for instance, can be considered as a measure to defend food sovereignty and self-sufficiency, which some authors refer to as post-pandemic neo-nationalism.

For example, for a highly water-intensive activity such as livestock, there was an increase in the Brazilian cattle herd by 23% between 2000 and 2010, when there was a widespread decrease in the number of cattle herds in the European Union and the United States (FAOSTAT, 2015).

Brazil is an important soybean exporting country. Additionally, according to the Department of Rural Socio-Economic Studies (2007), a significant replacement of animal greases by vegetable oils has been observed worldwide, due to factors associated with health, production costs, industrial development, and versatility of this type of raw material. Furthermore, the increase in the Brazilian soybean production will continue for several reasons, including the increase of world population (especially in China); also the soybean potential as a raw material in biodiesel, paint, lubricant, and plastic industries; and a growth in the consumption of soybean meal to meet the rising meat industry worldwide and in Brazil (Dall'Agnol and Hirakuri, 2008).

Maize represented 76% of the production of cereals, the second largest group in WF associated with FLW, being an important commodity among Brazilian exports (Figure 4). This food group holds the largest blue WF and grey WF when compared to the others, both in absolute and relative terms. This highlights the importance of maize production as a potential competitor for water allocation in the future.

The other trend is the growth of domestic demand for fruits and animal products, while the consumption of roots and tubers decreases, associated with the increase in *per capita* income. The world's *per capita* income is also expected to grow 4.5 times by 2050 compared to 2008 (Lundqvist et al., 2008). According to Benett's Law, cited by Parfitt et al. (2010), income growth leads to a transition in diet, i.e., an increase in the consumption of meat, fruits and vegetables, milk and dairy products, as well as a reduction in consumption of roots and tubers. In Brazil, meat consumption multiplied by 3.7 over the last 50 years; milk and dairy products, by 2.0; fruits and vegetables, by 1.5; cereals, by 1.2; and roots and tubers, by 0.7 (Figure 5).

With the growth of meat consumption in Brazil and the expectation of meeting the growing demand for agricultural products abroad, the impacts on water resources tend to worsen.

In addition to human activities, water waste can lead to a decrease in the number of species or number of individuals of the same species, affecting the balance of ecosystems (Figueiredo et al., 2017).

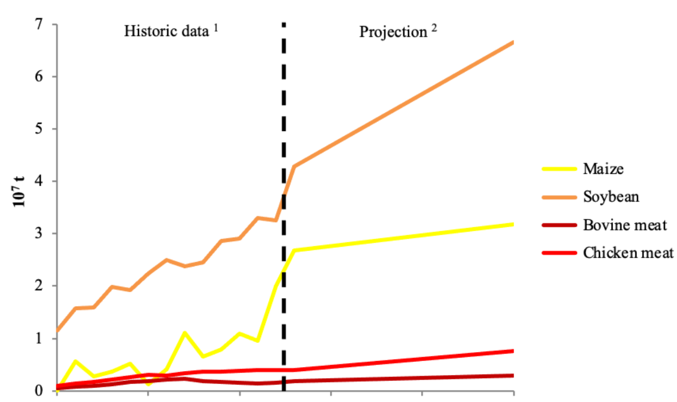

Figure 4 – Evolution of commodity exports by Brazil.
Source: FAOSTAT (2015).

Thus, greater attention must be paid to the efficiency in water use throughout the entire food production chain, from the agricultural production to the consumption stage, considering several factors that imply in this care, highlighting climate change notably.

Therefore, increasing the efficiency of the food production chain is imperative for all its extension, from the most efficient management of the rainfed agriculture, with better use of rain and the reduction of loss and waste, and even the incentive to the adoption of a diet based on fewer consumption of animal products.

Conclusion

Food loss and waste in Brazil were estimated at 49 million tonnes in 2013, according to FAO methodology (Gustavsson et al., 2013), with serious implications for water management in the country.

They result in a water loss of 87 billion cubic meters per year, or about 2,768 m^3/s, equivalent to 96% of the average flow of the river São Francisco in the source. This volume would be enough to produce food for 180 million people.

The largest water losses are associated with meat and cereals, corresponding to 49 and 19% of the total, respectively. This scenario can be aggravated by the increase in exports, whose agenda prioritizes maize, soybeans, and meats. Another aggravating factor is the change in eating habits of the Brazilian population, with increasing consumption of animal products, accounting for 61% of the entire volume of water lost.

Green water is the main resource for agricultural production, with a share of 96%. This flow is associated with land use and has a direct influence on the maintenance of ecosystem services, although it is ignored in water management. Greater attention should be given to this resource with the adoption of techniques that increase and retain soil moisture for longer, increase the efficiency of rainfed agriculture, and reduce the demand to expand irrigated areas.

The blue water footprint totals 53.6 m^3/s, which is equivalent to 52% of the consumptive use of the urban sector in 2013, which evidences the conflict between urban and agricultural uses. It should be highlighted that, for urban supply, blue water is the only option.

Grey water footprint accounts for 82.9 m^3/s and, although it does not necessarily require a quantitative reduction, it draws attention to the high volume of degraded water, as well as its environmental and economic implications, including from the eutrophication of water bodies to a greater energy demand for human and/or industrial use.

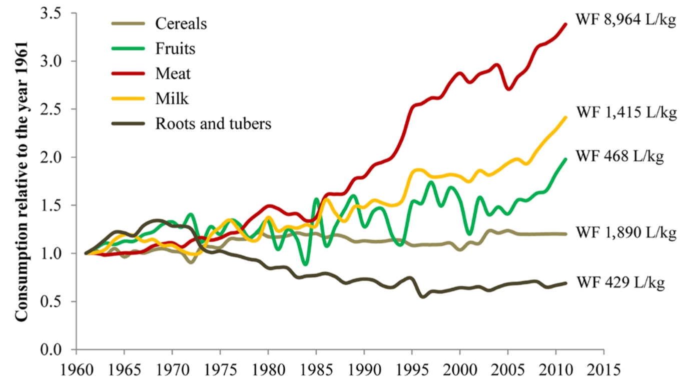

Figure 5 – Evolution of per capita consumption of some food groups in Brazil.
Source: FAOSTAT (2015).

Meeting goal 12.3 of the Sustainable Development Goals (SDGs), halving food loss and waste by 2030, would mean, alone, the availability of 458.6 m^3/s of freshwater. Key measures to achieve this goal are: improving agricultural practices, running campaigns aimed at consumers, improving transport infrastructure, and producing perishable products closer to the places of consumption.

Investment in improving water management in irrigated properties and sustainable intensification of rainfed agriculture would contribute to an additional gain.

Finally, the adoption of policies that redirect the model of primary products exports and campaigns to reduce the consumption of animal products would be an important advance in the preservation of water in Brazil.

A continuation of this work should address the water loss associated with food loss and waste considering specific regions throughout the years, including seasonal variations in agricultural activities such as drought (greater blue water demand) or excess rain (green water). A given water consumption has different effects in one region with adequate water availability and, in another, with a lack of this resource.

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Cohim, E.B.: Conceptualization, Methodology, Data curation, Validation, Writing — original draft, Formal analysis. Leão, A.S.: Methodology, Writing — original draft, Formal analysis. Silva Neto, H.A.: Methodology, Validation, Writing — original draft, Formal analysis. Santos, G.S.: Formal analysis.

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Study of the biogas potential generated from residue: peanut shells

Estudo do potencial de geração de biogás a partir de resíduos: casca de amendoim

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ABSTRACT

The increase in agricultural production generates a large volume of waste, which may lead to concerns about its proper destination. The main economic activity in Herculândia City, Western region of São Paulo State, Brazil, is the production and processing of peanuts. In this process, a large volume of peanut shells is generated. Following the current movement of using waste for energy purposes, in compliance with what was established by the Sustainable Development Goals (SDGs), this work aimed to carry out a study on the biogas potential generated from peanut shells. To this end, a low-cost biodigester prototype was built, which, over a period of 108 days, produced biogas and biofertilizer. The results showed that there was production of biogas from peanut waste; however, the volume produced did not provide savings in electricity costs when compared to the production of biogas from animal waste. Nevertheless, the work demonstrated the importance of providing solutions to the disposal of peanut shells, effectively mitigating future environmental problems, and serving as an alternative for generating sustainable and low-cost energy, especially for small producers.

Keywords: biogas; peanut shells; electricity; waste.

RESUMO

O aumento da produção agrícola gera um grande volume de resíduos, podendo levar a preocupações quanto à sua adequada destinação. O município de Herculândia, no oeste do estado de São Paulo, tem como principal atividade econômica a produção e processamento de amendoim. Nesse processo é gerado um grande volume de cascas. Seguindo a tendência da utilização de resíduos para fins energéticos, atendendo ao estabelecido pelos Objetivos de Desenvolvimento Sustentável (ODS), este trabalho teve como objetivo realizar um estudo sobre o potencial de geração de biogás a partir da casca do amendoim. Para tal, foi construído um protótipo de biodigestor de baixo custo que, em um período de 108 dias, produziu biogás e biofertilizante. Os resultados demostraram que houve produção de biogás a partir do resíduo; entretanto, o volume produzido não propiciou economia no custo de energia elétrica, quando comparado à produção de biogás oriunda de dejetos animais. No entanto, o trabalho demonstrou a importância de prover soluções ao descarte de resíduos da casca de amendoim, efetivamente mitigando futuros problemas ambientais e servindo como alternativa geradora de energia sustentável e de baixo custo, principalmente para pequenos produtores.

Palavras-chave: biogás; casca de amendoim; energia elétrica; resíduo.

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Introduction

Agricultural activities, as well as the processing of agricultural products, have been causing several environmental problems related to the waste generated during this process (Pelissari et al., 2010). The concern involves slurry, which is a result of decomposition of organic residues that presents a high contaminating factor. Slurry has the following main characteristics: dark color, unpleasant odor, and high toxicity (Miyagawa et al., 2016), with potential risk to contaminate the subsoil and groundwater with heavy metals and other substances highly harmful to human health (Milhomem Filho et al., 2016).

The use of renewable energy to meet the demands of the country's energy matrix is urgent and necessary, since the inputs used in agricultural production are finite, and the practice of monoculture exhausts the soil. In addition, agricultural residues and by-products, in some cases, may have a polluting potential, damaging the environment (Pelissari et al., 2010). Moreover, the use of renewable energy is one of the most discussed and globally relevant issues, part of the 2030 Agenda for Sustainable Development, proposed by the United Nations (UN), seeking to eradicate poverty, protect the planet, and ensure that people achieve peace and prosperity (UN, 2015). The restricted space and the increasing need to meet the demand for clean energy production, water, and food indicate that some paradigms need to be overcome, aiming to meet energy and environmental challenges of the global community (Brasil, 2018).

In line with the concept of sustainability, the Brazilian National Policy on Solid Waste (PNRS, as its acronym in Portuguese) was established in 2010, regulated by Law 12.305 / 2010. The PNRS is an important instrument with the purpose of regulating the management of solid waste, thus leading the country to follow paths that aim at improving the quality of life and environmental preservation (Brasil, 2010).

Industries in general, including those inserted in agricultural activities, are responsible for returning the residues to the productive centers and giving them the correct destination (Azevedo, 2014). An example of proper destination is in the sugar and alcohol sector, that carries out the recycling of straw and sugarcane bagasse, practices which contribute to sustainable development (Verri et al., 2017). According to Barbieri et al. (2010), the great challenge is to promote organizations that innovate efficiently in all three dimensions of sustainability: economic, social, and environmental.

For Polachini et al. (2016), one of the biggest environmental concerns is related to the intense use of fossil fuel. For that reason, new technologies and renewable sources for producing energy from agro-industrial residue are relevant due to its low-cost and availability, mainly in countries with agricultural potential, like Brazil. The increasing generation of residue is a major challenge, especially for developing countries that still face difficulties to correctly treat its residues (Feil et al., 2015). This problem happens because, in addition to leachate, another aspect must be considered about the disposal of organic residue when it does not receive the proper treatment: the increase in the emission of greenhouse gases (GHG). Zanoni et al. (2015) point out that the decomposition of materials produces mainly methane (CH_4). According to Chizzotti et al. (2012), this gas can pollute 23 to 25 times more than carbon dioxide (CO_2), requiring from nine to 15 years to be eliminated.

Even when considering specific questions regarding the aggravations caused by organic residue from agricultural production, the economic importance of agribusiness in Brazil should not be ignored. Agribusiness accounted for 21.4% of the Brazilian Gross Domestic Product (GDP) in 2019 (CEPEA/ESALQ; CNA, 2020), in addition to being responsible for producing staples and commodities for domestic and foreign markets. This leads to a reflection on the potential of such residue from countless activities present for its reuse.

Correa et al. (2019) point out that one use for the residues consists in transforming biomass into energy sources, contributing to supply the demands of the country's energy matrix, which seeks to increase the contribution of renewable energy.

Aziz and Hanafiah (2020), in their study on the production of biogas from solid organic residue in Malaysia, emphasize that this conversion appears as a promising technology, being able to achieve sustainable development through clean energy and sustainable consumption, in line with the SDGs (UN, 2015).

Bilotta and Ross (2016) point out that the agribusiness, in general, is a major source of residue, such as vegetables from the harvest, besides the processing residues, shells, straw, bark, and seeds, which have properties in their composition that allow them to be reused to generate energy. However, in Brazil, less than half of these materials are reused for this purpose, with more than 200 million tons of agro-industrial residue being discarded without reuse (Almeida, 2012).

The processing of peanuts generates a large volume of shells. Seeking to better treat residue associated with the use of new technologies, previous attempts were made to use peanut shells for producing bioethanol through the decomposition of hemicellulose, which passes in the fermentation process, ethanol production and, later, distillation, thus converting agricultural residue into biofuels (Polachini et al., 2016).

Slorach et al. (2019) emphasize the importance of studying anaerobic digestion in environmental sustainability. For this, rethinking the current development model, adopting sustainable practices, and visualizing new means of production is needed. Hence, the economic, social, and environmental dimensions must be considered, looking for new technologies associated with the production of renewable energy that causes less impact on the environment (Awasthi et al., 2018).

Based on this, the biodigester emerges as a tool capable of transforming residue into renewable energy (Oliveira et al., 2018). Biasi et al. (2018) mention that the anaerobic digestion carried out by biodigesters is a strategy with great potential for treating agro-industrial residue, since they do not require large areas for their construction and allow the reduction of residue that have a predisposition to pollute the environment. Thus, the energy sector, for being capable of providing energy and biofertilizers, should consider this (Campos et al., 2011; Kunz et al., 2019).

In this sense, this work sought to study the potential of biogas generation from peanut shells. Such residue is generated in large amounts in the processing of peanuts and can be applied in several ways (Zhao et al., 2012).

The *locus* of analysis was the Tupã region. Located in the Midwest of São Paulo State, it is considered one of the main producing regions for peanut crop in Brazil. The great relevance of this production is evident both in economic and social aspects, since it generates a large number of jobs in the agro-industrial system.

Herculândia City, located in the Tupã region, is inserted in this pole producer; and peanut crop is the main source of its economy, from cultivation to seed processing. According to data from the Brazilian Institute of Geography and Statistics (*Instituto Brasileiro de Geografia e Estatística* - IBGE, 2017), 29 farms that produce peanut are located within the perimeter of Herculândia, ranging from large to small farms. In 2015, the city stood out in the ranking of planted and harvested areas in São Paulo State, assuming the first position; in terms of volume produced, it ranked third, accounting for an amount of 17.8 thousand tons of peanuts (IBGE, 2017).

According to Araújo et al. (2014), 30% of all agricultural peanut production correspond to shells. Estimates say that 1,350 kg/ha shells are generated. Such residue, due to its large volume and difficulty of storage, is considered difficult to use for other purposes. Lora and Andrade (2009) add that, if agricultural residue were destined for energy transformation, it could contribute significantly to sustainability and to the energy matrix of the country.

Materials and Methods

For research development, three 120-liter containers with removable cover and sealing system were used. The cylindrical containers are of opaque blue color to prevent the incidence of sunlight on the material stored inside. These cylinders simulate the conditions of the Indian and Canadian biodigesters (Bezerra et al., 2014).

To allow the anaerobic fermentation process, the containers were interconnected. In addition to the 120-liter containers, 50-liter container with removable cover, PVC pipes, connections, glues, sealing materials, purifying filter for sulphidic acid (H_2S), carbon dioxide (CO_2) and humidity, thermometer, and container for biogas storage were used, as described in Table 1.

After the identification of the materials necessary for the preparation of the biodigester, construction began as shown in Figure 1.

Figure 2 shows the prototype of the biodigester that was built at Universidade Estadual de São Paulo "Júlio de Mesquita Filho" (UNESP), Campus of Tupã.

The prototype was installed on a farm located in Herculândia City, São Paulo State. To speed up the biodigestion process, the prototype was buried so that the temperature was kept between 25 and 35°C, since the temperature influences the decomposition of organic matter and the amount of methane (CH_4) present in the biogas (Mota et al., 2019).

After installing the biodigester, peanut shells were crushed with a forage crusher with a gasoline engine, power of 5.5 HP, and a 3 mm sieve. Crushing is an extremely important step in biodigestion. Galbiatti et al. (2011) point out that the difference between the use of whole residues and crushed ones can reach an increase of 53% of the methane gas present in the biogas produced using peanut shells and poultry litter.

Then, the barrels were filled with a ratio of 1 liter of crushed shell to 3 liters of water. The mixing process was carried out prior to the barrel feeding. All the material was stored inside the feeding container; after mixing the materials, the valve was opened, and all the compost was sent to the first container.

In this way, the volume of 72 liters of crushed peanut shells (11.4 kg) was mixed with 216 liters of water. After the feeding process, all valves were closed to start the anaerobic fermentation process inside the biodigester.

After 45 days of feeding, considering that the minimum period of biogas production is between 45 and 60 days (Galbiatti et al., 2011), three filters were installed, with the objective of removing hydrogen

Table 1 – Main materials used to build the prototype

Material	Dimension	Quantity (Unit/meters)
Drum (plastic drum barrel)	120 liters	3
Drum (plastic drum barrel)	50 liters	2
Flange	½"	1
Flanges	2"	9
45° Curves	½"	1
45° Curves	2"	8
PVC Pipe	2"	5
Valve	½"	1
Valve	2"	2
Quick Connect / Quick Connect Connector	½"	10/10
Thread Seal	Medium	1
PVC Lid	4"	6
PVC Pipe	4"	2
T PVC	½"	2
Transparent Flexible Hose	½"	2
Pivot	½"	10
Clamp	½"	10
Tap	½"	1

sulphide, carbon dioxide, and the humidity present in the gas, as illustrated in Figure 3.

In the first filter, a steel wool roll was added, with the purpose of removing sulfate compounds, such as hydrogen sulfide, which impairs the storage of the gas and its burning (Brancoli, 2014). Seeking a functional and accessible technology, the steel wool roll was used due to its low-cost acquisition. When the biogas encounters the various layers, the H_2S reacts with the iron oxide and iron hydroxides, forming the iron sulfides that are fixed on the material, causing its oxidation (Ryckebosch et al., 2011).

In the second filter, a mixture of water and chlorine (bleach) with an approximate volume of 1.5 liters was added. The second filter has different characteristics from the others. The biogas inlet and outlet are located at the top, causing the produced gas to pass through the mixture and go to the next filter. To avoid degradation of the chlorine present in the mixture, after installing the filter, the replacement of the compound occurred every 10 days. The main function of the mixture is removing carbon dioxide produced from anaerobic biodigestion (Brancoli, 2014).

In the third filter, silica gel was placed to remove the moisture present in the biogas and increase the calorific value of the compound (Baldacin and Pinto, 2015). To assist in the removal process, approximately 1 kg of material was introduced into the container. In order to identify whether the removal of moisture from the gas was taking place, changes in the pigmentation of the silica gel were observed. After that, thread sealant was used in the caps, and silicone in the inlets and outlets of the filters, seeking to reduce the risk of leakage of the produced biogas (Table 1).

To close the cycle, an air chamber was installed in the last filter to store the generated biogas. Two gas valves were used, the first on the outlet hose of the last filter, the second on the nozzle of the chamber (Table 2).

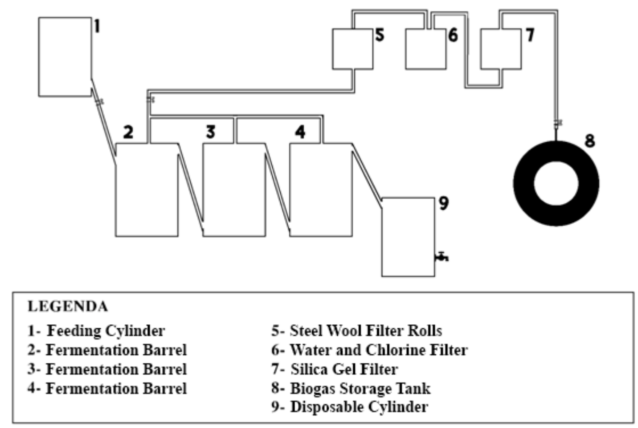

Figure 1 – Sketch of the biodigester prototype

1: feeding cylinder; 2: fermentation barrel; 3: fermentation barrel; 4: fermentation barrel; 5: steel wool filter rolls; 6: water and chlorine filter; 7: silica gel filter; 8: biogas storage tank; 9: disposable cylinder.

Figure 2 – Biodigester prototype

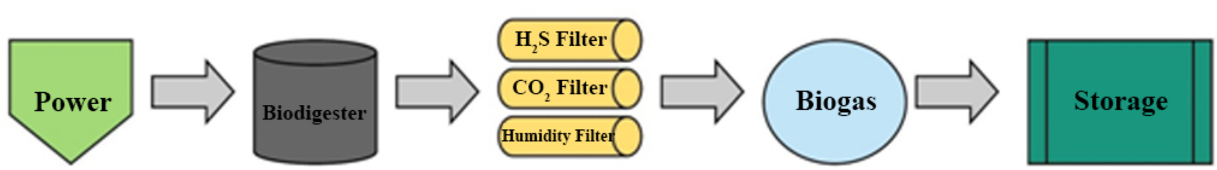

Figure 3 – Flowchart of biogas production
Source: adapted from Bonfim et al. (2019).

Table 2 – Summary of the filters used in the biogas purification

Filter	Utilized Material	Description
Filter 1	Steel Wool	Steel wool filter roll. Objective: to remove sulfate compounds, such as hydrogen sulfide, which impairs the storage of the gas and its burning (Brancoli, 2014)
Filter 2	Water and Chlorine	Approximately 1.5-liter mixture. Objective: to remove carbon dioxide produced from anaerobic digestion (Brancoli, 2014).
Filter 3	Silica Gel	Packaged Silica gel. Objective: to remove the moisture present in the biogas, thus increasing the calorific value of the compound (Baldacin and Pinto, 2015)

Table 3 – Biogas energy equivalence

Fuel	1 m^3 Biogas Equivalence
Gasoline	0.6 L
Kerosene	0.57 L
Diesel	0.55 L
Liquefied Petroleum Gas	0.45 kg
Ethanol	0.79 L
Firewood	1. 536 kg
Electric Power	1. 428 kWh

Source: adapted from Almeida (2016).

For estimating the savings in electricity consumption generated by biogas production, energy equivalence parameters were used, according to Masson et al. (2015), who relate the equivalence of 1 m^3 of biogas produced with the other types of fuels, as shown in Table 3.

Results

During the entire process of biodigestion of the residue, tests on the barrels and connections were carried out to identify possible leaks. The first indication of the production of biogas occurred 72 days after the feeding process, after opening the regulator that connected the buried system to the filters. At that moment, the appearance of bubbles inside the second filter, composed of chlorine and water, was seen.

Subsequently, the regulators were closed to increase the pressure inside the biodigester. For biogas storage, an air chamber with an approximate height of 0.22 m, external radius of 0.40 m, and internal radius of 0.18 m was used. Equation 1 was adopted to identify the filling volume of the chamber:

$$V = \pi . h(R^2 - r^2) \quad (1)$$

Where:
V = Volume;
π = Pi;
h = Height;
R = External radius;
r = Internal radius.
Likewise, chamber volume was identified according to Equation 2.

$$V = 3{,}14.0{,}22(0{,}4^2 - 0{,}18^2) = 0{,}088 \text{ m}^3 \quad (2)$$

Figure 4 – Air chamber filled with biogas

Table 4 – Filling the packaging chamber

Number of days	Accumulated m^3
72	0.08
94	0.16
101	0.04
108	No production
Total	0.2

After knowing the volume of the biogas storage container, regulators were opened, observing the movement of the liquid in the second filter. In sequence, the air chamber used to store the produced biogas began to inflate. Due to the low pressure of the system and the low resistance of the filters, the regulators were closed when filling the volume of a chamber, so that the gas would not return and break the chambers. Lastly, the chamber was emptied.

In the following week, steel wool roll of the first filter was oxidized, and so, it was replaced. The second opening of the regulators took place 94 days after the feeding process, following the same method as before. After 101 days, when opening the regulators, a decrease in biogas production was detected. When analyzing the air chamber, a volume corresponding to only half of its capacity was observed (0.04 m^3). After that, the regulators were closed, and the chamber was emptied. Figure 4 shows the first biogas production.

Table 4 shows the number of days on which gas production was verified, making up an amount of 0.2 m^3 of gas generated from the mixture.

Following the conclusion of the biodigestion process, there was no biogas production after 115 days. On this date, the unloading barrel was opened and the biofertilizer was accessed to identify its properties.

At the end of the process, a volume of 22 liters of biofertilizer was produced with the following characteristics: potential hydrogen (pH) of 6.4 and temperature of 21°C. According to Santos (1991), the pH of the biofertilizer can vary from 7.0 to 8.0 or be lower when fermentation is incomplete.

In the sensorial analysis, the presence of odor in the biofertilizer was observed. However, it did not present a putrefaction smell. As to its color, it presented light brown pigmentation. The quality of the biofertilizer is verified by means of color and odor, which is of low quality when it presents a putrefying odor and the foam that forms on the surface tends to be black (Gonçalves et al., 2009; Oliveira Filho et al., 2020). In such cases, it is suggested that the biofertilizer be discarded.

Table 5 presents a summary of the results obtained regarding gas production parameters.

Peanut shells stowed in the digester presented a methanogenic potential corresponding to 0.2 m^3 produced from 11.4 kg of residue, taking into account the 108-day conditioning period and external circumstances (rain and temperature).

The last evaluation of the biogas production occurred 108 days after the feeding process, and a minimum production was noted and considered irrelevant, so the total cycle of biogas production with peanut shells is comprised in a period of 108 days. Figure 5 presents the behavior of biogas production.

Table 5 – Biogas production parameters

Units / Parameters	Values
Peanut shells quantity (liters)	72
Peanut shells quantity (kilos)	11.4
Water (liters)	216
Conditioning time (days)	108
Biogas production (m^3)	0.2
Biofertilizer outlet temperature (°C)	21
ph	6.4
Odor	Yes
Color	Light brown

In order to carry out the economic analysis calculations of energy generated by biogas production, the final residue from peanut production is equivalent to approximately 1,275 $kg.ha^{-1}$. The amount of electricity consumption of BRL 0.57202 was also used for each 1 kWh employed by CPFL Energia, a non-state-owned Brazilian group of electric energy generation and distribution (CPFL, 2019). Table 6 presents a series of comparisons for producing biogas, taking into account the production *per hectare*.

As to the production of Herculândia City, if all this residue were used for producing biogas, 85,000 cylinders and 121,380 Kwh would be produced from each harvest.

Regarding biogas production on small farms, the reduction of electricity consumption can be equivalent to BRL 16.16 saved *per hectare* in the harvest, in the conditions shown in Table 7.

When compared to studies that estimate the production of biogas from swine residue (Souza et al., 2004; Martins and Oliveira, 2011) and cattle residue (Coldebella, 2006), it is evident that the amount of biogas generated exclusively from peanut shells is much less than that from animal residue. However, based on the results presented, these data are close to those generated from acerola cherry pulp, which has a volume of 0.1 m^3 produced from 52.10 kg of pulp (Bonfim et al., 2019).

Vintila et al. (2019) point out that, in Cameroon, Africa, after processing avocado, cocoa, and peanut crops, a large amount of residue is produced, which, when subjected to experiments, are transformed in gaseous (biogas) and liquid (ethanol) biofuels. The authors also estimate that if peanut skins are used to produce biogas, a volume of 30,376.960 m^3 would be produced from the residue generated in the country.

Peanut shells are an abundant and efficient resource for the biotechnological production of renewable fuels. Dahunsi et al. (2017) performed a previous treatment of the residue using the combination of mechanical and thermo alkaline procedures to optimize the retention time by increasing the temperature and pressure on the mixture. At the end of the process, a yield of 1739.20 m^3/ kg was obtained. For Liu et al. (2014), other applications can be developed with peanut shells. The authors used a mixture of 1,800 grams of fish remains from the local industry and available in large quantities, with 200 g of peanut shells, and produced an amount of 33.99 L in 20 days of biodigestion.

Further evidence suggests that animal residue can increase the biogas generating capacity of peanut shells, as suggested by Junqueira et al. (2011) and Paes et al. (2016). Thus, an amount of animal residue can be added next to the barrel, if available on the property, with the aim of increasing the biogas generating capacity. Family farming is characterized by the diversification of agricultural activities, which may favor the use of more than one residue to produce biogas.

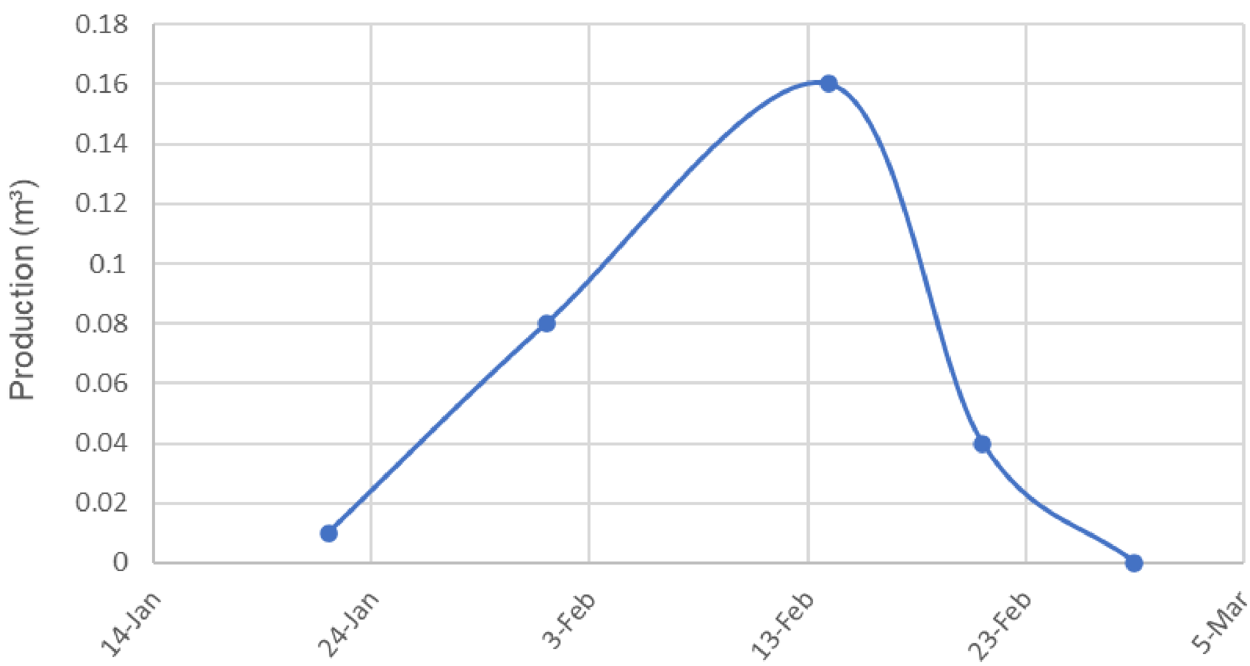

Figure 5 – Monitoring of biogas production

Table 6 – Comparison of residue production with energy resources

	Quantity (tonnes)	Biogas m³	Number of cylinders	Kwh/month
Residue *per hectare*	1.275	22.3	4	31.84
Residue in Herculândia City	4,845.0	85,000	15,740	121,380
Residue in São Paulo State	168,858,450.0	14,812,145	2,742,989	21,151,742.68

Source: Prepared by the authors, based on the data from the experiment; IBGE (2018); CPFL (2019).

Residue *by hectare*	1,250 kg	22.3 m³
Biogas equivalence	1 m³	1.428 kW/h
Energy equivalence (hectare per harvest)	130 days	31.84 kW/h
Price of kWh (CPFL, 2019)	1 kW/h	0.50742
Savings *per hectare* in the harvest		BRL 16.16

Source: Prepared by the authors, based on the data from the experiment; IBGE (2018); CPFL (2019).

Conclusion

The use of biodigesters to produce clean energy is in line with the SDGs, which seek to substantially increase the share of renewable energy in the global energy matrix to achieve sustainability. According to the analysis obtained in this work, peanut shells presented a production of approximately 0.2 m³ of biogas in a mixture of 11.4 kg of ground peanut shells with 216 liters of water. Regarding production *per hectare*, residues have the potential to generate an amount of 22.3 m³ of biogas, which corresponds to a generated energy of 31.84 Kwh per month.

Even though peanut shells present low potential in the generation of biogas when compared to animal residue, they have the potential to be used in small rural properties, since the energy consumption of family farms tends to be low. However, the use of peanut shells to produce biogas conveys the generation of electric energy from renewable sources in a decentralized manner, minimizing the use of energy from the concessionaires.

Contribution of authors:

Santos, C.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. Lourenzani, A.: Supervision, Validation, Visualization, Writing – review & editing. Mollo, M.: Supervision, Validation, Visualization, Writing – review & editing. Lopes, A.: Data curation, Formal analysis, Investigation, Project administration, Resources. Santos, P.: Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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Arabica coffee and cedar tree: integrating biotic and abiotic drivers

Arborização de cafezais arábica com cedro: integração de fatores bióticos e abióticos

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ABSTRACT

Agroforestry systems are important forms of sustainable farming, providing several ecosystem services. However, characterization and management of factors such as thermal and light heterogeneity, as well as interactions between trees and coffee plants, are determinants for achieving the desired sustainability. This study aimed to verify whether different distances between *Coffea arabica* L. and Australian red cedar can change soil and microclimate characteristics and how they alter morphological and physiological attributes of coffee plants over the rainy season and a prolonged drought period (*veranico*) in Summer. The trial was carried out in the municipality of Barra do Choça, in an area with Australian red cedar trees (*Toona ciliata* M. Roem), distributed in two hedges, spaced 19.8 × 3 m apart, in a northeast-southwest direction, and coffee plants var. Catucaí Vermelho (3.3 × 0.5 m). Treatments were defined by the distance between the coffee plants and the first row of the Australian red cedar hedge (3.3 m, T1; 6.6 m, T2; 9.9 m, T3; 13.2 m, T4; 16.4 m, T5). Morphology and physiology of coffee plants, soil temperature, incident light on coffee plants, and the allelopathic potential of Australian red cedar leaf extracts were assessed in the wet and dry season of the 2016–2017 Summer. Temperatures fluctuated less in experimental units close to the hedge. The reduced growth of coffee plants close to the hedges was related to self-shading associated with light restriction by the trees. The experiment showed the allelopathic potential of Australian red cedar leaves.

Keywords: agroforestry system; *Coffea arabica*; *Toona ciliata* M. Roem; allelopathy; shading.

RESUMO

Os sistemas agroflorestais são importantes formas de cultivo sustentável, gerando diversos serviços ecossistêmicos. Entretanto, a caracterização e o manejo de fatores como a heterogeneidade térmica e de radiação luminosa, e a interação entre componente arbóreo e os cafeeiros são determinantes para atingir a sustentabilidade almejada. O objetivo deste trabalho foi verificar se diferentes distâncias entre *Coffea arabica* L. e cedro australiano podem alterar as características do solo e do microclima e como isso altera os atributos morfológicos e fisiológicos das plantas de café durante a estação chuvosa e um período prolongado de seca (veranico) de verão. O ensaio foi conduzido no município de Barra do Choça, em área composta por árvores de cedro australiano (*Toona ciliatta* M. Roem) dispostas em dois renques subsequentes, com espaçamento de 19,8 × 3 m, sentido Nordeste-Sudoeste, e cafeeiros arábica var. Catucaí Vermelho (3,3 × 0,5 m). Os tratamentos foram definidos pela distância dos cafeeiros em relação à primeira linha do renque de cedro (3,3 m, T1; 6,6 m, T2; 9,9 m, T3; 13,2m, T4; e 16,5 m, T5). A morfofisiologia dos cafeeiros, a temperatura do solo, a radiação luminosa incidente nos cafeeiros de cada parcela e o potencial alelopático dos extratos de folhas do cedro australiano foram avaliados na estação úmida e seca do verão 2016/2017. Menor amplitude da variação térmica foi verificada nas áreas próximas ao renque. A redução do vigor de crescimento dos cafeeiros próximos aos renques de cedro foi relacionada ao autossombreamento associado à restrição de luz pelas árvores. Observou-se o potencial alelopático das folhas de cedro australiano.

Palavras-chave: sistema agroflorestal; *Coffea arabica*; *Toona ciliatta* M. Roem.; alelopatia; sombreamento.

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Introduction

Agroforestry systems are important forms of sustainable land use, with activities that may produce substantial environmental benefits, such as reducing the risk of extreme temperatures (Moreira et al., 2018; Coltri et al., 2019), promoting carbon sequestration and biomass accumulation (Meireles et al., 2019), nutrient cycling by means of a higher litter deposition (Galetti et al., 2018), and improving water use efficiency (Moreira et al., 2018; Padovan et al., 2018).

Sunlight availability is generally studied by assessing the intensity of incident radiation, using shade net houses as an empirical basis (Dias et al., 2017; Bote et al., 2018; Ribeiro et al., 2019). Under this artificial environment, light distribution and intensity are predominately homogeneous, much different from what is observed in field conditions. A tree-based system is characterized by uneven sunlight exposure and fluctuating temperatures throughout the day when compared to crops grown in full sun (Alves et al., 2016; Coltri et al., 2019), and this difference is more pronounced when trees are planted in hedges. With this type of tree arrangement, shading is tied to the positioning of the hedges in relation to the solar path as the Earth rotates and orbits the Sun. The hedge orientation determines the different shade lengths over the coffee field, and monitoring the seasons and daytime weather changes is of utmost importance (Moreira et al., 2018).

There is a dynamic interaction between the incidence of light and canopy trees throughout the seasons, conditioning plant growth and microclimatic conditions arranged in an agroforestry system (Schwerz et al., 2020). Tree lines planted in an east-west direction promoted self-shading with different intensities over the seasons (da Silva Neto et al., 2019).

When dealing with the tree component in agroforestry systems, the main focus has been light interception; however, other factors, such as allelopathy, have been pushed into the background when composing agroforestry systems. The Australian red cedar, *Toona ciliata* M. Roem, has been used as a shade tree in coffee fields due to its high-quality wood (Oliveira et al., 2019), much appreciated for carpentry, decoration, and making musical instruments (Bhardwaj et al., 2018). This species is easily adaptable to different soils and climates (Divakar, 2017) and has low susceptibility to insects, such as *Hypsipyla grandella* Zeller (Oliveira et al., 2019).

This study aimed to verify whether different distances between *Coffea arabica* L. and Australian red cedar can change soil and microclimate characteristics and how they alter morphological and physiological attributes of coffee plants over the rainy season and a drought period (*veranico*) in Summer.

Materials and Methods

The trial was carried out on a farm in Barra do Choça, Bahia State, Brazil (14°41'15"S, 40°26'15"W, altitude 943 m), from September 2016 to March 2017.

The site has a hot, temperate climate, with mean a annual temperature of 19.9°C. Mean temperatures ranged from 26.8°C in the warmest month to 12.2°C in the coldest month (Cfb according to the Köppen and Geiger climate classification). Mean annual rainfall is 741 mm, with the wettest months being December and January and the driest months being August and September. In this region, a prolonged drought occurs from February to April, the so-called *veranico* season (CONAB, 2016). During the trial, the daily mean temperature was 21.7°C, and rainfall was 385.8 mm (Figure 1).

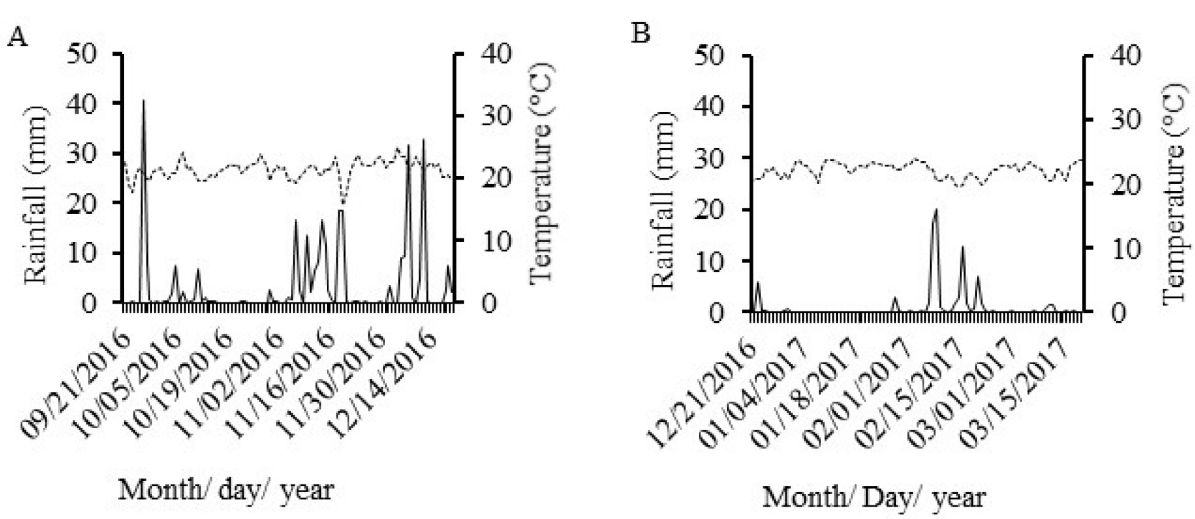

Figure 1 – Mean rainfall and temperature in the (A) rainy season 1 and (B) Summer season 2.

Australian red cedar trees and *Coffea arabica* cv. Catutaí Vermelho were transplanted to the experimental area in April and October 2014, respectively. The trees were planted in two hedges, spaced 19.8 × 3 m apart, in a northeast-southwest direction. Five coffee plant rows were grown between the hedges, spaced 3.3 × 0.5 m apart. Treatments were defined by the distance (D) between the coffee plants and the first row of the Australian red cedar hedge (3.3 m, T1; 6.6 m, T2; 9.9 m, T3; 13.2 m, T4; 16.4 m, T5), under rainfed conditions. Each experimental unit consisted of five coffee plants laid out in five blocks, totaling 25 plants per treatment.

Samples were collected from September to December 2016 (season 1, rainy season — S1) and from December 2016 to March 2017 (season 2, Summer *veranico* — S2).

For the morphological characterization of coffee plants, the following characteristics were measured: stem diameter (SD) (0.03 m above ground), measured on orthotropic stems, using a digital caliper (model DC-6); lateral shoot number (LSN) and leaf number (LN), both by direct counting; and plant height (H), from stem base to plant apex. Specific leaf area (SLA) was calculated with one leaf per experimental unit using a leaf area meter (model 3100, LI-COR, USA).

Leaf water potential (Ψ_w) was determined according to the method described by Scholander et al. (1964). The first fully expanded leaf was collected from the stem located in the middle third of the plant, in the morning ($\Psi_{wpd,}$ at 6:00 a.m.) and in the afternoon (Ψ_{wmd}, at midday and 6:00 p.m.). The readings were taken using a pressure chamber (PMS 1000, PMS, Corvallis).

Relative water content (RWC) was estimated according to Čatský (1960). Ten leaf discs measuring 12.65 mm in diameter were collected using drills. The discs were weighed (fresh weight) and then placed in Petri dishes filled with deionized water. The Petri dishes were kept in a biological oxygen demand (BOD) incubator at a constant temperature of 25°C for 24 h. After, the discs were weighed again (turgid weight) and oven-dried at 65°C to obtain the dry weight. RWC was calculated using Equation 1:

$$RWC = [(\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight})] * 100 \quad (1)$$

In which:
values = expressed as percentages.

Leaf greenness was determined using a portable chlorophyll content meter (SPAD 510, Minolta Camera Co., Osaka, Japan). The mean of three readings taken from each plant composing a plot was recorded.

Leaf temperature (LT) was measured on fully expanded leaves located in the middle third portion of the plant. Soil temperature (ST) was measured 50 cm above soil level. Both LT and ST were monitored at 6:00 a.m., 9:00 a.m., midday, 3:00 p.m., and 6:00 p.m. using a portable infrared thermometer (Multitemp, Radiant, China). Photosynthetic active radiation (PAR) was measured using a ceptometer (EMS-1, PP Systems, UK) at the same times of temperature readings, in both full sun (1.40 m above soil level) and above the plant canopy. PAR values were expressed as percentage.

For the bioassay of allelopathy, mature leaves were collected from the trees to prepare the extract by mixing 200 g of fresh leaf with 100 mL of heated distilled water. The resulting mixture was filtered. Aqueous solutions of polyethylene glycol (PEG) were prepared for concentrations (Brixº) similar to those determined for the red cedar leaf extracts (5, 10, 15, and 20% and a control). Lettuce seeds (*Lactuca sativa*) were used as target species, following da Silva et al. (2019). This trial adopted a completely randomized design, with 4 replicates, each consisting of 25 lettuce seeds sown on germination test papers placed on Petri dishes, totaling 20 experimental units. The following characteristics were determined: germination speed (GS), germination percentage on the fourth day (G4), and response index (RI), following the procedure reported by Lungu et al. (2011).

Means were tested for homogeneity of variances (Cochran) and normality (Lilliefors). Afterward, a combined analysis of multiple experiments was performed using a 1:7 ratio constraint when grouping mean squared errors of single analyses. For the combined analysis, the mean square of each main factor was contrasted with the mean square of the interaction. Interactions were verified using the ratio of the mean square of the interaction to the mean square of the residual. When a significant difference was identified for the main factor — season (S) —, the analysis of variance of the regression was used for the ratio of evaluated traits to the independent quantitative factor D, with fitted models based on the coefficient of determination ($R^2 \geq 50$) and biological behavior. Data analyses were performed using the statistical software SAEG (Statistical and Genetic Analysis System, version 9.1).

Results and Discussion

Almost all environmental factors, including ST and PAR, as well as morphological and physiological responses of coffee plants, were affected by S. As for ST, LT, and RWC, higher values were found in S1 compared to S2. For most morphological characteristics, Ψ_w and PAR measured in S2 were higher than those measured in S1 (Table 1).

Summer *veranico* reduced leaf RWC, although changes in Ψ_w were less pronounced. According to Peloso et al. (2017), when leaves are subjected to water deficit, RWC decreases; thus, RWC is a good indicator of plant water status. In S1, higher LT and ST associated with increased vapor pressure deficit and decreased coffee leaf hydraulic conductance were related to partial stomatal opening and reduction in Ψ_w (Rodrigues, W.P. et al., 2016).

Stomatal opening and closing mechanisms are strongly linked to light fluctuations over the day. Therefore, in the early morning, regardless of shading conditions, stomata remain partially closed due to the low radiation intensity, thus restricting the stomatal function of attenuating LT.

Table 1 – Morphophysiological characteristics of shade-grown Arabica coffee plants associated with toona trees (*Toona ciliata* M. Roem) cultivated in rainy Summer and Summer *"veranico"*.

Characteristic	Time (h)	Rainy Summer	Summer "veranico"	ANOVA Pr>F			
				Season (S)	Distance (D)	S*D	CV (%)
SD (mm)		18.90B	23.40A	*	*	ns	12.8
LSN		32.05A	35.88A	*	*	ns	12.2
H (cm)		69.61B	97.40A	**	**	ns	7.2
SLA (cm^2)		23.55B	50.83A	*	ns	ns	16.1
LN		330.12B	484.12A	**	*	ns	21.5
Ψw (MPa)	6	-0.94B	-0.40A	*	ns	**	27.9
	12	-1.89B	-0.99A	*	ns	*	28.6
	18	-1.29B	-0.31A	**	ns	**	26.7
RWC (%)		80.80A	68.85B	**	ns	ns	8.1
SPAD		67.06A	64.63A	ns	ns	ns	7.2
LT (°C)	6	17.95A	17.10A	ns	ns	**	3.5
	9	29.53A	23.43B	**	ns	*	9.4
	12	43.05A	26.32B	**	ns	*	11.6
	15	33.66A	24.84B	*	ns	**	9.1
	18	23.05A	20.36B	**	ns	ns	4.5
ST (°C)	6	19.90A	17.85B	*	ns	**	4.2
	9	39.03A	28.67B	*	ns	**	10.9
	12	58.44A	39.39B	**	ns	*	12.5
	15	39.11A	25.37B	**	ns	**	11.7
	18	25.77A	20.36B	**	ns	ns	3.8
PAR (%)	6	59.88B	87.39A	*	ns	**	27.2
	9	82.03A	66.54B	*	ns	ns	46.0
	12	90.18B	100.00A	*	ns	**	27.4
	15	77.27A	90.34A	ns	ns	**	27.3
	18	75.20B	99.33A	*	ns	ns	35.2

*Significance at $p \leq 0.05$; **Significance at $p \leq 0.01$; ns: not significant. Means followed by different letters in the same line are significantly different ($p < 0.05$, F-test); ANOVA: analysis of variance; CV: coefficient of variation; SD: shoot diameter; LSN: lateral shoot number; H: height; SLA: specific leaf area; LN: leaf number; ψ_w: predawn leaf water potential; RWC: relative water content; SPAD: soil plant analysis development index; LT: leaf temperature; ST: soil temperature; PAR: photosynthetic active radiation.

Coupled with this fact, the least difference in air temperature between S1 and S2 took place from 4:00 a.m. to 5:00 a.m., close to data collection at 6:00 a.m. (Figure 2).

Air temperature amplitude was lower between S1 and S2 compared with the interaction between S and D. This difference was linked to the lack of effect of S and D on LT in readings taken at 6:00 a.m. Trees are important for stabilizing the temperature within the agroforestry system, as they increase minimum mean temperatures and decrease maximum mean temperatures (Moreira et al., 2018). Nevertheless, air temperature and solar radiation showed higher spatial variability in shade-grown coffee fields, with modulations over the different seasons of the year (Petit-Aldana et al., 2017).

Soil plant analysis development (SPAD) readings were homogeneous (Table 1) due to a possible effect of the time of reading and to changes in leaf anatomy, thereby interfering with reading accuracy (Xiong et al., 2015; Padilla et al., 2019). Changes in light quality and intensity may affect the chloroplast position and, therefore, change real-time chlorophyll meter readings compared to chlorophyll content determined by destructive methods (Mamrutha et al., 2017). Although a close relationship between specific leaf area and chlorophyll meter readings has been reported and subsequently proposed as a procedure for plant breeding (Lisboa et al., 2019), no agreement has been reached on whether shade-induced morphological changes are always linked to increased leaf greenness.

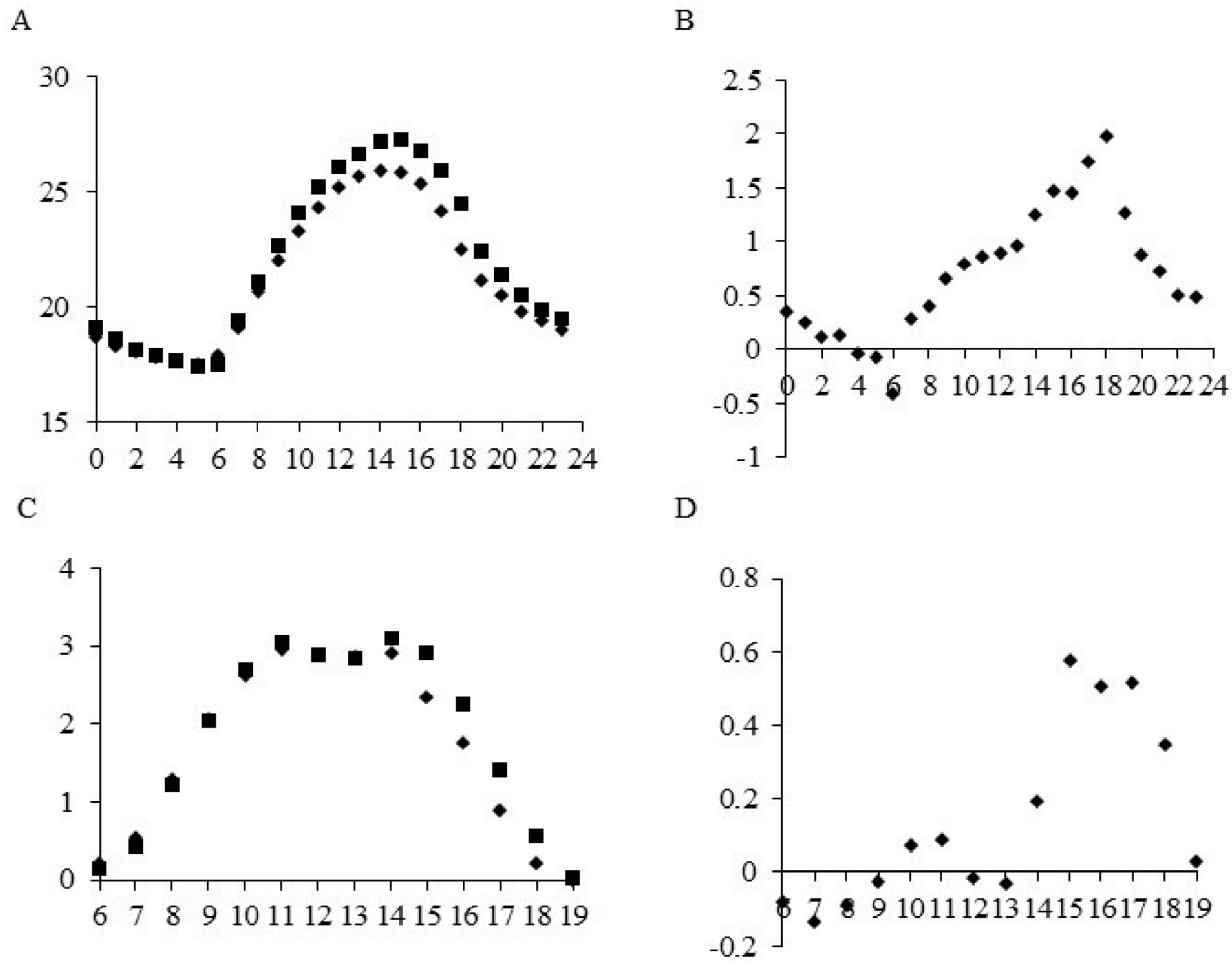

Figure 2 – (A) Fluctuations in daily temperature; (B) difference in thermal fluctuations over season 1 — rainy season — (♦) and season 2 — Summer *veranico* — (▪) in 2016/2017, measured every 60 minutes; (C) fluctuations in daily radiation; and (D) difference in radiation fluctuations between seasons 1 and 2.

Growing trees among crop plants modifies the microclimate; however, factors such as tree arrangement, species, age, and management are determinants of these changes in tree-based systems.

As for the difference in PAR readings between S1 and S2, readings from S2 tended to be higher than those from S1 (Figure 2C). In field conditions, variations in relative PAR indices are common, and both arrangement and composition of the system are determinants of such fluctuations (Araújo et al., 2016).

The main factors S and D induced morphological changes in coffee plants. D had no impact on temperature, light, and water status; nonetheless, an interaction between S and D was observed for these characteristics (Table 1). These subtle interactions between factors altered environment characteristics but were not intense enough to induce morphological changes. Therefore, the integrated nature of morphological measurements differed from the specificity of measurements done for the environment and plant water status.

An interaction between D and S was identified for LT, ST, and PAR from 6:00 a.m. to 3:00 p.m., except at 9:00 a.m. (Table 1). The absence of interaction between S and D at 9:00 a.m. for PAR was associated with the radiation homogeneity at this time in both seasons (Figure 2D).

The relationship between morphological traits (SD, LSN, H, SLA, and LN) and D in S1 and S2 was fitted to a quadratic model that showed a less vigorous growth of coffee plants located closer to tree hedges (Figures 3A, 3B, 3C, and 3D). Despite the similarity of the models for both seasons, the lower rainfall during S2 (*veranico*) intensified the shade effect on crop growth, thus resulting in more pronounced changes in growth traits (SD, LSN, H, and LN) for plants located farther from the hedge (Figures 3A, 3B and 3D). In S2, limited water and high temperatures (lower Ψ_w and higher LT) in coffee plants located closer to the hedges resulted in lower leaf expansion and smaller SLA (Figure 3C).

Generally, decreases in SD, NL, and LSN are common plant responses to shading; however, decreases in H and SLA are not common in shade-grown coffee fields. Intense self-shading due to increased plant density coupled with the shade cast by the cedar hedges reduced light availability, reaching, at times, as low as 20% PAR. This scenario impaired growth and some photomorphogenesis processes. Although *C. arabica* is originally from forest understory, these plants are highly sensitive to limited light, and decreased vigor has been reported by many studies on increasing plant density in shaded areas.

Allelopathic interactions were also studied in bioassays carried out in the laboratory, showing that as the concentration of Australian red cedar extracts increases, lettuce seed germination decreases (Figures 4A, 4B, and 4C).

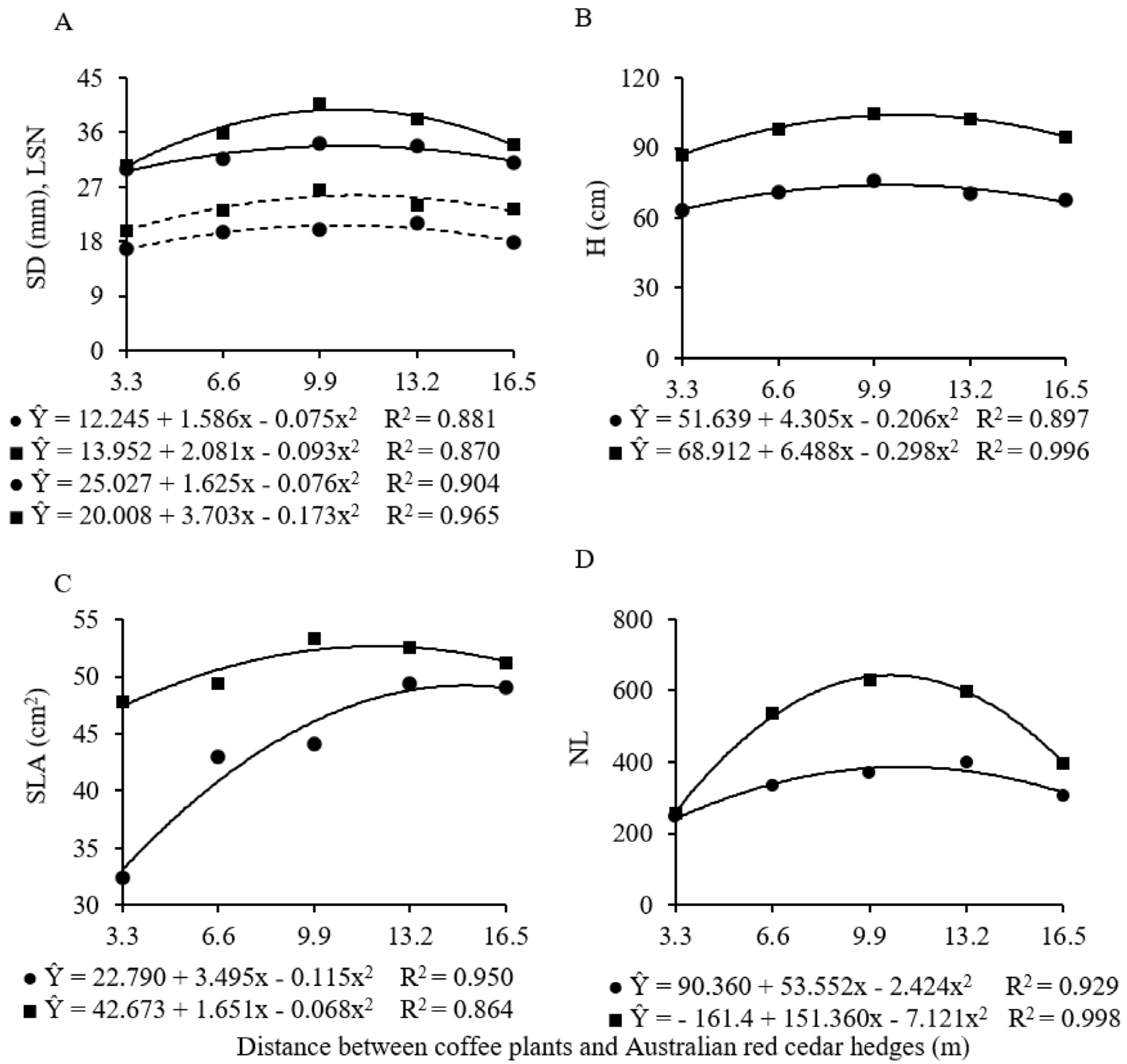

Figure 3 – (A) Stem diameter (---), stem number (–); (B) height; (C) specific leaf area; and (D) leaf number determined in the rainy season (●) and Summer *veranico* (▪) in coffee plants (*Coffea arabica* L.) shaded by Australian red cedar (*Toona ciliata* M. Roem).

This effect on germination was associated only with allelopathic effects since the soluble solids concentration of the extracts did not have an osmotic effect on germination, as shown by tests with PEG.

Tree litter can release phytotoxic exudates into the soil, restricting germination (Rawat et al., 2017) and growth vigor of surrounding plants (Garima and Devi, 2017). Limonoids (De Leo et al., 2018; Zhang et al., 2019) and several phenolic compounds (Samaradivakara et al., 2016; Tandon and Sand, 2016) predominate among metabolites of Australian red cedar. Limonoids extracted from several species belonging to the family Meliaceae have phytotoxic properties that inhibit germination of many crops, such as maize, common bean, gourd, and lettuce (Parmar et al., 2019). According to Nebo et al. (2015), limonoids isolated from species of the family Meliaceae are important sources of allelochemicals with potential for agriculture.

A quadratic model relating leaf Ψ_w to D was defined for measurements done at 6:00 p.m. in S1 and S2. In S1, the highest Ψ_w occurred mostly in coffee plants halfway between the Australian red cedar hedges (T3). The opposite trend was observed in S2 (Figures 5A and 5B).

Light intensity has a low impact on hydraulic responses of shade-grown coffee plants since *C. arabica* is original from shaded habitats where, from an evolutionary standpoint, the development of strategies regulating the plant hydraulics was limited (Miniussi et al., 2015). The presence of trees, however, modulates other environmental components that may contribute to the water status of coffee plants in a positive (presence of tree litter, reduced thermal amplitude, and limited soil evaporation) or negative way (superficial root development, competition between coffee plants and trees due to the reduced soil volume for the roots to explore).

In the S1 of this study, the lowest Ψ_w and RWC in coffee plants nearer the red cedars was related to the decrease in soil volume exploration by coffee plant roots due to reduced crop vigor and the presence of trees (Figures 5A and 5C). Root volume associated with improvements in hydraulic conductance is an important factor determining water availability to plants. In S2, Ψ_w had lower variation amplitude in response to D, with lower variations in LT and ST as well. The lowest leaf Ψ_w observed in T3 (9.9 m from the hedges) compared to those nearer the trees was associated with the water deficit induced by the higher shoot vigor (greater number of leaves), hence, representing a greater sink.

In S2, the relationship between leaf greenness (SPAD) and D was fitted to a quadratic model, showing an increase in leaf greenness up to 14.33 m (Figure 5D), while Campa et al. (2017) reported an opposite trend. These authors verified that the shaded condition increased leaf greenness in coffee plants. Factors that may affect the relationship between SPAD readings and shading are time of reading and changes in specific and total leaf area (Xiong et al., 2015).

The relationship between RWC and D was fitted to similar models in S1 and S2 (Figure 5C). An upward trend in RWC was detected for coffee plants located halfway between hedges in S1, and a downward trend was identified for coffee plants located close to one of the hedges in S2. The occurrence of such differences was attributed to changes in the Earth's revolution around the Sun between S1 and S2.

Increases in evapotranspiration rates were associated with the reduction in RWC, which was indirectly affected by the higher LT (Figure 5C). A similar relationship was reported by Thioune et al. (2017).

For S1, LT, ST, and PAR responses to D, in nearly all times of reading, were fitted to a quadratic model (Figures 5E, 5F, 6A, 6B, 6C, and 6D). These models were characterized by lower values for coffee plants close to the hedges, except for measurements taken at 6:00 a.m. In the early morning, these values were higher in coffee plants located nearer the trees than in those farther away from the hedges. In S2, an upward linear trend in the relationship between LT and D was observed only at 3:00 p.m.

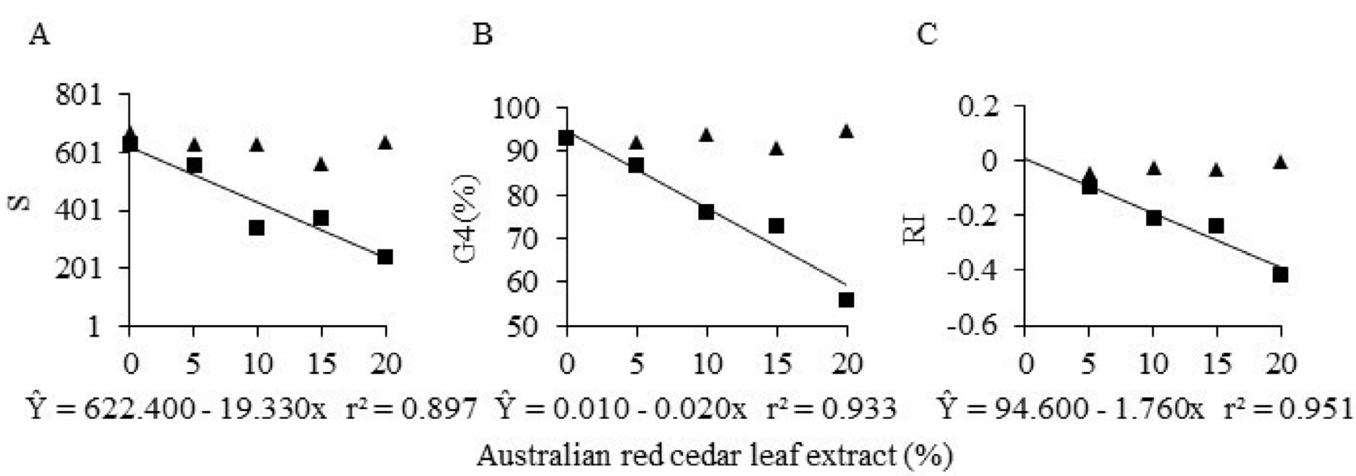

Figure 4 – (A) Emergence speed index; (B) germination on the fourth day; and (C) response index in lettuce seeds (*Lactuca sativa*) subjected to Australian red cedar leaf extract (▲) and polyethylene glycol solution (▪).

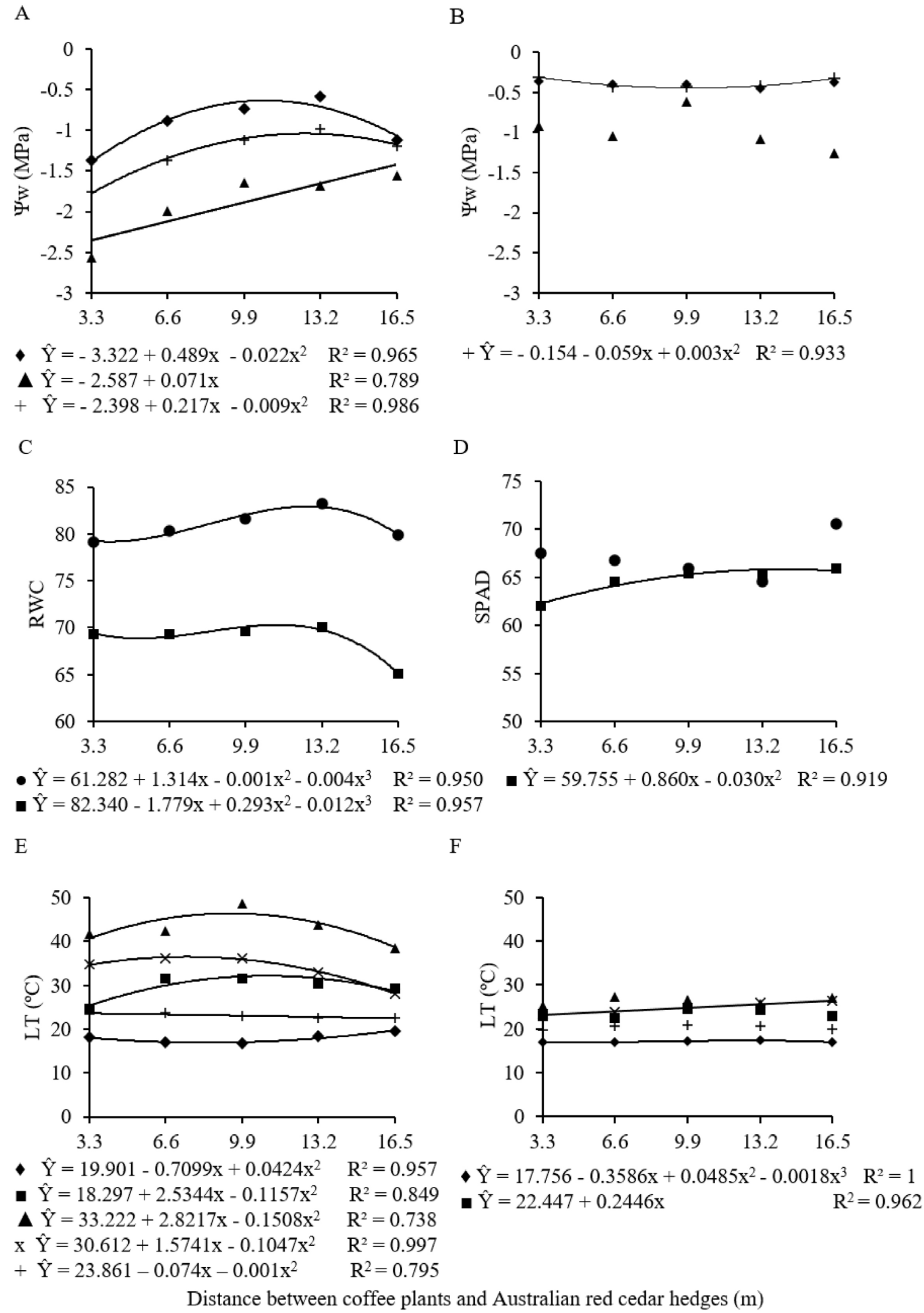

Figure 5 – Water potential (Ψw) in (A) the rainy season and (B) Summer *veranico* measured at 6:00 a.m. (♦), 12:00 a.m. (▲), and 6:00 p.m. (+); (C) relative water content (RWC) and (D) soil plant analysis development (SPAD) readings in the rainy season (●) and Summer *veranico* (▪). Leaf temperature (LT) in (E) the rainy season and (F) Summer *veranico* measured at 06:00 a.m. (♦), 9:00 a.m. (▪), 12:00 a.m. (▲), 3:00 p.m. (x), and 6:00 p.m. (+) in coffee plants (*Coffea arabica* L.) shaded by Australian red cedar (Toona ciliata M. Roem).

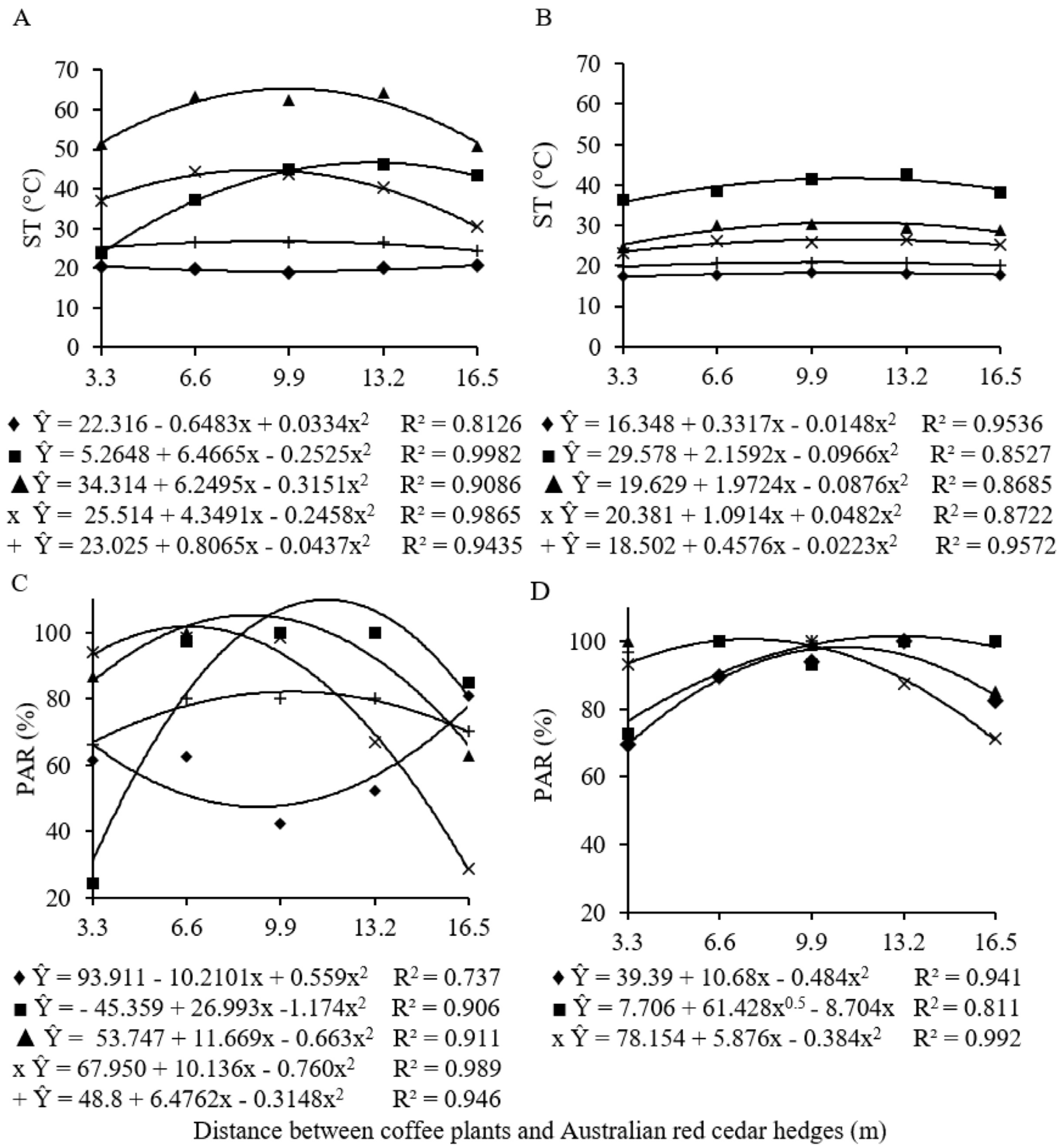

Figure 6 – Soil temperature (ST) in the (A) rainy season and (B) Summer *veranico*; photosynthetic active radiation (PAR) in the (C) rainy season and (D) Summer *veranico* measured at 6:00 a.m. (♦), 9:00 a.m. (▪), 12:00 a.m. (▲), 3:00 p.m. (x), and 6:00 p.m. (+) in coffee plants (*Coffea arabica*) shaded by Australian red cedar (*Toona ciliata* M. Roem).

Light and temperature modulation by trees are important for coffee fields from an ecosystem point of view, especially in the prospect of climate change (Da Matta et al., 2018; Coltri et al., 2019).

The thermal attenuation caused by distances between trees and coffee plants was previously reported by Moreira et al. (2018). We underline the elevated plant and soil temperatures registered in Summer at midday, in agreement with Oliosi et al. (2016), who described a thermal index as high as 40°C, at 12:00, in Summer and Autumn, in coffee plants maintained at 4.5 m of distance from cedar trees.

Conclusions

Environmental variations that occurred in the wet season and *veranico* in Summer affected the morphology, water relations, temperature, and light incidence in coffee plants associated with Australian red cedar hedges.

The distance from the hedge as a main effect has an impact on the morphology of coffee plants; however, it does not interfere with water relations, temperature, and PAR of coffee plants associated with red cedars.

The allelopathic effect of Australian red cedars and the excessive self-shading are factors that reduce the growth of coffee plants located near the tree hedge.

The presence of trees attenuates fluctuations in temperature and PAR. Distance from the hedge and season are factors that determine to what extent these fluctuations are modulated.

Contribution of authors:

Pereira, L.F.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing — original draft, Writing – review & editing. Matsumoto, S.N.: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. Oliveira, U.S.: Data curation, Investigation, Project administration, Visualization, Writing – review & editing. Viana, A.E.S.: Data curation, Formal analysis, Resources, Visualization, Writing – review & editing. Teixeira, E.C.: Investigation, Project administration, Visualization, Writing – review & editing.

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How long is long enough? Decreasing effects in *Aedes aegypti* larval mortality by plant extracts over time

Quanto tempo é tempo suficiente? Redução dos efeitos na mortalidade larval de *Aedes aegypti* por extratos de plantas ao longo do tempo

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ABSTRACT

Aedes aegypti has overcome all kinds of mosquito control attempts over the last century. Strategies for population control resorts to the use of synthetic insecticides, which can lead to problems like human intoxication and environmental contamination. The effects of *Bacillus thuringiensis* var. *israelensis* (Bti), *Ilex paraguariensis* (yerba mate), and *Ilex theezans* (caúna herb) extracts against *A. aegypti* larvae were evaluated. The bioassays were conducted under controlled laboratory conditions of temperature (27 ± 3°C) and photoperiod (12 h). Hydroalcoholic extract of the leaves of *I. theezans* displayed better residual effect compared to the aqueous extract of *I. paraguariensis* fruits. The strongest residual effect of *I. theezans* was probably due to the presence of certain chemicals in its leaves, such as coumarins, hemolytic saponins, and cyanogenic glucosides, which were absent in *I. paraguariensis*. The results herein contributed to the prospection of natural insecticides and opened the possibility for subsequent studies on the use of plant extracts in field situations in a short-time scale.

Keywords: dengue; vector control; inseticide; entomology; mate herb.

RESUMO

Aedes aegypti superou todos os tipos de tentativas de controle do mosquito pelo homem no último século. Estratégias para controle populacional recorrem ao uso de inseticidas sintéticos, que podem levar a problemas como intoxicação humana e contaminação ambiental. Foram avaliados os efeitos de *Bacillus thuringiensis* var. *israelensis* (Bti), extratos de *Ilex paraguariensis* (erva-mate) e *Ilex theezans* (erva-caúna) contra a mortalidade de larvas de *A. aegypti*. Os bioensaios foram conduzidos sob condições laboratoriais controladas de temperatura (27 ± 3°C) e fotoperíodo (12 h). O extrato hidroalcoólico de folhas de *I. theezans* apresentou melhor efeito residual quando comparado ao extrato aquoso de frutos de *I. paraguariensis*. O efeito residual mais forte de *I. theezans* provavelmente ocorreu devido à presença de substâncias químicas em suas folhas, tais como cumarinas, saponinas hemolíticas e glicosídeos cianogênicos, ausentes em *I. paraguariensis*. Nossos resultados contribuíram para a prospecção de inseticidas naturais e abriram a possibilidade de estudos subsequentes sobre o uso de extratos vegetais em situações de campo em um curto espaço de tempo.

Palavras-chave: dengue; controle vetorial; inseticida; entomologia; erva-mate.

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Introduction

Over the last century, the mosquito *Aedes aegypti* (Linnaeus, 1762) has overcome all mosquito control attempts. *A. aegypti* females are well known by their capacity of naturally and/or under laboratory conditions replicate and transmit over 100 kinds of viruses (Weaver and Reisen, 2010). As an example, the viruses of Dengue, Chikungunya, Zika, and, most recently, the Mayaro (Weaver and Reisen, 2010) virus can be listed in Brazil, which represent a real threat to public health (Lopes et al., 2014). Therefore, its medical importance requires the population control of this species to reduce virus transmission and, consequently, its epidemic status. Although several chemical and natural products have been extensively used on attempts to reduce the population of adults and larvae (Liu, 2015; Apaire-Marchais et al., 2016), adequate mosquito control is not even close to become true, especially due to the genetic resistance selectivity because of the incorrect use of natural products and chemicals (Sun et al., 2019). As a consequence, the most effective disease prevention method still focus on targeting the mosquito population by eliminating mosquito breeding places (Brasil, 2002).

A very promising field for reducing the mosquito population is to focus on mosquito control strategies that target immature aquatic stages, when the insect is more vulnerable (Brasil, 2001; Crivelenti et al., 2010). For this purpose, the use of synthetic insecticides is well known for its efficacy, causing mosquito larval mortality (Busato et al., 2015; Govindarajan et al., 2018). However, those chemicals might affect humans, resulting in intoxication and environmental contamination, and affecting biodiversity (Busato et al., 2015; Tahir et al., 2015; Baskar et al., 2018; Govindarajan et al., 2018). Regarding the environment, the continuous use of synthetic insecticides may present undesirable effects, such as the long-term permanence in the environment, selection of resistant populations, and the appearance of new pests (Tahir et al., 2015; Baskar et al., 2018; Govindarajan et al., 2018). As to human health, the presence of such synthetic chemicals on the environment can cause neurological damage and is associated with a wide range of symptoms, with significant deficits in the nervous system function (Araújo et al., 2007).

Alternatively to the use of synthetic chemicals, biological control plays an important role on mosquito management (Zara et al., 2016; Coelho et al., 2017). The use of bacteria spores as a mosquito larvicide has stood out among the several components that are part of mosquito-integrated management programs (Zara et al., 2016). Over the last decade, the use of inactivated spores of the bacteria *Bacillus thuringiensis* var. *israelensis* (Bti), spread in the water of mosquito breeding places, has met the expected results, reaching mortality rates above 99% (Soares-da-Silva et al., 2017; Nakazawa et al., 2020). Additionally, during the last few years, plant-derived compounds have been extensively used as an alternative method for controlling mosquitoes, not only because this is a new insecticidal agent, but also because it has been described as being environmentally friendly (Gomes et al., 2016; Guarda et al., 2016; Knakiewicz et al., 2016; Rosa et al., 2016). The use of natural insecticides has some advantages over traditional synthetic products, because natural products are potentially less toxic to the environment. Environmentally-friendly compounds are less concentrated, have faster degradation, and are specific to certain insect groups, resulting in less occupational exposure and less environmental pollution (Krinski et al., 2014).

Ilex paraguariensis A. St.-Hil (Aquifoliaceae), known as *mate*, is an abundant plant, native of South America, 20 m tall, with a dense crown, and very branched (Souza, 2009). After processing, its leaves are traditionally used in a regional tea known as *mate* in Argentina, Brazil, Paraguay, and Uruguay (Souza, 2009). *I. paraguariensis* is commercially important due to the presence of caffeine and theobromine, both recognized as having a stimulant effect in the nervous and cardiocirculatory systems (Castaldelli et al., 2011). The described pharmacological activities for *I. paraguariensis* leaf extracts include antioxidant, hypolipidemic (Gao et al., 2013; Messina et al., 2015), and hypoglycemic effects (Conceição et al., 2017). Besides that, *Ilex theezans* Mart. Ex Reissek (Aquifoliaceae), popularly known as caúna-herb, is commonly found in Southern Brazil (Souza, 2009). It is well known due to the physiological characteristics of its leaves as an adulterant of *I. paraguariensis* (Athayde et al., 1999). It is an evergreen tree, early secondary or late secondary species (Souza, 2009), 20-m tall and 70-cm diameter, on average (Athayde et al., 1999). Both the *I. paraguariensis* fruit extract and the *I. theezans* hydroacoholic leaf extracts have larvicidal effect against *A. aegypti* larvae (Busato et al., 2015; Knakiewicz et al., 2016).

Some studies showed that *Ilex* spp. leaves and fruit extracts kill *A. aegypti* larvae within a 24-h observational time (Busato et al., 2015; Knakiewicz et al., 2016). However, there are no studies in the current literature evaluating the effects of time on the bioinsecticide lethal activity. How long is long enough for the bioinsecticide to maintain its ability to kill? (Resende and Gama, 2006; Santos et al., 2007; Guirado and Bicudo, 2009). In this context, the lethal residual effect of *B. thuringiensis* var. *israelensis,* and leaf and fruit extracts of *I. theezans*, and *I. paraguariensis*, respectively, against *A. aegypti* larvae were evaluated. Time would positively affect *A. aegypti* larvae survival due to decay of the lethal compounds, as a hypothesis, but the mortality caused by *I. theezans* was higher when compared to *I. paraguariensis*, due to the difference in physical and chemical characteristics.

Material and Methods

Animal source

The *A. aegypti* larvae used in this experiment were provided by Laboratório de Entomologia Ecológica (LABENT-Eco). A filter paper holding about 300 eggs was placed in a plastic tray (30 × 20 cm) holding 1 L of tap dechlorinated water. After hatching, the larvae were distributed among three plastic trays of equal size and fed with 2 g of fish food. The mosquito larvae were raised for about 4-5 days until reaching 3rd and 4th instars.

Plant source and extract preparation

Fruits and leaves were obtained from native trees located at the Marechal Bormann district (27°19'05"S; 52°65'11"W), Chapecó City (Santa Catarina State), in December 2016. Plant parts were dehydrated at room temperature (± 20°C), pulverized in a knife mill (Cielamb®, CE 430), and stored away from light and humidity. Plant extracts were prepared according to Busato et al. (2015) and Knakiewicz et al. (2016). Samples of 20 g of *I. paraguariensis* dehydrated fruits and *I. theezans* leaves were used. Both samples were extracted by turbolysis, using 200 mL of distilled and deionized water and a hydroalcoholic solution (90% ethanol; 200 mL) as solvent, respectively (ANVISA, 2019). The extracts were filtered in Büchner funnel, concentrated by rotavapor under reduced pressure, lyophilized, weighed, identified, and stored in a freezer at -20°C. Hydroalcoholic and aqueous extracts were prepared using *I. theezans* and *I. paraguariensis* leaves and fruits, respectively. Leaves at a concentration of 1,000µg/mL were used, and fruits were diluted to 2,000 µg/mL. *B. thuringiensis* var. *israelensis* (Bti), strain WG®, was used in a concentration of 0.004 g/L, the lethal dose specified by the manufacturer.

Experimental microcosms and design

Plastic cups of 300 mL with 100 mL of dechlorinated water plus the treatment proposed were adopted. In each individual sample, 20 3rd and 4th instar *A. aegypti* larvae were added. Each container was covered with a mosquito net held by a rubber elastic band. Tests for the effects of Bti spores; *I. theezans*, and *I. paraguariensis* leaves and fruits, respectively, were conducted; clean aged water (control) on the *A. aegypti* larval mortality after seven days of exposure was also performed (Nakazawa et al., 2020). Before running the mortality test, each experimental treatment aged from one to eight weeks. Each week was considered as one age block with each treatment replicated six times. With this experimental design, the independence of each set of treatments was assured. The aged treatments were used to test for larval survival in each experimental week. At the end of the 7th day, larval survival was recorded, with both pupae and emerged adults being considered as survivals. The experiment was performed for eight weeks (56 days) and carried out between April and May 2017 at the LABENT-Eco mosquito colony room, under controlled conditions of temperature and photoperiod (27 ± 3°C, 12h D:L).

Statistics

Since both negative (Bti) and positive (tap water) control survival rates were 0.16% and 100%, respectively, the data were analyzed in both ways, with (complete model) and without (simple model) these two categories. In order to evaluate differences in the percentage of larval mortality (response variable) between simple (*I. paraguariensis* and *I. theezans*) and the complete models (only water, Bti spores, *I. paraguariensis* and *I. theezans*), regarding week (1 to 8) and week-treatment interaction (explicative variables), we used factorial GLM, with binomial correct to quasi-binomial (link = logit, test = Chi-square) distributions (Crawley, 2007). All analyzed GLMs were corrected for cases of under- or overdispersion.

Differences among the categorical variables were assessed with a contrast analysis (Crawley, 2007). In this analysis (orthogonal), the dependent variables (different treatment and weeks) were ordered increasingly and tested pairwise (with the closest values); sequentially, adding to the model values with no differences and testing with the next values in a stepwise model simplification (for more details see also Chapter 9 of Crawley, 2007). All analyses were performed using the R program (Venables et al., 2019).

Results

The *A. aegypti* larval mortality was not affected by the water age (positive control). In contrast, the Bti resulted in the death of all the larvae until the age of seven weeks, with only 6.6% of larvae alive on the age of eight weeks (negative control). In this way, due to these extreme results in controls (0% of mortality in the positive control and 100% of mortality in the negative control), mortality data were analyzed only between treatments (*I. paraguariensis* and *I. theezans*).

Larval mortality was significantly different between treatments (*I. paraguariensis* and *I. theezans*), weeks (1 to 8), and interaction factors (week:treatment) for both GLMs models (with and without positive and negative controls; Table 1). The highest larval mortality was found in the Bti treatment (negative control), followed by *I. theezans, I. paraguariensis*, and positive control (Table 1; Figure 1A). In addition, the *I. theezans* hydroalcoholic leaf extract, regardless of extract's age, killed significantly more *A. aegypti* larvae than the aqueous *I. paraguariensis* fruit extract (Table 1; Figure 1B).

A positive relationship between the survival of the larvae and the plant extract age was observed. In general, both *I. paraguariensis* and *I. theezans* killed less (mainly after seven weeks) mosquito larvae as the plant extracts aged (Figure 2). A higher significant larval mortality was found in week 1, followed by weeks 2 and 3, weeks 4 and 6, week 5, and weeks 7 and 8 (Figures 1B and 1C).

Residual deviance (estimate of the variance of the tested variables) in GLM with positive and negative controls, showed that differences in all treatments (74%) was the main responsible for larval mortality, followed by extracts' age (18%; Table 1). On the other hand, residual deviance in GLM, without positive and negative controls, showed that differences between all weeks (68%) was the main responsible for larval mortality, followed by treatments (6%; Table 1).

Discussion

Transformation of the larvicide effect into food resource over time

I. theezans and *I. paraguariensis* extracts are promising against mosquito larvae (if applied and monitored in the first weeks). The potential of using these plant extracts as larvicides for *A. aegypti* may be an advantage, since they are natural extracts and do not leave toxic waste in the environment. These extracts are an abundant and accessible alternative in Southern Brazil, where *A. aegypti* infestation and dengue cases have been observed in the last decade (Busato et al., 2015). However, extracts' age should be considered; the main objective of the present study is not to discourage the use of such alternative method, but to warn about the importance of extract aging before using it for mosquito-control purposes. Better results may also be obtained with the development of additional studies, evaluating the larvicidal activity of pure compounds isolated from these plants. Furthermore, better results might be obtained by evaluating if there is a supporting effect of more than one active principle with larvicidal action against *A. aegypti*.

Plant extracts may degrade as time goes by, and these organic compounds with previous larvicidal activity may become food for *A. aegypti* larvae (explaining the residual deviance percentage in GLMs models). The transformation of larvicides into food probably took place, especially in those treatments with seven- and eight-weeks old plant extracts, which presented the highest survival rate. Therefore, the age of plant extract should be considered (Albeny-Simões et al., 2015).

Aedes aegypti larval mortality between plant extracts

Plant extract age plays an important role on mosquito larvae mortality (mainly with positive and negative controls). Despite the plant species, plant parts, and extraction method, mosquito larval mortality decreases with the aging of plant extracts. However, the extracts tested were highly efficient in the first weeks of the experiment (high mortality). The higher larval mortality found in the *I. theezans* extracts can be partially explained by the use of solvents during the extraction process (Lee and Houghton, 2005). The hydroalcoholic extraction method used to obtain the *I. theezans* extracts removed low polarity chemical constituents from plant tissues, and these molecules have a higher ability to penetrate mosquitoes' larvae cells and modify their metabolic activities.

On the other hand, aqueous extraction, used for the *I. paraguariensis* fruits, preferentially removes high-polarity chemical compounds, which are not able to easily penetrate such cells (Lee and Houghton, 2005). Moreover, the susceptibility of *A. aegypti* larvae to *I. theezans* may be explained by the presence of secondary metabolites of the coumarin class and absence of alkaloids when compared to *I. paraguariensis* (Valduga et al., 1997). Coumarins are part of the secondary metabolism of several plants, being well known for presenting insecticidal activities, acting as a repellent of adult insects, preventing oviposition, impairing feeding and growth, promoting morphogenetic and hormonal system alterations, sexual behavior changes, and adult sterilization, among other effects (Dietrich et al., 2011). Furthermore, *I. paraguariensis* has a higher content of caffeoyl derivatives and flavonoids than *I. theezans*. Flavonoids are recognized for their potent larvicidal activity, which may partially explain the results obtained herein (Filip et al., 2001; Garcez et al., 2013). In raw plant extracts, the active constituents are usually found in small concentrations (Krinski et al., 2014).

Larvae mortality and extract age of *Aedes aegypti*

In both *I. theezans* and *I. paraguariensis,* the mortality of *A. aegypti* larvae exposed to one-week plant extracts was 100%.

Table 1 – Generalized linear models (GLM), degrees of freedom (Df), Residual Deviance (total and in), and *p* values, comparing the percentage of *Aedes aegypti* larvae mortality after exposure to treatments (water control, *Bacillus thuringiensis israelensis* — Bti, hydroalcoholic dried leaves extract of *Ilex theezans*, and aqueous *Ilex paraguariensis* fruits extract), time (8 weeks) and interaction among treatments and weeks, under laboratory conditions.

GLM	Df	Resid. Dev.	Resid. Dev. %	Pr(>Chi)	Analysis of contrast
a. With positive and negative controls					
Treatments	3	132.9	74.1	< 0.001	Control < *Ilex paraguariensis* < *Ilex theezans* < Bti
Weeks	7	33.4	18.6	< 0.001	Week 8 = 7 < 5 < 4 = 6 < 3 = 2 < 1
Treatment: weeks	21	3.0	1.7	< 0.001	
Residual	160	10.0	5.6		
b. Without positive and negative controls					
Treatments	1	3.1	6.6	< 0.001	*Ilex paraguariensis* < *Ilex theezans*
Weeks	7	32.3	68.7	< 0.001	Week 8 = 7 < 5 < 4 = 6 < 3 = 2 < 1
Treatment:weeks	7	2.4	5.1	0.002	
Residual	80	9.2	19.6		

However, the plant extracts for both species reduced the mortality of mosquito larvae as the plant extracts aged. These results pointed out the need for carefully selecting the right age for an *Ilex* spp. plant extract before using it to control mosquito larvae.

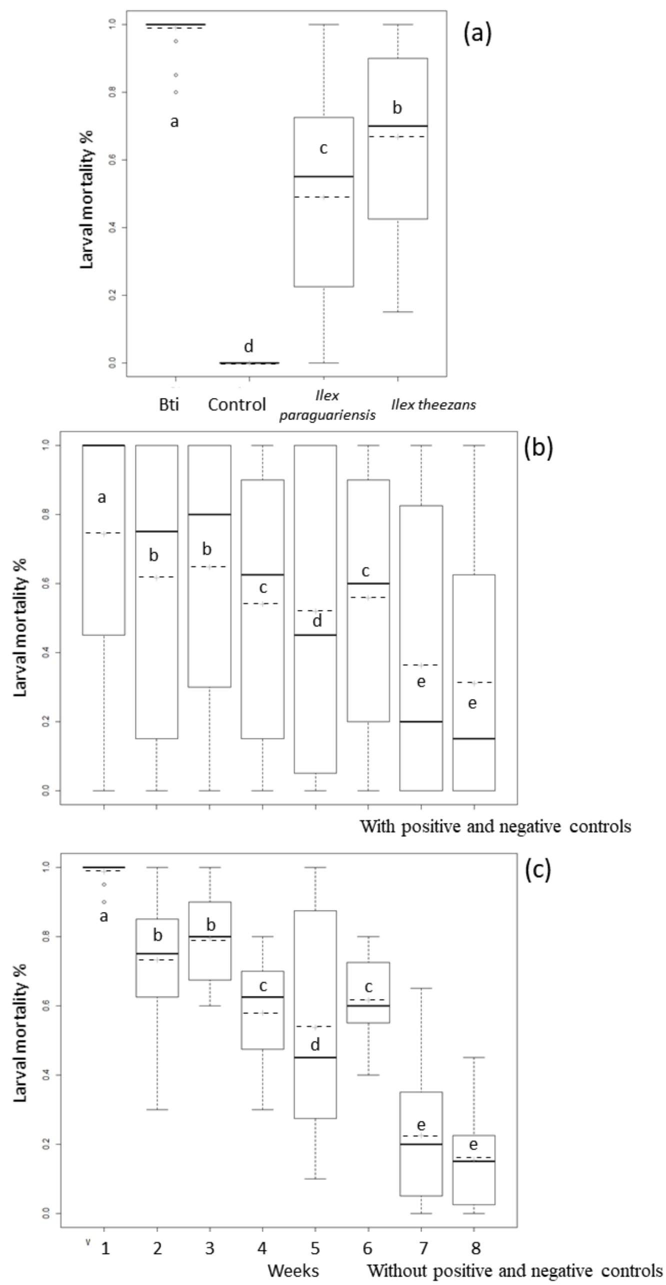

Figure 1 – (A) *Aedes aegypti* larvae mortality among treatments, (B) sample weeks with positive and negative controls, and (C) sample weeks without positive and negative controls among them*.
*Different letters ("a", "b", "c", "d", and "e") indicate significant differences. Boxes represent the quartiles, bold line represents the median, horizontal dashed line represents the mean, vertical dashed line represents the upper and lower limits, and circles represent the outliers.

This is especially true since a product is considered efficient for pest population control when it reduces individuals above 80%, otherwise resistance genes are selected (Jagadeesan et al., 2016).

In addition, besides the decaying of lethal chemical compounds which were toxic for mosquito larvae on the first week, as time goes by, the organic compounds present on the plant extracts may act as a useful food source for mosquito larvae. In this way, since organic compounds are well known for being an important component of several larval habitats, forming the basis of many food webs (Merritt et al., 1992; Moore et al., 2004), microorganisms such as bacteria play an important role in the cycling and breaking of large organic molecules (Sinsabaugh and Linkins, 1990). Therefore, microorganisms may act making them more easily absorbed by aquatic organisms, such as mosquito larvae, especially those belonging to the Culicidae family (Merritt et al., 1992). As a result, decomposing microbial communities present a relevant contribution to the diet of culicid larvae, being ingested with the organic remains over time (Merritt et al., 1992; Cochran-Stafira; Von Ende, 1998; Kaufman et al., 1999; Eisenberg et al., 2000).

The Bti of the present study resulted in 93.4% mortality in the eight-week solution. The residual effect described in the technical manual of the manufacturer is 30 days. Moreover, the values obtained herein were much higher than those described in the literature, with a lethality of 100% for 49 days and 6.6% of larval survival in up to 56 days. In this way, evaluating the effectiveness of the products that are already being used by state programs to control and combat *A. aegypti* could be performed. The use of the methodology without water renewal in the experiment resulted in a longer residual effect. Thus, not evaluating the effect of this renewal in reducing the residual effect of larvicidal substances (Pontes et al., 2005). Other studies in the field should encourage this water renewal by constantly emptying and replacing water, which would probably contribute to the reduction of the residual effect for all treatments tested.

Conclusions

The present study emphasized the need to implement alternative methods for vector control, since they represent a long-term risk. Furthermore, in the short term, it reported potential alternative pathways for mosquito-population control using natural products originated from the native flora. The plants presented a high larvicidal effect against *A. aegypti*, contributing to the maintenance of the quality of life and well-being of the population, since they are easily accessed by the local populations, reducing public expenditure with vector control and treatment of confirmed cases of dengue. Finally, time positively affected the survival of *A. aegypti* larvae due to the decay of lethal compounds from plant extracts, corroborating our first hypothesis. We also found that mortality by *B. thuringiensis* var. *israelensis* was constant throughout the experimental period and *A. aegypti* larvae survival was lower in the treatments with plant extracts than with *B. thuringiensis* var. *israelensis*. High mortality was observed in extracts of *I. theezans* compared to *I.*

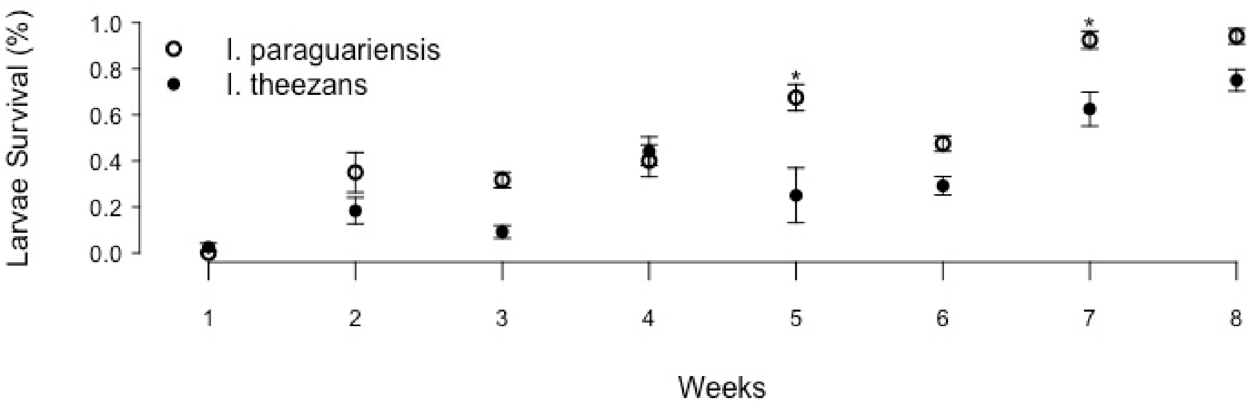

Figure 2 – *A. aegypti* larvae survival's mean as a function of plant extracts' age. Extracts' age are represented by weeks. The circles represent, aqueous *I. paraguariensis* fruits extract (open) and *I. theezans* hydroalcoholic leaves extract (closed).
*Significant statistical difference between *I. paraguariensis* and *I. theezans* affecting larvae survival.

paraguariensis, corroborating our second hypothesis. The strongest residual effect of *I. theezans* was probably due to the presence of chemicals on their leaves, such as coumarins, hemolytic saponins, and cyanogenic glucosides, which were absent in *I. paraguariensis*.

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Contribution of authors:

Cozzer, G.D.: Investigation, Data Curation, Writing – original draft. Rezende, R.S.: Software, Formal Analysis, Writing – review & editing. Lutinski, J.A.: Conceptualization, Methodology, Supervision, Writing – review & editing. Roman Júnior, W.A.: Methodology, Writing – review & editing. Busato, M.A.: Writing – review & editing. Simões, D.A.: Supervision, Formal Analysis, Funding Acquisition, Writing – review & editing.

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Influence of rainfall on wind power generation in Northeast Brazil

Influência da precipitação na geração de energia eólica no Nordeste do Brasil

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ABSTRACT

Wind power has been emerging as one of the main renewable energy sources in Northeast Brazil, which concentrates 87% of the country's installed wind capacity, especially in recent years, due to water scarcity and its seasonal energy complementarity to hydraulic generation. The objective of this article is to present a method to evaluate the influence of rainfall on the behavior of wind power generation, considering rainfall anomaly index and extreme climatic indices of precipitation. We utilized daily rainfall data from cities located near wind farms CE1 and CE2 in the state of Ceará — Aracati, in the 1974-2016 period, and Trairi, in the 1976-2016 period —, as well as daily wind power generation data for the same period, provided by the Electric System National Operator (ONS). The RClimdex software was used to calculate 11 indices of climatic extremes dependent on rainfall. The capacity factor for wind power generation was calculated for the period from 2011 to 2016 for the CE1 and CE2 wind farms. The application of this method found an inversely proportional relation between rainfall anomaly index (RAI) and the wind power capacity factor, with a decrease in total rainfall and a greater number of consecutive dry days and concentrated rain in the short term. From 2012 to 2016, the rainfall anomaly index was negative. However, wind power factors were higher than in 2011. The developed methodology can be applied to other wind farms, contributing to the medium and long term energy planning of the National Interconnected System.

Keywords: extreme events; energy planning; wind power factor; RClimdex.

RESUMO

A energia eólica vem despontando como uma das principais fontes renováveis de energia no Nordeste do Brasil, que concentra 87% da capacidade eólica instalada no país, especialmente nos últimos anos, devido à escassez hídrica e à sua complementariedade energética sazonal à geração hidráulica. O objetivo deste artigo é apresentar um método para avaliar a influência da precipitação no comportamento da geração de energia eólica. Foram utilizados dados diários de precipitação pluviométrica de cidades localizadas perto das usinas eólicas CE1 e CE2 no estado do Ceará — Aracati, no período 1974-2016, e Trairi, no período 1976-2016 —, bem como dados diários de geração eólica do mesmo período, fornecidos pelo Operador Nacional do Sistema Elétrico (ONS). Utilizou-se o software RClimdex para calcular 11 índices de extremos climáticos dependentes da precipitação pluviométrica. Com isso, determinou-se o fator de capacidade de geração de energia eólica para o período de 2011 a 2016 nas estações eólicas CE1 e CE2. A aplicação desse método constatou a existência de uma relação inversamente proporcional entre o Índice de Anomalia de Chuva (IAC) e o fator de capacidade da geração de energia eólica, com predominância de tendência de diminuição da precipitação total, com maior número de dias secos consecutivos e chuvas concentradas em curto período de tempo, embora os fatores eólicos tenham sido superiores a 2011. A metodologia desenvolvida pode ser aplicada a outros parques eólicos, contribuindo para o planejamento energético de médio e longo prazo do Sistema Interligado Nacional.

Palavras-chave: eventos extremos; planejamento energético; fator de capacidade da geração eólica; RClimdex.

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Introduction

Annually, more than 19,000 billion kWh of electricity are produced worldwide. About 70% comes from burning fossil fuels (41.5% from coal), followed by hydroelectric, nuclear, and other sources. One of the great challenges of the global energy sector is to satisfy the increased demand for energy, diversifying the energy matrix in order to reduce greenhouse gas emissions. In this context, the importance of renewable sources in the world energy matrix is growing. At least 45 countries, including 10 developing countries, have political goals to increase their use of renewable energy sources (GWEC, 2010).

Since 2014, Brazil is on the list of the 10 countries in the world with the largest installed capacity of wind energy, and has become a leader in the wind energy market in South America (GWEC, 2016). In 2019, the country reached a total of more than 15 GW of installed capacity. In 2024, Brazil's installed wind capacity will increase to 19 GW, representing an 11.1% growth from 2019 to 2024 (ONS, 2020). These values refer to the installed capacity onshore. Luz et al. (2020) point out that offshore wind projects are less impactful to the environment than onshore ones. So far, there are no plans to install offshore wind farms in Brazil.

Wind power has numerous benefits that make it an attractive energy source for both large energy consumers and small production applications distributed. The main benefits of this type of energy include: clean and inexhaustible energy; modular and scalable technology; energy price stability; reduced dependence on imported fuels; and economic development (Reeves and Beck, 2003).

Renewable energy, represented by small hydroelectric, biomass, wind, and solar plants, encompasses options to diversify the Brazilian electric matrix. However, it should not be seen only as an environmentally sustainable choice for a clean generation system, but also as an approach that addresses other social needs. Among these needs, there is greater reliability and security of supply, promoting seasonal energy complementarity to hydraulic energy — responsible for the largest portion of the installed capacity of electricity generation in the country —, improvement of maintenance of energy security, decrease in environmental impacts resulting from the use of fossil fuel, and reduction of climate change (Brasil, 2009; Wang et al., 2018).

Wind power generation cannot be addressed without discussing the issue of climate change, since it has caused changes in temperature and rainfall in different regions, with emphasis on the Northeastern region of Brazil, which has a high degree of climate vulnerability (Brasil, 2007). A warmer atmosphere is estimated, with more occurrences of extreme events of drought and rainfall, in frequency and intensity, causing more recurrent droughts and more severe floods (Marengo, 2007).

The consumption of fossil fuels has been increasing over the years and, along with them, the increase in greenhouse gases in the atmosphere has also been greater. However, in order to avoid environmental degradation, the use of renewable energy sources has increased visibly in recent years. Incentives were created for the production of renewable energy by the European Union, which, in July 2007, decided to reduce greenhouse gas emissions by 20%, increase the use of renewable energy systems by 20%, as well as increase energy efficiency by 20% by the end of 2020 (Ribeiro et al., 2014).

Climate change puts enormous pressure on water resource management. Floods and their consequences and severe droughts are resulting in natural disasters that reduce or destroy people's livelihoods in many regions of the world (Munichre, 2017). There has been a considerable increase in extreme weather events that cause deaths, economic loss, and destruction of infrastructure over the last decades, in addition to major impacts on energy generation (Souza et al., 2014).

In different regions of South America, extreme weather events have become increasingly common. The great droughts that occurred in the Amazon, from 2005 and 2010 (Lewis et al., 2011) and the great floods of 2008/2009 (Marengo et al., 2012), 2012 (Espinoza et al., 2013), and 2014 (Espinoza et al., 2014) can be mentioned, as well as the drought in Southeast Brazil in 2014/2015 (Coelho et al., 2016). According to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC AR5), published in 2013, climate change has been observed across the planet. Marengo (2008) points out that changes in rainfall patterns deserve great prominence among the observed climate changes, considering that they may change the availability of rain in one region or another. To verify these changes, indicators of climatic extremes, which indicate the intensity and frequency of such extreme events across the globe, can be used (Sillmann et al., 2013a).

In this context, the analysis of the influence of rainfall on the behavior of wind power generation is essential to guarantee a more robust forecast in the short and medium terms, particularly in the coast of Northeast Brazil, which concentrates 87% of the country's installed wind capacity, being one of the regions with the greatest potential for wind power generation in Brazil.

Thus, the present article aims to analyze the extreme climatic indices of rainfall in the state of Ceará, as well as rainfall anomaly index and its relation with the behavior of wind power generation through the analysis of the existing correlation between the capacity factor and rainfall anomaly index.

Climate change process

The Northeastern region of Brazil faces serious problems related to rainfall irregularity, resulting in severe and prolonged droughts, as well as extreme rainfall and floods (Ferreira et al., 2017). In this region, events like La Niña have been associated with the occurrence of rainy seasons that are more humid than normal, whereas El Niño has been associated with the occurrence of seasons drier than normal (Rodrigues et al., 2017). Many renewable energy sources for electricity generation, such as hydroelectric and wind power, depend on climatic factors. In the Northeastern region of Brazil, the installed capacity of wind power generation has increased substantially in recent years. The planned future increase in installed wind power capacity may change this picture; the demand for electricity generated by thermoelectric and imported plants will decrease (Koch et al., 2018).

According to Souza (2010), when choosing energy generation alternatives, the focus on sustainable development becomes necessary. Issues related to air pollution, greenhouse effect, depletion of fossil fuel reserves, security of supply, and equity are some of the drivers.

Managing the variability of wind power is a key factor in minimizing the cost of integrating wind-based power generation into grid systems, while maintaining the extremely high level of reliability required. The short-term forecast for wind energy is that it is one of the most economical and easy to implement investments available to system operators (Zack et al., 2016).

Several meteorological systems affect the climate in the Northeastern region of Brazil. The Intertropical Convergence Zone (ITCZ) is one of the systems that govern the rainfall trend in the northern part of the Northeastern region of Brazil. The ITCZ reaches its position further south between February and April, and further north between August and October (Oliveira and Costa, 2011). In addition to this annual oscillation, it presents oscillations with higher frequencies, varying from weeks to days. The influence of this positioning on rainfall is addressed in several studies (Lyra et al., 2019). The ITCZ is more significant over the oceans, and therefore, Sea Surface Temperature (SST) is one of the determining factors in its position and intensity (Ferreira and Mello, 2005; Carvalho, 2020).

The seasonal variation of ITCZ's positioning influences the generation of wind energy. In their analysis, Barros et al. (2017) found that the variation of ITCZ's position negatively influenced wind generation in the state of Ceará in February 10 and 11, 2017. In these days, wind generation was practically zero at some points of the day. Due to the high installed capacity of wind farms in the Northeastern region of Brazil, understanding the influence of meteorological systems on the behavior of wind generation is of fundamental importance for the operation of the electrical system.

Wind power generation in Brazil and in the Northeastern region of the country

The National Interconnected System (*Sistema Interligado Nacional* – SIN) is a hydro-thermo-wind system of continental dimensions, with an installed capacity of more than 162 GW, with 83% of the generation coming from renewable sources (hydroelectric + wind + solar), of which about 73% are hydraulic plants, which makes Brazil a world reference in renewable energy.

The Brazilian generating complex is undergoing a process of transformation and transition. Hydroelectricity will continue as the main source of energy generation, although its share in the total installed power of SIN will be reduced from 66.7%, in 2018, to 62.1%, in 2024 (ONS, 2020). Inserting future wind farms in SIN will promote beneficial effects for the entire system, especially for the Northeastern region, where the complementarity of wind generation in relation to the period of low inflows is registered.

In Brazil, SIN's installed generation capacity is mainly composed of hydroelectric plants distributed in 16 drainage basins, in different regions of the country. In recent years, the installation of wind farms, mainly in the Northeastern and Southern regions, has grown considerably, increasing the importance of this generation for serving the market. Due to the increase in the installed capacity of wind generation since 2014, its participation has grown over the years. In 2019, wind and solar generation served 10% of the SIN load, hydraulic generation served 73%, and thermal generation served 17%.

Since 2016, wind generation has been serving the highest percentage of the load in the Northeast, with its peak in 2019, when it served 50% of the load. Despite the water crisis, the Northeast exported roughly 1,500 MWmed from July to December 2019. The growth in the share of the solar source is also remarkable in the last three years, meeting 3% of the demand in the Northeast in 2019 (ONS, 2020).

Material and Methods

The method used to assess the influence of rainfall on the behavior of wind power generation was based on the consideration of the rainfall anomaly index and its relation with the capacity factor, as well as the assessment of extreme climatic precipitation indices, considering the following steps.

Selection of the study area

To select the study area, we sought a region in the Northeast with a large number of wind farms in operation, as well as pluviometric stations close to wind farms with good quality historical series available.

The coast of the state of Ceará was selected, as it has several wind farms in commercial operation with potential for expansion. The existence of nearby pluviometric stations with available historical series is also important. The plants in the coastal region of the state of Ceará were grouped into two located groups (CE1 and CE2), which have a higher concentration of plants, as shown in Figure 1.

Wind power plants (CE1 and CE2) were selected in two different areas of the coast of the state of Ceará, as they are representative of the plants operating on the northeastern coast. Trairi and Aracati rain stations were selected, due to the proximity to the selected wind farms, CE1 and CE2, respectively. The selection was made after analyzing the distance between the selected wind farms and the automatic meteorological stations of the National Institute of Meteorology (INMET) and Ceará's Foundation for Meteorology and Water Management (FUNCEME), as well as the National Water Agency's (ANA) rainfall stations (Brasil, 2016).

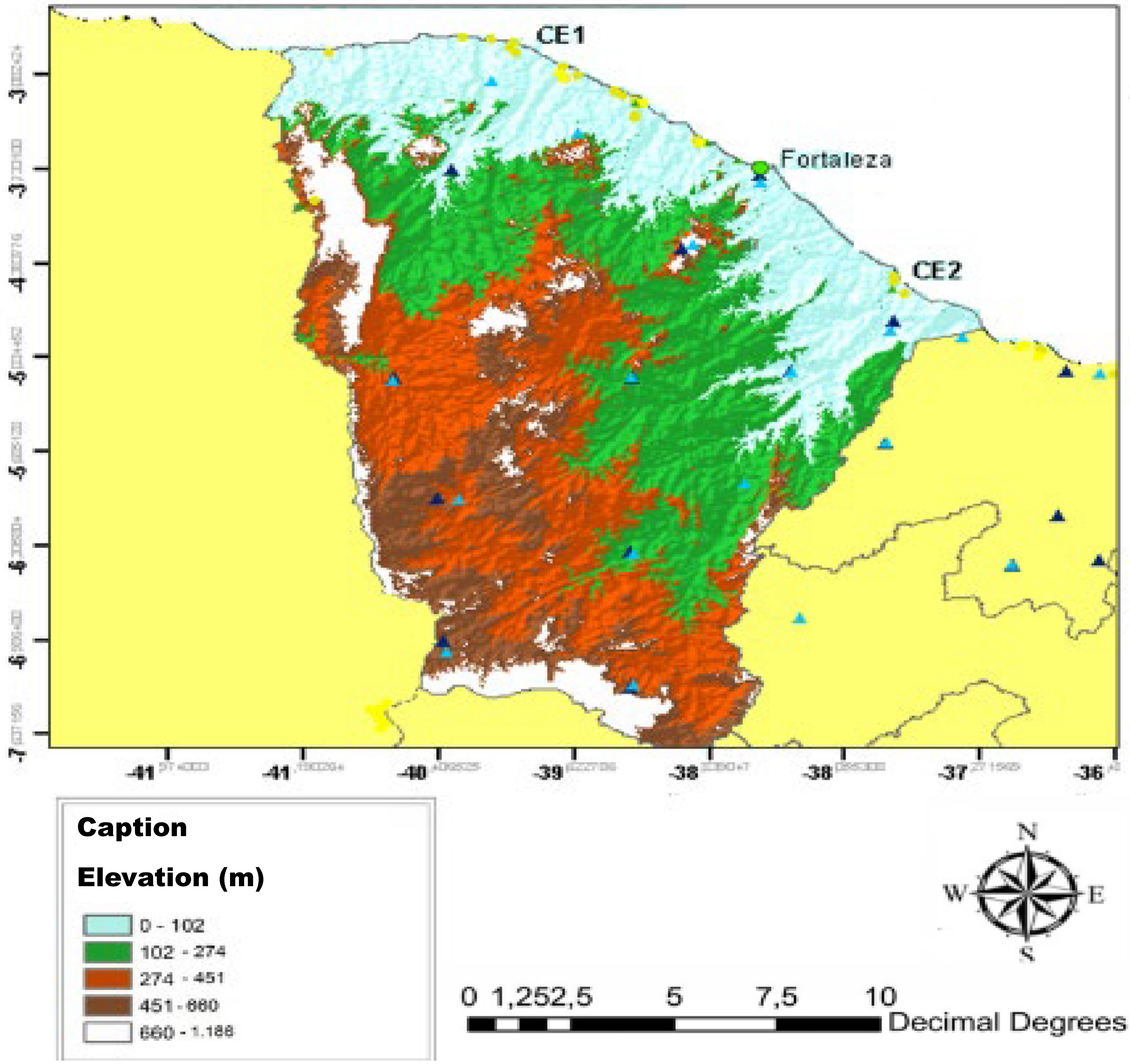

Figure 1 – Digital elevation map and location of wind farms in the state of Ceará.
Source: created with data from ONS (2020) and IBGE (2011).

Treatment and analysis of rainfall data

The consistency of the historical series and the filling of gaps in the daily rainfall data were performed using the simple linear regression method, which correlates the gaps in the station and in a neighboring station with gaps, as proposed by Tucci (2004).

The series consistency analysis was performed using the double mass method, developed by the United States Geological Survey (USGS), commonly adopted in Brazil. The methodology consists of selecting stations in a region and accumulating the monthly values for each of them. Then, the accumulated values corresponding to the station to be validated (in ordinates) and to another reliable station adopted as a basis for comparison (abscissa) were plotted on a Cartesian graph. With this method, identifying systematic errors (change in slope or trend), transcription errors, or stations subject to different rainfall regimes is possible (Tucci, 2004).

Rainfall Anomaly Index

Rainfall anomaly index (RAI) analyzes the frequency of dry and rainy years and the intensity of events. Based on Van-Rooy's methodology (1965) and adapted to the Northeast of Brazil by Freitas (2005), the climatic variability is evaluated through the creation of climatic indices spatialized in time and space, detecting periods considered extremely humid or dry. The assessment of the degree of severity and duration of the dry and wet periods was carried out by calculating the RAI (Freitas, 2005), obtained from Equations 1 and 2:

$$IAC = 3\left[\frac{(N-\overline{N})}{(M-\overline{N})}\right], for\ positive\ anomalies \quad (1)$$

$$IAC = -3\left[\frac{(N-\overline{N})}{(\overline{X}-\overline{N})}\right], for\ negative\ anomalies \quad (2)$$

Where:
N = annual precipitation (mm);
$\overline{N}$ = average annual precipitation of the historical series (mm);
$\overline{M}$ = average of the 10 largest annual rainfall in the historical series (mm);
$\overline{X}$ = average of the 10 lowest annual rainfall in the historical series (mm).

Positive anomalies are values above the historical average and negative anomalies are below the historical average of precipitation. Based on the methodology proposed by Freitas (2005) and Araújo et al. (2009), the classification of dry and wet years was used as a climatic indicator for the intensity of these anomalies, as shown in Table 1.

The average monthly rainfall was calculated with the history of the selected weather stations for January to May, from 2011 to 2016.

Wind generation capacity factor

In the present study, the capacity factor used is understood as a function of wind generation. The capacity factor of wind power generation is calculated through the relation between the average wind power generation and the installed capacity of the wind power plant, as shown in Equation 3.

$$FC = \left[\frac{(EOL\ generation\ (MWmed)}{Installed\ Capacity\ (MW)}\right] \quad (3)$$

Power generation is a function of wind speed, whose behavior varies over the day and over the months. The topographic characteristics of a region also influence the behavior of winds, since, in certain areas, differences in speed may occur, causing a reduction or acceleration in wind speed (Custódio, 2009). Among the main influencing factors in the wind regime, the following stand out: variation in speed with height; terrain roughness, which is characterized by vegetation, land use, and occupation; presence of obstacles in the vicinity; and the terrain, which can cause an effect of acceleration or deceleration in air flow (Varejão-Silva, 2006).

Calculation of extreme rainfall climate indices

With the purpose of detecting trends of climatic extremes indices in the analyzed stations, the software RClimdex 3.2.1, which is a program recommended by the World Meteorological Organization (WMO) for the calculation of climatic extremes indices aiming at monitoring and detecting climate changes, was used. This software was developed in R by Byron Gleason, a researcher at the National Oceanic and Atmospheric Administration's National Climate Data Center (NCDC), and has recently been used in CCI/CLIVAR (International Research Programme Climate Variability and Predictability) climate index workshops, according to Karl et al. (1999).

RClimdex 3.2.1 calculates all 27 basic indexes (11 related to precipitation and 16 related to temperature) recommended by the Expert Team on Climate Change Detection Monitoring and Indices (ETCCDMI) and provides, for each calculated index, statistical information, such as linear trend calculated by the method of least squares, level of statistical significance of the trend (p value), coefficient of determination (R^2), and standard error of the estimate, in addition to the graphs of the annual series (RCLIMDEX, 2004). In this work, only the 11 indices derived from rainfall were used, as shown in Table 2.

The climatic indices can be used to create graphs of the annual series, composed by the trends and calculated by the method of linear least squares regression, with statistical significance, showing the adjustments of these linear trends to the graphs statistically.

Rainfall and wind generation data used

The data from the 1974-2016 period from Aracati station, as well as data from 1976 to 2016 from Trairi station, was used. Daily wind generation data for 2011 to 2016, provided by ONS for the wind farms CE1 and CE2, were also used.

Table 1 – Intensity levels of rainfall anomaly index (RAI).

RAI Range	Intensity Level
Above 4	Extremely humid
2 to 4	Very humid
0 to 2	Humid
0 to -2	Dry
-2 to -4	Very dry
Below -4	Extremely dry

Source: Freitas (2005).

Table 2 – Climatic indices depending on daily rainfall with definitions and units.

Index	Indicator Name	Definition	Unit
PRCPTOT	Total annual rainfall on wet days	Total annual rainfall on wet days (RR ≥ 1 mm)	mm
CDD	Consecutive dry days	Maximum number of consecutive days with RR < 1 mm	days
CWD	Consecutive wet days	Maximum number of consecutive days RR ≥ 1 mm	days
R10mm	Number of days with precipitation above 10 mm	Number of days per year when rainfall was ≥ 10 mm	days
R20mm	Number of days with precipitation above 20 mm	Number of days per year when rainfall was ≥ 20 mm	days
R50mm	Number of days with rainfall above 50 mm	Number of days per year when rainfall was ≥ 50 mm	days
SDII	Simple daily intensity index	Total annual rainfall divided by the number of wet days (defined by PRCPTOT ≥ 1 mm	mm/day
Rx1day	Maximum amount of rainfall in one day	Maximum monthly amount of rainfall in one day	mm
Rx5day	Maximum amount of precipitation over five consecutive days	Maximum monthly amount of rainfall over five consecutive days	mm
R95p	Very humid days	Total annual rainfall in which RR > 95 percentile	mm
R99p	Extremely humid days	Total annual rainfall in which RR > 95 percentile	mm

*RR indicates daily precipitation.
Source: RCLIMDEX 1.0 (2004).

In the analysis of the extreme climatic indices of pluviometric precipitation, the daily observed data from the Trairi pluviometric station, from 1976 to 2015, is considered.

Correlation analysis

Pearson's correlation was also known as the Product-Moment Correlation Coefficient (Figueiredo Filho and Silva Júnior, 2009). The population correlation coefficient (parameter) ρ and its sample estimate are closely related to the normal bivariate distribution, whose probability density function is given by Equation 4:

$$f_{X,Y}(X,Y) = \frac{1}{2\pi\sigma_X\sigma_Y\sqrt{1-\rho^2}}\exp\left\{-\frac{1}{2(1-\rho^2)}\left[\left(\frac{X-\mu_X}{\sigma_X}\right) - 2\rho\left(\frac{Y-\mu_Y}{\sigma_Y}\right) + \left(\frac{Y-\mu_Y}{\sigma_Y}\right)^2\right]\right\} \quad (4)$$

In which:
$\rho_{X,Y} = \rho = \frac{COV(X,Y)}{\sigma_X\sigma_Y} = \frac{\sigma_{X,Y}}{\sigma_X\sigma_Y}$ = the population parameter:
$COV(X,Y)$ = the covariance between X and Y;
σ_X = the standard deviation of X;
σ_Y = the standard deviation of Y.

The Maximum Likelihood Estimator is given by Equation 5:

$$\hat{\rho}_{X,Y} = \hat{\rho} = \frac{\sum_{i=1}^{n}(p-\bar{X})(Yi-\bar{Y})}{n\sqrt{\sum_{i=1}^{n}\frac{(Xi-\bar{X})^2}{n}}\sqrt{\sum_{i=1}^{n}\frac{(Yi-\bar{Y})^2}{n}}} = \frac{\sum_{i=1}^{n}(Xi-\bar{X})(Yi-\bar{Y})}{n\hat{\sigma}_X\hat{\sigma}_Y} \quad (5)$$

In which:
n = the number of observations in the sample;
$\bar{X}$ = the arithmetic mean of X;
$\bar{Y}$ = the arithmetic mean of Y;
$\hat{\rho}$ = the standard deviation;
X_i = observation of variable x;
Yi = observation of variable y.

The correlation coefficient can also be interpreted in terms of $\hat{\rho}^2 = R^2$, called the determination or explanation coefficient. When multiplied by 100, $\hat{\rho}^2 = R^2$ gives the percentage of variation in Y (dependent variable), which can be explained by the variation in X (independent variable), that is, how much variation is common to both variables. The determination coefficient is the relation between the variation explained by the linear model ($\hat{Y} = \hat{\alpha}+\hat{\beta}X$, in which $\hat{\alpha}$ and $\hat{\beta}$ are constant) and the total variation.

The significance of the correlation coefficient will be assessed using the Student's t-test, for the significance levels of 1, 5, and 10%, and degrees of freedom of (n-2). Rejections to null hypotheses h0 will identify the existence of a linear correlation between the combinations performed. To test the hypothesis that the linear correlation coefficient is equal to zero, there must be: H0: ρ = 0 and H1: ρ ≠ 0, according to Equations 6 and 7:

$$t = \frac{\hat{\rho}\sqrt{n-2}}{\sqrt{1-\hat{\rho}^2}} \sim t_{n-2} \quad (6)$$

In which:
t_0 = the test statistic;
$\hat{\rho}$ = the standard deviation;
n = the sample size;
r = the estimate of the linear correlation coefficient.

Under the assumption of the null hypothesis H0: ρ = 0. The null hypothesis is rejected if:

$$|t_0| > t\alpha_{/2(n-2)} \tag{7}$$

t_0 = the test statistic;

n = the sample size;

The correlations that presented statistical significance at the 5% level, obtained by the Pearson method, were discussed. Pearson's correlation method (p) is a measure of linear association between two variables, expressed by Equation 8:

$$= \frac{1}{n-1}\sum\left(\frac{x_i-\overline{X}}{x}\right)\left(\frac{y_i-\overline{Y}}{y}\right) \tag{8}$$

Significance was obtained with the Student's t-test applied to a series that represents N degrees of freedom, corresponding to the years of the historical series of data analyzed. The significance level (α) is understood as the probability of making a certain estimate wrong. The lower the level of significance, the higher the confidence level (1 – α) of the correlation index obtained. For a significance level of α = 0.005 (5%), the confidence level is 0.95 (95%). The most frequently confidence level used as a limit for climatology is 95%.

Results and Discussion

Analysis of Rainfall Anomaly Index and its relation with the capacity factor of wind power generation

For the correlation analysis of the IAC × capacity factor of wind generation, average rainfall was used, calculated using the arithmetic average method, which consists of the average of the records of precipitation values. According to Tucci (2004), this method is influenced by extreme values and is satisfactory when sample distribution is uniform.

Figure 2 shows the capacity factor as a percentage of the wind farm CE2, and the rainfall anomaly index of the Aracati (CE) meteorological station from January to May, 2011 to 2016.

In 2011, the months of January, February, April and May showed positive anomalies in precipitation, and the average capacity factor (CF) of the period was 14%. In 2012, the months from January to May showed negative rainfall anomalies. The average CF of the period was 42%, that is, three times higher than the average CF recorded in 2011. The negative results of the IAC for 2012 are in line with the results obtained by Assis et al. (2015) in the climatic analysis of rainfall in the sub-basin of the São Francisco River basin, based on rainfall anomaly index performed. In their analysis, there was no positive RAI in 2012. All indices were classified as dry and extremely dry.

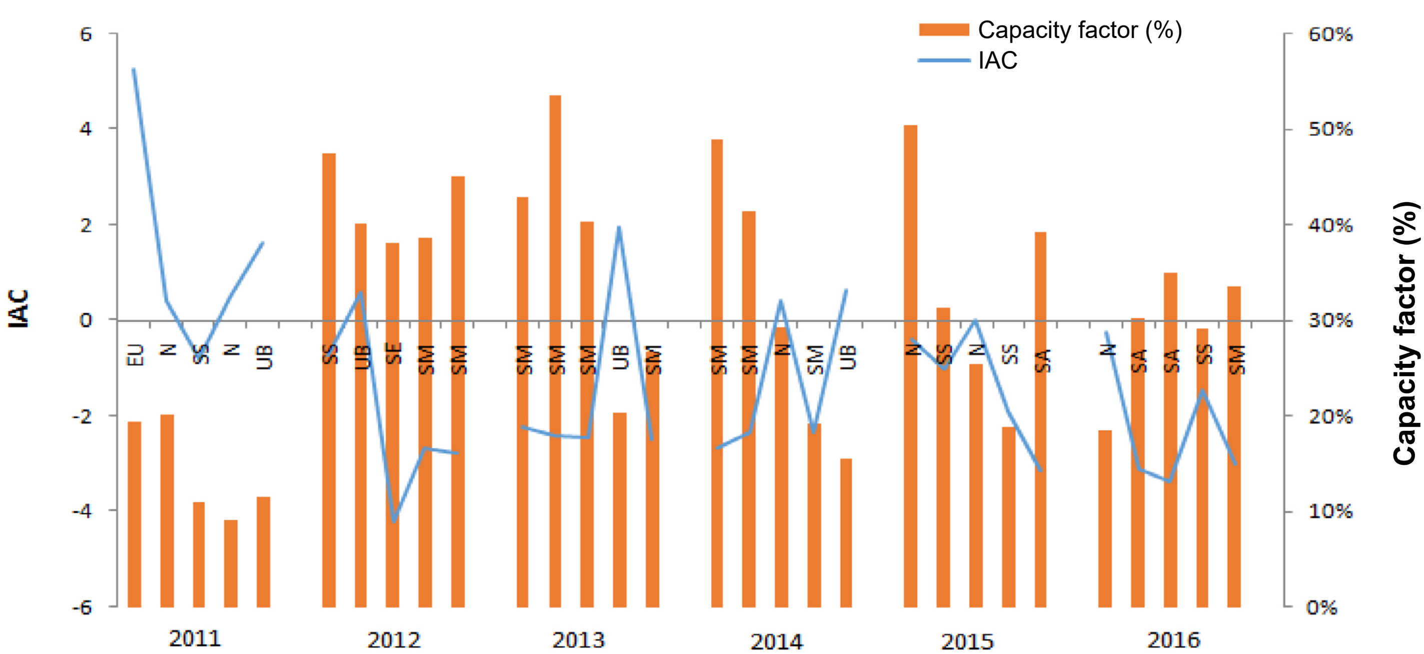

Figure 2 – Relation between the rainfall anomaly index and the capacity factor of wind generation in CE3, from January to May, 2011 to 2016. EH: extremely humid; HH: high humidity; MH: moderate humidity; LH: low humidity; N: normal; MD: mild drought; MD: moderate drought; HD: high drought; ED: extreme drought.

In 2013, the months of January, February, March and May showed negative anomalies in rainfall, and the average capacity factor (CF) of the period was 41%. In April, rainfall anomaly was positive and the CF dropped to 20%. In 2014, the months of January, February and April presented negative rainfall anomaly and the average CF was 37%. In March and May, 2014, with a positive rainfall anomaly, the average CF dropped to 22%.

In 2015, the months of January, February and March presented negative rainfall anomaly and the average CF was 36%. In May, rainfall anomaly was positive and the CF dropped to 39%. In 2016, January presented rainfall anomaly close to the average, with the CF equal to 19%. In the months of February, March, April and May, in which rainfall anomaly was negative, the average CF was 32%.

Figure 3 shows the average RAI for the period from January to May, 2011 to 2016. According to the analysis carried out, the CF tends to be higher in dry years. In the last five years, 2012 to 2016, negative rainfall anomalies with high capacity factors were observed. According to Marengo et al. (2016), the drought that plagued the semi-arid Northeastern region from 2012 to 2015 had an intensity and impact not seen in several decades. Changes in atmospheric circulation and rainfall detected since the summer of 2012 suggest a more active role of surface waters, cooler than the normal in the equatorial Pacific, and an Intertropical Convergence Zone displaced anomalously north from its regular position, with increased subsidence over the Northeastern region of Brazil. In the Northeast of Brazil, signs of drought began to appear in December 2011, and intensified during the summer and autumn of 2012, generating water deficiency in almost the entire semi-arid region, from central-southern Bahia state to the states of Rio Grande do Norte and Ceará in 2011-2014.

For the annual analysis of the linear correlation between the rainfall anomaly index (RAI) and the capacity factor (CF), there was a strong negative linear correlation, whose value was -0.87. It shows a reduction in CF in the months with positive rainfall anomalies. Although the period of analysis is short, from 2011 to 2016, this is the history available considering that the plants started their commercial operation in 2010. Despite this limitation, the results obtained are relevant because they present an analysis of rainfall impacts on the behavior of wind generation on the coast of Northeast Brazil. This study may be updated as the historical series is expanded.

According to the analysis carried out, there was a smaller capacity factor in the years that presented positive rainfall anomalies. However, in the years in which rainfall anomaly was negative (rain below the historical average), the capacity factor was higher. The analysis reveals that when the registered rainfall is below the historical average, the capacity factor for generating wind energy increases; on the other hand, when rainfall is above climatology, the capacity factor decreases.

Figure 4 illustrates the relation between the average daily wind generation verified × observed wind × observed rainfall of CE1 for the period from 03/29/2014 to 05/30/2016.

On the days in which daily rainfall was higher (above 20 mm), the average daily wind speed was below 6 m/s. As a consequence, wind generation was lower on these days. In this region, the main responsible factor in the occurrence of rainfall is the Intertropical Convergence Zone (Lyra et al., 2019).

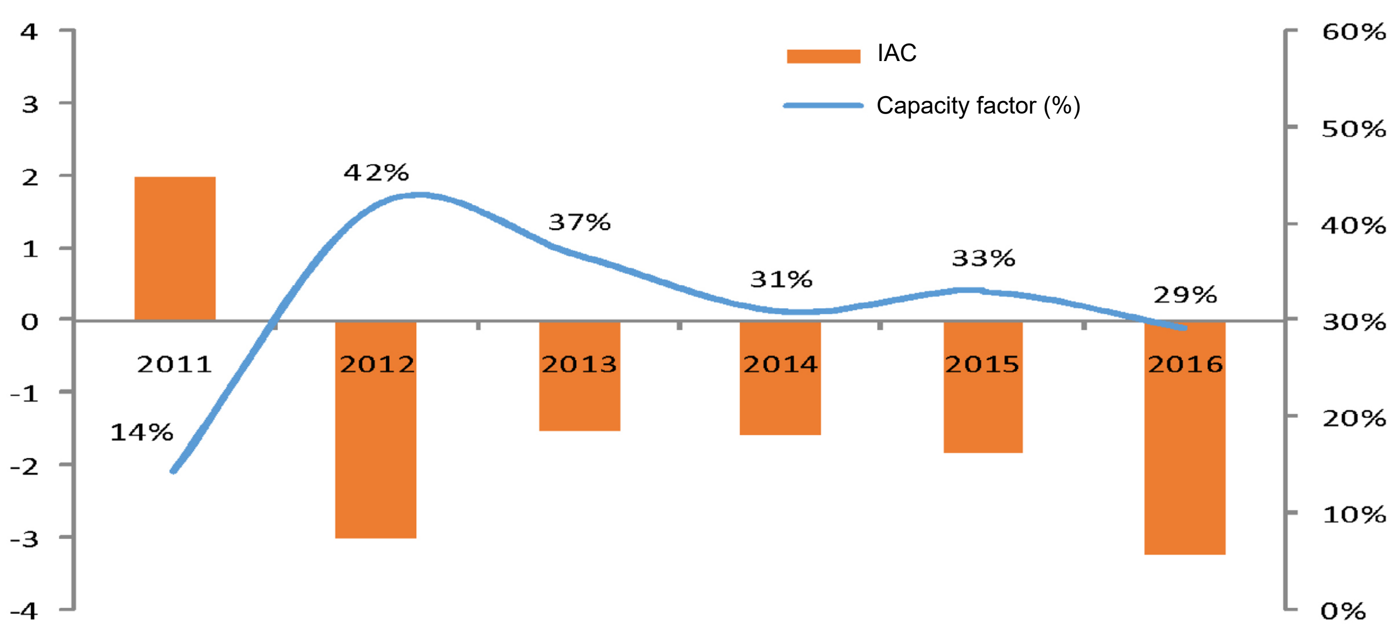

Figure 3 – Rainfall anomaly index for the period from January to May and the average capacity factor for the period.

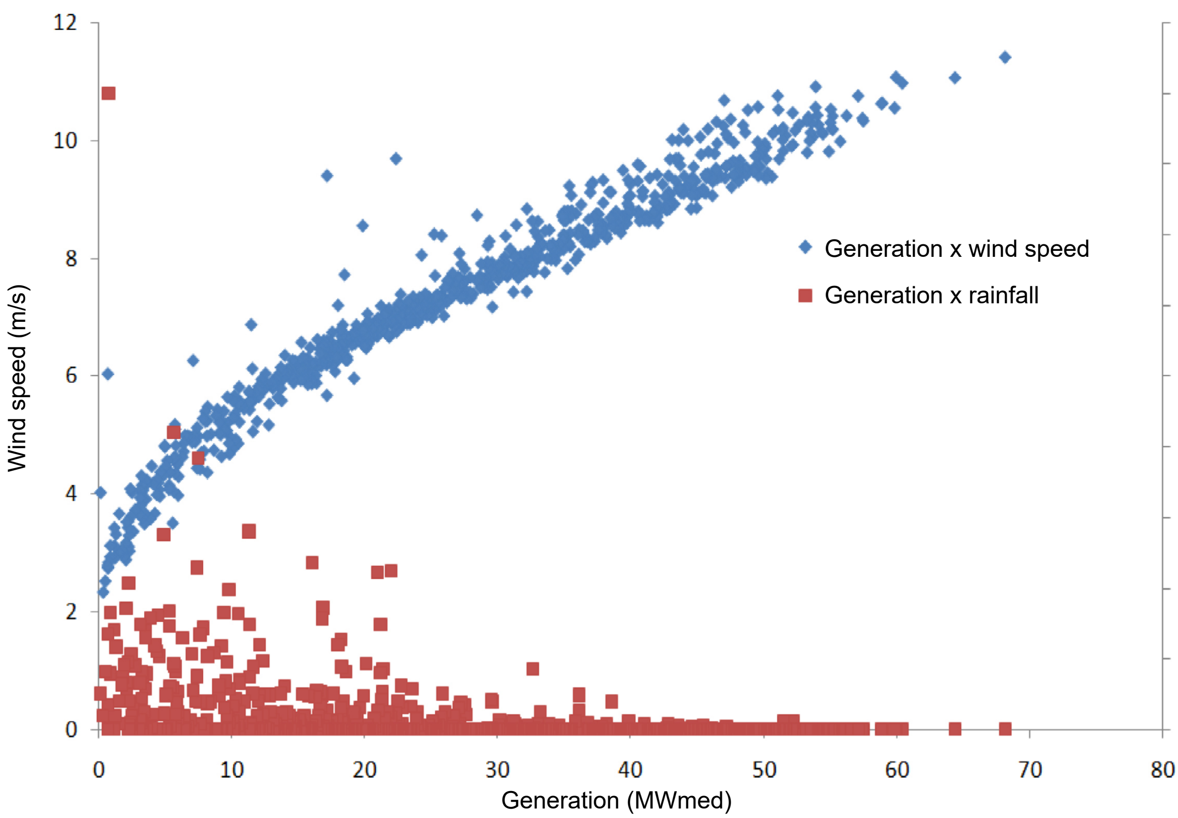

Figure 4 – Relation between wind generation, wind speed, and rainfall (CE1).

Calculation of climatic extremes indices using RClimdex

The results of climatic extremes indices using RClimdex are presented below.

Linear trend of total annual rainfall

Figure 5 shows the total annual rainfall index (PRCPTOT) of Aracati station, from 1974 to 2015. According to this index, there is a linear decreasing trend in the annual rainfall pattern. For the analyzed period, annual rainfall reduction rate was 19.7 mm.year^{-1}, that is, a reduction of 862 mm in precipitation over the last 42 years. The years 1983, 1993, 1998, and 2012 had the lowest total rainfall, with 1993 being the most critical year, with a total annual rainfall of 220 mm. The years 1993 and 1998 are associated with the strong-intensity El Niño phenomenon. The year 2012 did not constitute an occurrence of El Niño, however, it was an extremely dry year due to a Sea Surface Temperature (SST) anomaly in the Pacific Ocean (Rodrigues et al., 2017).

Figure 6 shows the total annual precipitation index (PRCPTOT) of the Trairi station. According to this index, there is a linear trend, which is not too sharp, in the annual rainfall pattern. For the analyzed period, the rate of annual rainfall increase was 10.5 mm.year^{-1} , that is, an increase in total annual rainfall of 420 mm over the last 40 years.

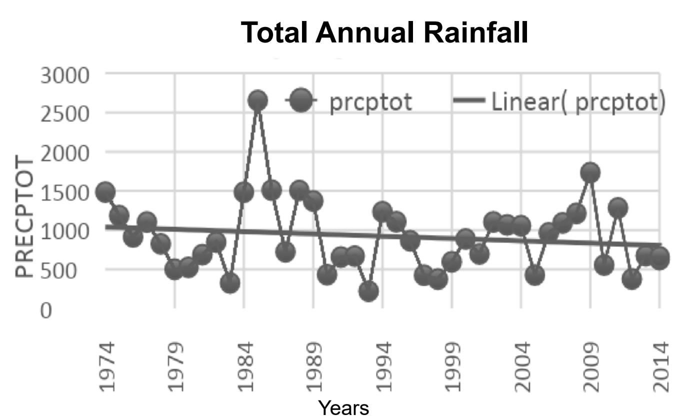

Figure 5 – Linear trend of total annual rainfall (PRCPTOT) of the Aracati station mm/year (1974–2015).

In the analysis of the temporal trend of the PRCPTOT index in Trairi, an alternation between rainy and dry years is observed, with annual rainfall above and below average. The years 1979 and 1993 presented the lowest total rainfall, with a total annual rainfall of 500 mm. The years 1977, 1985, 2002, 2003, and 2009 presented the highest annual rainfall volumes with annual values above 1,500 mm.

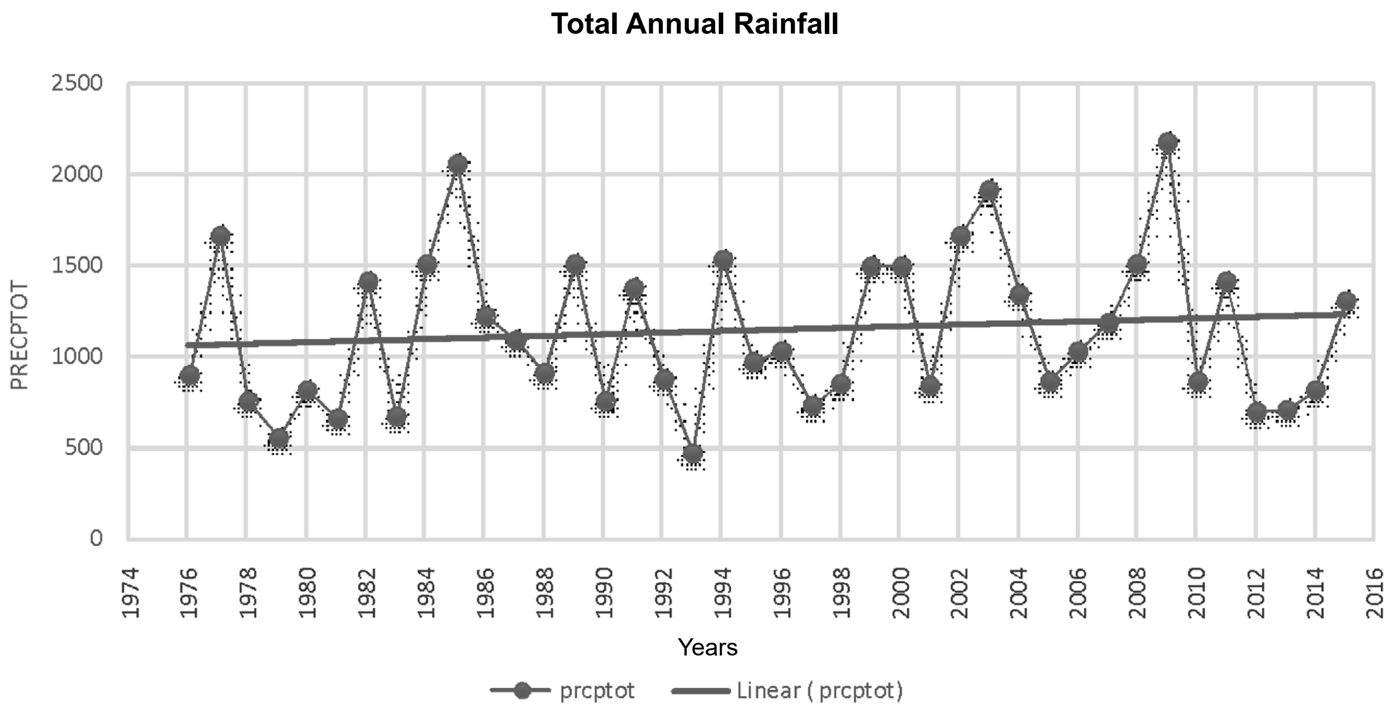

Figure 6 – Linear trend of total annual rainfall (PRCPTOT) of the Trairi station mm/year (1976–2015).

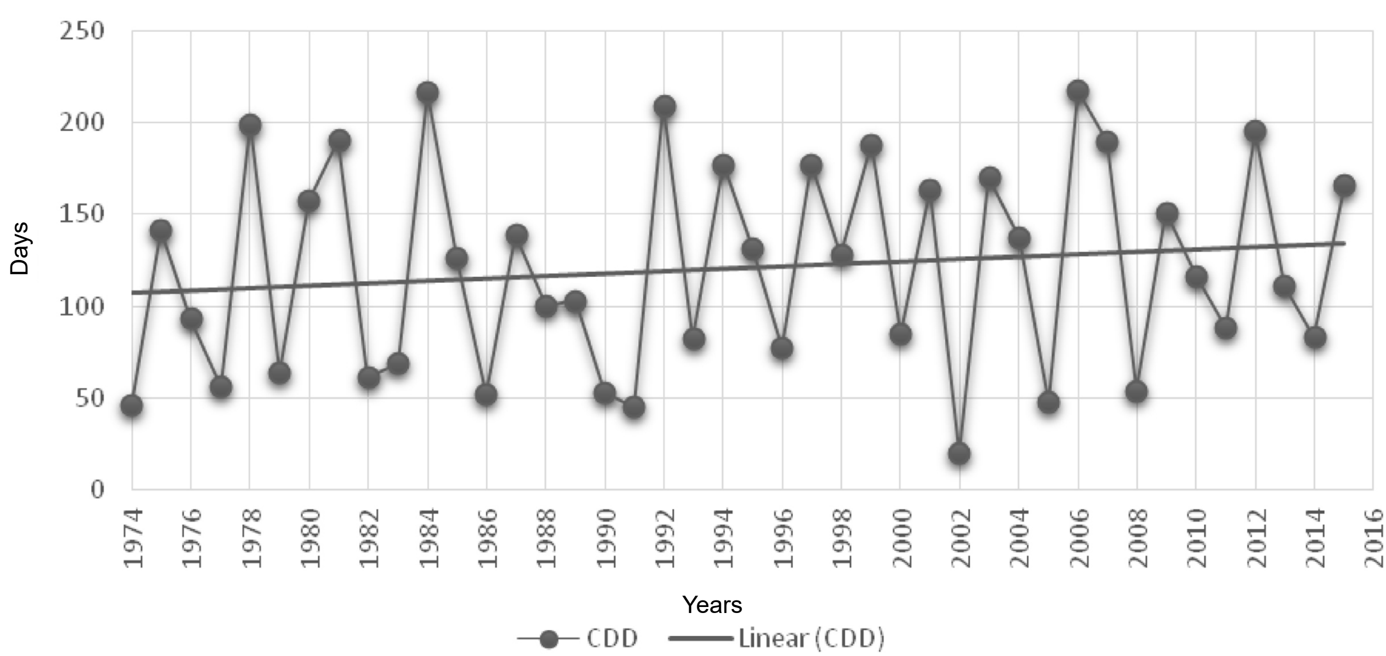

Figure 7 – Linear trend in the number of consecutive dry days (CDD) at the Aracati station (1974–2015).

The results found for Aracati station, unlike Trairi, show a trend of annual reduction in rainfall. In a study of the state of Ceará, Moncunill (2006) found negative trends in annual rainfall, using 23 pluviometric stations from 1974 to 2003. Santos et al. (2009), in a study of the entire state of Ceará, found results that point to a trend of increasing rainfall between 1935 and 2006.

Figure 7 shows the linear trend of the consecutive dry days (CDD) index for Aracati meteorological station from 1974 to 2015. There is a positive trend, indicating an increase in the number of consecutive dry days. The years 1984, 1992 and 2006 had more than 200 consecutive days without rain, that is, more than six months. The year 1993 was marked by a strong-intensity El Niño phenomenon, the most severe event of this magnitude recorded in the 1990s (Pereira et al., 2017; Rodrigues et al., 2017).

Linear trend of the number of consecutive dry days

This increase in consecutive days without rain is linked to the result of the index of total annual rainfall (PRCPTOT), presented above, since, with the decrease of the rainfall regime, there are fewer days with rain. This result indicates that, not only is it raining less in the region, but rainfall is increasingly sporadic, and is sometimes concentrated in a shorter period of time.

There is a great variability of rainfall, which often reaches peaks of four consecutive months or 120 days without precipitation. The year 2006 recorded the largest number of consecutive days without rain (217 days), that is, more than seven months.

Figure 8 shows the linear trend of the CDD index for the Trairi meteorological weather station, from 1976 to 2015. There is a negative trend, indicating a decrease in the number of consecutive dry days. The years 1982 and 1999 had more than 200 consecutive days without rain, that is, more than six months.

The divergent results between Aracati and Trairi pluviometric stations are justified by the divergence found in the calculation of the total annual precipitation index. When there is a decrease in the total annual rainfall, the days with rainfall tend to decrease as a consequence, generating an increase in the consecutive number of dry days and vice versa. Santos et al. (2009), who studied the state of Ceará, showed a variation between their pluviometric stations in the calculation of the CDD index, where there are both negative and positive trends in the number of consecutive dry days.

Linear trend in the number of consecutive days with rainfall > 1 mm

Figure 9 shows the trends of consecutive wet days (CWD) index of Aracati station (1974–2015). Considering the entire historical series, there was no trend of above increase or decrease as to the extreme climatic index of consecutive wet days, which corresponds to the maximum number of consecutive days in the year with rainfall above 1 mm. However, in recent years (2012–2015) there has been a reduction in the number of consecutive wet days.

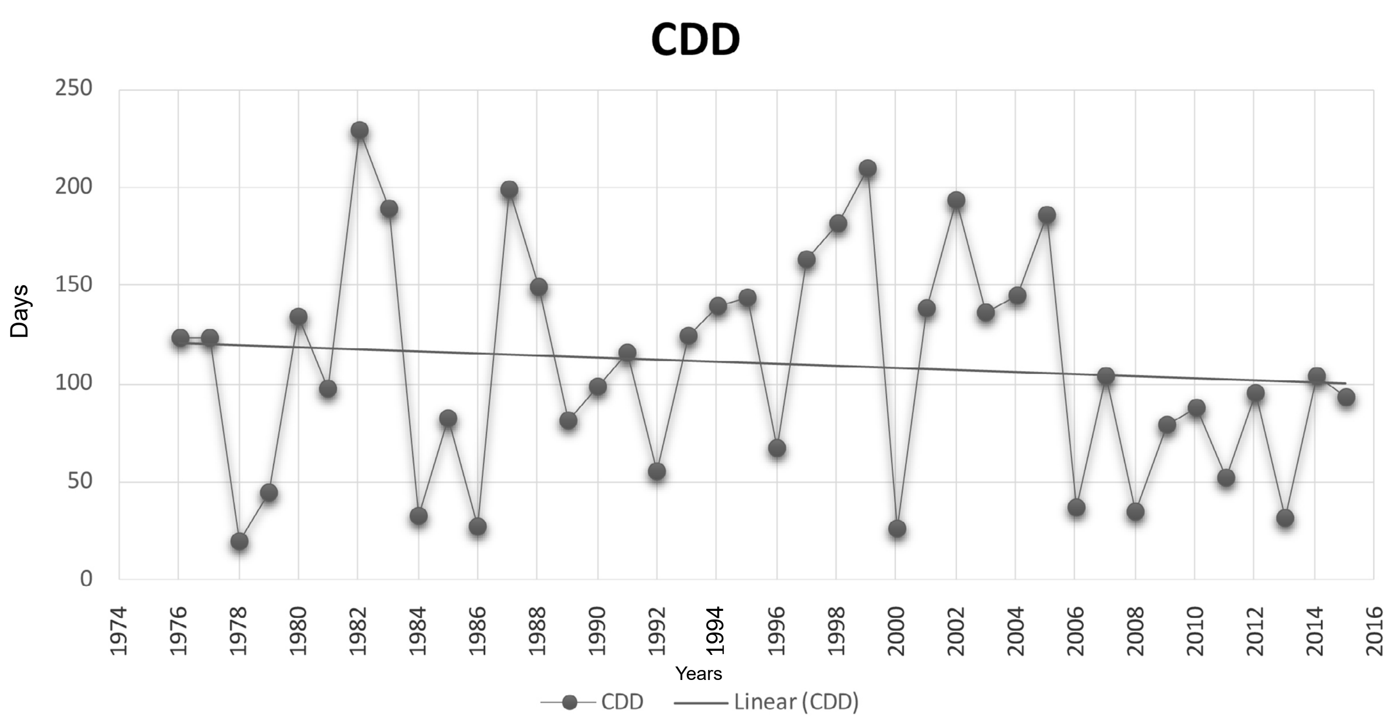

Figure 8 – Linear trend in the number of consecutive dry days (CDD) at the Trairi station (1976–2015).

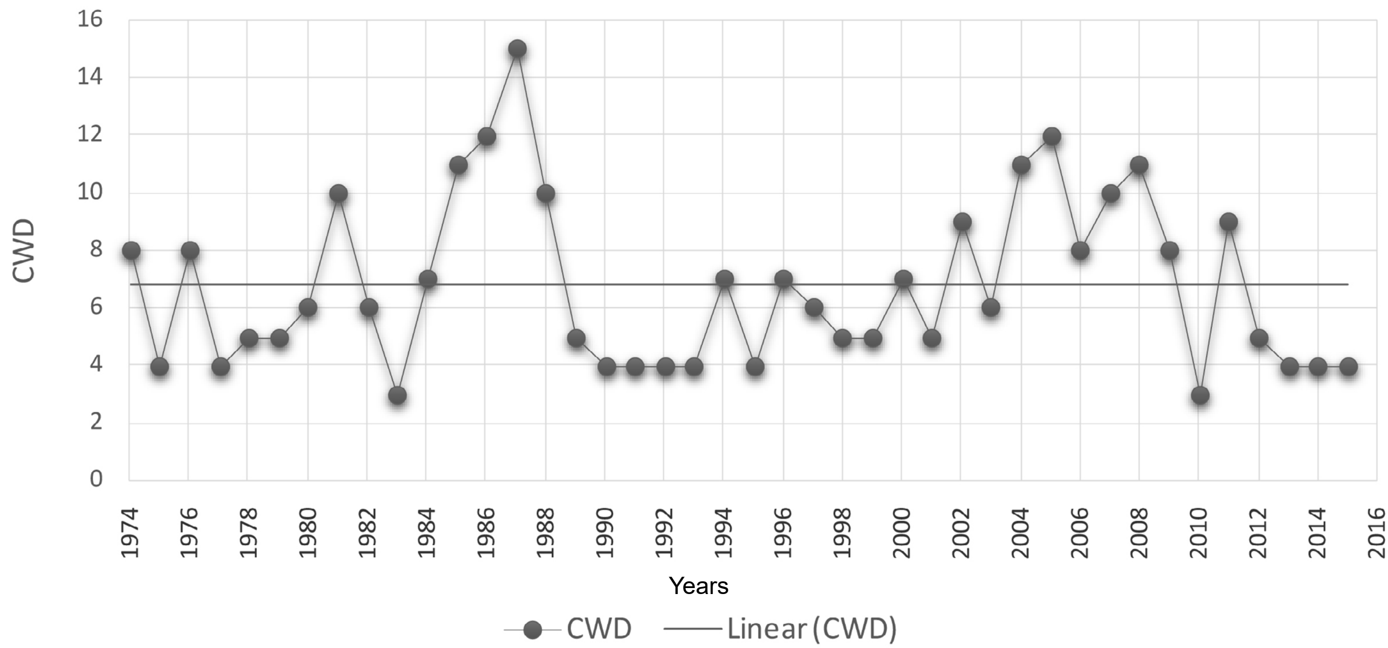

Figure 9 – Linear trend of the number of consecutive wet days (CWD) at the Aracati station (1974–2015).

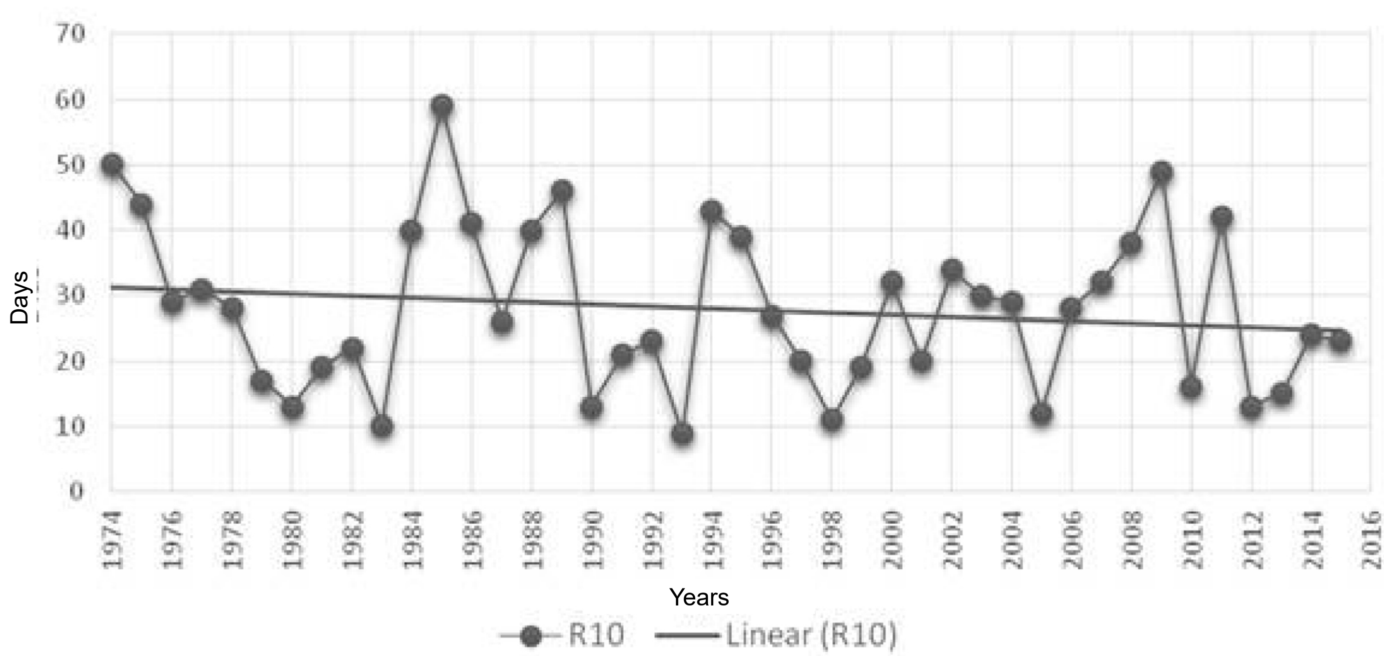

Figure 10 – Linear trend in the number of days/year in which rainfall was ≥ 10 mm (R10mm) at the Aracati station (1974–2015).

Figure 10 shows the linear trend of the extreme climate index R10mm at Aracati station, which represents the number of days per year in which rainfall was greater than 10 mm. The years 1985 and 2009 were those with the highest peaks; in these years, there were 59 and 49 days, respectively, when rainfall exceeded 10 mm. There is a linear trend to reduce the number of days with rainfall above 10 mm.

For the Trairi station, the analysis of the linear trend of the extreme climate index R10mm (Figure 11) shows that the years 1977,

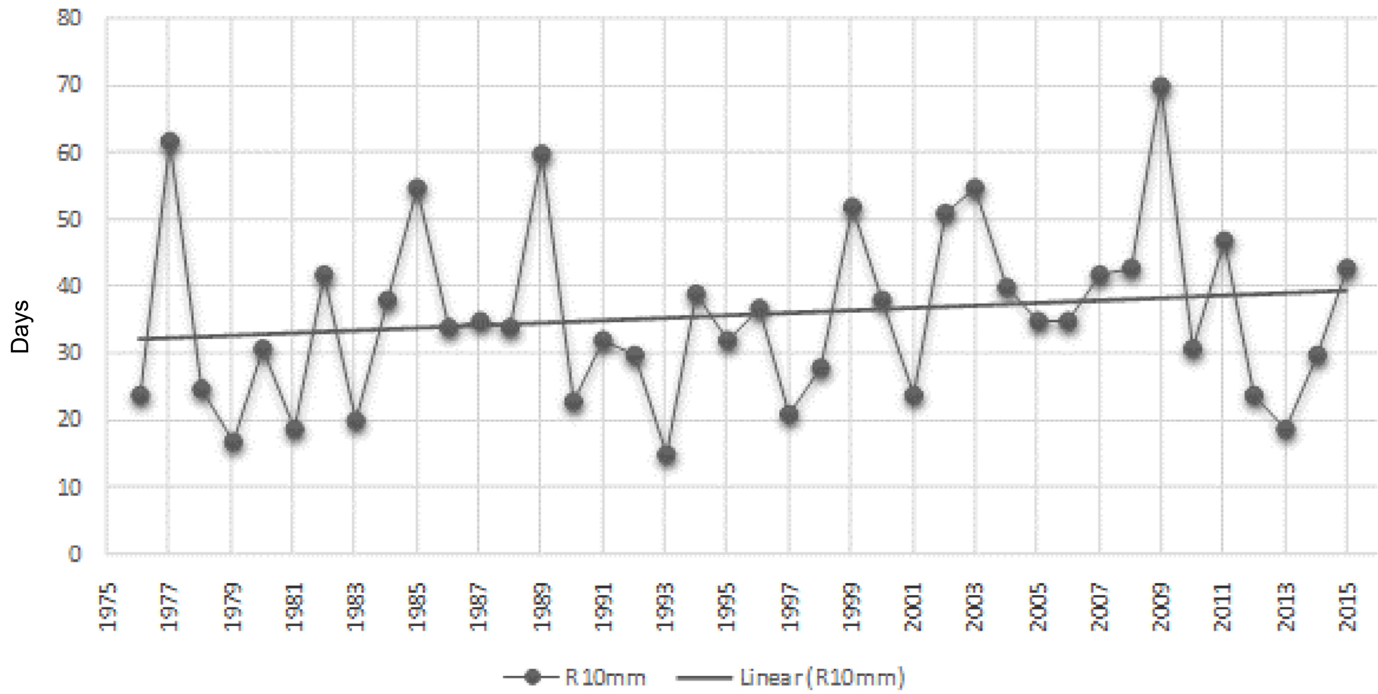

Figure 11 – Graph of the linear trend of the number of days/year in which rainfall was ≥ 10 mm (R10mm) at the Trairi station (1976–2015).

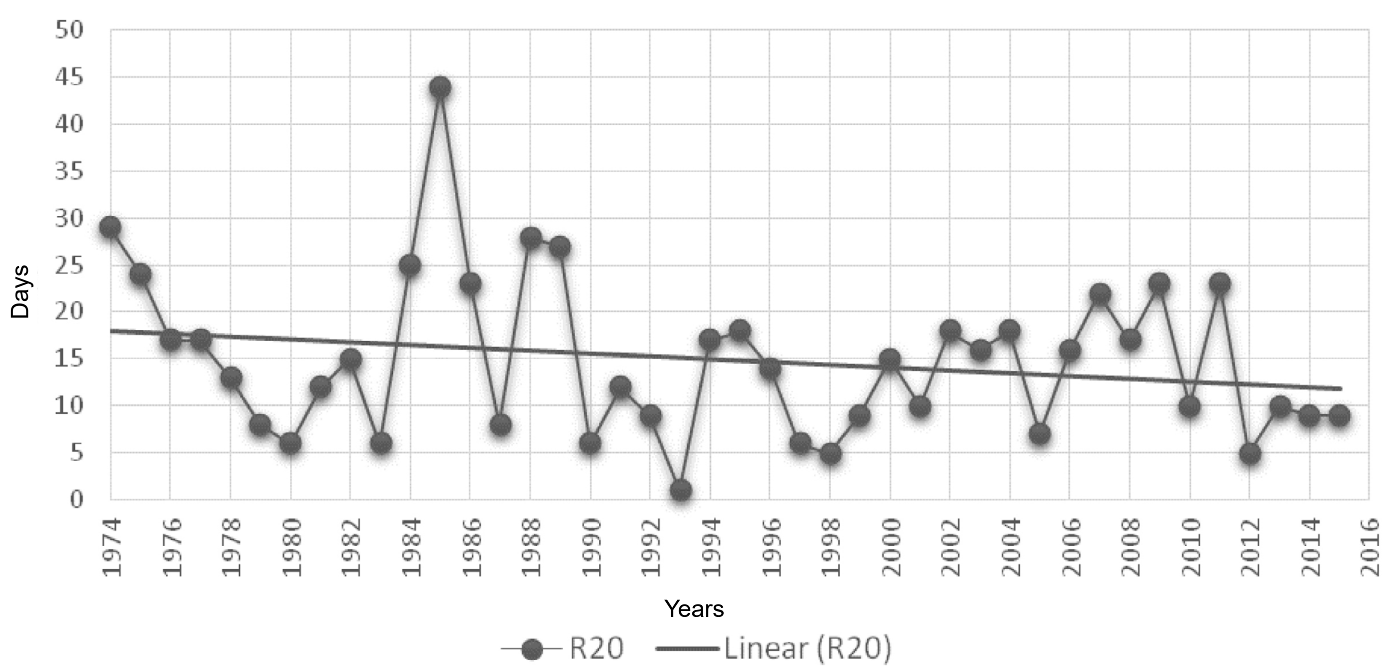

Figure 12 – Graph of the linear trend of the number of days/year in which rainfall was ≥ 20 mm (R20mm) at the Aracati station (1974–2015).

1989, and 2009 had the highest peaks; in these years, there were 60 and 70 days, respectively, in which rainfall exceeded 10 mm. There is a linear increasing trend in the number of days with rainfall above 10 mm.

Linear trend of number of days with rainfall > 20 mm

The R20mm index for the Aracati station (Figure 12) showed similar trends to the R10mm index, one of the biggest peaks during the first half of the historical series. From 2012 to 2015,

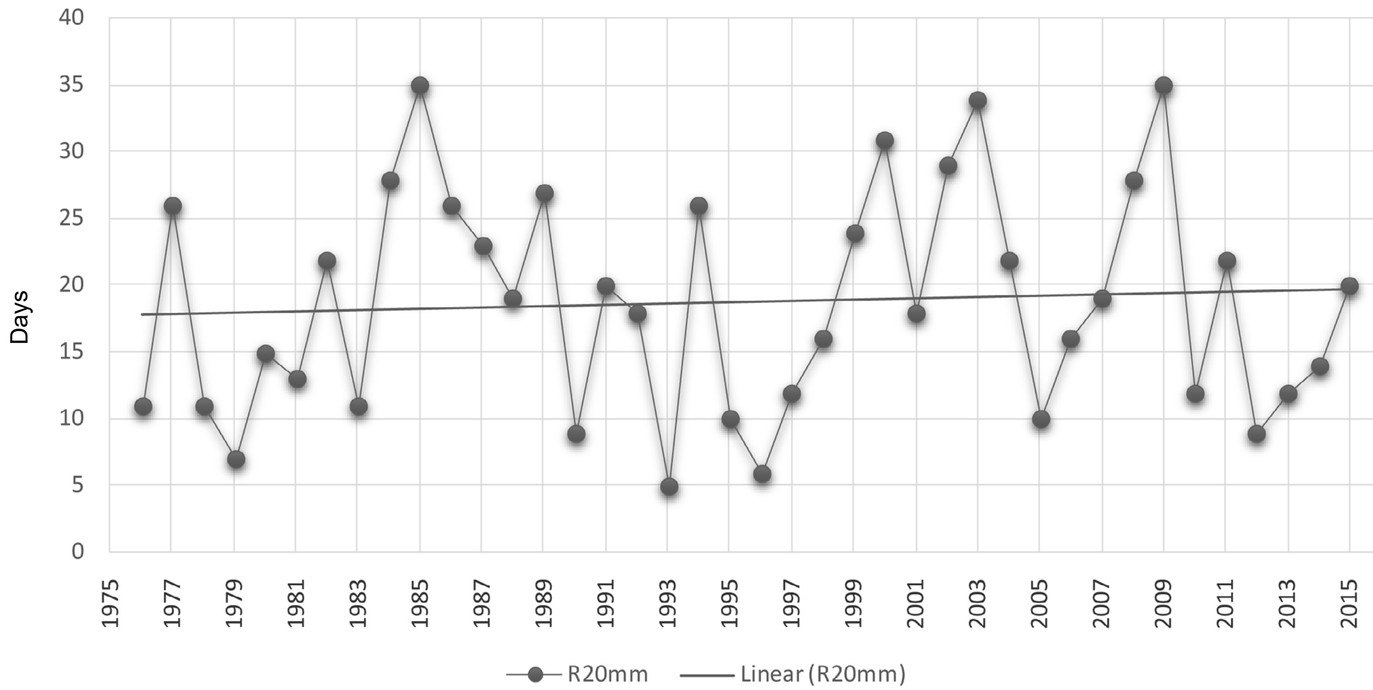

Figure 13 – Graph of the linear trend of the number of days/year in which rainfall ≥ 20 mm (R20mm) at the Trairi station (1976–2015).

the number of days with rainfall above 20 mm ranged from five to ten days.

For the Trairi station, the linear trend of the R20mm index (Figure 13) was also similar to that observed for the R10mm index. From 2012 to 2014, the number of days with rainfall above 20 mm was below average, varying from nine to 14 days.

Corroborating with the results previously shown, concerning the PRCPTOT and CDD climate indices, the analysis is consistent, since Aracati, which presented a decrease in rainfall and an increase in dry days, showed a decreasing trend in the number of days with rainfall up to 10 mm and up to 20 mm. The opposite occurred with Trairi, since, in this location, positive and negative trends were found for the PORCPTOT and CDD indices, consecutively.

Linear trend of the number of days with rainfall > 50 mm

The linear trend of the R50mm index at Aracati and Trairi stations (Figures 14 and 15) showed trends similar to the R10mm and R20mm indices.

For the Aracati station, the biggest peaks occurred during the first half of the historical series. The years 1985 and 2009 showed 11 and nine days, respectively, with rainfall above 50 mm. For Trairi station, the biggest peaks occurred during the second half of the historical series. The years 2000, 2003, and 2009 presented 11, 10 and nine days, respectively, with rainfall above 50 mm.

In the analysis of the indices of climatic extremes R10mm, R20mm, and R50mm, negative trends are observed in the three indices for the Aracati station, and positive trends for the Trairi station. For both stations, values below the historical average were recorded in the last four years, indicating that there was a decrease in the number of days per year with rainfall greater than 10 mm, 20 mm, and 50 mm, respectively. These indices are directly associated with the previous indices (PRCPTOT, CDD and CWD), since the results presented show a decrease in rainfall, an increase in the number of consecutive days without rain, and a decrease in the number of consecutive days with rain.

Linear trend of maximum rainfall in one day and five consecutive days

Analyzing the RX1day and RX5day indices at the Aracati station (Figures 16 and 17), there is neither a negative nor a positive trend. These indices correspond to the maximum rainfall recorded in one day and in five consecutive days, respectively. RX1day and RX5day are consistent with the PRCPTOT and R50mm indices, since there is a decreasing trend for both total rainfall and the number of days with heavy rain. Consequently, there is a decrease in these indices, both concentrated in one day and in five consecutive days.

Regarding the Trairi station, RX1day and RX5day indices (Figures 18 and 19) showed a slight negative trend, that is, a decrease in the maximum rainfall volume in one day and in five consecutive days,

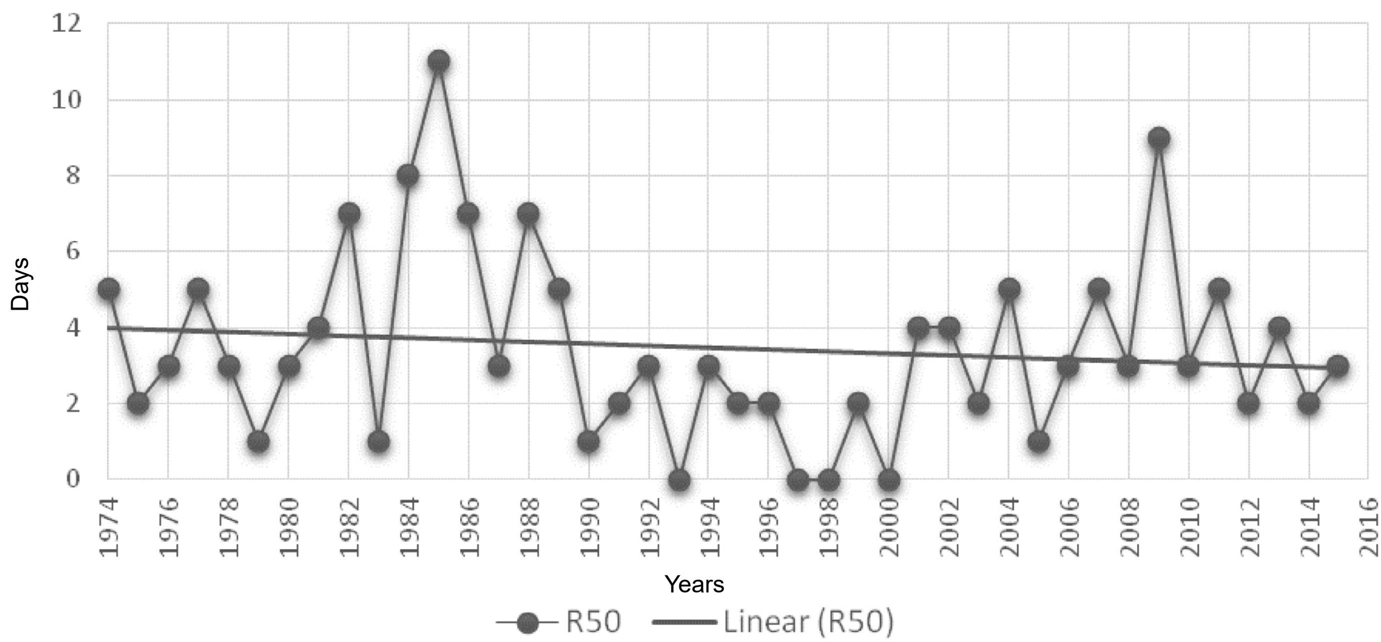

Figure 14 – Linear trend of the number of days/year in which rainfall was ≥ 50 mm (R50mm) at the Aracati station (1974–2015).

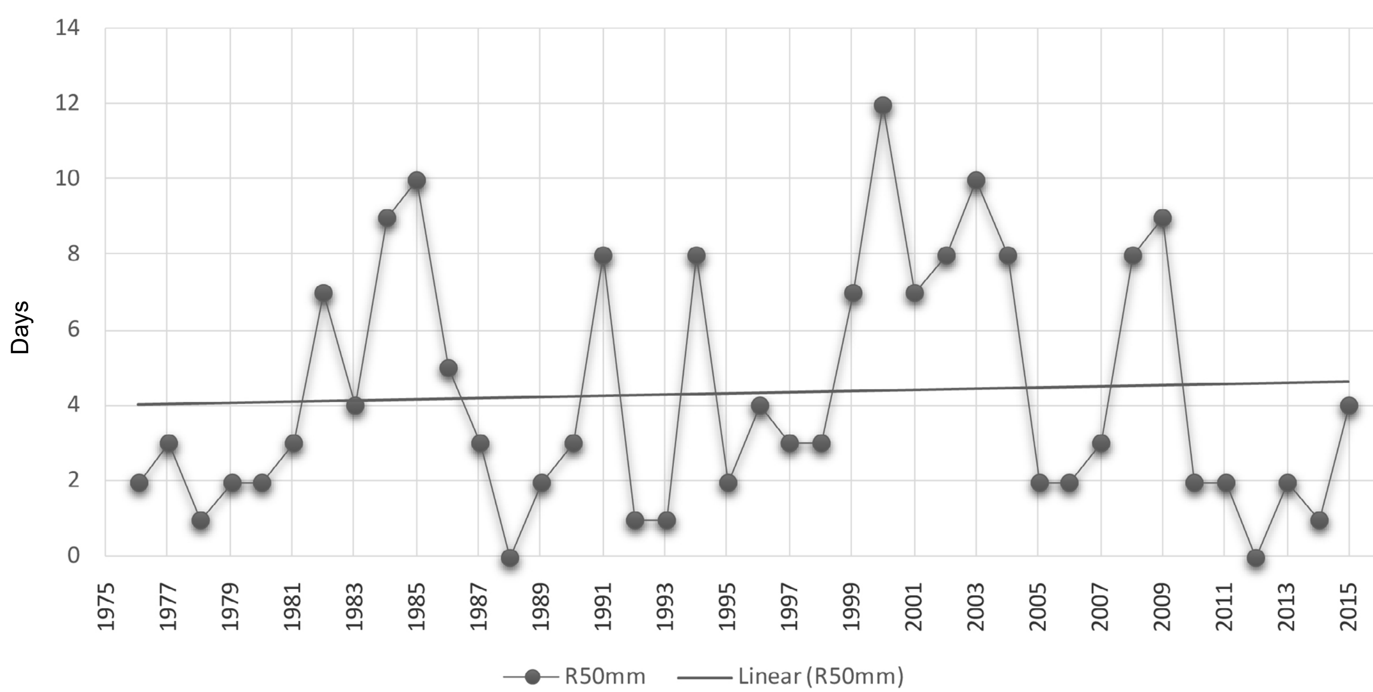

Figure 15 – Linear trend of the number of days/year in which rainfall was ≥ 50 mm (R50mm) at the Trairi station (1976–2015).

respectively. The maximum rainfall recorded in one day was 190 mm, and it occurred in 1976.

The predominance of negative trends in RX1day and RX5day indices shows a decrease in the maximum volume of rainfall in one day and in five consecutive days. The predominance of negative trends in these indices reinforces the decreasing trends found in total rainfall, the decrease in the daily intensity of rainfall and the decrease in the number of days with heavy, moderate, and intense rainfall.

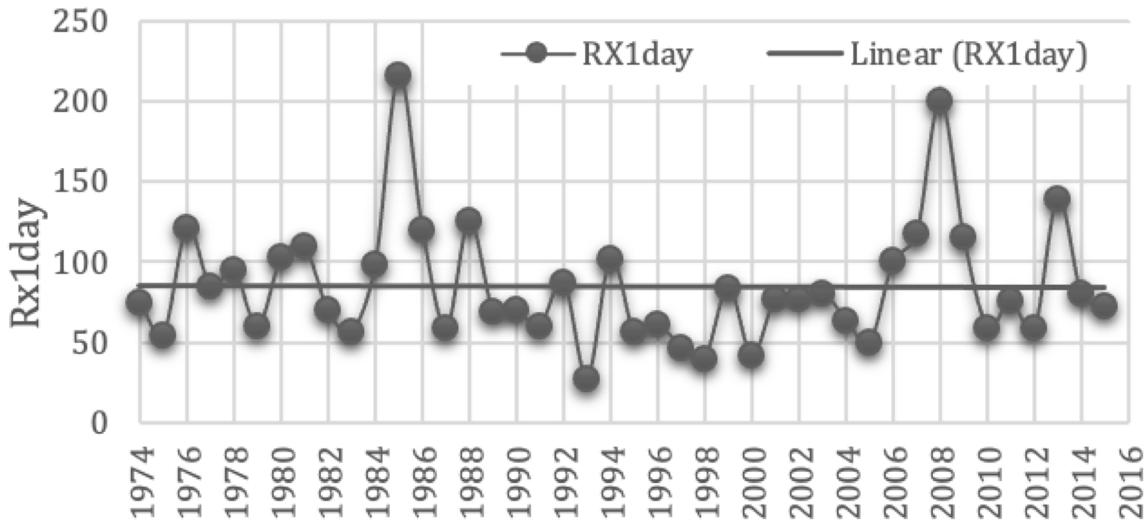

Figure 16 – Linear trend of the extremely humid days (RX1day) of the Aracati station (1974–2015).

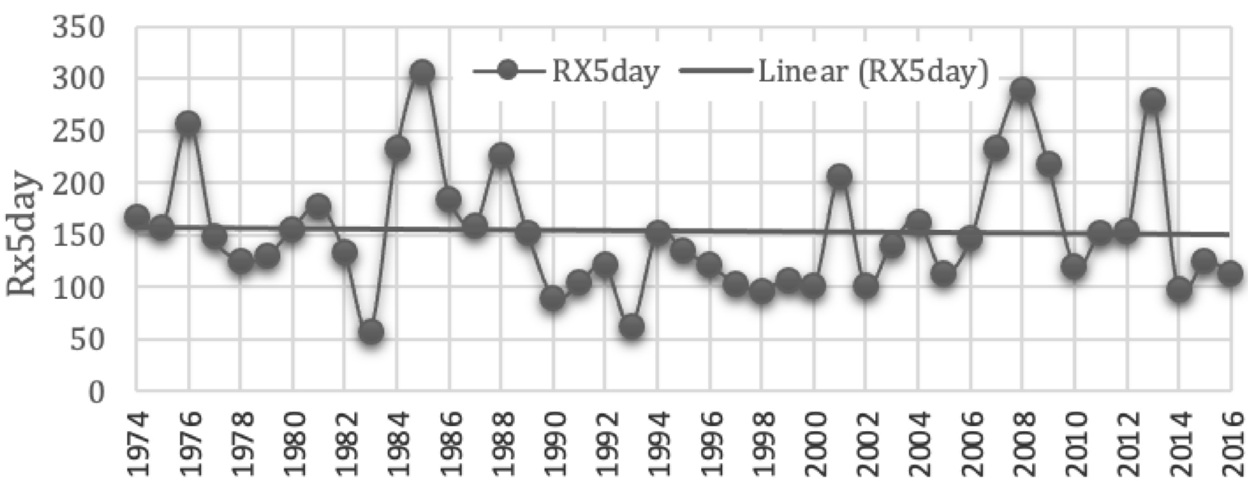

Figure 17 – Linear trend of the RX5day index of the Aracati station (1974–2015).

Considering the entire period of analysis for rainfall indices, there are few occurrences of significant trends of increase or decrease in the analyzed stations. However, it is clear that this result corroborates the work of Sillmann et al. (2013b), in which minor changes in rainfall rates for South America are present.

Analyzing the common period of rainfall and wind generation data from 2011 to 2015, the lowest capacity factor of wind generation is observed in 2011. In this same year, there was an increase in the number of consecutive wet days, as well as an increase in the number of days with rainfall above 10, 20, and 50 mm. However, from 2012 to 2015, capacity factors are higher and the exact opposite occurred, that is, there was a reduction in the number of consecutive wet days and in the number of days with rainfall above 10, 20, and 50 mm.

Final Considerations

The analysis of the climatic extremes indices carried out for the rainy seasons of Trairi and Aracati, located in the state of Ceará, revealed a predominance of negative decreasing trends in total rainfall, showing that rainfall is increasingly scarce and concentrated in a shorter period of time, as indicated by the CDD and R50mm indices.

In the analysis of the climatic extremes indices R10mm, R20mm, and R50mm, negative trends are observed in the three indices for the

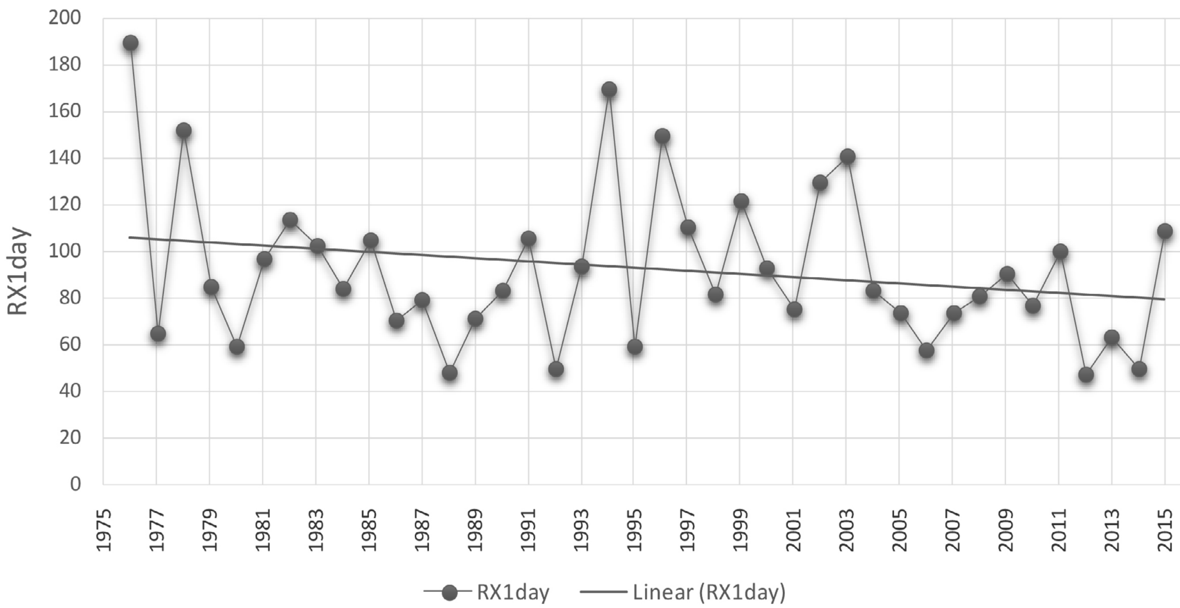

Figure 18 – Linear trend of the RX1day index of the Trairi station (1976–2015).

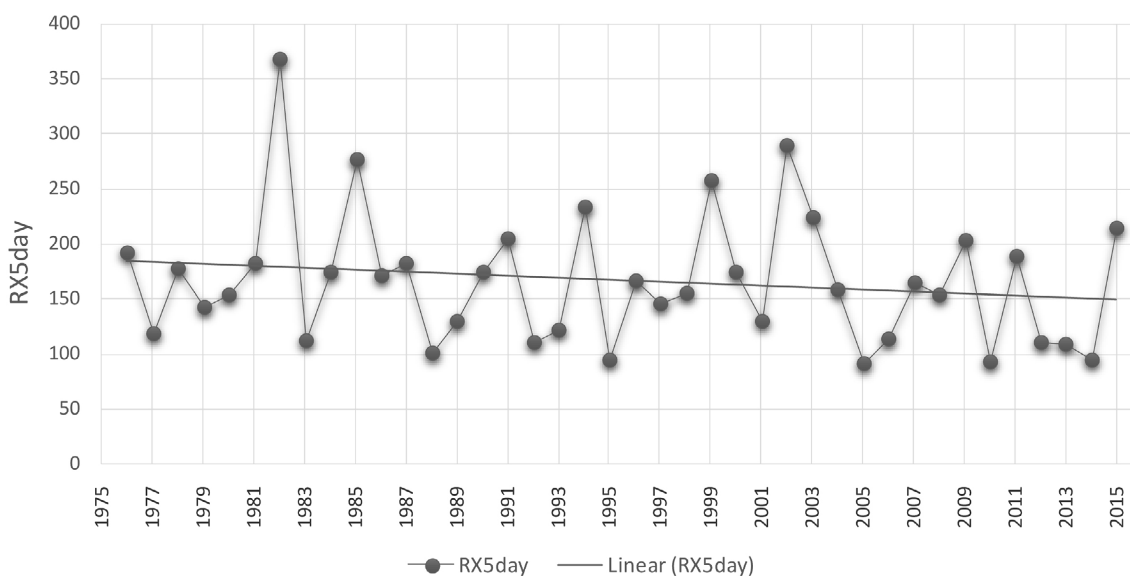

Figure 19 – Linear trend of the RX5day index of the Trairi station (1976–2015).

Aracati station, and positive trends for the Trairi station. For both stations, values below the historical average were recorded in the last four years, indicating that there was a decrease in the number of days per year with rainfall greater than 10 mm, 20 mm, and 50 mm, respectively. These indices are directly associated with the previous indices (PRCPTOT, CDD and CWD), since the results presented show a decrease in rainfall, an increase in the number of consecutive days without rain, and a decrease in the number of consecutive days with rain.

Analyzing the common period of rainfall and wind generation data from 2011 to 2015, the lowest capacity factor of wind generation is observed in 2011. In this same year, there was an increase in the number of consecutive wet days, as well as an increase in the number of days with rainfall above 10, 20, and 50 mm. However, from 2012 to 2015, capacity factors are higher and the exact opposite occurred, that is, there was a reduction in the number of consecutive wet days and in the number of days with rainfall above 10, 20, and 50 mm. Thus, the period of negative rainfall anomalies showed high wind generation capacity factors that may be associated with the occurrence of rainfall below the region's historical average, with extremely dry years.

For the analyzed period, there was an inversely proportional relationship between the rain anomaly index and the capacity factor of generation of the wind farm CE2, located close to the Aracati rain station, indicating that, in the months with negative precipitation anomalies, there was an increase in the capacity factor of wind generation in the months with negative rainfall anomalies. Due to the short history period of available data on verified wind generation, the need to repeat the analyzes for a period with a longer history is emphasized. For this region, verified wind generation data start in 2010.

In view of the climatic forecast of increased extreme weather events in the Northeastern region of Brazil, the present study must be expanded to other wind farms in the state of Ceará, as well as in different geographical regions and complex terrains, such as the states of Bahia, Rio Grande do Norte, Pernambuco, and Piauí. The results presented in research cannot be generalized for the entire Northeast, since it was applied only to stations in the state of Ceará, with a reduced historical series.

The growing participation of wind generation in the Brazilian energy matrix, specifically in the Northeastern region, requires further studies aimed at improving the forecast of wind energy generation for other regions in different horizons. Therefore, with the accomplishment of this work, we expect to contribute to the scientific society, mainly to Brazil and specifically to the Northeast, with a greater understanding and detailing of the trends of extreme climatic indices of rainfall and its relation with wind energy generation.

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Contribution of authors:

BARROS, A.M.L: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft; SOBRAL, M.C.: Conceptualization, Methodology, Writing – original draft, review & editing; SOUZA, W.M.: Methodology, Analysis, Investigation, Validation; ASSIS, J.M.O.: Methodology, Data curation, Software, Validation.

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Tertiary treatment of dairy industry wastewater with production of *Chlorella vulgaris* biomass: evaluation of effluent dilution

Tratamento terciário de efluente de indústria de laticínios com produção de biomassa de *Chlorella vulgaris*: avaliação da diluição do efluente

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ABSTRACT

Secondary wastewaters from the dairy industry may cause eutrophication of water bodies when not properly treated, mainly because they contain nutrients such as phosphorus and nitrogen. Tertiary treatment using microalgae could be an adequate solution for Minas Gerais State, the largest Brazilian milk producer, contributing to the reduction of environmental impacts, as well as providing biomass for oil extraction, and obtaining active compounds and inputs (including proteins) for animal feeding. In this work, dilutions (with distilled water) of the secondary wastewater from the dairy industry were evaluated to cultivate *Chlorella vulgaris* in a bench-scale tubular photobioreactor. Theresults indicate the feasibility of using wastewater from the dairy industry, after secondary treatment, to cultivate microalgae, showing cell growth like that obtained in control cultures (Bold basal medium). The secondary wastewater without dilution (100% wastewater) provided the best condition for biomass production. The biomass obtained in wastewater showed no differences from the biomass obtained in the Bold basal medium (control) in terms of protein, lipid content, or fatty acid profile.

Keywords: microalgae; biomass; tertiary wastewater treatment; dairy products; lipids.

RESUMO

Efluentes secundários da indústria de laticínios, quando não tratados adequadamente, podem provocar eutrofização de corpos d'água, principalmente por conter nutrientes como fósforo e nitrogênio. O tratamento terciário empregando microalgas poderia ser uma solução adequada para o estado de Minas Gerais, maior produtor brasileiro de leite, contribuindo na redução de impactos ambientais, bem como fornecendo biomassa para extração de óleos e obtenção de compostos ativos e insumos (incluindo proteínas) para nutrição animal. Neste trabalho, avaliaram-se diluições (com água destilada) do efluente secundário da indústria de laticínios para cultivo de *Chlorella vulgaris* em fotobiorreator tubular em escala de bancada. Os resultados encontrados indicam a viabilidade do uso de efluente de indústria de laticínios, pós tratamento secundário, para o cultivo de microalgas, apresentando crescimento similar àquele obtido em cultivos padrões (meio basal Bold). O efluente secundário sem diluição (100% efluente) foi o que apresentou melhor desempenho na produção de biomassa. Além disso, a biomassa obtida em efluentes não apresentou diferenças em relação àquela obtida em meio basal Bold (controle), no que se refere a teores de proteínas, lipídios ou perfil de ácidos graxos.

Palavras-chave: microalga; biomassa; tratamento terciário de efluente; laticínios; lipídios.

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Introduction

Currently, the dairy industry represents an activity of great importance in the world economy, with Brazil standing out with an annual production exceeding 35 billion liters (EMBRAPA, 2018). In this country, Minas Gerais State is its main producer, accounting for approximately 26% of the national milk production (CONAB, 2018).

The high milk production in Minas Gerais State can cause a problem related to the generation of liquid wastewater by the dairy industries, since the amount of generated residual water can significantly exceed the volume of processed milk, varying from 1 to 6 liters of water/kg of milk received (Maganha, 2006).

This type of wastewater originates from different dairy industry operations, such as cleaning equipment and surfaces, sanitizing, heating, and cooling. Consequently, this wastewater has a high BOD load (biochemical oxygen demand), COD (chemical oxygen demand), suspended solids (including oils and fats), dissolved organic compounds (mainly lactose and proteins), besides nutrients such as ammonia and phosphates (Sarkar et al., 2006).

When the amount of nutrients in these wastewaters presents high values, mainly nitrogen and phosphorus, it can cause the eutrophication phenomenon if dumped in a water body without additional treatment. It results in the accelerated proliferation of aquatic macrophytes, microalgae, and cyanobacteria, producing toxic substances, besides causing fish mortality, reducing species diversity, among other serious environmental problems (Maganha, 2006; Barreto et al., 2013).

To meet the environmental requirements, dairy industries can perform:

- preliminary treatments, such as via coarse screens (removal of coarse solids), grit chambers, and grease traps;
- secondary treatments involving biological processes, such as activated sludge, anaerobic filter, up flow anaerobic sludge blanket reactor (UASB), and stabilization ponds (Machado et al., 2001).

Besides these two treatments, there is a tertiary treatment, involving the removal of carbonates, ammonium, nitrate, and phosphate. However, it is rarely performed due to the high cost of the techniques that must be applied. Thus, a way to solve this problem would be through treatment involving microalgae cultivation, using residual water from stabilization ponds as a growth medium (Lourenço, 2006).

In general, biological wastewater treatment is considered more advantageous over chemical treatment, both ecologically and economically. In this context, the use of microalgae can have great potential for application, given the efficiency in the assimilation of carbon dioxide, as well as in the removal of nutrients such as nitrogen and phosphorus (Chinnasamy et al., 2010). Microalgae can remove up to 90% nitrogen and 96% phosphorus from liquid wastewaters (Kothari et al., 2013).

The cultivation of microalgae is an efficient option in the tertiary treatment of wastewaters due to their ability to rapidly develop in environments with high loads of nitrogen and inorganic phosphorus and in mitigating the greenhouse effect caused by excessive CO_2 emissions. Moreover, the applicability of the biomass resulting from this process is a promising opportunity since, in addition to removing nutrients, this biomass contains compounds with commercial interest, e.g., pigments and lipids. Therefore, these biomolecules provide us with additional gain, e.g., obtaining inputs for food supplements, drugs, and biofuels (Borowitzka, 1999; Derner et al., 2006; Venkatesan et al., 2006). Besides that, microalgae biomass, along with the effluent from stabilization ponds, can be applied in agriculture and fish farming (Sousa, 2007; Mata et al., 2010).

Microalgae can be grown in open (race-way systems and tanks) or closed systems (photobioreactors). Closed systems have been increasingly studied more recently, due to the effectiveness in controlling these microorganisms' growth and promoting better monitoring of their physical and chemical parameters (Carvalho et al., 2014).

Chlorella species have been successfully employed in several studies regarding wastewater treatment (Gupta et al., 2016; Choi et al., 2018; Rodrigues-Sousa et al., 2021). Kothari et al. (2012) observed not only the possibility of producing *Chlorella pyrenoidosa* biomass in pre-treated dairy industry wastewater, but also the efficiency of this microalgae in removing nitrogen and phosphorus. Moreover, Peng et al. (2019) observed that the organic compounds, present in wastewater, increase the microalgae biomass productivity through the mixotrophic growth and Bellucci et al. (2020) employed different microalgae species community (including *Chlorella* spp.) for the tertiary treatment municipal wastewater, indicating that these photosynthetic microorganisms also contributed to the disinfection of wastewater.

In this context, the present work evaluated the use of secondary wastewater from the dairy industry (after primary and secondary treatments) for cultivating the microalgae *Chlorella vulgaris*, having the wastewater dilution as an independent variable and comparing the data of cell growth, biomass productivity, and biochemical composition of biomass with cultivations in standard Bold basal medium (UTEX, [s.d.]).

Methodology

Microorganism

In this study, *Chlorella vulgaris* (CCMA-UFSCar 689) was employed. It was isolated at the Juréia Itatins Ecological Park (Peruíbe City, São Paulo State) (Matsudo et al., 2020), and kept in Erlenmeyer flasks containing Bold basal medium (UTEX, [s.d.]).

For preparing the inoculum, a small part of the cell suspension was aseptically added to other Erlenmeyer flasks containing the same sterile culture medium. The microorganism was kept in batch-type cultures, under light intensity of approximately 40 µmol photons m^{-2} s^{-1}, temperature of 25°C, initial pH of 7.0, and agitation of 100 RPM. The initial biomass concentration was between 50 and 100 $mg.L^{-1}$.

Tubular photobioreactor and culture conditions

The photobioreactor was built in the laboratory (Rodrigues-Sousa et al., 2021), adapting the one described by Ferreira et al. (2012), consisting of 20 transparent glass tubes (50 cm long and 1 cm internal diameter), with 2% inclination (1.15°) to facilitate the liquid flow, interconnected with silicone hoses of the same internal diameter. The illuminated volume corresponds to 1.26 L, and the total volume of the system was 2 L. There is a T-shaped tube in the lower part of the reactor tubes, in which compressed air enters to move the cell suspension into a flask on the top. In this flask, a porous stone is attached to a hose with an internal diameter of 4 mm, in which CO_2 enters to maintain pH when the solenoid valve opens, controlled by a programmed timer. Fluorescent lamps of 18 Watts were used to provide light at the intensity of 40 µmol photons m^{-2} s^{-1}.

Secondary wastewater from a dairy industry located in Southern Minas Gerais State was used. This wastewater results from primary treatment (with sieve and grease traps) and secondary treatment, carried out by the industry itself, through stabilization ponds (one anaerobic pond and two facultative ponds). In the laboratory, wastewater was filtered and frozen until it was used to not lose its original characteristics.

Different dilutions of secondary wastewater with distilled water were evaluated, obtaining the ratios 1:3 (25%), 1:1 (50%), and 3:1 (75%). These cultures were compared with those carried out in secondary wastewater without dilution (100%) and Bold basal medium (control).

Since a low concentration of total nitrogen was detected in the wastewater, and considering the concentration of residual phosphate, supplementation of this nutrient was carried out, in the form of sodium nitrate, to obtain the same proportion (N:P) present in the Bold medium.

Analytical methodologies

Determining biomass concentration

Biomass concentration was determined by turbidimetry at 550 nm (Becker, 2004). To do so, a calibration curve was constructed, correlating absorbance (550 nm) and biomass concentration (dry mass). Dry mass was gravimetrically determined in filters with a pore diameter of 1.2 µm.

Nutrients and chemical oxygen demand analyses

Both the Bold basal medium and secondary wastewater from the dairy industry were submitted to the following nutrient analyses, before and after cultivation: total inorganic nitrogen (nitrate, nitrite, and ammonium) and phosphate. For such analyses, the samples were previously filtered through a glass fiber membrane (0.45 µm) to remove organic matter.

Nitrogen in the form of nitrate was quantified by spectrophotometric method, according to APHA (2005). After acidification with HCl, to avoid interference with $CaCO_3$ concentrations, the samples were subjected to absorbance measurements at 200nm, subtracting the absorbance values at 275nm (interference from organic matter). A calibration curve was drawn up using KNO_3.

Nitrogen in the form of nitrite was quantified according to Mackereth et al. (1978) and Carmouze (1994). It is a spectrophotometric method that involves reacting the nitrite with $C_6H_8O_2N_2S$ and $C_{12}H_{14}N_2$.2HCl in an acid medium. The absorbance is measured with at 543 nm, and the calibration curve was drawn up with KNO_2.

Ammonium concentration was obtained by a spectrophotometric method involving the Berthelot reaction, using phenol and dichloroisocyanuric acid. Absorbance was measured at 630 nm, and NH_4Cl was used to draw up the calibration curve (Koroleff, 1976; Carmouze, 1994).

Phosphate was also quantified by spectrophotometric method, involving reaction with $(NH_4)_8Mo_7O_{24}.4H_2O$, $K_2Sb_2(C_4H_2O_6)_2$, and $C_6H_8O_8$ in acid medium. Absorbance is measured at 885 nm, and solutions with different concentrations of KH_2PO_4 were used for the calibration curve (Strickland and Parsons, 1960; Carmouze, 1994).

Secondary wastewater from the dairy industry was also submitted to COD (chemical oxygen demand) analysis by colorimetric method, using potassium dichromate as an oxidative agent, in accordance with the *Standard methods for the examination of water and wastewater* (APHA, 2005).

Analysis of the biochemical composition of biomass

At the end of each cultivation, the resulting biomass was centrifuged and dried at 60°C for approximately 12 hours. The pulverized dry biomass was submitted to the determination of total lipids and total proteins. Then, the lipid fraction was submitted to the analysis of fatty acids profile.

The quantification of total proteins was performed by the classic Kjeldahl method, adopting 6.25 as conversion factor based on the total nitrogen content (AOAC, 1984).

The quantification of total lipids was performed by the *Soxhlet* methodology, based on extraction with organic solvent (Chloroform:-Methanol; 2:1 v/v) (Pelizer et al., 1999).

Finally, the lipid fraction was recovered in petroleum ether. After the conversion of fatty acids into their corresponding methyl esters (Hartman and Lago, 1973), the analysis of fatty acid methyl esters was carried out in a gas chromatograph, model 7890 (Agillent Technologies, USA), equipped with a split/splitless injector and FID detector (*flame ionization detector*) in accordance with Pérez-Mora et al. (2016). The identification of fatty acids in the samples was carried out by comparing the retention times with those obtained in standards present in "37 Component FAME Mix" (Supelco).

Data analysis

Cultures were evaluated in terms of maximum biomass concentration (Xm), and this data was considered to calculate biomass productivity (Px), according to Equation 1:

$$Px = \frac{Xm - Xi}{t} \quad (1)$$

In which:

Xi = the initial biomass concentration;

t = the cultivation time.

Such data, as well as the lipid and protein contents, were compared by analysis of variance (ANOVA), with a significance level of 0.05, and Tukey's test, using the software Minitab 17.

Results and Discussion

Cultivation of *Chlorella vulgaris* in a tubular photobioreactor

In microalgae biomass production, the culture medium's choice is extremely important, combining low cost and adequate conditions for growth and obtaining the biochemical composition of interest. In the present work, the use of secondary wastewater from the dairy industry was evaluated in different ratios with distilled water: 1:3 (25%), 1:1 (50%), and 3:1 (75%). Wastewater was also used without dilution (100%), and cultivation in Bold basal medium was carried out as control.

When analyzing the concentrations of nitrogen and phosphorus in secondary wastewater from the dairy industry, the concentration of phosphorus (in the form of phosphate) was equal to 14 $mg.L^{-1}$. In contrast, the total inorganic nitrogen concentration (sum of nitrogen in the forms of nitrate, nitrite, and ammonium) was lower than 1 $mg.L^{-1}$. Thus, wastewater was supplemented to maintain the same nitrogen/phosphorus ratio as the Bold medium, which is 0.77. That is, 10 $mg.L^{-1}$ of nitrogen in the form of $NaNO_3$ was added. Regardless of dilution, all cultures with wastewater had the same initial supplementation with the nitrogen source.

Table 1 presents the results of maximum cell concentration (Xm), and biomass productivity (Px) obtained in the four different conditions using wastewater, as well as in the standard culture, using the Bold basal medium. Figure 1 shows the average growth curves (resulting from tests in duplicates) obtained for the four cultures in wastewater and compared with the standard Bold medium (control).

Table 1 – Maximum Biomass Concentration (Xm) and Biomass Productivity (Px) for *Chlorella vulgaris* cultures in wastewater from the dairy industry

Run	Xm* ($mg.L^{-1}$)	Px* ($mg.L^{-1}.d^{-1}$)
Control (Bold)	970.60 ± 48.90 ^A^	112.70 ± 8.54 ^A^
Wastewater 25%	224.30 ± 77.90 ^C^	36.40 ± 15.60 ^B^
Wastewater 50%	545.70 ± 63.70 ^BC^	114.79 ± 6.21 ^A^
Wastewater 75%	667.20 ± 126.00 ^AB^	126.16 ± 5.60 ^A^
Wastewater 100%	742.60 ± 114.60 ^AB^	92.58 ± 1.16 ^A^

*Average value obtained by the duplicate; A,B equal letters do not differ statistically, according to Tukey's test, considering a 95% confidence interval for Xm and Px.

After analyzing Table 1 and Figure 1, the growth of microalgae was found to occur satisfactorily in wastewater, even with the lowest concentration of nutrients, especially nitrogen and phosphorus (N = 41.17 $mg.L^{-1}$ and 10 $mg.L^{-1}$ and P = 53.25 $mg.L^{-1}$ and 14 $mg.L^{-1}$, in the Bold basal medium and the wastewater, respectively).

However, the highest dilutions (Wastewater 25% and Wastewater 50%) led to a reduction in the maximum biomass concentration (Xm = 224.30 and 545.70 $mg.L^{-1}$, respectively), which was lower than the values obtained in the control culture (Xm = 970.60 $mg.L^{-1}$) and in the wastewater without dilution (Xm = 742.60 $mg.L^{-1}$). The analysis of variance (ANOVA) confirms that the different experimental conditions significantly influenced this parameter ($p = 0.004$).

Despite the lower concentration of inorganic nutrients dissolved in wastewater, compared with the Bold basal medium, satisfactory microbial growth was probably benefited by the presence of organic compounds, since the COD (chemical oxygen demand) analysis resulted in 524 mg. $O_2.L^{-1}$. This organic compounds promoted the mixotrophic metabolism of *C. vulgaris*, which allows the reduction of biomass loss during respiration, increasing productivity (Yeh and Chang, 2012; Safi et al., 2014).

Experimental conditions also significantly influenced biomass productivity (ANOVA, $p = 0.001$). In fact, only the highest dilution led to a reduction in this parameter (Px = 36.40 $mg.L^{-1}.d^{-1}$). Although the 50% wastewater culture resulted in significantly lower biomass concentration (compared with the control culture), the shorter cultivation time resulted in statistically similar Px (Px = 112.70 and 114.79 $mg.L^{-1}.d^{-1}$, for control and Wastewater 50%, respectively). As shown in Figure 1, in Wastewater 100% and Control, growth stabilization started on days 7 or 8. In other cultures (diluted wastewaters), this stabilization started between the 4th and 6th days of cultivation.

Therefore, the use of wastewater 100% (without dilution) is recommended, avoiding the increase in volume and reducing water use for dilution. Kothari et al. (2012) suggest using Wastewater 75% to cultivate *Chlorella pyrenoidosa*. Tests carried out in our laboratory (Rodrigues-Sousa et al., 2021) show that in the cultivation using Erlenmeyer flasks, undiluted wastewater (Wastewater 100%) leads to a faster pH increase, inhibiting microalgae growth. In the present work, however, in a tubular photobioreactor, pH control with automated addition of pure CO_2 was probably the factor that favored the growth of *Chlorella vulgaris* even in undiluted wastewater.

Another factor to be highlighted here is nitrogen supplementation efficiency (in the form of $NaNO_3$) to guarantee the N:P ratio present in the Bold basal medium (N:P = 0.77). McGinn et al. (2011) point out that when growth is limited by a certain nutrient, the consequence is a decrease in the absorption of others. Therefore, the the medium components ratio can interfere in the yield of cultures, biomass biochemical composition, and the accumulation of certain nutrients in the extracellular medium.

Nutrient analysis

Nutrient analyses were carried out to verify the consumption of inorganic nutrients, total inorganic nitrogen (sum of nitrogen in the forms of nitrate, nitrite, and ammonium), and phosphorus (in the form of phosphate) after the cultivation as a way of tertiary treatment of industrial wastewater. It enabled calculating the efficiency of these nutrients' consumptions in each culture. The results of total inorganic nitrogen analyses for the cultures are shown in Table 2, and the results of the phosphorus analyses are shown in Table 3.

The results presented in Table 2 show a satisfactory efficiency in terms of total inorganic nitrogen consumption in all cultivation with wastewater with respect to the control, all of which have consumption efficiency of 96 to 98%. This efficiency in the consumption of total nitrogen is of great interest for wastewater tertiary treatment.

Through the results presented in Table 3, a high efficiency also in the removal of phosphorus in those cultures using wastewater supplemented with nitrogen can be observed, which occurs due to the low initial concentration of this nutrient in these media, when compared to the concentration found in the standard medium.

Similar results are obtained in the cultivation of *Botryococcus braunii* in diluted (50%) livestock wastewater, a condition in which the microalgae removed, on average, 88% of total nitrogen and 98% of total phosphorus (Shen et al., 2008).

Based on these results, wastewater after cultivation of the microalgae *Chlorella vulgaris* could be discharged in bodies of water, since NT values were in accordance with CONAMA resolution 357/2005, which states that — for freshwater from classes 1 and 2, in which nitrogen is a limiting factor for eutrophication, under the conditions established by the competent environmental agency — the total nitrogen value (after oxidation) should not exceed 1.27 mg.L^{-1} for lentic environments and 2.18 mg.L^{-1} for

Table 2 – Total inorganic nitrogen concentration values at the beginning and end of all cultures, besides their consumption efficiency

Medium	Total nitrogen		
	Initial (mg.L^{-1})	Final (mg.L^{-1})	Efficiency
Control (Bold)	65.25	2.32 ± 0.35	96%
Wastewater 25%	23.88 ± 12.66	0.33 ± 0.03	98%
Wastewater 50%	23.88 ± 12.66	0.58 ± 0.16	97%
Wastewater 75%	23.88 ± 12.66	0.49 ± 0.11	97%
Wastewater 100%	23.88 ± 12.66	0.92 ± 0.03	96%

Table 3 – Initial and final values of phosphorus concentration and the efficiency of its consumption in all cultures

Medium	Phosphorus		
	Initial (mg.L^{-1})	Final (mg.L^{-1})	Efficiency
Control (Bold)	113.10	84.98 ± 7.06	25 %
Wastewater 25%	3.50	0.02 ± 0.02	99%
Wastewater 50%	7.00	0.05 ± 0.03	99%
Wastewater 75%	10.50	0.23 ± 0.21	97%
Wastewater 100%	14.00	0.07 ± 0.01	99%

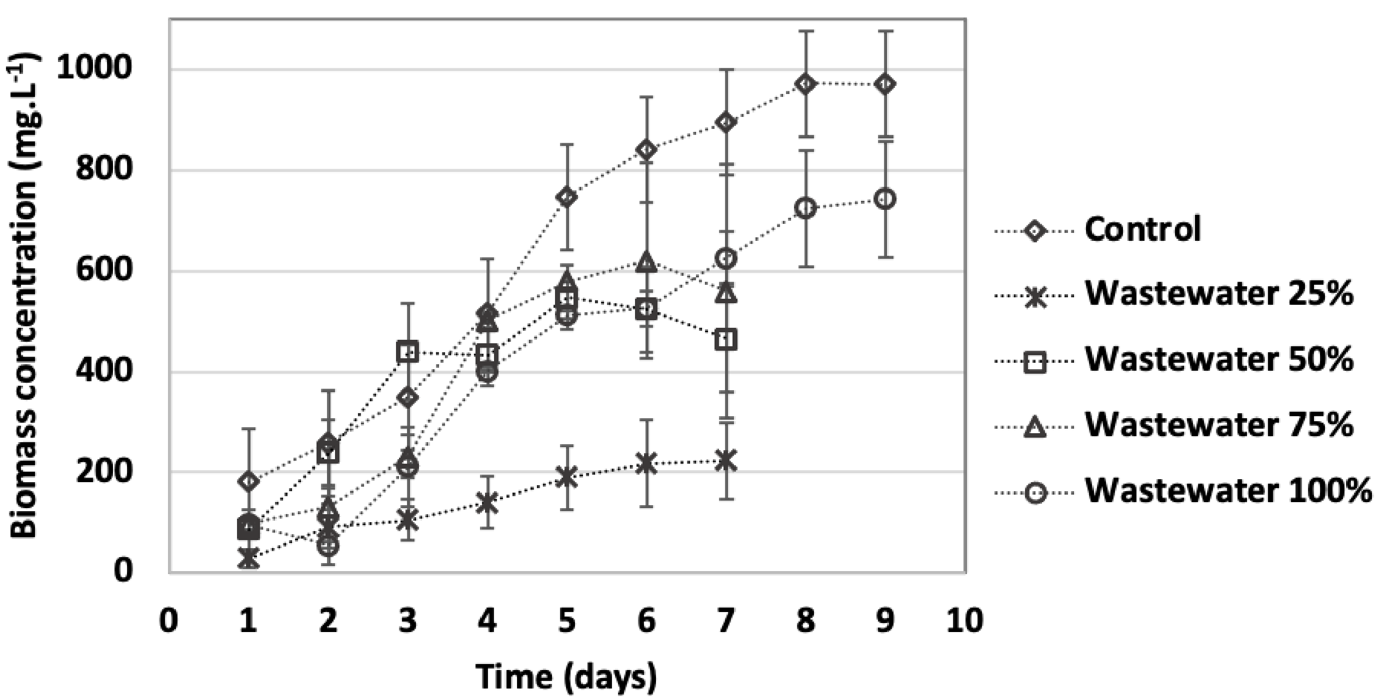

Figure 1 – Average growth curves for *Chlorella vulgaris* cultures in different proportions of wastewater from the dairy industry

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lotic environments at the reference stream flow. Nonetheless, if only the concentration of phosphorus is considered, no wastewater could be discharged into a lentic water body, since the resolution establishes for these class 1 and 2 environments that the total phosphorus value should be less than 0.020 $mg.L^{-1}$ P, but this treated wastewater could be discharged in lotic and tributary streams (of intermediate environments), since the total P value, in this case, must be less than 0.1 $mg.L^{-1}$ P (Brasil, 2005).

Considering that microalgae can effectively grow in waters containing nitrate and phosphate and also accumulate nutrients and metals from wastewaters, these attributes make them attractive and efficient tools beneficial to the environment, allowing wastewater treatment at a low cost (Kothari et al., 2012).

Analysis of the biochemical composition of biomass

The choice of a suitable medium is extremely important, since, in addition to its composition influencing the growth rate, it can also influence the biochemical composition of microalgae, which may favor certain later biomass applications (Lourenço, 2006). Thus, the biomass obtained in the cultures were subjected to analyses of total lipids and total proteins (Table 4), and fatty acid profile (Table 5).

Table 4 shows that the lipid content varied from 38.45 to 43.85%. According to ANOVA, there was no statistically significant influence between different culture conditions on this dependent variable (p = 0.945). Several authors state that it is possible to manipulate culture conditions to alter the biochemical composition of biomass, for example, by increasing the lipid content. Regarding lipids, their increase could be induced by the addition of sodium thiosulfate (reducing agent), osmotic stress (by the addition of NaCl, for instance), or nutritional stress (nitrogen starvation, for instance) (Takagi et al., 2006; Avila-León et al., 2020; Rodrigues-Sousa et al., 2021). The values found herein are quite promising, if comparing with data obtained with *Botryococcus braunii* biomass (32.6 ~ 36.9%) (Pérez-Mora et al., 2016) and *Ankistrodesmus braunii* biomass (38 ~ 39%) (Bresaola et al., 2019).

Table 4 – Content of lipids and proteins in *Chlorella vulgaris* biomass grown in dairy industry wastewater

Medium	Lipids (%)	Proteins (%)
Control (Bold)	38.45 ± 0.73 ^{A}	13.05 ± 2.80 ^{A}
Wastewater 25% Supplemented	39.04 ± 9.20 ^{A}	12.95 ± 3.32 ^{A}
Wastewater 50% Supplemented	39.76 ± 9.92 ^{A}	14.16 ± 2.31 ^{A}
Wastewater 75% Supplemented	38.56 ± 9.81 ^{A}	11.71 ± 0.64 ^{A}
Wastewater 100% Supplemented	43.85 ± 4.47 ^{A}	11.73 ± 0.50 ^{A}

Moreover, concerning the protein content, the different conditions of the culture medium did not significantly influence this parameter (ANOVA, p = 0.784), with mean values between 11.71 and 14.16%. These reduced values of total proteins can be justified by the low residual value of nitrogen, which is of great importance for the biosynthesis of amino acids and, consequently, of proteins (Markou et al., 2014); the stress caused by the lack of this nutrient may have induced the accumulation of lipids and the reduction of protein content (Wang et al., 2011). If the objective is obtaining biomass with high protein content, supplementing nitrogen throughout cultivation would be possible, as it has been well observed by different studies cultivating microalgae or cyanobacteria (Matsudo et al., 2009; Carvalho et al., 2013; Bresaola et al., 2019).

Under unfavorable or stressful environmental conditions, many algae alter their biosynthetic pathways to form and accumulate lipids, especially in the form of triacylglycerols, which serve mainly as carbon and energy storage. The fatty acid composition of typical microalgae oil is mainly composed of a mixture of unsaturated fatty acids, such as palmitoleic (16:1), oleic (18:1), linoleic (18:2), and linolenic (18:3) (Khan et al., 2009).

Table 5 shows that saturated fatty acids, such as palmitic (16:0) and stearic (C18:0), and unsaturated fatty acids, such as palmitoleic (16:1), heptadecenoic (17:1), oleic (C18:1n9), linoleic (C18:2n6), and γ-linolenic (C18:3n6) were present in all cultivation conditions of *Chlorella vulgaris*. Palmitic (16:0) and oleic (C18:1n9) acids had the highest per-

Table 5 – Fatty Acids Profile (%) of *Chlorella vulgaris* grown in wastewater from the dairy industry

Fatty acid (%)*	Control	Wastewater 25%	Wastewater 50%	Wastewater 75%	Wastewater 100%
C16:0	30.45 ± 0.31	36.55 ± 5.21	30.78 ± 0.14	31.21 ± 5.20	31.60 ± 0.31
C16:1	1.74 ± 0.13	0.81 ± 0.25	0.88 ± 0.07	1.24 ± 0.12	0.86 ± 0.02
N.I.**	1.93 ± 0.09	1.09 ± 0.25	0.69 ± 0.05	0.36 ± 0.00	0.74 ± 0.08
C17:1	3.22 ± 0.13	2.65 ± 0.59	2.33 ± 0.33	3.72 ± 0.90	2.50 ± 0.11
N.I.**	1.42 ± 0.05	1.44 ± 0.91	2.55 ± 0.23	3.63 ± 1.23	2.39 ± 0.11
C18:0	3.12 ± 0.09	4.03 ± 0.91	2.98 ± 0.15	5.06 ± 3.95	4.15 ± 0.12
C18:1n9	38.54 ± 0.40	37.66 ± 13.44	39.22 ± 1.69	28.60 ± 0.50	37.13 ± 0.55
C18:2n6	9.80 ± 0.04	8.90 ± 2.77	7.98 ± 0.21	10.35 ± 1.29	9.31 ± 0.28
C18:3n6	9.57 ± 0.32	9.60 ± 2.13	12.68 ± 0.78	16.18 ± 5.47	11.59 ± 0.19

*Percentage of fatty acids in relation to the total content (mass/mass); **unidentified compound, absent in the standard 37 FAME mix; C16:0 palmitic acid; C16:1 palmitoleic acid; C17:1 cis-10-heptadecenoic acid; C18:0 stearic acid; C18:1n9 oleic acid; C18:2n6 linoleic acid; C18:3n6 γ-linolenic acid.

centage in all cultures (30 ~ 36% and 27 ~ 39%, respectively), which agrees with results obtained by Converti et al. (2009).

Linoleic acid (C18:2n6c), considered essential for the human organism (Teitelbaum; Walker, 2001), was also detected in all cultures, reaching values between 7.89 and 10.35%. Finally, γ-linolenic acid (C18:3n6) was present with values between 9.57 and 16.18%. The importance of these two fatty acids (γ-linolenic acid and linoleic acid) is justified because they are precursors of arachidonic acid (Verlengia and Lima, 2002) and can, therefore, serve in the production of fatty acid supplements (Tallima and El Ridi, 2018).

Biodiesel is currently produced from oilseed plants (soy, rapeseed, and palm), but microalgae are presented as sustainable alternative feedstock for its production. Fatty acids methyl esters represent the main component of biodiesel, and the chemical structure of these molecules has a strong influence on the properties of this fuel (Lu et al., 2015). Although high levels of polyunsaturated fatty acids reduce the cold filter plugging point, they also reduce the oxidative stability of the product (Schenk et al., 2008). Therefore, as it can be seen in Table 5, the predominance of palmitic acid (saturated) and oleic acid (monounsaturated) makes the biomass obtained as suitable for this purpose, which was also observed in *Chlorella* sp. U4341 and *Monoraphidium* sp. FXY-10 grown in monoculture or co-culture by Zhao et al. (2014).

The biomass of *Chlorella vulgaris*, produced in the tertiary treatment of dairy industry wastewater, could be mainly employed as source of feedstock for biodiesel and animal feed production (Rodrigues-Sousa et al., 2021). Therefore, besides helping to mitigate an environmental problem (eutrophication), the use of *Chlorella vulgaris* in the tertiary treatment of dairy industry wastewater could allow reducing the costs to produce biomass for bioenergy and animal feed, serving as an alternative source, mainly during extreme dry seasons.

Conclusions

Microalgae can be an excellent solution for the tertiary treatment of dairy industry wastewater, allowing to minimize environmental problems, such as eutrophication, and generating biomass for the extraction of oils, bioactive compounds, as well as proteins and carbohydrates for animal feed.

When using secondary wastewater from a dairy industry, there was no need for dilution, as long as CO_2 wass added for pH control, reaching values of maximum biomass concentration and biomass productivity similar to control culture in Bold basal medium. However, quantifying phosphorus and nitrogen levels, and supplement in case of lacking one of them is important, adjusting the proportion similar to that found in the Bold basal medium (N:P = 0.77).

These results are promising in a Brazilian state where the dairy industry is of great economic and social importance. Besides their environmental benefits, the different biomass applications could bring economic benefits, even serving as an input (including proteins) for animal feed, for example.

Contribution of authors:

Nunes, I.V.O.: Investigation, methodology, formal analysis, writing – original draft. Inoue, C.H.B.: Investigation, methodology, writing – review and editing. Sousa, A.E.R.: Investigation, methodology. Carvalho, J.C.M.: Conceptualization, resources, funding acquisition, visualization. Gomes, A.M.A.: Methodology, visualization, writing – review and editing; Matsudo, M.C.: Conceptualization, funding acquisition, supervision, project administration, writing – review and editing.

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