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Chia seed mucilage: Extraction methods and potential application in food matrix



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Chia seed mucilage: Extraction methods and potential application in food matrix



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ABSTRACT

Chia (*Salvia hispanica* L.) is a herbaceous plant native to southern Mexico and northern Guatemala,

with a high nutrient content and technological properties. Currently, its seed has stood out, since it has 5 to 6% soluble fibers, which are exuded when the seeds are placed in water, forming a transparent capsule around the seeds. The extraction of chia mucilage involves three processes: hydration, extraction, and drying. The amount of mucilage extracted can be affected by several factors related to extraction conditions, such as chia ratio: water, extraction temperature, hydration time, agitation, and separation. In addition, its incorporation into food contributes to sensory and physical characteristics, with regard to texture, stabilization, formation of gels and emulsions, volume, inhibition of syneresis among other factors. Thus, the present study aimed to analyze studies that differ with regard to the extraction methodology, in addition to the application of this gel in food products. The review was carried out in July 2023, on the Periódicos CAPES electronic platform of scientific articles, which used chia mucilage as a substitute for commercial additives in foods. The selected articles were published between 2018 and 2023 and the following terms were used for the search: "mucilage", "chia", "applications". In the studies analyzed, it was possible to observe a variation in yield of 3.4 to 8.5% depending on the methodology used by the authors, which shows the relationship in the change of the variables: seed/water, time, temperature, and use of ultrasound. Regarding the application of mucilage in food formulation, the analyzed studies provided good results in the physicochemical and sensory characteristics of food products.

Keywords: Fat, Gum, Gel, Seeds, Texture, Stabilization.

1 INTRODUCTION

Plant seeds are rich in nutrients, fiber, and fatty acids, which make them an important food for human nutrition. Due to these functional properties, the *U.S. Food and Drug Administration* has considered plant seeds to be a dietary supplement (CHIANG et al., 2021; ROSAS-RAMÍREZ et al., 2017).



An example is chia (*Salvia hispanica L.*), a herbaceous plant, native to southern Mexico and northern Guatemala, which was used by many ancient civilizations as a food source. The nutritional compounds present in chia have gained importance in research in recent years, as it is a source of oil rich in polyunsaturated fatty acids (omega-3 and omega-6), proteins, fiber, minerals, and antioxidant compounds (CAPITANI et al., 2018; FERNANDES et al., 2020).

In addition to its high nutrient content, chia has technological properties that are gaining prominence today, due to the fact that it has 5 to 6% soluble fibers, which are exuded when the seeds are placed in water, forming a transparent capsule around the seeds, with gel-like characteristics (CAPITANI et al., 2016; MUTLU; KOPUK; PALABIYIK, 2023).

This gel is known as mucilage, considered as a tetrasaccharide with high branches. Its incorporation into food contributes to sensory and physical characteristics, with regard to texture, stabilization, formation of gels and emulsions, volume, inhibition of syneresis.

As they have in their composition other nutritional components such as proteins, oil and carbohydrates, it becomes an alternative in the replacement of fats, gelling agents, thickeners and stabilizers. (FERNANDES; MOLLADO, 2018; MUTLU; KOPUK; PALABIYIK, 2023; HIJAZI, 2022). This substitution is in line with the population's growing search for low-energy foods, which consequently challenges the industry to seek strategies for the replacement of fatty components, without changing the characteristics of the products (CHAVES et al., 2018).

The objective of this study was to explain the application of chia mucilage in the substitution of commercial agents in food, as well as to analyze the different conditions of extraction, performing a literature review.

2 RESEARCH METHOD

The present study addresses a systematic review carried out in July 2023 on the *Periódicos CAPES* electronic platform of scientific articles that used chia mucilage as a substitute for commercial additives in foods. The articles considered for this study were published between 2018 and 2023, in order to carry out a review of recent studies, in addition to the following terms were used in the search: "mucilage", "chia", "applications". For the choice of articles, we considered 08 (eight) that presented at least two combinations of keywords in the title, disregarding articles that were not in accordance with the research. A total of 61 articles were found. The research constituted the search for promising applications in the production of safe and healthy foods that replace commercial agents in their formulation.



3 RESULTS AND DISCUSSIONS

3.1 REASONS FOR THE APPLICATION OF CHIA SEED MUCILAGE (MSC)

Isolated chia seeds have been increasingly investigated; many studies are focused on optimizing the extraction yield, as well as its application in the food industry, due to its good ability to retain oil and have a strong emulsifying and stabilizing property, which makes it promising in replacing synthetic compounds (CHIANG et al., 2021; SILVA et al., 2022).

3.2 EXTRACTION AND PROPERTIES OF MUCILAGINOUS GEL

Basically, the extraction of chia mucilage involves three processes: hydration, extraction, and drying. The amount of mucilage extracted can be affected by several factors related to the extraction conditions, such as chia ratio: water, extraction temperature, hydration time, agitation and, especially, the way the mucilage is separated from the seeds (CHAVES et al, 2018). As a result, the studies analyzed in this review differ in terms of extraction time and temperature, and water/seed ratio. Some studies perform additional steps, such as ultrasound treatment, in order to increase the extraction yield. These changes in methodology are compared in Table 1.

The lowest yield observed in the table was in Feizi's work (3.4%). However, he worked with a low seed:water ratio, and low hydration time. Silva also had a low yield, as he worked at room temperature. Antigo et al. (2020) obtained a yield of 4.68% under extraction conditions of 1:30 (w/w) for the proportion of chia: water, 50 °C and 2h of hydration. Fernandes' work, Mellado (2018), on the other hand, shows a yield of 5.81% when using a proportion of chia:water higher, however, a lower extraction temperature (25 °C).

However, the highest yield was observed in the work of Chaves et al. (2018) who, in turn, extracted chia mucilage with the intention of obtaining a high moisture content by using wet mucilage, which resulted in 18.25% yield in relation to wet mass. However, for the purpose of comparison of yield with the literature, they dried and obtained a powder mucilage yield equal to 8.49%.

With the use of ultrasound in the work of Silva et al. (2022), it was noted that the mucilage fibers formed denser aggregates compared to samples that were only agitated. The increase in the viscosity of the solutions occurred mainly after 10 min, which resulted in a greater compaction of the mucilage and there was a greater loss of the gel due to its adhesion in the filtration. The timing is interesting, as longer ultrasound applications (30min and 6h) resulted in less viscous solutions, which facilitated the separation process and consequently increased the yield. However, the samples that were not sonicated had lower or similar yields to the samples that were not ultrasounded. The author obtained apparent viscosity values at 100 s⁻¹ between 0.200 and 0.280 Pa.s for the samples that were not sonicated, and between 0.057 and 0.273 Pa.s for the sonicated samples. In addition, thermal stability has increased with the use of ultrasound.



Table 1. Comparison of mucilage extraction of the articles selected for the research.

Seed/water ratio (p/p)	Time	Temperature °c	Ultrasound	Drying	Average yield (%)	Author/year
1:40	2h	25	N/A	Lyophilization	5,8	FERNANDES; MELLADO, 2018
1:30	2h	50	N/A	Oven drying with forced air circulation 50 °c for 96 hours / freeze drying	4,7	ANTIGO et al., 2020
1:30	3h 4,5 6h	25	Yes	Lyophilization	3,4	SILVA et al., 2022
1:40	2h	80	N/A	Lyophilization	8,5	CHAVES et al., 2018
1:20	20min	50	N/A	Lyophilization	3,4	FEIZI et al., 2021
3:30	3h	60	N/A	Lyophilization	-	RIBES et al., 2022
1:20	2h	30-50	N/A	Lyophilization	-	AK; CARDER, 2021
1:50, 2:25, 1:10	15h	37	N/A	Lyophilization	7,6	TOMIC et al., 2022

Source: authors, 2023.

3.3 APPLICATION OF MUCILAGE AS A FAT SUBSTITUTE AND/OR STABILIZERS IN FOOD PRODUCTS

Chia mucilage has characteristics that enhance its use as an alternative in replacing fat in a range of foods. In the work of Fernandes, Mellado (2018), different formulations of mayonnaise with freeze-dried chia mucilage were prepared, replacing the oil or egg yolk. Among the results obtained by the authors, the substitution of the oil for the mucilaginous gel increased the stability and texture of the mayonnaise, while the addition of the hydrogel in place of the egg yolk presented lower values in which they resemble the control mayonnaise. When sensorially analyzed, the result differed, the mayonnaise with egg substitution showed greater acceptability than the mayonnaise with oil substitution.

Arnak and Tarakçi (2021) applied powdered mucilage as a substitute for commercial stabilizers and emulsifiers, as these ingredients incorporate several physicochemical benefits into the final product, such as improved viscosity, stability, texture, shelf life, and melting properties. In this way, the authors evaluated chia mucilage as a substitute for the thickener salep, a product obtained from dried orchid tubers, widely used in Turkey, but of high cost and limited by law. The authors used ice cream as a food matrix. Due to this substitution, there has been a significant increase in melting. In addition, higher concentrations of mucilage powder reduced the hardness and viscosity of the ice cream. However, no significant effects were observed on water content, pH and acidity; In addition, ice cream made with 0.4% mucilage was considered the most promising to maintain quality, reducing



costs and still being sensorially attractive. Another point was the analysis of air incorporation (*overrun*), which resulted in higher values as the concentration of chia in the formulation increased.

On the contrary, the study by Feizi et al. (2021) applied mucilage as a fat substitute in ice cream and observed that the incorporation of air (*Overrum*) decreased as the concentration of chia mucilage in the formulation increased. The highest *overrun* value was for formulations containing 0.1% w/w of mucilage and the lowest for 0.2% w/w, obtaining respectively 108 and 96% of air incorporation. Regarding rheological properties, it was observed that ice cream mixtures containing the addition of mucilage had an increase in apparent viscosity compared to control samples. Regarding melting, performed at 20°C, the effect of the addition of mucilage reduced the melting rate compared to the other samples without mucilage; This is a result of fat aggregation and/or partial coalescence in the emulsion, partially coalesced fats provide an ice cream with desirable characteristics such as firmness, texture, melting and creaminess, which was observed with the addition of the mucilaginous gel. In short, the ice cream produced presented desirable characteristics and, according to the results obtained in the sensory analysis, the best ice cream contained 0.2% mucilage (w/w).

Chaves et al. (2018) used the combined effect of chia mucilage with carob gum (*Ceratonia siliqua L.*) in a kind of frozen yogurt, that is, a frozen goat's milk dessert. The formulations, at higher levels of chia mucilage, led to increased moisture content, apparent viscosity and texture; however, they reduced the *overrun*, because there was difficulty in the incorporation of air. With regard to melting, a critical parameter for a frozen product, even formulations with lower fat content showed a reduction in melt rate, which was desirable. The fat reduction was significant, since the formulations provided a reduction of 56.33% of fat in the sample composed of 100% chia mucilage, when compared to the sample with 0% mucilage. According to the authors, although fat is an important ingredient in the incorporation of air in frozen products, the alternative of replacing chia presented characteristics and values close to the original formulation.

Tomic et al. (2022), when evaluating chia seed mucilage as a fat substitute in the formation of gluten-free biscuits, observed that biscuits exhibited desirable physical and sensory properties. The incorporation of mucilage favored the composition of fatty acids, not significantly affecting hardness, weight and volume. Thus, the use of the hydrogel did not alter the structure and texture of the biscuit. In this work, it was observed that the viscosity of the hydrogel increases with higher concentrations and all samples showed characteristics of weak gels. One point analyzed is that 5% chia seed hydrogels were more susceptible to deformation than 10% chia seed hydrogels, which were too rigid to replace fat, so biscuits with 8% hydrogels had higher quality.

The work of Ribes et al. (2022) evaluated the addition of chia seed mucilage in the production of a chicken and vegetable soup, as an alternative to the use of thickeners. An analysis of the rheological properties of the soup showed that the product showed characteristics of a weak gel. In



addition, the soup showed greater consistency and firmness when compared to the control and with modified starch. Regarding the rheological properties, the viscosity of the mash (at $10s^{-1}$ and $50s^{-1}$) increased with the addition of mucilage, as expected due to the higher content of total solids.

4 CONCLUSION

This review addresses different conditions of chia mucilage extraction, where the seed/water ratio, time, temperature, and use of ultrasound were varied. When comparing the selected studies, it was possible to observe a variation in yield of 3.4 to 8.5% depending on the methodology used by the authors. In addition, the application of chia mucilage to replace commercial agents in food has been explored. Thus, the analyzed studies provided good results in the use of mucilage with regard to the physicochemical and sensory characteristics of the food products mentioned in the present study, such as ice cream, frozen dessert, chicken puree with vegetables, gluten-free biscuits and mayonnaise.



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Fauna and flora of the Brazilian Atlantic Forest: Educational theories and practices in favor of environmental preservation



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ABSTRACT

This article discusses a study proposal, elaborated through concrete activities, in direct contact with nature, about a contribution to the resolution of specific situations of environmental damage, in the

spaces close to the educational center, to reinforce the role of practice in the formation of values and in the development of proactive attitudes concerning the problems in question. whose vision lies in the urgency to act collectively in sensitizing and raising awareness about the importance of preserving the environment (fauna and flora), which has a remarkable relevance in the Brazilian Atlantic Forest.

The didactics presented integrates the study of the characteristics, conditions, and benefits of an ecological reserve in the Atlantic Forest of the Southeast region, where the purposes are analyzed according to the level of education of the participants, emphasizing the formation of children and adolescents. At this stage of life, the primacy for the construction of environmental values corresponds to several pedagogical concepts, because as learning at this early stage is produced in greater depth, children learn more through motivations and obtain better practical skills.

Keywords: Environmental Education, Awareness and Preservation.

1 INTRODUCTION

In view of the various challenges concerning the relationship between man and nature in contemporary society and the impacts on the environment caused by the action or omission of human beings, Environmental Education has become a necessity associated with the very existence of the human species. Thus, it is important to emphasize that the principles of EE are related to the knowledge of problems, the awareness of possible solutions and motivation to carry out transformations that generate real responses at a local and global level.

In Brazil, Law 9.795, of April 27, 1999, which provides for Environmental Education and institutes the National Policy for Environmental Education, by dealing with the responsibilities of public policies in relation to the engagement of society in the conservation, recovery and improvement of the environment; it makes educational institutions responsible for promoting Environmental Education in an integrated manner with the educational programs developed by the school itself.



Today's society lives a constant reflection on the attitudes of human beings in relation to the preservation of the environment, due to the transformations that modernity offers to society, thus provoking a socio-environmental emergency that brings out the need to rethink human activities, to provoke a significant change, aimed at the preservation of the environment. and that in the future, man knows how to live, coexist and enjoy natural resources in a conscious way.

Seeing the importance of having an education more focused on the environment and with the growing environmental imbalance that we are witnessing, renowned researchers in Ecology and Environment emerge who in their work come to clarify and justify what has been happening over the centuries, with serious research and studies centered on real facts about the environmental changes in their abiotic and non-abiotic environment and how man has been interfering with it.

The main objective here is not to establish predetermined fixed rules and norms about the environment and how to keep it preserved and intact, but to know how to enjoy it in the best possible way within an ecologically coherent balance.

In the PCNs, there is already talk about Environmental Education and that it should be put into practice in schools, not only as a concept and content, but also with attitudes.

With this project, the school in question seeks to raise this awareness, initially, in the students who make up Elementary School I and who are in the fourth and fifth year of this respective modality of education. Such students will be the propagators of this action, with the purpose of provoking a change in self-destructive social behaviors and in the incessant process of degradation of nature and people's quality of life. In addition, the participants aim to show that the responsibility towards the environment will have a greater or lesser social impact, defining the standard of development, commitments, control and limitation of the damage caused to the environment.

Finally, that man can enjoy, in an attentive way, all that nature provides, without harming it or preventing it from its potential for self-regeneration and collaborating for its full development, under the understanding that we are the environment in action.

2 ENVIRONMENTAL PRESERVATION: FAUNA AND FLORA OF THE ATLANTIC FOREST

The Atlantic Forest, a national heritage provided for in article 225 of the Federal Constitution, is one of the richest biomes in Brazil in terms of biodiversity, similar to the Amazon Rainforest, with lush vegetation, accentuated hygrophytism and ecological diversities that allow spontaneous regenerations. The abundant fauna has a large number of endemic species (which cannot be found anywhere else on the planet).

Law No. 11,428/2006, known as the Atlantic Forest Law, regulated by Decree No. 6,660/2008, which provides for the implementation of the Municipal Plan for the Conservation and Recovery of



the Atlantic Forest (PMCRMA), is the main instrument for the management and conservation of this Biome. It is relevant to clarify that although such regulations provide for restrictions pertinent to the conservation and preservation of fauna and flora, unfortunately today such riches are harmed by human actions, among them: deforestation, illegal trade and pastures, resulting in the extinction of several species, thus revealing the great problem of this wonderful set of ecosystems. According to statistical data released by the IBGE News Agency (2020); in 2014, in Brazil there were 3,299 species of animals and plants threatened with extinction, with the Atlantic Forest having the most threatened species; 1,989 or 25% of the biome's species in the study.

In the sense of the relationship between man and the environment (biotic and abiotic) they are part of an integrated whole and interacting together in this huge organized and self-sustaining ecosystem.

Thus, Reigota (2001) exemplifies his work on three social categories of the Environment.

With the Modern Era, and its technological, scientific, social and cultural developments, comes the advances of the population with its disorganized and highly devastating growth for the biotic and non-biotic environment, as misery, diseases, unemployment, families living in precarious houses and/or even on the streets appear, underemployment appears, socially and culturally it can be said that there is in this century a sub-race of human beings that does not have the slightest condition to understand, understand and solve the basics of the problems that surround them.

This social culture dates back to the Industrial Revolution and the disorganized growth of urban centers and the emptying of rural areas.

To better understand these "new problems" there is a science that comes to solve these problems, which is **Ecology**, which means house or home, is derived from the Greek *oikos* (Ricklefs, 2003). However, it was the German zoologist Ernest Haeckel (1834 -1919) who gave this word a new meaning, for him ecology is the body of knowledge concerning the economy of nature, and the investigation of the total relations of animals with their inorganic environment, including their friendly relations with those animals and plants with which they are directly or indirectly present to them. thus, ecology is the study of all the complex interrelationships determined by Darwin with the conditions of struggle for existence (Ricklefs, 2003).

Ecology is a new science with several definitions, from the science that studies living beings and their natural world, as well as the science that studies these organisms in terms of their distribution and abundance in the environment in which they are found (Boff, 1999).

According to Boff (1999), ecology takes place in four ways:

I - Environmental Ecology, is concerned with the Environment so that it does not suffer excessive disfigurements, thinking about having a better quality of life, here nature sees



itself outside the human being, seeks new technologies less polluting and ecologically less aggravating for nature.

II- Social Ecology, which inserts the human being within nature (wants the whole environment and just something fragmented), is concerned not only with having a more beautiful and modern urban environment, but also prioritizes solutions to current social problems, since citizens are and are part of a portion of nature.

III – Mental Ecology, or deep ecology, says that the causes of the Earth's deficit are not only to be found in the type of society, but in the type of mentality, whose roots go back to times before our modern history, these minds consider themselves masters of nature and that it should serve them, they live in an anthropocentric idea.

IV – Integral Ecology, is a new vision of the Earth, they see the Earth from outside the Earth, in this case the human being is the Earth itself while thinking, feeling, loving, crying, trying to accustom the human being to the global and holistic vision, capitalizing on the organic totality, being one and diverse in its totality, but uniting them in a globalized whole.

In the last decades Ecology, as a science, has been presenting a growing development in the media, intensifying its ideas, proposals and how to improve "our Environment" and with it our Planetary Ecosystem in a sustainable way for all who integrate this planet Earth (biotic and non-abiotic environments, together with man), but it has been happening with this flag raised under the name Ecology and its Sustainability, erroneous interpretations arise that distort the true meaning and essence of the word Ecology and its real importance.

Thus, environmentalists emerge who see in the "wave" of Modern Ecology with their discourses about the protection and well-being of nature, but they are people who distort the real concept of Ecology and its importance in the balance of the Ecosystem, which has been suffering a gradual and constant imbalance for decades of expansion and evolution of society. cultural, social and technological.

Ecology studies the structure and dynamics of biotic organisms with the abiotic environment, in a relationship of equilibrium, having support to respond and understand the imbalance that exists today with so much force and destruction (Boff, 1999).

Although ecologists have been discussing the effects of man's actions on the environment for more than half a century, the theme is recent in classrooms. Socio-environmental education is the most used term in recent times to define the concept for discussing the responsibilities of individuals and the consequences of their actions. (Rodrigues and Malzoni, 2010, p.15).

This project is focused on the critical current, since it has a greater approximation to Environmental Education, moving not only a group, but an entire community that, with significant attitudes, will involve other communities and so on. This paradigm is based on scientific and solid



arguments, with sustainable, ethically correct and responsible use of nature's resources, to ensure the satisfaction of current and future growing human needs (FUNIBER, 2022).

3 TRAINING OBJECTIVE

The main objective is to sensitize students to the development of skills in the promotion of values, attitudes and capacities for a transformative action of social life, in the natural environment and other spaces of society, situating the circumstances in the context of the student to carry out the experience in the praxis, in the world of causes and effects, of the scientific explanation related to previous knowledge about the origins and consequences, and environmental problems, making adaptations according to the particular specificities of each student, since every human being has several capacities that are expressed in a different way. Although they have common traits, they have distinct personalities and identities, such as culture, ethnicity, beliefs, and sexuality.

The CGEA (General Coordination of Environmental Education) provides the training of environmental educator teachers, dedicating efforts both to initial training in the degrees of the former magisterium, as well as in continuing education; the promotion of Environmental Education projects in schools, stimulating and developing teachers, students, management, employees, and the community, having an environmental educational praxis, proposing a permanent ecological education with all citizens who are members of the community (Trajber, 2007).

For this, it is necessary to have teachers who assume the commitment to the environment, its importance both in prevention and in the protection of humanity, having a self-critical look at their own values, running the risk of transmitting to the new generations - in the same way that we transmit social prejudices - erroneous concepts about the environment and the attitudes to be taken (FUNIBER, 2022, p. 112).

4 METHODOLOGY AND PROPOSED ACTIVITIES

Based on the theme addressed and the proposed objectives with the intention of carrying out an active and significant action, with the students being protagonists and the teacher the mediator of this process, in the development of this work a structured teaching methodology was used through an interdisciplinary project, based on a sequence of activities, distributed in the following stages:

1st Moment: In the introduction of the theme to the class, the teachers organized, at the school, an Awareness Lecture on the importance of preserving and conserving the Environment existing in the "Atlantic Forest"; as it is a comprehensive theme, the faculty had the support of professionals in the area who work in an NGO, which works with specific issues (Environment), environmental agents from the region itself that compose data, images and videos, among other resources, which will be



used to present the theme to the students, promoting their commotion and awareness, regarding the preservation of the environment in which they live.

2nd Moment: After the above activity is carried out in the school environment, the group goes to a field practice in which the students carried out a trail in an Ecological Park of the Municipality, near the school center, accompanied by the teachers and supervised by an environmental monitor who is available to give all the information to the possible questions related to the fauna and flora of the region. such as: What animals are part of this region? Which animals went extinct? Is there an illegal trade in animals? And what are these animals? What type of vegetation predominates in the region? Is it primary/secondary vegetation or virgin forest? The most significant and relevant data about the route traveled were recorded through photos and/or notes in notebooks.

3rd Moment: After the trail, already in the classroom, the students were guided by the teacher to produce a drawing, representing the rereading of the tour carried out. Each drawing consists of presenting a phrase that demonstrates the importance of having an "Environment" preserved.

4th Moment: Divided into groups, the students carried out a more specific research on the fauna and flora of the region in which they live. In addition, to make better use of the work, printed and digital materials (books, magazines, videos, documentaries, websites with reliable information) were used. Each group was responsible for a certain environmental area (fauna/flora), and made thematic environments for the exhibition of their work, through models, panels, reuse of scrap metal, mini lectures on garbage and the importance of the 4 Rs (recycle, reuse, reduce and rethink) and construction of a mini sensory trail with scraps and aromatic plants.

5th Moment: The presentations of the results of the activities developed by the students took place in the sports court and in the schoolyard, with an exhibition of materials made, focused on the theme: "**Environmental Exhibition of the Atlantic Forest of Brazil**". The exhibition was open to the public of the region and to the other students of the school, so that everyone could be sensitized and understand the relevance of the Atlantic Forest, its positive points for the population, for the city's surroundings, for commerce (such as tourism), job creation, and also the preservation of river springs.

The closing of the Environmental Exhibition took place through a theatrical presentation, with the participation of teachers and some students, who portrayed the knowledge acquired by the class throughout the activities, demonstrating how important it is to preserve the environment in which one lives, so that there can be harmony between man and nature. Such action is an invitation for the community in general to think more about the issue of preservation and also to become a partner of the school, assisting in the activities to be promoted, related to environmental issues

6th Moment: The approaches of this project were evaluated in two stages:

1st stage - In the exhibition "Environmental Exhibition of the Atlantic Forest of Brazil", where each visitor can register their comment, on a mural located at the exit of the site, referring to the work



carried out by the students with the supervision of the teachers and the school team, positive or negative comments, giving the opportunity for each visitor to evaluate the school events of their community, as well as possible suggestions for other future activities on the Environment;

2nd stage - The registration and dissemination of the results occurred through the production of an Informative Ebook (with online or printed access), which was built by the students and teachers who effectively participated in the project, also containing the opinions of the community who attended the exhibition "Atlantic Forest Environmental Exhibition".

5 FINAL THOUGHTS

When dealing with the purposes of social actions in different socio-environmental contexts and formal educational spaces, the urgency of an active citizenship is seen, guided by collective and organized actions, which seek to understand and act in decision-making related to environmental problems, quality of life and sustainability.

Educational practices such as this promote a dialogue of knowledge that allows the formation of a critical, creative thinking and attunement to the need to propose answers for the future, capable of analyzing the complex relationships between natural and social processes and of acting in the environment respecting sociocultural diversities, educating students for knowledge, so that they can politically insert themselves in the world. consciously, responsibly and in solidarity, since it was possible to achieve a positive result with the development of this proposal.

Education that works with interaction, with the protagonism of students, articulated with a social change, transforms pedagogy into a political practice, as suggested by Giroux (2003), with cooperation between educators and other cultural subjects engaged in social and environmental struggles, creating critical spaces for learning inside and outside the school, seeking union with organized social movements. Thus, changes will not occur from the top down, but with the direct participation of students, teachers, the community, and the environment, in a complex dynamic (Tristão, 2007, cited by Jacobi, 2009, p. 09).



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Pepper pseudocaryophyllus (Myrtaceae): An integrative review



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ABSTRACT

The *Pimenta pseudocaryophyllus* (Gomes) Landrum among the species of the genus Myrtaceae is the only Brazilian representative. Craveiro-domato, as it is popularly known in the Southern Region of Brazil, is an aromatic tree species, with popular use for medicines, condiments, and flavoring of beverages due to its flavor very similar to cloves. The Brazilian species has been widely used in traditional medicine for the treatment of various ailments, in addition to being linked to other traditional practices, such as the use of wood for internal carpentry works. However, there are not enough studies on its potential through biological activity. This study highlighted the botanical characteristics as well as the importance of expanding research regarding the chemical and biological activities of the species *Pimenta pseudocaryophyllus* (Gomes) Landrum.

Keywords: *Pepper pseudocaryophyllus*, Medicines.

1 INTRODUCTION

The Myrtaceae family is composed of vegetation considered pantropical, that is, they belong to the tropical regions of some continents. With about 140 genera, approximately 3000 species and two subfamilies classified as Myrtoideae and Leptospermoideae, its location stands out in the Americas and Australia. In Brazil, its varied number of species occupies a large part of the Atlantic Forest and, some rarely, the Cerrado (Silva; Mazine, 2016). Plants in this group are classified as angiosperms, considered the second largest Brazilian biome (Ribeiro et al., 2023).



Among the 140 genera of this family, the genus *Eucalyptus* stands out, which is considered the most famous group, its use in modern medicine is vast for having the ability to treat diseases related to the respiratory system, since it has expectorant, anti-inflammatory, and antiseptic properties (Hua et al., 2022).

The representatives of the Myrtaceae family have greater notoriety for the fact that they produce abundantly the essential oils that are produced due to the existence of secondary metabolites in their composition (Silveira, 2023). This group of plants has a great variability of secondary compounds, but its main composition is made up of tannins, flavonoids, and terpenes.

One of the groupings also relevant belongs to the genus *Myrcia*, in which among the main species are: *Myrcia uniflora* and *Myrcia palustris*. The first is popularly known as stone-ume-caá or vegetable insulin, and helps in the treatment of patients suffering from diabetes through the infusion of its dried leaves. The second, on the other hand, has the vernacular of pitangueira-do-mato, culturally it is used to treat dyspepsia and other gastric problems. In addition, its essential oil has antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis* (Santos, 2019).

Pepper species play multiple roles in everyday life, whether in the medicinal, gastronomic or pharmacological field. In addition, they are present in aromas, flavors or even in colors that are sometimes vivid and, therefore, have been part of societies for millennia (D'Angelis, Negrelle, 2021).

Most species belonging to the genus *Pimenta* have arboreal characteristics, reaching heights of up to 20 meters. Some variations within this genus can manifest in the form of shrubs. The geographic distribution of these species is mainly concentrated in the Caribbean and Central America, with the exception of the species *Pimenta pseudocaryophyllus*, which is native to the southeastern region of Brazil (Paula et al., 2010).

1.1 GENUS PEPPER

The genus *Pimenta* has the following general characteristics: Indumentum and hair: The indumentum can vary between species, and density is useful to distinguish them. The hairs can be unicellular, simple or symmetrically dibrachial, with whitish, yellowish or reddish-brown hues. Leaves: The leaves are generally persistent, usually leathery, although in some situations they may be submembranous. The lateral ribs, in most cases, are prominent and often form a right angle with the midrib (Paula et al., 2008)

Peppers are notable for their ability to cause sensations of heat and stinging in the mouth when consumed. These sensations are predominantly attributed to two groups of chemical compounds: capsaicinoids and volatile compounds (Pinto; Dick; Maidens, 2013).

Capsaicinoids are mainly responsible for the heat of chili peppers. Capsaicin is the most notable capsaicinoid and is responsible for much of the burning sensation we feel when consuming chili



peppers. However, other capsaicinoids, such as dihydrocapsaicin and nordihydrocapsaicin, also obeyed this sensation. The concentration of capsaicinoids varies from one variety of pepper to another, and even between fruits on the same plant. This variability is one of the reasons why some peppers are much spicier than others (Rivera, 2018).

In addition to capsaicinoids, chili peppers also contain a variety of volatile compounds that have created for their distinctive flavor. These compounds include terpenes, aldehydes, ketones, and alcohols, which impart aromatic, floral, and citrus notes to pepper varieties. Volatile compounds are responsible for the aromas we associate with peppers, from the fruity and sweet aroma of habanero peppers to the earthier aroma of jalapeño peppers. The presence of these compounds contributes significantly to the sensory richness of peppers, making them an essential ingredient in many cuisines around the world (Cruz and Carneiro, 2012).

Peppers, with their wide variety of colors, shapes, and levels of spiciness, are more than just spices to flavor foods. They also have a fascinating biological activity that has aroused the interest of the scientific community. The secret behind the "hotness" of chili peppers lies in a substance called capsaicin. This molecule, found mainly in the spicier varieties, plays a key role in various biological responses. Studies show that capsaicin may have influential effects on human health (Brito; Silva; Fluminhan, 2019).

1.2 CHARACTERISTICS OF THE SPECIES

Pimenta pseudocaryophyllus (Gomes) Landrum is the only Brazilian representative species of this genus of *Myrtaceae*. Its use is popularly used in the treatment of ailments, condiment and flavoring of beverages due to its flavor very similar to cloves. To a lesser extent, it is used in carpentry and urban afforestation. This biological heritage is a source of genetic, symbolic, and economic material resources for these peoples, who, in turn, possess rich knowledge of the natural world (D'angelis, A. S. R.; Negrelle R. R. B., 2014).

The Brazilian species is popularly known as Cataia ("leaf that burns" in Tupi-Guarani) on the coast of Paraná and in the Ribeira Valley (SP); tea-de-bugre, carnation-of-the-wood, laurel, carnation laurel, pau-cravo - Paraná and São Paulo; pink peel, lemon from the state of Goiás; tea-of-the-earth, cloves-of-the-earth, laurel-of-the-earth, false laurel, false-carnation - São Paulo; Craveiro-Paraná. (D'angelis, A. S. R.; Negrelle R. R. B., 2014).

The carnation, *Pimenta pseudocaryophyllus*, is an aromatic tree species of 4-10m in height, endowed with a very characteristic rounded crown. Trunk, usually erect 10-30cm in diameter, with fissured bark. Axillary inflorescences in simple dicases or composed with two to three very fragrant white flowers. Fruit, subglobose berry, dark when ripe and containing one to two seeds. It blooms from



October to January and bears fruit from May to September. It produces a moderate amount of viable seeds annually (Brandão *et al.*, 2006)tag.

There is also the *Pimenta pseudocaryophyllus* var. *pseudocaryophyllus*, which is a small tree typical of forests and mountainous areas of southeastern Brazil; *Pimenta pseudocaryophyllus* var. *Fulvescens* (A. P. de Canolle) Landrum is a small tree or shrub that is located in dry regions in south-central Brazil, with some specimens in Bolivia; and *Pimenta pseudocaryophyllus* var. *Hoehnei* (Burret) Landrum is a small tree confined to the coastal forest region of southeastern Brazil, from Santa Catarina to São Paulo. (Paula, J.A.M *et al.*, 2010).

The three varieties differ especially by the size of the leaves and petiole, with the largest measurements found in *Pimenta pseudocaryophyllus* var. *fulvescens*, and the smallest in *Pimenta pseudocaryophyllus* var. *hoehnei*. They are also differentiated by the number of flowers in the inflorescences, and in the *varieties pseudocaryophyllus* and *fulvescens* the inflorescences are in a dicasium or panicle of usually seven to fifteen flowers, while in the *Hoehnei* variety the inflorescences are in a digase with a maximum of three flowers (Landrum, 1986., Paula, J.A.M *et al.*, 2010).

This family has a diversity of species with medicinal applications already studied. This statement is illustrated by the work of Gottlieb *et al.* (1970), Suárez *et al.* (2000), Fernández *et al.* (2001), García *et al.* (2004), Silveira *et al.* (2005), Apel *et al.* (2006) and Biavatti *et al.* (2007), (Brandão *et al.*, 2006).

The species *Pimenta pseudocaryophyllus* (Gomes), L. R. Landrum has been widely used in traditional medicine for the treatment of various diseases, in addition to being linked to other traditional practices, such as the use of its fruits to season food and wood for internal carpentry works (Morgante *et al.*, 2010). Its leaves are widely used by the population of the interior of the state of Minas Gerais, Brazil, in the preparation of anti-flu teas (Paula *et al.*, 2008) and, in the municipality of Campos do Jordão, São Paulo, Brazil, in the preparation of soothing teas that regulate digestion and menstruation (Nakaoka-Sakita *et al.*, 1994).

Another important use of the species, which began in the 80s, was the flavoring of brandy with leaves of the plant, now commercialized along the coast of the states of Paraná and São Paulo. In general, the use of the species is based on extractive action in forest remnants where it occurs spontaneously, a fact that can compromise the population dynamics and the maintenance of the natural stock of the species, in addition to the quality of the product, since the chemical composition is influenced by climatic and edaphic variations (Morgante *et al.*, 2010).

The berries of the species, after being dried, are used as a substitute for cloves in jams and jellies, according to a custom dating back to the mid-1800s (Lullez, 1991), due to the similarity of smell and flavor of these two species (Farias, 2009). Since the nineteenth century there have been correlations between these species, and the specific name of the plant itself refers to the similarity



between them, pseudocaryophyllus means something like "false cloves", given that the scientific name of cloves is *Caryophyllus aromaticus* (Farias *et al.*, 2009; Neves *et al.*, 2009).

Carnation wood is heavy (density 1.00 g/cm³), hard, with a fine to medium texture, used by the small dimensions available only locally for internal carpentry works, as well as for firewood and charcoal. The tree has ornamental qualities that recommend it for urban afforestation, especially for narrow streets and under electrical networks. It is also recommended for energy and preservationist reforestation (Lorenzi, 1998).

2 FINAL THOUGHTS

There is little study about the species analyzed in this study. An attempt was made to elucidate the taxonomic classification and importance of *Pimenta pseudocaryophyllus* (Gomes) Landrum, not only in the botanical context, but also in relation to potential applications in several areas, including medicine, gastronomy and biodiversity conservation.



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Similarities between native and restored forest remnants in hydroelectric reservoir surroundings in Paraná



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ABSTRACT

Introduction: Due to the demand for the implementation of hydroelectric projects to enrich the Brazilian energy matrix, there is an inevitable need to restore the new Permanent Preservation Areas (APPs) resulting from the formation of the reservoirs of these projects. **Objective:** The main objective of this study is to evaluate and compare the characteristics of the remaining vegetation formations (middle stage secondary forest) and restored ones (reforested areas) that extend along the margins of the reservoir of the Salto Santiago Hydroelectric Power Plant, located in the municipality of Bonito do Iguaçu River - PR. The purpose of the analysis is to identify variations in the diversity and structure of the studied communities, in addition to defining the stages of succession of the evaluated communities. **Methodology:** To meet the objective of the study, the analyzes were performed using temporary plots. Three 20x100m plots were installed in the restored areas and 5 plots of 20x100m in the remaining forest. In these plots, all individuals with DBH

(diameter at breast height) greater than or equal to 10 cm were measured and identified. Still, smaller plots were allocated to evaluate the regenerating individuals, in which the individuals were identified and counted. Initially, the phytosociological parameters of both communities were estimated and, later, for the analyzes of similarity and indicator species, the plots were divided into subplots of 10x10m, totaling 160 plots. **Main Results:** In total, 52 species belonging to 25 botanical families were recorded in the remaining areas, compared to 37 species distributed in 17 families in the restored areas. The estimated basal area for the remaining areas was 26.7 m². ha⁻¹, while in the restored areas it was 6.8 m². ha⁻¹. The density of individuals was higher in the remaining areas (904 individuals/ha) in relation to the restored areas (310 individuals/ha). The dominant species were identified as *Cupania vernalis* in the remaining areas and *Mimosa bimocrunata* in the restored areas, with the latter being native to the region and frequently used during the restoration process. **Conclusion:** The results of this study highlighted significant differences between the remaining and restored areas in relation to species composition, density and basal area. The low similarity between communities, as demonstrated by the Jaccard index, does not exclude an overlapping trend as observed in the NMDS analyses. The dominant presence of indicator species suggests promising progress in restoration, while the remaining areas remain in a post-disturbance stage of succession. The research contributes to the understanding of the dynamics of regeneration of plant formations in areas of hydroelectric reservoirs, emphasizing the viability of restoration with species native to the region.

Keywords: Native, Forest, Hydroelectric.

1 INTRODUCTION

The process of forest recovery is quite dynamic, resulting from a series of biotic and abiotic factors of the environment, in which the complementary requirements of each species must be



observed. The regeneration of mixed forests involves the concept of secondary succession, understood as the orderly replacement of species over time, in a given location, until the formation of a generally stable plant community (MARTINS, 2005).

Investigating the patterns and dynamics of heterogeneous reforestation with native species is important in streamlining restoration processes (natural regeneration), aiming to reduce efforts related to the recovery process of degraded areas, especially those related to flora and fauna interactions.

According to Sousa Júnior (2005), in the case of artificial reservoirs, the new bank is considered a permanent preservation area and must be revegetated with native species. It is noteworthy that even species native to the site may present different behaviors depending on the seed matrices, in addition to the fact that the vegetation around the reservoir ends up being subjected to greater amounts of humidity, due to the proximity of the water resource, therefore, research with species and matrices to be implemented in these places is necessary to expand the possibilities of success in the restoration process.

Depending on the difference in level reached by the reservoir, the new banks are located in areas of slopes with soil types and original vegetation cover composed of another type of vegetation that does not forest riparians (SOUSA JÚNIOR, 2005). In these new banks created by the reservoir, the environment is sometimes not favorable to the growth of tree species, due to characteristics such as small depth and the presence of impediment layers, combined with water deficit and low fertility, according to Davide and Botelho (1999). In the riparian condition, the physical factors of the soil, determined directly by the hydrological behavior of the site, are the main determinants of the distribution and composition of species, in contrast to the chemical factors of the sediment, determined indirectly by the dynamics of the river (JOHNSON *et al.*, 1985). In addition, the variability of soils that occur in forest areas is very high, and sometimes the area has undergone degradation processes and may have lost its previous properties or will be highly altered, not supporting the implantation of the same previously existing species (MARTINS, 2005).

Among the factors that influence tree growth are the chemical and physical characteristics of the soil, the water regime, soil moisture, topography and competition with weeds. In addition to these factors, the vegetation present in the area is a good indicator of the conditions of the site (BOTELHO *et al.*, 1995). According to Faria (1996), the success of projects for the recovery of these areas through mixed reforestation depends, among other things, on the correct choice of species.

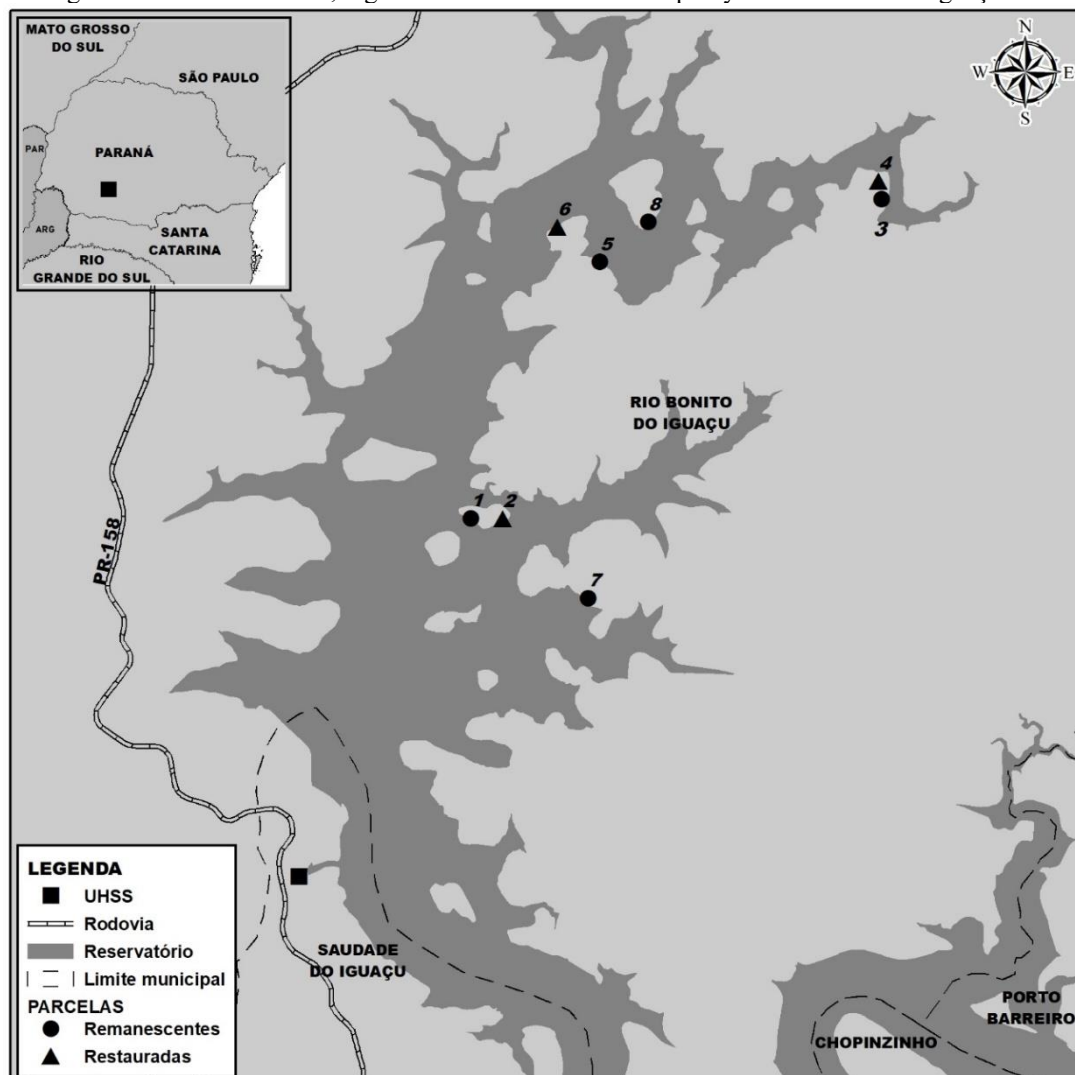
Thus, the objective of the present study was to compare the remaining and restored vegetation formations of the banks of the reservoir of the Salto Santiago Hydroelectric Power Plant, in the portion that surrounds the municipality of Rio Bonito do Iguaçu-PR., to evaluate the restoration process carried out in relation to diversity and similarity with the remaining vegetation.

2 METHODOLOGY

The study area is located on the banks and islands of the reservoir of the Salto Santiago Hydroelectric Power Plant, which is located in the middle course of the Iguaçu River, in the State of Paraná. It is located 340 km west of Curitiba, between the municipalities of Rio Bonito do Iguaçu and Saudade do Iguaçu, with geographic coordinates of 25°36' south latitude and 52°37' west longitude. It is located downstream of the Segredo Hydroelectric Power Plant and upstream of the Salto Osório Hydroelectric Power Plant. The reservoir has a perimeter of approximately 770 km and bathes the territory of 8 municipalities.

The plots were randomly distributed (Figure 01), with the objective of covering the diversity in terms of physiognomic and structural characteristics in each forest fragment. This was carried out in the section of the reservoir that comprises exclusively the municipality of Rio Bonito do Iguaçu. In this area, there were the most advanced restored areas in terms of development, with plantations of 9 to 10 years old. In addition, for comparison purposes, there were also well-preserved remaining areas with secondary forests in the middle stage of regeneration (Brasil, 1994).

Figure 01: UHSS reservoir, region that involves the municipality of Rio Bonito do Iguaçu-PR.





The sampling and data collection for the phytosociological floristic studies took place in 2016, which was divided into three levels of approach. The vegetation analyses were obtained using rectangular plots with an area of 2000 m² (20 m x 100 m), with the longest length being parallel to the reservoir margin. At this level of approach, called **Stock 1**, all tree individuals with diameters at 1.30 m from the ground (DBH) greater than or equal to 10 cm were identified and measured for the estimation of phytosociological parameters. Also, in a second level of approach, subplots of 100 m² (10 m x 10 m) were installed to evaluate the stock of individuals with DBH < 10 cm and with a total height equal to or greater than 3 m, it was called **Stock 2**. Finally, at the third level of approach, subplots of 10 m² (10 m x 1 m) were installed to evaluate the stock of individuals with total height less than 3 m and greater than 0.3 m and was called **Stock 3**. In the case of stock 2 and 3, the density of regenerating individuals was evaluated. These procedures were indicated by Figueiredo Filho *et al.* (2013).

In all, three plots were sampled in the restored community, totaling 6,000 m² and five plots in the remaining area, totaling 10,000 m². In relation to stock 2, 500 m² (five subplots) were sampled in the remaining area and 300 m² in the restored area (three subplots). In stock 3, 50 m² (five subplots) were sampled in the remaining area and 30 m² (three subplots) in the restored area.

To evaluate the horizontal structure of the forest, the traditional phytosociological parameters (Absolute and Relative Density, Absolute and Relative Dominance, Absolute and Relative Frequency and Importance Value Index) of the tree community (Stock 1) were estimated. The similarity between the two ecosystems was evaluated by subdividing the plots into subplots of 10x10m and the Jaccard Similarity Index was used, which, according to Durigan (2012) is one of the most used indices in surveys that aim to evaluate floristic seedings between communities and indicates the proportion of species shared between the samples in relation to the total number of species.

Also, to better evaluate the dissimilarity (or similarity) of species composition, multivariate analysis of nMDS (Non-Metric Multidimensional Scaling) was used. This method uses a distance coefficient to construct the similarity matrix, which, in this case, the Bray-Curtis distance was used because it presents the abundance of species per plots of each phytophysiognomy. nMDS is an iterative technique that aims to minimize STRESS (Standard Residuals Sum of Square), a measure of how much the positions of objects in a three-dimensional configuration deviate from the original distances or similarities after scaling. After the randomization of scores and axes and the calculation of the distance matrix between the sampling units, there is a correlation with the distance matrix constructed from the original data (PROVETE *et al.*, 2011).

Finally, in order to find species that characterize the evaluated communities, the *Indicator Value Method (IndVal)*, developed by Dufrene & Legendre (1997), was used, which combines measures of the degree of specificity of a species in an ecological state (e.g., a type of habitat), and the fidelity (or



frequency of occurrence) of this species within that state. This method provides an indicator value for each species, in the form of a percentage. According to McGeoch and Chown (1998), this method is robust to differences in methodology and sample size, in addition to deriving indicators from any site classification. It is noteworthy that, for the multivariate analyses and the Indicator Value, the plots were divided into subplots due to the extensive size of each plot, and the number of individuals was used for processing.

3 RESULTS

Considering all stocks, 52 species belonging to 25 botanical families were observed in the remaining community, with only one exotic species found (*Hovenia dulcis*). In relation to Stock 1, the remaining area presented 46 species, belonging to 41 genera, distributed in 27 botanical families.

On the other hand, 37 species belonging to 17 families were recorded in the restored areas. It is noteworthy that in these areas, 3 exotic species (*Eucalyptus* sp., *Hovenia dulcis* and *Psidium guajava*) were recorded. In relation to Stock 1 of the restored area, it presents an arboreal stratum with 18 species, belonging to 16 genera, distributed in 12 botanical families.

Table 01 shows the species richness found in the 3 strata for the two communities evaluated and the collection records are stored in the HUCO collection between numbers 7,321 and 7,368.

Table 01. Species richness for the two communities evaluated

Family	Species	Phyto	Rem.	Res.
Anacardiaceae	<i>Schinus terebinthifolia</i> Raddi	FES/FOM	X	X
Annonaceae	<i>Annona sylvatica</i> A.St.-Hil.	FES	X	
Araucariaceae	<i>Araucaria angustifolia</i> (Bertol.) Kuntze	FES/FOM		X
Arecaceae	<i>Syagrus romanzoffiana</i> (Cham.) Glassman	FES/FOM	X	X
Asparagaceae	<i>Cordyline spectabilis</i> Kunth & Bouché	FES/FOM	X	
Boraginaceae	<i>Cordia americana</i> (L.) Gottschling & J.S.Mill.	FES	X	
Cannabaceae	<i>Celtis iguanaea</i> (Jacq.) Sarg.	FES/FOM	X	
Cannabaceae	<i>Trema micrantha</i> (L.) Blume	FES/FOM		X
Cardiopteridaceae	<i>Citronella paniculata</i> (Mart.) R.A.Howard	FES/FOM	X	
Celastraceae	<i>Monteverdia ilicifolia</i> (Mart. ex Reissek) Viral	FES/FOM	X	
Euphorbiaceae	<i>Sapium glandulosum</i> (L.) Morong	FES/FOM	X	
Euphorbiaceae	<i>Gymnanthes klotzschiana</i> Müll.Arg.	FES/FOM	X	X
Euphorbiaceae	<i>Sebastiania brasiliensis</i> Spreng.	FES/FOM	X	
Fabaceae	<i>Albizia Edwallii</i> (Hoehne) Barneby & J.W.Grimes	FES/FOM	X	
Fabaceae	<i>Anadenanthera colubrina</i> (Old.) Brenan	FES	X	X
Fabaceae	<i>Ateleia glazioviana</i> Members.	FES/FOM	X	
Fabaceae	<i>Bauhinia forficata</i> Link	FES/FOM	X	
Fabaceae	<i>Erythrina crista-galli</i> L.	form	X	
Fabaceae	<i>Erythrina falcata</i> Benth.	FES/FOM	X	
Fabaceae	<i>Inga virescens</i> Benth.	form	X	
Fabaceae	<i>Muelleria campestris</i> (Mart. ex Benth.) M.J. Silva & A.M.G. Azevedo	FES/FOM	X	X



Family	Species	Phyto	Rem.	Res.
Fabaceae	<i>Machaerium stipitatum</i> Vogel	FES/FOM	X	
Fabaceae	<i>Mimosa bimucronata</i> (DC.) Kuntze	FES/FOM	X	X
Fabaceae	<i>Mimosa regnellii</i> Benth.	form		X
Fabaceae	<i>Mimosa scavenges</i> Benth.	form		X
Fabaceae	<i>Myrocarpus frondosus</i> Allemão	FES	X	
Fabaceae	<i>Parapiptadenia rigida</i> (Benth.) Brenan	FES/FOM	X	
Fabaceae	<i>Peltophorum dubium</i> (Spreng.) Taub.	FES/FOM	X	X
Fabaceae	<i>Senegalia tenuifolia</i> (L.) Britton & Rose	FES/FOM	X	X
Lamiaceae	<i>Vitex megapotamica</i> (Explosive.) Moldenke	FES/FOM	X	X
Lauraceae	<i>Cryptocarya aschersoniana</i> Mez	FES/FOM	X	
Lauraceae	<i>Nectandra lanceolata</i> Nees	FES/FOM	X	X
Lauraceae	<i>Nectandra megapotamica</i> (Spreng.) Mez	FES/FOM	X	X
Lauraceae	<i>Ocotea puberula</i> (Rich.) Nees	FES/FOM		X
Malvaceae	<i>Luehea Spread</i> Mart.	FES/FOM	X	X
Meliaceae	<i>Cedrela fissilis</i> Vell.	FES/FOM		X
Meliaceae	<i>Trichilia catigua</i> A.Juss.	FES	X	
Meliaceae	<i>Trichilia elegans</i> A.Juss.	FES/FOM	X	
Myrtaceae	<i>Campomanesia guazumifolia</i> (Cambess.) O.Berg	FES/FOM	X	
Myrtaceae	<i>Xanthocarpa Campomanesia</i> (Mars.) O.Berg	FES/FOM	X	X
Myrtaceae	<i>Eucalyptus</i> sp.	-		X
Myrtaceae	<i>Eugenia pyriformis</i> Cambess.	FES/FOM		X
Myrtaceae	<i>Eugenia uniflora</i> L.	FES/FOM	X	X
Myrtaceae	<i>Psidium cattleyanum</i> Sabine	FES/FOM		X
Myrtaceae	<i>Psidium guajava</i> L.	-		X
Phytolaccaceae	<i>Phytolacca dioica</i> L.	FES/FOM	X	
Phytolaccaceae	<i>Seguieria langsdorffii</i> MOQ.	FES/FOM	X	
Rhamnaceae	<i>Hovenia dulcis</i> Thunb.	-	X	X
Rosaceae	<i>Prunus myrtifolia</i> (L.) Urb.	FES/FOM	X	
Rutaceae	<i>Balfourodendron riedelianum</i> (Engl.) Engl.	FES	X	X
Rutaceae	<i>Citrus aurantium</i> L.	-		X
Rutaceae	<i>Helietta apiculata</i> Benth.	FES	X	
Rutaceae	<i>Zanthoxylum rhoifolium</i> Lam.	FES/FOM	X	X
Salicaceae	<i>Casearia decandra</i> Jacq.	FES/FOM	X	X
Salicaceae	<i>Casearia sylvestris</i> Sw.	FES/FOM	X	X
Sapindaceae	<i>Allophylus edulis</i> (A.St.-Hil. et al.) Hieron. ex Niederl.	FES/FOM	X	X
Sapindaceae	<i>Cupania vernalis</i> Cambess.	FES/FOM	X	X
Sapindaceae	<i>Diatenopteryx sorbifolia</i> Radlk.	FES/FOM	X	X
Sapindaceae	<i>Matayba elaeagnoides</i> Radlk.	FES/FOM		X
Simaroubaceae	<i>Castela tweedii</i> Planch.	FES/FOM	X	
Solanaceae	<i>Cestrum intermedium</i> Sendtn.	FES/FOM	X	
Solanaceae	<i>Solanum granulosoleprosum</i> Dunal	FES/FOM	X	X
Solanaceae	<i>Solanum paniculatum</i> L.	FES/FOM		X
Urticaceae	<i>Cecropia pachystachya</i> Trécul	FES	X	X
Verbenaceae	Ruiz & Pav.) Juices.	FES	X	

Legend: Phyto = Naturally occurring phytophysiology; Rem. = Forest remnant; Res. = Restored area; FES = Seasonal Semideciduous Forest; FOM = Mixed Ombrophilous Forest.



In a survey carried out in the Iguazu National Park, by Souza (2015) in plots allocated in an ecotone area between Mixed Ombrophilous Forest and Seasonal Semideciduous Forest, 90 species, 57 genera and 34 families were observed (adopting the inclusion criterion of trees with DBH above 5 cm). It is noteworthy that the Iguazu National Park has been a conservation unit without interference for decades, hence the high species richness, different from the areas surrounding reservoirs where the influence of the adjacent community may occur. In this sense, Pezzatto (2004), working on the bank of the reservoir of the Salto Caxias Hydroelectric Power Plant, downstream of the UHSS, also on the Iguazu River, divided his research area into compartments, and in compartment A (the same inclusion criterion as stock 1 of this work) 51 species, 45 identified genera and 26 families were identified, using the inclusion limit of 10 cm DBH. This value is very close to the present study, as mentioned in the work by Isernhagen *et al.* (2001) who, in a floristic survey carried out in ecotone environments between Mixed Ombrophilous Forest and Seasonal Semideciduous Forest at the Santa Clara Hydroelectric Power Plant, Pinhão-PR, recorded 44 species from 26 families.

In the case of Recovered Permanent Preservation Areas in the vicinity of reservoirs, it is worth highlighting the work of Melo and Durigan (2007) in restoration reforestation (aged 7, 9 and 13 years) in the Middle Paranapanema Valley, where the original vegetation of the region is classified as Seasonal Semideciduous Forest, which found between 20 and 29 species of tree characteristics per plots of 500 m². In other words, the species richness of the present work is superior to that of the aforementioned author, contributing to the natural process of ecosystem restoration.

Analyzing the parametric estimates of the horizontal structure of the two ecosystems evaluated, a Basal Area of 26.7 and 6.8 m².ha⁻¹ were recorded for the area of forest remnant and restored, respectively. Regarding the density of individuals, 904 individuals per hectare were estimated for the forest remnant area and 310 individuals per hectare for the restoration area, as can be seen in Table 02.

Table 02. Estimation of the phytosociological parameters of the two communities evaluated with emphasis on the five main species.

Species	OF	DR	AGO	FR	DoA	Dor	ENERGY
	N.ha ⁻¹	%	%	%	m ² . ha ⁻¹	%	0 - 100%
Forest Remnant							
<i>Cupania vernalis</i>	326	36,06	70	11,25	8,77	32,85	26,70
<i>Muelleria campestris</i>	132	14,6	68	10,93	3,06	11,48	12,34
<i>Myrocarpus frondosus</i>	63	6,97	36	5,79	1,67	6,25	6,33
<i>Nectandra megapotamica</i>	32	3,54	36	5,79	1,65	6,19	5,17
<i>Luehea spread apart</i>	40	4,42	32	5,14	1,50	5,60	5,06
<i>Other species</i>	311	34,41	380	61,10	10,05	37,63	44,4
Total	904	100	622	100	26,7	100	100
Restored Area							
<i>Mimosa bimucronata</i>	128	41,40	60,00	21,18	2,55	37,49	33,36
<i>Mimosa scabrella</i>	45	14,52	53,33	18,82	1,49	21,81	18,38



Species	OF	DR	AGO	FR	DoA	Dor	ENERGY
	N.ha ⁻¹	%	%	%	m ² . ha ⁻¹	%	0 - 100%
Forest Remnant							
<i>Schinus terebinthifolia</i>	48	15,59	40,00	14,12	0,68	10,00	13,24
<i>Anadenanthera colubrina</i>	15	4,84	16,67	5,88	0,45	6,67	5,80
<i>Peltophorum dubium</i>	10	3,23	20	7,06	0,12	1,81	4,03
<i>Other species</i>	64	20,42	93,00	32,94	1,51	22,22	25,19
Total	310	100	283	100	6,8	100	100

Legend: DA = absolute density; DR = relative density; AF = absolute frequency; RR = relative frequency; DoA = absolute dominance; DoR = relative dominance; IVI = importance value index; N.ha⁻¹ = number of trees per hectare; m². ha⁻¹ = square meters per hectare

Regarding the main species found, it is noteworthy that the three main species of each community were also suggested as indicator species by the results of the methodology analyzed in the present study, since only these presented the IndVal higher than 50% in a significant way to 1% probability (pvalue < 0.01, as shown in Table 03).

Table 03. Significant indicator species of the evaluated communities

Community	Species	IndVal (%)	p-value
Remaining	<i>Cupania vernalis</i>	77,5	0,005**
	<i>Muelleria campestris</i>	73,5	0,005**
	<i>Myrocarpus frondosus</i>	50,0	0,005**
Restored	<i>Mimosa bimucronata</i>	76,0	0,005**
	<i>Mimosa scabrella</i>	66,3	0,005**
	<i>Schinus terebinthifolia</i>	57,8	0,005**

Legend: Indval = Indicator Value Method; p-value = probability value (significance)

According to Klein (1972), *Cupania vernalis* is common in secondary associations, such as capoeiras, capoeirões and semi-devastated forests. Also, according to Lorenzi (2002), this species is characteristic of high-altitude semideciduous forest and Atlantic rain forest, occurring in almost all forest formations in the South and Southeast regions of Brazil.

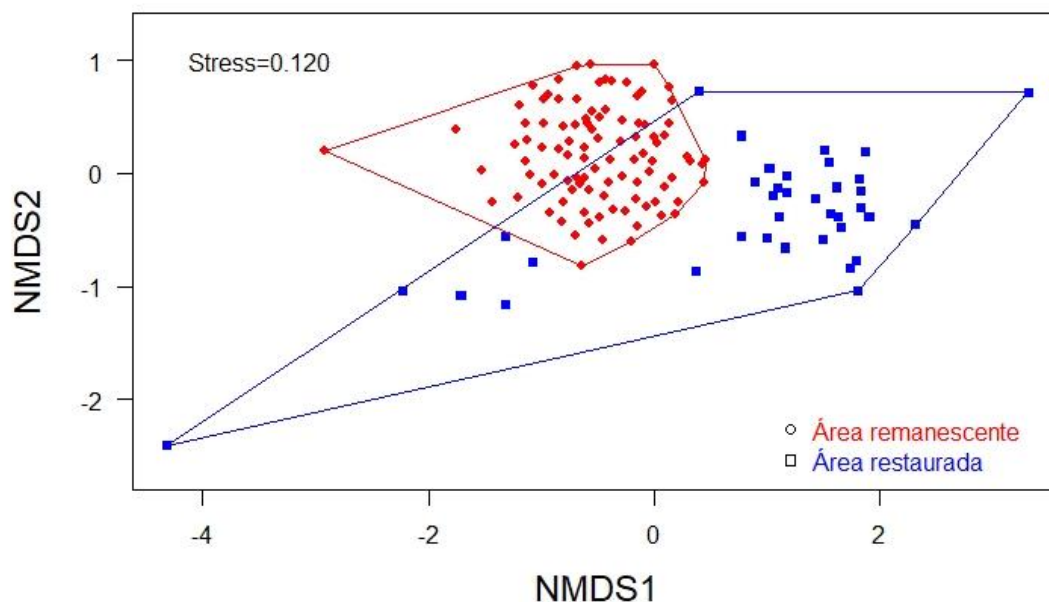
The species *Mimosa bimucronata* and *Mimosa scabrella* are legumes of great importance in the recovery of degraded areas, as they are part of the Fabaceae family and have symbiotic rhizobacteria in their roots, which stimulate the production of nitrogen in the soil, expanding the supply of vital nutrients to plants, contributing to the improvement of soil structure and fertility. as reported by several authors (BITENCOURT *et al.*, 2007; SILVA *et al.*, 2011; BARBOSA, 2014; LAURINDO *et al.*, 2023; RUIZ, *et al.*, 2023). Among the arboreal legumes occurring in plant formations in Brazil, *Mimosa bimucronata*, popularly known as maricá or hawthorn, is considered a typical species of the Atlantic Forest, with a wide distribution in this biome being widely cultivated in the Southeast region of Brazil for the formation of defensive hedges, due to the abundance of thorns on its branches (LORENZI, 2008). Being a pioneer species, *Mimosa bimucronata* is of great importance in the recovery of degraded areas, in which it is an indicator of the initial stage of regeneration, being a great



nucleating species, as it provides shelter to the fauna, functions as a perch for avifauna, in addition to protecting the seedlings of several species from the trampling of cattle (BITENCOURT *et al.*, 2007). In relation to *Mimosa scabrella*, popularly known as bracatinga, Carpanezi (1988) stated that this species is suitable for the recovery of degraded areas, maintaining a reasonable growth and with significant depositions of organic material and nitrogen in the soil. The predominance of pioneer species (maricá and bracatinga) in the arboreal stratum is justified by the fact that, according to the company's planting records, these were planted in a higher proportion than the non-pioneers, in order to maintain the natural dynamics of forest succession.

Analyzing the similarity between the two ecosystems, the value found for the Jaccard Similarity Index was 0.22, which is considered a low value. When analyzing the NMDS Graph, it is noticeable that although the two communities form distinct groups, there is an overlap between the two polygons formed because the use of ecological succession in the implementation of mixed forests is an attempt to give artificial regeneration a model following the conditions with which it occurs naturally in the forest (KAGEYAMA and GANDARA, 2001). In relation to the NMDS analysis, the Stress value found was 0.12, which is considered a considerable ordering, according to Clarke (1993), as shown in Graph 01.

Graph 01. NMDS graph of similarity between the two communities evaluated.



4 CONCLUSIONS

In view of the above, the importance of the species *Mimosa bimucronata* and *Mimosa scabrella* in the context of the rehabilitation of degraded areas is highlighted, since they play a fundamental role due to their association with nitrogen-fixing bacteria in the soil. In addition, it is important to



emphasize that the results of this research can provide significant assistance for the formulation of projects for the recovery of these environments, collaborating to accelerate the process of environmental restoration, constituting a new forest, an element of the common good.

In addition, it is also important to consider the introduction of the species *Cupania vernalis* in these environments, due to the importance of the species in this context, mainly due to the fact that it is the main species in the forest remnant areas of the study region.

It is also concluded that the restored forest is in an initial stage of succession with a development converging with nearby forest remnants, mainly with species typical of the region. It is noteworthy that the surrounding forest remnants are in the process of post-disturbance development, as evidenced by the characteristics of the community. Despite the low similarity calculated, it is evident that temporally the forests are distant, mainly due to the fact that the main species of the restored areas are typical of early succession, which favors the later propagation of later species. This study corroborates the planning of restoration projects, especially in ecotone regions between these two phytophysiognomies, which are widely occurring in the State of Paraná, also contributing to research on this theme.

Finally, it is noteworthy that, due to the high size of the new permanent preservation areas coming from the reservoir of the hydroelectric plant, the sample reflects part of the reality of the place, and there may be regions with different behaviors. Still, for more effective conclusions, the monitoring of the areas should be carried out, as well as the correlation with abiotic factors and the development of the ecosystem.



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Evaluation of the physical and chemical quality of the soil in different land uses, Frutal, MG



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ABSTRACT

Soils in areas of agricultural production, in the Cerrado region, when under intense cultivation, can present alterations in the chemical and physical constitution. This work aimed to evaluate the physical and chemical quality of the soil, at different depths in different land use systems (SUT): sorghum, rubber tree, pasture and native Cerrado in the municipality of Frutal-MG. In each SUT area, three samples were collected, at two depths. The design used was completely randomized 4x2, with four cropping systems and two depths, in three areas with the same SUT. The native forest system at a depth of 20-40 cm showed better values for electrical conductivity. For soil moisture the depth 0-20 cm were higher in the pasture, native forest and rubber tree systems, and 20-40 cm was native forest, between the depths the pasture system was greater in 0-20 cm. SUTs pasture showed higher soil density at depth 0-20 cm within and between depths. In the chemical analysis of the soil, regardless of the depth, the native forest has higher acidity. The copper content in the 20-40 cm depth, within the SUTs and depths, is higher for pasture, native forest, and rubber trees. In the sum of the bases within the 0-20 cm depth, the SUTs with the highest value were pasture, native forest and sorghum.

Keywords: Macro and micronutrients, Land use system, Agricultural sustainability.

1 INTRODUCTION

Grain production in Brazil may break records in the 2022/2023 harvest, with production of 312.2 million tons, 40.8 million tons more than in the 2021/22 harvest, representing a 15% increase in production, with the state of Minas Gerais being one of the largest grain producers in the country with an expected increase of 8.5% (CONAB, 2023). On the other hand, the areas destined for pastures have decreased, but productivity has been increasing, reaching 149.7 million hectares (IBGE, 2018).



Planted forests in Brazil extend over about 7 million hectares, and the state of Minas Gerais leads in planted area, with 1.49 million hectares, the main species being: pine, eucalyptus and rubber tree (CNA, 2020). These production areas are mostly located in areas that were originally Cerrado.

These soils, under natural conditions and corrected acidity, reveal favorable conditions for agriculture and livestock through their physical attributes. However, when under intense cultivation, they undergo changes in their chemical constitution (nutrients, organic matter, pH) and physical constitution (texture, structure, density, porosity). Thus, soils submitted to different management systems will tend to a new stable state, which may present a reduction in soil quality (SILVA, 2013; D'ANDRÉA, 2018).

The effects on the physical and chemical attributes of the soil in each management system depend on the type of tillage, the intensity of turning, the traffic of machinery, the types of equipment used, the management of plant residues and the soil moisture conditions at the time of tillage (VIEIRA; MUZILLE, 1995).

In general, the intensity of cultivation with soil turning has been observed by several authors as the main responsible for the increase in soil density and resistance to penetration and reduction of porosity (ALVARENGA; DAVIDE, 2009; D'ANDRÉA, 2018). The degradation of soil structure leads to a reduction in soil quality, consequently reduces plant development and increases the predisposition to accelerated water erosion, leading to loss of organic matter.

With the adoption of the appropriate cultivation system, seeking soil conservation and improvement of the system, conventional cultivation techniques are changed. Among these, the non-turning of the soil is always the first to be adopted. However, the absence of soil turning after several years under conventional management (plowing and harrowing) leads to a greater face-to-face rearrangement of soil mineral particles, resulting in a less favorable condition for cultivation than the conventional system previously adopted, since there will no longer be a mechanical increase in soil porosity due to turning (SANTOS et al., 2018b).

However, depending on the sustainable management practices adopted and the climatic conditions of the site, results are observed that indicate improvements in soil quality in the system after 3 to 4 years of implementation (MATA et al., 2021), such as increased porosity and carbon stock, in addition to reduced soil density and resistance to penetration.

Thus, it is important to continuously monitor these changes in order to produce information that supports interventions in these systems, aiming at improving soil quality with a consequent increase in crop productivity.

The objective of this study was to evaluate the physical and chemical quality of the soil at different depths in different land use systems (SUTs): sorghum, rubber tree, pasture and native Cerrado in the municipality of Frutal-MG.



2 THEORETICAL FRAMEWORK

The study of the variation of soil attributes over time allows us to quantify the magnitude and duration of changes caused by different management systems (SILVEIRA et al., 2011). These attributes are important to establish whether there has been degradation or improvement of soil quality in relation to a given management system (REICHERT et al., 2009).

The physical properties of the soil are of paramount importance due to the fact that they are linked to root growth; storage and supply of water and nutrients, gas exchange, and biological activity (ARSHAD; MARTINS, 2002). With the mechanization of labor in the field, soil compaction has become much more intense due to the fact that the machines have a large mass concentrated in small points (wheels), thus making it necessary to manage the soil with appropriate implements for soil decompaction.

However, agricultural machinery and implements, depending on soil moisture can increase compaction, this is also linked to the type of management and crop to be applied in the area, so it is necessary to study the cultivation environment, indicators and soil attributes.

The main physical indicators that have been used and recommended are texture; thickness (A horizon; solum); soil density; resistance to penetration; porosity; water holding capacity; hydraulic conductivity; and aggregate stability (ARAÚJO et al. 2012).

In the agricultural areas that are used for the first time, the soil has favorable conditions for the cultivation of plant species and presented an excellent production result. With the passage of time and the frequent use of the area, in addition to physical changes, the soil will also present chemical changes, causing productivity to reduce, no longer being interesting for the producer. In order for this not to happen, the owner must alternate crops (crop rotation/succession) so that the nutrients and properties of the soil are not depleted.

Knowledge about the particle size distribution of solid soil particles is essential in several situations, such as in the determination of texture, in studies on compaction and water movement and, consequently, for understanding the erosive processes that involve transport and deposition of particles, including nutrients essential to the development and productivity of plants (SILVA; FILE; ZUCOLOTO, 2011).

Soil attributes influence plant development, such as soil density, texture, porosity and stability of soil aggregates (MONTANARI *et al.*, 2015). The main changes are evidenced by the decrease in gas exchange, the rate of water infiltration into the soil and the increase in penetration resistance (DALCHIAVON *et al.*, 2014). Thus, these attributes can be considered as indicators of soil quality.

To improve the physical, chemical and biological quality of the soil, it is essential to know the damage caused by the different management systems, Soares et al. (2016) report that the inappropriate use of the soil, such as excessive turning or the use of poor conservation practices, can cause an



increase in density, a decrease in macroporosity and total porosity, among other damages. Different soil attributes have been employed to characterize the modifications resulting from the adoption of different soil management. The commonly used parameters are soil density and porosity (CARVALHO et al., 2014), soil resistance to penetration (TAVARES et al., 2014), soil moisture (LIMA et al., 2015), nutrient accumulation and organic matter (MARTINS et al., 2015; GOMES et al., 2015), particle density (BATISTA et al., 2017).

3 MATERIALS AND METHODS

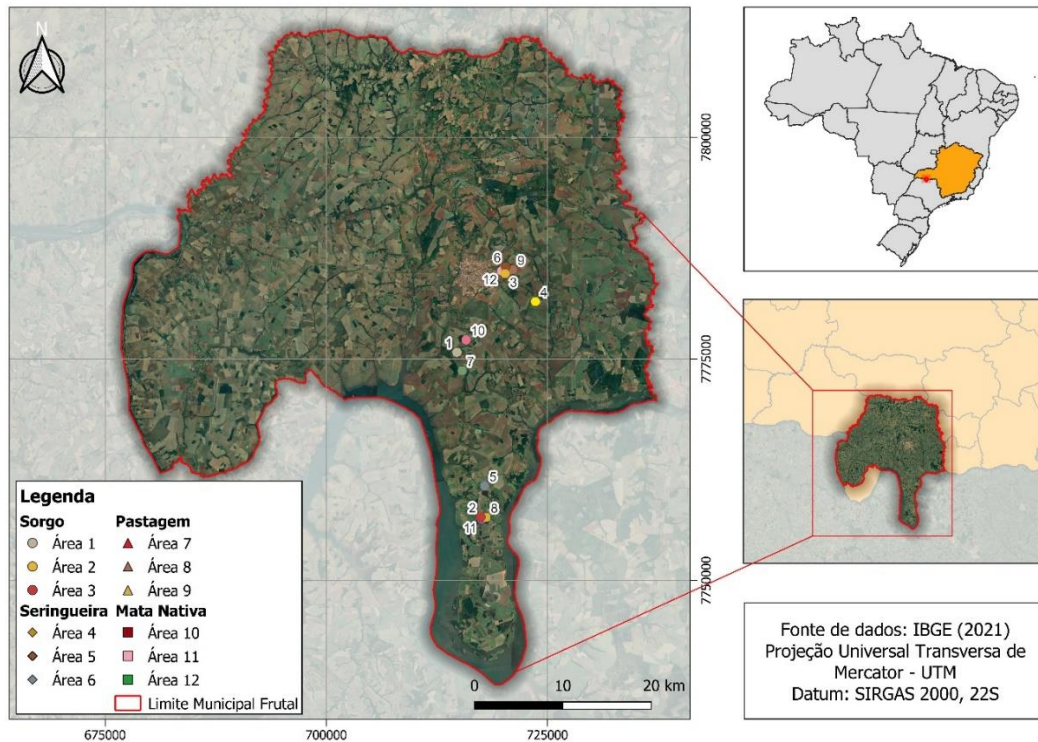
3.1 AREA OF STUDY

The study was carried out in the municipality of Frutal, in the Triângulo Mineiro region of the State of Minas Gerais. The climate of the region is defined as Aw, according to the Köppen-Geiger classification, tropical with the dry and cold season occurring in winter, and summer presents the season with the highest rainfall indices (DUBREUIL et al., 2018), with average annual temperature and precipitation of 23.8°C and 1626.9 mm, respectively, with precipitation concentrated between the months of November and April.

Samples were collected in four land use systems (SUT): pasture, rubber tree, sorghum and native savannah, totaling 12 areas (Figure 1). Each system had three replicates with areas at least 1.0 km apart. In each replica of the system, a 300 m transect was traced with three equidistant points at 100 m, maintaining a distance of 50 m from the edges of the area.

In the areas, an analysis of the landscape that is occupied in the ecosystem was carried out, where it was verified that it is in the Cerrado biome, convex, low slope and production area without trees and shrubs, with the exception of the legal reserve areas.

Figure 1. Location map of the experimental areas, municipality of Frutal-MG.



Source: Prepared by the authors, 2023.

3.2 CHARACTERIZATION OF LAND USE

The characterization and classification of soils in land use systems followed that proposed by Santos et al. (2018a). The spatial data of the locality were recorded with GPS and processed in the QGIS 3.22.7 software (QGIS Development Team, 2020). The classification of land use cover was carried out using the database of the Annual Project for Land Use and Mapping of the Brazilian MapBiomias - Collection 7, with a collection at a scale of 1:250,000 and the standardized RGB legend (MapBiomias, 2021). The platform is based on Landsat satellites (5-TM, 7-ETM+ and 8-OLI), with a spatial resolution of 30 m.

3.3 EXPERIMENTAL DESIGN

The experimental design was completely randomized (DIC) 4x2, with four cropping systems and two depths, in three areas with the same SUTs, with three replications in each area. Soil samples were collected during the cultivation of the systems, at depths of 0-20 and 20-40 cm. For the collections in each SUT, nine mini-trenches (considered as repetitions) were opened between the rows of each selected management system at random points.

After the collections, the soils were sent to the Soil Physics Laboratory of the State University of Minas Gerais – Fruiting Unit, where it underwent a sieve process to obtain the air-dried fine earth (TFSA), where they were later used for soil physics and chemical analyses. The samples were manually



de-disturbed and spread on 180 g m⁻² kraft paper trays and placed in a dry and ventilated place, exposed to the sun, until complete air desiccation.

3.4 PHYSICAL CHARACTERIZATION OF THE SOIL

For the determination of soil density (Ds) (g cm⁻³), by the volumetric ring method, undisturbed samples were collected with a *Uhland* sampler in cylinders with an average volume of 79 cm³ and for particle density (Dp) (g cm⁻³) the deformed samples were made with the Dutch auger. The total porosity (PT) of the soil was calculated by the soil density/particle density ratio using the formula adapted by the authors: $PT = (Ds - Dp) / Ds \times 100$ (TEIXEIRA et al., 2017).

Following the methodology of the previous author, soil texture was analyzed by the textural triangle, after the separation of the fractions: sand, silt and clay, by means of particle size sieves and symphonization.

Electrical conductivity (EC) and humidity (U) were measured in situ using the *Moisture Probe Meter* (MPM-160-B 12-bit) developed by *ICT International Pty Ltd*.

To determine the soil color of the respective samples, the classical method was used by Munsell®'s Color Chart, in which it is expressed in three components: hue, value and chroma (MUNSELL, 1994).

3.5 CHEMICAL CHARACTERIZATION OF THE SOIL

The pH and organic carbon analyses were performed at the UEMG soil laboratory, where they were performed according to Teixeira et al. (2017). The carbon stock (EstC) was calculated using the expression proposed by Costa et al. (2009): $EstC = (CO_{total} * Ds * e) / 10$; where: EstC: organic carbon stock in the studied layer (Mg ha⁻¹); CO_{total}: total organic carbon in the studied layer (g kg⁻¹); Ds: bulk density of the studied layer (kg dm⁻³); e: thickness of the studied layer (cm). The carbon balance was determined by the formula: $BC = (EstCCN - EstC) / TC$, where BC: carbon balance (Mg ha⁻¹ year⁻¹); EstCCN: carbon stock of each system studied (Mg ha⁻¹); TC: cultivation time (years).

For the chemical analysis of the soil, potassium (K) was read in the B 262 microron flame photometer, phosphorus (P) in the Ultraviolet Analytik Jena and calcium (Ca), magnesium (Mg), aluminum (AL), hydrogen (H), zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) in the Atomic Absorption Spectrometer (Perkin-Elmer Corp) and organic matter (MO) following the methodology of Teixeira et al. (2017).



3.6 DATA ANALYSIS

The effect of depth, land use system and system-depth interaction was analyzed by analysis of variance, and qualitative data were evaluated by Tukey's test at the level of 5% probability, using the statistical program SISVAR (FERREIRA, 2009).

4 RESULTS

The soils of the study areas were classified as a typical dystrophic Red Latosol, with dark reddish-brown colors (Table 1).

About the Red Latosol, cropping systems were studied under different land use systems, found in the region, always compared with the reference system, which in this study was considered the characteristic native Cerrado in the region.

Table 1. Staining of soil collection points by Munsell's color chart.

Area	Depth	Cor (Munsell)	Cor Manual tec. IBGE	Color (reference)
Sorghum				
1	0-20 cm	2.5YR 3/3	Dark reddish bruno-brown	
	20-40 cm	2.5YR 3/3	Dark reddish bruno-brown	
2	0-20 cm	2.5YR 2.5/3	Dark reddish bruno-brown	
	20-40 cm	2.5YR 2.5/3	Dark reddish bruno-brown	
3	0-20 cm	2.5YR 3/4	Dark reddish bruno-brown	
	20-40 cm	2,5YR 2,5/4	Dark reddish bruno-brown	
Rubber tree				
4	0-20 cm	2,5YR 3/4	Dark reddish bruno-brown	
	20-40 cm	2,5YR 3/4	Dark reddish bruno-brown	
5	0-20 cm	2,5YR 2,5/4	Dark reddish bruno-brown	
	20-40 cm	2,5YR 2,5/4	Dark reddish bruno-brown	
6	0-20 cm	2,5YR 2,5/4	Dark reddish bruno-brown	
	20-40 cm	2,5YR 3/4	Dark reddish bruno-brown	
Grass				
7	0-20 cm	2,5YR 3/4	Dark reddish bruno-brown	
	20-40 cm	2,5YR 3/4	Dark reddish bruno-brown	
8	0-20 cm	2,5YR 3/4	Dark reddish bruno-brown	
	20-40 cm	2,5YR 2,5/4	Dark reddish bruno-brown	
9	0-20 cm	2,5YR 3/4	Dark reddish bruno-brown	
	20-40 cm	2,5YR 3/4	Dark reddish bruno-brown	
Native Bushes				
10	0-20 cm	2.5YR 3/4	Dark reddish bruno-brown	
	20-40 cm	2,5YR 3/4	Dark reddish bruno-brown	
11	0-20 cm	2,5YR 3/4	Dark reddish bruno-brown	
	20-40 cm	2,5YR 3/4	Dark reddish bruno-brown	
12	0-20 cm	2,5YR 3/4	Dark reddish bruno-brown	



	20-40 cm	2,5YR 3/4	Dark reddish bruno-brown	
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Legend: Color (Munsell) = Color determined according to the Munsell chart; Color Manual Tec. IBGE = Color described according to IBGE (2015); Color (reference) = colors obtained by RGB.

Source: Prepared by the authors, 2023.

The soil texture in the sorghum, rubber tree and pasture areas was predominantly sandy clay loam, while in the native forest the texture was loam (Table 2). These variations in soil texture in different areas may be related to natural, topographic, or even erosive processes that have occurred over the years (FROZZI et al., 2018). As highlighted by Alifa et al. (2020), this variation indicates a homogeneity in soil pedogenetic processes and their approximation to the source materials.

Table 2. Description of soil texture according to fractionation, at different depths and areas on the cultivation of sorghum, rubber tree, pasture and native forest, municipality of Frutal-MG.

Area	Depth	Medium Sand	Medium Clay	Silte Media	Texture
Sorghum					
	 cm..... %.....		
1	0-20	70,4	15,4	14,2	Sandy loam
	20-40	76,1	20,2	3,7	Argyl-sandy loam
2	0-20	63,7	26,8	9,4	Argyl-sandy loam
	20-40	63,0	24,2	12,8	Argyl-sandy loam
3	0-20	42,7	15,9	41,5	Franca
	20-40	47,8	16,5	35,7	Franca
Rubber tree					
4	0-20	75,9	20,4	3,7	Argyl-sandy loam
	20-40	65,1	12,8	22,1	Sandy loam
5	0-20	44,9	18,8	36,3	Franca
	20-40	50,4	19,2	30,3	Argyl-sandy loam
6	0-20	63,2	24,5	12,4	Argyl-sandy loam
	20-40	48,2	19,2	32,5	Franca
Grass					
7	0-20	75,5	20,7	3,8	Argyl-sandy loam
	20-40	42,5	9,3	48,3	Franca
8	0-20	68,2	23,4	8,4	Argyl-sandy loam
	20-40	67,1	25,2	7,7	Argyl-sandy loam
9	0-20	45,0	31,2	23,7	Argyl-sandy loam
	20-40	47,2	29,6	23,2	Argyl-sandy loam
Native Bushes					
10	0-20	64,6	21,7	13,7	Argyl-sandy loam
	20-40	67,6	16,2	16,2	Sandy loam
11	0-20	64,3	16,1	19,7	Sandy loam
	20-40	49,5	17,2	33,3	Franca
12	0-20	48,7	19,6	31,7	Franca
	20-40	50,4	17,8	31,8	Franca

Source: Prepared by the authors, 2023.

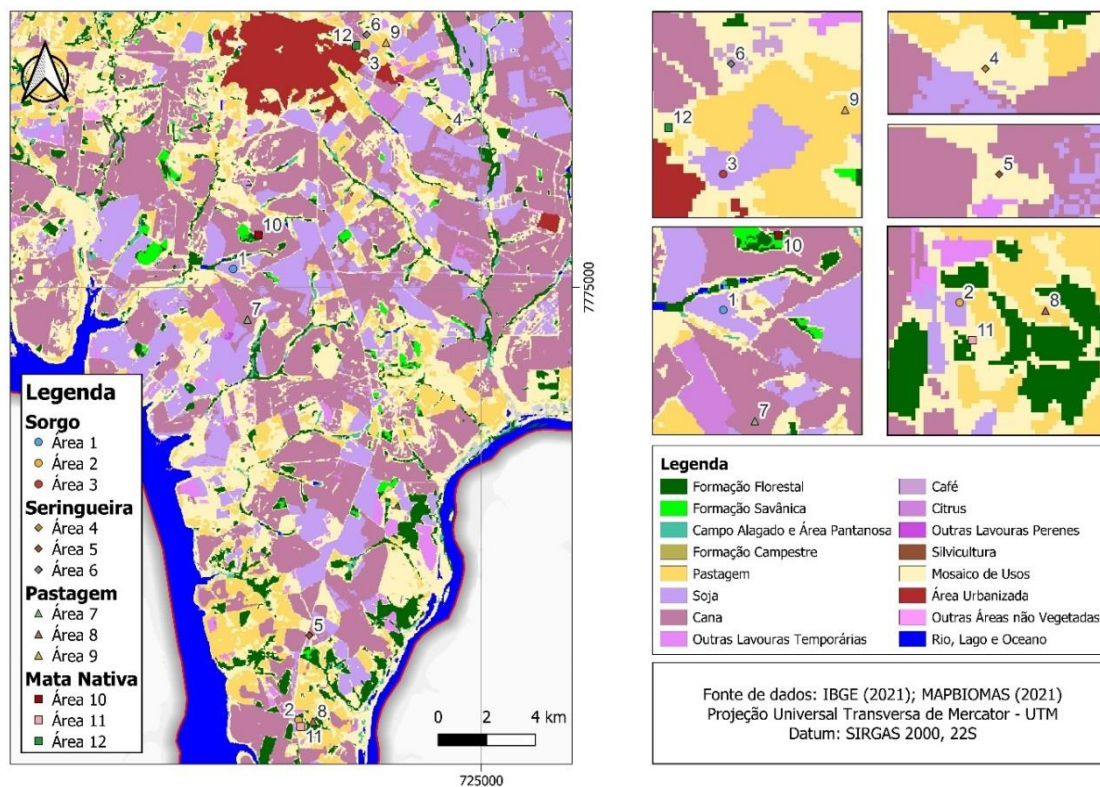
Figure 2 shows the map of land use and occupation, areas 1, 2 and 3, the occupation is soybean (eudicot), however the three SUTs met with the sorghum crop, being also an annual crop, however a monocot. In areas 4, 5 and 6 with occupation mosaic of uses (cultivated vegetation), being rubber tree SUTs, considered a cultivated vegetation.

For area 7 with sugarcane, 8 and 9 with pasture, all three having a pasture SUTs, as much as sugarcane and pastures are monocots, their size and management and cultivation system are very different. And areas 10 savannah formation (Cerrado biome), 11 and 12 use mosaic (Cerrado biome), these with SUTs Native Forest (Cerrado biome).

Although the images were taken in the same year of the study, there are divergent readings between the land use and occupation map with the on-site collection of the SUTs, where this is due to the crop rotation/succession and area renewal, since the survey soil collection may have occurred in different months from the image collected.

However, it is verified that the soils of the cultivated areas in use and occupied are suitable for the growth and development of crops.

Figure 2. Location map of the experimental areas, municipality of Frutal-MG.



Source: Prepared by the authors, 2023.

The physical properties of the soil are of paramount importance because they are linked to root growth, storage and supply of water and nutrients, gas exchange and biological activity (ARSHAD; MARTINS, 2002).

There was no significant difference for the variables particle density and soil porosity between SUTs and depths (Table 3). However, differences were observed for electrical conductivity (EC), moisture (U) and soil density (SD) (Table 3).



For the EC variable at the depth of 20-40 cm, the native forest presented the highest value. The decomposition of organic compounds present in the soil results in the release of organic salts into the soil, which may explain this result. Electrical conductivity (EC) is used to measure, by means of electric current, the amount of salts present in the soil solution, and the greater the amount of salts present in the solution, the higher the EC value obtained (BRANDÃO; LIMA, 2002).

Regardless of the ions present, excessive accumulation of salts in the root zone of plants can impair plant germination, development, and productivity. This accumulation causes the plant to expend more energy to be able to absorb water (due to the osmotic effect), impairing its essential metabolic processes (MAIA, 2017). It is important to note that each plant species has a maximum level of tolerance to excess salts.

Analyzing soil moisture at depth 0-20 cm, the SUTs with the highest values were native forest, pasture and rubber tree, and at depths 20-40 cm was native forest. When checking the humidity at the different depths in each SUT, it was found that pasture has the highest humidity in the 0-20 cm layer. Among the systems, the humidity showed higher values in the native forest, this can be justified by the existence of litter covering the surface layer of the soil, which helps to maintain moisture, in addition, it helps to increase the rates of organic matter over time, thus retaining greater moisture (GONÇALO FILHO et al., 2018).

For SD, higher values were observed for pasture, rubber and sorghum at the depth of 0-20 cm. When comparing the depths in the pasture system, the greatest increase was in the 0-20 cm layer. Through the statistical analysis, it was possible to demonstrate that the native forest areas expressed lower SD, pointing out that the existence of vegetation and soil cover helped in the increase of humidity, causing a decrease in density, certainly due to the greater contribution of organic matter.

It is important to highlight that higher SD values demonstrate that the area is compacted and, according to Mito et al. (2020), the compaction process is one of the fundamental reasons for soil degradation. Similar results were found by Custódio et al. (2015) and Bezerra et al. (2019) in pasture areas, where there was an increase in SD when compared to native forest and other systems. Compaction in pasture is associated with the topmost layer of the soil, however, it is not even considered a limiting factor for plant development (BONETTI et al., 2015).

According to Kiehl (1979), the larger the SD, the smaller its physical structure will be and in general less permeable, in which it may present limitations for the growth and development of plants.



Table 3. Average values of physical attributes in pasture, native forest, rubber tree and sorghum systems in the municipality of Frutal-MG.

Cultivation systems	CE1		U1		DP1		DS1		PO1	
 mv.....	% g cm ⁻³%.....			
 Depth (cm)									
	0-20	20-40	0-20	20-40	0-20	20-40	0-20	20-40	0-20	20-40
Grass	175,67aA	153,00bA	3,07aA	1,86bB	2,95	2,62	3.17abA	2.40aB	39,56	47,66
Native Bushes	216,56aA	235,78aA	4,81aA	4,97aA	2,66	2,56	2,52bA	2,70aA	36,02	36,84
Rubber tree	192,75aA	188,80bA	3,16aA	3,21bA	2,59	2,76	2.74abA	2,60aA	39,15	37,94
Sorghum	191,22aA	192,44bA	2,98bA	2,89bA	2,80	2,81	2.85abA	2,75aA	35,87	31,07
CV (%)	17,32		37,26		19,90		18,73		14,94	
Causes of variation	Fc									
SUTs.	10,36**		0,79ns		0,51ns		0,61ns		1,35ns	
Prof.	0,05ns		12,55**		0,20ns		3,02ns		0,04ns	
SUTs. X Prof.	1,20ns		1,15ns		0,66ns		2,76*		0,59ns	
Prof. X SUTs.	1,20ns		1,15ns		0,66ns		2,76**		0,59ns	

¹Means compared in the columns (lowercase letters) and row (uppercase letters) with the same letter do not differ from each other, by Tukey's test, at the level of 5% probability. * and ** significant at 5 and 1% probability, respectively. ns – not significant. CV – Coefficient of variation. Prof. – Depth; SUTs - System.

Source: Prepared by the authors, 2023.

In the variables in Table 4, there is a significant difference for pH, SB and Cu. For pH, the crops do not differ from each other, but all differ from the Native Forest (NM), because in crops of economic interest, there is alkalization of the soil through the mechanical application of lime and gypsum; Looking at the depths, there is not a significant difference between them. However, there is a difference between each depth when comparing the SUTs at the depth 0-20 cm, the highest values were found for rubber trees, with a pH of 5.98. At a depth of 20-40 cm, pasture, rubber tree and sorghum had a pH of 5.63; 5.56 and 5.11, respectively.

In general, in the most superficial layer (0-20 cm) the areas of agricultural cultivation obtained higher pH values, this fact can be justified as highlighted by Oliveira et al. (2001) due to the soil being subjected to deep plowing with greater turnover, thus bringing a higher concentration of bases to the surface.

For the variable Copper (Cu), there is a difference within the depth of 20-40 cm in the SUT, with higher values for pasture, native forest and rubber tree, while among the depths there are higher values at the depth of 20-40 cm in native forest. This is evident that SUTs are able to modify the amount of this element in the soil. This behavior differs from the study carried out by Silva et al. (2016), where the concentration of Cu was higher in cultivated soils than in areas with natural vegetation, however the author corroborates when he found that Cu increased at greater soil depths.

Regarding the sum of the bases (SB), there was a significant difference in depth of 0-20 cm between the SUTs, with higher values for the pasture, native forest and sorghum systems. It is justified



that at this depth these SUTs presented lower amounts of Ca, Mg and K in the soil. Carvalho et al. (2002) and Bernadi, Rassani and Ferreira (2012) point out that the values of BS in most Oxisols are generally low in the deeper layers, when comparing the superficial layers. The authors' statement is confirmed in this study, since at the depth of 20-40 cm, the BS values were lower than those found at the depth of 0-20 cm.

To improve the physicochemical quality of the soil, it is essential to know the damage caused by the different management systems, Soares et al. (2016) report that the inappropriate use of the soil, such as excessive turning or the use of poor conservation practices, can cause an increase in density, a decrease in macroporosity, total porosity, alteration in the amount of minerals in the soil, among other damages.

The study of the variation of soil physical and chemical attributes over time allows us to quantify the magnitude and duration of changes caused by different management systems (SILVEIRA et al., 2011). These attributes are important to establish whether there has been degradation or improvement in soil quality in relation to a given management system (REICHERT et al., 2019).

Table 4. Average values of the chemical attributes of the soils pasture, native forest, rubber tree and sorghum systems at different depths, in the municipality of Frutal-MG.

ATRIBUTO SI	CV (%) LAND USE SYSTEMS – SUTs								FV				
		Grass		Native Bushes		Rubber tree		Sorghum		Fc				
	 Depth (cm).....										Prof	SUTs	Prof X SUTs
		0-20	20-40	0-20	20-40	0-20	20-40	0-20	20-40					
pH (H ₂ O)	11,13	5,53bA	5,63aA	4,43bA	4,78bA	5,98aA	5,56aA	5,55bA	5,11abA	0,54n s	13,34 **	1,98n s		
MO (g dm ⁻³)	17,54	4,52aA	13,98aA	5,58aA	16,74aA	13,19aA	10,67aA	8,92aA	7,32aA	1,38n s	0,25n s	1,04n s		
P (mg dm ⁻³)	303,5 2	8,16aA	26,43aA	3,91aA	19,43aA	6,79aA	4,09aA	9,32aA	3,55aA	0,70n s	0,17n s	0,74n s		
K (mmol _c dm ⁻³)	128,3 9	2,15aA	2,60aA	1,31aA	1,94aA	1,19aA	1,07aA	1,67aA	1,58aA	0,14n s	1,02n s	0,15n s		
Ca (mmol _c dm ⁻³)	93,61	22,33aA	11,95aA	10,37aA	9,61aA	8,14aA	10,56aA	12,54aA	11,41aA	0,96n s	1,70n s	1,02n s		
Mg (mmol _c dm ⁻³)	76,00	4,33aA	2,99aA	3,18aA	2,87aA	2,51aA	4,66aA	2,91aA	2,54aA	0,01n s	0,67n s	1,59n s		
Al (mmol _c dm ⁻³)	105,0 0	0,83aA	3,06aA	3,72aA	3,33aA	3,75aA	4,50aA	3,00aA	3,89aA	1,28n s	1,35n s	0,39n s		
H+Al (mmol _c dm ⁻³)	41,77	13,44aA	13,56aA	20,56aA	19,33aA	17,38aA	18,90aA	17,22aA	15,89aA	0,01n s	2,67n s	0,16n s		
COrg (g dm ⁻³)	17,66	4,43aA	6,40aA	5,56aA	5,51aA	3,45aA	6,75aA	3,08aA	5,28aA	2,54n s	0,28n s	0,36n s		
SB (cmol _c dm ⁻³)	76,70	28,80aA	17,54aA	14,86ab A	14,42aA	11,84bA	16,29aA	17,11ab A	15,53aA	0,59n s	1,79*	1,10n s		
T (cmol _c dm ⁻³)	55,22	29,64aA	20,59aA	18,58aA	17,75aA	15,59aA	20,79aA	20,11aA	19,42aA	0,31n s	1,48n s	1,98n s		
T (mmol _c dm ⁻³)	39,50	42,25aA	31,09aA	35,42aA	33,75aA	29,24aA	35,19aA	34,34aA	31,42aA	0,64n s	0,36n s	1,19n s		
V (%)	53,08	64,00aA	55,59aA	34,14aA	39,39aA	40,76aA	42,28aA	45,10aA	43,48aA	0,02n s	2,98*	0,27n s		
M (%)	109,6 1	4,68aA	23,46aA	35,70aA	26,28aA	29,99aA	28,40aA	23,12aA	33,41aA	0,51n s	1,38n s	0,88n s		
Zn (mg dm ⁻³)	85,33	0,27aA	0,30aA	0,25aA	0,16aA	0,31aA	0,21aA	0,24aA	0,28aA	0,42n s	0,46n s	0,64n s		
Cu (mg dm ⁻³)	84,82	0,18aA	0,17abA	0,19aB	0,36aA	0,14aA	0,17abA	0,16aA	0,15bA	1,39n s	2,19*	1,38*		
Fe (mg dm ⁻³)	35,87	105,06a A	112,71a A	127,96a A	105,24a A	127,33a A	103,36a A	113,52a A	122,32a A	0,17n s	0,60n s	0,89n s		



Mn (mg dm ⁻³)	54,31	2,03aA	1,83aA	2,14aA	1,80aA	1,17aA	1,76aA	1,98aA	1,59aA	0,15n _s	0,90n _s	0,94n _s
EstC (Mg ha ⁻¹)	13,74	15,15aA	17,35aA	13,48aA	13,48aA	9,04aA	17,92aA	9,60aA	16,01aA	1,63n _s	0,20n _s	0,34n _s

¹Means compared with lowercase letters on the line at the same depths between the SUTs, uppercase letter on the same line between the depths in each SUTs, do not differ from each other, by Tukey's test, at the level of 5% probability. * and ** significant at 5 and 1% probability, respectively. ns – not significant. Fc – F calculated. CV – Coefficient of variation. Prof. – Depth.

Source: Prepared by the authors, 2023.

5 CONCLUSION

The soil class was a typical dystrophic Red Latosol with dark reddish haze, and the predominant texture in the areas of sorghum, rubber tree and pasture was sandy clay loam, and loam texture for native forest.

The native forest system at a depth of 20-40 cm showed better values for electrical conductivity. For soil moisture, the depth 0-20 cm was greater in the pasture, native forest and rubber tree systems and 20-40 cm was native forest, among the depths the pasture system was greater in 0-20 cm. The pasture SUTs showed higher soil density at the depth 0-20 cm within and between the depths.

In the chemical analysis of the soil, regardless of the depth, the native forest presents higher acidity. The copper content at the depth of 20-40 cm, within the SUTs and depths, is higher for pasture, native forest and rubber trees. In the sum of the bases within the depth 0-20 cm, the SUTs with the highest value were pasture, native forest and sorghum.

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Influence of coffee packaging on consumer purchase decision



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ABSTRACT

Packaging, aside from its primary role in containing food products, serves the crucial function of shaping expectations for a brand or product through its visual appeal, significantly influencing purchasing decisions. In the context of coffee, this

has gained heightened relevance given the evolving market dynamics and the increased emphasis on the product's positioning and differentiation in recent years. This study aimed to identify the key elements consumers prioritize when evaluating coffee packaging and examine the impact of both packaging and labels on the purchasing decisions of consumers. Through the administration of questionnaires (n = 100), the research revealed that color choices significantly influence consumer attraction, with red, dark brown, orange, and black standing out as particularly captivating options. The study also noted a substantial receptivity to innovative packaging, featuring distinctive graphic or technological elements. Conversely, aspects such as packaging format, ergonomics, vacuum/QR Code technologies, and packaging material demonstrated limited discernible impact on consumer preferences. The findings not only offer valuable insights for coffee-producing companies seeking to standardize packaging based on consumer preferences but also contribute to future research endeavors. By uncovering factors that may not resonate with consumers, this study lays the groundwork for further inquiries and the validation of hypotheses within the realm of coffee packaging research.

Keywords: Consumer behavior, Market research, Packaging design, Coffee.

1 INTRODUCTION

Coffee is one of the most consumed beverages in Brazil today, and the consumer has been demonstrating an emotional and affective relationship with the product, associating the experience of consuming coffee as one of life's pleasures, in addition to being a way to improve mood and mood. During the COVID-19 pandemic (2020-2021), a survey was conducted where it was possible to observe that consumers started to drink more coffee and claimed that social isolation and staying at home had a great influence on the increase in desire for the drink. Most respondents (72%) stated that coffee helped them overcome the worst moments of the pandemic (DOMINGUES, 2021).



Food packaging, according to the definition presented in RDC 91/2001 (ANVISA), is the article that is in direct contact with food, intended to contain it, from its manufacture to its delivery to the consumer, to protect it from external agents, alterations and contamination, as well as from adulteration. Therefore, by the definition of packaging, it is possible to perceive some of the main functions, such as: containment, protection, communication and convenience.

Blessa (2012) apud *Oliveira (2018)* , adds that the packaging must be designed to attract and attract the consumer and then cause the sale of the product. Being an important marketing tool at the point of sale, it must catch the consumer's attention through the colors, shapes, textures, and typography used in its design. Faced with this reality, companies become increasingly competitive, seeking a differential between the various products offered with similar characteristics. Thus, the packaging, together with the label, gain significant representation not only to store and transport the product offered, but also to position it on the shelves and seduce the consumer through an eye-catching design (OLIVEIRA, 2018). Consequently, it is necessary to improve the visual attractiveness of the packaging, since market globalization promotes an increasing supply of products with the high quality standard required, making industries need to use all possible resources to remain in the market.

In view of this, it is important to understand which attributes are observed by the consumer when buying a coffee, which information they will consider essential and which will not be so relevant. In this context, packaging elements such as: design, font, colors, application of new technologies, ergonomics, content, material, quality seals, legibility, illustrations, phrases, recyclable material, among others, can be evaluated. As the demands increase due to the improvement of packaging in terms of design, so does the need to produce packaging that is convenient and competitive. Several studies have been conducted with the purpose of observing the role of the packaging or factors contained in it in the consumer's purchase intention, however, there are few studies that address the reasons that lead the consumer to choose a certain type of coffee by analyzing the items that appear on the package. Thus, with this work, we aim to expand the understanding of the coffee consumer's purchase decision process. These results can help companies producing roasted and ground coffee powder to determine the best combination of packaging aspects, and consequently, facilitate the development of packaging that is attractive, making research important for the coffee industrial sector.

2 METHODOLOGY

The work was a cross-sectional research, of a self-applied nature, with a quantitative and descriptive approach, and the data were collected through an online questionnaire, which was made available from April to November 2022. The questionnaires were disseminated mainly through social networks and messaging applications, randomly to individuals aged between 15 and 71 years.



The questionnaire was structured in two sections, containing a total of 28 structured or semi-structured questions. However, in order for the questionnaire to take place, it was necessary to answer the main question about coffee consumption; If the individual answered no, the questionnaire would be closed, if he answered yes, he would continue.

a) Influence of the characteristics of the coffee packaging: in this section, 23 questions were addressed in Likert-type degree of agreement, constituting questions such as; Colors that refer to childhood influence or not the purchase decision, if the color of the packaging is a determining factor, innovative product, with colors different from the conventional would draw the consumer's attention, packaging format, ergonomic packaging, vacuum packaging, packaging that appears to have greater resistance, smelling the coffee before buying, if it would be a determining factor, contents of the package, packaging material, presence of a logo of a recognized brand, clarity of information, presence of quality seals, font of the letter, font size, illustrations, phrases, economical packaging, originality and exclusivity, packaging produced from recyclable material, use of *QR Code* and *Zip Lock*. Finally, a question evaluating which color the consumer has the most affinity or prefers when buying a coffee (red, yellow, orange, green, black, dark brown and white).

b) Sociodemographic issues: 5 questions were addressed with the objective of characterizing the respondent population, being the questions related to gender, age, level of education, monthly income and state of residence. It is important to emphasize that each participant had their information anonymous, directing all data and information obtained in an ethical manner.

3 RESULTS AND DISCUSSION

3.1 SOCIODEMOGRAPHIC PROFILE

Table 1 shows the data on the sociodemographic profiles of the 100 participants.

Table 1 - Sociodemographic profile of the research participants (n=100).

Variable	Absolute frequency (n)	Relative frequency (%)
Gender		
Female	52	52
Male	48	48
Age group		
15 to 18 years old	7	7
19 to 25 years old	40	40
26 to 32 years old	21	21
33 to 39 years old	13	13
40 to 50 years old	10	10
51 to 60 years old	6	6
61 to 70 years old	3	3
71 years or older	0	0
Schooling		



No schooling	0	0
Incomplete elementary school	0	0
Complete Elementary School	4	4
Incomplete high school	2	2
Completed high school	33	33
Incomplete tertiary education	32	32
Completed higher education	19	19
Postgraduate studies	10	10
Monthly income		
No income	18	18
Up to 1 minimum wage	20	20
From 1 to 3 minimum wages	33	33
From 3 to 6 minimum wages	24	24
From 6 to 9 minimum wages	5	5
From 9 to 12 minimum wages	0	0
From 12 to 15 minimum wages	0	0
More than 15 minimum wages	0	0

Source: Prepared by the author, 2022.

It is possible to note that the gender was divided between 48% of the male public and 52% of the female public, most individuals (40%) were between 19 and 25 years old, 33% had completed high school and 33% had an income of 1 to 3 minimum wages.

In a survey conducted by Domingues (2021), on the habits and preferences of coffee consumers in Brazil, the sample obtained was 4200 people interviewed and the gender was divided between 45% of the male audience and 55% of the female audience, which was similar to that of this study. The profile of the participants was also relatively similar to that of the audience in the study by Della Lucia et al. (2007), in which 75% of the participants were aged between 20 and 39 years, and 42.4% had incomplete higher education.

Regarding salary, according to data obtained in the Continuous PNAD (2021), 10% of Brazilians earn more than 3,359.00 reais, but in this survey it was obtained that 33% of respondents



receive from 1,212.00 to 3,636.00 reais. The knowledge of the monthly income is of great importance so that the consumer's profile can be analyzed.

3.2 FEATURES OF COFFEE PACKAGING

Table 2 shows the results obtained in relation to the characteristics of the coffee packaging.

Table 2 - Characteristics of the coffee packaging (scale from 1 = Strongly Disagree to 5 = Strongly Agree)

Statement	Average	Standard deviation
Coffee packaging colors refer to childhood memories and can influence the purchase decision	3,29	1,45
The color of the packaging is a determining factor	3,27	1,50
Of all the colors, which one catches the most attention	3,24	2,14
Innovative product, would draw attention and could lead to buying the product	4,08	1,22
Format is a determining factor	3,15	1,34
Packaging ergonomics influences the purchase decision	3,28	1,24
Vacuum packaging draws more attention than conventional packaging	2,83	1,40
Packaging that appears to be more resistant influences the purchase decision	3,95	1,26
Possibility to smell the coffee before buying	4,49	0,94
Package Contents	4,57	0,78
Packing material	3,05	1,30
Presence of a logo of a recognized brand	4,05	1,18
Preference for packaging that clearly shows all information	4,64	0,69
Presence of quality seals	4,01	1,17
Spelling is a determining factor	3,61	1,48
Readability	3,89	1,33
Illustrations	3,71	1,49
If the illustrations do not attract attention, it can lead to not choosing the product	3,53	1,26
Presence of sentences	3,7	1,46
Cost-effective packaging	3,89	1,34
Originality	4,11	1,19
Recyclable material	3,42	1,52
QR Code <i>Technology</i>	2,66	1,50
Zip Lock <i>Technology</i>	4,13	1,33

Source: Prepared by the author, 2022.



The statement that presented the highest agreement in this topic was: "Preference for packages that clearly show all information", reached a mean of 4.64 and a standard deviation of 0.69, followed by the statement "Package contents" obtained a mean of 4.57 and 0.78 standard deviation, while the statement "Possibility of smelling the coffee before buying" reached a mean of 4.49 and a standard deviation of 0.94.

The *Zip Lock* Technology with the mean and standard deviation respectively (4.13 and 1.33), originality of the product (4.11 and 1.19), innovative product (4.08 and 1.22), presence of a recognized brand logo (4.05 and 1.18), presence of quality seals on the packaging (4.01 and 1.17), were important statements for the research, as they reached the averages between 4.13 and 4.01, which refers to the average number of answers marked in the alternative: "partially agree".

On the other hand, statements such as "Packaging that presents itself to be more resistant", "Presence of the phrase economical packaging", "Legibility", "Illustrations", "Presence of phrases", "Spelling", "If the illustration does not arouse attention, this can lead to not choosing the product", "Recyclable material", "Colors of the coffee packaging refer to childhood memories", "Ergonomics", "Packaging color", ranged from 3.95 to 3.27 on average, that is, respondents marked the alternatives: "Neither agree nor disagree", "Partially agree" and "Strongly agree".

In a survey conducted by Esteves (2012), in relation to the color of the water bottle, it was asked if it arouses sensations that can attract or drive away the consumer, to which 1.8% of the respondents answered that they totally disagreed; 2.7% disagreed; 23.6% neither agree nor disagree; 38.2% agree; and 33.6% strongly agreed. On the other hand, in this research it was possible to observe a difference in relation to the public that totally disagrees with the statement (22.5%), different from what was expected at the beginning of the work, considering the difference in the product analyzed.

Regarding the colors that most attract the attention of the respondents (mean and standard deviation: 3.24 and 2.14), red was the dominant color with 40% of the votes, followed by dark brown with 22%, black (18.6%) and orange with 12%.

Della Lucia et al. (2009) point out that most of the consumers evaluated in the survey (a total of 75%) reported preferring brown and reddish colors for coffee packaging, claiming that "the color for coffee is brown with red". The possibility was raised by some consumers (37.5%) that green was associated with the organic product, since all the labels on the organic coffee packages had this color, at least in some details. This was similar in this study, where the colors that most caught the attention of the respondents were red and dark brown, followed by orange and black.

In addition, it is worth emphasizing the importance of conducting this type of research prior to product development, since understanding the factors that lead consumers to choose a particular product enables the industry to develop, innovate and define marketing strategies for it (DELLA LUCIA et al., 2009).



4 CONCLUSION

It is possible to conclude that, in the sample studied, coffee consumers are young, mostly female, with complete high school education, with an income of 1 to 3 minimum wages. Regarding the colors of the coffee packaging, there was a significant influence, and the colors that most attract the consumer's attention are red, dark brown, followed by orange and black. The aspect of an innovative product, which consists of different colors from the conventional one, i.e., a coffee package composed of graphic or even technological innovations, would be well accepted in relation to the respondent public, without taking into account the price and the answers regarding the acceptance of technological applications.

The shape of the coffee packaging, ergonomics, vacuum/*QR Code* technologies and packaging material did not bring significant results. It was also observed that factors such as: perception of resistant packaging, ability to smell the coffee before buying it, content (net weight), clarity of information and readability, were elements that constituted a high acceptance of the respondents. The illustrations contained in the coffee packaging, such as heart, cup, farm and gate, were factors that influenced the consumer. The presence of phrases on the packaging, the term "economical packaging", the originality/exclusivity factor and the "*Zip Lock*" technology were also relevant aspects in the research.

Finally, to collaborate in future studies, considering the questions used in this research. By establishing a direct and objective questionnaire, it will be possible to help coffee manufacturers to prepare a standardized booklet with all the characteristics that were relevant in relation to the consumer's choices, facilitating the development of a new coffee package, saving time and money with research and prototypes, obtaining a greater chance of success in the development of a new packaging.



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Biological control: Sustainably controlling pests in the vegetative phase of corn



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ABSTRACT

Brazil is considered the country with the greatest agricultural potential in the world, as it is in a region with a tropical climate, and this contributes positively to the increase in agriculture. Among the numerous segments of the production chain, corn stands out as one of the main grains produced in the country. The continuous cultivation of grass leads to a high incidence of pests, significantly affecting the productive potential of the crop and great losses to producers. A viable alternative to sustainably control these undesirable insects is biological control, allowing to reduce the population density of another organism, through living beings or natural substances, thus acting as pest control agents in agriculture, promoting balance in the crop and contributing to the reduction of agrochemicals in the field, favoring sustainability. since chemical pesticides are harmful to crops and human health.

Keywords: Sustainability, Corn, Pests.

1 INTRODUCTION

Biological control promotes balance between insects, pests and natural enemies, in addition to reducing and even replacing the use of agrochemicals in the field, contributing to sustainability (BORGES, 2011).

Brazil is a world leader in the use of biological products in crops and already exports technologies to other countries (LOBATO, 2019). According to Alexandre de Sene Pinto, "All the technology of biological products that other countries are using for large areas is coming from Brazil." The growth of biologics in the last two decades is notorious, and in number of registrations, they have already surpassed chemical products in the same period (LOHBAUER, et. al, 2022).

In 2020, an annual record was registered in Brazil, 96 new biological products registered, reaching in March 2022, 484 registrations since the year 2000, they are classified as low impact, which can be natural chemical substances, called semiochemicals, or biochemical compounds that induce behavioral responses in target organisms, such as pheromones and allelochemicals, which have a natural origin and control pests and diseases, as hormones regulating growth. There are



microbiological control agents on the market, which are viruses, bacteria, protozoa and fungi, and macrobiological agents, conferred by insects, mites, and even nematodes (LOHBAUER, et. al, 2022).

Maize (*Zea Mays*) is among the oldest crops in the world, having been cultivated for over 5,000 years. The importance and diversity of the use of grain are characterized in several agro-industrial segments, from human to animal food, as a direct source of food for the low-income population, even if in small proportions, and as a raw material in the formulation of feed and other segments of the chain, requiring high technological investment in order to maintain the health of crops (FRANCHU, 2021).

Brazil has consolidated its third position in the world among the largest corn producers in the world (EMBRAPA, 2022). In the 2022/23 harvest, Brazil reached a total production of 130 million tons of corn, attributed to the increase in planted areas of second crop corn, and recovery of productivity in the third crop (CONAB, 2023). However, one of the factors that most influence the yield of production is the attack of insect pests, which are divided into three main groups. The first group includes soil insects, also called initial pests, the second group is leaf area and stem insects, and the third group is composed of end-of-harvest insects that cause damage to ears (VALICENTE, et. al, 2015), requiring management in seed treatment and during the vegetative development of the crop.

In view of the facts mentioned, the importance of the corn crop in Brazil is observed, and based on this the following problem arises, do all producers have access to assertive information about the use and efficiency of biological products to control insect pests in corn crops, in addition to it being a means of contributing to the ecosystem?

Thus, this work is justified by the lack of reliable and accessible information about biological products and their efficiencies in controlling insect pests in the vegetative phase of corn, meanwhile, they are new products in the market that have been gaining prominence only in the last decades, and many producers are still afraid to use or replace them with chemical control. even though it has already proven its efficiency.

The general aim of this article is to report on the biological control of insect pests in the vegetative phase of maize crop. Presenting the following specific objectives: To address the importance of biological control, to inform the means of action of biological control and to present the main pests present in the vegetative phase of corn, in addition to demonstrating the main types of biological control, and presenting the main pests in the vegetative phase of the crop.

The work was developed based on a descriptive bibliographic research that includes exploratory research, referring to the use of biological control in the fight against pests in the vegetative phase in the corn crop, always thinking about contributing to a sustainable ecosystem. The research was developed with the help of books, magazines, links and scientific articles on the referred topic. The keywords used were: Sustainability, Corn and Pests.



2 LITERATURE REVIEW

2.1 BIOLOGICAL CONTROL IN BRAZIL

Brazil is considered the country with the greatest agricultural potential in the world (VANTINI, 2017), as it is located in a region with a tropical climate, a fact that contributes positively to the increase in agriculture, in addition to being extremely favorable for the cultivation of numerous crops throughout the year (MONNERAT, et. al, 2020), however the continuous cultivation and the increasing expansion of agricultural areas cause a high incidence of pests (VALICENTE, Et. al, 2015).

One of the viable alternatives to sustainably control these insects is biological control, which consists of reducing the population density of another organism. This alternative has been used since the third century, by the Chinese, with the manipulation of ants released in citrus to control pests (MONNERAT, et. al, 2020), although the management technique is old, in Brazil, only in recent decades has it been conquering the market, due to its cost, efficiency, and increased productivity (MONDIN, et. al, 2022).

In order for biological control to be solidified, it is necessary to invest more and more in education and knowledge transfer, because there are still many rural producers in the country who are unaware of the benefits and efficiency of biologicals as well as encouraging continuous development and innovation in this sustainable sector, contributing to the environment and human health (MONDIN, et. al, 2022).

Biological control is used to control sanitary problems, such as pests, diseases, and can be applied by four means, the natural one which consists when the populations of organisms are kept in balance, by natural occurrence, the conservationist environment, which man acts stimulating the prevention and natural increase of beneficial agents in the field, the classical means that is based on the collection of natural enemies usually in the region of origin, to release in desired areas and increase biocontrol, and augmentative, which consists of the application of parasitoids, predators, and entomopathogens, which is already known and used by farmers with inoculation in seed treatment (PIERO et. al, 2022).

2.2 MEANS OF ACTION OF BIOLOGICAL CONTROL

Biologics are living organisms or derived from them and are employed as active ingredients in formulations. In addition to inoculating, natural predatory enemies, which are organisms that feed on others to survive, are also considered biopesticides, especially ladybugs and earwigs (MONDIN, et. al, 2022).

Parasitoids, on the other hand, necessarily need a living host to complete their life cycle, and belong to the order Hymenoptera, in which wasps act positively on crops by parasitizing eggs, egg-larvae, larvae, pupae and aphids, such as *Trichogramma pretiosum*, *Chelonus insularis*, *Cotesia*



marginiventris, Tetrastichus Howard and Rhopalosiphum maidis, respectively, and are efficient means to control insect pests (CRUZ, 2022).

Entomopathogens contribute positively to the elimination of insect pests through viruses, bacteria and fungi, which contaminate the hosts, penetrating the integument and colonizing their body by the hemolymph, thus causing epizootics, that is, diseases that lead to death in addition to interfering with the feeding and reproduction of insects and mites, Beauveria bassiana, Metarhizium anisopliae and Baculoviridae are some of the most studied organisms for pest control (ROHRIG, 2021).

This shows that biological control is effective in reducing populations of agricultural pests, thus maintaining a population below the control level, in addition to minimizing damage caused by pests, and reducing the cost of chemical products to control them, because natural enemies, or agents have the ability to reproduce and survive in the environment, do not leave residues of agricultural products and do not cause resistance, benefiting the environment and consumers (PASSOS, MENDONÇA, 2020).

2.3 PESTS IN THE VEGETATIVE PHASE OF CORN

The occurrence of insect pests in corn cultivation significantly affects the productive potential of the crop, and during its cycle it is attacked by numerous pests and at different stages, and in the vegetative phase edaphoclimatic conditions, phenological stage, cropping system and biotic factors, directly influences the occurrence of these organisms (PARENTONI, et. al, 2022).

Of the pests of the vegetative phase, stem pests are mentioned, such as the sugarcane borer (*Diatraea saccharalis*) (Figure 1a), which in its larval stage feeds on the stalk, thus reducing the translocation of photoassimilates (CEREZA, et. al, 2021). The elasm caterpillar (*Elasmoplus lignosellus*) (Figure 1b), which destroys the growth point, thus causing wilting and death of the central leaves (VALICENTE, et. al, 2015). The screwworm (*Agrotis ipsilon*) (Figure 1c), which has a negative phototropic characteristic, because during the day it remains in the soil, and at night it is capable of sectioning several plants (CAMARGOS, et. al, 2021). And the green-bellied stink bug (*Dichelops melacanthus*) (Figure 3d), which feeds on sap in the xylem vessels, and injects toxic substances through the sheath to the inner leaves, causing lesions, in addition to causing curling of the younger leaves that do not open, forming a kind of "cigar" (MIRANDA, et. al, 2021).

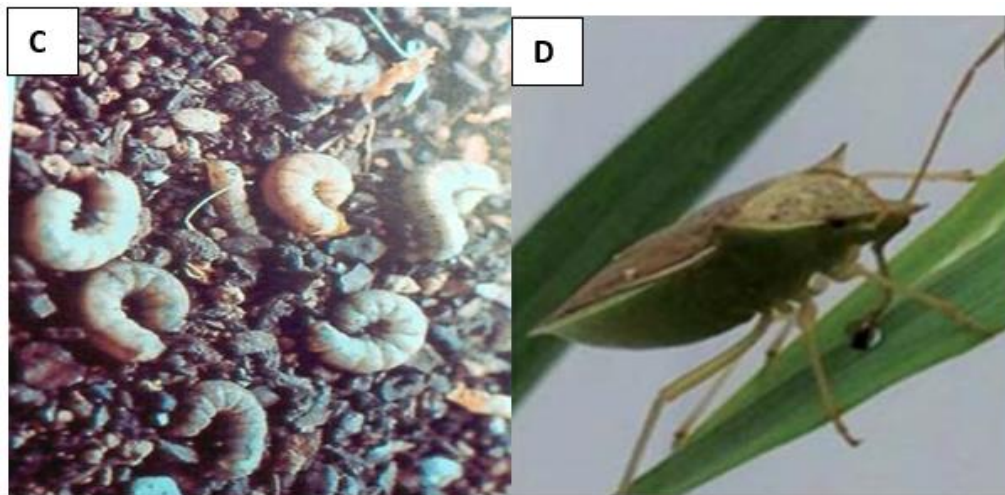


Figure 1: Stem pests (A) Sugarcane borer - *Diatraea saccharalis*; (B) Elasm caterpillar - *Elasmoplus lignosellus*; (C) Screwworm - *Agrotins ipsilon*; (D) Green-bellied stink bug - *Dichelops melacanthus*.



Source: Cana online (2020).

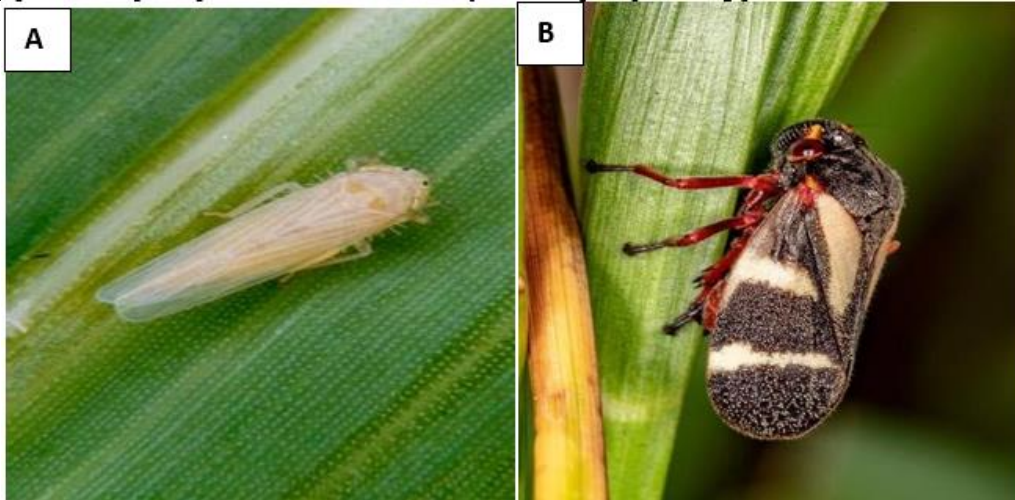
Source: Embrapa (2022).



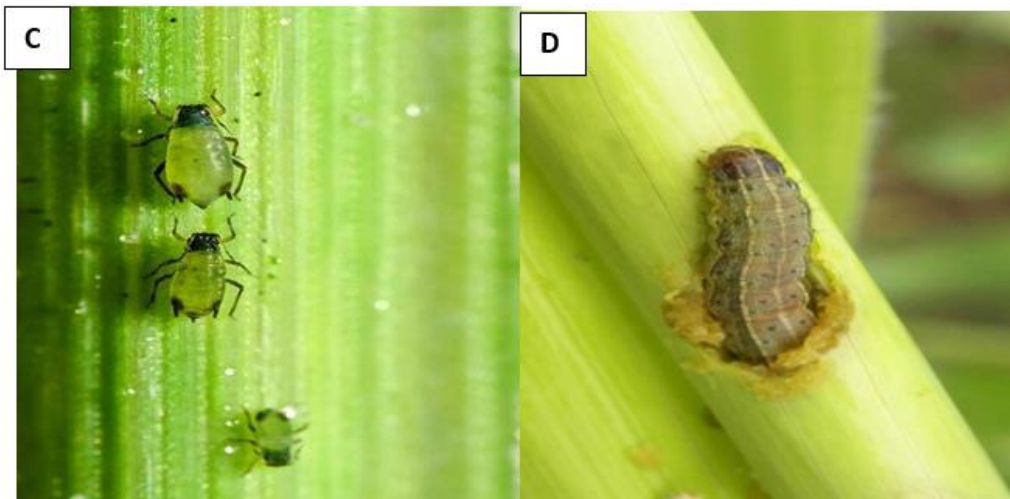
Source: Embrapa (2022). Source: Embrapa (2015).

In the leaf part, there are significant pests in economic damage to the corn crop, sucking insects such as the corn leafhopper (*Dalbulus maidis*) (Figure 2a), which sucks sap from the phloem, reducing the development of the root system, in addition to transmitting phytopathogens, as well as the pasture leafhopper (*Deois flavopicta*) (Figure 2b), which feeds on the leaves causing chlorosis, yellowing, necrosis, and can cause the death of the entire plant (CRUZ, et. al, 2015). On the other hand, the corn aphid (*Rhopalosiphum maidis*) (Figure 2c) sucks the sap of the plant. and eliminates sugary liquid that contributes to the formation of smoke on the leaves, hindering photosynthetic actions, in addition to being vectors of various viruses (SIEG, et. al, 2021). In the case of chewing insects, the fall armyworm (*Spodoptera frugiperda*) (Figure 2d) feeds on leaf tissue, leaving holes in the plant cartridge, and produces the characteristic of a row of perforations on the leaves (VALICENTE, et. al, 2015).

Figures 2: Leaf area pests (A) Corn leafhopper - *Dalbulus maidis* (B) Pasture leafhopper - *Deois flavopicta* (C) Corn aphid - *Rhopalosiphum maidis* (D) Fall armyworm - *Spodoptera frugiperda*.



Source: Embrapa (2020). Fonte: Aegro (2019).



Source: Rural Magazine (2020). Fonte: 3rlab (2021).

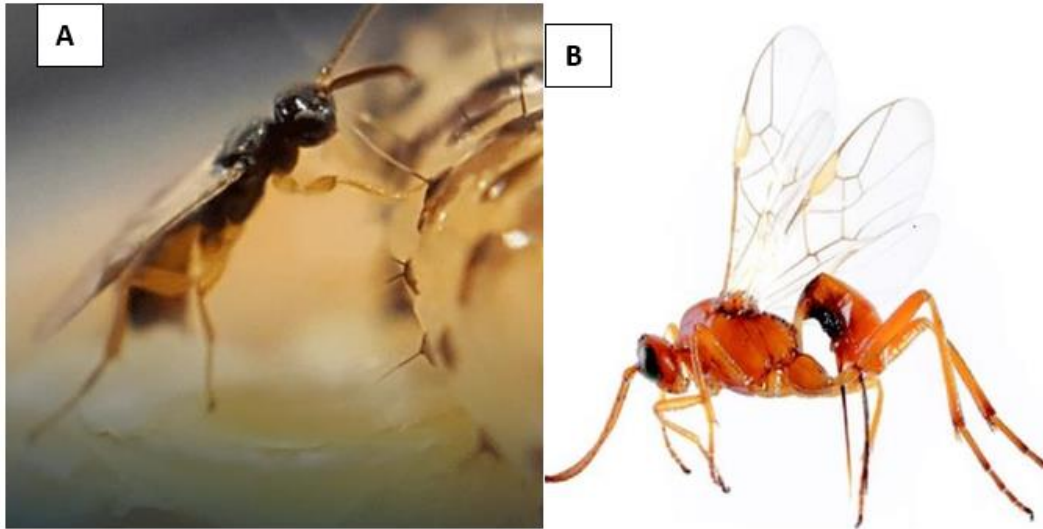
Pest attacks compromise the leaf area, significantly affecting corn crop productivity, and with this it is important to use assertive management, and the identification and monitoring of pests, as they are essential factors to facilitate decision-making, aiming at reducing losses and increasing profitability in corn crops (FILHO, et. al, 2016).

2.4 BIOLOGICAL CONTROL APPLIED TO PESTS IN THE VEGETATIVE PHASE OF MAIZE CROPS

Pest attack compromises the leaf area, significantly affecting the it is notorious that new technologies have been adopted, and through them pests can be controlled in a sustainable way, and biological control fits as a key piece in this category (VALICENTE, et. al, 2015), the use of living beings and natural substances as pest control agents in agriculture, and considered harmful to crops, through the use of other species due to their antagonism, through it, biological techniques in

agricultural production, applying only natural resources, have been present in society for centuries (MONDIN, et. al, 2022).

Figures 3: Biological control by means of parasitoid: (A) *Cotesia flavipes*, parasitoid of the sugarcane borer, (B) *Meteorus laphygmae*, parasitoid of the screwworm (C), *Ectophasiopsis* sp, parasitoid of the green-bellied stink bug.



Source: Genic, 2022

Fonte: Research Gate, 2020



Source: Embrapa, 2015

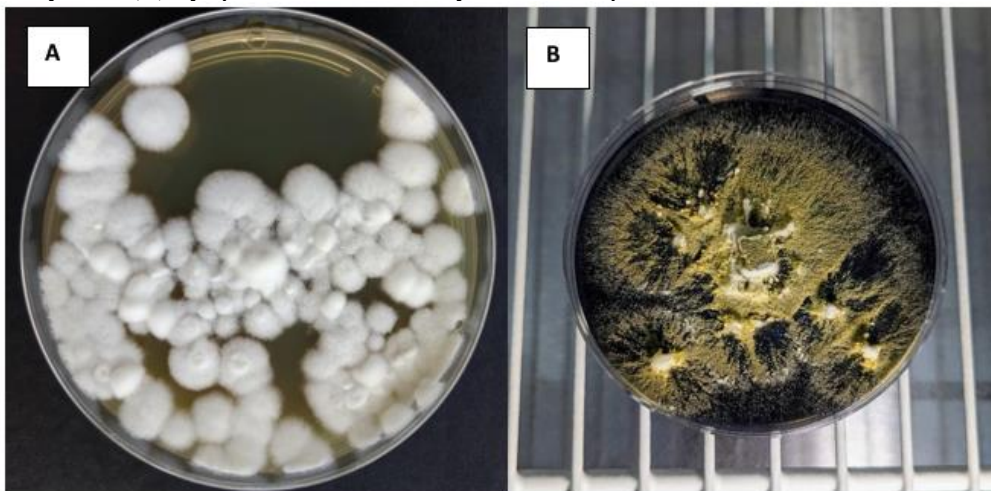
However, the use of biological control in crops to control the grazing of the vegetative part of corn is found in the case of the parasitoid *Cotesia flavipes* of the borer (*D. saccharalis*) (Figure 3a), this parasitism occurs through the ovoposition of the wasp inside the caterpillar, these eggs hatch larvae and they feed on the tissues of the caterpillar that dies exhausted (NAVA, Et. al, 2009), the control of the screwworm can be done by beneficial insects through microhymenoptera and dipterans (Figure 3b) (FILHO, GUIMARÃES, MOURA, 2022), and in the case of the green-bellied stink bug, there is *Ectophasiopsis* sp (Figure 3c), a species of parasitoid fly of the adult stink bug, which lay eggs on the pest's body, characteristic of its light coloration, that after penetrating the insect body they



become darkened, so the larva enters the digestive tract, and feeds on the pest, killing it (PANIZZI, et. al, 2015).

The corn leafhopper, pasture leafhopper and elasmobranch caterpillar can be controlled by entomopathogenic fungi, *Beauveria bassiana* (Figure 4a) and *Metarhizium anisopliae* (Figure 4b) have the ability to infect these insects and use them as hosts for germination of their spores and release of conidia in their corpses after 72 hours of infection (NOGUEIRA, et. al, 2022), (MATIOLI, 2020), (VIANA, 2009), in addition, among several means of sustainable control of fall armyworm, the biological insecticide Baculoviridae (Figure 4c) is mentioned, which after contact with the pest, reduces its feeding and causes death (CRESPO, et. al, 2021), and the aphid can be controlled by parasitizing wasps of the genus *Lysiphlebus*, (Figure 4d) that lay eggs inside the pest, and the larvae feed on the inside of the insect, causing death, a mode of action similar to *Cotesia flavipes* (VICENTE, JUNIOR, 2011).

Figure 4: Biological control of pests, through entomopathogens and natural enemy (A) *Beauveria bassiana*, control of the corn leafhopper and elasmobranch caterpillar (B) *Metarhizium anisopliae*, control of the grassland leafhopper (C) Baculoviridae, control of fall armyworm (D) *Lysiphlebus*, natural enemy of the corn aphid.



Source: Embrapa (2017). Source: Embrapa (2017).



Source: Embrapa (2016). Source: Cultivar Magazine (2023).

The release of biological control is determined by the release of a large amount of beneficial insects into the so-called flooded field, and in small areas of the environment quantities and calcified as an inoculant, and it is important to stress that these insects are originated in laboratories (CRUZ, 2022).

3 CONCLUSION

Biological control in the fight against pest infestations in the vegetative phase of corn is a promising alternative for producers, as it reduces the population of agricultural pests, leaving production areas with a population below the control level, thus optimizing decision-making, and causing reduction or replacement of chemical insecticides in crops, favoring agricultural balance and sustainability.

Brazil is among the largest producers of corn in the world, and pests are key to the drop in crop yield, and biological control is relevant, because control agents have the ability to reproduce and survive in the environment, if well managed, thus contributing to farmers, reducing production costs, and assisting in future harvests.

Biological control is a broad means that consists of controlling pests by different means and agents, thus being able to vary in pest control, as different formulations and natural enemies are found on the market for the same cause of damage, helping producers to make a vast decision, in addition, it does not leave residues to employees, consumers and the environment.



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Report on the use of experimental statistics applied to research in an area of the Amazon Region



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ABSTRACT

We understand that the act of research is essential in the learning process and, mainly, in the construction of autonomous and critical individuals. Understanding the appropriate use of statistics leads to fewer errors in reporting results and interpreting conclusions. What we observe today are analyzes of poorly performed experiments and erroneously interpreted results. This justifies that the student

himself, when constructing his article, is capable of carrying out the analyzes and interpreting the results. The project was developed with two students from the third period of the Higher Aquaculture Technology Course at the Federal Institute of Roraima. This work aimed to make students aware of the application of designs used in agricultural experimentation and their applications, through solving a list of exercises and to present the SISVAR computer system with diverse applications in the statistical analysis of experiments, due to its ease of access and use. The students were able to use data obtained from practical examples to: suggest the best design for each exercise, analyze the data and see if they followed the assumptions for carrying out the analysis of variance, formulate hypotheses for the validity of the F test, perform the analysis of variance and apply the F test. Using the F test, they were able to carry out the study of splits and find the best treatment in each exercise based on the Tukey test study or based on the regression study. We concluded that the students acquired the ability to interpret, analyze and resolve the information arising from the data collected during the research and made them aware of the different aspects of applying statistical methods proposed in the project.

Keywords: Experimentation, Aquaculture, Statistics, Amazônia, Fish, Teaching.

1 INTRODUCTION

The planning of strategic development programs in the most diverse areas, in order to be carried out efficiently, must create mechanisms in which data are collected, stored and studied in order to produce information that will guide the necessary guidelines in the appropriate management of such area. In relation to fishing, it could not be different. Unfortunately, Brazil lacks a set of regular monitoring statistics focused on fishing, according to what was mentioned on the Mar Sem Fim (2020) website.



We understand that the act of research is essential in the learning process and, mainly, in the construction of autonomous and critical individuals. Statistical analysis, present in scientific research and reported in original articles, allows the student to interpret the information from the data collected during the execution of the research and thus use it for the benefit of society. The comprehension of the proper use of statistics leads to fewer errors in the reporting of the results and in the interpretation of their conclusions (SILVA, 1995).

Agricultural research aims to improve the performance of agricultural systems in terms of their economic and social implications. The striking characteristic of agricultural systems is their complexity. The consequent complexity of agricultural research is more markedly high in a country with a large geographic scope and high social and economic heterogeneity, such as Brazil.

Environmental variation is a complex problem in achieving the expressiveness of agricultural research, greatly increasing the difficulties in inferences with the purpose of generating recommendations to farmers.

In the particular case of training in statistics, the program aims to enable researchers to use scientific methodology that contributes to the development of more effective research, which can have a relevant impact on the performance of agriculture.

In addition to the knowledge of his/her specific area, the researcher needs to have the appropriate mastery of the methods, techniques and procedures inherent to scientific research, indispensable to the effective development of research in his/her area. These include the statistical method, which is an integral part of the scientific method (SILVA, 1995).

For Stigler (1986), statistical methods were developed as a mixture of science, technology and logic in the solution and investigation of problems in various areas of human knowledge.

The understanding of the proper use of statistics leads to fewer errors in the reporting of the results and in the interpretation of their conclusions.

In recent decades, statistical calculations have been greatly facilitated by the use of computational applications. This allowed complex and time-consuming methods to be routinely applied. However, many researchers have replaced these applications by consulting a professional in the field of statistics. What is observed today are analyses of poorly performed experiments and erroneously interpreted results (SILVA, 2007). This justifies that the student himself, in the construction of his TCC or in an article, is able to perform the analyses and interpret the results.

Several statistical packages are available for the analysis of experiments, such as SAS – Statistical Analysis System – (Sas Institute Inc., 2000), which is, in general, one of the most used programs worldwide in the analysis of data in the agronomic, biological and social areas; STATGRAPHICS – Statistical Graphics System – (STATGRAPHICS, 1999); STATISTICA for Windows (STATISTICA, 2002), among others. National programs can be found in which the reader



can have easier access, among them: SANEST – Statistical Analysis System for Microcomputers – of the Federal University of Pelotas (ZONTA & MACHADO, 1991); SISVAR – Analysis of Variance System – of the Federal University of Lavras (FERREIRA, 2000a); the SAEG – System of Statistical Analysis (RIBEIRO JÚNIOR, 2001) and the GENES – Computational Application in Genetics and Statistics (CRUZ, 2001), both from the Federal University of Viçosa.

This work aimed to make the students of the Aquaculture Technology course of IFRR/CAM, aware of the application of three types of design widely used in agricultural experimentation, namely: completely randomized design - DIC, randomized block design - DBC and Latin square design - DQL and its applications, through resolution of list of exercises and to present the SISVAR computer system with diverse applications in the statistical analysis of experiments, due to its ease of access and use.

2 METHODOLOGY

2.1 Project Proposal

The project was developed with two students from the third period of the Higher Course in Aquaculture Technology at the Federal Institute of Roraima.

It was proposed to the students, as a form of training, that they solve lists of exercises by Banzatto and Kronka (1992). The examples taken from the books served only as a means of training in calculations. It was up to the students to indicate how the planning was thought by the author, indicating the reason for the adopted design; It was also up to them to elaborate the hypotheses to be accepted or rejected in the Sir Ronald Fisher test (F-test) of the analysis of variance, perform the analysis of variance, test the hypotheses and depending on the results of the F-test, followed by a resolution of the unfolding of the test: Tukey's test for qualitative data or regression study in the case of quantitative data. They first solved it using only a scientific calculator and then plotted the data in Microsoft Office Excel and solved it in the SISVAR software.

In the resolution of the exercises, the designs have mathematical models and, to perform the analysis of variance, we must consider whether the mathematical model accepts some hypotheses necessary for its validity of the analysis.

2.1.1 Completely randomized design - DIC

The resolution of a list with 5 exercises involving the design entirely at random was proposed, whose mathematical model is:

$$A - \text{DIC} - y_{ij} = m + t_i + e_{ij}$$

where:



y_{ijk} is the observed value of the trait studied, in treatment i ($i = 1, 2, \dots, I$) and in repetition j ($j = 1, 2, \dots, J$);

m is the overall mean of the experiment;

t_i is the effect of treatment I ;

e_{ij} is the error associated with the observation

y_{ij} or effect of uncontrolled factors on y_{ij} observation.

2.1.2 Randomized Block Design - DBC

At this stage, it was proposed to solve a list with 4 exercises involving the design in randomized blocks. The mathematical model of the randomized block design

$$B - DBC - y_{ji} = m + t_i + B_j + e_{ij}$$

where:

y_{ij} is the observed value of the trait studied, in treatment i ($i = 1, 2, \dots, I$) and is found in block j ($j = 1, 2, \dots, J$);

m is the overall mean of the experiment;

t_i is the effect due to treatment i , which has been applied to the plot;

B_j is the effect due to the block j in which the plot is located

e_{ij} is the effect of uncontrolled factors on the plot that received treatment I in block J .

2.1.3 Latin Square Design - DQL

At this stage, the resolution of a list with 5 exercises involving the Latin square design was proposed. The mathematical model of the Latin square design.

$$C - DQL - y_{jik} = m + l_i + c_j + tk(ij) + e_{ijk}$$

where:

y_{ijk} is the value observed in the parcel that received treatment k and which is found in row i and column j ;

m is the overall mean of the experiment;

l_i is the effect of line i in which the plot is located;

c_j is the J column effect, which has been applied to the plot;

$tk(ij)$ is the treatment effect k and which is found in row i and column j ;

e_{ijk} The effect of uncontrolled factors on the plot that received treatment I in block J .



It is not uncommon to find that one or more of the basic hypotheses is not verified, and then, before proceeding to the analysis of variance, the experimental data must be transformed in such a way that the basic assumptions are met (BANZATTO and KRONKA, 1992).

The basic hypotheses to be valid in analysis of variance are:

A- Additivity: the effects of factors occurring in the mathematical model must be additive.

B- Independence: errors or deviations due to the effect of uncontrolled factors must be independent.

C- Homogeneity of variances: errors or deviations because of uncontrolled factors must have a common variance σ^2 .

D- Normality: errors or deviations due to the effect of uncontrolled factors should have a normal distribution of probabilities.

This implies that the experimental data fit the normal probability distribution.

One of the most frequent cases of non-satisfaction of the basic hypotheses is the one in which there is no homogeneity, that is, the variance is not the same in the different treatments (BANZATTO and KRONKA, 1992).

2.1.4 Simple linear regression and correlation study

When it is desired to study the simultaneous behavior of two or more variables, Regression and Correlation analysis are used to evaluate the desired information. In regression, we estimated the relationship of one variable with another, expressing the dependent variable as a function of the independent variable. Regression studies sets of variables that are supposed to be in a cause-and-effect relationship (AZEVEDO, 2016). It is the study of the behavior of a dependent variable (Y_i) as a function of the variation of one or more independent variables (X_i, Z_i, W_i, \dots) assuming that these variables are in a cause-and-effect relationship (Azevedo, 2016). Correlation, which is sometimes confused with regression, studies the degree to which two or more variables vary simultaneously. That is, the degree of interrelationship between the variables: dependent variable and the independent variable. Regression and correlation methods cannot be applied to qualitative variables (attributes). The variables need to be continuous. According to the regression function, we can have: Rectilinear, Exponential, Parabolic, Potential Correlation (AZEVEDO, 2016).

2.2 USE OF THE SISVAR PROGRAM

The Sisvar program is a statistical analysis system that can also be used in teaching. SISVAR is one of the most widely used Brazilian statistical analysis programs in the country, either directly in the statistical analysis of scientific works from the most different areas of scientific knowledge or in the teaching of basic and experimental statistics. Sisvar has a number of competitive advantages, the



main one being its great interactivity with the user, providing a simple, efficient, powerful, very robust and accurate environment. These are the main reasons for its enormous use by a large part of the scientific community (FERREIRA, 2000).

3 RESULTS AND DISCUSSION

In the IHD design, the students performed the resolution of Banzatto and Kronka (1992).

In an experiment aimed at controlling the aphid (*Apis gossypii* Glover) in cucumber culture, Macedo (1970) used 6 replicates of the following treatments:

A - Witness; B - Ethyl azines; C - Supracid 40CE dose 1; D - Supracid 40CE dose 2 and E - Diazinon 60CE.

The data obtained regarding the number of aphids collected 36 hours after spraying are shown in Chart 1.

Table 1. Number of aphids collected 36 hours after spraying.

Kicked.	Repetitions						S2
	1	2	3	4	5	6	
A	2.370	1.687	2.592	2.283	2.910	3.020	233.749,60
B	1.282	1.527	871	1.25	824	920	75.558,80
C	562	321	636	317	485	842	40.126,17
D	173	127	132	150	129	227	1.502,27
E	193	71	82	62	96	44	2.791,87

Source: Banzatto and Kronka, 1992.

As the data were counts, it was suggested that the Hartley test be performed to verify the assumption of homogeneity of the variances.

$$H_c = S^2 \max / S^2 \min = 233.749,60 / 1.502,27 = 155,60^{**}$$

According to the Hartley test table, for 5 groups and 5 degrees of freedom, $H_{5\%} = 16.3$ and $H_{1\%} = 33.0$ were obtained. Since $H_c (155.60) > H (5.5)$ 1% (33.0) were rejected, the hypothesis of homogeneity of variances was rejected, i.e., the data did not follow the normal distribution; Then the data transformation was done. The data in Table 3.2.1 were transformed using the transformation $(\ln x)$. After the transformation of the data, the Hartley test was performed again with the transformed data, and it was found that the data then had a normal distribution after its transformation $H_c (5.62^{ns}) < H (16.3)$ 5% and $H (33.0)$ 1% , so the analysis of variance can be performed (table below).



Table 2. Analysis of variance with transformed data (ln x), aiming at the control of aphids in cucumber culture.

Sources of variation	G.L	Q.M.	Fc
Treatments	4	11,4785	103,92**
Error	25	0,1105	

Source: Banzatto and Kronka, 1992. **significant at 1% probability by the F-test.

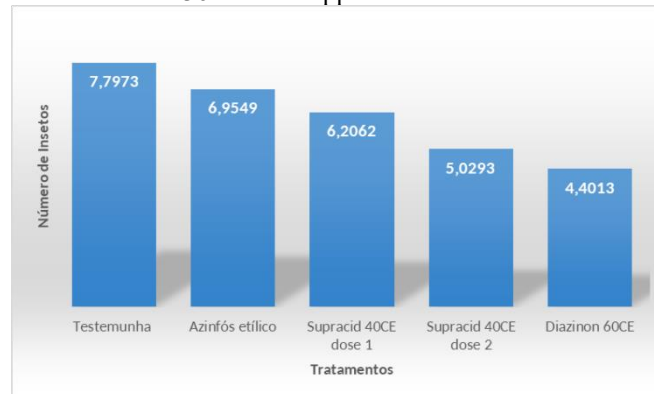
As the Fc value exceeds the critical value at the level of 1% probability, it is significant ($P < 0.01$). Chart 2, it is concluded that the insecticides tested have different effects on the number of aphids collected 36 hours after spraying in the cucumber crop. The mean of the treatments was calculated and the Tukey test was performed on the mean of treatments to verify which of the treatments controlled the number of aphids in the cucumber culture the most, chart 3.

Table 3. Average of treatments and Tukey's test

Treatments	Transmolded media	Unmolded Medium
Witness	7,7973 a	2.434
Ethyl azinfos	6.9549 B	1.048
Supracid 40CE dose 1	6.2062 C	496
Supracid 40CE dose 2	5,0293 d	153
Diazinon 60CE	4.4013 and	82
DMS	0,1357	
D	0,5645	

Means followed by the same letter did not differ from each other according to Tukey's test ($P > 0.05$); DMS minimal significant difference, Δ Tukey (5%). Source: author's elaboration

Figure 1. Aphid numbers after 36 hours of application of the treatment on cucumber plants.



Source: author's elaboration

Chart 3 and Figure 1 show that the insecticide that best controlled the number of aphids in the cucumber crop was diazinon 60CE (Trat. E), which had the lowest number of aphids. The coefficient of variation statistic was also calculated, resulting in $c.v.=5.47\%$. This coefficient of variation of 5.47%, very low in agriculture, indicates small variation of the data or great precision when we affirm that the insecticide was Diazinon 60CE was the one that best controlled the aphid population in cucumber plants under the conditions studied.



In the same way, it was suggested to the students to solve four more exercises in a completely random design and to perform the analyses using a scientific calculator, Microsoft Office Excel and SISVAR software.

Next, the students were asked to perform the analysis of the experiment in a randomized block design. The experiment by Cunha (2019) was used as a practice, chart 4. Effect of supplement use in cow feed on milk production in kg.

Table 4. Milk production values (kg)

Blocks	Treatments			
	No add-on	Cassava	Araruta	Sweet Potato
Gear	6,4	10,9	12,0	11,2
Dutch	6,2	11,6	10,9	11,6
Jersey	6,2	11,4	11,5	10,9
Nellore	7,1	10,4	11,1	12,1
Guzerá	6,6	12,4	11,8	10,1

Source: Cunha, 2019.

The students used the raw data (CUNHA, 2019) to formulate the hypotheses of the model (DBC), performed the analysis of variance, tested the hypotheses through the F test and made the unfolding through the Tukey test, once they accepted the hypothesis that there are different responses in milk production with the use of supplements, which was observed in the F test of the analysis of variance, Table 5.

Table 5. Analysis of variance for milk yield (kg).

Sources of variation	G.L	Q.M.	Fc
Treatments	3	29,187	60,06**
Blocks	4	0,0305	
Error	12	0,486	

Source: Cunha, 2019. **significant at 1% probability by the F-test.

After the F statistics, the critical values of F were searched in the tables at the level of 1% probability, obtaining: for 3.12 g.l. (1% = 5.95). Comparing the F stats with the critical values, it is concluded that the F test was significant at the level of 1% probability; hypothesis H_1 was accepted and it was concluded that at least two of the treatments differed from each other in relation to milk production (kg). As the treatments are qualitative and the F test was significant, the Tukey test was performed to find out where this difference was between treatments in Frame 6.



Table 6. Average of treatments and Tukey's test

Treatments	Transmolded media
Twelve-Beat	11,46 a
Araruta	11,34 a
Cassava	11,18 a
No add-on	6,50 b
DMS	1,31
D	4,20

Means followed by the same letter did not differ from each other according to Tukey's test ($P>0.05$); DMS significant minimal difference, Δ Tukey. Source: author's elaboration

By Tukey's test at 5% probability, it was observed that milk production increased with the supplementation of roots and tubers in the animals' diet. The three treatments with supplementation were equal to each other in increasing milk production and superior to the treatment without supplementation. The coefficient of variation statistic was also calculated, $c.v. = (\text{standard deviation/overall mean}) * 100$. The coefficient of variation statistic was also calculated, resulting in $c.v.=6.89\%$. This coefficient of variation of 6.89%, which is very low in agriculture, indicates a small variation in the data or great precision, when we affirm that the supplementation in the diet of the animals increased milk production.

As previously reported, the students performed the calculations using a scientific calculator, then plotted the data in Microsoft Office Excel and performed the analyses again in the SISVAR software. And also, as a form of training, they solved four more exercises.

Another very common type of design used in animal areas is the Latin square design - DQL, when there are two experimental conditions (physical or biological) heterogeneous enough to interfere with the inferences of the treatments to be tested. In this case, the blocks are arranged in two different ways, one constituting rows and the other columns.

It was proposed to the students to perform the analyses based on experiments Toledo Del Pino (2017).

In a forage cane competition experiment, Toledo Del Pino (2017), 5 varieties of forage cane arranged in a 5x5 Latin square were used. Forage cane varieties: A - CO290, B - CO294, C - CO297, D - CO299 and E - CO295.

The control was carried out by means of horizontal and vertical blocks and aimed to eliminate influences due to differences in fertility in two directions. The yields, in kg/plot, were as follows: Table 7:



Table 7. Production of 5 varieties of forage cane in kg/plot

Lines	Columns				
	1	2	3	4	5
1	432(D)	518(A)	458(B)	583(C)	331(E)
2	724(C)	478(E)	524(A)	550(B)	400(D)
3	489(E)	384(B)	556(C)	297(D)	420(A)
4	494(B)	500(D)	313(E)	486(A)	501(C)
5	515(A)	660(C)	438(D)	394(E)	318(B)

Source: Toledo del Pino, 2017.

The students formulated the hypotheses of the model, performed the analysis of variance, first in the calculator and then in the SISVAR application, and drew the conclusions.

Table 8. Analysis of variance for milk production (kg/plot).

Sources of variation	G.L	Q.M.	Fc
Treatments	4	34372,06	12,09**
Lines	4	7620,16	
Blocks	4	13910,16	
Error	12	2842,8933	

Source: Toledo del Pino, 2017. **significant at 1% probability by the F-test.

They concluded that there were differences in yield between the five varieties of forage cane test F at 1% probability Chart 8. They performed Tukey's test at 5% probability frame 9, and calculated the coefficient of variation, c.v. = 11.33%.

Table 9. Average of treatments and Tukey's test.

Forage cane varieties	Transmolded media
CO297	604,8 a
CO290	492,6 b
CO294	440,8 b
CO299	413,4 b
CO295	401,0 b
DMS	107,5
D	4,51

Means followed by the same letter did not differ from each other according to Tukey's test ($P > 0.05$); DMS significant minimal difference. Source: author's elaboration

Conclusion: the forage sugarcane variety CO297 was the one with the highest production and the others had statistically lower production than CO297 and similar among themselves.

Next, an analysis of variance was proposed, studying linear regression with data from Ragazzi (1979).

Effect of gypsum doses on common bean (*Phaseolus vulgaris* L.) Ragazzi (1979) used a completely randomized experiment with 4 replications to study the effects of 7 gypsum doses: 0, 50, 100, 150, 200, 250 and 300 kg/ha on several characteristics of the common bean plant 10.



Table 10. Weight of 1,000 seeds, in grams.

Gypsum doses kg/ha	Repetitions			
	1	2	3	4
0	134,8	139,7	147,6	132,3
50	161,7	157,7	150,3	144,7
100	160,7	172,7	163,4	161,3
150	161,3	168,2	160,7	161,0
200	165,7	160,0	158,2	151,0
250	171,8	157,3	150,4	160,4
300	154,5	160,4	148,8	154,0

Source: Ragazzi (1979).

Analysis of variance was performed, Chart 11. Since F_c was significant, and since the treatments were quantitative, a regression study was performed using the SISVAR program.

Table 11. Analysis of variance for milk production (kg/plot).

Sources of variation	G.L.	Q.M.	F_c
Treatments	6	302,055	7,39**
Error	21	40,84	
C.V.%	4,09	Overall Average	156,09

Source: Ragazzi (1979). **significant at 1% probability by the F-test.

Chart 12 shows that the first and second degree equations were significant, at 1% probability by the F test.

Table 12. Sequential Sums of Squares - Type I.

Sources of variation	G.L.	S.Q.	Q.M.	F_c	Pr>F
b1	1	423,154375	423,154375	10,361**	0,004
b2	1	1156,25601	1156,25601	28,312**	0,000
Regression deviation	4	232,915595	58,228899	1,426	0,260
Error	21	857,650000	40,840476		

Source: author's elaboration

Observing the results, chart 13, it can be seen that there is a tendency for an increasing response to a certain extent, and then decreasing.

Table 13. Independent variable, observed mean and estimated means.

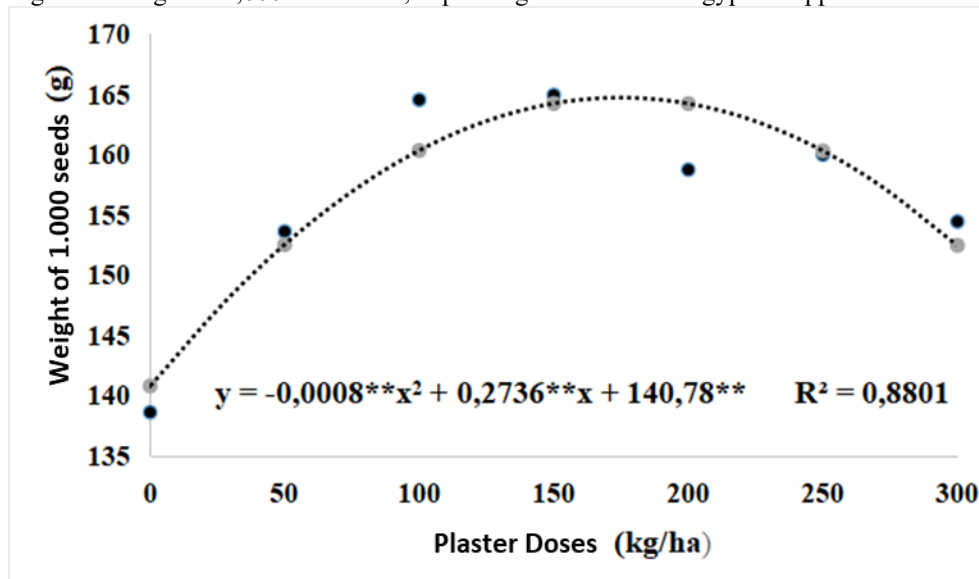
Values of the independent variable	Observed averages	Estimated averages
0,000	138,6000	140,9863
50,000	153,6000	152,2053
100,000	164,5250	159,7143
0,000	162,8000	163,5131
200,000	158,7250	163,6018
250,000	159,9750	159,9803
300,000	154,4250	152,6488

Source: author's elaboration



As the treatments are quantitative, the study was carried out using regression analysis as shown in figure 2.

Figure 2. Weight of 1,000 bean seeds, depending on the doses of gypsum applied to the soil.



Source: author's elaboration

Since the first derivative of equation $y = -0.0008x^2 + 0.2736x + 140.78$ is negative, the equation passes as maximum. The gypsum dose in the soil, of 171 kg/ha, was the one that produced the highest weight of 1,000 bean seeds of 164.1728 grams

4 CONCLUSIONS

The students were able to understand the difference between each of the designs: DIC, DBC and DQL, since in the resolution of each proposed exercise it was necessary to understand the type of design involved, since the form of resolution changes according to the most appropriate design. Once the exercise was solved, the students arrived at the analysis table called the analysis of variance table, in which they had to confront the hypotheses mentioned above. And depending on the accepted hypothesis, the exercise continued with the development of other calculations called Tukey's test or regression study, calculation of the standard error and calculation of the coefficient of variation; with resolution and interpretation of what each of the statistics means. We conclude that the students acquired the ability to interpret, analyze and solve the information from the data collected during the execution of the research and made them aware of the various aspects of the application of statistical methods proposed in the project.



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Chemical composition of brazilian pennyroyal essential oil (*Mentha pulegium* L.) and evaluation of the antimicrobial effect on *Staphylococcus aureus* and *Escherichia coli*



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ABSTRACT

The extraction of the EOs was first obtained by hydrodistillation, then their chemical composition was determined by GC/MS and their antimicrobial activity was evaluated. In the analysis and identification of the hydrodistilled essential oil of *Mentha pulegium* L. by gas chromatography and mass spectroscopy, 12 compounds were separated, representing 99.31% of the total mass of the essential oil. The majority component was pulegone (80.553%), other components present in appreciable levels were; Piperitone (5.430%); Cyclohexanone,5-methyl-2-(1-methyl)-,(2R-cis) (3.304%); trans-Isopulegone (2.945%); α -Humulena (1.950%). In addition, the screening of the essential oil reveals that there were appreciable results regarding its antibacterial activity against *S. aureus* 20 $\mu\text{g/mL}$ and *E. coli* 2.5 $\mu\text{g/mL}$. This result was obtained by the undiluted essential oil. However, more research on the factors that influence the biosynthesis and bioactivity of essential oils is needed as essential oils gain



important applications in the food and pharmaceutical industry.

Keywords: Antioxidant, Lamiaceae, Antibacterial properties, CBA, CG/MS.

1 INTRODUCTION

A variety of different chemical and synthetic compounds have been used as antimicrobial agents. However, the indiscriminate and widespread use of antimicrobials has led to a number of ecological and medical problems. Thus, one of the most important therapeutic challenges involves the treatment of infectious diseases caused by bacteria. After the discovery of penicillin and the subsequent expansion of its application in treatment, new antibiotics are continuously developed to fight infections. This has resulted in the expansion of the use of natural products such as synthetic antibiotics in the therapy of infections. However, the overuse of these antimicrobials is associated with increased resistance to different antibiotics in most bacteria (SALES *et al.*, 2015; SALES *et al.*, 2017). Several authors have tested essential oils from aromatic plants to extend the shelf life of foods (KOSTAKI *et al.*, 2009), while others have focused on antioxidant and antimicrobial action, and the properties of plant extracts and essential oils (CAO *et al.*, 2009).

The Lamiaceae family is known for harboring several species of menthol plants, mainly in temperate countries, with a global distribution, containing 7,886 species and are appreciated for their antibacterial, antifungal, antioxidant, antiviral, and biopesticide properties (SANTOS, 2022; SCHELLENBERGER *et al.*, 2023). In Brazil, this group is represented by 71 genera and 589 species, mainly found in open plant formations (OLIVEIRA *et al.*, 2023). This botanical family contains many species that possess distinct organoleptic properties due to the presence of secondary metabolites, especially essential oils found in the leaves, flowers, and fruits. This gives this family a great economic appeal, especially in sectors such as the food industry. In addition, in the perfume industry, species such as lavender (*Lavandula angustifolia* Mull.) and mint (*Mentha spp.*) are highly valued for their aromatic essential oils (OLIVEIRA, 2022; ANTAR *et al.*, 2023).

The genus *Mentha* has 30 species that grow in temperate regions of Eurasia, Australia, South America, and South Africa. Mint species have great relevance both from a medicinal and commercial point of view. The leaves, flowers, and stems of *Mentha spp.* are often used in herbal teas or as additives in commercial spice blends for various foods, providing aroma and flavor. In addition, *Mentha spp.* has been employed as a folk remedy for the treatment of conditions such as nausea, bronchitis, flatulence, anorexia, ulcerative colitis and liver problems, due to its anti-inflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue and anticatarrhal properties (DESCHAMPS *et al.*, 2006; HADJLAOUI, 2009; VILAR *et al.*, 2023).

Pennyroyal (*Mentha pulegium* L.) is a herbaceous, perennial plant that has evergreen leaves. The flowers, with a typically bilabiate gamopetala corolla, are grouped in small clusters. The fruit is a



small hard tetra-achene, the secretory structures are located on the leaves and stems, can reach up to half a meter in height, is naturalized in America and thrives in Western, Southern and Central Europe, Asia, Iran, Arab countries and Ethiopia (ALAPETITE, 1981; GRUENWALD *et al.*, 2000). It is a kind of great popularity and has already had a wide use in the food industry as a preservative ingredient. The antimicrobial properties of this plant are due to the potent compounds pulegone and 1-8 cineole (MAHMUDI *et al.*, 2011). In addition, it is known for its perfume and flavor in traditional medicine (RODRIGUES *et al.*, 2013). The essential oil and dry parts in the form of infusions, teas or powder, are used in medicine to treat digestive disorders, liver and gallbladder problems, amenorrhea, gout, colds, increased urination, skin diseases and also as abortifacients (AHMED *et al.*, 2018). They are also used in gastronomy, aromatherapy and the cosmetics industry, highlighting their versatility and applicability in different areas (GRUENWALD *et al.*, 2000; AGNIHOTRI *et al.*, 2005).

The essential oil of *Mentha pulegium L.* has demonstrated several pharmacological effects, including abortifacient activity in the myometrium of rats, cytotoxicity against different human cell lines, and antioxidant properties, the antibacterial effect of *M. pulegium* in Gram-positive bacteria is greater than in Gram-negative bacteria (MAHBOUBI; HAGHI, 2008; JAVANMARD *et al.*, 2018).

Detailed studies have revealed that the qualitative composition of *Mentha pulegium L.* oils depends on the geographical origin and the specific ecological sites from where the plant material is collected for distillation. Additionally, the quantitative composition of the oil can vary greatly depending on the growing region and the country of origin. For example, *Mentha pulegium L.* oil from Bulgaria was found to have a high percentage of pulegone (42.9-45.4%), while oil from Uruguay contained mostly pulegone (73.4%) and isolontone (12.9%). In Egypt, the oil contained pulegone (43.5%) and pipestone (12.2%), while in Tunisia, the main constituents were pulegone (41.8%) and isolontone (11.3%). In Iran the main constituents of *M. pulegium* were piperitone (38.0--%), piperitenone (33.0%). These studies identified three distinct chemotypes of *Mentha pulegium L.*, characterized by the following main components of the oil: (1) pulegone, (2) piperitenone and/or piperitone and (3) isomentone/neoisomenthol, in Algeria the marjoritarian compounds were pulegone (38.81 %) and menthol (19.24 %) (BIGO DE GROSSO; MOYNA, 1985; STOYANOVA *et al.*, 2005; EL-GHORAB, 2006; MKADDEM *et al.*, 2007; MAHBOUBI; HAGHI, 2008; TOPALOV; DIMITROV 1969, COOK *et al.*, 2007; BOUKHEBTI *et al.*, 2011).



TABLE 1 – MAIN COMPONENTS OF THE ESSENTIAL OIL OF *M. pulegium* IN DIFFERENT COUNTRIES OF CULTIVATION

Compound	Country of origin	Author
the moon (73.4%)	Uruguay	Lorenzo <i>et al.</i> (2002)
The moon (43.5%)	Egypt	El-Ghorab (2006)
power (42,9–45.4%)	Bulgaria	Stoyanova <i>et al.</i> (2005)
The moon (41.8%)	Tunisia	Mkaddem <i>et al.</i> (2007)
the moon (38.81%)	Algeria	BoukhebtI, <i>et al.</i> (2011)
piper (38.0%)	Iran	Mahboubi; Haghi (2008)

Source: Author's production (2023).

Biologically active compounds (BAC) found in plants have aroused increasing interest as highly desirable natural agents in various applications (LUGO, 2010). CBAs found in plants are characterized by their significant amounts of organoleptic and biochemical properties and play a key role in sensory quality in their health benefits (CODEVILLA, 2015). In addition, the secondary metabolites of plants are widely used in traditional medicine due to their well-established potential in terms of pharmaceutical and biological properties, including anti-inflammatory, neuroprotective, antioxidant, and antidiabetic activities. (PACHECO; ALVES, 2020).

The use of essential oils (EOs) by humans dates back more than 60,000 years, with its main focus on medicinal practices and religious rituals. Essential oils are aromatic and volatile liquids, which means that they evaporate quickly when exposed to rising temperatures. They consist of a complex mixture of organic compounds, containing only carbon, hydrogen, and oxygen atoms. These components can belong to a variety of classes of compounds, but the classes commonly found are terpenes and phenylpropenes. They are often extracted from plant materials such as leaves, flowers, roots, bark, and seeds. These oils are known for their distinctive and generally pleasant scents, and they have a wide range of applications in aromatherapy, the fragrance industry, health care, and more (SILVA *et al.*, 2009; SILVEIRA *et al.*, 2012),

The antibacterial properties of essential oils and their components are explored in several commercial products, such as dental root canal sealants, antiseptics, and feed supplements for lactating sows and weaned piglets. Some food preservatives containing EO are already commercially available (BURT, 2004).

The antimicrobial activity of EOs depends on their chemical composition. They are usually characterized by two or three main components in fairly high concentrations (up to 80%) compared to other components present only in trace amounts (FERNANDES, 2017). However, there is evidence that EOs are more strongly antimicrobial than is explained by the additive effect of their main antimicrobial components (LIMA, 2022). The smaller components seem to play a significant role. Thus, it is not clear which constituents or mixtures of them are mainly responsible for their antimicrobial activity (SANTOS, 2020). Thus, this study investigated the chemical composition of the



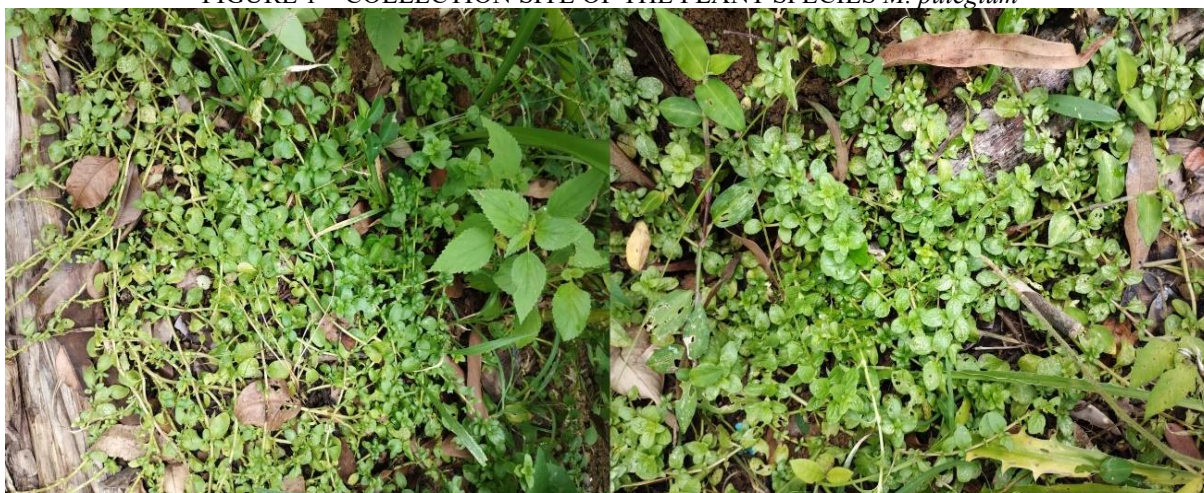
essential oil of the leaves and stems of *Brazilian M. pulegium* and the antibacterial effects on *Staphylococcus aureus* and *Escherichia coli*.

2 MATERIALS AND METHODS

2.1 COLLECTION OF PLANT MATERIAL

The pennyroyal plants were collected at the Jaqueira Agroecology Site (20°45'S - 41°31'W), about 287m above sea level, located in the municipality of Alegre/ES. On August 26, 2023, at 7:30 a.m., with cloudy skies and overcast by rain, on the new moon. The material was collected and stored in a black plastic bag and taken to the Chemistry Laboratory 4 of the Federal University of Espírito Santo – Alegre Campus.

FIGURE 1 – COLLECTION SITE OF THE PLANT SPECIES *M. pulegium*



Source: Authors' collection (2023).

2.2 EXTRACTION OF *MENTHA PULEGIUM* ESSENTIAL OIL

The plant parts were then washed under running water to eliminate soil and other surface contaminants. After removing the excess water with paper towels, the plant material was cut into small pieces with the help of scissors. 910g of leaves and stems of *M. pulegium* were used.

FIGURE 2 – PREPARATION OF THE RAW MATERIAL FOR THE EXTRACTION OF THE EO



Source: Authors' collection (2023).

The methodology used in this study for EO extraction was adapted to the technique employed by Behbahani *et al.*, (2013), in which the hydrodistillation technique was used for 16 hours with 1,500 ml of distilled water in a modified Clevenger device. The chopped parts were placed in natura in the 1 L round bottom flask and covered by distilled water, as a heat source an electric blanket was used and the distillation process was carried out by steam drag to extract the essential oil of *M. pulegium*. The oil obtained was collected and stored in an amber glass flask with screw cap in a 5°C refrigerator before the analyses.

FIGURE 3 – STEAM DRAG DISTILLATION PROCESS



Source: Authors' collection (2023).



2.3 CHEMICAL COMPOSITION OF PENNYROYAL ESSENTIAL OIL BY GAS CHROMATOGRAPHY (GC/MS)

The essential oil of *M. pulegium* was analyzed by gas chromatography-gas coupled to mass spectrometry (GC/MS) in the Shimadzu QP2010-Plus device, located in the Analytical Center of the Food and Nutrition Engineering Building. The following chromatographic conditions were used in both analyses: fused silica capillary column (30 m x 0.25 mm) with Rtx-5MS® stationary phase (0.25 µm film thickness); N₂ (in the GC/FID analysis) and He (in the GC/MS analysis) as carrier gas with a flow rate of 3.0 mL/min; the oven temperature followed a schedule in which it remained for 3 minutes at the initial temperature of 40 °C and then gradually increased by 3 °C/minute until it reached 240 °C, remaining at this temperature for 5 minutes; injector temperature of 250 °C; detector temperature 280°C; Split ratio of 1:30.

GC/MS analyses were performed on equipment operating with electronic impact with impact energy of 70 eV, scan speed of 1000, scan interval of 0.50 fragments/second and detected fragments from 29 to 400 (m/z). The identification of the chemical components of the essential oil was performed by comparing its mass spectra with those available in the Willey7, NIST05 and NIST05s spectrolibraries databases with the co-injection of standards and by the RI (Linear Temperature Programmed Retention Indexes) retention indexes. A mixture of linear n-alkanes (C₇ to C₄₀) was used to calculate Id. The RI calculated for each compound was compared with values in the literature (ADAMS, 2007). The relative percentage of each compound was calculated for all constituents of the sample. Gas chromatography analyses obtained data with a flame ionization detector (GC/FID). Compounds with a relative area greater than 1% were considered for the essential oils under study.

FIGURE 4 – SHIMADZU QP2010-PLUS DEVICE



Source: Authors' collection (2023).



2.4 BACTERIAL STRAINS

The bacterial strains used in this study were *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923).

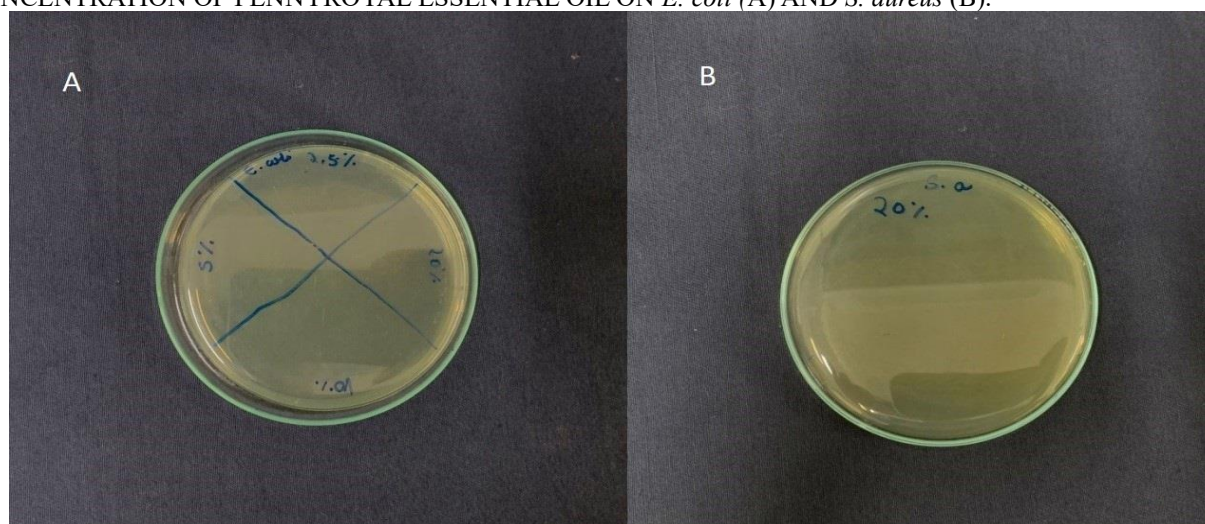
2.5 DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

The determination of the MIC was performed by the modified broth microdilution method (CLSI, 2015). The assays were performed in sterile polystyrene microplates with 96 round-bottomed wells, in triplicate. The final volume of the final reaction mixture was 200 μ L, the culture medium used was Mueller Hinton Broth (CMH) and the final concentration of the inoculum in the test was standardized to 5×10^5 CFU/ml. The tested EO concentrations ranged from 0.01% to 20% (v/v) [(the final concentration was 0.005% to 10% (v/v)]. The EO solutions were added with 2.5% DMSO to facilitate the solubilization of EO in aqueous solution. After assembly, the microplates were incubated at $35 \pm 2^\circ\text{C}$ for 20-24 h. The MIC was considered to be the lowest EO concentration capable of completely inhibiting bacterial growth (absence of precipitate or turbidity in the culture medium or precipitate $\leq 2\text{mm}$) after the 24-hour incubation period. The microplate had a sterility control (no inoculum), growth control (inoculum + culture medium), solvent control (inoculum + culture medium with DMSO) and a positive control (inoculum + culture medium with DMSO + gentamicin), all subjected to the same growing conditions.

2.6 EVALUATION OF THE ACTION OF ESSENTIAL OIL IN THE INHIBITION OF BACTERIAL GROWTH: BACTERICIDAL OR BACTERIOSTATIC ACTION

After the microdilution test in liquid medium, the MIC wells were plated, 2 concentrations higher than the MIC of the essential oil and the positive control, in Petri dishes containing 25 ml of nutrient agar. The plates were incubated at $35 \pm 2^\circ\text{C}$ for 24 hours. The result was defined by the presence or absence of colony formation, and the action was classified as bacteriostatic or bactericidal, respectively.

FIGURE 5 – NUTRIENT AGAR SUBCULTURE TO DETERMINE THE MINIMUM BACTERICIDAL CONCENTRATION OF PENNYROYAL ESSENTIAL OIL ON *E. coli* (A) AND *S. aureus* (B).



Source: Authors' collection (2023).

3 RESULTS AND DISCUSSION

3.1 CHEMICAL COMPOSITION OF *MENTHA PULEGIUM* ESSENTIAL OIL

12 compounds were separated by GC/MS, representing 99.31% of the total mass of essential oil of *M. pulegium*. The general chemical profile of the essential oil, retention time, retention index, and oil area are summarized in (Table 2).

TABLE 2 – CHEMICAL COMPOSITION OF *M. pulegium* essential oil

COMPOUND	TR*	GO**	%***
1. Pulegona	1265	1233	80,553
2. Piperitenona	1363	1340	5,430
3. Ciclohexanona,5-methyl-2-(1-methylethyl)-,(2R-cis)	1172	0	3,304
4. trans-Isopulegona	1185	1179	2,945
5. α -Humulena	1478	1452	1,950
6. D-Limonene	1028	1024	1,501
7. β -cariofileno	1442	1417	1,269
8. 3-octanol	1000	0	1,036
9. α -Pinene	929	932	0,588
10. Cyclohexanone	1186	0	0,582
11. β -Pinene	973	974	0,418
12. β -methylpropyl	796	721	0,415

*Retention time (default)

**Retention rate
Area %

Source: Authors' production (2023).

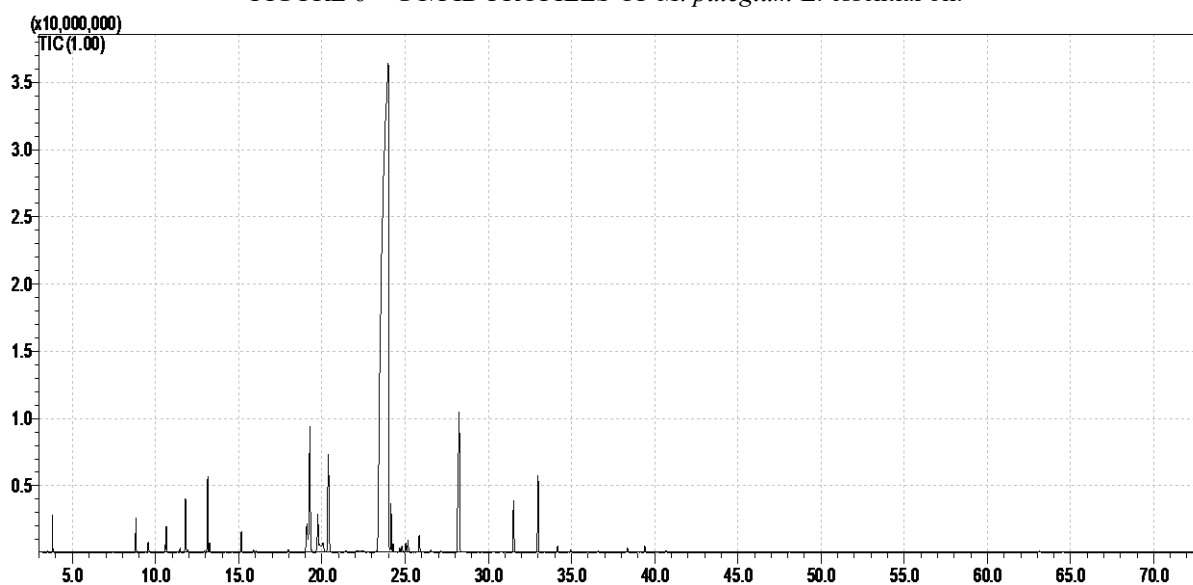
The antimicrobial activity of pennyroyal essential oil has been attributed to its main compounds, such as the high content of pulegon, iso-mentone, menthol, and piperite as reported by Hadjlaoui *et al.*, 2009, or to the high concentration of piperite and the synergistic effects of other constituents (MAHBOUBI; HAGHI, 2008). Even the content of α -Humulena has been presented as an explanation for the antimicrobial activity of this EO (INOUYE *et al.*, 2001). Whatever the case, in



general, oxygenated monoterpenes are significantly more active than monoterpene hydrocarbons (CARSON; RILEY, 1995), have generally been found in significant concentrations in *M. pulegium* EOs.

In the analysis by gas chromatography coupled to mass spectrometry (GC/MS) (Figure 6), it was evidenced that the essential oil extracted from *M. pulegium* used in this study contains the following major components: pulegone (80.553%); Piperitone (5.430%); Cyclohexanone,5-methyl-2-(1-methyl)-,(2R-cis) (3.304%); trans-Isopulegone (2.945%); α -Humulena (1.950%).

FIGURE 6 – GC/FID PROFILES OF *M. pulegium* L. essential oil.



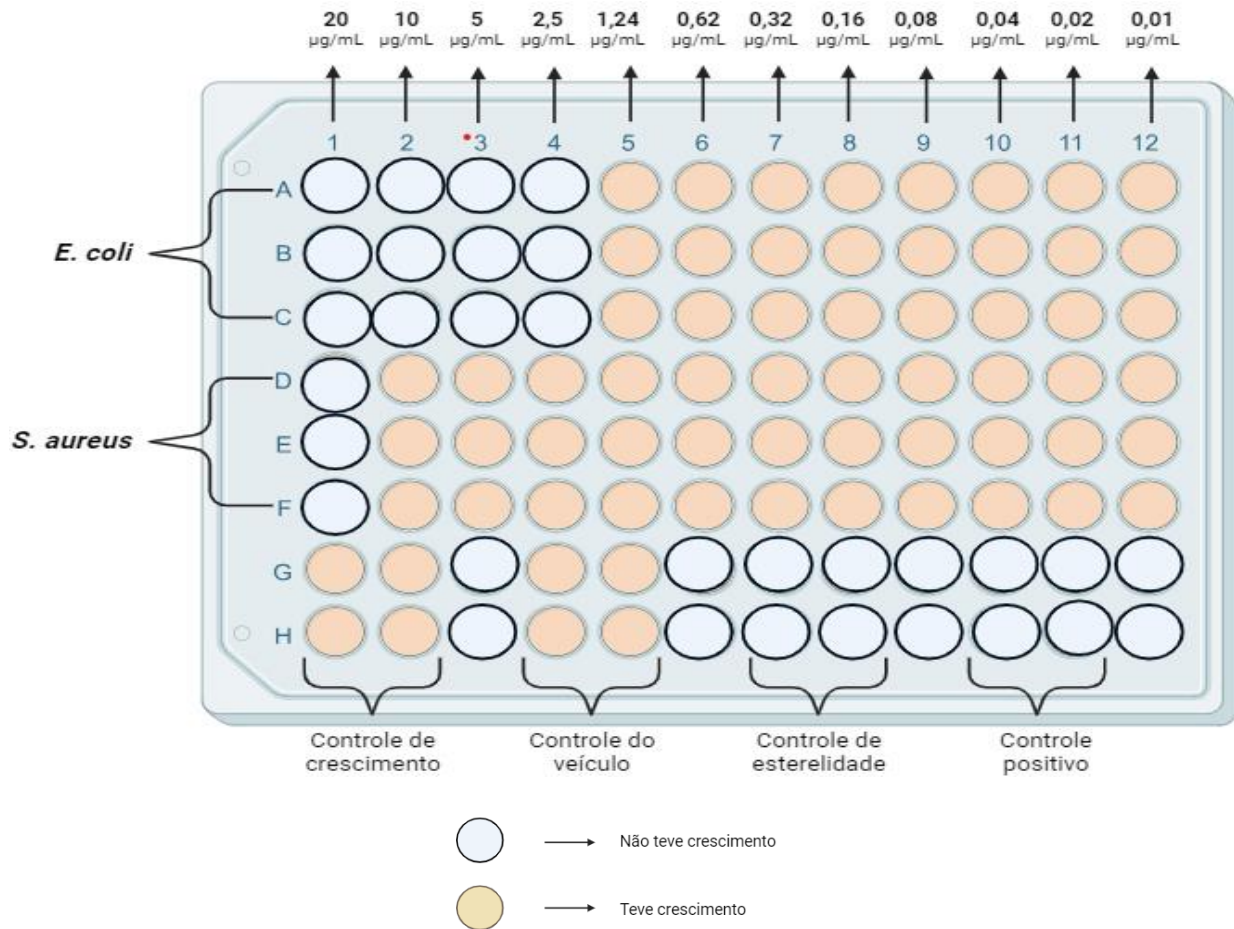
Source: Authors' production (2023).

Regarding the compounds found, a series of studies already published on *M. pulegium* reveal a great variability in its chemical profile. The results of the study identified which chemotype studied is familiar to those observed, with pulegone as the main marjoritarian component. In Uruguay (Lorenzo et al., 2002), Tunisia (Hajlaoui et al., 2009) and Morocco (Benayad, 2008) with pulegone content 73.4%, 61.11% and 73.33% respectively. In addition, results published by Vian *et al.* (2008) reported that pulegone (83.70%) was the major component of *M. pulegium*'s essential oil, while Mahboubi; Haghi (2008) reported that pipestone (38.0%) is the main constituent. Compared to oils tested with published data on the oil composition of other *M. pulegium* L. samples, there are some quantitative and qualitative differences. These chemical differences can probably be explained by the existence of different chemotypes, as well as the geographical distribution of this plant significantly influences the chemical composition of its essential oils.

3.2 ANTIMICROBIAL ASSESSMENT

The antibacterial activities of *M. pulegium* essential oil against Gram-negative *E. coli* bacteria and against Gram-positive bacteria *S. aureus* are described in (Figure 7).

FIGURE 7 – SCHEMATIC REPRESENTATION OF THE MICROPLATE AFTER 24 HOURS OF INCUBATION



Source: Authors' production (2023).

The results of the MIC obtained in this assay were 2.5 µg/mL for *E. coli* and 20 µg/mL for *S. aureus*. MBC was coincident with MIC, which highlights the bactericidal activity of pennyroyal essential oil.

TABLE 3 – MINIMUM INHIBITORY CONCENTRATION AND MECHANISMS OF ACTION OF PENNYROYAL ESSENTIAL OIL AGAINST *E. coli* AND *S. aureus* bacteria

Micro-organism	MIC (µg/mL)	CBM (µg/mL)
<i>E. coli</i>	2,5	2,5
<i>S. aureus</i>	20	20

Source: Authors' production (2023).



According to CLSI standards, microbial sensitivity is observed when, in conventional therapies, a drug is able to stop bacterial growth. On the other hand, a resistant microorganism becomes more challenging to control, even with the use of normally effective concentrations (WAYNE, 2005). The data from this study indicated that *E. coli* was the most sensitive strain tested in the EO of Brazilian *M. pulegium* and also indicate that the EO of *M. pulegium* may be an effective inhibitor agent of the strains studied. *Mentha pulegium* is considered a medicinal plant due to its pharmacological and biological properties (GHAZGHAZI *et al.*, 2013).

The results showed that the essential oil of *Mentha pulegium* L. brasileiro has higher antibacterial activity against *E. coli* 2.5 ($\mu\text{g/mL}$) than against *S. aureus* 20 ($\mu\text{g/mL}$). Boukhebt *et al.* (2011), reported that Gram-positive bacteria are more susceptible to essential oils than Gram-negative bacteria, but the results shown in this study showed that *E. coli*, which is a Gram-negative, was more susceptible to the essential oil of Brazilian *M. pulegium* L., using a microdose of lower inhibition concentration than for *S. aureus*.

Pennyroyal could be a center of interest in the food industry with flavoring, antioxidant and antimicrobial properties (GHAZGHAZI *et al.*, 2013). It is notable that the effect of *M. pulegium* L. essential oil against some bacterial strains is close to or superior to that of standard antibiotics.

4 CONCLUSIONS

The search for new products to control pathogens is a promising area of research. *Mentha pulegium* L. may be a good candidate for the development of new antimicrobials and can be used in important applications in the food and pharmaceutical industry. Despite increasing research in this area, more studies on the antimicrobial activity or chemical composition of essential oils are still needed.



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Chemical and biological study of the plant species *Tithonia diversifolia*



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ABSTRACT

With the advance of agriculture and the growing need for ways to control plagues, the large-scale use of synthetic herbicides has been causing some problems, such as soil pollution by persistent herbicide molecules, causing the emergence of resistant weed populations to control methods. Therefore, a lot of studies has been done aiming to find new compounds less toxic and persistent on the environment. The vegetable species *Tithonia diversifolia* is widely known for its invasive behavior. It is a plant native to Central America that spread across South and North America and across the world as an ornamental plant. A lot of reports on literature discourse about its allelopathic effect on many plant species, causing inhibition of growing and germination of seeds. Such effects are associated to sesquiterpene lactones, characteristic secondary metabolites from Asteraceae family, in which *T. diversifolia* belongs. In the present work, the extract of leaf washing of *T. diversifolia* using dichloromethane as solvent, from which the majoritarian compound was isolated and identified. The crude extract of leaf washing was submitted to in vitro phytotoxicity essay with *Lactuca sativa* seeds. The majoritarian compound was isolated though fractionation of the crude extract on silica flash column followed by a series of dilutions of fractions 1 and 2, and identified though nuclear magnetic resonance as being Tagitinin C. The signals of hydrogen and carbon were confirmed by comparison with data found on the literature. It was obtained a yield of 47,26% from the crude extract. The phytotoxicity essay with the crude extract of leaf washing reduced the growth of seedlings of *L. sativa*, however, exhibit less activity when compared with the herbicide Flumyazin®.

Keywords: Herbicide, Asteraceae, Natural products, Sesquiterpene lactones, Phytotoxicity.



1 INTRODUCTION

With the increasing demand for mass food production, agriculture has become one of the most important human activities. Several techniques have been developed throughout history, with the aim of producing more and more food to supply a population in constant ascendancy. That said, there is a lot of investment in studies that seek to optimize crop production yields and minimize the costs associated with them. Among the adversities faced by producers, weeds stand out as the main cause of crop losses, exceeding all other forms of pests, such as insects, nematodes, diseases and rodents (ABOUZIENA; HAGGAG, 2016). It is estimated that about 20 to 30% of crop expenditures are allocated to weed control (ARANTES, 2007; SANTOS, 2009). Weeds reduce yields, hinder harvesting, and reduce food quality due to contamination with seeds and impurities (ARANTES, 2007), which is why herbicides are the most consumed type of pesticide in Brazil, representing more than 50% of this total (SANTOS, 2009; OFOSU *et al.*, 2023).

Research dedicated to the study of herbicides between the 1950s and 2000s focused only on synthetic herbicides (VYVYAN, 2001). However, the large-scale use of synthetic herbicides has presented a number of problems for both human health and the environment. Among these problems, the effect of the herbicide on non-target plants, residues in soil and water, toxicity to non-target organisms, emergence of resistant plant populations, and harm to human health and safety can be highlighted (ABOUZIENA; HAGGAG, 2016). Among these problems, the emergence of resistant plants stands out, which has become the target of research related to the study of synthetic herbicides. The emergence of resistant weeds is closely linked to the accumulation of synthetic herbicide residues that are difficult to degrade. These substances exert a selection pressure on plant populations, selecting biotypes that are resistant.

Herbicide resistance is a natural and heritable characteristic of some biotypes present in a given population, which allows individuals who have such biotypes to be able to grow and reproduce after exposure to a dose of a herbicide that would be lethal to a normal population (CHRISTOFFOLETI; LÓPEZ-OVEJERO, 2003). There are five primary mechanisms of herbicide resistance: 1) Target-site resistance, the result of a mutation that alters the herbicide's binding site, often in an enzyme, preventing or reducing the herbicide's ability to bind; 2) Metabolism enhancement, so as to increase the plant's ability to metabolize the herbicide compound; 3) Decreased uptake and/or translocation, which may result in restriction of the herbicide's movement towards the site of action; 4) Sequester of the herbicide compound into the cell wall or vacuole, reducing the concentration of herbicide that reaches the site of action; 5) Amplification or overexpression of the gene for the production of the target enzyme, which generates the need for higher concentrations of herbicide to cause the death of the plant (HEAP, 2014). According to Heap (2014), the classes of herbicides that most selected resistant biotypes are acetolactate synthase inhibitors (ALS), photosystem II inhibitors, acetyl-CoA carboxylase



inhibitors (ACCase), synthetic auxins, bipyrilliums, and glyphosate. On the other hand, Ofosu *et al.* (2023) reports cases of resistance to 5-enol pyruvylxikimate-3-phosphate synthase (EPSP) inhibitors and fatty acid synthesis inhibitors, in addition to those mentioned above. ALS inhibitors are especially resistant in weed species. They have an active site in the enzyme acetolactate synthase, which is an enzyme vulnerable to mutations that confer resistance by the mechanism of alteration of the target site (EBERLEIN *et al.*, 1999; HEAP, 2014).

In addition to the mechanism of action, factors related to the bioecological characteristics of the plant species can also define the potential for the development of herbicide resistance. Such factors are a short life cycle, high seed production, low seed dormancy, several reproductive generations per year, extreme susceptibility to a particular herbicide, and great genetic variety (CHRISTOFFOLETI; LÓPEZ-OVEJERO, 2003).

Many synthetic herbicides, like pharmaceuticals, are based on natural compounds. About 70% of new pesticides are registered with ingredients that originate from natural product research. However, only 8% of conventional herbicides are derived from natural compounds and 7% are natural compounds (DAYAN; DUKE, 2014). Such compounds originate from the secondary metabolism of plants, which produce them to protect against herbivory, fungi, bacteria, viruses, nematodes and give a competitive advantage to the species, increasing the chance of survival (BALBINOT-JÚNIOR, 2004).

Allelopathy is defined as the effects, usually negative, exerted by one species of plant on the development of other species. Such effects are caused by secondary metabolites produced by the invasive species and released into the environment. Allelochemicals, as they are called, can be released by the plant by the processes of volatilization, root exudation, leaching, and decomposition of the plant body (MIRANDA *et al.*, 2015). The understanding of allelopathic relationships between plant species is a great ally in determining strategies to combat invasive plants (LOPES, 2016).

There is a growing concern for new herbicide formulations that are safer, both for human health and the environment, and allelochemicals may represent good substitutes as synthetic herbicides. In general, compounds of natural origin are less aggressive due to easy chemical and biological degradation (BALBINOT-JUNIOR, 2004; DAYAN; DUKE, 2014). Not to mention that working with natural compounds has the advantage that their biological activity is already established and, generally, they have a diversity of biosynthesized carbon skeletons that exhibit a range of biological activities associated with them (DAYAN; ROMAGNI; DUKE, 2000). For this reason, research on the isolation and phytotoxicity testing of allelopathic compounds from invasive plants has gained ground, with the objective of obtaining new compounds as potential natural herbicides and in the elucidation of new mechanisms of action (DAYAN; DUKE, 2014; MIRANDA *et al.*, 2015).



The plant species *Tithonia diversifolia*, belonging to the Asteraceae family, tribe Heliantheae, is an invasive plant that has a high growth rate (ORSOMANDO *et al.*, 2016). This species has great allelopathic potential and is capable of dominating hectares of agricultural and non-agricultural land, becoming a weed and disturbing native plant communities. *T. diversifolia* has allelopathic potential in the germination and growth of several plant species (KATO-NOGUCHI, 2020) and, as an invasive plant, it is a possible source of allelochemicals with phytotoxic potential (ALVES *et al.*, 2011). The present work discusses the potential of natural products derived from the plant species *T. diversifolia* as phytotoxic agents.

1.1 FAMILY ASTERACEAE

The Asteraceae family is the largest family of angiosperms, comprising 1,600 genera present on the planet. In Brazil, there are about 180 genera and 1,900 species distributed across different biomes (ROQUE; BAUTISTA, 2008; BROOK; SILVA; CASTRO, 2010). It represents 10% of the world's angiosperm flora and has been intensively studied in terms of anatomy, morphology and ecology, as well as in terms of its phytochemistry, metabolomics and macromolecular structure (NAKAJIMA; SEMIR, 2001). The family is represented by plants with very varied characteristics, which occupy a wide range of habitats, prevailing in areas with a tropical climate. They are represented by small plants, which can be herbaceous or shrubby, rarely arboreal (VERDI; BRICHENTE; PIZZOLATTI, 2005). A striking feature of the family is the capitulated inflorescences, whose flowers are inserted in a wide and rounded receptacle with a discoid shape containing bracts (SANTOS, 2019). Chapter cover can contain from one to more than 500 flowers whose ovary is inside the receptacle and is uniovulated (ROQUE; BAUTISTA, 2008; SANTOS, 2019).

Species of the Asteraceae family are widely studied from a phytochemical point of view because they present a diverse range of biologically active secondary metabolites, already presenting some compounds that provide the development of drugs, insecticides and other natural products (VERDI; BRICHENTE; PIZZOLATTI, 2005). The ethnobotanical use of Asteraceae species is widely reported in various parts of the globe for various purposes (HEINRICH *et al.*, 1998; MARTUCCI, 2016). In addition to their therapeutic use, they are also explored in food, cosmetic production and as ornamental plants (ROQUE; BAUTISTA, 2008).

Of the therapeutic compounds associated with plants of the Asteraceae family, flavonoids, polyacetylenes, coumarins, terpenoids and sesquiterpene lactones (ROQUE; BAUTISTA, 2008; MARTUCCI, 2016; KATO-NOGUCHI, 2020). Sesquiterpene lactones are compounds that have a diversity of biological activities, among them they have high phytotoxic potential. They are one of the classes of compounds associated with the evolutionary success and invasiveness of species, which have developed a unique chemical defense system (ROQUE; BAUTISTA, 2008). Therefore,



sesquiterpene lactones may be good candidates for the study of new herbicides based on natural products (ARANTES, 2007; SANTOS, 2009; MIRANDA *et al.*, 2015).

1.2 TRIBO HELIANTHEAE

The tribe Heliantheae is the most diverse and largest tribe of the family Asteraceae, considered by many taxonomists to be one of the most primitive taxa of Asteraceae (CHRISTENSEN; LAM, 1990; OLIVEIRA; SILVA; BARROS, 2007). The species that make up the tribe are characterized by having alternate or opposite leaves, usually trinervated, with a terminal inflorescence that can be paniculiform, corymbiform or in a capitulum with bisexual flowers (ALVES; ROQUE, 2016). They are found in regions with tropical and subtropical climates, with few representatives in regions with a temperate climate (OLIVEIRA; SILVA; BARROS, 2007). The tribe is composed of 113 genera comprising 1,460 species spread throughout Central and South America, and in Brazil there are about 60 genera and 374 species (OLIVEIRA; SILVA; BARROS, 2007; ALVES; ROQUE, 2016).

The relationships between Heliantheae species, as well as the relationships of the tribe with other closely related taxonomic groups, were proposed from chemotaxonomic studies, which revealed different classes of compounds found in the tribe, such as flavonoids, acetylenes and sesquiterpene lactones (CHRISTENSEN; LAM, 1990). The sesquiterpene lactones characteristic of this tribe are produced and stored in the glandular trichomes present in the aerial parts of plants (ROCHA, 2009, SILVA *et al.*, 2017). Such compounds occur in species of the tribe Heliantheae with an enormous variety of carbon skeletons (STEFANI, 2006), which opens space for several studies on natural products that the species of this tribe can provide, in view of the diversity of biological activities presented by sesquiterpene lactones (SCHMIDT, 2006). Among the most well-studied species of the tribe Heliantheae, the present work deals with the species *T. diversifolia*.

1.3 TITHONIA DIVERSIFOLIA

The plant species *T. diversifolia* is a shrubby plant that can reach up to 4 meters in height, has erect and very branched branches, alternate, petiolate leaves, usually divided into 3 to 5 lobes. The flowers, 12 to 14, are inserted peripherally in a capitulum inflorescence that is surrounded by the corolla whose sepals resemble petals of a simple flower of bright yellow color (PÉREZ *et al.*, 2009). The inflorescence and leaves of *T. diversifolia* are shown in figure 1.

It is the best-studied species in the genus *Tithonia*. It exhibits highly invasive behavior and has a high rate of biomass production (ORSOMANDO *et al.*, 2016). This plant can produce 80,000 to 160,000 seeds per m², with germination rates between 18 and 56% at 25°C (AJAO; MOTEETEE, 2017). However, there are several reports of the use of *T. diversifolia* for agricultural and therapeutic purposes. The ability of this species to adapt to nutrient-poor soils and to recover these soils is reported



in the literature (OLABODE *et al.*, 2007), in addition to being able to grow and colonize soils polluted by heavy metals, being able to remove metals from the environment and store them in their tissues (AYESA; CHUKWUKA; ODEYEMI, 2018). The species also has a high content of nutrients such as nitrogen, potassium and phosphorus, making it a good fertilizer alternative for soil management (OLABODE *et al.*, 2007). In addition, the high nutrient content makes the species good to be used as fodder for animals (ORSOMANDO *et al.*, 2016; AJAO; MOTEETEE, 2017).

Figure 1 – Specimen of *T. diversifolia*



Source: The Authors (2023)

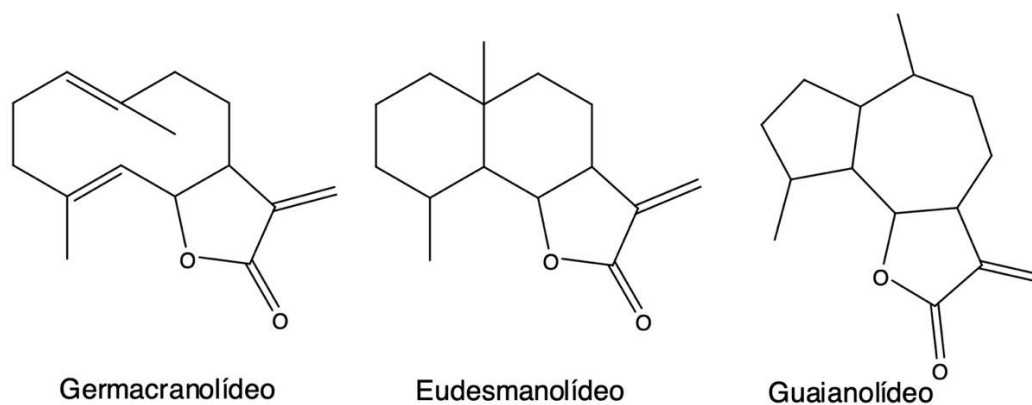
Several studies seek to elucidate the chemical constituents of *T. diversifolia*. The presence of flavonoids, sesquiterpenoids, diterpenoids and, in smaller amounts, phytosterols, xanthans, coumarins, ceramides, chromones and chromenes has been reported (CHAGAS-PAULA *et al.*, 2012). There are also reports of the presence of tannins, alkaloids and saponins (HERRERA; VERDECIA; RAMÍREZ, 2020). Orsomando *et al.* (2016) identified 161 volatile compounds in *T. diversifolia*, with monoterpenes standing out, which accounted for 46.9% of the volatile oil composition. The most present compounds in volatile oil are α -pinene, limonene, and cis-chrysentenol (ORSOMANDO *et al.*, 2016). The most representative class of secondary metabolites of the species is sesquiterpene lactones (CHAGAS-PAULA *et al.*, 2012; PASSONI *et al.*, 2013; SILVA *et al.*, 2017). Such compounds give *T. diversifolia* a high allelopathic potential, which influences the germination and growth of several plant species (CHAGAS-PAULA *et al.*, 2012). Many secondary metabolites of *T. diversifolia* have been studied regarding their biological activities and applicability. Due to the high production of biomass and biologically active metabolites, *T. diversifolia* has the potential to be exploited as a source of natural products for therapeutic and agricultural purposes (CHAGAS-PAULA *et al.*, 2012). Tagitynin C is a secondary metabolite of *T. diversifolia*, of the sesquiterpene lactone class, produced in the glandular trichomes of leaves (SILVA *et al.*, 2017).



1.3.1 Sesquiterpene Lactones

Sesquiterpene lactones are highly distributed secondary metabolites of plants, which have a great diversity of structures and metabolic activities (SCHMIDT, 2006). This class of compounds is a striking characteristic of the Asteraceae family and its diversity of structures is used as markers for chemotaxonomic studies of the groups belonging to this family (CHAGAS-PAULA *et al.*, 2012). This class of molecules is characterized by having a 15-carbon skeleton that is classified according to its arrangement. The main groups of STLs are the germacranolides, eudesmanolides, and guaianolids, and the most representative minor subgroups are the heliangolides and pseudoguayanollids (PADILLA-GONZALEZ; SAINTS; DA COSTA, 2016). Figure 2 represents the carbon skeletons of the major classes of STLs.

Figure 2 – Carbon skeletons of the main classes of sesquiterpene lactones

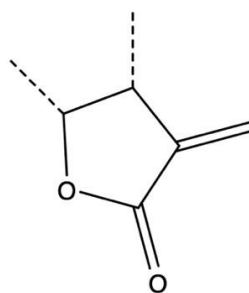


Fonte: Santos (2009)

The γ -lactone ring is present in the vast majority of STLs, containing an exocyclic methylene conjugated with a carbonyl (figure 3), and this part of the molecule is responsible for the diversity of biological activities (PADILLA-GONZALEZ; SAINTS; DA COSTA, 2016). The biological activities associated with STLs often occur through a mechanism of action based on interference in the function of cellular macromolecules through covalent bonds of the Michael addition type between the electrophilic part of the lactone and the nucleophilic center of the biological targets, which leads to the alkylation of the biological molecules (SCHMIDT, 2006). Figure 3 shows a common structure of the lactone ring. One of the activities presented by STLs is phytotoxicity, many of which affect the germination and development of some plant species, so they can be explored as models for new herbicides (ARANTES, 2007).



Figure 3 – General Chemical Structure of the Lactone Ring



Fonte: Santos (2009)

The plant species *T. diversifolia* produces large amounts of the sesquiterpene lactones Tagitynins A and C in its glandular trichomes (STEFANI, 2006). It has been reported that such compounds reduce seed germination and seedling growth, suggesting the possibility that these compounds are responsible for the invasive behavior and high allelopathic potential of the species (MIRANDA et al., 2015; KATO-NOGUCHI, 2020). The present work aims to explore the phytotoxic activity of extracts and compounds of *T. diversifolia*, and to evaluate the potential of this plant species as a source of natural products for agricultural purposes.

1.4 SESQUITERPENE LACTONES DERIVED FROM *TITHONIA DIVERSIFOLIA* AS AN ALTERNATIVE TO SYNTHETIC HERBICIDES

Herbicides are the most consumed class of pesticides among farmers. In 2019, the global pesticide market reached values close to \$84.5 billion, and accounts for 51.9% of agricultural product sales (OFOSU *et al.*, 2023). However, the large-scale use of synthetic herbicides is leading to a problem faced by rural producers, which is the emergence of weed populations resistant to commonly used products.

Heap (2014) conducted a data survey through the website called "The International Survey of Herbicide-Resistant Weeds", in which registered users and scientists from all over the world can register cases of pesticide resistance occurring in agriculture. According to the author, in 2013, there were 404 unique cases of a relationship between plant species and site of action of the herbicide. 220 plant species showed resistance to one or more herbicide mechanisms, 130 dicots and 90 monocots. The site presented data on resistant populations in 61 countries, with the countries with the highest records of occurrence being the United States, Canada, Australia, and France, respectively. In the year of publication of the article, Heap (2014) found that Brazil ranked eighth in the ranking of countries with the most cases of herbicide resistance, with 31 cases.

In a new search on the website's page carried out by the author in 2023, a decade after the one reported by Heap (2014), the page had 523 unique cases of species x herbicide action site relationship, with 269 plant species that showed resistance to one or more herbicide mechanisms of action, 154



dicotyledonous and 115 monocot. In 2023, the site presented cases of resistant populations in 72 countries and, in addition, Brazil came to occupy fourth place among the countries with the highest occurrence of resistant populations with 49 cases, behind only the United States, Canada, and Australia (<http://www.weedscience.org>). Comparing the data presented by Heap (2014) and those presented by the page after a decade, it is possible to observe that the problem of resistant populations is something that occurs all over the world, and there is a notable increase in the number of species that are becoming resistant to commercial synthetic herbicides. This poses a major problem for food production in the world because weeds compete for space and nutrients with cultivated plants, which reduces yield and crop quality. According to Hussein (quoted by ABOUZIENA; HAGGAG, 2016), 0.19 kg of weed dry matter results in 1 kg of loss of onion bulb yield, and allowing weeds to grow close to harvest can lead to the removal of 36.9; 9.6 and 57.0 kg per acre of soil nitrogen, phosphorus, and potassium, respectively.

That said, there is a great incentive to the search for natural products, extracted from plants, which can be as effective as synthetic herbicides, but which present less damage to environmental health (BALBINOT-JUNIOR, 2004). Allelochemicals originating from plants are a promising source in the search for new herbicidal compounds or new mechanisms of action (ARANTES, 2009). The invasive behavior and allelopathic potential of *T. diversifolia* makes the species the target of research regarding its allelochemicals and its potential as a source of herbicidal compounds.

2 METHODOLOGY

2.1 COLLECTION AND DRYING OF PLANT MATERIAL

The leaves of *Tithonia diversifolia* were collected in the municipality of Alegre – ES, Latitude -20.7633 Longitude -42.5339 (20° 45' 48" South and 41° 32' 2" West), and identified by researchers from the Federal University of Espírito Santo, *Alegre campus*. A total of 1,905 kg of plant material were collected. The material was dried in an oven at 50°C for 48 hours. After drying the plant material, the leaves were taken to the Laboratory of Organic Chemistry and Pharmacognosy, located at UFES *Alegre campus*, to obtain the dichloromethane extract.

2.2 EXTRACT PRODUCTION AND COMPOUND ISOLATION

The extract was prepared from the washing of the leaf surface of *T. diversifolia*, since in the literature it is reported that the substances of interest, the sesquiterpene lactones, are produced and stored in the glandular trichomes of species of the tribe Heliantheae (ROCHA, 2009; PAULA *et al.*, 2018). The solvent used was dichloromethane, chemical formula CH₂Cl₂. The choice of solvent was based on polarity studies of sesquiterpene lactones, which have good solubility in nonpolar solvents, such as acetone, ether and dichloromethane (SCHMIDT, 2006; SILVA *et al.*, 2016). The extract was



obtained by washing the leaf surface using 6L of solvent for a period of 3 minutes, until the glandular trichomes disappeared, and then it was filtered and concentrated in a rotaevaporator. After all the solvent has dried, the crude extract was ready.

The isolation of the compounds occurred through several chromatographic techniques in stationary phases of silica and a series of dilutions in solvents with extreme polarities. Initially, the crude extract was analyzed by thin layer chromatography (CCD) in order to analyze the best combination of organic solvents that could be used to conduct the fractionation of the extract. The solvent combination selected was Methanol:Ethyl Acetate:Dichloromethane acidified with acetic acid. After analysis by CCD, a column of normal-phase flash silica gel was performed at moderate pressure, following the concentrations described in Table 1. The fractions obtained were analyzed by CCD, in order to identify whether the fraction was a mixture or a pure compound.

Table 1 – Concentrations used in the fractionation of the crude extract of dichloromethane from *T. diversifolia*

Methanol	AcOEt	CH₂Cl₂
*5%	*0%	*95%
5%	0%	95%

Note: * Fraction not acidified with acetic acid (0.01%).

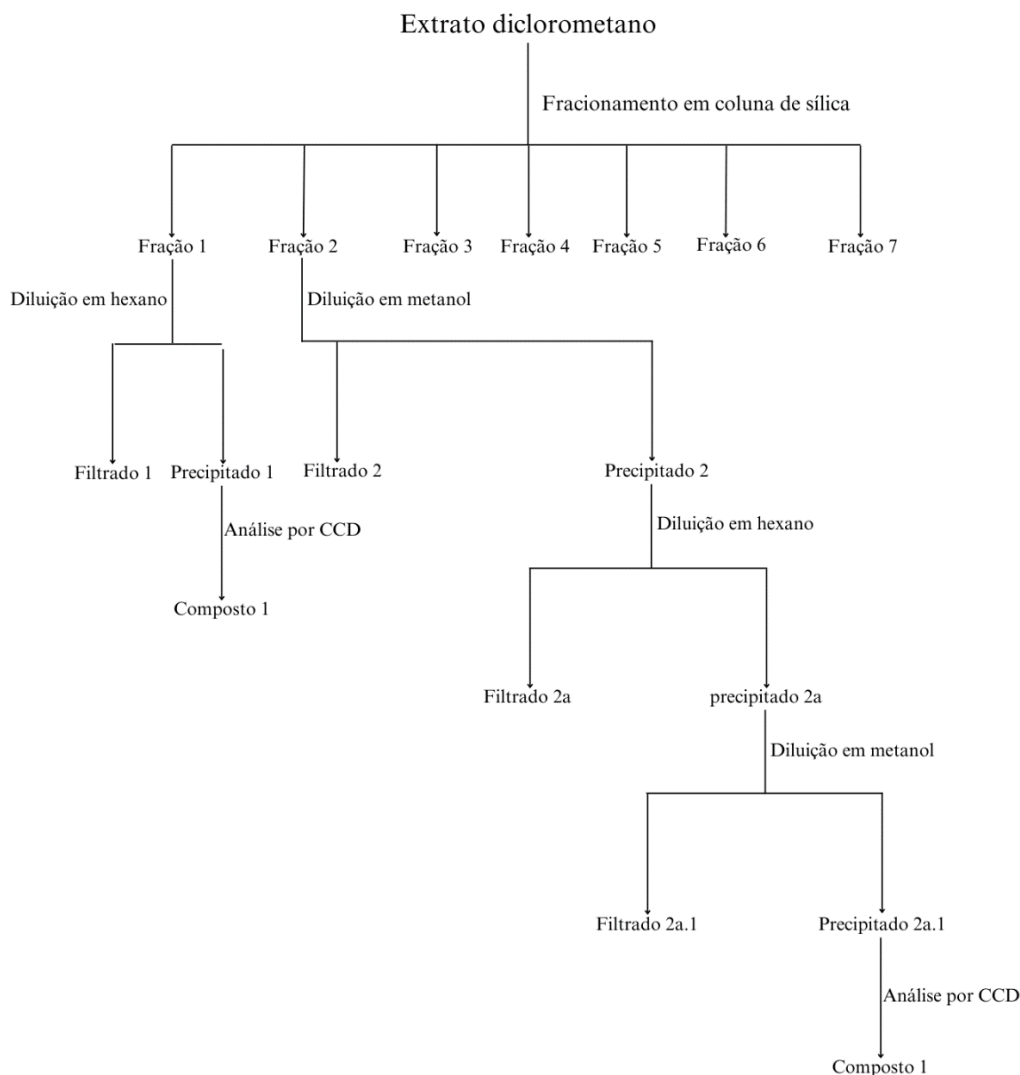
Source: The Authors (2023)

The first fraction, Methanol:CH₂Cl₂ (5%:95% - without acetic acid) was diluted in Hexane forming a precipitate. The liquid phase was separated from the precipitate by filtration with a glass and cotton pipette. The precipitate was analyzed by CCD revealing the pattern of only one stain, thus being a pure compound.

The second fraction, Methanol:CH₂Cl₂ (5%:95% - acidified with acetic acid) formed a precipitate when diluted in Methanol for analysis. The liquid phase was separated from the precipitate with a glass and cotton pipette, and the precipitate was subjected to dilution in hexane, forming a new precipitate. The new precipitate was again diluted in Methanol, generating a third precipitate. This was analysed in CCD revealing only one stain. In a comparative analysis by CCD, it was possible to observe the same pattern of the compound obtained from the first fraction, being called compound **(1)**. The flowchart shown in figure 4 shows the path taken to obtain the compost (1). Therefore, the fractions of compound 1 were mixed and taken to be identified by physical methods of compound identification.



Figure 4 – Flowchart of the path taken to obtain the compost (1)



Source: The Authors (2023)

2.3 IDENTIFICATION OF THE MAJOR COMPOUND

The identification of the isolated majority compound through the technique described in the previous item took place at the UNIFAL campus, in the city of Alfenas – MG, Brazil. The equipment used was a Tesla Bruker 7.05 spectrometer, model AC-300 located in the Nuclear Magnetic Resonance Laboratory, operated at 300 MHz in the hydrogen frequency and 75 MHz in the carbon frequency. The solvent used was deuterated chloroform.

2.4 PHYTOTOXICITY BIOASSAY

The phytotoxicity test was carried out at the Chemistry Laboratory of the Federal Institute of Espírito Santo, located in Rive, municipality of Alegre. Five solutions made at 70:30, water:dichloromethane, with concentrations of *T. diversifolia* crude extract μ equal to 3000, 1500, 750, 375 and 187.5 ppm ($\text{g}\cdot\text{mL}^{-1}$) in vitro seeds were tested. A solution of distilled water with the solvent

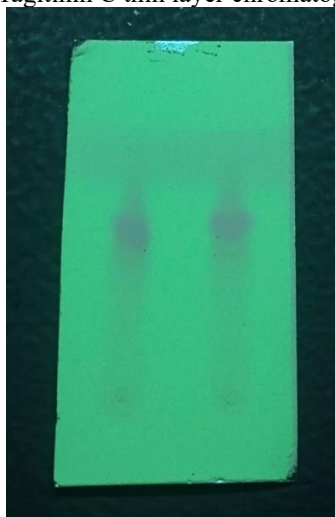


dichloromethane (70:30) was used as a negative control (C-), and the herbicide Flumyazin® was used as a positive control (C+) at the concentration of use indicated by the manufacturer. Each treatment was performed on *L. sativa* seeds arranged in Petri dishes lined with filter paper moistened with the crude extract solutions. The Petri dishes were arranged in a completely randomized design and deposited in a BOD incubator at 24°C throughout the experiment. The percentage of germinated seeds was observed after 8, 16, 24, 32, 40 and 48 hours of exposure to the treatments. Root and shoot growth were determined after 48 and 96 hours, respectively, of exposure to the concentrations used, with the aid of a digital caliper. From the data obtained, the following variables were evaluated: percentage of germination after 48 hours (%F), germination speed index (IVG) calculated according to Maguire (1962): $IVG = G1/N1 + G2/N2 + \dots + Gn/Nn$, where G1, G2, Gn= number of seedlings germinated from the first to the last count and N1, N2, Nn= number of days from the first to the last count.

3 RESULTS AND DISCUSSION

The major compound was isolated and identified as Tagitinin C, which corroborates data in the literature (ROCHA, 2009; PASSONI *et al.*, 2013; MIRANDA *et al.*, 2015; SILVA *et al.*, 2017; KATO-NOGUCHI, 2020). Tagitinin C was supplied by the first and second fractions from the fractionation of the crude extract of *Tithonia diversifolia* in a flash silica column with moderate pressure, using a 95:5 solution of CH₂Cl₂:MeOH as eluent, the first fraction without the addition of acetic acid and the second with the addition of acetic acid. Tagitinin C was identified as a pure compound by thin layer chromatography analysis, which revealed only one stain. The compounds isolated from fractions 1 and 2 were identified as the same compound because they had the same retention factor (Figure 5). The eluent used was 50:30:20 hexane:ethyl acetate:dichloromethane with the addition of acetic acid.

Figure 5 – Tagitinin C thin layer chromatography plate

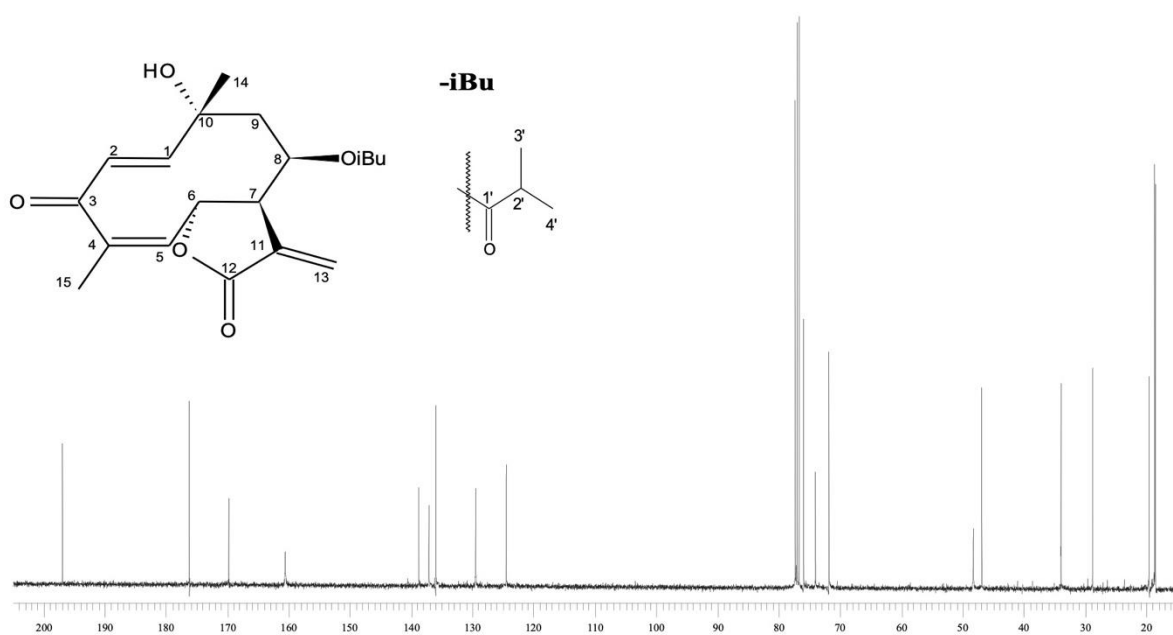


Source: The Authors (2023)



Confirmation of the chemical structure of Tagitinin C was achieved by nuclear magnetic resonance analysis of ^{13}C and ^1H . Figures 6 and 7 show the spectrum of ^{13}C and ^1H , respectively. The carbon spectrum showed the values of displacement of the carboxyl carbons in the conjugated ketone δ , resonating at C 197.0 (C-3), in the ester group, resonating at C 176.2 (C-1') and in the lacton ring, resonating at C 170.0 (δ C-12 δ). In addition to them, it also showed the quaternary carbon attached to the methylene group in the lacton ring resonating at C 136.0 (C-11), another quaternary carbon resonating at C 72.0 attached to the hydroxyl group and a methyl δ (C-10), and another quaternary carbon δ in the double bond. The carbons and hydrogens of the double bonds showed displacement values equal to C 160.5/H 5.82 (C-1), C 129.5/H 6.25 (C-2) and C 137/ δH 5.42 δ (C-5), C δ 139.0 δ ($\delta\delta$ δ C-4). The spectra show the remaining carbons of the lacton ring resonating at C δ 76.0/H 6.99 for the oxygen-bound carbon (C-6) and C 48.3/H δ 3.58 for the tertiary δ_{carbon} (δC -7). The chiral carbon attached to the ester group has a carbon and hydrogen signal resonating at C δ 74.0/H δ 5.35 (C-8). The chiral center is attached to a methylene whose hydrogens showed different displacement signals, resonating at C δ 47.0/H δ 2.00 and 1.10 (C-9). The chiral center also influences the displacement values of methylene bound to the lacton ring, whose signs were present in C δ 129.5/H 6.35 δ and 5.80 (C-13). The spectra obtained for the compound (1) were compatible with the data presented in the literature for Tagitinin C (SANCHÉZ-MENDOZA *et al.*, 2011). Table 2 shows all the ^{13}C and ^1H displacement signals presented by the Tagitinin C nuclear magnetic resonance spectrum.

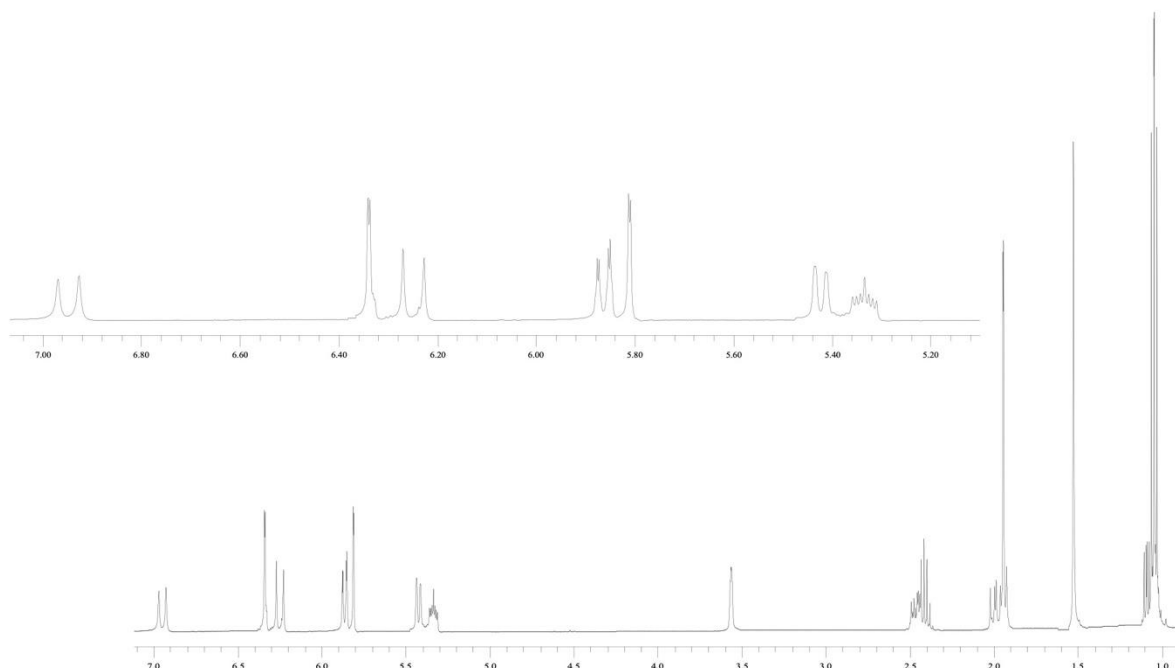
Figure 6 – Molecular structure of Tagitinin C and ^{13}C nuclear magnetic resonance spectrum



Source: The Authors (2023)



Figure 7 – ¹³C nuclear magnetic resonance spectrum of Tagitinin C



Source: The Authors (2023)

Table 2 – ¹³C and ¹H shift signals from Tagitinin C nuclear magnetic resonance spectra

Position	¹³ C (75 Hz)	¹³ C (Lit.)	¹ H (300 Hz)	¹ H (Lit.)
1	160,5	160,1	5.82 (dd, 15Hz)	6,94 (d, 17.1 Hz)
2	129,5	129,6	6,25 (d, 15Hz)	6,25 (d, 17.1 Hz)
3	197,0	196,7	-	-
4	139,0	138,8	-	-
5	137,0	137,2	5,42 (d, 8Hz)	5,87 (d, 9.0 Hz)
6	76,0	75,9	6,99 (d, 8Hz)	5,41 (d, 9.0 Hz)
7	48,3	47,0	3.58 (quartet, 3Hz)	3,54 (m)
8	74,0	73,9	5.35 (septeto, 3Hz)	5,30 (m)
9.a	47,0	48,3	2,00 (f, 16 e 8Hz)	2,42 (f, 14.1 e 4.2 Hz),
9.b	47,0	48,3	1,10 (f, 2 e 8Hz)	2,02 (f, 14.1 e 4.2)
10	72,0	71,9	-	-
11	136,0	136,0	-	-
12	170,0	169,7	-	-
13.a	129,5	124,9	6,35 (f, 8Hz)	6,35 (d, 1.8)
13.b	129,5	124,9	5,80 (f, 8Hz)	5,81 (d, 1.8)
14	19,8	19,0	1,51 (s)	1,54 (s,
15	29,0	29,0	1,92 (s)	1,95 (s)
1'	176,2	176,2	-	-
2'	34,0	30,0	2,50 (m, 6Hz)	
3'	18,4	18,8	1,05 (d, 8Hz)	1,05 (d, 6.9)
4'	18,6	18,6	1,05 (d, 8Hz)	1,07 (d, J 6.9 Hz)

Source: The Authors (2023)

Tagitinin C is a sesquiterpene lactone of the heliangolide type (PAULA, 2018) whose molecule is lipophilic (ROCHA, 2009), which explains the large amount of this compound in extracts prepared from less polar solvents, such as dichloromethane. This is demonstrated in the study carried out by Passoni *et al.* (2013) by performing the identification and quantification of the classes of compounds

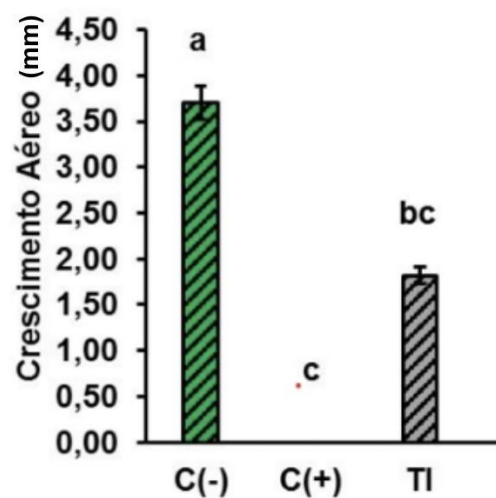


in different extracts of *T. diversifolia*, resulting in an extraction of a large amount of Tagitinin C in the extract of the plant's leaf wash with acetone and, on the other hand, there was no detection of this compound in the polar extract. A mass of 2.5 grams of the compound was obtained from 5.29 grams of crude extract, obtaining a yield of 47.26%, presenting a satisfactory yield.

For the phytotoxicity bioassay, the extract made from the washing of the leaf surface of *T. diversifolia* with dichloromethane was used. On the leaf surface is where the glandular trichomes are found, which are the structures that produce and store sesquiterpene lactones such as Tagitinin C (SILVA *et al.*, 2017), whose inhibitory activity on the development of several plant species is reported in the literature (MIRANDA *et al.*, 2015; KATO-NOGUSHI, 2020). In the present work, the germination of *Lactuca sativa* (lettuce) was used to demonstrate the phytotoxic effect of the extract. The percentage of germinated seeds was observed every 8 hours until the completion of the first 48 hours, and the measurement of roots and aerial parts occurred after 48 and 96 hours of exposure to the treatment. Figure 8 represents the result of the inhibition of germination and growth of *L. sativa* by the crude extract of *T. diversifolia* compared to the negative control (water:dichloromethane solution 70:30) and the positive control (Flumizyn® herbicide).

It is possible to observe that the crude extract of *T. diversifolia* showed inhibition of the development of *L. sativa* seedlings. However, the inhibition was about 50% lower when compared to the positive control, meaning that the extract has reasonable phytotoxic activity. The study was conducted during the initial development of the target plant, as they are more sensitive at this stage (LOPES, 2016).

Figure 8 – Result of the bioassay of phytotoxicity of *T. diversifolia* extract against the development of *L. sativa*



Source: The Authors (2023)



Sesquiterpene lactones, in general, are known for their potential as phytotoxic agents that can be exploited as models for new herbicides (ARANTES, 2007; RIAL *et al.*, 2016; DE OLIVEIRA, 2020). Santos (2009) investigated the phytotoxic activity of several sesquiterpene lactones synthesized on the root growth of *Sorghum bicolor* (sorghum) and *Cucumis sativus* (cucumber), whose percentage of inhibition was higher than 50% for some lactones. In addition, it has been shown to increase the efficacy of inhibition when the compound is encapsulated with carriers, such as SO₃Hex6 and β -cyclodextrin, whose encapsulated lactone increased from an inhibition percentage of 24.7% to 62.2% (SANTOS, 2009). Martins (2022) observed a dose-dependent inhibition of root growth of *Bidens pilosa* by the lapidolid sesquiterpene lactone extracted from *Lapidia aplicifolia*, another plant species belonging to the Asteraceae family. Rial *et al.* (2016) tested the phytotoxicity of several sesquiterpene lactones, isolated from the genera *Decachaeta*, *Salvia* and *Podachaenium*, against the development of barnyardgrass and brachiara, and concluded that 4 of the 5 sesquiterpene lactones that showed better inhibition were of the heliangolid type.

The results shown in the present work were obtained through the guided experiment at the Federal Institute of Esp rito Santo and represent preliminary results of a series of bioassays to be carried out, using new forms of extraction and also using isolated compounds, such as Tagitynins C, A and F. Through the results obtained it is possible to observe that the extract has phytotoxic potential, despite being smaller when compared to herbicide. However, in the literature, it is possible to verify the difference in the herbicide potential of different extracts, produced from different solvents and forms of extraction of *T. diversifolia*. Miranda *et al.* (2015) observed differences in the phytotoxic activity of extracts from different parts of the plant made from ethyl acetate and methanol, finding that the leaf extract produced with AcOEt showed higher phytotoxicity, which corroborates the fact that phytotoxic compounds are produced and stored in aerial parts. Kato-Noguchi (2020) conducted a survey in which phytotoxic activity of aqueous extracts of the leaves and shoots of *T. diversifolia* was reported against the growth and germination of several plants, such as rice, *B. pilosa*, corn, *Amaranthus cruentus*, *barley*, *cabbage*, *cucumber*, *onion*, *radish*, *tomato*, *Sorghum bicolor*, among others.

The target species selected for the phytotoxicity bioassay was *Lactuca sativa*, which is a standard target species for this type of experiment. Growth-inhibiting activity of *L. sativa* is widely reported by extracts of *T. diversifolia* (MIRANDA *et al.* 2015; KATO-NOGUSHI, 2020). Henzel (2022) found, through experiments, that aqueous extracts of *T. diversifolia* in different concentrations delayed the germination of *L. sativa* seeds, decreased the germination speed index, and caused morphological changes in germinated seedlings. On the other hand, Miranda *et al.* (2015) observed an inhibition rate equal to that of the herbicide Logran® against *L. sativa* for extracts produced with methanol and AcOEt of *T. diversifolia*, being the most active AcOEt extract. Therefore, it is possible



to say that the extracts of the plant species *T. diversifolia* have herbicidal potential and can be explored as candidates for natural products for agricultural purposes.

Several factors related to the procedures performed in the production of extracts and isolation of substances can interfere with the degree of biological activity of the natural product. The extraction process must be designed in order to select the metabolites of interest, taking into account the extraction efficiency, stability of the substances, availability of the media, process costs, and purpose of the extract (DE OLIVEIRA, 2020). Silva *et al.* (2017) conducted a comparative study of two forms of extraction of Tagitinin C taken from *T. diversifolia*, ultrasound-assisted extraction and dynamic maceration, and observed that ultrasound-assisted extraction was able to remove greater amounts of Tagitinin C. In addition, the solvent used to obtain the extract is also a key piece for determining the degree of activity of the extract, as can be seen in the work of Miranda *et al.* (2015). This relationship can also be observed in the work of Passoni *et al.* (2013), in which the extract made from acetone presented large amounts of the active ingredient Tagitinin C, while in the polar extract the compound was not detected. For the sesquiterpene lactones of *T. diversifolia*, the best solvents are those that are relatively nonpolar, such as chloroform, ethyl ether, benzene, acetone, dichloromethane, and ethyl acetate (SILVA *et al.*, 2017; DE OLIVEIRA, 2020). That said, further research should be carried out in order to carry out more tests using other solvents and forms of extraction, in addition to the elucidation of the other active compounds and tests of their phytotoxic activity.

4 CONCLUSIONS

The extract of the leaf lavage of *Tithonia diversifolia* presented Tagitinin C as the major compound. Through the phytotoxic assay it was possible to observe that the extract made by washing the leaves of *T. diversifolia* showed inhibition of the development of *Lactuca sativa*, however, the action of the extract was lower than that of the herbicide Flumyazin® used as a positive control. Finally, the potential of *T. diversifolia* extracts as natural products for pest control purposes is evident, so the present study should be continued in order to carry out more tests with extracts obtained from different forms of extraction, under different conditions and using different solvents, and also to carry out a study on the compounds responsible for phytotoxicity.



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Agroforestry systems in the semi-arid region of Minas Gerais



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ABSTRACT

Environmental education plays a fundamental role in contributing to the formation of conscious

citizens who can understand the relationship between humans and the natural environment. To achieve this, it is important to have the necessary means to build effective changes that contribute to the autonomy of the population in the Semi-arid region. In view of this, university extension plays an important role in the inclusion of values, skills, knowledge, responsibilities and aspects that promote improvement in the quality of life of society. In this context, the objective of this report is to describe the activities carried out in the workshop "Agroforestry Systems in the Semi-arid Region of Minas Gerais" held at the Tabocal Agricultural Family School (EFAT) in the municipality of São Francisco-MG. The workshop was conducted by undergraduate students from the Federal University of Minas Gerais' Agronomy program, and the following topics were covered: the definition of Agroforestry Systems (AFS); the difference between AFS and agricultural intercropping; the importance of AFS for food production; advantages and disadvantages; and management and implementation. The workshop was conducted dynamically and with active participation from the students, through questions and ideas raised, facilitating more in-depth discussions on the subjects.

Keywords: University Extension, Agrobiodiversity, Soil conservation, Environmental education.

1 INTRODUCTION

Environmental education is of fundamental importance because it contributes to the formation of conscious citizens, who can understand the relationship between men and the natural environment, the ways to conserve and preserve it and manage its resources properly (UNESCO, 2005 p. 44). In this sense, in relation to the Brazilian semi-arid region, characterized by presenting climatic adversities, associated with other historical, geographical and political factors, it is necessary to develop actions that promote behavioral changes.



To this end, it is important to have the necessary means to build effective changes that contribute to the autonomy of the population living in the semi-arid region. In this way, university extension plays an important role with regard to the inclusion of values, skills, knowledge, responsibilities and aspects that promote improvements in the quality of life of society.

The extension identifies social demands, promoting exchange between the knowledge of the university and society, benefiting both sides, as stated by Rodrigues et al. (2013). Therefore, this work allows the dissemination of the knowledge produced in the university while enabling academics to absorb the teachings and culture existing in external communities (CORRADI et al., 2019). Providing them with personal and professional development based on observation, questioning and the search for solutions to existing social problems.

It is worth mentioning the posture that extension workers should adopt in the face of educational activities aimed at the external community. For there to be an environment in which the exchange of knowledge takes place, it is necessary that respect and dialogue between the parties prevail. In this sense, in order to achieve the intended knowledge about agrobiodiversity in the semi-arid region, it is important to know the reality of the place, identifying collective potentialities and methodologies that will solve the problems experienced.

In view of these assumptions, the objective of this work was to describe the workshop "Agroforestry Systems in the Semiarid Region of Minas Gerais", developed with students from the Tabocal Agricultural Family School (EFAT) in São Francisco, MG. In view of the importance of this system for food production and nutritional security of low-income families, as well as for soil conservation and biodiversity.

2 METHODOLOGY

The workshop was planned and executed by undergraduate students of the Agronomy course at the Institute of Agrarian Sciences of the Federal University of Minas Gerais (ICA-UFMG), as part of the Rural Extension course. The workshop entitled "Agroforestry Systems in the Semi-arid Region of Minas Gerais" was held at the Tabocal Agricultural Family School (EFAT), based in the municipality of São Francisco – MG, on June 29, 2023. The target audience was high school students from the technical course in agriculture.

To teach the theoretical content, posters were made addressing the topics to be discussed. These posters featured various images, to make it easier for students to understand. An easel was used to support and present the posters.

Initially, the concept of Agroforestry Systems and its contribution to mitigating the negative effects caused by climate change and combating desertification was presented. For this, a poster



containing an image of an environment in the process of desertification was used, asking questions in relation to the actions that caused changes in that environment.

Then, the difference between Agroforestry Systems and agricultural consortia was addressed, emphasizing the importance of agroforestry for food production and the tree component for the system. Then, the main advantages and disadvantages of SAFs were presented.

Subsequently, aspects related to the management of SAFs were presented, such as the choice and disposition of species, irrigation, tree pruning and reuse of organic matter from the system itself to improve physical attributes and maintain soil fertility.

In the practical part of the workshop, seedlings of forest species were presented, including native ones such as pequi tree (*Caryocar brasiliensis*), coquinho-azedo (*Butia capitata*), jatobazeiro (*Hymenaea stigonocarpa*) and baruzeiro (*Dipteryx alata* Vog.). The importance of these for the biome, environmentally, and for the population, economically, was highlighted, since these species, in many municipalities in the North of Minas, contribute to the income of countless families.

The workshop ended with a dynamic, in which the students set up a SAF scheme, choosing the plant species and allocating them in the area according to their particular characteristics. To this end, printed figures of various vegetables, medicinal plants and tree species found in the semi-arid region of Minas Gerais were made available.

3 RESULTS AND DISCUSSIONS

The workshop was given to two classes, with 11 and 14 students, respectively, with the age group of students ranging from 15 to 17 years old, covering all years of high school.

In the development of the workshop, it was noticed that the group presented good acceptance and performance, as the participation of the students was observed throughout the discussion of the contents. The questions asked, both by the extension workers and by the students, enabled an intense exchange of knowledge and reflections on the themes.

Initially, as a means of introducing the concept and the importance that Agroforestry Systems play in the context of the semi-arid region, it was proposed that the students start thinking in front of an image that presented an environment in the process of desertification. In response, the students said, for example, that factors such as removal of native forest and erosion are responsible for this intense process of degradation.

Soon after, the concept of SAFs was built with the students, where, based on the visualization of an image, they said, based on the elements present, what characterized such systems. It was proposed to reflect on the role that trees and other elements that they identified in these images play in the prevention of degradation processes, as well as in the mitigation of greenhouse gas emissions. In this



sense, many associated, for example, that the permanence of plant species in the area favors the reduction of erosion and emission of polluting gases.

When asked what would be the differences between the Agroforestry Systems and the agricultural consortium, represented in the poster, many students answered that in the image of the SAF there was a greater variety of plants, as well as the presence of trees. The fundamental characteristic of agroforestry, which differentiates it from other agricultural crops, is the presence of a forestry component. While the association of annual plants, such as corn and beans, for example, is considered only an agricultural intercropping (GONÇALVES; MAN; MATIAS, 2016).

To complement the students' answer to the previous question, the importance of the forestry component in SAFs for income generation, biodiversity and soil conservation was discussed. The presence of woody species in agroforestry systems, as Macedo (2007) points out, plays an important role in favoring both the productivity and sustainability of these systems.

The importance of agroforestry in food production in the semi-arid region was also discussed. The group cited what can be produced for food – fruit trees, vegetables and annual crops such as corn and beans. In addition, the importance of this system for animal production was also highlighted.

The disposition of the species in the cultivation, pruning and irrigation areas was widely discussed with the EFAT students. The planting of species in rows was highlighted as being the most advantageous, as it facilitates cultural treatments, observing the peculiar characteristics of each plant component, defining spacing, intercropping and succession over time, respecting the physiology of the species and the use of resources in the environment.

In the practical part of the workshop, the seedlings of the species present in the semi-arid region were shown. Most of the students knew the species presented, demonstrating a close knowledge about the biodiversity of the semi-arid region, probably due to the fact that they all came from the region.

At this time, there was also a questioning of the interaction between species in agroforestry. One of the students questioned the fact that other plants hardly develop near the mastic tree (*Myracrodruon urundeuva* Allemão). In this way, the allelopathic effect related to this plant on some cultivated species was explained. Bonadio et al. (2014), for example, found a negative allelopathic effect of the extract of the leaves and stems of *M. urundeuva* on the germination and development of *Urochloa decumbens* and *U. brizantha*.

In addition, the role of universities and scientific research in the investigation of factors related to phenomena similar to the one raised in relation to allelopathy was also emphasized. Thus, university extension shows the importance of the relationship between institution and society, as it enables the generation of new knowledge and identification of society's needs and desires, for the creation of new research modalities (SANTOS et al., 2016).



At the end of the workshop, the dynamics in which the students actively participated in the assembly of a SAF. It was possible to observe the understanding they obtained about the theme of the workshop, especially with regard to the disposition of the species in the cultivation area aiming at the best reuse of resources.

In the extension activities that are developed, the students have the opportunity to associate theory and practice, presenting the knowledge acquired in the classroom and absorbing the popular knowledge rooted in the communities. In the workshop in question, the extension workers were able to obtain new perceptions on subjects related to this theme, which served as a basis for the improvement of knowledge and teaching methodology.

Figures 1 and 2 – Groups of EFAT students who participated in the workshop.



Source: Jordan (2023)



4 FINAL THOUGHTS

The workshop "Agroforestry Systems in the Semi-arid Region of Minas Gerais" was developed with intense participation of students, who interacted through questions and answers to the questions raised. It was also possible to observe the knowledge that the students already had about some of the subjects addressed in the workshop, which may be related to their insertion and coexistence in the semi-arid region.

In addition, the academics involved in conducting this workshop had the opportunity to share knowledge and increase their personal and professional experiences through the dialogue provided, based on respect, questioning and observation. Seeking, in this way, ways that contribute to the solution of existing problems.



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The impacts caused by drainage channels in wet areas of the Gravataí River – Rio Grande do Sul - Brazil and in Praia da Coronilha – Rocha – Uruguay



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ABSTRACT

Throughout history, many watercourses have undergone transformations to meet the demands for land and water use for agriculture and human supply. In both Uruguay and Rio Grande do Sul – Brazil, the human action that most contributes to the

degradation of wetlands is the cultivation of irrigated rice, through the drainage of areas, the use of pesticides and fertilizers, the removal of water for crops. In the scientific literature, there are few studies addressing the topic of anthropogenic impacts related to drainage channels in coastal Wet Areas (UAs) located in the south of Brazil and north of Uruguay. This work involves showing some impacts caused by the opening of drainage channels in AUs in different locations, which result in environmental impacts such as erosion processes, fires, changes in vegetation, degradation of wetlands, interventions in the dynamics of marine life, especially turtles. In summary, it is essential to adopt integrated and sustainable approaches to the management of AUs, considering the diverse impacts of human activities and interventions on the environment, to ensure the resilience and health of these areas in the long term.

Keywords: Drainage and irrigation channels, Andreoni Channel, Big Bath.

1 INTRODUCTION

Wetlands (UAs) are defined as lands with predominantly hydric soil, flooded or saturated by surface or groundwater at a frequency and duration sufficient to support vegetation adapted to saturated soil conditions (COVINGTON et al., 2003).

Historically, UAs were seen as sites of slimy swamps that harbored disease. This idea led to the drainage and conversion of these sites into areas of intensive agriculture, aquaculture, industry and housing. However, in recent years, there has been awareness that natural UAs perform important functions, such as mitigating floods, recharging aquifers and retaining pollutants. The number of countries that have adopted a policy of containing the destruction or degradation of AUs is significant, recognizing that these areas must be used sustainably, encouraging research that qualifies and quantifies their values, highlighting the importance of their preservation (EDWARD, MIKE and DUNCAN, 1997).



Regarding Brazilian AUs, there are still many divergences regarding the definition criteria which, in turn, compromise the processes for protection and sustainable management of these systems of great socio-environmental relevance (GOMES and MAGALHÃES, 2017).

One of the most important physical components of AUs is the soil. Through its depth, mineral composition, organic matter content, humidity regime, temperature regime and chemistry, it is possible to justify a greater or lesser incidence of the types of plants and species of organisms that adhere to the soil. For these and other reasons, it is essential that the soil is considered in the classification of AUs (COWARDIN et al., 1979).

Within the characteristics of Aus, *Banhado* is a typical name from Rio Grande do Sul with etymological terminology from the Spanish “*bañado*”. Wetlands are formed where fresh water is dammed and flows slowly, and the water that supplies the wetlands comes from nearby water bodies, such as lakes, lagoons, rivers and/or the outcrops of the water table and rainfall (BURGER, 2000; CARVALHO and OZORIO, 2007).

As awareness of UA ecosystem services grows, so does public interest in their protection. Among the fundamental purposes of classifying AUs is improving the management of natural resources. By better understanding the functional attributes of each unit, society is more likely to manage its AUs to improve the management of these environments (BRISON, 2004; MITSCH and GOSSELINK, 2015).

Throughout history, many watercourses have undergone transformations to meet the demands for land and water use for agriculture and human supply. Of the changes established by the introduction of irrigated agriculture, the rectification of river channels and the drainage of wetlands sought to expand the cultivated area and allow for irrigation (BRENNER, 2021).

In both Uruguay and Rio Grande do Sul, the human action that most contributes to the degradation of wetlands is the cultivation of irrigated rice, through the drainage of areas, the use of pesticides and fertilizers, the withdrawal of water for crops and the return of these waters with waste to natural systems (BRASIL, 2002). In addition to destroying and fragmenting habitats, cultivation requires a significant volume of water for irrigation and the systematic use of fertilizers, insecticides and herbicides, substantially impacting natural ecosystems (DIAS and BURGER, 2005).

The drainage and irrigation works in *Banhado Del Este* in Rocha – Uruguay, date back to the first half of the century, in 1920, with the plan to “recover” land for agricultural purposes. From this perspective, plans and projects began to be drawn up with important water regulation and drainage works. This includes projects led, in the first instance, by the Uruguayan State (1930-1935) and Lagoa Mirim Commission (1967-1972). Thus, in 1979, by decree the drainage works of the wetlands of the department of Rocha for agricultural use were declared of national interest. In the period between 1979



and 1981, important drainage and irrigation works were carried out by the Uruguayan State (BARILANI, 2011).

The drainage process of AUs in Brazil, such as in the Coastal Plain of Rio Grande do Sul, occurred in a similar way to the neighboring country. Drainages intensified in the 1980s, with encouragement from the Federal Government, the National Program for the Use of Irrigated Floodplains - PROVÁRZEAS NACIONAL was created, with the purpose of promoting the rational and gradual use of national floodplain areas at rural property level, through of decree no. 86,146, of 1981 (BRASIL, 1981).

After the promulgation of the Federal Constitution, and the consecration of the Socio-Environmental Rule of Law, environmental legislation has evolved and strengthened over the years, aimed at due environmental protection (GASPERINI and REZENDE, 2020). However, the State itself throughout history has promoted works that impacted fragile environments such as Australia.

The appropriation of environments through human occupation results in a change in pre-existing dynamics (SILVA, 2019). The long-term alteration of natural resources has major impacts, such as drainage works in wetlands and AUs.

For Silva (2019), changes in natural environments carried out through artificial channels can promote changes in local dynamics and adjacencies and recognizing the behavior of these features is essential to understanding the changes throughout an entire basin, especially in areas downstream of the changes. and its repercussions on landscapes.

In southern Brazil, the Gravataí River has part of its sources in the AU known as Banhado Grande. This river underwent changes that began with the diversion of the channel at its mouth in 1955 (BOHRER, 2001). In the 1960s, the middle and upstream stretches of the Gravataí River underwent a rectification process, the initial objective of which was to drain the basin's humid areas for agricultural expansion and human supply. The rectification work was planned and carried out by the extinct National Department of Sanitation Works (DNOS), with no responsibility for the liabilities generated (BRENNER, 2021).

Still on the changes resulting from the opening of canals in AUs, the case of La Coronilha beach in Uruguay portrays the impacts of these works. With the aim of optimizing rice cultivation in the region and livestock farming, the canalization caused an adverse and irreversible impact that has continued over the years due to the artificial drainage of fresh water into the ocean, adding suspended material and agrochemicals (LEICHT, 2014) .

This study will address state-funded works in coastal AUs covering two different countries, Uruguay and Brazil. In Brazil, the canalization work on the Gravataí River in the Banhado Grande Environmental Protection Area (APABG) and the diversion of its mouth, changing its position from



west to south; and in Uruguay the opening of the Andreoni drainage channel, in the Lagoa Mirim watershed, which flows into Praia da Coronilha.

In the scientific literature, there are few studies addressing the topic of anthropogenic impacts related to drainage channels in coastal AUs located in the south of Brazil and north of Uruguay. Such areas, according to Sell (2017), are located in the Pampa Atlântico. An eco province¹ constituted in the lowest and flatest portion of the Pampa, coinciding with the Coastal Plain of Rio Grande do Sul (TOMAZELLI and VILLWOCK, 2000). In this way, they have the same geomorphological formation and are similar in their natural characteristics.

Therefore, the aim is to identify environmental impacts and liabilities generated by these works. Since the State invests in short-term works that benefit society and, at the same time, impact the environment in the long term. With the advancement of environmental legislation and studies focused on this topic, the State can intervene to ensure the rational use of these environments, in addition to the possibility of their restoration, recovery, maintenance and preservation.

2 LOCALIZAÇÃO E CARACTERIZAÇÃO DA ÁREA DE ESTUDO

2.1 ARTIFICIAL CANAL - MOUTH OF THE GRAVATAÍ RIVER

According to Hanke et al., (2013), the flow of the Gravataí River into Guaíba in the 1940s occurred through a channel heading north. In the 1950s, a canal was opened leaving directly to the south, cutting the area called Humaitá into two parts: one the current Humaitá neighborhood and the other the Humaitá Island. With this work, the river began to flow into Saco do Cabral.

In the 1940s, with the extension of the urban fabric of Porto Alegre, work began on Cais Navegantes, continuing, in the north direction, with Cais Mauá. Starting at Largo da Conceição until Sertório Avenue, it was 2600 meters long. This work was only completed in 1955 during the construction of the Gravataí canal, which transformed the tip of the floodplain into Humaitá Island (BOHRER, 2011).

2.2 DNOS CHANNEL - BANHADO GRANDE

As described by Etchelar and Guaselli (2018), interventions resulting from agricultural activities in the Gravataí River Hydrographic Basin (BHRG) began in the 1960s, with the execution of a macro-drainage channel by the extinct National Department of Works and Sanitation (DNOS). The work aimed to improve the flow conditions and drainage of the wetlands with the intention of expanding the rice production areas (DNOS, 1985).

¹ Eco provinces, whose delimitations are important because they help to understand the limits of resilience of each landscape and, in the specific case of tourism, the attractive potential of each of them (SELL, 2017).



Another existing channel on this river is located in the Banhado Grande area and was built at the end of the 1960s by the surrounding farmers. This channel connects to the channel built by DNOS and was intended to rectify and drain the wetlands for agricultural expansion aimed at planting irrigated rice (ETCHELAR, 2014; BRENNER, 2016; BELLOLI, 2017 and SIMIONI, GUASSELLI and ETCHELAR, 2017) .

Banhado Grande is part of a Sustainable Use Conservation Unit, the Banhado Grande Environmental Protection Area (APABG), which was created in 1998 through State decree n°. 38,971. The central objective is to protect an area of permanent preservation represented by the wetlands present in the region and its important water regulatory function (Rio Grande do Sul, 2021).

2.3 ANDREONI CANAL – CORONILHA BEACH

According to Méndez (1991), the Andreoni canal is one of the canals built in the Lagoa Mirim Basin – Uruguay, with the purpose of conveying surplus waters from the lowlands located south of the Serra de São Miguel and the Lagoa Negra basin to the ocean. , on Coronilha beach, Figure 1.3. The aforementioned canal has existed since the 1920s, where its length was 3 km, then in 1959 there was an extension of 13 km and in 1965 the Andreoni canal connected with the Laguna Negra canal.

In the 1970s, the Andreoni canal collected the contribution of a network of canals built for the desiccation of AUs, which negatively affected the environmental quality of this area (SCARABINO, 2004).

However, it was the works carried out from the 1980s onwards, during the period of the military dictatorship in Uruguay, directed by General Abdón Raimúndez, which caused profound damage to the ecosystem by extending it to a total of 78 km (RUBIO, 2013).



Figure 1 - Location Map, 1.1. Canal da Foz do Rio Gravataí – Porto Alegre – Rio Grande do Sul - Brazil, 1.2. Banhado Grande – Glorinha – Rio Grande do Sul -Brasil e 1.3. Canal Andreoni, La Coronilha – Rocha – Uruguai.



Source: Prepared by the author.

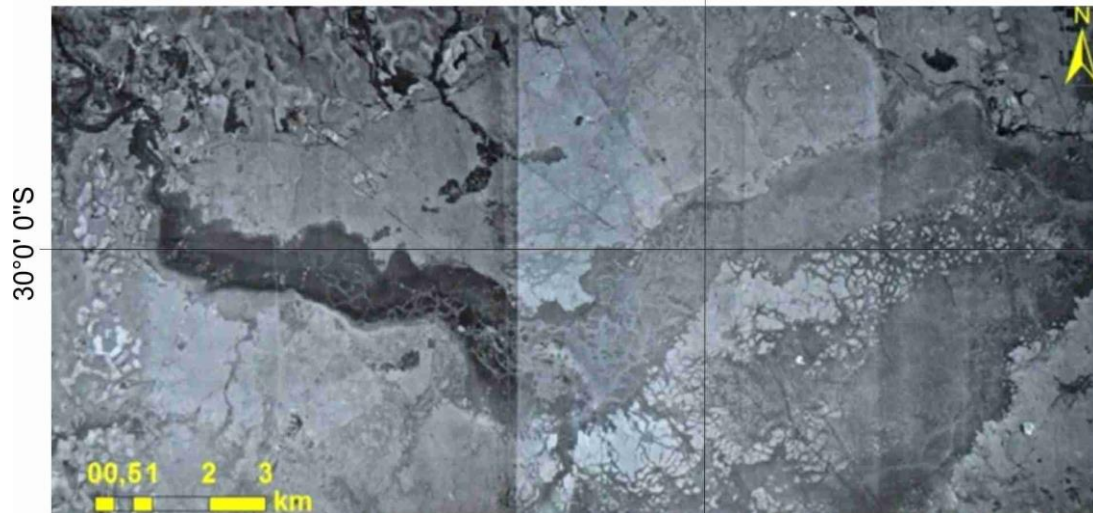
3 IMPACTS BY DRAINAGE CHANNELS

3.1 EROSION IN BANHADO GRANDE

Originally the Gravataí was a river with meandering features, which favored a low speed flow. Photograph in Figure 2, from 1960, illustrates a flooding event in the Gravataí River floodplain area, in darker tones. It is noteworthy that the floodplain is completely flooded and that the old meanders are connected by the flood event.



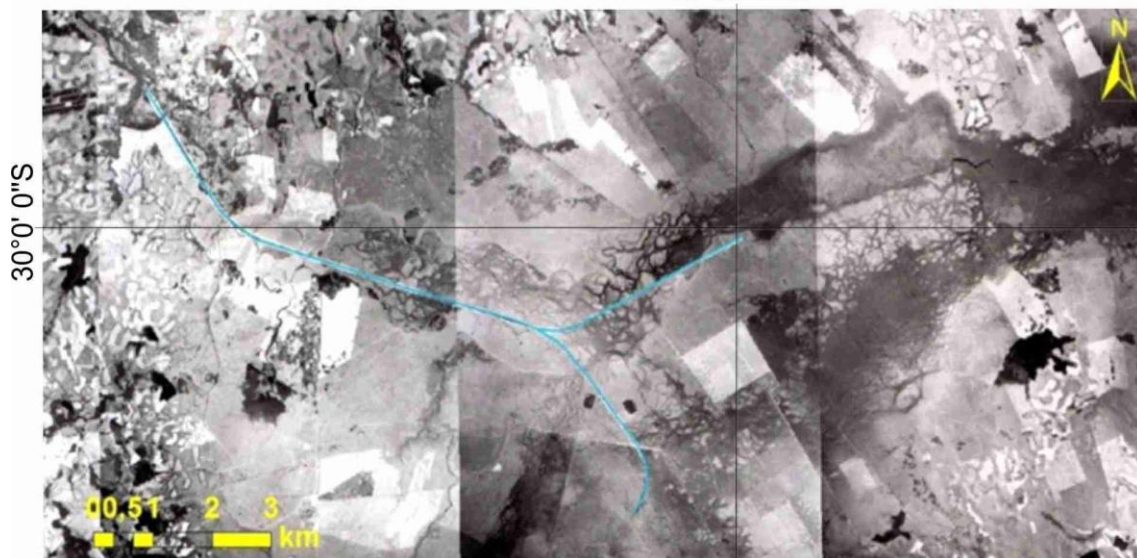
Figure 2 - Flooded area and connectivity of the old meanders of the Gravataí River floodplain.
50°50' 0"W



Aerial photograph supposedly dating from the 1960s. Source: Image provided by - Association of former scholarship holders in Germany (AEBA). Belloli, 2016.

The 1975 aerial photograph (Figure 2) shows the straightened stretch of river. Highlight is the meander network, to the north of the straightened section, which is largely disconnected from the Gravataí River. In this section, the speed of the water becomes different in relation to the natural condition of the Gravataí River (BRENNER, 2016).

Figure 3 - Straightened section of the Gravataí river.
50°50' 0"W



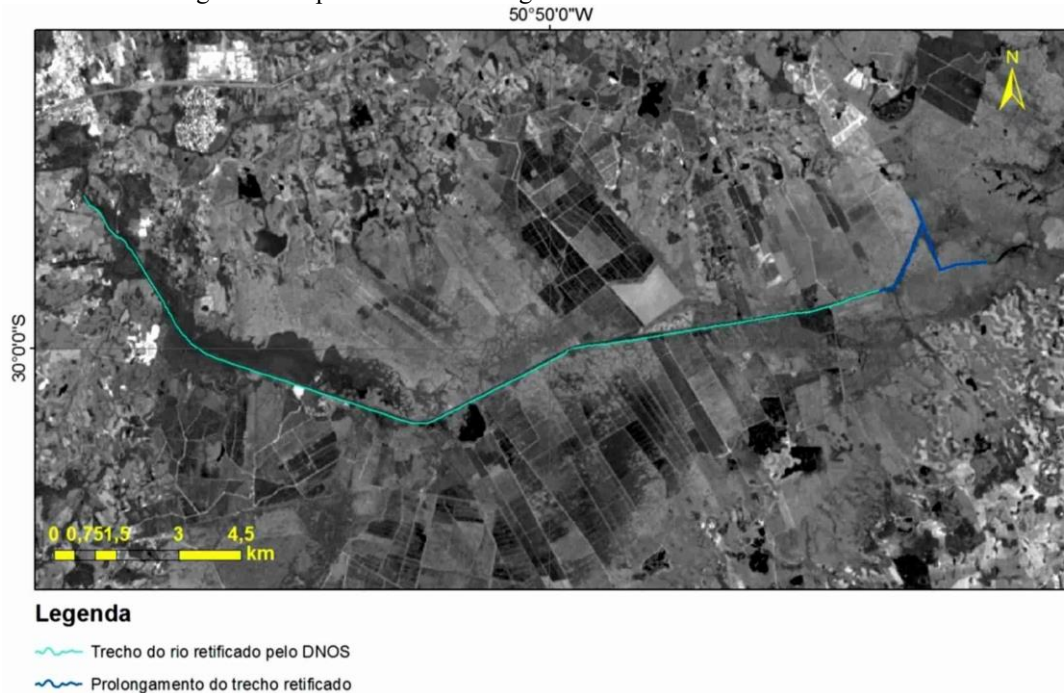
Source: Mosaic of georeferenced aerial photographs from 1975. Belloli, 2016.

The Landsat 8 satellite image from January 24, 2015, Figure 4, shows the enlargement of the straightened section to the east, highlighted in sky blue. According to Engineer Paulo Muller from the Gravataí Environment Foundation (FMMA), this expansion of the straightened section, next to Banhado Grande, was at the initiative of local rice producers, since works by the public sector had been suspended and were not had covered their production areas.



The work carried out by rice producers does not follow the original layout of the project, which provided for the execution of a main channel, straightening meanders in the upper course of the Gravataí river and entering the Banhado Grande for 35 km, to which two other secondary channels were connected. , totaling 66 km, whose objective was to “recover areas for agriculture”. However, the project was not fully implemented, with 37.5 having been built.

Figure 4 - Expansion of the straightened section of the Gravataí river.



Source: Landsat 8 OLI sensor image from 01/24/2015. Belloli, 2016.

The presence of water erosion in Banhado Grande is directly associated with land use and occupation. The straightening of the Gravataí River and the expansion of the straightened section for irrigating rice crops increased surface runoff and consequently lowered the water table (BRENNER, 2017; BELLOLI, 2016). Once physical variables are disturbed by human activities, advanced processes of water erosion begin to be triggered, although the process is not common in wetland areas.

The physical characteristics of the groundwater flow (RUBBO, 2004) and periods of high precipitation that cause flood pulses (SIMIONI, GUASSELLI and ETCHELAR, 2017) become an agent with erosive potential, once the gully begins. It should be noted that, regardless of the precipitation regime, there is a permanent flow of water in the gully gutter, even during dry periods. The water table resurfaces at all times of the year, typical of wetland areas. As a result, there is a continuous dragging of sediments, where in periods of high precipitation the dragging of soil particles intensifies. The advancement of the gully may be associated with groundwater dynamics, as the flow of the water table inside the gully maintains continuous erosion of sediments (ETCHELAR, 2017). Figure 5, with an image of the gully erosion process in Banhado Grande.

Figure 5 - Segment with the greatest widening of the gully in Banhado Grande.

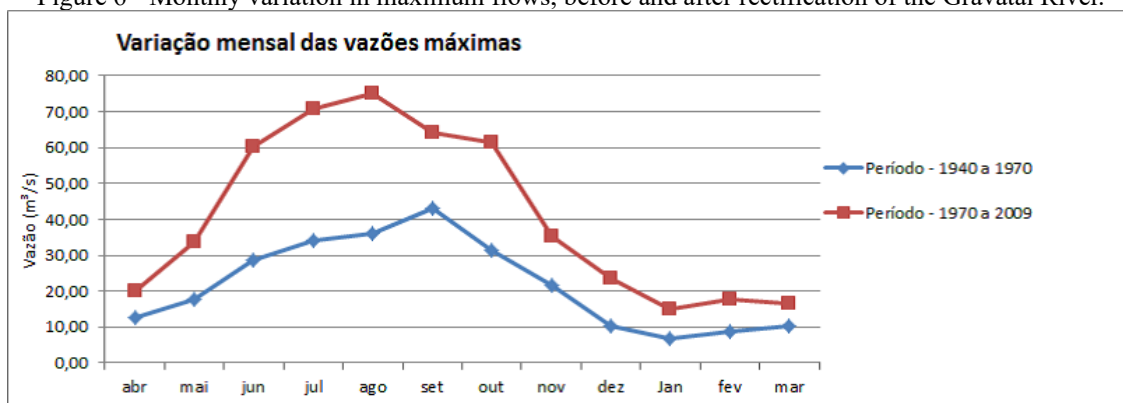


Source: Cecilia Balsamo Etchelar, 06/28/2017, personal archive

According to Belloli (2016), the analysis of maximum monthly flows Figure 6, between the years 1940-2009, shows a large increase in flow values, especially between the months with the highest precipitation in the basin (June to October). The maximum flow recorded from the years 1940-1970 for the pre-rectification period was 42.89 m³/s, in the month of September and the minimum flow was 6.27 m³/s in the month of January. In the post-rectification period, from 1970-2009, the maximum flow recorded was 74.93 m³/s in September and the minimum was recorded in January, with 15m³/s.

According to the author, the amplitude between the month with the lowest maximum flow (January) and the month with the highest maximum flow (September) was 36.62m³/s. In the post-rectification period, the amplitude between the month with the lowest maximum flow (January) and the month with the highest maximum flow (August) was greater, 59.93m³/s.

Figure 6 - Monthly variation in maximum flows, before and after rectification of the Gravataí River.

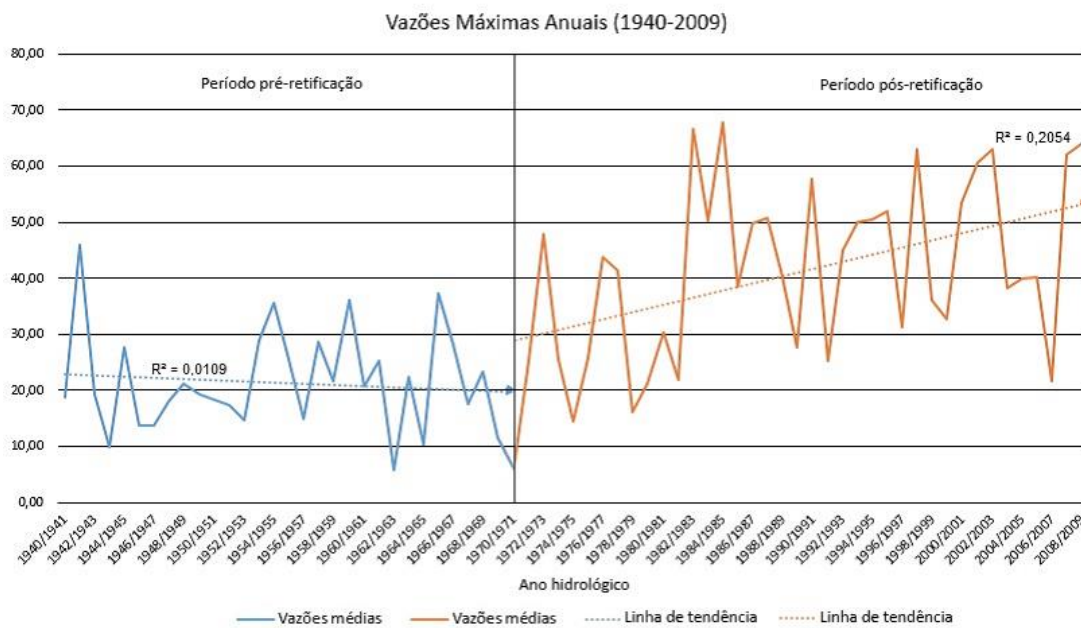


Source: Belloli, 2016.



The analysis of maximum annual flows (Figure 7), according to Brenner (2016), shows a tendency for flow values to increase in the post-rectification period. Maximum flows increased due to the ease of flow provided by rectification, as the course of the river previously circulated a pattern of curves that dampened and reduced the flow speed and with rectification the straight course favored an increase in water flow speed. The highest value of annual flow found refers to the hydrological year 1984/1985 with 68.51 m³/s, while the lowest value in the series of maximum flows was found in the hydrological year 2006/2007 with 21.28 m³/s .

Figure 7 - Maximum Annual Flows for the period 1940-2009.



Source: Brenner, 2017.

The analysis of flow patterns makes it possible to prove the increase in flows after the straightening of the Gravataí River. To the extent that the initial objective of DNOS in rectifying the Gravataí River was precisely to increase the speed of water flow and drainage of the Banhado Grande, we can affirm that this intervention has faithfully fulfilled its creation objectives (BRENNER, 2016).

Also according to Brenner (2017), when analyzing the standard behavior of flows on a longer time scale (69 years), an increase in flows after the rectification of the Gravataí River is confirmed. Such an extreme variation in flow rates gives rise to the need to rethink the route of the rectified course of the Gravataí. The straight section further favors accelerated flow from upstream to downstream, preventing the Banhado Grande from fulfilling its function as a “sponge” basin, by absorbing large peaks of rainfall and releasing water slowly and gradually.

Some considerations in the report prepared by a working group designated to evaluate the environmental impacts resulting from drainage works in the Banhado Grande (LUSCOSA, 1979) mentioned the increase in flooding downstream of the river, after the start of drainage works, and



predicted consequences in long term, such as more drastic changes in the landscape, such as: the intensification of secondary drainage for rice farming and the use of pesticides and fertilizers, predictions that have become reality.

Another relevant factor in erodibility processes concerns pedology. Etchelar (2014), collected soil samples in January 2014 at coordinates 29° 58' 29.65" S and 50°44'19.71", inside the gully. These samples were analyzed at the Center for Coastal and Oceanic Geology Studies (CECO), at the Federal University of Rio Grande do Sul, through the PANICOM/SAG program. This granulometric analysis made it possible to verify the percentages of Sand, Silt and Clay in each horizon of the profile.

Through the granulometric analysis of the soil (Table 2), in the horizons exposed on the slope of the gully, it indicates the first three samples of the profile, ordering from top to base, a predominance of sandy soil. The fourth sample demonstrates a large percentage of clay, in this horizon the base level of the gully is reached. It can be said that, as it has a more sandy particle size fraction, this type of soil would be more susceptible to the action of erosive processes, which justifies the erosive process reaching the base level, when it reaches the horizon called clay-silt-sandy (ETCHELAR, 2014).

Table 2 - Particle size analysis of the gully soil

	Sample 01 Arenosiltargiloso	Sample 02 Arenoargilosiltoso	Sample 03 Arenoargilosiltoso	Sample 04 Argilosilearenoso
Sand	63,96%	74,80%	52,24%	16,75%
Silt	29,87%	10,39%	18,11%	22,61%
Clay	6,16%	14,80%	29,63%	60,62%

Source: Etchelar (2014).

Once the gully-shaped erosion process has begun, soil particle size samples in the wetland area indicate greater susceptibility to erosion processes in the first three horizons that have a higher percentage of sand, unlike the fourth horizon, corresponding to the fourth sample that contains the highest index. of clay and indicative of the base level of the gully. When the verticalization of erosion inside the gully reaches the base level, the gully tends to evolve towards the horizontalization of this erosion process (ETCHELAR, 2014).

For Etchelar (2014), another important factor that leads to the formation of gullies is through erosion caused by subsurface flow, which gives rise to ducts, pipings or pipes. Gullies have steep side walls and, in general, a flat bottom, with water flowing inside them during rainy periods. By deepening their channels, gullies reach the water table and constitute a process of accelerated erosion and instability in landscapes (SUERTEGARAY et al., 2008). This type of erosion process called ducts or



pipings, are channels in the form of tunnels carved into the subsurface, with great oscillation in size and extension, with diameters varying from a few centimeters to several meters.

The flow of water that percolates through these pipes transports large quantities of material into the subsurface. As this material is removed, the river channel in the straightened section increases, which could result in the collapse of the soil above. Thus, leading to a significant evolution of a gully (SUERTEGARAY et al., 2008).

Erosion in tunnels occurs under the effect of surface and subsurface runoff water, which penetrates biogenic holes or cracks of different origins. If the water reaches the tunnel via a subsurface route, the forces and factors that act on erosion due to leakage predominate; If water originates from surface flows, turbulent flows predominate, thus, erosion by pipelines constitutes an excellent example of interaction between different fundamental mechanisms that can generate erosive features (GUERRA et al., 1999).

These subsurface erosion processes were identified in the wetland area, both on the slopes of the gully and on the slopes of the straightened section, as we can see a pipeline with water flow in Figure 8.

Figure 8 - Presence of an active pipeline with water flow on the riverbank, in the straightened section.

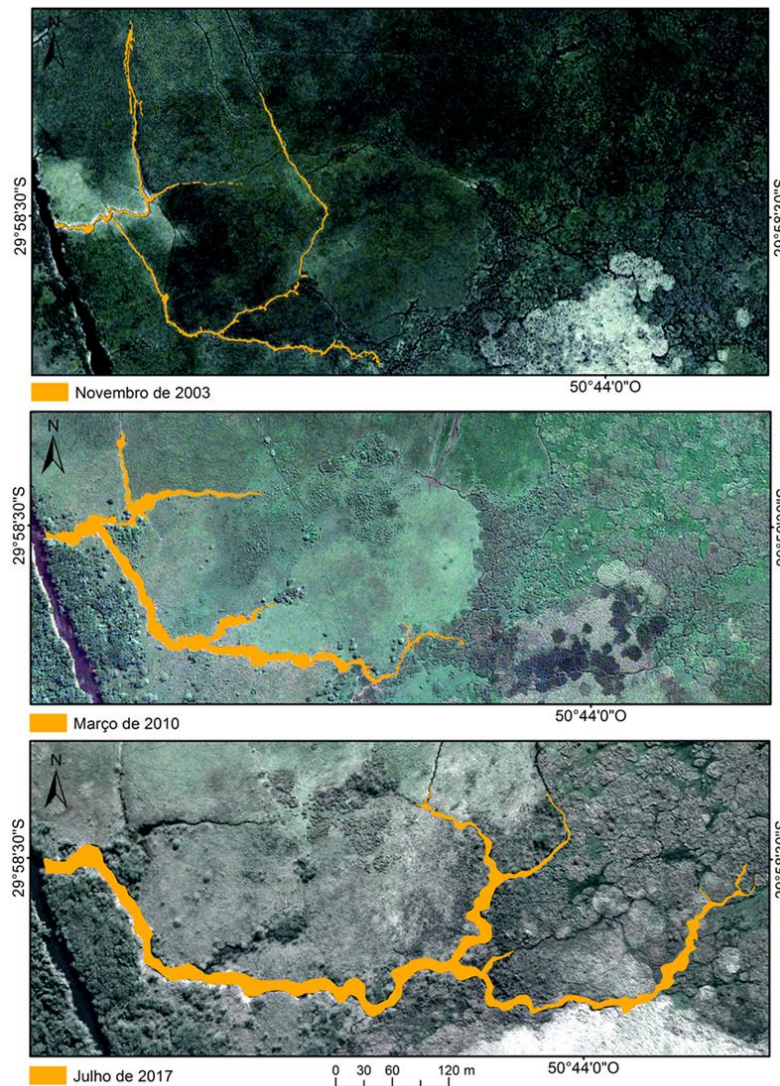


Source. Cecilia Balsamo Etchelar, 08/17/2018, personal archive.

The mapping of the gully, Figure 9, highlights the advancement of the erosion process, as well as the stabilization of erosion in some points. This fact may be associated with the advancement of shrub vegetation, identified in the field as a phytophysognomy with a predominance of Maricás (*Mimosa bimucronata*), which developed in the area surrounding the erosion process. The stabilization of part of the erosion can be observed to the north, in images from 2003 and 2010. The time series of

images shows the existence of an intense process of lateral widening of the gully, in addition to the process of remounting erosion, towards the interior of Banhado Grande, as shown in the 2017 image.

Figure 9 - Mapping of the temporal advance of the gully in Banhado Grande.



Elaboration: Etchelar and Guasselli (2018) based on images from 2003 and 2010, referring to the Google Earth image mosaic. Image from 2017, ARCGIS base map.

The time series of Google Earth images, in the analysis of the gully's features, made it possible to map and quantify the dynamics of the evolution of its erosion process. The eroded area increased from 2,909 m² in 2003 to 13,663 m² in 2017, Figure 10.

Figure 10 - Temporal evolution of the gully.



Source: Etchelar and Guasselli (2018).

According to Augustin and Aranha (2006), for a channel to evolve into a gully, the necessary condition is, in addition to erosion, the presence of a set of processes, including soil undermining and pipelines. These destabilize the walls and head of the channel, causing its widening and its evolution upstream, characterizing a gully.

Gully-shaped erosion in Banhado Grande and erosion in the straightened section cause negative environmental impacts of significant importance in this important and fragile ecosystem. Variables that cause environmental imbalance include: the lowering of the water table associated with the increase in flow and water drainage from the wetland towards the floodplain, the loss of soil and the silting of the river. There is also the possibility of access by hunters, from the gully, which becomes an access route to the interior of the marsh, as the place serves as a refuge for a great diversity of native and migratory fauna (ETCHELAR, 2017).

Through the visual interpretation of images for a time scale of the years 2003, 2012 and 2019, Figure 11, the impact that the gully causes on the typical vegetation of the wetland is identified. In the section upstream of the gully in the image: a) corresponding to the year 2003, when there is no presence of the erosion process; b) dated in 2012, the evolution of the gully and the retreat of vegetation can already be seen. In 2019: c) the gully reaches a stage that isolates part of the vegetation, leading to its replacement by another type of vegetation.

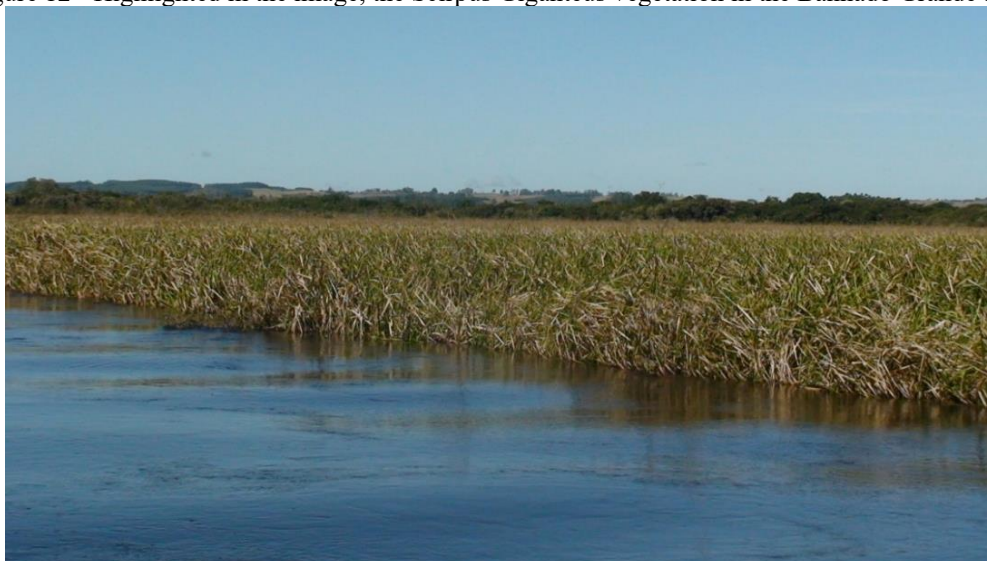
Figure 11 - Temporal analysis of a gully area in Banhado Grande where the conversion of vegetation type is observed as a result of gully expansion.



Elaboration: Etchelar (2020).

This vegetation is a species of large size, up to approximately 2 m in height, predominantly formed by *Scirpus Giganteus*, known as Tiririca or Palhão, Figure 12. The importance of these aquatic macrophytes is related to their metabolic activity, which develops micro-organisms associated in the collaboration of water purification by oxidizing the organic matter it contains (FZB, 1983).

Figure 12 - Highlighted in the image, the *Scirpus Giganteus* vegetation in the Banhado Grande area.



Source: Cecilia Balsamo Etchelar, June 25, 2015.



In addition to the damage to the water balance, the erosion processes triggered by the drainage dynamics of wetlands can drastically alter the vegetation adapted to the conditions of hydromorphic soils. For FZB (1983), the richness of the submerged vegetation in the wetlands provides the maintenance of inocula that will feed the river. If the base of the food chain, made up of these synthetic vegetables, capable of producing organic matter, is not maintained, the other components of the system will be threatened, such as: fish, amphibians, crustaceans, molluscs, insects and other consumers.

Wetlands have high nutrient availability and high vegetative/food production, but are highly vulnerable to drainage, landfills and rectification. Surely, because these cause changes in soil humidity that reflect on vegetation; since the fauna is dependent on it for refuge, shelter and food, both outside and inside the water (FZB, 1983).

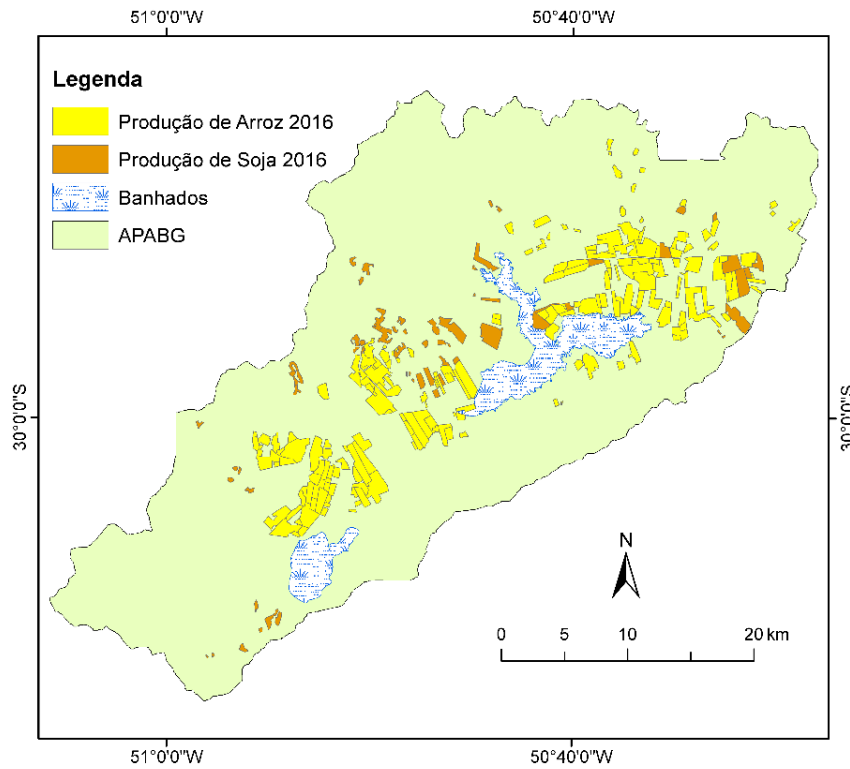
3.2 RICE AND SOYBEAN PRODUCTION IN APABG WETLANDS

In APABG, irrigated rice cultivation predominates in the flat areas next to the Gravataí River. According to Mertz (2002), the first large irrigated rice field in the Metropolitan Region of Porto Alegre was established in the municipality of Gravataí, in 1905. Rice production is, traditionally, the main temporary cycle agricultural production, due to the topographic, hydrological and pedology in the area. More recently, soybean cultivation was inserted into the agricultural rotation cycle, occupying representative areas.

According to the mapping of rice and soybean production at APABG, Figures 13, 14 and 15, rice production decreased by 852.69 ha between the 2016, 2018 and 2020 harvests, while soybean production increased by 2,722.36 ha in the same period, demonstrating that the increase in soybeans is not equivalent to the decrease in rice area. This does not show an inversion of culture in the area, but rather an increase in agricultural production in the same area that is probably used for rotation and fallow areas.

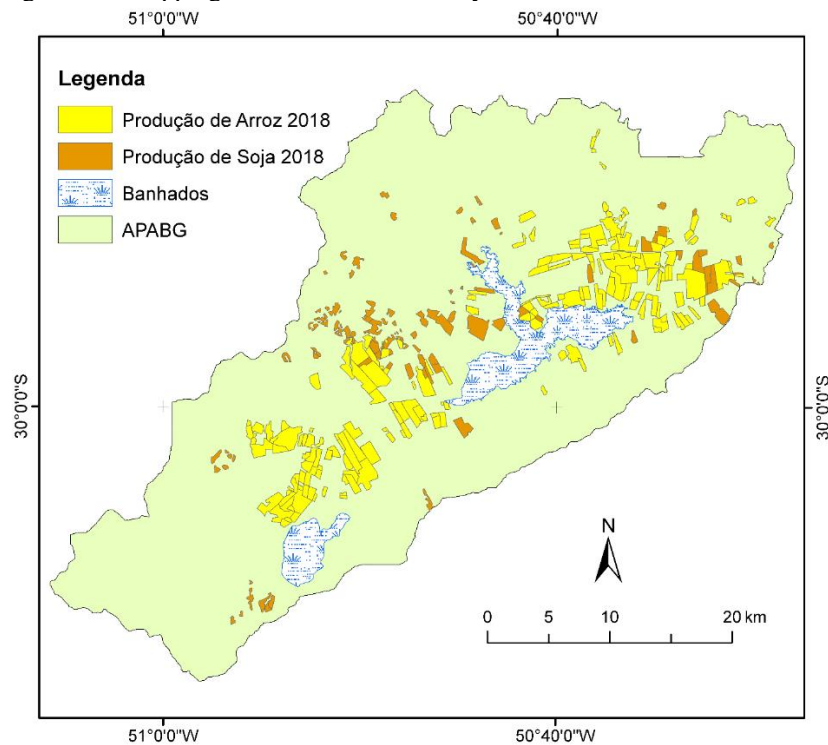


Figure 13 - Mapping of the 2016 rice and soybean harvest in the APABG area.



Prepared: Etchelar (2022) Source: Belloli and Etchelar (2020)

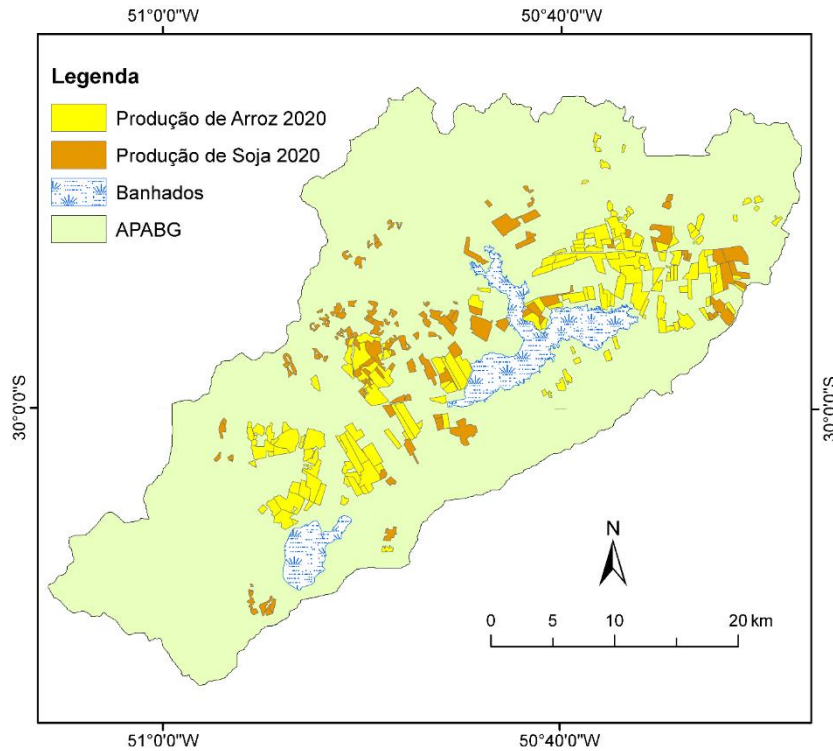
Figure 14 - Mapping of the 2018 rice and soybean harvest in the APABG area.



Prepared: Etchelar (2022) Source: Belloli and Etchelar (2020).



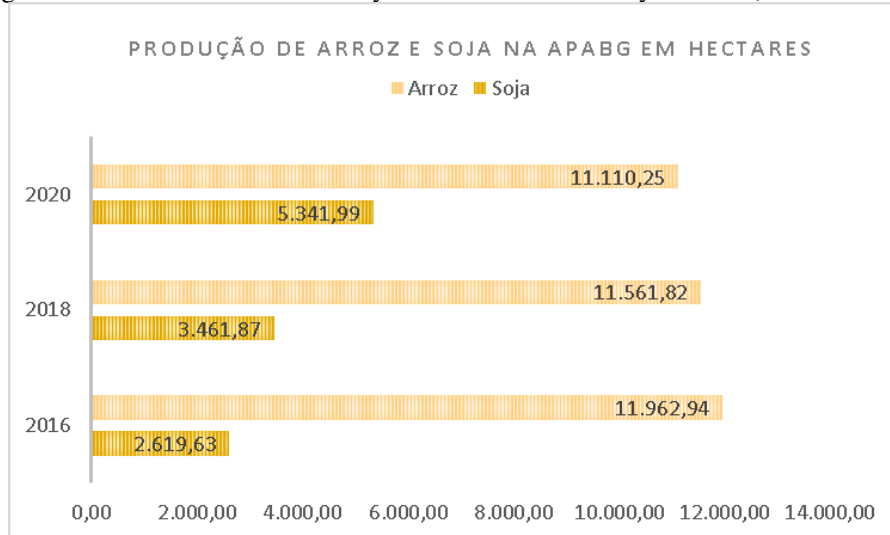
Figure 15 - Mapping of the 2020 rice and soybean harvest in the APABG area.



Prepared: Etchelar (2022) Source: Belloli and Etchelar (2020).

We noticed the increase in soybean cultivation in APABG in the mapping presented in the previous figures, which are highlighted in orange. However, when we transfer this data to the graph, Figure 16, we can see the progression of soybean production and a small decrease in rice cultivation.

Figure 16 - Production of rice and soybeans at APABG in the years 2016, 2018 and 2020.



Prepared: Etchelar, 2022.

3.3 FIRES IN BANHADO GRANDE

Ecosystems and society must be better prepared for the new scenario of extreme fires. Climate change is the present and the future and it is urgent to take adaptation and mitigation measures to reduce the expected impacts (Hernández, 2020).

In April 2020, a large fire impacted Banhado Grande, as seen in Figure 17. One of the difficulties encountered in controlling this fire, in addition to difficult access, were the several fires that occurred in the subsurface in peat deposits. Peatlands have not yet been studied according to Accordi et al. (2003)

.Figure 17 - Vegetation burning at APABG in April 2020.



Source: Banhado Grande APA Collection. Photo: Cecília S. Nin (2020).

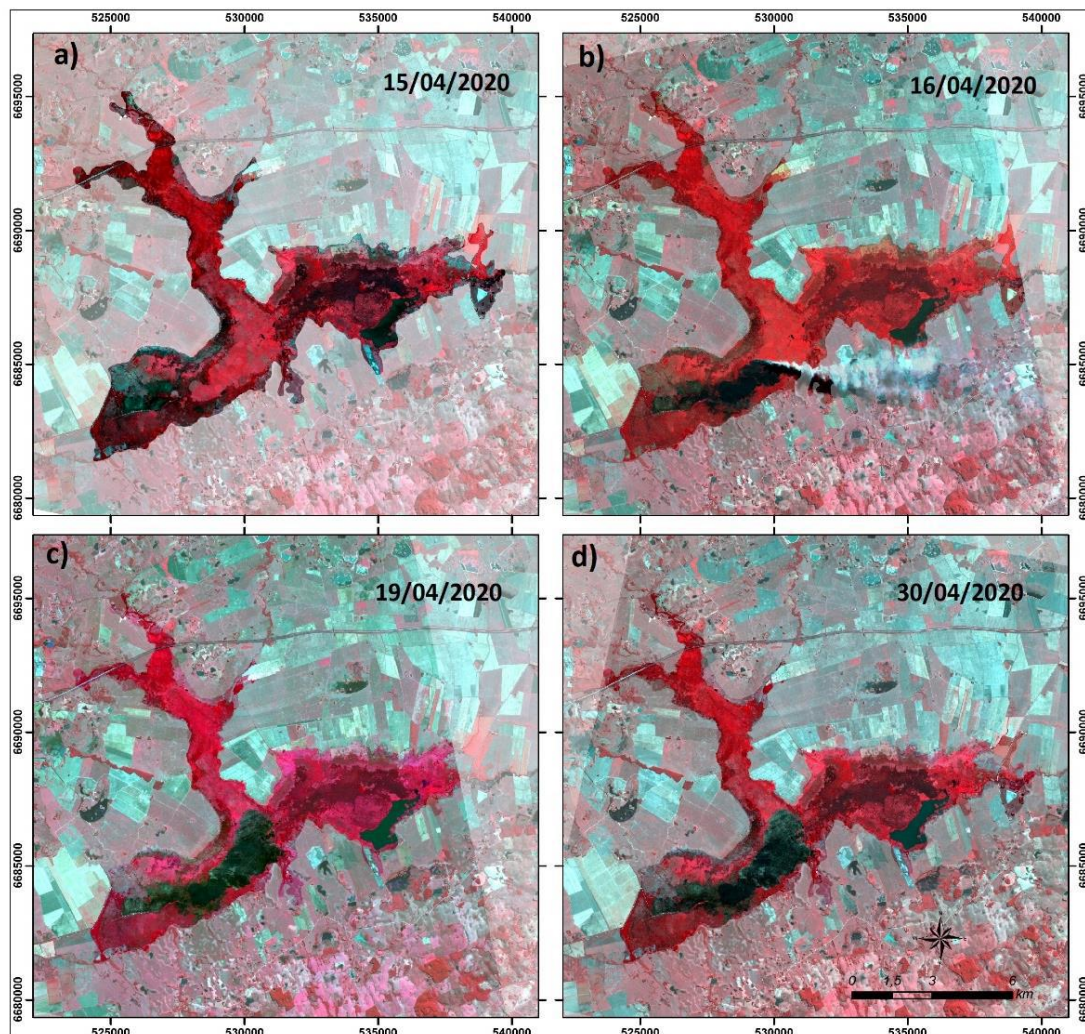
The APABG AUs are home to the two largest peat bog areas in the Coastal Plain and a paleo-environmental archive with a geological history of more than two billion years that is intertwined with the history of the formation of the continent itself and, particularly, the events that formed the continent. Atlantic coast of South America (ACCORDI et al., 2003).

According to Simioni (2021), the fire devastated approximately 12% of the total area of the wetland, where the areas with the presence of emerging vegetation were the most devastated. Through Planet Scope images, we observed that in Figure 18a, on 04/15/2020 the fire had not yet reached the emerging plants. On 04/16/2020, the image captured the advance of the fire over the emerging plants, Figure 18b, shows that the fire had consumed more than 60% of the area with the occurrence of emerging plants. The image from 04/19/2020, Figure 18c, shows that the surface fire had been controlled in the area of emerging vegetation, with the work of firefighters and volunteers and also the presence of a water body that prevented the fire from advancing to the east and southwest arms of the BG. However, although the surface fire was controlled, the so-called underground fire continued, with



the burning of peat, which lasted until the end of April, when the fire was completely controlled, Figure 18d.

Figure 18 - Temporal variation of the area burned by fire in Banhado Grande.



Source: Planetscope image, RGB 432 bands. Prepared: SIMIONI, 2021.

The lack of precipitation during this period was another major aggravating factor in the fire situation. From January to April 2020, rainfall levels were well below the monthly averages, with the months of March (12.2 mm of rainfall) and April (13.2 mm of rainfall) coinciding with the growth of the COVID pandemic -19, further aggravating the need for water for the municipalities supplied by this water source – at a time of emergency in terms of sanitation and public health (VERDUM and VIEIRA, 2020).

3.4 MOUTH OF THE GRAVATAÍ RIVER

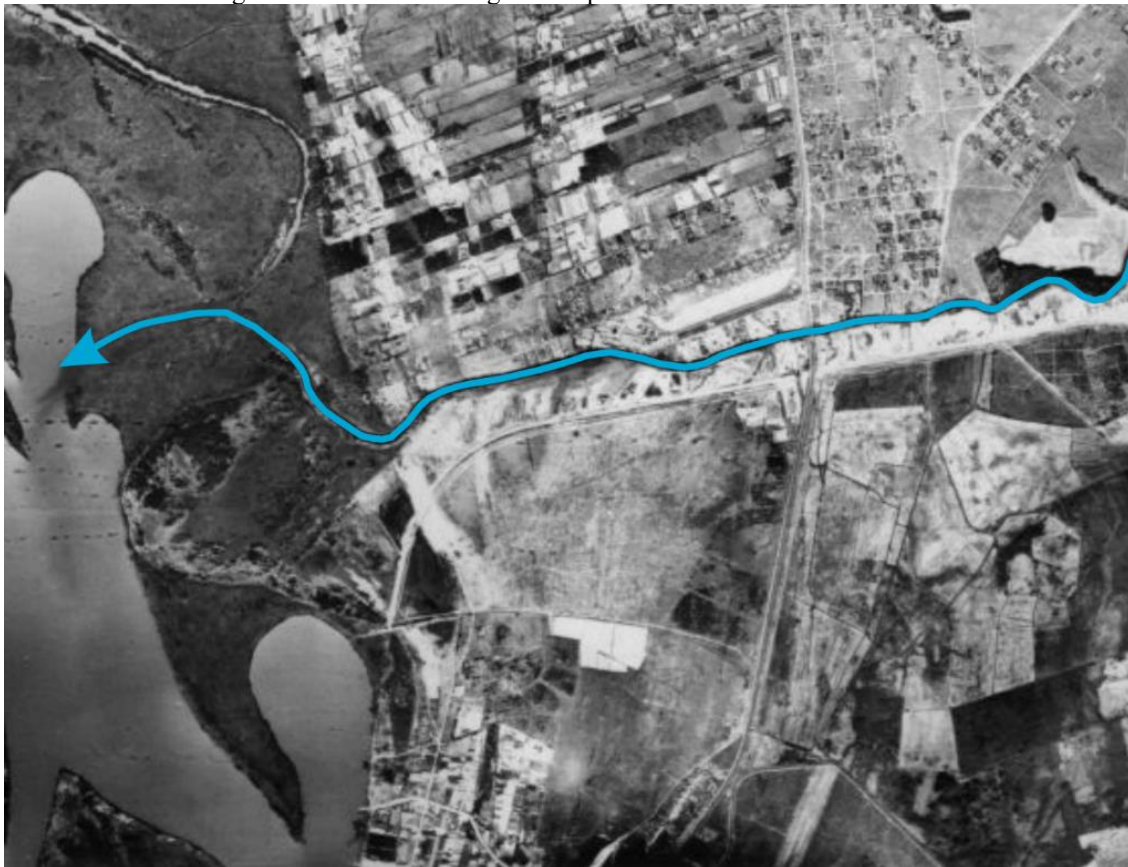
The 1950s period in Porto Alegre was marked by urban growth, which took on a very rapid pace, with the construction of commercial buildings, the installation of the railway that connects Porto Alegre to São Leopoldo and the construction of the new Cais do Porto, with the embankment of an



extensive strip between Avenida Voluntários da Pátria and Lake Guaíba. Landfills are established as alterations caused by human action, in the form of borrow areas (DIAS, 2011).

According to a work presented by Hanke et al., (2013), we must analyze the consequences of a rectification work carried out in the 1950s, at the mouth of the Gravataí river, which caused problems and changes in the functioning of the entire river basin, as Before this work, the Gravataí River flowed into Guaíba through a channel heading north/west, next to the stream in the Jacuí delta, as seen in Figure 19.

Figure 19 - Historical image of the preserved Gravataí river mouth.



Source: Image without defined date, HANKE et al., (2013). Prepared by the author.

In Figure 20, from 1956, we can see the opening of the canal leaving directly to the south, cutting through the area called Humaitá, where the river started to flow into Saco do Cabral. In Figure 21, we detail that the canal in 1956 was not yet fully open. In Figure 22 we see the fully consolidated channel with its darker waters of the Gravataí River meeting the lighter waters of the Jacuí River.



Figure 20 - Image from 1956 that shows the incomplete opening of the channel that changes the mouth of the Gravataí river.



Source: Aersurvey of 1956 1: 2000. Coordination of Geoprocessing and Urban Information – CGIU, Municipal Secretariat for the Environment and Sustainability – SMAMS. Prepared by the author.

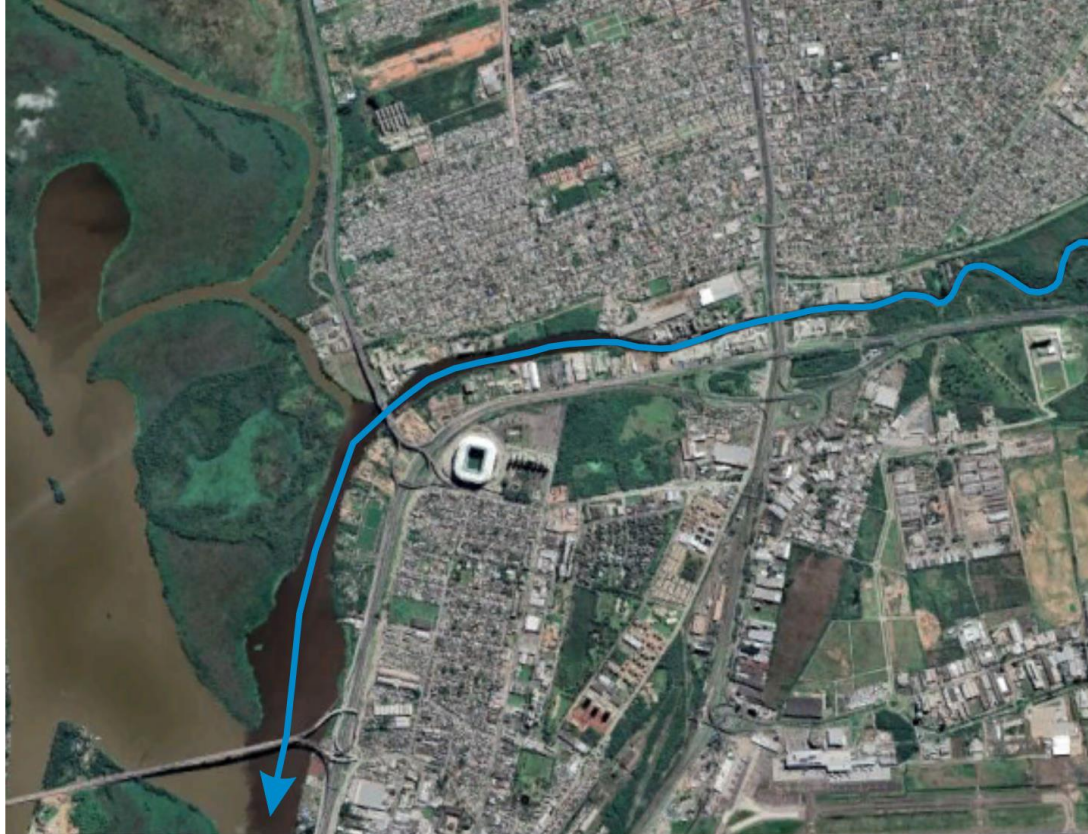
Figure 21 – Detail of the incomplete opening of the channel in the 1956 image.



Source: Aersurvey of 1956 1: 2000. Coordination of Geoprocessing and Urban Information – CGIU, Municipal Secretariat for the Environment and Sustainability – SMAMS. Prepared by the author.



Figure 22 - Satellite image showing the current condition of the channel at the mouth of the Gravataí river.



Source: Image from August 5, 2022 from Google Earth Pro. Prepared by the author.

3.5 CANAL ANDREONI

3.5.1 Impact on the beach

In October 2019, with a large amount of plant debris that was coming out with the fresh water from the Andreoni channel added to the oceanic algae, it formed a kind of very dense blanket that did not allow the turtles to swim, and caused them to rush into this vegetation (Figure 23). A similar phenomenon had already been recorded in 2012 and in 2016. The rescue was carried out in the Coronilla beach area (Figure 24), where there is a rehabilitation center that cleaned, hydrated and checked the physical conditions. Fortunately, this action managed to rescue all the turtles alive (URUGUAY VISIÓN MARÍTIMNA, 2019).

Figure 23 - Typical wetland vegetation on the edge of Coronilha beach – Uruguay.



Source: elpais.com.uy (2019).

Figure 24 - Turtles rescued on the edge of Coronilha beach – Uruguay after becoming trapped in vegetation coming from the AUS mixed with algae.

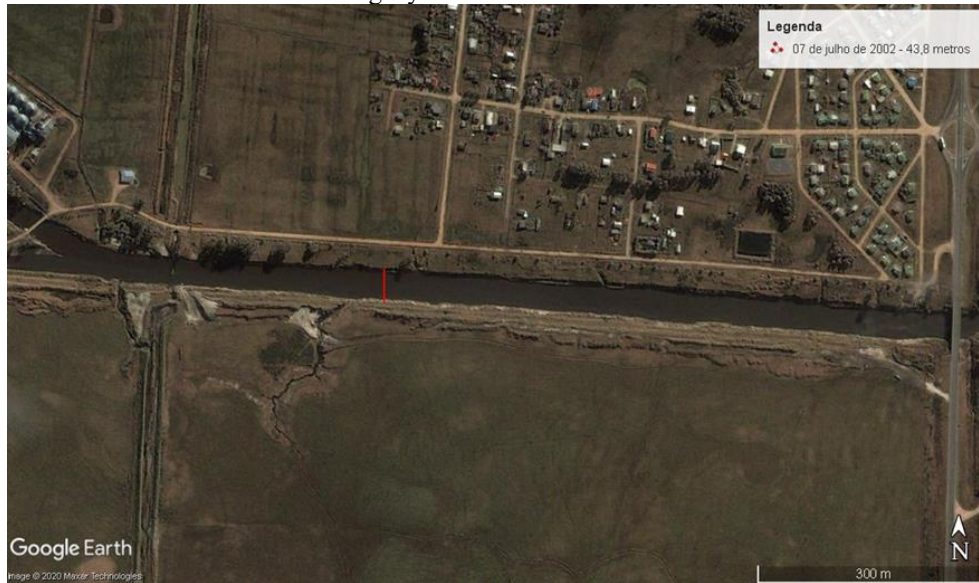


Source: Uruguay maritime vision, (2019).

3.5.2 Erosion of the Andreoni channel

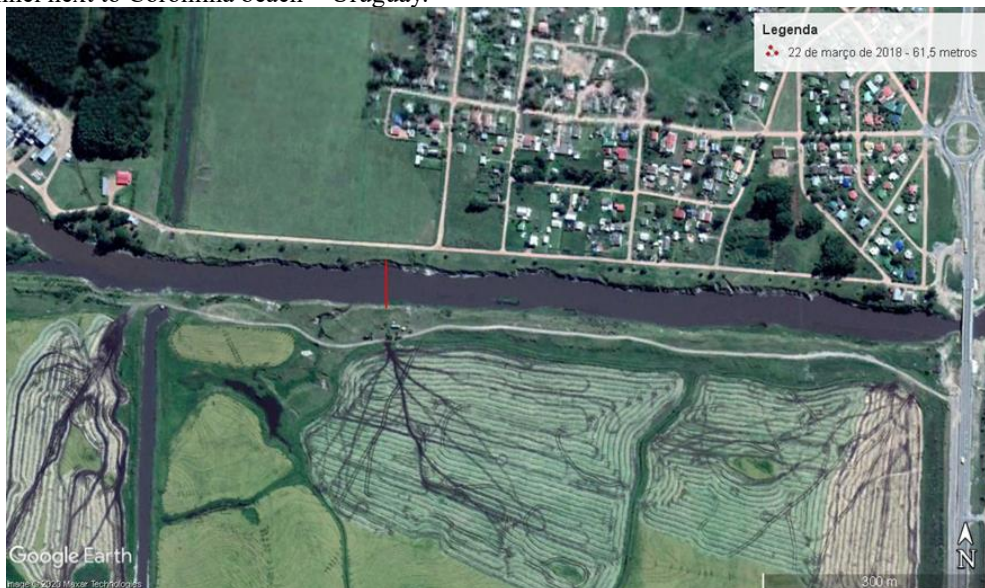
The temporal evolution of the stretch of the Andreoni channel, next to Coronilha beach, shows a progressive widening in this section demarcated by the red line in Figures 25 and 26. Between the years 2002 and 2018, it increased from 43.8 m to 61.5 m , resulting in a widening of 17.7 m over a period of 16 years.

Figure 25 - Satellite image of July 7, 2002 – Representation in the red line of the width of 43.8 m in this section of the Andreoni channel next to Coronilha beach – Uruguay.



Source: Google Earth Pro image. Prepared by the author.

Figure 26 - Satellite image of March 22, 2018 – Representation in the red line of the width of 61.5 m in this section of the Andreoni channel next to Coronilha beach – Uruguay.



Source: Google Earth Pro image. Prepared by the author.

4 FINAL CONSIDERATIONS

The study of the works on the Gravataí River drainage channel at APABG and the Andreoni drainage channel in the Lagoa Mirim Basin – Uruguay, together with the change in direction of the mouth of the Gravataí River in the 1950s, reveals significant impacts on urban areas (AUs) and agricultural areas of these regions.

Canalization and drainage interventions alter the natural course of rivers, affect local topography and drainage, and influence the environmental conditions of surrounding urban areas. The change in the direction of the mouth of the Gravataí River may have disturbed natural water flow



patterns, impacting local ecosystems and communities dependent on them.

The mapping of areas of agricultural use for crops such as rice and soybeans in APAGB and in the Lagoa Mirim hydrographic basin highlights the pressure exerted on these areas for agricultural purposes, and the increase in soybean production around the wetlands, which may have implications significant environmental impacts, such as loss of natural habitat and soil degradation. As well as the increase in fires and the destruction of the natural vegetation of this environment.

The canalization of the Gravataí River triggered major erosion processes in the Banhado Grande area in the long term. The impacts caused by the Andreoni channel on Coronilla beach in Uruguay are similar in terms of erosion to those that occurred on the Gravataí River, in addition to causing a major impact on the beach line with the deposits of waste from the wetland on marine life, such as turtles.

In summary, it is essential to adopt integrated and sustainable approaches to the management of AUs, considering the diverse impacts of human activities and interventions on the environment, to ensure the resilience and health of these areas in the long term.



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Multitemporal analysis of land use and occupation with a focus on agriculture, livestock and pebble extraction in the municipality of Ourém/PA, from 2016 to 2020



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ABSTRACT

The studies of the forms of use and coverage of the earth's surface are essential to provide subsidies for the environmental monitoring of a given area. The present work aimed to analyze the dynamics of land use and land cover in the municipality of Ourém/PA in the period from 2016 to 2020, generating representative information of the landscape dynamics, influenced by the physical and socioeconomic aspects inherent to this municipality. Land use and land cover classes were quantified and mapped with Google Earth Engine from 2016 to 2020. Data processing was performed using the ArcgisPro software to classify land use and land cover classes and to make maps. With the products generated, an image was selected for each year, in order to determine the areas for each thematic class of urban area, vegetation, agriculture and livestock, and pebble extraction, so that it was possible to quantify the class measurements. The results of each year were compared and a thematic map was prepared with the images selected to determine the use and occupation of the land in the municipality. The results revealed that the agriculture and pasture classes are predominant during the analyzed period, with a slight reduction in vegetation cover and a slight increase in the area of agriculture and pasture. Therefore, the identification and analysis of the classes of use and occupation provide both the understanding of these modes of use and the degradation to the environment, understanding the function of Man as a potential cause of this degradation.

Keywords: Transition Dynamics, Amazon rainforest, Geotechnologies.



1 INTRODUCTION

For the evaluation of the occupation process of a given area, it is essential to use Geotechnology resources such as Remote Sensing and Geographic Information System, which provide the generation of updated and reliable information (FACCO et al., 2017), allowing periodic mapping to monitor phenomena of change in land use and land cover.

These resources, according to Vaeza et al., (2010) provide greater efficiency in the process and update data at a lower cost, making them useful and indispensable for monitoring the dynamics of land use and occupation. In view of this, the survey of land use in a given region becomes a fundamental aspect for understanding the patterns of spatial organization and planning of public policies (PRADO; NOVO; PEREIRA, 2009).

In the temporal analysis of land use and land cover in Remote Sensing images of a municipality, through digital processing techniques, it provides the recognition of changes in agricultural and livestock activities, as well as changes in native forest areas. In tropical forests, these changes have occurred mainly due to anthropogenic pressures associated with the exploitation of raw materials (SOUSA et al., 2017). For Facco et al., (2017), this type of mapping provides a systematic inventory of the land surface, making it possible to categorize different uses into classes, which, associated with the geographic information system (GIS), enables the knowledge of the evolution of the spatial dynamics of land use with the production of time series of maps for the same area.

The authors Cunha et al., (2012), highlight that the use of geoprocessing techniques through GIS is efficient in detecting changes in land use and land cover, as well as changes caused in landscapes in a given place and time scale, making it possible to diagnose anthropogenic and natural changes in the landscape.

The increase in urban agglomerations, the exploitation of natural resources and mining activities cause rapid changes in landscapes that, combined with continuous and poorly planned development, generate various environmental and socioeconomic impacts. Therefore, it is necessary to obtain precise information about the geographic space for regional and local planning, in order to identify and classify the changes that have occurred in the landscapes (ARAÚJO FILHO; MENESES; SANO, 2009).

According to Sousa et al. (2017), this identification is carried out through the photointerpretation of the image captured by the sensors through the analysis of the elements associated with the targets such as color, shade, size, shape, texture, pattern, context, and presence of shadows. In order to understand the information in the image, it is necessary to characterize the data contained in it. Thus, digital classification techniques are applied to the image provided by the satellite in order to assign each pixel of the image under study to a category of a group of classes (BAKR; WEINDORF; BAHNASSY, 2010; NOVO, 2011).



Bolfe, Batistella and Ferreira (2012) explain that the brightness factor is directly related to the amount of radiation reflected in a certain range of the electromagnetic spectrum. The ratio of the radiation, which is reflected, to the radiation falling on the target is known as reflectance. Thus, the different classes of land cover (soil, vegetation, water, etc.) present an identifiable pattern with the application of photointerpretation and/or image classification techniques (AMARAL et al., 2009). We can cite several studies that perform analyses on multitemporal scales, with the aim of monitoring land use and occupation, such as those of Fujaco, Leite and Messias (2010), Vascellos, Terra and Cardoso (2013) and Santos et al., (2015).

Therefore, temporal analysis studies allow the seasonal monitoring of the earth's surface, such as, for example, the monitoring of the evolution of deforestation, urban growth, agricultural monitoring, among other applications (CARVALHO JUNIOR et al., 2005). Through the use of satellite images, multitemporal analysis is obtained, which allows the exploration of changes in the analyzed area, presented in the form of a map (BENEDETTI, 2010).

In view of the above, the objective of this work was to analyze the dynamics of land use and land cover in the municipality of Ourém in the State of Pará in the period from 2016 to 2020, generating representative information of the landscape dynamics, influenced by the physical and socioeconomic aspects inherent to this municipality.

2 MATERIALS AND METHODS

2.1 AREA OF STUDY

The municipality of Ourém is located in the state of Pará, in the Northeast Pará mesoregion, microregion of Guamá (Figure 1), whose headquarters are at coordinates $1^{\circ} 33' 07''$ S and $47^{\circ} 06' 52''$, with an area of 562,388 km², with an estimated population of 17,842 inhabitants. It neighbors the municipalities of Santa Luzia do Pará, Capitão Poço and Bonito, located 23 km north-west of Capitão Poço, the largest city in the vicinity (IBGE, 2010).



the municipal headquarters (SOUZA; FEATHER; SILVA, 2016), having as one of the factors that contribute to this problem the extraction of pebbles for civil construction, which, for decades, has been consolidated in the economic base of the municipality (GEOVANE; STOLEN; SOUZA, 2012; LOBELL et al., 2015).

2.2 PROCEDURES

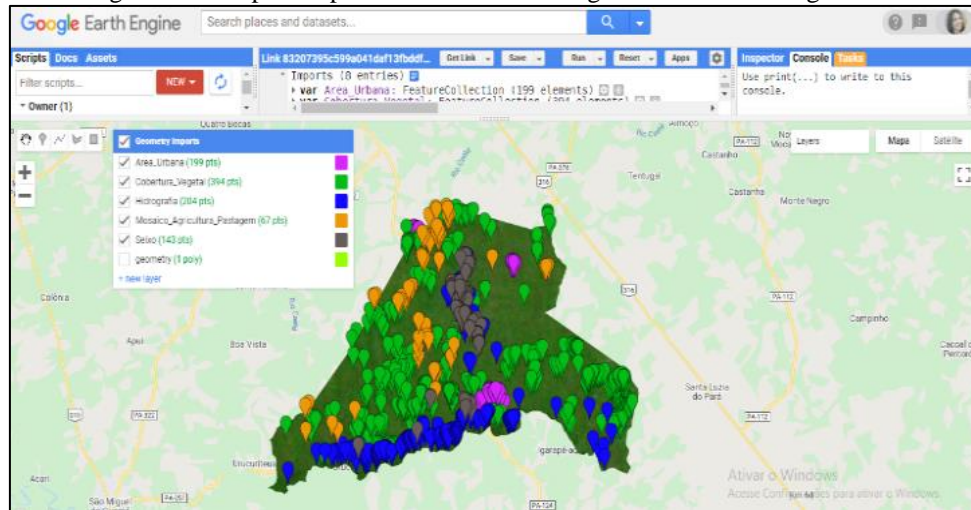
For the acquisition of satellite images for the municipality of Ourém, the *Google Earth Engine* application was used, from images, Landsat 8, OLI sensor, calibrated, atmospherically corrected and georeferenced in the Mercator Transverse Projection Plane Coordinate System (UTM) Datum Sirgas 2000 Zone 23S.

The data from the OLI sensor provides very important data and information, with 30 m of spatial resolution, which can be used in environmental studies for decision-making (ZHANG et al., 2015; SOULARD et al., 2016; CUTLER et al., 2007).

The land use and occupation classification procedure adopted by *Google Earth Engine* was based on the filtering of percentages of cloud cover and composed in mosaics of the spectral bands for the generation of the indices. The classification was supervised, where the user's prior knowledge of the area was obtained. Samples of pixels from each class were collected to generate their respective spectral signatures to classify all other pixels in the image, applying the *Random Forest* algorithm known as "decision trees". According to (GISLASON; BENEDIKTSSON; SVEINSSON, 2006), is a very effective statistical classifier with wide application. Very efficient in the classification of satellite images (BREIMAN, 2001), I have put together a set of methods, which uses several independent "decision trees", distributed in a similar way by random vectors according to an input pattern (ROUSE et al., 1973), with the aim of producing the most accurate classification possible (GISLASON; BENEDIKTSSON; SVEINSSON, 2006) (Figure 2).



Figure 2 – Samples of pixels of each class using Random Forest algorithm.



Elaboration: The author(s) (2021).

Its classification of satellite imagery according to (GISLASON; BENEDIKTSSON; SVEINSSON, 2006) is recommended, because in addition to including metrics that highlight the importance of variables, and the similarity between points, which can be grouped at different levels, classifying the entire image, assigning values when non-existent, producing a graphic result.

Five classes of land use and occupation were mapped between 2016 and 2020, divided into samples according to the variability of the spectral signature:

- a) Urban area;
- b) Vegetation cover;
- c) Hydrography;
- d) agriculture and livestock, and
- e) pebble extraction.

From the junction of the spectral bands of interest, mosaics of spectral bands were created for the generation of the indices.

To calculate the vegetation indices, NDVI (Normalized Difference Vegetation Index), in *Google Earth Engine*, from the OLI sensor, the red B4 (640 – 670 nm) and near infrared B5 (850 – 880 nm) bands were used, its index varies from -1 to 1.

The NDVI, proposed by Polidorio, Imai and Tommaselli (2004), was calculated by the following Equation 1:

$$NDVI = \frac{(NIR - R)}{(NIR + R)} \quad (1)$$



Where NIR is the reflectance of vegetation in the near-infrared band and R is the reflectance of vegetation in the red band.

For the calculation of the NDWI (Water Normalized Difference Index), the bands in green B3 and mid-infrared B6 were used, and this is since the spectrum of radiation reflected by water generally occupies the range of wavelengths between 400-900 nm, a fact that allows the water present in the pixel to be easily highlighted. being able to identify and separate water from soil particles (SILVA et al., 2019).

The index was calculated based on Equation 2 (POLIDORIO; IMAI; TOMMASELLI, 2004):

$$NDWI = \frac{(G - NIR)}{(G + NIR)} \quad (2)$$

Where G is the reflectance in the Green spectrum band and NIR is the reflectance in the mid-infrared band.

In this study, the calculation of the NDBI (Built-up Area Difference Index) was based on the application of the method in the approach of the NDBI studies developed by Zha, Gao and Ni (2003). To calculate the NDBI, the near-infrared B4 (760 - 900 nm) and mid-infrared B5 (1550 - 1750 nm) bands were used, according to the following Equation 3:

$$NDBI = \frac{(B5 - B4)}{(B5 + B4)} \quad (3)$$

The result of the ratio of the near-infrared and mid-infrared bands produces an image with values from -1 to 1. Where the highest values are expected to represent pixels contained in built-up areas and negative values represent pixels contained in unbuilt areas, such as vegetated surfaces.

After the image generation stage with the five classes, they were processed in ArcgisPro, including its plugins and extensions. The matrix files (raster) containing the classification of land cover use in a clipped mosaic, were converted into a vector file of the polygon type. The vector files containing the classification for 2016, 2017, 2018, 2019 and 2020 were used to define a color range for the classification of land cover use, assuming the color standards adopted for each class.

The land cover use classes were quantified, and the data were properly organized for the analysis of each area and year within Ourém (FERNANDES et al., 2021). It is at this stage that it validates the classification generated through the land use and land cover qualification process. According to Monteiro (2015), it is the phase responsible for the treatment of the knowledge obtained by the process of classification and interpretation of data by the user, whether through the elaboration of graphs, diagrams, tables, among others.

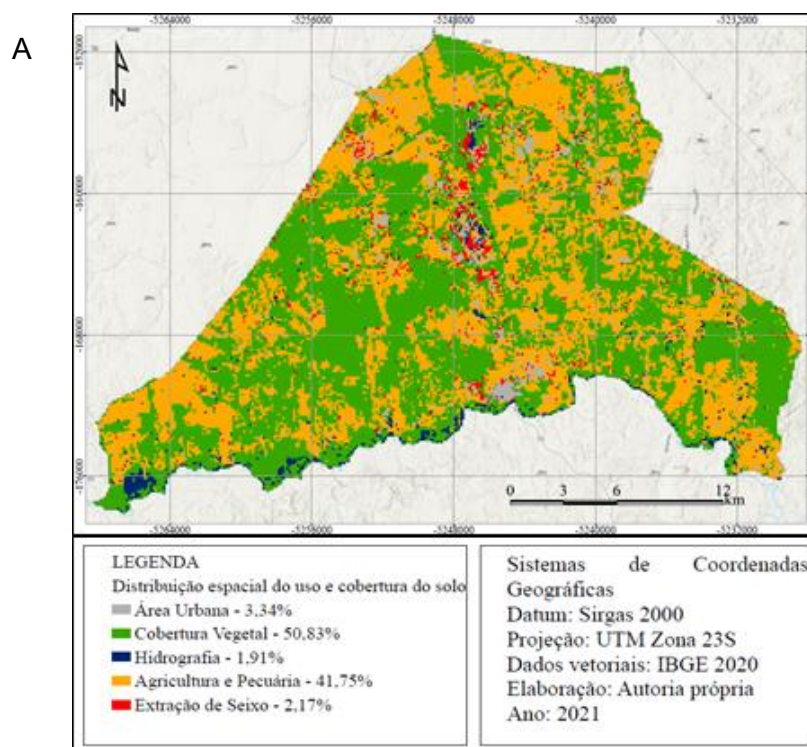


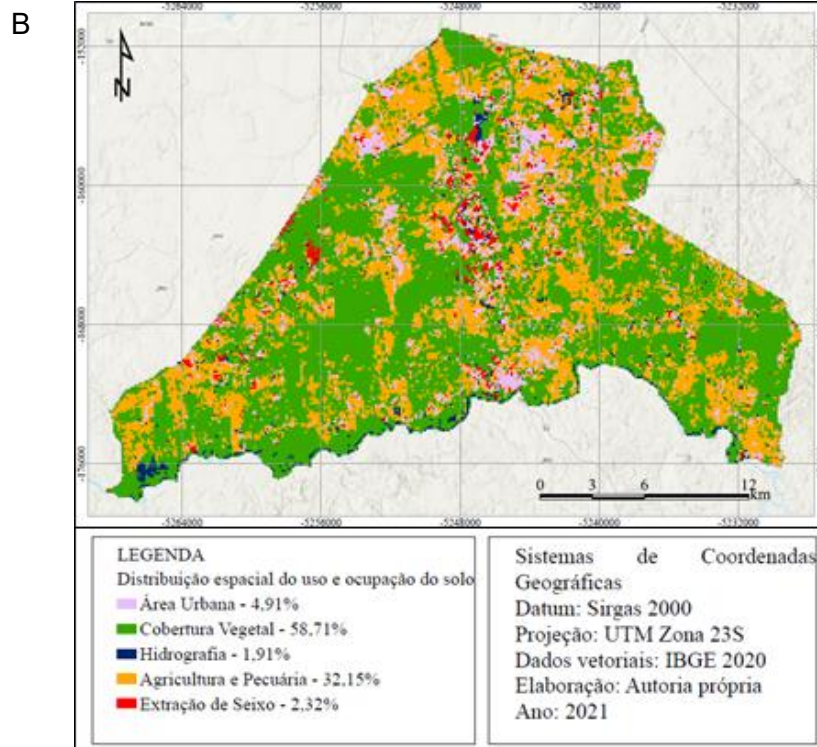
3 RESULTS AND DISCUSSION

From the survey of the acquired images, it is evident the change that occurred in this municipality, in relation to the use and occupation of the land, between the years 2016 and 2020. This time interval was chosen due to the high rate of silting of the municipality's main river and streams in the Amazonian summer.

Figure 3A shows the variation of the five classes analyzed in 2016. Predominant areas in the municipality were Vegetation cover (50.83%) and Agriculture and livestock (41.75%). As in Figure 3A, Figure 3B presents the five classes of land use and land cover distribution for the year 2017.

Figure 3 – Spatial distribution of land use and land cover for the years 2016 (A) and 2017 (B) in the municipality of Ourém, PA.





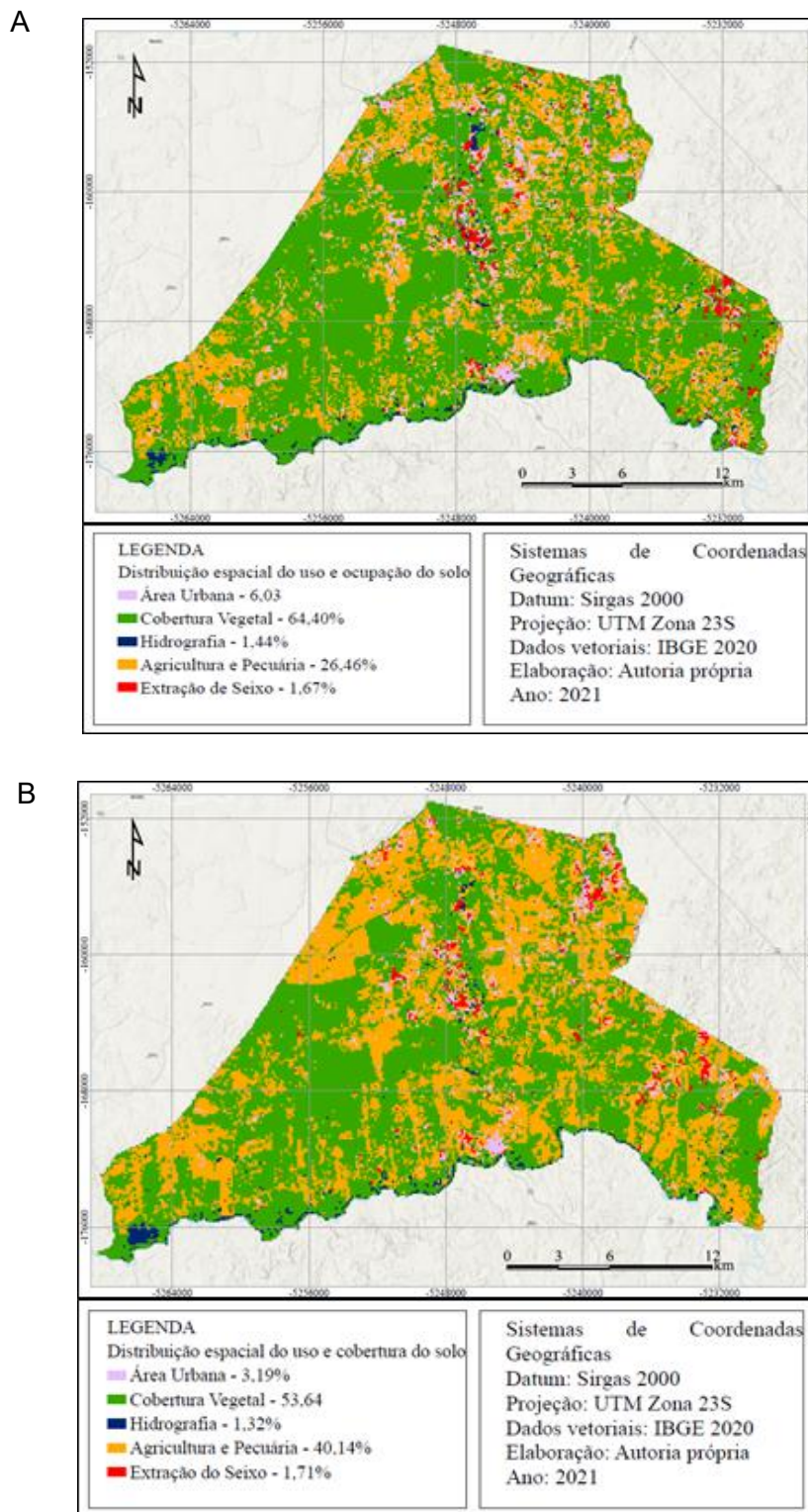
Source: IBGE (2020).

It was observed that there was an increase in the vegetation cover when compared to the area of the previous year (7.88%). In the same period, there was a reduction in agricultural activities in the region (9.6%), such as the cultivation of cassava, beans, corn and pasture. In addition, there was a significant increase of 1.57% in the urban area. As highlighted by Costa (2003), it is essential to know the history of the places, to understand the dynamics that transform the space, creating features that alternate over time.

In 2018, it was verified in the spatial distribution map (Figure 4A) that the vegetation cover area increased in relation to 2017 (5.69%) and agricultural activities decreased (5.69%), because the inactivity of pasture classes that encompasses agriculture can generate vegetation regeneration area, which will later be characterized as secondary vegetation (NASCIMENTO; FERNANDES, 2017). Unlike what happened in 2018, in 2019 the area of agriculture and pasture was larger than the area of vegetation cover. Agriculture grew by 13.68% while vegetation cover decreased by 10.76%. This may be related to the inefficiency of Brazilian environmental legislation, in the lack of inspection of protected environmental areas that could not have been altered (DIEGUES, 2001) (Figure 4B).



Figure 4 – Spatial distribution of land use and land cover for the years 2018 (A) and 2019 (B) in the municipality of Ourém, PA.



Source: IBGE (2020).

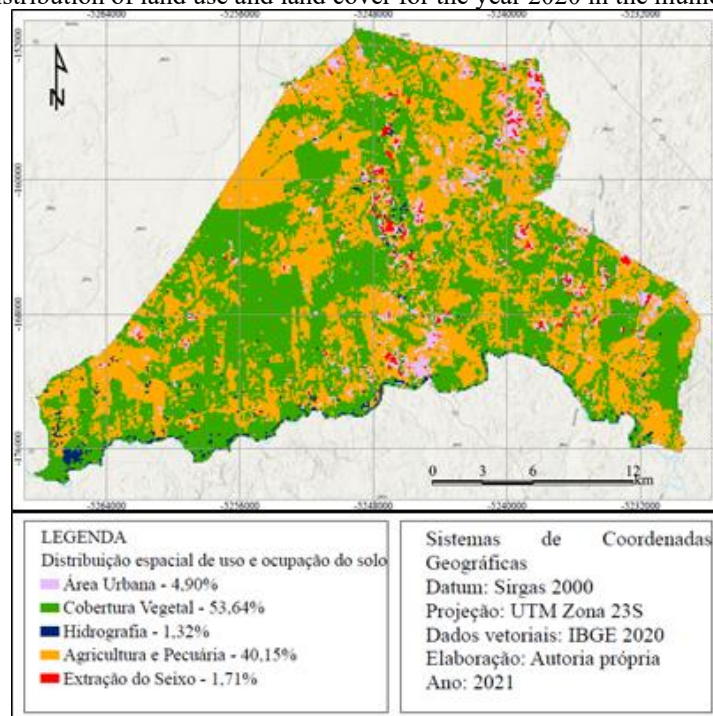
For Coelho et al., (2014), the physical characteristics of Brazilian municipalities have suffered from changes in land use and occupation, since such changes are directly related to the environmental



quality of the most diverse matrices that make up a city. The present study is able to identify these changes in the municipality of Ourém.

It is observed that in the year 2020 (Figure 5), of the five classes analyzed, the changes occurred in the classes of urban area, vegetation cover, and agriculture and pasture are very evident. In a study on the dynamics of vegetation areas, Lemos and Cruz Junior (2013) point out that climatic variables are a major intervening factor in the changes in vegetation in an area, clearly reflecting on the use and occupation of a soil. This fact has been evidenced in the municipality of Ourém in recent years.

Figure 5 – Spatial distribution of land use and land cover for the year 2020 in the municipality of Ourém, PA.



Source: IBGE (2020).

This may be related to the expansion of the Brazilian agricultural frontiers, which evidenced the absence of proposals for regional development of family farming and strategies for the development of the domestic market. However, the economic focus was to ensure the expansion of the extensive margin of large enterprises aimed at the foreign commodity market, a trajectory marked by low economic efficiency, social imbalance and inadequate use of natural resources (MATTOS, 2011).

Table 1 and Figure 6 show the areas of land use and occupation classes between the years 2016 and 2020 in the municipality of Ourém, both in hectare (ha) and in percentage (%), respectively.

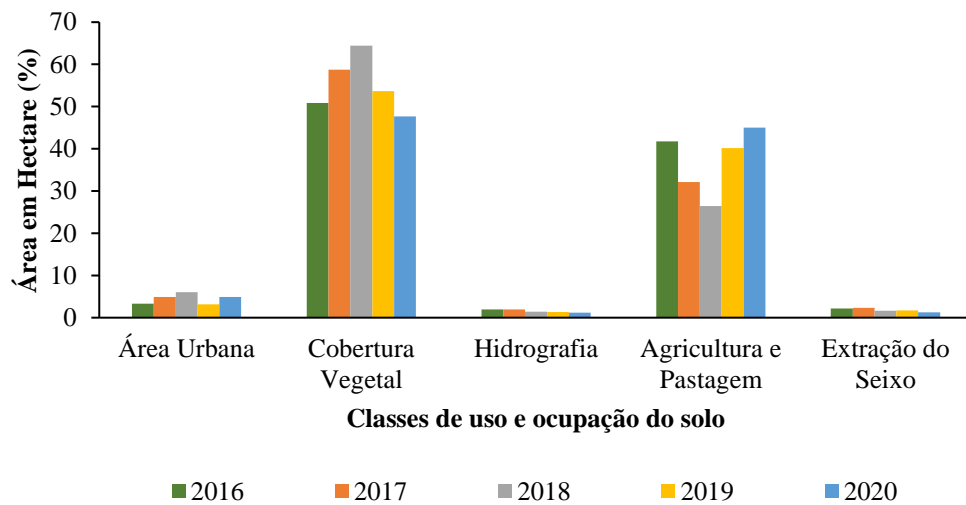


Table 1 – Variation of the areas of the land use and occupation classes between the years 2016 and 2020, in the municipality of Ourém.

Class	2016	2017	2018	2019	2020	Final balance of each class
	----- a -----					
Built-up area	1,88	2,77	3,40	1,80	2,76	880
Mulch	28,67	33,12	36,33	30,26	26,88	- 1,79
Hydrography	1,08	1079	815	743	664	- 413
Agriculture/Livestock	23,55	18,13	14,93	22,65	25,41	1,86
Pebble extraction	1,23	1,31	943	968	697	- 530

Elaboration: The author(s) (2021).

Figure 6 – Histogram of the percentage variation of land use and occupation classes in the municipality of Ourém, from 2016 to 2020.



Source: IBGE (2020).

When comparing the areas of the five classes analyzed in this study between 2016 and 2020, there was a reduction in the areas of vegetation cover (6.26%), hydrography (38.35%) and pebble extraction (43.19%) and an increase in the urban (46.73%) and agriculture and pasture (7.88%) areas (Figure 6). When checking the balance of areas of the classes at the end of the five years analyzed, it can be seen that urban areas and agriculture and livestock were the only classes with a positive balance, while the others had negative balances (Table 1).

These results, of land use and occupation for the municipality of Ourém, make it possible to understand in a deeper way the consequences to the environment, caused, mainly, by anthropic actions. The identification and analysis of the classes of use and occupation provide both the understanding of these modes of use and the degradation to the environment, understanding the role of human beings as a possible potential cause of negative impacts. For Pereira Neto and Fernandes (2015), this type of analysis, together with the understanding of its ecodynamics, would therefore be of fundamental importance for territorial and environmental planning.



We know that there are several forms of anthropogenic interference developed in the municipality of Ourém, such as agriculture and pasture, and pebble extraction. This, for the most part, is inserted, mainly, close to the places that are preserved, such as the APP's.

In this work, even verifying a sharp drop in the area of pebble extraction from 2016 to 2020, its activity is very intense and harmful to the environment, as research carried out by Souza, Pena and Silva (2016), Carvalho et al., (2013) and Coelho, Lucas and Sarmiento (2020), in Ourém, pointed out serious environmental consequences from pebble extraction: The "mountains" of sterile waste, from the washing of the pebble, suffer the actions of wind and rain, such as: the silting of water bodies; the random dumping of pebble washing waste; the depletion of soil fertility, resulting from the movement of horizons; damage to the forest restoration process; the suspension of dust in pits and on roads, among others.

As pointed out by these authors, about the silting of water bodies. In this work, there was a sharp drop in the water bodies of the municipality of Ourém, 38.34% of area, from 2016 to 2020, reflecting on the use and occupation of its soil.

In general terms, what can be observed is that currently the indices of anthropic interference in the areas of the municipality of Ourém are intensifying more and more, with longer periods of drought and drought. The data generated from this research may provide subsidies for the implementation of actions for the development of measures to benefit the environment.

4 CONCLUSIONS

Through the analysis of the results, it was possible to obtain considerations about the current situation of agriculture and livestock, and pebble extraction under the use and occupation of the soil, detecting the transformations that occurred over five years. During this period, the use and occupation of the soil in the municipality of Ourém underwent changes, especially in the decrease of vegetation cover and water bodies, caused mainly by the activity of pebble extraction, causing silting of its main river (Guamá River) and tributaries (Igarapés), dumping of waste from the washing of the pebble forming the "mountains" of sterile waste, depletion of soil fertility, among others. The information presented is shown to be important subsidies to the government as a basis for environmental planning and zoning actions, linked to the search for a more balanced coexistence between society and the environment. In addition, it is a low-cost and easy-to-apply methodology, whose results can minimize the cost and time of the prospecting and research phase.



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Chemisorption of toxic effluents from electroplating in residual gypsum from civil construction



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ABSTRACT

Some industrial sectors have an index of pollutants that are more degradable to the environment, for example, the electroplating industries. The treatment of its effluents is essential before it is

released into water bodies. The purpose of this work is to test construction waste as possible adsorbents of toxic metal ions. Plaster was used as study material. Specimens were made with plaster moistened with contaminated solutions of Cu^{2+} and Pb^{2+} ions. The specimens were submitted to flexural strength tests and the results showed a small decrease in the strength of this type of material when compared to gypsum moistened with drinking water. Adsorption tests were also carried out using gypsum board residues. Kinetic studies showed an adsorption of the Pb^{2+} ion in a time of approximately 30 minutes. The adsorption isotherm tests showed an adsorptive capacity of approximately 88 mg/g. New analyses should be carried out to verify the efficiency of the adsorption of other metal ions such as Cr, Ni and Cd from gypsum as adsorbent and other construction residues.

Keywords: Electroplating, Brass, Plaster.

1 INTRODUCTION

Galvanic activities are considered potentially polluting, especially when it comes to water sources, as this is a sector that promotes the generation of a large amount of waste and for the handling and treatment of these, investment and more and more technical innovations from scientific research and analysis are needed (Franco *et al.*, 2011).

The need for the development of durable materials and adaptable manufacturing processes, widely used in automotive and industrial sectors, are increasingly indispensable due to the constant evolutions that human beings have been presenting. But such great evolutions cannot occur in a disorderly way, taking into account that each and every action of man has social and ecological impacts, positive or negative.

In the macro region of Cariri, in the south of the state of Ceará, the city of Juazeiro do Norte stands out for its intense electroplating sector since 1990, with more than 100 factories specialized in



the manufacture of jewelry and semi-jewelry (Costa *et al.*, 2008). This process, which involves various electrochemical techniques, ends with the release of wastewater with high concentrations of metals, which are extremely polluting to the environment, and cannot be disposed of immediately. For the treatment of this aqueous waste, a precipitation process is commonly carried out using sodium hydroxide, which reduces the concentration of these metals in the water residue, however, it does not make it clear to the point of consumption or direct return to nature (Moreira, 2010).

As a result of these facts, associated studies are needed to promote an improvement in the treatment of these wastes, so that they do not become harmful effluents for the population.

As in other industrial sectors, Civil Construction also has a considerable share in the production of waste that is intolerable to the environment, with long degradation time and deteriorating effects on the ecosystem.

Especially in large cities, which have a very high population development, there are constant landscape changes, whether in public or private environments, which come from socioeconomic development, improvement in urban mobility, tourism development or greater political notoriety. With these constant changes, many tons of construction waste are produced year after year and often do not have an adequate destination, thus being dumped in landfills or inappropriate places. The reuse of this waste has become a point of concern for developed cities and an object of study, in order to contribute to nature and develop more sustainable production routes (Park and Tucker, 2016).

2 OBJECTIVE

The proposal of this study is based on a qualitative and quantitative analysis of the influence caused in civil construction waste, in this case, gypsum waste, when it is prepared from aqueous solutions that contain minimal and considerable amounts of metals, potentially dangerous to human health, which are present in wastewater from galvanic processes.

Another focus will be the direct use of construction waste as possible adsorbents of metal ions in solution. From pre-limited analyses, it will be possible to identify whether, under such circumstances, the residual material of civil construction can be reused, without compromising its resistance or the safety of those who handle it.

3 METHODOLOGY

3.1 OBTAINING SAMPLES

For the programmed analyses, it was necessary to choose a type of material, commonly used in civil construction. Due to the ease of handling and the many possibilities of applications in the sector, the material initially analyzed was gypsum, Calcium Sulfate hemihydrate extracted from gypsum.



Two different analyses were carried out, one with the virgin material (acquired in construction material deposits) and the other with a sample of residual construction plaster.

3.2 ENDURANCE TEST

With the virgin material, blocks were prepared in triplicate with a solution of Copper II (Cu^{2+}) and Lead III (Pb^{2+}) in different concentrations. The blocks were standardized so that there were no variations in the results that would induce analytical errors.

The preparation of the gypsum followed the standard used in the industries, with a proportion of 1/2 for water, that is, for a given mass of gypsum, the proportional half of water (50%) was used.

The solutions prepared with the metals initially varied their concentration from 10 to 100 ppm and in a second moment the analyses were redone with concentrations from 150 to 400 ppm. For each analysis, blank samples were used for comparisons.

For the first stage, with lower concentrations, the blocks were standardized in a metal shape, with each block being 2.5 cm wide, 7.6 cm long and 0.5 cm thick. In the second stage, with higher concentrations, the blocks were standardized into wooden molds with a proportion of 6.0 cm in width, 12.0 cm in length and 0.8 cm in thickness. These analyses were qualitative.

The blocks were prepared on a smooth, uniform countertop and dried for 72 hours. After the drying period, the molds were removed and the blocks were subjected to a tension test with a manual press and with the precise measurement of the force. Figure 01 shows the flexure test performed using an IP-90DI (500kgf) dynamometer.

FIGURE 01 – ENDURANCE TEST



Source: the authors (2023).



3.3 REACTION KINETICS ANALYSIS

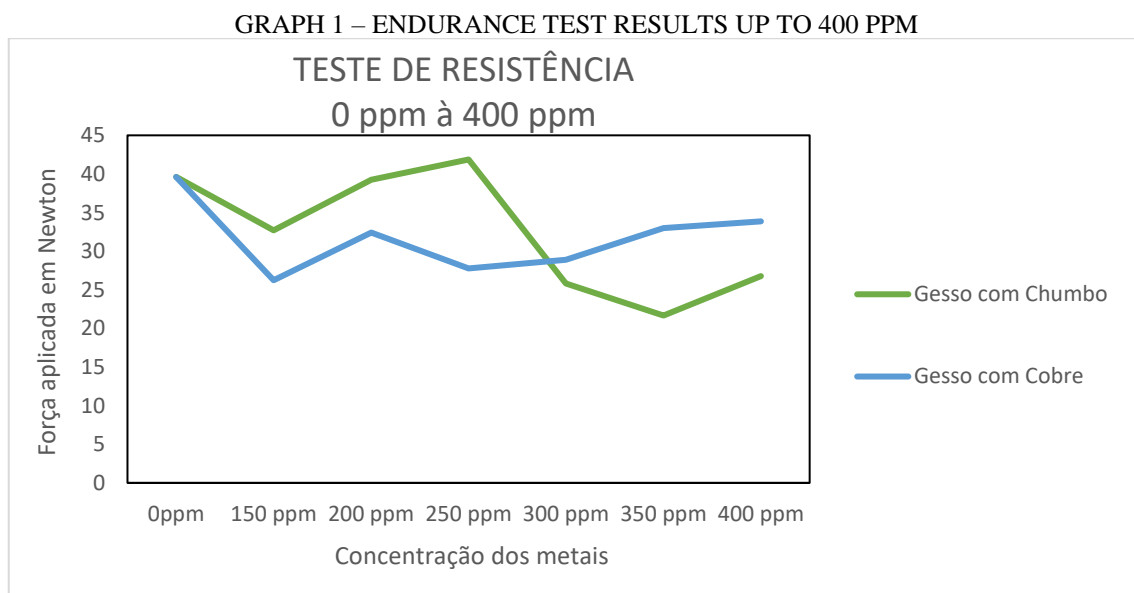
For the reaction kinetics and isotherm analyses, gypsum residue from civil construction was used. It was crushed and sieved before being submitted to the procedures. The average grain size of the material used ranged from 0.074 to 0.105 mm.

The kinetic analysis is done based on the variation of time, aiming to obtain the speed at which the reaction occurs and the minimum time necessary for it to happen. This analysis is carried out in two stages, being the submission of the study material to a buffered solution at pH 5.5 with a standardized concentration of 100 ppm of the metal ions. For every 10 mL of the solution containing the metal ions, approximately 0.0500 g of gypsum residue were used.

The predetermined times for reaction analysis in minutes were 1, 2, 4, 8, 16, 30, 60, 90, 120 minutes.

4 RESULTS

For the measurement of resistance, the results obtained were satisfactory in what was expected, both for the first stage, with lower concentrations (10 to 100 ppm of both metals), and in the second stage, with very high concentrations (150 to 400 ppm of both metals). The force measured in newton, used to break the block, did not show considerable variation due to the increase of the concentration of the metal in the preparation solution, which can be seen in graph 01. This tells us that the presence of the metal does not promote the loss of its resistance when compared to the value of the white realized.



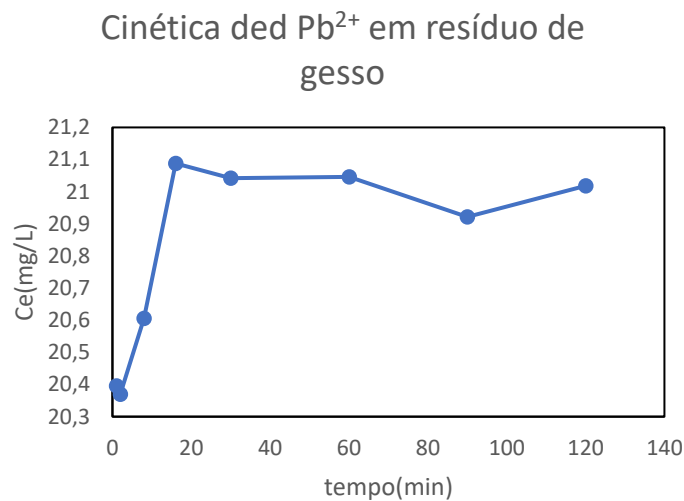
Source: the authors (2023).



For the reaction kinetics test, the results for copper and lead showed significant differences. The equilibrium time was approximately 30 minutes. The experimental data were compared with the pseudo-second-order model and were highly correlated with this model.

Graph 02 shows the experimental results of the kinetic study of adsorption of the Pb^{2+} ion against the gypsum residue.

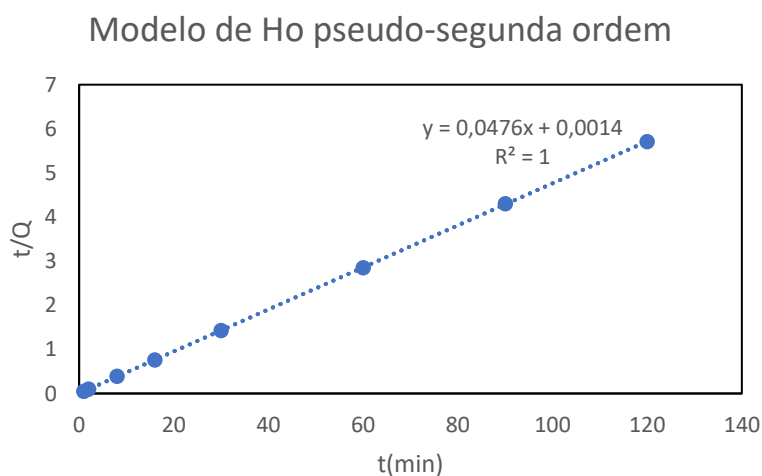
GRAPH 02 – RESULTS OF THE ADSORPTION KINETIC EQUILIBRIUM STUDY. SOLUTION CONCENTRATION OF 100 PPM AT PH 5.5



Source: the authors (2023).

Graph 03 shows the linearity correlation of the experimental data with the pseudo-second-order model

GRAPH 03 – EVALUATION OF THE REACTIONAL ORDER FROM THE PSEUDO-SECOND ORDER MODEL

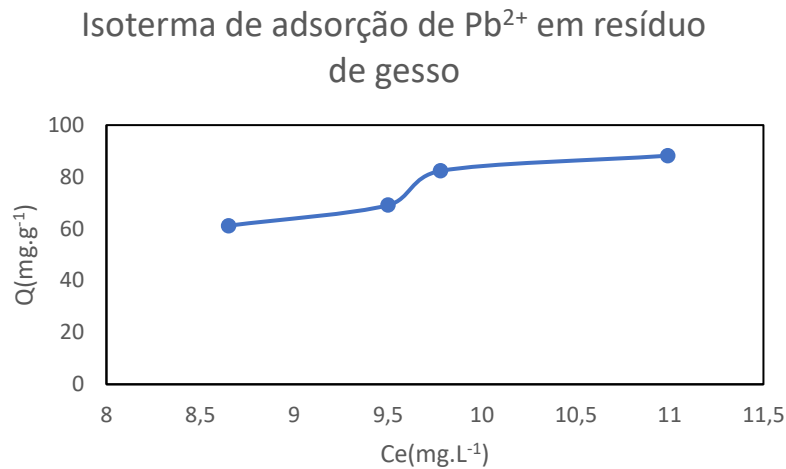


Source: the authors (2023).



The adsorption isotherm study showed a maximum adsorption capacity of the Pb^{2+} ion of 88 mg/g. This result is shown in graph 04, where the results of Q x CE are also listed.

GRAPH 04 – EVALUATION OF ADSORPTION ISOTHERM (Q X CE)



Source: the authors (2023).

5 CONCLUSION

The preliminary results show that the construction gypsum residue has a good adsorptive capacity against the Pb^{2+} ion. The kinetic study showed a rapid adsorption process. The adsorption isotherm study showed a capacity of around 88 mg/g. The resistance tests of the specimens using waters contaminated with Cu^{2+} and Pb^{2+} ions in the manufacture of gypsum pieces showed that the incorporation of these ions, even in high concentrations, did not significantly compromise the resistance of this material.

As a suggestion for further work, it is proposed that other analyses be carried out that will indicate the veracity of the preliminary results that, a priori, are quite satisfactory, and also a new stage of study, which will identify whether other construction waste, such as cement and mortar, also have the ability to absorb metals in solutions.



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Environmental accounting for the sustainable development of a plastics factory



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ABSTRACT

The research "Environmental Accounting for the Sustainable Development of a Plastics Factory" focused on the relevance of accounting around plastics factories. Unit I investigated the environmental impact of the plastics industry at a global and local level, addressing its evolution, types, economic influence and environmental effects. Unit II defined sustainable development, breaking down its concept, components and objectives to achieve economic, social and environmental balance. Unit III explored environmental accounting, addressing its concept, utility, costs, applications, reporting, and environmental performance indicators. In the legal framework, considering laws, decrees, resolutions and ordinances in the plastics industry and environmental management, including local regulations. The research took a quantitative approach, relying on data collection and analysis to assess quantifiable aspects of environmental accounting. Using a descriptive approach, conclusions were drawn that highlighted the essentiality of accounting to drive sustainable development in factories. A questionnaire with closed questions was proposed, which reflected an understanding of the importance of Environmental Accounting in the business environment and its impact on sustainable development. Respondents demonstrated a willingness toward accounting practices and environmental disclosures.

Keywords: Environmental accounting, Factory, Sustainable development.

1 INTRODUCTION

In a world in constant search of solutions to face environmental challenges and promote sustainable development, environmental accounting emerges as a valuable and relevant tool in various industrial sectors. The plastics industry, due to its preeminence in the global economy and its involvement in environmental issues, becomes a crucial field for implementing responsible practices and mitigating its impact on the natural environment.



The main objective of this final degree project is to analyse and understand the role of environmental accounting in the sustainable development of a plastics factory. To this end, an exhaustive analysis of the production processes and their relationship with environmental aspects was carried out, in order to describe in detail the impacts generated.

In addition, the relevance of environmental accounting in the context of current business practices and its contribution to the achievement of the Sustainable Development Goals (SDGs) established by the United Nations 2030 Agenda was considered. Environmental accounting is presented as a strategic tool to promote corporate social responsibility and compliance with environmental commitments.

At present, one of the main concerns at the district level is the deterioration of the environment. The pollution generated by a plastics factory, without the application of accounting methods to have accurate knowledge of how and how much to invest to cushion the environmental impact generated, so the following problem arises: What is the importance of applying environmental accounting for sustainable development in a plastics factory? From which the objective was generated: to describe the importance of environmental accounting for sustainable development in a plastics factory.

2 METHODOLOGY

2.1 RESEARCH FOCUS

The study method applied in this research is the quantitative approach, as it is based on the collection and analysis of data to evaluate the quantifiable and measurable aspects of environmental accounting in the plastics factory. This makes it possible to measure and compare the factory's environmental impacts, identify trends, and assess progress towards sustainable development goals.

According to Sampieri R. et al. (2014), quantitative methods are based on a methodical and logical approach whose purpose is to express research questions and hypotheses so that they can be tested later.

2.2 RESEARCH DESIGN

The level of research design that is applied is descriptive, as this design determines the existing environmental accounting practices in the plastics factory, identifies the environmental performance indicators used, analyzes the implemented policies, and evaluates the sustainable development initiatives underway. This allows you to get a clear and detailed overview of the situation at that time and establish a solid basis for future analyses.

According to Guevara et al. (2020), "The purpose of descriptive research is to understand prevailing situations, customs, and attitudes through accurate descriptions of activities, objects, processes, and people."



2.3 FIELD OF ACTION

The research is carried out in Ciudad de Este, Alto Paraná department, where surveys are analyzed that help in the research on environmental accounting for the sustainable development of a plastics factory.

2.4 POPULATION AND SAMPLE

Plastics factories located in Ciudad del Este.

Population 1. Five (5) plastics factory managers.

Population 2. Five (5) CPAs.

100% of the population is selected for the sample.

2.5 SAMPLING TECHNIQUE

Non-probabilistic sampling, also known as purposive sampling or trial sampling, is a sampling technique where the researcher selects items in the sample based on their judgment and the purpose of the study, rather than choosing them at random.

2.6 DATA COLLECTION TECHNIQUE

To achieve the objectives proposed in this research, we opted for the implementation of a questionnaire in digital format. This methodology is widely recognized and valued in the field of research, due to its ability to facilitate the collection and processing of data in an agile and efficient way.

The structured questionnaire includes a series of closed-ended questions, all with personalized multiple-choice answer options developed by the group. According to Tamayo (1994), there are various forms of observation that researchers carry out to gather information and data.

The research data collection tool is an online form. To be more specific, the survey is conducted using Google Form, an app that allows you to collect data online. According to Hernández Sampieri (1997), questionnaires are probably the most widely used method for data collection, as they consist of a set of questions about one or more variables to be investigated.

2.7 ANALYSIS OF COLLECTED DATA

The analysis of the collected data involved several steps and techniques, taking into account the objectives of the study and the nature of the questions included in the questionnaire. It began with data cleansing, which consisted of reviewing the responses collected to identify and correct errors or inconsistencies. Coding and visualizing data for the creation of graphs and visualizations was able to help interpret the results and communicate them effectively. Ending with the interpretation of results,

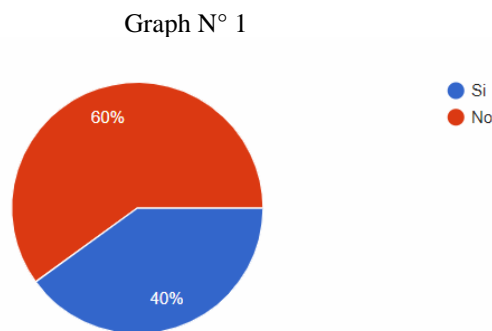


this step involved interpreting the statistical results in the context of the research question and the objective of the study. Finally, the results of the analysis were compiled into a report, where the methods of analysis were detailed, the main findings are presented with graphs, with a brief discussion and the implications of the results.

3 RESULTS

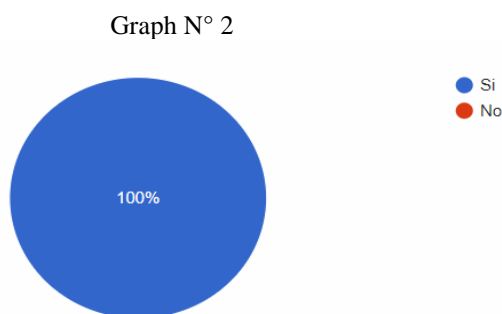
3.1 SURVEY OF PLASTICS FACTORIES IN CIUDAD DEL ESTE

3.1.1 Are you familiar with the concept of environmental accounting?



It is observed that most of the companies surveyed are not familiar with the concept of Environmental Accounting, while a significant percentage do have knowledge about Environmental Accounting.

3.1.2 Would you be willing to implement an environmental management system?

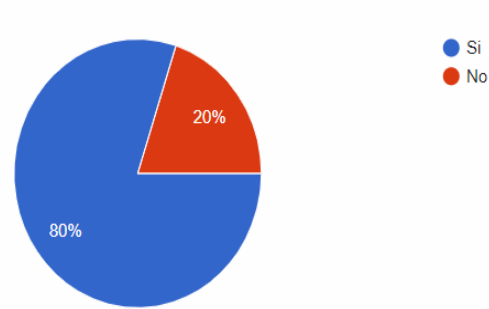


It is clear that all the companies in question would be willing to implement an environmental management system.

3.1.3 Do you think environmental accounting can help reduce your company's environmental impact?



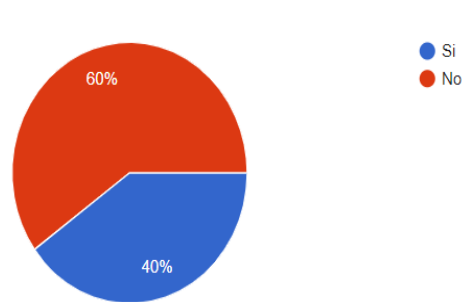
Graph N° 3



Most participating companies see the potential of environmental accounting to reduce environmental impact, although a minority group is less optimistic about it.

3.1.4 Do you know if there are any specific regulations or laws in Paraguay related to environmental accounting?

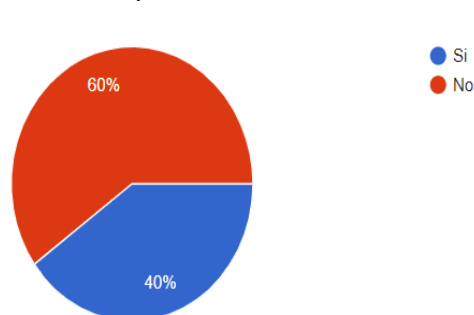
Graph N° 4



According to the results, it stands out that a majority of them are not familiar with environmental regulations in Paraguay, while a minority segment claims to be aware of them.

3.1.5 Has your company budgeted exclusively for environmental activities?

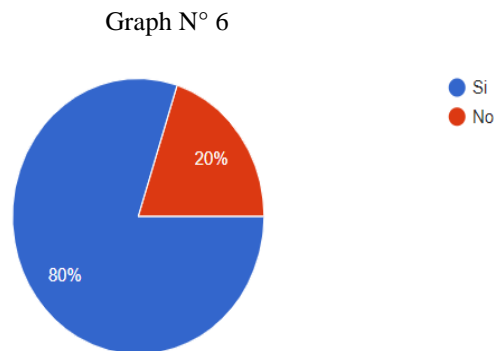
Graph N° 5





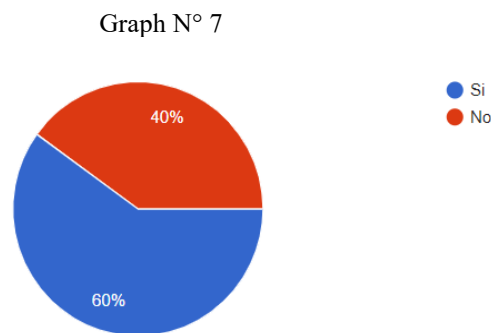
This graph shows that the majority of companies surveyed do not have a budget allocated to environmental activities, while a minority group does allocate resources for these initiatives.

3.1.6 Have you carried out an environmental impact assessment?



It should be noted that most of the companies have carried out the environmental impact study, while a smaller group has not yet complied with this obligation established by Law 294 on Environmental Impact Assessment.

3.1.7 Do you think companies should disclose information on their environmental performance?

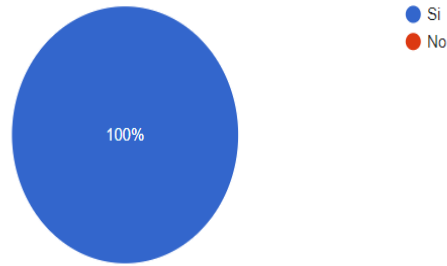


There is a tendency towards the idea of disclosing information on the environmental performance of companies, although there is also a contrary position in a segment of the group.

3.1.8 Do you think environmental accounting can contribute to sustainable development?



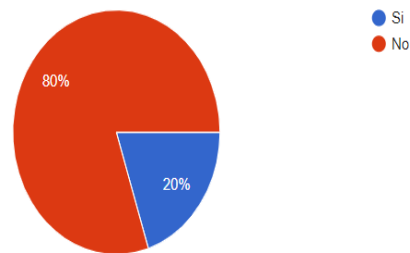
Graph N° 8



According to the graph, all the companies surveyed express belief that the implementation of Environmental Accounting can contribute to sustainable development.

3.1.9 Are you familiar with the environmental performance indicators used in environmental accounting?

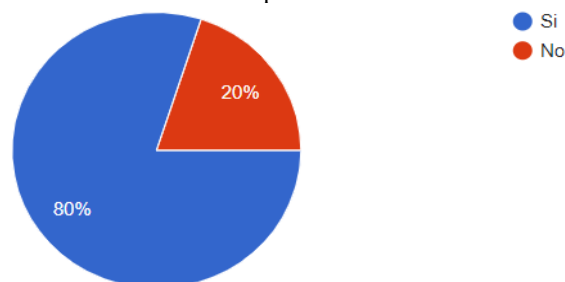
Graph N° 9



It can be seen that the minority is aware of the existence of environmental performance indicators that are used for environmental accounting.

3.1.10 Do you think the government should require the use of environmental accounting in companies?

Graph N° 10

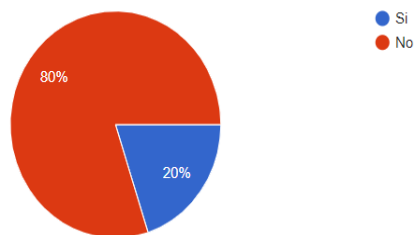




The majority of companies show support for the national government to promote the implementation of environmental accounting in companies, while a minority have a different opinion.

3.1.11 Do you know if there are institutions that provide support or advice on environmental accounting?

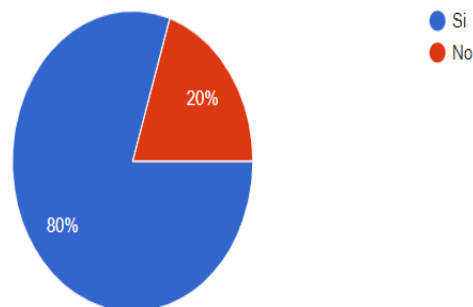
Graph N° 11



The majority lack knowledge about support institutions or advice on environmental accounting, although a minority are informed about this.

3.1.12 Would you be willing to invest in an Accounting-Environmental Management system?

Graph N° 12

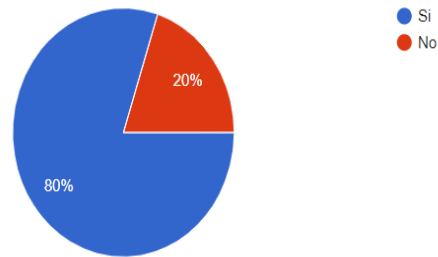


The graph shows that the majority show willingness to invest in an accounting-environmental management system, while the rest are not interested.

3.1.13 Do you think environmental accounting can help improve your company's image and reputation?



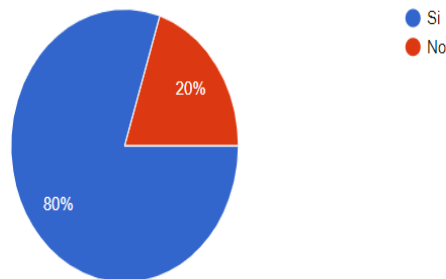
Graph N° 13



It should be noted that a large number of companies recognize that environmental accounting helps to improve the image and reputation of the company, although some differ in this opinion.

3.1.14 Do you think companies should allocate financial resources to implement environmental accounting?

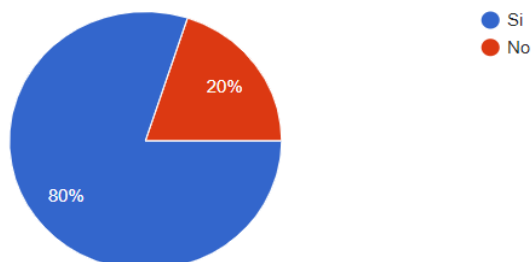
Graph N° 14



It is noted that most companies are willing to allocate financial resources to implement environmental accounting, although a smaller percentage do not share this perspective.

3.1.15 Do you think environmental accounting can foster innovation and efficiency in your company?

Graph N° 15

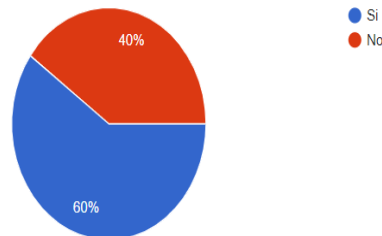




The majority of companies surveyed believe that environmental accounting can drive innovation and efficiency in their operations, while a smaller group has a different perspective.

3.1.16 Do you agree that companies should be transparent about their environmental impact?

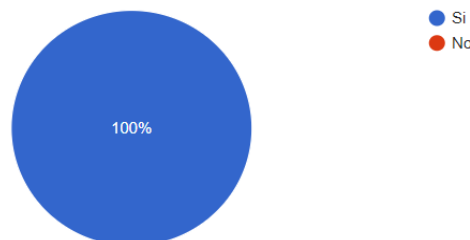
Graph N° 16



In this instance, the majority of companies are in favor of the idea that companies should be transparent about their actual environmental impact, although a smaller percentage have a contrary opinion.

3.1.17 Do you think that environmental accounting can contribute to more sustainable decision-making in companies?

Graph N° 17



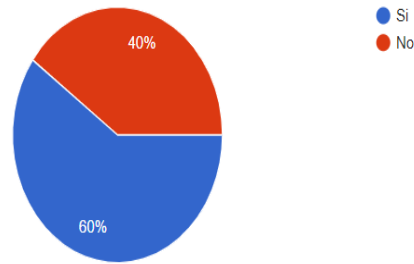
In this final graphical representation, it is clear that all respondents agree that environmental accounting could be instrumental in driving more sustainable decision-making in companies.

3.2 SURVEY OF CHARTERED ACCOUNTANTS

3.2.1 Are you familiar with the concept of environmental accounting?



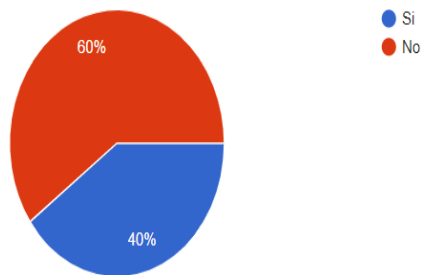
Graph N° 18



It can be seen that a majority is familiar with the concept of environmental accounting, while others are not.

3.2.2 Have you ever provided or could you provide advice for a company to implement environmental accounting?

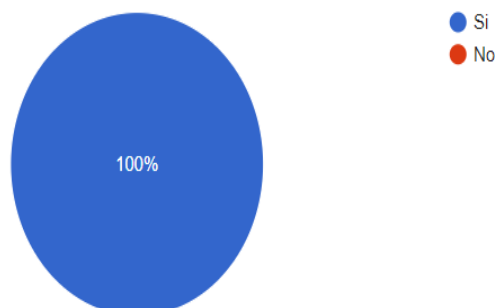
Graph N° 19



It is highlighted that the majority of accountants have never provided advice on environmental accounting, while a minority group claims to have performed this service.

3.2.3 Do you think that applying environmental accounting in a company can help reduce or remedy its environmental impact?

Graph N° 20

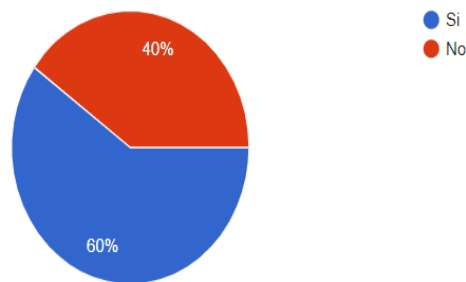




It is highlighted that all respondents believe that the implementation of environmental accounting in a company can contribute to the reduction or mitigation of its environmental impact.

3.2.4 Are you aware of the regulations or laws in force in Paraguay related to environmental impact?

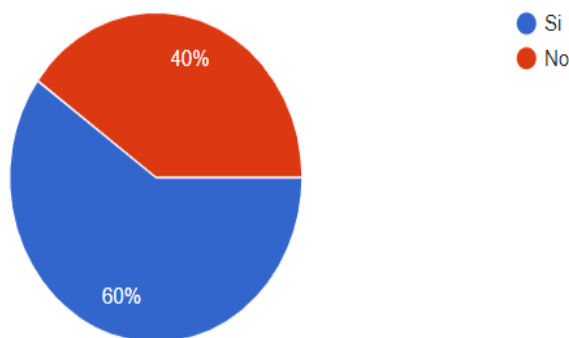
Graph N° 21



The majority of respondents are familiar with the regulations and laws related to environmental impact in our country, while others express ignorance in this regard.

3.2.5 Do you know environmental accounting accounts?

Graph N° 22

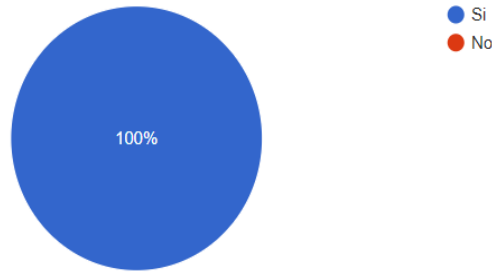


It is clear that some of the respondents have knowledge about environmental accounting accounts, while another group shows a lack of knowledge in this area.

3.2.6 Would you invest in a course or specialization in environmental accounting?



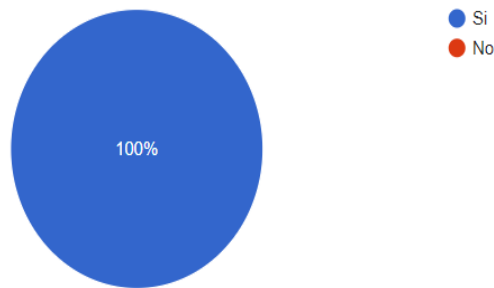
Graph N° 23



It is highlighted that all respondents are willing to invest in an environmental accounting course.

3.2.7 Do you think companies should disclose information on their environmental performance with an accounting approach?

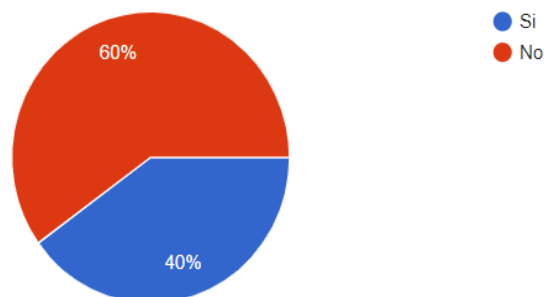
Graph N° 24



In this instance, all respondents agree that companies should share information about their environmental performance through an accounting approach.

3.2.8 Are you familiar with Environmental Reporting?

Graph N° 25

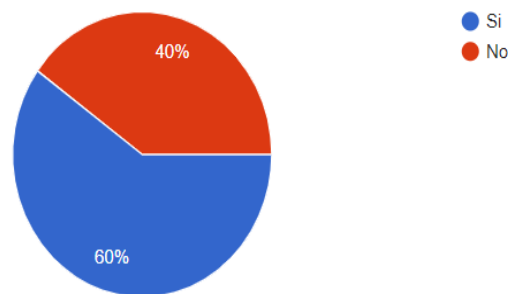




Most of the accounting professionals indicated a lack of knowledge of the concept of Environmental Reporting, while others are familiar with the term.

3.2.9 Are you aware of any environmental performance indicators used in environmental accounting?

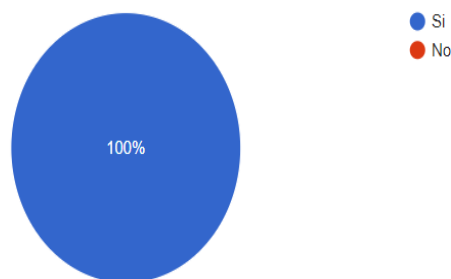
Graph N° 26



It can be noted that a portion of respondents are familiar with some environmental performance indicators that are used in environmental accounting, while others are not knowledgeable about them.

3.2.10 Do you think the government should require the implementation of environmental accounting?

Graph N° 27

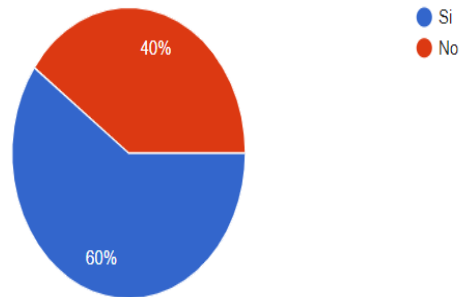


It can be seen that all of them find viable the possibility of the government requiring the implementation of environmental accounting.

3.2.11 Do you know if there are any institutions that provide support or advice on environmental accounting?



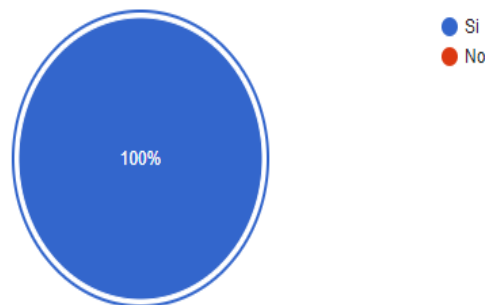
Graph N° 28



The majority of respondents claim to be aware of these institutions, while a smaller proportion say they are not aware of their existence.

3.2.12 Would you consider the implementation of an Accounting-Environmental Management system useful?

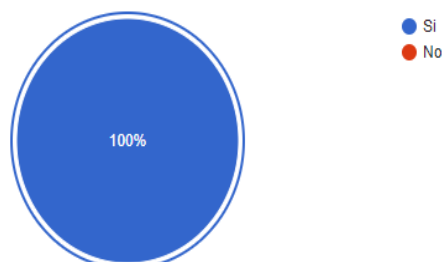
Graph N° 29



All respondents stated that they consider the implementation of an Accounting-Environmental Management system to be extremely useful.

3.2.13 Do you think that environmental accounting would be a good strategy to improve a company's image and reputation?

Graph N° 30

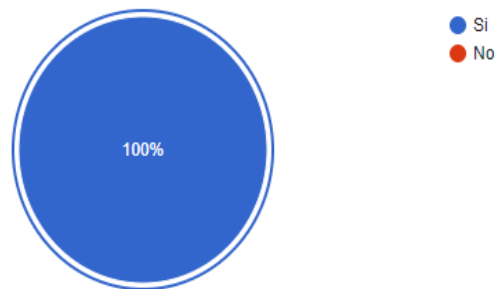




All respondents share the conviction that the implementation of environmental accounting would be an extremely beneficial measure to improve a company's image and reputation.

3.2.14 Do you think companies should allocate financial resources to implement environmental accounting?

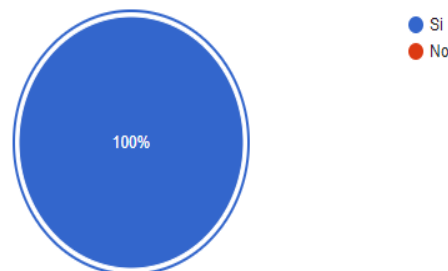
Graph N° 31



The opinion of the respondents is unanimous: all participants are convinced that companies should allocate financial resources to carry out the implementation of environmental accounting.

3.2.15 Do you think that environmental accounting can foster innovation and efficiency in companies?

Graph N° 32

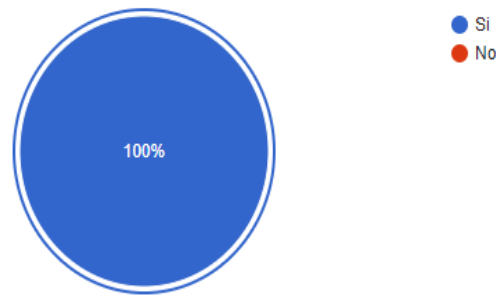


All respondents believe that environmental accounting can promote innovation and efficiency in companies.

3.2.16 Do you think that environmental accounting can contribute to more sustainable decision-making in companies?



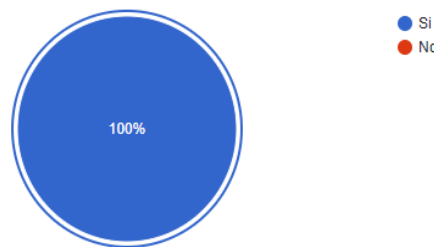
Graph N° 33



There is unanimity of thought among all participants: respondents considered that environmental accounting can indeed contribute to more sustainable business decision-making.

3.2.17 Do you think there is a lack of information and implementation regarding Environmental Accounting in our country?

Graph N° 34



All the participants share a solid conviction that in our country there is a clear lack of information and implementation with regard to environmental accounting.

4 CONCLUSION

Thus, this research on "Environmental Accounting for the Sustainable Development of a Plastics Factory" highlights the essential importance of integrating environmental accounting practices in plastics factories. The results emphasize that these practices not only reduce environmental impact, but also optimize operational efficiency and elevate the reputation of companies in this sector. The objectives set have been exhaustively met. How to apply environmental accounting in a plastics factory was defined and explored, highlighting its relevance.

In addition, it was determined how environmental accounting improves the operation of a plastics factory, providing a complete perspective. The identification of environmental accounting accounts highlights the relevance of categorizing and quantifying environmental aspects in the accounting framework, facilitating informed decisions and accountability. Finally, mentioning the



MADES standards establishes a crucial connection between accounting and the regulatory framework, underscoring the importance of alignment. This research demonstrates that environmental accounting not only quantifies and manages the environmental impact in a plastics factory, but also promotes sustainable development through conscious management of resources and environment in the industrial field.

The application of environmental accounting in a plastics factory is essential to harmonize economic goals with environmental responsibility. In doing so, the factory not only improves its efficiency and competitiveness, but also actively contributes to sustainable development, ensuring the long-term viability of its operations in a world increasingly aware of the importance of sustainability.

5 PROPOSALS

In the current context of growing environmental awareness and the need to promote sustainable practices in industries, this thesis proposes the implementation of environmental accounting for sustainable development in a plastics factory. The main objective of this proposal is to establish a strategic scheme to assess, control and remedy the environmental impacts of plastics production with an accounting approach, with a view to achieving a balance between industrial activity and environmental conservation.

This will include the assessment of natural resource utilization, pollutant emissions and waste generation. The goal is to have a detailed understanding of the company's current environmental footprint. The collection and analysis of accurate data will facilitate informed and well-informed decision-making, which will promote a culture of sustainability within the factory, involving employees and managers in the importance of environmental management. Training and education on responsible practices will be provided and active participation at all levels will be encouraged.

A transparent and periodic environmental reporting system will be established, documenting the progress and improvements made in environmental terms. Effective communication, both internally and externally, will be critical to showing the company's commitment to sustainability.

This proposal seeks to integrate environmental accounting as a strategic tool in a plastics factory, in order to promote its sustainable development. Through a comprehensive approach that encompasses diagnosis, measurement, implementation and education, the aim is to reduce the environmental footprint and establish a production model that is more responsible and harmonious with the natural environment.



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Use of plants of the genus *kalanchoe* as a potential treatment for inflammatory diseases



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ABSTRACT

Medicinal plants have been used throughout civilizations, accumulating empirical knowledge for treatment and prevention of diseases and often incorporate plant derivatives into treatments. Plants contain a variety of chemical compounds, classified as primary and secondary metabolites. These substances have applications in the pharmaceutical industry, colorants, and flavorings due to their beneficial properties. Thus, the genus *Kalanchoe* has several applicabilities in ethnomedicine. The objective of the work is to show the medicinal use of the extract of plant species of the genus *kalanchoe*, as treatments of diseases in articles from the years 2013 to 2023. It has shown anti-inflammatory, antitumor, healing, antidiabetic, antiulcerogenic, antihypertensive, detrusor relaxant, neuroprotective, antiviral, antimicrobial, antiasthmatic, antitussive and nephroprotective action.

Keywords: *Kalanchoe*, Phytochemical compounds, Inflammation, Pathology.

1 INTRODUCTION

Medicinal plants have always been used in the daily life of civilizations, both ancient and modern, so that, over time, empirical and effective knowledge have been acquired for the relief of diseases. This practice helps the human being a lot over time in relation to the search for treatment and



also prevention (Borges *et al.*, 2020). Currently, traditional methods with herbal plants remain constant, as a large part of the population sees this habit as safe, especially in emerging countries. Physicians also use the derivatives of these plants in prescriptions (Firmo *et al.*, 2011).

Plants have a wide variety of chemical compounds, which can be grouped into primary and secondary metabolites. Primary metabolites are substances produced by all plants and play a direct role in their growth and development. In contrast, secondary metabolites have a high specificity and a crucial role in the evolution of plants along with other living organisms, e.g., protection of plants against biotic and abiotic stresses. These secondary metabolites generally fall into three main classes of molecules: terpenes, phenolic compounds, and nitrogenous. In addition, they are widely used in the biopharmaceutical and various commercial industries, due to their beneficial properties, such as the production of medicines, dyes, and flavorings (Borges; Amorim, 2020).

With industrialization, herbal medicines, processed medicines derived from medicinal plants, came to the fore, which with current technology managed to enter the market, but with the chemical synthesis of drugs and the lack of scientific proof in the efficiency of these substances, brought a drop in the use of herbal medicines (Gadelha *et al.*, 2013). However, the association of the popular history and the clinical results of the use of herbal medicines collaborates in the satisfaction of people and doctors about the efficacy of the product. And it is even more indispensable, because politically it becomes recommended in the Unified Health System (SUS), mainly reaching primary care in which it ensures symptoms in the preliminary phase of diseases, and also aims at a more sustainable system and social participation (ROSA *et al.*, 2011).

Second classification of the *Angiosperm Phylogeny Group IV* (APG IV) the genus *Kalanchoe* It has a number of 145 species. Thus, it has a complex taxonomy with numerous synonyms of species, thus establishing the integration of the genus *Bryophyllum* to gender *Kalanchoe* (Descoings, 2006) soon *Bryophyllum* It is considered a heterotypic synonym, an example of which we have the species *Calancho Creta* (Andrews) Haw, sinonímia *Kalanchoe brasiliensis* Larrañaga e *Pencho Pinnata* (Lam.) Pers., synonymy *Bryophila Kalisinama* Salisb, all exotic species in Brazil (Goebel; Caddah; Giuffrè, 2020).

The genus *Kalanchoe*, in which it is part of the Crassulaceae family and spreads to continents in tropical and subtropical zones, such as Africa, Asia and America, contributes greatly to the treatment of diseases such as gastric ulcers, ear infections, arthritis, cough, skin lesions and inflammations as well as periodontals. In ethnomedicine, the crude extract or juice points to anticancer and healing properties, as an example, in the State of Rio Grande do Norte, Brazil, the leaves of *Kalanchoe pinnata* Lam. are used in direct application to wounds in the search for healing, as well as in the treatment of gastritis and ulcers. (De Araújo *et al.*, 2019; Stefanowicz-Hajduk *et al.*, 2020)tag.



Thus, the objective of this work was to carry out a bibliographic review of the articles published between the years 2013 and 2023, which show the medicinal use of the extract of plant species of the genus *Kalanchoe*, focusing on the treatment of diseases in general, as well as the identification of these plants and secondary metabolites responsible for the biological activity against pathologies, in addition to the experimental models used to evaluate the efficiency of these active compounds.

2 MATERIAL AND METHOD

The work is defined with a literature review, and is based on the following question: Which inflammatory diseases can be treated with plant species of the genus *kalanchoe* ?

In the review search, articles were searched in the following databases: *Scopus*, *PubMed*, and *Scielo*, based on a pre-defined period from January 2013 to May 2023. The keywords used were "*kalanchoe and disease or pathology or treatment*". The selection of articles was carried out in June 2023, where, through the reading of the titles and abstracts of the papers, the classification was made, and in a situation of insufficiency of this measure, the article was read in full. It is noteworthy that the analysis of the articles was only possible under conditions of easy access and in English, Spanish and Portuguese.

After being classified, the articles were fully read and selected according to the criteria (Chart 1). The selected works had to present a method of treatment for any disease or pathology that used the extract or herbal medicine of some species of the genus *Kalanchoe*. Thus, the following main information was extracted from the articles: scientific name of the plant, active compound, disease, region of the plant for the extraction of the compounds, type of extract and induction of the disease.

Table 1: Selections of articles (Exclusion and Inclusion Criteria), Teresina – PI, 2023

Database searches (<i>Scopus</i> , <i>PubMed</i> and <i>Scielo</i>)	
Exclusion Criteria	Inclusion criteria
<ul style="list-style-type: none"> - Work be a Thesis or Dissertation; - Work be a review article, patent document, congress abstract, book chapter, and editorials - Use a human clinical trial model; - The article must be written in languages other than English, Spanish and Portuguese. 	<ul style="list-style-type: none"> - Use of herbal medicines and/or compounds isolated from the plant as a form of treatment for diseases in general; - Conducting tests in animal experiments (<i>in vivo</i>) and <i>in vitro</i>

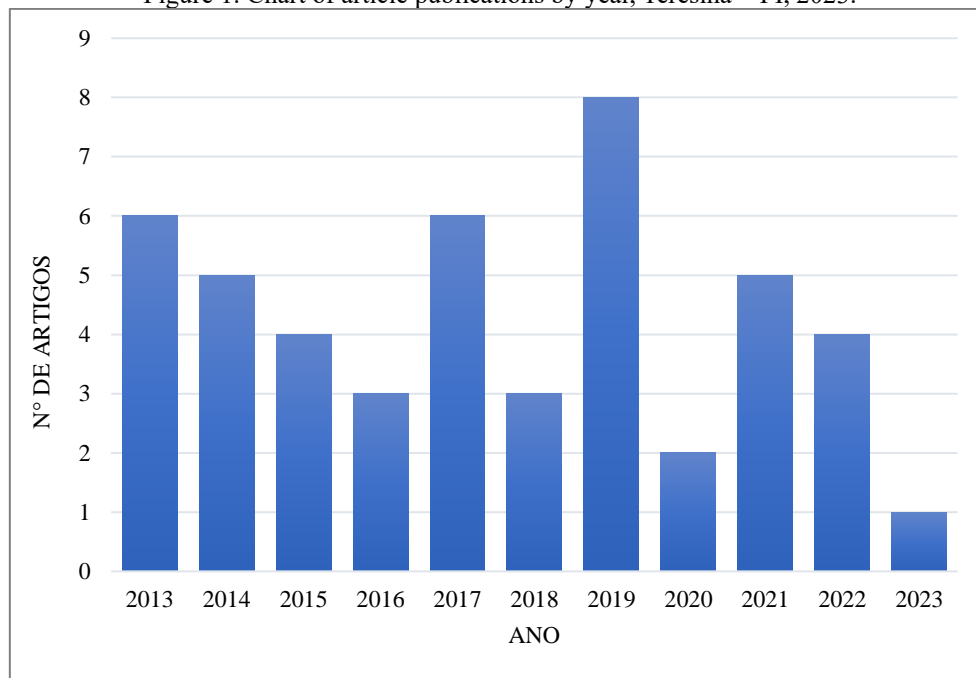
Source: The author



3 RESULT AND DISCUSSION

Initially, the searches totaled 329 articles in the *Scopus*, *PubMed* and *Scielo* databases. After this stage, 283 files that were duplicates or were not aligned with the focus of this review were excluded, resulting in a total of 46 studies (Table 1), which were used for the elaboration of this article. In terms of distribution by year, it was observed that 2019 had the highest number of publications, followed by 2017 and 2013, with a total of 8, 6 and 6 articles, respectively. On the other hand, the year 2023 had a single published paper, representing the lowest number of contributions in the period analyzed (Figure 1).

Figure 1. Chart of article publications by year, Teresina – PI, 2023.

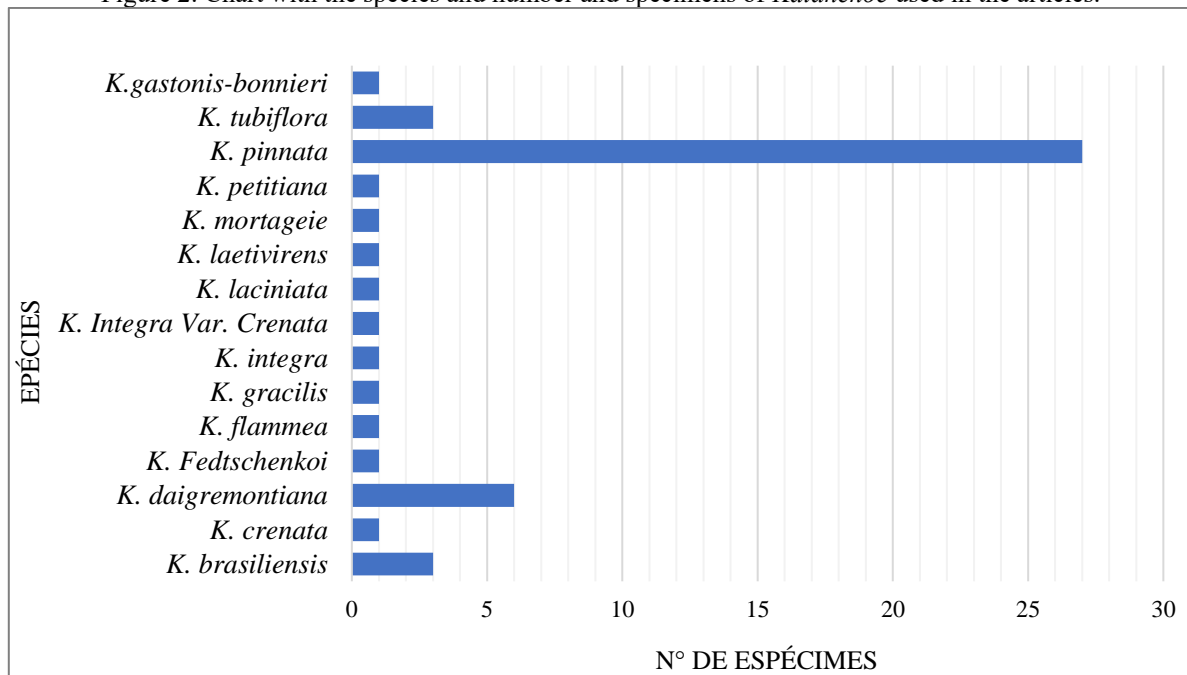


Source: The author

According to the researched works, 14 species and a variety of *Kalanchoe*. In all, the number of specimens was totaled 50, taking into account studies that had more than one species in the research. The species with the highest number of articles was *Pencho Pinnata* (Lam.) Pers. [sin. *Bryophila pinnacle* (Lam.) Kurz] with a number of 27 articles, being the best-known representative of the genre (Bogucka-Kocka *et al.*, 2018). Figure 2 shows that the *K. pinnata* was the most abundant and followed by the *K. daigremontiana*, *K. brasiliensis*, *K. tubiflora* with 6, 3 and 3 specimens respectively. And the other species were recorded in only one article.



Figure 2. Chart with the species and number and specimens of *Kalanchoe* used in the articles.



Source: The author

There is a specific interest in some species because of their unique health properties. So a variety of plant parts are used to make various types of extracts. And among the selected works, the botanical genus had a wide applicability in several diseases, as an anti-inflammatory effect (Chibli *et al.*, 2014; De Araújo *et al.*, 2019; Ferreira *et al.*, 2014) antitumor (Arias-Gonzalez *et al.*, 2018; Hseu *et al.*, 2019; Hsieh *et al.*, 2016; Huang *et al.*, 2013; Kayuppaipoo *et al.*, 2014; Palumbo *et al.*, 2019; Stefanowicz-Hajduk *et al.*, 2020; Stefanowicz-Hajduka *et al.*, 2022) healing (Mekonnen *et al.*, 2013) antidiabetic (Agüero-Hernández *et al.*, 2020; Religion-Neverred *et al.*, 2021; Menon *et al.*, 2015; Patil *et al.*, 2013; Ramon *et al.*, 2023), antiulcerogenic (Braz; Olive tree; Viana, 2013; De Araújo *et al.*, 2018; Shaffer *et al.*, 2017), anti-hypertensives (Bopda *et al.*, 2014), detrusor relaxant (Bachmann *et al.*, 2017; Fürer *et al.*, 2015) Neuroprotective (Anadozie, S.O. *et al.*, 2019; storeman *et al.*, 2021; Kukuia *et al.*, 2015; Mora-Pérez *et al.*, 2016), antiviral (Aoki *et al.*, 2014; Cryer *et al.*, 2017; Wang *et al.*, 2013), antimicrobial (Elizondo-Luévano *et al.*, 2021; The desire to be forgotten *et al.*, 2017; Lebedeva *et al.*, 2017; Mayorga *et al.*, 2019; Richwagen *et al.*, 2019; Rivero-Pérez *et al.*, 2022; Zakharchenko *et al.*, 2017) anti-asthmatic and antitussive (Salami *et al.*, 2013) and nephroprotectant (Anadozie *et al.*- 2018 Dighade *et al.*, 2021; Hewagama *et al.*, 2022; Kamgang *et al.*, 2015; Sohgaura *et al.*, 2019).



Table 1. Description of the articles selected in the review, Teresina – PI, 2023

Species and active compound(s)	Pathology	Starts from planta	Type of Extrato	Induction of pathology	Reference
<i>K. pinnata</i> kaempferitrin e kaempferol	Type 2 diabetes	Leaves	Hydroalcoholic	Oral administration of starch – <i>in vivo</i>	(Agüero-Hernández <i>et al.</i> , 2020)
<i>B. pinnatum</i> Antioxidants	Short-term memory loss	Leaves	Aqueous	Intraperitoneal injection of carbon tetrachloride – <i>in vivo</i>	(Anadozie <i>et al.</i> , 2019)
<i>B. pinnatum</i> Antioxidants	Renal oxidative damage	Leaves	Aqueous	Intraperitoneal injection of carbon tetrachloride – <i>in vivo</i>	(Anadozie <i>et al.</i> , 2018)
<i>K. pinnata</i> Quercetin and gallic acid	Viral infection	Leaves	Methanolic	Hepatitis C virus cell infection – <i>in vitro</i>	(Aoki <i>et al.</i> , 2014)
<i>K. flammaea</i> Coumaric Acid and Palmitic Acid	Prostate Cancer	Leaves	Ethyl Acetate	Human prostate cells – <i>in vitro</i>	(Arias-González <i>et al.</i> , 2018)
<i>K. Integra</i> var. <i>Crenata</i> Flavonoids	Cardiotoxicity	Leaves	Ethanollic	Doxorubicin injection – <i>in vivo</i>	(Asiedu-Gyekye <i>et al.</i> , 2022)
<i>B. pinnatum</i> Bufadienolids and flavonoid aglycones	Bexiga hiperativa	Leaves	Methanolic	K ⁺ solution in porcine urinary bladders – <i>in vitro</i>	(Bachmann <i>et al.</i> , 2017)
<i>B. pinnatum</i> Flavonoids, alkaloids, phenols, tannins and saponins	Memory loss	Bark and Leaves	Methanolic	Intraperitoneal injection of scopolamine hydrobromide – <i>in vivo</i>	(Bhandari <i>et al.</i> , 2021)
<i>K. pinnata</i> Flavonoids and polyphenols	Hypertension	Leaves	Aqueous	Oral administration of NaCl solution – <i>in vivo</i>	(Bopda <i>et al.</i> , 2014)
<i>B. pinnatum</i> Flavonoids and tannins	Gastric ulcer	Leaves	Aqueous	Intraperitoneal injection of indomethacin – <i>in vivo</i>	(Braz; Oliveira; Viana, 2013)
<i>B. pinnatum</i> Rutin, quercetin, and luteolin	Acute and chronic skin inflammation	Leaves	Ethanollic	Topical application of Croton oil, arachidonic acid, phenol, capsaicin and ethyl phenylpropiolate – <i>in vivo</i>	(Chibli <i>et al.</i> , 2014)
<i>K. pinnata</i> KPB-100 and KPB-200 (Unidentified)	Viral infection	Roots	Methanolic	Infection with human herpesvirus alfa and vaccinia virus in Vero cell culture – <i>in vitro</i>	(Cryer <i>et al.</i> , 2017)



<i>K. brasiliensis</i> e <i>K. pinnata</i> The bow of the bow; The throne, the throne of the throne, the throne of the throne	Topical inflammation	Leaves	Aqueous	Induction of ear edema by croton oil and paw edema induced by carrageenan – <i>in vivo</i>	(De Araújo <i>et al.</i> , 2019)
<i>K. brasiliensis</i> e <i>K. pinnata</i> The bow of the bow; the throat, the throat, the throat, the throat	Gastric lesions	Leaves	Aqueous	Oral induction with ethanol and indomethacin – <i>in vivo</i>	(De Araújo <i>et al.</i> , 2018)
<i>B. pinnatum</i> Flavonoides, polifenóis e saponinas	Multifatorial urolithiase	Leaves	Aqueous	Oral administration of ethylene glycol – <i>in vivo</i>	(Dighade <i>et al.</i> , 2021)
<i>K. daigremontiana</i> Quercetin	Parasitic infection	Leaves	Methanolic	Microassay with <i>Entamoeba histolytica</i> and <i>Trichomonas vaginalis</i> – <i>in vitro</i>	(Elizondo-Luévano <i>et al.</i> , 2021)
<i>K. pinnata</i> Quercithin	Nausea, vomiting, pain, and inflammation	Flowers	Aqueous (ethyl acetate and butanol fractions)	Induction of abdominal contortions by subcutaneous acetic acid with saline solution or indomethacin; Subcutaneous carrageenan-induced pleurisy with saline or dexamethasone in the pleural cavity; Ear edema induced by croton oil or dexamethasone – <i>in vivo</i>	(Ferreira <i>et al.</i> , 2014)
<i>B. pinnatum</i> Flavonoids	Overactive bladder syndrome	Leaves	Methanoid (fractions of flavonoids, bufadienolides and polar)	Electrical mediation by organ bath in porcine bladder detrusor muscle – <i>in vitro</i>	(Fürer <i>et al.</i> , 2015)
<i>K. laciniata</i> Flavonoids, tannins and polyphenolic compounds	Urolitidasis and nephrolithiasis	Leaves	Aqueous, ethanolic and hexane	CaOx induction in synthetic urine system – <i>in vitro</i>	(Hewagama; Hewawasam, 2022)
<i>K. tubiflora</i> Kalantuboside B	Cancer	Whole Plant	Ethanolic	Human melanoma cell and murine tumor – <i>in vitro</i> ; subcutaneous injection into the flanks of tumor cells suspended in matrix gel – <i>in vivo</i>	(Hseu <i>et al.</i> , 2019)



<i>K. tubiflora</i> (Unidentified)	Cancer	Whole Plant	Aqueous	Human lung adenocarcinoma cell line – <i>in vitro</i> ; subcutaneous inoculation into the flank of lung cancer cells – <i>in vivo</i>	(Hsieh <i>et al.</i> , 2016)
<i>K. tubiflora</i> Cardenolideos and Glycosideos Bufadienolideos	Cancer	Whole Plant	Ethanollic (n-hexane, EtOAc and n-BuOH fractions)	Human cell linhas: pulmonary adenocarcinoma epithelial; oral adenoescumous carcinoma; melanoma; Myelocytic leukemia – <i>in vitro</i>	(Huang <i>et al.</i> , 2013)
<i>B. laetivirens</i> (Unidentified)	Cancer	Leaves	Metalnolic (fractions of water and dichloromethane)	Human Lung Cancer Cell Line – <i>In Vitro</i>	(Kaewpiboon <i>et al.</i> , 2014)
<i>K. crenata</i> Tannins, triterpenes and polyphenols	Renal insufficiency	Whole Plant	Methanolic	Intravenous injection of adriamycin – <i>in vivo</i>	(Kamgang; Fondjo; Oyono, 2015)
<i>K. daigremontiana</i> Bufadienolids	Oxidation	Roots	Aqueous methanol (bufadienolide fraction)	Exposure of peroxy nitrite in human blood plasma – <i>in vitro</i>	(Kołodziejczyk-Czepas <i>et al.</i> , 2016)
<i>B. pinnatum</i> Alcaloides, fenóis, flavonoides, taninos, antraquinonas e esteroides	Bacterial infection	Leaves	Methanolic and ethyl acetate	Oral inoculation of a <i>Helicobacter pylori</i> suspension – <i>in vivo</i>	(The desire must be <i>et al.</i> , 2017)
<i>K. Integra</i> (Unidentified)	Depression	Leaves	Aqueous	Treatment with reserpine, α -methyl paratyrosine (AMPT) or p-chlorophenylalanine – <i>in vivo</i>	(Kukuia <i>et al.</i> , 2015)
<i>K. pinnata</i> (Wild and Transgenic) Flavonoids or lectins	Bacterial infection in wounds	Leaves	Aqueous	Modelling of purulent infection with <i>Staphylococcus aureus</i> and/or <i>Pseudomonas aeruginosa</i> – <i>in vivo</i>	(Lebedeva <i>et al.</i> , 2017)
<i>K. daigremontiana</i> Ascorbic acid, phenols, gallic acid, flavonoids and quercetin	Diabetes mellitus	Leaves	Gross	Dieta <i>ad libitum</i> líquida hiperglicêmica rica em sacarose – <i>in vivo</i>	(Madariaga-Navarrete <i>et al.</i> , 2021)
<i>K. brasiliensis</i> Flavonoids	Bacterial infection	Leaves	Hydroethenolic	Assay with 5 strains of <i>Salmonella spp.</i> – <i>in vitro</i>	(Mayorga <i>et al.</i> , 2019)
<i>K. petitiana</i> Polyphenols, alkaloids, flavonoids and tannins	Wound healing	Leaves	Hydroalcoholic (aqueous, methanolic and chloroform fractions)	Skin excision and incision with scissors – <i>in vivo</i>	(Mekonnen <i>et al.</i> , 2013)



<i>K. pinnata</i> Flavonoides, polifenóis, triterpenoides, fitoesteróis e zinco	Diabetes mellitus	Leaves	Aqueous	Single intraperitoneal injection of streptozotocin – <i>in vivo</i>	(Menon; Sparks; Omoruyi, 2015)
<i>K. pinnata</i> Sterols and terperes	Convulsion	Root or Stem	Methanolic	Oral induction of pentylene tetrazole – <i>in vivo</i>	(Mora-Pérez; Hernández- Medel, 2016)
<i>Q. Gastonis- Bonniéri</i> Glycositized lignans	Benign prostatic hyperplasia	Leaves, flowers and underground parts	Aqueous	Human cell culture of benign prostatic hyperplasia – <i>in vitro</i>	(Palumbo <i>et al.</i> , 2019)
<i>K. pinnata</i> (Unidentified)	Diabetes mellitus	Leaves	Petroleum ether, chloroform, dichloroethane and aqueous fractions	Intraperitoneal injection of streptozotocin in citrate buffer – <i>in vivo</i> ; Incubation of rat pancreatic islets in glucose – <i>in vitro</i>	(Patil <i>et al.</i> , 2013)
<i>K. pinnata</i> Quercetin, kaempferol, apigenin, epigallocatechin gallate and avicularin	Diabetes Mellitus	Leaves	Dimethyl sulfoxide	Human Diabetic Muscle Myoblast Cell – <i>In Vitro</i>	(Ramon <i>et al.</i> , 2023)
<i>K. mortageie</i> K . <i>Fedtschenkoi</i> Kaempferol, quercetin, phenolic compounds, caffeic acid, p- coumaric acid and ferulic acid	Bacterial infections	Leaves, flowers and stem	Aqueous and ethanolic	Assay with ESKAPE bacterial strains – <i>in vitro</i>	(Richwagen <i>et al.</i> , 2019)
<i>K. Daigremontiana</i> Flavonoids, flavones, saponins, alkaloids, xanthenes, polyphenols, tannins and derivatives of pyrazol-5- carboxamide	Parasite and bacterial infections	Leaves	Hydroalcoholic	Ensaio com ovos de <i>Haemonchus contortus</i> ; e cepas de <i>Staphylococcus aureus</i> , <i>Bacillus subtilis</i> , <i>Salmonella typhimurium</i> , <i>Pseudomonas aeruginosa</i> , <i>Listeria monocytogenes</i> e <i>Escherichia coli</i> – <i>in vitro</i>	(Rivero-Pérez <i>et al.</i> , 2022)
<i>Bee. Hindsight</i> Flavonoids	Asthma and coughing spells	Leaves	Aqueous	Histamine Aerosol Exposure for Asthma and Citric Acid for Cough – <i>In Vivo</i>	(Salami <i>et al.</i> , 2013)
<i>K. pinnata</i> Quercetin	Peptic ulcers	Leaves	Hydroethanolic (ethyl acetate fraction)	Oral induction of gastric lesions by gavage with ethanol HCl – <i>in vivo</i>	(Sobreira <i>et al.</i> , 2017)
<i>K. pinnata</i> Flavonoids and polyphenols	Nephrolithia	Leaves	Hydroethanolic	Induction of ammonium chloride and ethylene glycol administered via	(Sohgaura; Bigoniya; Shrivastava, 2019)



					drinking water – <i>in vivo</i>	
	<i>K. daigremontiana</i> Bufadienolids	Cancer	Leaves	Ethanollic (aqueous, dichloromethanelic, and bersaldegenin 1,3,5-orthoacetate fractions)	Human cervical adenocarcinoma, ovarian cancer, breast cancer, malignant melanoma and keratinocyte cell lines – <i>in vitro</i>	(Stefanowicz-Hajduk <i>et al.</i> , 2020)
	<i>K. daigremontiana</i> Bufadienolideos	Cancer	Leaves	Aqueous	Human ovarian cancer cell line and human keratinocyte cells – <i>in vitro</i>	(Stefanowicz-Hajduk, Justyna <i>et al.</i> , 2022)
	<i>K. gracilis</i> The Quercitina	Viral infection	Stem	Aqueous (Ethyl acetate and n-butanol fractions)	Enterovirus 71 and Coxsackievirus A16 strains in RD cells cultured in Eagle medium – <i>in vitro</i> ; Intraperitoneal infection with Enterovirus 71 – <i>in vivo</i>	(Wang <i>et al.</i> , 2013)
	<i>K. pinnata</i> (unidentified)	Fungal infection in wounds	Leaves	Aqueous	Infection with <i>Candida albicans</i> in wounds – <i>in vivo</i>	(Zakharchenko <i>et al.</i> , 2017)

Source: The author

The genus presented a wide range of applications, this is linked to the chemical composition, where its secondary metabolites, such as flavonoids, polyphenols, bufadienolides, tannins, saponins, acids, alkaloids, exert important biological activities (Rivero-Pérez *et al.*, 2022; Stefanowicz-Hajduk *et al.*, 2022).

At work Chibli *et al* (2014), three anti-inflammatory flavonoids were identified: rutin, quercetin, and luteolin, which possibly reduce vasodilation, leukocyte infiltration, and edema. These compounds are antioxidants that prevent oxidative stress due to inflammatory processes. The anti-inflammatory activity of ethanolic extract of *K. pinnata* It can also be given by the presence of steroid derivatives. De Araújo *et al.* (2019) showed that aqueous extract of *K. brasiliensis* It can inhibit the inflammatory mediators released in the two phases of inflammation, showing better performance, while the *K. pinnata* It can inhibit only the mediators released in the first phase. They were able to reduce the activity of the cellular digestion enzyme, a result that suggests that the flavonoids present in the extract were able to inhibit the infiltration of neutrophils and consequently the response to inflammation. And Ferreira *et al.* (2014) corroborates, mentioning that flavonol, aglycone quercetin has an immunomodulatory effect through the regulation of inflammatory mediators.

In research *in vitro*, findings indicate that the ethanolic extract of *K. flammaea* It induces the generation of oxidative stress when prostate cells are exposed, in which this cytotoxic action associated



with coumaric acid and palmitic acid present in the extract, which could lead to the activation of an apoptotic stimulus of the extract, inducing cell cycle arrest and antiproliferative activity (Arias-Gonzalez *et al.*, 2018). Another study shows that mitochondrial function was critically impaired by early apoptosis mediated by Kalantuboside B, a natural derivative of bufadienolid extracted from *K. tubiflora*, in melanoma cells *in vitro* (Hseu *et al.*, 2019; Huang *et al.*, 2013). And the study *in vivo* corroborated, showing the reduction of tumor size in mice (Hseu *et al.*, 2019). And Hsieh *et al.* (2016) has also had results where treatment *in vivo* It significantly slowed the growth of the lung tumor, with extract of the same species. Similar activity occurred with *K. Lativirens*, in which it exhibited significant antiproliferative effects against lung cancer cells (Kaewpiboon *et al.*, 2014).

Other compounds important in the antitumor activity of the genus *Kalanchoe* are the bufadienolides, where they are usually characterized as toxic components with strong cytotoxic activity, among them are 16-hydroxybersaldegenin acetate; Bersaldegenin acetate-4 and 5; Bryophyllin A and B; Bersaldegenin acetate-1 and 2; Daigremontianina; and bersaldegenin-1,3,5-orthoacetate (Stefanowicz-Hajduk *et al.*, 2020). Studies done with the species *K. daigremontiana*, show that the main bufadienolide, bersaldegenin-1,3,5-orthoacetate, has the potential to be responsible for the cytotoxic activity of the plant. Different types extracts showed strong antiproliferative and cytotoxic activities in ovarian cancer cells and significantly inhibited the cell cycle (Stefanowicz-Hajduk *et al.* 2022; Stefanowicz-Hajduk *et al.* 2020).

4 FINAL THOUGHTS

It is verified that the genus *Kalanchoe* is a potential botanical group in the treatment of several pathologies, namely: anti-inflammatory, antitumor, healing, antidiabetic, antiulcerogenic, antihypertensive, detrusor relaxant, neuroprotective, antiviral, antimicrobial, antiasthmatic, antitussive and nephroprotective action. Many secondary composites of plants of this genus, when used in adequate concentrations, have several medicinal effects.



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Use of organominerals in agriculture



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ABSTRACT

Animal production systems generate a high amount of organic waste, which if disposed of irrationally can cause contamination of both soil and water.

Aviary litter is one of these examples, which, due to the high amount of organic matter and nutrients, has the potential to be applied as an organic fertilizer, totally or partially replacing chemical fertilizers, which are essential for plant development. The use of organic fertilizers can also reduce fertilization costs, which are high, mainly due to dependence on imports. Treatments such as composting are important to reduce the pathogenic load of the material, ensuring greater safety in its use. Many positive responses have already been scientifically proven, bringing benefits such as soil improvement and increased yields, both of annual crops and perennial pastures. The recommendation of the dose of the organic fertilizer can be carried out in order to meet the demand for P, which is found in low concentrations in Brazilian soils. To increase the concentration of nutrients in organic fertilizers and make them more balanced, they can be complemented with chemical fertilizers, generating organominerals. The proper use of residues, especially poultry litter, combined with chemical fertilizers can contribute satisfactorily to farmers, due to the possibility of reducing the operational cost of production and chemical fertilizers.

Keywords: Aviary litter, Phosphorus, Organic fertilization.

1 INTRODUCTION

Activities related to food production are in broad development in Brazil, with emphasis on poultry farming. This chain provides numerous benefits, being the promoter of various jobs, food, among other advantages for the region. However, this high production generates waste with a high pollutant load, which, if not disposed of properly, causes serious environmental impacts.

One of the ways to mitigate the impacts on the environment is the use of residues in agriculture, such as fertilizers and poultry litter is one of the residues with potential for use for this purpose.

At the same time, it is important to infer that the acquisition of chemical fertilizers has been suffering increases in acquisition values, impacting exorbitant increases in fixed costs; something that



undermines the sustainability and economic efficiency of agricultural production (ALLAM et al., 2022). Thus, one strategy to reduce production costs and make agricultural production sustainable is the use of organic fertilizers.

The avian compost has satisfactory results when compared to other compounds, as they influence the physical, chemical and microbiological characteristics. In addition to increasing base saturation and raising levels of nutrients such as calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P) and zinc (Zn) (SCHALLEMBERGER et al., 2019). According to Santos and Camargo (2008), organic sources can replace all or part of the P required by plants.

With the combination of minerals with organic fertilizer, such as poultry litter, organominerals originate. This tool meets the current need for sustainability reconciled with socioeconomic development.

The enrichment of organic fertilisers, mainly such as P, allows them to be used mainly in the basic fertilisation of both annual crops and perennial pastures.

It is worth mentioning that P is one of the nutrients responsible for the development of the root system at the beginning of the development of the plant organism, responsible for the increase in vigor, better use of water, resistance to pathogens and among others (MALAVOLTA, 2006).

2 DEVELOPMENT

2.1 P DEFICIENCY IN CERRADO OXISOLS

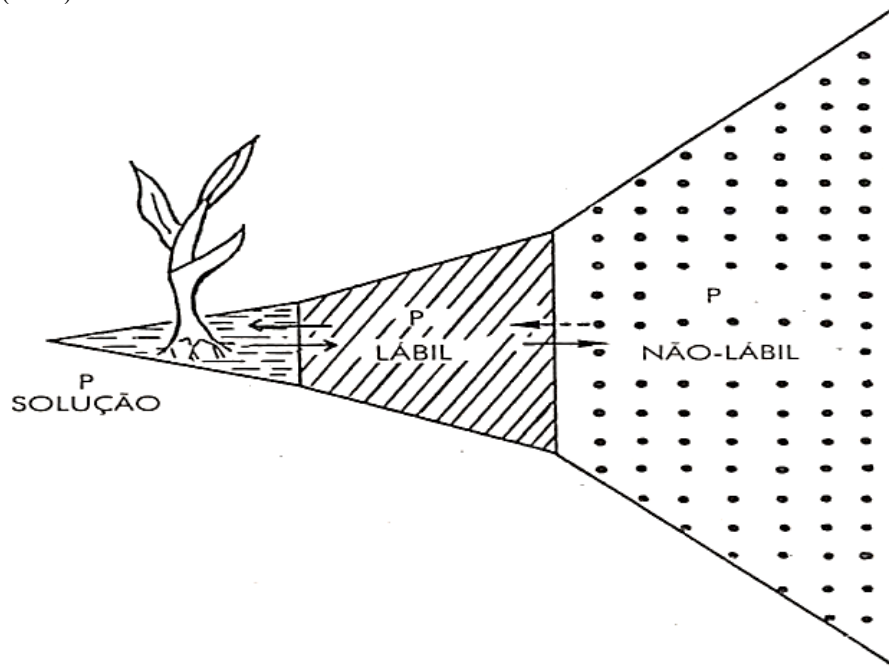
The soils of the Cerrado, for the most part, are formed by Oxisols, characterized by being highly weathered, porous, deep and deficient in some essential minerals for crops of agronomic interest. However, they have a great capacity for technified agriculture and livestock, due to the favorable climate for crop development. It has privileged relevance for the expansion of agriculture specialized in grains, due to the ease they offer to the traffic of agricultural machinery (MAROUELLI, 2003).

The limiting factors for agricultural use in these soils are the deficiency of P, Ca, nitrogen (N) and high content of aluminum (Al), a toxic element for most commercial crops, since it becomes necessary to apply correctives and fertilization, establishing means that make it possible to synchronize the release of nutrients with the time of greatest demand for the crops. thus avoiding their immobilization or accelerated mineralization (SANZONOWICZ, 2010; PITTA et al., 2012; BOTTEGA et al., 2013).

The amount of total P in Oxisols is relatively high, however, it is not found in its labile form, i.e., accessible to plants in the soil (SANTOS, 2009), as can be seen in Figure 1.



Figure 1. Diagram showing the relationship between non-labile phosphorus fractions and phosphorus in soil solution. Adapted from Rajj (1991).



P is an essential macronutrient, effectively participates in several metabolic processes in the organism of plants, has a lot of influence with other soil nutrients, such as Al, iron (Fe) and Ca. It is also present in organic forms and its low rate of propagation in the soil makes it less available for soil/plant (ALMEIDA et al., 2016).

Thus, the application of high doses of P is essential, as it is essential for the success of crops, whether grain or forage. P is one of the most important nutrients and deserves greater concern during management, due to its enormous adsorption capacity in the mineral phase of the soil (SCHONINGER et al., 2013).

Paiva et al. (2012) observed that N and P doses increased their availability in the soil, positively influencing the weight of green corn ears. The crop has an excellent response to phosphate fertilization more than to nitrogen fertilization, demonstrating that in Cerrado soils, P is more limiting to corn production than N.

In the no-tillage system, the maximum use of P by the plants prevails, a factor attributed to the reduction of contact between the fertilizer and soil particles, resulting from the absence of turning and the presence of vegetation cover or straw on the soil surface, providing moisture retention (ROSIM et al., 2012).

Nunes et al. (2011) found that soybean yield in the 14th year of cultivation in the area was not affected by the source of phosphate fertilizer or by the mode of application, but by the cultivation system. The highest yield was obtained in the no-tillage system, due to higher P availability when compared to the conventional system.



The increase in the organic matter (OM) content, which occurs in a no-tillage system, increases the availability of P in the soil, which is mainly due to the competition of organic anions and functional groups of humic substances by the adsorption sites, which increases the concentration of the element in the soil solution

The positive effect of phosphate fertilization on forages has also been proven by Oliveira et al. (2012), with an increase in the number of tillers and dry mass of shoots and roots of Mombasa grass; by Almeida et al. (2013), also with Mpmbaça grass, which found an increase in the production of green and dry matter in the aerial part and also in the number of forage tillers; By Carneiro et al. (2017), who obtained positive effects on height and average number of tillers.

2.2 USE OF ORGANIC WASTE IN AGRICULTURE

Animal production chains generate a high amount of organic waste with a high degree of polluting agents and the irrational disposal of these in the environment causes serious ecological imbalances, however it can be minimized through environmental liabilities, such as its use in agriculture. When effluents are properly treated, they become interesting due to the enormous supply of excreted nutrients that were not used in the diet, these with high levels of N, P and K. Not to mention the enormous amount of organic material (KARUNANITHI et al., 2015).

Organic fertilizers from animals fed a diet richer in concentrate tend to have a faster availability of nutrients than those animals fed with a diet richer in roughage, thus creating a very broad spectrum in relation to the release time of these nutrients, this is all related to the carbon/nitrogen ratio (C/N) present in the organic compost (SOCIEDADE BRASILEIRA DE CIÊNCIA DO SOLO, 2016).

The use of organic fertilizer provides significant changes in soil characteristics, providing greater porosity, increased water retention, greater formation of soil aggregates, and increased CEC. For this, it is necessary to transform this material into humus, or mineralization, through composting, which promotes a reduction in the C/N ratio of the organic compost (BATISTA et al., 2018)

In order to make the nutrients present in the organic fertilizer available to the plants, it is necessary to transform the organic fraction to the inorganic fraction mediated by the microorganisms present in the soil, a process called mineralization, which varies according to the composition of the organic fertilizer, the activity of the biota, soil characteristics and edaphoclimatic conditions (VANEGA CHACÓN et al., 2011).

2.3 POULTRY BEDDING

The poultry litter integrates part of the waste from the production system. In the house, it has the purpose of preventing the friction of the bird with the surface, absorbing moisture, incorporating



feces, feathers, skin peeling and food remains. It is usually composed of wood shavings, rice husks or straw (VIEIRA, 2011).

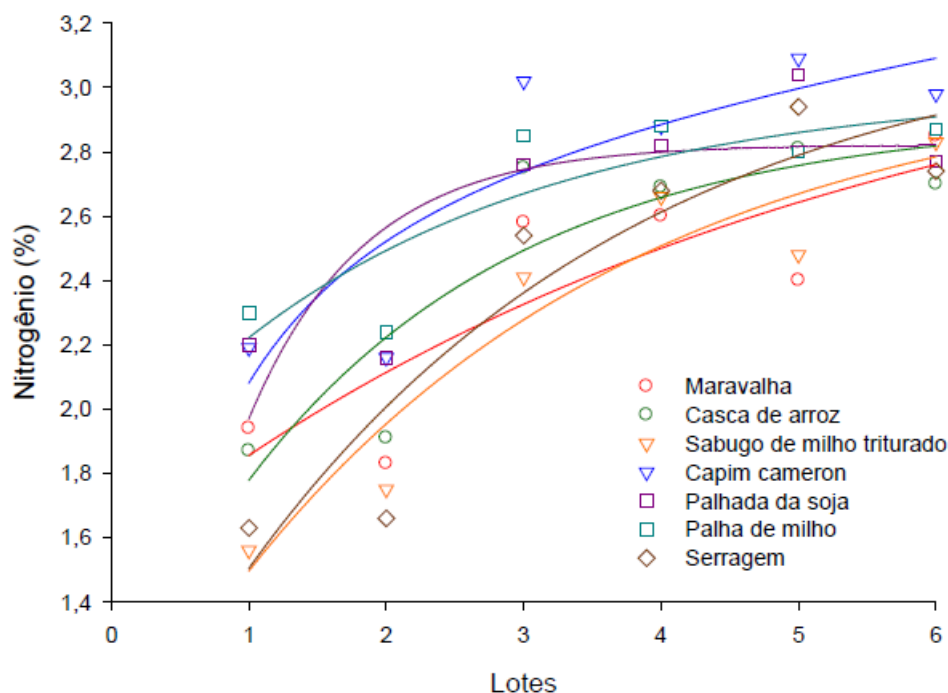
In order to be able to reuse the litter, it is necessary that it is handled and treated correctly, in order to mitigate the population of microorganisms that may harm the following batches. There are several types of management aimed at the inactivation and control of pathogens between batches. In the country, the most commonly used are windrow fermentation, the addition of lime to the litter and flat fermentation, which is summarized in the covering of the litter with canvas along the entire length of the aviary (MACKLIN et al., 2006).

It is important to highlight that when the occurrence of severe sanitary cases is observed, all material must be removed for incineration and cleaning of the shed, followed by a sanitary vacuum before the accommodation of the next batch (SILVA, 2012).

Reuse can be done for up to 12 batches, however, it is mostly used for six batches in a row. At the end of each batch, the crusts and pasted materials are removed, and if the remaining litter is not very thick, a new substrate is incorporated (MENDES et al., 2004).

Both the materials used as bedding and the number of passes influence the availability of nutrients. Avila (2007) found that with the increase in the number of passes, the N content increased and Cameron grass, crushed corn cob, soybean straw and sawdust provided greater availability (Figure 2). These results emphasize the need to understand the composition of poultry litter before recommending doses for application in the field.

Figure 2. Nitrogen (N) contents in various poultry litters varying according to the raw material used in relation to the number of flocks of birds.



Fonte: Adapted from Avila (2007).



Table 1 shows that with the increase in the number of broiler passes (3-4 to 7-8 batches), there is an increase in nutrients. From the data presented, the increase was 16% of N, 12% of P₂O₅, 28% of K₂O, 11% of Ca and 20% of Mg. The type of bird also influences the concentration of nutrients.

Table 1. Average values of nutrients and dry matter content in different amounts of chicken litter reuse and different categories.

Organic material	C-org.	N ₂	P ₂ O ₅	K ₂ O	Ca	Mg	Dry Matter
	----- % (m/m) -----						
Frango Bed (3-4 lots)	30	3,2	3,5	2,5	4,0	0,8	75
Frango Bed (5-6 lots)	28	3,5	3,8	3,0	4,2	0,9	75
Frango Bed (7-8 lots)	25	3,8	4,0	3,5	4,5	1,0	75
Peru Bed (2 lots)	23	5,0	4,0	4,0	3,7	0,8	75
Layer bed	30	1,6	4,9	1,9	14,4	0,9	72

Source: Adapted from BRAZILIAN SOCIETY OF SOIL SCIENCE (2016).

Chicken litter was widely used in cattle feed in Brazil. After several outbreaks in other countries by Bovine Spongiform Encephalopathy, commonly known as "Mad Cow Disease", in 2001, the Ministry of Agriculture, Livestock and Supply (MAPA) prohibited its use throughout the country through Normative Instruction (IN) No. 15, in its 2nd article (BRASIL, 2009).

Normative Instruction No. 15 was annulled by IN No. 7 of March 2004, declaring exclusively the importation of products that could cause Bovine Spongiform Encephalopathy and did not refer to any residue of national origin. However, in the same month of 2004, IN No. 8 came into force, condemning throughout the national territory the production, commercialization and use of products intended for the feeding of ruminants that contain proteins and fats of animal origin, including poultry litter (JÚNIOR, 2010).

2.4 POULTRY LITTER TREATMENT THROUGH COMPOSTING

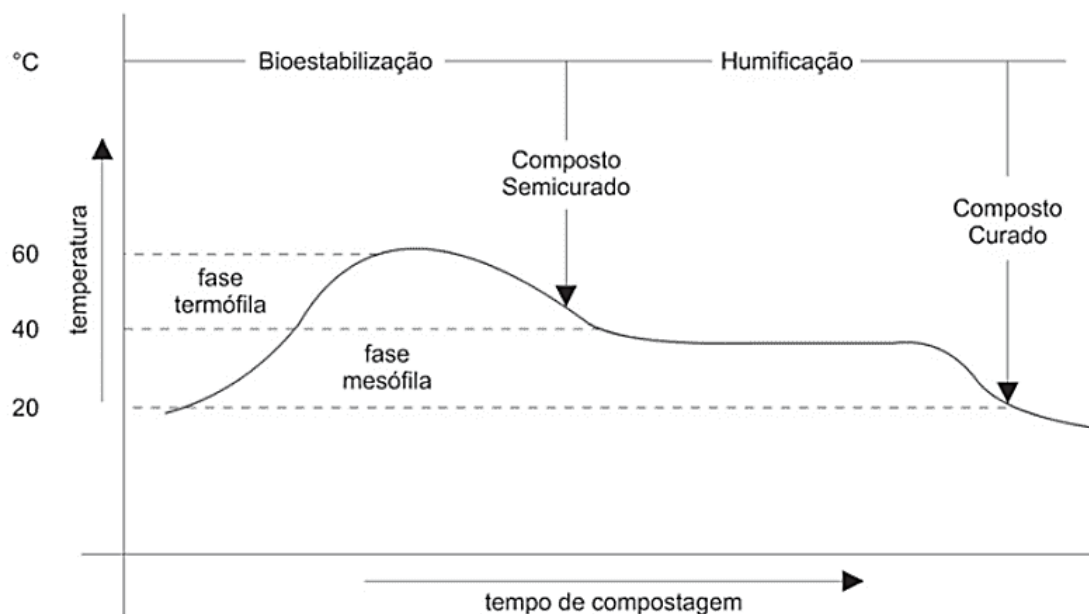
Composting is a set of techniques characterized by the biological process of decomposition, where microorganisms convert organic matter into carbon dioxide, biomass, heat, and humic substances. After the composting process, the final product tends to improve the physical, chemical and biological properties of the soil, without causing environmental problems. Composting enables an effective disposal of organic waste, preventing its agglomeration in landfills or in the environment (HAO, 2016).

The composting process can be summarized in phases (SILVA, 2010; KIMURA, 2014) (Figure 3).



- **Mesophilic phase:** defined by the increase in temperature up to 40°C as a result of the exponential development of microorganisms and degradation of easily degradable compounds, such as sugars and proteins.
- **Thermophilic Phase:** thermophilic microorganisms replace mesophilic microorganisms, the temperature exceeds 40° C, increasing the rate of biodegradation of lipids, hemicellulose, cellulose and lignin, reducing the mass and volume of the rakes.
- **Cooling phase:** decrease in microbial activities, thermophilic populations are replaced by mesophiles, due to the decline in temperature.
- **Maturation phase:** this is the last stage of the transformation of complex molecules into humic substances.

Figure 3. Summary of the phases of the composting process, highlighting the interaction between temperature and time.



Source: D'ALMEIDA; VILHENA, 2000.

To differentiate the temperature phases, simply introduce rebar to the bottom of the windrows, until the end of the composting process. These iron bars should be removed to check the temperature every two or three days until the first turn, and once a week, until the end of the process.

The main factors that interfere with composting are microorganisms, aeration, humidity, temperature, C/N ratio, chemical and physical characteristics of the materials involved, pile dimensions and the stability of the biodegradability of the microbial population, these when misconducted, can lead to low composting efficiency resulting in a compost of inferior quality (XI et al., 2015).

Table 2 shows the appropriate and desirable amplitude of each of these items, in order to have an efficient composting process.



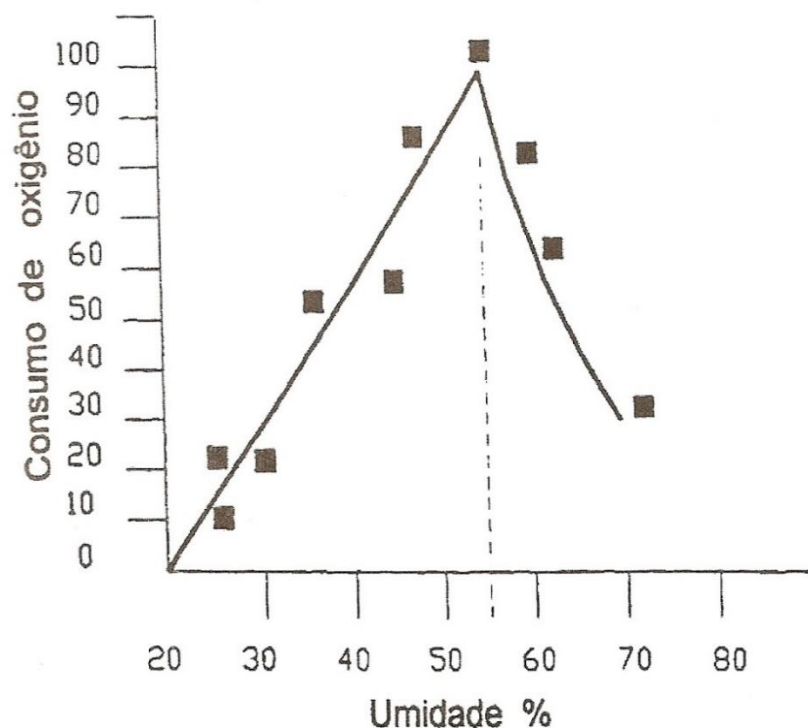
Table 2. Recommendations for efficient composting.

Conditions	Adequate amplitude	Desirable breadth
Oxygen Concentration	Greater than 5%	Much higher than 5%
Moisture	40 – 65%	50 – 60%
Temperature (°C)	43,5 – 65,5	54,5 – 60,0
Carbon:Nitrogen Ratio	20:1 – 40:1	25:1 – 30:1
Moisture	40 – 65%	50 – 60%
ph	5,5 – 9,0	6,5 – 8,0

Source: Adapted from Soares e Silva (2021).

High levels of humidity leave the material soaked, impairing the aerobic decomposition of the material, due to the lack of oxygen, generating bad odors. Oxygen consumption, which is the digestion of organic matter, increases considerably with the increase in moisture content from 25% to 55%, falling almost vertically when it exceeds this last value (Figure 4).

Figure 4. Effect of humidity on oxygen consumption in household solid waste composting.



Fonte: GOLUEKE (1975).



In order to achieve the desired values of the C/N ratio, it is often necessary to mix materials according to the different compositions of the residues (Table 3).

Table 3. C/N ratio of some wastes.

Material	C/N Ratio
Bovine manure	18:1
Bird Manure	10:1
Pig manure	19:1
Sheep manure	15:1
Equine manure	18:1
Aviary bedding	14:1
Rice: husk and straw	39:1

Fonte: Adapted from Kiehl (1998).

Composting has numerous advantages, as it enables the decomposition of carcasses remaining in poultry litter, thus an excellent technique for treating solid waste (CESTONARO et al., 2010; ORRICO JUNIOR et al., 2012; PAIVA et al., 2012).

Orrico Júnior et al. (2010), in the study evaluating the effectiveness of the composting process in the treatment of poultry litter waste and poultry carcasses, observed that the percentage of bones in relation to the initial amount of carcasses, which resisted the composting process, was 2.95%, most of them with rustic structures.

Composting programs are in focus, especially in the treatment of poultry waste such as chicken litter and layer waste. Due to environmental pollution and the requirements of MAPA (Annex IV, IN n°25/2009), for biosafety reasons, waste of animal origin must be treated (BRASIL, 2009).

Properly conducted composting is able to mitigate most of the pathogenic microorganisms present in the organic matter, thus reducing the risk of contamination. The mechanisms of pathogen elimination are understood by the combination of temperature, competition between microorganisms and exposure time. Among the parameters that are easy to monitor is temperature (LONGHURST et al., 2010).

However, during the composting process, a considerable amount of N is lost by volatilization in the form of ammonia (NH₃). Studies indicate that the most significant losses occur during the initial stages of composting, when there is a greater amount of easily decomposed organic material and a rapid increase in temperature due to the activity of microorganisms (JANCZAK et al., 2017).

The high temperature during the composting process is the result of the biodegradation of organic matter by microorganisms, which can become a threat to the process if the temperature exceeds 75°C, leading to a reduction or even a halt in microbial activity (MASSUKADO, 2008). Studies show that the temperature should be maintained up to 60°C, being able to efficiently reconcile the mitigation of pathogens and high levels of biodegradation (FIALHO, 2007).



It was observed in a study conducted by Ferreira (2021) that composting was effective in significantly reducing the population of thermotolerant coliforms in poultry litter waste, both organic and conventional. In addition, composting was effective in eliminating viable helminth eggs in all animal waste tested, reinforcing its efficiency as a method of organic waste treatment.

2.5 USE OF POULTRY LITTER AS ORGANIC FERTILISER

Due to the high percentage of organic matter contained in poultry litter, this is considered an interesting residue from an agronomic point of view, for commercial crops. However, this use must have a technical basis and be consistent with the reality of each producer, knowledge of the needs of the soil, plants and especially the chemical composition of these compounds.

Noce et al. (2014) using poultry litter as fertilizer found that it provided positive effects on corn yield for silage and, depending on the market climate and regional availability of the product, there is the possibility of replacing chemical fertilization.

The use of poultry litter as an organic fertilizer for pasture during the off-season period on the corn crop demonstrates viability, as it allows gains in crop productivity due to its high level of nutrients (NOVAKOWISKI et al., 2013).

Portugal et al. (2009) observed the effects of the use of different doses of chicken litter for two consecutive years on soil chemical changes and dry matter accumulation of *Urochloa brizantha* cv. Marandu. The use of this residue significantly increased the dry matter production (8 t ha^{-1}) when compared to the treatment that did not receive the residue (4 t ha^{-1}).

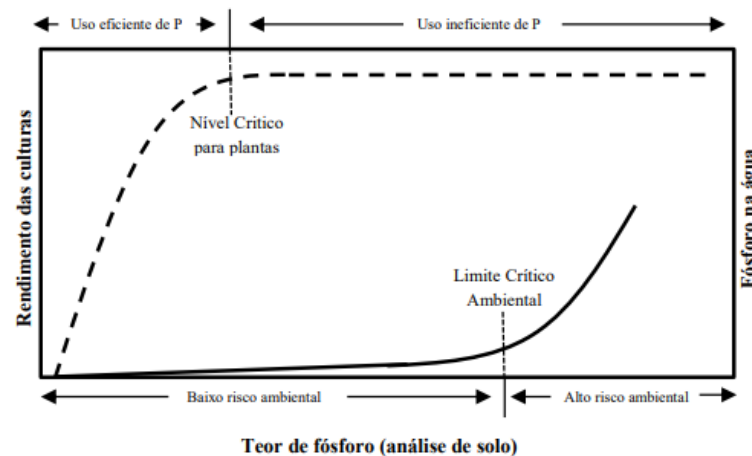
Silva et al. (2013) using only laying hen manure, found an increase in the production of green mass and in the length of *Urochloa grass. brizantha* cv. Thus, the level of soil organic matter is highly correlated with the production potential of pastoral systems, especially in systems where fertilization is not used. The recovery and effective maintenance of adequate OM levels are essential for the sustainable development of livestock in tropical regions.

For the use of poultry litter as fertilizer in pastures, it is absolutely necessary to verify and analyze its source material, it is essential to have easy biodegradation and to be free of pathogenic microorganisms that may contaminate the soil and the water table (BENITES, 2011).

The systematic application of chicken litter in the soil can cause accumulation of P. This accumulation occurs due to the concentration of N, P₂O₅ and K₂O which is practically 1:1:1 (Table 1), while for most crops the requirements of N, P₂O₅ and K₂O are 6:1:4; 6:1:3 respectively. One of the precautions when recommending the appropriate dose is to use the critical nutrient for the calculation basis. In addition to the agronomic response, which is the effect on crop yields, it is necessary to observe the environmental response



Figure 5. Schematic representation of the relative yield of crops and the amount of P in the water as a function of the available P content in the soil, highlighting the critical level of P for the crops and the critical environmental limit.



Cast iron: Gatiboni et al. (2014).

2.6 ORGANO-MINERAL FERTILIZERS AND THEIR USE

When organic fertilizers are complemented with mineral fertilizers, they give rise to organo-mineral fertilizers (SOUSA, 2012). Solid organo-mineral fertilizers must contain at least 8% organic carbon, 10% primary macronutrients N, P and K alone (BRASIL, 2009).

According to NORMATIVE INSTRUCTION No. 23, OF AUGUST 13, 2005, of the Brazilian Legislation, organomineral fertilizer is defined as a product resulting from the physical mixture or combination of mineral or organic fertilizers, which can be in crumbled, granulated or pelleted form (ALANE, 2015).

Organic compounds, together with mineral sources, are a tool that intensifies the efficiency of mineral fertilizers, minimizing crop fertilization costs and providing improvements in soil characteristics, through a greater supply of nutrients, which causes greater activity of microorganisms present in the soil, increasing the stability and sustainability of the system (RABELO, 2015; ULSENHEIMER et al., 2012).

Organo-mineral fertilizers have great potential for agricultural application in annual and perennial crops. This encompasses valuable commodities such as corn, soybeans, beans, wheat, cotton, as well as perennial pastures. From a sustainable production perspective, its use is economically viable, reducing dependence on mineral fertilizers and contributing to environmental conservation (MALAQUIAS; SANTOS, 2017).

According to Andrade et al. (2012), organo-mineral fertilizers are superior to chemical and organic fertilizers, because the absence of some essential nutrients for plants through the combination of fertilizers can be easily supplied, and the absence of one nutrient can be found in greater quantities in the other.



Tiritam and Santos (2012), studying the influence of fertilization with organomineral fertilizer, observed satisfactory results for off-season corn, as the treatment that used organomineral fertilizers was superior in relation to the others that did not use fertilizer, with higher productivity in corn production and improvements in soil properties.

According to Borges et al. (2015), the use of organomineral fertilizer in soybean planting provided superior productivity when compared to mineral fertilization treatments, and may be a viable possibility from an agronomic and economic point of view in crop management.

Silva et al. (2015) found that organomineral fertilizers increase the levels of nutrients such as Ca and Mg from 6.8 and 3.3 cmolc dm^{-3} to 8.2 and 4.6 cmolc dm^{-3} in the soil layers that were studied and reduced aluminum saturation from the lowest dose (60 kg ha^{-1} of N; 90 kg ha^{-1} of P_2O_5 and 100 kg ha^{-1} of K_2O).

3 CONSIDERATIONS

1 DUE TO THE FACT THAT IT IS A GOOD SOURCE OF NUTRIENTS AND ORGANIC MATTER, CHICKEN LITTER HAS THE POTENTIAL TO BE USED IN AGRICULTURE. HOWEVER, IT IS NECESSARY FOR THIS WASTE TO GO THROUGH A COMPOSTING PROCESS, WHICH IMPROVES ITS AGRONOMIC CHARACTERISTICS, IN ADDITION TO ELIMINATING PATHOGENIC MICROORGANISMS WHEN THE TEMPERATURE OF THE COMPOST PILES IS CONTROLLED. THERE IS STILL THE POSSIBILITY OF ENRICHING THIS MATERIAL WITH CHEMICAL FERTILIZERS, GIVING RISE TO ORGANOMINERALS.

Both composted poultry litter and organominerals, if used rationally, have great potential to be used in agriculture, promoting soil improvements and reducing production costs.



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Environmental ethics at an intercultural garden (Scientific initiation in sustainability)



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ABSTRACT

This chapter represents the historical record of the stages of the third management of the intercultural didactic garden (medicinal and sensory) in a courtyard used by the Homeopathy Service, located in the 7th Infirmary of the Hospital Geral Santa Casa da Misericórdia in Rio de Janeiro/Brazil, whose elaboration was inter - transdisciplinary and intersectoral, voluntary and cooperative, with the purpose of cultural appreciation and intercultural and interethnic experience, as a way of promoting well-being and mental health through the preservation of intangible heritage, which is the knowledge of the people. The doctor and naturalist, who brought Homeopathic Medicine to Brazil at that time, cared for members and families of traditional ethnic communities and enslaved indigenous peoples or, when they no longer served, those left to fend for themselves. Therefore, from this stage completed in primary care, scientific initiations were carried out with the plant elements of the Benoit Jules Mure medicinal garden, ranging from in situ morphological knowledge to the pharmaceutical process using the Hahnemannian method of multiple bottles, to investigate circumscribed environmental issues. Scientific investigations were started with *Calendula officinalis*, *Euphorbia tirucalli*, *Annona muricata*. For the present stage described here, the



germination of Cowpeas (*Vigna unguiculata*) was investigated, originating from Africa and used to this day by Quilombola Traditional Knowledge, selected based on a bibliographical review. Cowpea grown in this garden in cups with an high ultra-diluted and energized (HUD) 5DH Sulphur solution, as directed for organic garden cultivation in Rio de Janeiro, has broad potential for clinical scientific research in nutrition and the environment, also for phytoremediation. This study, with the use of streamlined solutions, thus contributed to expanding the perception regarding the positive and

enriching role of cultural diversity and also biodiversity, helping both to reduce the risk of food shortages for those in urban situations in Rio de Janeiro, as well as in awareness of ethical behavior of care for everything that makes up the environment (Environmental Ethics) and Cultural Competence.

Keywords: Didactic garden management, *Euphorbia tirucalli*, *Vigna unguiculata*, HUD 5DH Sulphur, Family psychosocial care for traditional communities.

1 INTRODUCTION

For 21 years, plant oxidative stress has been investigated by our research group in the face of nature's harsh elements, which lead to the demineralization of plants and, sometimes, lead to a cancerinic with desmineralization state that triggers weakening, which, in turn, allows the entry of nematodes and other organisms, not only causing gall diseases but several others. So, as there's no solution for so many cases, novel models of investigation were introduced with high ultradiluted and succussioned – HUD - solutions (KUSTER et al., 2002; VARRICCHIO et al., 2006; 2022; GASPAR et al., 2022).

At the same time, after vegetal biotechnological research (VARRICCHIO et al., 2008), as a retributive task in bioethics, educational measures to environmental health for the community were carried out (GASPAR et al., 2017; GASPAR, 2018; GASPAR et al., 2020; GASPAR & RONCARATI, 2021; GASPAR et al., 2023; DELAUNAY DE SOUZA et al., 2023), through Environmental Health, Parasitology, Bioethics Project of the Immunoparasitology and Toxicological Analysis Laboratory of Faculty of Pharmacy of Federal University of Rio de Janeiro (SAPB/LIPAT/FF/UFRJ Project - <https://sites.google.com/view/lipat/sapb>), the team focused on actions in primary health care via health education, also to members of traditional ethnic communities and to indigenous (originary people) – in urban situations in Rio de Janeiro (MUSMANNO et al., 2019; WASIM et al., 2020, 2021).

In accordance with higher education (DCN, 2014) and the environmental aspects mentioned in the new Code of Medical Ethics (2018), over years we have taken this traditional knowledge to universities for intercultural changes in cultural didactic events held in jointly by SAPB-LIPAT/FF/UFRJ Project, by the professor of the curricular unit Traditional Knowledge and Associated Rights of the INPI postgraduate course, and by the Laboratory of Studies on the Aging Process of the Institute of Psychiatry of the University of Brazil/UFRJ (PROVE – MEPPSO/IPUB /UFRJ (VARRICCHIO & LAGE, 2020; VARRICCHIO, 2022).

Society lives under the oppression of a great discomfort (WHO, 1986; IBGE, 2020). Sensibilization environmental activities were carried out at the local level with Homeopathy professionals, based on the understanding of paragraph 9 of the Organon of the Art of Healing written



by Samuel Hahnemann (1831 In PUSTIGLIONE & CARILLO JR., 1994). Through creation of an intercultural garden which once social spread occurred through citizen actions and could bring or, even rescue, the subjective feeling of well-being for many “differents”, in society, at Homeopathy Service at 7a Ward in General Hospital Santa Casa da Misericórdia do Rio de Janeiro/Brazil (HGSCM_RJ) with it’s garden, a health space.

2 GOAL

Describe the steps taken in education for sustainability through the planning of the teaching garden, Intercultural Garden Dr. Benoit Jules Mure (“Garden from everywhere to everyone”) that raise awareness and develop environmental ethical competence.

3 METHODOLOGY

Case study (report of the historical process over the years – VENTURA, 2007).

4 RESULTS

During three years of studies, other issues related to traditional knowledge people raised and were also addressed (KOTTOW, 2011; GASPAR et al., 2017; GORINI et al., 2020). Since 2018, at the Homeopathy outpatient clinic at HGSCM-RJ, we have taken part of this previously formed traditional work group, so that they could be part of the construction of this intercultural experience based on the principles of clinical bioethics: The intercultural didactic garden (GASPAR et al., 2022). Other two gardens previously prepared by our group within other traditional communities served as inspiration for the first garden planned and organized for the courtyard of the Hospital Geral Santa Casa da Misericórdia in Rio de Janeiro, used by the Homeopathy Service of the 7th Ward opened in 2020.

However, the movement of nature itself after the return of the period of social isolation during the pandemic, determined a second one better designed garden, which emerged in the same place because the environmental awareness activities and the education for sustainability and environmental ethics had started in person and continued through online hybrid teaching at hidden curriculum (MACHADO-Duigó-TUKANO et al., 2019). Therefore, we went through the stages of Intercultural Garden I (2020) and II (2021) (GASPAR et al., 2022).

5 INTERCULTURAL GARDEN III - DR. BENOIT JULES MURE - 2023:

Environmental educator Sandra Ávila Gaspar and her team of employees were responsible for adapting the soil conditions, gracefully to Santa Casa, aiming for the necessary replanting. In parallel, work was carried out to revitalize the site (painting renovation and restoration of installations from the colonial period) so that, on April 10, 2023, the work on the garden designed with people and ethnic



communities in an urban situation since 2018, updated during this period, was reopened and received the name of honorable Dr Benoit Jules Mure, a french physician who took care of slavery people in Rio de Janeiro/Brazil (GASPAR et al., 2024).

The stages of revitalization of Intercultural Garden III show the dynamics of this type of work process, perceived by us as a continuum of cultural exchanges and adjustments to the climatic and environmental conditions that arise. The soil was cared for and enriched in accordance with the cultivation guidelines in agrohomeopathy (DUTRA, 2012 In GASPAR et al., 2022; 2024).

Objective of this article, the following species with established medicinal actions were sown and, some, replanted, between 2023 February and March (Table 1), regarded the potential for local topical application as already described in the scientific literature, but this time, also considering plant biotechnological potential to investigations on environmental health, such as mosquito and nematodes control, besides environmental restoration (natural products and HUD as fertilizers, to germination):

Table 1: Cultivation of the Intercultural Garden III - Dr. Benoit Jules Mure - in February-March 2023. Plant biotechnological potentials of the selected species.

Popular Name	Scientific Name	Source/ Etnobothanic Traditional Use	Medicinal Use	Topic Use	Environmental Potential
Açucena	<i>Hippeastrum hybridum</i>	South America /Indígenous Amazon	NF	Skin lesions Boils	NF
Alecrim	<i>Rosmarinum officinalis</i>	Mediterranean, Central Europe/Italian	Digestive	Antitinflamator y	NF
Alfavaca	<i>Ocimum basilicum*</i>	Africa,Asia/Indigenous Brazil N e NE	Digestive	NF	NF
Alfazema	<i>Lavandula latifolia</i>	Mediterran/ Temperate regions	Antiespasmodic	NF	NE
Aroeira	<i>Schinus terebenthifolius</i>	Native - SP - Ribeira Valley/ Indigenous	Antimicrobial	Healing	NF
Aveloz	<i>Euphorbia tirucalli</i>	Africa and the Middle East	Antineoplastic	Anti-warts	NF
Babosa	<i>Aloe vera</i>	Africa and the Middle East	Antioxidant	Strengthening hair strands	NF
Beijo	<i>Impatiens walleriana</i>	Africa/Etnias	Diuretic, Catartic	NF	NF
Boldo de quintal	<i>Plectranthus barbatus</i>	Africa Asia	Digestive	NF	NF
Boldo do Chile	<i>Peumus boldus</i>	Africa, Asia, Europe, Andes, Brazil/Indígenous	Digestive	Antiparasitic	NF
Capim limão	<i>Cymbopongo citratus</i>	India	Muscle relaxant	Antifungal	Repelente insetos
Confrei	<i>Symphytum officinalis</i>	Europe, Mediterranean	NF	Antiinflammatory	NF
Cúrcuma	<i>Curcuma longa</i>	Asia	Imunomodulator, Antiviral	Antiinflammatory	NF
Elevante	<i>Mentha viridis</i>	Europe	Antiespasmodic	NF	Antihelmíntica
Erva-cidreira	<i>Melissa officinalis</i>	Mediterranean, Middle East/Arabian	Digestive, sedatif	NF	NF
Erva doce	<i>Pimpinella anisum/ Foeniculum vulgare</i>	Mediterranean, Asia, Africa, Europe	Anti-espasmodic	Muscle relaxant	NF



Gengibre	<i>Zyngiber officinalis</i>	India, China	Imunomodulator, Adaptogen	NF	NF
Graviola	<i>Annona muricata</i>	Central America, Peruan Valley, Brazil	Pain. Constipation. Antioxidant	NF	Seeds: Anthelmintic Insecticide
Guaco	<i>Mikania glomerata</i>	Native	Respiratory system	NF	Venomous animal bites
Guiné	<i>Petiveria tetrandra</i> *	Americas/Indígenous	NF	Healing Antifungal Insect bites Antiofidic	NF
Hortelã pimenta	<i>Mentha piperita</i>	NF	Digestive Analgesic Vermifugous	Oral Antiseptic Insect bites	NF
Jaboticaba	<i>Plinia cauliflora</i>	South America	Diarrheia	Oral Antiseptic	NF
Louro Grego	<i>Laurus nobilis</i>	Mediterranean, Europe	Digestive	NF	NF
Mangueira	<i>Mangifera indica</i>	India	Anemia Cough	Oral Antiseptic Varicous ulcers	NF
Manjeriçao	<i>Ocimum basilicum</i>	Asia, Africa	Cough Headache	Antiseptic	Antifungal
Marcelão	<i>Achyrocline satureioides</i> *	LatinAmerica/Indigenous	Abdominal pain	Hair lightener	NF
Menta	<i>Mentha spp</i>	Asia	Antiviral Expectorant	Antifungal Analgesic	Antifungal
Mirra	<i>Commiphora myrrha</i>	Africa, Israel, Middle East, India, Thailand	Antiinflammatory	Oral Antiseptic	NF
Neem	<i>Azadirachta indica</i>	Asia	Imunomodulator, Antiviral	Eczema, Psoriasis	Control pests Anthelmintic Fertilizer
Noni	<i>Morinda citrifolia</i>	Asia	Urinary infections	Boils	Pediculosis Tickicide
Onze horas	<i>Portulaca grandiflora</i>	South American	Anemia	Burns	NF
Ora pro nobis	<i>Pereskia aculeata</i>	Americas	Anemia	Healing	Tripanocide
Orégano	<i>Origannum vulgare</i>	Mediterranean, Europe, Asia	Adaptogen Antimicoplasm, Anti <i>Listeria monocytogenes</i>	Antifungal	NF
Pimenta biquinho	<i>Capsicum chinense</i>	Americas	Analgesic	NF	NF
Pinha (Fruta de Conde)	<i>Annona squamosa</i> *	Americas	Antiinflammatory Muscle relaxant	Muscle relaxant	NF
Pitanga	<i>Eugenia uniflora</i>	Native Atlantic Forest /Tupi Indigenous	Fever, Headache	NF	NF
Poejo	<i>Mentha rulecium</i>	Asia	Respiratorysystem	Antiseptic	NF
Romã	<i>Punica granatum</i>	Oriental Mediterranean Middle East,	Imunomodulator, Antineoplastic, Memory Stimuly	Healing Oral Antiseptic	NF
Saião	<i>Kalanchoe pinnata</i>	SouthAfrica, <u>Madagascar</u> , Asia	Antiinflammatory Respiratorysystem	Skin ulcers	NF
Salsa	<i>Petroselinum crispum</i>	Central Mediterranean	Digestive; Anti-hipertensive,	Antiinflammatory	NF
Terramicina	<i>Alternanthera brasiliana</i>	South, Latin America	Respiratorysystem	Antiseptic	NF
Tomilho limão	<i>Thymus citriodora</i>	Europe, North Africa, Asia	Respiratorysystem	Acne	NF



Urucum	<i>Bixa orellana</i>	Tropical America	Antioxidant Vermifugous Antitinflamatory	Sun Protector, Insect Bites	NF
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Source: MURE* (1849); BRANCH et al. (1983) & BORGEA (1991) In LAMEIRA (2011); BR/MS/SUS (2006), Phytotherapeutic Formulary of the Brazilian Pharmacopoeia (FFFB, 2021). EMBRAPA. GONÇALVES et al. (2021). Legend: NF – Not Found.

So, Intercultural Garden III (also referred to as Ethnic Garden, Hope Garden, Health Garden) was reopened on 10.04.2023 in honor to disciple of Samuel Hahnemann and naturalist, Benoit Jules Mure, the first to produce books on homeopathic medicines obtained from Brazilian biodiversity (MURE, 1849). So, scientific initiation of undergraduated students had began. Sensorial intercultural didactic garden offers bean, fruits (mango, conde fruit, jabuticaba, lemon and soursop), flowers and bouquet, besides well being (GASPAR et al., 2024).

The crude latex, the total aqueous extract from a specimen of *Euphorbia tirucalli* (Fire stick) from garden of Product Natural Research Institute *situ* at Ilha do Fundão Campus of Federal University of Rio de Janeiro (IPPN/UFRJ) and HUD 30CH from the total aqueous extract from a specimen *situ* at UFRRJ/Seropedica Campus were not cytotoxic against the MCF7 lineage of human breast adenocarcinoma and nor to Melan A lineage of normal melanocytes. Worthy of note, HUD 5CH, 15CH and 30CH obtained from crushing the latex of the IPPN/UFRJ specimen, previously tested in the same lineages, were cytotoxic and still capable of selective action only in tumor lineage, including a significant ($p < 0,05$) modification in glycolytic enzymes pathway metabolism detected (AQUINO et al., 2008).

There were no action of these pharmacotechnical preparations on the apoptosis pathways. Very important was lack of activity in protein expression at Bcl-2 pathway because it excluded the anti-apoptotic action of the three extracts tested. The Bcl-2 family of proteins plays a critical role in regulating apoptosis in both physiological and pathological conditions. Some of these proteins, such as Bcl-2 and bcl-XL, bcl-w, mcl-1 and A1, are anti-apoptotic, while others, such as bax, bad and bid are pro-apoptotic (THOMPSON, 1999). So, these extracts didn't were able to induce cell proliferation. Bax pathway, pro-apoptotic through the mitochondrial route, was negative for the three pharmacotechnical preparations tested (VARRICCHIO et al., 2019).

While the CD95 pathway was negative for raw latex in a total of three experiments. However, cell membrane labeling was positive in the first assay analyzed by confocal microscopy and subsequently negative for the other two. The detection of chemical components and their variable concentration through HPLC/UV analysis could then explain such variation in plant chemical production as a secondary effect to seasonality (VARRICCHIO et al., 2019), thus justifying the following micropropagation step. It's well known that quality control of a herbal medicine is not found in the laboratory like synthetic medicine, but in its plant chemical production obtained through the chosen and used crop (VARRICCHIO et al., 2019a).



Returning to the writings of Hippocrates (460-370 BC) who already stated: “Let food be your medicine and the medicine is its food”, scientific initiations were initiated with *Euphorbia tirucalli* – stick of fire (MUSMANNO et al., 2019) and went on with *Calendula officinallis* (MARTINS et al., 2022, a), *Annona muricata* - soursop/graviola (GASPAR et al., 2024) besides other aspects related to fruits and nutrology (OLIVEIRA et al., 2019; GONÇALVES et al., 2021; BELLIZZI et al., 2022; WENDLING DA SILVA et al., 2023; CRUZ FILHO et al., 2023; HANSEL – MARTINS et al., 2023; 2024).

Soursop has medicinal characteristics in several of its compounds, marked its notorious anti-inflammatory and antitumoral action. Numerous studies aim to isolate the compounds present in the fruits, peels, seeds and roots of soursop with the purpose of identifying new molecules with antitumor action. To date, there are 212 bioactive compounds, such as acetogenins and alkaloids that have anticancer, anthelmintic and insecticidal action, however, in high doses it has neurotoxic action. Several studies involving soursop compounds, such as acetogenins, alkaloids and phenols, have revealed chemopreventive and chemotherapeutic reactions against cancer cells. According to the historical context of use of this fruit, there is potential future participation in the prevention and treatment of cancer (DAL'COL FROIS & LEIRIA, 2019).

Not listed as HUD solution in Brazilian homeopathic pharmacopeia (FHB, 2022), nevertheless, this review suggested the broad potential for studying this fruit cultivated in the intercultural didactic garden III, for guidance for monographs and course completion works, both: in its anti-inflammatory potential for local application, or in its environmental, antiparasitic and insecticidal, potential (GASPAR et al., 2024).

As plan research, we intend to evaluate biological effects in the development of this fruit besides black bean and Cowpea bean, cultivated in pots at this garden when under some HUD solutions prepared from minerals as official guides (DUTRA, 2012), each mineral per time evaluated (BELLIZZI et al., 2022). What do we know about Cowpea bean?

Cowpea (*Vigna unguiculata* (L.) Walp.) has African origin, arriving in Brazil through colonizers in the state of Bahia in the middle of the 16th century (FREIRE et al., 2018). It has great relevance from a socioeconomic point of view, with its culture being quite common in the Northeast and North regions of the country (SOUZA, 2018), being a component of the population's usual diet, especially in rural areas, where its culture aims to constitute the income and guarantee own consumption of producing families (FREIRE FILHO et al., 2005; FROTA et al., 2008).

It also stands out for its great nutritional value, high amount of proteins, as well as its functional role due to its dietary fibers, which help regulate the lipid and glycemic profile. It also presents complex carbohydrates and phenolic compounds, producing high antioxidant activity (SILVA et al., 2009). The phenolic compounds (phenolic acids and tannins) present in it are factors that limit its consumption by



reducing the solubility of some minerals by forming some complexes, but these same compounds are of great benefit, as they act as antioxidants, preventing cardiovascular diseases and cancer of such prevalent diseases, whose incidence has been rising (OLIVEIRA & MAIA, 2022).

Cowpea (*Vigna unguiculata* (L.) Walp.) is a bean that stands out for its great nutritional value, in addition to having phenolic compounds that act as antioxidants, and can contribute to the prevention of various diseases, including Cancer. Oncological disease and its treatment cause changes that affect the patient's intake and eating habits. According to the WHO, it is estimated that new cases of Cancer will increase by up to 70% in the coming decades (SOUSA DA SILVA et al., 2022).

Beans contain a wide range of bioactive compounds such as flavonoids, anthocyanins, proanthocyanidins and isoflavones, as well as some phenolic acids. The antioxidant mechanism of phenolic compounds can be summarized as a transfer based on hydrogen atoms or a single electron transfer through protons. The ability of polyphenols to reduce the risk of developing several chronic non-communicable diseases linked to oxidative stress, including cancer, can be explained both by the presence of reducing polyphenols and their metabolites in plasma (SOUSA DA SILVA et al., 2022).

However, it is a bean that is difficult to germinate and survives little, often acquiring diseases, the most worrying being the Severe Mosaic Disease virus, which kills the crop, so, a proposal in plant biotechnology had been established by our research group (VARRICCHIO, KUSTER, LAGE & BRIOSO, 2007 In GASPAR et al., 2017). Nevertheless, there are other interesting biotechnological research to application of *Cowpea mosaic virus* (CPMV).

Cowpea mosaic virus (or CPMV), is a comovirus that affects the flowers of the cowpea plant, is harmless when inserted into the body of a mammal - and by extension, into that of a human. However, the pathogen evokes a potent immune response, stronger than other viruses of plant origin. At a certain point, the immune system realizes that the cowpea virus is inside a tumor and begins to fight the cancer cells (DUVAL et al., 2020).

Injected directly into cancer cells, it works as a danger signal so that the body, believing it is fighting infected cells, acts against the carcinoma. CPMV has only been tested in dogs. The viral treatment was applied to 6 cases of mouth cancer, a condition that can lead to the death of the animal and whose chances of recurrence are high - 85% of cases. Combined with radiotherapy, the team injected four doses of 200 micrograms of a modified version of the virus (three times more concentrated than a flu vaccine) into their canine patients over the course of two weeks. None of the six presented the tumor again (DUVAL et al., 2020).

In 2015, a study led by immunologist Steve Fiering had observed good results from the same comovirus in models of melanoma, breast, ovarian and colon cancer in mice. The pathogen works as a warning against cancer cells and is capable of making the immune system also fight metastasis. For



now, only three oncological viral treatments have the approval of health departments around the world - two against melanoma, and one that acts on cases of neck and head cancer (DUVAL et al., 2020).

This immunotherapy has been shown to be capable of launching a coordinated immune response against any metastatic and future tumors. Furthermore, it was positive in laboratory rats and dogs with different types of cancer, from melanoma (skin) to sarcoma (connective tissues). Thus, combining HFRT with immunoactive agents such as CPMV could enhance the overall therapeutic effect. In particular, we hypothesize the combination will lead to a larger cytotoxic immune response, ultimately improving efficacy *via* up-regulation of apoptotic pathways and immune cell activation, both cytotoxic natural killer cells and cytotoxic T cells (DUVAL et al., 2020).

It was not understood why the cowpea virus was a potential weapon against cancer and other pathogens affecting legumes were not. Then, its action was compared to that of the cowpea severe mosaic virus and the tobacco ringspot virus. The three were injected into mice in three doses given seven days apart. As expected, those who received the cowpea virus had a higher survival rate and the tumor stopped growing four days after the second dose. Immune cells were extracted from mice for analysis. The cowpea virus triggers a more intense inflammatory response, which evokes the immune system's response to the tumors (BEISS et al., 2022).

5.1 ATTENDED CULTIVE OF *VIGNA UNGUICULATA* ELICITED WITH *E.TIRUCALLI* EXTRACT AND HUD 5CH SULPHUR AS ELICITORS: BIOTECHNOLOGICAL POTENTIAL ON BIOREMEDIATION?

Euphorbia tirucalli L. is a succulent native to the African continent but it's widespread for all other continents, used since 8000 b.C. Despite toxic latex, it's used by original peoples as analgesic and tumoral reductor, and it's antioxidant, antineoplastic and immunomodulatory activities were already verified (BETANCUR-GALVIS et al., 2002; 2003). Varricchio (2005) verified citotoxicity to B16F10 Melanome murine MDR (resistent to multiple drugs) to both: fresh raw latex and ethanolic extract, but it's toxicity is related to strong oxidative stress caused by latex, which is able to induct cellular membrane and vegetal wall lesions (VARRICCHIO et al., 2019). In turn, *Vigna unguiculata* (Caupi Bean) is the bean with the highest concentration of iron and proteins still used by quilombolas and northeastern people in Brazil (GASPAR et al., 2017; MUSMANNO et al., 2019).

The assay aimed to evaluate the biological effect of the total ethanolic extract 30% *E. tirucalli* and HUD 5DH Sulfur on the germination of cowpea (*V. unguiculata*). As methodology, assisted cultives of *V. unguiculata* in 200 ml of mineral water were carried out. Three seeds per pot, in three pots, in triplicate per row, with one more pot (N = 30). It was administered separately: 1 drop of total extract of *E. tirucalli*, HUD5DH, and respective controls prepared by the Hahnemannian method of multiple flasks. The action/time curve was verified on the first, third and seventh day of cultivate: pH,



free chlorine, total alkalinity, color, turbidity and presence of cyanuric acid were measured by the colorimetric method. Temperature measured by an Infrared thermometer B-Max. Morphology and germination were observed by ANOVA statistical programme.

Results showed that it had an inhibition on the germination of *V. unguiculata* ($p < 0,05$), curiously associated with engorgement of the seeds, an evidence of metabolic route deviation. Hormetic ponderal phytoextracts with also HUD 5DH Sulfur test solutions, induced cyanuric acid at water where seeds were suggestive of protein breakdown, possibly due to erosion of the bean skin. Cyanuric acid is commonly used as pesticide, mosquito insecticide and mainly as a repellent of invasors plant species and their microbiological pathogens, through allelopathy mediated by radicle secretions emissions by roots (BEZERRA, 2015; HANSEL – MARTINS et al., 2023).

Cyanuric acid is also able to compete with some chemicals. It is a precursor to polyesters, polyurethanes, bleaches, disinfectants and herbicides. This last function, in turn, can be used in plant biotechnology in the bioremediation of soils contaminated with herbicides, used to combat invasive weeds. So, this experiment will be repeated separately to further study the mechanisms. Under this vision, the use of herbal extracts and diluted solutions (infinitesimals) seeks to become an alternative for the cultivation of small farmers, reducing their exposure to insecticides and pesticides currently used in industry, such as organochlorines, which are considered endocrine disruptors (HANSEL – MARTINS et al., 2023) that can cause serious health problems, including mental disorders (HANSEL – MARTINS et al., 2024).

To conclude, a biotechnological potential for bioremediation of soils contaminated with herbicides, through nitrogen oxidative stress, was evidenced. Assays on this route will be carried out in phytopathology laboratory and cyanuric acid will be the pathogenetic marker of biological activity for HUD solution tested (GASPAR et al., 2024), as already evidenced at this present assay. Furthermore, herbal extracts and high ultradiluted succussioned solutions may be an alternative to the cultivate of small farmers, reducing exposure to pesticides.

Novel assays with raw extracts obtained from Cowpea bean of different assisted cultivate and micropropagation will go on being evaluated, about their mechanisms of action at MCF7 and Melan A lineages.

6 DISCUSSION

There are some definitions about Ethnobotany. Ethnobotany addresses the way people incorporate plants into their cultural practices and traditions (BALICK & COX, 1997). Ethnobotany studies the interrelationships between humans and plants in dynamic systems (ALCORN, 1995).

“Ethnobotanical approaches
can provide
important answers



so much for trouble
biological conservation
as for targeted questions
for local development”.
(HANAZAKI, 2006, a)
“

Recapitulating the path of structuring work in ethnobotany: Since 2003 we have worked in contact with people, listening to popular, regional and traditional reports, as a function of bioethics feedback from academic research with plant species of cosmopolitan dissemination, since Rio de Janeiro/Brazil is home to representatives of several peoples and ethnicities (VARRICCHIO et al., 2008; OLIVEIRA et al., 2019).

Due to this prolonged contact with the country's biodiversity and, especially, with the existing cultural diversity, it was realized how much both are disrespected or not valued, so, two lines of extension work were started. Research for Environmental Health, seeking remove local problems brought by the community and with their pro-active participation; in parallel the second line, with joint tasks to raise environmental awareness, through the teaching of environmental ethics through the master's feedback product prepared in partnership with an interdisciplinary group of teachers and community members, Environmental Awareness Primer (VARRICCHIO et al., 2003 In VARRICCHIO & PYRRHO, 2017; <https://sites.google.com/view/lipat/sapb>).

Since 2008, scientific initiations have been carried out, also for members of traditional and ethnic groups in urban situations in Rio de Janeiro, in cooperative and collaborative partnerships (MACHADO/Duigó-TUKANO et al., 2019; 2023). After years of listening to the needs highlighted by leaders and also their suggestions, protocols and strategies were developed in accordance with their worldviews and eco-perceptions (VARRICCHIO & LAGE, 2020), prioritizing this knowledge as an end in itself (MACHADO/Duigó-TUKANO et al., 2019; VACITE et al. , 2023; MACHADO/Duigó-TUKANO et al., 2023; CLER & VARRICCHIO, 2023; FREIRE SOUZA SILVA et al., 2023; KATHAR et al., 2023; CRUZ FILHO et al., 2023; DELAUNAY DE SOUZA et al., 2023, a; HANSEL MARTINS et al., 2023; 2024).

By this way, young, mature and elderly representatives of peoples and ethnicities who attend the school clinic: Pakistanis, Roma, Japanese, Hebrews, originary indigenous of the Tukano people, Satere-Mawe people, Caete people, Guarani people, together with physicians, all team cared by Jungian art therapists Cristiane Gerolis and Fabio Tavares, were able to show that they are beyond those spaces previously occupied during the period of colonization - exile and slavery (TAVARES & GEROLIS, 2019).

Culmination of this journey by the cultivation carried out by everyone together was recorded in videos (Homeopathy Service, JTL, 2021 - Part 1, Part 2, Part 3). So, there was an understanding of the



concrete return of the multisensory and multidimensional apprehension of their eco-perceptions regarding environmental ethics (MORAES et al., 2021; BELLIZZI et al., 2022a).

In this feedback perspective from intercultural didactic clinical bioethics (KOTTOW, 2011), the team of art therapists voluntarily developed a project to raise awareness for inner reflection for the entire multi, inter and transdisciplinary team of the Benoit Mure Center at Homeopathy Service, employing common elements of plant species, working on their uses and the individual perspective on their subjective, singular meaning and worldview, such as corn, urucum, beans (VARRICCHIO & LAGE, 2020). The agronomic and cultural procedures and products generated in intersectoral partnership have already been communicated (GASPAR et al 2022).

7 INTERCULTURAL GARDEN III (2023):

From 2018 onwards, teaching outpatient clinic supported by Benoit Mure for Care, Assistance, Research and Studies Nucleus studied HUD from *Punica granatum* (Pomegranate), HUD from *Elaies guinensis* (Dendê) HUD from *Bixa orellana* (Urucum), among others. All of these homeopathic drugs exist in the Brazilian Homeopathic Pharmacopoeia, even authorized by MS/ANVISA. Indeed, they were used also as functional foods (BELLIZZI et al., 2022).

As a territorialized and intersectoralized service, it became a teaching outpatient clinic also aimed at people and ethnic communities in urban situations in Rio de Janeiro/Brazil, who reside around it (VARRICCHIO & LAGE, 2020; MACHADO/Duigó-TUKANO et al., 2023; HANSEL MARTINS et al., 2024), and whose proactive participation of its leaders stood out (VACITE et al., 2023; MACHADO/Duigó-TUKANO et al., 2023; 2024).

Creation of a sensory garden with pharmacognostic utilities for plant studies and investigations with HUD drawing comparisons of effect in basic research, as already carried out (VARRICCHIO et al., 2006): botanical certification of plant species for research in plant cultivates, inclusion and integration of people, welcoming and respecting the knowledge and their self-direction, process of choosing and sowing selected species, as *Calendula officinalis* (GASPAR et al., 2022; MARTINS et al., 2022, a ; HANSEL-MARTINS et al., 2023; 2024).

It is clear that today this intercultural garden does not correspond to a medicinal garden for the preparation of medicinal extracts (neither oral via, nor local use), since if it were, it would meet the requirements for a “living pharmacy” requiring the participation of a pharmaceutical professional for monitoring the chemical marking of plant production because of edaphoclimatic fluctuations. However, the cultivation organic standards for medicinal plants in the State of Rio de Janeiro were respected (DUTRA, 2012) as they were intended for research with HUD obtained from the pharmacotechnical preparation of these present specimens, for investigations of local environmental



solutions, evaluated both by biological markers and pathogenetical markers (HANSEL-MARTINS et al., 2022, a; 2024).

Socio-cultural and environmental activities were developed from a transcultural perspective to raise awareness among these garden users (VARRICCHIO et al., 2021). Previously, intersectionality in health was investigated to develop this work (MELLO & GONÇALVES, 2010): social class, color, gender, ethnicity (GONÇALVES et al., 2019; 2020; 2021; MENDES et al., 2021; 2022; VARRICCHIO et al., 2023). While regarding social determinants and their impacts on health: invisibility, inequity, racism, they were discussed by the representatives of ethnicities, peoples and collectives themselves (VACITE, 2016; 2018; ZAFAR et al., 2019; MUSMANNO et al., 2021; VACITE et al., 2023; DE LUNA et al., 2021; CLER & VARRICCHIO, 2023).

The purpose of serving the user population through primary health care was fulfilled, with a view inserted to the principles of homeopathic philosophy, bioethics and principlialist ethics (“*primo mal nun facere*” – HIPÓCRATES In BEAUCHAMP & CHILDRESS, 2002). This facilitated matrix support and citizen approximation and integration during the joint planning of the garden, represented a unique opportunity for everyone involved (LEAL et al., 2022). Aspects about intercultural education such as awareness of Environmental Ethics were communicated (FREIRE SOUZA SILVA et al., 2023; CRUZ FILHO et al., 2023).

Ethno-knowledge was valued through awareness of environmental ethics through ethno-centered education, without compromising professional medical ethics performed based upon bioethical clinic (BELLIZZI et al., 2022, b; 2023; HANSEL – MARTINS et al., 2023; 2024), besides academic and didactic-cultural communications: pomegranate, açaí, beans, among adaptogens (PANOSSIAN et al., 1999) and other functional foods – papaya, grape, coconut (VARRICCHIO et al., 2019; MUSMANNO et al., 2019; WASIM et al., 2020; ALMEIDA et al., 2022; BELLIZZI et al., 2022).

Health education to balance nutritional status of the host aimed metabolic stimulation, to evoke immunomodulation responses (BELLIZZI et al., 2023). After registering the hybrid education process via hidden curriculum for the Leagues and Medical Residency students at UNIFASE-FMP (MUSMANNO et al., 2023), the intercultural garden III Benoit Mure was then presented under several different aspects, recently thought as an open-air library (HANSEL-MARTINS et al., 2024; GASPAR et al., 2024).

The cumulative effects of different carcinogens or carcinogens are responsible for the initiation, promotion, progression and inhibition of the tumor (INCA, 2018). According to the World Health Organization (WHO), the unrestrained growth of malignant cells results from the interaction between a person's genetic factors and three categories of external agents, which include physical carcinogens such as ultraviolet and ionizing radiation; chemical carcinogens, such as the components of tobacco smoke, aflatoxin (food contaminant, e.g. peanuts) and arsenic (drinking water contaminant); and still



biological carcinogens, such as infections with certain viruses and bacteria and chronic infestations with parasites (WHO, 2013). According to the WHO, it is estimated that in addition to the high prevalence, the incidence of new cancer diagnoses will increase by up to 70% in the coming decades. 35% of cancers are related to modifiable risk factors to which the population is exposed, representing a problem with a high impact on public health (RIVERA-MEZA et al., 2018).

Childhood cancer accounts for 2 to 3% of all malignant neoplasms in most populations. Both in Brazil and in developed countries, cancer already represents the leading cause of death from disease among children and adolescents aged 1 to 19, in all regions of the country (CURVO et al., 2013). Leukemia is the most common type in this age group, representing between 25% and 35% of all types, with Acute Lymphoid Leukemia (ALL) being the most common in the world. Tumors of the Central Nervous System (CNS) represent around 8% to 15% of pediatric neoplasms worldwide and solid tumors are the most common (VIDOTTO et al., 2017).

According to current evidence, between 30% and 50% of cancer deaths could be prevented by modifying or avoiding key risk factors, including avoiding tobacco products, reducing alcohol consumption, maintaining a healthy body weight, exercising physical exercises regularly and address risk factors related to the development of cancer (WHO, 2023).

Noteworthy, the odor and flavor of lemon is provided by the terpenoid limonene, which enhances anti-inflammatory and immunomodulatory actions (BOIK, 2001). Ascorbic acid is found in citrus foods such as lemon, capable of producing proteins such as collagen and has antioxidant properties (SANTOS et al, 2021), anti-inflammatory effects and increases the action of the immune system, therefore, its consumption can be favorable in the prevention and treatment of cancer (ABIRI & VAFA, 2021).

Another phenolic compound found in several natural sources, such as plants, fruits and vegetables, is gallic acid, with antioxidant, anti-inflammatory and antitumor activity and cardiovascular protection (SALAS et al., 2013). Gallic acid present in *E. tirucalli* from IPPN/UFRJ (VARRICCHIO et al., 2019) may explain the death of tumor cells by apoptosis (TANG & CHEUNG, 2019 In SOUSA DA SILVA et al., 2022).

Beans, in addition to being consumed throughout the country, contain many nutrients, including antioxidant compounds, with Caupi beans being very rich in phenolic compounds, essential for health (FREITAS et al. 2019). It is a food of great socioeconomic importance and one of the most important food sources for society in many regions of the world (BEZERRA, 2015).

Beans involve an enormous abundance of bioactive compounds such as flavonoids, proanthocyanidins, anthocyanins, isoflavones, and also some phenolic acids (CHOUNG et al., 2003). There are approximately five thousand phenols, including flavonoids, phenolic acids, simple phenolics, coumarins, condensed tannins, lignins and tocopherols. They are responsible for the color, astringency,



aroma and oxidative stability of foods (ANGELO; JORGE, 2007; NACZK; SHAHID, 2004). Bean phenolic compounds are located in the seed coat, confer antioxidant properties, reduce the amount of pro-oxidant agents and the action of free radicals, preventing oxidative modifications (BOATENG et al., 2008; MARATHE et al., 2011) . In cowpea, 5 classes of anthocyanins were observed: delphinidin 3-glucoside, cyanidin 3-glucoside, petunidin 3-glucoside, peonidin 3-glucoside and malvidin 3-glucoside. Anthocyanins protect leaves from ultraviolet radiation, in certain types of plants they help fight pathogens and improve and regulate photosynthesis (BEZERRA, 2015).

The anticancer effects of grain legumes may be associated with antioxidant activities. Cowpea seeds are a good source of antioxidant compounds considered anticancer (THUMBRAIN et al., 2020). Cowpea is rich in phenolic compounds with potential protective properties on the risk of developing cancer, such as antiproliferative action and cell death induction properties (TEIXEIRA-GUEDES et al., 2019).

Reactive oxygen species are involved in several stages of tumor transformation and progression, such as self-sufficiency in growth signals, insensitivity to anti-proliferative signals, apoptosis, angiogenesis, metastasis, metabolism and inflammation (SILVA & JASIULIONIS, 2014). Protect cells from damage caused by free radicals; these capture, stabilize or deactivate reactive oxygen species (ROS) before reaching the cells (BECERRIL-SÁNCHE et al, 2021) due to the presence of reducing polyphenols and their metabolites in plasma, as well as their effect on the absorption of pro-oxidative components of food, such as iron (CIPOLLETTI et al., 2018). So, foods rich in iron and vitamins are guided and investigated by us (ALMEIDA et al., 2022).

Gutiérrez-Urbe et al (2011 In SOUSA DA SILVA et al., 2022) evaluated the inhibitory effects of phenolic extracts using human breast cancer cells (hormone-dependent MCF-7), showing an inhibition of the growth of hormone-dependent human breast cancer cells of hormones (MCF-7) by phenolic compounds from cowpea. Siddhuraju and Becker (2007) reported a good scavenging activity of cowpea seed extracts on the hydroxyl radical, in addition to greater scavenging activity of these radicals. Therefore, the antioxidant activity of phenolic compounds is attributed to their ability to eliminate free radicals by donating hydrogen atoms, electrons or metal cations; This ability to interact with free radicals is due to its structure, particularly due to the number and positions of the hydroxyl groups and the nature of the substitutions in the aromatic rings (BECERRIL-SÁNCHE et al, 2021).

Garden and functional food nutritional experiences of Benoit Mure Nucleus at Homeopathy Service were submitted to the rigor of health service evaluation by managers, as recommended by Donabedian (PROVE & LIPAT & LAFFH, 2021; MUSMANNO et al., 2022, a; LIPAT/SAPB Project & Institutions, 2022-1) and, once again, was submitted to peer view (WASIM et al., 2022; VARRICCHIO et al., 2022; WENDLING DA SILVA et al., 2023; BELLIZZI et al., 2022, a; 2023). At the current context of great climate instability and uncertainty, consistent rational methodological



investigations are encouraged to be communicated, for evaluation and reflection by scientific community (GASPAR et al., 2023a; MACHADO, C. V. da S. M./Duigó-TUKANO et al., 2023a).

Furthermore, as a result of pandemic SARS-Cov-2 – Covid19 professional contribution, a *strictu sensu* qualitative ethnographic research has been officially developed regarding the repercussions of social isolation on these ethnic and indigenous leaders in an urban situation in Rio de Janeiro/Brazil by the Psychosocial Care Program of the Institute of Psychiatry of the University of Rio de Janeiro. Brazil – MEPPSO - IPUB/UFRJ. This entire route of bioethical returns and professional experience to families from traditional, ethnic and indigenous originary people, was made through partnership among a school multidisciplinary league LAFFH/UNIFASE-FMP, by discipline at INPI, by the SAPB-LIPAT/UFRJ Project, Homeopathy Service and by the PROVE-IPUB/UFRJ Laboratory. All procedures were included in the documentation submitted to Brazil Platform approved by the CEP/CONEP System/National Health Council (BR/MS/CNS), in Brasília/DF (VARRICCHIO, 2022) and were already communicated (VARRICCHIO, 2022a MACHADO/Duigó-TUKANO et al., 2024; LOPES et al., 2024).

Authors are aware that what they have published is not directly related to Homeopathic Medicine in its prescriptive way. But actions were based upon the philosophical principles of the Organon of the Art of Healing by Samuel Hahnemann (1831, In PUSTIGLIONE & CARILLO JR., 1994), when he referred to professional intelligence contained in hygienic measures removing noxia. Here, it was carried out through health education, in a dialogue with what had been already recommended by the Ottawa Charter, about health promotion at society (WHO, 1986).

Education for sustainability promoted biodiversity, just an ethnocentric education promoted cultural diversity. With this memory recorded, we moved on to discuss other stages of construction in environmental ethics, based on paragraph 9 of Samuel Hahnemann, cooperating that individuals and their families to reach the highest goals of their existence even at certain (some critical) periods of their existence (VARRICCHIO et al., 2023). Vitalism is so generous that always function, always work at Cartesian model, the beginning of research, going into complexity models (GASPAR et al., 2023).

That's our understanding upon Law of Similarity, which can be presented to students (also undergraduates), based on experiments during the short contacts possible, as Scientific Weeks, Congress, etc. Mainly: through correct and pertinent controls used, it was allowed relevant discussion distinguishing the HUD effect from those one of solvent controls effects, nanoeffects, hormetic effects and even the effects of quantum fields generated by luminous fluences, such as the incidence of sunlight during assisted cultivates. So, it also emphasized the distinction from the effects triggered by the classical Hahnemannian method of HUD. Plant cell wall and cellular membrane receptors are able to answer to infinitesimales also named HUD solutions (VARRICCHIO et al., 2006; GASPAR et al., 2023, a).



Very relevant, the controls bring the strength of the evidence of statistical results to the fore, observing the effect of HUD on a given biological system under investigation, chosen rationally and carefully, contributing to effective learning and respect for everything that science can still do not explain, but it can move towards this understanding. The methodological commitment positively impacts people's perception, mainly due to the fact that it was adapted to the current model. Most professionals in general still think that this is not possible in any way, because they didn't have access to these type of research during undergraduation period.

University centers are not in the field of doxia. Scientific thinking evolves through complexity, innovation and perplexity. Institutions that host research, support ethical and rational methodological approach to novel research themes. The rational attitude is to investigate carefully (GASPAR et al., 2024).

Therefore, it is urgently to be emphasized that there is still much to be studied in epistemology, the philosophy of science, and in terms of the rigor of the scientific methodology to be adopted, to visit the various consistent studies that exist, in Brazil and abroad, officially published. That's why this group only worked HUD on basic research, this way aiming to environmental rebalancing. Indeed, defining conceptual environmental criteria were respected (GASPAR et al., 2024). It is recommended to study Marcos Zulian Teixeira - PhD (2018; 2020), especially that which deals with epistemology (TEIXEIRA, 2023).

The key points of the chapter stand out:

- This chapter addressed the evolutionary history of the planning and implementation of the intercultural and interethnic garden as a health space, but also as the correct beginning of basic research on plant extracts and ultra-diluted solutions succussioned by the Hahnemannian method of multiple flasks. Scientific initiations were carried out and are in progress.
- Addressed the characterization, general aspects and socioeconomic importance of an intercultural garden, focusing on Cowpea bean (*Vigna unguiculata* (L.) Walp.) as well as consumption.
- The relevance and nutritional properties of Cowpeas were addressed, as well as the compounds present.
- Some mechanisms of action of phenolic compounds were explained, such as antioxidant effects promoting the reduction of the risk of developing cancer, suggesting it as a preventive functional food to be methodological investigated.



8 CONCLUSION

This work, which began in 2003 with the Environmental Awareness Guide, matured and was completed with the implementation of the third intercultural garden linked to the Homeopathy Service at the 7th Ward in the courtyard of the Hospital Geral Santa Casa da Misericórdia in Rio de Janeiro/Brazil. It was decided to record the care, teaching and research stages of this primary attention care project for families from originary people (indigenous), ethnic and traditional communities in this territory.

The possible beneficial health effects that the bioactive compounds present in Cowpeas can promote in reducing the risk of developing cancer, a serious public health problem. The choice of this theme is mainly due to the high consumption of this food, mainly in the North and Northeast regions, being a frequent component in the diet of almost entire Brazilian population.

As researchers, we unite in favor of rational methodological investigations and to reaffirm principles and values that build well being in society and to environment. Planet as a whole appeals for help.

To conclude, it was understood that the greatest learning resided in respectful, peaceful and harmonious human coexistence, thus resulting in the expression of a healthy garden in balance between its constituent elements and as a health space whose multiple purposes allowed us to appreciate the fragrant aroma it exudes, inviting birds, bees, butterflies and other living beings to contemplate and learn in nature.



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The evolution of sustainable awareness through key historical milestones



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ABSTRACT

The research aimed to analyze the trajectory of environmental movements throughout history, highlighting their impacts, public policies and awareness initiatives in the promotion of environmental conservation and sustainable practices. To this end, a bibliographic research was carried out through the survey of articles on the Scopus, SciELO and Google Scholar platforms. As a result, it has been found that, from the Industrial Revolution to recent events such as Rio+20 and the



Paris Agreement, the historical context has shaped these movements and influenced global sustainability policies. The emergence of environmentalism as a political and social force was a response to the challenges of environmental degradation and the unbridled exploitation of natural resources. Events such as the Love Canal disaster and the Stockholm Conference boosted environmental activism, culminating in Rio-92, which laid solid foundations for international cooperation and important environmental agreements. The Kyoto Protocol was crucial in the fight against climate change, while the

Johannesburg Earth Summit and Rio+20 reaffirmed the commitment to sustainable development and global cooperation. Despite advances, challenges such as climate change require collaborative approaches involving governments, the private sector, and civil society. Environmentalism and sustainability represent commitments to the future, requiring continuity in awareness, responsible public policies, and sustainable practices to achieve an equitable and environmentally sound future.

Keywords: Environment, Environmental movements, Sustainability.

1 INTRODUCTION

Sustainability and environmentalism represent fundamental pillars in the search for a more balanced and harmonious future between human activities and the environment. While sustainability encompasses the idea of ensuring the responsible use of natural resources, reducing environmental impact, and promoting the social and economic well-being of present and future generations, environmentalism emerges as a social and political movement that advocates for the preservation of ecosystems, the conservation of biodiversity, and the mitigation of climate change (MENDES, 2022).

As Santos et al. (2020) reiterate, sustainability and environmentalism are intertwined, with environmentalism often serving as the basis and inspiration for sustainable policies and practices, while sustainability provides the theoretical and practical framework for achieving the goals sought by environmentalism.

In recent years, humanity has witnessed a significant increase in awareness and engagement on issues related to sustainability and environmentalism, both from civil society and the private and government sector. Environmental movements have been pushing for stricter environmental protection policies, while sustainability has been increasingly incorporated into business strategies and public policies. This convergence of efforts is essential to address global environmental challenges, such as biodiversity loss, pollution, scarcity of natural resources and climate change, and to promote fairer and more sustainable development for all (GIACOMETTI; DOMINSCHEK, 2018).

In this context, the objective of this research was to analyze the trajectory of environmental movements throughout history, investigating the impacts of environmental movements, public policies and awareness initiatives in the promotion of environmental conservation and the adoption of sustainable practices. To this end, a bibliographic search was carried out through the survey of articles on the Scopus, SciELO and Google Scholar platforms.



2 DEVELOPMENT

Since the early nineteenth century, with the rise of the Industrial Revolution, the world has witnessed rapid economic and social transformation. However, along with industrial advances have also come severe environmental impacts, such as air and water pollution, rampant deforestation, and the degradation of ecosystems. These problems aroused growing concerns among the thinkers and activists of the time, who began to warn about the dangers of the unbridled exploitation of natural resources (ROCHA; FILE; WALDMAN, 2020).

The indiscriminate use of coal as an energy source in factories and locomotives has contributed to the emission of large amounts of air pollutants, such as sulfur dioxide and soot, generating air quality problems in industrial urban areas. In addition, the waters of rivers and lakes have been contaminated by industrial waste, resulting in water pollution and destruction of aquatic habitats (ROCHA; FILE; WALDMAN, 2020).

Deforestation was also a direct consequence of the Industrial Revolution, as demand for wood increased for the construction of factories, homes, and urban infrastructure, as well as for the production of charcoal used in the steel industry. These activities have contributed to the loss of natural habitats and the fragmentation of ecosystems, affecting biodiversity and ecosystem services (MENDES, 2022).

Thus, the context of the Industrial Revolution marked the beginning of an era of significant environmental impacts, which shaped the basis for the later emergence of the environmental movement. The environmental consequences of this historical period highlight the importance of considering economic development in a sustainable way, seeking to reconcile industrial progress with the protection and conservation of the environment (ROCHA; FILE; WALDMAN, 2020).

However, it was only in the twentieth century that environmental movements gained greater visibility and organization, driven by landmark events such as the Love Canal environmental disaster in the United States in the 1970s. The Love Canal environmental disaster is one of the most iconic episodes in U.S. environmental history. It took place in the city of Niagara Falls, New York, during the 1940s to 1970s. Love Canal was a residential area built over an old navigation canal that had been excavated and abandoned in the late nineteenth century (RABELO, 2019).

In the 1920s, the chemical company Hooker Chemical Company used the canal to deposit toxic industrial waste, including chemicals such as hexachlorobenzene and dioxin. In the 1950s, the area was sold to the City of Niagara Falls at a symbolic price, and a residential neighborhood was built over the site. However, residents began to report health problems, such as congenital malformations, cancer, and respiratory diseases, in addition to noticing the appearance of toxic substances in the soil and water (STEINMETZ; BURMANN; BURGEL, 2023).



In 1978, U.S. President Jimmy Carter declared a national state of emergency in Love Canal, and more than 800 families were relocated to safe areas. The Love Canal disaster had a significant impact on the U.S. environmental movement, leading to the enactment of federal environmental protection laws such as the National Environmental Policy Act (NEPA) and the Superfund Act, which established a program to clean up toxic contaminated sites across the country (STEINMETZ; BURMANN; BURGEL, 2023).

According to Rabelo (2019), the Love Canal disaster highlighted the dangers of uncontrolled industrial pollution and the need for stricter environmental regulations to protect public health and the environment. It has also served as a powerful example of the power of community mobilization and environmental activism in the pursuit of environmental justice and holding corporations accountable for environmental damage.

According to Dellagnezze (2022), another factor that contributed to the intensification of environmental movements was the United Nations Conference on the Human Environment in Stockholm in 1972. The United Nations Conference on the Human Environment, held in Stockholm in 1972, was a historic milestone in the development of global environmental policies. The event brought together representatives from 113 countries, as well as non-governmental organizations and observers, to discuss emerging environmental issues and seek solutions to the environmental challenges facing the planet.

The Stockholm Conference was the first global gathering of world leaders dedicated exclusively to the environment. Its main objective was to sensitize governments and public opinion on the importance of environmental protection and to promote international cooperation to address global environmental problems (DELLAGNEZZE, 2022).

During the conference, a variety of topics were discussed, including air and water pollution, deforestation, biodiversity conservation, and the impact of human activities on climate and ecosystems. One of the most significant outcomes of the conference was the adoption of the Declaration on the Human Environment, which emphasized the urgent need to protect and preserve the environment for present and future generations (ZECA, 2022).

In addition to the Declaration on the Human Environment, the conference also resulted in the creation of the United Nations Environment Programme (UNEP), which is the leading global authority responsible for environmental issues within the United Nations system. The Stockholm Conference laid the groundwork for future international environmental negotiations and agreements, including the Convention on International Trade in Endangered Species (CITES) and the United Nations Convention on Climate Change (UNFCCC) (ZECA, 2022).

With the increased awareness of global environmental issues, non-governmental organizations and social movements dedicated to protecting the environment and promoting sustainability have



emerged. Movements such as Greenpeace, founded in 1971, have become icons of the global environmental struggle, carrying out awareness campaigns, protests, and direct actions in defense of biodiversity and against environmental degradation (ZECA, 2022).

According to Candido, Rédua, and Kato (2021), the 1990s marked an important advance in the field of sustainability, with the elaboration of Agenda 21 at the United Nations Conference on Environment and Development (Rio-92), which established principles and guidelines for sustainable development at the global level. Agenda 21 was a comprehensive document that outlined strategies to promote sustainable development in different areas, including the economy, society, and the environment. This agenda recognized the interconnectedness between these aspects and emphasized the need to address them in an integrated and collaborative manner.

Rio-92 was an important milestone because it brought together leaders from around the world to discuss and negotiate concrete actions to address environmental challenges. This has led to increased international cooperation and the adoption of important environmental agreements and protocols, such as the United Nations Framework Convention on Climate Change and the Convention on Biological Diversity. These efforts have been instrumental in laying a solid foundation for the promotion of sustainability globally and in laying the groundwork for future environmental initiatives and agreements. However, they also highlighted the continued need for action and commitment to address evolving environmental challenges (CANDIDO; REDUOUS; KATO, 2021).

After the historic United Nations Conference on Environment and Development, better known as Rio-92, the global scenario of environmental movements began to evolve significantly. This event marked a crucial turning point by bringing together leaders from around the world to discuss and negotiate concrete actions to address the urgent environmental challenges facing the planet. Rio-92 not only fostered greater awareness of issues such as climate change, pollution, and biodiversity loss, but also catalyzed growing international cooperation. This has been reflected in the adoption of important environmental agreements and protocols, such as the United Nations Framework Convention on Climate Change and the Convention on Biological Diversity (SCOVAZZI, 2022).

Thus, after Rio-92, a series of landmark events continued to boost environmental movements and influence global sustainability policies. Among them, the Kyoto Protocol, established in 1997, was a crucial step in the fight against climate change, setting emission reduction targets for industrialized countries. This protocol was an important development of the United Nations Framework Convention on Climate Change (UNFCCC), established at Rio-92 (SOUZA, 2008).

The Kyoto Protocol had as its main objective to reduce greenhouse gas emissions, particularly from industrialized countries, recognizing their historical responsibility for climate change. To achieve this goal, the protocol established mandatory emission reduction targets for developed countries in relation to 1990 levels (SOUZA, 2008).



One of the distinguishing features of the Kyoto Protocol was the Emissions Trading mechanism, which allowed countries meeting their emissions reduction targets to purchase carbon credits from countries that exceeded their targets or invest in mitigation projects in other countries. This mechanism aimed to promote a more efficient and cost-effective global reduction of emissions (CHAVES, 2015).

However, despite its ambitious goals, the Kyoto Protocol has faced significant challenges, including a lack of buy-in from some of the largest emitters of greenhouse gases, such as the United States, which has not ratified the agreement. In addition, there were concerns about the fairness of the targets, as developing countries had no obligations to reduce emissions. Despite its limitations, the Kyoto Protocol has set an important precedent for international cooperation in the fight against climate change and has influenced the global environmental agenda. He highlighted the need for concrete and binding actions to address one of the most pressing challenges facing humanity and laid the groundwork for future climate agreements such as the Paris Agreement (CHAVES, 2015).

Years later, the Johannesburg Earth Summit held in 2002 reviewed progress since Rio-92 and highlighted the need to intensify efforts to achieve sustainable development goals. The event aimed to review the progress made since Rio-92 and assess emerging challenges related to sustainable development. The summit brought together world leaders, non-governmental organizations, and civil society representatives to discuss environmental, social, and economic issues. It provided an opportunity for participants to share experiences, exchange knowledge, and make renewed commitments to promote sustainable development on a global scale (AQUINO et al., 2022).

One of the main outcomes of the Johannesburg Earth Summit was the reaffirmation of the principles and commitments established at Rio-92, including the importance of integrating economic, social and environmental development. In addition, the summit highlighted the need to intensify efforts to achieve the Sustainable Development Goals (SDGs), subsequently established by the United Nations (UN) (AQUINO et al., 2022).

The Johannesburg Summit, however, also faced criticism, particularly regarding the lack of significant progress in implementing previous commitments and the absence of binding agreements to tackle pressing environmental issues such as climate change and biodiversity loss. Despite the limitations, the Johannesburg Earth Summit played an important role in maintaining the focus on the sustainable development agenda and bringing together leaders and key actors to promote global dialogue and cooperation in pursuit of a more equitable, prosperous, and environmentally sound future (AQUINO et al., 2022).

As Sugahara and Rodrigues (2019) point out, twenty years after Rio-92, the World Summit on Sustainable Development, known as Rio+20, brought together global leaders to adopt the document "The Future We Want", outlining a collective vision for sustainable development. The event brought together world leaders, representatives of governments, non-governmental organizations, the private



sector and civil society to discuss and take action on global challenges related to sustainable development. The main objective of Rio+20 was to renew the political commitment to sustainable development and to assess the progress made since Rio-92, as well as to identify new ways to move towards a more sustainable future. The event also aimed to strengthen global environmental governance and promote integration between the economic, social and environmental dimensions of development.

At the Rio+20 event, the document entitled "The Future We Want" was adopted. This document outlined a collective vision of world leaders on sustainable development, highlighting key principles and priority areas for action. Among the topics covered were the eradication of poverty, the promotion of sustainable patterns of production and consumption, the protection and management of natural resources, and the promotion of gender equality and women's empowerment. In addition to the outcome document, Rio+20 also promoted a series of thematic dialogues, high-level panels and side events, which provided opportunities for exchanging experiences, sharing good practices and mobilizing resources for sustainable development initiatives (SUGAHARA; RODRIGUES, 2019).

One of the most significant events was the Paris Agreement in 2015, a milestone in the fight against climate change. This agreement set global goals to limit global temperature rise and strengthen resilience to climate change. It represented a renewed response to the challenges identified since Rio-92, highlighting the importance of international cooperation and the mobilization of civil society in the search for a more sustainable future (GUEDES, 2021).

Today, environmentalism and sustainability remain central themes in the public and political debate, in the face of urgent challenges such as climate change, biodiversity loss and ecosystem degradation. Local and global environmental movements play a crucial role in pushing for environmentally responsible public policies and promoting sustainable practices in society (COSTA; FERREZIN, 2021).

The emergence of new technologies and innovative approaches has contributed to driving the sustainability agenda forward, offering creative solutions to the environmental challenges facing the planet. In this sense, environmentalism and sustainability represent not only a concern for the present, but also a commitment to the future of the next generations and of planet Earth itself (COSTA; FERREZIN, 2021).

3 FINAL THOUGHTS

In view of the above, the research aimed to analyze the trajectory of environmental movements throughout history, focusing on the impacts of such movements, public policies and awareness initiatives in the promotion of environmental conservation and the adoption of sustainable practices. Through retrospective analysis, it was possible to understand how the historical context, from the



Industrial Revolution to more recent events such as Rio+20 and the Paris Agreement, has shaped and influenced environmental movements and global sustainability policies.

In examining the evolution of these movements, it became clear that the emergence of environmentalism as a significant force in the political and social sphere was a response to the growing challenges of environmental degradation and the harmful consequences of the rampant exploitation of natural resources. From events such as the Love Canal disaster and the Stockholm Conference in 1972, environmental activism gained prominence, culminating in Rio-92, which established a solid foundation for international cooperation and the adoption of important environmental agreements and protocols.

The Kyoto Protocol, for example, was a significant milestone in the fight against climate change, highlighting the need for concrete and binding action by industrialized countries. Similarly, the Johannesburg Earth Summit and Rio+20 reaffirmed the commitment to sustainable development and promoted global dialogue and cooperation to address emerging environmental challenges.

However, it is important to note that despite the progress made, there is still much to be done. The emergence of global challenges such as climate change and biodiversity loss require a more comprehensive and collaborative approach, involving not only governments, but also the private sector, civil society, and citizens at large.

In this sense, environmentalism and sustainability represent not only a concern for the present, but also a commitment to the future of the next generations and of planet Earth itself. Therefore, it is essential to continue promoting awareness, adopting environmentally responsible public policies, and encouraging sustainable practices at all levels of society. Only through coordinated and committed efforts can we achieve a more equitable, prosperous and environmentally sound future for all.



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Sustainability and the historical trajectory of environmental movements



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ABSTRACT

The present research aimed to analyze the evolution of sustainable awareness over time, focusing on the main historical milestones, initiatives and practices adopted, as well as the results achieved so far. To carry out this study, the methodology of bibliographic research was used through a survey of data in articles, theses, dissertations and books. As a result, it was found that sustainable awareness has been an increasingly relevant topic over the years, driving initiatives and practices aimed at preserving



the environment and promoting sustainable development. In addition, it was possible to observe that the theme has been addressed by various sectors of society, such as business, government, and education, demonstrating the importance of sustainable awareness for the construction of a

fairer and more balanced future for the next generations.

Keywords: Sustainability, Environment, Awareness.

1 INTRODUCTION

Since the Industrial Revolution, humanity has had a significant impact on the environment, which has been worsening over time. The increasing exploitation of natural resources, the mass production of goods and the emission of polluting gases have put the health of the planet and the survival of several species at risk. The concern with sustainability is increasingly pressing, as humanity is approaching the limits of what the planet can support (GUEDES, 2021).

Over the years, sustainable awareness has evolved, involving various sectors of society, from the private sector to the governmental sphere and civil society. In this scenario, sustainable awareness has gained increasing importance both in the academic and business spheres, as it is a way capable of contributing to the reduction of the impact of human activity on the environment and promoting a fairer and more responsible development (ANTUNES, 2021).

According to Ribeiro and Cherobim (2017), sustainable awareness has been shown to be fundamental for the promotion of a more sustainable and balanced development in different areas of society. However, there are still many gaps in knowledge about how awareness has been promoted and what have been the results achieved to date. In addition, the practices adopted in the search for more sustainable development are often not effective or do not achieve the desired impact.

In this context, this research aimed to analyze the evolution of sustainable awareness over time, in order to ascertain the main historical milestones, initiatives and practices adopted, as well as the results achieved so far. To this end, the bibliographic research method was adopted, as the data were collected from different sources of information, including official documents, scientific articles, theses, dissertations, books and reports from governmental and non-governmental organizations.

Based on the results of the research, it is expected to contribute to the advancement of knowledge on the evolution of sustainable awareness and to the promotion of sustainable practices, whether in the governmental, business or social sphere. Thus, research can have important impacts on decision-making in different sectors of society, providing relevant information and data for the formulation of public policies, business strategies, and civil society actions.



2 DEVELOPMENT

2.1 THE EVOLUTION OF SUSTAINABLE AWARENESS THROUGH KEY HISTORICAL MILESTONES

In this topic, the development of the evolution of sustainable awareness through the main historical milestones over time is presented, thus contemplating the initial milestones from the Industrial Revolution to the contemporary scenario.

2.1.1 Initial milestone of sustainable awareness

The environmental process after the Industrial Revolution began in the nineteenth century, when people began to notice the negative impacts that industrialization was having on the environment and public health. With increasing urbanization and mass production, there has been increasing air, water, and soil pollution, as well as degradation of natural ecosystems (POTT; ESTRELA, 2017).

The concern with sustainable awareness emerged at the end of the twentieth century, in a context of growing environmental awareness and recognition of the need to protect the environment and the planet's natural resources. This movement arose in response to several environmental problems that began to become more visible, such as air and water pollution, the destruction of ecosystems, and climate change (MAGRINI, 2001).

From this perspective, the environmental movement emerged, according to Alexandre (2000), as a way to minimize the intensification of anthropic actions on nature. The aim of this movement was to promote awareness and protection of the environment, so as to seek sustainable development capable of meeting the needs of the present without compromising future generations. This aimed to bring about changes in public policies, in society's consumption habits and in the behavior of companies and industries.

In the early twentieth century, the first environmental laws emerged, such as the Water Protection Act of 1901 in Germany and the Soil Conservation Act of 1935 in the United States. However, it was only in the 1960s that the modern environmental movement took hold, driven by events such as the publication of Rachel Carson's book "Silent Spring" in 1962, which warned of the dangers of pesticides and other chemicals to wildlife and human health (BONZI, 2013).

Rachel Carson's book was important in raising awareness of the dangers of chemical pollution and led to the creation of stricter laws and regulations for the use of pesticides and other chemicals. Carson's work had a major impact on public opinion and led to greater pressure for the creation of stricter laws and regulations for the use of these chemicals. As a result, several government agencies created stricter regulations for the use of pesticides and other chemicals, and the book was instrumental in the emergence of the modern environmental movement (SANTOS, 2022).



2.1.2 Earth Day 1970

In the following years, several important events took place that helped promote environmental awareness, such as Earth Day in 1970. On April 22, 1970, millions of people across the United States participated in demonstrations, protests, and educational events to draw attention to the growing pollution of air, water, and soil. The idea for Earth Day came from U.S. Senator Gaylord Nelson, who was concerned about increasing pollution and the government's lack of action to address these issues (LEFF, 2001).

Earth Day 1970 was an astonishing success, with an estimated 20 million people participating across the country. The event inspired the creation of the United States Environmental Protection Agency (EPA) and the enactment of several important environmental protection laws, including the National Air Pollution Act, the Clean Water Act, and the Endangered Species Act (MORAES, 2018).

In addition to the United States, Earth Day 1970 also had a significant impact around the world. Demonstrations and educational events took place in many countries, including Canada, Australia, the United Kingdom, Japan, and many others. Through these events, awareness of the need to protect the environment and reduce pollution has been amplified around the world (GROHS; MATTHIES, 2021).

2.1.3 United States Environmental Protection Agency of 1970

Also in 1970, the United States Environmental Protection Agency was created. The U.S. Environmental Protection Agency (EPA) was created in 1970, during the administration of President Richard Nixon, as a result of the environmental movement that gained momentum at the time. The EPA is a federal agency responsible for protecting the environment and human health by regulating air, water, and soil pollution (CAPELLARI; CAPELLARI, 2015).

According to Colacios (2014), the creation of the EPA was an important milestone in the history of environmental protection in the United States, as it consolidated several environmental functions of the federal government into a single agency. Prior to the creation of the EPA, environmental protection functions were distributed across several agencies, including the Department of Health, Education, and Welfare, the Department of the Interior, and the Department of Agriculture. The EPA is responsible for developing and enforcing environmental laws and regulations, as well as providing grants and technical support to state and local governments to help them implement environmental protection programs.

Among the most important laws the EPA is tasked with implementing are the *Clean Air Act*, the *Clean Water Act*, the *Endangered Species Act*, and the *Resource Conservation and Recovery Act* (COLACIOS, 2014).

From the perspective of Guedes (2021), EPA has played a key role in environmental risk assessment and management, including the identification and assessment of toxic and hazardous



chemicals, as well as the assessment of risks to human health and the environment. In addition, it was responsible for ensuring the compliance of companies and industries with environmental laws and regulations, monitoring and applying penalties and sanctions when necessary.

2.1.4 1972 United Nations Conference

According to Pimenta and Nardelli (2015), awareness of sustainable development gained greater notoriety in 1972, through the United Nations Conference on the Human Environment in Stockholm. The United Nations Conference on the Human Environment was held in Stockholm, Sweden, from June 5 to 16, 1972. This conference was the first international gathering of world leaders to discuss environmental issues and marked the beginning of the global environmental protection movement.

As Guimarães and Fontoura (2012) point out, the Stockholm Conference brought together representatives from 113 countries and non-governmental organizations to discuss global environmental issues, including air and water pollution, biodiversity loss, and climate change. The main goal of the conference was to find ways to balance economic development with the protection of the environment.

During the Conference, a number of important resolutions and agreements were adopted. The most important was the Stockholm Declaration on the Human Environment, which set out the basic principles for protecting the environment and promoting sustainable development. The declaration affirmed that the protection of the environment is essential for human well-being and called on governments around the world to cooperate to protect the environment (GUIMARÃES; FONTOURA, 2012).

In addition to the Stockholm Declaration, the conference also led to the creation of the United Nations Environment Programme (UNEP), which is the main UN agency tasked with dealing with global environmental issues. UNEP aims to promote sustainable development and environmental protection around the world (AGUIAR, 2016).

The Stockholm Conference also established, according to Aguiar (2016), the agenda for future international conferences on global environmental issues, including the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992, and the United Nations Conference on Climate Change, held in Paris in 2015.

2.1.5 Our Common Future Report 1983

From the 1980s onwards, the concern with sustainability began to become, according to Lopes et al. (2017), more important on the global political agenda. The World Commission on Environment and Development, established by the UN in 1983, published a report in 1987 titled "Our Common



Future," which popularized the concept of sustainable development. The report defined sustainable development as "that which meets the needs of the present without compromising the ability of future generations to meet their own needs."

Goldemberg (2015) reiterates that the report "Our Common Future" was an important milestone in the history of sustainability, as it was the first document to establish the idea of sustainable development as a central concept for social and economic progress. The report highlighted the need to strike a balance between the economic, social and environmental dimensions of development and proposed a set of strategies to achieve this goal.

In addition, the report also emphasized the importance of international cooperation to address global environmental issues such as climate change, pollution and land degradation. This cooperation requires integration between civil society and the public and private sectors in the search for sustainable solutions. The document stated that sustainable development requires the integration of policies and actions in all areas, including energy, transport, agriculture, industry and trade (JAPIASSÚ; GUERRA, 2017).

2.1.6 Agenda 21 (1992)

In the following years, several initiatives and movements emerged in defense of sustainability, such as Agenda 21, which was a global action plan for sustainable development adopted during the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992. The objective of Agenda 21 was to promote economically, socially and environmentally sustainable development around the world, considering the global challenges of poverty, environmental degradation and social inequality (MARTINS et al., 2015).

According to Dias (2018), the Agenda 21 action plan encompassed 40 chapters covering a wide variety of topics, including biodiversity, energy, transport, urban development, agriculture, water resources, health, education, and public participation. Agenda 21 highlighted the importance of international cooperation, civil society participation, and public and private sector engagement to achieve the sustainable development goals.

As mentioned by Jannuzzi and Carlo (2018), among the main goals established by Agenda 21, we can mention the eradication of poverty, the reduction of social inequalities, the improvement of the health and well-being of the population, the promotion of sustainable production and consumption patterns, the conservation of biodiversity and natural ecosystems, the reduction of pollution and greenhouse gas emissions, and strengthening institutional capacity to deal with environmental and development issues.

Agenda 21 is considered an important milestone in the history of sustainability and has influenced policies and actions around the world since its adoption. In addition, Agenda 21 inspired



the creation of other international sustainability agreements, such as the Sustainable Development Goals (SDGs) adopted in 2015 by the UN (JANNUZZI; CARLO, 2018).

2.1.7 1997 Protocol of Kyoto

In the late 1990s, the Kyoto Protocol was created in 1997, where an international agreement was signed in 1997 during the third Conference of the Parties to the United Nations Framework Convention on Climate Change (COP3) held in Kyoto, Japan. The protocol established quantified commitments to reduce greenhouse gas (GHG) emissions by industrialized countries, with the aim of combating global warming (SOUZA, 2008).

Chaves (2015) points out that the signatory countries of the Kyoto Protocol agreed to reduce their greenhouse gas emissions by at least 5.2% below 1990 levels in the period between 2008 and 2012. To achieve this goal, individual GHG emission limits were established for each industrialized country that signed the agreement. These limits are known as emission reduction targets.

In addition, the Kyoto Protocol created the Clean Development Mechanism (CDM), which allows industrialized countries to finance emission reduction projects in developing countries as a way to achieve their own emission reduction targets. The protocol also established an emissions trading system, allowing countries that exceed their reduction targets to purchase emissions credits from other countries that have achieved reductions beyond their own targets (CHAVES, 2015).

The Kyoto Protocol entered into force in 2005 and initially involved the participation of 38 industrialized countries. The United States, one of the world's largest emitters of greenhouse gases, signed the agreement in 1998 but never ratified it. In 2012, during the Conference of the Parties held in Doha, Qatar, the signatory countries agreed to extend the period of the Kyoto Protocol until 2020 and to adopt a second commitment period covering the period from 2013 to 2020 (SEIFFERT, 2013).

The Kyoto Protocol is considered an important milestone in the fight against climate change, as it established for the first time legally binding commitments to reduce greenhouse gas emissions for industrialized countries. Since then, other international agreements have been established to address climate change, including the 2010 Green Fund (SOUZA, 2018).

2.1.8 ISO 14040:2006

According to the Brazilian Association of Technical Standards (2006), ISO 14040:2006 is a standard developed in 2006 that establishes guidelines and principles for life cycle assessment (LCA) of products and services. The standard is used to assess the environmental impacts of a product or service throughout its entire life cycle, from the extraction of raw materials to their disposal.



ISO 14040:2006 defines four steps for LCA, which are: defining objectives and scope, analyzing life cycle inventory, assessing life cycle impact, and interpreting the life cycle. Each of these steps is explained in Chart 1.

Table 1. Product and Service Life Cycle Assessment

<u>Definition of objectives and scope</u>
At this stage, the objectives of the LCA are defined and the scope of the study is established. It is important to identify the boundaries of the study and define which environmental impacts will be assessed.
<u>Lifecycle inventory analysis</u>
In this step, data is collected on all inputs and outputs of materials, energy, and other resources throughout the life cycle of the product or service. This includes the extraction of raw materials, transportation, manufacturing, use, and disposal.
<u>Life Cycle Impact Assessment</u>
In this step, the data collected in the inventory analysis is evaluated in relation to environmental impacts. This includes assessing greenhouse gas emissions, toxicity, and waste generation, among other impacts
<u>Life Cycle Interpretation</u>
In this step, the results of the impact assessment are interpreted and analyzed to identify opportunities for improvement. The conclusions are used to make informed decisions about the product or service being evaluated

Source: ISO 14040 (2006).

Based on the above, it turns out that ISO 14040:2006 is an important tool for companies to assess the environmental impact of their products and services. The standard helps businesses identify opportunities for improvement and make informed decisions about the sustainability of their products and services. In addition, LCA can also be used to support public policy decision-making and help guide actions to promote sustainability.

2.1.9 Green Fund 2010

The Green Climate Fund (GCF) is a financial mechanism created by the United Nations Framework Convention on Climate Change (UNFCCC) to help developing countries cope with the impacts of climate change and promote low-carbon sustainable development (PEREIRA, 2022).

The GCF was established in 2010 during the 16th Conference of the Parties (COP) to the UNFCCC, and became operational in 2015. Its main objective is to mobilize significant financial resources from public and private sources to support developing countries in implementing climate change mitigation and adaptation actions (ANTUNES, 2021).

Pereira (2022) reiterates that the Green Fund's resources are directed to projects and programs in areas such as renewable energy, energy efficiency, sustainable transport, water resources and forest



management, sustainable agriculture, climate change adaptation, and strengthening the capacity of developing countries to deal with these challenges.

The GCF is considered one of the key financial mechanisms for the implementation of the Paris Agreement and aims to achieve annual funding of \$100 billion by 2025 to support climate action in developing countries. The Green Fund is managed by a board, with representatives from both developed and developing countries, and is responsible for making decisions about the financing of projects and programs (CASTRO et al., 2019).

2.1.10 ISO 14006:2011

ISO 14006 (2011) is an international standard developed in 2011 that establishes guidelines for incorporating environmental aspects into product *design*. The standard aims to help companies integrate sustainability into all stages of the product life cycle, from design to disposal (ABNT, 2011).

ISO 14006:2011 defines environmental *design* as the process of incorporating environmental considerations into the design of products and services, with the aim of improving their environmental performance throughout their life cycle. The standard establishes guidelines for the implementation of an environmental design system in an organization, which includes the steps outlined in Chart 2.

Table 2. Guidelines for incorporating environmental aspects into *product design*

<u>Establish an <i>environmental design</i> policy</u>
The organization should establish an <i>environmental design</i> policy that reflects its commitment to sustainability and sets clear goals for incorporating environmental considerations into <i>product design</i> .
<u>Integrate <i>environmental design</i> into the product development process</u>
The organization must integrate environmental considerations into the product development process, from conception to the end of the product lifecycle. This includes establishing environmental criteria for evaluating and selecting materials, manufacturing processes, and technologies.
<u>Develop skills and knowledge for <i>environmental design</i></u>
The organization must ensure that its design team has the necessary skills and knowledge to incorporate environmental considerations into <i>product design</i> . This can include training in life cycle assessment, <i>eco-design</i> , and environmental technologies.
<u>Conduct Life Cycle Assessment</u>
The organization should conduct a life cycle assessment of its products to identify opportunities for environmental improvements at all stages of the product lifecycle.
<u>Communicate environmental information throughout the supply chain</u>
The organization must communicate relevant environmental information throughout the supply chain to ensure that its suppliers and partners also incorporate environmental considerations into their design and manufacturing processes.

Source: ISO 14006 (2011).



ISO 14006:2011 is an important tool to help businesses incorporate environmental considerations into the *design* of products and services. The standard can help businesses reduce their environmental impact, improve their efficiency, and increase competitiveness in the market. Additionally, environmental design can also be an opportunity for businesses to innovate and develop more sustainable products that meet consumer demands for greener products.

2.1.11 ISO 14031:2013

As pointed out by the Brazilian Association of Technical Standards (2013), ISO 14031 (2013) is a standard published in 2013 that establishes guidelines for the evaluation of an organization's environmental performance. Its goal is to help organizations measure, evaluate, and improve their environmental performance through a performance-based environmental management system.

The standard is based on the Plan-Do-Check-Act (PDCA) continuous improvement cycle and defines environmental performance as the measurable outcome of an organization's actions in relation to the environment, based on its environmental policy, objectives and targets. This means that the standard establishes guidelines for the implementation of an environmental management system that allows organizations to assess and measure their environmental performance against their environmental objectives and targets.

In this sense, the environmental management system requires the implementation of four stages, which are: planning, implementation, verification and critical analysis. Table 3 provides more details of these steps.

Table 3. The steps of the environmental management system

<u>Planning</u>
The organization must establish an environmental policy and establish measurable environmental objectives and targets. In addition, the organization must identify and evaluate the environmental aspects of its activities, products, and services.
<u>Implementation</u>
The organization must implement its environmental management system and identify the processes and procedures necessary to achieve its environmental objectives and goals. The organization should also provide training and awareness of environmental issues to its employees.
<u>Verification</u>
The organization must evaluate its environmental performance on a regular basis by measuring and monitoring its environmental impacts. The organization must identify the root causes of environmental problems and implement corrective measures.
<u>Critical analysis</u>
The organization should conduct regular critical reviews of its environmental management system, assessing whether its environmental objectives and goals are being achieved and identifying areas for continuous improvement.

Fonte:ISO 14031 (2013).



2.1.12 ISO 14001:2015

The ISO 14001:2015 standard is an internationally recognized standard for environmental management systems (EMS). It defines the requirements for establishing, implementing, maintaining and continuously improving an effective environmental management system (ABNT, 2015).

The purpose of ISO 14001:2015 is to help organizations minimize the negative environmental impacts of their activities, products, and services. The standard encourages companies to responsibly and sustainably manage environmental issues, including the use of natural resources, greenhouse gas emissions, waste management, and other aspects relevant to the environment.

The standard is applicable to all organizations, regardless of industry or size, that wish to implement an effective environmental management system. It provides a framework for identifying the environmental aspects and impacts of the organization's activities, establishing environmental objectives and targets, and implementing an action plan to achieve them.

ISO 14001:2015 also requires organizations to take a risk-based approach to environmental management. This means that organizations must identify the environmental risks and opportunities associated with their activities, products, and services and implement measures to minimize those risks and take advantage of those opportunities.

In addition, the standard requires organizations to establish a monitoring, measurement, and analysis program to assess the effectiveness of their environmental management system. This allows organizations to identify areas that need improvement and implement corrective actions to ensure that their environmental management system is always in compliance with the requirements of the standard.

2.1.13 2015 Paris Agreement

In addition to the Green Fund, another important milestone for sustainable awareness was the 2015 Paris Agreement, which established ambitious GHG emission reduction targets for all signatory countries. Such an agreement was an international treaty on climate change, which was adopted in December 2015 at the Conference of the Parties (COP21) to the United Nations Framework Convention on Climate Change (UNFCCC), held in Paris, France. The primary objective of the agreement is to limit global warming to below 2 degrees Celsius above pre-industrial levels and to pursue efforts to limit it to 1.5 degrees Celsius (REI; GATES; SOUZA, 2017).

According to Aubertin and Kalil (2016), the signatory countries of the agreement committed to reducing their greenhouse gas emissions and to present, on a regular basis, national action plans to deal with climate change. In addition, the Paris Agreement provides for international cooperation to help the most vulnerable countries cope with the impacts of climate change and the transition to a low-carbon economy. The agreement entered into force in November 2016 and so far has 197 signatories.



In the year 2015, the Sustainable Development Goals (SDGs) were established by the United Nations in 2015, during the United Nations Summit on Sustainable Development. The SDGs consist of 17 interlinked and ambitious goals that aim to eradicate poverty, protect the planet and ensure prosperity for all. Table 4 shows the 17 goals established by the United Nations in 2015.

Table 4. The 17 goals set by the United Nations in 2015.

<ul style="list-style-type: none">● Poverty Eradication: End poverty in all its forms, everywhere.● Zero Hunger and Sustainable Agriculture: End hunger, achieve food security and improved nutrition, and promote sustainable agriculture.● Health and Well-Being: Ensuring healthy lives and promoting well-being for all ages.● Quality Education: Ensure inclusive, equitable and quality education, and promote lifelong learning opportunities for all.● Gender Equality: Achieve gender equality and empower all women and girls.● Drinking Water and Sanitation: Ensure the availability and sustainable management of water and sanitation for all.● Affordable and Clean Energy: Ensure access to clean, affordable and sustainable energy for all.● Decent Work and Economic Growth: Promote sustainable, inclusive economic growth and sustain decent employment for all.● Industry, Innovation and Infrastructure: Build resilient infrastructure, promote inclusive industrialization and foster innovation.● Reducing Inequalities: Reducing social, economic and regional inequalities everywhere.● Sustainable Cities and Communities: Making cities and communities inclusive, safe, resilient and sustainable.● Sustainable Consumption and Production: Ensure sustainable consumption and production patterns, including sustainable waste management.● Action Against Global Climate Change: Take urgent action to combat climate change and its effects.● Life in Water: Protect marine life and the oceans, and promote the sustainable use of marine resources.● Life on Land: Protect, restore and promote the sustainable use of terrestrial ecosystems, manage forests sustainably, combat desertification and biodiversity loss.● Peace, Justice and Strong Institutions: Promote peaceful, just and inclusive societies and strengthen strong institutions.● Partnerships and Means of Implementation: Strengthen the means of implementation and revitalize the global partnership for sustainable development.

Source: United Nations (2015).

The process of formulating the SDGs involved a broad process of consultation and negotiation involving governments, civil society organizations, the private sector, and other relevant actors around the world. The goal was to develop a global agenda for sustainable development that could guide the policies and actions of governments, international organizations, and other actors for the next 15 years (ANTUNES, 2021).

The SDGs replaced the Millennium Development Goals (MDGs), which were established in 2000 with the aim of fighting poverty and improving the living conditions of people around the world. The SDGs are more comprehensive than the MDGs, addressing issues such as gender equality, climate change, sustainable use of natural resources, and peace and justice (REI; GATES; SOUZA, 2017).

2.1.14 ISO 14004:2016

ISO 14004:2016 is, according to the Brazilian Association of Technical Standards (2016), an internationally recognized standard for guidelines on environmental management systems (EMS). It



provides information on the principles, requirements and guidelines for the implementation of an effective EMS in accordance with ISO 14001:2015.

ISO 14004:2016 is applicable to all organizations, regardless of size or industry, that want to implement an effective EMS. The said ISO offers detailed guidance to help organizations understand and implement the requirements of ISO 14001:2015 effectively. The guidance provided by ISO 14004:2016 is based on fundamental principles of environmental management, including senior management commitment, risk-based approach, employee and stakeholder involvement, and continuous improvement.

The standard addresses several aspects of environmental management, including planning and implementation; monitoring and measuring; auditing and review; and communication and stakeholder involvement, as shown in Table 5.

Table 5. Elements of environmental management

<u>Planning & Implementation</u>
ISO 14004:2016 provides guidance on identifying the environmental aspects of the organization, setting environmental objectives and targets, and developing an action plan to achieve them. Monitoring and measurement: The standard offers guidance on how to establish a monitoring and measurement program to assess the effectiveness of the EMS and identify areas that need improvement.
<u>Monitoring & Measurement</u>
The standard offers guidance on how to establish a monitoring and measurement program to assess the effectiveness of the EMS and identify areas that need improvement.
<u>Audit & Review</u>
ISO 14004:2016 provides guidance on how to conduct internal and external EMS audits and reviews by management to ensure that the system complies with the standard.
<u>Communication and stakeholder engagement</u>
The standard offers guidance on how to engage stakeholders, including employees, customers, suppliers, and the local community, and communicate the results of the EMS.

Source: ISO 14004 (2016).

In summary, ISO 14004:2016 is an important standard for organizations that want to implement an effective EMS in compliance with ISO 14001:2015. It provides detailed guidance to help organizations understand and implement the requirements of the standard effectively, and to responsibly and sustainably manage the environmental issues associated with their activities, products and services.

2.1.15 GSR

For Costa and Ferezin (2021), the acronym ESG refers to a set of environmental, social, and corporate governance criteria that companies must consider in their operations and business strategies.



These criteria have gained more and more importance in recent years, as companies are increasingly held accountable for their social responsibility and environmental impact.

In this case, the "E" refers to environmental aspects, such as the emission of greenhouse gases, use of natural resources, waste management, among others. The "S" refers to social issues such as labor relations, diversity and inclusion, employee health and safety, among others. And the "G" refers to corporate governance issues, such as transparency in operations, business ethics, independence of the board of directors, among others (COSTA; FERREZIN, 2021).

As Irigaray and Stocker (2022) reiterate, incorporating these ESG criteria into a company can lead to tangible benefits, such as better risk management, greater resilience, talent attraction and retention, and a positive reputation. In addition, there is evidence that companies that take their ESG commitments seriously tend to perform better financially in the long run.

Currently, investors, regulators, and other stakeholders are increasingly requiring companies to adopt ESG practices and disclose information related to these criteria in their financial and sustainability reporting. As a result, many companies are incorporating these criteria into their strategy and management, aiming to create long-term value for their shareholders and other stakeholders (GUEDES, 2021).

3 CONCLUSION

Over the last few decades, sustainable awareness has become increasingly important around the world, and this research aimed to analyze the evolution of this awareness over time, as well as the main historical milestones, initiatives and practices adopted, and the results achieved to date. Based on the bibliographic research carried out, it was possible to verify that sustainable awareness began in the 1960s and 1970s, with the emergence of the environmental movement and the concern with the degradation of the environment.

Since then, sustainable awareness has become increasingly strong, and various initiatives and practices have been adopted by governments, businesses, and non-governmental organizations to promote sustainability and protect the environment. Among the most important initiatives are the creation of environmental laws and regulations, the promotion of conscious consumption practices, the development of cleaner and more sustainable technologies, the adoption of environmental management practices in companies, and the awareness of the general population.

The results achieved so far are quite significant, with the reduction of greenhouse gas emissions, the preservation of natural areas and the promotion of recycling and the use of renewable energy sources. However, there is still much to be done to ensure long-term sustainability, and it is critical that sustainable awareness continues to be promoted and encouraged around the world.



Therefore, this research found that sustainable awareness has evolved significantly over time, with the adoption of various initiatives and practices to promote sustainability and protect the environment. While important results have already been achieved, it is critical that this awareness continues to be promoted and encouraged around the world in order to ensure long-term sustainability and protect our planet for future generations.



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Evaluation of the effect of Gastoxin® B57 on maize seed quality by germination and cold test



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ABSTRACT

Gastoxin® B57 is used to control stored grain pests. The objective of this study was to evaluate the effect of different doses of Gastoxin® B57 on seed quality of 18 maize lines through germination and cold testing. The seeds were treated with Gastoxin® B57 for 72 hours in a 1m³ environment. The dosages used were: dosage recommended by the manufacturer; twice the recommended dosage and control. After treatment with the insecticide, germination and cold tests were performed. The experimental design was randomized blocks in a 3x18 factorial scheme (3 doses and 18 strains), followed by Tukey's test of comparison of means. Analysis of variance showed that there was a difference in treatments as a function of the doses. Lines 2, 3, 4, 14 and 16 showed higher germination and vigor potentials. The dose recommended by the manufacturer and the control did not affect the seed quality of the corn lines in the cold test and in the germination test.

Keywords: *Zea mays*, Insecticide, Germination, Vigor.

1 INTRODUCTION

In the process of seed production and commercialization, one of the main factors is the preservation of seed quality throughout the storage period, and seed quality must be maintained at least until the sowing time Carvalho (1992).



Physiological quality, in particular, can be affected by the action of different agents. Among them, the storage pests *Sitophilus zeamais*, *S. oryzae*, *Rhyzopertha dominica* and *Sitotroga cerealella*, which may be largely responsible for the deterioration of the stored seed lot (LORINI et al.2003). The control of these pests depends practically on liquid chemical insecticides (preventive treatment) and fumigants (purge).

Gastoxin® B57, the main product used on the market today, is effective at all stages of insect development.

In addition to quantitative losses, pest attacks on seeds can cause losses in germination power and vigor (BARNEY et al. 1991).

As corn seed purging is routinely performed, and due to the few studies reported in the literature on its effect on seeds, it is of great importance to evaluate the effects of commercially available insecticides on seed quality.

Based on the few studies found in the literature, Andrade & Nascimento (1987) on the effect of purging on the physiological quality of maize seeds, this study was developed. The objective of this study was to evaluate the effect of Gastoxin® B57 on maize seed quality by germination and cold tests.

2 MATERIAL AND METHODS

Seed samples from 18 lines from the breeding program were used. The seeds were treated with phosphine gas using Gastoxin® B57 for 72 hours in a 1m³ environment.

The dosages used were: a) Manufacturer's recommended dosage – 1 g of Gastoxin® B57 per m³ (1 Gastoxin tablet per m³), b) Double the recommended dosage – 2 g of Gastoxin® B57 per m³ (2 Gastoxin tablets per m³) c) No dosage (control).

After insecticide treatment, germination tests were performed according to Brazil (2009) and cold test, according to Barros et al. (1999) and Vieira & Krzyzanowski (1999).

The experimental design was randomized blocks, in a 3x18 factorial scheme. To meet the assumptions of the analysis of variance, the transformation of the germination and cold test data was performed using a logarithmic scale (log).

The mean clustering test proposed by Scott-Knott (1974) was used. Statistical analyses were performed using the R program (R Development Team, 2006)

3 RESULTS AND DISCUSSION

It was verified by the analysis of variance that there was a difference in treatments as a function of the doses of Gastoxin® B57, considering the germination and cold tests, i.e., at least one treatment differs from another as a function of dosage.

By grouping the means by the Scott-Knott test at 5% probability, of the 18 corn lines



considering each dose of the product, it is observed that the dosages of 0 and 1 gram of Gastoxin® B57 were combined in the same group, i.e., they did not affect the germination of the seeds of the strains (Table 1)

Table 1. Averages of the 18 maize lines in the germination test at each dose of the product Gastoxin® B57

Dose	Average ¹
0	3.04 to
1	3.04 to
2	2.90 B

¹Averages followed by the same letter do not differ from each other by the Scott-Knott test at 5% probability.

Their vigor was also not affected (Table 2)

Table 2. Averages of the 18 corn strains in the cold test at each dose of Gastoxin® B57 product

Dose	Average ¹
0	3,08 a
1	3,05 a
2	2,69 b

¹Averages followed by the same letter do not differ from each other by the Scott-Knott test at 5% probability.

The lines differed into two and three groups in the cold and germination tests, respectively, and lines 2, 3, 4, 14 and 16 showed higher germination and vigor potentials (Table 3)

Table 3. Average, in the three doses of phosphine, of the transformed data of the 18 treatments in the germination and cold tests

Lineage	Germination Test ¹	Cold Test ¹
1	2.78 c	3.07 a
2	3.16 a	3.13 a
3	3.20 a	3.05 a
4	3.13 a	3.04 a
5	3.03 b	3.06 a
6	2.89 c	2.94 a
7	2.85 c	3.10 a
8	2.98 b	2.88 b
9	2.76 c	2.96 a
10	3.17 a	2,87 b
11	2.84 c	2.84 b
12	3.04 b	2.84 b
13	2.89 c	2.71 b
14	3.18 a	2.95 a
15	3.01 b	2.81 b
16	3.18 a	3.07 a
17	2.86 c	2.92 a
18	2.82 c	2.67 b

¹Averages followed by the same letter do not differ from each other by the Scott-Knott test at 5% probability.



4 CONCLUSION

The doses of 0 and 1 gram of Gastoxin® B57 did not affect the seed quality of the corn lines in the cold test and in the germination test.



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Use of digitized images to evaluate the quality of coffee seeds



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ABSTRACT

The analysis of digital images consists of the generation of dimensional data, such as width, length and area, or attributes, such as color and texture of a captured image, and their measurement by means of counting or frequency methods. The use of image analysis to evaluate the physiological quality of seeds has been considered an objective, economical and practical method. Thus, this work was carried out with the objective of comparing the results of the evaluation of the physiological quality of coffee seeds obtained in the germination tests with those obtained from the analysis of digitized images. Thirty-two lots of coffee seeds were used, which were submitted to conventional analysis and image analysis using the computer program WINFOLIA.® Although additional research is needed to confirm the results, it has been observed that the image analysis method can accurately provide the germination and vigor of coffee seeds in a more practical, economical and anticipated way than the conventional test.

Keywords: *Coffea arabica*, Physiological, Technology.

1 INTRODUCTION

Coffee growing in Brazil is one of the most significant agricultural activities, both because of the volume of financial resources involved and because of the use of a significant amount of labor, with a decisive contribution to the development of municipalities in the producing regions (Rena & Maestri, 1986).

Coffee plantations are implanted from seedlings and one of the critical points for their production is to obtain quality seeds, especially vigor. Many steps and care must be taken during seed production, which must be completed with the analysis to evaluate the final quality of the seeds in order to ensure high viability and vigor for the formation of seedlings with the necessary quality for the implementation of vigorous and productive crops. However, the evaluation of the quality of coffee



seeds is a laborious and time-consuming step, since it depends on the removal of the parchment to perform the germination test, which lasts at least thirty days, or the removal of the embryos, an activity that requires a lot of skill and time from the laboratory, when faster results are desired using the tetrazolium test.

The evaluation of seed quality by means of image analysis is a technique studied for several crops and has shown promise for automating the evaluation of viability and vigor tests in seeds, with the potential to reduce the time needed to issue the results (Hoffmaster, 2003).

According to Teixeira, Cícero & Dourado Neto (2003), there has been a favorable evolution for the improvement of computerized techniques, which are more sensitive for capture and more precise for the processing and extraction of useful information for the seed industry, defining an amplification of sensitivity by digital means.

The WINFOLIA® software, owned by Regent (2005) was developed to measure the leaf area of seedlings, from the capture of images in scanners and subsequent analysis of them, with the aid of a computer program.

Therefore, the objective of this research was to evaluate methodologies for the determination of the physiological quality of coffee seeds, using the image analysis technique using the WINFOLIA software.

2 MATERIAL AND METHODS

Thirty-two lots of coffee seeds (*Coffea arabica* L.), with different levels of physiological quality, obtained from UFLA plantations were used. The seeds were submitted to the germination test, where the percentage of root protrusion, normal seedlings at fifteen and thirty days old (Brazil, 2009), strong and weak normal seedlings, and shoot and root dry mass were evaluated.

At the end of the germination test, images of the hypocotyls of the seedlings were captured, which were removed from the seedlings with the aid of a scalpel. The images were processed and digitized using the WINFOLIA® software, specific for the measurement of leaf area, resulting in measurements of the mean area per seedling, total area of the hypocotyls, mean perimeter and total perimeter of the hypocotyls. These results were then correlated with the results obtained in the germination test.

The ASSISTAT software was used for correlation analysis.

3 RESULTS AND DISCUSSION

From the analysis of the results, it was observed that there was a significant correlation between the values of the total area of the hypocotyls of the seedlings, obtained from the correlated image analysis, and all the other results of the evaluation of the physiological quality of the coffee seeds,



obtained through the germination test. The high correlation obtained between this variable and the percentage of normal seedlings at 30 days of age, from 80% to the 1% level of significance, is noteworthy. The value of the mean hypocotyl area per seedling showed a significant correlation only with the percentage of normal seedlings at 30 days, of weak normals, although with lower correlation coefficients.

The total perimeter values of hypocotyls were also correlated with the results of all conventional tests to evaluate the physiological quality of the coffee seeds used, highlighting the high correlation coefficient of this variable with the percentage of normal seedlings at 30 days and root protrusion. The mean hypocotyl perimeter values of the seedlings did not correlate with any of the other results of quality evaluation by means of the germination test. The result of the analysis of digitized images that best correlated with the final result of the germination test, i.e., the percentage of normal seedlings at thirty days, was the total area of hypocotyls of the seedlings evaluated in this study (Table 1)

Table 1. Correlation coefficients between the results of conventional and image analysis tests. SDA-total area of hypocotyls. MDA-Mean Hypocotyl Area. SDP-total perimeter of hypocotyls. MDP-mid-hypocotyl circumference. MSA-seedling shoot dry mass. MSR-dry mass of seedling roots. Seedling-seedling dry mass. N30-percentage of normal seedlings. N Weak-percentage of weak normal seedlings. N Strong - percentage of strong normal seedlings. Dead-percentage of dead seedlings. FLSCO-percentage of seedlings with open cotyledonary leaves at 45 days. Significance levels: ns – not significant; * - significant at the level of 5%; ** - significant at the level of 1%.

	SDA	MDA	SDP	MDP	MSA	MSR	Seedling
SDA		0.6667**	0.8912**	0.3328**	0.1855*	0.1599*	0.1889*
MDA	0.6667**		0.5792**	0.6725**	0.0480ns	0.1424ns	0.1472ns
SDP	0.8912**	0.5792**		0.5966**	0.0682ns	0.0934ns	0.1031ns
MDP	0.3328**	0.6725**	0.5966**		-0.1836*	0.0141ns	-0.0193ns
MSA	0.1855*	0.0480ns	0.0682ns	-0.1836*		0.0602ns	0.2384**
MSR	0.1599*	0.1424ns	0.0934ns	0.0141ns	0.0602ns		0.9837**
Seedling	0.1889*	0.1472ns	0.1031ns	-0.0193ns	0.2384**	0.9837**	
Prot Rad	0.7706**	0.3362**	0.6643**	0.0115ns	0.1388ns	0.1166ns	0.1384ns
N30	0.8065**	0.2747**	0.6846**	-0.0295ns	0.1503*	0.1137ns	0.1377ns
N Weak	0.3120**	0.1784*	0.1732*	-0.1167ns	0.0889ns	0.2225**	0.2325**
N strong	0.3037**	0.0326ns	0.3477**	0.0922ns	0.0264ns	-0.1324ns	-0.1240ns
Dead	-0.7856**	-0.3270**	-0.6743**	0.0039ns	-0.1341ns	-0.1224ns	-0.1432ns
Abnormal	-0.2103**	0.0645ns	-0.1608*	0.0698ns	-0.0744ns	-0.0284ns	-0.0410ns
FLSCO	0.5975**	0.4574**	0.4325**	0.1088ns	0.2448**	0.1647*	0.2043**
	Prot Rad	N30	N Weak	N strong	Dead	Abnormal	FLSCO
SDA	0.7706**	0.8065**	0.3120**	0.3037**	-0.7856**	-0.2103**	0.5975**
MDA	0.3362**	0.2747**	0.1784*	0.0326ns	-0.3270**	0.0645ns	0.4574**
SDP	0.6643**	0.6846**	0.1732*	0.3477**	-0.6743**	-0.1608*	0.4325**
MDP	0.0115ns	-0.0295ns	-0.1167ns	0.0922ns	0.0039ns	0.0698ns	0.1088ns
MSA	0.1388ns	0.1503*	0.0889ns	0.0264ns	-0.1341ns	-0.0744ns	0.2448**
MSR	0.1166ns	0.1137ns	0.2225**	-0.1324ns	-0.1224ns	-0.0284ns	0.1647*



Plantula	0.1384ns	0.1377ns	0.2325**	-0.1240ns	-0.1432ns	-0.0410ns	0.2043**
Prot Rad		0.8846**	0.4332**	0.2437**	-0.9534**	-0.0337ns	0.4650**
N30	0.8846**		0.3604**	0.4024**	-0.9098**	-0.3810**	0.5141**
N Weak	0.4332**	0.3604**		-0.7089**	-0.4265**	0.0675ns	0.2059**
N strong	0.2437**	0.4024**	-0.7089**		-0.2693**	-0.3544**	0.1866*
Dead	-0.9534**	-0.9098**	-0.4265**	-0.2693**		-0.0097ns	-0.4761**
Abnormal	-0.0337ns	-0.3810**	0.0675ns	-0.3544**	-0.0097ns		-0.1836*
FLSCO	0.4650**	0.5141**	0.2059**	0.1866*	-0.4761**	-0.1836*	

4 CONCLUSION

The use of image analysis with the aid of the WINFOLIA® program is a promising method for the evaluation of the viability and vigor of coffee seed lots, especially using the total area of seedling hypocotyls.



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Influence of fertigation on seed quality coffee



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ABSTRACT

Water availability and mineral nutrition are factors that affect the coffee crop and, when poorly managed, can cause excessive plant growth, delayed fruit maturation, leaching of soluble nutrients (mainly nitrogen), flower drop, higher occurrence of soil diseases and physiological disorders, higher energy costs and wear and tear of the irrigation system. This work was developed with the purpose of studying, among others, the effects of fertigation on the physical and physiological quality of coffee seeds produced in irrigated coffee plantations under different doses and installments of fertilization. In addition to the traditional system without irrigation and fertilized four times a year during the rainy season (control), five fertilization rates were studied, 70, 100, 130, 160 and 190% of the recommended for coffee fertigated by a drip system and two installments, twelve and four applications during the year. After harvesting the fruits at the cherry stage and drying, the seeds without parchment were submitted to analyses to evaluate the physiological quality, by means of the germination and vigor tests, and the physical quality, by the sieve retention test. It was concluded that the fertigation treatments used affect the physical quality of coffee seeds and do not affect the physiological quality.

Keywords: *Coffea arabica* L., Quality, Water.

1 INTRODUCTION

In areas where the dry season is well defined, such as the Brazilian Cerrado, the use of irrigation in coffee cultivation is essential, since in this region there are problems of water deficit (Silva et al., 2000). According to Santinato and Silva (2001), in order to obtain a crop formation with economic success, it is necessary to have an adequate formation of the seedlings, since it is a perennial crop where it will be conducted for many years. Nutrition is one of the determining factors in the development of coffee plants (Matiello et al., 2006). Water availability and mineral nutrition are vital factors during some phenological periods of the coffee plant, and can cause excessive plant growth, delayed fruit maturation, leaching of soluble nutrients (mainly nitrogen), flower drop, higher occurrence of soil



diseases and physiological disorders, higher energy costs and wear and tear of the irrigation system. Studies have shown, for example, that carbohydrate metabolism is very sensitive to changes in the water status of plants, and the accumulation of assimilates is affected by the availability of water and nutrients, among other factors.

Mengel & Kirkby (1982) observed that when an external water stress translates into an internal water deficit, there are a number of physiological and metabolic changes, reducing growth. Within this context, this work was developed with the purpose of studying, among others, the effects of fertigation on the physical and physiological quality of coffee seeds produced in irrigated coffee plantations under different doses and installments of fertilization.

2 MATERIAL AND METHODS

The drip irrigation system was equipped with simultaneous control of fertilizer application in the three replications, and soil moisture was indirectly monitored by tensiometers installed at depths of 10, 25, 40 and 60 cm, whose readings were used to calculate water depths. In addition to the traditional system without irrigation and fertilized four times a year during the rainy season (control), five fertilization rates were studied, 70, 100, 130, 160 and 190% of the recommended for coffee fertigated by a drip system and two installments, twelve and four applications during the year.

To evaluate the quality of the seeds produced in this crop, after harvesting the fruits at the cherry stage, they were pulped and demucilated by submersion in water for 24 hours and washed in running water. After drying, the seeds without parchment were submitted to analyses to evaluate the physiological quality, by means of germination and vigor tests, and of the physical quality, by the sieve retention test. The experimental design was randomized blocks in a factorial scheme, with three replications.

3 RESULTS AND DISCUSSION

It was found that the mean percentage of root protrusion was not influenced by fertilization levels (Table 1)



Table 1 – Average percentage of root protrusion of coffee seeds obtained from fruits harvested at different levels of fertilization in two installments

Doses	Installment	
	4 months	12 months
70 %	89.17 Aa α	90.50 Aa α
100 %	90.17 Aa α	89.67 Aa α
130 %	91.33 Aa α	87.83 Aa α
160 %	90.33 Aa α	87.67 Aa α
190 %	92.67 Aa α	83.83 From α
Test	87α	

Averages followed by the same uppercase Arabic letter in the column and lowercase in the row do not differ from each other by the Scott Knott test at 5%. Treatment with the same Greek letter as the control in the spine does not differ from the same by the 5% Bonferroni t-test.

It was verified that these levels, even the lowest level of fertilization, did not affect the percentage of root protrusion of the seeds, not differing from the control. In the same table, it was observed that the 12-month installment at the 190% dose provided a lower percentage of root protrusion.

No effects of the treatments were found on the percentage of normal seedlings, strong normal seedlings, cotyledonary leaves and average root dry matter weight (Tables 2, 3, 4 and 5).

Table 2 – Average percentage of normal seedlings of coffee seeds obtained from fruits harvested at different levels of fertilization in two installments.

Doses	Installment	
	4 months	12 months
70 %	82.83 Aa α	81.50 Aa α
100 %	81.77 Aa α	80.83 Aa α
130 %	75.17 Aa α	77.33 Aa α
160 %	83.33 Aa α	75.50 Aa α
190 %	83.67 Aa α	76.00 Aa α
Test	76 α	

Averages followed by the same uppercase Arabic letter in the column and lowercase in the row do not differ from each other by the Scott Knott test at 5%. Treatment with the same Greek letter as the control in the spine does not differ from the same by the 5% Bonferroni t-test.

Table 3 – Average percentage of strong normal seedlings of coffee seeds obtained from fruits harvested at different levels of fertilization in two installments.

Doses	Installment	
	4 months	12 months
70 %	36.33 Aa α	50.17 Aa α
100 %	27.67 Aa α	39.50 Aa α
130 %	28.33 Aa α	31.17 Aa α
160 %	52.83 Aa α	34.50 Aa α
190 %	35.50 Aa α	39.83 Aa α
Test	24 α	

Averages followed by the same uppercase Arabic letter in the column and lowercase in the row do not differ from each other by the Scott Knott test at 5%. Treatment with the same Greek letter as the control in the spine does not differ from the same by the 5% Bonferroni t-test.

In general, it is observed in the average percentage of cotyledonary leaves a trend of higher averages in the 4th installment in the increasing doses.



Table 4 – Average percentage of cotyledonary leaves of coffee seeds obtained from fruits harvested at different levels of fertilization in two installments.

Doses	Installment	
	4 months	12 months
70 %	46.00 Aa α	42.00 Aa α
100 %	44.67 Aa α	42.67 Aa α
130 %	59.33 Aa α	51.33 Aa α
160 %	52.67 Aa α	40.67 Aa α
190 %	47.33 Aa α	42.00 Aa α
Test	42 α	

Averages followed by the same uppercase Arabic letter in the column and lowercase in the row do not differ from each other by the Scott Knott test at 5%. Treatment with the same Greek letter as the control in the spine does not differ from the same by the 5% Bonferroni t-test.

Table 5 – Average root dry matter weight of coffee seeds obtained from fruits harvested at different fertilization levels in two installments

Doses	Installment	
	4 months	12 months
70 %	0.53 Aa α	0.53 Aa α
100 %	0.49 Aa α	0.55 Aa α
130 %	0.59 Aa α	0.51 Aa α
160 %	0.53 Aa α	0.46 Aa α
190 %	0.48 Aa α	0.55 Aa α
Test	0,45 α	

Averages followed by the same uppercase Arabic letter in the column and lowercase in the row do not differ from each other by Scott Knott's test at 5%. Treatment with the same Greek letter as the control in the spine does not differ from the same by the 5% Bonferroni t-test.

These results differ from those found by Mauri et al. (2005), who found that a better mineral nutrition of the mother plants can positively influence the germination and vigor of the seeds and, consequently, provide more vigorous and better quality seedlings. However, they corroborate Lima et al. (2003) who did not observe the effect of fertilization on the physiological quality of coffee seeds.

Although no significant difference was observed in the percentage of normal seedlings (Table 2), there is a trend towards better results in the four-month installment plan and higher doses. It is also verified that for the 12-month installment plan, the higher the dosage, the lower the values of normal seedlings.

Differently from the other variables related to physiological quality, the mean hypocotyl dry matter weights (Table 6) showed significant differences between treatments. Higher mean hypocotyl dry matter weights were obtained using the 130% dose and 12-month installments. Laviola et al. (2007) found that coffee cultivars have statistically equal leaf dry matter weights, regardless of the level of fertilization to which the mother plants were submitted.



Table 6 – Average dry matter weight of the hypocotyl of coffee seeds obtained from fruits harvested at different levels of fertilization in two installments.

Doses	Installment	
	4 months	12 months
70 %	0.65Aa α	0.65 A
100 %	0.63Aa α	0.71No α
130 %	0.68From α	0.83Aa α
160 %	0.69Aa α	0.67The α
190 %	0.51Bb α	0.62Ba α
Test	0,61 α	

Averages followed by the same uppercase Arabic letter in the column and lowercase in the row do not differ from each other by the Scott Knott test at 5%. Treatment with the same Greek letter as the control in the spine does not differ from the same by the 5% Bonferroni t-test.

All the variables studied related to the physiological quality of the seeds did not differ statistically from the control, unlike the variables related to the physical quality. It was found that, for sieve retention, there were significant differences between the treatments and the control (Tables 7 and 8). The control had a sieve percentage of 20 statistically higher than all the doses of the installment plan (Table 7). This indicates that, perhaps in this splitting, the higher doses may favor the obtaining of seeds from this sieve and not favor the physiological quality.

Sá (1994) commented that fertilization is one of the factors that can affect the size, weight and vigor of seeds, and, in many situations, these effects can be linked to the permeability and integrity of seed tissue membranes, since several nutrients act as enzymatic activators or as components of these membranes.

Table 7 – Average percentage of sieve retention 20 of coffee seeds obtained from fruits harvested at different levels of fertilization in two installments UFLA, Lavras - MG, 2011.

Doses	Installment	
	4 months	12 months
70 %	48.45Aa β	52.18Aa α
100 %	40.60 Aa β	55.30Aa α
130 %	47.17Aa β	55.21Aa α
160 %	50.65Aa β	55.12Aa α
190 %	53.72Aa β	54.18Aa ab
Test	63,74 α	

Averages followed by the same uppercase Arabic letter in the column and lowercase in the row do not differ from each other by the Scott Knott test at 5%. Treatment with the same Greek letter as the control in the spine does not differ from the same by the 5% Bonferroni t-test.

It was observed that in the 160% dose treatment, in addition to the sieve percentage values 20 not differing within the 4 and 12-month installments, they also did not differ statistically from the control (Table 8)



Table 8 – Average percentage of sieve retention 18 of coffee seeds obtained from fruits harvested at different levels of fertilization in two installments

Doses	Installment	
	4 months	12 months
70 %	36.96Aa α	18.84From β
100 %	45.92Aa α	24.52From β
130 %	32.15Aa β	25.32Aa β
160 %	34.65Aa α	33.23Aa α
190 %	22.04Aa β	31.31Aa α
Test	20,16 β	

Averages followed by the same uppercase Arabic letter in the column and lowercase in the row do not differ from each other by the Scott Knott test at 5%. Treatment with the same Greek letter as the control in the spine does not differ from the same by the 5% Bonferroni t-test.

The results of this study showed the importance of adequate fertilization in the production of coffee seeds, since the results obtained so far are few and inconsistent, requiring further studies related to adequate doses and interference in seed production and quality (Imolesi et al., 2001). The recommendation of fertilizers for planting crops for seed production is generally similar to that used for grain production (Maeda et al., 1986).

4 CONCLUSION

The fertigation treatments used affect the physical quality of coffee seeds and do not affect the physiological quality.



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Survey of fungi present in plant species of the dry forest in the microregion of Januária – MG



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ABSTRACT

The dry forest is characterized by presenting a seasonal rhythm qualified by leaf fall during the dry period followed by recovery of the plant canopy in the rainy season. In this environment, there are studies related to the plant characterization of the area, but its microbiota is little studied. Thus, the objective of this work was to carry out a survey of phytopathogenic and endophytic fungal species found in plant species of the dry forest of the microregion of Januária-MG. Plants with typical disease symptoms were collected and the fungi isolated in the laboratory. To obtain the endophytic fungi, healthy tissues were collected. Morphological analyses were implemented to characterize the genus. In addition, the mycelial growth velocity index, characterization of mycelial color and texture were calculated. Thirty-three colonies of phytopathogenic fungi and thirteen colonies of endophytic fungi were isolated from the thirteen plant species collected. It was possible to identify the phytopathogenic fungus of the genus *Pestalotia* sp in cashew (*Anacardium humile*), jatobá (*Hymenaea courbaril*) and parakeet (*Cochlospermum orinocense*) plants and the fungus *Penicilium*, endophytic in castor bean plants (*Esenbeckia febrifuga*).

Keywords: *Febrifuge esenbeckia*, Etiology, Endophytic fungus, *Pestalotia*.

1 INTRODUCTION

The dry forest or seasonal deciduous forest is characterized by a sharp drop of leaves during the dry season. In the north of Minas Gerais, in the region of Januária, a typical dry forest can be found, on the limits of the municipality with Cônego Marinho and Bonito de Minas. Interest in the knowledge of fungal species with potential employment in agricultural practices has increased significantly with the discovery of plant growth promoting microorganisms, biological control agents for pests, pathogens, and weeds. Studies in the area of biological control invest in the use of microorganisms as



potential substitutes for chemical products in crops, thus favoring the preservation of the environment (Peixoto Neto et al., 2002).

Due to the fact that the dry forest is a little-explored biome, the chances of finding new microorganisms associated with plant species are high. The present work proposed to isolate and identify phytopathogenic and endophytic fungi present in dry forest species in the microregion of Januária/MG from the description morphological characterization of the genus, definition of mycelial growth rate, color and texture of the mycelium. In addition, the objective of this study was to set up a mycotheque and a herbarium with injured tissues to record the patterns of injury caused by phytopathogenic fungi.

2 MATERIAL AND METHODS

The work was carried out at IFNMG – Campus Januária – Laboratory of Phytopathology and at the State University of Montes Claros – Laboratory of Epidemiology and Biocontrol of Microorganisms. The samples were collected in the dry forest in a locality located on the banks of the municipal highway between Brejo do Amparo and the interchange that gives access to Cônego Marinho and Bonito de Minas.

Samples were collected between February and April 2012, during the rainy season. The material was taken from stems and leaves of plants with necrotic and healthy lesions. The trees were georeferenced. In the laboratory, part of the material was separated to make exsiccata. The indirect isolation of the fungus was performed in potato dextrose agar (PDA) medium and the material was kept at 25°C and photoperiod of 12 h.

In order to isolate endophytic fungi, it was proposed to use different culture media in order to select the medium that would allow greater recovery of these microorganisms. Thus, the following were used: acidified PDA, Sabouraud, Nutrient Agar and Plate Count Agar (PCA). For three days, the number of distinct colonies developed on each plate was counted. One colony was considered different from the other according to the color and texture of the mycelium it presented. The isolates obtained in pure culture were transferred to test tubes containing BDA and stored at 4°C. The identification of the genus was performed based on morphological characters according to dichotomous keys for imperfect fungi (Barnett, 1998).

To define the mycelial growth velocity index (MCVI), a disk of PDA medium with fungal mycelium (0.5 cm) was deposited in the center of each Petri dish with PDA medium, and three plates were used to evaluate the IVCM per isolate. Daily, for seven days, measurements were made in two perpendicular directions of the colony with the aid of a digital caliper. The mean diameter of each colony/evaluation was calculated. These data were used in the calculation of the MCVI, according to



the formula: $MCVI = \Sigma (D - Da) \times N - 1$, where: D = current mean diameter of the colony; Da= average diameter of the previous day's colony; N= number of days after inoculation.

The experimental design was completely randomized, with three replications. To determine the texture and color of the colonies, fifteen isolates were placed to grow in PDA medium under the incubation conditions described above. After seven days, the colonies were analyzed to define the texture according to the methodology of Nobles (1948) cited by Silva (2006).

To evaluate the color of the mycelium of the surface and reverse of the colony, the Munsell Soil Color Charts was used.

Samples were collected from twelve plant species from the dry forest in the region of Januária/MG, among them periquiteira, cagaita, joá, cajuí, jatobá and jacarandá do cerrado. The choice of plant species was made according to their economic or medicinal importance. Of these, 33 colonies of phytopathogenic fungi were obtained.

To obtain the endophytic fungi, the castor bean (*E. febrifuga*) was selected, because it is a medicinal species and did not present any typical lesion of pathogens, demonstrating that, for some reason, it remained resistant to the attack of microorganisms even though it was in an environment where several other species presented.

3 RESULTS AND DISCUSSION

About thirteen colonies of endophytic fungi were obtained from castor bean samples. The Nutrient Agar medium showed the lowest performance in the recovery of fungal colonies.

Table 1. Morphological characterization of 15 isolates from plant species from the dry forest of the region of Januária/MG based on the mycelial growth velocity index (IVCM), mycelium color and texture.

Isolado	Hospedeiro	IVCM	Cor micélio aéreo	Cor reverso colônia	Textura
AM 01	Periquiteira	1,224	Branco	Marrom amarelado escuro	Camurça
AM 04	Cajui	1,093	Branco	Branco	Camurça
AM 05	Cajui	1,329	Cinza esverdeado claro	Cinza Claro	Cotonosa
AM 06	Cajui	1,200	Branco	Cinza Claro	Lacunosa
AM 07	Jatobá	1,091	Marrom oliva	Cinza muito escuro	Aveludada
AM 10	Jatobá	1,399	Branco	Branco	Camurça
AM 18	Cagaita	0,988	Cinza esverdeado muito escuro	Cinza esverdeado escuro	Camurça
AM 23	Joá	0,998	Marrom escuro acinzentado	Cinza esverdeado muito escuro	Crostosa
AM 24	Joá	1,089	Branco	Cinza	Farinácea
AM 25	Mamoninha	0,721	Cinza esverdeado	Cinza esverdeado claro	Camurça
AM 31	Mamoninha	0,893	Branco	Cinza claro	Aveludada
AM 32	Mamoninha	2,435	Branco	Preto	Flocosa
AM 34	Jacarandá do Cerrado	1,383	Branco	Marrom oliva claro	Cotonosa
AM 35	Jacarandá do Cerrado	1,008	Cinza	Preto	Plumosa
AM 36	Jacarandá do Cerrado	1,116	Amarelo	Vermelho escuro	Lacunosa

The endophytic isolate AM 25 was identified as *Penicillium* sp. This genus is very common as a causal agent of molds or blue molds, however, it has been reported as an endophytic fungus producing



compounds with interesting characteristics for various purposes (Mathew et al., 2010; Intaradom et al., 2012).

The isolates presented different MVI, with BF 25 and AM 32 being the ones with the lowest and highest MVI, with 0.721 and 2.435 cm/day, respectively (Table 1). The color of the mycelium ranged from greenish, grayish, whitish and darkish tones to suede, cottony, lacunous, velvety, crusted, floury, flaky and feathery (Table 1). The exsiccates were of good quality, allowing the observation of the lesion pattern in the plant tissue.

4 CONCLUSIONS

The fragment of Mata Seca near the municipality of Januária-MG, finds many plant species affected by phytopathogenic fungi.

The genus *Pestalotia* was identified in three plant species and the genus *Penicilium* was identified as an endophytic fungus in castor bean.

The other isolates obtained should be identified, because it is believed, based on the morphological characterization of the colonies, that there is a wide variety of pathogenic and endophytic species in the collected material.

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Sustainable energy in Brazil: Challenges and opportunities for the coming years



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ABSTRACT

Over the last few decades, Brazil has demonstrated a privileged position in the global energy scenario. The country's energy matrix is diversified, with emphasis on the participation of renewable energies, which represent a significant portion of total energy production. Thus, the present research aimed to analyze the main challenges and opportunities for Brazil in the future as a potential energy generator. To this end, a literature review was carried out and, as a result, it was possible to verify that Brazil has opportunities and challenges in the sustainable energy sector. The main energy sources analyzed were hydroelectric, biomass, solar, wind and tidal. In this scenario, the diversification of the energy matrix, with a focus on renewable sources, is important to reduce greenhouse gas emissions and boost sustainable development in the country. The country has a biodiversity that contributes to the promotion of sustainable energies, such as an extensive coastline and regions with favorable winds, mainly in the Northeast and South. However, overcoming challenges such as start-up costs, infrastructure, and regulation is essential for the success of the



country's energy transition, which can attract investment, drive innovation, and strengthen global competitiveness.

Keywords: Energy, Sustainability, Brazil.

1 INTRODUCTION

Over the last few decades, Brazil has demonstrated a privileged position in the global energy scenario. The country's energy matrix is diversified, with emphasis on the participation of renewable energies, which represent a significant portion of total energy production. Hydroelectric power, for example, is one of the main sources used, taking advantage of the abundant hydraulic potential present in several regions of the country. In addition, biomass, wind energy and other renewable sources have gained space in the Brazilian energy matrix, contributing to the reduction of polluting gas emissions (DUPONT; GRASSI; ROMITTI, 2015).

As Bondarik, Pilatti and Horst (2018) point out, energy is a fundamental resource for the economic and social development of a country. In the global context, the search for sustainable and renewable energy sources has gained more and more prominence, due to concerns about climate change and the need to reduce greenhouse gas emissions. In this scenario, Brazil stands out as a major producer of energy, especially from renewable sources, such as hydroelectric, biomass and wind.

Hydroelectric power plays a key role in the Brazilian energy matrix. With a vast hydraulic potential present in several regions of the country, hydroelectric plants have been responsible for a significant portion of the total production of electricity. Brazil has large water reservoirs, such as those of the Itaipu, Belo Monte and Tucuruí power plants, which can generate significant amounts of electricity (JOURDA, 2016).

In addition to hydropower, biomass is another important source of renewable energy in Brazil. The country has a vast agricultural production, which results in a large amount of organic waste. These residues, such as sugarcane bagasse and agricultural crop residues, can be transformed into biomass and used for energy generation. Biomass plants have expanded in the country, contributing to the diversification of the energy matrix and to the reduction of polluting gas emissions (ALMEIDA; GUIMARÃES, 2022).

Wind energy has also gained significant ground in Brazil. With an extensive coastline and favorable wind conditions in several regions, the country has great potential for generating energy from wind. Wind farms have been built in different Brazilian states, especially in the Northeast, where wind conditions are more favorable. Wind energy has proven to be a viable and sustainable alternative, contributing to the country's energy matrix and to the reduction of greenhouse gas emissions (CUNHA et al., 2019).

In addition to these sources, other renewable energies are also gaining ground in Brazil. The country has been investing in technologies such as solar energy, biogas, and tidal energy, exploring



their potential in different regions. Solar energy, for example, has shown significant growth in recent years, driven by government incentives and the reduction in the costs of solar panels. This diversification of the Brazilian energy matrix contributes to increasing the country's energy security, reducing dependence on non-renewable sources, and mitigating environmental impacts (REIS; FATIGUES; CARVALHO, 2009).

According to Freire (2014), Brazil has faced challenges in the energy area, such as the need for investments in infrastructure and the search for sustainable solutions for energy generation and consumption. The transition to a cleaner and more sustainable energy model requires adequate public policies, incentives for the development of renewable technologies, and investments in research and development. However, the country has significant potential to further expand the use of renewable sources and play an important role in the global transition to a more sustainable energy matrix.

Brazil's prominent position as a major energy producer not only impacts the national economy, but also has environmental and social implications. The construction of large hydroelectric power plants, for example, can cause the displacement of traditional communities and generate significant environmental impacts, such as the flooding of environmental preservation areas. The use of biomass as an energy source, in turn, can generate concerns related to sustainability and the proper management of natural resources (DUPONT; GRASSI; ROMITTI, 2015).

In this context, this study sought to investigate, as a problematization, the following research question: "What are the main challenges and opportunities in Brazil in the coming years for the expansion and consolidation of a sustainable energy matrix, considering the available renewable sources and their environmental and social impacts?".

The proposed problematization is based on the need to understand the challenges and opportunities that Brazil will face in the coming years to promote the expansion and consolidation of a sustainable energy matrix. Although the country has a privileged position in the global energy scenario, with emphasis on the participation of renewable energies, there are a number of issues to be addressed to ensure the sustainability of this sector.

Thus, the present research aimed to analyze the main challenges and opportunities for Brazil as a potential future energy generator. To this end, the bibliographic research method was applied, which is, according to Gil (2011), a survey of documents already published, such as books, scientific articles, dissertations, theses, technical reports, among others, which address the theme in question. This research method allows obtaining up-to-date information based on previous studies, contributing to the deepening of knowledge on the subject.

Through this literature review, it is expected to contribute to the deepening of knowledge about Brazil as a major energy producer, highlighting its importance in the global energy scenario and the issues related to this position. The analysis of energy sources, their impacts and the public policies



adopted will allow a broader understanding of the opportunities and challenges faced in the Brazilian energy sector. In this way, strategies and guidelines can be identified for an efficient and sustainable management of energy resources, aiming at the economic and social development of the country, in line with the protection of the environment.

2 DEVELOPMENT

2.1 SUSTAINABLE ENERGIES AND OPPORTUNITIES FOR BRAZIL IN THE COMING YEARS

Sustainable energies, also known as renewable and clean energies, are those obtained from natural sources that regenerate or are virtually inexhaustible. Brazil has enormous potential for the development of sustainable energies in the coming years. The country is characterized by having a favorable climate, vast natural resources, and a growing demand for energy. The diversification of the energy matrix and the adoption of renewable sources have become strategic priorities for Brazil, aiming to reduce greenhouse gas emissions, increase energy security and boost sustainable development (LIMA, 2017).

One of Brazil's main natural resources is, according to Lopes and Taques (2016), its abundance of rivers and watersheds, which enables the generation of energy through hydroelectric plants. Hydroelectric power is already a significant source in the Brazilian energy matrix, representing approximately 60% of the installed capacity. The country's hydroelectric potential is still vast, with numerous opportunities for the construction of new plants and the use of small hydroelectric plants, especially in the Amazon region.

The Amazon has a large number of rivers and a favorable topography, which enables the implementation of medium and large hydroelectric plants. Projects such as the Belo Monte Hydroelectric Power Plant, located on the Xingu River, are examples of the region's hydroelectric potential. In addition to the Amazon, other regions of the country also have great potential for hydroelectric power generation. The rivers that cross the Brazilian territory, such as the São Francisco, Paraná, Tocantins, Madeira and others, offer favorable conditions for the construction of plants of different sizes. Hydroelectric power plants contribute to the stability of the national electricity system by providing clean, renewable, and low-cost energy (LOPES; TAQUES, 2016)..

Biomass is also a relevant opportunity for Brazil. The country is a major agricultural producer and has a vast amount of agricultural and forestry waste that can be used as biomass for energy generation. The production of biogas from animal waste and organic waste is also a promising area. The expansion of the use of biomass contributes to the use of waste, the reduction of emissions and the development of sustainable production chains (KITAYAMA, 2008).

The use of biomass as an energy source is a viable and environmentally friendly alternative, as it allows the use of waste that could otherwise become an environmental problem, such as crop



residues, tree bark, sawdust and sugarcane bagasse, among others. These residues can be transformed into solid, liquid or gaseous fuels for the production of heat, electricity or biogas (ALMEIDA; GUIMARÃES, 2022).

In addition, the production of biogas from animal waste and organic waste is another promising area in Brazil, as pointed out by Tachizawa (2011). The anaerobic decomposition of these materials generates biogas, composed mainly of methane, which can be used as fuel for the generation of electricity and heat. The expansion of the use of biogas contributes to the reduction of greenhouse gas emissions, since methane is a more potent greenhouse gas than carbon dioxide.

The use of biomass as an energy source offers several advantages. In addition to reducing dependence on fossil fuels and mitigating the environmental impacts associated with these fuels, biomass contributes to the development of sustainable production chains. The use of agricultural and forestry residues as biomass generates jobs in the production, transportation and processing chain of these materials (KITAYAMA, 2008).

Brazil has great potential to expand the use of biomass as an energy source, due to its vast agricultural and forestry production. This requires the development of efficient and sustainable technologies for the conversion of biomass into energy, as well as appropriate policies and incentives to promote their use. The expansion of biomass will contribute to the use of waste, the reduction of greenhouse gas emissions and the development of a more sustainable and circular economy (MIRANDA; MARTINS; LOPES, 2019).

In addition, it is worth highlighting Brazil's potential in relation to solar energy. The country has one of the best solar irradiations in the world, especially in the Northeast and Midwest regions. These regions are privileged with excellent solar irradiation conditions, which means that they receive high levels of solar radiation throughout the year (PINHO; GALDINO, 2014).

In the Brazilian Northeast, the solar incidence is, according to Machado and Miranda (2015), particularly favorable. The region is known for its tropical and semi-arid climate, with sunny days for most of the year. The dry climate and low cloud cover rates provide a high availability of direct solar radiation, ideal for the production of solar photovoltaic energy. Cities such as Fortaleza, Natal and Recife are among those with one of the highest rates of solar irradiation in Brazil.

The Midwest region, on the other hand, is characterized by a tropical climate, with well-defined seasons and a period of drought during the winter. These climatic conditions favor the generation of solar energy, since the sky is usually open and the solar incidence is intense during most of the year. Cities such as Brasília, Cuiabá and Goiânia have high levels of solar irradiation, making them suitable places for the installation of photovoltaic solar energy systems (PINHO; GALDINO, 2014).

From the perspective of Lana et al. (2015), the presence of these favorable conditions in the Northeast and Midwest regions of Brazil creates an environment conducive to the expansion of solar



energy. The abundant availability of solar radiation allows for the maximum use of photovoltaic solar energy, making it a viable and attractive source for electricity generation.

In addition, these regions are also known for their vast territorial extension, which makes it possible to install large centralized generation solar plants. These projects can take advantage of the availability of land and the favorable climate for the large-scale production of solar energy, contributing to the diversification of the energy matrix and the supply of electricity to adjacent regions (LANA et al., 2015).

The expansion of solar PV has been significant in recent years, driven by a combination of factors such as reduced equipment costs, government incentives, and energy auctions. With the potential still untapped, Brazil can continue to grow as a leader in solar energy, both in centralized generation and in distributed systems (MACHADO; MIRANDA, 2015).

The Brazilian government has implemented incentives and public policies that stimulate the development of solar energy. Among these measures, Normative Resolution 482/2012 of the National Electric Energy Agency (ANEEL) stands out, which established the rules for the electricity compensation system, allowing consumers to generate their own solar energy and exchange the surplus with the local distributor (DUPONT; GRASSI; ROMITTI, 2015).

As pointed out by Reis, Fadigas and Carvalho (2009), another important factor for the growth of solar energy in Brazil is the energy auctions promoted by the government. These auctions aim to contract new renewable energy generation projects, including solar photovoltaics. With the competition among entrepreneurs and the search for competitive rates, auctions have boosted the expansion of solar energy in the country.

Wind energy also presents great opportunities for Brazil. The country has an extensive coastline and regions with favorable winds, especially in the Northeast and South. Brazil's extensive coastline, which stretches for more than 7,000 kilometers, offers a great opportunity for offshore wind energy exploration, i.e., offshore wind power generation. Coastal areas are known to experience strong and constant winds, which is ideal for the installation of offshore wind farms. These farms can be built close to the coast, where the sea depths are suitable for attaching wind turbines to bases on the seabed (MACHADO; MIRANDA, 2015).

In addition to the coast, Brazil also has regions with favorable winds in the Northeast and South of the country. The Northeast stands out as one of the regions with the greatest wind potential in Brazil. This region has constant winds, driven by the trade winds that blow from the Atlantic Ocean. States such as Bahia, Rio Grande do Norte, Ceará and Piauí have a large capacity for wind power generation, with wind farms already installed and in operation. In the South of Brazil, the states of Rio Grande do Sul and Santa Catarina stand out, which also have favorable winds for wind power generation. The



southern region is influenced by cold wind currents coming from Antarctica, creating favorable conditions for the installation of onshore wind farms (LANA et al., 2015).

Alves (2010, p. 165) emphasizes that:

Faced with the global scenario of climate change, the importance of renewable energies is growing more and more in the spaces of government planning about their energy matrices. In Brazil, the wind potential has aroused the interest of several manufacturers and representatives of the main countries involved with wind energy. It is worth remembering that the Northeast region was one of the pioneers in the installation of wind energy for use in the generation of electricity. The facilities already in operation show an important initiative both by the Brazilian concessionaires responsible for the experimental projects and by the self-producing energy companies that, within the new scenario of the electricity sector, invest in the development of wind power for energy generation. For the field of wind energy development in Brazil, the figure of the self-producer and the independent producer are fundamental in the expansion of this sector, and in the promotion of a sustainable energy matrix.

The exploration of Brazil's wind potential has been driven by government incentives, such as wind energy auctions, which promote the contracting of new projects and enable the expansion of the sector. These initiatives have contributed to the significant growth of installed wind energy capacity in the country. The use of wind resources in Brazil has the potential to boost the economic and social development of the regions where wind farms are installed. The construction of these projects generates direct and indirect jobs, stimulates the local production chain and attracts investments in the areas of infrastructure and services (ALVES, 2010).

The installed capacity of wind energy has increased considerably in recent years, with successful auctions and technological advancements. The continuous expansion of wind energy contributes to the diversification of the energy matrix, reducing dependence on non-renewable sources and offering opportunities for job creation and regional development (NASCIMENTO; MARIE; CUNHA, 2012).

According to Nascimento, Mendonça and Cunha (2012, p. 647):

[...] The environmental benefits that innovations in the Brazilian wind sector have been experiencing are related to the exploration of new sources of raw material for energy production, contributing to the reduction of polluting gases and reducing dependence on fossil fuels. In addition to structuring a complementary form for hydraulic energy, with a view to increasing the potential for wind energy production in periods of drought. It was also possible to verify that the institutional issues that govern the national energy system and the wind energy sector system have enabled and encouraged the feasibility of wind projects, highlighting the economic, environmental and social benefits that the strengthening of this industry can provide to the country.

Finally, it is worth mentioning the future opportunities that Brazil has in relation to tidal energy. Brazil has an extensive coastline, with a coastline that stretches for about 7,400 km. This represents great potential for tidal power generation. The North and Northeast regions of the country, in particular, have favorable geographical characteristics for the exploitation of this form of energy, with large areas of estuaries, bays and deltas (FISCHER, 2014).



The opportunities for Brazil in tidal energy in the coming years are significant. The country has a great potential for generating electricity from this source, which is still in the early stages of development. The implementation of tidal energy projects would bring several benefits, such as the diversification of the energy matrix, the reduction of greenhouse gas emissions, and the promotion of technological and industrial development (NETO et al., 2011).

According to Oliveira (2016), there are different technologies available for tidal energy generation. One of them is the installation of dams or dikes in favorable coastal areas, where seawater enters and leaves according to the tides. This movement of water drives hydraulic turbines that generate electricity. This technology is known as tidal energy of impoundage.

Another technology under development is the use of submerged turbines or tidal current turbines, which are installed on the seabed and capture the kinetic energy of ocean currents. This technology is known as tidal tidal energy. Brazil has great potential for the implementation of this technology, especially in the Amazon region, where the mouths of rivers generate strong sea currents (SESMIL, 2013).

However, it is important to highlight that tidal energy presents technical, environmental, and economic challenges. The construction of offshore structures requires significant investments and the technology is still in the development and improvement phase. In addition, careful environmental impact studies need to be carried out to minimise the effects on marine ecosystems (SILVA et al., 2018).

To seize the opportunities in tidal energy, Brazil should encourage research and development in this area, promote public-private sector partnerships, establish appropriate regulatory frameworks, and offer financial incentives. Collaboration with countries that already have experience in this form of energy, such as the United Kingdom and France, can be beneficial for mutual development and learning (TOLMASQUIM, 2016).

According to Fischer (2014), tidal energy can contribute to the diversification of Brazil's energy matrix, reducing dependence on non-renewable sources and mitigating the environmental impacts associated with electricity generation. In addition, the implementation of tidal energy projects can boost regional economic growth, generate jobs, and strengthen domestic industry.

2.2 SUSTAINABLE ENERGIES AND THE CHALLENGES FOR BRAZIL IN THE COMING YEARS

Brazil is a privileged country in terms of water resources, with a vast potential for the generation of electricity through hydroelectric plants. However, the development of this type of energy is not without significant challenges.

According to Freire (2014), one of the main challenges faced by hydroelectric power in Brazil is the environmental issue. The construction of large dams to create reservoirs can result in significant



environmental impacts, such as the displacement of local communities, the flooding of forest areas, and the loss of natural habitats. In addition, the formation of reservoirs can lead to the accumulation of organic matter and the consequent decomposition process, releasing greenhouse gases, such as methane, into the atmosphere. These environmental impacts are the subject of debates and controversies regarding the viability and sustainability of hydropower.

In addition, Lopes and Taques (2016) point out that another challenge to be considered is climate variability. Brazil is a country of continental dimensions and has different river basins, with different rainfall regimes. In years of scarce rainfall or hydrological imbalance, hydroelectric power generation may be compromised, resulting in a decrease in electricity production capacity. This can lead to the activation of thermal power plants, which use fossil fuels, increasing production costs and greenhouse gas emissions.

The over-reliance on hydropower also makes the sector vulnerable to extreme weather events, such as prolonged droughts or intense floods. In addition, hydropower requires large financial investments and a long construction time. The implementation of large hydroelectric power plants requires considerable resources, involving the construction of dams, turbines, transmission lines and associated infrastructure. These investments can be risky, as demand projections and energy prices can fluctuate over time, affecting the profitability of these projects. In addition, bureaucracy and environmental licensing processes can delay the construction and start-up of new hydroelectric plants (ALMEIDA; GUIMARÃES, 2022).

Another important challenge is the need to diversify the energy matrix. While hydropower is an important and renewable source, over-reliance on it can make the Brazilian energy sector vulnerable to fluctuations in water supply and the impacts of climate change. For this reason, it is essential to explore and develop other energy sources, such as wind, solar, biomass and even nuclear energy. The diversification of the energy matrix contributes to the security of supply and the reduction of dependence on a single source (NETO et al., 2020).

According to Lopes and Taques (2016, p. 91):

With a water potential that represents more than 12% of the world's surface fresh water, conducive to the installation of hydroelectric plants and SHPs, disregarding the burden of the environmental impacts of its facilities, with the convenience of relying on sugarcane harvests in periods of drought, combined with the high incidence of winds on the north and northeast coasts, The lack of effectiveness of the Brazilian government's actions is evident. The Brazilian energy matrix, therefore, ends up concentrated in the use of fossil fuels and hydroelectric power, despite the favorable scenario for its diversification.

Biomass energy also faces some significant challenges in its implementation and expansion in the country. This is because one of the main challenges of biomass energy in Brazil is the availability and logistics of raw material supply. Although the country is rich in agricultural and forestry resources, the collection, transportation, and storage of biomass waste can be complex and costly. The energy



efficiency of biomass can also vary depending on the type of material used, the way it is processed, and the technology employed. Proper logistics and infrastructure are essential to ensure a continuous and sustainable supply of biomass (LOPES; TAQUES, 2016).

Tabatabaei and Ghanavati (2018) point out that another challenge is related to the environmental sustainability of biomass energy. Although it is a renewable source, burning biomass for power generation can generate greenhouse gas emissions such as carbon dioxide and nitrogen oxides. In addition, the expansion of biomass production can generate additional pressure on natural resources, such as forest areas and agricultural crops, which can lead to deforestation and biodiversity loss. It is essential to implement sustainable practices, such as the use of appropriate forest management techniques and the encouragement of biomass production from agricultural and industrial waste, in order to minimize environmental impacts.

It is also worth highlighting the economic competitiveness of biomass energy in relation to other energy sources. Production costs, especially those related to transportation, processing, and storage of biomass, can be high, affecting the economic viability of projects. In addition, uncertainty regarding stimulus policies and volatile fossil fuel prices may affect the attractiveness of biomass energy investments. A stable and enabling regulatory environment, along with adequate incentives, is needed to boost the development and competitiveness of this energy source (TABATABAEI; GHANAVATI, 2018).

The need for advanced technologies and investments in research and development is another important challenge in biomass energy in the country. The search for more efficient processes for converting biomass into energy, such as gasification and pyrolysis, can provide an increase in efficiency and use of resources. In addition, the development of carbon capture and storage technologies could further reduce emissions associated with biomass burning (UCZAI, 2009).

Lopes and Taques (2016, p. 92) argue that:

The lack of effectiveness, therefore, is not a sign of non-evolution of environmental policies practiced in the country. Evaluating only actions, without analyzing their results, even though there are opportunities for the implementation of wind farms, greater incentives for ethanol, use of biomass, all of which can be used in a complementary way, it is possible to affirm that there is a concern in Brazil with its environmental policies and its sustainable growth. However, the advances in terms of policies and control do not reflect the inconsistent results, leveraged by the complexity in the definition of the set of energy sustainability indicators, and demonstrate at certain times that their complexity, despite all the incentives, inhibits investors and they spend energy on the means and not on the end goal.

As for solar energy, it faces several important challenges in Brazil. One of the main obstacles is the high initial cost of installing solar systems. While solar panel prices have decreased over time, considerable investment is still required to purchase and install a PV system. This makes it difficult for many people to access solar energy, especially those with low incomes (MACHADO; MIRANDA, 2015).



According to Almeida and Guimarães (2022), there is still a lack of awareness and education about the benefits of solar energy. Many people in Brazil are unaware of the technical aspects and the environmental and economic benefits of this form of energy. It is essential to invest in awareness and education campaigns to inform the population about the advantages of solar energy, highlighting its potential to reduce electricity costs and its positive impact on the environment.

Also according to the aforementioned author, Brazil faces challenges related to infrastructure and regulation. Connecting solar systems to the existing power grid requires investments in power distribution and transmission infrastructure. It is also necessary to establish clear and stable regulations that encourage distributed solar power generation and establish transparent rules for grid connection and compensation of excess generated energy. It is important to create an enabling environment for solar energy investments, with consistent policies and incentives that attract businesses and investors.

The variability of solar generation is another challenge to be addressed. Solar energy production depends on the availability of sunlight, which varies throughout the day and throughout the year. This requires the use of energy storage systems or supplementation with other energy sources to ensure a continuous supply. The expansion of energy storage capacity and the development of load management technologies are key to overcoming this variability and ensuring an efficient integration of solar energy into the country's electricity matrix (LANA et al., 2015).

Bureaucracy and permitting processes also pose significant challenges for solar energy in Brazil. Obtaining licenses and permits for the installation of solar systems can be a time-consuming and complex process, which discourages potential investors and hinders the growth of the industry. Simplifying and streamlining licensing processes, reducing bureaucracy, are measures that can boost the development of solar energy in the country (DANTAS; POMPERMAYER, 2018).

Regarding wind energy, Ferreira (2019) points out that infrastructure is one of the main challenges of this type of energy in the country. The construction of wind farms requires significant investments in terms of installing turbines, transmission grids, and energy storage systems. This infrastructure needs to be planned and implemented in an efficient manner to ensure that the energy generated by wind farms is integrated into the national grid in a stable and reliable manner.

It is also necessary to consider the availability of suitable areas for the installation of wind farms. Although Brazil has a huge wind potential along its extensive coastline and in some inland regions, it is necessary to identify locations with consistent and favorable wind speeds to ensure the economic viability of the projects. In addition, it is necessary to consider the environmental and social impact of these facilities, ensuring the preservation of sensitive areas and involving local communities in the development process (FERREIRA, 2019).

The issue of energy storage is also a major challenge for wind energy in Brazil. Unlike thermal or hydroelectric power plants, wind generation depends directly on wind conditions. This means that



energy production is intermittent and varies over time. Therefore, it is essential to develop efficient storage solutions, such as batteries or pumped storage systems, to address variability and ensure a continuous and stable supply of electricity.

The regulatory issue and incentive mechanisms are also challenges for wind energy in Brazil. Although the country has adopted policies to stimulate the expansion of renewable energies, such as energy auctions and the creation of the incentive program called *RenovaBio*, there are still bureaucratic obstacles and a lack of clarity in the rules and guidelines for the implementation of wind farms. A stable, predictable and favourable energy policy is key to attracting investment and boosting the sector (BARROSO, 2022).

According to Alves (2010, p. 186):

As long as renewable technologies are at a small stage of development, with high cost and small market share, it is necessary to have a solid legal, regulatory and institutional apparatus in order to reduce risks for financiers. In addition to all these points raised, a change in the international context should be kept in mind, with the increase in the price of oil and the ratification of the Kyoto Protocol.

Nevertheless, the maintenance and operation of wind farms represent an ongoing challenge. Wind turbines are complex machines that require regular and specialized maintenance to ensure their efficiency and longevity. The lack of skilled labor and the need for adequate support infrastructure can affect the performance and availability of wind farms (ALVES, 2010).

Last but not least, it is worth mentioning the challenges for Brazil in the coming years in relation to tidal energy. Authors such as Fischer (2014) emphasize that there is a need for technology for the capture and conversion of tidal energy. There are different methods to harness this energy, such as tidal turbines, floating buoy systems, and wave energy systems. Each method presents its own technical complexities and requires significant investments in research and development to adapt them to the specific conditions of the Brazilian coast.

In addition to technology, there is a lack of adequate infrastructure for the installation of tidal energy projects, given that it is necessary to build structures such as dams, dikes, and power stations to capture and convert tidal energy into electricity. This requires careful planning and substantial investments in civil engineering (TOLMASQUIM, 2016).

Environmental permitting is a significant challenge for tidal energy projects. The process of obtaining environmental licenses in Brazil is known to be complex and time-consuming. Tidal energy projects involve environmental impacts and, therefore, must undergo rigorous evaluation to ensure sustainability and preservation of coastal ecosystems (TOLMASQUIM, 2016).

According to Neto et al. (2011), another point of attention is the cost of tidal energy. At the current stage of development, tidal energy projects can be more expensive than conventional energy sources. Initial investments are high due to the need for specialized technology and specific



infrastructure. However, it is important to highlight that as technology advances and the scale of production increases, costs are expected to gradually decrease.

An additional challenge is the integration of tidal energy into the existing power grid. Tidal energy is intermittent, depending on the tidal cycle, which requires energy storage systems or a flexible power grid capable of handling generation variations. Construction of transmission lines and upgrading of electrical infrastructure may be necessary to accommodate tidal energy efficiently and reliably (NETO et al., 2011).

Finally, another challenge faced by tidal energy in Brazil is public awareness and acceptance. Most people are not yet familiar with this renewable energy source and may have concerns about the environmental and socioeconomic impacts of the projects. It is essential to educate the population about the benefits of tidal energy, as well as to promote dialogue and public participation to ensure the necessary support for the development of these projects.

3 FINAL THOUGHTS

Based on the accomplishment of this bibliographic research, which aimed to analyze the main challenges and opportunities for Brazil as a future potential generator of sustainable energy, it is possible to conclude that the country has a solid and favorable base for the development of renewable sources in the coming years. The diversification of the energy matrix, with an emphasis on sustainable energies, is an important strategy to reduce greenhouse gas emissions, increase energy security and boost sustainable development.

Hydroelectric energy emerges as a significant source in the Brazilian energy matrix, with an enormous potential for use, especially in the Amazon region. The construction of new power plants and the use of small hydroelectric plants can contribute to the stability of the national electricity system by providing clean, renewable and low-cost energy. However, such energy faces challenges related to environmental issues, climate variability, large investments and bureaucracy.

Biomass also stands out as a relevant opportunity for Brazil, which has a vast amount of agricultural and forestry waste. The expansion of the use of biomass for energy generation will contribute to the use of waste, the reduction of emissions and the development of sustainable production chains. As challenges, biomass energy faces obstacles regarding the availability and logistics of raw materials, environmental sustainability, and economic competitiveness. It is necessary to invest in advanced technologies, research and development to increase efficiency and reduce the environmental impacts of this energy source.

In the field of solar energy, Brazil has one of the best solar irradiations in the world, especially in the Northeast and Midwest regions. The abundant availability of solar radiation enables large-scale photovoltaic solar power generation, both in distributed systems and in centralized generation.



Government incentives and energy auctions have boosted the growth of this source, which has increasingly competitive costs compared to conventional energy sources. In the case of solar energy, it is necessary to overcome the challenges related to the high initial cost, lack of awareness, infrastructure and regulation.

Wind energy also presents great opportunities for Brazil, taking advantage of the extensive coastline and regions with favorable winds, especially in the Northeast and South of the country. Wind energy auctions have stimulated the expansion of the sector, contributing to the diversification of the energy matrix and to the economic and social development of the regions where wind farms are installed. However, the expansion of wind energy requires efficient planning, identification of suitable areas, development of storage solutions, and clear regulatory policies.

Finally, it highlights the existing opportunities and challenges in relation to tidal energy, which emerges as a promising future opportunity for Brazil, due to its extensive coastline. The exploitation of this form of energy is still at an early stage of development, but it has the potential to diversify the energy matrix, reduce greenhouse gas emissions, and boost technological and industrial development. However, this energy requires technological advancements, adequate infrastructure, and appropriate environmental licensing processes.

In short, addressing the challenges related to sustainable energy in the coming years requires a comprehensive approach, which involves diversification of the energy matrix, technological improvement, investments in infrastructure, and adequate regulation. The search for sustainable solutions and the development of a resilient and efficient energy sector are essential to ensure energy security, reduce environmental impacts, and boost Brazil's economic growth in the global context.

The search for sustainable solutions and the development of a resilient and efficient energy sector are essential to ensure energy security, reduce environmental impacts, and boost Brazil's economic growth in the global context. By investing in sustainable energy, the country will be able to reduce its dependence on fossil fuels, mitigate climate change, improve air quality, and create green jobs. In addition, the transition to a cleaner and more diversified energy matrix can attract foreign investment, boost technological innovation, and strengthen the country's competitiveness on the international stage.



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