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Effects of Exhaustion Gases from Biodiesel Blends on the Lichen *Cladonia verticillaris*: A Complimentary Evaluation

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A B S T R A C T

The objective of this study was to evaluate the effects of gases emitted by blends of biodiesel with petroleum diesel at intermediate concentrations (between 10 % and 40 %) on the biochemistry of the lichen *Cladonia verticillaris* (Raddi) Fr. Samples of the respective species were exposed to the gases during a period of sixty minutes, using a stationary engine, with blends of 20 % (B20), 25 % (B25) and 30 % (B30) of biodiesel, from the oil from cotton, to petroleum diesel. In the period of 30, 60 and 90 days, the concentrations of primary metabolites (chlorophylls and pheophytins) and secondary (protocetraric acid and fumarprotocetraric acid) were determined. A control group was used for comparison purposes. The gases emitted during the combustion process were also evaluated at three times (0 minutes, 30 minutes and 60 minutes). The results did not show significant differences for chlorophylls, when all treatments and control materials were compared, however, variations were observed for the other metabolites. The concentration of protocetraric acid was higher compared to fumarprotocetraric acid, indicating that there were blocks in the synthesis of the metabolic pathway. Cluster analyzis showed that for groups B20 and B25, pheophytins are related to CO_2, while for B30 their chlorophylls are related to NO_2. The results demonstrate that the respective mixtures do not affect the integrity of the species, confirming the recommendation to use mixtures of biodiesel with petroleum diesel up to 40 %.

Keywords: biofuels, environmental impact, living organisms, lichen substances, biomonitoring.

Efeitos dos Gases da Exaustão das Misturas de Biodiesel no Líquen *Cladonia verticillaris*: Uma Avaliação Complementar

R E S U M O

O objetivo deste estudo foi avaliar os efeitos dos gases emitidos pela mistura de biodiesel com óleo diesel de petróleo em concentrações intermediárias (entre 10 % e 40 %) na bioquímica do líquen *Cladonia verticillaris* (Raddi) Fr. Amostras da respectiva espécie foram expostas aos gases durante um período de sessenta minutos, utilizando um motor estacionário, com misturas de 20 % (B20), 25 % (B25) e 30 % (B30) de biodiesel, a partir do óleo de algodão, ao diesel de petróleo. Nos períodos de 30, 60 e 90 dias, foram determinadas as concentrações dos metabólitos primários (clorofilas e feofitinas) e secundários (ácido protocetrárico e ácido fumarprotocetrárico). Um grupo de controle foi usado para fins de comparação. Os gases emitidos durante o processo de combustão também foram avaliados em três tempos (0 minutos, 30 minutos e 60 minutos). Os resultados não mostraram diferenças significativas para clorofilas, quando todos os tratamentos e materiais de controle foram comparados, porém, variações foram observadas para os demais metabólitos. A concentração de ácido protocetrárico foi maior em comparação com o ácido furmarprotocetrárico, indicando que houve bloqueios na síntese da via metabólica. A análise de agrupamento mostrou que para os grupos B20 e B25 as feofitinas estão relacionadas ao CO_2, enquanto para o B30 suas clorofilas estão relacionadas ao NO_2. Os resultados demonstram que as respectivas misturas não afetam a integridade da espécie, confirmando a recomendação de utilização de misturas de biodiesel com óleo diesel de petróleo até 40 %.

Palavras-chave: biocombustíveis, impacto ambiental, organismos vivos, substâncias liquênicas, biomonitoramento.

Introduction

Fossil fuels represent 81 % of global primary energy consumption, with 92 % applied in the transport sector, causing concerns due to a possible reduction in supply and serious environmental impacts (Phuang et al., 2022). Petroleum oil, one of the main raw materials for fossil fuels, still has a significant world consumption, even with several short and long-term market uncertainties, mainly due to the sanctions imposed on Russia (IEA, 2022). Brazil's road transport sector is still highly dependent on fossil fuels, even with the policy of mixing biodiesel in all diesel-like fuels, generating a higher greenhouse gas (GHG) emission than all thermoelectric plants from the country (Julio et al., 2022). This same problem can be seen in Mexico, where fossil fuels constitute almost 85 % of the national energy matrix, with a percentage of 43 % for the transport sector, causing serious problems due to high GHG emissions (Masera and Rivero, 2022).

The need for an energy transition to replace fossil fuels has drawn attention to the use of biodiesel as a promising biofuel alternative (Gholami et al., 2020). However, since the engines are designed to operate with diesel oil, it is necessary to use blends of biodiesel with petroleum diesel (Loo et al., 2021) for a good performance. In the world, 66 countries, 14 in the Americas, have adopted a mixture of biofuels with fossil fuels to promote the use of more sustainable fuels (Dey et al., 2021). These factors led to studies aimed at monitoring the gases emitted during the combustion process and their implications (McCarthy et al., 2011; Schröder et al., 2013; Abu-Hamdeh and Alnefaie, 2015, Evtyugina et al., 2023).

Brazil has a history of production and use of biofuels, with an emphasis on biodiesel, mainly produced from raw materials from agricultural lands, such as sugarcane and soy, among others (Grangeia et al., 2022; Júlio et al., 2022). The current Brazilian legislation requires a mixture of 12 % of biodiesel to commercial petroleum diesel, with a forecast for this percentage to increase to 15 % in 2026 (CNPE, 2023). However, using biomass from agricultural land implies serious problems, such as high GHG emissions compared to other biofuels, environmental impacts, and community conflicts, among others (Khan et al., 2021). The need to monitor the gases from the blends derived from biodiesel produced from this group of biomasses also becomes essential for planning the national energy policy.

It is known that engine exhaust results in the emission of gases that impacting the biota of natural and/or urban ecosystems, in addition to humans. In the case of biodiesel, produced from agricultural biomass, a recent study by Da Silva et al. (2021) reports that a high concentration (50 % and 70 %) of its blends with petroleum diesel can negatively impact the environment. The authors used the lichen *Cladonia verticillaris* (Raddi) Fr. as a biomonitor, whose responses are already standardized, and this species is certified as a standard biomonitor for atmospheric pollutants in Northeastern Brazil (Silva and Pereira, 2002). Da Silva et al. (2021) study also addresses the importance of reviewing the biodiesel blend policy for better efficiency in the ecological energy transition.

The changes in their chemical composition are the main markers for assessing environmental quality and responses are observed mainly through the determination of their primary (chlorophylls and pheophytins) and secondary (phenols) metabolites (Silva and Pereira, 2002; Da Silva et al., 2021; Silva et al., 2021, Da Silva et al., 2022). Chlorophylls are related to their nutritional aspect, having as a degradation product the formation of pheophytin; phenols are associated with defense mechanisms against the action of pollutants and living organisms that may interfere with the vitality of lichen species (Pereira et al., 2020). Studies that sought to assess environmental quality from the biochemical parameters of *C. verticillaris* are found in the literature (Silva and Pereira, 2002; Mota-Filho et al., 2007; Da Silva et al., 2021, Silva et al., 2021).

Regarding pioneering tests involving engines powered by different blends of biodiesel with petroleum diesel, Da Silva et al. (2021) demonstrated that the different proportions of these blends greatly influence the metabolism of C. verticillaris. Thus, it was suggested the need to expand the assessment of the impact of these mixtures in different percentages using the same species in biomonitoring. With due to the detection of damage to the metabolism and structure of the lichen in concentrations higher than 40% of the biodiesel blends, it was questioned whether intermediate concentrations, with intervals of 5% between 10% and 40%, respectively with lower limit to the absence of damage and, higher than the damage verified by Da Silva et al. (2021), would also impact organisms used in biomonitoring.

This observation becomes important due to studies carried out by Evtyugina et al. (2023), which indicate lower emissions of pollutants and organic particles in biodiesel blends in the percentages of 10 %, 20 %, and 30 % compared to diesel. However, a study carried out to evaluate the impacts caused by biodiesel blends on the quality of the United States points to an increase in the emission of hydrocarbons and carbon monoxide and a lack of reduction of particulate matter to the percentage of 20% (O'Malley and Searle, 2021). It is possible that intermediate concentrations do not cause significant impacts on lichen biochemistry, confirming the use of biodiesel blends up to the percentage of 40 %. However, due to issues reported in the previously mentioned studies, it is essential to carry out the individual evaluation. In this context, this work aimed to evaluate the effects of three blends of biodiesel (from cottonseed oil) to petroleum diesel, intermediate to the borderline between inert and producing damage to the metabolism of lichen *C. verticillaris*, from the production of photosynthetic pigments and secondary metabolites, complementing the study carried out by Da Silva et al. (2021). The results obtained may serve as an indicator of ways to use biofuels without further damage to living organisms.

Material and methods

Collection of lichen material and exposure to biodiesel blends

Samples of the lichen species *Cladonia verticillaris* were collected in a conserved area in the Mamanguape, state of Paraíba, Brazil. After the collection process and packaging in paper bags, the material was transported to the Laboratory of Environmental Geography of the Federal University of Pernambuco (LAGEAM-UFPE) for removal of particulates (branches, leaves, and the sand) and packaged (portions of 10g) in perforated nylon bags. These packages were positioned at a distance of 1.5 m from the exhaust of the stationary engine, model FIAT C78 ENT, F2B, for 60 minutes of exposure. Blends of biodiesel with petroleum diesel B20 (20 % biodiesel and 80 % diesel), B25 (25 % biodiesel and 75 % diesel), and B30 (30 % biodiesel and 70 % diesel) were used for the combustion process and exposure of the lichen material. The biodiesel used came from cottonseed oil.

During the combustion process, measurements of carbon monoxide-CO, carbon dioxide-CO_2; oxygen gas - O_2; nitric oxide-NO; nitrogen dioxide-NO_2 in 0 minutes (start of combustion), 30 minutes, and 60 minutes (end of discharge), using the Seitron Chemist 400 Combustion Analyzer probe were determined.

After exposure, the samples submitted to the gases were transported to the LAGEAM-UFPE, placed in glass boxes with lids, and kept in the laboratory at room temperature (28 ± 3 °C). Throughout the experiment, 2 mL of deionized water was sprayed twice a week. After 30, 60, and 90 days post-exposure, samples (2 g) were collected for determination of primary (chlorophylls and pheophytins) and secondary (protocetraric acid and fumarprotocetraric acid). Analyzes were performed in duplicate to determine each compound. For comparison purposes, a control group was used, unexposed to engine material, being conditioned in the same way, and the same compounds were determined with the exposed material.

Determination of primary metabolites (phenolic compounds)

The chlorophyll and pheophytin contents of all samples were determined from the extraction of 0.5 g of the lichen thallus, which was later crushed and infused in 5 mL of dimethylsulfoxide (DMSO), remaining for 72 h in a dark environment. Subsequently, they obtained the extracts after filtration using a qualitative paper filter. The extracts were analyzed by a spectrophotometer (BIOCHORM, model Libra S22), at wavelengths of 630, 647, 664, and 691 nm, for quantification of chlorophylls (Pompelli et al., 2013) and 536 and 666 nm for quantification of pheophytins (Vernon, 1960). The equations for calculations used to obtain the final values in mg L^{-1} are described in detail in the study by Silva et al. (2021).

Determination of secondary metabolites

The fumarprotocetraric acid (FUM) and protocetraric acid (PRO) were quantified from extracts obtained by successive extractions of the thallus with ether, chloroform, and acetone (2 mL of solvent / 1 g of macerated lichen material). The material was infused at 18 °C for two h for each solvent used. The extract was filtered and placed in test tubes at each solvent change interval. The organic extract was obtained after evaporating the solvents at room temperature (28 ± 3 °C). The organic extract was diluted in acetone, and the FUM and PRO contents were determined by reading the liquid extracts in a spectrophotometer at wavelengths of 210, 254, and 366 nm (Silva and Pereira, 2014). The equations for calculating the content of FUM and PRO in μg mL^{-1} are described in the study by Silva and Pereira (2014).

Statistical analyzis

To analyze the results, analyzis of Variance (ANOVA) and Tukey's test were applied to compare means. As the chlorophyll tests, at 90 days, did not meet the principles of homogeneity and normality, verified with the application of the Bartlett and Shapiro Wilk tests, the non-parametric Kruskal-Wallis test and Dunn's post-test were used for this analyzis. Cluster analyzis (unsupervised clustering) was applied to assess a possible relationship between the gases emitted during the combustion period and the secondary pigments and metabolites. The data were analyzed using the free software R (i386 4.1.2), using the EXPDES.PT. (Ferreira et al., 2021), DPLYR (Wickham et al., 2022), RSTATIX (Kassambara, 2021) packages and the function cluster.

Results

The results of gases emitted during the combustion process of the different biodiesel mixtures did not report significant differences for all treatments ($p>0.05$), presenting similar values in all treatments for each determined parameter (Table 1).

Table 1- Determination of gases emitted during the combustion process of blends of biodiesel (from cottonseed oil) added to petroleum diesel at different concentrations.

Determined parameters	Biodiesel Blends		
	B20	B25	B30
		ppm	
NO_2	23 ± 2.9	19.7 ± 3.5	25.3 ± 0.7
CO	132.3 ± 23	119.3 ± 15.9	149 ± 1
NO	109 ± 14.5	109 ± 20.2	106 ± 2.6
		%	
CO_2	2.6 ± 0.1	2.7 ± 0.1	2.6 ± 0.1
O_2	17.3 ± 0.1	17.2 ± 0.1	17.4 ± 0.1

Legend: Means are the measurements of the three measurement time intervals (0 minutes, 30 minutes and 60 minutes) ± standard error. B20 (20 % biodiesel and 80 % diesel), B25 (25 % biodiesel and 75 % diesel) and B30 (30 % biodiesel and 70 % diesel). Carbon monoxide-CO, carbon dioxide-CO_2; oxygen gas - O_2; nitric oxide-NO; nitrogen dioxide-NO_2.

The analyzing the primary metabolites, treatments and control material, showed no significant differences for each post-exposure analyzis period (Table 2). The pheophytins showed variations, with B25 showing the highest concentration for all periods. However, pheophytin concentrations in all treatments and control material remained lower compared to chlorophylls. Comparing the periods of analyzis for all treatments, the period of 30 days reported the highest concentrations of chlorophylls, with a decay occurring for the control material and group B25 at 60 and 90 days. For this same period, there was a decrease in pheophytins (60 days) and stabilization at (90 days), with an increase in this pigment occurring in the last analyzis time only for B25.

In regarding secondary metabolites (Table 2), for PRO, the control and B25 showed the highest concentrations in all periods, however an increase in concentration at 60 days is observed for B30, with a decrease at 90 days. For FUM, B25 showed the highest concentrations at 30 and 90 days, in which B30 showed the highest concentration at 60 days. However, for all treatments, the contents of this metabolite were lower than PRO.

Table 2- Concentrations of primary (photosynthetic pigments) and secondary (phenolics) metabolites in the thallus of *Cladonia verticillaris* (Raddi) Fr. exposed to the blends of biodiesel added to diesel oil in different concentrations and control material.

	Photosynthetic Pigments		Phenolic Compounds	
Treatments	Chlorophyll mg L^{-1}	Pheophytin mg L^{-1}	PRO µg mL^{-1}	FUM µg mL^{-1}
		30 Days		
Control	42.5 ± 4.4 aA	20.8 ± 1.6 aA	40.4 ± 0.1 bB	42.1 ± 2.1 bA
B20	42.5 ± 6.6 aA	13.4 ± 0.2 bA	27.3 ± 2.9 bA	27.8 ± 4.8 bA
B25	57.0 ± 0.8 aA	23.2 ± 0.4 aA	100.8 ± 1.6 aA	85.8 ± 0.8 aA
B30	36.4 ± 1.3 aA	12.7 ± 0.2 bA	34.8 ± 3.6 bB	32.8 ± 2.8 bB
		60 Days		
Control	27.5 ± 2.8 aAB	8.4 ± 0.2 aB	62.5 ± 6.5 abAB	51.3 ± 4.0 bA
B20	28.2 ± 7.4 aA	7.4 ± 1.6 aAB	35.0 ± 2.2 bA	29.1 ± 1.3 cA
B25	23.1 ± 0.8 aC	10.1 ± 2.0 aB	51.0 ± 0.5 abB	31.0 ± 0.6 cC
B30	22.5 ± 3.2 aA	5.4 ± 0.7 aB	74.8 ± 10.6 aA	67.6 ± 1.1 aA
		90 Days		
Control	15.1 ± 0.2 aB	5.6 ± 0.7 bB	68.3 ± 2.6 aA	53.6 ± 5.9 aA
B20	15.8 ± 6.5 aA	4.9 ± 1.6 bB	26.0 ± 0.3 cA	23.3 ± 0.2 bA
B25	41.3 ± 0.0 aB	19.0 ± 0.1 aA	56.4 ± 0.7 bB	45.8 ± 0.5 aB
B30	18.1 ± 4.7 aA	6.2 ± 0.9 bB	28.3 ± 0.4 cB	25.3 ± 1.1 bB

Legend: Comparison of means with 95 % confidence ($p<0.05$). Lowercase letters represent the comparison between treatments and control material for each analyzis period. Values are means (n=2) ± standard error. Capital letters represent the comparison between the analyzis periods for each treatment. B20 - 20 % biodiesel and 80 % diesel; B25 - 25 % biodiesel and 75 % diesel; B30 - 30 % biodiesel and 70 % diesel. PRO: protocetraric acid; FUM: fumarprotocetraric acid.

The construction of dendrograms made it possible to visualize the grouping of the data of the gases determined during the combustion and the respective metabolites (primary and secondary) of *C. verticillaris*. Treatments B20 and B25 showed the same behaviour, highlighting the relationship between pheophytin and CO_2. For B30, chlorophyll was related to NO_2. For all treatments, PRO is related to FUM and NO to CO (Figure 1).

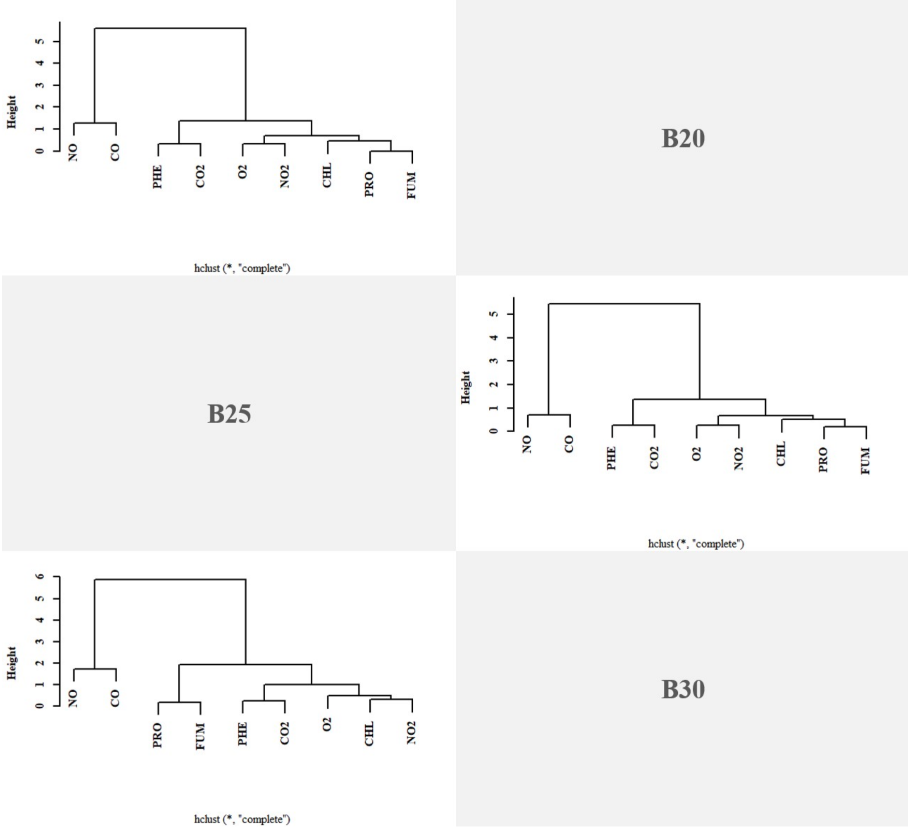

Figure 1- Cluster analyzis of gases emitted during the combustion of the biodiesel mixture with petroleum diesel at different concentrations and metabolites (primary and secondary) of the lichen thallus *Cladonia verticillaris* (Raddi) Fr. using as a biomonitor. A: B20- 20% biodiesel and 80% diesel; B: B25- 25% biodiesel and 75% diesel; C: B30- 30% biodiesel and 70% diesel. CHL: chlorophyll; PHE: pheophytin; PRO: protocetraric acid; FUM: fumarprotocetraric acid; CO: carbon monoxide, CO_2: carbon dioxide; O_2: oxygen (gas); NO: nitric oxide; NO_2: nitrogen dioxide.

Discussion

The studies carried out by Jalaludin et al. (2020) report an increase in CO_2 and NOx, as well as a decrease in CO with the increment of biodiesel to petroleum diesel, at intervals of 10% of the mixture of biodiesel to petroleum diesel (B10, B20 and B30). Da Silva et al. (2021) also observed that a higher value in the emission of gases occurred with a mixture of 70% of biodiesel with petroleum diesel. However, the gas emissions carried out by the concentrations used in this study are similar to a mixture of 70 % biodiesel to diesel (B70). Studies by Zhang et al. (2021) point out that blends of biodiesel with petroleum diesel can benefit from fewer emissions of some gases. However, these parameters have associations between biodiesel viscosity and engine parameters. The results of CO emissions in this study being similar to B70 may be linked to high engine cylinder temperatures, an important apparatus in the combustion process. Variations in gas emission concentrations related mainly to engine performance and the oxygen levels present in the mixture were also reported in the studies by Abed et al. (2019). Evtyugina et al. (2023) note that the literature results on the emission of gases by biodiesel blends are complex and inconclusive. However, some devices can reduce the oxygen content in the engine cylinder, providing lower emissions of some gases, such as nitrogen oxides. The respective studies show that gas emissions can be influenced mainly by the devices that make up the engine, especially those created to operate only with diesel oil. This observation was also made by Chen et al. (2023) when evaluating that the use of the exhaust gas recirculation valve (EGR) at a rate of 15 % can bring benefits in the reduction of gas emissions.

In regarding primary metabolites, Houri et al. (2020) approach that the excess of pollutants increases the levels of pheophytin, and there may also be an increase in chlorophyll as a mode of adaptation of the species. Silva et al. (2021) also report that the increase in chlorophyll synthesis in

C. verticillaris occurs as compensatory mechanisms for atmospheric pollutants. By relating the results of the literature with this study, it can be seen that lichen did not suffer major impacts when exposed to different blends of biodiesel and possibly had no impacts on its chloroplasts, the main site of attack by atmospheric pollutants (Sewelam, 2016; Houri et al., 2020), for reporting similar concentrations of chlorophyll, which are superior to pheophytins. The impacts on chlorophyll production occur mainly when the pollutant impacts cell division, causing the development of deformed cells and the decrease of living cells (Santos et al., 2022). Because the results show chlorophyll contents similar to the control group, it is possible to infer that biodiesel blends up to 30 % do not cause damage to the cell structure of *C. verticillaris*. In this study, analyzis of the ultrastructure of the thalli were not carried out, however, based on the observations made, it is highly encouraged that such tests be carried out in research on the respective theme. The significant differences between pheophytins in the period of 30 and 90 days after exposure can be explained by the behavior of chlorophyll levels. Silva et al. (2021) observed that pheophytins follow fluctuations in chlorophyll levels and, even though this metabolite did not report significant differences, greater variations in its concentrations occurred at 30 and 90 days after exposure.

For secondary metabolites, even with a higher concentration of PRO in relation to FUM, caused by a probable blockage in the metabolic pathway reported by other studies with the same species (Mota Filho et al., 2007), it was observed that there were no differences high. This process demonstrated that the different blends did not significantly affect the synthesis of FUM, according to Ahti et al. (1993) the major compound of the species. Higher concentrations of PRO and FUM were observed for the control and in the mixtures B25 and B30. Da Silva et al. (2021) also reported that *C. verticillaris* showed higher concentrations of PRO compared to FUM when exposed to high concentrations of biodiesel mixture with diesel (40%, 50% and 70%), which is a defense mechanism of the species against pollutants, in an attempt to maintain their vitality. The high concentrations of the respective metabolites for the control may also be associated with the change in the place of origin, which probably caused a stress in the species, affecting its functioning (Williams et al., 2017). The low variation in the production of secondary metabolites may also be an adaptation of the species to the pollutants emitted during the combustion process of the mixtures, as it does not significantly affect its vitality, with *C. verticillaris* being a resident species in the environmental pollution, as reported by Da Silva et al. (2022). Studies performed by Osyczka et al. (2021) also report on the adaptation of lichens to pollutants, mainly through the production of ribitol, forming a pool of intermediate products that can serve as precursors for the production of secondary metabolites.

It is important to emphasize that the gases evaluated in this study were similar to those reported by Da Silva et al. (2021). However, the emission rates for mixtures between 20 % and -30 %, identical to the 70 % mixture, did not negatively impact the biochemistry in C. verticillaris. This factor demonstrates that the gases reported in both studies may have less influence on primary and secondary metabolites. The emission of other compounds, such as distributed water-soluble organic particles (WSO), may have greater effects on to impact the lichen when exposed to mixtures of 50 % (B50) and 70 % (B70) (Da Silva et al., 2021; Evtyugina et al., 2023). In this study, it was impossible to evaluate several gases and compounds that can be emitted during the combustion process of biodiesel blends and their effects on lichen. However, it is essential to carry out more research from this perspective.

The researchers that seek to evaluate the relationship of potentially toxic compounds and effects on lichen metabolites is highly encouraged, given the lack of work on this topic. The association of pheophytin with CO_2 for B20 and B25 can be explained by the fact that this gas affects the production of chlorophyll, with a study showing a negative impact of the respective gas on gene expression in the tangerine fruit and, consequently, causing the degradation of the respective pigment (Lu et al,. 2020), which causes the formation of pheophytin (Silva et al., 2021). Takahashi et al. (2013), when conducting a study with the species *Arabidopsis thaliana* L., mentioned that NO_2 regulated their organ growth, controlling cell proliferation and increase, as it has different sets of genes induced by the respective gas. This cell proliferation can regulate the photosynthetic rate and, consequently, the production of chlorophyll, and this factor may be associated with the relationship between chlorophyll and NO_2 in the B30 treatment. The authors also commented that NO_2induces an increase in photosynthesis. Frati et al. (2006) when carrying out a study in the environment with NO_2 and the lichen *Evernia prunastri* reported that this compound did not negatively affect the chlorophyll content, corroborating the results shown in the cluster analyzes seen in this study.

Conclusion

The results showed that lichen *C. verticillaris* did not suffer relevant impacts when exposed to biodiesel mixtures at concentrations of 20 %, 25 %, and 30 %. Chlorophylls did not differ significantly from the control material in all analyzis periods. Pheophytins remained at their lowest levels concerning chlorophyll. This process demonstrates that the gases did not impact the metabolic functioning of the species. This information is confirmed by PRO and FUM concentrations which did not report much change. This study demonstrates that concentrations of biodiesel mixed with petroleum diesel up to 40% are recommended when it comes to environmental pollution resulting from the emission of gases produced by these products.

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