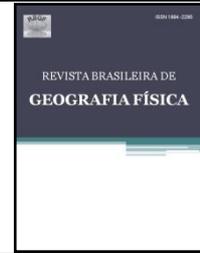




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Interaction of Cladoniaceae Lichens With Quartzarenic Neosols in Northeastern Brazil: A Mini Review

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ABSTRACT

Lichens are organisms capable of interacting with the environment, playing an important role in the modification of their substrate. In coastal areas of northeastern Brazil with sandy soils, as an extension of Atlantic rainforest, or in an interface with semi-arid Caatinga biome and high mountain humid forests, named *brejos*, lichens of the family Cladoniaceae are found forming pillows on these soils. They interact with their substrate, influencing the chemical composition and biota. In this study, we summarize data about the interaction of these lichens with Quartzarenic Neosols, which occurs in this phytophysiology named *tabuleiros* or *cerrados*.

Keywords: nutrient cycle; lichen substances; weathering; sandy soils

Introduction

Lichens are exceptional organisms in their interactions with environmental factors and play an important role in ecosystem functioning (Nash III, 2008).

In this context, in natural environments, lichens can be found inhabiting different tree branches (Figure 1), barks (Figure 2), leaves (Figure 3),

rocks (Figure 4), and soils (Figure 5), in addition to other, man-made substrates. In the case of human interference, they have demonstrated the ability to adapt to different substrates, such as walls and roofs, including more unusual ones like glass and shells found in garbage thrown into the environment, or even mailboxes and fire hydrants (Figure 6).



Figure 1. Examples of fruticose lichens on tree branches. A *Usnea* sp from humid forest of Chile, B Usneaceae from coastal desert of Chile, C *Teloschistes exilis* from “matorral” vegetation, Chile. Photos from EC Pereira



Figure 2. Lichens that occur on tree bark. A green Parmeliaceae with dark Cyanolichen (blue-green algae) from Paudalho, Pernambuco State, B crustose lichens from Carolina, Maranhão State, C fruticose lichens (genus *Ramalina* and *Parmelia*) in Florida State – USA. D foliose lichen from Paudalho, Pernambuco State, E foliose Parmeliaceae on dead wood in Mamanguape, Paraíba State. Photos from EC Pereira



Figure 3. Foliicolous lichens from Atlantic Rainforest. A on deciduous and B on Melastomataceae leaf, C several species of lichens on *Anthurium* leaves, D on leaf of *Persea Americana*. Figures A, C, D show parts of the leaves with magnification for better observation of lichen colonies. Photos from EC Pereira



Figure 4. Lichens using rocks as substrate. A Crustose lichens in semiarid region of Pernambuco, B Fruticose lichens on rocks of coastal desert in La Serena (Chile), C *Rhizocarpon geographicum* in desert of Iquique (Chile). Photos from EC Pereira



Figure 5. Edaphic savannah (tabuleiro) of Paraiba and examples of Cladoniaceae species that naturally occur in the area. Left to right side: *Cladina dendroides*, *Cladonia salzmanii*, *Cladonia verticillaris*, *Cladonia substellata*. Photos from EC Pereira



Figure 6. Lichens on man-made substrates. A on iron fence, and B on fire hydrant, both at the Universidade Federal de Pernambuco, Recife, C on plastic mail box at Paudalho, Pernambuco, D on roof tiles with clay and E asbestos cement found in Talca and La Serena, respectively (Chile), F on shells of dead mollusks, in area of solid residue accumulation in coastal desert, Chile. Photos from EC Pereira

Despite these adaptations, lichens are extremely sensitive to environmental impacts and degradation, which can lead to their disappearance, and in severe cases, even their extinction (Nash III, 2008; Pereira et al., 2012). This dilemma remains unsolved, due to lichens' slow growth and ability of entrapping and retaining pollutants and

radionuclides, which when added to the destruction of natural vegetation, leads to an almost impossible reposition replacement of these organisms in nature (Silva et al., 2010; Pereira et al., 2017).

Due to their interactions with the environment, lichens can act as bioindicators for conservation, as well as ecological continuity in tropical rainforests,

as in the case of the foliicolous life form (Rivas-Plata et al., 2008). Their effects on ecological succession (Nash III, 2008), soil development, biota, and chemical transformation (Silva et al., 2012; Santiago et al., 2018) have been reported. In these interactions, nutrient cycling has also been mentioned. In addition to the biogeochemical cycles that occur in nature, for lichens, principally those that use soil as a substrate, a microcycle has been reported. Since lichens can acquire ions dissolved in humid air, or when elements volatilize from soil, they can capture and use them in their metabolism. Some of these elements allow for the production of secondary compounds, whereas others can block their synthesis. In this way, any contaminant in the air or in the soil can affect the metabolism and vitality of lichens.

For this reason, researchers are focusing on the role of secondary metabolites of lichens in ecosystem functioning, since they can act in rock weathering (Silva et al., 2012) and soil development through the percolation of phenolic compounds which result from their secondary metabolism, interfering in the ecosystems' biota and chemical composition. As an example, *Cladonia verticillaris* was found to be capable of enhancing the chemical attributes of degraded soils in the semi-arid region of Brazil (*caatinga*), increasing the content of Ca (418.87 %) and Mg (208.3 %), while the characteristic phenolics from the species were found to have degraded minerals such as albite and microcline. For these reasons, *C. verticillaris* is considered a bioremediator of soils in the process of salinization, as occurs with Chromic Luvisols, or Fluvisols in this area, considered a core driver of Brazilian desertification. Nonetheless, when considering the main problem facing these soils, that is, a high concentration of Na, this lichen was not able to remove it, despite being capable of meliorating the quality of soil, making it more fertile, by releasing nutrients (Silva, 2014).

C. verticillaris produces fumarprotocetraric and protocetraric acids as major compounds (Ahti et al., 1993); these substances enhance the quality of salinized soils without lowering Na. Similarly, when *C. substellata* that produces usnic acid (Ahti et al., 1993; Silva, 2018) was used, this lichen and its extracts were verified to have remediated Fluvisols, while the addition of organic matter resulted in a decrease of Na under laboratory conditions.

This has bearing for the Brazilian Northeast, where a characteristic phytogeography called *tabuleiros* or *cerrados*, resembles a savannah, but due to edaphic, not climate characteristics. In these

areas, the characteristic sandy soils support the occurrence of tufts (or pillows) of lichens of the Cladoniaceae family (Ahti et al., 1993; Pereira, 1998), surrounded by sugarcane plantation (Vasconcelos, 2013). The suppression of vegetation and use of chemical fertilizers in the surrounding agricultural matrix are problems facing *tabuleiros*, leading to a loss of species/biodiversity and soil fertility, as well as contamination of ecosystems.

In this study, we emphasize the role of lichens from the Cladoniaceae family in these ecosystems, and how human action can affect the functioning and survival of these organisms.

It is worth pointing out that the studies mentioned in this text are the only ones found in the literature, since this subject is a new focus of investigation for lichenologists in Brazil. Manuscripts describing lichens and their relationships to weathering rocks, or to developing soils can be found, principally among old texts, some of them from decades or even a century ago. This way, a review demonstrating a new paradigm for geography and lichenology is worthy of attention and can contribute to scientific knowledge.

The Cladoniaceae Family

Lichens considered cladoniform make up part of a terricolous (occurring on soil) habit, belong to the Discomycetes Division, and produce a dimorphic thallus. The Cladoniaceae family is the most representative of this group of lichens having cladoniform characteristics (Pereira, 1998).

Cladoniaceae belongs to the Order Lecanorales, Class Ascomycetes, and their photobionts constitute unicellular green algae. They produce a thallus, also called podetium, with a dimorphic nature, starting as a squamulose or crustose form, and evolving into an erect thallus (Nash III, 2008).

Due to the ecological adaptation of Cladoniaceae, populations of this family are generally confined, with periods of expansion and/or contraction, depending on successional cycles. For example, *C. cristatella* from North America shows rapid maturation, and is often found fertile, which enables it to rapidly disperse spores and develop new populations; in contrast, species of boreal forests typically maintain relatively stable growth and are slow to occupy new places for a long time (Pereira, 1998). In addition, species of this family are often found inhabiting sandy and acidic soils, as in boreal forests (Piznak and Backor, 2019), whose covering can be over 60 %, with emphasis in areas with

reindeer lichen, contrasting the floristic between sandy and clay areas in those forests (Walker et al., 2019). These populations are also considered as capable of occupying microclimate niches, inhabiting driest places than mosses and vascular plants (Haughian and Burton, 2018).

The chemical study of lichens is highly important to understanding their phylogeny and taxonomy. Cladoniform species follow the standard composition of the *Cladonia* genus, with prevalence of substances from β -orcinol series, besides some specific compounds of some species, which also help distinguish genera (Pereira, 1998).

Genera and species occurred in the Brazilian Northeast

The first survey of the Cladoniaceae in *tabuleiros* of the Brazilian Northeast was published by Ahti et al. (1993). The authors mentioned species from the coastal zone and suggested the existence of this formation is restricted to an extension of the Atlantic Rainforest.

Later, Cladoniaceae species were also found in *tabuleiros* (or *cerrados*) of the interior parts of the region, with the same soil structure (sandy), and also other edaphic characteristics, as in Saloá and Catimbau National Park (Fonseca, 2012; Santos et al., 2014), or in Cariri Plateau in Ceará state (unpublished data).

According to Ahti et al. (1993) in *tabuleiros* (or *cerrados*) of Paraíba, Pernambuco and Sergipe states, 22 species have been registered, most of them new records for the region, or species new to science, some of them considered by the authors as confined to the Brazilian Northeast. On the other hand, other states have not yet been visited, and these geographical formations have only been considered for the coast. With this in mind, we consider there to be a need to update this information, especially considering the importance of lichens in remaining *tabuleiro* vegetation.

Quartzarenic Neosols from *tabuleiros/cerrados*

Tabuleiros/cerrados from coastal to interior (west) areas of the Brazilian Northeast can also occur in other part of the country, including the Amazon. They result from a slow and gradual modification of the climate during glacial cycles, mainly during the Quaternary (Santos et al., 2014).

When found in the coastal zone, they are associated with the Atlantic Rainforest, where the relief is somewhat high and plain, capable of

retaining a sandy layer that covers the clay portion of the soil. In a local scale, variation on micro-relief can conditioning different water flux, that associated to sand characteristics of this soil, can favor the deposition of clay in deeper horizons. Assays with oxalate extracts of these soils pointed out it as in podsolization process (Silva et al., 2019).

On the other hand, *tabuleiros* found in the west of the region (*tabuleiros* of the interior or *cerrados*) are patches of vegetation growing in plain relief, between a semi-arid depression and a higher altitude, humid area (Santos et al., 2014). In both cases, the phytophysognomy is similar to a climatic savannah. This kind of biome (*cerrado senso stricto* or savannah) with climatic characteristics (such as marked rainy and dry seasons, among others) is found in the Brazilian Central-West and Southeast regions.

The existence of edaphic *cerrados* (*sensu lato*) in areas with a climate different to that found in *cerrado senso stricto* is supported by the refuge theory, which explains the retraction of vegetation in periods of dry climate, while maintaining some spots of vegetation in their original locations, due to physical and environmental conditioning (Haffer and Prance, 2002).

In such areas lichens are abundant, where species of Cladoniaceae occur on sandy soil and have a clear relationship with the substrate (Vasconcelos et al., 2013; 2015).

In a world biome context, the phenolic compounds synthesized from secondary metabolism of these lichens, play an important ecological and biological role in the ecosystem, acting as allelochemicals, or supplying energy to soil microorganisms (Piznak and Backor, 2019). As a local example, *Cladonia verticillaris* was mentioned as producer of compounds considered as potent bio-herbicide, or as phyto-stimulator, depending on the class of substance extracted from its thallus (Tigre et al., 2012; 2015), or *C. salzmannii* whose interaction with soil microorganisms was demonstrated by Santiago et al. (2018).

Action of Cladoniaceae phenolics on soils from Northeast *tabuleiros/cerrados*

Studies of lichens and their compounds acting on Quartzarenic Neosols from *tabuleiros* started in the search for products which would enhance the production of lichen phenolics capable of modifying the chemical and biological properties of these soils. Since these compounds have

chelating properties, the existence of lichens using soil as a substrate, would explain the modifications achieved under these tufts of Cladoniaceae (Figure 5).

Vasconcelos (2009) has submitted the thalli of *C. verticillaris* to soil from areas of its occurrence (Quartzarenic Neosol) under laboratory conditions, using a closed system. The author also tested an exogenous source of potassium phosphate for enhancing the production of both protocetraric (PRO) and fumarprotocetraric (FUM) acids. The main goal was to identify if these phenolics would react with salts in the soil, increasing its fertility, and if the source of phosphate would influence the biosynthesis of PRO and FUM. The results showed that different concentrations of phosphate solutions lead to a hyperproduction of phenolics, and these products percolate into the soil, transforming it by increasing its cationic exchange capacity, and decreasing its pH values, which are good results, since this soil is poor and acidic. In the closed system, the water added to the soil had solubilized the salts in it, and after evaporation, the ions contained in the vapor could be absorbed by the lichen thallus.

To enhance the production of FUM and PRO by *C. verticillaris*, Silva et al. (2012) used solutions of urea for inducing the biosynthesis of these compounds, since the nitrogen salt after enzymatic hydrolysis, produces carbon dioxide and ammonia. The former is used in photosynthetic procedures, while the latter in metabolism (Vicente et al., 1984; Silva, 2011). In this study, the lichen enhanced the production of FUM, as well as intermediary compounds of its biosynthesis. All of which were found in soil subjacent to lichen samples, including compounds in oxidized forms, due to reactions with soil elements. The analysis of the soil after the experiments revealed modification of pH values and of H and Al contents, with increases in fertility, especially in treatments with urea added to lichen samples.

Studies were also carried out with *C. salzmännii* that produces barbatic acid (BAR) as a principal compound (Ahti et al., 1993; Pereira, 1998). This species and its compounds are capable of increasing pH, total carbon and nitrogen of soil, especially when lichen samples are submitted to gamma radiation. This exogenous source also induces a differentiated synthesis of BAR, and its consequent percolation into the soil (Melo, 2011). The action of BAR and *C. salzmännii* was studied in more detail by Santiago et al. (2018). The authors found that the species can influence the formation of mycorrhizae, mainly in samples of soil covered by lichen tufts, consequently

improving the development of vascular plants of *tabuleiros*. BAR was detected in all soil samples where *C. salzmännii* occurred, influencing positively its chemical properties. In greenhouse experiments using seedlings of *Genipa americana*, which occurs naturally in the area, soil inoculated with mycorrhizae and covered by lichen thalli was shown to provide better development of plants with biometric parameters superior to the control, or in comparison to application of the thallus or mycorrhizae alone. The authors concluded that BAR can stimulate associations between plants and mycorrhizae, through root and soil systems.

In this context, Zraik et al. (2018) had also studied boreal lichens, by determining the relationship between soil characteristics and lichen covering, through the action of their secondary metabolites percolated to this soil. The authors found that the form of the sand particle influences on species composition, varying from round to angular, and pH, organic matter and sand content had influenced on atranorin occurrence.

This way, the mechanism of interaction between lichen and soil, in *tabuleiros* of Brazilian Northeast should be investigated, with emphasis to the protection of these remaining areas. As the surrounding areas of this vegetation are dominated by sugar cane plantation (Silva et al., 2019), the role of nitrogen can elucidate the mechanism of action of this product used as chemical fertilizer, as well as the damage that can be caused to the lichens and to the environment.

In this sense, Vasconcelos (2013) investigated the relationship with exogenous nitrogen sources using *C. verticillaris* as an experimental model in laboratory conditions. It was found that soil humidity influences the retention or percolation of phenolics and proteins from the lichen, as well as the chemical processes that result from this transport. Findings included interactions with ions of the soil and partial or total hydrolysis of lichen substances (Vasconcelos, 2013), and also that the lichen captures and uses nitrogen as a source for increasing urease activity, increasing the production of modified soil substances (Vasconcelos et al., 2013; 2016).

These experiments simulated the influence of contamination of preserved habitats, or remaining ecosystems embedded in plantation areas, mainly on a large scale, where significant amounts of chemical fertilizers are added, including in the form of urea (Vasconcelos et al., 2016).

In addition to the parameter evaluated (contamination by agricultural products), luminosity, influenced by the extraction of natural vegetation, and seasonality represented by greater

intensity of rain, can also result in the destruction of this ecosystem (Vasconcelos, 2013).

Corroborating these data, Vasconcelos et al. (2015) documented how intense are these lichen-soil interactions, through assays of soil samples by X-ray diffractometry and scanning electron microscopy, where particles of the soil are trapped in the inner thallus, including a portion of a fossil dinoflagellate.

On the other hand, the mechanism of interaction between lichen compounds and the soil used as their substrate should be an important tool for interpreting the mode of action of these substances on both chemical and biological transformation of Quartzarenic Neosols. In this context, de Armas et al. (2016) used columns packed with the mentioned soil, to which were added extracts of *Cladonia verticillaris* that naturally occur in this soil. The experiments were carried out using columns with pre-hydrated or non-hydrated soil, followed by addition of these extracts. The main objective was to determine the effect of moisture on retention or percolation of phenolics (fumarprotocetraric – FUM and protocetraric – PRO acids) and proteins contained in the extract. The results showed that FUM was strongly retained in the soil, and its hydrolysis increased as humidity was enhanced. The study showed that FUM, PRO and proteins

could be leached, retained, transformed or left to percolate to the deeper layers of the soil, and this procedure depends on the amount of rainfall, as shown in treatments with pre-hydrated soil.

During ongoing research to improve our comprehension of the action of lichen metabolites on Quartzarenic Neosols, and using the column method (Figure 7), Silva (2018) studied the effect of both thallus or extracts obtained from *Cladonia substellata*, another member of the Cladoniaceae which occurs on this soil. The author also conducted experiments under domes (Figure 8) for six months in a long-term evaluation. The results showed that the lichen produced its main compound, usnic acid, in all treatments, especially under humid conditions, simulating rainy episodes, preserving the functioning of their cells. On the other hand, the chemical composition of the soil was not strongly affected, except for the pH values. The microbial population of the soil samples was highly variable, and decreased in treatments with an organic extract containing usnic acid, probably due to the antibiotic action of this substance. The author concluded that both the extract and thallus of *C. substellata* are capable of influencing the chemical composition and biota of Quartzarenic Neosols, improving their properties.

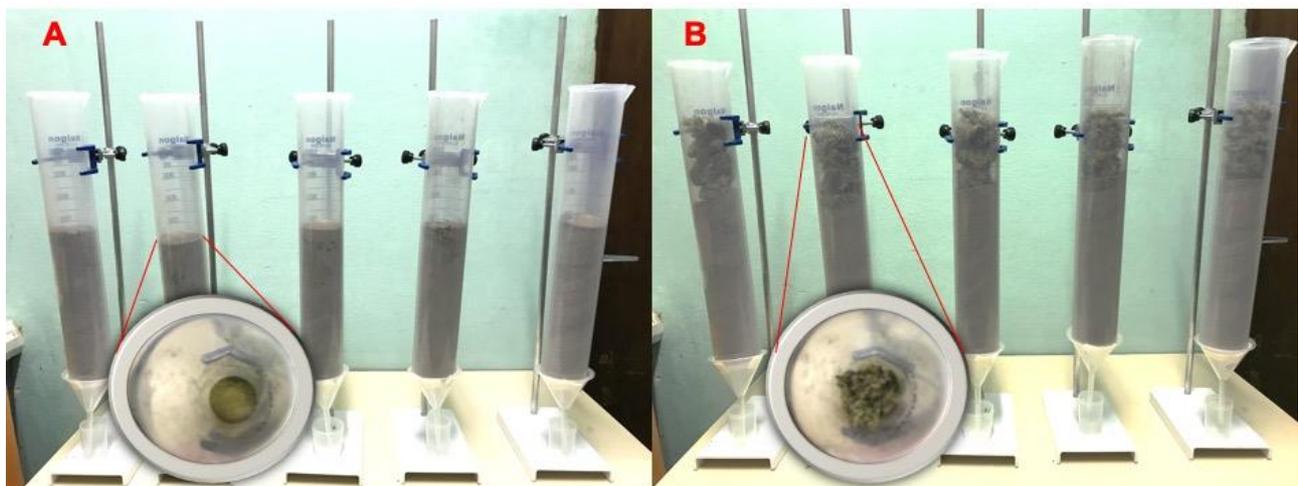


Figure 7. Experiments with Quartzarenic Neosol in columns for establishing the retention of lichen compounds into the soil, and their possible action as chelating agent, using crude extract (A) or natural thallus (B), simulating dry or rainy season, through pre or post hydration.



Figure 8. Long term experiments in domes with soil covered by lichen thallus, for determining the capacity of liberation of chelating agents to the substrate.

Conclusion

The tight Cladoniaceae lichens – Quartzarenic Neosol relationship is confirmed, since the lichen species tested are capable of interacting with and modifying the substrate.

Further studies are required with other compounds and species, and with more information of action mechanism, for a better contribution to the knowledge about the role of these organisms in the ecosystem, which would facilitate conservation plans.

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