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FROM BIOLOGICAL COLONIZATION TO THE SOIL AND CRUST FORMATION: THE ROLE OF CYANOBACTERIA AND SMALL ANIMALS IN A KARSTIC LANDSCAPE, NORTH OF MINAS GERAIS STATE, BRAZIL

DA COLONIZAÇÃO BIOLÓGICA À FORMAÇÃO DE BOLSÕES DE SOLOS E CROSTAS: O PAPEL DE CIANOBACTÉRIAS E PEQUENOS ANIMAIS NA PAISAGEM CÁRSTICA DO NORTE DE MINAS

DE LA COLONIZACIÓN BIOLÓGICA A LA FORMACIÓN DE SUELOS Y COSTRAS: EL PAPEL DE LAS CIANOBACTÉRIAS Y PEQUEÑOS ANIMALES EN UN PAISAJE KÁRSTICO, AL NORTE DE LA PROVINCIA DE MINAS GERAIS, BRASIL

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ABSTRACT: The susceptibility of limestones to biological colonization can be a significant role in the development of karst relief, since the microbial biofilms formation and exopolymeric compounds produced by different metabolic activities, and can influence the dissolution processes and precipitation of minerals, boosting specific transformations at karst. Study about biological performance in limestone outcrops in Brazil are still scarce, so it was aimed to analyze the role of the cyanobacteria and small animals (rodents and marsupials) in the superficial transformation of limestones and soil formation in the North of Minas Gerais state. Thus, the methods used were the total organic carbon content with fractionation of humic substances, potential acidity, and micromorphological and microchemistry characterization. The results showed a low recovery of total organic carbon, with a predominance of humin, followed by humic and fulvic acids. The potential acidity presented values classified as very low to very high, and the micromorphological and microchemistry images showed some specific features of the limestone. The conclusion is that the cyanobacteria, rodents and marsupials are part of the karst relief evolution, and possibly the effects of their colonization were able to promote the formation of biofilms, which through complex metabolic interactions, stimulated the processes of biokarstification, favoring the surface transformations at limestone and the soil and crust formation, that contribute to the karst relief.

Keywords: Biokarstification; Limestones; Biofilm.

RESUMO: A suscetibilidade das rochas calcárias à colonização biológica pode desempenhar um papel significativo no desenvolvimento do relevo cárstico, uma vez que a formação de biofilmes microbianos e compostos exopoliméricos produzidos por distintas atividades metabólicas, podem influenciar os processos de dissolução e precipitação de minerais, estimulando transformações específicas no carste. Tendo em vista que os estudos sobre a atuação biológica em afloramentos de calcário no Norte de Minas Gerais ainda são escassos, o presente estudo teve como objetivo analisar o papel de cianobactérias e de pequenos animais (roedores e marsupiais) na formação bolsões de solos, crostas e feições em calcários do Norte de Minas Gerais. O método utilizado para investigar a interação das espécies em estudo com a rocha calcária e formação de solos e crostas foi a análise do teor de carbono orgânico total, fracionamento de substâncias húmicas, acidez potencial e caracterização micromorfológica e microquímica. Os resultados apontaram uma pequena recuperação do carbono orgânico total, com predomínio da humina, seguida pelos ácidos húmicos e fúlvicos; a acidez potencial variou de muito baixa a muito alta; e as imagens micromorfológicas e microquímicas revelaram algumas feições específicas no calcário. Foi possível concluir que as cianobactérias, roedores e marsupiais estão inseridos dentro de uma evolução do relevo cárstico e possivelmente os efeitos da colonização foram capazes de promover a formação de biofilmes, que por meio de complexas interações metabólicas, estimularam os processos de biocarstificação, favorecendo as transformações superficiais na rocha calcária e a gênese de solos, crostas e feições que auxiliam na evolução do relevo cárstico.

Palavras-chave: Biocarstificação; Rochas Calcárias; Biofilme.

RESUMEN: La susceptibilidad de las rocas calizas a la colonización biológica puede jugar un papel importante en el desarrollo del relieve kárstico, ya que la formación de biopelículas microbianas y compuestos exopoliméricos producidos por diferentes actividades metabólicas pueden influir en los procesos de disolución y precipitación de minerales, estimulando transformaciones específicas en el karst. Teniendo en cuenta que los estudios sobre la actividad biológica en afloramientos calcáreos en el norte de Minas Gerais aún son escasos, el presente estudio tuvo como objetivo analizar el papel de las cianobacterias y pequeños animales (roedores y marsupiales) en la formación de bolsas de suelos, costras y rasgos en calizas en el norte de la provincia de Minas Gerais, Brasil. El método utilizado para investigar la interacción de las especies estudiadas con la roca caliza y la formación de suelos y costras fue el análisis del contenido de carbono orgánico total, fraccionamiento de sustancias húmicas, acidez potencial y caracterización micromorfológica y microquímica. Los resultados mostraron una baja recuperación de carbono orgánico total, con predominio de la humina, seguida de los ácidos húmicos y fúlvicos. La acidez potencial presentó valores clasificados de muy bajos a muy altos, y las imágenes micromorfológicas y microquímicas mostraron algunas características específicas de la caliza. Se pudo concluir que las cyanobacteria, roedores y marsupials se insertan dentro de una evolución del relieve kárstico y posiblemente los efectos de la colonización pudieron promover la formación de biopelículas que, mediante complejas interacciones metabólicas, estimularon los procesos de biokarstificación, favoreciendo las transformaciones superficiales en la roca caliza y la génesis de suelos, costras y elementos que ayudan en la evolución del relieve kárstico.

Palabras clave: Biokarstificación; Rocas Calizas; Biopelícula.

1. INTRODUCTION

The Brazilian territory is formed of 5 to 7% of karst landscapes, and in the Minas Gerais state, about 3 to 5% of area, is constituted by carbonate rocks (TRAVASSOS, 2010). In the municipality of Montes Claros, north of Minas Gerais, the karst relief shapes the landscape with limestone outcrops in the form of Karren, which are colonized by different organisms, including cyanobacteria, rodents and marsupials.

Cyanobacteria are photosynthetic beings with peculiar physiological characteristics capable of colonizing the most different environments. These microorganisms constitute one of the essential elements in the microbial biofilms' formation, as they excrete extracellular polysaccharides that increase microbial diversity, thus interacting with the mineral substrate (GOLUBIC; SEONG-JOO; BROWNE, 2000; OSORIO-RODRIGUEZ; SANCHEZ-QUINONEZ, 2018; WILLIAMS; BÜDEL; WILLIAMS, 2018) and stimulating the establishment of successional stages of other species such as lichens and mosses (RONCERO-RAMOS et al., 2020).

Rodents and marsupials has a physiological characteristic that allow to adapt well at high temperatures zones and poor of water and food zones, especially in extreme periods of drought (OLIVEIRA et al., 2011). They live especially in crevices or cracks of rocky outcrops at Brazilian semiarid region and some locals of northern Minas Gerais, bordering with the cerrado biome. These places serve as a refuge against predators and collective latrines at strategic points, usually in high areas of the rocks (PARENTE; CAVALCANTI, 2017).

Many studies have observed the role of organisms in processes that influence the transformation of surface rocks, soil formation and relief evolution. Doddy and Roden (2018) examined the potential of microorganisms as bioerosion agents and showed that cyanobacteria are involved in soil formation and the transformation of Burren limestone outcrops in Ireland into an environment favorable to ecological succession. Lü et al. (2019) observed that the microbial carbonic anhydrase enzyme plays an essential role in carbonate precipitation and the morphology of modern speleothems. Levett et al. (2020) demonstrated how the participation of microorganisms is fundamental in the dissolution and precipitation of Fe in outcrops of ferruginous rocks (cangas), guaranteeing the dynamics of degradation and formation of these materials at landscape.

It is very well documented that many organisms can work to transform an entire landscape. Thus, only general studies, non-specific, were found in the Brazilian literature about the contribution of the cyanobacteria, rodents and marsupials to the process of soil and crust formation from the dissolution of carbonate rocks and the emergence of karst features in a landscape context. Brazilian research is generally limited to the morphological, physiological or behavioral adaptations of these species, as pointed by Aro et al., (2019), Souza et al. (2020), and Alvarenga et al. (2020).

Thus, the study aimed to analyze the role of the cyanobacteria, rodents and marsupials in the formation of soil pockets, crusts, and features in limestones at northern Minas Gerais, Brazil.

2. MATERIALS AND METHODS

2.1. Study area

The study was carried out in the municipality of Montes Claros, located in the Upper Middle São Francisco River basin, northern Minas Gerais (Figure 1).

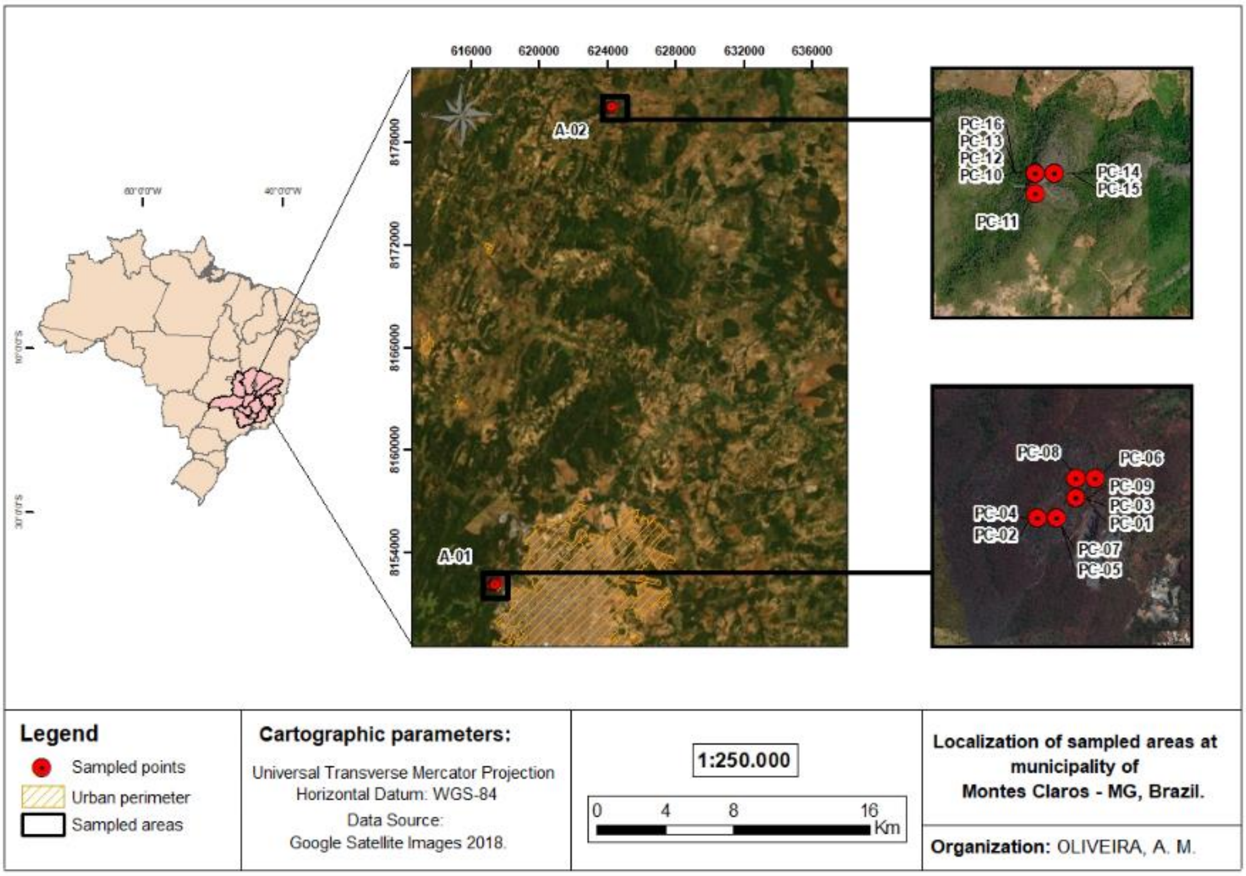

Figure 1 - Location of the studied areas and sampled points. Oliveira et al. (2023).

According to the Köppen classification, the climate is type Aw, described as humid tropical savannah with dry winter. The average annual rainfall is approximately 1,000 mm, with rainfall concentrated between October and March, while the dry season occurs from June to August, with an annual average temperature of 23 °C (CLIMATE DATA, 2022). Even with irregular rainfall, the region is home to several wellspring and springs of the São Francisco River basin, which help in the sculpting process of the karst features.

The natural vegetation cover at Montes Claros is constituted by cerrado biome covering areas of tops and plateaus, by Seasonal Semideciduous Forest present in the drainages of slopes, and, with reduced cover, by Macaubais in more flattened portions of the landscape. In this region there are contacts with the caatinga biome (semiarid region) (CAMINHAS; FONSECA, 2020).

In the sampled areas located in limestone outcrops (Figure 2), there are fragments of rocky vegetation rich in species of the Cactaceae and Bromeliaceae families, many with restricted occurrences, such as the bromeliad *Encholirium Luxor* L. B. Sm. & R. W. Read, interspersed with Deciduous Seasonal Forest, known as “dry forest” (PEREIRA et al., 2020).

Figure 2 - Limestone outcrops and Karren formation, Montes Claros - MG, Brazil. Note: A) Area 01: Highlight for the Karren; B) Area 02: Landscape overview. Oliveira et al. (2023).

Montes Claros is part of a karstified carbonate domain in the province of the São Francisco Craton, specifically associated with terrigenous and carbonate metasedimentary rocks of the Neoproterozoic age of the Bambuí Group, which were deposited in a foreland basin of exclusively marine sedimentation. In the study area, rocks of the Lagoa do Jacaré Formation outcrop, where slim crystallized limestones, stylolites, gray-green and pyritic siltstones are found; marly, placid, dark limestones, sometimes recrystallized oosparites and breccia-like, pyritic, with nodules of fetid black calcite, interspersed with siltstones and rare marls (IGLESIAS; UHLEIN, 2009).

The lithological characteristics in line with the exogenous weathering processes contributed to sculpting the karst scenarios in Montes Claros, which present altimetric elevations between 500 and 1,075 m. The areas with lower altitudes correspond to about a third of the planning surfaces of the São Francisco River depression, and those with higher altitudes make up the rest of the area, which facilitates the flow of groundwater and the evolution of the karst relief present in the Residual Plateau of São Francisco (LEITE et al., 2011).

2.2. Sampling

The collections were carried out regarding the rocky outcrops visibly influenced by the occupation of the cyanobacteria, which usually occurs in Kamenitza, and by the rodents and marsupials droppings on the limestone rock, indicating its presence at the site (Figure 3).

Figure 3 - Colonized limestone rocks, Montes Claros - MG, Brazil. Note: A) Kamenitza with presence of cyanobacteria; B) Latrine containing rodent/marsupials feces. Oliveira et al. (2023).

Sixteen samples were collected, including rocks, soil pockets, crusts, and excrements, in two limestone outcrops: one near the city of Montes Claros and another in the Samambaia Community. From them, thirteen samples refer to materials under the influence of the cyanobacteria, and three are samples influenced by rodents and marsupials. The samples were collected using a hammer, chisel, spatula, and plastic bags for packaging, which, after identification, they were sent for laboratory analysis. The Table 1 shows the characteristics of the samples collected.

Table 1 - General characteristics of the samples collected

Collection area	Altitude	Collect point	Geographic coordinate	Characteristics of the material collected	Analyzes performed
A1 Close to the urban perimeter of Montes Claros	816 m	P01	16º42'36" S 43º53'52" O	Limestone rocks and crusts without rodents and marsupials excretions	(H+Al) and MICRO(*) (**)
		P02	16º42'39" S 43º54'01" O	Limestone rocks and crusts without rodents and marsupials excretions	(H+Al) and MICRO(*) (**)
		P03	16º42'36" S 43º53'52" O	Rodents and marsupials excretions	(H+Al)
		P04	16º42'39" S 43º54'01" O	Cyanobacteria on limestone rock fragments	TOC, HS, (H+Al), and MICRO(*)
		P05	16º42'00" S 43º54'01" O	Limestone dust with cyanobacteria	TOC, HS, and (H+Al)
		P06	16º42'39" S 43º53'49" O	Soil pockets between limestone rock fragments and cyanobacteria	TOC, HS, and (H+Al)
		P07	16º42'00" S 43º54'01" O	Soil pockets between limestone rock fragments and cyanobacteria	TOC, HS, and (H+Al)
		P08	43º53'52" S 16º42'32" O	Soil pockets with cyanobacteria	TOC, HS, and (H+Al)
		P09	16º42'36" S 43º53'52" O	Soil pockets between limestone rock fragments and cyanobacteria	TOC, HS, and (H+Al)
A2 Samambaia Community	790 m	P10	16º27'25" S 43º50'09" O	Cyanobacteria on limestone rock fragments	TOC, HS, (H+Al), and MICRO(*)
		P11	16º27'28" S 43º50'09" O	Soil pockets with cyanobacteria	TOC, HS, and (H+AL)
		P12	16º27'25" S 43º50'09" O	Soil pockets with cyanobacteria	TOC, HS, and (H+Al)
		P13	16º27'25" S 43º50'09" O	Soil pockets between limestone rock fragments and cyanobacteria	TOC, HS, and (H+AL)
		P14	16º27'25" S	Soil pockets between limestone	TOC, HS,

		43º27’25” O	rock fragments and cyanobacteria	(H+Al) and MICRO(*) (**)
	P15	16º27’25” S 43º27’25” O	Soil pockets with cyanobacteria	TOC, HS, and (H+Al)
	P16	16º27’25” S 43º50’09” O	Soil pockets with cyanobacteria	TOC, HS, and (H+Al)

TOC: Total Organic Carbon. HS: Humic Substances. (H+Al): Potential Acidity. MICRO: (*) Micromorphology (*) and (**) Microchemistry. Oliveira et al. (2023).

2.3. Laboratory analysis

For the cyanobacterium domain, the total organic carbon content, fractionation of humic substances, potential acidity (P04 to P16), micromorphological characterization (P04, P10, and P14), and microchemical characterization (P14) were performed. For the rodents and marsupials domain, due it samples are excrements, crusts, and rocks with a small number of soil pockets, it was not possible to carry out the analysis of the carbon content and fractionation of humic substances, being analyzed the potential acidity (P01, P02, and P03) and micromorphological and microchemical characterization (P01 and P02).

The total organic carbon content was determined by oxidation using 0,5 g of air-dried fine earth (TFSA) in 5 mL of potassium dichromate solution ($K_2Cr_2O_7$) 0,167 mol L^{-1} and 7,5 mL of concentrated sulfuric acid (H_2SO_4), according to Yeomans and Bremner (1988). The chemical fractionation of humic substances was carried out according to the method of Swift (1996) adapted by Mendonça and Matos (2005). For extraction, 1,0 g of TFSA was used and 10 mL of sodium hydroxide (NaOH) 0,1 mol L^{-1} was added. The percentage of the humification index and the fulvic acid, humic acid, and humin fractions concerning the total organic carbon content was calculated.

Potential acidity, using 5 g of TFSA, was determined by the calcium acetate extraction method $Ca(CH_3COO)_2.H_2O$ 0,5 mol L^{-1} buffered at pH 7,0 and determined by titration with sodium hydroxide (NaOH) 0,025 mol L^{-1} (EMBRAPA, 2017).

For micromorphological characterization, thin and polished slides were made, according to Martins et al. (2002). The samples were impregnated with resin, transferred to an oven at a temperature of 55 ºC, sliced, and polished until reaching a dimension of 1,8 x 30 x 40 mm (30 μm thick). Following the criteria established by Stoops (2003) and Stoops, Marcelino e Mees (2018), the slides were described through the images obtained by the Trinocular Petrographic microscope, model Zeiss - Axiophot, with an attached camera. Representative areas were analyzed using the Scanning Electron Microscope (SEM), model VEGA3-TESCAN, and the microchemical maps and punctual analyzes were performed using Energy Dispersion Spectrometry (EDS).

3. RESULTS

3.1. Total organic carbon, fractionation of humic substances, and potential acidity

The collected soil pockets showed a small recovery of organic carbon ranging from 0,53 to 24,82 dag.kg^{-1}, in P10 and P15, respectively (Table 2). The highest values were obtained in samples P15 (24,82 dag.kg^{-1}), P14 (19,94 dag.kg^{-1}), P16 (19,33 dag.kg^{-1}), and P06 (18,72dag.kg^{-1}), and the lowest values in samples P10 (0,53 dag.kg^{-1}), P04 (1,36 dag.kg^{-1}), P07 (2,86 dag.kg^{-1}), and P05 (2,87 dag.kg^{-1}).

Table 2 - Total organic carbon content, humic substances, and potential acidity

SAMPLES	TOC(1)	HI(2)	FAF(3)	HAF(4)	HUM(5)	H+Al(6)
	dag.kg^{-1}	%				$cmol_c.kg^{-1}$
(*)(**)P01	-	-	-	-	-	-
(*)P02	-	-	-	-	-	0,16
(*)P03	-	-	-	-	-	23,9
(**)P04	1,36	52,2	0,07	0,27	4,04	-
P05	2,87	45,6	0,01	1,37	11,22	0,16
P06	18,72	14,5	5,17	6,08	4,50	0,64
P07	2,86	64,2	1,57	1,47	16,57	0,48
(**)P08	5,91	33,6	1,17	0,67	19,30	-
P09	6,52	32,1	1,27	2,17	17,71	0,32
(**)P10	0,53	3,6	0,07	0,01	0,06	-
P11	5,91	37,8	1,97	1,97	20,67	0,32
P12	10,18	5,6	3,17	6,18	1,65	1,60
P13	8,35	6,5	2,37	2,27	0,40	0,32
P14	19,94	26,1	5,37	5,88	15,43	2,14
P15	24,82	19,4	8,08	5,68	9,62	1,92
P16	19,33	18,7	9,28	6,98	6,89	2,08

(1)COT: Total organic carbon. (2)HI: Percentage of the humification index. (3)FAF: Percentage of the fulvic acids fraction in relation to the total organic carbon. (4)HAF: Percentage of humic acid fraction in relation to total organic carbon. (5)HUM: Percentage of the humin fraction in relation to total organic carbon. (6)H+Al: Potential acidity. (*): TOC results are not presented for samples P01 and P02, considering they are crusts and rocks with little/no soil, and P03, as they are rodent/marsupial excrement. (**): Unsatisfactory results for (H+Al). Oliveira et al. (2023).

The humification index presented values ranging from 3,6% (P10) to 64,2% (P07), with the highest values observed in P07 (64,2%) and P04 (52,2%), while P10, P12, and P13 had the lowest values, (3,6%), (5,6%), and (6,5%), respectively.

When analyzing the solubility of humic substances in an aqueous medium, it was observed that humin was the most significant fraction as a carbon reserve, ranging from 0,40 to 20,67%, followed by the humic acid fraction with values between 0,01 and 6,98%, and fulvic from 0,07 to 9,28%. The highest values for humin were observed in samples P11 (20,67%) and P08 (19,30%), and the lowest in P13 (0,40%) and P10 (0,06%).

Among the soluble fractions, humic acids showed the highest levels in samples P16 (6,98%), P12 (6,18%) and P06 (6,08%), while the lowest levels were observed in samples P10 (0,01%), P04 (0,27%), and P08 (0,67%). The fulvic acid fraction showed the highest contents in samples P16 (9,28%) and P15 (8,08%), and the lowest in samples P10 (0,07%), P05 (0,01%), and P04 (0,07%).

Potential acidity levels showed a wide range with values between 0,16 and 23,9 $cmol_c.kg^{-1}$, evidencing different intensities of buffering and cationic exchanges. When the results obtained with the classification proposed by Ribeiro, Guimarães e Alvarez V. (1999) for potential acidity are being considered, it was possible to identify samples with “very low” acidity (≤ 1,0 $cmol_c.kg^{-1}$) in P06 (0,64 $cmol_c.kg^{-1}$), P07 (0,48 $cmol_c.kg^{-1}$), P09, P11, P13 (0,32 $cmol_c.kg^{-1}$), and P02, P05 (0,16 $cmol_c.kg^{-1}$), “low” acidity (between 1,01 - 2,50 cmolc.kg^{-1}) in P14 (2,14 cmolc.kg^{-1}), P16 (2,08 cmolc.kg^{-1}), P15 (1,92 cmolc.kg^{-1}), and P12 (1,60 cmolc.kg^{-1}), and “very high” acidity (> 9,00 $cmol_c.kg^{-1}$) in P03 (23,90 $cmol_c.kg^{-1}$), for the rodent and marsupial excrement sample.

3.2. Micromorphological characterization

3.2.1. Thin sections associated with the cyanobacteria

The results indicated areas with a thin coverage of moderately to very humidified material, with a black to dark reddish-black hue, constituting a carapace that is discontinuously impregnated on the rock surface (Figure 4A). Similar reactions were not observed elsewhere in the rock (Figure 4B). Where the alteration cortex is thicker (approximately 2 mm thick on average), the rock surface acquires a more irregular appearance, suggesting selective dissolution. The penetration of the organic material occurs through the fractures, and the fillings follow the diaclase planes that internally reproduce the surface edge reactions (Figures 4C and 4D).

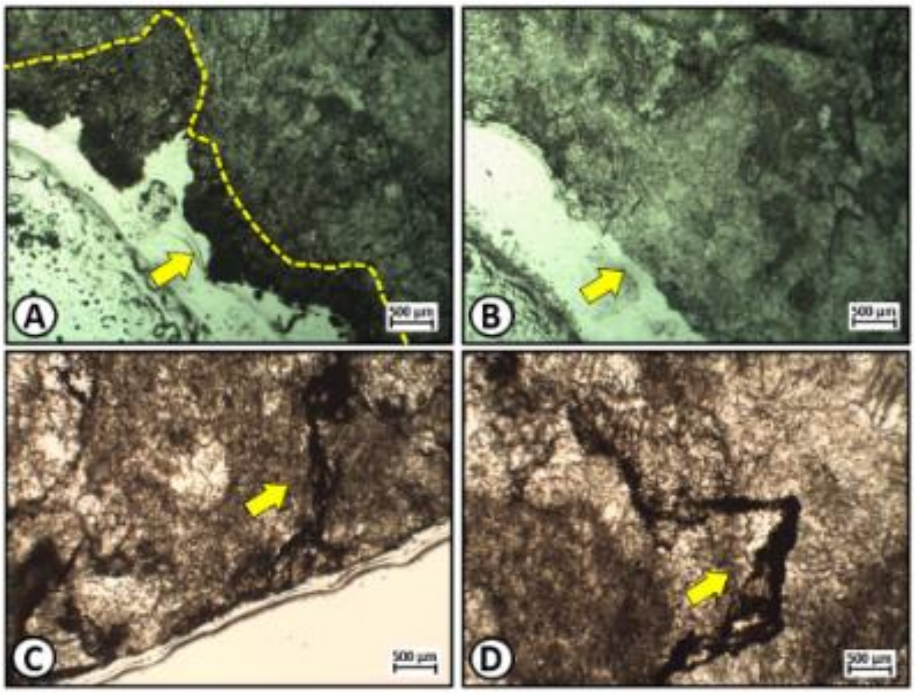

Figure 4 - Colonization areas with cyanobacteria, point P04. Note: A) With carapace; B) Without shell; C and D) Fractures with organic material. Oliveira et al. (2023).

When magnifying the image (magnification of 100x), the microscopic observations revealed the filamentous appearance on the surface of the limestone rock, as well as the detachment of small fragments of very altered rock and relatively soaked by the amorphous humified organic material (Figure 5).

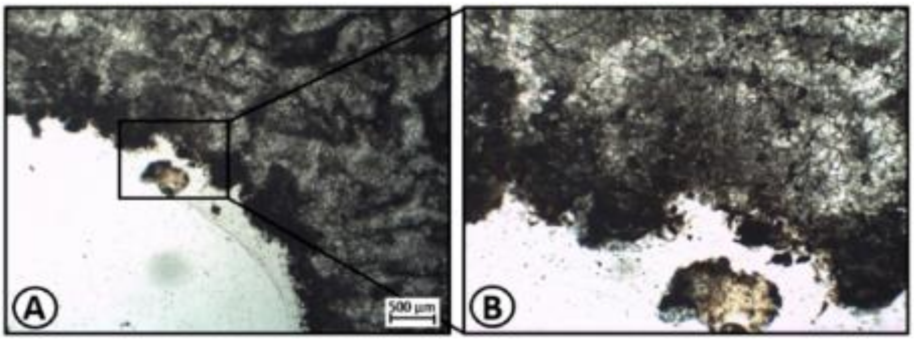

Figure 5 - Fragments of heavily altered rock, point P10. Note: A) Rock fragments; B) Detail of image A. Oliveira et al. (2023).

It was observed that the alteration reaction between the humified organic material and the carbonates seems to be very intense and with greater penetration. Thus, organic matter occurs in greater quantity, suggesting that this type of rock provides better conditions for the growth of microorganisms. The opening of the matrix occurs through the formation of irregularly shaped pores, typically of dissolution, which is connected, forming a simple packing-type network and isolating small fragments of rock that have not yet been degraded (Figure 6).

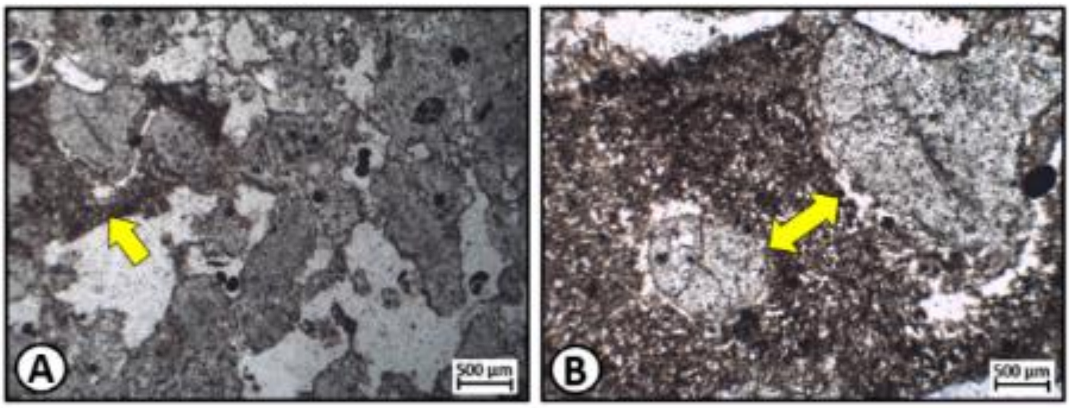

Figure 6 - Filling of pores by organic matter, point P14. Note: A) Irregular pores occupied by organic matter; B) Isolated rock fragments. Oliveira et al. (2023).

3.2.2. Slender sections associated with rodents and marsupials

The formation of crusts was the most expressive feature of influence of the colonization effects of the rodents and marsupials and are associated with the saline efflorescences encrusted on the limestone surface. They were observed to present macroscopic characteristics indicative of considerable coating with millimeter to centimeter thickness, showing rough surfaces with colors ranging from gray-white to yellowish, easily identified in the field.

In the thin sections, the crusts formed on the limestone surface presented a variety of micro facilities with different stages of biokarstification. The contact portion of the crust with the limestone surface is signaled by a reddish color change cortex, whose behavior suggests the formation of iron compounds and is marked by its degradation (Figure 7A1). The increasing precipitation of solutions over the rock leads to the formation of well-demarcated halos of calcified materials (Figure 7A2). In the upper part of the crust, it was possible to observe the discoloration of the substrate, resulting in the darkening of the surface (Figure 7A3). The pores are partially filled by by-products with amorphous aspects of precipitation, evidencing a higher microbial activity (Figure 7B4). Minerals with a needle-like habit are identified and confirmed by microchemical analysis to be needle-fiber calcite (Figure 7C5).

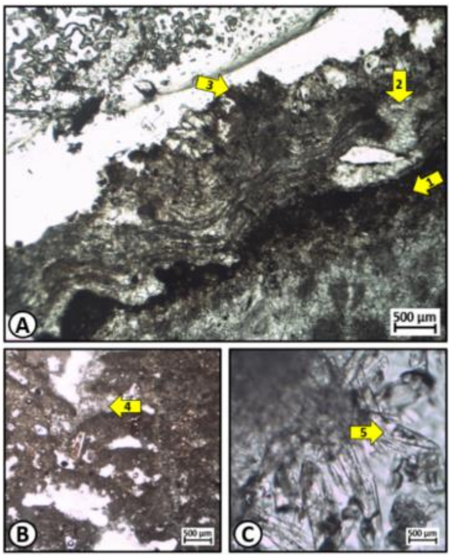

Figure 7 – Rodent/marsupial colonization. A and B: point 01; C: point 02. Note: Arrows indicate each stage of biokarstification; A1) Acidification; A2) Precipitation; A3) Discoloration; B4) precipitation; C5) Needle-fiber calcite. Oliveira et al. (2023).

3.3. Microchemical characterization

The microchemical results for the thin organic horizon (undisturbed sample) under the influence of the cyanobacteria revealed the presence of the elements Ca, O, C, and Si, significantly evidencing calcite as the main constituent and, in restricted points of the alteration cortex, it was possible to observe the occurrence of carbonate silicification processes (Figure 8).

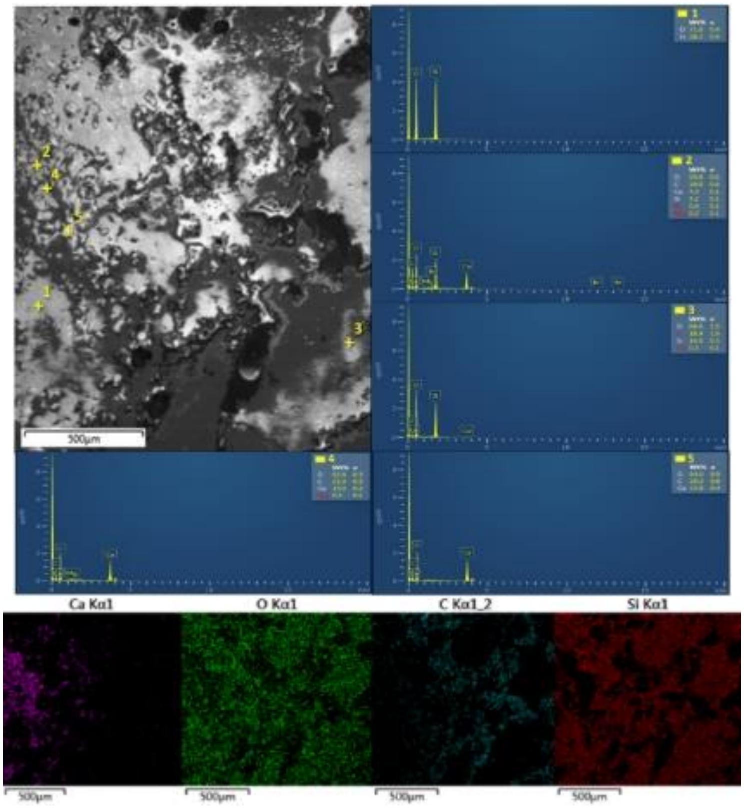

Figure 8 - Microchemical maps of the point P14 with backscattered electron images of the elements Ca, O, C, and Si obtained by EDS probe. The colored areas show the presence of the chemical element highlighted in the center of each image. Oliveira et al. (2023).

Regarding the alteration crust influenced by the colonization of the rodent/marsupial, the results showed the presence of the elements Ca, O, C, and Al, also significantly showing calcite as the main constituent. Due to its hydrolyzing tendency, the presence of Al in the solution suggests higher acidification of the medium, and in a poorly defined way, Fe was observed in the sample (Figure 9). The presence of this element, even if in a very subtle way, corroborates the visual and petrographic observations carried out in the sample.

As with layer P14, under the influence of the cyanobacteria, layer P01 highlights the carbonate silicification process at the edge of the limestone, demonstrating the formation of a secondary alteration material concentrating Si (Figure 10).

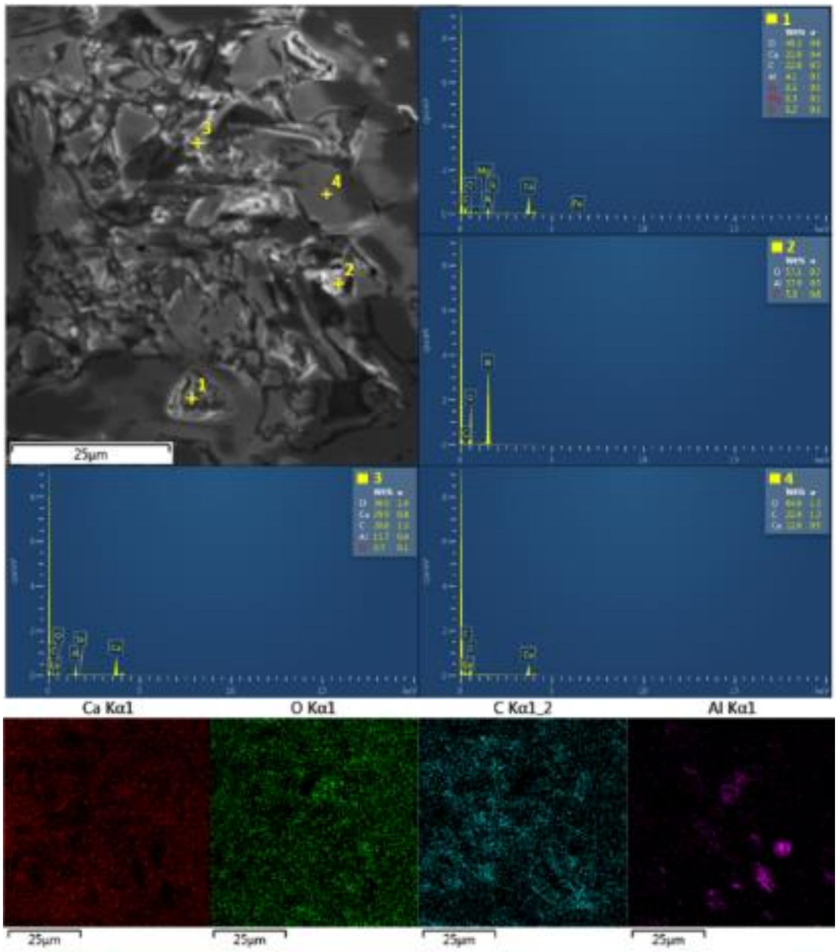

Figure 9 - Microchemical maps of the P02 point with backscattered electron images of the elements Ca, O, C, and Al obtained by EDS probe. The colored areas show the presence of the chemical element highlighted in the center of each image. Oliveira et al. (2023).

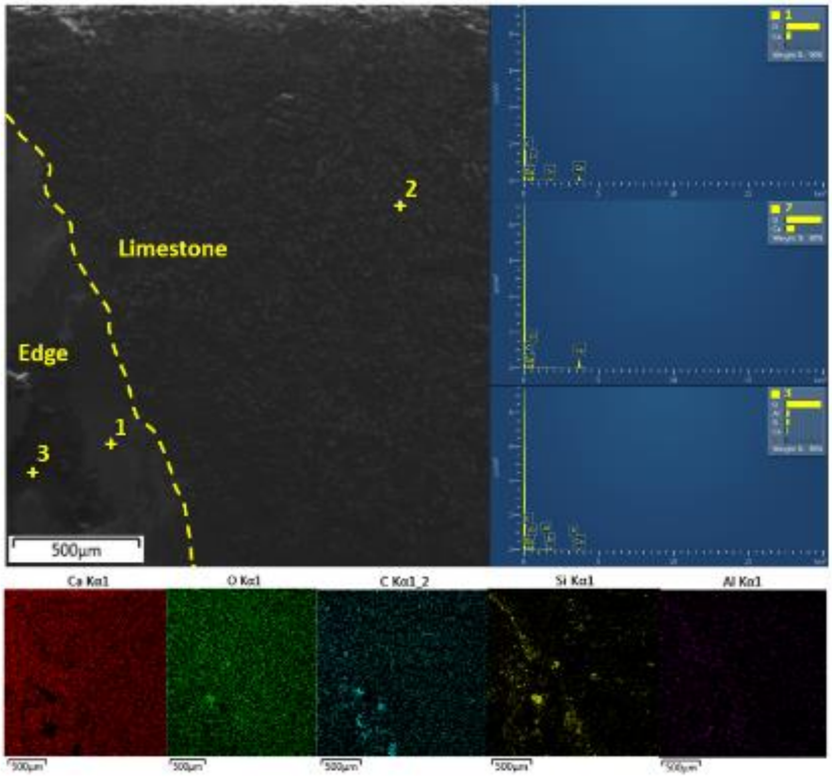

Figure 10 - Microchemical maps of point P01 with backscattered electron images of the elements Ca, O, C, Si, and Al obtained by EDS probe. The colored areas show the presence of the chemical element highlighted in the center of each image. Oliveira et al. (2023).

4. DISCUSSION

4.1. The role of the cyanobacteria in the superficial transformation of limestone and the genesis of karst features

Cyanobacteria are pioneers in the colonization process and favor the formation of microbial biofilms, which, through complex metabolic interactions, can precipitate or dissolve minerals (DUPRAZ et al., 2009). Possibly, the colonization mechanism begins with the fixation of the cyanobacteria in the lithological substrate that starts to secrete extracellular polysaccharides, which are responsible for maintaining adhesion to the surface and attracting different microorganisms, forming biofilms. Once adhered to substrates, microorganisms can form colonies with complex interactions and transform the physicochemical characteristics of rocks (GOLUBIC; SEONG-JOO; BROWNE, 2000; PINHEIRO et al., 2019).

Among these transformations, the results showed a close relationship between carbonates and microorganisms, suggesting that the different metabolic activities present in biofilms play a significant role in rock degradation. It is possible to consider that the trend toward higher levels of organic carbon is due to a more advanced stage of colonization carried out by cyanobacteria, which provides conditions for the formation of an organic matrix composed of different microorganisms that act in the production and mineralization of organic carbon. The lowest levels obtained are indicative of an initial stage of formation with low rates of microbial density, species richness, and metabolic activity, which may limit the progress of the humification process of organic matter.

It is very likely that the highest values obtained for the humification index are directly related to the development of biofilms since cyanobacteria interacts with the complex microbial matrix in the production of extracellular polysaccharides and multiple metabolisms intensify the decomposition of organic material, providing the formation of hydroxyl and carboxyl functional groups that can promote or inhibit the precipitation of carbonates (DUPRAZ et al., 2009). The heterogeneity between the source material (different types of limestone), the resistance to decomposition, and the complexity of the metabolic reactions that influence the intensity of the humification process may have motivated the different values found (MARTINS et al., 2015).

This study observed that humin was the most significant fraction of humic substances, followed by humic and fulvic acids, suggesting higher stability and interaction with the mineral fraction. Pizauro Júnior and Melo (1995) highlighted that the predominance of one fraction over the other indicates the advanced stage of mineralization of organic matter and the incorporation of nitrogen into the humic structure, as well as Nierop (1999), using ^{13}C, stated that 40-45% of the carbon from the humin fraction are extracellular polysaccharides that are preserved in the soil through interaction with the mineral fraction and formation of organometallic complexes.

As the cyanobacteria presents the heterocyst, a cell capable of fixing nitrogen, it probably, by converting nitrogen to forms available for assimilation, may be contributing to humin being the most expressive fraction of organic matter. In addition, the Aw climate of the studied area, due to its well-defined seasons, possibly contributes to the predominance of humin among the humic fractions (fulvic acid and humic acid) insofar as it allows the dehydration of organic compounds in the dry season, concentrated between from June to August, and the rapid formation of more stable organic compounds with high molecular weight in the rainy season, between October and March (SILVA, 2013).

The thin sections revealed that cyanobacteria seem to be part of an evolution of specific features in the limestone rock, with emphasis on the alteration cortex, fragmentation, and fillings. The presence of a thin organic layer suggests a direct relationship with the alteration cortex, being an indication that the alteration process in the limestone rock can be restricted to active microenvironments of intense metabolism and microbial diversity that occurs in consonance with the characteristics of the rock and environment, forming “microbial hotspots” (KUZYAKOV; BLAGODATSKAYA, 2015). In addition to the metabolism that drives the biogeochemical transformations, the selective dissolution is possibly due to the different solubility of the minerals, the acidic capacity of the solution, and the time spent in contact with the reactive limestone surface (EMBRAPA, 2004; FORD; WILLIAMS, 2007).

According to Pentecost and Whitton (2012), limestone outcrops serve as a source of dissolved carbon for the photosynthetic metabolism of the cyanobacteria, which grows preferentially in Kamenitzas. When colonizing the rocks, cyanobacteria in association with microorganisms that coexist in the biofilms, can favor an increase in pH and calcium concentrations, inducing the precipitation of carbonates (DUPRAZ et al., 2009) and stimulating chemical dissolution through the excretion of acids that slowly corrode the surface of rocks, releasing them in small fragments. Extracellular polysaccharides and pore expansion facilitate the retention of water and airborne clay particles, which favors the formation of soil pockets that accumulate organic biomass and serve as a source of nutrients for the development of subsequent organisms (LIAN et al., 2008; DODDY; RODEN, 2018), which can enhance the degradation of limestone rock.

The role of microorganisms in the dissolution of carbonates has been well documented due to their ability to excrete chelating substances and organic (oxalic, citric, pyruvic, lactic, and others) and inorganic (sulfuric, nitric, carbonic, and others) acids which, because they are very reactive, react with minerals and accelerate the dissolution process (PINHEIRO et al., 2019). In a study of karst forms, Shroder (2013) stated that the solubility of carbonate minerals in the presence of pure water is around 7 mg L^{-1}, very low, and increases rapidly as a function of the absorption of dissolved carbon dioxide and other acids. For this reason, acids resulting from excretions of the cyanobacteria in metabolic cooperation with the biofilm and, especially the rodents and marsupials, which presented "very high" potential acidity (RIBEIRO; GUIMARÃES; ALVAREZ V., 1999) and the presence of Al in the microchemical analyses, are indicative of the increase of chemically aggressive water to dissolve the carbonate rock and develop several karst features. Parente and Cavalcanti (2017) also confirmed a tendency for acidic substrate in rodent excretion samples. The wide range of values found for the potential acidity evidenced the different intensities of buffering power and maybe related to the lower content of organic matter, the different types of clay minerals, and the metabolic complexity that influences the decomposition of organic biomass (ALMEIDA JÚNIOR; NASCIMENTO; BARROS, 2015).

It is possible that the fragmentation process that generated small limestone sediments incorporated into the amorphous material occurred through the dissolution phase that led to the production of loose particles of rocks and carbonates in solution, which resulted in a constructive phase (DUANE; AL-MISHWAT; RAFIQUE, 2003), which bound to these particles through the matrix extracellular polysaccharides (ROSSI; DE PHILIPPIS, 2015). In addition, in periods of drought, the sheaths of cyanobacteria undergo considerable shrinkage, while in periods of rain, they can absorb water 20 times their dry weight, as stated by Satoh et al. (2002). This process of desiccation and hydration causes increased pressure on the pores and surface flaking of the rock, which may also have led to limestone fragmentation.

In the case of fills, the intense alteration reaction between the humified material and the carbonates seems to be directly linked to the hygroscopicity of the rock that, through fractured and very porous surfaces, allows for greater retention of water, essential to attract and condition microbial growth (GÓMEZ-CORNELIO et al., 2012).

4.2. The role of rodents and marsupials in the surface transformation of limestone and the genesis of karst features

The results revealed the possible participation of rodents and marsupials in mineral neogenesis from the influence of its excrements, which are related to a process of evolution of features identified in the limestone rock such as efflorescence, alteration cortex, discoloration, halos, crusts, and the fills. It is very likely that the high temperatures in the North of Minas Gerais, in addition to other factors such as urea and uric acid present in the urine of the rodents (ZOGNO, 2002), probably acted as catalysts to accelerate the dissolution/crystallization reactions of the more soluble minerals present in the limestone, causing salt efflorescence, which according to Saiz-Jimenez and Laiz (2000), Piñar et al. (2009), and Lepinay et al. (2018), form ideal habitats for the attachment of microorganisms that can promote the precipitation of minerals.

In a study on pathologies caused by urine in ornamental rocks composed of granite, marble, and limestone, Ribeiro et al. (2011) demonstrated the strong interaction on the mineral surface due to the acidity produced by urea and/or uric acid. Their results indicated more intense alterations in Cariri limestone due to the degree of porosity and subsequent iron oxidation. It suggests that the reddish color change cortex was formed by the percolation of the rodents/marsupials urine from the rock pores and fractures, which under more acidic conditions favored its probable oxidation (STEPHENS; ROSE; GILBERTSON, 2017). Although the dissolution and precipitation process of Fe compounds occur by the action of oxygen (which reacts with Fe under the influence of several factors such as pH, temperature, and mainly humidity) (FIGUEIREDO, 1999), it is also influenced by several species of microorganisms (STEPHENS; ROSE; GILBERTSON, 2017) that to obtain energy or catalyze iron without energy benefit, their complex metabolic pathways can favor the biogeochemical cycle of iron oxidation and reduction, transforming Fe^{2+} into insoluble Fe^{3+}, which precipitates (RODEN et al., 2004; EMERSON, 2019).

It seems, however, that the presence of fungi and bacteria caused the discoloration in the limestone rock since the increased exposure to UV radiation induces the synthesis of dark melanin pigments as a form of defense by the microorganism (ROSSI; DE PHILIPPIS, 2015). The darkening of the substrate reduces the albedo, which can significantly interfere with the rock surface temperature and cause the mechanical stress of expansion and contraction of the pores, resulting in structural damage to the substrate (WARKE; SMITH; MAGEE, 1996). This alteration is intensified by bacterial and fungal hyphae that, when penetrating between the cleavage planes of the minerals, can cause mechanical stresses and destabilize the rock structure. The influence of seasonal cycles, alternating dry and wet periods, the population fluctuation of rodents and marsupials, and the complex microbial metabolism may have led to the development of alteration halos. Wet periods favor the deposition of precipitated carbonates, as the higher availability of moisture stimulates the formation of new microbial colonies and the rapid production of exopolymeric substances that accelerate the development of slightly laminated substrates over several seasons (DUANE, 2001).

Castanier, Métayer-Levrel and Perthuisot (1999) demonstrated that the metabolites involved in photosynthesis, methanogenesis, the sulfur cycle, and the nitrogen cycle induce several chemical changes in the microenvironment that can promote carbonate precipitation. In this study, the degradation of urea present in the rodents and marsupials urine has likely driven the formation of crusts with precipitated carbonate. The urease enzyme, synthesized by several microorganisms when it catalyzes the hydrolysis of urea, produces ammonia and carbamate, which naturally hydrolyses to produce ammonia and carbonic acid. When balanced in the aqueous medium, these products form bicarbonate, ammonium, and hydroxides, causing an increase in pH. With the reduction of hydrogen ions and the increase of the hydroxyl concentration, the bicarbonate balance is altered, favoring the formation of carbonates that, in the presence of free calcium, promote the precipitation of carbonates (CASTANIER; MÉTAYER-LEVREL; PERTHUISOT, 1999; PHILLIPS et al., 2013; KRAJEWSKA, 2018).

The analysis of this process suggests that the microbial capacity to promote alkalinity increase and create localized precipitation conditions could explain the initial pore filling with amorphous and probably hydrated by-products, which progressively formed more crystalline structures of growth (CASTANIER; MÉTAYER-LEVREL; PERTHUISOT, 1999), resulting in needle-fiber calcite, as confirmed by microchemical analysis. The origin of this calcite may be closely associated with the extracellular matrix of polysaccharides or the presence of microbial cell surfaces, which served as nucleation models, facilitating precipitation and crystal growth (DUPRAZ et al., 2009).

5. CONCLUSION

The thin organic layer and the alteration crusts were the most notable effects that indicated biokarstification processes in the karst relief, in which the cyanobacteria, rodents and marsupials were involved.

The cyanobacteria, when colonizing the lithological substrate, possibly made initial changes that created conditions to attract microorganisms and form biofilms with complex metabolisms that stimulated the humification processes.

The various metabolic activities released acidic substances that gradually dissolved and fragmented the limestone rock, forming soil pockets that favored the presence of new microorganisms, intensifying biokarstification in the limestone.

The presence of rodents and marsupials indicated that different biokarstification processes were involved. In this case, urine was an essential agent for mineralogical dissolution, which eventually created pores/microenvironments favorable to the development of microorganisms and intensified the reaction sites in the rock, giving rise to a crust of precipitates that influenced the ecological succession and, consequently, the karst relief.

The features identified in the limestone rock, which despite being associated with a slow and (often) imperceptible evolution of the karst relief, are fundamental for the understanding and study of colonizing organisms, soils, and rocks, which even within a context point in the landscape allow a better understanding of how the complex karst system evolves.

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