The first occurrence of the biotic mineral guanine in Brazil, a nitrogen-bearing molecule found in a cavernicolous environment

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Abstract

Caves on Earth can be studied as analogous environments on Mars, like underground structures, including Martian lava tubes. On Earth, these environments can sustain large communities hidden from the Sun, thriving especially from chemolithoautotrophic metabolisms. Among the main elements for life as we know it (CHONPS), understanding the nitrogen cycle is of great importance. During sampling of caves for metagenomic studies in the region of Serro and Morro do Pilar, state of Minas Gerais, small deposits of a white/cream color organic material were found on the walls of a shelter. Using techniques like RAMAN and FTIR, it was possible to confirm that the substance found was guanine, a nitrogen-bearing mineral and nitrogen source. However, it has not yet been possible to perform sample diffraction (XRD) due to the low amount of material collected. It is proposed that guanine comes from the nitrogenous excreta of arthropods. This is the first record of mineral guanine in Brazil, with preservation similar to that of the mineral guanine from bat guano. This discovery can broaden our knowledge about minerals associated with caves in Brazil, expand the metabolic possibilities and ecological processes associated with cavernicolous environments, and bring new knowledge in an astrobiological context.

Résumé

Les grottes terrestres peuvent être étudiées comme des environnements analogues sur Mars, comme les structures souterraines, y compris les tubes de lave martiens. Sur Terre, ces environnements peuvent abriter de vastes communautés cachées du soleil, qui prospèrent notamment grâce à des métabolismes chimiolithoautotrophes. Parmi les principaux éléments de la vie telle que nous la connaissons (CHONPS), la compréhension du cycle de l'azote est d'une grande importance. Lors de l'échantillonnage de grottes pour des études métagénomiques dans la région de Serro et Morro do Pilar, dans l'État de Minas Gerais, de petits dépôts de matière organique de couleur blanche/crème ont été trouvés sur les parois d'un abri. En utilisant des techniques telles que RAMAN et FTIR, il a été possible de confirmer que la substance trouvée était de la guanine, un minéral azoté et une source d'azote. Cependant, il n'a pas encore été possible d'effectuer une diffraction d'échantillon (XRD) en raison de la faible quantité de matériel recueilli. Il est proposé que la guanine provienne des excréments azotés des arthropodes. Il s'agit du premier enregistrement de guanine minérale au Brésil, avec une préservation similaire à celle de la guanine provenant du guano de chauve-souris. Cette découverte peut élargir nos connaissances sur les minéraux associés aux grottes du Brésil, élargir les possibilités métaboliques et les processus écologiques associés aux environnements cavernicoles, et apporter de nouvelles connaissances dans un contexte astrobiologique.

1. Introduction

Although several studies have explored microbial communities in different subterranean ecosystems, little is known about the diversity of their metabolic processes and survival strategies. The mineralogy of caves is intimately linked with living beings that thrive in this environment, and recent studies have uncovered the potential relationships between microbial genomes and their ecological processes, like chemolithoautotrophic metabolisms (e.g. BENDIA et al. 2022). This provides support for discussing its implications for the search for life forms existing outside our planet, in environments rich in Fe, especially on Mars (e.g. SILVA et al., 2024).

The organisms in these caves and shelters have already been shown to have a high diversity of genes involved in different biogeochemical cycles, including pathways reducing, and oxidative processes related to C, S, N and Fe. These metabolisms are closely linked to the availability of chemical species in caves and shelters, so local geochemistry and mineralogy are of great importance. Thus, the interpretation of the mineralogical mapping of the environment could reveal the metabolic potential and diversity in cave ecosystems, contributing to elucidate the potential metabolisms in analogous extraterrestrial oligotrophic systems, such as underground environments on Mars, such as lava tubes (LÉVEILLÉ & DATTA 2010; PUŞCAŞ et al. 2010; SAURO et al. 2020).

The presence of nitrogen-bearing minerals is common in caves and shelters, and it usually comes from the percolation of water receiving the contribution of organic matter or microorganisms that act to produce species like ammonium, nitrite and nitrate (such as *Nitrosomonas* and *Nitrobacter*) (ONAC & FORTI 2011; DE SOUZA et al. 2023). Other organic minerals can form from reactions of organic deposits, being the bat guano the most important, although bone breccias and the excrements of various animals may occasionally be the source. More than 100 secondary minerals are known as guano derivatives, while some organic compounds, like guanine and urea, segregate directly from the guano itself (ONAC & FORTI 2011).

Guanine, as a mineral occurrence, was recorded only in Peru (where it was discovered 1844), Australia, Chile, Mexico (2 different places) and South Africa (MINDAT 2025). To date, there are no reports of the observation of the mineral guanine in Brazil. Here, we describe the discovery of small deposits of a white organic material found on the walls of a shelter in the region of Serro and Morro do Pilar, state of Minas Gerais. This finding corresponds to the first discovery of guanine as a mineral in Brazil.

2. Materials and methods

Caves and shelters were visited in the region between Serro and Morro do Pilar, in the state of Minas Gerais, in July 2023. Samples of the wall and ceiling of a small shelter (-18.406673, -43.419928, 1315 m) containing mineral deposits (Fig.1) were initially collected during a prospecting for a genomic and geochemical study of the caves in the area.

The Raman measurements were carried out using a Renishaw InVia Reflex equipment coupled to a Leica DM2500M microscope, using a 785 nm (diode laser, Renishaw)-500 mW- with 5% of its power. Each individual spectrum was collected using 30 accumulations of 1 s each, with the removal of cosmic rays. The software Wire 4.4, Fityk 1.3.1, and OriginPro 8 were used to collect, analyze, and plot the data, respectively. Standards of adenine, cytosine, guanine, thymine, and uracil (Sigma Aldrich) were also measured and used as a comparison to help the identification of the mineral deposits.

Samples of the collected material and a commercial standard of guanine (Sigma Aldrich) were analyzed without prior treatment by infrared absorption spectroscopy (FTIR) in attenuated total reflectance (ATR) mode in a Bruker FTIR-ATR spectrometer, model ALPHA, equipped with a DTGS detector. The spectra were obtained with a resolution of 2 cm^{-1} and 128 accumulations.

Scanning Electron Microscopy (SEM) was performed at low pressure, on a Quanta 250 model equipment (FEI), with an Aztec EDS system, 82 mm^2 window, from Oxford Instruments Ltd, with 3 detectors - Secondary electron imaging (SEM), cathodoluminescence (CL) and backscattered electrons (DBS/BSE) for compositional characterization by EDS of various materials. Due to the size and shape of the whole samples, a small amount of the mineral had to be removed from the matrix and placed on a carbon tape for microscopy.

Figure 1: Images displaying a large cream-colored deposit (~1 mm diameter) on the dark matrix (mag. ~40x).

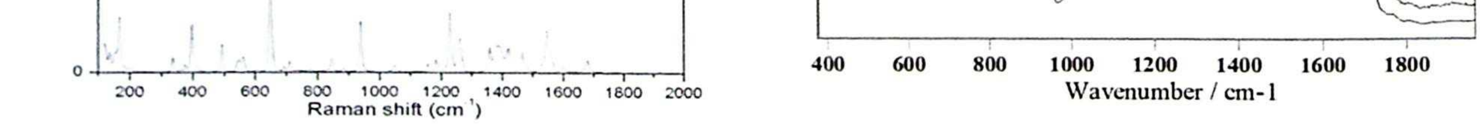

Figure 2: (Left) Raman spectra of the material studied: the upper spectrum (Sample) corresponds to the mineral deposits collected in the quartzite shelter, and the lower spectrum (guanine) corresponds to the guanine standard. (Right) FTIR-ATR spectra (a) of the collected sample and (b) of the commercial guanine standard.

3. Results

The expedition in the region between Serro and Morro do Pilar focused on collecting samples for a study to investigate the microbial communities that colonize iron-rich caves. However, during the prospecting, caves, and shelters in quartzite rocks were also located and visited. Although these sites were not the primary interest of the expedition, samples of those places were also collected and mineral characterizations were carried out. Wall and ceiling samples collected from one of those quartzite shelters showed small deposits of a white/cream color organic material on the matrix, a presence that was only recognized during the Raman characterization of the sample, days after the collection and return to the laboratory.

The deposits were present on a dark, almost black matrix. It is important to note that during sample collection, there was an interest in characterizing this black matrix rock from the shelter, generating a bias during the collection process. On the matrix, it is possible to find small white and transparent crystals identified by Raman as quartz, probably originating from the quartzite rock on which this dark matrix was formed. The mineral samples were also spread on the dark matrix, and have varied sizes and shapes, generally measuring around 1 mm in diameter.

Their coloration varies and can be described as ranging from a light cream color, almost white, to a yellowish color (Fig.1).

The Raman spectrum of the mineral sample was consistent with the guanine standard spectrum, showing peaks with similar shifts and relative intensities (Fig.2). The only two prominent differences between the spectra are the presence of a peak at 109 cm^{-1} in the guanine standard, absent in the mineral sample, and the presence of a much more intense peak at 202 cm^{-1} in the mineral sample than in the guanine standard.

The FTIR-ATR spectra recorded for the sample showed correspondence with the commercial standard of guanine in powdered solid form, with bands observed at 3312, 3167, 3110, 3060, 2988, 2903, 2844, 2783, 2689, 2639, 1693, 1667, 1651, 1634, 1563, 1548, 1474, 1461, 1415, 1370, 1360, 1258, 1213, 1172, 1148, 1117, 1044, 874, 848, 837, 776, 773, 726, 701, 688, 667, 644, 570, 555, 537, 514, 501, 419 and 397 cm^{-1} (Fig.2) (LOPES et al. 2012). The bands at 2379, 2349, and 2324 cm^{-1}, in turn, were observed exclusively for the investigated sample, related to the presence of carbon dioxide (CO_2) (CULP et al. 2010) probably adsorbed in the sample.

SEM showed the high purity of the sample, only demonstrating the presence of small amounts of Al and Si, probably originating from the matrix. In higher magnifications, it is possible to observe the crystalline structure of guanine on a small scale (Fig.3), similar to that observed in other guanine depositions of biological origin (HU et al. 2023).

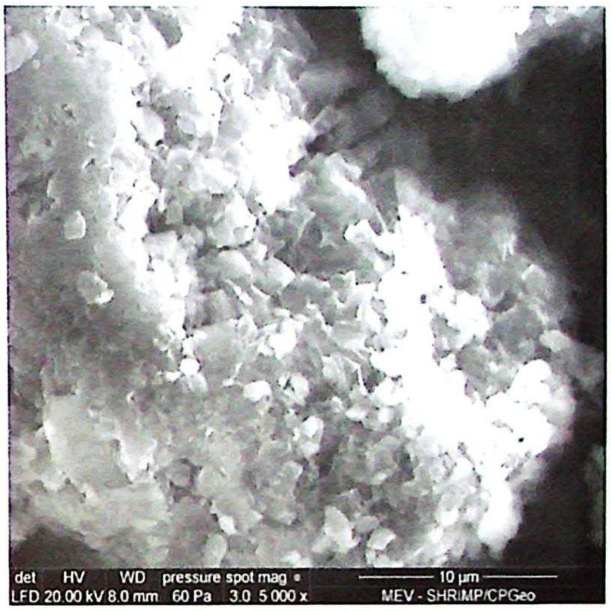

Figure 3: *SEM image of guanine crystals of the sample (mag. 5,000x).*

4. Discussion

With the aid of micro-Raman and FTIR spectroscopy, it was possible to confirm that the mineral sample that was collected was guanine. Guanine occurs mainly in three distinct forms or polymorphs: guanine monohydrate (GM), anhydrous guanine α form (AG α), and anhydrous guanine β form (AG β). Other three phases are also known: the dehydrated form of guanine monohydrate (synthesized at 150°C), the amorphous hydrated form of guanine, and the anhydrous guanine γ form (AG γ), sometimes interpreted as one type of twinning of AG β (HU et al. 2023). The most straightforward way to solve this doubt regarding the exact polymorph of these guanine deposits is using the X-ray diffraction technique (XRD), being fundamental for the description of the mineral.

Although not ideal for characterizing the type of guanine, Raman spectroscopy can provide some hints about the polymorph that was found. The absence of some intense peaks (e.g., 1474 cm^{-1}) and the presence of others (e.g., 1233, 1265, and 1552 cm-1) greatly reduces the likelihood that this sample is GM (PILÁTOVÁ et al. 2022). The Raman spectrum is in agreement with that expected for anhydrous guanine (LOPES et al. 2012), however, the presence of the peak at 202 cm^{-1}, associated with skeletal ring torsions of the molecule (LOPES et al. 2012), could be able to differentiate the AG α phase (without peak) from the AG β phase (with peak) (HU et al. 2023). Thus, the sample collected at the shelter would correspond to the AG β phase, while the standard guanine would be the AG α phase.

The FTIR-ATR spectra for the investigated sample and the analyzed commercial standard showed correspondence with FTIR spectra reported in the literature for the anhydrous form of guanine, in agreement with the FTIR spectrum expected for the tautomeric form 7H-ketoamine (LOPES et al. 2012). The distinction between the possible tautomeric forms of guanine is important to determine since they significantly influence the stability of the compound depending on the environment in which it is found (SHUKLA & LESZCZYNSKI 2006). The origin of the CO_2 adsorbed in the guanine sample investigated, in turn, may be related to decomposition processes still underway in the collected material, possibly resulting from microbiological activity. The decomposition of guanine by bacteria was demonstrated by RAKOSKY & BECK (1955), with the release of ammonia, CO_2, and acetic acid as final products. Taking this hypothesis into account, the non-observation of ammonia and acetic acid in the recorded spectra may be due to the low concentration of these species in relation to the guanine concentration.

Through SEM, it is possible to perceive the homogeneity and crystalline structure of guanine in the collected sample, which appears to have a high degree of purity. However, it is not possible to determine which crystalline phase is present, which is expected to be obtained in the future with XRD.

Studies indicate that guanine, as well as other nitrogenous compounds and organic materials, are associated with arthropod excreta in general (SOUZA et al., 2017; SANTOS & ANTUNES, 2018; CABEZAS-CRUZ, 2023). The products of such components contribute significantly to the ecology and diversity found in cave environments (TRAJANO, 2000; FERREIRA et al., 2007). Unfortunately, at the time of collection, no arthropods were observed on the walls of the shelter studied, nor were any signs (such as exuviae) that could suggest which arthropod produced the guanine deposit. However, the possibility of identifying the populations that produce such excreta opens doors for the conservation of species and preservation of cavities where they occur (AULER & PILÓ, 2014; NITZU et al., 2018).

5. Conclusion

This study characterized deposits found in a shelter in the region between Serro and Morro do Pilar, state of Minas Gerais. During the characterization, it was possible to demonstrate, thanks to similarities between the sample and a commercial standard observed during Raman spectroscopy and FTIR-ATR, that the material is a biomineralization of the substance guanine. This is the first description of the presence of this mineral in Brazil, only the sixth country in the world.

Guanine is found in three main phases, with the monohydrate phase being the least likely. Between the two anhydrous crystalline phases of guanine, the most likely one in this case would correspond to the AG β phase. However, only through XRD can the doubt be resolved, an analysis that has not yet been performed due to difficulties with the small quantity of the sample.

Due to the lack of light, the cave environment has a low capacity to maintain photosynthetic organisms, making the external supply of organic material and the internal production of excreta through their organisms essential in these environments (TRAJANO, 2013). In this sense, the microbial communities found in caves, often generated through guano (bat excreta) or other arthropod excreta, for example, are essential for the functioning and maintenance of cave communities (NORTHUP, 2011; TRAJANO, 2013; SAKOUI et al., 2020). Thus, in the present study, it was possible to observe samples of nitrogenous waste of arthropods, relating them to the presence of guanine. Thus, after excretion, guanine underwent a natural process of organization and crystallization, becoming a mineral originating from a biogenic substance.

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