Sulfur on Salitre Formation, Bahia Karst: Origin, microbiology, speleogenesis and mineral deposits

Tom Dias Motta Morita (1), Ivo Karmann (2) Renato Gamba Romano (2),Rebeca Lizárraga (2), Lucas Padoan de Sá Godinho (3), Vivian Pellizari (2)

(1) Instituto de Geociências da Universidade de São Paulo, 562 Lago Street São Paulo, SP, 05508-080, Brazil, tomdmmorita@gmail.com

(2) Instituto Oceanográfico da Universidade de São Paulo, 191 Oceanográfico Square, São Paulo, SP, 05508-120, Brazil

(3) Instituto de Geociências da Universidade Federal de Minas Gerais, Av. Pres. Antônio Carlos, 6627 - Pampulha, Belo Horizonte - MG, 31270-901

Abstract

In recent decades, research has increased on hypogenic caves and karst aquifers formed by sulfuric acid, which are estimated to comprise a quarter of the world's caves. This paper focuses on the role of sulfuric acid in shaping karst aquifers in Brazil, specifically through the generation of H2SO4 within the Irecê Basin. This area is noted for its diverse gypsum speleothems and unique groundwater chemistry, hosted by the Neoproterozoic limestones of the Salitre Formation. The study employs chemical groundwater analysis, and metagenomengene sequencing. Findings indicate a significant variation in SO42- levels (2.1 to 1542.1 mg/L) and a stronger correlation with major cations Ca2+ and Mg2+ than with HCO3-, suggesting H2SO4 as the main corrosive agent in limestone dissolution. The analysis also categorizes sampling points into different hydrochemical facies and identifies a slight depletion of the 34S isotope in deep aquifer waters, pointing to pyrite oxidation as a significant sulfate source. Taxonomic analysis reveals diverse microorganisms involved in sulfur oxidation, indicating biogenic influences on sulfuric acid production.

1. Introduction

Sulfuric acid speleogenesis (SAS) in karst aquifers, compared to epigenic karst systems, involves a more intricate hydrochemical framework requiring a known sulfur source for completeness. In Brazil, studies on SAS are limited to a few regions where sulfur primarily derives from the oxidation of sulfides, especially pyrite, due to the scarce evidence of evaporite or hydrocarbon deposits in or beneath the host rock of these caves. The prevailing theory posits that sulfuric acid formation via sulfide oxidation is driven and facilitated by microbial activity. This study seeks to expand our understanding of microbial roles in speleogenesis by examining the karst system in the Una Group, located in the southern part of the Irecê Basin in central Bahia, Northeastern Brazil. This region is recognized for its high sulfate concentrations in groundwater, documented over several decades (e.g., GUERRA, 1986; VALLE, 2004), and for the extensive diversity and size of gypsum speleothems in Iraquara's caves.

SAS has been proposed by AULER (1999) as a potential hypogenic initiation mechanism before epigenic and paragenetic processes in these caves. VALLE (2004) attempted to identify connections between major cations and anions, noting a strong correlation between SO42- and (Ca2+ + Mg2+), indicating that the chemical dynamics likely involve another corrosive agent in addition to epigenic carbonic acid, namely sulfuric acid. Based on the detection of sulfur cycle bacteria, such as the Thiobacillus genus, VALLE (2004) suggested a biogenic origin for this acid.

The karst in question forms part of the Neoproterozoic limestones of the Salitre Formation, which overlies the Mesoproterozoic pelites and conglomerates of the Bebedouro Formation, both components of the Una Group. The limestones, due to the region's general synclinal structure with a north-south axis, are flanked (east, west, and south) by the quartzite and conglomerate hills of the Chapada Diamantina Group (Fig. 1), which are stratigraphically beneath the Una Group (MAGALHÃES et al., 2016). Notably, the limestones of the Salitre Formation contain sulfide occurrences, such as galena, pyrite, and chalcopyrite (MISI & SOUTO, 1975).

The geomorphological configuration of the area affects the type of water recharge in the karst system. In the southern region, where rainfall is more abundant, allogeneic recharge from the hills predominates. In contrast, the northern part of the study area, bounded by the Jacaré River and characterized by lower rainfall, comprises extensive carbonate terrains with primarily autochthonous recharge further from the hills.

2. Materials and methods

Field works were carried out in the southern region of the Irecê Basin in January/February 2021 (rainy season) and, August and October 2021 (dry season) enabling seasonal comparison. It was dedicated for water sampling from wells and caves for hydro-chemical and microbiological analysis and rock and speleothem sampling to chemical, mineralogical and isotope analysis.

Physicochemical parameters were determined *in situ* using multiparameter probes while ion contents were obtained by various analytical techniques as shown in figure 1. The hydrochemical data was then statistically processed using the *Aquachem*, *Origin* and *PHREEQC* softwares.

Analysed elements	Analytical methods
Ba, Ca, Sr, Fe, Mg, Mn, K, Na	SMEWW, 23rd Ed. 2017, Method 3120 B / USEPA 6010C - 03:2007, SMEWW 23rd Ed. 2017 Method 3030E
Cl, F, PO_4, NO_3, NO_2, SO_4	EPA SW - 846 - 300.1 - 1999
H_2S	SMEWW, 23rd Ed. 2017, Method 4500-S2-, H / G
N-NH_3	SMEWW, 23rd Ed. 2017, Method 4500-NH3, B e F

Figure 1: Hydrochemical analytical methods.

SIAGAS database (CPRM, 2020), known caves and previous researches conducted by VALLE (2004) and MORITA (2018) were used as a base for choosing the sampling sites (Fig. 2) for new and accurate hydrogeochemical microbiological data.

The microbiology sampling process involved collecting 15 liters of water in a pre-sterilized container, followed by filtering through 0.22 µm Sterivex membranes, retaining particulate material and microorganisms. Samples were then preserved with RNAlater™ to prevent genetic degradation and stored frozen for transport to the University of São Paulo's Microbial Ecology Lab. DNA extraction used the DNeasy PowerWater Kit and manufacturer's protocol, modified for RNAlater use. DNA integrity was checked via gel electrophoresis, and quantified with the Qubit dsDNA HS assay. Sequencing of the 16S rRNA gene V4 and V5 regions was conducted using Illumina MiSeq, providing detailed phylogenetic information. Data processing and statistical analysis were performed with QIIME2 and DADA2 plugins, with taxonomic classification through the Silva v. 138 database. The FAPROTAX tool was used to infer metabolic activities of the identified microorganisms, linking them to specific biochemical functions.

All cave samples are authorized by ICMBio n° 76989-1.

3. Results

The distribution of wells with sulfate concentration data (extracted from the SIAGAS platform CPRM 2020) along the study area is shown on figure 2. The boreholes were compared by its depth, sulfate content and geographical location, indicating an increase of sulfate content heading to the northern portion of the karst aquifer.

The analytical results show a wide range of contents in major cations and anions, and based on the piper diagram plot a predominance of calcium bicarbonated and calcium sulfated waters were identified. Both display calcium as the major cation, provided by the host rock, meanwhile the anions, HCO3- or SO42-, could be provided by the corrosive agent of the system, carbonic or sulfuric acid, respectively. Sulfate concentrations, if generally observed, covering all samples, vary between 2 and 1542 mg/L, with an average of 391 mg/L, median of 131 mg/L and a non-normal distribution

Comparing both of these anions individually with the sum of the Ca2+ and Mg2+ (Fig. 3), we identified that, within a wide overview of all the samples collected, that sulfate presents a correlation R2=0.91 while bicarbonate presents a much lower value of R2= 0.17. Furthermore, the plot of Figure 3, shows that there are different groups of samples that must be identified and studies separately.

Samples groups were divided according to hydrochemical facies into phreatic cave lakes, boreholes (deep-seated aquifer), karst springs, and vadose cave water. It was observed that the deep-seated aquifer water samples collected from boreholes tend to present higher concentrations of ions related to the dissolution of the rock. This factor may also be related to the geographic location, since not many cavities are known in the northern region, thus the northern region samples were collected from boreholes, with the exception of a vadose cave sample in Lapa do Sumidouro.

The results of the chemical analyses align with the tubular well database available on the SIAGAS platform (CPRM, 2020 - Figure 4.1) and obtained during the pre-field phase. As depicted in Figure 4.9, the lower concentrations of the main ions related to the dissolution of the host rock (SO4, HCO3-, Ca2+, and Mg2+) are primarily found in the southern region of the Irecê Basin, where precipitation is more intense and the karst aquifer is more influenced by allogenic recharge from the quartzites of the Espinhaço Supergroup.

It is interesting to note the relationship established by Worthington and Ford (1995) between bicarbonate and sulfate anions in karst springs, and how this can be applied to understand the origin of the main cations and anions in karst aquifers (figure 4). According to these authors, the tendency for high sulfate concentrations in the water from deeper aquifer levels, accessed via tubular wells in this case, suggests the possibility of factors other than carbonic acid action contributing to rock dissolution. This includes the action of sulfuric acid, which may be linked to the presence of evaporitic minerals or sulfides interspersed in the limestones, as mentioned by the authors.

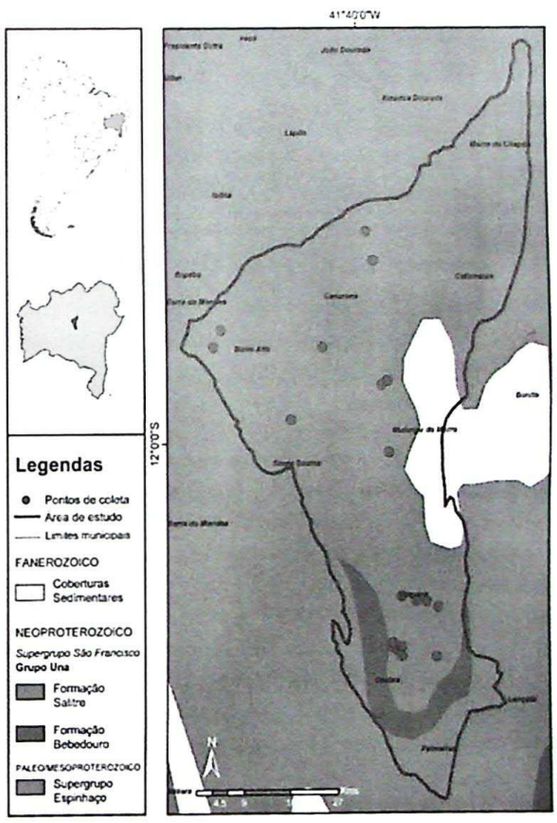


Figure 2: Location of the study area and collection points (indicated by the polygon and red circles, respectively), on the geological map, taken from Gómez et al. (2019).

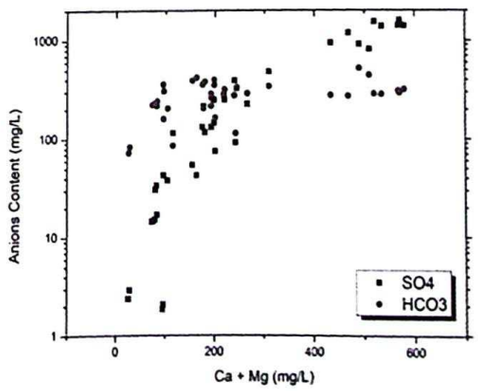


Figure 3: Scatter plot comparing HCO3- and SO42- with Mg2+ and Ca2+ sum.

In the chemical system of karst, it is crucial not only to understand the chemical reactions involved in the dissolution of the host rock and the precipitation of secondary minerals but also to assess whether these reactions are in equilibrium. The Saturation Index (SI) of minerals serves as a vital indicator of this equilibrium. Based on the physicochemical parameters of water, the SI helps determine whether aqueous solutions are undersaturated, saturated, or supersaturated concerning specific minerals. Negative SI values indicate undersaturation, suggesting a tendency for the mineral to dissolve from the host rock. In contrast, zero values suggest saturation and likely equilibrium, while positive values indicate supersaturation and a propensity for chemical deposition.

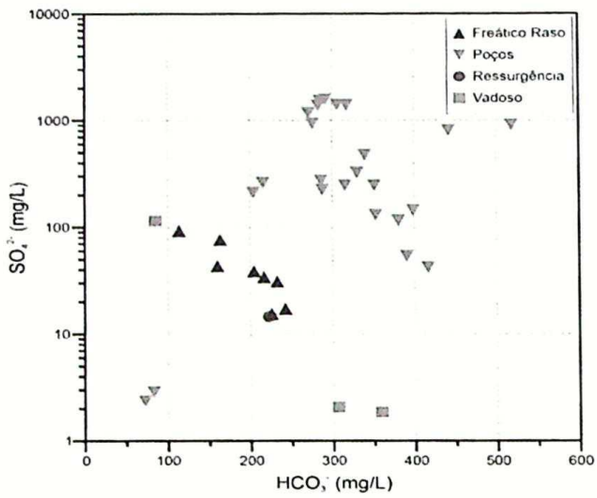


Figure 4: Correlation on a monologarithmic scale between the anions HCO3- and SO42-. The X-axis indicates the bicarbonate content, and the Y-axis (in logarithmic scale) indicates the sulfate content. Both concentrations are presented in mg/L.

Analytical and physicochemical data were used to calculate the saturation indices for key minerals such as calcite, aragonite, and gypsum. These calculations employed water samples from shallow aquifers and tubular wells, chosen for the larger number of samples available. The shallow aquifer showed SI values close to zero, ranging from 0.05 to 0.45 with an average of 0.23 for calcite, and from -2.49 to -1.47 with an average of -2.03 for gypsum. Tubular well samples exhibited a broader range of SI values, from -1.58 to 1.59 with an average of 0.01 for calcite. For gypsum, the indices ranged from -3.70 to -1.11 with an average of -0.20.

To expand the hydrogeochemistry of sulfur in the system, X-ray diffraction analyses were conducted on rock samples and chemical deposits to identify potential sulfur-bearing minerals and enhance understanding of the diversity and dimensions of sulfate speleothems in the Iraquara caves.

Samples of speleothems identified in the field as gypsum, or similarly habituated and locally identified as gypsum by guides, were collected for verification. Analyses included speleothems from Gruta do Didi, Gruta Torrinha, Lapa da Umburana, Lapa Doce, a mineral observed in the rock at Lapa Doce, and two crusts precipitated in irrigation wells.

For the analysis of microbial diversity in the samples, data were filtered to display only groups involved in the sulfur cycle. Proteobacteria, known for reducing sulfur compounds such as thiosulfate, elemental sulfur, and DMSO (Fukuyama et al., 2020), includes species like Sulfurospirillum carboxydovorans MV. Previous studies identified related organisms such as Thiobacillus and Thiothrix, linked to sulfuric acid speleogenesis and geospeleology (Hill, 1985; Auler and Smart, 2003; Valle, 2004; Engel et al., 2010). Crenarchaeota members breathe elemental sulfur, with the Sulfolobales order utilizing it as an energy source (Leight & Whitman, 2001; Liu et al., 2021). Planctomycetota can use elemental sulfur as an electron acceptor in anaerobic conditions (Kaboré et al., 2020). Bacteroidota are noted for their sulfur-reducing ability, producing sulfur sulfide (Hahn et al., 2022). Campilobacterota members oxidize sulfur compounds, coupled with nitrate reduction or molecular oxygen use (Xiao et al., 2023). FAPROTAX is a bioinformatics tool used to infer bacterial metabolic functions from 16S rRNA gene sequencing, predicting functional groups based on literature data. Figure 5 illustrates the metabolic functions associated with microorganisms in the samples, indicating their role in sulfur compound respiration and oxidation under aphotic conditions. The samples from Lagoa Preta and Poço do Luisão show high metabolic intensities related to sulfur and its compound's respiration and oxidation in dark environments.

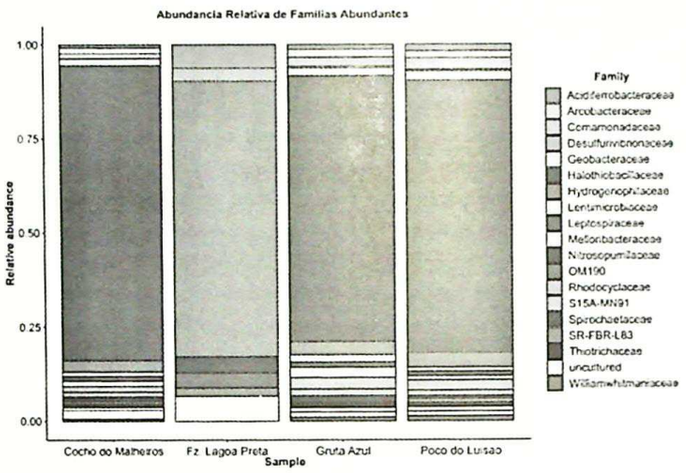


Figure 4: Relative abundance of dominant families (>1%) by station. Each column represents a sample, and the Y-axis indicates the proportion of each family (denoted by colors) for the analyzed samples.

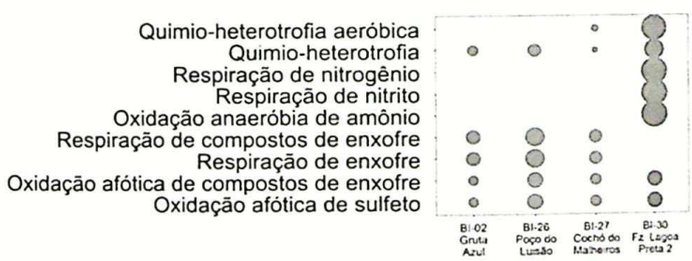


Figure 5: Relative abundance of the predicted functional groups for the communities present at each station. The size of the circles represents the percentage of relative abundance, ranging from 1% to 100%. The circles are distributed in the figure according to the sample (X-axis) and metabolic function (Y-axis).

4. Discussion

The hydrochemical results indicate that the northern region of the study area tends to exhibit the highest levels of sulfates in groundwater when compared to the southern region. These values when compared to the HCO3- contents, in which no correlations were found, is an indicative that, under the SAS hypothesis for the Irecê Basin, the bicarbonate present in the water does not come exclusively as a product of H2CO3 carbonate rock dissolution. Towards south, the sulfate content decreases, indicating greater epigenic activity, as evidenced by greater cave density (Fig. 1) as well as morphological and sedimentological characteristics of the caves. Considering the speleogenetic model suggested by AULER

(1999) and the predominance of epigenic karst over hypogenic according to the denudation rate and annual rainfall (AULER & SMART, 2003), it is understood that the hypogenic features, responsible for the initial stage of speleogenesis, are superimposed by epigenetic features.

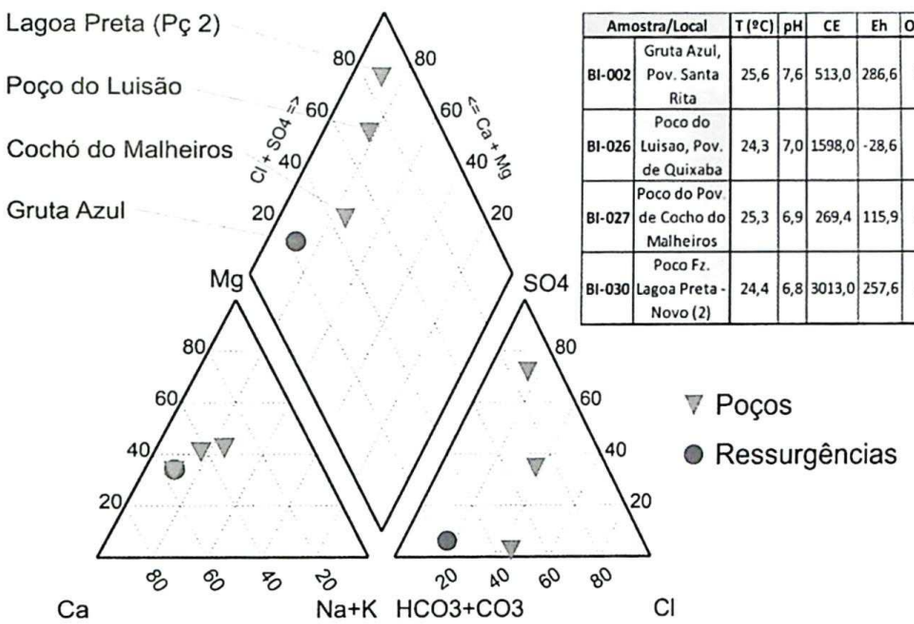
The southern region of the Irecê Basin has higher rainfall and higher allogenic recharge, which enables the exposure and development of caves closer to the surface and less sulfated groundwater. On the other hand, the northern region represents an earlier stage of karst development at greater depth and a more significant performance of H2SO4 as a corrosive agent, originated in the host rock itself.

The microbial diversity results highlight the significant presence of various microorganism groups linked to the sulfur cycle in the Irecê Basin's karst aquifer. Despite a low sample size due to research limitations and the non-retrieval of genetic material in several samples, interesting observations can still be made. Notably, only the sample from Gruta Azul was collected during the first field campaign, but this did not significantly alter the microbial community compared to the hydrochemical results. Ionic contents remained consistent across field campaigns, likely due to unusual drought conditions during the supposed rainy season of late 2020 and early 2021. Thus, temporal variations are considered less crucial than hydrochemical facies for differentiating hydrochemical environments.

Particularly, the microbial diversity of the sample from Fazenda Lagoa Preta stands out, evidenced by the relative abundance of 20 taxonomic families associated with the sulfur cycle. This distinction aligns with hydrochemical results (Figure 6)) where high sulfate levels are highlighted, making it the most sulfated sample among the four analyzed.

The likely link between hydrochemical and microbiological results is due to the primary sulfur source in the geochemical system, typically metallic sulfides, mainly pyrites. The microbial activity highlighted by the presence of microorganisms related to the sulfur cycle facilitates the oxidation of these sulfides, releasing sulfur species in aquatic environments.

Additionally, the other samples also showed the same metabolic functions related to sulfur. Notably, the Poço do Luisão sample, located in the Quixaba settlement, also exhibited significant sulfate levels, though not as prominent as those in the Lagoa Preta sample. This variation is likely due to the geographic locations of the sampling points, as indicated by the hydrochemical results, with the southern region of the study area having higher rainfall and greater influence from allogenic recharge from the quartzites bordering the Irecê Basin to the west, south, and east.



Amostra/Local		T (ºC)	pH	CE	Eh	OD (%)	SO4	HCO3	Ca+Mg
BI-002	Gruta Azul, Pov. Santa Rita	25,6	7,6	513,0	286,6	58,2	14,5	222,3	73,1
BI-026	Poco do Luisao, Pov. de Quixaba	24,3	7,0	1598,0	-28,6	7,6	276,0	286,7	217,6
BI-027	Poco do Pov. de Cocho do Malheiros	25,3	6,9	269,4	115,9	23,4	2,9	82,8	27,9
BI-030	Poco Fz. Lagoa Preta - Novo (2)	24,4	6,8	3013,0	257,6	34,3	1406,7	317,8	579,4

Figure 6: Piper diagram and simplified table of physical-chemical parameters and analytical results for the samples presented in the microbiology results.

5. Conclusion

Although important data are still lacking for the consolidation of the SAS hypothesis in the karst system of the Irecê Basin and mainly to the biogenic production of this acid, the sulfate present in groundwater are in notable concentrations among Brazilian karst.

Correlations found between SO_4^{2-} and the major cations, geographic location and relative abundance of Thiothrix and Thiobacillus genus bacteria corroborate to the understanding that sulphur has an effective participation in the local speleogenesis.

Acknowledgments

We thank Lívia Rocha, Aghata Zarelli and Gabriela Duarte for helping in our field works, the LECOM-IO and Karst System Lab - Igc teams for the contributions throughout the research and ICMBio-CECAV/Ferro Puro (TCCE 01/2020) for the research funding We would also like to thank Laurent Bruxelles and Lionel Barriquand for the support.

References.

AULER A. (1999) S. Karst evolution and paleoclimate of eastern Brazil. 264p. PhD, University of Bristol.

AULER A., SMART P. L. (2003) The influence of bedrock-derived acidity in the development of surface and underground karst: evidence from the Precambrian carbonates of semi-arid northeastern Brazil. Earth surface processes and landforms, 28, 157-168.

COMPANHIA DE PESQUISA DE RECURSOS MINERAIS. CPRM. (2020) Sistema de Informações de Águas Subterrâneas. SIAGAS. Brasília: CPRM. Avaiable on: http://siagasweb.cprm.gov.br/layout/index.php

Guerra, A. M. (1986) Processos de carstificação e hidrogeologia do Grupo Bambuí na região de Irecê-Bahia. PhD. Instituto de Geociências, Universidade de São Paulo.

GÓMEZ J., SCHOBBENHAUS C., MONTES N. E., compilers. (2019) Geological Map of South America 2019. Scale 1:5,000,000. Commission for the Geological Map of the World (CGMW), Servicio Geológico Colombiano, y Serviço Geológico do Brasil, Paris.

MAGALHÃES A. G. C., G. P., RAJA GABAGLIA C. M. S., SCHERER M. B., B´ALLICO F., GUADAGNIN E., BENTO FREIRE L. R., SILVA BORN O., CATUNEANU (2016) Sequence hierarchy in a Mesoproterozoic interior sag basin: From basin fill to reservoir scale, the Tombador Formation, Chapada Diamantina Basin, Brazil: Basin Research, v. 28, no. 3, 393–432,

MISI A., SOUTO P. (1975) Controle estratigráfico das mineralizações de chumbo, zinco, flúor e bário no Grupo Bambuí–parte leste da Chapada de Irecê (BA). Brazilian Journal of Geology, v.5, n.1, 30-45.

MORITA, T.D.M., KARMANN, I., ROMANO, R. G., PELIZZARI, V., VALLE, M. A., GODINHO, L. P. de S. (2019) Ácido sulfúrico como agente corrosivo no sistema cárstico de Iraquara (Grupo Una, BA). In: ZAMPAULO, R. A. (org.) CONGRESSO BRASILEIRO DE ESPELEOLOGIA, 35, Bonito. Anais: SBE, Campinas, 45-51.

VALLE, M. A. (2004) Hidrogeoquímica do Grupo Una (Bacias de Irecê e Salitre): um exemplo da ação de ácido sulfúrico no sistema cárstico. 122p. PhD. Universidade de São Paulo.