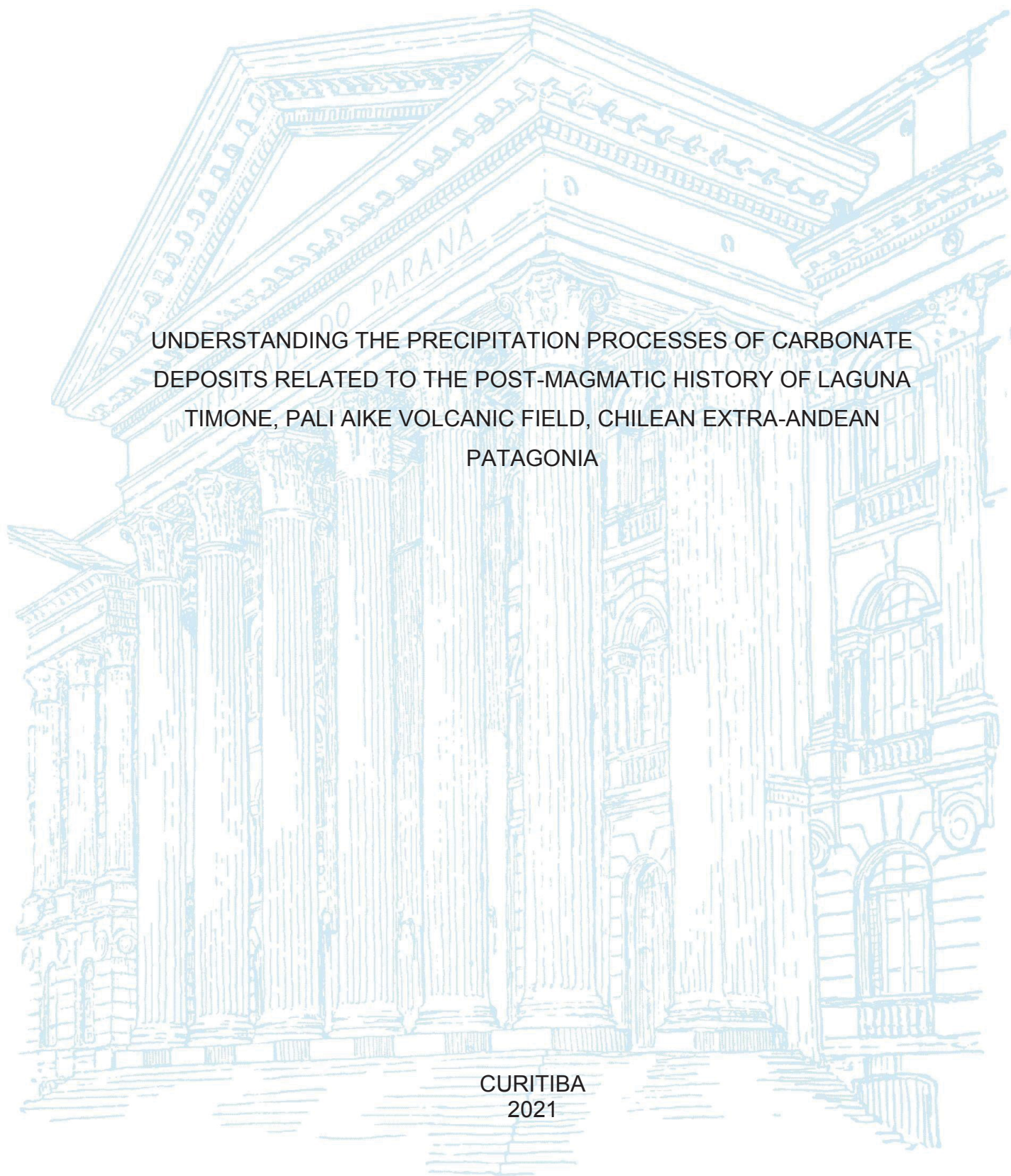


UNIVERSIDADE FEDERAL DO PARANÁ

CAROLINA HENRIQUEZ VALENZUELA

UNDERSTANDING THE PRECIPITATION PROCESSES OF CARBONATE
DEPOSITS RELATED TO THE POST-MAGMATIC HISTORY OF LAGUNA
TIMONE, PALI AIKE VOLCANIC FIELD, CHILEAN EXTRA-ANDEAN
PATAGONIA

CURITIBA
2021



CAROLINA HENRIQUEZ VALENZUELA

UNDERSTANDING THE PRECIPITATION PROCESSES OF CARBONATE DEPOSITS
RELATED TO THE POST-MAGMATIC HISTORY OF LAGUNA TIMONE, PALI AIKE
VOLCANIC FIELD, CHILEAN EXTRA-ANDEAN PATAGONIA

Dissertação apresentada ao curso de Pós-Graduação em Geologia, Setor de Ciências da Terra, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Geologia.

Orientadora: Profa. Dra. Anelize Manuela Bahniuk Rumbelsperger

CURITIBA

2021

V155u

Valenzuela, Carolina Henríquez

Understanding the precipitation processes of carbonate deposits relate to the post-magmatic history of Laguna Timone, pali aike volcanic field, chilean extra-andean Patagonia [recurso eletrônico] / Carolina Henríquez Valenzuela. – Curitiba, 2021.

Dissertação (mestrado) - Universidade Federal do Paraná, Setor de Ciências da Terra, Programa de Pós-graduação em Geologia, 2021.

Orientadora: Anelize Manuela Bahniuk Rumbelsperger.

1. Mineralogia. 2. Carbonetos. 3. Argilominerais. I. Universidade Federal do Paraná. II. Rumbelsperger, Anelize Manuela Bahniuk. III. Título.

CDD: 666.72

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação GEOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **CAROLINA SOLEDAD HENRÍQUEZ VALENZUELA** intitulada: **UNDERSTANDING THE PRECIPITATION PROCESSES OF CARBONATE DEPOSITS RELATE TO THE POST-MAGMATIC HISTORY OF LAGUNA TIMONE, PALI AIKE VOLCANIC FIELD, CHILEAN EXTRA-ANDEAN PATAGONIA**, sob orientação da Profa. Dra. ANELIZE MANUELA BAHNIUK RUMBELSPERGER, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestra está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 30 de Setembro de 2021.

Assinatura Eletrônica

01/10/2021 10:55:46.0

ANELIZE MANUELA BAHNIUK RUMBELSPERGER

Presidente da Banca Examinadora

Assinatura Eletrônica

09/11/2021 11:26:14.0

FRANCISCO JOSÉ FUENTES IZA

Avaliador Externo (UNIVERSIDAD DE CHILE)

Assinatura Eletrônica

06/11/2021 11:14:09.0

MAURICIO CALDERON NETTLE

Avaliador Externo (UNIVERSIDAD ANDRES BELLO)

ACKNOWLEDGMENT

The realization of this work would not have been possible without the support and help of various people and institutions, to whom I dedicate this work. First, I thank my counselor Prof. Dr. Anelize Bahniuk Rumbelsperger for inviting and trusting me the development of this work, and welcoming me in Brazil. Thank you for the opportunity, guidance, knowledge and above all the motivation you transmit to carry out this and all your projects. Thank you very much for your joy, good character, and energy that you spread to those around you. I admire you so much for your knowledge and authentic way of being.

To Prof. Dr. Leonardo Fadel Cury, for the invitation to be part of his teamwork, his motivation, and for the great support and guidance in fieldwork that were essential for the development of this project. To Prof. Dr. Mauricio Calderón for pushing me to this challenge, for his support, friendship, and guidance that were essential for the development of this project. To Prof. Dr. Gustavo Barbosa for his collaboration with the LPH analyzes, his knowledge, joy, and great support in fieldwork. I thank Dr. José Pérez-Donoso for the discussions and guidance that were essential for the development of this project. I thank Nicolás Bruna for his help in the analyzes, his patience, and his motivation for the experiments to be successful.

I thank the technicians and officials of the LAMIR Institute team who were essential for the development of the various analyzes and data presented in this work, especially Inaria, Fran, Keiji, Joyci, and Diego. Thanks to everyone who was part of this process, Belo, Sergio, Lari, Poldie, Profe FF, Cristina Rodrigo, Adri, Edi, Joana, Adelita, Vale, Vicho, Paulo, Veleda.

To my friends in life for always being with me, supporting me, giving me trust and love, Gustavo, Oscar, Jesús, Jeshu, Ana Karen, Jenny, May, Maca. Being away from your country is not always easy, without you this experience would not have been so beautiful. To my family in Curitiba, Niedja, Keiji, Felipe, Tereza, Iana, Tati, Guido, Elias, Emi, Ibra, Andrea, Matheus, Bianca, thank you for making me feel at home, for your love, company, and adventures.

I appreciate the love, tenderness, and unconditional support from my parents, brothers Felipe and Jorge, sisters-in-law Magy and Xime, nephews Rafa, Arturo, Santiago, and Horacio. They are my inspiration and admiration in life. Thank you, dad and mom, for always letting me be free, fulfilling my dreams, teaching me non-judgment, and enjoying the simple things in life. I love them. I also thank my godparents for the support they have always given me.

Finally, I would like to thank Project Diagenesis (ANP 20129-3), financed by Shell and LAMIR for the scholarship funds and support for this research. Also, thank the postgraduate program in Geology at UFPR for the opportunity to complete a postgraduate degree. Also, Fondecyt projects (J.M.P-D) and (M.C.) supported part of this research in Chile.

RESUMO

A Patagonia Austral Chilena é um dos poucos locais em que se pode encontrar precipitação de carbonatos em uma cratera maar. A cratera maar da Laguna Timone está localizada na região, mais precisamente no Campo Vulcânico de Pali-Aike, um complexo vulcânico-tectônico do Quaternário presente no sul da Patagonia, com fonte de magmatismo associada ao fluxo da astenosfera *sub-slab* através das janelas de laje. A Laguna Timone é preenchida por um corpo d'água com alta salinidade e alcalinidade, com baixa temperatura e rico em minerais como o cálcio, magnésio, sódio, potássio, nitrato, nitrito, sulfato e fosfato. A precipitação média anual baixa, em torno de 200 mm, e os ventos fortes são responsáveis pelas altas taxas de evaporação e pelo déficit hídrico anual. Microbialitas retrabalhadas, com crosta carbonática, argilominerais e atividade microbiana são observados em regiões próximas da costa da lagoa. Os carbonatos, sedimentados e o biofilme foram analisados e caracterizados. A mineralogia das microbialitas e da crosta era formada por calcita com valores de $\delta^{13}\text{C}$ VPDB (-0.43 ‰ to 2.50 ‰) que indicam a ocorrência de processos físico-químicos e bioquímicos nem sua precipitação. Enquanto que os valores de $\delta^{18}\text{O}$ VPDB (-6.52 ‰ to 2.28 ‰) estão associados com os processos de evaporação e com as influências meteóricas. A fração de argilominerais do sedimento é composta, em sua maior parte, por illita, clorita e esmectita. Apartir das análises HRTEM pode-se relacionar o amorfo Mg-argila aos cristais de calcita. A biodiversidade bacteriana e fúngica foi examinada usando o sequenciamento Illumina do PCR-amplificado rRNA 16s e genes ITS do DNA total extraído. Os resultados apresentam que os filos Proteobacteria, Bacteroidetes, Cyanobacteria e Verrucomicrobia dominaram as comunidades bacterianas e os gêneros *Pseudomonas*, *Rhodobacter*, *Brevundimonas*, e *Oscillatoria* estão relacionados com a precipitação de carbonato registrada em condições ambientais similares. Em relação às comunidades fúngicas, os gêneros predominantes foram os de *Ilyonectria*, *Tetracladium*, *Thelebolus*, *Acrostalagmus* e *Plectosphaerella*, e tem sido encontrada no ambiente Antártico. As bactérias foram isoladas do biofilme e tapetes microbiais e um isolado bacteriano, capaz de precipitar o carbonato de cálcio in vitro, foi obtido. Os cristais precipitados foram analisados e os resultados foram consistentes com a mineralogia da rocha carbonática. Os precipitados estão associados com os tapetes microbianos, permitindo a formação de organominerais. Como conclusão, a combinação do intemperismo de silicato e processos físico-químicos, bioquímicos e climáticos levam a precipitação de carbonatos.

Palavras- chave: Microbialitos. Carbonates. Lagoas alcalinas. Argilominerais.

ABSTRACT

The Chilean Patagonia Austral is only a few sites where carbonate precipitates in a maar crater. The Laguna Timone maar crater is situated in the Pali Aike Volcanic Field, a Quaternary volcano-tectonic complex in southern Patagonia, where the source of magmatism is associated with sub-slab asthenosphere flow through slab windows. Laguna Timone is filled by a body of water with high salinity and alkalinity, cold, and rich in calcium, magnesium, sodium, potassium, nitrate, nitrite, sulfate, and phosphates. The annual precipitation is around 200 mm, and the strong winds are responsible for high evaporation rates per year. Microbialites, carbonate crust, mud, and microbial activity are documented near the shore of the lagoon. The carbonates, sediment, and biofilm were analyzed and characterized. The mineralogy of reworked microbialites and crust was calcite. The $\delta^{13}\text{C}$ VPDB (-0.43 ‰ to 2.50 ‰) values indicate physico-chemical and biochemical processes in the precipitation. The $\delta^{18}\text{O}$ VPDB values (-6.52 ‰ to 2.28 ‰) are associated with evaporation processes and meteoric influences. The clay fraction of sediment was characterized and is dominated by illite, chlorite, and smectite. HRTEM analysis shows that the amorphous Mg-clay has a relation with calcite crystals. Bacterial and fungal biodiversity was examined using Illumina sequencing of PCR-amplified 16s rRNA and ITS genes from total extracted DNA. The results show that the Proteobacteria, Bacteroidetes, Cyanobacteria, and Verrucomicrobia phyla dominated the bacterial communities. *Pseudomonas*, *Rhodobacter*, *Brevundimonas*, and *Oscillatoria* genera found in the samples have been associated with carbonate precipitation recorded under similar environmental conditions. Regarding fungal communities, *Ilyonectria*, *Tetracladium*, *Thelebolus*, *Acrostalagmus*, and *Plectosphaerella* were the predominant genera determined in samples found in the Antarctic environment. Bacteria were isolated from biofilm and mats and a bacterial isolate capable of *in vitro* precipitate calcium carbonate *in vitro* was obtained. The crystals precipitated were analyzed and the results were consistent with mineralogy of carbonate rock. The precipitates are associated with microbial mats allowing organomineral formation. In conclusion, the combination of silicate weathering and physicochemical, biochemical and climate processes lead to carbonate precipitation.

Key words: Microbialites. Carbonates. Saline-alkaline Lake. Clay minerals.

LIST OF FIGURES

Figure 1. Schematic tectonic setting of southern South America, showing the location of the Pali Aike Volcanic Field (PAVF) (Source: adapted from Ross et al., 2011).	5
Figure 2. Location of the area: Laguna Timone (red star) on Pali-Aike Volcanic Field (PAVF). The legend shows the three units of the volcanic sequence that constitute the PAVF (Source: adapted from D'Orazio et al., 2000).	6
Figure 3. A) Schematic maar-diatreme system (source: Ross et al., 2011). B) Drone image on Laguna Timone maar crater.	7
Figure 4. Location of samples in the study area	15
Figure 5. Methods used for each type of sample for the development of the research.	16
Figure 6. Isolation and cultivation of TIMO sample to microbiologically induced calcite precipitation experiment	27
Figure 7. Location and geologic context of the study area. The Laguna Timone maar is located near the SW border of the Pali-Aike Volcanic Field (Source: adapted from Ross et al., 2011).	31
Figure 8. Location of samples in study area	33
Figure 9. A) Arborescent texture with a micrite to microsparite matrix, small peloids, and organic material. B) Growth coupled to clast. C) Structures like ramifications with high porosity. D) Massive texture, intercalation of micrite and microsparite with crenulated internal lamination. E) Micro CT image confirming a shrub-like form with high porosity. F) SEM imagen show few laminations with neo-crystal of calcite	37
Figure 10. A) Botryoidal structure showing coalescent oncoid and a lumpy texture with micrite, sparite and microsparitic matrix, small peloids, subhedral and equant crystal form. Intracrystalline microporosity, vuggy and fenestral porosity. B) Oncoid Filled with microcrystalline aggregates of calcite showing concentric sheets around a nucleus associated. C, D) Spheroidal form. E, F) Micro CT images show nano-scale calcite crystals coalescing in spherical shapes.	38
Figure 11. TESCAN analyses of carbonates A) LT01 composed by 99.85% of calcite B) LT02 composed by 99.45% of calcite c) Botroidal crust composed by 99.78% of calcite. All the samples contain detrital material less than 1%.	39
Figure 12. Stable isotope data of oxygen and carbon of Laguna Timone samples.	40

Figure 13. Graphic shows elemental composition of the bulk sediments. A) Element concentration of Gray-mud (LTS01). B) Element concentration of Green- mud (LTS02).	42
Figure 14. XRD patterns of the clay fraction of Green- mud (LTS02) sample of peak positions and intensities in patterns obtained from air-dried (AD), ethylene glycol (EG) and heating 550°C (H) treatments. Smectite group was identified by AD: 14,42 Å, EG: 17,55 Å, H: 10,09 Å to 001 peak positions. Illite group was identified by AD: 10,03 Å, EG: 10,04 Å, H: 10,09 Å (d001) and 5,03A° (d002). Chlorite group was identified by AD: 7,10 Å, EG: 7,10 Å, H: collapse (d002) and 3.54 Å (d004). The quartz is represented by 4,26 Å (d001) and 3,35 Å (d002) and 3,21 Å (d110) suggest feldspar.	43
Figure 15. XRD patterns of the clay fraction of Gray-mud (LTS01) sample of peak positions and intensities in patterns obtained from air-dried, ethylene glycol and heating 550°C treatments. Illite group was identified by AD: 10,04 Å, EG: 10,01 Å, H: 10,06 Å (d001) and 5,03A° (d002). Chlorite group was identified by AD: 7,11 Å, EG: 7,09 Å, H: collapse to 002 and 3,54 Å 004 peak position. The quartz is representedby 4.26 Å (d001) and 3,34 Å (d002).	43
Figure 16. Scanning electron microscope images. A) Thin sheet of clay minerals covering the elongate calcite crystals in Grey-mud (LT01) sample. B) EDX analysis shows calcite. C) Thin sheet of clay minerals covering partially crystal and dissolved diatom in Green-mud (LT02) sample. D) EDX analysis of thin sheet show clay mineral composition of EDX point in C. E, F) Partially dissolved diatoms. G) “Cotton like” clays associated with Mg-clay and elongated crystal of calcite. H) Clay coatings on diatom structures. I) Thin packets of mixed-layer with spacings corresponding to the sum of illite- and smectite-like layer spacings (21–22 Å periodicity). J) Chlorite/smectite interstratification (24 Å periodicity).	45
Figure 17. The Schoeller-Berkaloff diagram shows the variation on ion concentration between may (2017) and september (2019) seasons. The red lines represent May and blue lines represent September.	47
Figure 18. Image show the main source of availability elements in the system	49
Figure 19. Location and geologic context of the study area: Laguna Timone in the Pali Aike Volcanic Field (PAVF) (source: adapted from Corbella, 2002).	58

Figure 20. Carbonate samples. A) Microbialite (LT01). B) Microbialite with botryoidal facies (LT02). C) Carbonate crust (LT03) found around the maar crater.	58
Figure 21. Location of the microbial samples around the Laguna Timone maar crater. TIMO 1 corresponds to biofilm viscous material of green color with bubbles showing current activity. TIMO 2 and TIMO 3 have boomerang shapes and are greenish to brownish.....	59
Figure 22. Fluorescence microscopy images of biofilm and microbial mats. A, B) Images correspond the TIMO 1 sample showing the filamentous (red fluorescence) and mineral sphere (blue fluorescence) in different planes. C, D) Shows filamentous with morphology characteristic of cyanobacteria and red autofluorescence interacting with amorphous crystals and diatoms in TIMO 2 sample. E, F) Images of TIMO 3 sample also show clearly relationship of microorganisms and blue mineral associated to calcite. Filamentous (F), Mineral (M) structures, Diatoms (D).....	62
Figure 23. Scanning electron microscope images of the biological samples. (A) EPS with filamentous cyanobacteria and diatom from TIMO 1 sample. (B) Images reveal unicellular cyanobacteria. (C) Coccoid bacteria and (D) Diatoms frustules from TIMO 2 sample. (E) Filamentous interconnected forming ramifications, (F) Diatom with coccoid bacteria from TIMO 3 sample	63
Figure 24. (A) Bacterial genera composition of Biofilm (TIMO 1). (B) Bacterial genera composition of microbial mats (TIMO 2). (C) Bacterial genera composition of microbial mats (TIMO 3).....	64
Figure 25. Relative abundance of fungal classes in TIMO 1, TIMO 2, and TIMO 3 samples.....	69
Figure 26. Relative abundance of fungal genera in TIMO 1, TIMO 2, and TIMO 3 samples.....	69
Figure 27. Alpha diversity indices of TIMO 1, TIMO 2, and TIMO 3 samples	70
Figure 28. Digital image from an optical microscope CCD camera – TCC-5.0ICE showing oval CaCO_3 crystals precipitated from bacteria after 15 days of growth at 28°C . A, B) Carbonate crystals precipitated in the culture medium from the TIMO 1 sample. C, D) Carbonate crystals precipitated in the culture medium from the TIMO 2 sample, indicating characteristics of calcite. SEM images and EDS analysis exhibit the crystal precipitated. E) Oval-shaped crystal from TIMO 2, and EDS spectra showing Ca, C, O peaks associated with calcite composition. F) Crystalline crystal	

aggregates and biofilm from TIMO 3, and EDS spectra showing Ca, C, O peaks associated with calcite composition.....	71
---	----

LIST OF TABLES

Table 1. Reaction and by-products involved in different metabolic pathways leading to MPC (source: adapted from Zhu and Dittrich, 2016).	13
Table 2. The image shows physicochemical characteristics of water sample. PHREEQC calculated (*) saturations states.	36
Table 3. Mineralogical composition XRD and TESCAN- TIMA analysis	40
Table 4. Comparative table of microbial diversity from TIMO 1 and their register in the world.....	65
Table 5. Comparative table of microbial diversity from TIMO 2 and their register in the world.....	66
Table 6. Comparative table of microbial diversity from TIMO 3 and their register in the world.....	68

SUMARY

1 CHAPTER I: GENERAL CONSIDERATIONS	1
1.1 INTRODUCTION	1
1.2 GOALS OF THE THESIS	2
1.3 STRUCTURE OF THE THESIS	3
1.4 GEOLOGICAL SETTING	4
1.5 CLIMATIC CONDITIONS	8
1.6 TERMINOLOGY	10
1.6.1 Geomicrobiology - An Overview	10
1.6.2 Organomineralization – An Overview	10
1.6.3 Extracellular polymeric substances (EPS) – An Overview	11
1.6.4 Metabolic pathway to carbonate precipitation - An Overview	12
1.6.5 Microbial carbonate precipitation in hypersaline lakes.....	13
1.6.6 Chemical weatharing vs. Carbonate precipitation.....	14
2 CHAPTER II: SAMPLING AND METHODOS.....	14
2.1 FIELD METHODOLOGY	14
2.2 WATER CHEMISTRY	16
2.3 X-RAY DIFFRACTION (XRD)	17
2.4 X-RAY FLUORESCENCE (XRF)	18
2.5 CHEMICAL AND MINERALOGICAL ANALYSIS (TESCAN- TIMA)	18
2.6 PETROGRAPHY	19
2.7 SCANNING ELECTRON MICROSCOPY (SEM).....	19
2.8 HIGH RESOLUTION TRANSMISSION ELECTRON MICROSCOPY (HRTEM) .	20
2.9 X-RAY MICROTOMOGRAPHY (MICRO-CT).....	20
2.10 C&O ISOTOPY.....	21
2.11 FLUORESCENCE MICROSCOPY.....	22
2.12 DNA EXTRACTION FOR 16S RRNA AND ITS REGION ANALYSIS	22
2.13 16S RRNA GENE SEQUENCING FOR BACTERIAL IDENTIFICATION	24
2.14 LABORATORY EXPERIMENT	25
2.14.1 Microorganisms Induce Carbonate Precipitation	25
3 CHAPTER III: RESULTS	28
3.1 FIRST ARTICLE	28
3.2 SECOND ARTICLE	55

4 CHAPTER IV: CONCLUSIONS OF THE WORK.....	87
5 REFERENCES.....	88

1 CHAPTER I: GENERAL CONSIDERATIONS

1.1 INTRODUCTION

The extreme climate conditions and the interaction of ice-sheet dynamics, volcano-tectonic and marine events offer the southernmost part of the South American continent a unique geological and geobiological site. There are only a few modern environments where primary carbonate precipitate associated to microbial activity occurs under these conditions. One such setting corresponds to a crater maar located about 50 km east of the Andean Mountain chain and north of the Strait of Magallanes denominated Laguna Timone. It is immersed in Pali Aike Volcanic Field (PAVF), a Quaternary volcano-tectonic complex.

Basalts and alkaline basalts, including mantelic xenoliths (Mazzarini and D'Orazio 2003) characterize the study area. The rocks are associated with mafic and ultramafic magmatism related to the opening of an asthenospheric window under South America in response to the subduction of the Chile Ridge under the continent (Dickinson y Snyder, 1979; D'Orazio et al., 2000, 2001). In addition, Tertiary marine sediments, Miocene fine-grained molasse sediment, fluvio-glacial deposits are present related to different events (Mazzarini and D'Orazio 2003; Zolitschka et al., 2006).

The volcanic stage of PAFV formed hundreds of maars. These body of waters are exposed to semi-arid, humid, and cold climates, high salinity, high alkalinity ($\text{pH} > 9$), low temperatures (closed to 0°C), strong winds, and low annual precipitation (ca. 200 mm per year) (Weischet, 1996). Therefore, carbonate precipitation influenced by microorganisms is limited.

The carbonate precipitation is induced by diverse metabolic capacities of microbes, especially by oxygenic photosynthesis, that increase pH in the surrounding environment promoting the carbonate precipitation (Ludwig et., al 2005; Visscher and Stolz, 2005; Riding, 2006; Dupraz et al., 2009; Shiraishi, 2012). Microbialites are

abundant in hypersaline lakes and can be associated with microbial mats, which are common in these lakes (Glunk et al., 2011). Also, several studies suggest that under these conditions, alkaliphilic and halophilic species, sulfate-reducing, and ureolytic bacteria may play a role in carbonate precipitation (López-García et al., 2005). The development of microorganisms in hypersaline environments is interesting because biodiversity is limited. In general, these lakes are also exposed to other conditions such as low nutrient, alkalinity, high UV irradiance, and toxic compounds as heavy metals (Rodríguez Valera, 1988; Ventosa, 2006). Hypersaline environments are considered settings where life emerged on the Earth (Dundas, 1998). Alkaline and saline lakes present exceptionally high rates of photosynthesis, serving as a temporary sink for dissolved CO₂ (Melack and Kilham 1974; Paul and Mormile, 2017).

On the other hand, silicate weathering is a crucial process of Earth's long-term carbon cycle (Berner et al., 1983). This process has a unique place in the geological cycle that involves the interface between the atmosphere, the hydrosphere, and the biosphere (Wilson, 2004). The dissolution of minerals from weathering will produce dissolved cations like Ca²⁺, Mg²⁺, and Fe²⁺, and bicarbonate to increase the alkalinity and pH of the aqueous system suitable for carbonate minerals precipitation, or secondary minerals as Mg-clay from bedrock enriched in ferromagnesian minerals (Ferris et al., 1994; Kelemen et al., 2011; Bundelewa et al., 2014; Oelkers et al., 2015; Pozo and Calvo, 2018).

The exceptional conditions offer Laguna Timone as an open laboratory with unique characteristics to understand the mineral precipitation processes. Thus, this study aims to furnish new insights to enhance the understanding of specific bacterial composition and carbonate precipitation in an unusual scenario.

1.2 GOALS OF THE THESIS

Diverse techniques were applied, such as geochemical, petrographic and biological analysis to the development of the research. Also, laboratory experiments were performed in order to understand what potential precipitation has the

microorganisms. Thus, the main goal of this dissertation is determinate the influence of hydrochemical, environment, climate conditions and microbial activity in carbonate precipitation.

The specific objectives are:

- Identify the influence of climatic conditions on mineral precipitation
- Verify the influence of hydrochemical properties on mineral precipitation
- Verity the influence of lithology on mineral precipitation
- Identify the influence of microorganisms on mineral precipitation
- Furnish the understanding of autochthonous bacterial community composition within this volcanic lake ecosystem of extreme and complex conditions
- Simulate conditions in a laboratory experiment to induce organo-precipitation
- Compare the bacterial community of the samples of microbial mats of Laguna Timone from other sites.

1.3 STRUCTURE OF THE THESIS

The thesis was structured in 4 chapters. Chapter 1 contains introduction, main goals, geological setting and climatic conditions of the study area, and general terminology. The methodology and description of each technique that were developed and used in this work is presented in Chapter 2.

Chapters 3 and 4 corresponds to results and discussions exposed as a scientific manuscript. The first article, titled: "Microorganism Composition Associated to Mineral Precipitation on Microbial Mats from Laguna Timone Maar Crater, Pali Aike Volcanic Field, Chilean Extra-Andean Patagonia" is submitted in *Frontiers in microbiology Journal*. The manuscript investigates the influence of microbial consortium in calcite formation through of a laboratory experiment and the characterization of microorganisms.

The second article, titled: "The role of physicochemical and biochemical

processes on carbonate precipitation within the Laguna Timone maar in the Pali Aike Volcanic Field, southernmost extra-Andean Patagonia,” in preparation to be submitted in Sedimentology journal. The article presented different process, such as physicochemical, climatic, and environmental conditions that lead to carbonate precipitation. Finally, Chapter 4 show the main findings of this research and future perspectives of work.

1.4 GEOLOGICAL SETTING

The geodynamic evolution (Figure 1) in the southernmost area of South America is mainly due to the interaction between three tectonic plates: Nazca, South American, Antarctic, and Scotia. It results from: (1) the Subduction of the Nazca and Antarctic Plates beneath the South American Plate and (2) transcurrent movements between the Scotia and South American Plates. Cande and Leslie (1986) postulated that the active oceanic ridge (Chile Ridge) collided with the Chile Trench at the latitude of Tierra del Fuego at about 16 Ma and generating a triple junction. The subduction of the Chile Ridge under the continent produced the opening of a slab window beneath South America, generating the Cenozoic mafic plateau magmatism in extra-Andean Patagonia (Dickinson y Snyder, 1979; D’Orazio et al., 2000, 2001).

The complex geotectonic configuration of plate boundaries near the PAVF at 52°S latitude resulted in distinctive tectonic provinces (Winslow, 1983). These units corresponds (west to east):(1) the Paleozoic metamorphic basement, intruded by the Jurassic-Miocene Patagonian Batholith and exposed in the Pacific Archipelago (Herve et al., 2007); (2) the Patagonian Cordillera, constituted of silicic volcanic rocks of Upper Jurassic to Lower Cretaceous (Tobifera Formation) and an ophiolitic/volcaniclastic assemblage associated to Early Cretaceous ‘Rocas Verdes’ back-arc basin (Calderon et al., 2007); (3) The thin-skinned ‘fold-and-thrust belt’ originated since the Late Cretaceous to the Late Miocene (Fosdick et al., 2011); (4) Finally, the Magallanes foreland basin, influenced by Neogene extensional tectonics generating to graben systems, that consist of the Jurassic-Miocene volcano-sedimentary formations of the Magallanes Basin (Diraison et al., 1997).

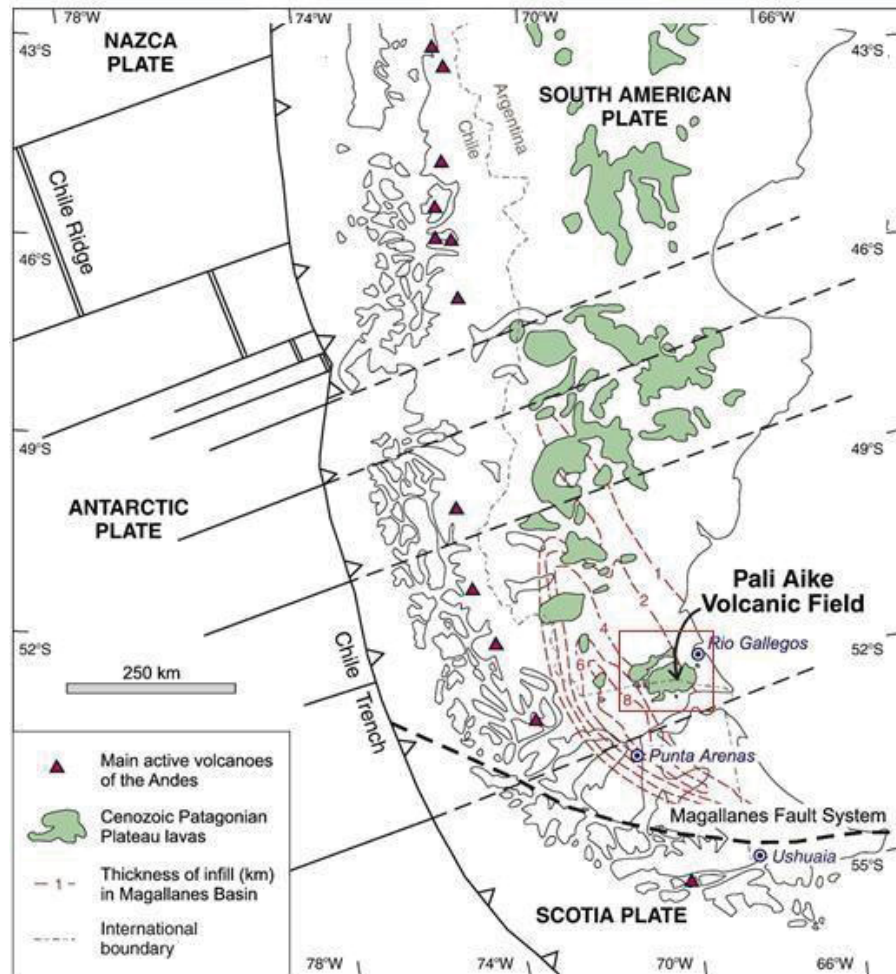


Figure 1. Schematic tectonic setting of southern South America, showing the location of the Pali Aike Volcanic Field (PAVF) (Source: adapted from Ross et al., 2011).

The last two units essentially were affected by the activity of the Pali Aike Volcanic Field, which the volcanic products were deposited on the volcano-sedimentary sequences of the Austral-Patagonian rift (Corbella and et., 1996). The Late Miocene to Quaternary PAVF (3.8 to 0.17 Ma) extends over approximately 4500 km² in Argentina and Chile. Three volcanic pulses were proposed related to different volcanic events. According to D'Orazio et al. (2000), the PAVF stratigraphy was divided into three stratigraphic units, U1, U2, and U3 (Figure 2). Unit U1 is the oldest (~3.78 Ma) and the most extensive unit, covering 83% of the total area, consists of a planar lava flow that forms a plateau. This succession presents 120 m in thickness. Unit U2 consists of over 450 volcanic edifices that include spatter, scoria cones, maars, tuff rings and are associated with basaltic lava flows (Corbella, 2002),

occupying 15% of the total area of the PAVF. The maar Laguna Timone discussed in this work belongs to unit U2. The preservation of these morphologies is affected due to erosion. Finally, unit U3 is the youngest (~ 0.17 Ma) and consists of well-preserved scoria cones and lava flows. The U3 occurs only in the SE part of the field and occupies only 2% of the total area.

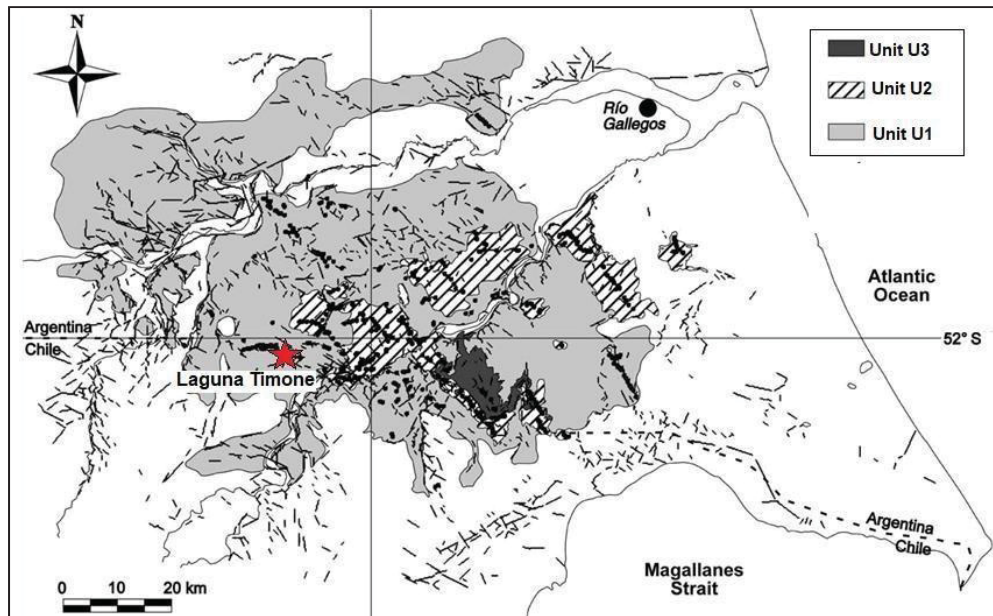


Figure 2. Location of the area: Laguna Timone (red star) on Pali-Aike Volcanic Field (PAVF). The legend shows the three units of the volcanic sequence that constitute the PAVF (Source: adapted from D'Orazio et al., 2000).

The rocks are mainly basalts and alkaline basalts, including mantelic xenoliths (Mazzarini and D'Orazio, 2003). In addition, Tertiary marine sediments (sandstone and shale) are related to a marine transgression (Patagonia Formation), and corresponds to the oldest outcropping geological strata of the study area, Miocene fine-grained molasse sediments from the Lower Miocene tectonic uplift of the Andes and fluvio-glacial deposits from the Pliocene and Pleistocene glaciations are present. The volcanic field represents the contrasting topography of a recently built eruptive area due to glacial and periglacial phenomena, geomorphological agents of the fluvial cycle, and wind activity (Zolitschka et al., 2006).

The Pali Aike Volcanic Field contains about 100 maars (500–4000 m in diameter). Maar–diatreme (Figure 3a) originates from explosive phreatomagmatic eruptions when ascending magma interact with groundwater or ground ice (Beget et al., 1996). The collapse structure propagates to the surface and results in the initial maar crater (Lorenz, 2003), and a tephra ring forms outside the crater during the phreatomagmatic activity.

The maars in the PAFV are different in size and morphology. For example, Laguna Timone (Figure 3b) is shallow volcanic edifices above diatremes with a 1960 m of diameter. Several studies indicate that the ratio of maar diameter to depth (D/d) can indicate the age of a maar. Therefore, this maar could be the oldest maars in the study area due to this ratio (Coronato et al., 2011).

Maar tephra rims contain materials generated during the eruptions (ash, lapilli, and basalt bombs), juvenile fragments, and material derived from unconsolidated deposits as glacial-fluvial gravels, sands, silts, and lithic clasts from the Tertiary due to the excavation of the maar crater and underlying diatreme by the phreatomagmatic explosions (Corbella and Gagliardini, 1997).

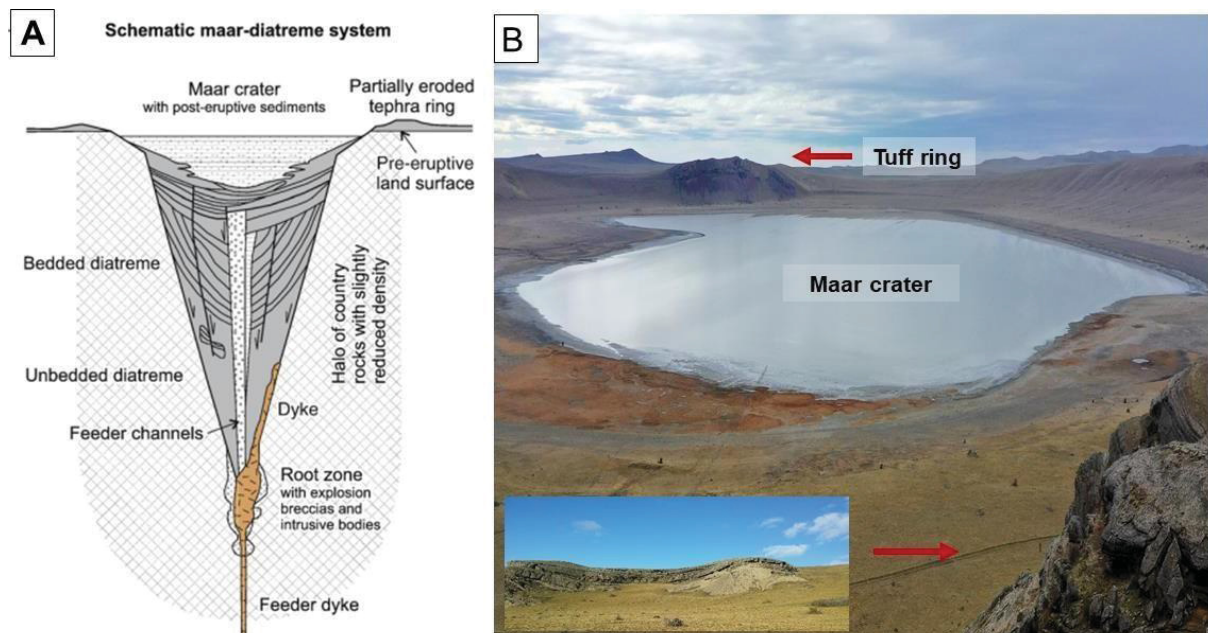


Figure 3. A) Schematic maar-diatreme system (source: Ross et al., 2011). B) Drone image on Laguna Timone maar crater.

The approximately 450 monogenetic cones in Pali-Aike have a spatial distribution with a preferential alignment and elongation. The observation realized by Mazzarini and D'Orazio (2003) through satellite image, topographic maps, field surveys, and aerial photos determines four main lineaments families: N - S (azimuth range 350 °-010 °), NE - SW (021 ° - 060 °), E-W (080 °-100 °) and NW - SE (110 ° – 150 °). The NW - SE and NE - SW are the most common structural orientations in PAVF. The Laguna Timone maar shows a slight elongation in NE-SW. The principal trends are related to the subduction between the Antarctic and South American Plates and the strike-slip motion between the South American and the Scotia Plates, generating preferential orientations NE-SW and ENE-WSW (D'Orazio et al.,2000).

The recent volcanism is consistent with high temperatures and the existence of a positive thermal anomaly on a regional scale. Based on thermometry and barometry, Stern et al. (1989) concluded that the lithospheric mantle in this sector has a high geothermal gradient of $> 10^{\circ} \text{C} / \text{km}$ between 50 and 100 km deep, reaching temperatures greater than 1300°C at depths less than 100 km. In addition, the occurrence of mantle xenolith at Pali Aike suggests the existence of fault systems that connected deep magmatic reservoirs with the surface, this could be associated in currently to channeling geothermal fluids (Emil, 2018).

1.5 CLIMATIC CONDITIONS

Climatic conditions of southern Patagonia are controlled by Antarctic ice mass and winds circulation from the west (Weischet, 1996). High solar radiation melts extensive ice fields during the summer. Moreover, Humboldt Current (west coast) and Falkland Currents (east coast) transport cold water to the north along the Patagonian coasts, simultaneously reducing atmospheric heating. The cold-air masses advection arrive from the continent generates cold summers, and the proximity to the ocean is the cause of moderate winters (Barros et al., 1979). In general, southern Patagonia is characterized by extreme climatic conditions. Moreover, westerly winds that transport humid air from the Pacific Ocean to the Southern Andes lead to annual rainfall values between 4000-6000 mm on the coast and Andean territory.

On the other hand, the Andean mountains behave as a topographic barrier for humid winds and generate the rain shadow on the eastern slopes of the Andes, where the Pali Aike volcanic field is located. It causes an abrupt decrease in precipitation to less than 300 mm per year, with a regular distribution throughout the year, generating semi-arid and desert climates (Zolitschka et al., 2006).

In addition, the decrease in relative humidity related to the increase of mean annual temperature is another factor that influences the reduction in precipitation east of the Andes (Weischet, 1996). Eastern Patagonia tends to have a continental climate dominated by the SWW, with highly evaporative conditions at the surface (Garreaud et al., 2013).

A strong characteristic of the Patagonian climate is the predominance and intensity of westerly winds that are present during the year (Paruelo et al., 1998), with mean annual wind speed values of 7.4 m/s at Rio Gallegos with more intensity in summer than in winter (Baruth et al., 1998; Paruelo et al., 1998). The satellite images taken at different seasons during the year showed that many of the lakes in this area are desiccate at the end of the austral summer and deflated to form and deposit wind streaks (Zolitschka et al., 2006).

The regional hydrology is unexplored in this area, with the exceptions of Laguna Potrok Aike on the Argentine border. This lake, in general, is fed by precipitation and groundwater and shows levels rapidly fluctuating within during the year. However, have no permanent tributaries and no surface outflow (Mayr et al., 2007).

Glaciations in the Pali Aike Volcanic Field area occurred during the Pliocene and Pleistocene (3.5 and 1.0 Ma) (Zolitschka et al., 2006). Glaciers from the south covered the area, originating from the Magellan Strait, Seno Skyring, and Seno Otway. Caldenius (1932) called Initioglacial the most extensive glacial advance, and Mercer (1976) used the term Greatest Patagonian Glaciation. Meglioli (1992) established regionally different terms for the same event: Bella Vista Glaciation for the Rio Gallegos Valley and Sierra de Los Frailes Glaciation for Cabo Virgenes. The radiometric age range of the PAVF volcanism is approximately 3.78 to 0.17 Ma, but most of the volcanism is younger than 1.5 Ma (Mazzarini and D'Orazio, 2003).

1.6 TERMINOLOGY

1.6.1 Geomicrobiology - An Overview

Geomicrobiology studies the role that microorganisms play in geological processes from the moment of their first appearance on Earth to the present. This discipline combines geological, chemical, and biological approaches through methodologies used in microbiology (genetics, physiology, culture, microscopy) and geology (chemical analysis of water, mineralogy, isotopic geochemistry) (Ehrlich, 2006; Gadd, 2010; Druschel and Kappler, 2015).

Microorganisms shape their geochemical environment through their growth and metabolic needs and, therefore, exert significant geochemical and mineralogical control over their environment. This indicates that microbial evolution has coincided with variations in the geosphere, driving essential changes in the chemistry of the ocean, continent, and atmosphere (Druschel and Kappler, 2015).

Since their origin, about 4 billion years ago, microorganisms have had a profound influence on the configuration of our planet (Konhauser, 2007). There are many events throughout the history of the Earth, and one is the "Great Oxidation Event" (GOE) that occurred 2.45 Ga ago, where cyanobacteria raised the level of atmospheric oxygen through oxygenic photosynthesis, which caused the diversification of species minerals (Konhauser et al., 2011).

1.6.2 Organomineralization – An Overview

The organomineralization is a process that can be intrinsically (e.g., microbial metabolisms) or extrinsically driven (e.g., degassing, evaporation). Organomineralization can therefore be either an active (biologically- induced) or passive (biologically-influenced) process (Dupraz et al., 2009). Mineral deposits resulting from organomineralization are called microbialites (Burne and Moore, 1987; Perris et al., 2007; Dupraz et al., 2009). Microbialites are one of the first pieces of evidence of life on Earth. These changed the atmosphere conditions and biosphere

through geological time, and they are the basis for the existence of modern life (Solari, 2010). Microbial communities lived in the primeval waters of the planet in extreme environmental conditions and adapted to these conditions (Dupraz et al., 2009).

Microbialites are organo-sedimentary deposits that accrete by the action of benthic microbial communities, which trap and bind detrital sediment and/or form the locus of mineral precipitation (Burne and Moore, 1987; Riding, 1991). Microbialites may be composed of distinct mineral assemblages, and carbonate is the most common construction component. However, it is also possible to find microbialites made of silica, carbonate, or phosphate throughout the geologic record. The morphogenesis of these structures is a function of environmental influence, biological and ecological controls, processes, and lithification rates (Riding, 1991). Microbialites can be classified into four general types based on their internal fabric: stromatolites, thrombolites, dendrolites, and travertines (Riding, 1991). It is possible to apply to many additional authigenic accumulations in which microbes are locally evident, such as, some tufa, travertine, speleothem, springs, seeps and vent deposits (Riding, 2011).

Living microbialites are complex colonies of microorganisms, including photosynthetic filamentous cyanobacteria, eukaryotic microalgae, and autotrophic and heterotrophic microbes (Bauld, 1986). The microbial communities dominated by prokaryotes that show intimate interaction between the microbes, the colonized surface. The essentially aqueous medium where they develop is called microbial mats or mats. Microbial mats are a succession of biofilms that contain complex communities or ecosystems with chemical gradients, an abundance of phototrophic organisms, and stratification in levels (Stolz, 2000). Likewise, biofilm is composed of microorganisms that colonize solid surfaces, and by the extracellular products of the matrix secreted by them (Marshall, 1992).

1.6.3 Extracellular polymeric substances (EPS) – An Overview

Extracellular polymeric substances accumulate outside cells to form a protective and adhesive matrix that attaches them to substrates (Costerton et al., 1978; Costerton

and Lappin-Scott, 1995). Some of the physiological functions correspond to organic matter preservation (Pacton et al., 2012), stabilization of the mat by increasing the cohesiveness of the surface, protection against UV, reduction of grazing pressure, acting as a conduit for chemical communication, and the creation of specific micro-environments (Brouwer et al., 2002; Decho et al., 2005; Paterson et al., 2008).

EPS consists of polysaccharides, amino acids, and a variety of chemical functional groups that can also contain fragments of DNA, proteins, and other organic molecules (Perry et al., 2005; Lawrence et al., 2003). Determined by surface speciation of EPS, the bacterial and mineral surfaces, hydrophobicity, and electrostatic interactions drive the adsorption of bacteria on mineral surfaces (Yee et al., 2000). Photoautotrophic and heterotrophic bacteria can produce EPS, although cyanobacteria are recognized as the most important EPS producers (De Philippis et al., 2001). Extracellular polymeric substances also play an essential role in microbial calcification (Tourney and Ngwenya, 2014), inhibiting or promoting mineral precipitation (Dupraz et al., 2009).

Hypersaline conditions are conducive to microbial mat and microbialite formation, possibly through the exclusion of eukaryotes that graze or compete for light and space in these high salinity environments (Des Marais, 1995).

1.6.4 Metabolic pathway to carbonate precipitation - An Overview

In nature, a diversity of microorganisms induces the precipitation of carbonates, through the chemical alteration of the environment, through a variety of physiological activities, or by serving as a crystal nucleus. The carbonate precipitation by microorganisms can occur by different metabolic pathways, such as photosynthesis, ureolysis, ammonification, denitrification, sulfate reduction, anaerobic sulfide oxidation, and methane oxidation (Table 1) (Zhu and Dittrich, 2016).

Table 1. Reaction and by-products involved in different metabolic pathways leading to MPC (source: adapted from Zhu and Dittrich, 2016).

Microbial groups	Metabolism	Reactions	By-product
Cyanobacteria Algae	Photosynthesis	$2\text{HCO}_3^- + \text{Ca}^{2+} \rightarrow \text{CH}_2\text{O} + \text{CaCO}_3 + \text{O}_2$	O_2 CH_2O
Ureolytic bacteria	Ureolysis	$\text{CO}(\text{NH}_2)_2 + 2\text{H}_2\text{O} + \text{Ca}^{2+} + \text{Cell} \rightarrow 2\text{NH}_4^+ + \text{Cell-CaCO}_3$	NH_4^+
Nitrate-reducing bacteria	Denitrification	$\text{CH}_3\text{COO}^- + 2.6\text{H}^+ + 1.6\text{NO}_3^- \rightarrow 2\text{CO}_2 + 0.8\text{N}_2 + 2.8\text{H}_2\text{O}$ $\text{Ca}^{2+} + \text{CO}_2(\text{aq}) + 2\text{OH}^- \rightarrow \text{CaCO}_3(\text{s}) + \text{H}_2\text{O}$	Complete reaction: $\text{CO}_2 + \text{N}_2$ Incomplete reaction: $\text{NO} + \text{N}_2\text{O}$
Myxobacteria	Ammonification	–	NH_3
Sulfate reduction bacteria	Sulfate reduction	$\text{SO}_4^{2-} + 2[\text{CH}_2\text{O}] + \text{OH}^- + \text{Ca}^{2+} \rightarrow \text{CaCO}_3 + \text{CO}_2 + 2\text{H}_2\text{O} + \text{HS}^-$	CO_2 HS^-
Methanogens	Methane oxidation	Anaerobic oxidation: $\text{CH}_4 + \text{SO}_4^{2-} + \text{Ca}^{2+} \rightarrow \text{CaCO}_3 + \text{H}_2\text{S} + \text{H}_2\text{O}$ Aerobic oxidation: $\text{CH}_4 + 2\text{O}_2 \rightarrow \text{CO}_2 + 2\text{H}_2\text{O}$	H_2S

1.6.5 Microbial carbonate precipitation in hypersaline lakes

Hypersaline environments are considered probable settings where life emerged on Earth (Dundas, 1998), and the microorganisms we currently observe in hypersaline environments are probably the descendants of such primordial forms of life (DasSarma, 2012). Microbialites are the oldest fossil evidence for life on Earth. These were dominant in many environments but currently are limited to a few hypersaline lakes, such as Lake Clifton in Australia (Moore and Burne 1994) and Storr's Lake in the Bahamas (Paul et al., 2016).

Extreme conditions characterize hypersaline lakes. Nevertheless, organisms as bacteria have successfully grown and reproduced under high-salt conditions (DasSarma, 2012). The biodiversity is specific due to high salt concentration and low nutrient, oxygen availability, alkalinity, high UV irradiance (Arahal and Ventosa 2006). Microbialites are abundant in hypersaline lakes and can be associated with microbial mats, which are very common in these lakes (Glunk et al., 2011).

In hypersaline environments, the processes of ureolysis and photosynthesis by microorganisms dominate in carbonate precipitation (Zhu and Dittrich, 2016). The carbonate precipitation is associated with the increased concentration of CO_3 by photosynthetic bacteria (Ludwig et al., 2005). Nevertheless, several studies suggest that alkaliphilic and halophilic species, bacteria may play a role in carbonate precipitation (López-García et al., 2005).

1.6.6 Chemical weathering vs. Carbonate precipitation

Chemical weathering of silicate minerals is responsible for approximately 50–75% of CO₂ consumed on the continents (Berner, 1991). Silicate minerals, such as olivine, pyroxene, and plagioclase, can react with carbon dioxide and form carbonate minerals (Ferris et al., 1994; White et al., 1995). Thus, chemical weathering can lead to understanding the global carbon cycle (White, 2003). This process is conditioned by many factors such as dissolution kinetics of minerals, mechanical erosion, lithology, tectonics, biota, climate, and hydrology (Donnini et al., 2016). The lithology is very important; for example, the basalts are among the more easily weathered than other crystalline silicate rocks (Louvat, 1997). Basalt and ultramafic rocks as peridotites are rich in Ca and Mg, and could drive carbonate precipitation (Harrison et al., 2012; Wilson et al., 2009).

In natural weathering processes, the calcium or magnesium containing minerals react with gaseous CO₂ and form solid calcium or magnesium carbonates (Huijgen, 2007).



2 CHAPTER II: SAMPLING AND METHODOS

2.1 FIELD METHODOLOGY

The field stage was carried out between September 8 and 22, 2019, where samples of water, biological, sediment, carbonate precipitates, and microbialites (Figure 4) were collected from the margin of the Lagoon. The general methodology applied to obtain samples is described below: (i) Record the geographical coordinate of the sampling point (GPS) (ii) Take a general photograph of the sector and (iii) Make a brief description of the place including general aspects such as petrography and some more specific aspects, such as the color of the sediment and the presence of organic matter at the sampling site.

In Laguna Timone, the carbonate facies are significantly different from Torres del Paine (Solari, 2010) and Laguna del Toro areas. The carbonate buildings, thick tuffs, and travertine deposits are absent. Instead, the main facies correspond to millimeter-scale crusts and microbialites fragments. In the distal part of the lagoon, at the level of the steppe, microbialites were observed showing thrombolites fragments and botryoidal structures. The samples were named LT01 and LT02.

In addition, the carbonate crust covers pebbles of different compositions. Some of the pebbles are cemented by carbonates forming blocks of carbonate breccia. The sediment was sampled according to two distinctive colors, green and grey. In the flow region associated with streams, a green mud is superimposed by a thin layer of well-crystallized carbonates and salts, and dissection cracks with botryoidal carbonate superimpose a gray mud. The collected samples were denominated LTS01-Green and LTS02-Gray.

The current microbial process was identified around the lagoon, associate with a biofilm with bubbles, and the microbial mats are greenish to brownish, and boomerang-shaped were sampled. The samples were named TIMO 1 (Biofilm), TIMO 2 (microbial mats), and TIMO 3 (microbial mats).

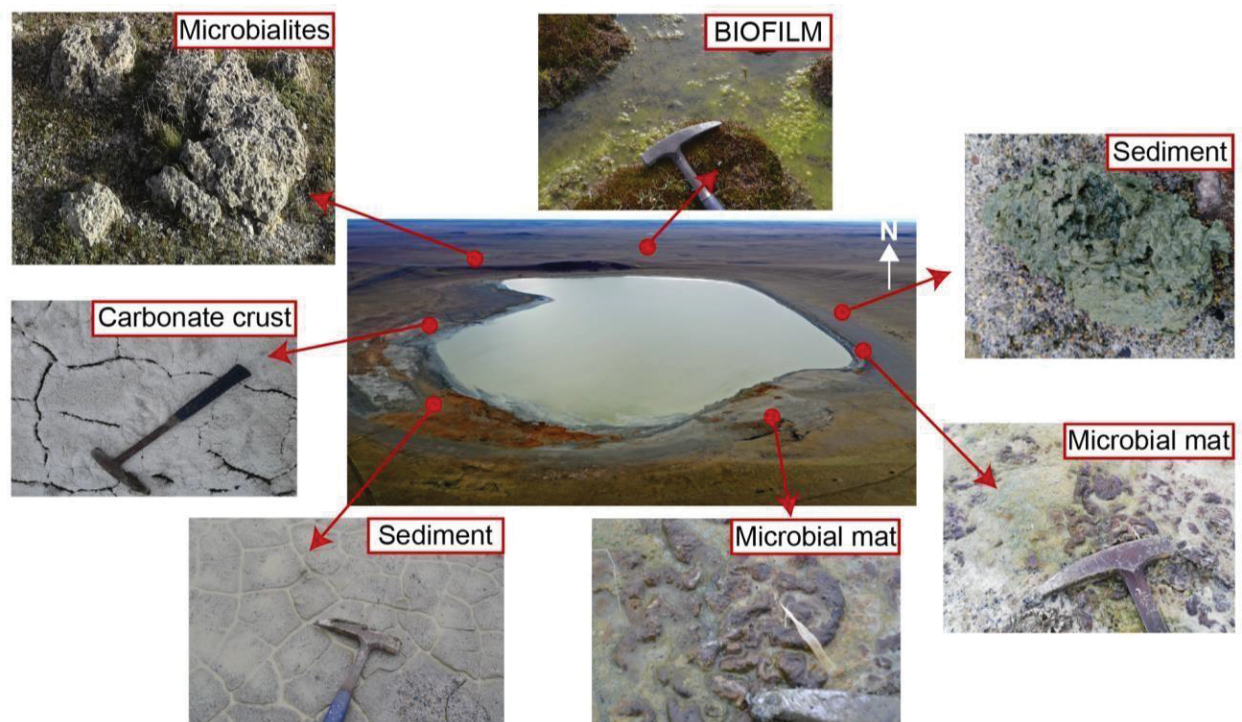


Figure 4. Location of samples in the study area.

The diversity methods were used to achieve the objectives (Figure 5) will be described below:

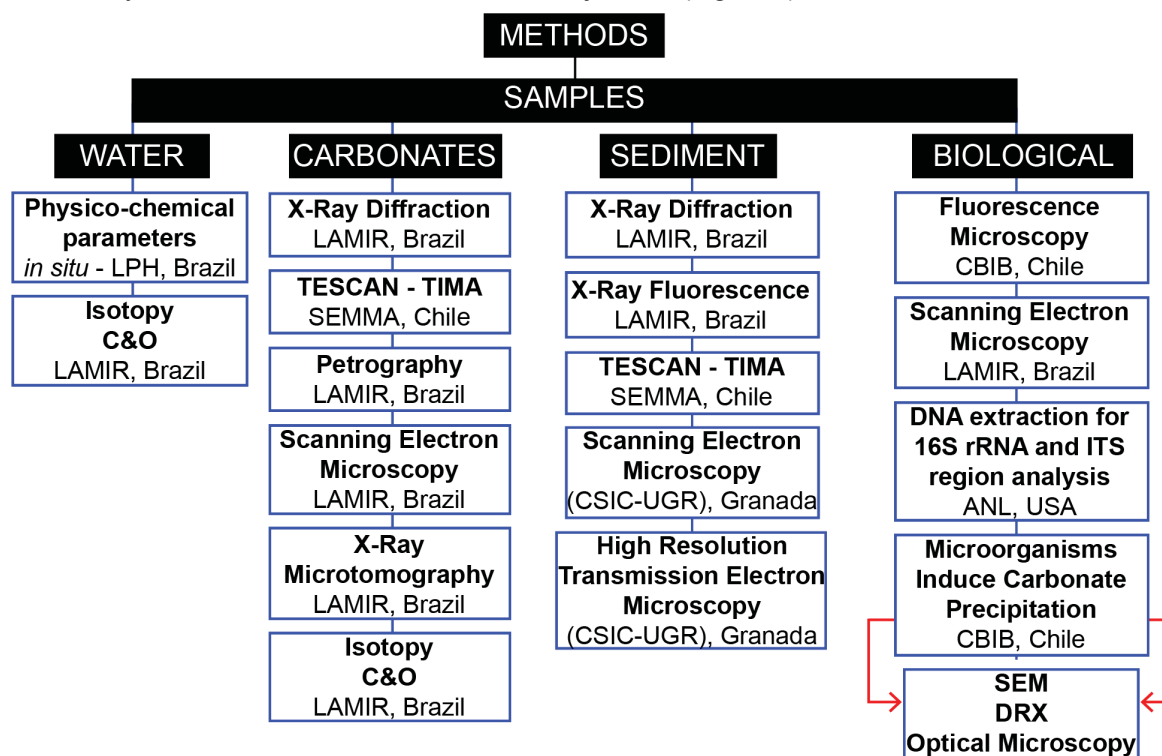


Figure 5. Methods used for each type of sample for the development of the research.

2.2 WATER CHEMISTRY

Water parameters such as temperature ($^{\circ}\text{C}$), pH, electric conductivity (EC mScm^{-1}) and total dissolved solids (TDS mgL^{-1}) through a multiparameter equipment Horiba were measured in situ during the field campaign. The water chemistry was analyzed at Laboratório de Pesquisas Hidrogeológicas (LPH), housed at UFPR. The sample in the field was filtered through $0.45\ \mu\text{m}$ SFCA (surfactant-free cellulose acetate) syringe filters, the procedure was repeated due to its high content of dissolved solids. In the laboratory, the sample were filtered using fiberglass membrane and a cellulose ester membrane. The concentrations were measured in mg / L and the abundance in milliequivalents per liter. The ions concentrations of sulfate, fluorine, phosphate, nitrites, nitrates, silica, iron and manganese using colorimetry were measured through of Spectrophotometer equipment (MN® UV/Vis II). Chlorine, Total alkalinity, Carbonate, Bicarbonate, Hydroxide, Calcium, Magnesium was using the analytical

method Titulometry through BRAND® Titrette® digital bottle-top burette. To measure sodium and potassium, the flame emission method was used by Photometer model FC 280 brand Celm. Gravimetry was used for total dissolved solids. The pH and conductivity were measured by potentiometry using sp2000 equipment Sensoglass brand. To geochemical simulations of the data, Phreeqc Interactive (Pfizer database) was used to calculate mineral saturation states. In addition, the data was analyzed in Piper diagram to classify the type of water.

2.3 X-RAY DIFFRACTION (XRD)

The X-ray diffraction technique was used to identify the mineral phases present in carbonate and clay fractions. The XRD analyzes were performed on the PANalytical X-ray diffractometer, model Empyrean with X-Celerator detector, with $\text{CuK}\alpha$ radiation conditions and readings with a scan rate of $0.5^\circ / \text{min}$, under a voltage of 40kv, current of 30mA. The carbonate rock and clays minerals scans were executed from angles of 2θ from 3° to 70° and from 3° to 30° , respectively, using a step size of a $0.016^\circ 2\theta$ and a count time of 10.16s per step. The mineral composition and semi-quantitative mineral abundances of the samples were determined using the HighScore software. The different carbonate minerals in the samples were calculated from their d -spacing in the X-ray diffraction spectra following Zhang et al. (2010).

The preparation for X-ray diffraction of the clay fraction was based on the procedures manual suggested by Kisch (1991). To analyze the mineralogy of the clay fraction, the samples were pulverized with a tungsten pot, and the clay fraction of the samples was obtained from the supernatant of the dispersion of the pulverized sample in distilled water in the proportion: 10 g of dry powder per 100 ml of deionized water, in beakers of 500 ml. For disaggregation, the samples were exposed to ultrasound for 1 minute with the addition of about 0.1 g of sodium pyrophosphate (deflocculant). After disaggregation, the samples were poured into 100 ml beakers for a 2-hour decantation period. After decanting, the supernatant fraction was removed with the aid of a pipette. The residue was smeared onto glass slides. To help in mineralogical identification of glass slides, different treatments were used for the

preparation: air-dried sample, ethylene glycol vapor saturation, and heating at 550°C in order to identify the different clay mineral species. These analyses were performed at LAMIR Institute, housed at UFPR.

2.4 X-RAY FLUORESCENCE (XRF)

The samples of carbonates and sediment were used for the quantitative chemical analysis by X-ray Fluorescence method to identify and quantify in the sediments the presence of 10 main oxides (SiO_2 , Al_2O_3 , Fe_2O_3 , CaO , MgO , K_2O , Na_2O , TiO_2 , MnO , and P_2O_5) and four trace elements (Sr, Ba, S, and Cl). For the analysis of the samples, a pressed tablet was prepared consisting of 7.0 g of the pulverized sample and 1.4g of organic wax aggregating material, which is subsequently pressed. To determine L.O.I. (loss on ignition), the samples were subjected to 1000 ° C for two h in a muffle. The equipment used corresponds to the X-ray Fluorescence Spectrometer, from the PANalytical brand, model AXIOS mAX with a rhodium tube, and the interpretation was carried out by the interpretation software SuperQ 5.3. These analyses were performed at LAMIR Institute, housed at UFPR.

2.5 CHEMICAL AND MINERALOGICAL ANALYSIS (TESCAN- TIMA)

Mineral and elemental mass of rocks and sediment samples were analyzed by an analytical technique known as automated mineralogical mapping by a TESCAN-TIMA instrument. Image analysis was performed simultaneously with SEM backscatter electron images combined with X-ray fluorescence. The operation is automated and has a robust database that transforms EDS chemical data to mineralogy. This equipment presents four types of detectors: a secondary electron detector, SE for high-resolution image acquisition at the micron level. A backscattered electron detector, BSE, for the acquisition in the grayscale of the different compositional phases. A Bruker brand X-ray detector for chemical microanalysis with its associated Quantax software. Three PulseTor brand X-ray detectors for automated mineralogy with their associated TIMA software. The

samples were prepared by mixing the pulverized rock and/or clay material with epoxy resin and powdered graphite (it provides conductivity to the sample for adequate conduction of the electron beam). The mixture is homogenized and poured into a 30 mm diameter cylindrical mold, then left to harden in a heat-curing pot, obtaining the briquettes, which are then polished and covered with a thin layer of carbon a graphite evaporator. The equipment is housed in Chile at *Soluciones en microscopía y Mineralogía Aplicada* (SEMMA).

2.6 PETROGRAPHY

In order to characterize, identify and understand the mineral composition, textures and sedimentary structures of the microbialites present in Laguna Timone, thin sections were made. For the description of the samples and photomicrography capture, the Zeiss Image A2m petrographic microscope was used and the images were processed in the Axio Vision software. These analyses were performed at LAMIR Institute, housed at UFPR.

2.7 SCANNING ELECTRON MICROSCOPY (SEM)

The morphological of rocks, microbial mats and chemical composition of specific points were obtained using a JEOL model 6010LA scanning electron microscope equipped with detectors for secondary electrons, backscatter electrons, and energy dispersive spectroscopy (EDS) model EX-94410T1L11 for images and operating equipment with voltage of 20 kV and the magnification range of this instrument is 5x to 300,000x. This equipment has a capability to operate this in low vacuum for biological samples and Secondary Electron Imaging (SEI) enables observation, characterization and imaging of microstructures of bacterial filaments specimens at LAMIR Institute, housed at UFPR. The rocks were subsampled by manual separation into a ca 2 mm thick surface of the most representative morphology. The microbial mat and biofilm were subsampled by manual separation into a ca 1,5 mm thick surface sample and to improve the overall integrity of material and preserve their

original microstructure the samples were air-dehydrated through Critical Point Drying (CPD) that to dry fixed and dehydrated biological samples before observation for scanning electron microscopy (SEM), Bal-tec model CPD030 equipment was used at *Centro de Microscopía Electronica* (CME), housed at UFPR.

2.8 HIGH RESOLUTION TRANSMISSION ELECTRON MICROSCOPY (HRTEM)

To obtain more morphological, structural, and chemical detail of the individual crystals, identifying the crystalline phases, Transmission Electron Microscopy (TEM) allows determining the internal structure of even biological materials. Furthermore, TEM provides detailed information on crystallography and chemistry of minimal areas such as square nanometers and features with 0.1 nm of space (Singh and Gilkes, 1995; Klein and Dutrow, 2012), useful in the identification of mineral clays. The samples were pulverized and solubilized in 99.9% alcohol and subsequently put in an ultrasound. To affix the particles was used carbon film 200 mesh - copper and later metalized. Images, qualitative and quantitative chemical analyses of clay fraction samples were obtained by High Resolution Transmission Electron Microscope – FEI TITAN G2, equipment operating at 300 kV at *Centro de Instrumentación Científica* (CIC), housed at the Universidad de Granada.

2.9 X-RAY MICROTOMOGRAPHY (MICRO-CT)

Microtomography is a type of physical analysis that allows the study of non-destructive cross-sections and three-dimensional models. It allows the visualization and quantification of specific parts of an internal structure of material or rock. Computerized tomography produces an image closer to the real thing, for these thousand shares of radiographs of different rotation angles, from 0 ° to 360 °, of the analyzed object are patinated (Cnudde et al., 2006). The previously selected rock samples were cut in a rectangular shape of approximately 1.0 cm x 3.0 cm. An X-ray microtomography, model 1172 from Skyscan, was used, with 90kV potential

parameters, 112 μA current, 12.8 μm / pixel resolution, and computer “clusters” for treatment processing of results, allowing microscale analysis.

2.10 C&O ISOTOPY

The use of stable carbon isotopes offers a possibility for distinguishing between inorganic and biologically influenced carbonates (Burne and More, 1987). The carbon isotopic composition of carbonate minerals depends on environmental conditions and biological activity during their formation. Therefore, the enrichment or impoverishment of an isotope concerning another indicates the material's geographical, biological, and geological origin. Therefore, it is possible to estimate the environmental conditions and biogeochemical processes acting in the precipitation of carbonates, reflects in the variations in the stable isotopes of ^{13}C and ^{18}O (Muccio and Jackson, 2009, Rumbelsperger, 2013).

Carbonates from the physical-chemical process will have a similar isotopic signature from the water where precipitation occurred, and carbonates generated by biological processes will present different isotopic data due to the fractionation of carbon isotopes. This differentiation is generated by the preferential use of mild isotopes (^{12}C) by microorganisms during photosynthesis. This process causes enrichment of ^{13}C in the aqueous solution, and then carbonate that precipitates inorganically from the evaporation of the water will be enriched in ^{13}C represented by positive $\delta^{13}\text{C}$ values, and biologically generated carbonates will be impoverished in ^{13}C by presenting negative $\delta^{13}\text{C}$ values (Burne and More, 1987).

Oxygen Isotope Composition from the precipitated carbonate in aquatic systems depends on the temperature and isotopic composition of the host water (Veizer and Hoefs, 1976, Kasting et al., 2006). Thus, variations of the values of $\delta^{18}\text{O}$ of carbonates are generally used to reconstruct paleoclimatic fluctuations, paleogeographic evolution, and diagenetic degrees (Burdett et al., 1990, Veizer et al., 1999, Kasting et al., 2006). Mass spectrometer model Delta V Advantage, brand Thermo Fischer Scientific, was used to bulk C and O isotopic composition. The

spectrometry is performed on CO₂ released from phosphoric acid digestion of carbonatic powder samples 'online' by reaction with 100% H₃PO₄ at 72 °C using gas preparation and introduction system Gas Bench II. Isotopic ratios were referenced in a standard international and are expressed in the 'δ' notation relative to Vienna Pee Dee Belemnite (VPDB). Data were processed by software Isodat 3.0. These analyses were carried out at LAMIR Institute, housed at UFPR.

2.11 FLUORESCENCE MICROSCOPY

To determine the presence of bacteria a fluorescence microscope was used since minerals and microorganisms in microbial mats show fluorescence when exposed to ultraviolet radiation (UV). These analyses were carried out at Centro de Bioinformática y Biología Integrativa (CBIB), housed at Universidad Nacional Andrés Bello (UNAB), Chile. Samples were examined with an epifluorescence microscope MF606 BW OPTICS with a 5 wave bands.

2.12 DNA EXTRACTION FOR 16S RRNA AND ITS REGION ANALYSIS

According to the manufacturer's instruction, DNA was extracted from the microbial mat and biofilm samples of Laguna Timone using PowerSoil® DNA Isolation Kit (Figure 5). The instructions mentioned below were followed (MO BIO Laboratories, 2014). The PowerSoil® DNA Isolation Kit comprises a novel method for isolating genomic DNA from samples utilizing our patented Inhibitor Removal Technology® (IRT). The kit is intended for use with environmental samples containing a high humic acid content, including difficult soil. In addition, the isolated DNA has a high purity level, allowing for more successful PCR amplification of organisms from the sample.

To the PowerBead Tubes provided, 0,25 grams of sample are added. The PowerBead Tube contains a buffer that helps disperse the particles begin to dissolve humic acids and protect nucleic acids from degradation, is mixture using the vortex. Then 60 µL of Solution C1 (contains SDS and other disruption agents required for complete cell lysis) was added and vortexed briefly, at maximum speed for 10

minutes, then tubes are placed in a centrifuge at 10,000 x g for 30 seconds at room temperature and were transferred supernatant to a clean 2 ml Collection Tube, avoiding the pellet. Next, the 250 µL of Solution C2 was added and vortexed for 5 seconds and incubated at 4°C for 5 minutes.

Then, the tubes were centrifuged at room temperature for 1 minute at 10,000 x g, 600 µL of supernatant transfer up 2 ml in a clean Collection Tube and 750 µL of supernatant was transferred into a clean, 2 ml Collection Tub, avoiding the pellet. Next, 1200 µL of Solution C4 was added to the supernatant and vortexed for 5 seconds. Approximately 675 µL was loaded onto a Spin Filter and centrifuged at 10,000 x g for 1 minute at room temperature. The flow was discarded and 675 µL of supernatant was added to the Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature. 500 µL of Solution C5 was added and centrifuged at room temperature for 30 seconds at 10,000 x g.

The flow was discarded and centrifuged again at room temperature for 1 minute at 10,000 x g, and the Spin Filter was placed in a clean 2 ml Collection Tube. Then, 100 µL of Solution C6 was added to the center of the white filter membrane and centrifuged at room temperature for 30 seconds at 10,000 x g, and the Spin Filter was discarded. Finally, the DNA in the tube is now ready for any downstream application. It is recommended to store frozen DNA (-20° to -80°C). These analyses were carried out at the *Center for Bioinformatics and Integrative Biology (CBIB)*, housed at *Universidad Nacional Andrés Bello (UNAB)*, Chile.

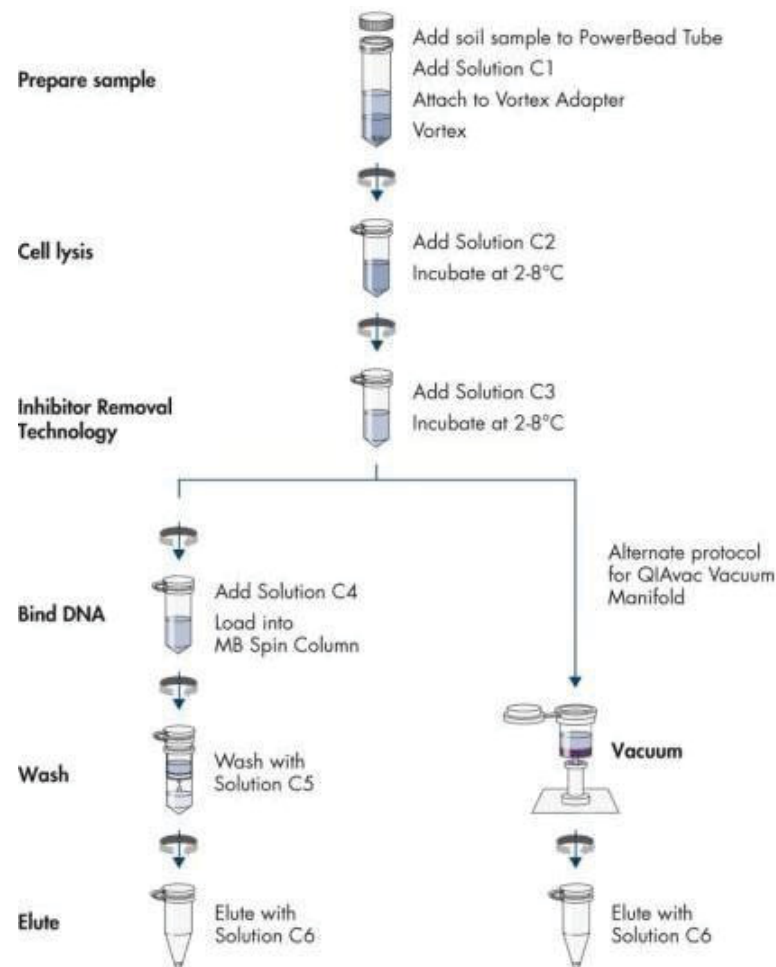


Figure 5. DNA PowerSoil Kit procedure.

2.13 16S RRNA GENE SEQUENCING FOR BACTERIAL IDENTIFICATION

PCR was performed on 1–5 ng of total extracted DNA, targeting the V4 hypervariableregion of the 16S rRNA bacterial genes using the universal primers 27F (5-AGA GTT TGA TCC TGG CTC AG-3) and 1492R (5-NGGT TAC CTT GTT ACG ACT T-3) (Lane et al., 1991). This process involves three main steps: denaturation, pairing, and extension. PCR reactions were performed using the following conditions: 94°C for 3 minutes to denature the DNA, with 35 cycles at 94 °C for 45 s, 50 °C for 60 s, and 72 °C for 90 s; with a final extension of 10 min at 72 °C to ensure complete amplification. Each 25ul PCR reaction contains 12ul of MoBio PCR Water (Certified DNA-Free), 10ul of 5 Prime HotMasterMix (1x), 1ul of

Forward Primer (5Uμ concentration, 200pM final), 1ul Golay Barcode Tagged Reverse Primer (5uM concentration, 200pM final), and 1ul of template DNA. Amplicons were quantified using PicoGreen (Invitrogen) and a plate reader. Once quantified, different volumes of each product are pooled into a single tube so that each amplicon is represented equally. This pool is then cleaned using the UltraClean® PCR Clean-Up Kit (MoBio) and then quantified using the Qubit (Invitrogen).

Samples were sequenced in the Argonne National Laboratories using the Earth Microbiome Project barcoded primer set, adapted for the Illumina HiSeq2000 and MiSeq (Caporaso et al., 2012; Caporaso et al., 2010). Data analysis of the 16s rRNA gene in bacteria, and the ITS region in fungi was performed using the DADA2 software package for Illumina sequenced paired-end fastq files (<https://benjjneb.github.io/dada2/>) (Callahan et al., 2016). Alpha diversity and abundance analysis were carried out using Phyloseq R Package (<https://joey711.github.io/phyloseq/>) (McMurdie et al., 2013). The phyloseq package is a tool to import, store, analyze, and graphically display complex phylogenetic sequencing data that has already been clustered into Operational Taxonomic Units (OTUs), especially when there is associated sample data, phylogenetic tree, and/or taxonomic assignment of the OTUs.

2.14 LABORATORY EXPERIMENT

2.14.1 Microorganisms Induce Carbonate Precipitation

The culture media corresponds to a liquid or solid preparation used in laboratories for the growth, transport, or maintenance of microorganisms (Madigan et al., 2003). A microorganism-induced carbonate experiment with an isolated bacteria was used to identify if microorganisms play a role in the precipitation processes. In this study, cultivation media were developed for the bacteria capable of precipitating carbonates can grow. 5g of each sample were used in 30 ml of sterile distilled water solution and stirred at 300 rpm for 48 h at a temperature of 28 ° C. There are several ways to

prepare cultures media. This procedure will use the technique of plating in quadrant streaks, inoculating 20 μL of each sample on one end of the agar-agar Petri dish (containing solid medium), with a sterile, and spreading in striae on the surface in one or more directions.

Individual cells are dislodged from the tip when rubbed on the surface and develop isolated colonies. In addition, a negative control is carried out by performing the same procedure, but instead of inoculating a sample, sterile distilled water is plated to verify that the method was carried out correctly and that there is no contamination (growth of some microorganism). Bacteria were isolated from microbial samples using R2A solid culture media (0.5 g of yeast extract, 0.5 g of Casamino acids, 0.5 g of Peptone, 0.5 g of glucose, 0.3 g of pyruvic acid, 0.3 g of K_2HPO_4 , and 0.05 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, per liter) (Reasoner and Geldreich, 1985) supplemented with 2.5 g calcium chloride. Then, the isolates obtained were grown at 28 °C and 11°C on B4 culture media (2.5 g of calcium acetate, 4 g of yeast extract, 10 g of glucose, and 20 g of agar per liter) (Cacchio et al., 2015). To evaluate the capacity to precipitate carbonates. The final pH was adjusted to 9 with NaOH- The colonies were evaluated every 48 h with light microscopy, and the formation of crystals on the surface of the culture medium were selected as macroscopically positive to observe the formation of crystals. These analyses were carried out at the Center for Bioinformatics and Integrative Biology (CBIB), housed at *Universidad Nacional Andrés Bello* (UNAB), Chile.

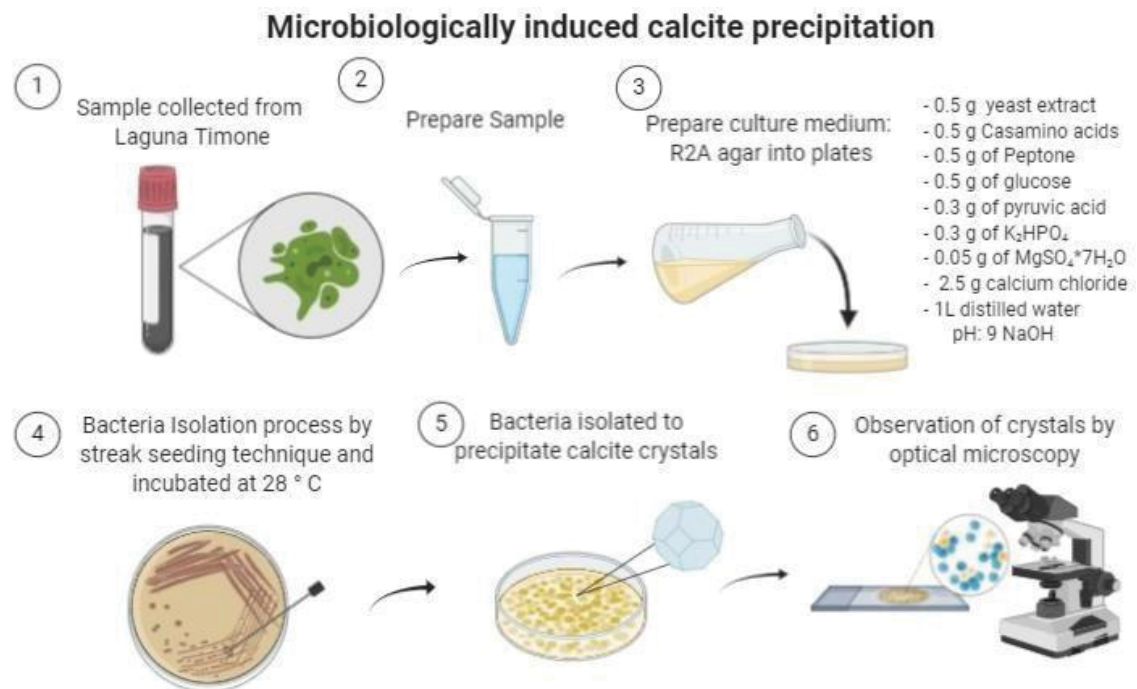


Figure 6. Isolation and cultivation of TIMO sample to microbiologically induced calcite precipitation experiment

3 CHAPTER III: RESULTS

FIRST MANUSCRIPT ^{*1}

The role of physicochemical and biochemical processes on carbonate precipitation within the Laguna Timone maar in the Pali Aike Volcanic Field, southernmost extra-Andean Patagonia

^{*1} This manuscript will be submit to Sedimentary Geology Magazine.

ABSTRACT

The Laguna Timone maar crater is situated in the Pali Aike Volcanic Field, a Quaternary volcano-tectonic complex in southern Patagonia and represent one of hundreds of “pools” of brines developed after explosive volcanic eruptions in a periglacial environment. The lagoon constitutes an endorheic hydrological system where processes leading to carbonate precipitation under extreme physicochemical and weather conditions can be explored. The Laguna Timone is recharged by groundwater and limited rainwater (annual precipitation rates of ca. 200 mm per year). The strong surface wind regimes are responsible for high evaporation rates, ranging between 1000 - 1600 mm per year. The brine waters contain high contents of Na (29.076 mg / L) and Cl⁻ (41.000 mg / L). Carbonate precipitation was studied in fragments of microbialite deposits and carbonate crust at the surface of pebbles located on the lagoon shore. The clay fraction of sediment was also characterized. The mineralogy of microbialites and crust mainly consists of calcite and traces of detrital minerals. The $\delta^{13}\text{C}$ VPDB (-0.43 ‰ to 2.50 ‰) values in texturally differentiated carbonates indicate physico-chemical and biochemical processes controlling their precipitation. The $\delta^{18}\text{O}$ VPDB values (-6.52 ‰ to 2.28 ‰) are associated to evaporation processes and meteoric influence. The clay fraction is dominated by illite, chlorite, and smectite. HRTEM analysis shows that the amorphous Mg- clay has a relation with calcite crystals. The carbonate precipitation is presumably originated from weathering of the surrounding basaltic rocks, biological influences, and climate conditions.

Key words: Microbialites; carbonate crust, alkaline-saline, clay mineral, weathering, basalt

1. Introduction

Silicate weathering is a key process of Earth's long-term carbon cycle (Berner et al., 1983). This process has a unique place in the geological cycle that involved the interface between the atmosphere, the hydrosphere, and the biosphere (Wilson, 2004). The mafic and ultramafic rocks, as basalts and peridotites, respectively, act as natural carbon sinks through weathering and/or subsequently carbonate mineralization (Dabirian et al., 2012). The dissolution of minerals from mafic rocks produces dissolved cations like Ca^{2+} , Mg^{2+} , Fe^{2+} and bicarbonate that increase the alkalinity and pH of waters and thus can lead to the formation of carbonates and clay mineral as smectites (Ferris et al., 1994; Kelemen et al., 2011; Bundelewa et al., 2014; Oelkers et al., 2015; Pozo and Calvo, 2018). On the other hand, there is a diversity of microorganisms that induce the nucleation and precipitation of carbonates, through chemical changes in the water system promoting the supersaturation state of the solution, increasing the availability of Ca^{2+} ion, through a variety of physiological functions (Wright and Oren, 2005; Braissant et al., 2007; Zhu and Dittrich, 2016). In addition, calcite has been found precipitating from silicates rich in Si and Mg in biological and chemical conditions (Bontognali et al., 2010; Burne et al., 2014; Pacton et al., 2015). Accordingly, the authigenic clay minerals could be related to calcite precipitation through biologically influenced mineralization (Calvo et al., 1999; Armstrong et al., 2020).

The Pali Aike Volcanic Field (PAVF) is a late Miocene to Quaternary volcano-tectonic complex in southern Patagonia located ca. 50 km east of the Andes Mountain chain and north of the Estrecho de Magallanes (Figure 7). The volcanic field overlies the km-thick sedimentary and volcanoclastic successions of the Upper Cretaceous to Cenozoic Magallanes Basin (Winslow, 1983; Mazzarini and D'Orazio 2003, Zolitschka et al., 2006). Volcanic rocks are basalts and alkaline basalts, including mantle xenoliths and xenocrysts sourced from the lithospheric mantle and incorporated at the site of generation and during the ascent of basaltic magmas (Skewes, 1979; Stern et al., 1991). A variety of volcanic-related morphologies such as spatter and slag cones, tuff rings, lava flows, and maars are distinguishable.

The origin of the PAVF involved three main volcanic stages, including a phase

dominated by explosive volcanism in a periglacial environment. Phreatomagmatic eruptions were triggered by the interaction of mafic magmas with groundwater and/or permafrost (Coronato et al., 2011) and formed hundreds of maars, which are relatively small craters and shallow depressions, commonly filled by a lake. The study object is the maar known as Laguna Timone, which is filled by body of water with high salinity and alkalinity. Maars can be envisaged as more or less circular pools brines and/or endorheic basins where the role of silicate weathering and physicochemical and biochemical processes on carbonate precipitation can be evaluated. Exceptionally, maars in southern Patagonia constitutes sites where carbonate precipitation proceed in a cold environment subject to episodes of freezing and strong winds. Additionally, they represent an opportunity to identify the former microorganisms colonizing lands after the retreat of ice sheets in sub-Antarctic regions (Henriquez et al., in prep).

2. Geological setting

The Cenozoic geodynamic evolution of southernmost South America is mainly due to the interaction between three tectonic plates: South American, Antarctic, and Scotia plates. It results from: (1) Subduction of the Nazca and Antarctic Plates beneath the South American Plate and (2) transcurrent movements between the Scotia and South American Plates. The occurrence of Cenozoic mafic magmatism associated with the Pali Aike Volcanic Field (PAVF) (Figure 7) is related to the opening of an asthenospheric window under South America in response to the subduction of the Chile Ridge under the continent (Dickinson y Snyder, 1979; D'Orazio et al., 2000, 2001). The complex geotectonic configuration of plate boundaries near the PAVF at 52°S latitude resulted in distinctive tectonic provinces (Winslow, 1983). From west to east, these units corresponds: (1) a Paleozoic metamorphic basement, intruded by the Jurassic-Miocene Patagonian Batholith exposed in the Pacific Coastal Archipelago (Hervé et al., 2007); (2) the Patagonian Cordillera, constituted of Upper Jurassic to Lower Cretaceous silicic volcanic rocks (Tobifera Formation) and ophiolitic complexes of the 'Rocas Verdes' back-arc basin (Calderón et al., 2007); (3) the eastern thin-skinned 'fold-and-thrust belt' developed

since the Late Cretaceous to the late Miocene (Fosdick et al., 2011); (4) Finally, the Magallanes foreland basin was affected by Neogene extensional tectonics and development of graben systems (Diraison et al., 1997).

The emplacement of the Pali Aike Volcanic Field, occurred on top of the Upper Cretaceous to Cenozoic Magallanes Basin (Winslow, 1983; Mazzarini and D'Orazio 2003, Zolitschka et al., 2006) along the southern Patagonian rift (Corbella et al., 1996). The late Miocene to Quaternary PAVF (3.8 to 0.17 Ma) extends over an area of approximately 4500 km² in Argentina and Chile. Three main volcanic stages have been proposed for the generation of the PAVF. According D'Orazio et al. (2000), the PAVF stratigraphy can be divided into three stratigraphic units, U1, U2 and U3. Unit U1 is the oldest and most extensive unit, covers 83% of the total area, consists of planar lava flows that form a plateau. Unit U2 consists of over 450 volcanic edifices. The edifices include: spatter, scoria cones, maars, and tuff ring associated with basaltic lava flows (Corbella, 2002). These occupy 15% of the total area of the PAVF. Finally, unit U3 is the youngest and consists of scoria cones, and lava flows well preserved, occurs only in the SE part of the volcanic field, and occupies only 2% of the total area.

The maar Laguna Timone discussed in this work belongs to unit U2. The preservation of these morphologies is affected due to erosion. Laguna Timone is the result of circular emission centers that reach up to two kilometers in diameter and are surrounded by a tuff ring resulting from a phreatomagmatic eruption, and the lagoon can be the oldest maars in the study area due to this ratio (Coronato et al., 2011).

The rocks are mainly basalts and alkaline basalts, including mantelic xenoliths (Mazzarini and D'Orazio 2003). In addition, Tertiary marine sediments (sandstone and shale) related to a marine transgression (Patagonia Formation) corresponds to the oldest outcropping geological strata of the study area. Miocene fine-grained molasse sediments from the Lower Miocene tectonic uplift of the Andes, and fluvioglacial deposits from the Pliocene and Pleistocene glaciations (Zolitschka et al., 2006) are present. The volcanic field represents the contrasting topography of a recently built eruptive area caused to glacial and periglacial phenomena, geomorphological agents of the fluvial cycle, and wind activity (Corbella and

Gagliardini, 1997).

The monogenetic cones in Pali-Aike have a spatial distribution with a preferential alignment and elongation. The observation realized by Mazzarini and D'Orazio (2003) through satellite image, topographic maps, field surveys, and aerial photos determines four main lineaments families: N - S (azimuth range 350° – 010°), NE - SW (021° - 060°), E - W (080° – 100°) and NW - SE (110° – 150°). The NW – SE and NE – SW are the most common structural orientations in PAVF. Recent volcanism is consistent with elevated geothermal gradients, suggesting that heat transport by advection can be related to hydrothermal fluids heated by deep and stagnant magmatic reservoirs within the continental crust.

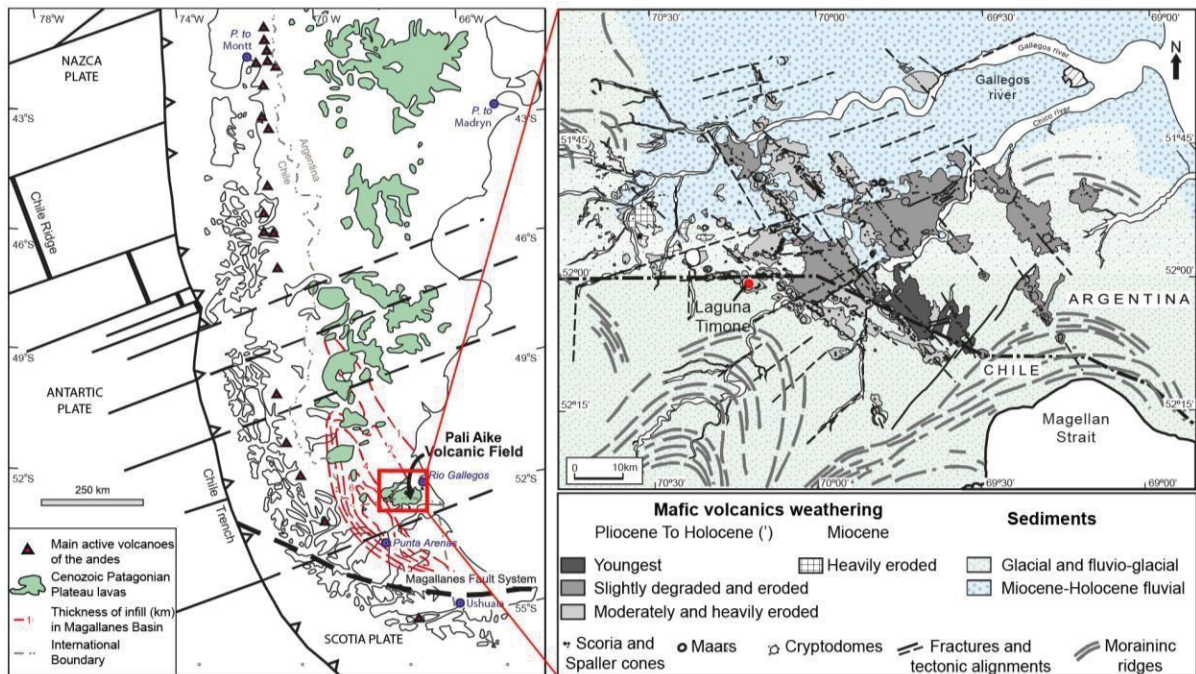


Figure 7. Location and geologic context of the study area. The Laguna Timone maar is located near the SW border of the Pali-Aike Volcanic Field (Source: adapted from Ross et al., 2011).

3. Climatic conditions

Climatic conditions of southern Patagonia are controlled by Antarctic ice mass and winds circulation from the west (Weischet, 1996). High solar radiation melts extensive ice fields during the summer. Moreover, Humboldt Current (west coast) and Falkland Currents (east coast) transport cold water to the north along the

Patagonian coasts, simultaneously reducing atmospheric heating. The cold-air masses advection arrives from the continent generate cold summers, and the proximity to the ocean is the cause of moderate winters (Barros et al., 1979). In general, southern Patagonia is characterized by extreme climatic conditions. Moreover, westerly winds that transport humid air from the Pacific Ocean to the Southern Andes lead to annual rainfall values between 4000-6000 mm on the coast and Andean territory. On the other hand, the Andean mountains behave as a topographic barrier for humid winds and generate the rain shadow on the eastern slopes of the Andes, where the Pali Aike volcanic field is located. This fact generates an abrupt decrease in precipitation to less than 300 mm per year, with a regular distribution generating semi-arid and desert climates (Zolitschka et al., 2006). In addition, the decrease in relative humidity related to the increase of mean annual temperature is another factor that influences the reduction of precipitation east of the Andes (Weischet, 1996). Eastern Patagonia tends to have a continental climate dominated by the SWW, with highly evaporative conditions at the surface (Garreaud et al., 2013). In summary, the area of Laguna Timone is characterized by a semi- arid and cold climate, with temperatures close to 0°C in winter, characterized by regimes of strong winds and low annual precipitation rates (ca. 200 mm/year).

4. Material and methods

For this study, samples of water, carbonate rocks, and sediments were collected during the field campaign (Figure 8). In Laguna Timone, the carbonate facies are significantly different from those report in Torres del Paine (Solari, 2010); basically, the carbonate buildings are absent. The main facies correspond to millimeter-scale crusts and fragments of microbialites. In the distal part of the lagoon, at the level of the steppe, microbialites were observed, showing botryoidal and thrombolites structures. The samples were named LT01 and LT02. The carbonate crust covers pebbles of different compositions. Some of the pebbles are cemented by carbonates forming blocks of carbonate breccia. The sample was named LT03- Botryoidal crust. The sediment was sampled according to two distinctive colors, grey and green. The

green mud is superimposed by a thin layer of well-crystallized carbonates and salts in the streams region. Dissection cracks and botryoidal carbonate crystal superimpose the gray mud. The sediment samples were named LTS01- Gray and LTS02-green.

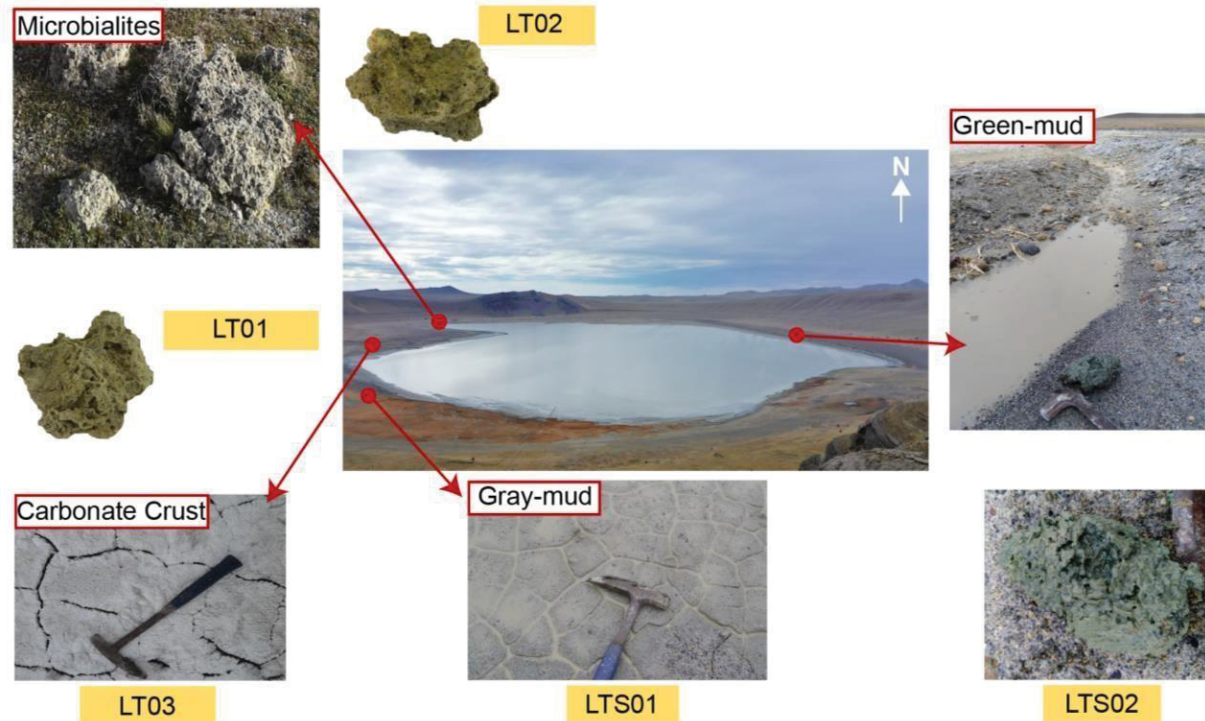


Figure 8. Location of samples in study area.

Water physiochemistry

Water parameters such as temperature ($^{\circ}\text{C}$), pH, electric conductivity (EC mScm^{-1}) and total dissolved solids (TDS mgL^{-1}) through a multiparameter equipment Horiba were measured in situ during the field campaign. The water chemistry was analyzed at *Laboratório de Pesquisas Hidrogeológicas* (LPH), housed at UFPR. The sample in the field was filtered through $0.45\ \mu\text{m}$ SFCA (surfactant-free cellulose acetate) syringe filters, the procedure was repeated due to its high content of dissolved solids. In the laboratory, the sample were filtered using fiberglass membrane and a cellulose ester membrane. The concentrations were measured in mg / L and the abundance in milliequivalents per liter. The ions concentrations of sulfate, fluorine, phosphate, nitrites, nitrates, silica, iron and manganese using colorimetry were measured through of Spectrophotometer equipment (MN® UV/Vis II). Chlorine, Total alkalinity, Carbonate, Bicarbonate, Hydroxide, Calcium,

Magnesium was using the analytical method Titulometry through BRAND® Titrette® digital bottle-top burette. To measure sodium and potassium, the flame emission method was used by Photometer model FC 280 brand Celm. Gravimetry was used for total dissolved solids. The pH and conductivity were measured by potentiometry using sp2000 equipment Sensoglass brand. To geochemical simulations of the data, PHREEQC Interactive (Pfizer database) was used to calculate mineral saturation states. The supersaturation state (Ω) of a solution was calculated through thermodynamic calculation as a function: $\Omega = ([Ca^{2+}] [CO_3^{2-}] / K^*_{sp})$ with ion activity product and K^*_{sp} the stoichiometric solubility product corresponding carbonate mineral (Stumm and Morgan, 1996). In addition, the data was analyzed in Piper diagram to classify the type of water.

Mineral and microstructure characterization of carbonates

The mineral and microfacies analyzes of the microbialites were performed using standard petrographic thin sections, examined under a Zeiss Image A2m microscope. The images were processed with the Axio Vision software. In addition, the morphology of carbonates was obtained using a JEOL model 6010LA scanning electron microscope equipped with detectors for secondary electrons, backscatter electrons, and energy dispersive spectroscopy (EDS) model EX-94410T1L11 for images and operating equipment with a voltage of 20 kV and the magnification range of this instrument is 5x to 300,000x. Microtomography was used to visualize specific parts of an internal structure of carbonates, and it allows the observation of non-destructive cross-sections and three-dimensional parts. Computerized tomography produces an image closer to the real thing, for these thousand shares of radiographs of different rotation angles, from 0 ° to 360 °, of the analyzed object are patinated (Cnudde et al.,2006). The previously selected rock samples were cut in a rectangular shape of approximately

1.0 cm x 3.0 cm. X-ray Microtomography, model 1172 from Skyscan, was used to analyze, with 90kV potential parameters, 112 μ A current, and 12.8 μ m / pixel resolution and computer “clusters” for analysis treatment and processing of results,

allows microscale analysis. These analyses were performed at LAMIR Institute, housed at UFPR.

Chemical composition of carbonates and sediment

The X-ray diffraction (XRD) analyses of carbonate rocks and sediments were performed with the PANalytical X-ray diffractometer, model Empyrean with X-Celerator detector, with $\text{CuK}\alpha$ radiation conditions with readings a scan rate of $0.5^\circ / \text{min}$, under a voltage of 40kv, current of 30mA. The different carbonate minerals in the samples were determined from their d -spacing in the X-ray diffraction spectra following Zhang et al. (2010). The preparation of the samples for X-ray diffraction of the clay fraction was based on the manual of the procedures suggested by Kisch (1991), and clay mineral identifications were based on methods described through the comparison of peak positions and intensities in patterns obtained from treatments: air-dried, ethylene glycol and heating 550°C (Moore and Reynolds, 1997). The mineral composition was determined using the HighScore software at LAMIR Institute, housed at UFPR.

The quantitative chemical analysis by X-ray Fluorescence (XRF) method was used to identify and quantify the presence of 10 main oxides (SiO_2 , Al_2O_3 , Fe_2O_3 , CaO , MgO , K_2O , Na_2O , TiO_2 , MnO , and P_2O_5) and trace elements (Sr, Ba, S, and Cl). The equipment used corresponds to the X-ray Fluorescence Spectrometer, from the PANalytical brand, model AXIOS mAX with a rhodium tube. The interpretation was conducted by the software SuperQ 5.3 at the LAMIR Institute, housed at UFPR.

Mineral and elemental mass of rocks and sediment were analyzed by an analytical technique known as automated mineralogical mapping by a TESCAN-TIMA instrument. Image analysis was performed simultaneously with SEM backscatter electron images combined with X-ray fluorescence. The operation is automated, and it has a robust database that transforms EDS chemical data to mineralogy. The equipment is housed in Chileat *Soluciones en microscopía y Mineralogía Aplicada* (SEMMA).

To obtain morphological structure and chemical detail of the individual crystals and crystalline phases of clay fraction. The samples were pulverized and solubilized in 99.9% alcohol and subsequently put in an ultrasound. To affix the particles was used carbon film 200 mesh - copper and later metalized. Images, qualitative analyses, and quantitative chemical analyses of clay fraction samples were obtained by High-Resolution Transmission Electron Microscope – FEI TITAN G2, equipped at 300 kV at *Centro de Instrumentación Científica (CIC)*, housed at the Universidad de Granada.

Isotope composition

A mass spectrometer model Delta V Advantage, brand Thermo Fischer Scientific, was used for the experimental acquisition of mineral C and O isotopic composition. The spectrometry is performed on CO₂ released from phosphoric acid digestion of carbonatic powder samples 'online' by reaction with 100% H₃PO₄ at 72 °C using gas preparation and introduction system Gas Bench II. Isotopic ratios were referenced in a standard international and are expressed in the 'δ' notation relative to Vienna Pee Dee Belemnite (VPDB). Data were processed by software Isodat 3.0. These analyses were carried out at the LAMIR Institute, housed at UFPR.

5. RESULTS

5.1 Water physiochemistry

Physico-chemical parameters (Table 2). The lagoon water is cloudy and dirty. Physico- chemical parameters show the pH range (ca.10), the electrical conductivity (111,000 uS/cm), and dissolved solids (110,567 ppm). The surficial water temperature of the lagoon is close to 0°C reaching a maximum of 15°C. The water chemistry shows Ca²⁺ (9 mg/L), Mg²⁺ (3 mg/l), and carbonate (29,997.43 mg/l). Comparing chloride (41,000 mg/l) ion average results with different types of water, the concentrations are very high, even exceeding the average content in seawater.

Sodium (29,076 mg/l), K (2,560 mg/L), nitrate (358,15 mg/l), sulfate (140 mg/l) and phosphates (110 mg/l) follow. Related to hydrochemical data, an analysis of the Piper diagram shows a strong tendency towards sodium chloride type. Geochemical simulations to calculate mineral saturation states show that aragonite, calcite and dolomite could precipitate under the current physicochemical conditions of the lagoon.

Table 2. The image shows physicochemical characteristics of water sample. PHREEQC calculated (*) saturations states.

Physiochemical analysis of water	
Physical Parameters	Water
Total Dissolved Solids (TDS) (m)	110,567
pH	9,7
Electric Conductivity (uScm-1)	111.000,0
Temperature (°C)	7
Chemical Parameters (mg/L)	Water
Calcium (Ca)	9
Magnesium (Mg)	3
Sodium (Na)	29.076,00
Potassium (K)	2.560,00
Iron (Fe)	0,42
Manganese (Mn)	0,25
Chloride (Cl)	41.000,00
Fluoride (F)	12,5
Sulphates (SO ₂)	140
Phosphorus (PO ₃)	110
Nitrates (NH ₃)	358,15
Nitrites (NO ₂)	0,272
Carbonates (CO ₃)	29.997,43
Hidroxides (OH)	1.018,32
Alkalinity Total	57.948,66
Saturation states	
Ω Calcite*	1.64
Ω Aragonite*	1.8
Ω Dolomite*	3.01

5.2 Microstructural characterization of carbonates

The reworked nature of microbialite deposit prevents a macroscopic facies characterization. However, features such as botryoidal and spherulitic forms, and laminations are recognized at the microscopic scale. The samples from Laguna Timone are highly porous, have interparticle, vugs, and fenestral types. The porosity of carbonates could have been increased by weathering effects, fragmentation, and desiccation.

LT01 show arborescent structures associated with coalescent oncoid with a

micrite to microsparite matrix that varies between dark and light colors with irregular contours (Figure 9a). The cloudy aspect is due to the high content of organic material. It is possible to observe around a clast as the material takes advantage of that substrate to start growing (Figure 9b).

Some areas of the thin section show high porosity (Figure 9c). The portions with massive texture are defined by intercalation of micrite and microsparite as layers with different tonalities, defining a millimeter crenulated internal lamination (Figure 9d). Micro CT and SEM images show ramification with high porosity (53%) (Figure 9e) and few laminations with neo-crystal of calcite (Figure 9f).

LT02 shows botryoidal structure defined by coalescent oncolite and a lumpy texture with micrite, sparite, and microsparitic matrix. Small peloids are observed. Crystal aggregates tend to develop a subhedral and equant form. Intracrystalline microporosity, vuggy, and fenestral porosity are distinguishing (Figure 10a). The oncolite are filled with microcrystalline aggregates of calcite (Figure 10b), showing concentric sheets around a nucleus. Spherical form is observed (Figure 10c), and SEM shows similar forms of calcite (Figure 10d). Micro CT images show nano-scale calcite crystals coalescing into spherical shapes, neo-forming calcite in botryoidal (Figure 10e). The porosity is less than LT01 sample (36%) (Figure 10f).

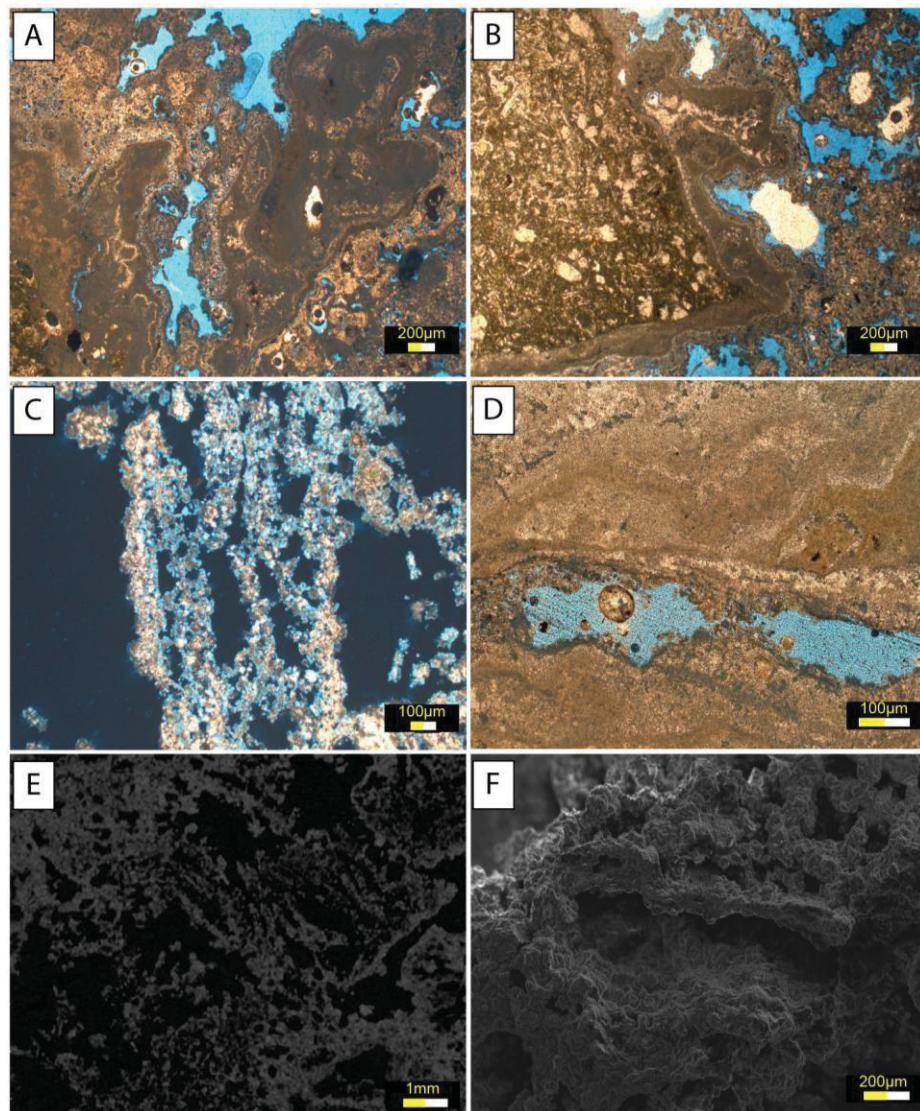


Figure 9. A) Arborescent texture with a micrite to microsparite matrix, small peloids, and organic material. B) Growth coupled to clast. C) Structures like ramifications with high porosity. D) Massive texture, intercalation of micrite and microsparite with crenulated internal lamination. E) Micro CT image confirming a shrub-like form with high porosity. F) SEM image shows few laminations with neo-crystal of calcite.

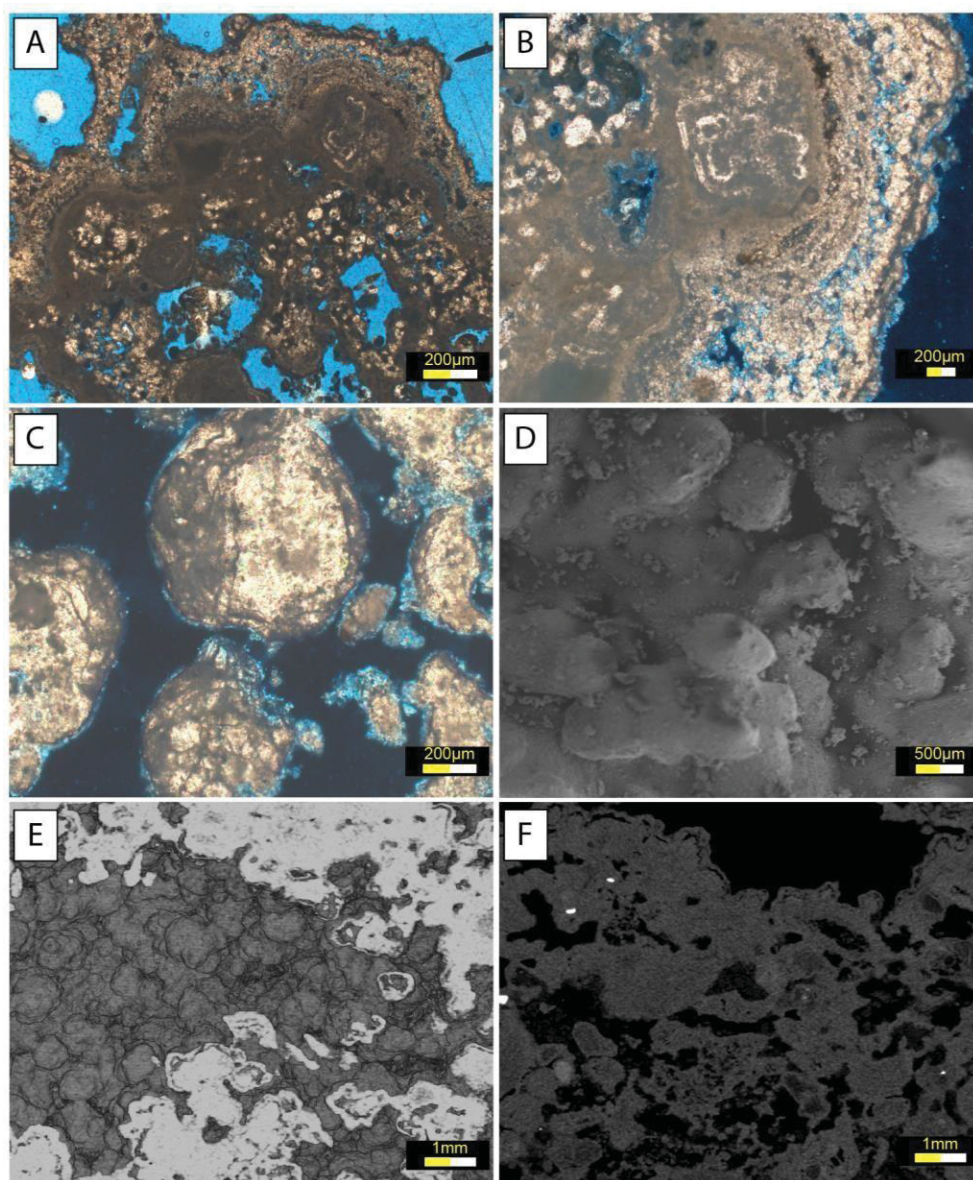


Figure 10. A) Botryoidal structure showing coalescent oncoidal and a lumpy texture with micrite, sparite and microsparitic matrix, small peloids, subhedral and equant crystal form. Intracrystalline microporosity, vuggy and fenestral porosity. B) Oncoidal Filled with microcrystalline aggregates of calcite showing concentric sheets around a nucleus associated. C, D) Spheroidal form. E, F) Micro CT images show nano-scale calcite crystals coalescing in spherical shapes.

5.3 Chemical composition of carbonates

TESCAN- TIMA results reveal that mass percentage close to 100% is calcite.

Traces of anorthoclase, labradorite, quartz, amphibole, muscovite, and biotite are accessory minerals, probably as particles carried by the wind (Figure 11a, b, c). In addition, the elemental analyzes in the samples reveal Ca, C and O (Figure 11d, e, f) as the main constituents, with low magnesium content ($> 1\%$), confirmed that the carbonates correspond to calcite. Mineralogical XRD analysis verified the results delivered by TESCAN-TIMA and showed that all samples, microbialites (LT01, LT02) and crust (LT03) are mainly composed of calcite with a bit of variation in proportions among the samples. The classification of carbonate was based on the d-spacing values in X-ray diffraction spectra, following the evaluation from Zhang et al. (2010) show calcite. (Table 3). Monohydrocalcite in the sample was detected in LT01, and fluorite is a trapped detrital mineral in carbonates LT02 and LT03.

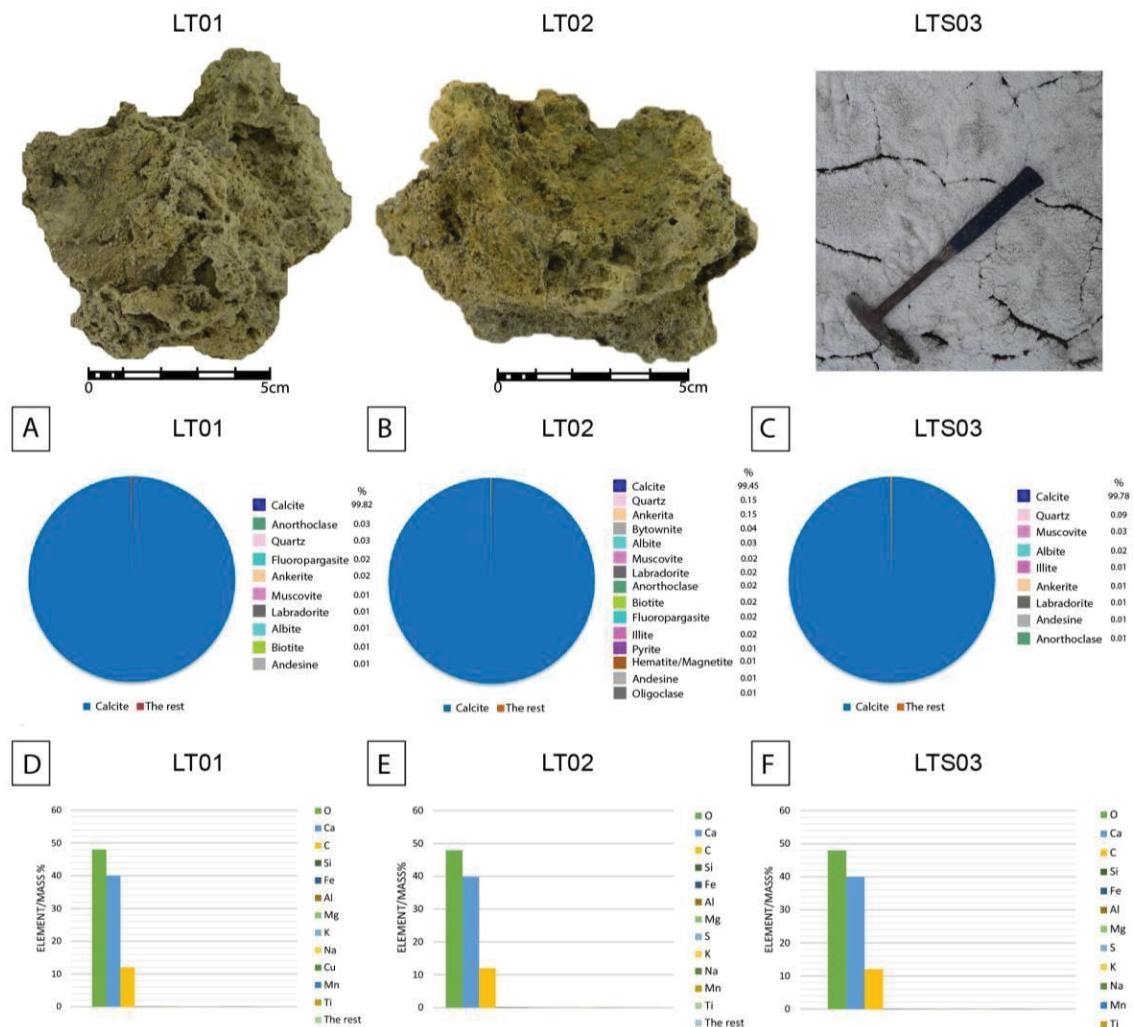


Figure 11. TESCAN analyses of carbonates A) LT01 composed by 99.85% of calcite B) LT02 composed by 99.45% of calcite c) Botroidal crust composed by 99.78% of calcite. All the samples contain detrital material less than 1%.

Table 3. Mineralogical composition XRD and TESCAN- TIMA analysis

Samples	XRD %	d(A°) Carbonates	Mineral (Zhang et al. 2010 classification)	Other mineral phases	TESCAN %	Mineral phases	Other mineral phases
LT01	98%	3,03	Calcite	Monohydrocalcite	99,82%	Calcite	Anorthoclase, quartz, fluoropargasite, Ankerite
LT02	96%	3,04	Calcite	Fluorita	99,45%	Calcite	Quartz, ankerite, Plagioclase
LT03	89%	3,03	Calcite	Fluorita	99,78%	Calcite	Quartz, muscovite, plagioclase

5.4 C&O isotopy analysis

The carbon and oxygen isotopic ratios of microbialite and crust samples were measured to better understand the environmental conditions. The microbialite samples show an average isotopic composition, with positive values for $\delta^{13}\text{C}$, varying from -0.5 to 2.5 ‰ VPDB and depleted values for $\delta^{18}\text{O}$, varying from -5.00 to -6.80 ‰ VPDB. In the other hand, the crust samples, showed little variation among the sample, with lightly negative values for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, where for $\delta^{13}\text{C}$ hanged from 0 to -0.5 ‰ VPDB and $\delta^{18}\text{O}$ has values around - 2.00 ‰ VPDB. Note the considerable difference between both facies (Figure 12).

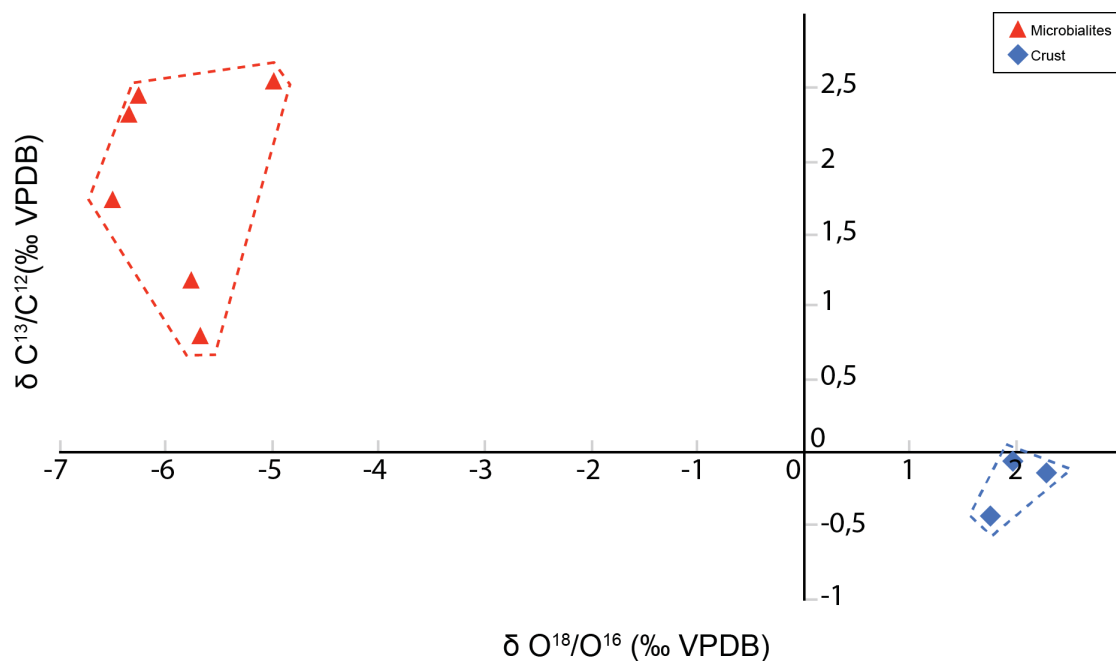


Figure 12. The stable isotope data of oxygen and carbon of Laguna Timone microbialite and cruts samples.

5.5 Chemical composition and microstructure of the clay minerals

TESCAN analysis showed chemical composition (%) of the following elements in the bulk sediments: O, Si, Ca^{2+} , Mg^{2+} , Fe, Al^{3+} , K⁺, Ti, C, Na⁺, F⁻, V, Mn, S, Cl, P, Cu, H, B, and REE (Figure 13). It is only possible to observe a significant variation of Ca concentration between grey-mud (LTS01) and green-mud (LTS02), 6.97% and 3.43%, respectively. The lithological characteristics of the parental area are critical to justify the inputs of these elements, mainly Ca^{2+} , Mg^{2+} and Al^{3+} associated with mafic rocks. The source of Mg and Ca is related to weathering of minerals in basaltic rocks, such as olivine and orthopyroxene, and plagioclase and diopside, respectively. Regarding Si, glacial-fluvial deposits, clays, diatoms, detrital material, and weathering of the silicate's minerals may be possible sources.

The XRF analysis shows that major oxides content in grey-mud (sample LTS01) is characterized by high contents of SiO_2 (51,5 wt%) and CaO (17,2 wt%) followed by Al_2O_3 (9,3 wt%), Fe_2O_3 (7,8 wt%), MgO (5,3 wt%), Na₂O (3,5 wt%), K₂O (1,9%) and Cl (1,1%). The mass of the trace elements is below that 1 wt%. The green-mud (sample LTS02) also indicates high contents of SiO_2 (51,5 wt%) but shows significant differences in Na₂O (17,9 wt%), CaO (7,1 wt%) concentration in relation to the grey-mud. Al_2O_3 (9,3 wt%), Fe_2O_3 (8,0 wt%) and MgO (5,3 wt%) are similar. The Cl (7,7 wt%) and K₂O (3 wt%) concentration is notably higher. The mass of the trace elements (P, Mn, Sr, Ba, Rb, Br) is below 0,5 wt%. TiO_2 is less abundant (1 wt%) and can be considered as a minor constituent, but Ti has been used as an indicator of the presence of clay minerals (Koinig et al., 2003).

Several studies show that K is used as an indicator of clay (Kylander et al., 2011) associated K with illite (Minyuk et al., 2007) related to K₂O, Na₂O, CaO composition. The high Fe_2O_3 and MgO values in the sediments are indicators of chlorite related to Mg and Fe. These are two of the major elements of chlorite formation (Brownlow, 1996). Smectite is supported mainly by Si, Al, Mg, Ca, and Fe (Drief and Nieto, 2000).

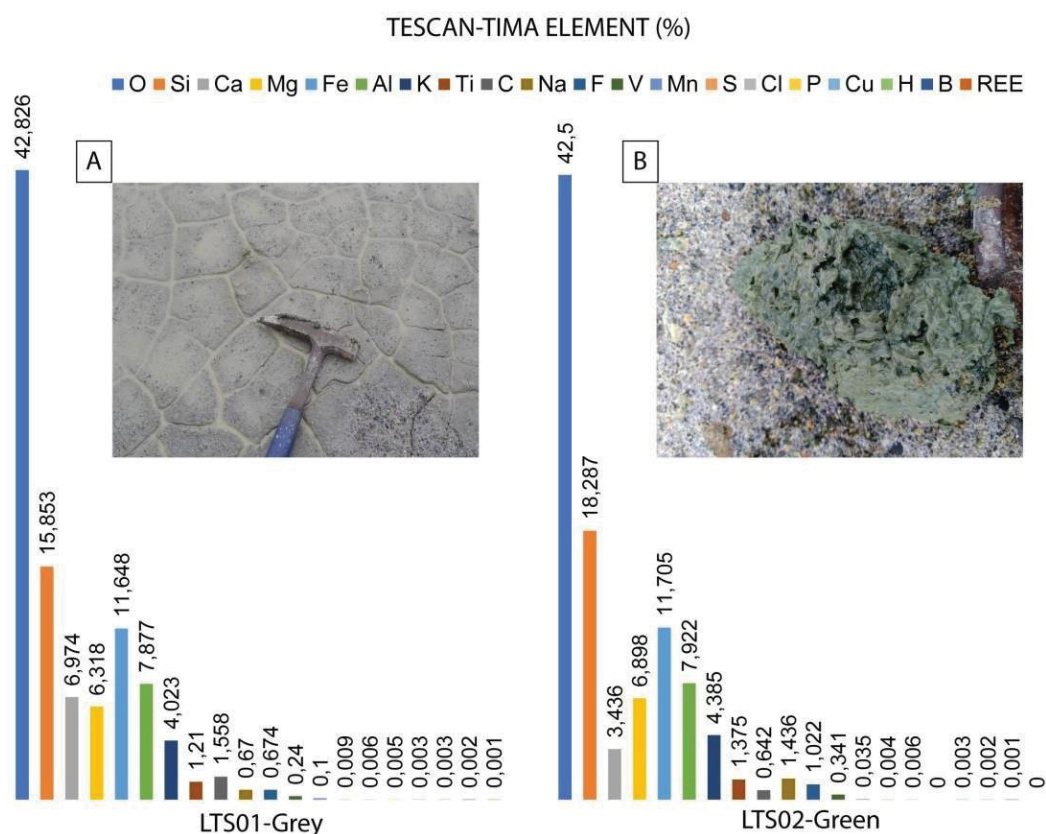


Figure 13. Graphic shows elemental composition of the bulk sediments. A) Element concentration of Gray-mud (LTS01). B) Element concentration of Green- mud (LTS02).

Individual crystals cannot be identified in SEM images because the clay appears flaky, partially covering grains of detrital quartz and calcite. Therefore, the precise identification of the clays fraction based on X-ray diffraction analyses, supported by TEM and EDX analyses showing the main elements involved. Clay mineral identifications were based on methods described by comparing peak positions and intensities in patterns obtained from treatments: air-dried, ethylene glycol, and heating 550°C (Moore and Reynolds).

Green mud (LTS02) (Figure 14a) showed a peak at 14.42 Å in air-dried conditions following treatment with ethylene glycol expanding to 17.55 Å through the 001 peaks and collapses to 10.09 Å after heating to 550°C, confirmed the presence of a clay mineral of the smectite group (according to USGS-flow chart). Other clays identified in the samples correspond to members of the illite group characterized by intense 10.03 Å° 001 peak and a 5.03Å° 002 peak unaltered by ethylene glycol and

heating to 550°C (Fanning et al., 1989). Since the reflection at 7Å° corresponds to the sum between kaolinite (d001) and chlorite (d002), it is difficult to quantify them because they do not vary with the ethylene-glycol treatment and when they are heated to 550 ° C collapses the crystalline structure. However, in this case, characteristic basal reflections of chlorite were represented by a 3.54 Å 004 peak. Therefore, in poly-mineral samples, a way to differentiate between kaolinite and chlorite is based on comparing the 3.58 Å kaolinite peak with the 3.54 Å chlorite peak (Biscaye, 1965), and peak position of 7.10 Å indicates chlorite (Biscaye, 1964).

The quartz is identified by the intensity peaks corresponding to the reflections of 4.26 Å (d001) and 3.35 Å (d002) and 3.21 Å suggest the presence of feldspar. Grey-mud (LTS01) (Figure 15) shows a difference in smectite peak absence. X-Ray Diffraction of the clay mineralogy of samples shows that smectite, chlorite, and illite to be the primary clay minerals, with quartz and potassium feldspar. Figure 15 shows that the difference in intensity and crystallinity of smectite can be related to authigenic minerals.

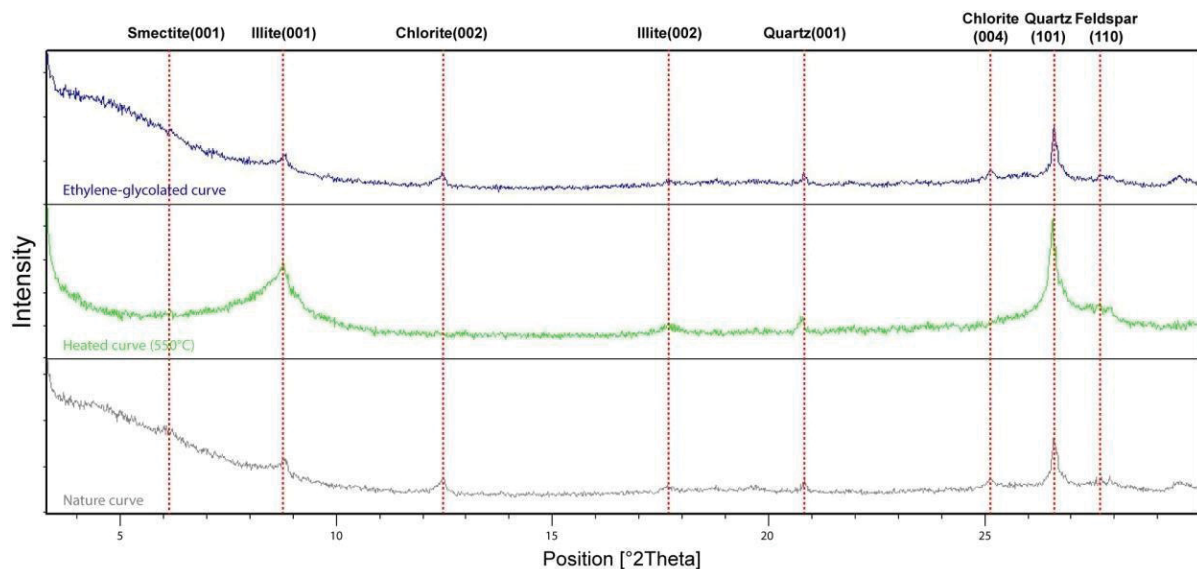


Figure 14. XRD patterns of the clay fraction of Green- mud (LTS02) sample of peak positions and intensities in patterns obtained from air-dried (AD), ethylene glycol (EG) and heating 550°C (H) treatments. Smectite group was identified by AD: 14,42 Å, EG: 17,55 Å, H: 10,09 Å to 001 peak positions. Illite group was identified by AD: 10,03 Å, EG: 10,04 Å, H: 10,09 Å (d001) and 5,03Å° (d002). Chlorite group was identified by AD: 7,10 Å, EG: 7,10 Å, H: collapse (d002) and 3.54 Å (d004). The quartz is represented by 4,26 Å (d001) and 3,35 Å (d002) and 3,21 Å (d110) suggest feldspar.

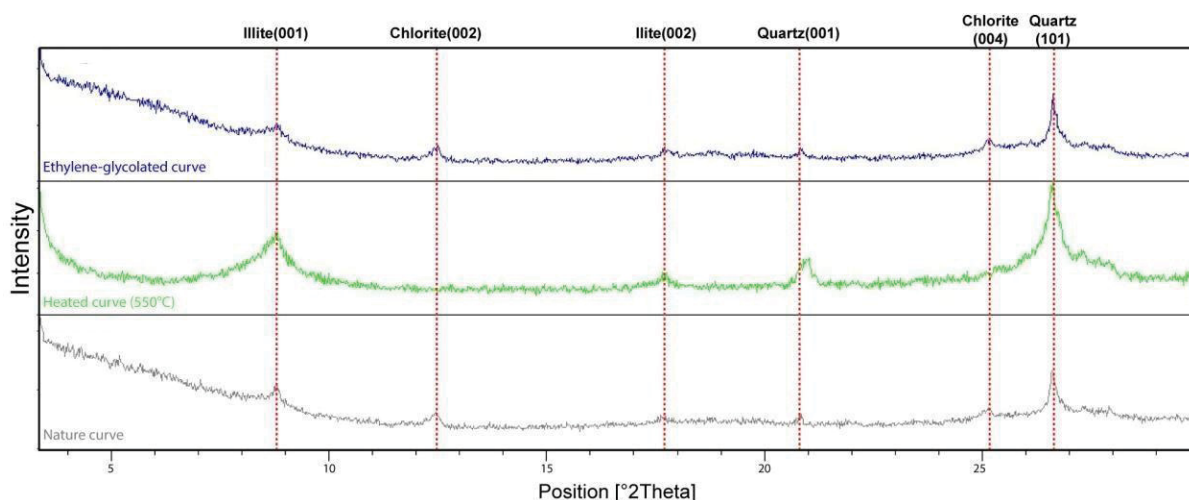


Figure 15. XRD patterns of the clay fraction of Gray-mud (LTS01) sample of peak positions and intensities in patterns obtained from air-dried, ethylene glycol and heating 550°C treatments. Illite group was identified by AD: 10,04 Å, EG: 10,01 Å, H: 10,06 Å (d001) and 5,03 Å (d002). Chlorite group was identified by AD: 7,11 Å, EG: 7,09 Å, H: collapse to 002 and 3,54 Å 004 peak position. The quartz is represented by 4.26 Å (d001) and 3,34 Å (d002).

Scanning electron microscopy (SEM images) shows mineral grains and an aggregate of fine crystals that generally have irregular morphologies. In some cases, spherical, angular, and tabular shapes were identified. Within the mineral, grain calcite is dominated (Figure 16a, b). In addition, a thin layer without defined morphology is covering the crystals (Figure 16c). The EDX spectra of the different samples generally indicate a similar trend in terms of Si, Mg, Al, Na, Fe, Ca, and K in their composition (Figure 16d). These characteristics are indicative of clay minerals. Also, the bulk sediment shows diatoms in the phase of dissolution inside (Figure 16e).

Imagen obtained from Transmission electron microscopy (TEM) shows irregular morphologies like "cotton". Also, elongated crystals composed of Ca and O associated with calcite were identified (Figure 16f, g). The "cotton" morphology is associated with authigenic processes and is common in smectites associated with reactive surfaces (Tosca, 2015; Tosca and Wright, 2015). Clay coatings on diatom structures (Figure 16h) were observed. High-resolution TEM lattice-fringe image of the small clay particle show mixed-layer I-S packets of layer spacing from 1.3 nm characteristic of smectite and layer spacing from 1.0 nm characteristic of illite (Figure 16i). The spacings correspond to the sum of illite and smectite-like layer (21.7–23 Å periodicity) (Figure 16i) (Drits et al., 1997). Layers to 24 Å (Figure 16j) are the result

of an ordered sum of one chlorite layer (14 Å) and one contracted smectite layer (10 Å) (Shau et al. 1990). TEM analysis shows indicate that clays minerals correspond mainly to illite, smectite, and chlorite.

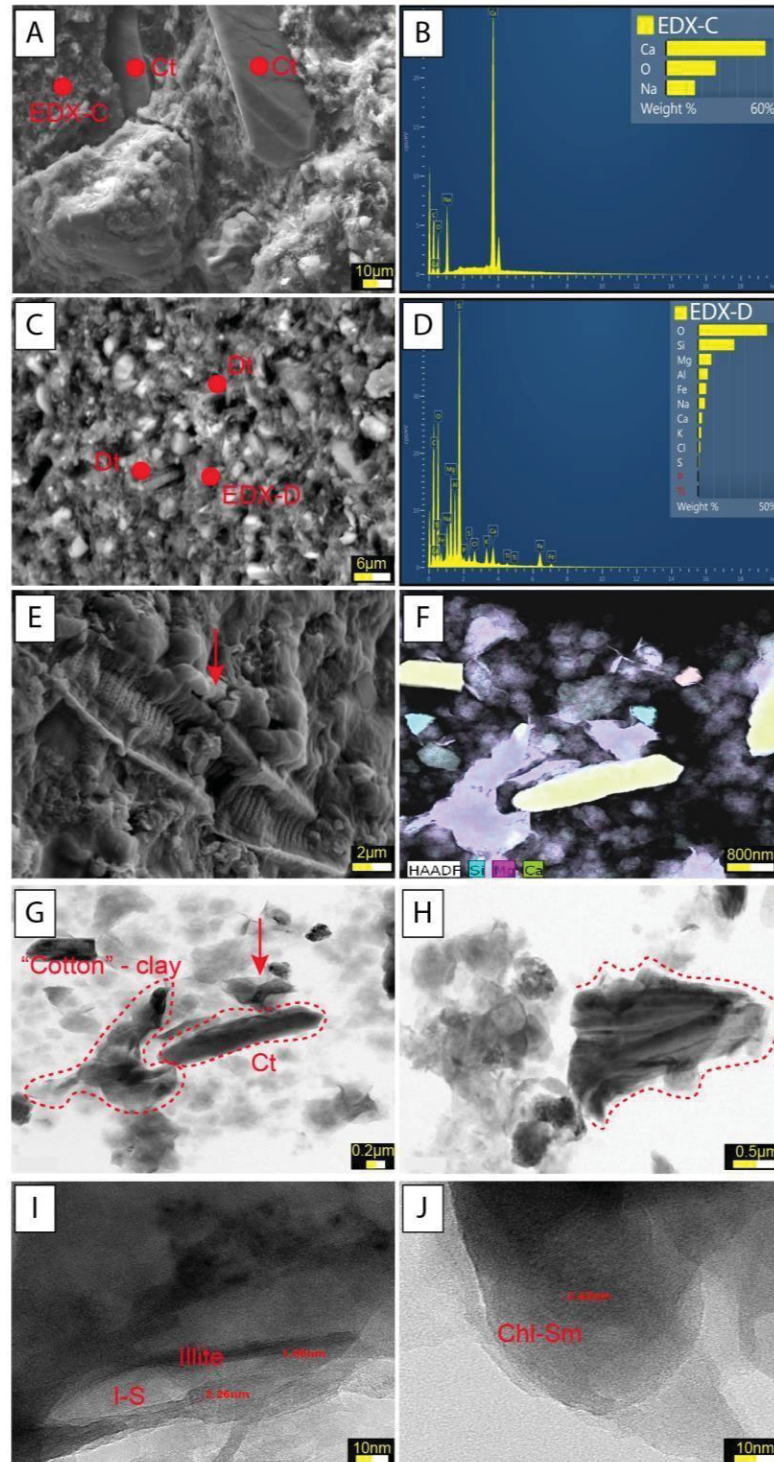


Figure 16. Scanning electron microscope images. A) Thin sheet of clay minerals covering the elongate calcite crystals in Grey-mud (LT01) sample. B) EDX analysis shows calcite. C) Thin sheet of clay

minerals covering partially crystal and dissolved diatom in Green-mud (LT02) sample. D) EDX analysis of thin sheet show clay mineral composition of EDX point in C. E, F) Partially dissolved diatoms. G) “Cotton like” clays associated with Mg-clay and elongated crystal of calcite. H) Clay coatings on diatom structures. I) Thin packets of mixed-layer with spacings corresponding to the sum of illite- and smectite-like layer spacings (21–22 Å periodicity). J) Chlorite/smectite interstratification (24 Å periodicity).

6. DISCUSSIONS

6.1 Role of climatic/hydrochemical conditions

The superficial and cold waters of Laguna Timone are characterized by high salinity and alkalinity, being rich in calcium, magnesium, sodium, potassium, nitrate, nitrite, sulfate, and phosphates. The supersaturation state (Ω) calculated from solution shows Ω ($= 1.64$) > 1 , indicating that is supersaturated with respect to CaCO_3 and thus suitable for carbonate precipitation. The high contents of chloride and sodium favors the high salinity in the lagoon system that exerted a key role in increase the solubility of minerals (O'Corner et al., 2001). The cations like Ca^{2+} , Mg^{2+} , and Fe^{2+} , and $\text{H}_2\text{CO}_3^{-2}$ are released from the crystalline structure when are exposed to saline solution increasing the alkalinity and pH of the aqueous system, which subsequently can lead to the formation of carbonate minerals (Ferris et al., 1994; Kelemen et al., 2011; Bundelewa et al., 2014; Oelkers et al., 2015; Dabirian et al., 2012).

The strong winds at the latitudes of PAVF are responsible for permanently mix the shallow water column and the high evaporation rates in the range of 1000 e 1600mm per year (Ohlendorf et al., 2013). This fact induces calcite supersaturation of water and subsequent precipitation. The evaporation/precipitation processes are so critical factors that affect saline lakes (Williams, 2002); usually these are restricted to regions of low rainfall and high evapotranspiration rates like Laguna Timone. Small changes in the climatic conditions are linked to variations in their hydrological balance producing changes on physical and chemical properties. At the same time, the evaporation event causes the brine been more concentrated.

Laguna Timone is exposed to extreme weather conditions showing a seasonal

fluctuation in the limnological parameters. The lagoon shows a noticeable temporal chemical variability between the months of may (2017) and september (2019). The Schoeller-Berkaloff diagram also indicate the seasonal variation of ion concentration and the consequence of high evaporation, decreasing the precipitation rates act on carbonate precipitation (Figure 17). Therefore, the hydrosphere-troposphere interaction is a potential controller of this system for carbonate precipitation.

Oxygen isotope values are consistent with the physicochemical process, associated limnological variations have influence on precipitation. The positive $\delta^{18}\text{O}$ VPDB values (2.28‰) of thin crust indicates that evaporation processes prevailed compared to meteoric influences, and the more negative $\delta^{18}\text{O}$ values (-6.52‰) observed in the microbialite shows possibly influence of meteoric water and/or groundwater as the fluid for carbonate precipitation.

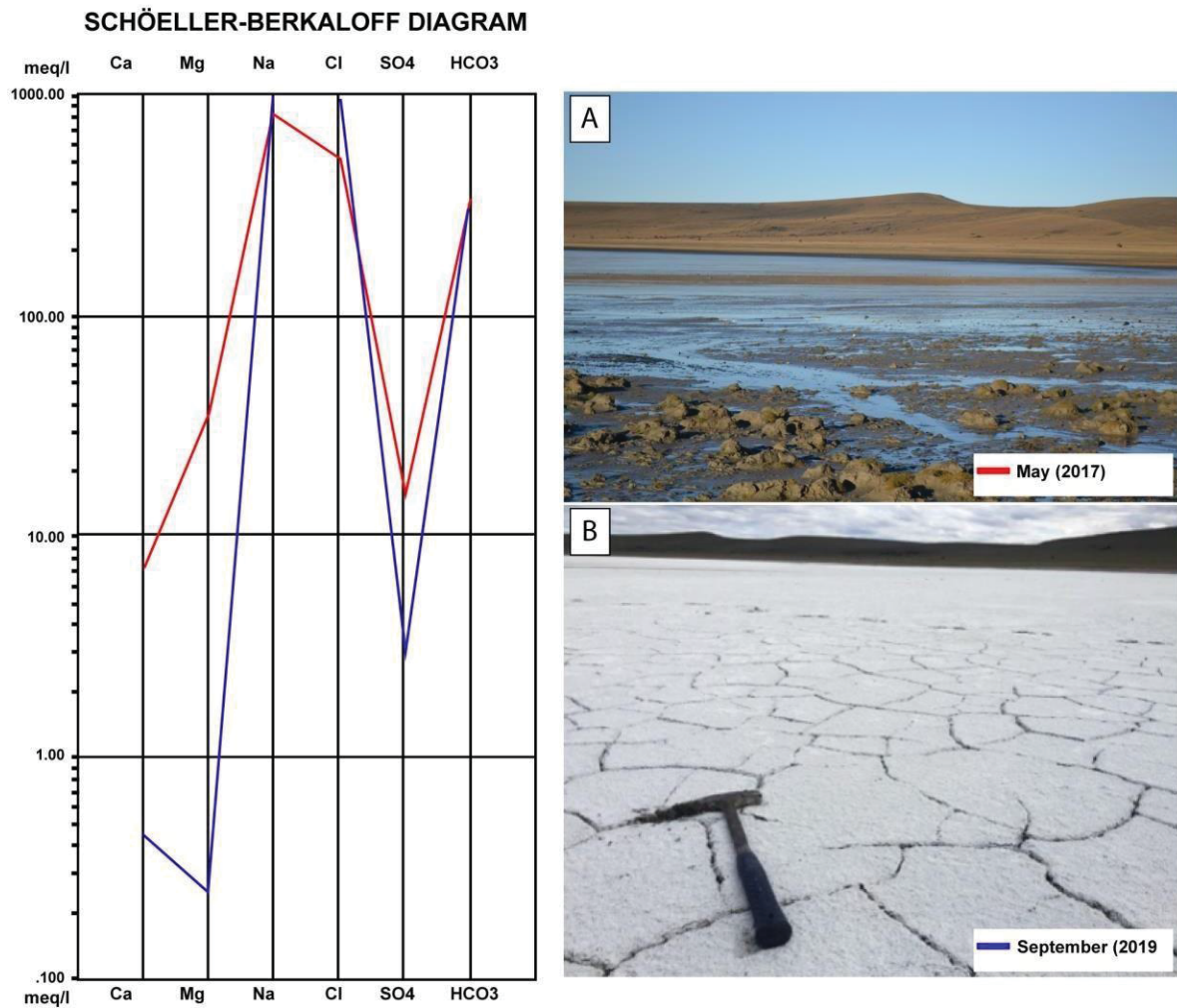


Figure 17. The Schoeller-Berkaloff diagram shows the variation on ion concentration between may (2017) and september (2019) seasons. The red lines represent May and blue lines represent September.

6.2 Significance of lithology in mineral precipitation

The weathering silicates greatly impacts the soil formation and the cation release (Figure 18). The enriched of chloride, sodium, fluoride, potassium, calcium, and magnesium compositions indicate that the chemical compounds of superficial waters at Laguna Timone is influenced, primarily, by chemical weathering of bedrock minerals, such as mafic rocks and ultramafic components of the PAVF. The physical and chemical disintegration of olivine, pyroxene, and plagioclase in basalts, and minerals in rare lherzolites and harzburgites xenoliths provides element availability as Ca, Mg, Fe, Al, Si, K, Na and, subsequently, saturation of waters and sediment

enrichment.

The presence of olivine could be important in this context because studies show that the ion leaching from this mineral can drive the subsequent precipitation of carbonate and have an important geochemical process on carbon cycle (White et al., 1995; Olsson et al., 2012).

The chemical composition of sediments at Laguna Timone show that Si is the main component. This is probably related to the occurrence of quartz grains that may originate from mechanical weathering of fluvioglacial sediments and basal tills described in PAVF (Zolitschka et al., 2006). Also, as a result of silicate weathering from mafic rocks, tuff ring, and scarce windblown detrital components. On the other hand, it could be associated to unconsolidated deposit from pre-eruptive maar deposits exposed on the surface before phreatomagmatic eruptions (Corbella and Gagliardini, 1997).

The clay fraction was characterized by illite, chlorite, and smectite. Several studies (Singer, 1966; Drief et al., 2001; Prudencio et al., 2002; Wilson, 2004; Dekayir et al., 2005; Needham et al., 2006), shows that the weathering of mafic rocks can be generated secondary mineralogy, as smectite like interstratified/mixed-layer forms, corrensite chlorite and illite. The Mg ions input from ferromagnesian minerals could lead to Mg-clays formation. Also, the cotton-like morphologies associated with smectites by transformation and authigenic processes (Welton, 2003) are related by the transformation of precursor minerals such as olivine, pyroxene chlorite and illite (Pozo and Calvo, 2018), all identified in Laguna Timone.

Some authors (Pozo and Calvo, 2018; Singer, 1984 and Calvo et al. 1999) postulated that the occurrence of clays belonging to the smectite group is favored in evaporitic environments and semi-arid climatic conditions, which are exposed to the contribution of ferromagnesian silicates and the alkaline and saline conditions promoting the precipitation of these minerals, consistent with what is reported at Laguna Timone. In addition, according to Tournassat et al. (2009), the clay minerals as smectites link hydrated Mg^{2+} within the interlaminar region stronger than ions as Na^+ and Ca^{2+} . Therefore, the cation exchange in smectite could reduce the inhibiting effect of Mg^{2+} on nucleation of $CaCO_3$ to favor the precipitation of calcite.

The Si available probably are related to weathering of feldspar from mafic

rocks, which is more rapidly than quartz to yield secondary minerals and silicic acid, as: $2\text{KAlSi}_3\text{O}_8 + 2\text{CO}_2 + 11\text{H}_2\text{O} \rightarrow \text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4 + 2\text{K}^+ + 2\text{HCO}_3^- + 4\text{H}_4\text{SiO}_4$ (McKeague and Cline, 1963; White, 1995). Therefore, the available Si as silicic acid could eventually be used for frustule formation of diatoms and/or subsequent clays.

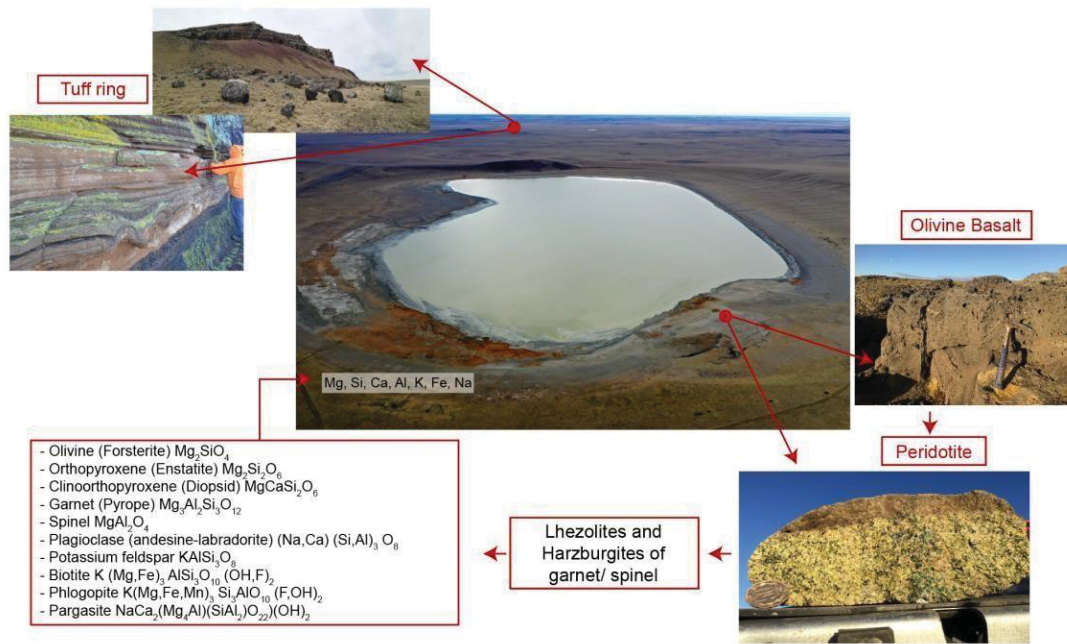


Figure 18. Image show the main source of availability elements in the system.

6.3 Clay formation and the relationship with carbonate precipitation

The SEM and TEM images show partially dissolved diatoms and clay coatings on diatom structures. In addition, images show the cotton-like morphologies associated with Mg- clay composition together with calcite. This fact could suggest that reactive Si provided by dissolved diatom fragments assist in clay precipitation. Therefore, dissolved silica from diatoms could promote the precipitation of Mg-Si substances, and several studies suggest that calcite can be precipitated from silicates rich in Si and Mg (Bontognali et al., 2010; Wright, 2012; Burne et al., 2014).

Cyanobacterial photosynthetic found in Laguna Timone (Henriquez et al., in prep) could influence the precipitation of Mg-Si substances through increases in pH producing undissociated H_4SiO_4 (Bischoff et al., 2020) and facilitating the

precipitation of Ca-carbonate through biological processes.

Also, diatoms produce EPS that binding of Fe, Al, Si, Ca, Mg, Na, K and other elements (Underwood, 2010; Urbani et al., 2012), and the nucleation of Mg-silicates comes from of pre-existing surface such as amorphous silica, detrital clay minerals and extracellular polymeric substance (EPS) (Tosca and Wright, 2015). Thus, the close association between carbonate crystals, EPS and Mg-Si clay could suggests that the precipitation also is microbially mediated.

7. Conclusions

The lakes in this region are essential from a hydro-sedimentological perspective because they offer an unusual opportunity to examine the genesis of a wide variety of chemical precipitates. This research reveals different processes associated with carbonate precipitation that occur under exceptional conditions that implicate the interaction of ice-sheet dynamics, volcano-tectonic events, and chemical weathering in a cold environment subject to episodes of freezing and strong winds. The concentration of available ions was associated with the weathering of the surrounding rocks, rainwater infiltration, and sediment-water interface that provides the necessary element saturation and consequently carbonate precipitation, which is favors by strong winds that can lead to evaporative processes. This fact showed the strong hydrosphere-troposphere interaction, where the variations are depending on environmental conditions. The geochemical data showed that the carbonates are affected by evaporative processes, meteoric and biological influences. Regarding, the clay minerals formation suggests that reactive Si could be provided by dissolved diatom fragments related to the authigenic process and from silicate weathering, and consequently, contribute to carbonate precipitation. Finally, of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values indicates that the carbonate precipitation is controlled by either physicochemical and/or biochemical processes.

**Microorganism Composition Associated with Mineral Precipitation in
Microbial Mats from Laguna Timone Maar Crater, Pali Aike Volcanic Field,
Chilean Extra-Andean Patagonia**

**1 This manuscript has been submitted to Frontiers in
Microbiology.*

Abstract

The Laguna Timone maar crater is a unique environment with extreme conditions situated in the Pali Aike Volcanic Field, Extra-Andean Patagonia, whose magmatism source is associated with sub-slab asthenosphere flow through slab windows. The low annual precipitations (~ 200 mm) and strong winds that characterize this zone are responsible for high evaporation rates —1000 – 1600 mm per year. The lagoon water is alkaline, hypersaline, cold (close to 10°C), and rich in calcium, magnesium, sodium, potassium, nitrate, nitrite, sulfate, and phosphates. Microbialites, carbonate crust, and microbial activity are documented near the lagoon shore. Biofilm and microbial mats developed around the maar were sampled and characterized. We studied the role of microorganisms in carbonate organomineralization. Bacterial and fungal biodiversity was examined using Illumina sequencing of PCR-amplified 16S rRNA and ITS genes from total extracted DNA. The results showed that the Proteobacteria, Bacteroidetes, Cyanobacteria, and Verrucomicrobia phyla dominated the bacterial communities. The *Pseudomonas*, *Rhodobacter*, *Brevundimonas*, and *Oscillatoria* genera found in the samples have been associated with carbonate precipitation recorded under similar environmental conditions. Regarding fungal communities, *Ilyonectria*, *Tetracladium*, *Thelebolus*, *Acrostalagmus*, and *Plectosphaerella* were the predominant genera in samples found in the Antarctic environment. In general, determined microbial communities correspond to those reported in environments with extreme living conditions. Bacteria were isolated from Biofilm and mats, and a bacterial isolate capable of precipitating calcium carbonate *in vitro* was obtained. The precipitated crystals were analyzed, and the results were consistent with the mineralogy of carbonate rocks. The precipitates were associated with microbial mats forming an organomineral calcite. Our findings support that Laguna Timone is a natural laboratory for understanding organomineral precipitation processes under unique conditions in a place still unexplored.

Keywords: microbial mat, biofilm, carbonates, organomineral, alkaline-saline lagoon, laboratory experiment.

1. Introduction

There are only a few modern environments where primary carbonate precipitates in a maar crater on the Earth's surface. One example is located in southern South America, in the Pali Aike Volcanic Field (PAVF) (Skewes and Stern, 1979), a Quaternary volcano-tectonic complex about 50 km east of the Andean Mountain chain and north of the Estrecho de Magallanes. The study area is the Laguna Timone maar crater, characterized by carbonate precipitation and microbial activity on the lagoon shore. The microbial mats and biofilm present in this unique environment tolerate extreme environmental and climatic conditions, including: high salinity, high pH (< 9), low temperatures (close to 0°C), high radiation, strong winds, semi-arid and cold climates with annual precipitation of approximately 200 mm (Weischet, 1996). The exceptional conditions of this site have extraordinary scientific value for research on microbiological characterization involved in carbonate precipitation because they implicate the interaction of ice-sheet dynamics, volcano-tectonic events, and chemical weathering under extreme climate conditions. These processes provide the necessary elements used by microorganisms to acquire many nutrients, such as C, N, P, Fe, various metals, and some organic compounds, from mineral surfaces (Luttge et al., 2005; Gadd, 2010). Bacterial cell surfaces trap cations of the system, like Ca^{2+} , and bind them to their cell surface, creating ideal nucleation sites for calcite deposition (Stocks-Fischer et al., 1999).

In addition, low temperatures slow the growth of most microorganisms and can even prevent growth altogether in some cases. This scenario is mostly due to the low activity of cell enzymes under this condition (Madigan et al., 2003). Microbial mats consist of different communities (Stolz, 2000), which vary according to the geochemical conditions of the system (Des Marais, 2003). For example, communities in microbial mats located in wet and warm climates are characterized by high bacterial abundance and diversity. On the other hand, microbial communities in cold and dry climates present lower abundance and diversity. For instance, variations in salinity are associated with significant changes in bacterial communities (Vuillemin et al., 2013). Hypersaline environments are considered one of the probable settings where life emerged on Earth (Dundas et al., 1998), and some microorganisms

currently inhabiting these hypersaline environments might be descendants of such primordial life forms (DasSarma, 2012).

Microbial communities are modulated by the harsh conditions present, such as high salt concentration, scarce nutrients, oxygen availability, alkalinity, and high UV irradiance (Ventosa, 2006). Microbialites are abundant in hypersaline lakes and can be associated with microbial mats, which are very common in these lakes (Glunk et al., 2011). Historically, these microbial mats were dominant in many environments, but currently, they are limited to a few hypersaline lakes, such as Lake Clifton in Australia (Moore and Burne, 1994) and Storr's Lake in the Bahamas (Paul et al., 2016). Several studies suggest that alkaliphilic and halophilic species, as well as sulfate-reducing and ureolytic bacteria, may play a role in carbonate precipitation (López-García et al., 2005). Based on the arguments above, hypersaline lakes represent unique environments for the development of microbial life and are considered hot spots of microbial diversity (Ley et al., 2006).

Extremophiles are organisms that optimally grow and reproduce under extreme conditions of temperature, pressure, pH, salinity, aridity, or radiation. The northern and southern parts of Chile have several lakes with these characteristics, where the unusual geochemistry supports the growth of an array of microorganisms. Among them, the still unexplored PAVF endorheic lagoons have unique extreme conditions, precipitated carbonates, and unknown microbial communities. To increase our understanding of the organomineralization process and the environmental conditions during carbonate precipitation in modern environments, we studied the relation between mineral-bacteria on carbonate precipitation in Laguna Timone. The maar crater represents an open laboratory to understand these processes, where microbial communities present in microbial mats have the capacity to promote carbonate precipitation in a saline aquatic system.

2. Geological and climatic context

PAVF corresponds to a Quaternary volcano-tectonic complex that covers an area of approximately 3000 km² (Skewes and Stern, 1979), located on top of the Mesozoic to Cenozoic sedimentary succession of the Magallanes Basin (Natland et al., 2014;

Fosdick et al., 2020), including fluvioglacial deposits from Pliocene and Pleistocene glaciations (Zolitschka et al., 2006). PAVF geologic evolution is mainly linked to fissural basaltic magmatism within an extensional setting (D’Orazio et al., 2000) with a distinguishable variety of volcanic-related morphologies, such as spatter and slag cones, tuff rings, lava flows, and maars. PAVF contains around a hundred maar craters formed during phreatomagmatic eruptions caused by the interaction of mantle-derived basaltic magmas with either groundwater, surface water, and/or permafrost in a proximal periglacial environment (Coronato et al., 2013). The Laguna Timone maar crater (Figure 19) results from a subcircular emission center that reaches up to two kilometers in diameter and is surrounded by a tuff ring and basaltic lava flows at some extent with mantle xenoliths. The associated phreatomagmatic eruption also generated two other neighboring craters located at higher topographic levels, where no water is currently available.

Regarding climatic conditions, the Andean Mountain chain behaves as a topographic barrier for humid winds, producing rain shadow in the eastern slopes of the Andes, where PAVF is located, and causing an abrupt decrease in precipitation to less than 200 mm per year, with regular distribution throughout the year, resulting in a semi-arid and desert climate (Zolitschka et al., 2006). In addition, the decrease in relative humidity associated with the increase in mean annual temperature is another factor that influences the reduction in precipitation east of the Andes (Weischet, 1996). Eastern Patagonia tends to have a continental climate dominated by the strong Southern Westerly Winds, with highly evaporative conditions at the surface (Garreaud et al., 2013).

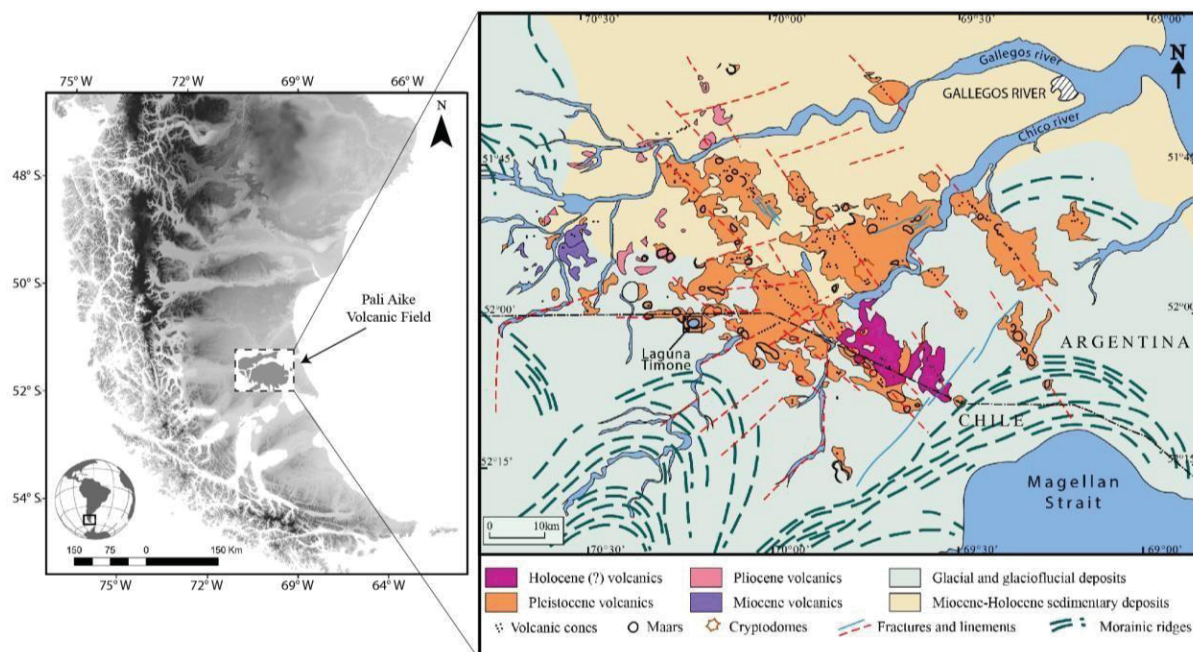


Figure 19. Location and geologic context of the study area: Laguna Timone in the Pali Aike Volcanic Field (PAVF) (source: adapted from Corbella, 2002).

3. Material and methods

In 2019, samples of water, carbonate rocks, and microbial mats around the lagoon were obtained during the field campaign. Biological samples were picked up with gloves and sterile scalpel and tweezers to minimize possible contaminations, placed in sterile bags, and kept at 4°C until processing. The samples (Figure 20) were named TIMO 1 (biofilm), TIMO 2 (microbial mats), and TIMO 3 (microbial mats) and used for genetic analyses. The carbonate samples were named LT01 and LT02 (microbialites), and LT03 (botryoidal crust) (Figure 21).



Figure 20. Carbonate samples. A) Microbialite (LT01). B) Microbialite with botryoidal facies (LT02). C) Carbonate crust (LT03) found around the maar crater.

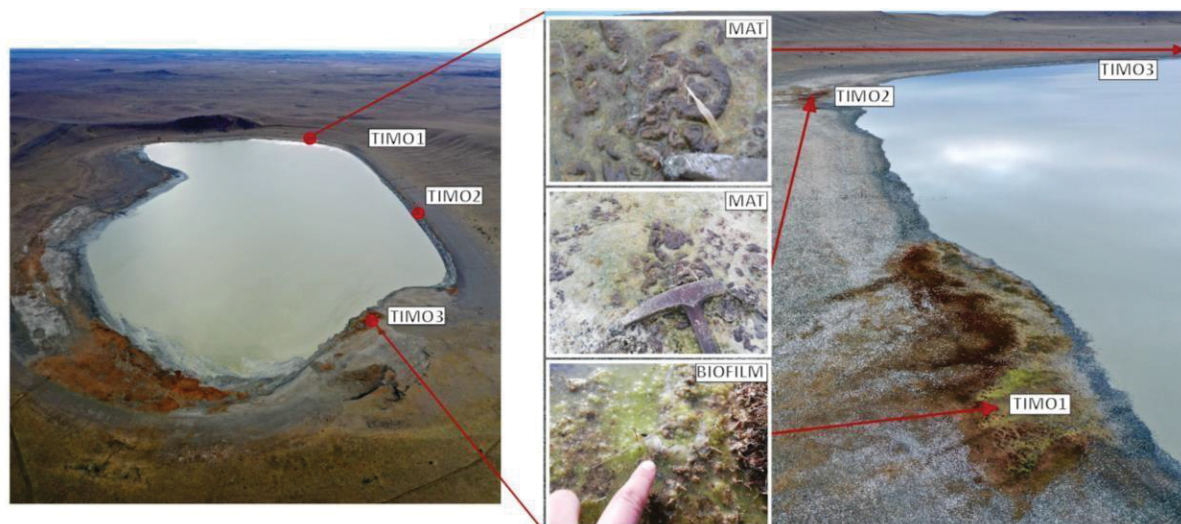


Figure 21. Location of the microbial samples around the Laguna Timone maar crater. TIMO 1 corresponds to biofilm viscous material of green color with bubbles showing current activity. TIMO 2 and TIMO 3 have boomerang shapes and are greenish to brownish.

Water chemistry

Water parameters, such as temperature ($^{\circ}\text{C}$), pH, electric conductivity (EC mScm^{-1}), total dissolved solids (TDS mgL^{-1}) through a Horiba multiparameter equipment, and total alkalinity using a photometer PF-12Plus, were measured in situ. Water chemistry was analyzed at Laboratório de Pesquisas Hidrogeológicas (LPH), housed at Universidade Federal do Paraná (UFPR). In the field, the sample was filtered through $0.45\text{ }\mu\text{m}$ SFCA (surfactant-free cellulose acetate) syringe filters, the procedure was repeated due to its high content of dissolved solids, and the product was stored in a cooler with artificial ice, keeping the temperature up to 4°C . In the laboratory, the sample was filtered using a fiberglass membrane and a cellulose ester membrane. Concentrations were measured in mg L^{-1} , and abundance was calculated in milliequivalents per liter. Ion concentrations of chlorine sulfate, fluorine, phosphate, nitrites, nitrates, silica, iron, and manganese were measured by colorimetry using a spectrophotometer (MN® UV/Vis II). Carbonate, bicarbonate, hydroxide, calcium, and hardness (and then magnesium) were calculated using the titration analytical method through BRAND® Titrette® digital bottle top burette. Sodium and potassium were measured based on the flame emission method with a Celm FC 280

photometer. Gravimetry was used for total dissolved solids.

Mineralogical analysis of carbonates using X-ray diffraction and TESCAN

Mineralogical composition of carbonates was assessed by an analytical technique known as automated mineralogical mapping, using a TESCAN-TIMA instrument. The operation is automated and has a robust database that transforms energy-dispersive spectroscopy (EDS) chemical data to mineralogy. The equipment is housed in Chile at Soluciones en Microscopía y Mineralogía Aplicada (SEMMA). Carbonate X-ray diffraction (XRD) analyses were performed by the PANalytical X-ray diffractometer, model Empyrean, with the X-Celerator detector. Mineral composition and semi-quantitative mineral abundances of the samples were determined using the HighScore software. The d-spacing of different carbonate minerals in the samples was obtained from XRD spectra (Zhang et al., 2010).

Bacterial microscopy characterization

Presence of bacteria was determined by a fluorescence microscope since minerals and microorganisms in microbial mats show fluorescence when exposed to ultraviolet radiation (UV). These analyses were carried out at Centro de Bioinformática y Biología Integrativa (CBIB), housed at Universidad Nacional Andrés Bello (UNAB), Chile. Samples were examined with the epifluorescence microscope MF606 BW OPTICS, with 5 wave bands. The morphology of microbial mats, minerals, and crystal precipitated by bacteria was obtained at the LAMIR Institute housed at UFPR by a JEOL 6010LA scanning electron microscope equipped with detectors for secondary electrons, backscatter electrons, and EDS model EX-94410T1L11 for images. The equipment operates with a voltage of 20 kV, and its magnification range is 5x to 300,000x. The microbial mat and biofilm were subsampled by manual separation into a ca 1.5 mm thick surface sample. To improve the overall integrity of the material and preserve the original microstructure, the samples were air-dehydrated through critical point drying (CPD) to dry fixed and

dehydrated biological samples before scanning electron microscopy (SEM) analysis using a Bal-tec CPD030 microscope at Centro de Microscopía Electronica (CME), housed at UFPR.

DNA extraction for 16S rRNA and ITS gene analysis

DNA extraction from microbial mat and biofilm samples of Laguna Timone was performed with the PowerSoil® DNA Isolation Kit (MO BIO Laboratories) following the manufacturer's instruction; next, total DNA was quantified using Qubit fluorometer (Invitrogen). Samples were sequenced in the Argonne National Laboratories with the Earth Microbiome Project barcoded primer set, adapted for the Illumina HiSeq2000 and MiSeq (Caporaso et al., 2012; Caporaso et al., 2010). Data analysis of the 16S rRNA gene in bacteria and the ITS sequence in fungi was performed in the DADA2 software package for paired-end Illumina sequencing fastq files (<https://benjjneb.github.io/dada2/>) (Callahan et al., 2016). Alpha diversity and abundance analysis were carried out using the Phyloseq R Package (<https://joey711.github.io/phyloseq/>) (McMurdie et al., 2013).

Microbially induced calcite precipitation experiment

Microbially induced calcite precipitation (MICP) evaluates the formation of calcium carbonate from a supersaturated solution produced by biochemical activities (Bosak, 2011). To identify whether microorganisms participate in precipitation processes in Laguna Timone, bacteria were isolated from microbial samples, and carbonate precipitation experiments were performed. Bacteria were isolated from microbial samples using R2A solid culture media (0.5 g yeast extract, 0.5 g casamino acids, 0.5 g peptone, 0.5 g glucose, 0.3 g pyruvic acid, 0.3 g K₂HPO₄, and 0.05 g MgSO₄·7H₂O, per liter) (Reasoner and Geldreich, 1985) supplemented with 2.5 g calcium chloride. Afterward, the isolates obtained were grown at 28°C and 11°C in B4 culture media (2.5 g calcium acetate, 4 g yeast extract, 10 g glucose, and 20 g agar per liter) (Cacchio et al., 2015) to evaluate their capacity to precipitate carbonates.

The experiments were carried out in triplicate with a blank control.

4. Results

4.1 Water chemistry

The lagoon water is cloudy, dirty, and gray-colored. Physicochemical parameters indicated an alkaline pH (ca.10), an electrical conductivity of 111,000 uS/cm, and 110,567 ppm of dissolved solids. The temperature of the lagoon is close to 10°C reaching a maximum of 15°C during the summer. Water chemistry shows Ca^{2+} (9 mgL^{-1}), Mg^{2+} (3 mgL^{-1}), and carbonate (29,997.43 mgL^{-1}). Comparing average chloride ion results (41,000 mgL^{-1}) with different types of water, the concentrations are very high, even exceeding the average content in seawater. Sodium (29,076 mgL^{-1}), fluoride (12.5 mgL^{-1}), potassium (2,560 mgL^{-1}), nitrate (358.15 mgL^{-1}), sulfate (140 mgL^{-1}), and phosphates (110 mgL^{-1}) were determined (Table 2). The microbial samples were exposed to the same physicochemical conditions around the lagoon due to the constant mixing of water caused by the strong winds (Henriquez et al., in prep).

4.2 Microorganisms present on mats and biofilm

The TIMO 1 sample consisted of a green and viscous material with some bubbles inside. The appearance of TIMO 2 and TIMO 3 samples presented no significant differences. At the macroscopic level, TIMO 2 and TIMO 3 samples were greenish to brownish, 3 mm thick, with the classical boomerang shape of microbialites (Figure 2). The samples were observed in a fluorescence microscope, showing filamentous microorganisms with a microscopic morphology characteristic of cyanobacteria and typical red autofluorescence indicative of photosynthetic pigments that reveal chlorophyll and phycobilin protein (Yokoo et al., 2015) (Figure 4). TIMO 1 sample had mineral structures emitting blue fluorescence that can be associated with calcite (Yokoo et al., 2015). In this sample, the mineral is more crystalline than that

observed in the other samples, and the mineral grains seem like small isolated grains of filamentous structures (Figures 22A, B). In TIMO 2 (Figures 22C, D) and TIMO 3 (Figures 22E, F), diatoms were identified between communities of photosynthesizing organisms. A high abundance of cyanobacteria (associated with red fluorescence) was observed in microbial mat samples, showing a close relationship with carbonate precipitates. The microscopy analysis revealed that mineral structures had amorphous forms.

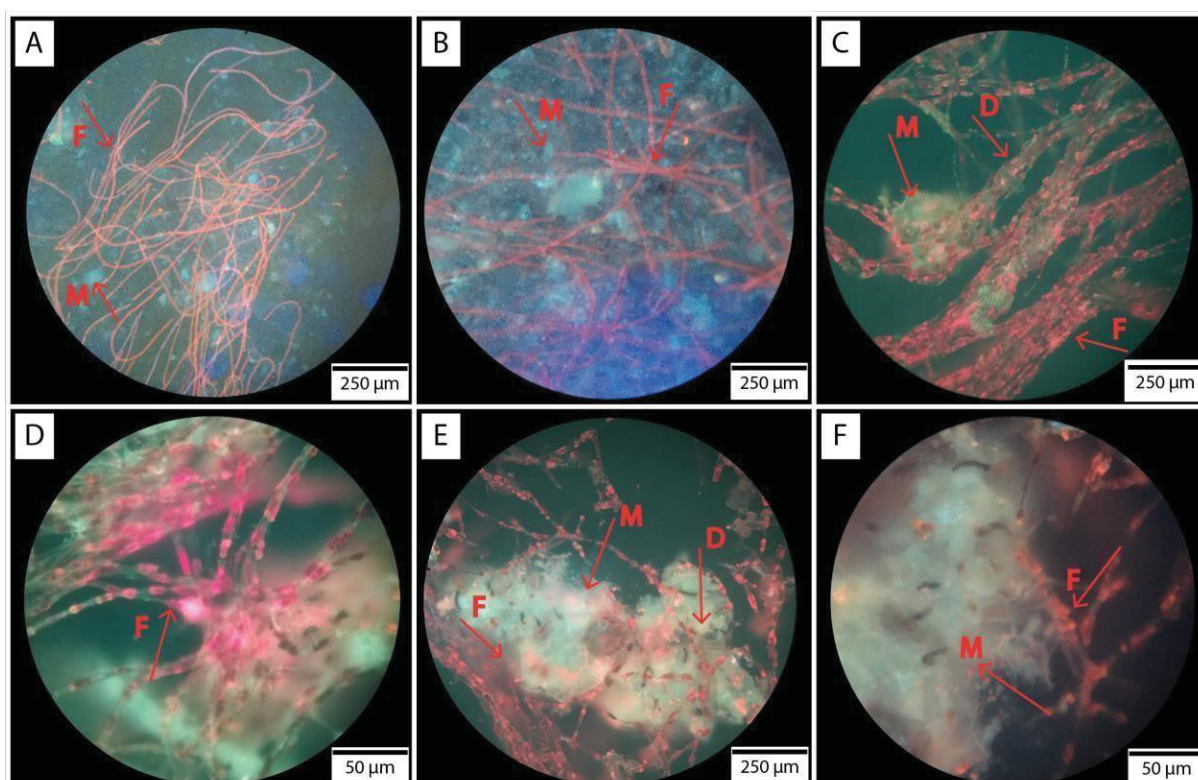


Figure 22. Fluorescence microscopy images of biofilm and microbial mats. A, B) Images correspond to the TIMO 1 sample showing the filamentous (red fluorescence) and mineral sphere (blue fluorescence) in different planes. C, D) Shows filamentous with morphology characteristic of cyanobacteria and red autofluorescence interacting with amorphous crystals and diatoms in TIMO 2 sample. E, F) Images of TIMO 3 sample also show clearly relationship of microorganisms and blue mineral associated to calcite. Filamentous (F), Mineral (M) structures, Diatoms (D).

SEM images of TIMO 1 showed a typical EPS cobweb-like appearance (Figure 23A) with filamentous structures. TIMO 2 sample presented different types of filaments forming ramifications (Figure 23B) and coccoid bacteria (Figure 5C). A great variety of diatoms were identified, most were partially dissolved, had broken valves, and only a few were well preserved (Figure 23D) and interacted with bacteria

and the matrix. TIMO 3 sample (Figure 23E, F) showed the same characteristics determined in TIMO 2 sample.

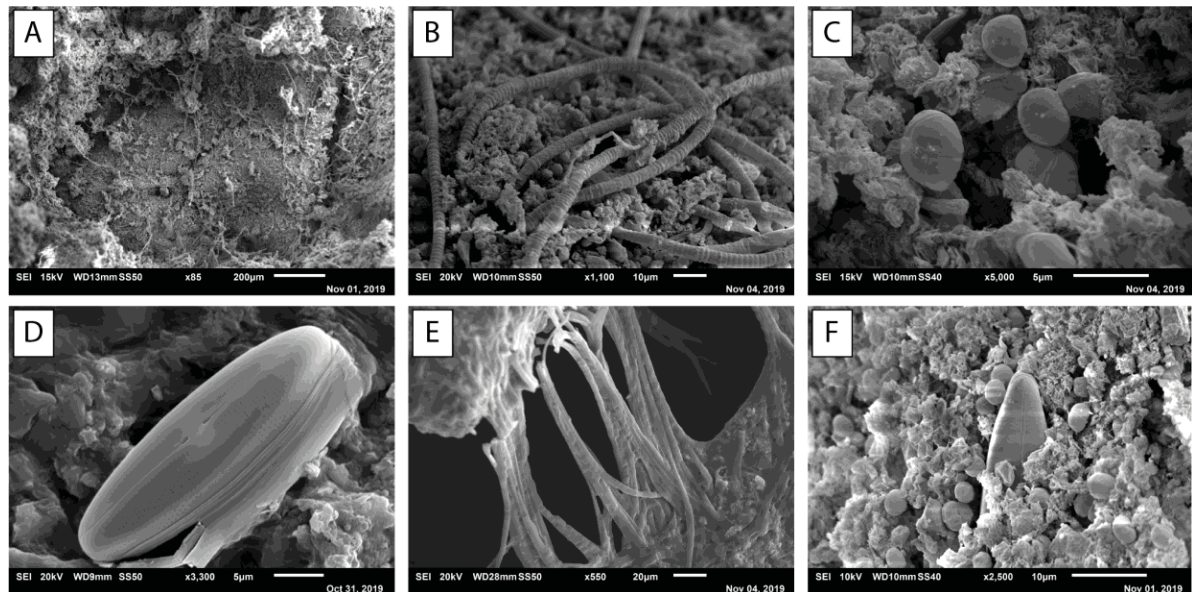


Figure 23. Scanning electron microscope images of the biological samples. (A) EPS with filamentous cyanobacteria and diatom from TIMO 1 sample. (B) Images reveal unicellular cyanobacteria. (C) Coccoid bacteria and (D) Diatoms frustules from TIMO 2 sample. (E) Filamentous interconnected forming ramifications, (F) Diatom with coccoid bacteria from TIMO 3 sample.

4.3 Carbonate deposit

TESCAN-TIMA and XRD analyses revealed a mass percentage of calcite close to 100%, with anorthoclase, quartz, fluoropargasite, ankerite, muscovite, labradorite, and biotite as accessory minerals, probably particles carried by the wind. Mineralogical composition XRD analysis confirmed the results delivered by TESCAN-TIMA and showed that microbialites (LT01, LT02) and crust (LT03) samples were mostly composed of calcite with a slight variation in proportion among the samples. Monohydrocalcite was detected in the LT01 sample, and fluorite trapped as detrital mineral in LT02 and LT03-Botryoidal crust samples. D-spacing varied from 3.03 to 3.04, showing a calcite peak (Table 3) (Henriquez et al., in prep).

4.4 Bacterial and fungal composition

4.5

The 16s metagenomic sequencing analyses revealed the diversity of bacterial

communities present in TIMO 1, TIMO 2, and TIMO 3 samples (Figure 24). TIMO 1 biofilm sample consisted mainly of *Pseudomonas* genera with ~30% relative abundance, followed by *Luteolibacter*, *Amoebophilus*, *Rhodobacter*, *Flavobacterium*, *Brevundimonas*, *Microcystis*, *Chthoniobacter*, *Rhizorhapis*, *Lewinella*, *Chryseolinea*, *Rubrivivax*, *Polymorphobacter*, *Nitrosomonas*, *Phreatobacter*, *Roseomonas*, and *Amaricoccus*. TIMO 2 microbial mat sample was characterized by *Oscillatoria* with ~17% relative abundance, followed by *Nodosilinea*, *Roseinatronobacter*, *Nodularia*, *Belliella*, *Anditalea*, *Aliidiomarina*, *Nitritalea*, *Loktanella*, *Indibacter*, *Porphyrobacter*, *Azoarcus*, *Owenweeksia*, *Aquiflexum*, *Salinarimonas*, *Halomonas*, *Wenzhouxiangella*, *Lunatimonas*, and *Paracoccus*. In the TIMO 3 microbial mat sample, *Belliella* was the most abundant genera with ~22% relative abundance, followed by *Nodularia*, *Loktanella*, *Aquiflexum*, *Wenzhouxiangella*, *Salinarimonas*, *Roseinatronobacter*, *Oscillatoria*, *Indibacter*, *Halodurantibacterium*, *Luteolibacter*, *Azoarcus*, *Porphyrobacter*, and *Marivirga*.

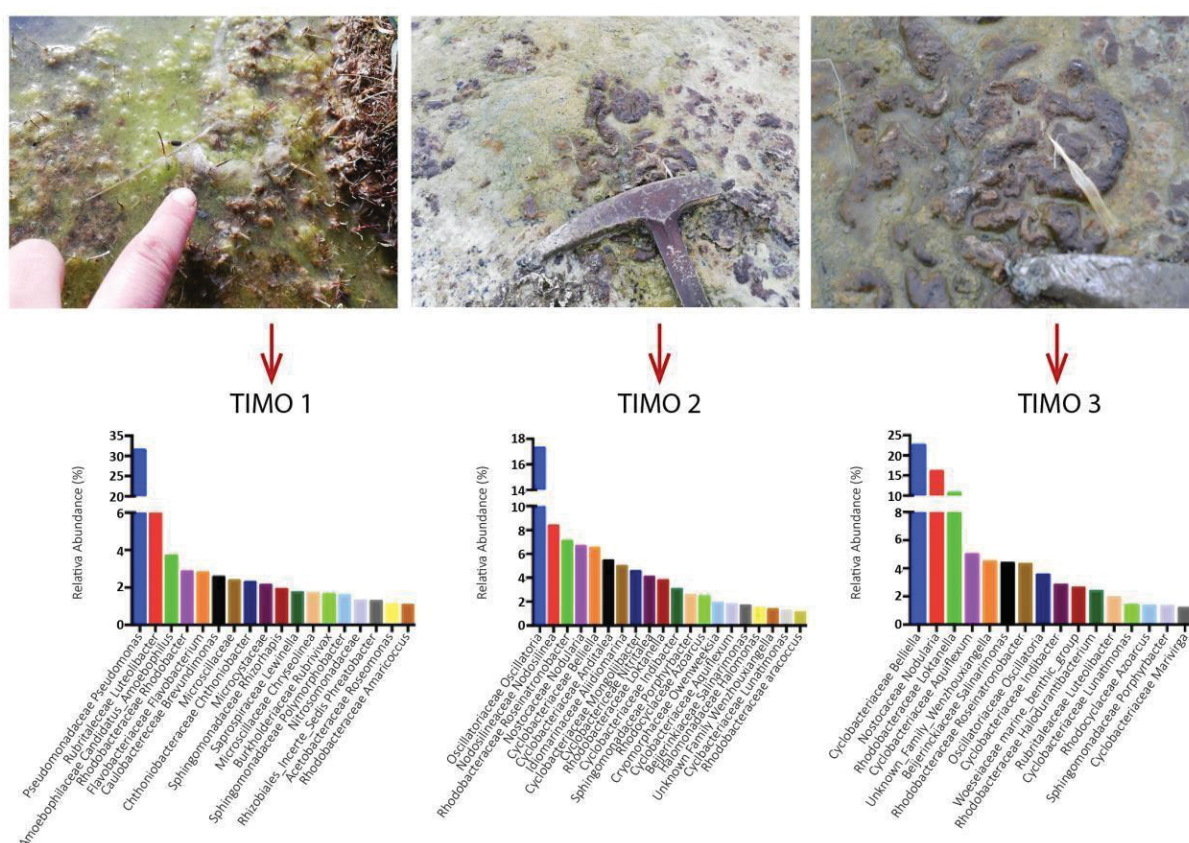


Figure 24. (A) Bacterial genera composition of Biofilm (TIMO 1). (B) Bacterial genera composition of microbial mats (TIMO 2). (C) Bacterial genera composition of microbial mats (TIMO 3).

The dominant phyla in the TIMO 1 sample were Proteobacteria, followed by Verrucomicrobia, Bacteroidetes, Amoebohilus, and Cyanobacteria (Table 4). In the TIMO 2 sample, the most abundant phylum was Cyanobacteria, followed by Proteobacteria and Bacteroidetes (Table 5). In the TIMO 3 sample, the most abundant phylum was Bacteroidetes and Proteobacteria, followed by Cyanobacteria and Verrucomicrobia (Table 6). Within the Proteobacteria phylum, representatives from Alphaproteobacteria, Gammaproteobacteria, and Betaproteobacteria were found. The Gammaproteobacteria sub-phylum was dominant in the TIMO 1 sample, followed by Alphaproteobacteria. Gammaproteobacteria were affiliated with *Pseudomonas*. The most abundant Alphaproteobacteria were *Rhodobacter*, followed by *Brevundimonas*, *Rhizorhapis*, *Polymorphobacter*, *Nitrosomonas*, *Phreatobacter*, *Roseomonas*, and *Amaricoccus*. Betaproteobacteria were represented by *Rubrivivax*. Regarding the TIMO 2 sample, the most abundant sub-phylum was Alphaproteobacteria,

represented by *Roseinatronobacter*, followed by *Loktanella*, *Porphyrobacter*, *Nitrosomonas*, *Salinarimonas*, and *Paracoccus*. Gammaproteobacteria corresponded to *Aliidiomarina*, *Halomonas*, and *Wenzhouxiangella*, while Betaproteobacteria were represented by *Azoarcus*. In the TIMO 3 sample, the most abundant sub-phylum was Alphaproteobacteria associated with *Loktanella*, followed by *Salinarimonas*, *Roseinatronobacter*, *Woeseiaceae*, *Halodurantibacterium*, and *Porphyrobacter*. Betaproteobacteria were represented by *Azoarcus*. Regarding the Bacteroidetes phylum, TIMO 1 sample was represented by *Flavobacterium*, followed by *Microscillaceae* and *Lewinella*; TIMO 2 was represented by *Belliella*, followed by *Anditalea*, *Mongoliibacter*, *Nitritalea*, *Indibacter*, *Owenweeksia*, *Aquiflexum*, and *Lunatimonas*. TIMO 3 sample was represented by *Belliella*, followed by *Aquiflexum*, *Indibacter*, and *Marivirga*. TIMO 1 sample showed a low abundance of cyanobacteria, and its only genus was *Microcystis*, while TIMO 2 was characterized by the predominance of cyanobacteria, with *Oscillatoria* as the most abundant, followed by *Nodosilinea* and *Nodularia*. TIMO 3 showed a high abundance of *Nodularia*, followed by *Oscillatoria* within the cyanobacteria group. The Verrucomicrobia phylum corresponded to *Luteolibacter* and *Chthoniobacter* in the

TIMO 1 sample. This phylum was absent in TIMO 2 and was represented by *Luteolibacter* in TIMO 3.

Table 4. Comparative table of microbial diversity from TIMO 1 and their register in the world

Sample	Name	Source	Reference	Phylum
TIMO 1	<i>Pseudomonas</i>	Has been documented how elaborate calcite and survive in extreme environments in cold saline and alkaline lakes (Norway).	Boquet et al., 1973; Anderson et al., 1992; Hamdan et al., 2011; Meyer et al., 2011; Chu et al., 2012.	Proteobacteria
	<i>Luteolibacter</i>	Has been documented in alkaline soils and cold (0.5 °C) climate (Norway), seawater (China) and in brackish and alkaline water with abundant SO_4^{2-} and Mg^{2+} .	Kim et al., 2015; Fang et al., 2015; Zhang et al., 2017.	Verrucomicrobia
	<i>Rhodobacter</i>	Has been reported inn microbialites of crater lake alkaline (Mexico), with high salinity and a high concentration of sodium, magnesium, chloride and bicarbonate. Also, in polyextreme ecosystem how a bacterial survival under extreme UV radiation (Chile) and in submarine ikaite columns, (Greenland) cold and alkaline place.	Vilaclara et al., 1993; Xiong and Bauer, 2002; Stougaard et al., 2002; Armienta et al., 2008; Couradeau et al., 2011; Glaring et al., 2015; Perez et al., 2018.	Proteobacteria
	<i>Flavobacterium</i>	Has been isolated from seawater and in saline evaporitic basins (northern Chile).	Dorador et al., 2009; Kristyanto et al., 2018.	Bacteroidetes
	<i>Brevundimonas</i>	Has been reported in 'Cave Without A Name' (USA) and the Pamukkale travertines (Turkey). Has been observed by precipitate calcium carbonate minerals.	Okyay et al., 2015.	Proteobacteria

<i>Chthoniobacter</i>	Has been documented in Thrombolites from Lake Sarmiento, Torres del Paine National Park of Chilean Patagonia.	Osman et al., 2020.	Verrucomicrobia
<i>Microcystis</i>	Cocoid cyanobacterium. Has been reported in freshwater lakes, ponds and in marine environments. In alkaline-saline lakes of East Africa and Pantanal wetland (Brazil).	Richardson et al.,1988; Naila et al., 2016; Nonga et al., 2017; Demoulin et al., 2019.	Cyanobacteria
<i>Rhizorhapis</i>	Has been reported in groundwater (Korea) and alkaline lakes at -11 and 12°C (Canada)	Chen et al., 2013; Fournier et al., 2021	Proteobacteria
<i>Lewinella</i>	Has been documented in marine environments (Korea) and in Antarctic, seawater.	Oh et al 2009; Jung, et al 2016.	Bacteroidetes
<i>Polymorphobacter</i>	Has been reported in Ice core drilled Glacier (China).	Xing et al 2017	Proteobacteria
<i>Nitrosomonas</i>	Thermophilic nitrifying bacteria reported in geothermal springs (Russia).	Golovacheva et al.,1976; Lebedeva et al 2005; Nejdat,2005.	Proteobacteria
<i>Phreatobacter</i>	Has been documented in a hot spring in a Tibetan geothermal field	Ming et al., 2020.	Proteobacteria
<i>Roseomonas</i>	Has been isolated from Arctic tundra soil (Norway),10 °C and at pH 9.0.	Kim et al., 2016.	Proteobacteria
<i>Amaricoccus</i>	Has been documented in activated sludge system.	Falvo et al., 2001.	Proteobacteria

Table 5. Comparative table of microbial diversity from TIMO 2 and their register in the world.

Sample	Name	Source	Reference	Phylum
TIMO 2	<i>Oscillatoria</i>	Has been reported in microbialite in alkaline Pyramid Lake (USA), in hypersaline microbial mat on Antarctica (USA), in Salar de Llamará, Atacama Desert (Chile), in stromatolites of saline-alkaline crater-lake (Indian Ocean), in microbialites how responsible for calcium carbonate precipitation. in submarine ikaite columns, (Greenland) cold and alkaline place.	Monty et al., 1976; Freytet et al., 1996; Freytet et al., 1998; Arp et al 1999; Nadeau et al 1999; Stougaard et al., 2002; Demergasso et al., 2003; Vasconcelos et al., 2006; Dupraz et al., 2009; Cellamare et al., 2018.	Cyanobacteria
	<i>Nodosilinea</i>	Has been documented in saline-alkaline lakes of extreme environments in Nhecolândia, Brazil and in soil Signy Island (Antarctica).	Andreote et al., 2014; Radzi et al., 2019.	Cyanobacteria
	<i>Roseinatronobacter</i>	Has been reported in alkaliphilic conditions, hypersaline soda Mono Lake, California (USA).	Boldareva et al., 2007.	Proteobacteria
	<i>Nodularia</i>	Has been reported in salt water with nitrogen-fixing abilities, in modern microbialites of alkaline crater Lake Alchichica (Mexico).	Lyra et al., 2005; Koskeniemi et al., 2007; Gerard et al., 2013.	Cyanobacteria

<i>Belliella</i>	Has been documented in alkaline, saline and thermal habitats, in mud-volcano with a saline and haloalkaline lake (Taiwan), in sediment of alkaline Lake Elmenteita (Kenya), in surface water of saline Lake Tuosu (China), in alkaline and brackish Lake Solenoe (Russia).	Arun et al., 2009; Zhang et al., 2015; Kozyreva et al., 2016.	Bacteroidetes
<i>Anditalea</i>	Has been reported in extreme alkali-saline soil (China).	Shi et al., 2012.	Bacteroidetes
<i>Aliidiomarina</i>	Has been reported in Saline-alkali farmland top soil (China).	Xu et al., 2016.	Proteobacteria
<i>Mongoliibacter</i>	Haloalkalitolerant bacterium. Has been documented in haloalkaline lake (China).	Wang et al., 2016.	Bacteroidetes
<i>Nitritalea</i>	Has been documented in saline and alkaline Lonar Lake (India).	Antony et al., 2013.	Bacteroidetes
<i>Loktanella</i>	Has been documented in seawater and microbial mats in the Dry Valley (Antarctica).	Van Trappen et al., 2004; Yoon et al., 2013.	Proteobacteria
<i>Indibacter</i>	Has been isolated from a haloalkaline Lonar lake (India).	Kumar et al., 2010.	Bacteroidetes
<i>Porphyrobacter</i>	Has been reported in sea water (Korea), in stromatolites at Shark Bay (Australia).	Yoon et al 2004; Goh et al., 2009.	Proteobacteria
<i>Azoarcus</i>	Has been documented in geothermal spring microbialites, Romania.	Coman et al., 2015.	Proteobacteria
<i>Nitrosomonas</i>	Thermophilic nitrifying bacteria reported in geothermal springs (Russia).	Golovacheva et al., 1976; Lebedeva et al., 2005; Nejdat et al., 2005.	Proteobacteria
<i>Owenweeksia</i>	Has been documented in sea-water in Hong Kong (China).	Lau et al., 2005.	Bacteroidetes
<i>Aquiflexum</i>	Has been reported in haloalkaline lake (China).	Wang et al., 2016.	Bacteroidetes
<i>Salinarimonas</i>	Has been documented in photosynthetic mat in salt mine in Yunna (China), in active microbialites obtained in Lagoa Vermelha (Brazil), in microbial mat of Hot Lake (USA).	Liu et al., 2010; Lindemann et al., 2013; Popall et al., 2020.	Proteobacteria
<i>Holomonas</i>	Has been reported in deep-sea hydrothermal-vent environments, in saline water (Korea), in Salt Lake on the Qinghai-Tibet Plateau and in a subterranean (China).	Kaye, 2004; Kim et al., 2007; Xu et al., 2007.	Proteobacteria
<i>Wenzhouxiangella</i>	Halophilic bacterium. Has been reported in Salt Lake in Xinjiang (China).	Han et al., 2019	Proteobacteria
<i>Lunatimonas</i>	Has documented in Lonar Lake (India).	Srinivas et al., 2014.	Bacteroidetes
<i>Paracoccus</i>	Has been reported in stromatolites at Shark Bay (Australia).	Goh et al., 2009.	Proteobacteria

Table 6. Comparative table of microbial diversity from TIMO 3 and their register in the world.

Sample	Name	Source	Reference	Phylum
TIMO 3	<i>Belliella</i>	Has been documented in alkaline, saline and thermal habitats, in mud-volcano with a saline and haloalkaline lake (Taiwan), in sediment of alkaline Lake Elmenteita (Kenya), in surface water of saline Lake Tuosu (China), in alkaline and brackish Lake Solenoe (Russia).	Arun et al., 2009; Zhong et al., 2015; Kozyreva et al., 2016.	Bacteroidetes
	<i>Nodularia</i>	Has been reported in salt water in modern microbialites of alkaline crater Lake Alchichica (Mexico).	Lyra et al., 2005; Koskenniemi et al., 2007; Gerard et al., 2014.	Cyanobacteria
	<i>Loktanella</i>	Has been documented in seawater and microbial mats in the Dry Valley (Antarctica).	Van Trappen et al., 2004; Yoon et al., 2013.	Proteobacteria
	<i>Aquiflexum</i>	Has been reported in haloalkaline lake (China).	Wang et al., 2016.	Bacteroidetes
	<i>Wenzhouxiangella</i>	Has been reported In Salt Lake in Xinjiang (China).	Han et al., 2019	Proteobacteria
	<i>Salinarimonas</i>	Has been documented in photosynthetic mat in salt mine in Yunna (China), in active microbialites obtained in Lagoa Vermelha (Brazil), in microbial mat of Hot Lake (USA).	Liu et al., 2010; Lindemann et al., 2013; Popall et al., 2020.	Proteobacteria
	<i>Roseinatronobacter</i>	Has been reported in alkaliphilic conditions, hypersaline soda Mono Lake, California (USA).	Boldareva et al., 2007	Proteobacteria
	<i>Oscillatoria</i>	Has been reported in microbialite in alkaline Pyramid Lake (USA), in hypersaline microbial mat on Antarctica (USA), in Salar de Llamará, Atacama Desert (Chile), in stromatolites of saline-alkaline crater-lake (Indian Ocean), in microbialites how responsible for calcium carbonate precipitation. in submarine ikaite columns, (Greenland) cold and alkaline place.	Monty et al., 1976; Freytet et al., 1996; Freytet et al., 1998; Arp et al 1999; Nadeau et al 1999; Stougaard et al., 2002; Demergasso et al., 2003; Vasconcelos et al., 2006; Dupraz et al., 2009; Cellamare et al., 2018.	Cyanobacteria
	<i>Indibacter</i>	Has been isolated from a haloalkaline Lonar lake (India).	Kumar et al., 2010.	Bacteroidetes
	<i>Woeseiaceae</i>	Has been documented in marine sediments how sulfide-oxidizers.	Mußmann et al., 2017.	Proteobacteria
	<i>Halodurantibacterium</i>	Has been reported in oil production mixture from the Daqing Oilfield (China).	Lv et al., 2015.	Proteobacteria
	<i>Luteolibacter</i>	Has been documented in alkaline soils and cold (0.5 °C) climate (Norway), seawater (China) and in brackish and alkaline water with abundant SO ₄ ²⁻ and Mg ²⁺ .	Kim et al., 2015; Fang et al., 2015; Zhang et al., 2017.	Verrucomicrobia
	<i>Azoarcus</i>	Has been documented in geothermal spring microbialites, Romania.	Coman et al., 2015.	Proteobacteria
	<i>Porphyrobacter</i>	Has been reported in sea water (Korea), in stromatolites at Shark Bay (Australia).	Yoon et al 2004; Goh et al., 2009.	Proteobacteria
	<i>Marivirga</i>	Has been reported in Mud volcano located in the Andaman Islands (India) and in surface seawater of the Atlantic Ocean.	Srinivas et al., 2011; Lin et al., 2015.	Bacteroidetes

Fungi were identified by ITS sequence analysis. Samples from Laguna Timone indicated the presence of fungi from the Leotiomyces and Sordariomycetes classes (Figure 25). The genera *Ilyonectria* and *Tetracladium* were identified in the TIMO 1 sample. In TIMO 2, the genus *Thelebolus* was identified, while *Acrostalagmus* and *Plectosphaerella* were detected in TIMO 3 (Figure 26).

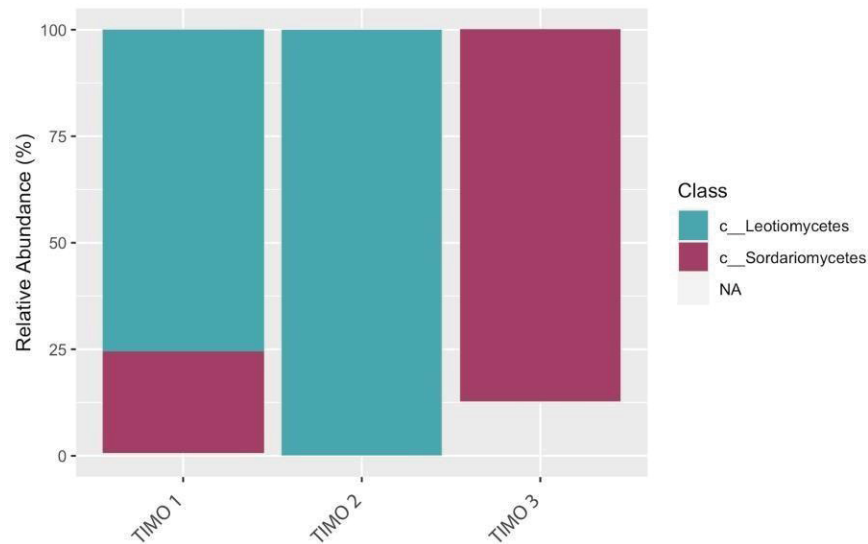


Figure 25. Relative abundance of fungal classes in TIMO 1, TIMO 2, and TIMO 3 samples.

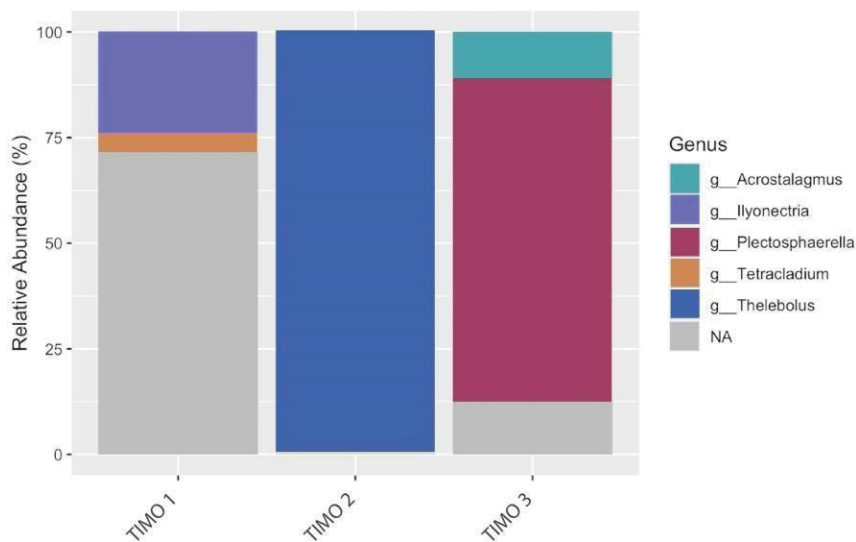


Figure 26. Relative abundance of fungal genera in TIMO 1, TIMO 2, and TIMO 3 samples

Alpha diversity was analyzed using the observed operational taxonomic units (OTUs) to find diversity indices and richness estimators of the bacterial community in TIMO 1, TIMO 2, and TIMO 3 samples. Chao1, Shannon, and Simpson indices were calculated (Figure 27). Alpha diversity showed the number of species observed, the

abundance of unique or rare individuals (Chao1), the richness and abundance (Shannon), and the dominance (Simpson). All samples showed low alpha diversity, in agreement with estimated species richness values, which exhibited lower Chao1 indices. TIMO 2 presented higher values compared to TIMO 1 and TIMO 3. Alpha diversity of TIMO 1, TIMO 2, and TIMO 3 samples was low, with Shannon indices ranging from 1.82 to 1.02, compared to true diversity, whose values vary between 2 and 3 in natural environments (Shannon, 1948). The Simpson index indicated high dominance in TIMO 1 and TIMO 3 (Simpson close to 1) and low dominance in TIMO 2 (Simpson close to 0) (Simpson, 1949).

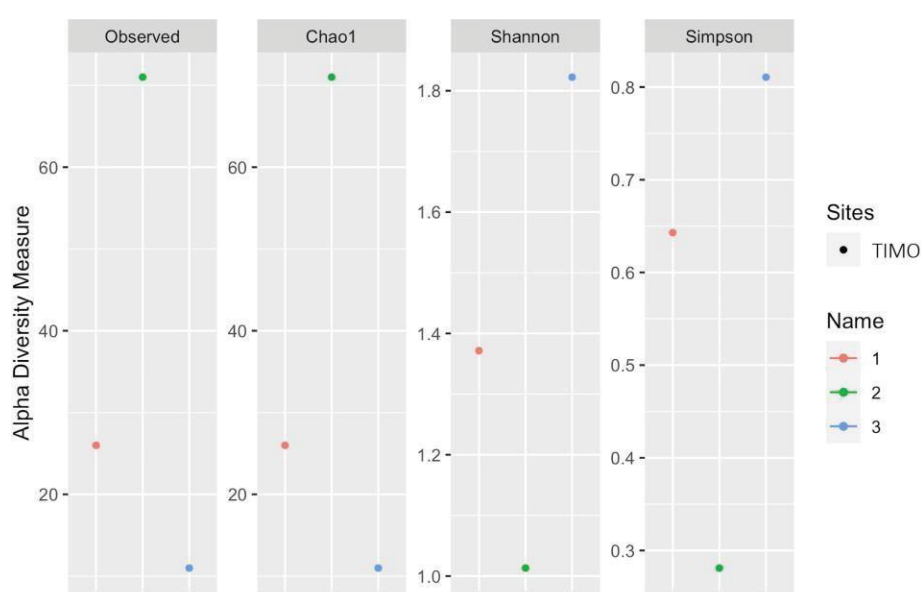


Figure 27. Alpha diversity indices of TIMO 1, TIMO 2, and TIMO 3 samples.

4.6 Microbially induced calcite precipitation

Bacteria from TIMO 1, TIMO 2, and TIMO 3 samples were isolated in R2A media. Next, the capacity to precipitate calcite of all isolates *in vitro* was determined (see methods). Isolated colonies were evaluated every 48 h with an optical microscope, and mineral precipitation was observed only after 15 days of inoculation, with very small crystals and oval shapes, which are difficult to distinguish macroscopically. Bacteria isolated from TIMO 2 (Figures 28A, B) and TIMO 3 (Figures 28C, D) samples could form crystalline CaCO_3 in R2A agar medium plates amended with calcium chloride after incubation at 28°C (Figure 28). However, in the TIMO 1 sample,

no crystal precipitation was observed. This sample had slightly fewer isolates, which may have influenced the results. The CaCO_3 precipitation occurred within the bacterial colony and not directly in the culture medium. In samples inoculated at 11°C , no colony growth occurred. SEM observations revealed the morphological characteristics of the mineral precipitated and the involvement of bacteria. Crystal shapes associated with irregular spheres were produced by isolates from TIMO 2 (Figure 28E). Irregular aggregates and grains with botryoidal habit were identified; nevertheless, most crystals had rough surfaces. SEM images showed bacterial cells related to CaCO_3 crystals from TIMO 3 (Figure 28F). EDS analyses showed Ca, C, O peaks; therefore, these grains have a carbon carbonate composition that can be associated with CaCO_3 bioprecipitation from bacteria. XRD was carried out to identify the crystal precipitated by bacteria, and the results revealed that calcite was a carbonate present in both samples.

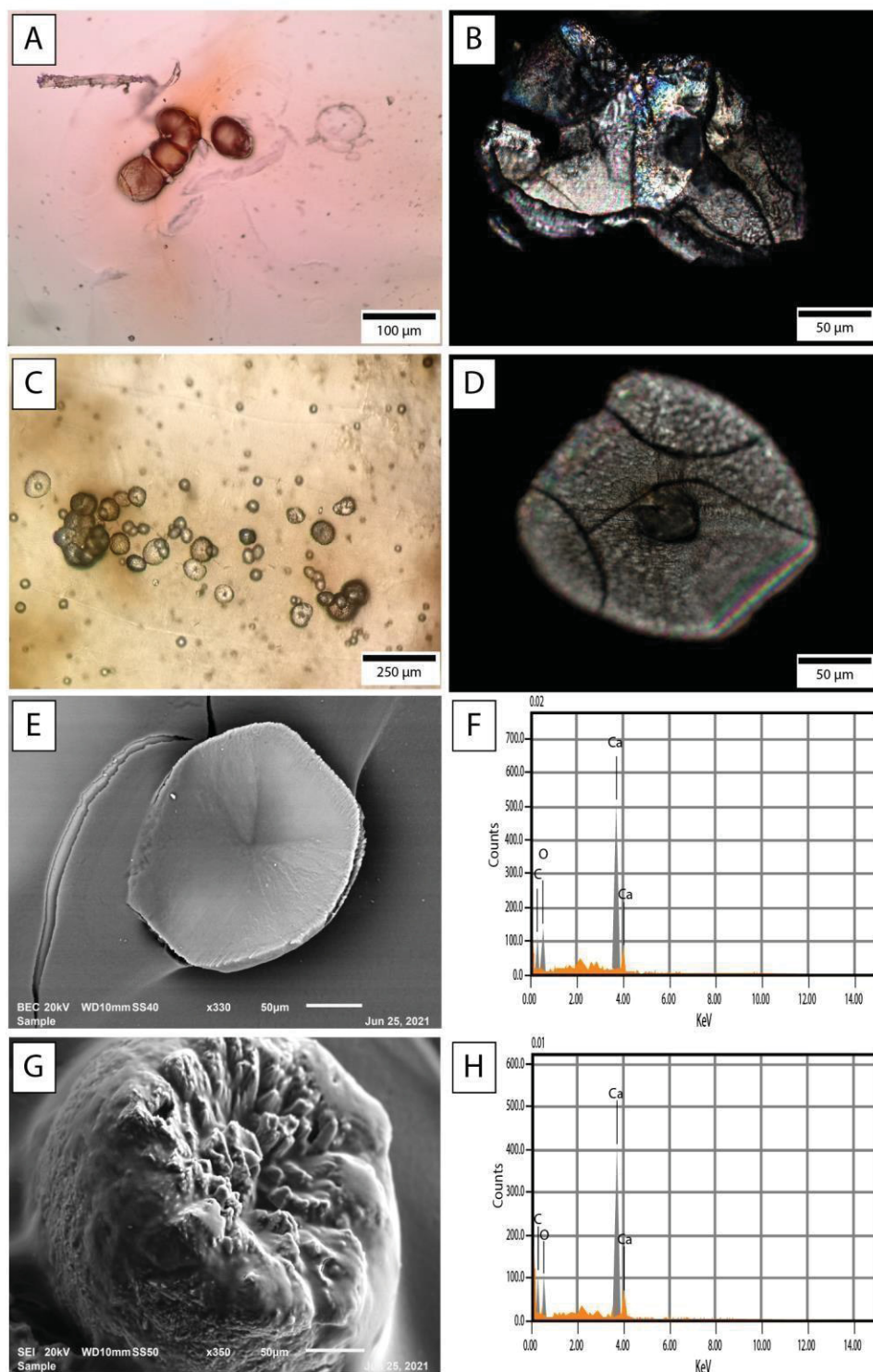


Figure 28. Digital image from an optical microscope CCD camera – TCC-5.0ICE showing oval CaCO_3 crystals precipitated from bacteria after 15 days of growth at 28°C . A, B) Carbonate crystals precipitated in the culture medium from the TIMO 1 sample. C, D) Carbonate crystals precipitated in the culture medium from the TIMO 2 sample, indicating characteristics of calcite. SEM images and

EDS analysis exhibit the crystal precipitated. E) Oval-shaped crystal from TIMO 2, and EDS spectra showing Ca, C, O peaks associated with calcite composition. F) Crystalline crystal aggregates and biofilm from TIMO 3, and EDS spectra showing Ca, C, O peaks associated with calcite composition.

5. Discussion

Bacterial communities from Laguna Timone were related to other sequences reported in different saline lakes, as well as dry and cold areas of the world. Aiming to summarize and better understand the available information in the literature, we built a database including all this information (Tables 3 and 4). At the genus level, the most abundant genera in TIMO 1 were *Pseudomonas* and *Luteolibacter*. *Pseudomonas* has been broadly described in extreme environments and has also been associated with the production of calcite (Anderson et al., 1992; Boquet et al., 1973; Hamdan et al., 2011; Meyer et al., 2011; Chu et al., 2012). *Pseudomonas* can develop under critical environmental conditions associated with alkaline and saline lakes at cold temperatures, such as in Norway. *Luteolibacter* has been documented in alkaline soils and cold (0.5°C) environments like Norway. It has also been detected in seawater in China and in brackish and alkaline water with high concentrations of SO_4^{2-} and Mg^{2+} (Jiang et al., 2012; Kim et al., 2015; Zhang et al., 2017). Bacterial communities of *Luteolibacter* have great ecological importance in soils due to their high relative abundance (Bergmann et al., 2011). In addition, members of this phylum have been reported in marine (Cardman et al., 2014) and hypersaline mat ecosystems (Spring et al., 2015). These characteristics are similar to those of Laguna Timone. These organisms are also capable of developing in environments with high concentrations of SO_4^{2-} and Mg^{2+} (elements that are part of water chemistry) in cold, saline, and alkaline conditions.

Rhodobacter has been documented as sulfur-oxidizing photosynthesizers and nitrogen fixers involved in thrombolite formation and accretion (Xiong and Bauer, 2002; Myshrall et al., 2010; Mobberley et al., 2013; Gleeson et al., 2016; Louyakis et al., 2017). This genus has been reported in microbialites of an alkaline crater lake in Mexico, under high salinity and high concentrations of sodium, magnesium, chloride,

and bicarbonate. Members of this genus are present in polyextreme ecosystems, surviving under extreme UV radiation (Chile), and in submarine ikaite columns, characterized by cold and alkaline conditions (Greenland) (Vilaclara et al., 1993; Xiong and Bauer, 2002; Stougaard et al., 2002; Armienta et al., 2008; Couradeau et al., 2011; Glaring et al., 2015; Perez et al., 2018). *Flavobacterium* has been isolated from seawater and saline evaporitic basins in northern Chile (Dorador et al., 2009; Kristyanto et al., 2018).

Brevundimonas has been reported in the Cave Without A Name (USA) and the Pamukkale travertines

(Turkey), showing an association with the precipitation of calcium carbonate minerals (Okyay et al., 2015). *Chthoniobacter* has been documented in thrombolites from Lake Sarmiento, located at the Torres del Paine National Park in the Chilean Patagonia (Osman et al., 2020). *Microcystis* (coccoid cyanobacteria) has been reported in freshwater lakes and marine environments, as well as alkalinesaline lakes of East Africa and the Pantanal wetland, Brazil (Naíla et al., 2016; Nonga et al., 2017; Demoulin et al., 2019). Mats of the *Microcystis* cyanobacterium have been associated with manganese oxidation in surface waters (Richardson et al., 1988). *Rhizorhapis* has been reported in Korean groundwater and Canadian alkaline lakes between -11°C and 12°C (Chen et al., 2013; Fournier et al., 2021). *Lewinella* has also been documented in Korean marine environments (Jung et al., 2016) and Antarctic seawater (Oh et al., 2009). *Polymorphobacter* has been identified in an ice core drilled glacier, China (Xing et al., 2017). *Nitrosomonas* has been reported as thermophilic nitrifying bacteria in geothermal springs in Russia (Golovacheva et al., 1976; Lebedeva et al., 2005; Nejidat, 2005). *Phreatobacter* has been documented in a hot spring from a Tibetan geothermal field (Ming et al., 2020). *Roseomonas* has been isolated from the Arctic tundra (at 10°C and pH 9.0) in Norway (Kim et al., 2016). *Amaricoccus* has been detected in an activated sludge system (Falvo et al., 2001).

At the genus level, the most abundant genera in TIMO 2 were *Oscillatoria* and *Nodosilinea*. *Oscillatoria* has been described in microbialites as responsible for calcium carbonate precipitation. In this context, *Oscillatoria* has been reported in microbialites in the alkaline Pyramid Lake, USA, in hypersaline microbial mats in

Antarctica, in Salar de Lllamará, Atacama Desert, Chile, in stromatolites from a saline-alkaline crater lake in the Indian Ocean, and in submarine ikaite columns (Greenland) (Monty et al., 1976; Freytet et al., 1996; Freytet et al., 1998; Arp et al., 1999; Nadeau et al., 1999; Stougaard et al., 2002; Demergasso et al., 2003; Vasconcelos et al., 2006; Dupraz et al., 2009; Cellamare et al., 2018). *Nodosilinea* has been documented in saline-alkaline lakes in extreme environments in Nhecolândia, Brazil, and in soil from Signy Island, Antarctica (Andreote et al., 2014; Radzi et al., 2019).

Roseinatronobacter is characterized by surviving in alkaliphilic conditions, such as the hypersaline soda Mono Lake, California, USA (Boldareva et al., 2007). *Nodularia* has been reported in saltwater with nitrogen-fixing abilities and in modern microbialites of the alkaline crater Lake Alchichica, Mexico (Lyra et al., 2005; Koskenniemi et al., 2007; Gerard et al., 2013). *Belliella* has been documented in alkaline, saline, and thermal habitats, in mud volcanoes with a saline and haloalkaline lake (Taiwan), in sediments of the alkaline Lake Elmenteita (Kenya), in surface water of the saline Lake Tuosu (China), and in the alkaline and brackish Lake Solenoe (Russia) (Arun et al., 2009; Zhong et al., 2015; Kozyreva et al., 2016). *Anditalea* has been identified in extreme saline-alkali soil, China (Shi et al., 2012). *Aliidiomarina* has been reported in saline-alkali farmland topsoil, China (Xu et al., 2017). *Mongoliibacter* has been detected in a haloalkaline lake, China (Wang et al., 2016). *Nitritalea* and *Indibacter* have been documented in saline and alkaline lakes, such as the Lonar Lake (India) (Kumar et al., 2010; Antony et al., 2013). *Loktanella* has been described in seawater and microbial mats in the Dry Valley (Antarctica) (Van Trappen et al., 2004; Yoon et al., 2013). *Porphyrobacter* has been reported in seawater (Korea) and stromatolites at Shark Bay, Australia (Yoon et al., 2004; Goh et al., 2009). *Azoarcus* has been documented in geothermal spring microbialites in Romania (Coman et al., 2015). *Nitrosomonas* was also identified in TIMO 2. *Owenweeksia* has been detected in seawater in Hong Kong, China (Lau et al., 2005). *Aquiflexum* has been reported in a haloalkaline lake in China. *Salinarimonas* has been described in photosynthetic mats in a salt mine in Yunnan, China, in active microbialites obtained from Lagoa Vermelha, Brazil, and in microbial mats from Hot Lake, USA (Liu et al., 2010; Popall et al., 2020; Lindemann et al., 2013). *Halomonas* has been found in deep-sea

hydrothermal-vent environments, saline water (Korea), Salt Lake in the Qinghai–Tibet Plateau (Kaye, 2004; Kim et al., 2007; Xu et al., 2007). *Wenzhouxiangella* has been reported in Salt Lake in Xinjiang, China (Han et al., 2019). *Lunatimonas* has shown nitrate-reducing activity documented in Lonar Lake, India (Srinivas et al., 2014). *Paracoccus* has been detected in stromatolites at Shark Bay, Australia (Goh et al., 2009).

The genus groups mentioned above for TIMO 2 are also present in TIMO 3; however, under different concentrations. In TIMO 3, the predominant genera were *Belliella* and *Nodularia* of the Cyanobacteria phylum, followed by *Loktanella*, *Aquiflexum*, *Wenzhouxiangella*, *Salinarimonas*, *Roseinatronobacter*, *Oscillatoria*, *Indibacter*, *Azoarcus*, and *Porphyrobacter*. In addition, it presented *Woeseiaceae*, which has been documented in marine sediments (Mussmann et al., 2017), as well as *Halodurantibacterium*, reported in an oil production mixture from the Daqing Oilfield (China) (Lv et al., 2015). All microorganisms of samples in Laguna Timone are reported as resistant to critical conditions, particularly saline and alkaline environments.

The bacterial phyla Proteobacteria, Cyanobacteria, Bacteroidetes, and Verrucomicrobia found in Laguna Timone have also been reported in modern microbialites and hypersaline lakes (Ley et al., 2006; Gleeson et al., 2016). TIMO 2 and TIMO 3 samples are dominated by cyanobacteria, which have been reported in microbial mats of cold, saline, and alkaline environment as responsible for carbonate precipitation (Monty et al., 1976; Arpet et al., 1999; Vasconcelos et al., 2006; Gerard et al., 2013; Cellamare et al., 2018). In addition, the Cyanobacteria phylum has great importance for the precipitation of organominerals such as calcite (Vasconcelos et al., 2006; Dupraz et al., 2009; Bontognali et al., 2014; Burne et al., 2014). Besides, carbonate precipitation is abundant in hypersaline lakes and can be associated with microbial mats (Glunk et al., 2011), whose increased concentrations of CO₃ are generated by photosynthetic bacteria (Ludwig et al., 2005).

Also, several studies suggest that alkaliphilic and halophilic species may play a role in carbonate precipitation (López-García et al., 2005). Among the genera found in the samples, *Salinarimonas* corresponded to halotolerant bacteria (Liu et al., 2010), while *Aliidiomarina* represented haloalkalitolerant bacteria (Xu et al., 2017). Some halotolerant types of *Flavobacterium* have been described (Kim et al., 2018).

Anditalea, *Mongoliibacter*, *Aquiflexum*, and *Lunatimonas* are alkaliphilic halotolerant bacteria (Liu et al., 2010; Shi et al., 2012; Srinivas et al., 2014; Wang et al., 2016). *Nitritalea* and *Indibacter* correspond to alkaliphilic bacteria (Kumar et al., 2010; Antony et al., 2013), which could be related to carbonate precipitation, together with photosynthetic bacteria in Laguna Timone. With respect to the metabolic capacities found in bacterial communities, the results obtained revealed the presence of microorganisms that participate in the nitrogen cycle, particularly for nitrate reducing microbial groups, such as *Lunatimonas*. The denitrification process increases the pH in the environment by consuming H^+ and produces CO_2 , which favors carbonate precipitation (Erşan et al., 2015b).

Regarding the fungal communities detected in Laguna Timone samples, the genus *Thelebolus* corresponded to psychrotolerant and halotolerant fungi that have been isolated from the Ross Sea and the Dry Valleys in Antarctica (Arenz et al., 2006; Connel et al., 2006). In addition, it has been reported in Antarctic lagoons associated with microbial mats; these conditions present environmental similarities (De Hoog et al., 2007) to those found in Laguna Timone. This finding could explain its presence in subantarctic environments exposed to temperatures below the freezing point of water for long periods. Furthermore, it has been isolated from soil samples from cold regions of Europe (Svalbard) and Asia (Himalayan mountains) (Anupama et al., 2011; Singh et al., 2016). Fungi of the *Acrostalagmus* genus have been described in saline and alkaline sites (Rojas et al., 2008; Grum Grzhimaylo et al., 2016). This information is very interesting because the water in Laguna Timone has high alkalinity (pH 9.7) and salinity (Table 1). Moreover, some reports indicate the presence of fungi of the *Acrostalagmus* genus in calcareous caves with alkaline pH (Nagai et al., 1998), which could suggest the participation of fungi in calcite bioprecipitation processes in the samples from Laguna Timone since the generation of calcium carbonate is favored at alkaline pH. The *Tetracladium* genus corresponds to psychrotolerant aquatic hyphomycetes of cosmopolitan distribution that have been isolated from tropical to cold areas such as the Himalayas or Antarctica (Tsuji et al., 2013; Zhang et al., 2014). These fungi contribute to the decomposition of leaves in streams, which could explain their participation in the decomposition of organic matter in Laguna Timone, thus helping to obtain nutrients for microbial communities

(Chauvet et al., 1988; Chergui et al., 1991; Weyers et al., 1996). Similarly, fungi of the *Plectosphaerella* and *Ilyonectria* genera are also associated with plants (plant pathogens) and can participate in the degradation of organic matter, especially leaves. Fungi of the *Plectosphaerella* genus have been isolated from various environments around the world, including soil, water, and plants (Velmurugan et al., 2011; Raimondo et al., 2018; Zhang et al., 2019), while fungi of the *Ilyonectria* genus have been identified and/or isolated from root and plant samples obtained from temperate and cold environments (Petit et al., 2005; Abreo et al., 2010; Cabral et al., 2012; Hirose et al., 2013).

Concerning alpha diversity analyses, comparison among the samples showed no significant differences in Chao 1, Simpson, and Shannon indices. The values reveal that all samples studied consisted of low diversity microbial communities. Nevertheless, the finding indicates the existence of microorganisms capable of surviving in high salinity and alkalinity, low temperatures, arid conditions, and strong winds. Results show a high degree of specialization due to the presence of very few predominant fungi and bacteria species according to the Shannon and Simpson indices, respectively, suggesting that these microbial communities are highly specialized and resistant to extreme conditions; therefore, environmental conditions reduce the possibility of species to survive and encourage the development of only some specialized microorganism.

Microbe–mineral relationships and carbonate precipitation experiment

Most carbonate precipitation occurs inorganically (Pentecost, 2003); however, microbial metabolism is a factor that also promotes carbonate precipitation (Dupraz et al., 2009). In Laguna Timone, the presence of precipitated CaCO_3 suggests a relationship with the microbial community. In the same way, water alkalinity, Ca^{+2} concentration, and evaporation related to weather conditions could all lead to CaCO_3 precipitation. The results obtained from Laguna Timone show that the constant evaporation of water saturated with CaCO_3 produces carbonate deposits, while the organoprecipitation of carbonates occurs associated with a complex combination of

physicochemical and biological parameters. Calcite precipitation from the microbial mat is biologically induced by microbial metabolism. Many studies have postulated that a specific microbial metabolism or the presence of microbes or/and their EPS can lead to the formation of calcite (Dupraz et al., 2009). The experiment of crystal precipitation from microbiologically-induced calcium carbonate precipitation supports this hypothesis. Organomineralization can result in CaCO_3 deposits in this type of environment. The CaCO_3 precipitation occurred within the bacterial colony and not directly in the culture medium. Therefore, the presence of crystallization nuclei is not sufficient for CaCO_3 precipitation, requiring the help of microbial metabolism. At 28°C , bacterial growth is faster; consequently, the probability of crystal precipitation is higher than at low temperatures. In general, bacteria growth and crystal precipitation were scarce. This scenario could be due to three main factors: (I) short time to form crystals; (II) slow kinetics in the growth process; (III) number of isolated colonies per plate. In addition, XRD and SEM (EDS) analysis from

precipitated crystals shows calcite mineralogy. This result coincides with the mineralogy present in carbonate rocks of Laguna Timone. The crystals appear as irregular aggregates and grains with botryoidal habit, similar to how they occur in the natural system. This finding reinforces the idea that systems such as Laguna Timone offer a natural and experimental laboratory to study the interactions that give rise to mineral precipitation.

Most microbial genera present in the samples are exposed to similar extreme living conditions. However, some have been found at higher temperatures. The chemical analyses of water samples were similar to those of hypersaline lake ecosystems in terms of salinity and mineral composition, indicating climatological and geological-tectonic influences. The hypersaline environment presents unique conditions to the life of microorganisms (Potts, 1994); high salinity results in a low taxonomic diversity because living under this condition is energetically costly (Jiang et al., 2007), requiring a balanced mix of nutrients. If an essential nutrient is absent, it affects the growth of microbial communities (Prescott et al., 2002; Lozupone and Knight, 2007). Water chemical elements provide the necessary nutrients for

biosynthesis and energy production, which are necessary for microbial growth (Druschel and Kappler, 2015); ions present in ecosystems, such as Ca^+ , Mg^{2+} , K^+ , Na^+ , SO_4^{2-} , CO_3^- , Cl^- , and NO_3^- , participate in biological cycles. The biological and physicochemical characteristics of the saline-alkaline water in Laguna Timone offer the conditions for carbonate mineral precipitation using the elements available in the system, such as Ca. Several studies have shown that microbial carbonate precipitation has a relationship with weathering of silicate minerals, providing a source of atmospheric carbonate dioxide in terrestrial environments (Schwartzman and Volk, 1989; Berner, 1990; Ferris et al., 1994). The structure of bacterial and fungal communities correlates with the chemical composition of water, determined by weathering rocks. In cold environments, rocks could produce reservoirs of microorganisms, protecting and supplying nutrients from environmental stresses; for example, elements released during this mineral dissolution, such as Si, P, and K, stimulate biological productivity (Kelemen et al., 2020). Hence, the importance of the geological environment of Laguna Timone. The weathering of silicates from basaltic rocks will provide the necessary elements to contribute to the chemical reactions essential for carbonate precipitation coupled to microorganisms. The following reaction: $(\text{Ca})\text{SiO}_3 + 2\text{H}_2\text{CO}_3 + \text{H}_2\text{O} \rightarrow (\text{Ca})\text{CO}_3 + \text{CH}_2\text{O}$ (organic matter) + $\text{H}_4\text{SiO}_4 + \text{O}_2$ shows the combination of expressions for the chemical weathering of silicate minerals, the process of photosynthesis, and, consequently, the carbonate precipitation (Ferris et al., 1994). The products of this reaction, such as silicic acid, could eventually be used by siliceous organisms like diatoms or other mineral phase formations, like clay. Calcium and bicarbonate ions could result in calcite precipitation in the system.

6. Conclusion

The results show an outstanding model system for studying microbe-mineral interactions under unique environmental conditions. This idea is supported by the presence of precipitated CaCO_3 associated with microbial activity in the study area and under laboratory conditions. The findings indicate a combination of

physicochemical and biological participation in carbonate mineral precipitation related to the geological context, which supplies a diversity of nutrients and protection to the microorganisms developed. In addition, the participation of bacterial genera such as *Pseudomonas*, *Rhodobacter*, *Chthoniobacter*, *Brevundimonas*, and *Oscillatoria*, as well as *Acrostalagmus* fungi, in carbonate precipitation have been previously reported. The relative abundance of Cyanobacteria in Laguna Timone suggests that photosynthesis may play a role in precipitating carbonate, although this is one of many metabolic processes likely contributing to carbonate precipitation in hypersaline lakes (Arp et al., 2001). The sequences detected herein were related to environmental sequences from sites with extreme living conditions, such as high alkalinity (alkaliphiles) and high salinity (halophiles — *Luteolibacter*, *Rhodobacter*, *Roseinatronobacter*, *Belliella*, *Anditalea*, *Mongoliibacter*, *Lunatimonas*, *Wenzhouxiangella*, *Nitritalea*, *Indibacter*, *Flavobacterium*, *Salinarimonas*, and *Halomonas*). The development of these microbial groups might indicate the presence of complex communities in the Laguna Timone maar crater, which could not survive without the nutrients provided by the environment. Water geochemical elements and the environment of Laguna Timone provide the necessary nutrients, like Ca, Mg, P, K, and Fe, for bacteria and fungi survival and can lead to carbonate precipitation. Bacteria and fungi found in microbial samples from Laguna Timone were closely related to other cold and arid regions, including the Arctic, Antarctica, and Nordic countries, suggesting that environmental limitations such as cold temperatures and alkaline and saline conditions may lead to similarities in microbial community composition. Currently, geomicrobiological studies are quite scarce in this type of system. During the past few years, more investigations on the geomicrobiology of the Extra-Andean Patagonia and Antarctica have been developed since these environments can provide important insights into the relationships between microorganisms and mineral phases, which ultimately can be associated with the origin of life.

4 CHAPTER IV: CONCLUSIONS OF THE WORK

This research reveals several processes associated with carbonate precipitation. The geochemical data showed that the carbonates are affected by evaporative processes and meteoric influences. In addition, the results provided insight into the dynamic by which these microorganisms could grow and adapt to ecosystems potentially extreme to live and precipitate carbonate. The precipitates occur in close association with microbial mats. The evidence suggests that the calcite was precipitated through biogenic processes. Thus, it shows an outstanding model system for studying microbe-mineral interactions under unique environmental conditions. The relative abundance of cyanobacteria, alkaliphiles and halophiles in Laguna Timone suggested that photosynthesis may have a role in precipitating carbonate. All the genera found were related to environmental sequences from sites with extreme life conditions, contributing new insight about communities from a hypersaline environment.

When evaluating precipitation processes, this scenario shows that geological and biological phenomena have a close relationship, especially crucial for life development. It is a combination of physiochemical and biological contribution on carbonate mineral precipitation related to geological context that furnish to diversity of nutrients and protection to microorganism developed. The geological conditions that mafic (and ultramafic rocks) provide, could have an essential role to provide the necessary elements for a system saturation, consequently creating a carbonate and clay precipitation, and necessary nutrients such as Ca, Mg, P, K, and Fe, for bacteria survival leading to carbonate precipitation.

Recent works have reported that mafic rocks like basalt and ultramafic rocks like peridotites, these rocks act as natural carbon reservoirs during weathering comparing to other rocks and eventually could carbonate precipitate. In addition, biological activity can significantly accelerate the weathering of mafic silicates under the uptake of CO₂. Therefore, it would be interesting for future stages to establish a relationship between olivine and carbonate precipitation through the capture of CO₂. The peridotites are present in specific locations in the world, resulting more attractive.

In addition, clumped and $^{87}\text{Sr} / ^{86}\text{Sr}$ isotopes could be used to understand the fluid origin of carbonates and paleotemperature. Finally, Pali Aike represents a natural laboratory to understand several processes.

5. REFERENCES

- Abreo, E., Martinez, S., Bettucci, L., and Lupo, S. 2010. Morphological and molecular characterization of *Campylocarpon* and *Cylindrocarpon* spp. associated with black foot disease of grapevines in Uruguay. *Australasian Plant Pathology*. 39, 446–452. doi: 10.1071/AP10021
- Anderson, S., Appanna, V. D., Huang, J., and Viswanatha, T. 1992. A novel role for calcite in calcium homeostasis. *FEBS Lett.* 308, 94–96. doi:10.1016/0014-5793(92)81059-U
- Andreote, A.P.D., Vaz, M.G.M.V., Genuário, D.B., Barbiero, L., Rezende-Filho, A.T. and Fiore, M.F. 2014. Nonheterocytous cyanobacteria from Brazilian saline-alkaline lakes. *Journal of Phycology*. 50, 675–684. doi: 10.1111/jpy.12192
- Antony, C. P., Kumaresan, D., Hunger, S., Drake, H. L., Murrell, J. C., and Shouche, Y. S. 2013. Microbiology of Lonar Lake and other soda lakes. *ISME J.* 7, 468–476. doi: 10.1038/ismej.2012.137
- Anupama, P. D., Praveen, K. D., Singh, R. K., Kumar, S., Srivastava, A. K., and Arora, D. K. 2011. A psychrophilic and halotolerant strain of *Thelebolus* microsporus from Pangong Lake, Himalaya. *Mycosp.* 2: 601-609.
- Arenz, B., and Blanchette, R. 2011. Distribution and abundance of soil fungi in Antarctica at sites on the Peninsula, Ross Sea Region and McMurdo Dry Valleys. *Soil Biol. Biochem.* 43, 308–315. doi: 10.1016/j.soilbio.2010.10.016
- Armienta, M. A., Vilaclara, G., De la Cruz-Reyna, S., Ramos, S., Cenicerós, N., Cruz, O., et al. 2008. Water chemistry of lakes related to active and inactive Mexican volcanoes. *J. Volcanol. Geotherm. Res.* 178, 249– 258. doi: 10.1016/j.jvolgeores.2008.06.019.
- Arp, D. J. 1999. Butane metabolism by butane-grown *Pseudomonas butanovora*. *Microbiology*. 145, 1173– 1180. doi: 10.1099/13500872-145-5-1173.
- Arp, G., Reimer, A., and Reitner, J. 2001. Photosynthesis-induced biofilm calcification and calcium concentrations in Phanerozoic oceans. *Science* 292, 1701–1704. doi:10.1126/science.1057204.
- Arun, A. B., Young, C. C., Chen, W. M., Hung, M. H., Lai, W. A., Chou, J. H., et al. 2009. *Belliella pelovolcani* sp. nov., isolated from a mud-volcano in Taiwan. *Int J Syst Evol Microbiol.* 59, 2534-7. doi: 10.1099/ijs.0.009753-0.
- Barros, V., Scian, B., Mattio, H. 1979. Campos de precipitación de la provincia de Chubut (1931- 1960). *Geoacta* 10, 175-192.
- Baruth, B., Endlicher, W., Hoppe, P. 1998. Climate and desertification processes

in Patagonia. *Bamberger Geographische Schriften*. 15, 307–320.

Baver, L.D., Gardner, W.H., Gardner, W.R. 1972. *Soil Physics*. John Wiley, New York: 4.

Beget, J.E., Hopkins, D.M., Charron, S.D. 1996. The largest known Mars on Earth, Seward Peninsula, Northwest Alaska. *Arctic*. 49, 62–69.

Bergmann, G. T., Bates, S. T., Eilers, K. G., Lauber, C. L., Caporaso, J. G., Walters, W. A., et al. 2011. The under-recognized dominance of *Verrucomicrobia* in soil bacterial communities. *Soil. Biol. Biochem.* 43, 1450–1455. doi: 10.1016/j.soilbio.2011.03.012.

Berner, R. A. 1990. Atmospheric carbon dioxide levels over phanerozoic time. *Science*. 249, 1382–1386. doi: 10.1126/science.249.4975.1382.

Berner, R. A., Lasaga, A. C. and Garrels, R. M. 1983. The carbonate–silicate geochemical cycle and its effect on atmospheric carbon dioxide over the past 100 million years. *Am. J. Sci.* 283, 641–683.

Biscaye, P. E., 1964. Distinction between kaolinite and chlorite in recent sediments by X-ray diffraction. *Am. Mineral* 49, 1281–1289.

Biscaye, P.E. 1965. Mineralogy and sedimentation of recent deep-sea clay in the Atlantic Ocean and adjacent seas and oceans. *Geol. Soc. Am. Bull* 76, 803– 832.

Bischoff, K., Sirantoine, E., Wilson, M.E., George, A.D., Mendes Monteiro, J., Saunders, M. 2020 Spherulitic microbialites from modern hypersaline lakes, Rottnest Island, Western Australia. *Geobiology*, 18: 725–741. doi: 10.1111/gbi.12400.

Boldareva, E. N., Bryantseva, I. A., Tsapin, A., Nelson, K., Sorokin, D. Y., Tourova, T. P., et al. 2007. The new alkaliphilic bacteriochlorophyll a-containing bacterium *Roseinatronobacter monicus* sp. nov. from the hypersaline Soda Mono Lake (California, United States). *Microbiology*. 76, 82–92. doi: 10.1134/S0026261707010122.

Bontognali T.R.R., Vasconcelos C., Warthmann R.J., Bernasconi S.M., Dupraz C., Strohmenger C.J., McKenzie J.A. 2010. Dolomite formation within microbial mats in the coastal sabkha of Abu Dhabi (United Arab Emirates). *Sedimentology*. 57, 824–844.

Bontognali, T. R., McKenzie, J. A., Warthmann, R. J., and Vasconcelos, C. 2014. Microbially influenced formation of Mg-calcite and Ca-dolomite in the presence of exopolymeric substances produced by sulphate-reducing bacteria. *Terra Nova*. 26, 72–77. doi: 10.1111/ter.12072.

Boquet, E., Boronat, A., and Ramos-Cormenzana, A. 1973. Production of calcite (calcium carbonate) crystals by soil bacteria is a general phenomenon. *Nature*. 246, 527–529. doi:10.1038/246527a0.

Bosak, T. (2011). “Calcite precipitation, microbially induced” in *Encyclopedia of earth sciences series*, eds J. Reitner and V. Thiel (Netherlands: Springer), 223–227.

Braissant, O., Decho, A. W., Dupraz, C., Glunk, C., Przekop, K. M., and Visscher, P. T. 2007. Exopolymeric substances of sulfate-reducing bacteria: Interactions with calcium at alkaline pH and implication for formation of carbonate minerals. *Geobiology*, 5, 401-411. doi: 10.1111/j.1472- 4669.2007.00117.x.

Brouwer, J.F.C., Wolfstein, K., Stal, L.J. (2002). Physical characterization and diel dynamics of different fractions of extracellular polysaccharides in an axenic culture of a benthic diatom. *Eur. J. Phycol.* 37: 37-44.

Brownlow, A.H. 1996. Geochemistry. Prentice Hall Publ., Upper Saddle River, NJ, 580.

Bundeleva, I. A., Shirokova, L. S., Pokrovsky, O. S., Benezeth, P., Menez, B., Gerard, E., et al. 2014. Experimental modeling of calcium carbonate precipitation by cyanobacterium *Gloeocapsa* sp. *Chem.Geol.* 374,44–60. Doi:10.1016/j.chemgeo.2014.03.007.

Burne, R.V., Moore, L. S., Christy, A. G., Troitzsch, U., King, P. L., Carnerup, A. M., et al. 2014. Stevensite in the modern thrombolites of Lake Clifton, Western Australia: A missing link in microbialite mineralization?. *Geology*. 42, 575-578. doi: 10.1130/G35484.1.

Cabral, A., Groenewald, J. Z., Rego, C., Oliveira, H., and Crous, P. W. 2012. Cylindrocarpus root rot: multi- gene analysis reveals novel species within the *Ilyonectria radiculicola* species complex. *Mycological Progress*. 11, 655-688. doi: 10.1007/s11557-011-0777-7.

Cacchio, P., Ercole, C., and Lepidi, A. 2015. Evidences for Bioprecipitation of

Pedogenic Calcite by Calcifying Bacteria from Three Different Soils of L'Aquila Basin (Abruzzi, Central Italy). *Geomicrobiology Journal*. 32, 701- 711. doi: 10.1080/01490451.2014.1001095.

Caldenius, C., 1932. Las Glaciaciones Cuaternarias en Patagonia y Tierra del Fuego. In: Ministerio de Agricultura de la Nación, vol. 95. Dirección General de Minas y Geología, Buenos Aires, 148.

Calderón, M., Fildani, A., Herve, F., Fanning, C.M., Weislogel, A., Cordani, U., 2007. Late Jurassic bimodal magmatism in the northern sea-floor remnant of the Rocas Verdes basin, southern Patagonian Andes. *Journal of the Geological Society of London*. 162, 1011–1022.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). Dada2: high-resolution sample inference from illumina amplicon data. *Nat. Methods*. 13, 581–583. doi: 10.1038/nmeth.3869.

Calvo, J.P., Blanc-Valleron, M.M., Rodríguez-Aranda, J.P. Rouchy, J.M.; Sanz, M.E. 1999. Authigenic clay minerals in continental evaporitic environments. In *Palaeoweathering, Palaeosurfaces and Related Continental Deposits*; Thiry, M., Simon-Coinçon, R., Eds.; Special Publications of the *International Association of Sedimentologists*, Oxford: 129–151pp.

Cande, S., y Leslie, R. 1986. Late Cenozoic tectonics of the Southern Chile Trench. *Journal of Geophysical Research*. 471-496.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., et al. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat. Meth.* 7, 335–336. doi: 10.1038/nmeth.f.303.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., et al. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* 6, 1621– 1624. doi: 10.1038/ismej.2012.8.

Cardman, Z., Arnosti, C., Durbin, A., Ziervogel, K., Cox, C., Steen, A. D., et al. 2014. Verrucomicrobia are candidates for polysaccharide-degrading bacterioplankton in an arctic fjord of Svalbard. *Appl. Environ. Microbiol.* 80, 3749–3756. doi: 10.1128/AEM.00899-14.

Cellamare, M., Duval, C., Drelin, Y., Djediat, C., Touibi N.,

- Agogu  H., et al. 2018. Characterization of phototrophic microorganisms and description of new cyanobacteria isolated from the saline-alkaline crater-lake Dziani Dzaha (Mayotte, Indian Ocean). *FEMS Microbiology Ecology*. 94. doi.org/10.1093/femsec/fiy108.
- Chauvet, E., and Merc , J. 1988. Aquatic Hyphomycetes: their role in the decomposition of leaf-litter. *Revue des sciences de l'eau/journal of water science. Paris*. 1, 203-216.
- Chen, H., Jogler, M., Rohde, M., Klenk, H. P., Busse, H. J., Tindall, B. J., et al. 2013. *Sphingobium limneticum* sp. nov. and *Sphingobium boeckii* sp. nov., two freshwater planktonic members of the family *Sphingomonadaceae*, and reclassification of *Sphingomonas suberifaciens* as *Sphingobium suberifaciens* comb. nov. *Int J Syst Evol Microbiol*. 63, 735-743. doi: 10.1099/ijs.0.040105-0.
- Chergui, H., and Pattee, E. 1991. An experimental study of the breakdown of submerged leaves by hyphomycetes and invertebrates in Morocco. *Freshwater Biology*. 26, 97-110. doi: 10.1111/j.1365- 2427.1991.tb00512.x.
- Chu, J., Stabnikov, V., and Ivanov, V. 2012. Microbially induced calcium carbonate precipitation on surface or in the bulk of soil. *Geomicrobiol. J*. 29, 544–549. doi:10.1080/01490451.2011.592929.
- Cnudde, V., Marcelino, V., Masschaele, B., Vlassenbroeck, J., Dierick, M., Van Hoorebeke, L., Jacobs, P. 2006. Non-destructive high-resolution X-ray CT as an aid for the quantification of soil compaction. *Geophysical Research Abstract* 8.
- Coman, C., Chiriac, C. M., Robeson, M. S., Ionescu, C., Dragos, N., Barbu-Tudoran, L., et al. 2015. Structure, mineralogy, and microbial diversity of geothermal spring microbialites associated with a deep oil drilling in Romania. *Front. Microbiol*. 6:253. doi: 10.3389/fmicb.2015.00253.
- Connell, L., Redman, R., Craig, S., and Rodriguez, R. 2006. Distribution and abundance of fungi in the soils of Taylor Valley, Antarctica. *Soil Biol. Biochem*. 38, 3083–3094. doi: 10.1016/j.soilbio.2006.02.016
- Corbella, H., Chelotti, L., Pomposiello, C. 1996. Neotect nica del rift Jur sico austral en Pali Aike, Patagonia Exrandina, Santa Cruz, Argentina. *XIII Congreso Geol gico Argentino y III Congreso de Exploraci n de Hidrocarburos, II*, 383–393.

Corbella, H., Ercolano, B., 2002. In: Cabaleri, N., Cingolani, C.A., Linares, E., López de Luchi, M.G., Osters, H.A., Panarello, H.O. (Eds.), 2002. Acerca del valle medio e inferior del río Gallegos. Patagonia austral -Argentina. *Actas del XV Congreso Geológico Argentino*, Buenos Aires, 2: 661- 666.

Corbella, H., Gagliardini, D. 1997. Caracterización morfológica de la faja volcano tectónica "Pali Aike" mediante imágenes ERS-1. Proceeding of an international seminar on the use and applications of ERS in Latin America, Viña del Mar, Chile.

Coronato, A., Rabassa, J. 2011. Pleistocene glaciations in southern Patagonia and Tierra del Fuego. In: Ehlers, J., Gibbard, P. (Eds.), Quaternary Glaciations e Extent and Chronology, Part IV e A Closer Look. *Elsevier Developments in Quaternary Science*.15, 715- 727.

Coronato, A., Ercolano, B., Corbella, H., Tiberi, P. (2013). Glacial, fluvial and volcanic landscape evolution in the Laguna Potrok Aike maar area, Southern Patagonia, Argentina. *Quaternary Science Reviews*. 71, 13-26. doi: 10.1016/j.quascirev.2012.06.019.

Costa, N., Kolman, M.A., and Giani, A. (2016). Cyanobacteria diversity in alkaline saline lakes in the Brazilian Pantanal wetland: a polyphasic approach. *Journal of Plankton Research*, 38, 1389-1403. doi: 10.1093/plankt/fbw066.

Costerton, J.W. and Lappin-Scott, H.M. 1995 Introduction to microbial biofilms. In: Microbial Biofilms (Eds. Costerton, J.W. and Lappin-Scott, H.M.), 1-11, Cambridge University Press, Cambridge.

Costerton, J.W., Geesey, G.G. and Cheng, G.K. 1978. How bacteria stick. *Scientific American*, 238, 86-95.

Couradeau, E., Benzerara, K., Moreira, D., Gérard, E., Kaźmierczak, J., Tavera, R., et al. (2011). Prokaryotic and Eukaryotic community structure in field and cultured microbialites from the alkaline lake alchichica (Mexico) ed. J. A. Gilbert. *PLoS ONE* 6: e28767. doi: 10.1371/journal.pone.0028767.

D'orazio, M., Agostini, S., Innocenti, F., Manetti, P., Haller, M., Mazzarini, F. 2001. Slab-window related magmatism from the southernmost South America: The Late Miocene mafic volcanics from the Estancia Glencross Area (~52°S, Argentina-Chile). *Lithos*, 57: 67-89.

D’Orazio, M., Agostini, S., Mazzarini, F., Innocenti, F., Manetti, P., Haller, M.J., et al. 2000. The Pali Aike Volcanic Field, Patagonia: Slab-window magmatism near the tip of South America. *Tectonophysics*, 321, 407- 427. doi:10.1016/S0040-1951(00)00082-2.

D’Orazio, M., Agostini, S., Mazzarini, F., Innocenti, F., Manetti, P., Haller, M.J., et al. (2000). The Pali Aike Volcanic Field, Patagonia: Slab-window magmatism near the tip of South America. *Tectonophysics*. 321, 407- 427. doi:10.1016/S0040-1951(00)00082-2.

Dabirian, R., Beiranvand, M., Aghahoseini, S. 2012. Mineral carbonation in peridotite rock for CO₂ sequestration and a method of leakage reduction of CO₂ in the rock. *Nafta* 63: 44– 48.

DasSarma, S., and DasSarma, P. 2012. “Halophiles,” in *eLS*, (Chichester: Wiley), 1–13. doi: 10.1002/9780470015902.a0000394.pub4

De Hoog, G. S., Nishikaku, A. S., Fernandez-Zeppenfeldt, G., Padín-González, C., Burger, E., Badali, H., et al. 2007. Molecular analysis and pathogenicity of the *Cladophialophora carrionii* complex, with the description of a novel species. *Stud. Mycol.* 58, 219-234. doi: 10.3114/sim.2007.58.08.

De Philippis R Sili C Paperi R Vincenzini M. 2001. Exopolysaccharide-producing cyanobacteria and their possible exploitation: a review. *J Appl Phycol.* 13, 293–299.

Decho, A.W., Visscher, P.T. and Reid, R.P. 2005 Production and cycling of natural microbial exopolymers (EPS) within a marine stromatolite. *Palaeogeography, Palaeoclimatology, Palaeoecology*. 219, 71-86.

Dekayir, A., Amouric, M., and Olives, J. 2005. Clay minerals in hydrothermally altered basalts from Middle Atlas, Morocco. *Clay Minerals*, 40, 67-77. doi:10.1180/0009855054010156.

Demergasso, C., Chong, G., Galleguillos, P., Escudero, L., Martinez-Alonso, M., and Esteve, I. 2003. Microbial mats from the Llamará salt flat, northern Chile. *Rev. Chil. Hist. Nat.* 76, 485- 499. doi: 10.4067/S0716-078X2003000300012.

Demoulin, C. F., Lara, Y. J., Cornet, L., François C., Baurain, D., Wilmotte, A., et al. (2019). Cyanobacteria evolution: Insight from the fossil record. *Free Radic Biol Med.* 140:206-223. doi: 10.1016/j.freeradbiomed.2019.05.007

Des Marais, D. J. 2003. Biogeochemistry of hypersaline microbial mats illustrates the dynamics of modern microbial ecosystems and the early evolution of the biosphere. *Biol. Bull.* 204, 160–167. doi: 10.2307/1543552.

Dias, I. A., Cury, L. F., Titon, B. G., Athayde, G. B., Fedalto, G., da Rocha Santos, L., ... & Manuela Bahniuk Rumbeslperger, A. 2020. The Occurrence of Authigenic Clay Minerals in Alkaline-Saline Lakes, Pantanal Wetland (Nhecolândia Region, Brazil). *Minerals*. 10, 718.

Dickinson, W. R., and W. S. Snyder. 1979. Geometry of subducted slabs related to San Andreas transform, *J. Geol.* 87, 609– 627.

Diraison, M., Cobbold, P., Gapais, D., Rossello, E. 1997. Magellan Strait: part of the Neogene rift system. *Geology* 25: 703-706.

Donnini, M., Frondini, F., Probst, J. L., Probst, A., Cardellini, C., Marchesini, I., & Guzzetti, F. (2016). Chemical weathering and consumption of atmospheric carbon dioxide in the Alpine region. *Global and Planetary Change*.136, 65-81.

Dorador, C., Meneses, D., Urtuvia, V., Demergasso, C., Vila, I., Witzel, K.-P., et al. (2009). Diversity of Bacteroidetes in high-altitude saline evaporitic basins in northern Chile. *J. Geophys. Res.* 114, G00D05. doi: 10.1029/2008JG000837.

Drief, A. and Nieto, E. 2000. Chemical composition of smectites formed in clastic sediments.

Drief, A., Nieto, F., Sanchez-Navas, A., 2001. Experimental clay-mineral formation from a subvolcanic rock by interaction with 1M NaOH solution at room temperature. *Clays Clay Miner.* 49, 92–106.

Drits, V.A., Lindgreen, H., Sakharov, B.A., Salyn, A.S. 1997. Sequential structure transformation of illite-smectite-vermiculite during diagenesis of Upper Jurassic shales, North Sea. *Clay Minerals* 33, 351-371.

Druschel, G. K., and Kappler, A. 2015. Geomicrobiology and microbial geochemistry. *Elements*. 11, 389–394. doi: 10.2113/gselements.11.6.389.

Dundas, I. 1998. Was the environment for primordial life hypersaline?. *Extremophiles*. 2, 375–77. doi: 10.1007/s007920050081

Dupraz, C., Reid, R. P., Braissant, O., Decho, A. W., Norman, R. S., and

Visser, P. T. 2009. Processes of carbonate precipitation in modern microbial mats. *Earth Sci. Rev.* 96, 141–152. doi: 10.1016/j.earscirev.2008.10.005.

Ehrlich, H. 2006. Geomicrobiology: relative roles of bacteria and fungi as geomicrobial agents. En Gadd, G. M., (eds) 2006. *Fungi in Biogeo-chemical Cycles*. Cambridge: Cambridge University Press, 1-10.

Erşan, Y. Ç., De Belie, N., and Boon, N. (2015b). Microbially induced CaCO₃ precipitation through denitrification: an optimization study in minimal nutrient environment. *Biochem. Eng. J.* 101, 108–118. doi: 10.1016/j.bej.2015.05.006.

Falvo, A., Levantesi, C., Rossetti, S., Seviour, R. J., and Tandoi, V. (2001). Synthesis of intracellular storage polymers by *Amaricoccus kaplicensis*, a tetrad forming bacterium present in activated sludge. *J. Appl. Microbiol.* 91, 299–305. doi: 10.1046/j.1365-2672.2001.01384.x.

Fanning, D.S., Keramidas, V.Z., El-desoky, M.A., 1989. Micas. In Dixon, J.B., and Weed, S.B., eds., *Minerals in Soil Environments*. Madison, WI: Soil Science Society of America, 551–634.

Ferris, F. G., Wiese, R. G., and Fyfe, W. S. 1994. Precipitation of carbonate minerals by microorganisms: Implications for silicate weathering and the global carbon dioxide budget, *Geomicrobiology Journal*. 12, 1- 13. doi: 10.1080/01490459409377966.

Fosdick, J.C., Romans, B.W., Fildani, A., Bernhardt, A., Calderón, M., and Graham, S.A. 2011. Kinematic evolution of the Patagonian retro-arc fold-and-thrust belt and Magallanes foreland basin, Chile and Argentina, 51°30'S: *Geological Society of America Bulletin*. 123,1679–1698- doi:10.1130/B30242.1.

Fosdick, J.C., VanderLeest R. A., Bostelmann, J. E. Leonard, J. S., Ugalde, R., Oyarzún, J. L., et al. (2020). Revised Timing of Cenozoic Atlantic Incursions and Changing Hinterland Sediment Sources during Southern Patagonian Orogenesis. *Lithosphere*. doi.org/10.2113/2020/8883099.

Fournier, I. B., Lovejoy, C., Vincent, W. F. 2021. Changes in the Community Structure of Under-Ice and Open-Water Microbiomes in Urban Lakes Exposed to Road Salts. *Front Microbiol.* 12. doi: 10.3389/fmicb.2021.660719.

Freytet, P., and Plet, A. 1996. Modern freshwater microbial carbonates: the *Phormidium* stromatolites (tufa- travertine) of southeastern Burgundy (Paris

Basin, France). *Facies*. 34, 219–237. doi:10.1007/BF02546166.

Freytet, P., and Verrecchia, E. P. 1998. Freshwater organisms that build stromatolites: a synopsis of biocrystallization by prokaryotic and eukaryotic algae. *Sedimentology*. 45, 535–563. doi:10.1046/j.1365- 3091.1998.00155.x.

Gadd, G. 2010. Metals, minerals and microbes: geomicrobiology and bioremediation. *Microbiology* 156: 609-643.

Garreaud, R., Lopez, P., Minvielle, M., Rojas, M. 2013. Large-scale control on the Patagonian climate. *J. Clim*, 26, 215- 230.

Gerard, E., Menez, B., Couradeau, E., Moreira, D., Benzerara, K., Tavera, R., et al. (2013). Specific carbonate- microbe interactions in the modern microbialites of Lake Alchichica (Mexico). *ISME J.* 7, 1997–2009. doi: 10.1038/ismej.2013.8.

Glaring, M. A., Vester, J. K., Lylloff, J. E., Al-Soud, W. A., Sørensen, S. J., and Stougaard, P. (2015). Microbial diversity in a permanently cold and alkaline environment in Greenland. *PLoS ONE* 10: e0124863. doi: 10.1371/journal.pone.0124863.

Gleeson, D. B., Wacey, D., Waite, I., O'Donnell, G. A., and Kilburn, M. R. (2015). Biodiversity of living, non- marine, thrombolites of Lake Clifton, Western Australia. *Geomicrobiol. J.* 33, 850-859. doi: 10.1080/01490451.2015.1118168.

Gleeson, D., Mathes, F., Farrell, M., and Leopold, M. (2016). Environmental drivers of soil microbial community structure and function at the Avon River Critical Zone Observatory. *Sci. Total Environ.* 571, 1407–1418. doi: 10.1016/j.scitotenv.2016.05.185

Glunk C., Dupraz C., Braissant O., Gallagher K. L., Verrecchia E. P., Visscher P. T. 2011. Microbially mediated carbonate precipitation in a hypersaline lake, big pond (Eleuthera, Bahamas). *Sedimentology*. 58, 720–736. doi:10.1111/j.1365-3091.2010.01180.x,

Goh, F., Allen, M. A., Leuko, S., Kawaguchi, T., Decho, A. W., Burns, B. P., et al. 2009. Determining the specific microbial populations and their spatial distribution within the stromatolite ecosystem of Shark Bay. *ISME J.* 3, 383–396. doi: 10.1038/ismej.2008.114.

Golovacheva, R. S. (1976). Thermophilic nitrifying bacteria from hot springs. *Microbiology*. 45, 329–331.

Grum-Grzhimaylo, A. A., Georgieva, M. L., Bondarenko, S. A., Debets, A. J. M., and Bilanenko, E. N. (2016). On the diversity of fungi from soda soils. *Fungal Divers*. 76, 27–74. doi: 10.1007/s13225- 716 015-0320-2.

Han, S. B., Hou, X. J., Yu, Y. H., Ju, Z., Zhang, R., Wang, R. J., et al. 2019. Description of *Wenzhouxiangella salilacus* sp. nov., a moderate halophilic bacterium isolated from a Salt Lake in Xinjiang Province, China. *Antonie van Leeuwenhoek*. 112, 847–855. doi: 10.1007/s10482-018-01216-w.

Harrison, A. L., Power, I. M., and Dipple, G. M. 2013. Accelerated carbonation of brucite in mine tailings for carbon sequestration. *Environmental science & technology*. 47, 126– 134. doi.org/10.1021/es3012854.

Herve, F.; Pankhurst, Robert; Fanning, C.M.; Calderon, M.; Yaxley, G.M. 2007 The South Patagonian batholith : 150 my of granite magmatism on a plate margin. *Lithos*. 97, 373-394.

Hirose, D., Tanabe, Y., Uchida, M., Kudoh, S., Osono, T. 2013. Microfungi associated with withering willow wood in ground contact near Syowa Station, East Antarctica for 40 years. *Polar Biol*. 36, 919–924. doi: 10.1007/s00300-013-1320-x.

Huijgen, W. J. 2007. *Carbon dioxide sequestration by mineral carbonation*. Implications for the smectite-illite transformation. *Clay Minerals*, 35: 665-678.

Jiang, F., Li, W. J., Xiao, M. C., Dai, J., Kan, W. J., Chen, L., et al. 2012. *Luteolibacter luojiensis* sp nov., isolated from Arctic tundra soil, and emended description of the genus *Luteolibacter*. *Int. J. Syst. Evol. Microbiol*. 62, 2259–2263. doi: 10.1099/ijs.0.037309-0

Jiang, H., Dong, H., Yu, B., Liu, X., Li, Y., Ji, S., and Zhang, C. L. (2007). Microbial response to salinity change in Lake Chaka, a hypersaline lake on Tibetan plateau. *Environmental Microbiology*. 9, 2603–2621.

Jung, Y. T., Lee, J. S., Yoon, J. H. (2016). *Lewinella aquimaris* sp. nov., isolated from seawater. *Int J Syst Evol Microbiol*. 66, 3989-399. doi: 10.1099/ijsem.0.001299.

Kaye, J. Z., Márquez, M. C., Ventosa, A., Baross, J. A. (2004). *Halomonas neptunia* sp. nov., *Halomonas sulfidaeris* sp. nov., *Halomonas axialensis* sp. nov. and *Halomonas hydrothermalis* sp. nov.: halophilic bacteria isolated from deep-sea hydrothermal-vent environments. *Int J Syst Evol Microbiol.* 54, 499-511. doi: 10.1099/ijs.0.02799-0.

Kelemen, P. B., Matter, J., Streit, E. E., Rudge, J. F., Curry, W. B., and Blusztajn, J. 2011. Rates and mechanisms of mineral carbonation in peridotite: natural processes and recipes for enhanced, *in situ* CO₂ capture and storage. *Annu. Rev. Earth Planet. Sci.* 39, 545–576. doi: 10.1146/annurev-earth-092010-152509.

Kelemen, P., McQueen, N., Wilcox, J., Renforth, P., Dipple, G., and Vankeuren, A. 2020. Engineered carbon mineralization in ultramafic rocks for CO₂ removal from air: Review and new insights. *Chemical Geology.* 550, 119628. doi: 10.1016/j.chemgeo.2020.119628.

Kim, K. K., Jin, L., Yang, H. C., and Lee, S. T. (2007). *Halomonas gomseomensis* sp. nov., *Halomonas janggokensis* sp. nov., *Halomonas salaria* sp. nov. and *Halomonas denitrificans* sp. nov., moderately halophilic bacteria isolated from saline water. *Int. J. Syst. Evol. Microbiol.* 57, 675–681. doi: 10.1099/ijs.0.64767-0.

Kim, M., Pak, S., Rim, S., Ren, L., Jiang, F., Chang, X., et al. (2015). *Luteolibacter arcticus* sp. nov., isolated from high Arctic tundra soil, and emended description of the genus *Luteolibacter*. *Int. J. Syst. Evol. Microbiol.* 65, 1922–1928. doi: 10.1099/ijs.0.000202.

Kisch J. 1991. Calibration of the anchizone a critical comparison of illite 'crystallinity' scales used for definition. *Journal of Metamorphic Geology.* 8, 31-46.

Koinig, K. A., Shotyk, W., Lotter, A.F., Ohlendorf, C., Sturm, M. 2003. 9000 years of geochemical evolution of lithogenic major and trace elements in the sediment of an alpine lake—the role of climate, vegetation, and landuse history. *J Paleolimnol.* 30, 307–320.

Konhauser, K., Lalonde, S., Planavsky, N., Pecoits, E., Lyons, T., Mojzsis, S., Rouxel, O., Barley, M., Rosiere, C., Fralick, P., Kump, L., Bekker, A. 2011. Aerobic bacterial pyrite oxidation and acid rock drainage during the Great Oxidation Event. *Nature* 478,369–373.

Konhauser, K.O., 2007. Introduction to Geomicrobiology. Blackwell, Oxford, 425.

Lawrence, J.R., Swerhone, G.D.W., Leppard, G.G., Araki, T., Zhang, X., West, M.M. and Hitchcock, A.P. (2003) Scanning Transmission X-Ray, Laser Scanning, and Transmission electron microscopy mapping of the exopolymeric matrix of microbial biofilms. *Applied and Environmental Microbiology*. 69, 5543-5554.

Koskenniemi, K., Lyra, C., Rajaniemi-Wacklin, P., Jokela, J., and Sivonen, K. (2007). Quantitative real-time PCR detection of toxic *Nodularia cyanobacteria* in the Baltic Sea. *Appl. Environ. Microbiol.* 73, 2173– 2179. doi: 10.1128/AEM.02746-06.

Kozyreva, L., Egorova, D., Anan'ina, L., Plotnikova, E., Ariskina, E., Prisyazhnaya, N., et al. (2016). *Belliella buryatensis* sp. nov., isolated from alkaline lake water. *Int J Syst Evol Microbiol.* 66, 137-143. doi: 10.1099/ijsem.0.000682.

Kristyanto, S., Nguyen, T.M., Chaudhary, D.K., Lee, S.-S., and Kim, J. 2018. Characterization of *Flavobacterium aquimarinum* sp. nov., a halotolerant bacterium isolated from seawater. *J Microbiol.* 56, 317–323. doi: 10.1007/s12275-018-7454-3.

Kumar, S., Karan, R., Kapoor, S., Singh, S. P., and Khare, S. K. (2012). Screening and isolation of halophilic bacteria producing industrially important enzymes. *Braz. J. Microbiol.* 43, 1595–1603. doi: 10.1590/S1517-83822012000400044.

Kylander, M. E., Ampel, L., Wohlfarth, B., Veres, D. 2011. High-resolution X-ray fluorescence core scanning analysis of Les Echets (France) sedimentary sequence: new insights from chemical proxies. *J Quat Sci*, 26:109–117.

Lau, K. W., Ng, C. Y., Ren, J., Lau, S. C., Qian, P. Y., Wong, P. K., et al. 2005. *Owenweeksia hongkongensis* gen. nov., sp. nov., a novel marine bacterium of the phylum 'Bacteroidetes'. *Int. J. Syst. Evol. Microbiol.* 55, 1051–1057. doi: 10.1099/ijms.0.63155-0.

Lebedeva, E. V., Off, S., Zumbärgel, S., Kruse, M., Shagzhina, A., Lückner, S., et al. 2011. Isolation and characterization of a moderately thermophilic nitrite-oxidizing bacterium from a geothermal spring. *FEMS Microbiol. Ecol.* 75, 195–204. doi: 10.1111/j.1574-6941.2010.01006.x.

Ley, R. E., Harris, J. K., Wilcox, J., Spear, J. R., Miller, S. R., Bebout, B. M., et al.

2006. Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. *Appl. Environ. Microbiol.* 72, 3685–3695. doi: 10.1128/AEM.72.5.3685-3695.2006.

Lindemann, S. R., Moran, J. J., Stegen, J. C., Renslow, R. S., Hutchison, J. R., Cole, J. K., et al. (2013). The epsomitic phototrophic microbial mat of Hot Lake, Washington: community structural responses to seasonal cycling. *Front. Microb. Physiol. Metab.* 4, 323. doi: 10.3389/fmicb.2013.00323.

Liu, J. H, Wang, Y. X., Zhang, X. X., Wang, Z. G., Chen, Y. G., Wen, M. L., et al. 2010. *Salinarimonas rosea* gen. nov., sp. nov., a new member of the alpha-2 subgroup of the Proteobacteria. *Int J Syst Evol Microbiol.* 60, 55-60. doi: 10.1099/ijs.0.006981-0.

Lopez-Garcia, P., Kazmierczak, J., Benzerara, K., Kempe, S., Guyot, F., Moreire, D. 2005. Bacterial diversity and carbonate precipitation in the giant microbialites from the highly alkaline Lake Van, Turkey. *Extremophiles.* 9, 263–274. doi: 10.1007/s00792-005-0457-0.

Lorenz, V., 2003. Maar-diatremes volcanoes, their formation, and their setting in hard-rock or soft-rock environments. *Geolines.* 15, 7283.

Louvat, P. 1997. Etude géochimique de l'érosion fluviale d'îles volcaniques à l'aide des bilans d'éléments majeurs et traces. PhD Thesis. Université Paris-7 Paris. 322.pp.

Louyakis, A. S., Mobberley, J. M., Vitek, B. E., Visscher, P. T., Hagan, P. D., Reid, R. P., et al. (2017). A study of the microbial spatial heterogeneity of Bahamian Thrombolites using molecular, biochemical, and stable isotope analyses. *Astrobiology.* 17, 413–430. doi: 10.1089/ast.2016.1563.

Lozupone, C. A., and Knight, R. (2007). Global patterns in bacterial diversity. *Proc. Natl. Acad. Sci. U.S.A.* 104, 11436–11440. doi: 10.1073/pnas.0611525104.

Ludwig, R., Al-Horani, F. A., De Beer, D., and Jonkers, H. M. 2005. Photosynthesis- controlled calcification in a hypersaline microbial mat. *Limnol. Oceanogr.* 50, 1836–1843. doi:10.4319/lo.2005.50.6.1836.

Lüttge, A., Zhang, L., and Nealson, K.H. (2005). Mineral surfaces and their implications for microbial attachment: Results from Monte Carlo simulations and direct surface observation. *American Journal of Science June.* 305, 766-790. doi:

10.2475/ajs.305.6-8.766.

Lv, X. L., Xie, B. S., Cai, M., Tang, Y.Q., Wang, Y. N., Cui, H. L., et al. 2015. *Halodurantibacterium flavum* gen. nov., sp. nov., a non-phototrophic bacterium isolated from an oil production mixture. *Curr Microbiol.* 70, 141-8. doi: 10.1007/s00284-014-0696-3.

Lyra, C., Laamanen, M., Lehtimäki, J. M., Surakka, A., and Sivonen, K. (2005). Benthic cyanobacteria of the genus *Nodularia* are non-toxic, without gas vacuoles, able to glide and genetically more diverse than planktonic *Nodularia*. *Int. J. Syst. Evol. Microbiol.* 55, 555–568. doi: 10.1099/ijs.0.63288-0.

Madigan, M, T., Martinko, J, M., Parker, J. 2003. Brock Biology of Microorganisms. Upper Saddle River, NJ: Prentice Hall, 1019.

Marshall, K.C. 1992. Biofilms: an overview of bacterial adhesion, activity, and control at surfaces. *ASM News.* 58, 202-207.

Mayr, C., Lücke, A., Stichler, W., Trimborn, P., Ercolano, B., Oliva, G., Ohlendorf, C., Soto, J., Fey, M., Habertzettl, T., Janssen, S., Schäbitz, F., Schleser, G.H., Wille, M., Zolitschka, B. 2007. Precipitation origin and evaporation of lakes in semi-arid Patagonia (Argentina) inferred from stable isotopes (d18O, d2H). *Journal of Hydrology.* 334, 53-63.

Mazzarini, F., and D’Orazio, M., 2003. Spatial distribution of cones and satellite-detected lineaments in the Pali Aike Volcanic Field (southernmost Patagonia): insights into tectonic setting of a Neogene rift system. *J. of Volcanol. Geotherm.Res.* 125: 291 – 305. doi: 10.1016/S0377-0273(03)00120-3.

McKeague, J.A. and Cline, M.G. 1963. Silica in Soil Solutions. I. The Form and Concentration of Dissolved Silica in Aqueous Extracts of Some Soils. *Canadian Journal of Soil Science* 43, 70-82. doi.org/10.4141/cjss63-010.

McMurdie, P. J., Holmes, S., Kindt, R., Legendre, P., and O’Hara, R. 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLOS ONE* 8: e61217. doi: 10.1371/journal.pone.0061217.

Meglioli, A. 1992. *Glacial geology and geochronology of southernmost Patagonia and Tierra del Fuego, Argentina and Chile*. PhD thesis, Leight University.

Melack, J.M. and Kilham, P. (1974) Photosynthetic Rates of Phytoplankton in East African Alkaline, Saline Lakes. *Limnology and Oceanography*, 19, 743-755.
Mercer, J. H., 1976: Glacial history of southernmost South America. *Quaternary Research*, 6:125-166.

Meyer, F., Bang, S., Min, S., Stetler, L., and Bang, S. 2011. Microbiologically-Induced Soil Stabilization: Application of *Sporosarcina pasteurii* for Fugitive Dust Control. *Geo-Frontiers*. 4002–4011. doi:10.1061/41165(397)409.

Ming, Y. Z., Liu, L., Xian, W. D, Jiao, J. Y., Liu, Z.T, Li., M. M., et al. (2020). *Rhabdaerophilum calidifontis* gen. nov., sp. nov., a novel bacterium isolated from a hot spring, and proposal of *Rhabdaerophilaceae* fam. nov. *International Journal of Systematic and Evolutionary Microbiology*. 70:2298-2304. doi: 10.1099/ijsem.0.004035.

Minyuk, P., Brigham-Grette, J., Melles, M., Borkhodoev, V.Y., Glushkova, O.Y. 2007. Inorganic geochemistry of El gygytgyn Lake sediments, northeastern Russia, as an indicator of paleoclimatic change for the last 250 kyr. *J Paleolimnol* 37:123–133.

Mobberley, J. M., Khodadad, C. L., and Foster, J. S. 2013. Metabolic potential of lithifying cyanobacteria- dominated thrombolitic mats. *Photosynth. Res.* 118, 125–140. doi: 10.1007/s11120-013-9890-6.

Monty, C. L.V. (1976) “The origin and development of cryptalgal fabrics”, in *Stromatolites: Developments in Sedimentology*, ed M.R Walter (Amsterdam: Elsevier). 20, 193-249. doi:10.1016/S0070-4571(08)71137-3.

Moore, D. M., Reynolds, J. R. R. C. 1997. X-Ray Diffraction and the identification and Analysis of Clay Minerals. 2 ed. Oxford University Press.

Moore, L. S., and Burne, R. V. 1994. “The modern thrombolites of Lake Clifton, Western Australia,” in *Phanerozoic Stromatolites II*, eds J. Bertrand-Sarfati and C. Monty (Boston,MA: Kluwer Academic Publishers), 3–29.

Muccio, Z., and Jackson, G. P. 2009. Isotope Ratio Mass Spectrometry. *The Analyst*, 134(2), 213–222. doi.org/10.1039/b808232d.

Mußmann, M., Pjevac, P., Krüger, K., and Dykma, S. 2017. Genomic repertoire of the *Woeseiaceae*/JTB255, cosmopolitan and abundant core members of microbial communities in marine sediments. *ISME J.* 11, 1276–1281. doi:

10.1038/ismej.2016.185.

Myshrall, K. L., Mobberley, J. M., Green, S. J., Visscher, P. T., Havemann, S. A., Reid, R. P., et al. (2010). Biogeochemical cycling and microbial diversity in the thrombolitic microbialites of Highborne Cay, Bahamas. *Geobiology*. 8, 337–354. doi: 10.1111/j.1472-4669.2010.00245.x.

Nadeau, T.-L., Howard-Williams, C., and Castenholz, R. W. 1999. Effects of solar UV and visible irradiance on photosynthesis and vertical migration of *Oscillatoria* sp. (Cyanobacteria) in an Antarctic microbial mat. *Aquat. Microb. Ecol.* 20, 231–243. doi: 10.3354/ame020231.

Nagai, K., Suzuki, K., and Okada, G. 1998. Studies on the distribution of alkalophilic and alkali-tolerant soil fungi II: Fungal flora in two limestone caves in Japan. *Mycoscience*. 39, 293. doi: 10.1007/BF02464011.

Natland, M. L., Gonzales, E. P., Cañon, A., Ernst, M. 1974. A system of stages for correlation of Magallanes Basin sediments. *Geological Society of America Memoirs*, 139.

Needham, S.J., Worden, R.H., Cuadros J. 2006. Sediment ingestion by worms and the production of bio-clays: a study of macrobiologically enhanced weathering and early diagenetic processes. *Sedimentology*, 53, 567-579.

Nejidat, A. 2005. Nitrification and occurrence of salt-tolerant nitrifying bacteria in the Negev desert soils.

Nonga, H. E., Mdegela, R. H., Sandvik, M., Lie, E., Miles, C. O., Skaare, J. U. 2017. Cyanobacteria and cyanobacterial toxins in the alkaline-saline Lakes Natron and Momela, Tanzania. *In Proceedings of the 34th scientific conference of the Tanzania Veterinary Association*.32, 108–116.

O’Corner, W.K., Dahlin, D.C., Nilson, D.N., Rush, G.E., Gerdemann, S.J., Walters, R.P., and Turner, R.C. 2001. Aqueous Mineral Carbonation Studies Using Olivine and Sepertine, *Albany Research Center*.

Oehlerich, M., Mayr, C., Griesshaber, E., Lucke, A., Oeckler, O.M., Ohlendorf, C., Schmahl, W.W., Zolitschka, B. 2013. Ikaite precipitation in a lacustrine environment—implications for palaeoclimatic studies using carbonates from Laguna Potrok Aike (Patagonia, Argentina). *Quat Sci Rev*, 71, 46–53.

Oelkers, E.H., Benning, L.G., Lutz, S., Mavromatis, V., Pearce, C. R., and Plummer, O. 2015. The efficient long-term inhibition of forsterite dissolution by common soil bacteria and fungi at Earth surface conditions. *Geochim. Cosmochim. Acta* 168, 222-235.

Oh, H. M., Lee, K., Cho, J. C. 2009. *Lewinella antarctica* sp. nov., a marine bacterium isolated from Antarctic seawater. *Int J Syst Evol Microbiol.* 59:65-68. doi: 10.1099/ijs.0.000794-0.

Okuy, T. O., Rodrigues, D. F., and Laanbroek, R. 2015. Biotic and abiotic effects on CO₂ sequestration during microbially-induced calcium carbonate precipitation. *FEMS Microbiol. Ecol.* 91: fiv017. doi: 10.1093/femsec/fiv017.

Olsson, J., Bovet, N., Makovicky, E., Bechgaard, K., Balogh, Z., Stipp, S.L.S., 2012. Olivine reactivity with CO₂ and H₂O on a microscale: implications for carbon sequestration. *Geochim. Cosmochim. Acta* 77, 86–97.

Osman, J.R, Viedma, P., Mendoza, J., and Cotoras, D. 2020. Bacterial and Geochemical Composition of Thrombolites from Lake Sarmiento, Torres del Paine National Park of Chilean Patagonia. *Geomicrobiology Journal.* 37, 376-388. doi: 10.1080/01490451.2019.1709107.

Pacton, M., Ariztegui, D., Wacey, D., Kilburn, M.R., Rollion-Bard, C., Farah, R. and Vasconcelos, C. 2012. Going nano: A new step toward understanding the processes governing freshwater ooid formation. *Geology*, 40, 547-550.

Paruelo, J.M., Beltrón, A., Jobbágy, E., Sala, E.O., Golluscio, R.A. 1998. The climate of Patagonia: general patterns and controls on biotic processes. *Ecologia Austral* 8, 85– 101.

Paterson D. M., Aspden R. J., Visscher P. T., Consalvey M., Andres M. S., Decho A. W., et al. 2008. Light dependent biostabilisation of sediments by stromatolite assemblages. *PLoS ONE* 3:e3176 10.1371/journal.pone.0003176.

Paul, V. G., and Mormile, M. R. 2017. A case for the protection of saline and hypersaline environments: a microbiological perspective. *FEMS microbiology ecology*, 93(8), 10.1093/femsec/fix091. doi: 10.1093/femsec/fix091.

Paul, V.G., Wronkiewicz, D.J., Mormile, M.R., and Foster, J.S. 2016. Mineralogy and Microbial Diversity of the Microbialites in the Hypersaline Storr's Lake, the

Bahamas. *Astrobiology*. 16, 282. doi:10.1089/ast.2015.1326.

Pentecost, A. 2003. Cyanobacteria associated with hot spring travertines. *Can. J. Earth Sci.* 40, 1447–1457. doi:10.1139/e03-075.

Pérez, V., Dorador, C., Molina, V., Yáñez, C., Hengst, M. (2018). *Rhodobacter* sp. Rb3, an aerobic anoxygenic phototroph which thrives in the polyextreme ecosystem of the Salar de Huasco, in the Chilean Altiplano. *Antonie Van Leeuwenhoek*. 8, 449-1465. doi: 10.1007/s10482-018-1067-z.

Perry T. D. IV., Klepac-Ceraj V., Zhang X. V., McNamara C. J., Polz M. F., Martin S. T., Berke N. and Mitchell R. 2005. Binding of harvested bacterial exopolymers to the surface of calcite. *Environ. Sci. Technol.* 39: 8770-8775.

Perry, R.S., Mcloughlin, N., Lynne, B.Y., Sephton, M.A., Oliver, J.D., Perry, C.C., Campbell, K., Engel, M.H., Farmer, J.D., Brasier, M.D., Staley, J.T. 2007. Defining biominerals and organominerals: Direct and indirect indicators of life. *Sedimentary Geology* 201, 157- 79.

Petit, E., and Gubler, W. D. (2005). Characterization of *Cylindrocarpum* species, the cause of black foot disease of grapevine in California. *Plant Disease*. 89, 1051-1059. doi: 10.1094/PD-89-1051.

Popall, R. M., Bolhuis, H., Muyzer, G and Sánchez-Román, M. (2020). Stromatolites as Biosignatures of Atmospheric Oxygenation: Carbonate Biomineralization and UV-C Resilience in a *Geitlerinema* sp. - Dominated Culture. *Front. Microbiol.* 11,948. doi: 10.3389/fmicb.2020.00948.

Potts, M. 1994. Desiccation tolerance of prokaryotes. *Microbiol. Rev.* 58, 755–805.

Pozo, M.; Calvo, J.P. 2018. An Overview of Authigenic Magnesian Clays. *Minerals* 8, 520. doi.org/10.3390/min8110520.

Prescott, L. M., Harley, J. P., and Klein, D. A. (2002). *Microbiology*. Boston, MA: McGraw-Hill.

Prudêncio, M.I., SequeiraBraga, M.A., Paquet, H., Waerenborgh, J.C., Pereira, L.C.J. and Gouveia, M.

Radzi, R., Muangmai, N., Broady, P., Wan Omar, W. M., Lavoue, S., Convey, P.,

et al. 2019. *Nodosilinea signiensis* sp. nov. (Leptolyngbyaceae, Synechococcales), a new terrestrial cyanobacterium isolated from mats collected on Signy Island, South Orkney Islands, Antarctica. *PloS one*, 14, e0224395. doi.org/10.1371/journal.pone.0224395.

Raimondo, M., and Carlucci, A. 2018. Characterization and pathogenicity of *Plectosphaerella* spp. collected from basil and parsley in Italy. *Phytopathologia Mediterranea*. 57, 284-295. doi: 10.14601/Phytopathol_Mediterr-23206.

Reasoner, D. J., and Geldreich, E. E. 1985. A new medium for the enumeration and subculture of bacteria from potable water. *Appl. Environ. Microbiol.* 49, 1–7.
Richardson, L. L., Aguilar, C., and Nealson, K. H. (1988). Manganese oxidation in pH and O₂ microenvironments produced by phytoplankton. *Limnol. Oceanogr.* 33, 352–363. doi: 10.4319/lo.1988.33.3.0352.

Riding, R. 1991. Classification of microbial carbonates. Calcareous algae and stromatolites. Berlin, *Springer-Verlag*. 21-51.

Riding, R. 2011. Microbialites, stromatolites, and thrombolites. In: Encyclopedia of Geobiology (Eds. Reitner, J. and Thiel, V. 635-654, Encyclopedia of Earth Science Series, Springer, Heidelberg.

Rodriguez-Valera, F. 1988. Characteristics and microbial ecology of hypersaline environments, in “Halophilic Bacteria” vol. 1 (Rodriguez-Valera, F. Ed.), 3–30. CRC Press, Boca Raton.

Rojas, N. L., Cavalitto, S. F., Cabello, M., Hours, R. A., and Voget, C. E. 2008. Alkaline polysaccharidases produced in solid state cultures by alkalophilic fungi isolated from Argentina. *J Pure Appl Microbiol.* 2, 1- 10.

Ross P-S, Delpit S, Haller MJ, Németh K, Corbella H. 2011. Influence of the substrate on maar diatreme volcanoes – An example of a mixed setting from the Pali Aike volcanic field, Argentina. *J Volcanol Geoth Res*, 201, 253-271.

Rumbelsperger A. M. B. 2013. Coupling Organic and Inorganic Methods to study Growth and Diagenesis of modern microbial carbonates, Rio de Janeiro State, Brazil: Implications for Interpreting Ancient Microbialite Facies Development. Tese de Doutorado. ETH Zurich, 160.

Schwartzman, D. W., and Volk, T. 1989. Biotic enhancement of weathering and the habitability of Earth. *Nature*. 340, 457–460. doi: 10.1038/340457a0.

Shannon, C. E. 1948. A mathematical theory of communication. *Bell Syst. Tech. J.* 27, 379–423. doi: 10.1002/j.1538-7305.1948.tb01338.x.

Shau, Y.H., Peacor, D.R., Essene, E.J. 1990. Corrensite and mixed-layer chlorite/smectite in metabasalt from northern Taiwan: TEM/AEM, EMPA, XRD, and optical studies. *Contrib. Mineral. Petrol* 105, 123–142. doi.org/10.1007/BF00678980.

Shi, W., Takano, T., and Liu, S. 2012. *Anditalea andensis* gen. nov., sp. nov., an alkaliphilic, halotolerant bacterium isolated from extreme alkali–saline soil. *Antonie van Leeuwenhoek*. 102, 703–710. doi: 10.1007/s10482-012-9770-7.

Shiraishi, F. 2012. Chemical conditions favoring photosynthesis induced CaCO₃ precipitation and implications for microbial carbonate formation in the ancient ocean. *Geochim. Cosmochim. Acta*. 77, 157–174.

Simpson, E. H. 1949. Measurement of diversity. *Nature*. 163:688. doi: 10.1038/163688a0.

Singer, A. 1966. The mineralogy of the clay fraction from basaltic soils in the Galilee (Israel). *J. Soil Sci.* 17:138-146.

Singer, A. 1984. Pedogenic palygorskite in the arid environment. In *Palygorskite-Sepiolite. Occurrences, Genesis and Uses, Developments in Sedimentology*; Singer, A., Galán, E., Eds. *Elsevier*, Amsterdam, The Netherlands, 37.

Singh, P., Roy, U., and Tsuji, M. 2016. Characterization of yeast and filamentous fungi from Brøggerbreen glaciers, Svalbard. *Polar Record*. 52, 442-449. doi: 10.1017/S0032247416000085 *situ* CO₂ capture and storage. *Annu. Rev. Earth Planet. Sci.* 39, 545–576. doi: 10.1146/annurev- earth-092010-152509.

Skewes, M., Stern, C. 1979. Petrology and geochemistry of alkali basalts and ultramafic inclusions from the Palei Aike volcanic field in southern Chile and the origin of the Patagonian plateau lavas. *J. Volcanol. Geotherm. Res.* 3.

Solari, M., 2010. Paleo-termometría y Evolución del Sistema Hidrológico del Parque Nacional Torres del Paine, Patagonia.

Spring, S., Brinkmann, N., Murrja, M., Spröer, C., Reitner, J., Klenk, H.-P. 2015. High diversity of culturable prokaryotes in a lithifying hypersaline microbial mat. *Geomicrobiol. J.* 32, 332-346. doi: 10.1080/01490451.2014.913095.

- Srinivas, T. N., Aditya, S., Bhumika, V., Kumar, P. A. 2014. *Lunatimonas lonarensis* gen. nov., sp. nov., a haloalkaline bacterium of the family *Cyclobacteriaceae* with nitrate reducing activity. *Syst Appl Microbiol.* 37, 10-6. doi: 10.1016/j.syapm.2013.10.003.
- Stern, C. 1991. Role of subduction erosion in the generation of Andean magmatism. *Geology*, 19: 78 – 81.
- Stocks-Fischer, S., Galinat, J. K., and Bang, S. S. (1999). Microbiological precipitation of CaCO₃. *Soil Biol. Biochem.* 31, 1563–1571. doi: 10.1016/S0038-0717(99)00082-6.
- Stolz, J. F. 2000. Structure of microbial mats and biofilms. In *Microbial sediments* (pp. 1-8). Springer, Berlin, Heidelberg.
- Stougaard, P., Jørgensen, F., Johnsen, M. G., and Hansen, O. C. 2002. Microbial diversity in ikaite tufa columns: an alkaline, cold ecological niche in Greenland. *Environ Microbiol.* 4, 487-93. doi: 10.1046/j.1462-2920.2002.00327.x.
- Stumm, W., and Morgan, J. J. 1981. Aquatic Chemistry. An Introduction Emphasizing Chemical Equilibria in Natural Waters. John Wiley and Sons, New York, New York.
- Tosca, N. J. 2015. Geochemical pathways to Mg-clay formation. In: Pozo M. & Galán E. (eds) *Magnesian Clays: Characterization, Origins and Applications. AIPEA (Association Internationale pour l'Etude des Argiles)*. 2, 283- 329.
- Tosca, N. J., Wright, V. P. 2015. Diagenetic pathways linked to labile Mg-clays in lacustrine carbonate reservoirs: a model for the origin of secondary porosity in the Cretaceous pre-salt Barra Velha Formation, offshore Brazil. *The Geological Society of London*, 14.
- Tournassat, C., Gailhanou, H., Crouzet, C., Braibant, G., Gautier, A., Gaucher, E. C. 2009. Cation Exchange Selectivity Coefficient Values on Smectite and Mixed-Layer Illite/Smectite Minerals. *Soil Science Society of America Journal.* 73, 928-942.
- Tourney J., Ngwenya B. T. 2014. The role of bacterial extracellular polymeric substances in geomicrobiology. *Chem. Geol.* 386 115–132. 10.1128/AEM.06568-11.

Tsuji, M., Yokota, Y., Shimohara, K., Kudoh, S., Hoshino, T. (2013). An application of wastewater treatment in a cold environment and stable lipase production of Antarctic basidiomycetous yeast *Mrakia blollopis*. *PLoS ONE* 8: e59376. doi: 10.1371/journal.pone.0059376.

Underwood, G., 2010, Exopolymers (extracellular polymeric substances) in diatom dominated marine sediment biofilms, in Seckbach, J., and Oren, A., eds., *Cellular Origin, Life in Extreme Habitats and Astrobiology*, p. 287–300, the Netherlands, Springer.

Urbani, R., Sist, P., Pletikapic, G., Radic, T.M., Svetlicc, V., and Zutic, V. 2012. Diatom polysaccharides: extracellular production, isolation and molecular characterization, in Karunaratne, D.N., ed., *The Complex World of Polysaccharides*: INTECH, Croatia, doi.org/10.5772/51251.

Van Trappen, S., Mergaert, J., and Swings, J. (2004). *Loktanella salsilacus* gen. nov., sp. nov., *Loktanella fryxellensis* sp. nov. and *Loktanella vestfoldensis* sp. nov., new members of the *Rhodobacter* group, isolated from microbial mats in Antarctic lakes. *Int. J. Syst. Evol. Microbiol.* 54, 1263–1269. doi: 10.1099/ijs.0.03006-0.

Vasconcelos, C., Warthmann, R., McKenzie, J. A., Visscher, P. T., Bittermann, A. G., and Van Lith, Y. (2006). Lithifying microbial mats in Lagoa Vermelha, Brazil: Modern Precambrian relics?. *Sediment. Geol.* 185, 175–183. doi: 10.1016/j.sedgeo.2005.12.022.

Velmurugan, N., Kalpana, D., Han, J. H., Cha, H. J., and Soo Lee, Y. (2011). A novel low temperature chitinase from the marine fungus *Plectosphaerella* sp. strain MF-1. doi: 10.1515/BOT.2010.068.

Ventosa, A and Arahall, D. R. 2009. Physico-chemical characteristics of hypersaline environments and their biodiversity. *Extremophiles*. 2, 1–6.

Ventosa, A. 2006. “Unusual micro-organisms from unusual habitats: hypersaline environments,” in *Prokaryotic Diversity: Mechanisms and Significance: Published for the Society for General Microbiology*, eds N. A. Logan, H. M. Lappin-Scott, and P. C. F. Oyston (Cambridge: Cambridge University Press), 223–254. doi: 10.1017/CBO9780511754913.015.

Vilaclara, G., Chávez, M., Lugo, A., González, H., and Gaytán, M. 1993. Comparative description of crater- lakes basic chemistry in Puebla State, Mexico.

Verhandlungen Internationale Vereinigung für Theoretische Angewandte Limnologie. 25, 435–440.

Visscher, P.T. and Stolz, J.F. 2005. Microbial mats as bioreactors: populations, process, and products. *Paleogeogr. Paleoclimatol. Paleoecol.*, 219, 87–100.

Vuillemin, A., Ariztegui, D., and the Pasado Science Team. 2013. Geomicrobiological investigations in subsaline maar lake over the last 1500 years. *Quat. Sci. Rev.* 71, 119–130. doi: 10.1016/j.quascirev.2012.04.011.

Wang, Y. X., Liu, Y. P., Huo, Q. Q., Li, Y. P., Feng, F.Y., Xiao, W., et al. 2016. *Mongoliibacter ruber* gen. nov., sp. nov., a haloalkalitolerant bacterium of the family *Cyclobacteriaceae* isolated from a haloalkaline lake. *Int J Syst Evol Microbiol.* 66, 1088–1094. doi: 10.1099/ijsem.0.000836.

Weischet, W. 1996. Regionale Klimatologie. Die neue Welt. Tcubner. Stuttgart, 468.

Welton, J. E., 2003. SEM Petrology Atlas: Methods in Exploration Series No. 4. *The American Association on Petroleum Geologists*, 2, 240.

Weyers, H. S., and Suberkropp, K. 1996. Fungal and bacterial production during the breakdown of yellow poplar leaves in 2 streams. *Journal of the North American Benthological Society*. 15, 408–420. doi: 10.2307/1467795.

White, A.F. 2003. Natural weathering rates of silicate minerals. Surface and Ground Water, Weathering, and Soils. Treatise on Geochemistry, Volume 5. Editor: J.I. Drever. Executive Editors: H.D. Holland and K.K. Turekian. Elsevier, p. 133–168.

White, A.F., and Blum, A.E. 1995. Effects of climate on chemical weathering in watersheds.

Williams, W. 2002. Environmental threats to salt lakes and the likely status of inland saline ecosystems in 2025. *Environmental Conservation*. 29, 154–167.

Wilson, M. 2004. Weathering of the primary rock-forming minerals: Processes, products and rates. *Clay Minerals*, 39, 233–266. doi:10.1180/0009855043930133.

Wilson, Siobhan A., Dipple, Gregory M., Power, Ian M., Thom, James M., Anderson, Robert G., Raudsepp, Mati, Gabites, Janet E., and Southam, Gordon.

2009. Carbon dioxide fixation within mine wastes of ultramafic-hosted ore deposits: examples from the Clinton Creek and Cassiar Chrysotile deposits, Canada. *Economic Geology*.104, 95-112.

Winslow, M. A. 1983. Clastic dike swarms and the structural evolution of the foreland fold and thrust belt of the southern Andes. *Geological Society of America Bulletin*, 1073-1080.

Wright, D.T., and Oren, A. 2005. Nonphotosynthetic Bacteria and the Formation of Carbonates and Evaporites Through Time. *Geomicrobiology Journal*, 22, 27 - 53.

Xing, T., Liu, Y., Wang, N., Xu, B., Shen, L., Liu, K., et al. (2017). *Polymorphobacter glacialis* sp. nov., isolated from ice core. *Int J Syst Evol Microbiol*. 67, 617-620. doi: 10.1099/ijsem.0.001672.

Xiong, J., and Bauer, C. E. 2002. Complex evolution of photosynthesis. *Annu. Rev. Plant Biol.* 53, 503–521.

Xu, L., Sun, J. Q., Wang, L. J., Liu, X. Z., Ji, Y.Y., Shao, Z. Q., Wu, X. L. 2017. *Aliidiomarina soli* sp. nov., isolated from saline-alkaline soil. *Int J Syst Evol Microbiol*. 67,724-728.

Xu, X. W., Wu, Y. H., Zhou, Z., Wang, C. S., Zhou, Y. G., Zhang, H., et al. 2007. *Halomonas saccharevitans* sp. nov., *Halomonas arcis* sp. nov. and *Halomonas subterranea* sp. nov., halophilic bacteria isolated from hypersaline environments of China. *Int. J. Syst. Evol. Microbiol.* 57, 1619–1624. doi: 10.1099/ijjs.0.65022-0.

Yee N., Fein J. B., Daughney C. J. 2000. Experimental study of the pH, ionic strength, and reversibility behavior of bacteria-mineral adsorption. *Geochim. Cosmochim. Acta* 64, 609–617.10.1016/S0016-7037(99)00342-7.

Yokoo R., Hood R.D., Savage, D.F. (2015). Live-cell imaging of cyanobacteria. *Photosynth Res.* 126, 33– 46. doi: 10.1007/s11120-014-0049-x.

Yoon, J. H, Jung, Y.T., and Lee, J. S. 2013. *Loktanella litorea* sp. nov., isolated from seawater. *Int J Syst Evol Microbiol*. 63,175–180.

Yoon, J. H., Lee, M. H., and Oh, T. K. 2004. *Porphyrobacter donghaensis* sp. nov., isolated from sea water of the East Sea in Korea. *Int. J. Syst. Evol. Microbiol.* 54, 2231–2235. doi: 10.1099/ijjs.0.63226-0

Zhang F., Xu H., Konishi, H., Roden, E. E. 2010. A relationship between d104 value and composition in the calcite-disordered dolomite solid-solution series. *American Mineralogist*. 95, 1650-1656.

Zhang, N., Xiao, X., Pei, M., Liu, X., and Liang, Y. 2017. Discordant temporal turnovers of sediment bacterial and eukaryotic communities in response to dredging: nonresilience and functional changes. *Appl. Environ. Microbiol.* 83. doi: 10.1128/aem.02526-16.

Zhang, X., Xu, S., Li, C., Zhao, L., Feng, H., Yue, G., et al. 2014. The soil carbon/nitrogen ratio and moisture affect microbial community structures in alkaline permafrost-affected soils with different vegetation types on the Tibetan plateau. *Research in Microbiology*. 165, 128-139. doi: 10.1016/j.resmic.2014.01.002.

Zhang, Z. Y., Chen, W. H., Zou, X., Han, Y. F., Huang, J. Z., Liang, Z. Q., et al. 2019. Phylogeny and taxonomy of two new *Plectosphaerella* (*Plectosphaerellaceae*, *Glomerellales*) species from China. *MycKeys*. 57, 47–60. doi: 10.3897/mycokeys.57.36628.

Zhong, Z. P., Liu, Y., Hou, T. T., Zhou, Y. G., Liu, H. C., Liu. Z. P. 2015. *Belliella aquatica* sp. nov., isolated from a saline lake. *Int J Syst Evol Microbiol*. 65, 1622-1627. doi: 10.1099/ijs.0.000148.

Zhu, T. and Dittrich, M. 2016. Carbonate Precipitation through Microbial Activities in Natural environment, and Their Potential in Biotechnology: A Review. *Frontiers in Bioeng. & Biotechnol.* 4, 1–21. doi: 10.3389/fbioe.2016.00004.

Zolitschka, B., Schäbitz, F., Lücke, A., Clifton, G., Corbella, H., Ercolano, B., et al. 2006. Crater lakes of the Pali Aike Volcanic Field as key sites of paleoclimatic and paleoecological reconstructions in southern Patagonia, Argentina. *J. South Am. Earth Sci.* 21, 294–309. doi: 10.1016/j.jsames.2006.04.001.