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Limnological characterization of the water, algae and mud resources used in Copahue Thermal Complex (Neuquén, Argentina)

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Abstract. We carried out the physicochemical characterization of extreme environments of acidic geothermal springs of the Copahue Thermal Complex, and isolated and cultivated algae used for therapeutic and medicinal purposes. Ecophysiological aspects, algal photosynthetic responses and potential toxicity of sediments were analysed. At the 15 sampling sites (pH: 2.0-6.7, conductivity: 283-3230 µS/cm, temperature: 22-60 °C), 11 Cyanobacteria species of a total of 24 algae were identified. The species richness was low with true inhabitants of highly acidic waters: *Cyanidium caldarium*, *Euglena mutabilis*, *Chlamydomonas acidophila*, *Achnanthidium minutissimum,* and *Eunotia exigua,* and cosmopolitan species of thermal springs: *Mastigocladus laminosus*, *Leptolyngbia boryana* and *Phormidium tergestinum*. All the species were well adapted to low light levels (15-55 µmol photon.m^{-2}.s^{-1}), and photosynthetic responses were similar to those in comparable environments. pH and temperature were important factors in algal distribution, and N:P relationship suggests that P is the limiting nutrient for algal growth. Fractions controlling P availability in the muds were those bound to organic matter, and Fe/Al oxyhydroxides. Healing muds have the ability to precipitate metals and would not be toxic. Cadmium and other potentially toxic metals were in very low concentrations and pose no risk for the human use as healing muds and bathing.

[Keywords: acidic algae, thermal algae, healing muds, hot springs, extreme environments]

Resumen. **Caracterización limnológica del agua, las algas y los barros utilizados en el Complejo Termal Copahue (Neuquén, Argentina)**. Se realizó la caracterización físico-química de los ambientes extremos (aguas termales y barros) del Complejo Termal Copahue, y se aislaron y cultivaron algas empleadas con propósitos medicinales y terapéuticos. Se analizaron aspectos ecofisiológicos de las especies y sus respuestas fotosintéticas, así como la potencial toxicidad de los barros. En los 15 sitios muestreados (pH: 2.0-6.7, conductividad: 283-3230 µS/cm, temperatura: 22-60 °C) se identificaron 11 especies de cianobacterias de un total de 24 especies algales. La riqueza específica fue baja, con especies representativas de ambientes ácidos: *Cyanidium caldarium*, *Euglena mutabilis*, *Chlamydomonas acidophila*, *Achnanthidium minutissimum* y *Eunotia exigua,* y especies cosmopolitas de aguas termales: *Mastigocladus laminosus*, *Leptolyngbia boryana* y *Phormidium tergestinum*. Todas las especies mostraron estar bien adaptadas a bajas intensidades lumínicas (15-55 µmol fotones.m^{-2}.s^{-1}) y sus respuestas fotosintéticas resultaron comparables a las de ambientes similares. El pH y la temperatura fueron factores importantes en la distribución de las algas, y la alta relación N:P sugiere que el P es el nutriente limitante del crecimiento algal. Las fracciones que controlaron la disponibilidad de P en los sedimentos fueron las de materia orgánica y de oxihidróxidos de Fe/Al. Los barros terapéuticos mostraron capacidad para precipitar los metales y no serían tóxicos. El Cd y otros metales potencialmente tóxicos estuvieron presentes en muy bajas concentraciones y no representarían un riesgo para uso humano como barros curativos y baños.

[Palabras clave: algas acidófilas, algas termales, barros terapéuticos, aguas termales, ambientes extremos]

INTRODUCTION

Thermoacidic water bodies are extreme environments scattered on all continents and on many islands belonging to geologically active regions that have geothermal activity and low pH levels, characterized by waters that severely limit the growth of photoautotrophic organisms (Toplin et al. 2008). Hot springs are mainly associated with current or recent volcanic activity and have characteristic environmental conditions such as temperatures that can range between 35 and 110 °C and low pH levels due primarily to the strong predominance of sulphur (Pentecost et al.

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2003; Toplin et al. 2008). Thermal springs are, therefore, biologically significant in that they provide critical habitat for clusters of species, many of which are ecologically restricted by narrow thermal tolerances. Studies of similar systems on different continents have revealed a dominance of microbial mats comprising a diversity of prokaryotic organisms, mainly cyanobacteria (Whitton and Potts 2002; Sompong et al. 2005).

Since early history thermal resources have been used as therapeutic agents all over the world, especially hot springs as healing baths (Monasterio 2012). Balneotherapy is the use of baths containing thermal mineral waters from natural hot springs for the treatment and cure of diseases. People have used geothermal water and mineral water for cooking, bathing, and medical purposes. It is known that the presence of various elements and ions, especially sulphur and sulphate ion make hot springs water suitable for medical purposes mainly for skin therapy (de Michele et al. 2008).

Peloids are natural muds that settle on the bottom of water bodies, with certain thermal, physico-chemical and antimicrobial properties (de Michele et al. 2008) deriving from geological, climatic, hydrological, biological and physicochemical factors. However, to acquire such a condition they must have the optimum properties for medical or cosmetic applications. For centuries healing thermal muds have been used as a treatment for psoriasis and acne, and the beauty industry has used the mud-clay for the preparation of musk soaps and baths (Rasskazov et al. 2017).

In Argentina, the Copahue Thermal Complex (CTC) is one of the most important thermal resources in the country with international reach due to the variety of its muds, fumes, algae and waters. In recent years, some studies showed the different mechanisms explaining the therapeutic effects of thermal resources in the Copahue region, especially for their benefit on different respiratory, dermatological, muscular and osteoarticular diseases (Monasterio 2012). De Michele et al. (2008) have focused on the bactericidal effects of the mud liquid phase on *Staphylococcus aureus*, *S. epidermis*, *Enterococcus faecalis* and *Candida albicans* strains from the skin, nasal, intestinal and vaginal microbiota. Ubogui et al. (2008) found a 40% improvement of the psoriasis in just 7 days using a suspension of sulfur gray peloid and thermophilic algae extracted from Laguna Verde. Some mineralogical, chemical and textural characteristics of natural clayey-sulphurous peloids were carried out (Baschini et al. 2010) as well as hidrogeochemical studies that compared the variations of SO_4^{-2}, SO_4^{-2}/Cl^- and rare earth composition in the water, before and after the last eruptive cycle of Copahue volcano in December 2012 (Gaviria Reyes et al. 2016). Other authors investigated the great biodiversity of chemolithoautotrophic and heterotrophic organisms: archaea, bacteria and fungi (Chiacchiarini et al. 2010; Urbieta et al. 2015). Although mat forming cyanobacteria are the dominant photoautotrophic organisms in Copahue thermal environments, there are still very few records about their diversity and ecophysiology (Flores Melo et al. 2019).

This paper presents information on the characterization of the thermal resources (muds, algae and water) in the CTC, which are used for balneotherapy and mud therapy. We have sought to contribute to the characterization of representative hot springs and ponds from a limnological point of view regarding the use of their resources. The objectives were to carry out the physico-chemical characterization of water bodies and muds, and to isolate and cultivate algae —acidic and thermophilic species— belonging to different groups of the autotrophic community. Algal photosynthetic response to experimental light conditions were evaluated, and ecophysiological aspects of Copahue acid-thermophilic phycoflora and sediments potential toxicity were also studied.

Materials and Methods

The CTC is located at Copahue Provincial Park, Neuquén province, northwest of Argentine Patagonia (Figure 1). The Thermal Complex region is located in a shallow canyon, at 2010 m above sea level, 37°49' S and 71°06' W, covering an extension of 600 m E-W and between 150 and 200 m N-S. The Park has numerous surface geothermal manifestations of volcanic origin, three of which are part of the CTC: boiling pools (olletas), fumaroles and small vents, in a muddy clay soil. The different environments present at CTC area are used differently from a medicinal point of view (Table 1). In general, water from hot springs is used for drinking purposes or for healing treatments, while ponds are used for public bathing or as a source of mud, algae or water for treatments inside the Complex. Algal and water samples were taken on February 2012 and muds were sampled on April 2018 (full description in Supplementary Material).

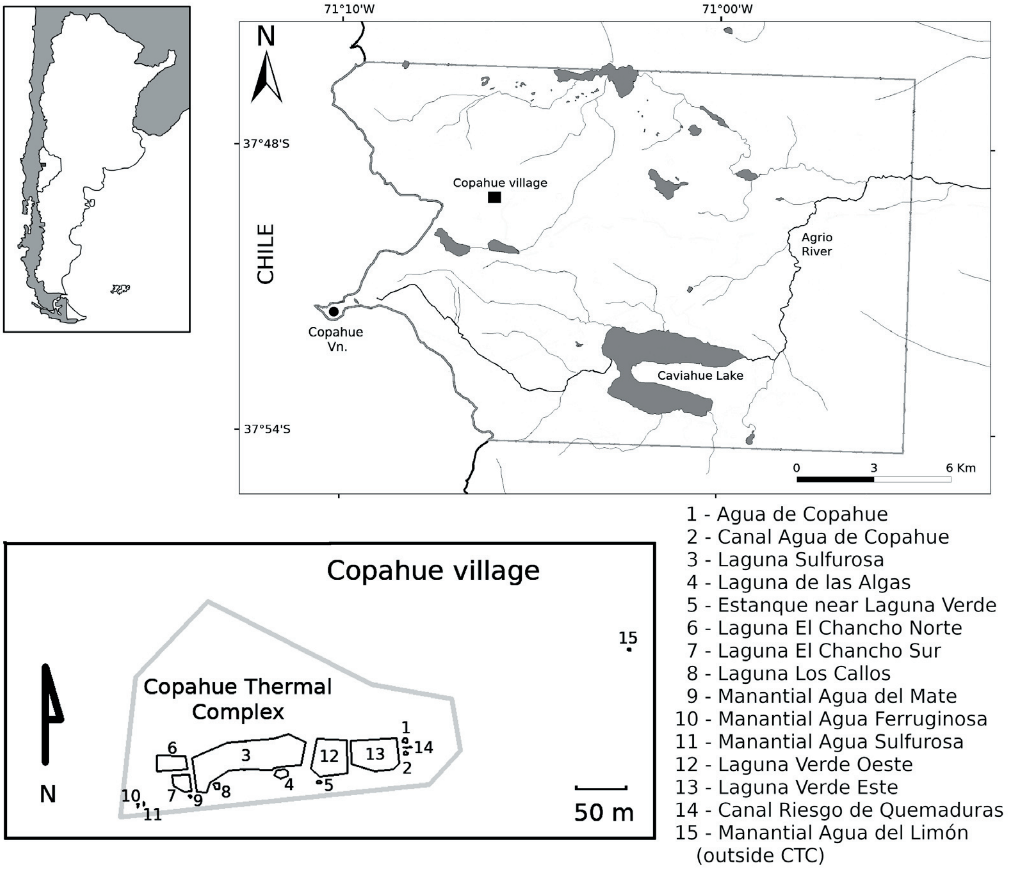


Figure 1. Map of CTC and its location in the Neuquén province, Argentina. The sampling sites are numbered and described in Table 1.

Figura 1. Mapa del Complejo Termal Copahue y su ubicación en la provincia de Neuquén, Argentina. Los sitios de muestreo están indicados con números y se describen en la Tabla 1.

Water and algae samples

In each thermal environment of the CTC (Figure 1), basic *in situ* variables were measured and algae samples were taken from all of them. Water samples for chemical characterization were taken from Laguna Verde Oeste and Laguna de las Algas (full description in Supplementary Material). In the laboratory, the following chemical variables were analysed in the water samples: soluble reactive phosphorus (SRP), total phosphorus (TP), total nitrogen (TN), nitrates ($N\text{-}NO_3^-$), nitrites ($N\text{-}NO_2^-$) and ammonium ($N\text{-}NH_4^+$). A full methodological description is given in Supplementary Material.

Algae samples were taken with spatula and/or with a 10 µm pore plankton net according to the characteristics of each site (full description in Supplementary Material). A total of 15 integrated samples were collected, one for each thermal manifestation, and were divided into two parts: one was fixed in 4% formalin for taxonomic identifications and the other remained unfixed for isolates and cultures. The isolated algae were grown in 50 mL Erlenmeyers in BG-11 culture medium for Cyanobacteria species (Andersen 2005), in Satake medium for *Cyanidium caldarium* and *Euglena mutabilis* (Satake and Saijo 1974) and Bold Basal medium (Andersen 2005) for *Parachlorella kessleri,* and kept under controlled conditions of light (90 µmol photons.$m^{-2}.s^{-1}$ of Photosynthetic Active Radiation [PAR]) and temperature (20±2 °C) in a NeoLine IncuLab 300 incubation chamber.

Algal species were identified under a light microscope (magnification 400 and 1000×) and with specialized literature for each algal group (full description in Supplementary Material). The AlgaeBase website (algaebase.org) was consulted for updated nomenclatural and systematics of taxonomic groups data. The samples were deposited in the herbarium of

Table 1. pH, electrical conductivity (EC) and water temperature at the sites sampled in CTC during February 2012. Uses: W1=drinking water with different purposes; W2=healing water; A=algae for different medical treatments; M=healing muds; B=bathing; X=not in use.

Tabla 1. Sitios muestreados en el Complejo Termal Copahue y valores de pH, conductividad y temperatura registrados durante el muestreo de Febrero 2012. Usos: W1=agua para consumo con diferentes propiedades; W2=agua para tratamientos curativos; A=algas para diferentes tratamientos médicos; M=barros curativos; B=baños curativos; X=no en uso.

N°	Sample Site	Environment	Coordinates	pH	EC (µS/cm)	T (°C)	Uses
1	Agua de Copahue o Vichy	Hot spring	37°49′5.1′′ S 71°05'48.4" W	6.1	874	40	W1
2	Canal Agua de Copahue	Hot spring	37°49′5.3′′ S 71°05'48.5" W	6.0	283	22	W1
3	Laguna Sulfurosa	Pond	37°49′5.9′′ S 71°05'54.8" W	3.4	2152	60	W2
4	Laguna de las Algas	Pond	37°49′6′′ S 71°05'54" W	5.8	1016	46	A
5	Estanque near Laguna Verde	Pond	37°49′6.2′′ S 71°05'51.9" W	5.5	752	50	A
6	Laguna El Chancho Norte	Pond	37°49′5.59′′ S 71°05'57.86" W	2.8	2911	35	M, B
7	Laguna El Chancho Sur	Pond	37°49′6.4′′ S 71°05'57.2" W	6.0	1266	57	X
8	Laguna Los Callos	Pond	37°49′6.4′′ S 71°05'56.1" W	5.9	1003	39	W2
9	Manantial Agua del Mate	Hot spring	37°49′6.8′′ S 71°05'57.3" W	6.3	1220	50	W1
10	Manantial Agua Ferruginosa	Hot spring	37°49′3.3′′ S 71°05'59.1" W	6.1	1722	55	W1
11	Manantial Agua Sulfurosa	Hot spring	37°49′7.1′′ S 71°05'59.7" W	6.7	1311	44	W1
12	Laguna Verde Oeste	Pond	37°49′5.44′′ S 71°05'51.45" W	2.6	2131	27	A, B
13	Laguna Verde Este	Pond	37°49′5.38′′ S 71°05'49.5" W	2.5	2242	25	X
14	Canal Riesgo de Quemaduras	Hot spring	37°49′5.1′′ S 71°05'48.45" W	2.3	3230	42	W1
15	Manantial Agua del Limón	Hot spring	37°49′0.65′′ S 71°05'41.2" W	2.0	1312	52	W1

the Centro Regional Universitario Bariloche (BCRU) (Universidad Nacional del Comahue, Argentina).

Photosynthesis measurements

Six algal species were isolated and cultivated: *Chroococcus membraninus*, *Mastigocladus laminosus*, *Kamptonema animale*, *Cyanidium caldarium*, *Parachlorella kessleri* and *Euglena mutabilis*, and respiration and photosynthesis rates (PS) were measured. PS were determined as oxygen exchange by using an oxygen micro-sensor (Microx TX3, Presens, Germany). Three replicates of each algal species assayed were incubated in a 2 mL chamber at 20 °C. To avoid oxygen micro-gradients inside the chamber, gentle stirring was provided by a magnetic stirrer. In order to estimate PS the samples were incubated at increasing levels of light irradiance, which ranged from 0 to 250 µmol photons.m^{-2}.s^{-1} of PAR, as determined with the LI-250A light-meter equipped with a spherical micro quantum sensor (US-SQS, Waltz, Germany). Each level of irradiance was applied until steady-state oxygen production was observed (c.a. 5 min).

Smith's equation (1936) was used to describe the relationship between photosynthesis and irradiance:

$$PS = (Pmax \cdot \alpha \cdot E) / (Pmax^2 + (\alpha \cdot E)^2)^{-1/2} + DR$$

In this equation, PS is the rate of photosynthesis per unit of chlorophyll a, Pmax is the maximum rate of gross photosynthesis, α is the initial slope of the P-E curve, E is the photon flux density, and DR is the rate of dark respiration. Ek was calculated as the ratio Pmax: α (Talling 1957) corresponding to the point at which the slope of the light saturation curve intersects the plateau.

Thermal muds

Thermal muds were obtained from the ponds used as a source of mud for therapeutic treatments: Laguna Verde Oeste and Laguna El Chancho Norte. Mud samples were extracted with 6-cm PVC pipes (one for each pond) and processed in the field laboratory one hour after they were obtained. Mud cores were sliced in three fractions of 2.5 cm and for each fraction pH and redox potential (Eh) values were recorded (ORION Model 920 A, with specifics electrodes). Each fraction was stored in 50 mL Falcon© tubes under nitrogen atmosphere and the dark at 4 °C and transported to INIBIOMA laboratories in Bariloche (71°18′ W - 41°9′ S). In the laboratory, mud samples were dried in an oven at 60 °C and subsequently filtered through a 500 µm mesh screen (Newark, ASTM Nº 36 USA Standard Series Sieves) to remove the less reactive coarse fraction. The following chemical analyses were performed on the fraction thus obtained: Total Phosphorus: digestion with concentrated H_2SO_4 and H_2O_2 30% (Carter and Gregorich 2006). After digestion, the phosphorus content was determined following the Murphy and Riley method (1962). Hieltjes and Lijklema method (1980) was used for the fractionation of phosphorus. Total Nitrogen and Total Carbon: with an automatic C and N analyser (Thermo Flash EA 1112). The elemental chemical composition was determined by using SEM-EDAX analysis (Philips 515-EDAX Genesis 2000), and mineralogy was determined by using X-ray diffraction using a PANanalitical diffractometer.

Simultaneously extracted metals and acid-volatile sulphides. This approach was used to assess the content of potentially toxic metals. Simultaneously extracted metals (SEM) and acid-volatile sulphides (AVS) extraction was performed with a corer from the three strata following US-EPA method (Allen et al. 1993). AVS content was determined immediately by KI titration (APHA 1998). Acid extracts were centrifuged at 4000 rpm, separated from the pellet with a Pasteur pipette and a 0.45 µm pore membrane filtration. The concentrations of Cu, Fe, Cr, Cd, Zn, Pb, Mn, Mg and Ca in the extracts were determined by flame atomic absorption spectroscopy (FIAS; Analyst 100, Perkin Elmer, USA).

Results

The geographical coordinates for all sites were recorded in Table 1. The physico-chemical variables measured *in situ* (Table 1) show that the different environments sampled in the CTC present a variety of manifestations characterized by the wide range of pH (2.0-6.7), electrical conductivity (283-3230 µS/cm) and temperature (22-60 °C), despite the relatively small area where they are located (3.4 ha).

The light measurements in Laguna Verde Oeste and Laguna de las Algas at different depths showed values close to 2500 µmol photons.$m^{-2}.s^{-1}$ of PAR in the air. The intensity decreases up to 8 times in the first centimetre of depth (205-376 µmol photons.$m^{-2}.s^{-1}$) and has values close to 140 µmol photons.$m^{-2}.s^{-1}$ at 60 cm depth. According to Secchi depth (reading of 25 cm), 1% of the light at surface penetrates to a depth of 75 cm.

Dissolved nutrients in pond water showed concentrations of 13 and 10 µg/L for SRP, 35 and 41 µg/L for $N\text{-}NO_3^- + N\text{-}NO_2^-$, and 17 and 13 mg/L for $N\text{-}NH_4^+$ in Laguna Verde Oeste and Laguna de las Algas, respectively. Total nutrients were 0.3 and 0.2 mg/L for TP, and 17 and 14 mg/L for TN, respectively.

The algal species found in each environment (Table 2) were mainly represented by 11 Cyanophyceae species of a total of 24. Other algal groups with at least one species were registered in the area: Bacillariophyceae, Cyanidiophyceae, Euglenophyceae, Trebouxiophyceae, Zygnematophyceae, Ulvophyceae and Chlorophyceae.

Photosynthesis

Changes in photosynthesis vs irradiance (P-E curves) for the six algae species isolated from the CTC and the parameters describing those curves are shown in Figure 2. Pmax varied 3 orders of magnitude ranging between 71 and 25529 (µmol O_2.(mg Chl *a*)$^{-1}$.h^{-1}) for *P. kessleri* and *C. membraninus*, respectively. The highest value of the initial slope (α) was found in *C. membraninus* (972.9 µmol O_2.(mg Chl *a*)$^{-1}$.h^{-1}.µmol photon.$m^{-2}.s^{-1}$), and the lowest one, in *M. laminosus* (1.7 µmol O_2.(mg Chl *a*)$^{-1}$.h^{-1}.µmol photon.$m^{-2}.s^{-1}$). Respiration rate (DR) followed the same pattern as Pmax, varying between 19 and 5234 µmol O_2.(mg Chl *a*)$^{-1}$.h^{-1} for *P. kessleri* and *C. membraninus*, respectively. Light intensity at which the slope of the light saturation curve intersects the plateau (Ek) was relatively low for all the species varying between 15 and 55 µmol photon.$m^{-2}.s^{-1}$ for *C. caldarium* and *M. laminosus*, respectively.

Thermal muds

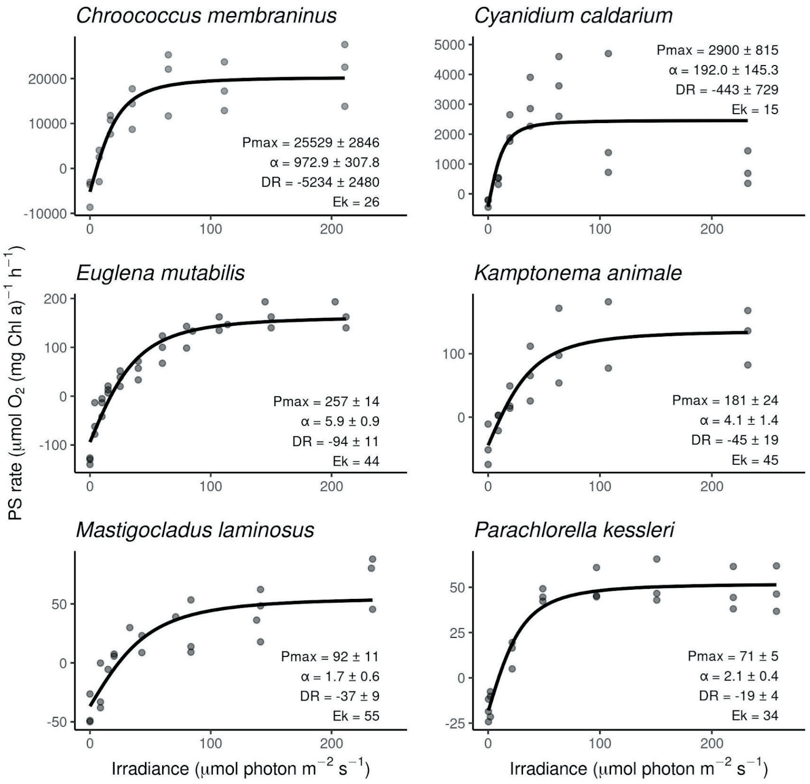
The total nutrient concentrations (P, N and C) in Laguna Verde Oeste mud was 1.3 mg/g dw for TP, 1 mg/g dw for TN and 9 mg/g dw for TC. The values in Laguna El Chancho Norte were of the same magnitude, 0.4, 12 and 72 mg/g dw for TP, TN and TC, respectively. The labile P in the mud and the fraction of P

Table 2. Relative abundance of species found in different environments of CTC in February 2012. ±: scarce; +: frequent; ++: abundant; +++: very abundant. The numbers in columns correspond to the environments shown in Figure 1 and listed on Table 1.

Tabla 2. Abundancia relativa de las especies encontradas en los diferentes ambientes del Complejo Termal Copahue en febrero 2012. ±: escasa; +: frecuente; ++: abundante; +++: muy abundante. Los números de las columnas se corresponden con los ambientes mostrados en la Figura 1 y en la Tabla 1.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Cyanophyceae															
Chroococcidiopsis thermalis Geitler	±	±			±						±				
Chroococcus membraninus (Meneghini) Nägeli				±											
Kamptonema animale (C. Agardh ex Gomont) Strunecký, Komárek and J. Smarda	±						+								
Kamptonema formosum (Bory ex Gomont) Strunecký, Komárek and J. Smarda				±			+				±				
Komvophoron jovis (Copeland) Anagnostidis and Komárek				±							±				
Leptolyngbya boryana (Gomont) Anagnostidis and Komárek				±	+					+					
Mastigocladus laminosus Cohn ex Kirchner	+	+		+	++		+		++	++	++				
Oscillatoria subbrevis Schmidle	+	+					+		+						
Phormidium tergestinum (Rabenhorst ex Gomont) Anagnostidis and Komárek	+	+		+	+										
Phormidium thermobium Anagnostidis				±	+		+	+++							
Spirulina gracilis Gruia		±		±											
Cyanidiophyceae															
Cyanidium caldarium (Tilden) Geitler			+++			+++							±	+++	+++
Euglenophyceae															
Euglena mutabilis F. Schmitz	±					+							±		
Chlorophyceae															
Chlamydomonas acidophila Negoro												±	±		
Scenedesmus sp.									±						
Oedogonium sp.									±						
Trebouxiophyceae															
Parachlorella kessleri (Fott and Nováková) Krienitz, E. H. Hegewald, Hepperle, V. Huss, T. Rohr and M. Wolf		±				±	±		+		+	+++	+++		
Zygnematophyceae															
Cosmarium sp.	±	±					±								
Ulvophyceae															
Ulothrix sp.	±											±	±		
Bacillariophyceae															
Achnanthidium minutissimum (Kützing) Czarnecki		±	±									±		±	
Eunotia exigua (Brébisson ex Kützing) Rabenhorst		±									±			±	
Eunotia spp.		±		±					±			±		±	
Nitzschia spp.		±		±							±			±	
Pinnularia spp.												±	±		

bound to the Al and Fe (9 and 225 µg/g dw) in Laguna Verde Oeste were of the same order of magnitude as those in Laguna El Chancho Norte (2 and 119 µg/g dw). On the other hand, the fraction of P bound to organic matter (OM) turned out to be the most important in both ponds (953 µg/g dw in Laguna Verde Oeste and 247 µg/g dw in Laguna El Chancho Norte). The fraction of P bound to Ca compounds was higher in the mud of Laguna Verde Oeste (138 µg/g dw) than that of Laguna El Chancho Norte (24 µg/g dw).



Pmax: µmol O_2.(mg Chl *a*)$^{-1}$ .h^{-1};
α: µmol O_2.(mg Chl *a*)$^{-1}$.h^{-1} .µmol photon.m^{-2}.s^{-1};
DR: µmol O_2.(mg Chl *a*)$^{-1}$.h^{-1};
Ek: µmol photon.m^{-2}.s^{-1}

Figure 2. Photosynthesis-Irradiance curves of different algae species from CTC. The lines represent the fitted data (grey dots) to Smith's equation (see text).

Figura 2. Curvas fotosíntesis-irradiancia para diferentes especies algales del Complejo Termal Copahue. Las líneas representan los ajustes a la ecuación de Smith (ver en el texto) de los datos (puntos).

Table 3. pH, redox potential (Eh), acid-volatile sulfide (AVS), simultaneously extracted metals (SEM), the ratio SEM/AVS and concentration of metals in each sediment stratum analysed. AVS, SEM, and metals were expressed as the mean ± SD of 3 replicates in samples taken in April 2018.

Tabla 3. Valores de pH, potencial redox (Eh), sulfuros ácido-volátiles (AVS), metales extraídos en simultáneo (SEM), relación SEM/AVS y concentración de metales en cada estrato de los sedimentos analizados. AVS, SEM y los metales se expresan como el promedio ± el desvío estándar de 3 repeticiones para las muestras tomadas en abril 2018.

	Laguna Verde Oeste			Laguna El Chancho Norte		
	0-2.5 CM	2.5-5 CM	5-7.5 CM	0-2.5 CM	2.5-5 CM	5-7.5 CM
pH	2.8	4.9	5.6	2.1	2.2	2.5
Eh (mV)	-8.0	-87.3	-229.2	109.0	38.8	-11.7
AVS (µg/g)	2562±407	663±63	121±25	97±6	667±182	247±33
SEM (µg/g)	7.5±3.3	14.2±0.5	5.3±1.4	4.1±1.3	3.3±0.7	4.5±1.1
SEM/AVS	3	21	43	42	5	18
Cu (µg/g)	1.2±0.1	1.3±0.1	1.6±1.2	<0.9	<0.9	<0.9
Fe (µg/g)	63±1	200±16	57±11	69±7	114±9	102±9
Pb (µg/g)	<5	<5	<5	<5	<5	<5
Cr (µg/g)	<4	56±6	33±4	8±4	11±2	18±4
Zn (µg/g)	2±0.1	11±0.3	2±0.1	2±0.3	2±0.2	2±0.5
Mg (µg/g)	112±12	164±3	96±3	64±3	98±3	75±3
Ca (µg/g)	29±1	821±12	515±37	18±1	14±1	16±1

The elemental composition of the peloids from Laguna Verde Oeste was represented by Si (47.4%)>>B>Al>S>Fe>K=Ti>Ca>Na>>Mg (0.3%), while it was completely different from those from Laguna El Chancho Norte: B=S (43 %)>>Si>Al>Fe>Ba>Ti>Ca=K>>Mg (0.1%). The main minerals that constitute Laguna Verde Oeste muds were covellite (CuS) in 79% and albite ($AlNa\ O_8Si$) 18%, and differ from the minerals found in Laguna El Chancho Norte thermal muds, which are sulphides (S_8) 79%, iron phosphate ($FePO_4$) 9%, lautite (AsCuS) 7% and pyrite (FeS_2) 5%.

The pH of Laguna Verde Oeste muds analysed by strata (Table 3) increased with depth from 2.8 to 5.6, while the Eh decreased inversely from -8 to -229.2 mV. AVS decreased with depth from 2562 to 121 μg/g, while SEM content was higher between 2.5 and 5.0 cm depth (14.2 μg/g) (Table 3). In the three strata studied, the SEM/AVS ratio was always significantly lower than 1. In Laguna El Chancho Norte thermal muds pH was similar in the three strata and Eh was positive in the upper strata and negative in the deepest one. The AVS in Laguna El Chancho Norte was lower than in Laguna Verde Oeste (Table 3) as well as the SEM, although the SEM/AVS ratio was equally low and much less than 1. The content of each metal was likewise very low (Table 3), including Mg, Ca and Fe. Lead was below the limit of detection in both muds, and Cu was below the limit of detection in Laguna El Chancho Norte and slightly above in Laguna Verde Oeste. Chromium was the most abundant trace metal.

Discussion

Water and algae

At the 15 sampling sites, species distribution is mainly determined by pH. The 11 identified cyanobacterial species predominated in environments with a pH higher than 5.8 (Laguna de las Algas), while the 13 eukaryotic algal species were present in water bodies along the pH range registered (2.0-6.7). The lowest pH required for cyanobacteria to develop is 4.0 (Whitton and Potts 2002). This is because at low pH, inorganic carbon is present as CO_2 with virtually no bicarbonate ($HCO_3^=$) ions and the fact that cyanobacteria are adapted to living in low concentrations of CO_2 and high $HCO_3^=$ (Gross 2000).

The other determinant of algal community structure in Copahue hot springs is temperature. The temperature range appropriate for Cyanobacteria growth is significantly wider than that for other algae and, in general, the optimum temperature is close to 35 °C (Fogg et al. 1973). The upper temperature limit is close to 73 °C, and it is less than the limit for non-photosynthetic bacteria. In the ponds and hot springs studied in Copahue, temperature did not exceed 61 °C. Eukaryotic algae were found in the colder waters except for rhodophyte *Cyanidium caldarium*, while thermotolerant prokaryotes were dominant in warmer waters. This is a species distribution well reported in volcanic areas around the world (Whitton and Potts 2002; Toplin et al. 2008).

Dissolved nutrients (P and N) and conductivity (EC) were unlikely to be as important as pH and temperature in algal species distribution in Laguna Verde Oeste and Laguna de las Algas (10-13 μg/L of SRP, 35-41 μg/L of $NO_3^-+NO_2^-$, 13-17 mg/L of NH_4^+ and EC: 283-3230 μS/cm). Most values are lower than those in similar volcanic regions around the world with thermo-acidic algae: Thailand: 0.13-1.5 mg/L of SRP, 0.3-8.4 mg/L of NO_3^-, 0.06-2.75 mg/L of NH_4^+, EC: 337-705 μS/cm (Sompong et al. 2005), Saudi Arabia: 8 mg/L of SRP, 7 mg/L of NO_3^-, 46 mg/L of NH_4^+ and EC: 3200 μS/cm (Mohamed 2008) or Bulgaria: 7 μg/L of SRP, 2 mg/L of NO_3^- and EC: 85 μS/cm (Lukavský et al. 2011).

The greatest TN contribution comes mainly from ammonium (94 and 99% of TN_{avg}=15.5 mg N/L), which resulted in a concentration three orders of magnitude higher, with respect to nitrates and nitrites, the latter representing a non-significant contribution of dissolved inorganic nitrogen. In Copahue hot springs, ammonium concentrations are both the result of natural volcanic contribution, as it occurs in Río Agrio sources (Pedrozo et al. 2008) where similar values of N were recorded, and the metabolic processes of the phycoflora in the lentic, natural or artificial systems of the thermal complex. Ammonium was an order of magnitude higher in Copahue (on average 15 mg N/L) than in other extreme environments as acidic mining lakes in Germany (3.3 mg N/L) (Nixdorf et al. 1998) or Yellowstone in USA (1.4 mg N/L) (Doemel and Brock 1971).

The dissolved N:P ratio is used to predict limiting macronutrients. An N:P <7 is indicative of N limitation and if N:P >7 then P is suggested as the limiting nutrient for algal growth (Reynolds 2006). The N:P ratio in Copahue environments showed values much higher than 7 (1364 in Laguna de las Algas and 1260 in Laguna Verde), mainly explained by the ammonium content, suggesting that the limiting nutrient for algal growth is P. The N:P ratio was very high in Copahue water bodies compared with values calculated for the natural volcanic environments of Yellowstone Park (1.5) (Doemel and Brock 1971), Saudi Arabia (6.6) (Mohamed 2008) or Bulgaria (0.1) (Lukavský et al. 2011), suggesting N is the limiting nutrient.

The species richness in Copahue area was low compared with the 453 cyanobacteria found in neutral or alkaline hot springs in Yellowstone National Park (Copeland 1936) and in most of the volcanic areas around the world (Lukavský et al. 2011). Some of the species found in Copahue are true inhabitants of highly acidic waters: *Cyanidium caldarium*, *Euglena mutabilis*, *Chlamydomonas acidophila* (Gross 2000), the diatoms: *Achnanthidium minutissimum, Eunotia exigua, Nitzschia* spp., *Pinnularia* spp. (DeNicola 2000; Urbieta et al. 2015) and cosmopolitan species of thermal springs: *Mastigocladus laminosus*, *Leptolyngbia boryana* and *Phormidium tergestinum* (Flores Melo et al. 2019). None of Copahue cyanobacteria have been mentioned in the literature as toxic but the toxin production of cyanobacteria in hot spring bath waters is poorly explored (Mohamed 2008).

One of the most used thermal resources in Copahue is the highly productive Laguna Verde Oeste, its warm waters for bathing and its algae for dermatological uses (Monasterio 2012). The dominant species *P. kessleri* maintains a natural monoculture with high cell abundance (20×10^6 cells/mL) throughout the year (Juarez and Vélez 1993). The strain of *P. kessleri* isolated from Copahue produces extracellular compounds with antimicrobial activity (Juarez and Accorinti 1995) and has antioxidant defences in its cells (Sabatini et al. 2009). Other strain of the species (CGMCC No. 4917) showed a highly efficient lipid production in the dark (Wang et al. 2012). Algae concentrates are used for the treatment of psoriasis, dermatitis and eczemas, which are alleviated by the antihistamine, anti-inflammatory and antibacterial properties of *P. kessleri* (Ubogui et al. 2008; Monasterio 2012).

Other algae concentrate used for relaxing, anti-stress and dermatological treatments is collected from Laguna de las Algas (Monasterio 2012). In this case, 8 cyanobacteria species intervene in the process, *M. laminosus* and *L. boryana* being the dominant. *M. laminosus* has been recommended to be used on the skin in phycotherapy for its antibiotic effects (Accorinti and Wenzel 1991) but little is known about the individual effect of the other 7 species (and the interaction with local bacterial community) used for dermatological treatments in CTC.

The spring waters of CTC are used to make healing drinks: Agua Sulfurosa, Agua Ferruginosa, Agua de Copahue, Agua del Mate and Manantial Agua del Limón (termasdecopahue.gob.ar/tecnicas-termales). An example of algae present in these environments is *C. caldarium*, the only inhabitant in the spring Manantial Agua del Limón. This hot spring waters are prescribed for patients with digestive and hypercholesterolemia problems (Monasterio 2012). The waters of the Agua del Mate spring, where at least six microalgae species develop, with a temperature of 50 °C, are used for infusions (Monasterio 2012).

Photosynthesis

The photosynthetic response of algae from the CTC generally showed similarities with what was reported by other authors in comparable environments, except for the cyanobacteria *Ch. membraninus* and the red algae *C. caldarium*. Values of Pmax obtained for *E. mutabilis* from Copahue were comparable with those recorded by Sittenfeld et al. (2002) for a closely related strain of *Euglena*, found in an acidic and thermal pool in a volcanic area in Costa Rica. Meanwhile, Pmax, α, DR and Ek values reported by Souza-Egipsy et al. (2011) for *E. mutabilis* from the Río Tinto (an extreme acidic river, Spain), were slightly higher than those obtained in our measurements. These authors reported photosynthetic parameters for a green alga (*Chlorella* sp. from the Río Tinto), which could be compared with *P. kessleri* of Laguna Verde, but its values were also somewhat higher than our measurements. When the photosynthetic response of *P. kessleri* from Copahue is compared with that of other green algae very frequently found in acidic environments, *Chlamydomonas reinhardtii* or *C. acidophila*, similar results are obtained although *P. kessleri* values were somewhat lower than those for *Chlamydomonas* (Neale and Melis 1986; Gerloff-Elias et al. 2005). In the two Copahue cyanobacteria, *K. animale* and *M. laminosus*, the photosynthetic responses were comparable with and in the same range as those for *E. mutabilis* or *P. kessleri*. All the species tested showed to be well-adapted to low light levels, since the Ek values obtained varied between 15 and 55 μmol photon.m^{-2}.s^{-1}, being below the results obtained for the periphyton of Río Agrio (Baffico et al. 2004), a nearby acidic environment influenced by the Copahue volcano. They were also somewhat lower than values reported for algae from Río Tinto (Souza-Egipsy et al. 2011), for cyanobacterial mats in a hot spring in Yellowstone National Park (Revsbech and Ward 1984), or for filamentous green algae (*Zygogonium ericetorum*) in a mining lake in Germany (Kleeberg et al. 2006). This adaptation to low light intensities could be related to the fact that they inhabit environments with significant light irradiance (located at 2000 m above sea level), but with high attenuation, as shown by our light measurements.

Values of Pmax, α and DR for *Ch. membraninus* and *C. caldarium*, were one or more orders of magnitude higher than those obtained for the other species, which was striking. The explanation would be found in the relationship between chlorophyll *a* and the accessory pigments that participate in the photosynthesis process or in an exceptionally greater photosynthetic efficiency. In the first case, chlorophyll *a* is commonly used as a measure of pigment content in algae; however, accessory pigments can also contribute significantly to light absorption. Part of the photosynthetic apparatus of red algae and cyanobacteria are phycobiliproteins that aggregate into phycobilisomes (Kremer 1982; Albertano et al. 2000). The main changes in the amount of pigment in phycobilisomes during photoacclimation have been shown to occur due to changes in phycobilin amount rather than chlorophyll *a* amount in the reaction centres (MacIntyre et al. 2002). In a work on pigments and light response in cyanobacterial mats, Jørgensen et al. (1987) demonstrated that phycobiliproteins were the dominant active antenna pigment during photosynthesis and that they could efficiently derive the captured energy to both photosystem I and II, displacing chlorophyll *a* in importance. Therefore, it is possible to think that the photosynthetic efficiency of both species from Copahue was influenced by the phycobilins amount they possess. Since this amount is not included in Pmax, α or DR calculations, then these parameters had particularly high values only because they were relativized to a small amount of chlorophyll *a*.

In the second case, both species might have a very high photosynthetic efficiency probably because they are adapted to grow under low light conditions: sediments in Laguna del Chancho (*C. caldarium*), mixed with other cyanobacteria in Laguna de las Algas (*Ch. membraninus*). In *C. caldarium* and other Cyanidiales, a higher photosynthetic efficiency at low light intensities and photoinhibiton at high light intensities has been documented (Doemel and Brock 1971; Ford 1986; Reeb and Bhattacharya 2010; Thangaraj et al. 2011). While in *Ch. membraninus* from Laguna de las Algas, which was observed in the mats formed by several algae, it is possible to think of a similar adaptation to that one observed in the blooms of mat-forming *Microcystis aeruginosa*. *Microcystis* occurs mainly as a colonial form under natural conditions and develops surface blooms creating a steep light gradient due to self-shading. Increased photosynthetic efficiency as adaptation to the high light intensity (Raps et al. 1983), no apparent photoinhibition and greater efficiency in colonial than in unicellular forms has been reported for this cyanobacteria (Wu and Song 2008).

Thermal muds

The muds studied present a fine texture: dark green for Laguna Verde, and grey for Laguna El Chancho. From the thermal point of view, mud temperatures were lower in Laguna Verde than in Laguna El Chancho, coinciding with the results reported by Armijo et al. (2008). Mud pH values were below 4.4 in Laguna Verde, being less acidic than in Laguna El Chancho. Mud pH will depend on the geochemical characteristics of the water. In this sense, the values measured are in relation to the water pH in these same ponds (Armijo et al. 2008). On the other hand, the pH values of the muds studied were more acidic than those reported by Santana et al. (2004), who characterized and analysed different geothermal deposits in Andean areas of northern Chile and Ecuador, and recorded average pH values of 5.1.

TP concentration in the mud of Laguna Verde presented similar values to those reported by Temporetti et al. (2014) for the sediments of Lake Caviahue, which is part of the same volcanic system. The autochthonous contribution of TP in the shallow lake is high due to the dead and precipitated algae. On the other hand, Laguna El Chancho mud presented very low concentrations of this nutrient when compared with the measurements in Laguna Verde and in Lake Caviahue. Labile P fraction in the muds, which represents the most readily available source of P for algal growth (Golterman 2004), was higher in Laguna Verde than in Laguna El Chancho, according to the TP values obtained and reflecting the high productivity of Laguna Verde. In both muds, the two predominant P fractions were (in order of importance) the one bound to OM and the Fe/Al oxy-hydroxides, indicating that these two fractions control P availability in both muds studied. Similar results were reported for Lake Caviahue sediments by Temporetti et al. (2014). TN and TC concentrations showed an inverse behaviour regarding TP. The concentrations of these two nutrients measured in Laguna El Chancho mud were in the same order of magnitude as those recorded by Temporetti et al. (2014) for Lake Caviahue, and higher than those measured in Laguna Verde mud.

The elemental composition reflected that the muds of both ponds are different. Laguna Verde mud was mainly characterized by silicon oxides and to a lesser extent by Al, Fe and sulphur oxides. On the other hand, Laguna El Chancho mud was characterized by the predominance of sulphur oxides and to a lesser extent by silicon, Al and Fe oxides. In this sense, Laguna Verde mud has a chemical composition similar to that reported for Lake Caviahue sediment (Temporetti et al. 2014; Cabrera et al. 2020). In the mineralogy of both muds studied, the minerals associated with sulphur compounds (covalite in Laguna Verde and sulphur in Laguna El Chancho) predominated, giving it the characteristic smell of 'rotten eggs'. Armijo et al. (2008) registered sulphur and pyrite minerals for Laguna El Chancho mud, in accordance with our results. In Laguna Verde, AVS concentration was very high in the surface decreasing to 5% in the deepest stratum. Since the waters are of sulphurous origin, this is likely a consequence of excess H_2S rather than biological activity; sulphides are expected to be in their protonated form. The SEM/AVS ratio was well below 1 in all cases, which means that the muds of both ponds still have the ability to precipitate metals (Ankley et al. 1996; Boothman et al. 2001), and should not be toxic for the human use as healing muds and bathing. This approach is used in the field of aquatic toxicology to assess the potential for metal ions found in sediment or mud to cause toxic effects in organisms.

The light grey colour of the muds agreed with the low concentration of FeS (black) found in all the strata studied. In addition, Fe, Ca and Mg concentrations were very low and comparable with those obtained by acid extractions from Lake Caviahue sediments (Cabrera et al. 2020). With regard to trace metals, Cd was below the limit of detection, and the rest of the potentially toxic metals studied were found in very low concentrations, posing no risk for the human use as healing muds and bathing. In this sense, Cr is expected to be soluble as Cr^{+3} and $CrOH^{+2}$ rather than as the hexavalent harmful forms (Berry et al. 2004).

Finally, the purpose of this work was to recognize and appreciate the many thermal resources of Copahue, word that means 'place of sulphur or place of healing' in Mapuche language (Álvarez 1960), and where more than 7300 people were treated from December to April in seasons 2017/2018 and 2018/2019 (termasdecopahue.gob.ar). Our contribution to the knowledge on the quality of muds and water, and their interaction with the algal species is added to the contribution by other researchers who have worked in the area (see Monasterio 2012, and the citations included). The CTC has thermal resources that, due to their diversity and simultaneity, are seldom found elsewhere on the planet: muds, algae, therapeutic vapours and mineral-medicinal waters. Our survey is the first step to study the species of algae —which often go unnoticed— and that are ingested, smeared on the skin and used in baths during medical treatments and that may be participating in the therapeutic effects. Therefore, future work should focus on deepening their study to assess their potential economic value in biotechnology, among other fields.

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