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Diatom of the Bacillariophyceae Class in Thermophilic Microbial Mats Present in Sulphurous Hot Springs and their Possible Biotechnological Application

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ABSTRACT

The diversity of diatoms in the hot springs of the Comanjilla geothermal zone in northern Guanajuato, Mexico was studied. Hot springs are extreme ecosystems that, despite having high temperatures, constitute an environment for many thermophilic microorganisms (bacteria, cyanobacteria, and diatoms). The thermal water studied is classified as hyperthermal water (45°C to 100°C), of deep origin, and with low mineralization, are of type sulfuric sodium chloride, since the dissolved content of hydrogen sulfide (H_2S) is found in concentrations higher than 1mg/L, and its smell is similar to the one of rotten eggs, presents a pH of 7.6 to 9.1 that represents neutrophilic to alkaliphilic environments, with a variable electrical conductivity (EC) (658-698 μs / cm) and total dissolved solids (TDS) (314-24 ppm). In the same way, these hot springs present microbial mats that consist of several stratified layers of green and orange color of 100 cm^2, each one, which are dominated by specific types of microorganisms such as bacteria, cyanobacteria, but mainly diatoms, the latter were studied applying the scanning electron microscope and the optical microscope. The morphological characteristics observed in the optical microscope and in the scanning electron microscopy indicate the presence of diatoms of the Bacillariophyceae class, represented by *Sellaphora disjuncta* (55%), *Achnanthes brevipes var. intermedia* (45%). This diatom present in thermophilic microbial mats in the sulphurous geothermal zone of Comanjilla represents: a) the first report of said microorganisms in the study area and Mexico; b) an ecosystem of great interest from the biotechnological and industrial point of view; c) an important taxon in terms of diversity and technology; d) an applications in biofuels, environmental monitoring, wastewater treatment, manufacture of fertilizers, production of secondary metabolites, medical compounds, energy sources and food industry and within nanotechnology. It is important to mention that the physical and chemical characteristics of thermal water such as temperature, pH, dissolved solids, electrical conductivity, hardness, alkalinity and silica concentrations, were the major environmental factors influencing the distribution of diatoms in sulphurous hot springs.

1. Introduction

Diatoms in thermophilic microbial mats are interesting study organisms for basic as well as for biotechnology application. In recent years, diatoms have been studied not only from the academic point of view but also within industrial and biotechnological applications. Proof of this are the applications that have been given to them within the industrial and commercial sector, such as carbon neutral, synthesis of fuels, pharmaceuticals, health foods, biomolecules, materials relevant to nanotechnology, and bioremediatory of contaminated water, biomonitoring, paleoecology, and forensics analyzes, which can be combined in the near future to make diatoms a lucrative source of novel substances with widespread relevance [1, 2]. Diatoms are unicellular, eukaryotic and photosynthetic organisms are unique microscopic algae that contain silica and have different geometric shapes. It is known that they form microbial mats and are produced in wet places where photosynthesis is possible. These photosynthetically active organisms are responsible for 20-25% of total terrestrial primary production, and approximately 40% of annual marine biomass production [3], making them the most dominant group of organisms sequestering carbon from the atmosphere. These microorganisms live generally in marine, freshwater and terrestrial ecosystems but there are few studies that describe them in an ecosystem of hot springs [4, 5, 6]. There are few species of heterotrophic diatoms that survive in dark conditions, some live as endosymbionts in some organisms or non-invasively on the body surface of organisms, for example, it is the foraminifera that host endosymbiotic algae such as diatoms and chlorophytes. So, each species of diatoms exhibits a specific environmental tolerance, forming sets of species corresponding to the condition of the habitat [7]. It is important to mention that in Mexico there are no studies related to the subject and the information about thermophilic diatoms found in hot springs is null, so the data obtained in said research will provide knowledge about the physical-chemical composition of thermal water and its importance in microbial diversity. In addition, the data obtained will be compared with studies carried out in other countries. Diatoms studied live in thermal springs usually located along fault lines where groundwater can be stored and heated up to reach a certain depth and then emerge in the form of hot water. The heated water contains a wide range of dissolved minerals (Fe, Mn, Mg, Ca, Na) and traces of gases (CO_2, H_2, H_2S, CO) [8, 9]. The chemistry of thermal waters varies a lot and depends mainly on the properties of the source rock. Due to the temperature gradients present in the thermal water flow channel, different microbial communities (halophiles, thermophiles, barophiles, pscryophiles and acidophiles) can be established in these channels [10]. Microbial diversity in microbial mats of sulphurous hot springs is a major resource for biotechnological products and processes because of the role of microbes in nutrient cycling, the production of large amounts of bio-based chemicals, polymeric substances, energy sources and biofuels, environmental detoxification, metabolic abilities in pharmaceuticals and industrial processes and act as a major resource for agricultural, industrial, and medicinal applications [11, 12]. The thermophiles microorganisms represent the most promising option as a source of extremozymes with extreme stability able to withstand drastic process condition [13]. The presence of thermal springs in the state of Guanajuato has been recognized by local inhabitants since before the arrival of the Spaniards. In the state of Guanajuato have been reported several thermal sources potentially exploitable from the energy point of view as medicinal and recreational. The presence to the outside of this type of waters is related to the existence of faults that connect the deep spring of thermal water, with the surface, which allows the ascent of the waters. The thermal springs are numerous (173) and are frequently distributed in several places in the state of Guanajuato, as a result of the tectonic activity and the phenomena subsequent to the intense volcanism of which its territory has been subject, since it belongs to the provinces physiographic of the Mesa del Centro, Sierra Madre Oriental and Eje Neovolcánico. The diatoms of the Guanajuato hot springs have been studied to obtain information on the diversity of genera and species that inhabit these waters and develop an understanding of the ecology and life cycle of diatoms in order to be able to use them for various commercial and industrial purposes. The main objective of this study was to describe the taxonomy of the community of diatoms that inhabit thermophilic microbial mats present in sulfurous hot springs, at the same time to analyze those physicochemical factors that affect their abundance, and to evaluate the biotechnological potential that they may have the diatoms described in this work. For this last objective, specialized databases on the subject were used. The knowledge obtained from this research will be used to create an inventory of diatom species in hot springs and compare them with those diatoms reported in different ecosystems (marine, freshwater and terrestrial), in the same way it will be the basis of a reference resource for the detection and application of these thermal water diatoms in industrial and biotechnological processes.

2. Description of the study area

Comanjilla is located 32 kilometers from the city of Guanajuato, Mexico. It is accessed by the federal highway number 45 that joins the cities of Silao and Leon with a branch of 10 km, at the height of the village of the Sauces that goes to the Aquatic Park of Comanjilla (Figure **1**). Due to the healing properties of its thermal waters, the Aquatic Park of Comanjilla was well known and appreciated. This thermal source corresponds to the Comanjilla geothermic zone of where 25 hydrothermal springs are located, some as hotbeds with deposition of sulfur and mineral salts with surface temperatures between 45° and 92°C, distributed in an area of 1.2 km2. The thermal source was studied by evaluating source emergency temperature, appearance, smell, taste, mineralization, hardness, turbidity, dissolved solids and cation concentrations (Na, Mg, Mo, Ca^{+2}, K^{+}, S, Al^{+3}), anions (H_2S, SO_4, Cl), oxides (SiO_2, Al_2O_3, MgO, K_2O, P_2O_5, SO_3, Fe_2O_3), and trace elements (Cu, Zr, Si, Ar, and Rh), allowing them to be classified, in terms of their chemical composition and thermalism as hyperthermal waters (45°C to 100°C) with a global chemical tendency of the ions towards an H_2S-Na type behavior (sodium sulphides), of deep origin and of low mineralization, observing a high content of silica and low in Calcium and Magnesium. From the geological point of view, the hot springs are in a depression filled with Cenozoic sediments, delimited, and cut by faults. The thermal waters are related to volcanic units of intermediate composition, basalts, rhyolites, conglomerate, and sandstones.

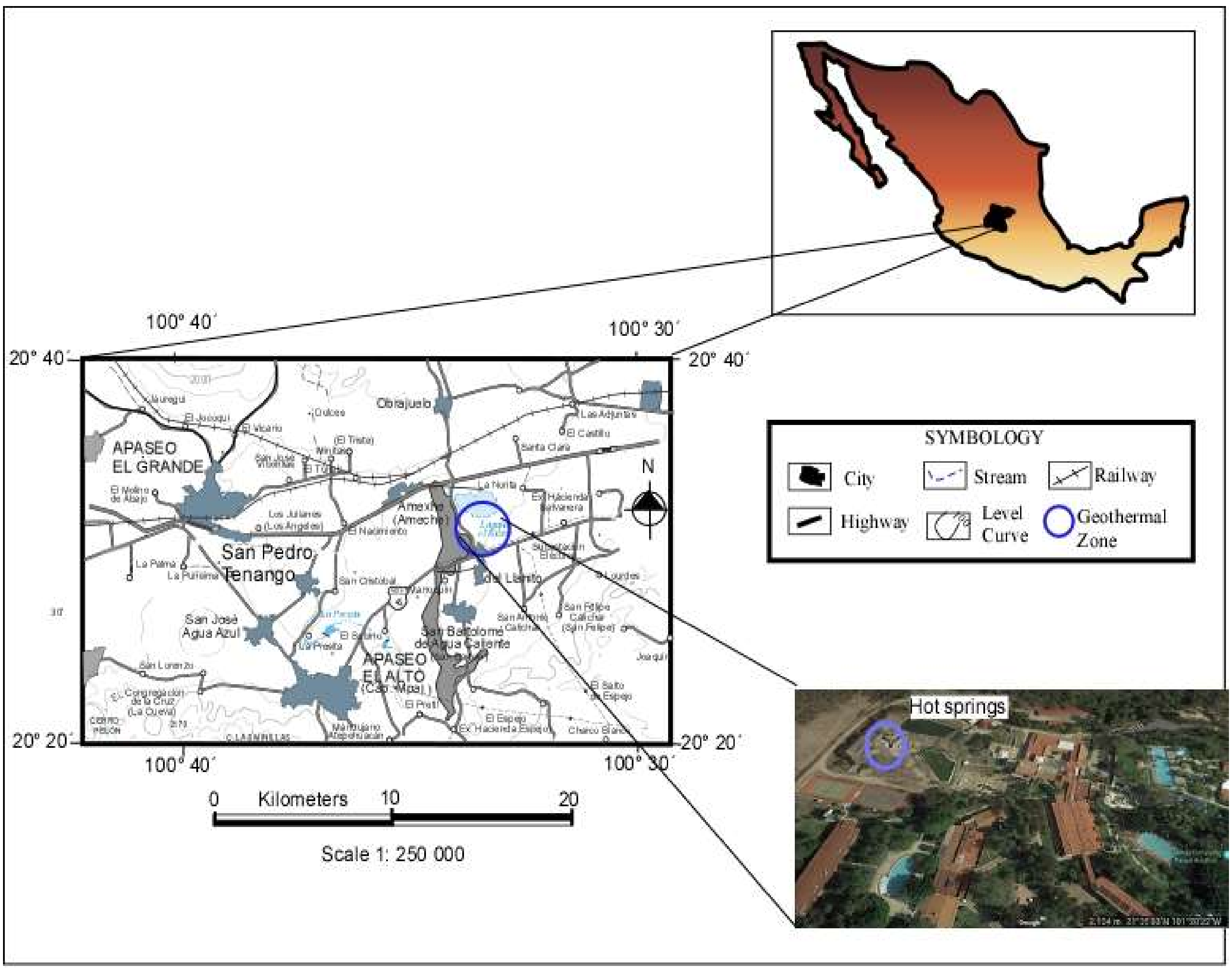

Figure 1: Location of area of study.

3.Methodology

3.1. Thermophilic Microbial Mats Sampling and Characterization

Seven hot springs in the Comanjilla geothermal zone in the state of Guanajuato possessing differing physical and chemical characteristics were chosen for this study. The samples (M1, M2, M3, M4, and M5) are collected from five hot springs out of the seven recorded in the study area during the months of December 2017 to April 2018 (Figure **2**). The samples were selected considering the alignment of hot springs to fault systems, which correspond to two families. The first family is characterized by a system NE-SW which cuts the second NW-SE fault system. These fault systems contribute to the different physical and chemical characteristics of the hot springs. The samples (M1, M2, and M3) belong to the NE-SW fault system, and the microbial mats have a brown coloration, while the samples (M4, and M5) belong to the NW-SE system and the coloration of the microbial mats is green. Samples were collected aseptically from one thermophilic microbial mats of hot springs Comanjilla in different thermo flask and processed immediately in the laboratory. Three replicates were taken for the sample from the same spot of the same location. For the sampling, preservation, and handling of the samples, the Official Mexican NOM [14], was followed. The samples taken were placed in coolers with cooling bags or ice bags closed for transport to the laboratory, at a temperature between 4°C and 10°C, taking care not to freeze the samples.

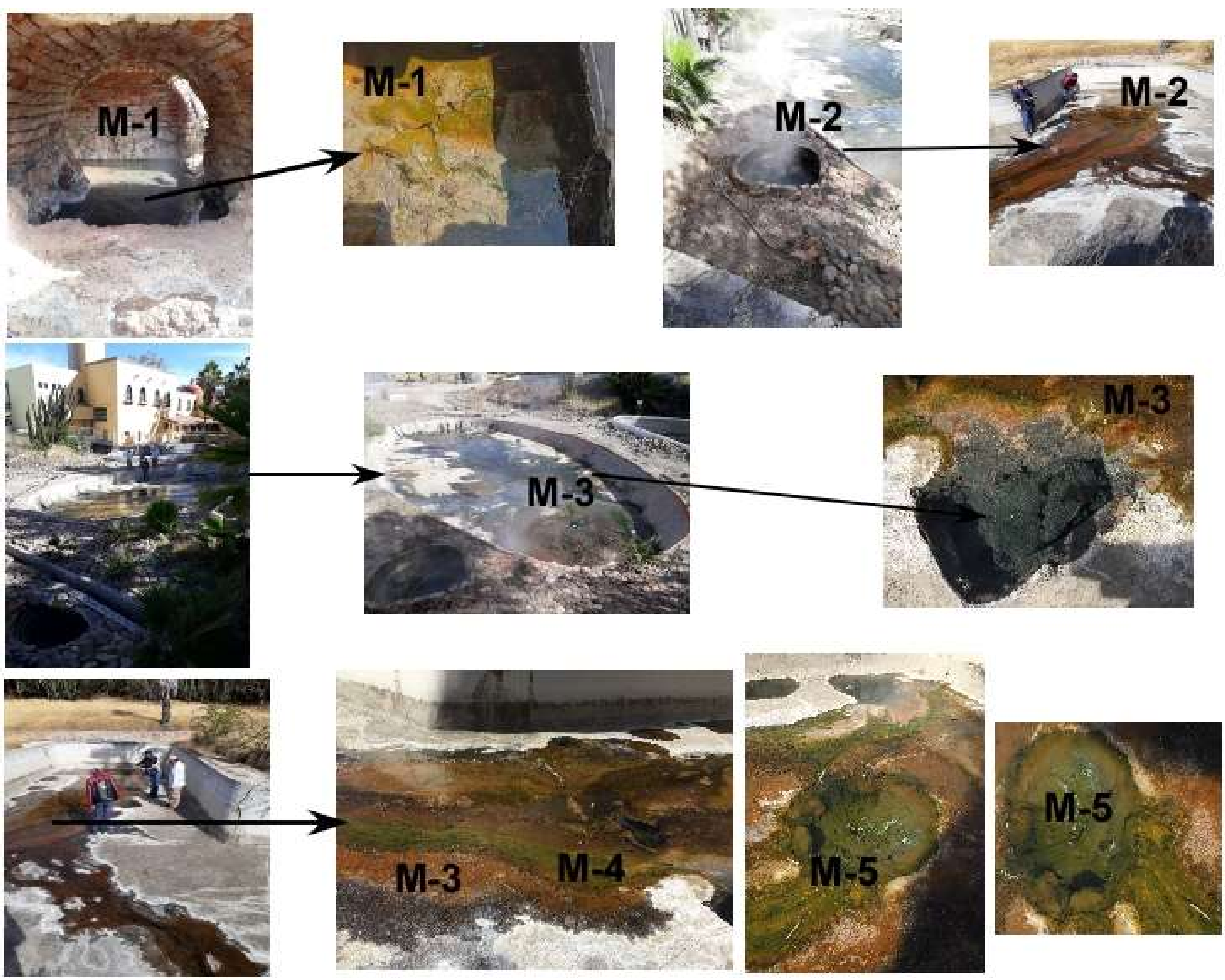

Figure 2: Photographs of five sulphurous hot springs in Comanjilla geothermic zone. M1, M2, M3, brown microbial mats; M4, M5 green microbial mats.

3.2. Physical Characteristics of Water in the Thermophilic Microbial Mats

Physical properties such as: a) temperature, b) pH c) electrical conductivity (EC), d) total dissolved solids (TDS) and e) water hardness of bacterial carpet was measured *in situ* using thermometer mercury with precision of 1°C. The pH was determined with a Corning potentiometer model 610 A. The electrical conductivity (EC) was measured with a Conductivity Meter 850037 SPER SCIENTIFIC. The total dissolved solids (TDS) were measured with a TDS PURIKOR PK-TDS3 and the hardness in the water was calculated based on the content of calcium and magnesium salts.

3.3. Chemical Characteristics of Water in the Thermophilic Microbial Mats

For the analysis of cation, anions and metals concentrations, the X-ray fluorescence (XRF) technique was used. Samples are analyzed with a Rigaku NEX CG X-ray fluorescence spectrometer using energy dispersion (EDXRF). The spectrometer has a Pd anode X-ray tube, a maximum power of 50 W with a maximum voltage of 50 kV -2 mA, and in an atmosphere of it. The analysis was carried out in the LICAMM UG laboratory of the Department of Mines, Metallurgy, and Geology of the Guanajuato University.

3.4. Identified Microorganisms in Thermophilic Microbial Mats

3.4.1. Diatoms Observation to the Scanning Electron Microscopy (SEM), Optical Microscope and Classification

The morphological aspects of the diatom were investigated by (SEM) observation with gold coating and with an optical microscope equipped with a digital camera (Olympus BX40). The SEM instrument (JEOL, JSM- 6010 PLUS/LA) was operated at 15 kV in a low vacuum, while the energy dispersion scanner spectrometer (EDS), attached to the SEM, and was used for semi-quantitative chemical analysis. The SEM-EDS analyses were carried out in the laboratory LICAMM of the Guanajuato University. For its observation the protocol of [15] was used, describing it below: 1. The sample was filtered with a filter that does not dissolve with organic solvent. 2. The filters were placed in suitable containers for drying at critical points. 3. Fixed with a 2.5% glutaraldehyde solution in 0.1M phosphate buffer prepared with filtered seawater. 4. To remove the salts, the samples were transferred to decreasing concentrations of seawater. 5. After fixing it was dehydrated in series of growing ethanol. 6. Finally the sample was dried in the desiccator to critical point. The guide of [16], was used for the generic allocation of diatoms.

4. Results

4.1. Characterization of the Samples

The Prospecting of five hot springs in Comanjilla between December 2017 and April 2018 was carried out. Thermal waters represent moderate to high thermophilic (45°C to 100°C) and neutrophil to alkalophilic (pH 7.6-9.1), transparent color, rotten egg odor, sulfurous taste, turbidity (60-70 NTU), environments with variable electrical conductivity (658 µS / cm to 704 µS / cm) and total dissolved solids (314 ppm-569 ppm), (Table **1**). The five hot springs are aligned transversely to the fault systems. Five samples (M1, M2, M3, M4, and M5) were obtained from the thermal sources of the geothermal zone of Comanjilla limited by two fault systems. Sample M1, M2, and M3 were obtained from the brown microbial mats, which has a pH 9.1, with a temperature that varies from 64°C to 92°C, classifying these waters as hyperthermal. Associated with the NE-SW fault system. Its pH indicates that it is slightly alkaline water. The hardness range in french degrees of this type of water is 32°f to 42°f, which indicates hard water. The electrical conductivity varies from 658µS/cm to 704 µS/cm, which indicates poor brackish water (Table **1**). According to its mineralogical composition, the type of water M1, M2, and M3 (Slightly alkaline), it is classified as chlorinated sulphurous water, since the content of hydrogen sulfide solution (H_2S) is found in concentrations higher than 1mg / L, and its smell is similar to rotten eggs. Similarly, they have low mineralization and low calcium (Ca) and Magnesium (Mg) and high silica (Si), Aluminum (Al), and chlorine (Cl) content, which indicates a deep origin (Table **2**). The sample M4 and M5 were obtained from the green microbial mats, which has a pH 7.6, with a temperature that varies from 45°C to 79° C, classifying these waters as hyperthermal. Associated with the NW-SE fault system. Its pH indicates that it is neutral water. The hardness range in french degrees of this type of water is 32°f to 42°f, which indicates hard water. The electrical conductivity is 690µS/cm to 698 µS/cm, which indicates poor brackish water (Table **1**). According to its mineralogical composition, the type of water M4, and M5 (neutral) is classified as chlorinated sulphurous water, since the dissolved content of hydrogen sulfide (H_2S) is found in concentrations higher than 1mg / L, and its smell is similar to of rotten eggs. Similarly, they have low mineralization and low calcium (Ca) and Magnesium (Mg) and high silica (Si), Aluminum (Al), and chlorine (Cl) content, which indicates a deep origin (Table **2**). Chemically both samples have similar values in Cu, S, Cl, being different in values of Al, Ca, Mg, Zr, Mo, Si, K, Ar, and Rh (Table **2**). Regarding the values of oxides, M1, M2, and M3 show higher concentrations in MgO, K_2O, CaO, and Fe_2O_3 compared with the M4 and M5. However, M4, and M5 show higher concentrations in SiO_2, Al_2O_3, P_2O_5, H_2S, SO_3, SO_4 (Table **2**).

Table 1: Organoleptic and physic characteristics of thermal water springs, of the Comanjilla geothermal zone.

Thermal Water Spring	Failure System	Type of Water	Color	Odor	Taste	Turbidity (NTU)	pH	T °c	SDT (ppm)	EC (µS/cm)	°f	Hardness
M1	NE-SW	slightly alkaline	Transparent	Rotten egg	Sulphurous	70	9.1	78	314	658	32-42	hard
M2	NE-SW	slightly alkaline	Transparent	Rotten egg	Sulphurous	70	9.1	64	340	704	32-42	hard
M3	NE-SW	slightly alkaline	Transparent	Rotten egg	Sulphurous	70	9.1	92	569	683	32-42	hard
M4	NW-SE	neutral	Transparent	Rotten egg	Sulphurous	60	7.6	79	324	698	32-42	hard
M5	NW-SE	neutral	Transparent	Rotten egg	Sulphurous	60	7.6	45	328	690	32-42	hard

Table 2: XRF analysis of thermal water springs, of the Comanjilla geothermal zone. ND no detected.

ELEMENT (ppm)	M1 Slightly Alkaline	M2 Slightly Alkaline	M3 Slightly Alkaline	M4 Neutral	M5 Neutral
CATIONS					
Na	ND	ND	ND	ND	ND
Mg	112	110	111	ND	ND
Mo	9.14	9.1	9.14	5.08	5
Ca^{2+}	22.1	21.9	22	26.2	26
K^{+}	18.1	17.9	18	30.8	30
S	24.2	24.1	24	25.6	25.5
Al^{3+}	238	235	236	247	246
ANIONS					
H_2S^-	27	25	26	55	54
$SO_4^{=}$	76	74	75	154	153
Cl^{--}	19.4	19.3	19.2	17.8	17.5
TRACE ELEMENTS					
Cu	2.9	2.8	2.7	2.9	2.8
Zr	244	243	245	312	311
Si	307	305	306	261	260
Ar	253	251	252	132	131
Rh	ND	ND	ND	892	891
OXIDES (mass %)					
SiO_2	48.6	48.4	48.5	49.4	49.3
TiO2	ND	ND	ND	ND	ND
Al_2O_3	23.2	23.1	23.1	29.4	29.3
MnO	ND	ND	ND	ND	ND
Na2O	ND	ND	ND	ND	ND
MgO	8.85	8.84	8.83	1.93	1.92
K_2O	4.31	4.30	4.30	0.018	0.017
CaO	4.06	4.05	4.04	ND	ND
P_2O_5	1.12	1.10	1.11	2.56	2.55
SO_3	9.26	9.22	9.25	16.7	16.6
Fe_2O_3	0.55	0.53	0.54	ND	ND

4.2. Identified Diatoms in Thermophilic Microbial Mats with Scanning Electron Microscopy (SEM) and Optical Microscope

A total of seven species of diatom were determined in thermophilic microbial mats present in sulphurous hot springs of geothermal zone of Comanjilla (Figures **3**, **4**, **5**. **6**, and **7**), predominantly Bacillariophyceae class. The name Bacillariophyceae has been used in several ways: to refer to all diatoms or to refer to the raphe-bearing pennate diatom or to refer to all pennate diatoms. In our case, we mean all the diatoms pennate.

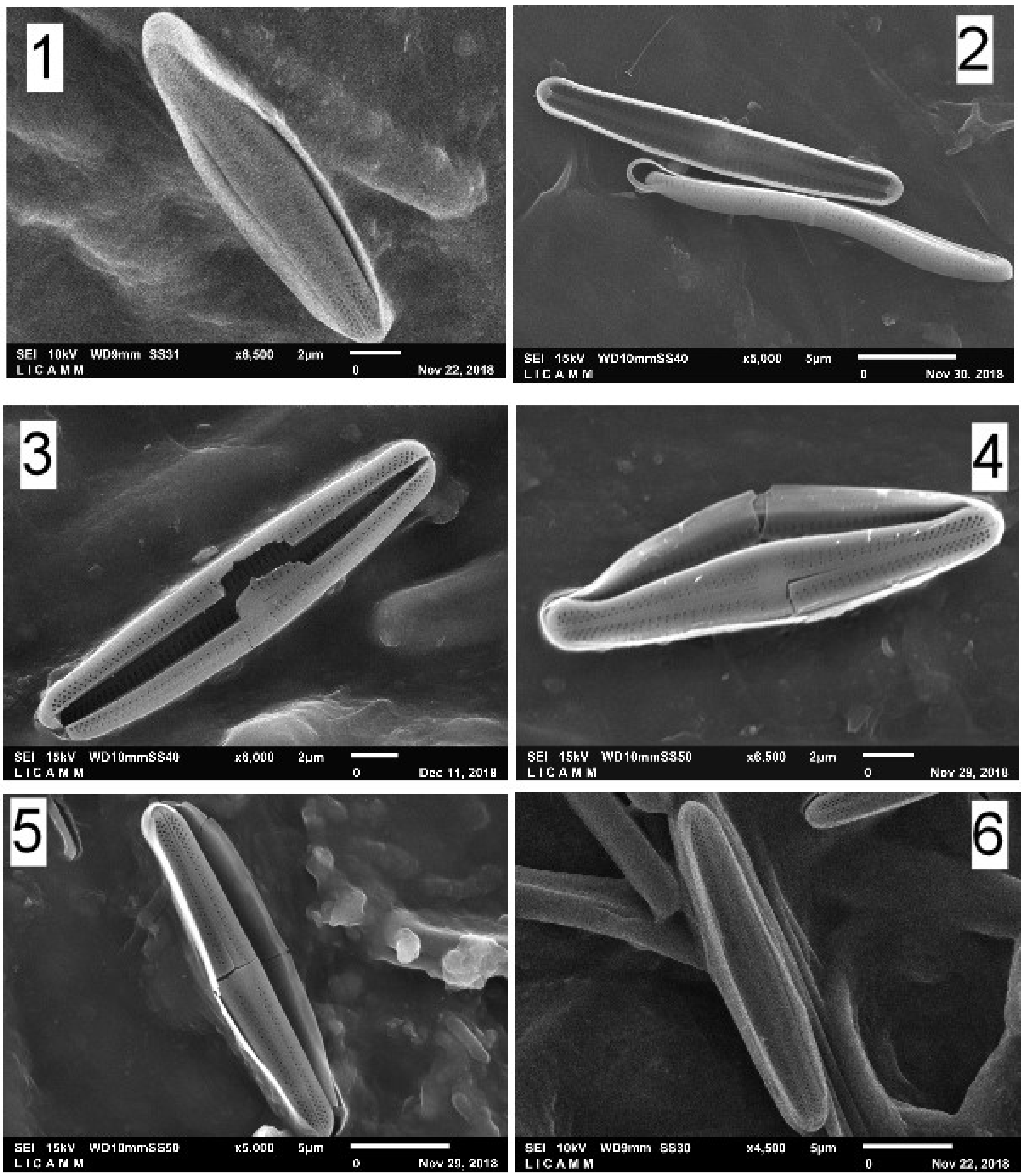

Figure 3: SEMs of *Achnanthes brevipes var. intermedia*. (1-5). External views of raphe valves. (6). Internal view of RV with valvocopula, showing the copilae with two rows.

4.2.1. Identified Diatoms in Thermophilic Microbial Mats with Scanning Electron Microscopy (SEM)

Achnanthes brevipes var. intermedia (Kützing) Cleve [17], were determined in thermophilic microbial mats present in sulphurous hot springs of the geothermal zone of Comanjilla (Figure **2**), belongs to the

Bacillariophyceae class. The Bacillariophyceae class is unicellular algae characterized by a silica outer shell or frustule [18]. They comprise one of the most abundant algal groups globally and are important in benthic and planktonic algal communities in freshwater, marine, and terrestrial habitats. *A. brrevipes var intermedia* belongs to the genus *Achnanthes* [19, 20]. Her basionym is *Achnanthes intermedia* Kützing 1833, and her synonym is *Achnanthes intermedia* Kützing 1833; *Achnanthes subsessilis* Kützing 1833; *Achnanthidium brevipes var. intermedia* '(Kützing) Cleve' VanLandingham 1967. Her Parent is *Achnanthes brevipes C*. Agardh, 1824. The general environment is marine, freshwater, and brackish. In the study area, these diatoms coexist with the genus *Bacillus* represented by *Brevibacillus agri and Paenibacillus* sp., to temperatures of 70°C and 92°C in sulphurous hot spring.

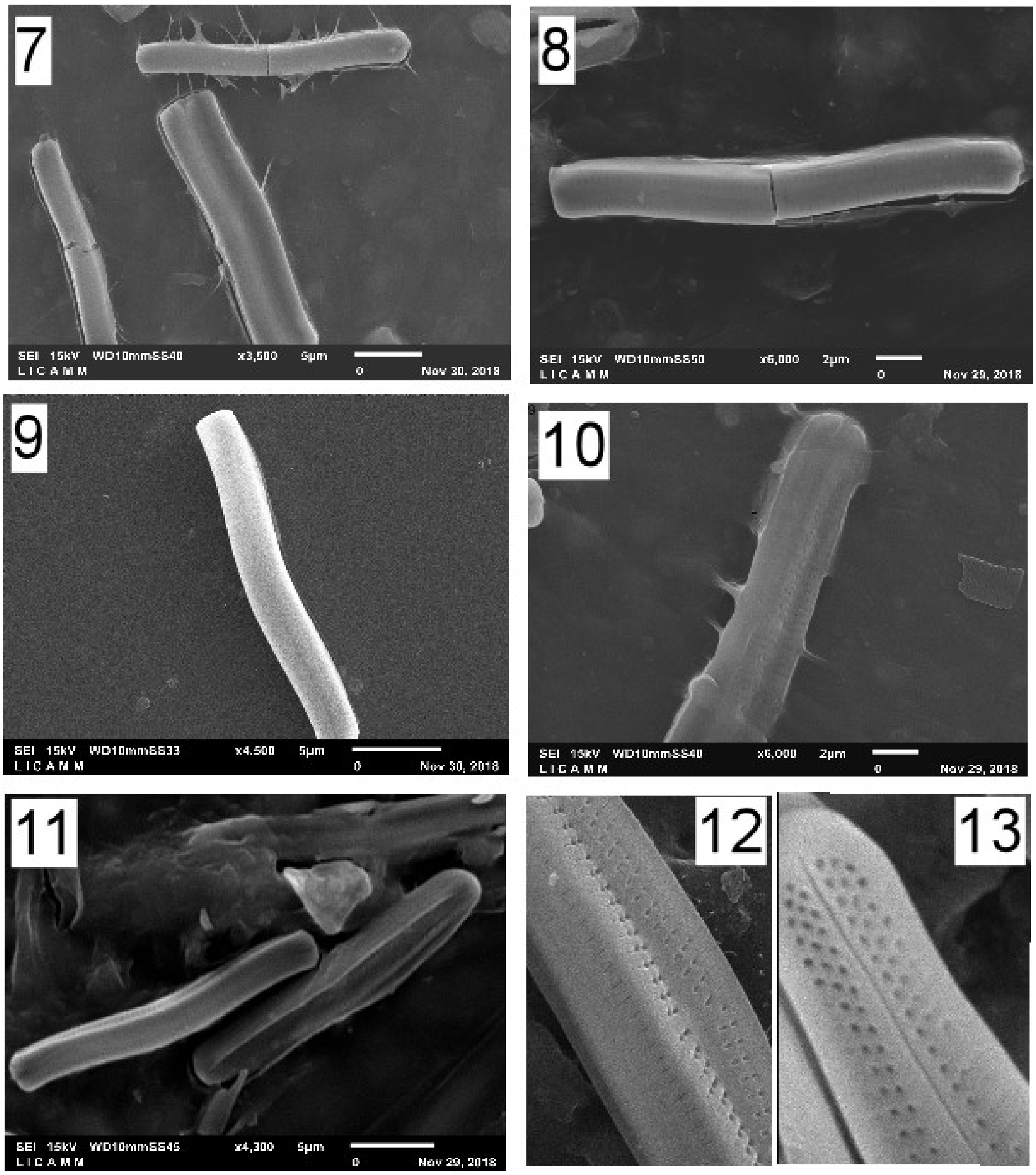

Figure 4: SEMs of *Achnanthes brevipes var. intermedia*. (7-11). Views of a girdle view. (12-13). Terminal fissure, curving in opposite direction to central raphe ending.

4.2.2. Description Scanning Electron Microscopy (Fig 3, 4, 5, 6)

Raphe valve (Fig. **3**): Raphe valve with fascia. The valves are shallow and arched with a narrow, eccentric sternum. The size of the valves is 20μm of long and 5μm of width, linear-lanceolate or linear, elliptical to elliptical, wedge-shaped, rounded at the apices, often slightly concave in the middle. The central area is quite narrow, reaching the edges forming a transverse fascia. Internal view of RV with valvocopula, showing the copulae with two rows, and its pars interior extending inside the valve.

Girdle view (Fig. **4**): The valve is slightly curved geniculately, with a convex ARV and a concave RV. Frustule usually consists of RV, ARV, and three or four copulae. Arcuate frustule forming a "V". Cíngulo made up of a reduced number of copulae. All copulae, including valvocopula, are open bands with the opening of the band alternately and connecting each other. The terminal fissures are deflected in the opposite direction to the central pores. The striae are uniseriate, with a radial arrangement Striae uniseriate, composed of almost rounded areolae with slightly recessed foramina, occluded by cribra with rounded perforations

Valve external (Fig. **5**): The areolae are usually round, often tending towards the square, slightly sunk below the basal siliceous layer in external view.

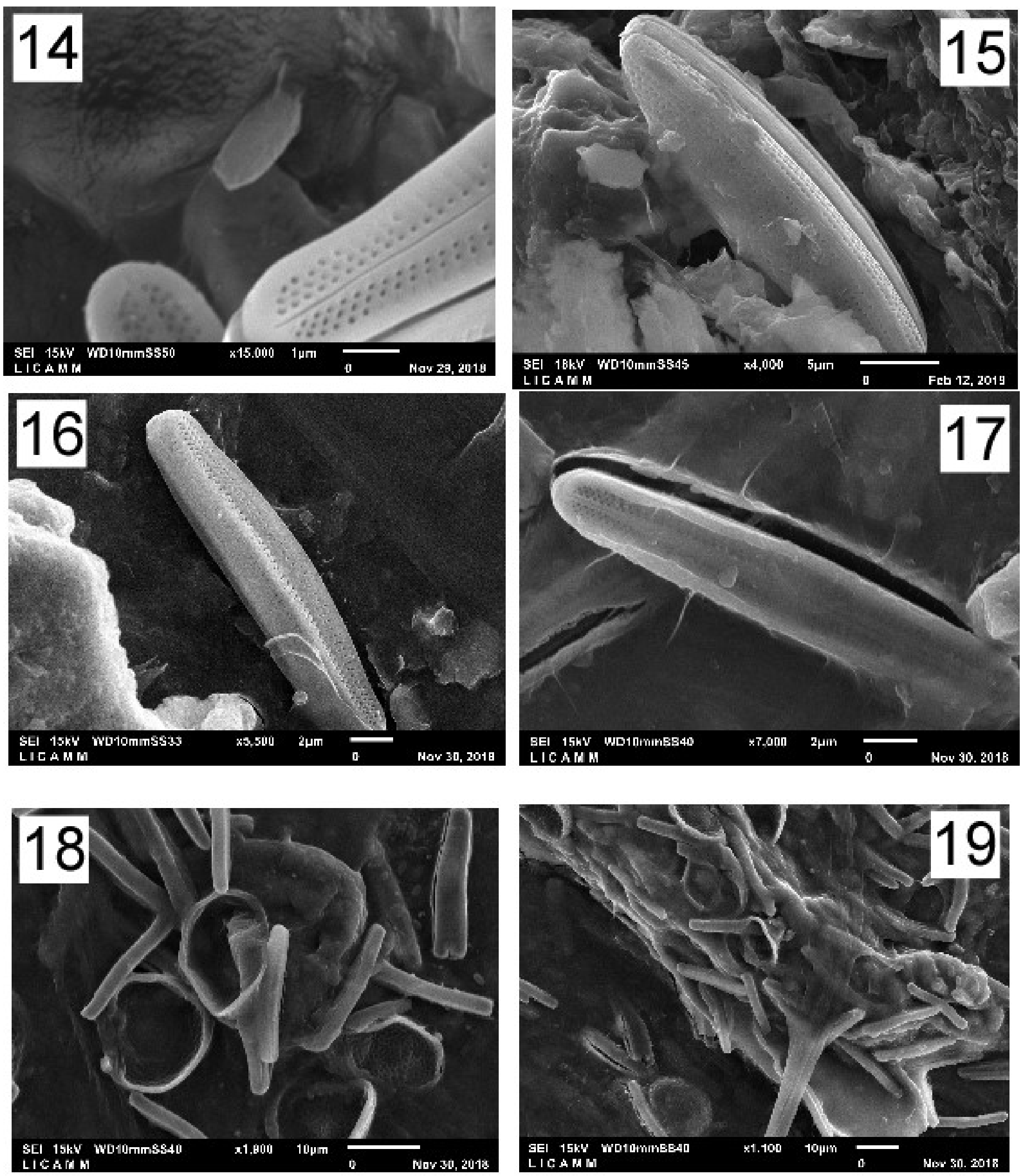

Figure 5: SEMs of *Achnanthes brevipes var. intermedia*. (14-17). External view of an entire raphe valves. (18-19). *Achnanthes brevipes var. intermedia* in thermophilic microbial mats.

Microbial mats (Fig. **6**): *Achnanthes brevipes var. intermedia* (Kützing) Cleve, coexist with the genus Bacillus represented by *Brevibacillus agri* and *Paenibacillus* sp., these thermophilic bacteria are of great interest due to their biotechnological importance as a source of thermostable enzymes and industrial products [21, 22].

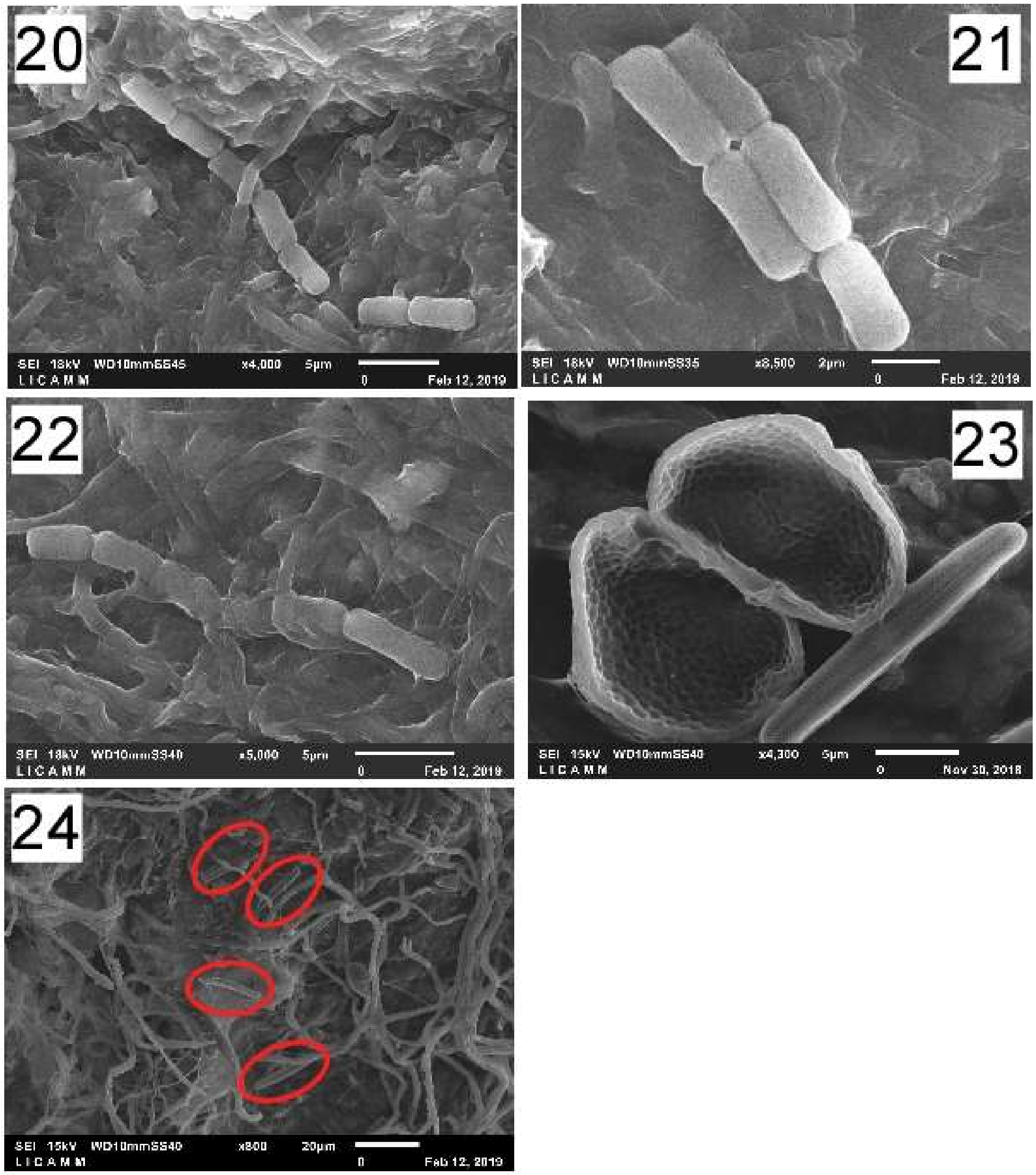

Figure 6: (20-22). SEMs of genus Bacillus represented by *Brevibacillus agri* and *Paenibacillus* sp. (23-24). *Achnanthes brevipes var. intermedia*, coexist with the genus Bacillus represented *Brevibacillus agri*, and *Paenibacillus* sp. In thermophilic microbial mats present in sulphurous hot springs.

4.2.3. Identified Diatoms in Thermophilic Microbial Mats with Optical Microscope (Fig.7)

Diatoms of the Bacillariophyceae class, represented by the Achnanthales, and Sellaphora orders, have elongate cells, shaped like boats, rods, spicules or bananas and it is sometimes stated that this, rather than the organization of the valve pattern, is the essential feature of the group. *Achnanthes brevipes var. intermedia*, in the optical microscope the cell is attached to the substrate: either via the raphe directly with the concave shell, or by means of a jelly stem. The second shell has no raphe. The cells have one or two H-shaped plastids. Some brackish water species have many lenticular plastids (Figure **7a**). *Sellaphora disjuncta*, in the optical microscope the axial area is straight; the raphe is filiform (Figure **7b**).

Figure 7: Photograph of diatoms in optical microscope in hot springs of the Comanjilla geothermal zone; (a) *Achnanthes brevipes var. intermedia*; (b) *Sellaphora disjuncta* (Hust.) G.G. Mann 1989.

4.3. Ecology and Distribution

The genus *Achnanthes* have been described in marine, fresh- and brackish waters [23]. According to [24], *Achnanthes brevipes var. intermedia*, are widely distributed and lives in waters of lower salt concentration. [25], mentioned that this species has a cosmopolitan distribution along with lowland areas even in estuaries.

5. Discussion

This is the first report of diatoms in thermophilic microbial mats that carpet hot springs in Mexico, where thermophilic bacteria as *Brevibacillus agri* and *Paenibacillus sp*, living in association with the diatoms of the Bacillariophyceae class represented by the genus *Achnanthales, and Sellaphora*. The results indicated that with the increase in the temperature of the water in the tanks above 90°C, the number of diatom species is impoverished, as is the case of the sample M1, M2, and M3. The diatom species recorded in the sample M1, M2, and M3 have a little abundance of the species *A. brevipes var* intermedia, probably due to the conditions of pH, slightly alkaline water, temperature, low concentrations in Ca, K, H_2S, and SO_4, higher concentrations in Mg, Mo, MgO, K_2O, CaO and Fe_2O_3, and absence of NaCl. While M4 and M5 show a greater abundance of the species *A. brevipes var* intermedia due to the temperature, pH, neutral water, absence of NaCl, Mg, and to the concentrations of H_2S, SO_4, Ca, K, SiO_2, Al_20_3, P_20_5 and SO_3 compared to M1, M2, and M3. This indicates that M1, M2, M3, M4, and M5, although they correspond to the same geothermal zone, have physical and chemical characteristics that differentiate them. These differences are due to the movement of hydrothermal fluids throughout the fault systems, which cause mineralogical changes due to the introduction of certain chemical elements from the rocks. Likewise, it is important to mention that the NE-SW fault system is younger and is the one that controls the thermal springs of pH 9.1, while the NW-SE fault system that is older and controls the thermal springs of pH 7.6. The increase in silica together with the pH, temperature, and type of water were important factors for the distribution and diversity of diatoms, example of the diatoms reported in Thailand [26]. The diatoms absorb the surrounding silicon at low concentration (<1 μM) and are actively transported through the membranes, such as silicic acid, then the insoluble silicon product for insertion into the mobile walls, this mechanism allows its silica biocaps (frustules) are used as nanomaterials [15, 27]. The SiO_2 content varies with the individual species and with the environmental conditions. The variables such as pH, electrical conductivity, hardness, alkalinity, temperature, total dissolved solids, and chemical composition are involved in the relationship and abundance of species [26] since diatoms responded better to changes in the physicochemical environment [28, 29], describes the environmental variables involved in the appearance of diatoms in an estuary. In this article, they report that the environmental factors for the *Achnanthes brevipes* are the electrical conductivity (100) and turbidity (61). In the geothermal zone of Comanjilla, this species presents similar values to those reported by [29]. Electrical conductivity (EC) is known to be an important factor for determining the composition of the epilithic diatom community [30]. However, some species remain at elevated temperatures and actively develop and even to form masses as *Sellaphora disjuncta*, so the temperature becomes an important factor. All taxa have a pH of neutral waters to some alkalines. *Achnanthes brevipes* var. intermedia to have a wide salt tolerance [31, 32]. *Achnanthes brevipes* var intermedia has been described by [33] in hypersaline waters, where this species presents double frustule an adjustment to hypersalinity. The measured values of Rhodium and Argon are possibly controlled by structural geology, namely the presence of previous fault systems, and by the lithology of the leached host rocks. Diatoms (*Bacillariophyceae*) are frequently a ubiquitous, highly successful, and distinctive group of single-celled algae, with the presence of siliceous cell walls, called frustules. Similarly, it is the richest in diatom species, representing great ecological, biotechnological, and primary production importance [3]. Diatoms contribute significantly to the productivity of many ecosystems, often forming the basis of aquatic food chains and ecological indicators [34], they are also used in biological monitoring as it responds quickly to environmental changes and provides early indications of the impacts of pollution and habitat restoration and as paleo-ecological significance [35, 30]. They have been extensively used as indicators of environmental change: eutrophication, acidification, salinification, sea-level change, and land-use change, [36]. In recent years, diatoms have been studied for their great potential in chemical devices, solar cell batteries, and electroluminescence devices [37]. Similarly, during the last years have been studied for bio-applications, the immobilization of biomolecules, the detection of gases, and biomasses [38]. Today, the major emphasis is focused on their application in nanotechnology and biotechnology including nanofabrication techniques, chemo and biosensing, particle sorting, control of particles in micro- and nano fluidics and also on their use in analyzing ecological problems; such as climate change, acidification, and eutrophication of aqueous ecosystems, biomineralization, synthesis of biomaterial, and waste degradation, [39]. *Achnanthes brevipes C. Agardh,* parent of *Achanathes brevipes* var intermedia (Kützing) Cleve, has been studied by [40], where they selected eight examples of diatoms in different Egyptian habitats and environments: *Aulacoseira granulata* (Ehrenberg) Simonsen, *Actinocyclus octonarius* Ehrenberg, *Cyclotella meneghiniana* Kützing*, Pleurosira laevis* (Ehrenberg) Compére, *Synedra ulna* (Nitzspes) Agardh, *Nitzschia amphibia* Grunow. *Nitzschia palea* (Kützing) W. Smith, to study in detail the ultra-structures of their frustules, the result was that it is possible to obtain mesoporous macroparticles and silica microparticles from the species. Based on this, *Achnanthes brevipes* var intermedia (Kützing) Cleve, represents a potential within biotechnological applications for obtaining porous materials (Table **3**). All these applications are referred to diatoms that inhabit marine ecosystems, rivers, estuaries, soils, with few studies made in thermal waters. Based on this, in this research work, we report diatom species that live together with bacteria thermophilic in an ecosystem whose temperature exceeds 90°C. Opening a great opportunity in the applications and uses of these diatoms in this new little-explored ecosystem.

6. Conclusion

The diatom species described in this work live in environments at pH 7.6–9.1 in thermophilic microbial mats derived from sulphurous hot springs from 45°C to 100 °C. The type of water is slightly alkaline to neutral. According to (Kroger & Poulsen, 2008) the pH influences in the structure of diatom communities, in diversity and in total biomass produced. The physical characteristics pH, temperature, turbidity, and chemical composition of water were significant factors affecting the relative abundance of *Achnanthes brevipes* var intermedia species. It is important to highlight the lack of sodium as an element in the samples, so the concentration of NaCl salts is not an indicative element for the distribution of diatoms in the studied hot springs, in the same way, the electrical conductivity and total dissolved solids are not shows as a key element for the composition of diatoms since the values obtained do not present significant variations. The diversity of thermophilic microorganisms (diatoms) found in the microbial mats of hot springs of the geothermal zone of Comanjilla: 1) can be further exploited for the large-scale benefit of humanity; 2) diatoms of the Bacillariophyceae class, represents a new scenario for biotechnological applications, especially in industrial processes; 3) the study of diatoms as a functional unit will help to better understand how these complex and integrated communities adapt to life around the thermal waters, where factors such as pH, electrical conductivity, hardness, alkalinity, temperature, chemical composition influences the relationship and abundance of these organisms; 4) diatoms are important taxa not only in terms of diversity and ecology but also due to their applications in environmental monitoring, nanotechnology, biofuels, medicine, agriculture, and food industry; 5) diatoms are considered as important elements for the synthesis of

Table 3: Diatom applications taking into account the database of other countries in biosynthesis, biodegradation, bioremediation, biomineralization, biosensor, biodiesel, biomedical, bio nutrition, and biotechnological.

Diatom Species	Bio Synthesis	Bio Degradation	Bio Remediation	Bio Mineralization	Bio Sensor	Bio Diesel	Bio Medical	Bio Nutrition	Bio Monitoring	Bio Technological	References
Achnanthes longipes	x						x	x			[41, 42]
Achnanthes oblongella Oestrup		x									[43]
Achnanthes brevipes Agardh										x	[40]
Aulacoseira granulata										x	[40]
Amphora coffeaeformis		x					x	x			[42, 44]
Cylindrotheca fusiformis							x				[2]
Cyclotella sp.					x						[45]
Cyclotella cryptica						x	x		x		[2, 46, 47]
Cyclotella meneghiniana	x			x						x	[40, 48]
Cylindrotheca fusiformis									x		[46]
Cylindrotheca closterium		x									[49]
Cymbella cistula	x										[41]
Cocconeis placentula Ehr.		x									[43]
Coscinodiscus concinnus					x						[50]
Coscinodiscus wailesii					x						[50, 51]
Coscinodiscus argus					x						[49]
Chaetoceros sp.								x			[42]
Chaetoceros cryptica	x										[43]
Chaetoceros muelleri								x			[52]
Chaetoceros muelleri var. subsalsum						x					[53]
Cheaters calcitran										x	[64]
Chaetoceros gracilis						x		x			[52, 55]
Diademis gallica	x										[42]
Ditylum birghtwellii					x						[56]
Fragilaria capucina Desm		x									[43]
Halamphora coffeaeformis						x					[57]
Haslea ostrearia	x										[43]
Isochrysis galbana								x			[58]
Melosira nummuloides				x							[43]
Navicula sp.							x	x		x	[42]
Navicula atomus	x										[42]

(Table 3 contd....)

Diatom Species	Bio Synthesis	Bio Degradation	Bio Remediation	Bio Mineralization	Bio Sensor	Bio Diesel	Bio Medical	Bio Nutrition	Bio Monitoring	Bio Technological	References
Navicula cincta						x					[59, 60]
Navicula saprophilia							x				[2]
Navicula minima	x			x							[61]
Nitzschia sp.	x	x	x				x	x			[42, 62, 63]
Nitzschia closterium						x					[55]
Nitzschia soratensis					x						[49]
Nitzschia laevis							x			x	[65]
Nitzschia obtusa	x			x							[61]
Nitzschia palea										x	[40]
Nitzschia navisvaringica										x	[15]
Nitzschia amphibia										x	[40]
Odontella aurita								x			[42]
Pavlova lutheri								x			[58]
Phaeodactylum tricornutum	x					x	x	x	x	x	[2, 46, 42, 65]
Pinnularia sp.				x						x	[43. 66]
Rhizosole sp							x				[42]
Stauroneis sp	x										[42]
Skeletonema sp.								x			[42]
Skeletonema costratum			x			x	x	x	x	x	[42, 46, 58-60]
Skeletonema marinoi										x	[42]
Stephanopyxis turris	x										[67]
Stephanodiscus hantzschii	x			x							[68]
Thalassiosira weissflogii				x			x				[2]
Thalassiosira pseudonana	x			x	x		x	x	x	x	[2, 46, 56, 58, 66]
Thalassiosira Rotula					x					x	[42]

biomaterials, for pollution problems, to rehabilitate sites and to determine the toxicity of a place by heavy metals; 6) *Achnanthes* genus presents applications in biosynthesis, biodegradation, bionutrition, biomedical, and biotechnology; 7) *Achnanthes brevipes* var intermedia, represents a potential within of the nanotechnology applications; 8) the thermal sources represent a new scenario for the study and use of thermal diatoms as an alternate application in nanotechnology and biotechnology; 9) this information may provide important tools to explain, ecological, biotechnological, and biodiversity phenomena in the hot spring.

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