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Short communication

Screening of thermophilic microalgae and cyanobacteria from Tunisian geothermal sources

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ABSTRACT

Six geothermal sources distributed in the north and south of Tunisia were examined monthly during the period from August 2009 to April 2010, in order to determine mats communities' abundance and biodiversity. Twenty-seven taxa, belonging to three taxonomic groups: Cyanobacteria, Bacillariophyta and Chlorophyta were identified. The Cyanobacteria group was more prominent with a particular dominance of two genera *Phormidium* and *Spirulina*. Their maximum abundance was respectively $919.2 \pm 44.2\ 10^3$ cells ml^{-1} and $652.8 \pm 92.6\ 10^3$ cells ml^{-1}. The dominant mats morphologies in prospected sites were filaments. Results demonstrate that biodiversity values ranged between 0.16 and 1.15 bits. cell^{-1}. The spatial variations showed that the regional specificity in mats species biodiversity and abundance between north and south were not signified, whereas, each site had specific species. Temporal variations showed an increase of microalgae and prokaryotes abundance in the dry season.

1. Introduction

The high temperatures associated with geothermal activity frequently result in surface and subsurface geothermal springs, commonly known as "hot springs". In general, these ecosystems inhabit with microbial mats, which are defined as microorganism communities that colonize surfaces. The hot spring mat communities were the unique adaptations of the microbial flora to these harsh environments (Zakaria and Abdulrahman 2007). Microbial group populate these mats may be a potential source of active molecules which can possess therapeutic properties. Several investigations of microalgae and Cyanobacteria flora for many sources of springs have reported a dominance of prokaryotic organisms (Castenholz, 1976; Roeselers et al., 2007; Sompong et al., 2005; Ward et al., 1998), and small eukaryotes (e.g., diatoms, unicellular algae) (Jonathan and John, 2009). Tunisian geothermal sources are of particular interest especially in the arid region of the south where we have a lack of surface water. In this region, mostly springs are of low enthalpy, with temperatures up to 70 °C and have a potential use for agricultural purposes; oasis irrigation, sericulture, aquaculture (Kairouani and Nehdi, 2005), and for drinking water supply. Sources located in the northeastern part of Tunisia, in the "Cap Bon" basin, characterized by geological faults with different thermal and hydraulic signatures (Bouri et al., 2008). These sources are used mainly for therapeutic hot bathing (hammams). In fact, geothermal Tunisian waters are quite known for treating different kinds of diseases such as skin diseases and rheumatism (Anonymous, 2004). Although therapeutic properties of Tunisian geothermal sources, there are no inventories or studies on the distribution and dynamics of mats species populate this ecosystem. The present work reports a first screening of mats community in Tunisian geothermal sources in the N-E and S-E. The purpose of this study is to investigate the composition, abundance, spatial and temporal distribution patterns of microalgae and Cyanobacteria species inhabit in these hot springs in order to assess the difference between microalgae and Cyanobacteria communities in the north and south of Tunisia and to prove the effect of season variation on the species richness and abundance.

2. Materials and methods

Two typical regions were selected one from North-East of Tunisia: Korbous (36°81′N, 36°56′E), and the other from the South: Bechima-El Hamma Gabes (33°91′N, 9°73′E) with two different climate and water properties. The region of Korbous is characterized by an annual average precipitation about 500 mm and average

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temperature of 18 °C. Thermal sources were characterized by hot mineralized water with high concentration of fluoride and sodium. Two representative sources were examined; Ain Atrous at a 5 m altitude and Ain Kalassira located at the sea level. The south region is influenced by an arid climate. The annual average precipitation is of 189 mm, occurring mainly during the winter. High temperatures, sometimes over 35 °C, are recorded during summer (Ketata et al., 2006). Geothermal sources were characterized by low mineralized water with temperature up to 70 °C. In this region, four sites were investigated: Bechima source, Bechima cooling tower, Khabyet cooling tower and Galeb cooling tower. Mats communities were collected between August 2009 and April 2010, by scratching of boulders and submersed plants from each spring. Samples were placed directly in sterile plastic tubes and fixed in 1% lugol's iodine solution for the identification and enumeration of microalgae cells. Temperature, salinity, dissolved oxygen and pH were measured three times, in situ, using portable meters, respectively, salinity scale (*WTW, LF 323*), oximeter (Lutron DO 5510) and pH-meter (Lutron 208). Chemical composition of water sources was reported by Tunisian Office of Thermalism (Anonymous, 2004). Microalgae and Cyanobacteria species enclosed the geothermal mats were studied under light microscopy (Zeiss, Axiostar) at a magnification of ×1000. Identification of the observed microalgae was based on morphological criteria, according to the taxonomic works of Anagnostidis and Komarek (1988, 1990), Reviers (2003), Ricard (1987) and Waterbury (2006). Cell dimensions were measured using a calibrated eyepiece micrometer, and from images of live and freshly preserved material using a digital camera (Yashica EZ F1027). Microalgae and Cyanobacteria in each sample were counted three times on Malassez hemocytometer using ten micro liters from homogenized solution of 25 g mats per liter. Density was estimated for natural taxonomic unit cells per ml of homogenized sample. The cells number of filament species was calculated and cell size was determined as well as filament mean length, by measuring the length of 30 filaments. Species diversity was calculated using the Shannon–Weaver index, in order to take into account both species diversity and relative abundance (Shannon and Weaver, 1963).

3. Results

The mean values of physical and chemical parameters measured in the six hot spring sources indicate that Ain Atrous and Bechima sources are characterized by high temperature (60 °C) and slight acidic waters (pH 6.4). These sources were distinguished by the highest mineralized waters (Table 1) as justified by the highest contents of sodium (3392.5 mg l^{-1}) and sulphates (4970 mg l^{-1}). The southern sources (Bechima source and cooling Tower, Khabyet and Galeb cooling Towers) were characterized by alkaline pH, and salty (1.9–2.3 g l^{-1}) waters. Sampling materials collected from selected geothermal sources were composed of microbial mat, dense with green or yellow color comprising a diversity of microalgae and prokaryotes varying from filamentous to unicellular form. Twenty-seven microalgae and prokaryotes species, mainly belonging to Cyanobacteria group, were registered (Table 2). The most frequent and widely distributed taxa were *Phormidium* sp. and *Spirulina* sp. In fact, four species of *Phormidium* sp. were distinguished. *Phormidium* spI. was the most abundant in Ain Atrous (919.2 ± 44.2 10^3 cells ml^{-1}) and Khabyet tower sites, whereas *Phormidium* spIII. was the most abundant in Bechima tower. The genus *Oscillatoria* represented by two species (*Oscillatoria* spI., spII.) was the most frequent in Kalassira. The contribution of the Cyanobacteria coccoid genera (*Synechococcus*, *Gleocapsa*, *Cyanothece*, *Synechocystis*) to the mats communities was poor. Diatoms species were identified essentially in Kalassira source (7 species), with the most abundant one was Melosira (4.81 10^3 cells ml^{-1}). Chlorophyta class was exclusively found at southern sites (Bechima source, Bechima cooling tower and Galeb Cooling Tower). This class was represented by *Cosmarium* and *Mougeotia* genera with low abundance respectively 0.51 ± 0.02 10^3 cells ml^{-1} (Bechima source) and 0.06 ± 0.08 10^3 cells ml^{-1} (Galeb Tower). The highest biodiversity index (1.92 ± 0.05 bits. $cell^{-1}$) was displayed by Bechima source despite its high temperature (60 °C). The lowest one was recorded in Ain Atrous (0.83 ± 0.03 bits. $cell^{-1}$). Seasonal fluctuation of mats community determined for all sampling sites showed that the highest abundance was recorded in dry season. This abundance was doubled in the site Ain Atrous, Kalassira and Bechima source (Table 3). Biodiversity indexes in wet and dry season for each prospected site (Table 3) ranged from 0.16 to 1.15 bits. $cell^{-1}$. Biodiversity indexes were more elevated during dry season in Ain Kalassira, Bechima cooling tower and Galeb cooling tower than those observed during wet season. However, these indexes were the highest during wet season in Ain Atrous and Khabyet cooling tower. In Bechima source, the biodiversity was stable in both seasons.

4. Discussion

In this work, we have investigated the microalgae and cyanobacteria communities of mats collected from six Tunisian geothermal sources. The taxa recognized in these prospected sites were also identified by Castenholz (1969), Peter (1988) and Kullberg (1971) in different hot springs distributed respectively in

Table 1
Physical and chemical parameters in the six prospected Tunisian geothermal sources (Anonymous, 2004).

	Ain Atrous	Ain Kalassira	Bechima source	Bechima cooling tower	Khabyet cooling tower	Galeb cooling tower
Temperature (°C)	61 ± 1.8	43.2 ± 0.4	60.3 ± 5.7	40.6 ± 1.1	40	40
Dissolved oxygen (mg l^{-1})	3.6 ± 0.05	3.5 ± 0.05	4.06 ± 0.05	2.03 ± 0.05	0.3 ± 0.2	5.1 ± 0.1
pH	6.4 ± 0.2	6.4 ± 0.1	7.15 ± 0.6	8.2 ± 0.9	8.4 ± 0.4	8.5 ± 0.5
Salinity (g l^{-1})	0.010 ± 5E-0.5	0.010 ± 5E-0.5	2.3 ± 0.05	2 ± 0	1.9 ± 0.05	2 ± 0.1
Flow rate (l s^{-1})	39	1.4	20	40	70	40
Calcium (mg l^{-1})	800	820	424.65	117.5	320	117.5
Magnesium (mg l^{-1})	240.76	182.40	74.20	88.2	82	88.2
Sodium (mg l^{-1})	2953.20	3392.5	506.57	287.3	350	287.3
Potassium (mg l^{-1})	89.70	45	99.13	24.7	51	24.7
Bicarbonates (mg l^{-1})	536.80	524.6	183	ND	97.60	ND
Sulphates (mg l^{-1})	2110.13	2214	1187.05	1012	920.36	1012
Chlorides (mg l^{-1})	4970.00	5325	888.83	663.8	710	663.8
Fluorides (mg l^{-1})	3.2	3.1	0.55	ND	1.55	ND
Nitrates (mg l^{-1})	ND	3.1	2.64	ND	0.48	ND

ND: Not determined.