



# **Extreme and Heterogeneous Conditions of the Desert Wetland Chott Ech Chergui (Algeria) Allow Isolating Halophilic, Alkalophilic and Thermophilic Bacteria**

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**Extreme and heterogeneous conditions of the desert wetland Chott Ech Chergui (Algeria) allow isolating halophilic, alkalophilic and thermophilic bacteria.**

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## Abstract

Wetlands concentrate biodiversity, particularly in arid regions. Chott Ech Chergui (Algeria), one of the biggest wetlands of the north African desert, presents extreme temperatures and salinity conditions. We initiated a physico-chemical and microbiological survey at seven sites encompassing various conditions (salt lakes, freshwater lakes and hot springs), through a one-year monthly sampling campaign. Four sites under the influence of the spring showed little temporal physio-chemical variation. Three sites displayed higher variation and the aridity level, high from late spring until autumn, was a strong predictor of water chemistry. We evidenced extremophile bacteria. Among 320 bacterial isolates tested for thermo-, acido- and alcalino- tolerance, 39, affiliated to Firmicutes and Proteobacteria, were successfully maintained and underwent physiological, biochemical and molecular characterizations. Thermotolerance was recorded up to 100°C for a water isolate affiliated to *Geobacillus stearothermophilus*. Halotolerance was recorded up to 30% NaCl (w:v) for soil isolates affiliated to *Salinicola zeshunii*, *Chromohalobacter beijerinckii* and *Virgibacillus halodenitrificans*. Acido- and alcalo- tolerance were recorded for a pH range from 5 to 11 for several soil and water isolates affiliated to *Exiguobacterium*, *Bacillus*, *Salinicola* and *Chromohalobacter* genus. Our results comfort the interest of studying extremophile diversity in wetlands of the highly arid Sahara region.

**Keywords:** 16S rDNA, Aridity, Conservation value, Extremophile bacteria, Sahara.

## 1. Introduction

Wetlands account for about 40% of the overall value of ecosystem services worldwide (Gardner and Finlayson 2018). As source and reservoirs of water, they support a rich biodiversity through nutrient allocation and resource provisioning. They also ensure protection from floods and droughts to surrounding people and globally, they are important carbon sinks. However, they are severely endangered by global change, which is now responsible for the loss of 21 percent of wetlands world's surfaces (Fluet-Chouinard et al 2023).

By contrast with temperate regions, wetlands of north Africa that are located at the door of arid deserts face extreme conditions of dryness and elevated temperature that make them even more vulnerable to climate change, with important consequences on biodiversity and ecosystem services they support. In Algeria, Chott Ech Chergui (34°16'N 00°33'E), one of the largest African wetlands covers an area of 855,500 hectares (altitude 1000 m) at the frontier of the Algerian desert between the north of the tellaric Atlas and the south of the Saharan Atlas. Its water table is made up of three interconnected aquifers (Tertiary continental, Seneonic and Bajo-Bathon aquifers) (Aoudia et al 2020) from which emerge natural thermal springs. These were exploited by drilling between 1985 and 1990 to create an irrigated area of 2850 ha and to allow agricultural expansion in such an inhospitable area. In parallel to increasing drought in the region, decrease of the hot springs flow was observed, dropping from 600 L.s<sup>-1</sup> in 1960 to 80 L.s<sup>-1</sup> in 2019, which evidenced the decrease of the water table level (Remini 2010; Direction Générale des Forêts Saïda, personal communication) and the vulnerability of such a large wetland facing land-use change (Winkler et al 2021), especially since Chott Ech Chergui is a shallow flat-bottomed depression locally called “sebkha”. Known to hold significant faunistic and floristic richness, Chott Ech Chergui was included in the List of Wetlands of International Importance of the Ramsar Convention in 2001 (<http://www.ramsar.org>). However, besides studies on land use cartography and floristic diversity showing that aridity and human activities threaten this ecosystem (Arabi and Mederbal 2016), the physicochemical characteristics of Chott Ech Chergui surface water, and the biodiversity it holds have not yet been documented, although these characteristics are essential to understand the wetland dynamics, its degradation and ultimately define conservation strategies.

Given its location, Chott Ech Chergui is a possible reservoir of microorganism holding physiological and biochemical characteristics typical of such arid environment that have high valorization potential in biotechnologies. Such extremophile organisms have evolved the ability to thrive in environment showing extreme values of temperature (>50°C), salinity (>5%, w:v) or pH (<6 or >8) such as in hot springs (Nabagye and Yatuha 2022), geothermal area (Belilla et al 2019), salterns (Leoni et al 2020) or salt water (Saibi et al 2022; Hassani et al 2022). In some situations, with overlaid environmental stresses, some microorganisms - that are called “polyextremophile”, have acquired surprising capacities to withstand superimposed environmental stresses (Egamberdieva et al 2018). Microbial communities living in such extreme conditions are given much attention as potential early Earth ecosystem analogs and sources of new genes and metabolites valuable for bioengineering (Ye et al 2023). Studies on such extreme microbial communities often combine cultivation and molecular approaches (Belilla et al 2019).

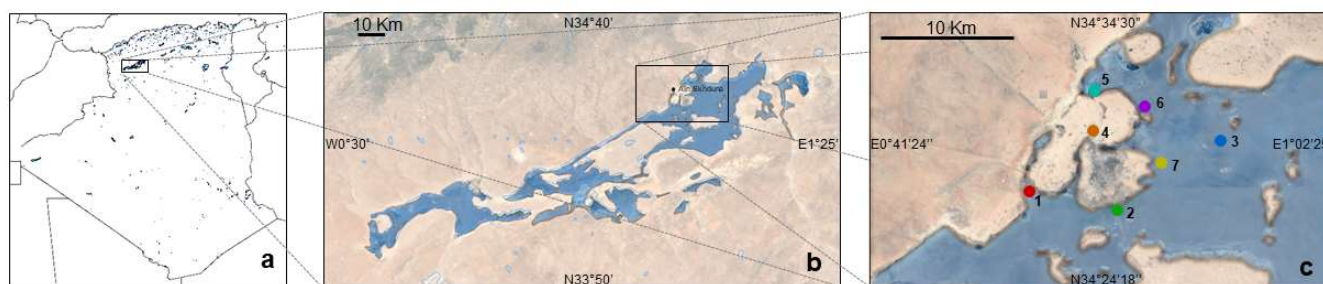
With the objective to gain knowledge on the physicochemical characteristics of Chott Ech Chergui wetland and its yearly dynamics, we achieved a one-year monthly monitoring of water physicochemical characteristics at several locations potentially uncovering distinct environmental situations as regards of temperature, salt and nutrient concentrations. Expecting that to the typical environmental conditions of Chott Ech Chergui should

correspond typical microbial life, we established a culture collection of water extremophile bacteria that were selected for particular thermo-, halo- or alkali- tolerance and we completed their phylogenetic identification.

## 2. Methods

### 2.1. Studied area and sample collection

Chott Ech Chergui presents several contrasted environmental conditions including thermal springs, freshwater, and saltwater. To initiate the physicochemical and microbiological survey, locations showing regular water availability and easy accessibility were chosen, leading to seven representative water sampling sites at proximity of the major thermal spring of Chott Ech Chergui (Fig.1 and Table 1). All sampling sites and mapping data were georeferenced using the mobile GPS GARMIN GPSmap 62. Cartography was generated using mapping software ArcGis 10.1 and image superposition in Google Earth Pro 7.3. Sampling campaigns were conducted monthly for one year from December 2018 to November 2019. At each site, samples of water and sediments were collected in triplicate at a maximum of 10 cm depth for both sample type and under aseptic conditions, using sterile 2.5 L opaque glass bottles for surface water and sterile 50 mL opaque glass bottles for sediments. Samples were preserved at 4°C until laboratory analysis. Water samples were filtered through sterile 0.22 µm pore-diameter filters (Whatman) before chemical analyses (except suspended matter and turbidity).



**Fig. 1** Geographical location of chott Ech Chergui and of sampling sites near Ain Skhoua. Map of the Algerian hydrological network, **a**; representation of Chott Ech Chergui wetland, **b**; and location of study sites, **c**. Study sites have been affected a number and a colour as follows: 1 (red), Source Zawia; 2 (green), Chott Ech Chergui; 3 (deep blue), Large sebkha; 4 (orange), Source Ain Skhoua; 5 (light blue), Bassin; 6 (purple), Small sebkha; 7 (yellow), Oued (see Table 1 for site characteristics). Latitude and longitude are displayed on the maps' borders.

### 2.2. Meteorology and hydrodynamics

Meteorological data were obtained from the Algerian website freemeteo (<https://dz.freemeteo.com/>). Rainfall readings (mm) were used to calculate monthly and annual precipitations. The minimum and maximum air temperature (°C) per day were used for descriptive statistics (monthly and annual, monthly maximum and minimum average temperatures). An ombrothermic diagram was drawn to determine rainfall and temperature variations during the year of field sampling (Fig. 2). To better understand the region's climatic characteristics, monthly and annual aridity index were determined according to De Martonne's classification (Pellicone et al 2019). For each site and date, water depth (cm) was determined using a measuring tape.

### 2.3. Physicochemical characteristics

pH, temperature, and salinity were measured *in situ* with Hanna probes HI991001, HI93530, HI96822 (0-150 ppm) in the salt site at 1/10 dilution in MilliQ water, respectively; dissolved oxygen and conductivity were measured with Hanna probes dedicated to extreme conditions, HI9145 and HI99301, respectively. The water turbidity was determined with a MARTINI Mi415 turbidimeter (0-1000 NTU). Within 24 hours of sampling and using the methods outlined in Rodier's technical manuals (Rodier et al. 2009, 2016). Ammonium, nitrate, nitrite and orthophosphate concentrations were determined by spectrophotometry (METERTECH SP8001) using indophenol blue, sodium salicylate, sulfanilamide and ammonium molybdate colorimetric methods, respectively. The determination of the hydrometric titer and calcium and magnesium contents were carried out using the EDTA titrimetric method. The water chloride concentration was determined with silver nitrates according to the Mohr Method and the organic matter concentration was determined through the reduction of potassium permanganate by the oxidable matter in the water. Mineralization was calculated from conductivity measures based on the calculation table described in the Rodier technical manuals (Rodier et al. 2009, 2016). Sulfate and bicarbonate contents were determined in January, April, July, October by volumetric titration (Metrohm 848 Titrino plus, Metrohm). Potassium and sodium contents were determined by atomic absorption spectrophotometry using the PFP7 Flame Photometer (Jenway). In addition, pH, salinity, and dry matter in sediments was measured each season by SEOR company.

### 2.4. Microbiological cultures and isolation

Following tests of several culture media, Nutrient Agar (NA) was chosen as the most suitable to isolate the different types of extremophiles and to ensure cost-effectiveness for future biotechnological developments. Culture duration and incubation temperatures were chosen according to (Rainey and Oren 2006). Bacterial isolation was performed using a serial dilution method up to  $10^{-3}$  in 0.85% sterile saline solution followed by an enrichment step in liquid NA (Elazzazy et al 2015), with pH adjusted to 7 at the exception of alkali-tolerant isolation, for which pH was adjusted to 11. For halo-tolerant isolation, sodium chloride was set to 250 g.L<sup>-1</sup>. The mediums were distributed in test tubes and sterilized by autoclaving 120°C for 20 min. Culture tubes were then inoculated with one gram of sampled sediment or one milliliter of sampled water, making five replicates per sample. Culture enrichments were incubated at 35°C for a period of 48 hours for alkalitolerant, one week for halo-tolerant and 48 h at 65°C for thermo-tolerant culture. Bacterial isolation was achieved following streaking of the enrichment cultures on NA slants and incubated as previously. Bacterial isolates were kept on agar slants by regular streaking on fresh medium and freeze-stored in a 40% glycerol solution at -80°C.

### 2.5. Biochemical, physiological and tolerance studies of bacterial isolates

Standard bacteriology methods were used to identify the isolated strains (Holt 1994; Collins et al 2004). The microscopic approach combined with Gram and spore staining methods was used as phenotypic characterization of bacterial cell. To examine halotolerance, bacterial growth was achieved at salt concentrations ranging from 0% to 30% (W/V), with 1% NaCl supplementation steps. To examine thermotolerance, bacterial growth was evaluated at temperature ranging from 4°C to 120°C with 3°C intervals. To examine pH tolerance, bacterial growth was measured at each pH unit from pH=3 to pH=12. Each bacterial isolate was subjected to the following

biochemical tests: amylase, lipase, citrate reductase, nitrate reductase, acetoin production, motility, mannitol, glucose, galactose, fructose, catalase, oxidase, gas and H<sub>2</sub>S production. The strains' ability to synthesize enzymes was tested under appropriate salt, temperature, and pH optimal range conditions for each strain.

## 2.6. Phylogenetic characterization of bacterial isolates

The bacterial cells were recovered from 1.5 mL culture subsamples following centrifugation at 1500 g for 20 min. Bacterial cell lysis was achieved following resuspension of the culture pellet in 200 mL buffer TE (Tris 10 mM, EDTA 1 mM pH 8), addition of 50 mL lysozyme (80 mg/mL) and incubation at 37° for 4 h. The mixture was then crushed with glass beads for 90 s at 30 Hz using the TissueLyser (Qiagen). Following centrifugation for 30 s at 11000 g, the supernatant was collected in 2 mL tubes, and the pellet was washed in 250 mL of TE buffer before being crushed and centrifuged once more. Both supernatants were combined and 30 µl of 10 mg/mL RNase was added and the mixture was incubated at 50°C for 1h30. 25 µl of 2X lysis buffer (1% SDS, 20 mM NaCl, 20mM Tris pH 8, 20 mM EDTA) and 2.5 µl of Proteinase K (20 mg/mL), followed by incubation at 50°C for 2 h. The DNA was then precipitated with sodium acetate and ethanol (0.1 V sodium acetate pH = 8 at 3 M and 3 V ethanol 100% at -20 °C). After overnight incubation at -20°C, the mixture was centrifuged at 16 000 g for 20 min at 4°C. The supernatant was discarded and the pellet was rinsed with 100 µl of 70% ethanol (-20°C) and soft manual stirring four times. Following centrifugation at 16000 g for 20 min at 4°C, the supernatant was discarded and the DNA containing pellet was eluted in 50 µl TE buffer after total evaporation of the ethanol. Nucleic acid assay and purity testing were performed using the NanoDrop™UV-visible spectrophotometer (Thermo Scientific).

PCR amplification of the bacterial 16S rDNA was achieved using forward primer 16S 27F: AGAGTTTGATCMTGGCTCAG (modified of Fd1 primer after (Winker and Woese 1991) and reverse primer 16S 1547 Rd1: AAGGAGGTGATCCAGCC (Winker and Woese 1991). PCR reactions were conducted in a volume of 50 µl, with 0.2 mM each dNTP (PCR nucleotide Mix, Promega), 0.1 µM of each primer, 5 µl 10X Buffer, 0.025 U/µL of DreamTaq DNA polymerase (ThermoScientific) and 10 pg to 1 µg genomic DNA was added to the reaction mixture. After initial denaturation at 95°C for 2 min, we ran 40 cycles of denaturation at 95°C for 30s, followed by annealing at 55°C for 30s, elongation at 72°C for 1 min and a final elongation at 72°C for 10 min. Amplicons were visualized following agarose (2%) gel electrophoresis and ethidium bromide staining under UV light transilluminator (Molecular Imager® Gel Doc™). Amplicons of ~1500 nt were Sanger-sequenced (Eurofins Genomics©).

16S rDNA sequences were manually edited using the BioEdit program (Hall et al 2011) based on visual examination of chromatograms. 16S rDNA query sequences were submitted to the Genbank database and provided with an accession number via the National Center for Biotechnology Information (NCBI; Table S1). Most similar reference sequences were retrieved using the BLAST program and were joined to our new Sanger sequences for phylogenetic reconstruction using the Mega X software (Kumar et al 2018). Query and reference sequences were aligned using the CLUSTALW program (Thompson et al 2003), and phylogenetic trees were reconstructed by the maximum composite likelihood method (Saitou and Nei 1987). Bootstrap test (500 replicates) was achieved to calculate the percentage of replicate trees in which the associated taxa clustered together, and the evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al 2004).

## 2.7. Statistical analyses

Physicochemical data were analyzed using JMP 9.0 (SAS Institute Inc.) for univariate analysis and PRIMER v7 program and (Primer-E Ltd, Plymouth, United Kingdom; Clarke and Gorley 2015) for multivariate analysis. Canonical analysis of principal coordinates was achieved using the CAP routine of the PERMANOVA+ program (Primer-E Ltd, Plymouth, United Kingdom) and the variability of physicochemical data across sampling sites, captured on CAP1 and CAP2 eigenvalues, was tested using between pair student t tests. In addition, the distribution of the points representing the anions and cations characterizing the chemical structure of the water of the Chott Ech Chergui was determined according to the piper diagram and using the Diagram software. The chemical facies of the waters of each sampling site at the Chott Ech Chergui level was illustrated from the average concentrations of calcium chloride, magnesium, sodium, potassium, sulphate, bicarbonates and nitrates after their classification by the Schoeller Berkloff diagram using the Diagram software.

## 3. Results

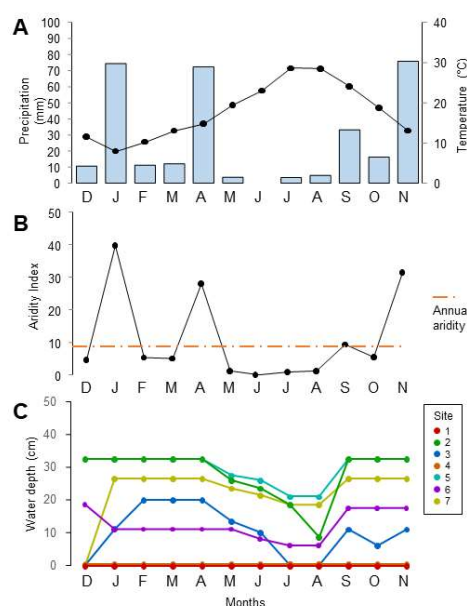
### 3.1. Local meteorology and hydrodynamics

The metrological data provided by Station Rghuis showed that mean monthly temperature varied between 11°C and 28°C. The highest temperature recorded in the area was 48°C in July, and the lowest temperature was 0°C in January. Large variations in the amount of rainfall were observed throughout the year, with ~60 mm recorded in January, April, and November and ~26 mm spike recorded in September following a three-month drought summer period (Fig. 2A). The aridity index displayed three peaks, mirroring the dynamics of precipitation throughout the year (Fig. 2B). Water depth varied consequently during the sampling year with higher values measured during rainy periods and lower values measured during the dry summer season (Fig. 2C). Among sampling sites, the highest water depth (~32 cm) was measured on sites n°2 and n°5, in the vast freshwater depression and at the beginning of a river fed by the second thermal source, respectively; the lowest values was recorded during the driest months of July and August, with full drying out at site n°3 in the vast arid depression fed by the 1st thermal spring and rainfalls.

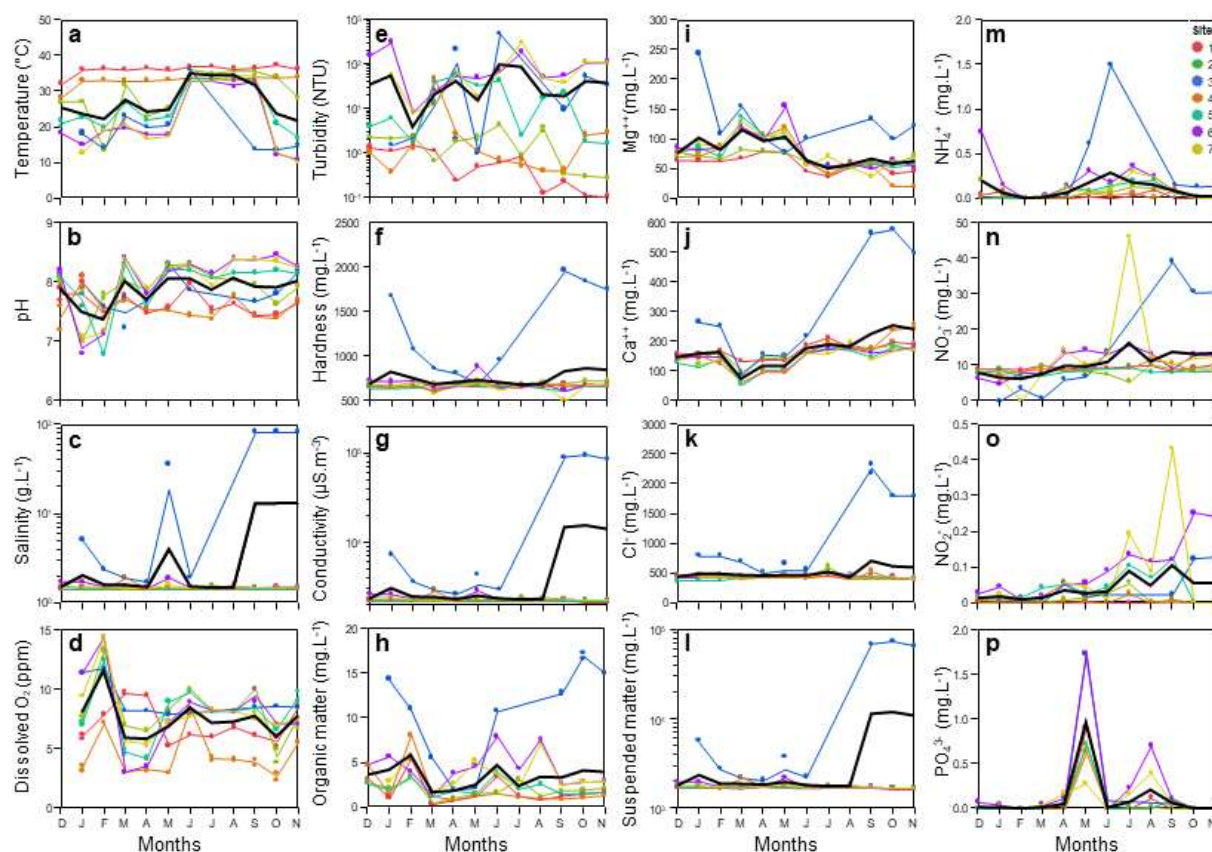
### 3.2. Temporal variation of water physicochemistry

Most water parameters measured at site n°3 (Fig. 3) showed highest values and variability across sampling dates (i.e. Salinity ~ 80g/L , Hardness ~2000 mg.L<sup>-1</sup> , Conductivity ~100000 µSm<sup>3</sup>, Organic Matter. ~17 mg.L<sup>-1</sup> , Mg<sup>++</sup> ~250 mg.L<sup>-1</sup> , Ca<sup>++</sup> ~600 mg.L<sup>-1</sup> , Cl<sup>-</sup> ~2400 mg.L<sup>-1</sup> and NO<sub>3</sub><sup>-</sup> ~40 mg.L<sup>-1</sup> ), which must be put in relation with sediments with high salinity content (Table S1) and the succession of drying – rewetting periods that prevented from correct water sampling in December 2018, July and August 2019. Also, sites n°1 and 4, that are in the direct stream of the thermal sources showed an average temperature of ~35°C all year. However, for all other sites, the temporal variation of water physicochemical parameters revealed seasonal patterns.





**Fig. 2** Meteorology of the region of Ain Skhoua and hydrological dynamics of the study sites during the sampling campaign (December 2018 to November 2019): Ombrothermic diagram, **a**; variation of the aridity index, **b**; variation of water depth at each study site, **c**.



**Fig. 3** Dynamics of water physicochemical parameters at the various sampling sites of Chott Ech Chergui between December 2018 and November 2019: temperature, **a**; pH, **b**; salinity, **c**; dissolved Oxygen, **d**; turbidity **e**; hardness **f**; conductivity, **g**; organic matter **h**; magnesium content, **i**; calcium content, **j**; chloride content, **k**; suspended matter, **l**; ammonium content, **m**; nitrate content, **n**; nitrite content, **o**; orthophosphates, **p**. Capital letters indicate successive sampling months on the X-axis. For convenience color legend of the sites is displayed in graph **m**. The thick black lines show across sites average values.

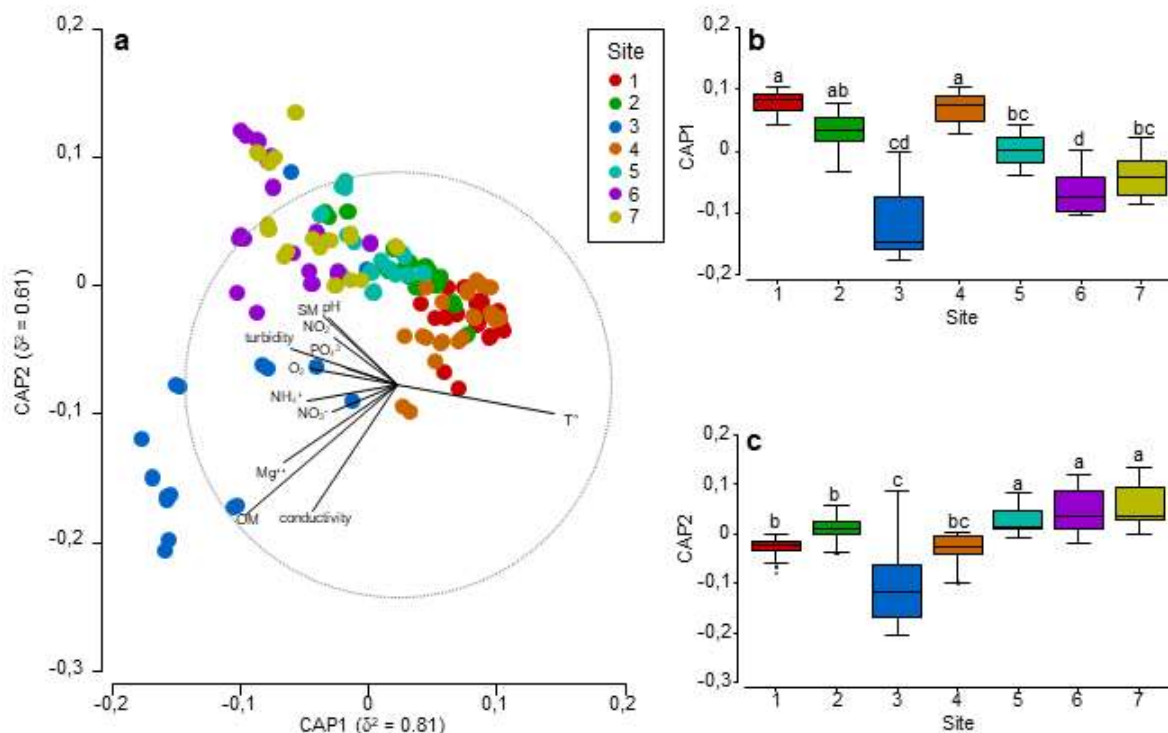
Winter was marked by low water temperature  $\sim 23^{\circ}\text{C}$ , with lower points at  $\sim 12^{\circ}\text{C}$  locally). This was associated with neutral pH values ( $\sim 7.5$ ), the highest values of dissolved oxygen ( $\sim 12$  ppm) and organic matter contents ( $\sim 5$  mg.mL<sup>-1</sup>). The arrival of spring came along with the slow increase of temperature (up to the maximum value of  $\sim 32^{\circ}\text{C}$  in June) and pH (reaching pH $\sim 8$  in May), while dissolved oxygen dropped importantly (down to  $\sim 6$  ppm in April) before rising up to a secondary peak late spring ( $\sim 8$  ppm in June). The organic matter content followed a similar pattern as that of oxygen, with an important decrease early spring (down to  $\sim 2$  mg.L<sup>-1</sup> in April) and a secondary peak late spring ( $\sim 5$  mg.L<sup>-1</sup> in June). The magnesium content reached its highest value ( $\sim 100$  mg.L<sup>-1</sup> in May) in contrast to the calcium content that reached its lowest value ( $\sim 80$  mg.L<sup>-1</sup> in March). Also, spring was characterized by a peak value of phosphate content ( $\sim 1$  mg.L<sup>-1</sup> in May).

From the late spring to early autumn, water temperature rises to the maximum value that characterize permanently site n°1 and 4 ( $\sim 32^{\circ}\text{C}$ ), at all other sites. This was associated with almost stable alkaline conditions (pH $\sim 8$ ), low and stable oxygen content ( $\sim 7.5$  ppm) and a weak increase of the organic matter content. While magnesium maintain at its lowest throughout summer ( $\sim 50$  mg.L<sup>-1</sup>), the calcium contents increased to reach its maximum value early fall ( $\sim 200$  mg.L<sup>-1</sup> in October). Interestingly, the nitrogen cycle was marked by successive rises of ammonium content (rising up to  $\sim 0.25$  mg.L<sup>-1</sup> in June), nitrates contents (rising up to  $\sim 15$  mg.L<sup>-1</sup> in July) and nitrites (rising up to  $\sim 0.1$  mg.L<sup>-1</sup> between July and September). Also, summer was marked by a secondary peak value of phosphate ( $\sim 0.2$  mg.L<sup>-1</sup> in August).

The third period of autumn was marked by water temperature dropping (down to  $\sim 20^{\circ}\text{C}$ ), lowest values of magnesium content ( $\sim 50$  mg.L<sup>-1</sup>) and highest value of calcium content (rising up to  $\sim 200$  mg.L<sup>-1</sup> in October and November).

### 3.3. Spatial variability of water physicochemistry

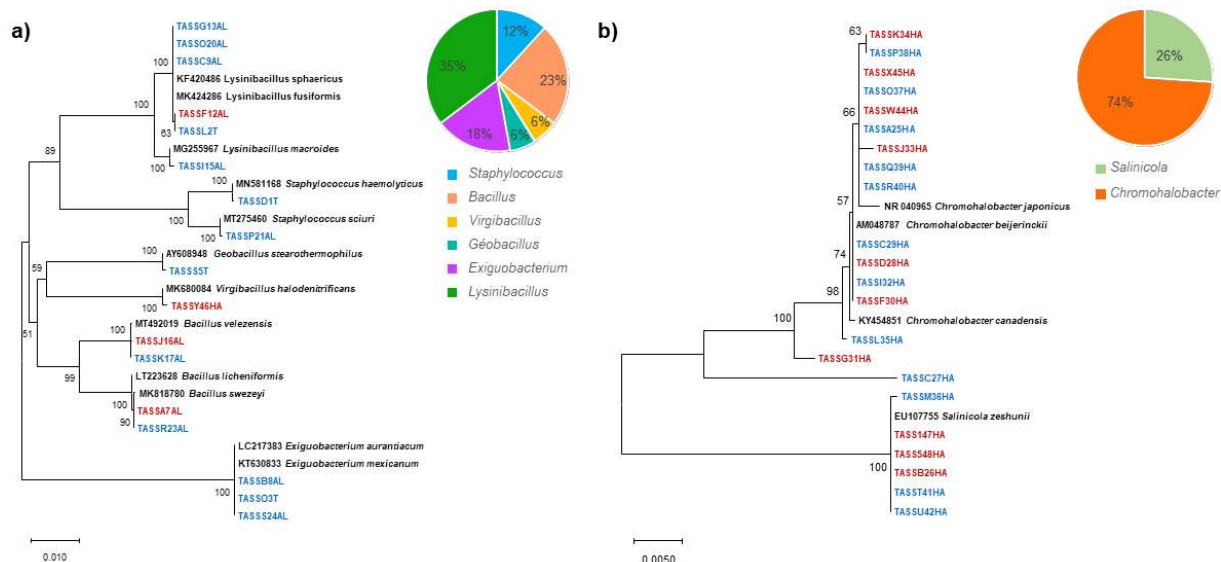
The first two axes of the CAP analysis of water physicochemical parameter allowed categorizing the sampling sites into three groups, showing their contrasted environmental conditions (Fig. 4). The superimposed correlation circle summarizes the variables that describe the factors explaining the variation across sites. Sites 1 and 4, corresponding to source Zaiwa and source Ain Skouna, respectively, were driven by elevated water temperature and low values for much of the considered environmental parameters, which point out the oligotrophy and low oxygenation of the thermal water resurging from the belowground water table. Sites 5, 6 and 7 and to a lower extend site 2, opposed to the sites 1 and 4 on the CAP 1 axis partly due to contrasted water temperature regime, but also because these sites showed high values of pH, suspended matter, nitrites, orthophosphate, turbidity and oxygen content, which typify these sites as eutrophic and alkaline. Site 3, corresponding to the vast arid depression of the Large Sebkhah also opposed on the CAP 1 axis to the sources site. But site 3 opposed to site 5, 6, 7 and 2 on the CAP 2 axis as it showed high values of ammonium, nitrate, magnesium, organic matter and conductivity (regrouping salinity, hardness, calcium content and chloride content). This latter site can thus be qualified as eutrophic and saline.



**Fig. 4** Constrained ordination from the canonical analysis of principal coordinates (CAP analysis) discriminating the various sampling sites of Chott Ech Chergui according to the water physicochemical parameters measured from December 2018 to November 2019 (a). The superimposed Pearson correlation circle show correlations vectors of individual physicochemical parameters: temperature (T°), pH, dissolved oxygen (O<sub>2</sub>), turbidity, organic matter (OM), magnesium content (Mg<sup>++</sup>), suspended matter (SM), ammonium content (NH<sub>4</sub><sup>+</sup>), nitrate content (NO<sub>3</sub><sup>-</sup>), nitrite content (NO<sub>2</sub><sup>-</sup>), orthophosphates (PO<sub>4</sub><sup>3-</sup>) and conductivity. The latter vector is representative for variation of salinity, hardness, calcium content and chloride content, showing correlation coefficient  $r > 0.84$ . Comparison of the physicochemical variability across sampling sites is shown on the two most discriminant axis CAP1 and CAP2 (b and c, respectively). Values affected with a different letter are significantly different for  $p < 0.05$ .

### 3.4. Isolation and characterization of bacterial extremophile isolates

Following microbiological analyses, 320 bacterial isolates from soil and sediments samples were tested for thermo-, acido- and alcalino- tolerance. Among them, only 39 were maintained in a culture collection (Table S1). Following sequencing of the 16S rRNA genes and phylogenetic affiliation with subject sequences retrieved from the NCBI GenBank, 17 isolates were affiliated to the phylum Firmicutes, class Bacilli, order Bacillales, family Bacillaceae within genus *Bacillus*, *Lysinibacillus*, *Virgibacillus*, *Geobacillus*, *Exiguobacterium*, and family Staphylococcaceae within genus *Staphylococcus* (Fig. 5A); 22 isolates were affiliated to the phylum Proteobacteria, class gamma-Proteobacteria, order Oceanospirillales, family Halomonadaceae within genus *Chromohalobacter* and *Salinicola* (Fig. 5B). Two isolates (OM301605 and OM301582) did not have closely related subject sequence in the database. Isolate OM301582 had a particularly weak percentage identity with the closest reference sequence in the NCBI database (<94%), which weaken its formal affiliation to the *Chromalobacter* genus.



**Fig. 5** Taxonomic affiliation of bacterial isolates through phylogenetic reconstruction of their 16S rRNA sequences with closest relative sequences collected from the NCBI GeneBank database. Phylogenetic reconstruction is shown separately for firmicutes (a) and for proteobacteria (b). For each bacterial group, pie charts show the proportional distribution of strains within genus.

The various surveyed water and sediment sites revealed contrasted phylogenetic affiliation across sites, so that bacteria affiliated to the Proteobacteria were only recovered at sites 3 (Large Sebkh) and 6 (Small Sebkh), while those affiliated to the Firmicutes were isolated at all sites to the exception of site 7 (Oued). The 16 bacterial isolates affiliated to the phylum Firmicutes could grow in the absence of salt but could tolerate 5 to 15% salt in the culture medium, these are considered “halotolerant”; and 23 bacterial isolates affiliated to the phylum Firmicutes (genus *Virgibacillus*) and to the phylum Proteobacteria that could not grow without salt, had a minimum requirement of 5% salt content in the culture medium, and could still grow at a high salt concentration of up to 30%, these are considered “halophilic.” All isolates showed the ability to thrive in both acidic and alkaline conditions, but 10 isolates could support only low alkalinity (pH 9), while 29 isolates could still thrive at high alkalinity (up to pH 10 and 12), these are considered “alkalitolerant” and “alkaliphilic”, respectively. Similarly, the 27 isolates showing the ability to grow at pH 5 are considered “acidotolerant”. All isolates had the ability to grow above 45°C and remarkably, one isolate required high temperature to grow (ranging from 50°C up to 100°C), these are considered “thermophilic”. Contrastingly, five isolates showed the ability to thrive in large temperature range (ranging from 4°C to 60°C), these are considered “psychrothermotolerant”.

The screening for enzymatic activities showed that all isolates expressed their activities in pH, salt- and temperature- ranges they were cultivated in. Over all isolates, 20 isolates from the genus *Chromohalobacter* and *Salinicola* could reduce citrate, 17 isolates affiliated to the genus *Staphylococcus*, *Bacillus*, *Exiguobacterium*, *Chromohalobacter*, *Salinicola* and *Virgibacillus* synthesize acetoin, 15 isolates affiliated to the genus *Staphylococcus*, *Lysinibacillus*, *Exiguobacterium*, *Geobacillus*, *Bacillus*, *Chromohalobacter* and *Salinicola* reduce nitrates, 11 strains bacteria isolates affiliated to the genus *Staphylococcus*, *Geobacillus*, *Bacillus*, *Lysinibacillus*, and *Chromohalobacter* produce lipase, and 14 isolates affiliated to the genus *Lysinibacillus*, *Exiguobacterium*, *Geobacillus* and *Bacillus* synthesize amylase. 32 isolates utilized sugar (mannose, glucose, fructose), and 11

used mannitol as main carbon source. 23 isolates were oxidase-positive, 31 had antioxidant catalase and interestingly, most of the enzyme producers were affiliated to the firmicutes, not to the proteobacteria.

#### 4. Discussion

Several wetlands with extreme environmental conditions, have been the focus of extensive research to describe microbial diversity and biotechnological potential. The arid and hypersaline Chott El Jerid (Tunisia), with hypersaline conditions, was studied to screen bacteria and archaea isolates for the accumulation of polyhydroxyalkanoates inclusions (Ben Abdallah et al 2020). The hypersaline geothermal area of Dallol in the desert of Danakil (Ethiopia), holding chaotropic conditions related to  $Mg^{++}/Ca^{++}$ -dominated brines, hyperacidity and elevated temperatures allowed identifying exceptionally diverse and abundant ultrasmall archaea (Belilla et al 2019). In Algeria, the salty to moderately salty wetland dayat Morsli, near Oran, facing many years of anthropogenic pollution, was investigated for physicochemical and biological yearly dynamics in the view of rehabilitation possibilities (Ben Bayer et al 2019); recent investigation of The Great Sebkhia of Oran, a hypersaline lake, allowed characterizing 17 halotolerant bacterial isolate as promising sources of important molecules (Saibi et al 2022); and the hypersaline Saharian chott Zehrez-Chergui was investigated using a combination of cultivable and non-cultivable method in search of soil halophilic archaea (Hassani et al 2022). The present study constitutes the first investigation of Chott Ech Chergui (Algeria) that aimed to provide one of the largest wetlands of the north African desert with physico-chemical and bacteriological characteristics. Water deficiency is thus the main constraint of this wetland, which occurs seasonally in some areas or constitutes a chronic stress in others, leading to dried out sites that prevented from appropriate sampling. At the exception of site n°3 (Large Sebkhia), that showed high level of organic matter contents, the surface waters of Chott Ech Chergui are generally oligotrophic and observed changes in water chemistry are mainly related to meteorological factors. The water nutrient dynamic is affected by soil leaching that follows periods of rainfalls (Loudière and Gourbesville 2020), and also by water evaporation at the dry summer season that concentrate nutrients (nitrate, nitrite, orthophosphate) as previously reported by the United Nation report (Hazzab 2011; Ouali et al 2018). Our study distinguishes four key habitats in the Chott Ech Chergui wetland based on contrasted physicochemical parameters. The first are the hot springs (sites n°1 and n°4), which have a constant water temperature of 37-40°C throughout the year, a high oligotrophy level with low contents of organic matter, nitrogen, and phosphorus. According to the classification of Skopljak (Skopljak et al 2006), these thermal waters are dominated by chlorides and calcium and, unlike other thermal springs in the region (Hazzab 2011; Ouali et al 2018), they also show elevated contents of sulfate and sodium. Among the eight thermal springs relevant of the public sector in Algeria, only one thermal spring (Hammam Charef, located ~200 km east of Chott Ech Chergui) has the most similar water chemistry (Ouali et al 2018; Fig. S1 and S2). This extreme habitat, characterized by high temperatures and by oligotrophic conditions, imposes strong physiological constraints that should limit bacterial metabolism, growth and multiplication. The second habitat is hypersaline (site n°3) showing high salinity levels in the sediment (up to 380 g.L<sup>-1</sup>; Table S2) that drive elevated but fluctuating salt contents in water (reaching 80 g.L<sup>-1</sup>) in relation to regular supply with freshwater, hot springs and rain. In this habitat, high concentrations of chloride, magnesium, calcium, nitrites, nitrates, orthophosphates, and ammonium should favor bacterial growth and sustain diversification of thermo- and halo- tolerant microbiota. In comparison to other hypersaline habitats of north Africa, the three permanent arid salt lakes in the Oued Righ valley (northeastern Algeria) showed the

same type of chlorinated and calcium sulphate waters (Gouasmia et al 2016); while the four arid salt lakes of Tunisia showed differences of water minerality and nutrient content, in relation to chlorides and sodium/potassium dominance (Kbir-Ariguib et al 2001). The third habitat (sites n°6 and n°7), in addition to hypersaline characteristics of both water and sediments (Table S2), is characterized by elevated pH values, suitable for the development of alkaliphile microorganisms. The fourth habitat (sites n°2 and n°5) given its condition at the confluence of all three previous habitats, showed average levels of nutrient, pH, salinity and temperature values, making of it the most hospitable habitat of Chott Ech Chergui that should hold abundant and diversified microbial life.

The relationship between the environmental constrain of the habitats defined above and microbial diversity should be addressed in further studies using new generation DNA sequencing technics. However, our sampling campaign successfully allowed the bacteriological, biochemical and molecular characterization of several extremophile isolates in relation to the key environmental constraints of Chott Ech Chergui. These were affiliated to the Proteobacteria (Table S1), with several strains regrouped to genus *Chromohalobacter* and *Salinicola* and to the Firmicutes, with several strains regrouped to genus *Bacillus*, *Geobacillus*, *Staphylococcus*, *Exigubacterium*, *Lysinibacillus* and *Virgibacillus*. Such bacterial taxa were also isolated from saline ecosystems of northeastern Algeria (Menasria et al 2019), from salterns in Korea (Lee et al 2012), from various saline habitats in India (Kumar et al 2012), from saline and hypersaline lakes in the Qinghai Province of China (Jiang et al 2018) and more closely, in The Great Sebkha of Oran (Saibi et al 2022).

All these bacterial isolates had the ability to thrive at alkaline pH and high salt concentrations. The bacterial isolates affiliated to the Firmicutes formed a group of halotolerant bacteria, to the exception of the *Virgibacillus* strain OM301613 considered halophilic, like all bacterial isolates affiliated to the Proteobacteria. According to the classification of alkaliphile (Mevada et al 2022), thirty bacterial isolates were considered facultative alkaliphiles, while nine were considered alkalitolerant.

Three bacterial isolates affiliated to the genus *Exiguobacterium* (strains OM301601, OM301581 and OM301607) can thrive at temperatures ranging from 4°C to 60°C and are thus considered psychro-thermotolerant. One bacterial isolate affiliated to the genus *Geobacillus* (strain OM301608) is considered thermophilic as it shows the ability to grow at 100°C, while all other bacterial isolates are considered meso-thermotolerant as they show abilities to grow at temperature ranging from 15°C to 60°C. The recovery of thermotolerant bacteria affiliated to the genus *Bacillus* and *Geobacillus* is not surprising as they have often been described as abundant members of microbial community living in thermal environments (Amin et al 2018; Narsing Rao et al 2018; Panosyan et al 2018; Xian et al 2018; Belilla et al 2019 and Saghatelian et al, 2021).

Although all bacterial isolates were polyextremophile, their site of origin determines their degree of tolerance to the three main constraint of Chott Ech Chergui wetland (temperature, alkalinity and salinity). For instance, strains that can grow at high salt concentrations were found in the second and third habitats (sites 3 and 6) characterized by salinity and alkalinity, respectively; and those that can grow at high temperature were found in the first habitat (sites 1 and 4) characterized by elevated temperatures and in the fourth habitat (sites 2 and 5) showing intermediate conditions. However, alkaliphilic and alkaline-tolerant strains were found in all four habitats.

Several bacterial isolates of our collection were shown to express enzymatic activities valuable in biotechnology (such as lipases and amylases) in a large range of temperature or salt concentrations. These initial bacteriological

test open perspective of biochemical investigation of the corresponding bioactive molecules these bacterial isolates produce (Boyadzhieva et al 2018; Qiu et al 2021; Pham et al 2021) and highlights the biotechnological potential of Chott Ech Chergui wetland as a reservoir of polyextremophile bacterial isolates.

## **5. Conclusion**

This study provides the first report of the physicochemical conditions of the Chott Ech Chergui wetland and a typification of the water habitats with extreme conditions it holds, including seasonal aridity, elevated thermal gradient, high salinity and high alkalinity. As a first step in the way to describe the biodiversity this unique ecosystem supports, we successfully isolated and identified halophilic, alkalophilic and thermophilic bacteria, some of which, polyextremophile, combine all three characters. This study constitutes the base of future programs of biodiversity monitoring of Chott Ech Chergui wetland and offers promising perspectives of valorization in biotechnologies as conservation values for wetlands with extreme environmental conditions. The remarked decrease of the watered surface at the dry summer season are primary symptoms of a more general drying up of Chott Ech Chergui wetland that is likely to emphasize with climate change and call for the improvement of management practices and the control of surrounding human activities.

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## **7. Statements and Declarations**

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### **Competing Interests**

The authors have no relevant financial or non-financial interests to disclose.

### **Author Contributions**

• Conceptualization: Abi Ayad Sidi Mohammed El Amine, Lamara Sid Ahmed Chawki, Mehrou Zouggar Aicha, Denis Francoise, Caruso Aurore; Methodology: Mehrou Zouggar Aicha, Pascal Mirleau, Chenuil Anne; Formal analysis and investigation: Mehrou Zouggar Aicha, Pascal Mirleau, Chenuil Anne; Writing - original draft preparation: Mehrou Zouggar Aicha, Pascal Mirleau; Writing - review and editing: Mehrou Zouggar Aicha, Pascal Mirleau, Chenuil Anne, Denis Francoise; Funding acquisition: Chenuil Anne, Abi Ayad Sidi Mohammed El Amine; Resources: Pascal Mirleau, Chenuil Anne, Abi Ayad Sidi Mohammed; Supervision: Pascal Mirleau, Chenuil Anne.

### **Data Availability**

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

573 **8. Tables**

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575 **Table 1.** Name, location and characteristics of the sampling sites in the wetlands Chott Ech Chergui.

Site	Name	Latitude	longitude	Brief description
1	Source Zawia	34°28'2.21"N	0°49'7.93'' E	First thermal source main point of the Chott Ech Chergui supply
2	Chott Ech Chergui	34°30'0.96 ''N	0°50'39.88''E	Vast freshwater depression
3	Large Sebkha	34°27'13.80"N	0°50'33.21"E	Vast arid depression salty soil fed by the 1st thermal spring and rain waters
4	Source Ain Skhouana	34°30'8.14 ''N	0°50'44.50''E	Second thermal source and second feeding point of the Chott Ech Chergui
5	Bassin	34°30'0.96 ''N	0°50'39.88''E	Beginning of a river fed by the second thermal source
6	Small Sebkha	34°29'42.12 ''N	0°50'54.36''E	Basin fed by the second thermal spring and rainwater forming a brackish lake
7	Oued	34°29'46"N	0°53'27''E	feeding point on the east side of the Chott Ech Chergui forming the continuation of the river

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