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AÏN BOU RKHISS AND AÏN KIBRIT, TWO SPRINGS FROM THE MERGUELLIL BASIN (KAIROUAN, CENTRAL TUNISIA): DIATOM ASSEMBLAGES, BIOLOGICAL POLLUOSENSITIVITY INDICES, HYDROGEOLOGY AND SOCIETAL ASPECTS

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ABSTRACT. – Surveys of the diatom assemblages from Aïn Bou Rkhiss and Aïn Kibrit, two springs that flow out into the Merguellil Wadi, and Aïn Ben Ali, a well located a few kilometers to the east, in the same geological formations (Kairouan district, Central Tunisia), were conducted in 2014 and 2016. The aim of this study was to characterize the species diversity of these water points, establish their ecological status and test the water quality using diatom indices standardized in France [Biological Diatom Index (BDI) and Specific Polluosensitivity Index (SPI)]. Several physical-chemical variables were measured in 2016. Aïn Bou Rkhiss and Aïn Kibrit are in close proximity to one another but differ in terms of their bio-geochemical characteristics and uses (drinking water for domestic use and sulfur-rich water). We discuss the population's growing anxiety with respect to their water supply and explore the traditional knowledge concerning the region's groundwater circulation. The BDI index, reveals a lower quality than that assumed by local users. This may be due to a lack of knowledge about some other local water supplies of good quality that would have served as reference, and inadequacy of these methods designed for European freshwaters without consideration of more southern diatoms. A new variety of *Luticola* is described.

INTRODUCTION

The Merguellil Basin (Fig. 1) is located in Central Tunisia and characterized by a semi-arid climate. The catchment area is about 1200 km^2, draining waters originating from the Tunisian Dorsal (eastern extension of the Atlas Mountains), toward the Kairouan plain (Kingumbi *et al*. 2007, Le Goulven *et al*. 2009). The geology of this part of Tunisia is complex but relatively well-documented (Pervinquière 1903, Castany 1951, Martinez *et al*. 1990). The area of interest is located in the Ouesletia anticline (Fig. 1). The heart of the anticline lies in the Jebil hill and is occupied by nummulitic limestones attributed to Ypresian age (lower Eocene). The Merguellil basin overlies three major aquifers: Bou Hafna, Haffouz Cherichira and Aïn Beidha, with complex hydrodynamic interactions that were described by Kingumbi *et al*. (2007) and Ben Ammar *et al*. (2006, 2009). Groundwater ever-increasing exploitation is also pointed out in the basin. The Bou Hafna and Haffouz aquifers show a declining water table for the last 40 years (about 30 m).

Flow rates (bimonthly data) of the Merguellil Wadi are reported by the 'Direction Générale des Ressources en Eau' (DGRE from the Kairouan CRDA 'Commissariat Régional au Développement Agricole'), but the specific flow rate of each spring is not documented. From 1970 to 2005, the flow rate of the Merguellil Wadi, in the sector of the Aïn Bou Rkhiss, ranged from 8.10^{-4} m^3/s to ca. 1 m^3/s (mean 0.085 m^3/s), with long periods of low rates and sudden increases every five or more years (not illustrated). Kingumbi (2006) cites the springs from Ktifet El Omrane and Cherichira as possible natural outlets of the Bou Hafna and Haffouz Cherichira aquifers. The Bou Hafna aquifer has been exploited since 1895, firstly through the capture of Bou Hafna springs and then directly through the installation of exploitation boreholes, from the 1960s (Massuel & Riaux 2017).

Taxonomic data on freshwater diatoms and cyanobacteria from North Africa, and particularly Tunisia, are relatively scarce (see Levanets & van Rensburg 2010), and are mainly turned within wetlands (or sebkha), lakes, lagoons and oases (e.g., Lanzi 1876, Petit 1895, Amossé

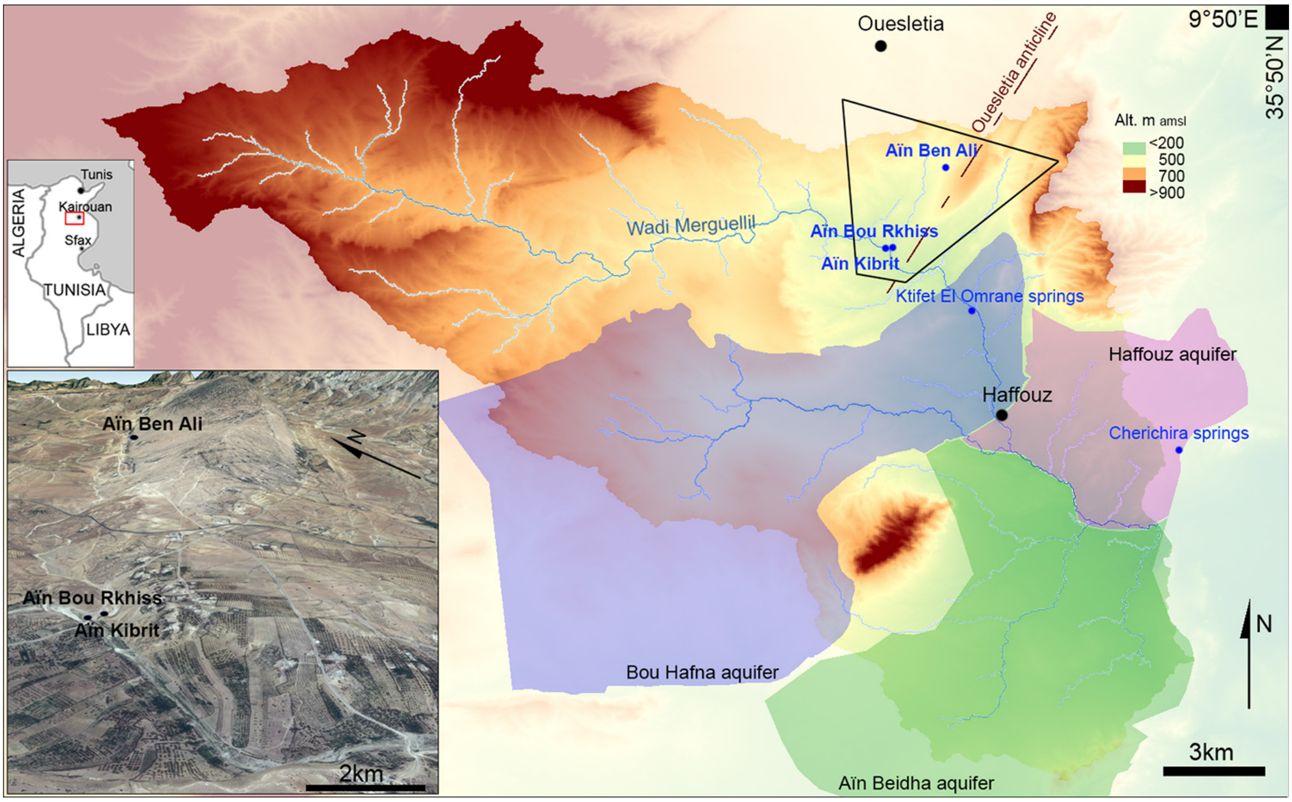


Fig. 1. – Merguellil Basin and sampling sites: Aïn Bou Rkhiss, Aïn Kibrit and Aïn Ben Ali.

1941, Gayral 1952, Hustedt 1953, D'Hollander & Caljon 1978, Flower *et al*. 2001), but also oueds and estuaries, with water quality assessment (e.g., Hammada *et al*. 1996, Chaïb & Tison-Rosebery 2012, Nehar *et al*. 2014, Ouchir *et al*. 2017). The diatom assemblages of springs from semi-arid countries from North Africa, and thermomineral springs, also received relatively low attention (Belloc 1893, 1895a, b, 1896, Ghozzi *et al*. 2013, Ouchir *et al*. 2017).

In the present study, two springs and a well are investigated, with a focus on their diatom and cyanobacteria assemblages: Aïn Bou Rkhiss, Aïn Kibrit and Aïn Ben Ali ('Aïn' refers to spring, in Arabic), located Northwest of Haffouz (Fig. 1, Figs 2-5). Aïn Bou Rkhiss emerges on the left bank from the Eocene nummulitic limestones (Figs 2-3). Aïn Kibrit flows directly in the Merguellil Wadi river bed through alluvium of Holocene age (Fig. 4). Aïn Ben Ali (Fig. 5) is tapped in the same formations but located 3 km northeast, in the Ouesletia anticline (Fig. 1, detailed insert).

Benthic diatoms are regarded as good ecological indicators for freshwater waterbodies (e.g., Descy 1979, Prygiel & Coste 1999, Prygiel *et al*. 1999, Coles *et al*. 2015) and also for springs (e.g., Wojtal & Sobczyk 2012). Diatoms are widely used in water quality monitoring programs (e.g., Coste *et al*. 2009, Besse-Lototskaya *et al*. 2011). The Biological Diatom Index (BDI) (Lecointe *et al*. 1993, AFNOR 2007, Coste *et al*. 2009), which is primarily sensitive to nutrient pollution and the Specific Polluosensitivity Index (IPS) (Coste in Cemagref 1982), both using diatoms, were used to assess the ecological status of the sites. These indices were first applied in France and later throughout Europe with improvements (Coste *et al*. 2009). Recently, the use of diatoms as indicators of ecosystem health was discussed by Ouchir *et al*. (2017) for Lake Ichkeul (Northern Tunisia). In South Africa, Taylor *et al*. (2007a, b) used diatom-based indices, among which BDI and SPI, in water quality monitoring. Such indices were also tentatively used and compared in remote localities, e.g., in China for river health assessment (Tan *et al*. 2013, 2017; Qu *et al*. 2014).

In the present study, physical-chemical variables were concurrently measured to characterize the reservoir signature of each water supply and to validate the BDI and SPI indices. Aïn Ben Ali, located upstream to Aïn Bou Rkhiss and Aïn Kibrit, was investigated to evaluate a possible connection between the reservoirs of the three water supplies.

To date, investigations have focused on the social aspects of access to water in the Merguellil Basin from a socio-historical perspective (Géroudet 2004, Mahafoudh *et al*. 2004, Belaïd & Riaux 2013, Riaux 2013, 2016, Riaux *et al*. 2015). In a study by Collard *et al*. (2015), the relation to recent developments in hydro-agriculture was also explored. As it is the case elsewhere in semi-arid countries, the Aïn Bou Rkhiss water is an important

Figs 2-5. – Sampling sites: Aïn Bou Rkhiss (2-3), Aïn Kibrit (4) and Aïn Ben Ali (5). Scale bars: 30 cm (Fig. 2), 2 m (Fig. 4).

Table I. – Samples details, slide numbers (in collection M. Coste, IRSTEA, Bordeaux).

Acronym/code	Site	Latitude Longitude (WGS84)		Substrate	Date	Slide n°
KIB1	Aïn Kibrit	9°35’53.87”E	35°43’31.61”N	Sediment and macroalgae	15/12/14	18573
KIB2	”	”	”	Sediment and macroalgae	”	18574
AKB1	”	”	”	On degassing points: sediment and green macroalgae	25/02/16	18737
AKB2	”	”	”	Pebbles scraping	”	18738
ALI1	Aïn Ben Ali	9°37”13.95”E	35°45”24.05”N	Phytoplankton and scraping	”	18730
ALI2	”	”	”	Phytoplankton and scraping	”	18731
ALI3	”	”	”	Phytoplankton	”	18732
BOU1	Aïn Bou Rkhiss	9°35”56.90”E	35°43”30.83”N	Turf and short cyanobacteria	”	18733
BOU2	”	”	”	Rock scraping	”	18734
BOU3	”	”	”	Adjacent exutory: sediment	”	18735
BOU4	”	”	”	Pebbles scraping	”	18736
ABK1	”	”	”	Rock scraping and cyanobacteria	15/12/14	18575
ABK2	”	”	”	Rock scraping and cyanobacteria	”	18576

resource for local inhabitants, providing a safe, high quality and perennial source of drinking water (the water is said to be of better quality than bottled water). The Aïn Bou Rkhiss and Kibrit springs are also important for the small-scale agriculture of this area as they supply the wadi's base flow which is used for irrigated cultures alongside the wadi's bank. For locals, myths, historical stories, anecdotes or memories-remembrances, form the basis for their theories-beliefs which explain the interconnection between the springs in their area. This is important because these stories carry forms of local claims of ownership of the territory and the waters that circulate there.

MATERIALS AND METHODS

Sampling sites and biological descriptors: The Aïn Bou Rkhiss (Table I, Fig. 1; 9°35'56.90"E 35°43'30.83"N) situated Northwest of Haffouz, 6 km from Douar Chaïb, is a set of springs flowing out through the fissured nummulitic limestone between 341 and 345 m a.s.l. The main outlet is a short fissure (20-25 cm long, 5 cm large) in the river-bed of a small brook confluent with the Merguellil wadi. The latter outlet is confined by a natural ridge that protects the spring from contamination by the brook. The edges of the opening are smooth. No mosses grow in the immediate vicinity of the spring, only some short tufts of filamentous cyanobacteria are present. Pebbles are almost absent. Beneath the opening of the spring, the cavity seems to be widening and connected to a set of small cracks. The massive limestone outcrop is not karstified and shows no sign of significant dissolution. The flow rate of the Aïn Bou Rkhiss never exceeds 5 l/s (M. Ayachi, DGRE-CRDA, comm pers). The first diatom survey (December 2014) focused primarily on the collection of benthic macroalgae for the study of epiphytes and bed-rock microphytobenthos. A second survey (February 2016) allowed for a more widespread sampling of the outlet (scraped with a blade) and pebbles immediately downstream of the outlet. An adjacent small outlet (BOU3 sample, 2016 survey, Table I) was sampled ca. 30 cm from the major spring (no chemistry available). BOU3 outlet drained from a water sheet possibly more superficial and polluted than the major outlet (cyanobacteria were, *de visu*, more abundant in BOU3 than near the major outlet).

The sulfur spring Aïn Kibrit flows out directly into the Merguellil river bed, ca. 80 m from Aïn Bou Rkhiss (upstream). This spring takes its name from the presence of hydrogen sulfide (Sulfur: 'kibrit' in Arabic). The Aïn Kibrit outflow is diffuse, with several degassing points and water outlets scattered within a circular area of ca. 5 m across. The area showed gray sulfurous sediments covered by abundant macroalgae in December 2014, while only filamentous short green algae, mainly concentrated on the degassing points, were present in February 2016. The macroalgae and sediments were sampled on each sampling date.

Aïn Ben Ali is a water-well (sort of well giving access to the Bou Hafna water table) located in a rugged landscape (Fig. 1, 433 m a.s.l.). The well is ca. 4.5 m deep (Fig. 5) and, according to local residents, never or rarely dries. Due to the difficult access, only two scrapings from the bottom and submerged parts of the well were made with a bucket. Some phytoplankton was collected with a plankton net (10 µm mesh).

All samples (IRSTEA Bordeaux, collection M. Coste, Table I) were preserved in formaldehyde (10% final concentration). For examination with a light microscope (LM), the samples were washed with distilled water to remove salts, treated with 35 % w/w H_2O_2 for 2 h at 70 °C to remove organic matter, rinsed several times in distilled water, alcohol-desiccated and mounted on glass slides using Naphrax® (refraction index 1.74; slides in collection M. Coste). The slides (Table I) were examined with a Leitz/Leica DMRD Phase Contrast Microscope System. For SEM examination, the samples were filtered through 1 µm Nuclepore® filters and rinsed twice with deionized water (milliQ) to remove salts. Filters were air-dried and mounted onto aluminum stubs before being coated with gold-palladium alloy (EMSCOP SC 500 sputter coater) and examined with a Carl Zeiss EVO50 LEO SEM and a Hitachi S-4500 SEM operated at 5 kV, calibrated with a Silicon grating TGX01.

Diatom species were identified with LM at 1000× magnification (with a screening at 200× for the biggest taxa), according to Krammer & Lange-Bertalot (1986-1991, 2004a, 2004b, 2007a, b), Metzeltin & Lange-Bertalot (1998), Lange-Bertalot & Genkal (1999), Krammer (2000, 2002, 2003), Hofmann *et al*. (2011). The synonymies prior to 1979 follow VanLandingham (1967-1979). More than 400 valves and frustules were identified per permanent slide, except for smaller samples, such as those from Aïn Ben Ali, where fewer valves were identified.

Two biological indices based on benthic diatoms were applied on the Merguellil data sets: the Specific Polluosensitivity Index (SPI; Coste in Cemagref 1982) and the Biological Diatom Index (BDI; established by Lenoir & Coste 1996), standardized in AFNOR (2007) and improved by Coste *et al*. (2009). 'These indices are based on the Zelinka & Marvan formula (Zelinka & Marvan 1961), which is a weighted average of species indicator values. A different number of species is taken into account by each index: BDI uses 209 taxa' […] 'and IPS uses all known taxa' (Gomà *et al*. 2004). The BDI calculation method is presented in detail by Prygiel & Coste (2000). The two indices range from 1 to 20, with 1 being the worst water quality and 20 the best. Input and calculations were performed on OMNIDIA software (Lecointe *et al*. 1993) version 5.5.

The filamentous macroalgae and adhering cyanobacteria were also identified for each water supply (Hasler *et al*. 2014, Komárek & Anagnostidis 1999, 2005, Ghozzi *et al*. 2013, Komárek 2013).

Physical-chemical descriptors: During the 2016 survey, water temperature was measured with a Testo 735 (± 0.05 °C), and conductivity and pH with calibrated portable conductivity and pH meter probes (WTW 3110 and WTW315i, respectively). Water samples for nutrient surveys were collected with a syringe and filtered through Whatman 0.45 µm GMF syringe filters. The samples were immediately poisoned with $HgCl_2$ (final concentration 0.6 mM), for later analyses in LOMIC-UMR 7621 (Banyuls/Mer, France). Nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^-) and silicic acid [$Si(OH)_4$] concentrations were measured (Autoanalyser Skalar San++ Flow Analyser), following Aminot & Kérouel (2007).

PCA, NMDS and clustering analyses: All statistical analyses were performed using the package 'vegan' in R (Oksanen *et al*. 2016). In order to identify samples or groups of samples with comparable diatom assemblages, and keeping in mind that data were limited, a Principal Component Analysis (PCA) of the relative abundance (diatoms and sites) in a two dimensional space with minimal loss of information was used. In addition, we performed a Non-Metric Multidimensional Scaling (NMDS) for the samples (2016) accompanied by environmental data (conductivity, salinity, pH and nutrients data). The aim of this analysis was to explore if environmental data could help in the interpretation of the observed clusters obtained with NMDS. NMDS was performed using Bray-Curtis dissimilarities between sites. Environmental data vectors were then fitted using the 'envfit' function and statistical significance was estimated by randomly permuting data 1000 times.

In addition to the latter ordination analyses, we performed a hierarchical classification analysis. Hierarchical clustering was performed using the 'hclust' function using on a Bray-Curtis dissimilarity matrix among sites (complete linkage clustering). This method defines the cluster distance between two clusters to be the maximum distance between their individual components. This method was chosen as no obvious outlier data points were detected in the data set and the alternative method (single linkage) is known to cause undesirable elongated clusters (Manning *et al*. 2008).

Study of water uses and local knowledge: The social study of water uses and local knowledge about the springs used an inductive reasoning following the grounded theory (Glazer & Strauss 1967). This qualitative approach required the collection of the following field data: observations, measurements, bibliography and interviews. Local knowledge of the three springs was gathered from residents, irrigators and well-owners with the help of a translator. Analysis of the discourse was iterative and a collaborative effort of anthropologists and hydrogeologists occurred from 2012 to 2016 (see Riaux & Massuel 2014, Massuel *et al*. 2018). Questioning and hypotheses were tested with new field data and constantly reformulated until a coherent interpretation was reached (Olivier de Sardan 2005, 2015).

RESULTS

Geochemical descriptors: signature of each water supply

The temperatures for the two springs are similar (i.e., slightly higher for Aïn Kibrit 23.6 °C versus 22.3 °C for Aïn Bou Rkhiss, Table II), while the conductivity of the sulfur spring is much higher (1232 versus 821 µS/cm). Nutrient concentrations also show differences among sites (Table II), with the highest PO_4 concentrations observed in Aïn Ben Ali (≥ 68 µg/l) and the highest NO_2 and $Si(OH)_4$ concentrations in Aïn Kibrit (5 µg/l and 7 mg/l, respectively). Similar concentrations of NO_3 were found in Aïn Bou Rkhiss and Aïn Ben Ali (ca. 6 mg/l), yet for Aïn Kibrit NO_3 remained below the detection limit.

Biological descriptors: macroalgae and diatom assemblages, diatom polluosensitivity indices (BDI, SPI)

Macroalgal and diatom assemblages

The relative mean abundance of dominant taxa for all samples from each site (2014 and 2016) is given in

Table II. – pH, temperature (T), and nutrients, in Aïn Kibrit, Aïn Bou Rkhiss and Aïn Ben Ali (25 February 2016). *: below detection limit; σ: standard deviation.

Site	T (°C)	pH	Conductivity (µS/cm)	Nutrients	NO_2 (µg/l)	NO_3 (mg/l)	PO_4 (µg/l)	$Si(OH)_4$ (mg/l)
Aïn Kibrit	23.6	7.7	1232	KIB1	4.14	*0	19.96	6.85
				KIB2	5.52	*0	26.60	7.18
				Mean	**4.83**	**0.00**	**23.28**	**7.02**
				σ	**0.98**	**0.00**	**4.70**	**0.23**
Aïn Bou Rkhiss	22.3	7.61	821	BOU1	0.46	6.27	23.75	5.23
				BOU2	0.92	6.10	24.70	5.27
				Mean	**0.69**	**6.19**	**24.23**	**5.25**
				σ	**0.33**	**0.12**	**0.67**	**0.03**
Aïn Ben Ali	20.3	7.59	625	ALI1	3.22	6.10	67.45	5.16
				ALI2	3.22	6.22	68.40	5.44
				Mean	**3.22**	**6.16**	**67.93**	**5.30**
				σ	**0.00**	**0.08**	**0.67**	**0.20**

Table III. – Dominant diatom species per site, expressed in relative abundance (all samples cumulated per site).

1) Aïn Kibrit (4 samples)	
Counts per slide: up to 950	
Dominant species, relative abundance ≥ 0.5 %	**%**
Brachysira neoexilis Lange-Bertalot	17.5
Nitzschia palea (Kützing) W. Smith	13.5
Nitzschia palea var. *debilis* (Kützing) Grunow in Cleve & Grunow	10.6
Navicula veneta Kützing	7.3
Achnanthidium minutissimum (Kützing) Czarnecki	6.5
Crenotia thermalis (Rabenhorst) Wojtal	4.1
Brachysira vitrea (Grunow) Ross in Hartley	3.2
Cymbopleura amphicephala Krammer	3.0
Nitzschia solita Hustedt	2.4
Sellaphora pupula (Kützing) Mereschkowksy	2.3
Rhopalodia gibba (Ehrenberg) O. Müller	1.6
Nitzschia capitellata Hustedt in A. Schmidt *et al.*	1.6
Nitzschia frustulum (Kützing) Grunow	1.5
Navicula recens (Lange-Bertalot) Lange-Bertalot	1.5
Nitzschia microcephala Grunow in Cleve & Möller	1.2
Nitzschia thermaloides Hustedt	1.2
Navicula rostellata Kützing	1.0
Tryblionella kuetzingii Álvarez-Blanco & S. Blanco	0.9
Nitzschia inconspicua Grunow	0.8
Encyonopsis microcephala (Grunow) Krammer	0.8
Caloneis macedonica Hustedt	0.7
Tryblionella hungarica (Grunow) D.G. Mann in Round *et al.*	0.7
Nitzschia gracilis Hantzsch	0.7
Craticula ambigua (Ehrenberg) D.G. Mann	0.6
Staurophora tackei (Hustedt) Bahls	0.5
Nitzschia intermedia Hantzsch ex Cleve & Grunow	0.5
Diatoma moniliformis (Kützing) D.M. Williams	0.5
2) Aïn Bou Rkhiss (6 samples)	
Counts per slide: up to 753	
Dominant species, relative abundance ≥ 0.5 %	**%**
Gomphonema parvulum Kützing	13.9
Nitzschia palea (Kützing) W. Smith	11.4
Planothidium rostratum (Oestrup) Lange-Bertalot	11.4
Ulnaria danica (Kützing) Compère & Bukhtiyarova	11.2
Luticola goeppertiana (Bleisch in Rabenhorst) D.G. Mann in Round *et al.*	10.9
Achnanthidium minutissimum (Kützing) Czarnecki	9.6
Nitzschia inconspicua Grunow	3.6
Ulnaria ulna (Nitzsch) Compère	3.6
Brachysira neoexilis Lange-Bertalot	3.6
Navicula veneta Kützing	2.7
Tryblionella hungarica (Grunow) D.G. Mann in Round *et al.*	2.1
Nitzschia filiformis var. *conferta* (P.G. Richter) Lange-Bertalot	1.7
Brachysira vitrea (Grunow) R. Ross in Hartley	1.5
Ulnaria lanceolata (Kützing) Compère	1.3
Nitzschia amphibia f. *amphibia* Grunow	1.3

Table III. The produced lists are therefore not exhaustive and some taxa present as rare, but with a particular ecological significance, are not listed in Table III, but they are here commented.

Aïn Ben Ali well: The restricted sampling did not allow to study the cyanobacteria. Among the dominant diatom taxa (Table III), *Navicula veneta* Kützing (1st rank) and *Nitzschia umbonata* (Ehrenberg) Lange-Bertalot (13th rank) are considered as signature for a low water quality (pollution indicators), whereas *Achnanthidium minutissimum* (Kützing) Czarnecki (6th rank) and *Aneumastus tusculus* (Ehrenberg) D.G. Mann & Stickle are considered to indicate good water quality.

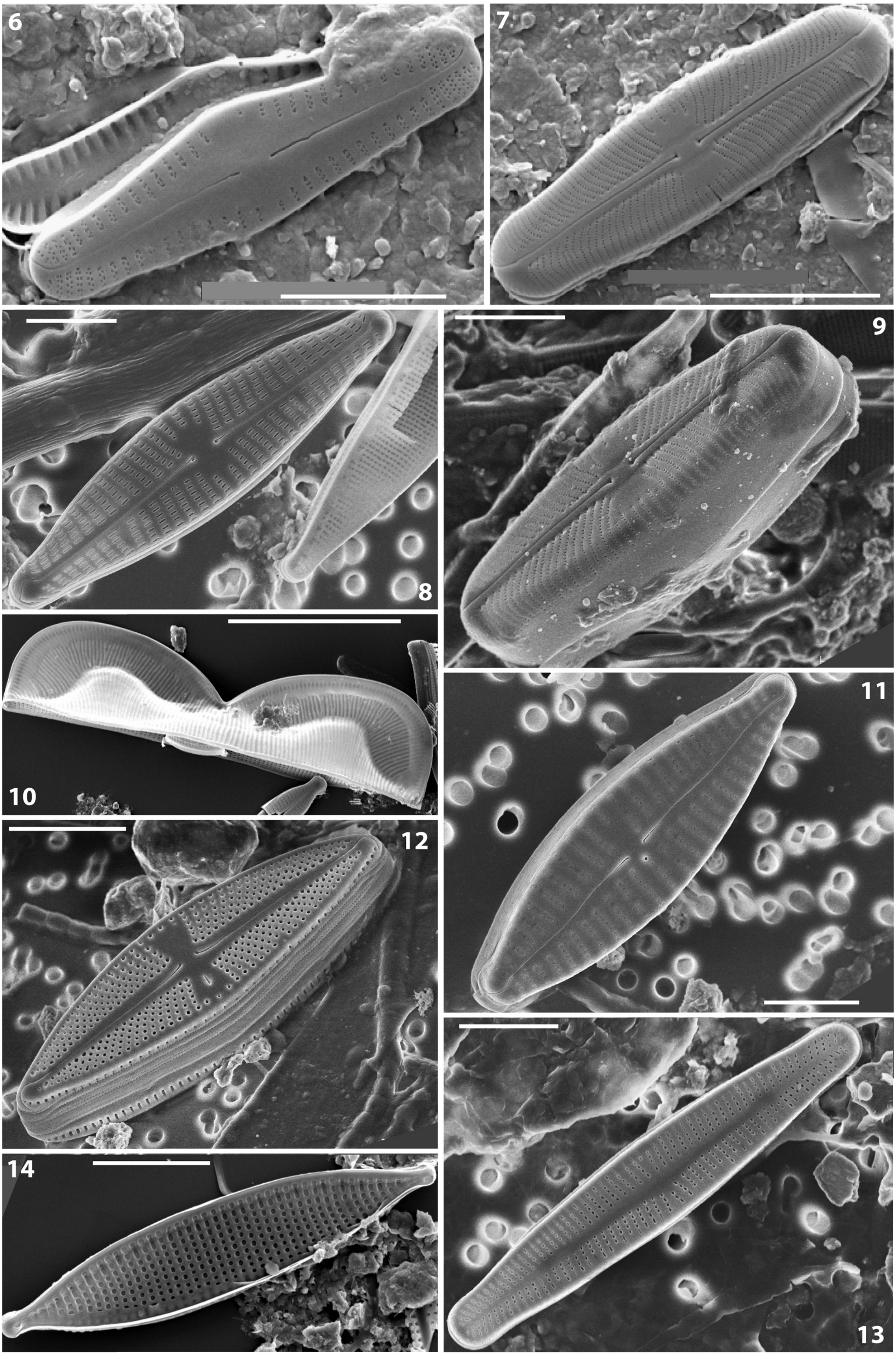
Aïn Kibrit: In 2014 the filamentous macroalgae assemblage from Aïn Kibrit (with sulfur-rich water, see above), is dominated by cyanobacteria including *Oscillatoria* spp. (among which *O. chalybea* Mertens), *Lyngbya* sp., *Phormidium* sp. (or *Leptolyngbya* sp.), *Pseudanabaena* (including *P. papillatterminata* (Kiselev) Kukk. and *P. catenata* Lauterborn), and *Ammassolinea* cf. *attenuata* Hasler (Hasler *et al*. 2014). Among the diatoms (Table III, Figs 6-14), *Brachysira neoexilis* Lange-Bertalot is the dominant taxon. *Brachysira vitrea* (Grunow) Ross in Hartley is also well represented (7th rank). Both are considered to occur in oligotrophic (Hofmann *et al*. 2011) to oligo-mesotrophic conditions. On the other hand, 20 % of the assemblage is considered as saprophilic or N-heterotrophic, with a low water quality signature (e.g., *Nitzschia palea* (Kützing) W. Smith, *Nitzschia palea* var. *debilis* (Kützing) Grunow in Cleve & Grunow, *Nitzschia capitellata* Hustedt in

Table III. – Continued.

Nitzschia solita Hustedt	1.1
Caloneis lancettula (Schulz) Lange-Bertalot & Witkowski	0.8
Nitzschia frustulum (Kützing) Grunow	0.8
Nitzschia perminuta (Grunow) M. Peragallo	0.7
Gomphonema exilissimum (Grunow) Lange-Bertalot & E. Reichardt	0.5
3) Aïn Ben Ali (3 samples)	
Counts per slide (see Mat. & Meth.): up to 89	
Dominant species, relative abundance ≥ 1 %	**%**
Navicula veneta Kützing	33.0
Fragilaria gracilis Østrup	9.6
Brachysira neoexilis Lange-Bertalot	3.7
Stauroneis siberica Lange-Bertalot & Krammer	3.7
Fragilariforma nitzschioides (Grunow) Lange-Bertalot in Hofmann *et al.*	3.5
Achnanthidium minutissimum (Kützing) Czarnecki	3.5
Mastogloia smithii Thwaites	3.1
Nitzschia palea (Kützing) W. Smith	2.7
Denticula kuetzingii Grunow	2.6
Gomphonema parvulum Kützing	2.6
Cymbella cymbiformis C. Agardh	2.0
Lindavia comta (Ehrenberg) Nakov, Guillory, M.L. Julius, E.C. Theriot & A.J. Alverson	1.9
Nitzschia umbonata (Ehrenberg) Lange-Bertalot	1.9
Luticola goeppertiana (Bleisch in Rabenhorst) D.G. Mann in Round *et al.*	1.8
Brachysira neglectissima Lange-Bertalot	1.8
Aulacoseira granulata (Ehrenberg) Simonsen	1.7
Amphora pediculus (Kützing) Grunow	1.6
Encyonema cespitosum Kützing	1.5
Nitzschia gracilis Hantzsch	1.5
Navicula radiosa Kützing	1.3
Nitzschia amphibia Grunow	1.3
Fragilariforma bicapitata (Ant. Mayer) D.M. Williams & Round	0.9
Achnanthidium nanum (F. Meister) Novais & Jüttner	0.9
Gomphonema pumilum (Grunow) E. Reichardt & Lange-Bertalot	0.9
Hantzschia amphioxys (Ehrenberg) Grunow in Cleve & Grunow	0.9
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	0.9

A. Schmidt *et al.*, *Sellaphora pupula* (Kützing) Mereschkowksy (Figs 7, 9), *Navicula veneta* Kützing (Fig. 8) and *Pinnularia brebissonii* (Kützing) Rabenhorst. The diatom assemblage includes several taxa associated with high conductivity (here due to high sulfur concentrations), such as *Craticula halophila* (Grunow *ex* Van Heurck) Mann, *Entomoneis paludosa* (W. Smith) Reimer, *Nitzschia aurariae* Cholnoky, *N. filiformis* (W.M. Smith) Van Heurck, *N. nana* Grunow in Van Heurck, *N. obtusa* W.M. Smith, *N. thermaloides* Hustedt, *N. vitrea* Norman, *Surirella ovalis* Brébisson, *Tryblionella hungarica* (Grunow) D.G. Mann in Round *et al*. The species richness (mean: 51 taxa) and specific diversity (Shannon-Weaver, mean: 4.0) are relatively high (Table IV). BDI and SPI scores (mean: 12.19 and 8.64 respectively) are relatively low, suggesting that the water masses are of low quality. The difference between both indices is essentially due to the fact that the BDI does not consider the most halophilous taxa.

Aïn Bou Rkhiss: The assemblage of filamentous macroalgae from Aïn Bou Rkhiss (considered by locals to be of high quality) is dominated by cyanobacteria and Chlorophyceae (e.g., *Spirogyra*). Non-heterocystous cyanobacteria are dominant, with *Phormidium* aff. *autumnale* and *Lyngbya* sp. These usually reflect a nitrogen enrichment, often considered a consequence of organic contamination (even if the homocysted cyanobacteria, in contrast to the heterocysted ones, are not considered nitrogen-fixing). Among the dominant diatom taxa (Table III), *Gomphonema parvulum* Kützing (1st rank) (Fig. 11) is considered as facultatively N-heterotrophic and *Nitzschia palea* (2nd rank) as obligately N-heterotrophic. *Planothidium rostratum* (Oestrup) Lange-Bertalot (3rd rank) is N-autotrophic but tolerant and *Ulnaria danica* (Kützing) Compère & Bukhtiyarova (4th rank) is considered as β-mesosaprobous (Van Dam *et al*. 1994). *Luticola goeppertiana* (Bleisch in Rabenhorst) D.G. Mann in Round *et al*. (5th rank) (Fig. 12) is a pollution-resistant species, able to grow at high conductivity and tolerant of temporary drying. On the other hand, the more sensitive *Achnanthidium minutissimum* (Fig. 13, rank 6th), and *Brachysira vitrea* (Grunow) R. Ross in Hartley only 13th. BOU3 (adjacent outlet, Table I) and BOU4 (pebbles close to the major outlet) show more halophilous diatoms (*Tryblionella hungarica* (Grunow) D.G. Mann in Round *et al*., *Nitzschia elegantula* Grunow in Van Heurck, *N. frustulum* (Kützing) Grunow, *N. inconspicua* Grunow). The Shannon-Weaver diversity of Aïn Bou Rkhiss (mean 2.9) is lower than that of Aïn Kibrit, (ca. 4.0, Table IV). A rare *Luticola* D.G. Mann in the samples from Aïn Bou Rkhiss is described here as *Luticola lancettula* var. *merguellilae* var. nov. (see below).



Figs 6-14. – *Crenotia thermalis* (6); *Sellaphora pupula*, valvar view (7); *Navicula veneta* (8); *Sellaphora pupula*, cingular view (9); *Entomoneis paludosa* var. *subsalina* (10); *Gomphonema parvulum* (11); *Luticola* aff. *goeppertiana* (12); *Achnanthidium minutissimum* (13); *Nitzschia desertorum* (14). Scale bars: 20 µm (Fig. 10), 10 µm (Fig. 7), 5 µm (Figs 6, 9, 12, 14), 4 µm (Figs 8, 11, 14).

Table IV. – Specific richness, Shannon-Weaver index and SPI (Cemagref 1982) and BDI (AFNOR 2007) indices, per individual sample. σ: standard deviation.

	2014	2014	2016	2016	2016	2016	Mean	± σ
Aïn Kibrit	**KIB1**	**KIB2**	**AKB1**	**AKB2**				
Species richness	58	22	60	64			**51.0**	**19.5**
Shannon-Weaver	3.7	2.6	4.7	5.0			**4.0**	**1.1**
SPI (Cemagref 1982)	8.6	8.8	9.3	8.0			**8.6**	**0.5**
BDI (AFNOR 2007)	12.9	14.4	11.2	10.3			**12.2**	**1.8**
Aïn Bou Rkhiss	**ABK1**	**ABK2**	**BOU1**	**BOU2**	**BOU3**	**BOU4**		
Species richness	18	31	15	10	31	44	**24.8**	**12.7**
Shannon-Weaver	2.3	3.4	2.5	2.2	2.8	4.3	**2.9**	**0.8**
SPI (Cemagref 1982)	3.5	8.0	9.5	14.5	12.2	9.5	**9.5**	**3.8**
BDI (AFNOR 2007)	8.2	9.1	11.6	15.2	13.0	10.1	**11.2**	**2.6**
Aïn Ben Ali			**ALI1**	**ALI2**	**ALI3**			
Species richness			17	20	27		**21.3**	**5.1**
Shannon-Weaver			0.9	4.1	4.4		**3.1**	**2.0**
SPI (Cemagref 1982)			13.7	12.8	15.0		**13.4**	**1.1**
BDI (AFNOR 2007)			13.2	14.0	16.4		**14.5**	**1.7**

***Luticola lancettula* var. *merguellilae* M. Coste & Riaux var. nov. (LM Figs 17-18, SEM Figs 15-16, 19-20, Table V)**

Description: Valves relatively small (length 32-38 µm) and narrow (7.8-8.3 µm wide), lanceolate to linear-elliptic/fusiform (length/width ca. 4.3) with bluntly rounded apices (Figs 16, 20). Raphe straight. Striae uniseriate (18-19.5 in 10 µm), strongly radiate, striae slightly denser on the apices. 25-29 areolae in 10 µm. Junction of the valve face and mantle well defined. One row of areolae on the mantle, with a density similar to that of the striae (Fig. 20). A single transapically elongated stigma on one side of the large asymmetrically bow-tie shaped stauros, marginally delineated by a single row of smaller areolae (Figs 15-18). Proximal raphe endings on the valve face bent opposite to the stigma and with small droplet-shaped terminations relatively far from each other (ca. 1.2 µm) (Fig. 19). Terminal raphe fissures strongly hooked to the stigma side (Fig. 20). Cingular bands (more than four) open and with ligulae. Each band has two rows of transapically elongate small puncta that become smaller near the apices (Figs 19-20 arrowheads, ca. 65 puncta in 10 µm).

Holotype: Slide 18576 ABK2 housed in BM (BM 101302).

Isotype (here assigned): Slide 18576 in collection M. Coste (IRSTEA, Bordeaux).

Paratype: SEM Stub 3-300617 ABK1 housed in BM (BM001166804).

Type locality: Aïn Bou Rkhiss, spring from the Merguellil Basin (Central Tunisia; 9°35'56.90"E 35°43'30.83"N). Collector: C. Riaux-Gobin.

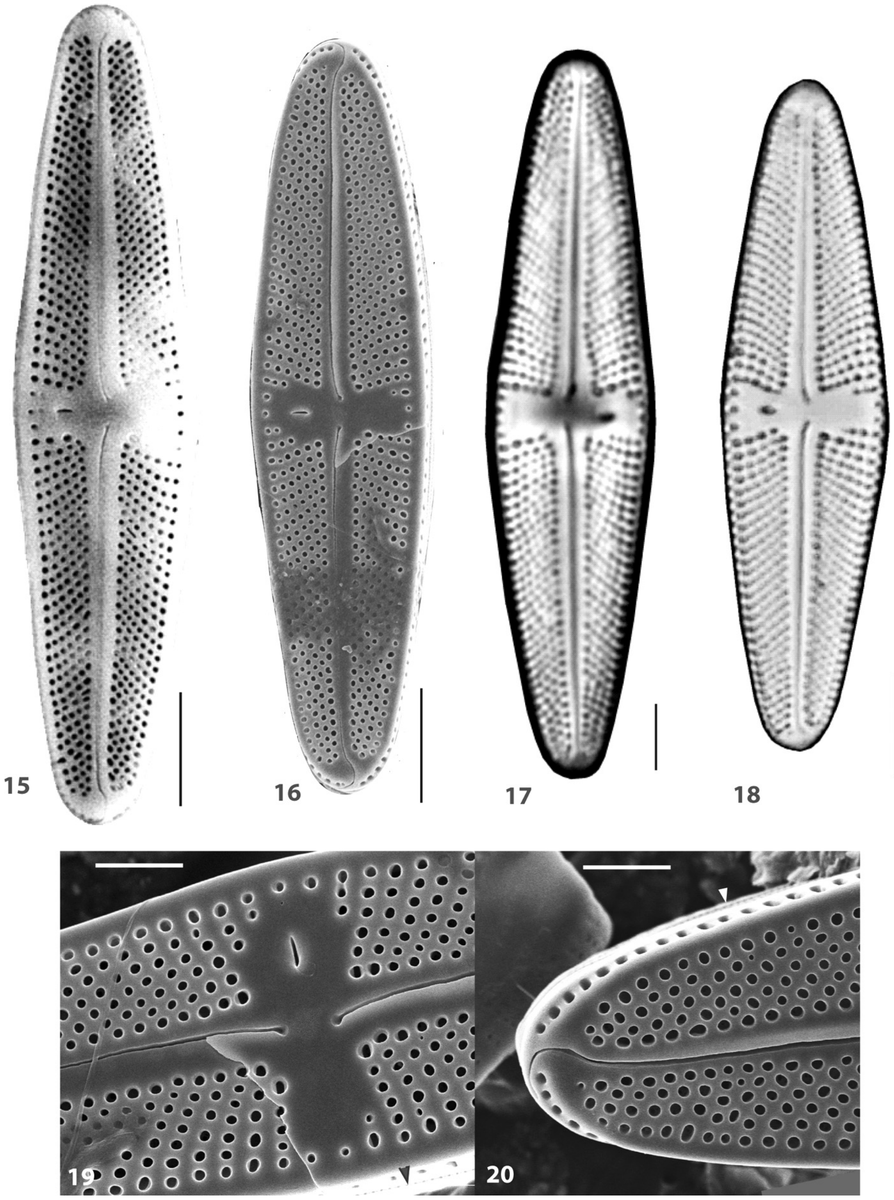
Etymology: The epithet *merguellilae* refers to the Merguellil Basin (Central Tunisia) where it was first observed.

Habitat: Filamentous Chlorophyta (e.g., *Spirogyra*).

Taxonomic note: *Luticola* D.G. Mann (Round *et al*. 1990) is an extremely diverse genus with up to 228 species names and 13 infra-specific names. *Luticola lancettula* var. *merguellilae* var. nov. is very similar to *L. lancettula* Levkov, Metzeltin & A. Pavlov in Levkov *et al*. (2013: 151, figs 73/1-30; 75/6-8), except for its more blunted apices and lower stria density (18-19.5 in 10 µm versus 22-24 in *L. lancettula*). The new variety can also be compared to the recently described *L. tujii* Levkov, Metzeltin & A. Pavlov in Levkov *et al*. (Levkov *et al*. 2013, Glushchenko *et al*. 2017), but the latter has smaller dimensions and a higher stria density than *L. lancettula* var. *merguelliliae* (Table V). *Luticola hunanensis* Bing Liu & D.M. Williams in Liu *et al*. (Liu *et al*. 2017) has also some similarities, but has a less lanceolate shape (lower L/W) and several particular structures that make it a different taxon (i.e., position and shape of the stigma, marginal row of areolae, Table V).

Diatomic polluosensitivity indices (BDI, SPI)

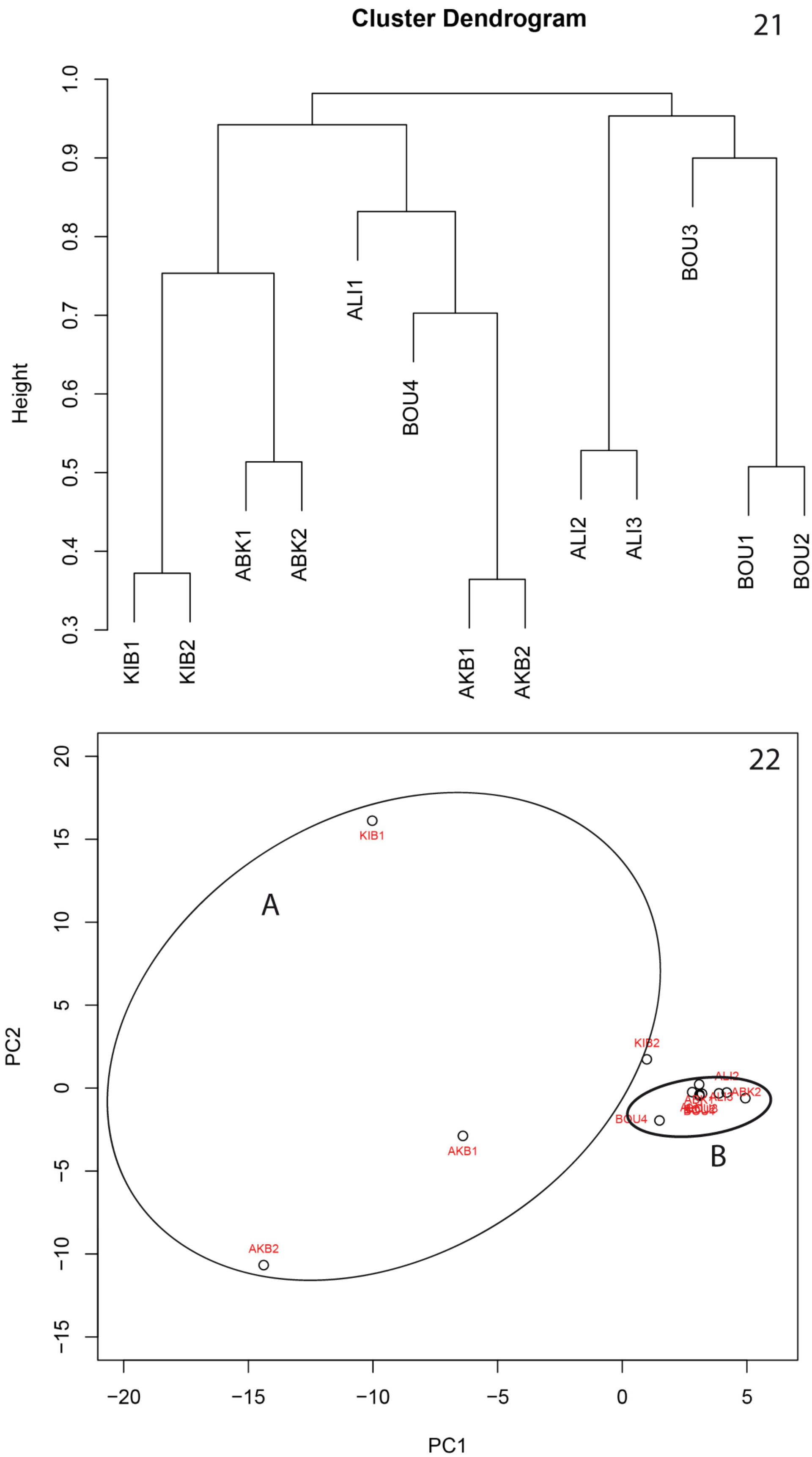
The BDI and SPI indices were calculated on all samples (3 Aïn Ben Ali, 6 Aïn Bou Rkhiss and 4 Aïn Kibrit, see sample details in Table I). Results are presented in Table IV (BDI and SPI indices, specific richness and Shannon Weaver index). All sites show a relatively high variability. The species richness is surprisingly high in Aïn Kibrit (up



Figs 15-20. – *Luticola lancettula* var. *merguellilae* var. nov. LM valvar views (17-18); SEM valvar views (15-16); SEM detail of the central area with the process opening (19); SEM detail of the apex with the terminal raphe fissure (20). Scale bars: 5 µm (Figs 15-18), 2 µm (Figs 19-20).

Table V. – Biometrics of the discussed *Luticola* taxa, following their original description.

	Luticola lancettula	***Luticola lancettula* var. *merguellilae* var. nov.**	***Luticola tujii***	***Luticola hunanensis***
Frustule outline	Lanceolate to elliptic-lanceolate, broadly rounded apices	Lanceolate to linear-elliptic/ fusiform, blunt apices	Elliptic-elongate, relatively acut apices to broadly rounded	Lanceolate to elliptic-lanceolate
Length (µm)	18-40	32-38	14-23	14.7-28.4
Width (µm)	6.5-9.0	7.8-8.3	5.0-6.5	6.3-8.3
Striae in 10 µm	22-24	18-19.5	22-28	22-25
Axial area	Narrow, linear	Straight, moderately large	Narrow	Large
Central area	Bow-tie-shaped	Bow-tie stauros, one row of marginal small areolae	Bow-tie, one row of marginal areolae	Rectangular 'to somewhat panduriform'
Stigma	Halfway between valve centre and margin	Far from the margin	Far from the margin	'Slit-like stigma positioned close to one marginal areola'
Habitat	Brazil	Central Tunisia	Laos and Cambodia	Central China
Reference	Levkov *et al.* 2013	This study	Levkov *et al.* 2013 Glushchenko *et al.* 2017	Liu *et al.* 2017



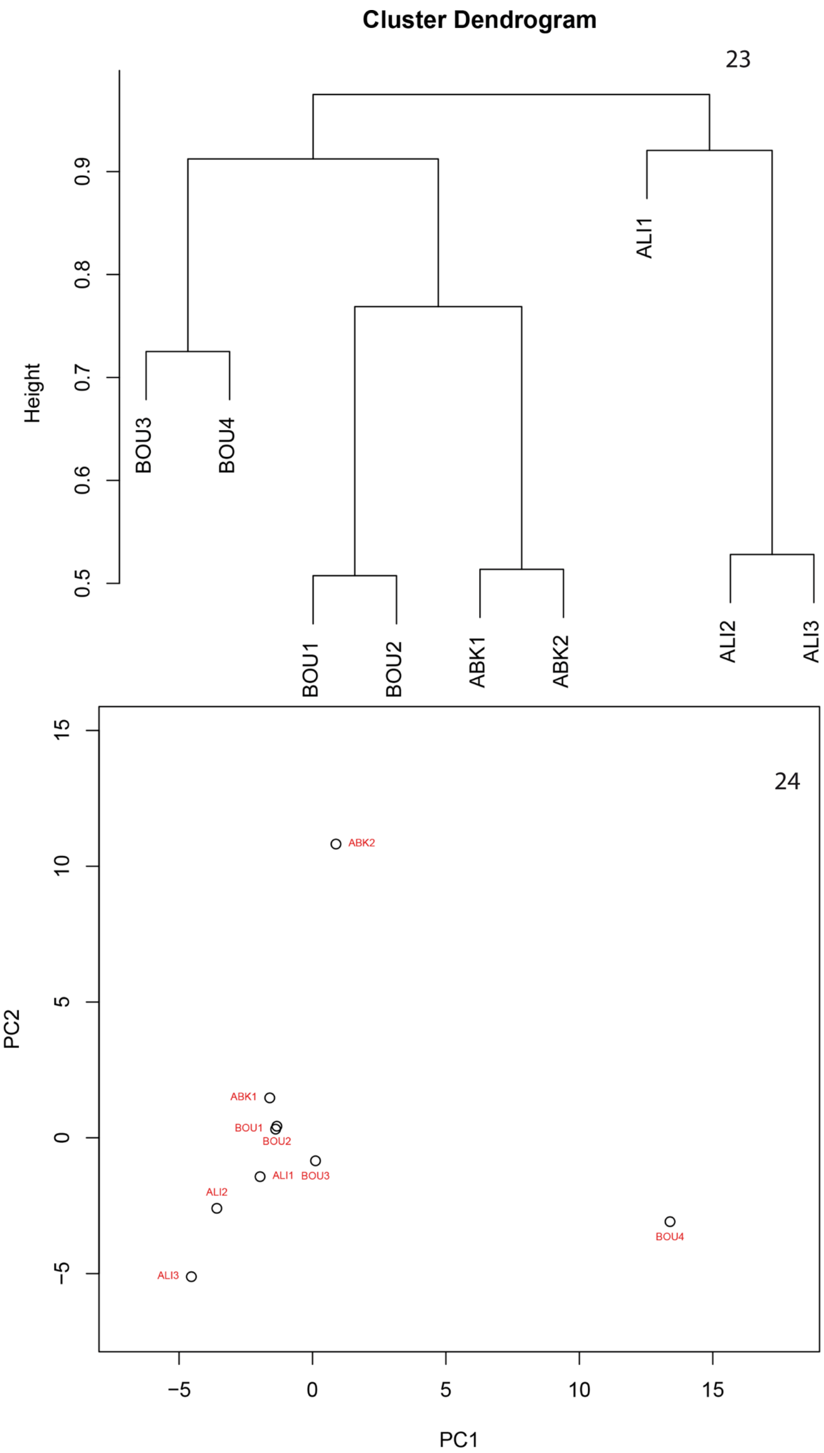
Figs 21-22. – PCA analysis (22 A–B) and Hierarchical clustering dendrogram (21), performed on the full data set (see text and acronyms in Table I). Heights represents the Bray-Curtis dissimilarities among sites.

to 64), while often low in such sulfur springs (Ghozzi *et al*. 2013).

PCA, NMDS and diatom characterization of each water supply

Aïn Kibrit water is rich in sulfur with chemical characteristics markedly different from those of Aïn Bou Rkhiss and Aïn Ben Ali (Table II), that may deeply influence the diatom assemblages. Therefore, two distinct PCA were performed, the first on the full data set (n = 13, Fig. 22) and the second on a subset obtained by eliminating the samples from Aïn Kibrit (n = 9, Fig. 24). A supplementary analysis (NMDS analysis, Fig. 25), was performed on the 2016 samples only (except for BOU3, an adjacent supply from which no geochemical measurements are available), with the associated geochemical descriptors as supplementary variables.

The first 2 factors of the first PCA analysis (Fig. 22) explained 43.5 % of the variance. The Aïn Kibrit samples (AKB2, AKB1, KIB1, and in a lesser extent KIB2) are well separated from the other samples (Fig. 22A). The first factor (PC1) is related to taxa present in Aïn Bou Rkhiss and Aïn Ben Ali and absent from Aïn Kibrit: e.g., *Luticola goeppertiana*, *Gomphonema parvulum*, *Planothidium rostratum*, *Ulnaria ulna* (Nitzsch)



Figs 23-24. – PCA analysis (24) and Hierarchical clustering dendrogram (23), performed on a subset of data (without Aïn Kibrit samples, see text and acronyms in Table I). Heights represent the Bray-Curtis dissimilarities among sites.

Compère and *Ulnaria lanceolata* (Kützing) Compère. The second factor (PC2) is strongly related to the halophilous taxa *Brachysira neoexilis* and *Nitzschia palea*, dominant in the samples KIB1 and KIB2, while the samples AKB2 and AKB1 contained *Achnanthidium minutissimum* (also present in the two other sites, though at different levels). All Aïn Kibrit samples contained taxa that are absent from the other two sites: e.g. *Craticula halophila* (Grunow *ex* Van Heurck) D.G. Mann, *Crenotia thermalis* (Rabenhorst) Wojtal (Fig. 2), *Entomoneis paludosa* var. *subsalina* (Cleve) Krammer, *Tryblionella calida* (Grunow in Cleve & Grunow) D.G. Mann in Round *et al*. These latter taxa, are only present in the sulfur spring and particularly in AKB2 (specific richness: 64), and give a particular position to Aïn Kibrit on the PCA (Fig. 22A). KIB2, with a lower specific richness (i.e., 22), is more similar to the other two sites, Aïn Bou Rkhiss and Aïn Ben Ali (Fig. 22B).

The dendrogram (using Bray-Curtis dissimilarity matrix) associated with the first PCA analysis (Fig. 21) clearly groups several pairs of samples from the same site (KIB1-2, ABK1-2, AKB1-2, ALI1-3, BOU1-2). These pairs all have Bray-Curtis (B-C) indexes lower than 0.51. Overall, three main clusters were defined, one defined by KIB1-2 and ABK1-2 (B-C index 0.75), one defined by AKB1-2, BOU4 and ALI1 (B-C index 0.83) and one cluster which encompassed the remaining sites: Ali-2-3, BOU1-2-3. However, this last cluster was highly heterogeneous with dissimilarities among some sites as high as 0.94 (Fig. 21).

The first 2 factors of the second PCA analysis (Fig. 24) explained 50.1 % of the variance. ABK2 (Aïn Bou Rkhiss), well separated along the PC2 axis, is characterized by *Ulnaria lanceolata* (also present, but with a lower score, on ABK1 and ALI1), and by a high abundance of *Gomphonema parvulum* and *Luticola goeppertiana*. BOU4 (Aïn Bou Rkhiss) with a positive score on the PC1 axis is characterized by *Caloneis lancettula* (Schulz) Lange-Bertalot & Witkowski (4.96 %), that is present only in this sample, and by significant scores for *Nitzschia filiformis* var. *conferta* (P.G. Richter) Lange-Bertalot in Lange-Bertalot & Krammer (9.9 %) and for the halophilous *Nitzschia inconspicua* (19.6 %). The three Aïn Ben Ali samples (ALI1, ALI2, ALI3), negatively grouped along the two axes, are characterized by several taxa that are absent from Aïn Bou Rkhiss: e.g., *Fragilariforma nitzschioides* (Grunow)

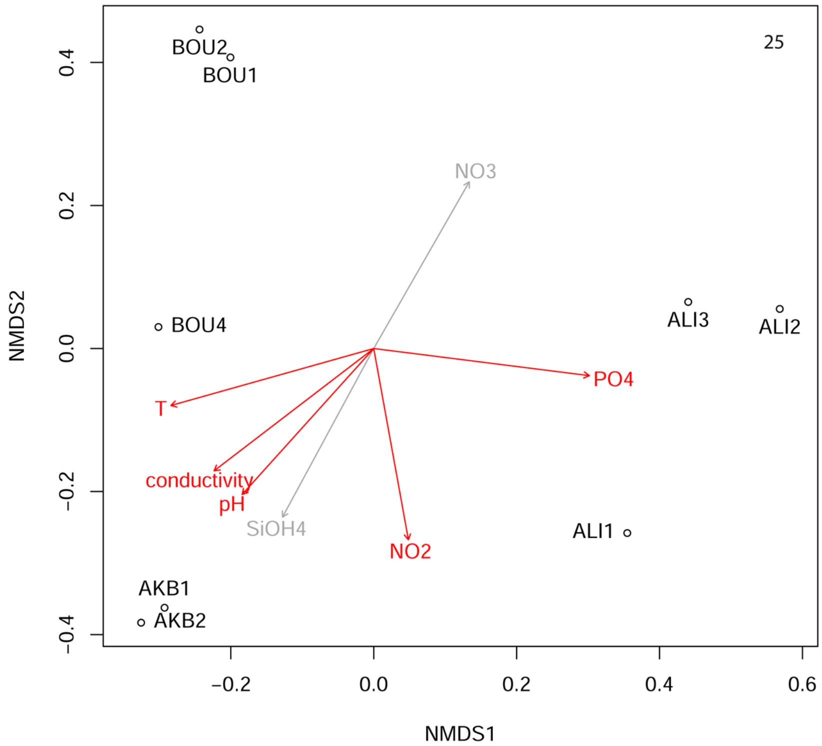


Fig. 25. – NMDS analysis, performed on a subset of data (samples conducted in 2016, geochemical data as supplementary variables, without BOU3, see text and acronyms in Table I). Vectors represent environmental variables. Arrow direction represents the direction of the gradient; the length of each vector is proportional to the correlation between ordination and environmental variable. Vectors in 'red' indicate significant correlations at $p < 0.05$ and 'grey' vectors indicate significant correlations at $p < 0.10$.

Lange-Bertalot in Hofmann *et al*., *Fragilaria gracilis* Østrup, *Stauroneis siberica* Lange-Bertalot & Krammer and *Nitzschia umbonata*, the last taxon serving as an indicator of low water quality.

The dendrogram associated with the second analysis (Fig. 23) groups together the Aïn Ben Ali samples, distinctly from the Aïn Bou Rkhiss samples. Furthermore, BOU3 (adjacent exutory, see Table I) and BOU4 are distinct from the other Aïn Bou Rkhiss samples, possibly due to their higher percentage of halophilous diatom taxa.

The third analysis (NMDS, Fig. 25) permits the position and weight of the geochemical descriptors associated with the samples conducted in 2016 to be visualized (except for BOU3, see above). The three sites are well differentiated/individualized, with Aïn Ben Ali (ALI1, ALI2, ALI3) associated with the higher PO_4 concentrations (positive on the NMDS1 axis), Aïn Bou Rkhiss (BOU1, BOU2, BOU4) enriched with NO_3 (positive on the NMDS2 axis), and Aïn Kibrit (AKB1, AKB2) associated with high temperature and conductivity.

Societal aspects: uses and local knowledge

Uses

Aïn Bou Rkhiss, Aïn Kibrit and Aïn Ben Ali are considered by communities living near the Merguellil Wadi as a crucial water supply. Water from the Aïn Bou Rkhiss is also considered to have a better quality than the water from the public fountain, and this spring is perennial. According to the people surveyed, Aïn Bou Rkhiss dried up only once during the summer of 2013. Thus, this spring is a drinking water supply that strongly supplements the public water service which regularly fails. Aïn Bou Rkhiss is one of the most abundant and perennial springs connected to the Merguellil stream bed. It contributes largely to the baseflow during the dry season (from April to September), which is also the period of highest water demand for local agriculture. Families settled on the riverbanks earn most of their livelihood from small-scale irrigated agriculture (tree crops and vegetables). Farmers got used to favor the natural mixing of water from the two springs and the stream runoff to improve the quality of the water for irrigation. However, people are worried that 'someone' (a state administration or a private operator) will commercialize the Aïn Bou Rkhiss spring for bottled water. At the moment, water is a state property and individuals do not have any formal rights to the water, which would protect their access to these springs.

Groundwater interconnection, via local knowledge

The second item of interest is what people think of these springs in relation to groundwater circulation. The first survey results show that many believe that Aïn Bou Rkhiss is connected to Aïn Ben Ali through an underground lake or wadi. This spring is symbolically central because it is located in the middle of the social group's territory: Aïn Ben Ali is located near a religious sanctuary (oulli/wally) where the community comes together every year for a ritual celebration. The hydrological link between these two springs could be viewed as a political discourse: the link between people living near Aïn Bou Rkhiss with their original territory is validated by physical observations.

In support of this hydrological relationship, people provide explanations through a story. One day, the 'grandfathers' decided to learn from the waters of Aïn Bou Rkhiss. Their hypothesis was that these waters were coming from Aïn Ben Ali. So, they put wheat bran in the Aïn Ben Ali spring, and some of them went to wait near the Aïn Bou Rkhiss. After one day and a night, wheat bran came out of Aïn Bou Rkhiss. They celebrated this day, and are convinced that Aïn Bou Rkhiss is the 'daughter' of Aïn Ben Ali. Hydrogeologists were initially skeptical of this since the fractures of the limestone aquifer of the Lower Eocene (Ypresian) were not supposed to be connected on such scale. However, the piezometric levels match perfectly and this interconnection is now a working hypothesis.

DISCUSSION

Sampling

The macro and microalgal samplings performed over a two-year interval on Aïn Bou Rkhiss and Aïn Kibrit show a high variability among sites and samples, but also among dates (see the PCA analyses conducted on all individual diatom samplings, Fig. 22). The three samples from Aïn Ben Ali also show high variability. The sampling methodology may introduce a bias: the 2014 samplings on Aïn Bou Rkhiss mainly concerned macroalgae and rock scraping, with no pebbles examined. In 2016, the samplings were more representative, with pebbles collected on Aïn Bou Rkhiss and Aïn Kibrit, in complement to rock scrapings, macroalgae and sediments. The comparison between the diatom assemblages from Aïn Bou Rkhiss and Aïn Ben Ali is biased by the fact that the Aïn Ben Ali benthic samplings were difficult to obtain and the phytoplankton was overestimated.

Geochemical and biological differences amongst sites

The three water supplies have different geochemical signatures, and it is not surprising that the Aïn Kibrit sulfur spring showed the most significantly different characteristics. Except for the PO_4 concentrations, Aïn Ben Ali and Aïn Bou Rkhiss show relatively similar chemistry. The diatom assemblages also are different among sites, with the Aïn Kibrit sulfur spring showing the greatest species richness and the most diversified halophilous assemblage. A great percentage of the taxa from Aïn Kibrit is not present on the other two supplies. Surprisingly, on each water supply, several taxa are signatures of high water quality while some others, often dominant, are signatures of low water quality. In support of the OMS normative classification http://www.lenntech.fr/applications/potable/normes/normes-oms-eau-potable.htm, up to 50 mg/l NO_3 in water is considered as drinking water. In Aïn Bou Rkhiss and Aïn Ben Ali the NO_3 concentrations (ca. 6 mg/l) are low, in spite of the presence of diversified cyanobacteria and low BDI scores.

The PCA on the complete data set (Fig. 22) shows a difference between Aïn Kibrit samples and the other two water supplies that are grouped together on an eccentric part of the analysis. The PCA also highlights the differences between all Aïn Kibrit samples. The flora (and dominant taxa) associated with the macroalgae are slightly different from those associated with pebbles and with sulfurous sediments. The PCA conducted on a subset (without Aïn Kibrit samples, Fig. 23) groups the Aïn Bou Rkhiss samples (if except the eccentric ABK2 and BOU4, see above), while the Aïn Ben Ali samples are negatively grouped.

Groundwater interconnection

The signature of the two water supplies Aïn Bou Rkhiss and Aïn Ben Ali (geochemistry and diatom assemblages) do not permit us to conclude with certainty about the groundwater interconnection, even if the chemistry of both sites is relatively similar. Concerning the biological signatures, further investigations are needed, e.g., with more appropriate diatom samplings for Aïn Ben Ali. The stories told by the people refer to a possible connection between these two water supplies, which is probably true. However, this connection may not be direct, and the Ypresian aquifer may also be connected to the adjacent or overlapping Oligocene sandstone aquifer where groundwater mixing and circulation can occur. A drain-tracing dye should be carried out to check the groundwater transfer time, and definitively validate these water myths.

CONCLUSION

The way diatoms are sampled is very important and may influence the results, since macroalgae such as cyanobacteria may host epiphytes with a low water quality signature, microphytobenthos colonizing pebbles or hard substrates in the same environment may give higher scores, and most biological methods use epilithic diatoms rather than other assemblages. In the present study, in the immediate vicinity of the Aïn Bou Rkhiss outlet there were no pebbles to be sampled in 2014, while in 2016 some were present (e.g., BOU4) but they gave a bad signature with several halophilous taxa present (see Results). Furthermore, when the counts per slide are too low (e.g., Aïn Ben Ali, see above) it can introduce bias into the calculation of the indices.

The BDI and SPI scores obtained for the three Merguellil water supplies would qualify the water masses as low quality, while the NO_3 concentrations in Aïn Bou Rkhiss and Aïn Ben Ali are neither worrisome, nor excessive (Table I, and OMS norms = WHO standards). On the other hand, the conductivity, even in Aïn Bou Rkhiss and Aïn Ben Ali are higher than recommended in the OMS normative limits ($\leq$ 250 µS/cm), and probably permitted the numerous halophilous taxa to develop (see Table III). Furthermore, the diatom taxa have to be carefully checked, since varieties or forms (i.e., cryptic diversity) with slightly different morphologies may have different polluosensitivity signatures, and it may bring bias in the calculation of the indices. In South Africa, Taylor *et al.* (2007b) demonstrated that these indices (BDI, SPI and several other indices also based on diatoms), are useful in water quality monitoring, but that a 'diatom index unique to South Africa including endemic species will have to be formulated'. Following Tan *et al*. (2017) 'The environmental variables associated with the diatom indices probably differ across geographic regions because the limiting factors for reproduction and growth are different'. It would be of interest to also measure ionic strength or other specific chemistry, and to evaluate the role of higher temperatures, that may influence the diatom optima. In conclusion, the BDI and PSI indices have to be applied with caution to non-European regions and may need to be carefully calibrated (Besse-Lototskaya *et al*. 2011, Kahlert *et al*. 2016 and refs therein).

The present geochemical analyses show that in a very restricted area (ca. 100 m^2) two springs (Aïn Bou Rkhiss and Aïn Kibrit) flow with different origin, or following different flow paths, with Aïn Kibrit coming from another aquifer reservoir or crossing evaporitic formations (gypsum). The diatom assemblages of these two springs attest of their different geochemical origins, with several halophilous taxa present in Kibrit that are absent from Bou Rkhiss. Otherwise, even if the diatom assemblages are relatively different in Aïn Ben Ali and Aïn Bou Rkhiss, partly due to the sampling at Ben Ali which under-represents microphytobenthos in favor of phytoplankton, the water of these two springs may share the same aquifer. The groundwater flow paths hypothesis need more investigations to be validated.

For communities living within a semi-arid climate such as in the Merguellil Basin (Central Tunisia), the water supplies are very limited while this water is essential, e.g., for the irrigation of the small and local agriculture (Collard *et al*. 2015, Riaux 2016). Furthermore, there is a precarious balance between subsistence agriculture and supply demands for coastal towns in the Sahel (Le Goulven *et al*. 2009). Potable water is particularly important for the local population. Therefore, the normative qualification of the water supplies (e.g., through biological indices such as the BDI or SPI) outside of the original systems where they first developed, has to be refined, taking into consideration local studies that may serve as reference.

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