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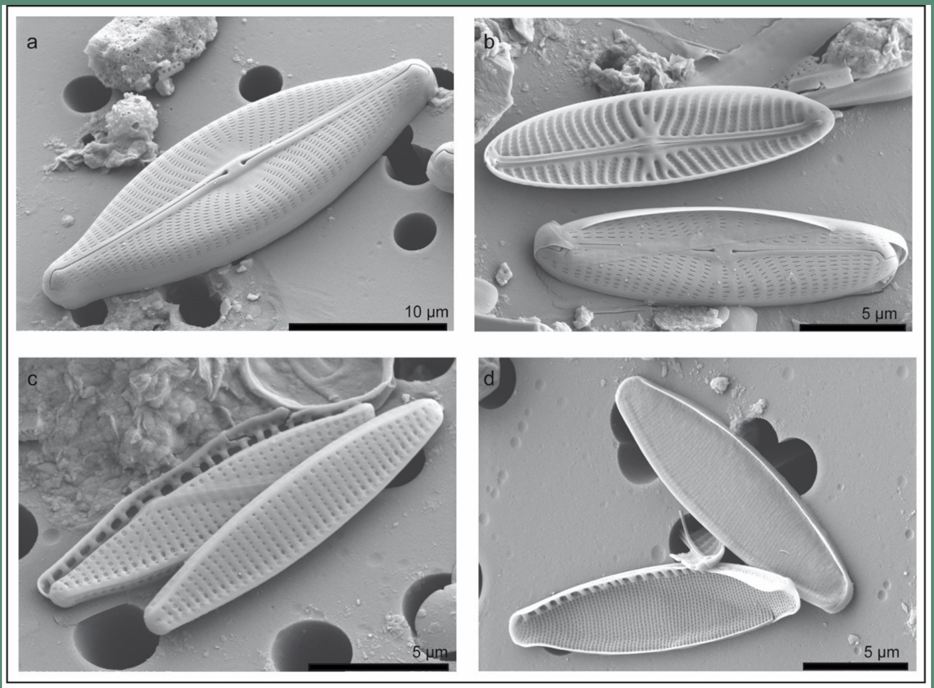

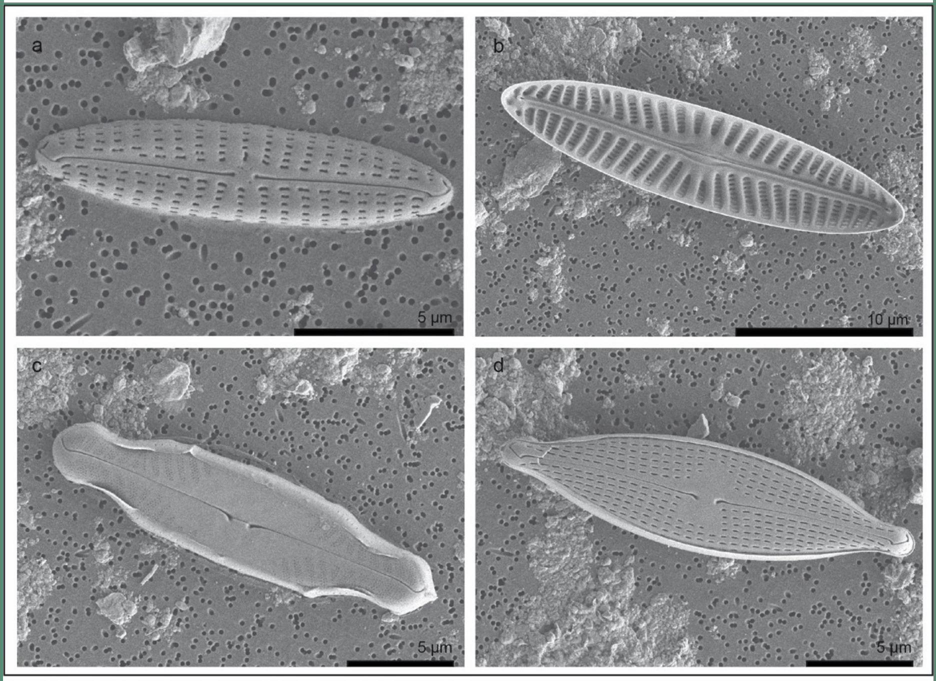

Brackish diatom species observed at the bituminous Poix spring: an island of curiosity

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Brackish diatom species observed at the bituminous Poix spring: an island of curiosity

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Résumé

La source de la Poix est une particularité géologique du Massif central français car elle est connue pour être bitumineuse associée à de l'eau salée, du méthane et des traces d'hydrogène sulfuré. Entre mars 2017 et juin 2020, trois prélèvements ont été effectués en raclant les diatomées sur la couche de bitume. De plus, les conditions physiques et chimiques ont été examinées. Il apparaît que la conductivité de l'eau salée est supérieure à celle de l'océan, entraînant le développement de certaines espèces de diatomées saumâtres, observées pour certaines d'entre elles sur les sédiments intertidaux ou dans les estuaires. Même si les conditions abiotiques étaient extrêmes pour ces algues, la biodiversité était élevée et révèle des espèces ayant une grande capacité à supporter des concentrations et des fluctuations de salinité élevées.

Mots-clés
Eau saumâtre
Biodiversité
Communautés de diatomées
Sources minérales

Abstract

The Poix spring is a geological particularity of the French Massif central as it is known to be bitumen associated with salt water, methane and traces of hydrogen sulphide. Between March 2017 and June 2020, three samples were taken by scraping the diatoms on the layer of bitumen. Moreover, the physical and chemical conditions were examined. It appears that the conductivity of the saline water is higher than the one of the ocean leading to the development of some brackish diatom species observed for some of them on the intertidal sediments or estuaries. Even if the abiotic conditions were extreme for these algae, the biodiversity was high and reveals species with high ability to withstand in high salinity concentrations and fluctuations.

Keywords
Brackish water
Biodiversity
Diatoms communities
Mineral springs

Introduction

Brackish water is defined as having a chloride content more than 400 mg.L^{-1} or an electrical conductivity greater than 1.5 dS.m^{-1} (Shevah 2014). Brackish environments are classically observed on coastal areas, estuary but also in inland habitats. When considering inland areas, these are habitats under the influence of saline waters that accompany the salt deposits, as well as areas exposed to water deficit in dry climate (Reimold & Queen 1974; Poljakoff-Mayber & Gale 1975). These brackish environments are relatively widespread throughout the world and cover ca. 10% of the land (O'Leary & Glenn 1994; Piernik 2012). Inland salt environments occur in limited areas, mostly on "fossil salt deposits and around salty springs" (Waisel 1972; Piernik 2012).

Among the springs, there a wide range of physical and chemical conditions with conductivity ranging from 10 to 70,000 µS.cm^{-1} (Sabater & Roca 1992; Angeli *et al.* 2010; Żelazna-Wieczorek 2011; Wojtal 2013; Beauger *et al.* 2015, 2016, 2017, 2019). When the conductivity is the highest with high sodium concentration, springs are associated with halophyte vegetation or micro-organisms adapted to high osmotic pressure. Among these micro-organisms, diatoms are present and distributed in this particular habitat. These microscopic algae are known to have ecological preferences for most of them. So, brackish diatom species are associated and grow in salty springs (Rakowska 1997; Wojtal 2013; Lai *et al.* 2016).

In the French Massif central, there are many salt marshes and among the 500 inventoried mineral springs (Lecoq 1864), some are salty springs that induced this brackish ecosystem. Indeed, an on-going survey on diatoms that began in 2015, reveals that the conductivity of the mineral springs ranges between 37 and 123,200 µS.cm^{-1} with an average of 4800 µS.cm^{-1} (Beauger, personal communication). The Poix spring at Clermont-Ferrand is the mineral spring with the highest conductivity and salinity of this region. Since at least antiquity, this spring has drawn attention particularly for the occurrence of bitumen with water. In 1575, the French author François de Belle-Forest described the area as a hill where bitumen and spring water flow in the book "La cosmologie universelle de tout le monde". Later, this spring was also mentioned by the doctor Jean Banc in 1604, the chemist Joseph de Tournefort in 1734, the geologist Jean-Etienne Guettard in 1759 and the famous naturalists Antoine Delarbre in 1749, Georges-Louis Leclerc de Buffon in 1799 and Henri Lecoq in 1864 (Nivet 1846; Truchot 1878; Legrand 2019). Focusing on the mineral Poix spring, the main aim of the study was to estimate the biodiversity associated with these harsh conditions by studying diatom communities during different seasons.

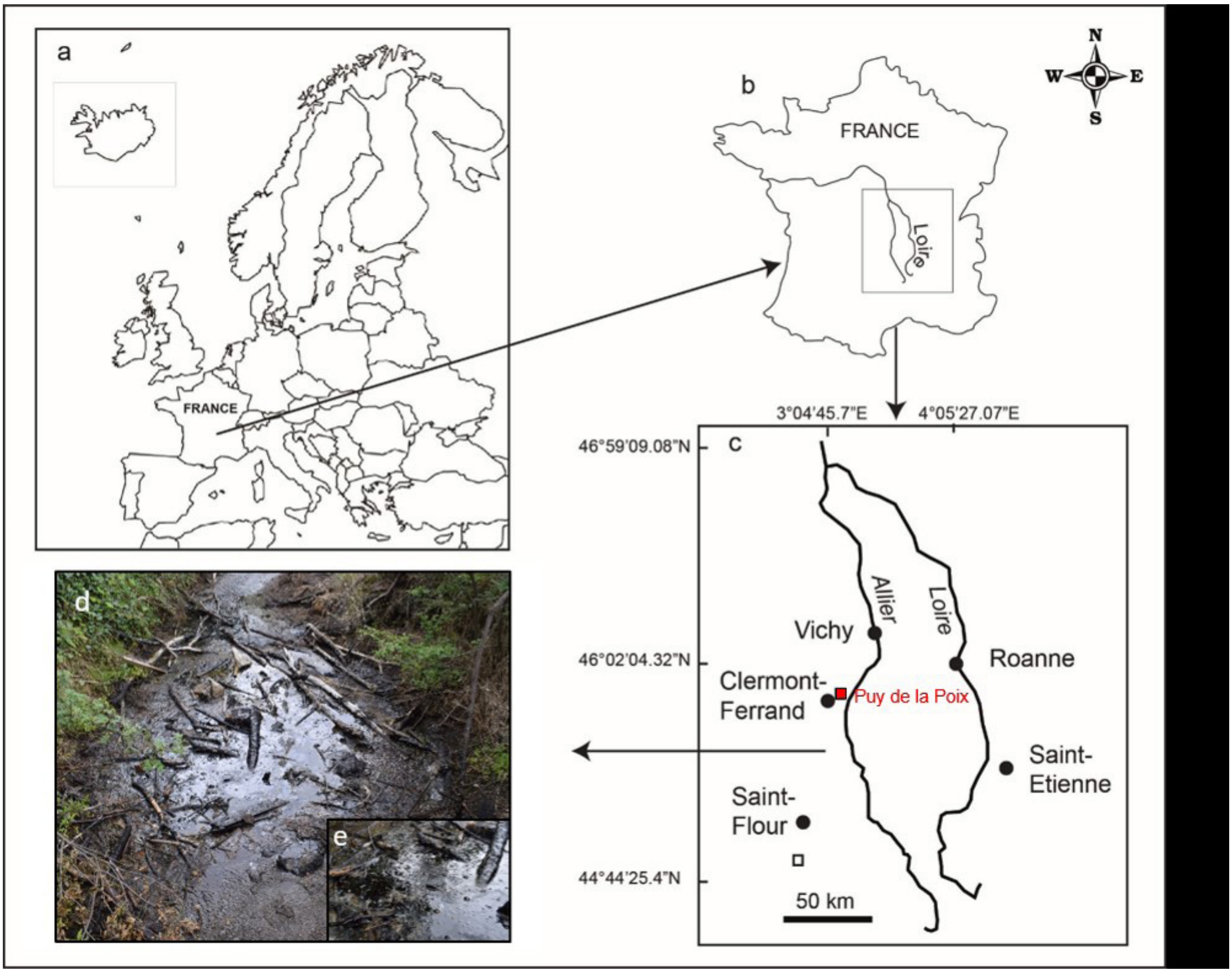

Figure 1 – Map of the studied mineral spring. a: map of Europe; b and c: position location of the Poix spring; d: photo of the Poix spring and the bitumen.

Materials and methods

Study site

The Poix spring is situated in the French Massif central and more particularly in the plain of Limagne near Clermont-Ferrand (Fig. 1) (E Lambert 93: 711392.04; N Lambert 93: 6520283.61; altitude: 337m). In the Limagne plain, the Oligocene sediments are naturally rich in hydrocarbons. Indeed, the former lake of Limagne was very rich in life, and the conditions of sedimentation preserved a large part of the organic matter. This organic matter has undergone diagenesis and has partly become bitumen. These hydrocarbons (less dense than the water and the surrounding rocks) rise through the rocks of the Limagne, by following the volcanic vents of the peperitic mini-volcano situated in the area (Thomas 2008). Moreover, this bitumen is associated with salt water, methane and traces of hydrogen sulphide (Gonnard 1906). Lecoq (1864) underlined that out of 82 g of material there is 70 g of sodium chloride, 7 g of de sodium sulphate, 0.1 g of bitumen and organic matter, a small amount of other salts, carbonate of lime, carbonate of magnesia, etc. and finally only traces of sulfur and silica. The place where the spring emerges is not shaded and takes full advantage of the sunshine, which is favourable for the development of diatoms.

Physical and chemical analyses and diatom sampling

The sampling survey was carried out on the 16th March 2017, on the 22nd October 2019 and on the 8th June 2020 (table 1) in order to make an inventory of the diatom flora of the site. The geographical position of the sampling site was georeferenced using a DGPS Trimble Geo7x, in Lambert 93. *In situ*, conductivity (µS.cm^{-1}), pH (pH units), water temperature (°C) and the dissolved oxygen (%) were measured using respectively a multi-parameter WTW FC probe 340i and an oximeter Ysi ProODO. A water sample was collected for further chemical analysis in the laboratory and was analysed using the high-pressure ion chromatography technique. First, these samples were filtered using Whatmann GF/C filters. For the cation analysis, a Thermo Scientific Dionex ICS1100 system was used, whereas for the anions, a Thermo Scientific Dionex Aquion system was used. The concentrations (mg L^{-1}) in lithium (Li^+), sodium (Na^+), ammonium (NH_4^+), potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}), fluorine (F^-), chloride (Cl^-), nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3-}) and sulphate (SO_4^{2-}) were measured in the laboratory. In addition, carbonate concentration (HCO_3^-) was measured using a Hach Carbonate AL-DT kit (Hach, Loveland, CO, USA).

Diatom sampling was possible at certain locations where the bitumen was submerged at the emergence of the spring. The sampling area was equivalent to a 30x30 cm area. Diatoms were collected by scraping the bituminous deposit at the bottom of the spring using a toothbrush and a pipette (Fig. 1d). Then, vial was named and dated.

Slide preparation, microscopy and analysis

For each sample collected at the Poix spring, a small fraction of the sample (2 ml) was prepared for light microscopy (LM) observation following the method described in Prygiel & Coste (2000). Samples were cleaned using hydrogen peroxide (H_2O_2, 35%) and hydrochloric acid (HCl, 37%), and rinsed several times with distilled water. A drop of the diluted cleaned material was dried on coverslips and mounted in Naphrax. LM observations and morphometric measurements were done using a Leica® DM2700M microscope with a 100x oil immersion objective using a differential interference contrast. For each slide, 400 valves were counted. Once the counting was complete, the relative abundances of diatom species were calculated.

Diatom species were identified according to Krammer & Lange-Bertalot (1997a, 1997b, 2000a, 2000b, 2004), Krammer (2000, 2002, 2003), Lange-Bertalot (2001), Werum & Lange-Bertalot (2004), Żelazna-Wieczorek (2011), Wojtal (2013), Levkov *et al.* (2016), Lange-Bertalot *et al.* (2017), associated to further articles (Spaulding & Stoermer 1997; Spaulding *et al.* 1999; Beauger *et al.* 2015, 2016, 2017, 2019; Wetzel *et al.* 2015).

For the scanning electron microscopy (SEM), parts of the oxidized suspensions were filtered with additional deionized water through a 3 or 0.2 µm Isopore polycarbonate membrane filter (Merck Millipore). Filters were mounted on stubs using adhesive carbon tabs and coated with carbon (Quorum Q150 TES**)**. An ultrahigh-resolution analytical field emission (FE) scanning electron microscope, Hitachi SU-70 for the sample taken in 2017 and Hitachi Regulus 8230 (Hitachi High-Technologies Corporation, Japan) for the two other samples, was operated at 2 or 5 kV and 10 mm distance for image analysis. SEM images were taken using the lower (SE-L) and upper (SE-U) detector signal.

Shannon index and evenness were calculated using "vegan" package version 2.6-2 (Oksanen *et al.* 2022) in R version 4.1.2. Ecological traits were calculated according to Van Dam *et al.* (1994) using OMNIDIA 6.0.8 software (Lecointe *et al.* 1993) and Lange-Bertalot *et al.* (2017).

Results

Physical and chemical environment

The physico-chemical data of the Poix spring measured during the three campaigns are presented in table 1. On the whole the conductivity and all ionic concentrations were very high contrary to the dissolved oxygen. In March 2017, the conductivity was the highest with 123,200 µS.cm^{-1} and at this period, the concentration in sodium and chloride was the double compared to 2019 and 2020 with 44,295.2 and 60,477.2 mg.L^{-1}. In 2019 and 2020, the conductivity was 46,260 and 53,300 µS.cm^{-1} respectively and other ionic concentrations were comparable except magnesium and calcium.

Diatom communities

On the whole, thirty-one different species were observed at the Poix spring (Table 2) with many species of *Navicula* and *Nitzschia*. The *Navicula* species sampled were *N. cincta* (Ehrenberg) Ralfs in Pritchard, *N. gregaria* Donkin, *N. meulemansii* A.Mertens, Witkowski & Lange-Bertalot (Figs 2a-e, 3b), *N. salinarum* Grunow in Cleve & Grunow (Figs 2f-i, 3a), *N. salinicola* Hustedt (Figs 2j-o, 4a) and *N. veneta* Kützing. The *Nitzschia* species observed were *Nitzschia inconspicua* Grunow (Fig. 3c), *Nitzschia* aff. *lacuum* Lange-Bertalot, *Nitzschia pusilla* Grunow (Fig. 3d), *Nitzschia* cf. *epithemioides* Grunow in Cleve & Grunow and *Nitzschia* aff. *liebethruthii* Rabenhorst.

As it is shown in Table 3, biodiversity was the lowest in 2017 and 2019 with 11 species and there were 26 species in 2020. Shannon index was 1.41 in 2017, 0.75 in 2019 and 2.51 in 2020. Evenness varied between 0.39 and 0.77. In March 2017, the dominant species were *Navicula salinarum* (54.4%) (Fig. 3a), *Nitzschia pusilla* (Fig. 3d) (20.5%) and *Navicula veneta* (9.7%). In October 2019, it was *Navicula salinicola* (84.0%) (Fig. 4a). At last, in June 2020, *Navicula veneta* (22.5%), *Navicula cincta* (Fig. 4b) (16.7%), *Nitzschia* cf. *liebetruthii* (11.4%), *Pinnularia joculata* (Manguin) Krammer (10.7%) (Fig. 4c), *Navicula meulemansii* (6.8%) (Fig. 3a) and *Crenotia thermalis* (Rabenhorst) Wojtal (4.7%). Some individual of *Muelleria terrestris* (J.B.Petersen) S.A.Spaulding & Stoermer were observed (Fig. 5). Some unknown species were also observed such as *Halamphora* sp. and two species of *Pinnularia* undetermined.

Related to the ecological trait "salinity", the community was dominated by brackish water species such as *Navicula salinarum* in 2017 (Fig. 6). In 2019, marine-brackish taxa dominated such as *Navicula salinicola*. At last, in 2020, it was a mix of halophilous, brackish and marine-brackish water species such as *Navicula veneta*.

Table 1 – Ionic concentrations (mg.L^{-1}), physical and chemical results of the measures done in-situ at the Poix spring.

Sampling date	3/16/2017	10/22/2019	6/8/2020
Li$^+$	171.3	56.4	59.5
Na$^+$	44,295.2	13,748.7	10,583.7
NH$_4^+$	2.9	5.4	17.1
K$^+$	228.0	146.8	107.8
Mg^{2+}	658.0	548.7	294.0
Ca^{2+}	622.1	1,072.9	504.4
F$^-$	2.5	1.2	0.2
Cl$^-$	60,477.2	22,929.4	17,417.5
NO$_2^-$	0.0	0.0	0.0
Br$^-$	230.5	73.8	56.4
NO$_3^-$	1.3	0.1	0.1
PO$_4^{3-}$	0.1	0.1	1.3
SO$_4^{2-}$	5,417.8	2,570.0	2,121.3
HCO$_3^-$	4,290.0	2,320.0	1,360.0
Conductivity (µS.cm^{-1})	123.2	46.26	53.3
pH (pH units)	7.2	6.8	7.5
O$_2$ (%)	6.3	2.8	7.8
Water temperature (°C)	10.6	12.9	13.6

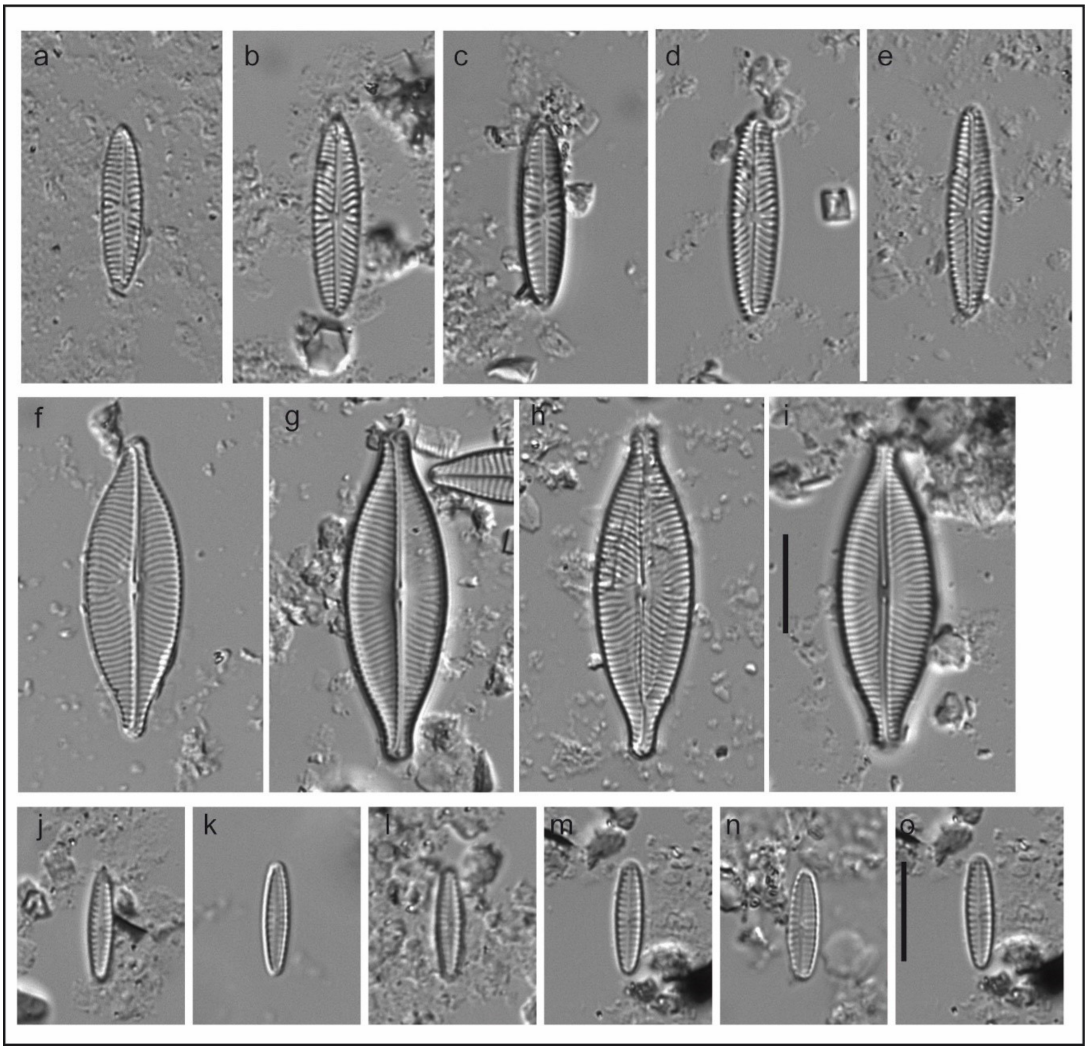

Figure 2 – Some *Navicula* species observed at the Poix spring. *Navicula meulemansii* **(a-e)**, *Navicula salinarum* **(f-i)**, *Navicula salinicola* **(j-o)**. Scale bar = 10 µm.

Table 2 – Relative abundances of the diatom species observed at each sampling period.

Sampling date	**3/16/2017**	**10/22/2019**	**6/8/2020**
Caloneis fontinalis (Grunow in Van Heurck) A.Cleve	0	0	1.1
Crenotia thermalis (Rabenhorst) Wojtal	0	0	4.7
Gomphonema parvulum Kützing	0	0	0.21
Halamphora sp.	0.71	0	0
Hantzschia amphioxys (Ehrenberg) Grunow in Cleve & Grunow	0	2.73	2.5
Hippodonta subtilissima Lange-Bertalot. Metzeltin & Witkowski	0	1.74	0
Humidophila brekkaensis (J.B.Petersen) R.L.Lowe. Kociolek. J.R.Johansen.Van deVijver. Lange-Bertalot & Kopalová	0	0	0.21
Humidophila contenta (Grunow) R.L.Lowe. Kociolek. J.R.Johansen. Van de Vijver. Lange-Bertalot & Kopalová	0.23	0.75	1.31
Luticola aff. *frequentissima* Levkov. Metzeltin & A.Pavlov	0	0	0.7
Luticola ventricosa (Kützing) D.G.Mann in Round *et al.*	0	0	0.6
Muelleria terrestris (J.B.Petersen) S.A.Spaulding & Stoermer	0	0	0.65
Navicula cincta (Ehrenberg) Ralfs in Pritchard	0.24	0.25	17.13
Navicula gregaria Donkin	5.25	0	0.21
Navicula meulemansii A.Mertens. Witkowski & Lange-Bertalot	4.53	0	7.01
Navicula salinarum Grunow in Cleve & Grunow	54.42	0	1.94
Navicula salinicola Hustedt	0	84.08	5.7
Navicula veneta Kützing	9.79	5.22	23.07
Nitzschia aff. *lacuum* Lange-Bertalot	0	0	1.97
Nitzschia aff. *liebethruthii* Rabenhorst	1.67	0	11.5
Nitzschia cf. *epithemioides* Grunow in Cleve & Grunow	0	0	1.1
Nitzschia inconspicua Grunow	2.39	0	0
Nitzschia pusilla Grunow	20.53	1.99	2.14
Pinnularia joculata (Manguin) Krammer	0	1.74	10
Pinnularia sp.1	0	0	2.2
Pinnularia sp.2	0	0	1.2
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	0.24	0	0
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	0	0.75	0.21
Pseudofallacia monoculata (Hustedt) Y.Liu. Kociolek & Q.Wang in Liu *et al.*	0	0	1.32
Pseudofallacia sp.	0	0	0.88
Sellaphora bacillum (Ehrenberg) D.G.Mann	0	0.5	0
Stauroneis tackei (Hustedt) Krammer & Lange-Bertalot in Kusber *et al.*	0	0.25	0.44

Table 3 – α-diversity of samples showing species richness, Shannon's index diversity and evenness.

	Richness	Shannon	Evenness
2017	11	1.41	0.59
2019	11	0.75	0.31
2020	26	2.51	0.77

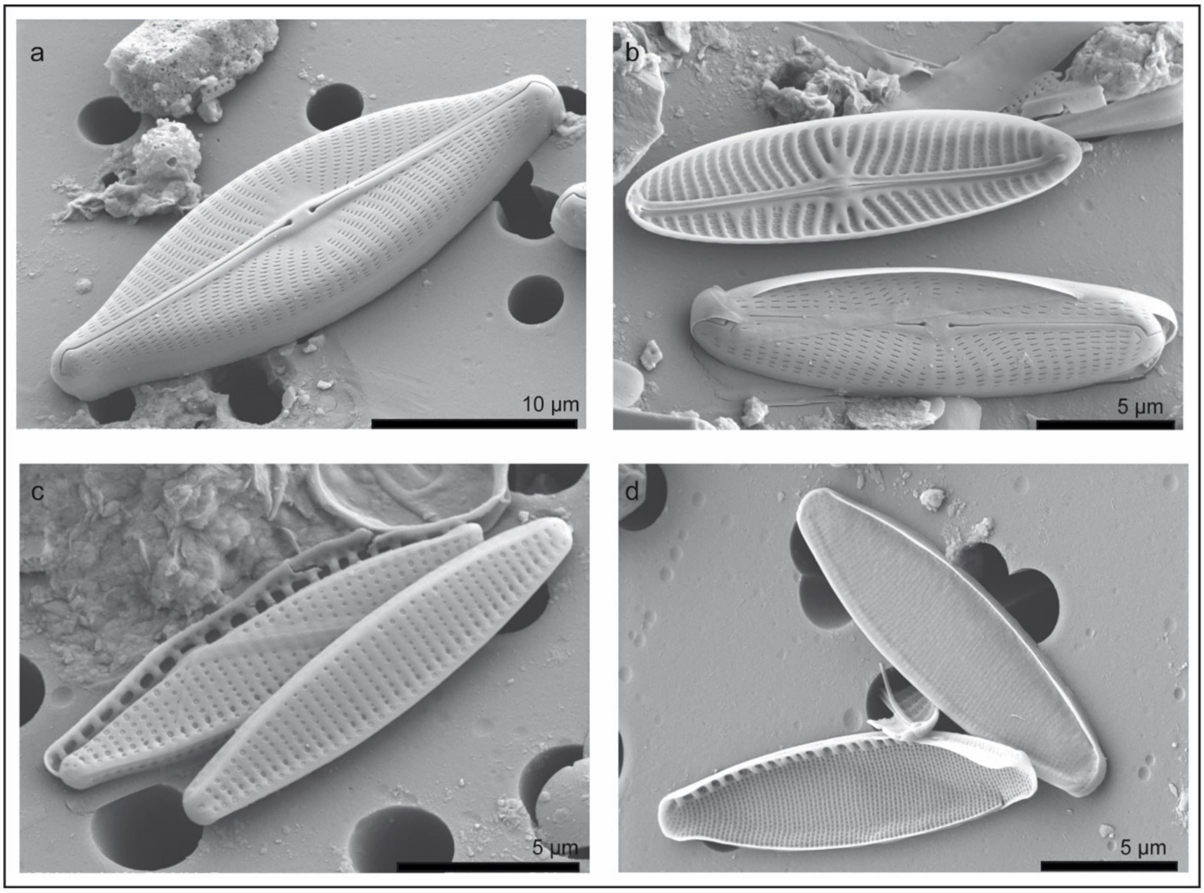

Figure 3 – Photographs at the scanning electron microscopy of some species: *Navicula salinarum* **(a)**, *Navicula meulemansii* **(b)**, *Nitzschia inconspicua* **(c)**, *Nitzschia pusilla* **(d)**.

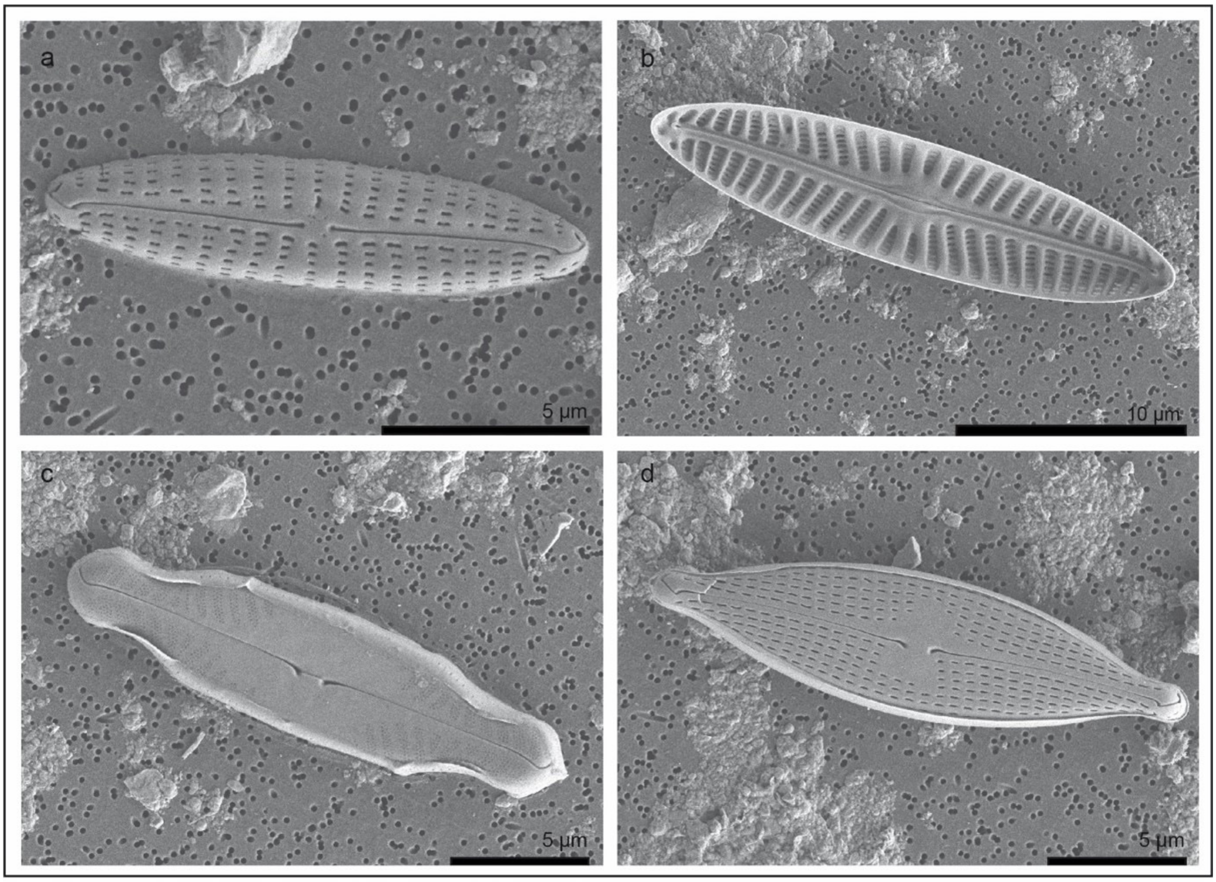

Figure 4 – Photographs at the scanning electron microscopy of some species: *Navicula salinicola* **(a)**, *Navicula cincta* **(b)**, *Pinnularia joculata* **(c)**, *Navicula gregaria* **(d)**.

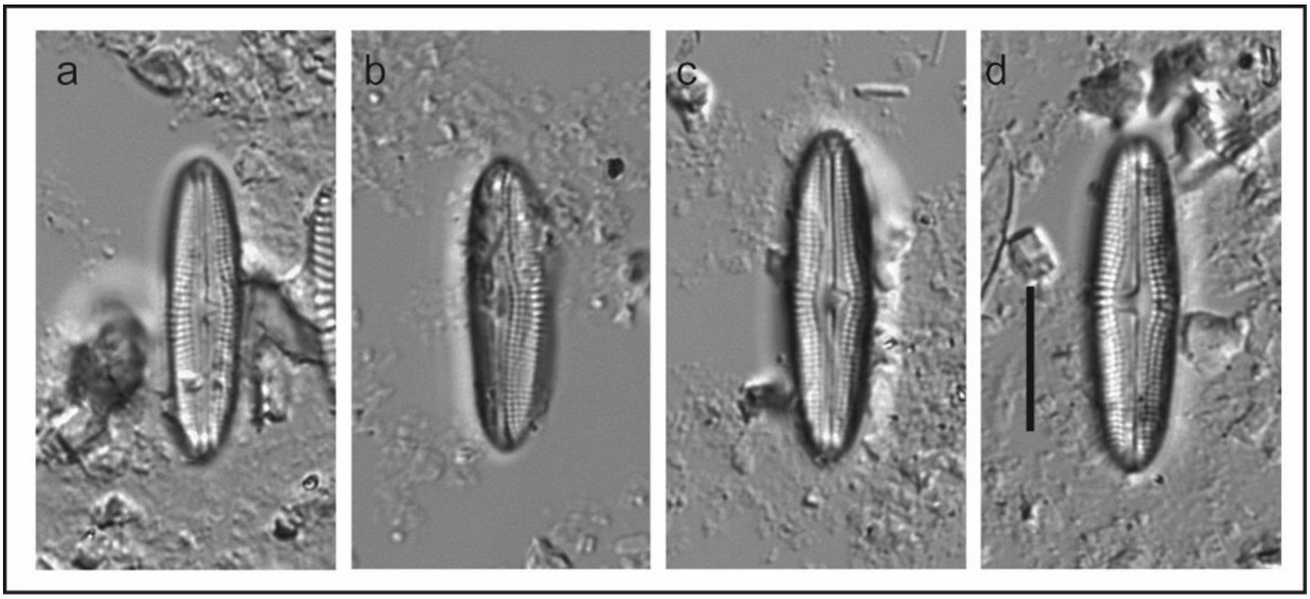

Figure 5 (a-d) – *Muelleria terrestris* observed at the Poix spring. Scale bar = 10 µm.

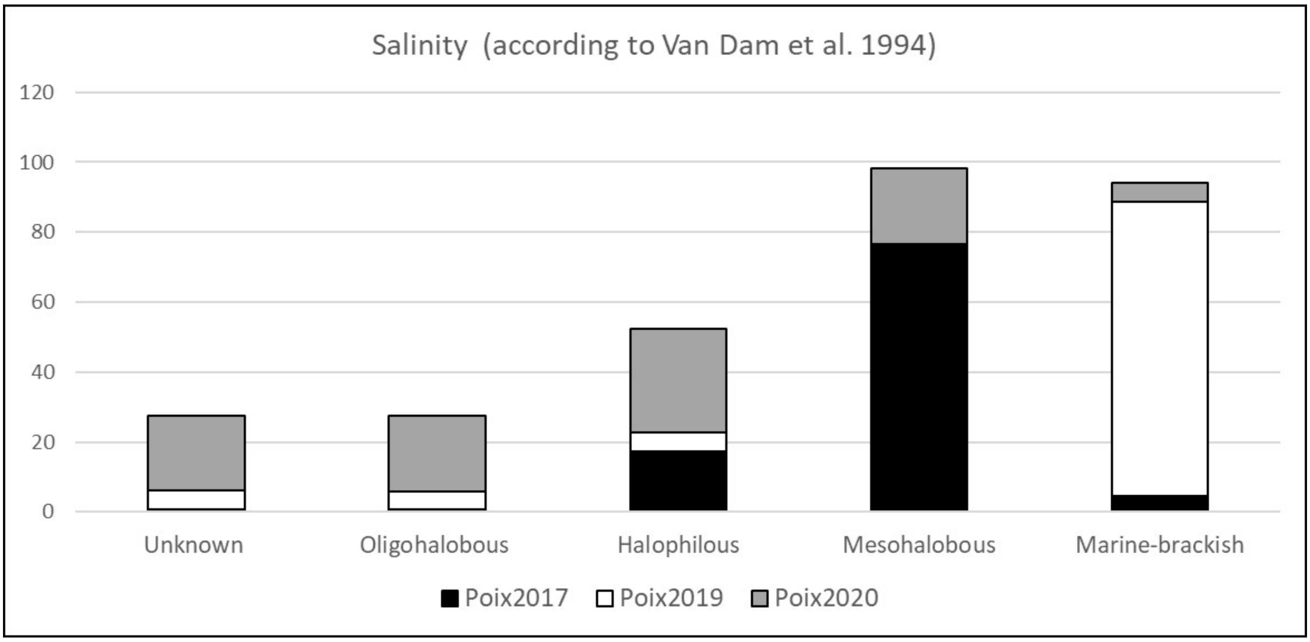

Figure 6 – Ecological traits retained: salinity at the Poix spring for the three samples.

Discussion

In the Poix spring, characterized by a high mineralization as underlined the high conductivity and salinity, the pH is about 7 and the oxygenation is low. Moreover, the concentration in Lithium and Bromine underlined the deep origin of the water (Michard 1990). The conductivity ranged between 46,260 and 123,200 µS.cm^{-1} and is consequently higher than ocean that was evaluated as 32,000 µS.cm^{-1} by some authors (Schnepf *et al.* 2014; Sabaka *et al.* 2015). Thus, the abiotic conditions lead to an extreme environment where diatoms were living. In such environment, only species with ecological preferences in adequation with the properties of the water could be encountered. Species richness in 2017 and 2019 was 11 and reached 26 in 2020. The estimated richness varies greatly depending on the spring. In Lai *et al.* (2019a), the species richness of mineral springs sampled in Auvergne and Sardinia varied between 2 and 14. Also in Beauger *et al.* (2020), 17 springs of the Auvergne region were sampled with a richness ranging between 2 and 21 (mean: 9). Lai *et al.* (2019c) sampled several Mediterranean karst springs, the richest spring was Sa Vena Manna-Sedini with 26 species detected while the mean of richness of the 8 springs sampled was 8. When Cantonati *et al.* (2012) collected diatoms in 110 springs of the Alps during summer, species richness ranged between 3 and 36 (mean: 13). These observations suggest that diatoms richness is high during 2020 in the mineral spring of the Poix. Shannon diversity was higher in 2020 and 2017 when evenness was higher also.

Diatom species richness is about twice as high in 2020 as in 2017 and 2019. The first hypothesis made to explain these observations is that it is a seasonal effect, as species richness is classically higher in the warmer seasons (Lavoie *et al.* 2003). Indeed, in 2020 diatoms were sampled in spring whereas in 2017 and 2019 they were sampled in winter and autumn respectively. The second hypothesis is that the stress conditions in the spring have decreased because the conductivity in the spring has decreased from 123,200 to 53,300 µS.cm^{-1}. However, in 2019, a decrease in conductivity did not lead to an increase in species richness.

The community was mainly dominated by different species of *Navicula* such as *N. cincta*, *N. gregaria*, *N. meulemansii*, *N. salinarum*, *N. salinicola* and *N. veneta* and also by different species of *Nitzschia* such as *N. inconspicua*, *N.* aff. *lacuum*, *N. pusilla*, *Nitzschia* cf *epithemioides* and *Nitzschia* aff. *liebethruthii*. Moreover, *Crenotia thermalis* in 2020 and *Pinnularia joculata* at the three campaigns, were also well represented in the diatom community.

The physical and chemical conditions encountered by diatoms in this spring induced the presence of several halophilous to brackish species such as *Crenotia thermalis* and the cosmopolitan *Navicula salinarum*, *N. salinicola* and *N. veneta* (Van Dam *et al.* 1994; Lange-Bertalot 2001). Indeed, the synthetic ecological spectra highlighted the dominance of species that prefer high concentrations of dissolved salts. *Crenotia thermalis* occurred in electrolyte-rich inland habitats, particularly thermal and mineral springs (Wojtal 2013; Lange-Bertalot *et al.* 2017). *Navicula salinarum* was abundant on the coasts, in inland brackish waters, including inland brackish lakes where salts are supplied mainly by rocks and soil weathering (athalassic ecosystems) such as in Slovenia, where it occurred in the only brackish lake (Krivograd Klemenčič *et al.* 2006). Even if this taxon is not classically encountered in springs, it was observed in some salt springs in Poland (Lange-Bertalot 2001; Wojtal 2013; Żelazna-Wieczorek *et al.* 2015). In athalassic habitats in Poland, *N. salinarum* was observed associated with *N. veneta* and *N. meulemansii* (Żelazna-Wieczorek *et al.* 2015). *Navicula veneta* is common in electrolyte rich to brackish waters (Lange-Bertalot 2001). In Poland, it was observed in the sulphide spring of slightly alkaline water of high specific conductivity but very low dissolved oxygen (Wojtal 2013). Moreover, *N. meulemansii* has a wide geographical distribution in coastal brackish waters (The Netherlands and Germany), European estuaries (Portugal), and the San Francisco Bay (U.S.A.) (Mertens *et al.* 2014). Indeed, it has been identified in samples collected from intertidal sediments from the Jadebusen near Dangast (Witkowski *et al.* 2004), the Tagus estuary (Ribeiro 2010), the San Francisco Bay area, and from inland brackish waters in the Netherlands (Mertens *et al.* 2014).

Among the other dominant species, *N. salinicola* was collected along the coast in brackish waters, in river estuaries, in inland salt springs and in freshwaters with a high electrolyte content (Targett *et al.* 1983; Lange-Bertalot 2001; Guiry & Guiry 2022). *Navicula gregaria* was also observed at the Poix spring (Fig. 4d). It is a species that was found on coasts and inland salt springs of different countries (Lange-Bertalot 2001; Owen *et al.* 2008; Żelazna-Wieczorek *et al.* 2015; Leira et al. 2017; Angel *et al.* 2018; Lai *et al.* 2019a, 2019b).

Navicula cincta was also present in the samples. It is considered as oligohalobous in Van Dam *et al.* (1994) whereas this species lives in electrolyte rich to brackish waters (Lange-Bertalot 2001). *Navicula cincta* was observed in athalassic habitats in Poland and also in mineral springs of a very high specific conductivity, in thermo-mineral springs in Sardinia and in Slovenia (Krivograd Klemenčič *et al.* 2006; Wojtal 2013; Żelazna-Wieczorek et al. 2015; Lai *et al.* 2019a, 2019b).

Nitzschia pusilla was observed in brackish waters (Krammer & Lange-Bertalot 1997b), saline ponds (Trobajo *et al.* 2011), a hypersaline coastal lagoon (Sylvestre *et al.* 2001), and a saline lake (Taukulis & John 2006).

At last, some taxa living in a wide amplitude of ecological conditions were present at the Poix spring, such as *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot, *P. lanceolatum* (Brébisson ex Kützing) Lange-Bertalot and *Nitzschia inconspicua* (Lange-Bertalot *et al.* 2017) (Fig. 3c) that is also known to extend into brackish waters (Kelly *et al.* 2015). *Hantzschia amphioxys* (Ehrenberg) Grunow in Cleve & Grunow that lives in prolongedly dry and only temporarily wetted (aerial) biotopes, such as soils and rock crevices, underlined that the level of water was low at this site (Lange-Bertalot *et al.* 2017). *Muelleria terrestris* was observed in soils (Petersen 1915; Spaulding *et al.* 1999; Foets *et al.* 2021), and on wet mosses in an intermittent spring of a village in Macedonia (Levkov *et al.* 2019). *Muelleria terrestris* was also present in a petrifying *Cratoneurion* spring in Belgium (Denys & Oosterlynck 2015). *Pinnularia joculata* was described in freshwater (Bourrelly & Manguin 1952; Krammer 2000) and was also observed in mineral springs (Quintela *et al.* 2013; Lai *et al.* 2019b).

Eight species *Humidophila contenta* (Grunow) R.L.Lowe, Kociolek, J.R.Johansen, Van de Vijver, Lange-Bertalot & Kopalová, *Navicula cincta*, *N. gregaria*, *N. meulemansii*, *N. salinarum*, *N. veneta*, *Nitzschia* aff. *liebetruthii*, and *Pinnularia joculata* were present during the three periods of sampling. These species seem to have great capacity to maintain in extreme environments with high fluctuations of conductivity and ion concentrations suggesting the presence of high salinity tolerance mechanisms named osmoregulation. These mechanisms allow cells to compensate osmotic and ionic stress.

Furthermore, the presence of the eight species and the majority of brackish species in the Poix spring as an isolated environment is remarkable and allows the emergence of a question on the origin of these species. This question was already addressed in the literature. Since 1940s, Remane proposed the idea that brackish species are freshwater or marine species adapted to brackish conditions or descendants of these two categories of species (Remane 1940). He also suggested that there are identifiable brackish assemblages (Cognetti & Maltagliati 2000; Potapova 2011). In contrast, Barnes (1989) suggested the inexistence of specific brackish assemblages but the presence of some populations of thalassogenous species in brackish environments (Cognetti & Maltagliati 2000). Each argument has its value depending on the environment studied. To go further, Round & Sims (1981) support the marine origin of diatoms and the colonisation of freshwaters by some lineages because of the high number of fossils and genera of marine diatoms found in the early Pliocene (Sims *et al.* 2006). The reconstruction of phylogeny of Thalassiosirales by Alverson *et al.* (2007) revealed more complicated schema and no unidirectional colonization. Indeed, in their study three lineages of Thalassiosirales seems to have colonized freshwater habitats independently. Another interesting result of this study is that there were at least three recolonizations of marine habitats by these lineages that led to speciation events resulting in the presence of these lineages in marine and freshwater habitats. The genus *Navicula* sensu stricto and *Nitzschia* found in the Poix spring are among the species of raphid diatoms that are found in marine and freshwater environments which agrees with Barnes propositions (Barnes 1989).

Thus, to explain the ability of some diatom taxa to live in high salinity concentration and fluctuation environments, Potapova (2011) made the hypothesis of the innate ability of diatoms to adapt to changing salinity or high salinity and the better conservation of this ability by some lineages. Indeed, ocean salinity was not modelled as stable (Hay *et al.* 2006), a great decrease of salinity was estimated during the Mesozoic because of the younger basin after the Pangée breaking. But it seems to have several other decreasing salinity events in the past that suggests that diatoms and other micro-organisms were confronted to several fluctuations of salinity in their evolution.

Conclusion

The studied of the Poix spring is known to be bitumen and associated with salt water, methane and traces of hydrogen sulphide. This environment with a conductivity higher than the ocean could be considered as extreme for diatom species. Moreover, this spring, as others, could be also considered as isolated aquatic ecosystem in the landscape whose conditions have certainly changed very little over the past millennia. The Poix spring showed a relatively high species richness with 31taxa observed during the three sampling campaigns. The diatom community was mainly dominated by different *Navicula* and *Nitzschia* species. The species are mainly halophilous to brackish such as *Crenotia thermalis*, *Navicula meulemansii*, *N. salinarum*, *N. salinicola* and *N. veneta* observed for some of them in coastal brackish environments or estuaries. Because of their living environment, these species maintain high ability of adaptation of high salinity concentrations and fluctuations which could be the result of well conserved ability of ionic and osmotic stress resistance. This inland brackish environment is a geological and also ecological particularity of the French Massif central. For these arguments, this type of environment needs to be preserved.

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