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Biodiversity and Environmental Factors Structuring Diatom Assemblages of Mineral Saline Springs in the French Massif Central

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Abstract: Springs are abundant and present worldwide and are among the most threatened ecosystems on Earth. The main aim of this study was to evaluate the biodiversity of seventy-nine (79) mineral saline springs situated in the French Massif Central, focusing on the species richness and the estimated richness and also on the diatom community composition. The influence of physical and chemical factors on both richness and communities was analyzed in order to have a better knowledge of the diatom ecological preferences and identify species typical of saline springs. Since December 2014, an on-going inventory of mineral springs has been in progress. For each spring, physical and chemical characteristics were measured, and benthic diatoms were sampled. The richness was the lowest in the springs presenting a man-made construction around the emergence. In the other springs, the highest richness was associated with the lowest lithium, sodium, total dissolved solid concentrations, and conductivity. Mineralization and some ions (bromine, calcium, chloride, fluoride, lithium, potassium, and sodium) were found to be the most critical drivers of diatom community composition. Some diatom species were typical of specific abiotic conditions, such as *Navicula sanctamargaritae*, which was associated with the highest potassium concentration. These species could appear as bio-indicators of these conditions.

Keywords: diatom communities; mineral saline springs; biodiversity; environmental factors

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1. Introduction

Springs are abundant and present worldwide, "with estimated numbers in the tens of millions" [1]. Spring types have been classified based on hydrology, geology, hydrochemistry, water temperature, biological assemblages, and human use [1–3]. Thus, for example, the springs feeding streams that are crenal (springs situated at headwaters according to the longitudinal models of running waters) [4] can be distinguished from mineral springs that are characterized by high ion concentrations. For this last group, displaying high mineralization, their hydrochemistry reflects the lithology of the aquifer.

Mineral and thermal springs are formed by hydrogeological processes linked to volcanic and tectonic activities. All spring water comes from precipitation that seep into the ground under the combined action of gravity and capillary action to the hydrostatic level [3]. A significant portion of rainwater feeds shallow aquifers, while elsewhere it slowly infiltrates faults up to several kilometers deep. Indeed, mineral springs coincide with the fault zones [5]. Hot waters circulating in the earth's crust will dissolve minerals from the rocks through which they pass [6]. Then, the water rises through hydrostatic or gaseous pressure with different major and trace elements linked to the deep warming, favoring their dissolution. The knowledge of aquifer temperature and the total electric charge of mobile elements allows us to predict these elemental concentrations in waters [7]. As springs are fed from thermally buffered underground sources, they show a slight variation in temperature [3] and other physical and chemical characteristics, such as the concentrations of sodium, calcium, and potassium ions [5,8].

Springs are ecotones that rely on groundwater to surface water [9], and they are known to be groundwater-dependent ecosystems [10] and isolated biotopes [9,11] capable of hosting specific biocenoses due to their discontinuous presence in the landscape [9]. The associated organisms and communities are often unique and have specific traits and distribution patterns [12–16]. These environments are generated by upwellings, and surface waters often host significant biodiversity and species adapted to the constraints imposed by these ecosystems [14,15].

Many species of algae appear to have developed adaptations to live in water characterized by high salinity and/or high concentrations of specific ions. Most alkaline hot springs contain between 1000 and 2000 mg of total dissolved solids (TDS) per liter; some have salinities higher than seawater [6,17]. Depending on the mineral elements present in the water, such as sodium, some springs create inland saline areas under the influence of saline waters [18]. According to the classification of Krieger [19], springs are moderately salty when TDS varies between 3000 ppm and 10,000 ppm. In this case, different ecosystems may develop where a halophilic flora grows [18,20], including benthic microscopic algae: the diatoms. These algae are characterized by a siliceous skeleton (frustule). They are found in almost every aquatic environment, including fresh and marine waters and terrestrial ecosystem. They are generally considered bioindicators of environmental quality because of their high sensitivity to elements of geogenic origin and the nutrient loading that can occur as a result of contamination [13]. In mineral springs, these microalgae are studied by diatomists all over the world (e.g., [21–31]). Many studies have been performed in Central Europe [21,22,24], others in other countries of Europe, e.g., France [14–16], Italy [25], and Portugal (Azores archipelago) [26]. In other parts of the world, investigations have to date been carried out in Turkey [23], Chile [27], the USA (California) [28], Africa [29], Thailand [30], Russia [31], Iceland [32,33], Canada [32], Japan [32], and New Zealand [33].

In the French Massif Central, numerous mineral saline springs are present and sodium bicarbonate waters form the main hydromineral province in France [5]. These waters have a dominant endogenous character. Their geographical distribution shows a relationship with recent volcanism. This province is made up of basins located on the major volcano-tectonic directions (N-S, N-W–S-E, N-E–S-W) or at the point of intersection of two of these directions [5]. In this part of France, few studies have been conducted on diatoms living in mineral springs [12,34–36] and on a limited number of mineral springs. Thus, these studies did not address the inventory of the biodiversity of these algae. As a consequence, since the survey initiated in 2014, new taxa have been recently discovered in these springs, such as *Navicula sanctamargaritae* Beauger [14], *Sellaphora labernardierei* Beauger, C. E. Wetzel & Ector [15], *Craticula lecohui* Beauger, C. E. Wetzel & Ector [16], *Pseudostaurosira bardii* Beauger, C. E. Wetzel & Ector [37], and *Chamaepinnularia salina* Beauger, C. E. Wetzel, Allain & Ector [38]. A new genus called *Fontina* Beauger, C. E. Wetzel & Ector in [39] was also observed and described in a mineral spring in that area. Moreover, preliminary research, focusing on diatom biodiversity from 17 springs in this area, was performed between November 2014 and April 2015 [40], and has provided some preliminary information on the ecology of some species encountered in these springs.

The main aim of the present study was to evaluate the biodiversity of the saline springs of the Massif Central related to the species richness and estimated richness of the diatom community. The influence of environmental factors on both diatom richness and communities was also analyzed in order (1) to increase our understanding on the ecological preferences of the observed diatom species, and (2) to identify species typical of saline springs.

2. Materials and Methods

2.1. Study Site

Between December 2014 and April 2022, an on-going inventory of mineral springs, and the diatoms inhabiting these habitats, in the French Massif Central was carried out. The area under study covers a zone of approximately 85,000 km² and comprises 15% of the territory of the metropolitan France. It is influenced by oceanic, continental, and Mediterranean climates. Each spring was sampled once only.

Among the mineral springs sampled since 2014 in the French Massif Central, we examined the saline springs for diatom biodiversity with a TDS range varying between 3000 ppm and 10,000 ppm, thereby retaining some 79 emergences according to Krieger [19]. The springs retained are situated in different places of the Massif Central (Figure 1; Table S1). Most are located in the same area (Puy-de-Dôme department). Among the 79 springs, 47 present an artificial construction around the emergence sites, such as Tennis and Lefort springs (Figure 2). For some sites, two or three springs were sampled, such as at Poix springs and Châtel-Guyon springs. Poix springs are peculiar in that they are known to contain bitumen associated with salt water, methane, and traces of hydrogen sulfide [41].

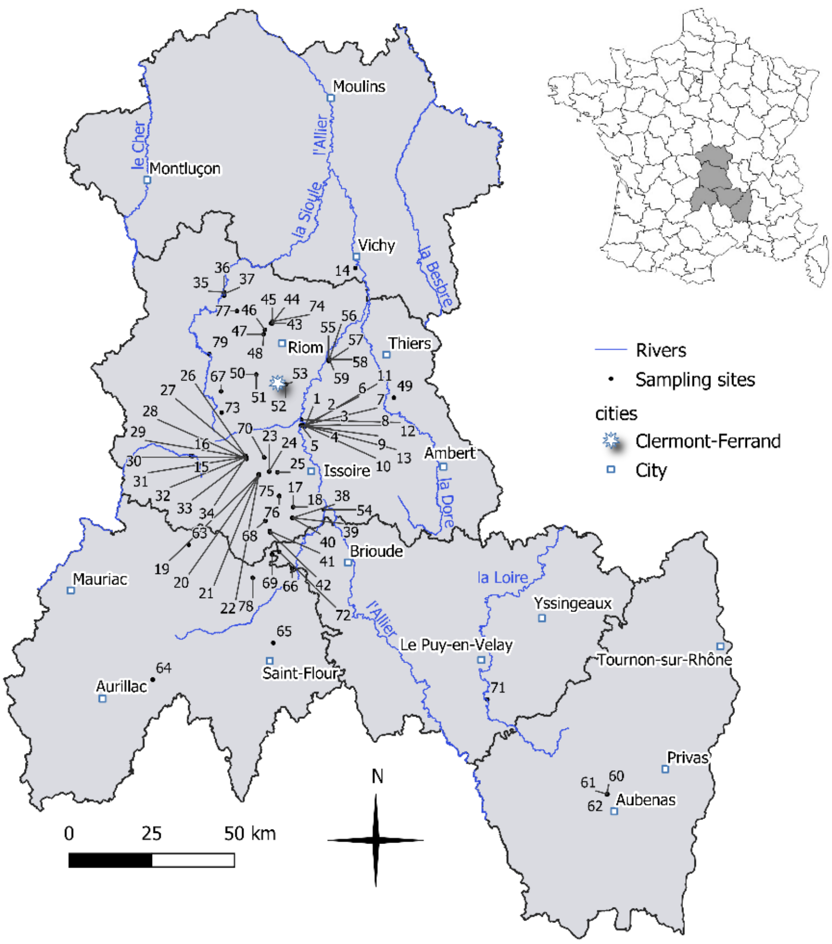

Figure 1. Map of the sampling sites (mineral springs). The correspondence between the spring number and its name is presented in Table S1.

Figure 2. Photography of some studied springs: Tennis (**a**) and Lefort (**b**).

2.2. Diatom Sampling and Physical and Chemical Analysis

In the field, the sampling sites were georeferenced using a DGPS Geo7x (Trimble, Sunnyvale, CA, USA) in Lambert 93. For each spring, several parameters were measured in-situ: conductivity (μS cm^{-1}), pH (pH unit), and water temperature (°C), using a multi-parameter WTW probe FC Multi 340i, and also dissolved oxygen (% and mg L^{-1}) using a ProODO oxygen probe (Ysi, Yellow Springs, OH, USA). In addition, carbonate concentration (HCO_3^-) was determined directly in the field using a HACH Digital Titrator, sulfuric acid (0.1600 N and 1.600 N), and Bromocresol Green-Methyl Red Indicator (Hach method 8203) (Hach, Loveland, CO, USA). Moreover, for each spring, a water sample was collected in clean, sterile, 180 mL polypropylene vials with polyethylene caps and was transported in a cooler to the GEOLAB laboratory in Clermont-Ferrand (CNRS UMR 6042, University Clermont-Auvergne), whereupon they were stored at 4 °C.

Diatoms were sampled in relation to the dominant substrate observed for each spring (Table S1). As underlined by Baker et al. [42], the substrate collected in springs was found to influence diatom community composition less than local environmental conditions. The sediments were recovered by scraping off the first few millimeters of mud directly within the vial, and the stones and travertine (a sedimentary rock) by brushing them with a toothbrush. Samples were fixed with 99% ethanol to a final concentration of 70%.

Once back in the GEOLAB laboratory, and following filtration of the samples, the concentrations of the various anions and cations present (as (mg L^{-1}): lithium, sodium, ammonium, potassium, magnesium, calcium, fluoride, chloride, nitrite, nitrate, phosphate, and sulphate) were analyzed within three days using high-pressure ion chromatography. For cations, a Thermo Scientific Dionex ICS1100 system was used, and for anions, a Thermo Scientific Dionex Aquion system (Thermo Fisher Scientific, Courtaboeuf, France) was used.

2.3. Slide Preparation and Microscopy

Diatom samples were prepared for light microscopic (LM) observation following the method of Prygiel and Coste [43] by cleaning a small sub-sample of epipelic and epilithic raw material with hydrogen peroxide (H_2O_2, 35% *v*/*v*; Caldic France, Cournon d'Auvergne, France) and hydrochloric acid (HCl 37% *v*/*v*; Fisher Scientific, Loughborough, UK). The cleaned material was then rinsed several times and subsequently diluted with distilled water to avoid excessive concentrations of diatom valves on the slides. Lastly, a drop of the diluted cleaned material was dried on coverslips and mounted in Naphrax®. LM observations and morphometric measurements were performed using a DM2700M microscope (Leica, Wetzlar, Germany) with a 100× oil immersion objective using differential interference contrast. Various taxonomic keys were used [44–58]. For each spring, ≈400 valves were enumerated on random transects, and the count data were converted to percentage relative abundance of the total count.

2.4. Data Analysis

2.4.1. Statistical Analyses on the Physical and Chemical Variables

The Piper diagram was created using the data derived from the 79 mineral spring samples [59]. It represents the anion and cation concentrations on two specific triangles, whose sides show the relative contents of each of the major ions compared to the total of these ions (cations on the left triangle, anions on the right triangle). The relative position of an analytical result on each of these two triangles makes it possible to specify the dominance of the variance anionic or cationic species present. For both triangles, a diamond is situated, on which is plotted the intersection of the two lines from the points identified on each triangle. This point of intersection represents the global analysis of the sample; its relative position allows one to specify the type of water concerned, such as bicarbonate-rich with sodium and potassium. For this, we used DIAGRAMMES software (version 6.59) [60].

The physical and chemical variables of the dataset were studied using principal component analyses (PCA). Analysis of NO_2^- ions was not performed as several concentrations were below the detection limit; nor was it performed on dissolved oxygen because of missing values due to a technical problem. Kruskal–Wallis tests were conducted on the physical and chemical variables to test whether the groups discriminated by the PCA were significant.

2.4.2. Diatoms Data Statistical Analyses

Because the identification of 300–400 individuals did not provide an exhaustive list of the species richness (S) of a spring [61], we extrapolated the richness values using the Chao1 index [62], which estimates the number of unobserved species based on the number of species observed once or twice in the sampling. Statistical analyses of local diversity were conducted using the species richness, S, and the richness estimated by Chao1, and also the Shannon (H) and Piélou (J) indices. Kruskal–Wallis tests were applied to test for significant differences between taxonomic metrics (S, estimated richness (Chao1) and Shannon–Wiener diversity and Piélou indices) among the groups of springs observed in the PCA.

A Canonical Correspondence Analysis was performed on the species with a relative abundance ≥ 1% of the community. The data used for CCA were log-transformed, after being tested with the Detrended Correspondence Analyses (DCA) [63]. According to DCA analyses, gradient lengths of three or higher values supported the suitability of the data for CCA application [64,65].

The environmental variables with a correlation above 0.5 in the CCA axes were transformed, using the k-means-based discretization method, to obtain classes of concentration used in the Multi-Response Permutation Procedures (MRPP) analysis [66]. MRPP tests were performed to assess differences between lithium, sodium, conductivity, and pH. MRPP is a nonparametric, multivariate procedure that tests whether there is significant difference between two or more groups. The method compares dissimilarities within and between groups. The output of MRPP includes the test statistic T, which indicates separation among groups (a large negative value of T indicates a high relative discrimination of groups), whilst the A statistics estimates the within-group homogeneity. For the A-statistic, if it approaches 1, the groups are completely different; if the A statistic approaches 0, the heterogeneity within groups equals what would be expected by chance; if the A statistic approaches −1, the groups are homogeneous [66]. The *p*-value describes the extent to which the separation between groups is probably due only to chance [66]. For the MRPP, the following options were used: Sorensen Distance Measure, n/sum (n) weighting of groups, and pairwise comparison was made. Lastly, Indicator Species Analysis was used to identify the most characteristic species of each group. The statistical significance of each species indicator value was tested using a Monte-Carlo permutation test (999 permutations, $p < 0.05$).

The analyses were performed using XLSTAT 2016 1.1 (Addinsoft, Paris, France) and PC-ORD 7 softwares (Gleneden Beach, OR, USA) [67,68].

3. Results

3.1. Physical and Chemical Characteristics

Based on the Piper diagram, several springs were bicarbonate-rich with sodium and potassium (blue diamond) (Figure 3). On the opposite side, the orange diamond represents the springs characterized by chlorinated and sulphated calcium waters, such as Font Salado 1 and 2 (numbers 50 and 51 on the map), Pont des Eaux (73), St Coust (46), and Châtel-Guyon 1 and 2 (47, 48). The mineral springs characterized by sodium and potassium chloride or sodium sulphate waters (pink diamond) were, for example, Poix 1 and 3 (52, 53), Croizat (15), and Félix (16). Some springs are intermediate.

Considering all the springs studied, the conductivity was high and varied between 2112 and 12,370 μS cm^{-1}, while TDS values varied between 1665 and 10,493 mg L^{-1} (Table 1). The water of the different springs was acidic. Cation concentrations were also high, such as lithium (between 1.04 and 25.73 mg L^{-1}) and sodium (between 185.77 and 2323.05 mg L^{-1}) (Table 1). Chloride concentration varied between 24.88 and 351 mg L^{-1} (Table 1). For the nitrates, concentrations were low, and the highest value was measured at the spring Poix 3 (24.5 mg L^{-1}). Without this value, the mean was low, at around 0.60 mg L^{-1} (±1.12).

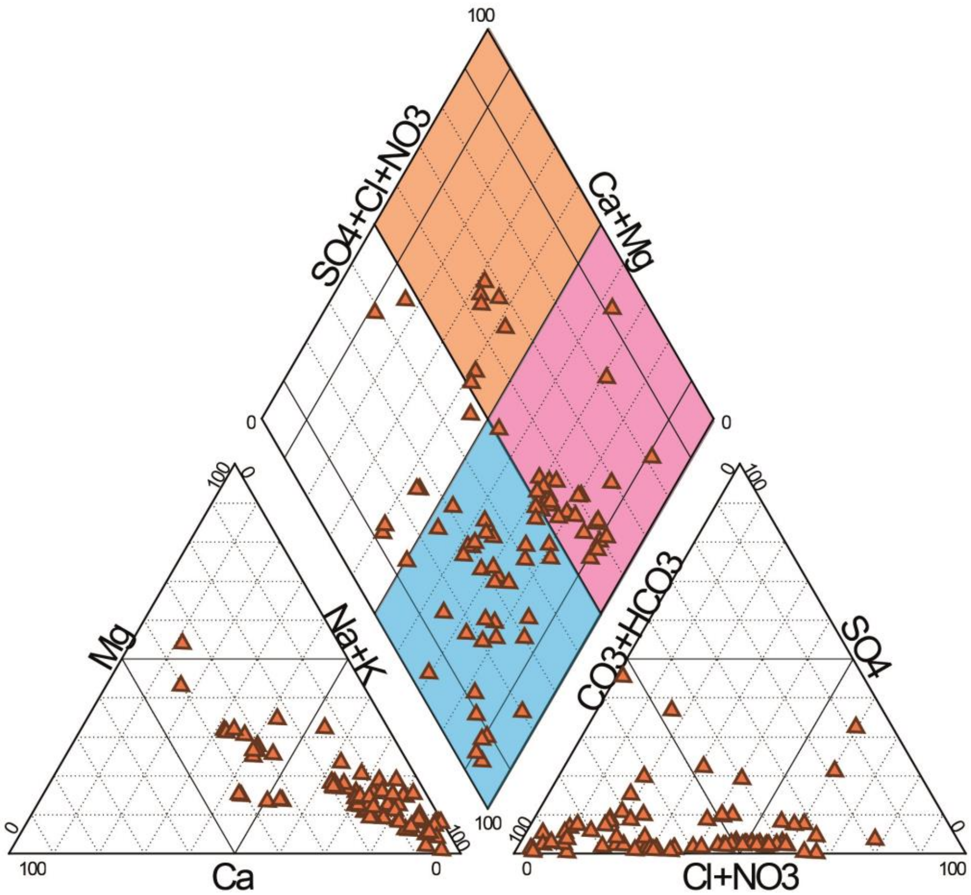

Figure 3. Piper diagram highlighting the main water types of the 79 mineral springs sampled. Several springs were bicarbonate-rich with sodium and potassium (blue diamond), others were characterized by chlorinated and sulphated calcium waters (orange diamond). At last, some mineral springs were characterized by sodium and potassium chloride or sodium sulphate waters (pink diamond).

Table 1. Environmental variables measured at the study sites: parameters measured in situ, TDS and cation and anion concentrations (in mg L^{-1}).

Environmental Variables	Mean (±SD)
Conductivity (µS cm^{-1})	6073.85 ± 2112.00
pH (pH unit)	6.87 ± 0.47
Water temperature (°C)	16.82 ± 8.24
TDS (mg L^{-1})	5025.80 ± 1665.47
Li^+	6.03 ± 4.08
Na^+	1065.66 ± 541.40
NH_4^+	1.12 ± 0.78
K^+	125.52 ± 63.71
Mg^{2+}	124.63 ± 81.93
Ca^{2+}	209.73 ± 147.31
F^-	1.18 ± 1.33
Cl^-	996.88 ± 774.70
Br^-	2.27 ± 1.88
NO_3^-	0.91 ± 2.92
PO_4^{3-}	0.15 ± 0.59
SO_4^{2-}	176.16 ± 243.02
HCO_3^-	2312.70 ± 749.37

Figure 4 shows the results of a Principal Component Analysis using the spring physical and chemical variables. PCA axes 1 and 2 explain a total of 38.2 and 16.1%, respectively, of the variance in the environmental data. In the left part of the first factorial plane, a first group (group 1) appears, with the springs highly mineralized with low nitrate and phosphate concentrations. These springs were also carbonated, with a high concentration of lithium, sodium, chloride, and bromine ions present. This group includes springs such as Font de Vie (number 65 on the map), Croizat (15), and the springs situated in the city of St-Nectaire (numbers 26 to 34) and in the area of the former thermal station of Ste Marguerite (numbers 1 to 13). The conductivity of this group was approximately 8000 µS cm^{-1} on average. Lys, the geyser situated in the city of Vals-les-Bains and Adrienne (14, 60, and 62) was associated with a high concentration of fluoride (5.8 mg L^{-1} on average) and carbonates (4482 mg L^{-1} on average). In the right part of the first factorial plane (group 2), there are less mineralized mineral springs (mean conductivity was approximately 4500 µS cm^{-1}). Lastly, Poix 3 (53) was apart and associated with high concentrations of magnesium, calcium, nitrates, and phosphates.

Kruskal–Wallis tests were performed on the physical and chemical variables to determine whether the two groups discriminated using PCA are significant. (Table 2). The two groups were significant for all abiotic variables, magnesium, calcium, fluoride, nitrates, and sulphates excepted.

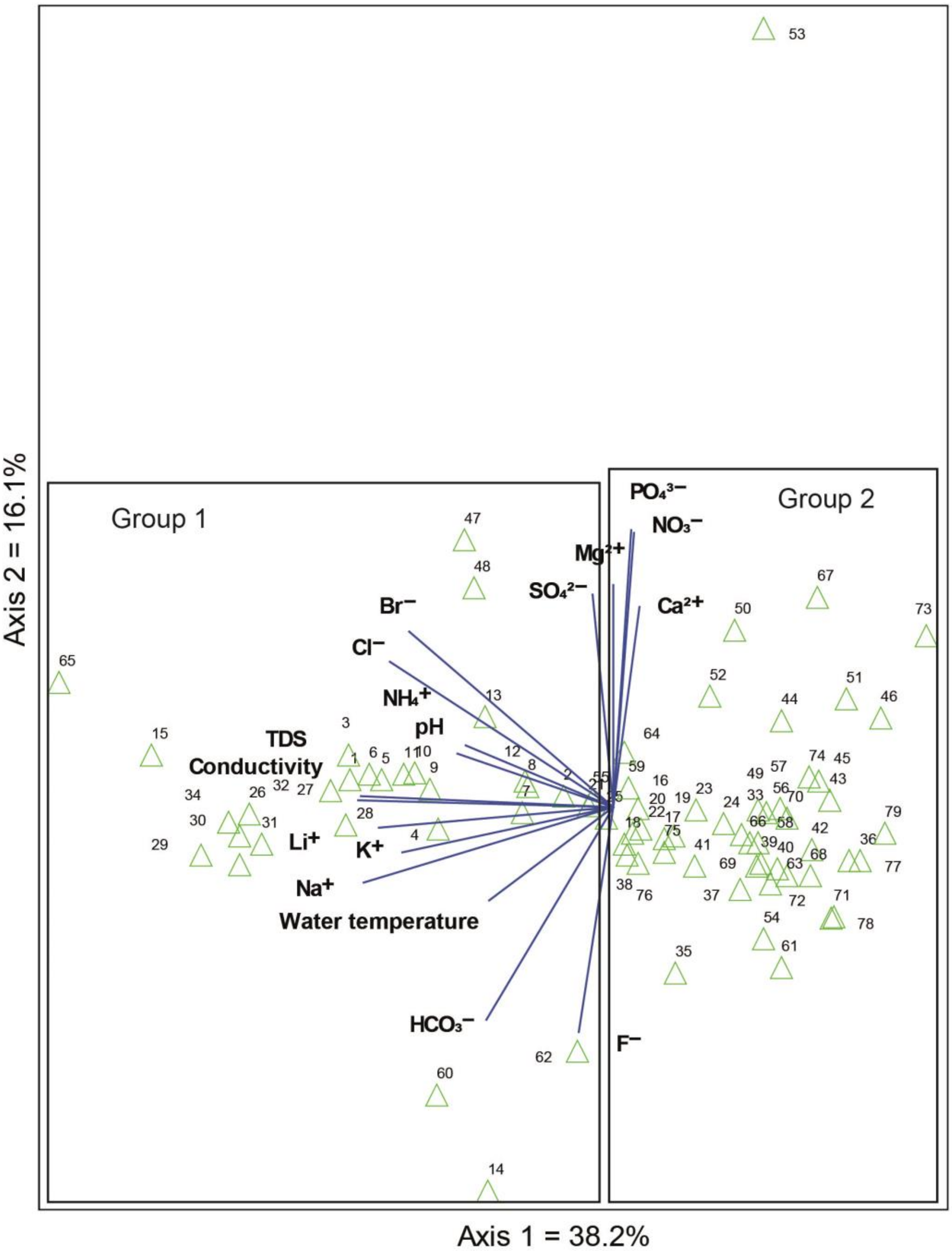

Figure 4. Ordination plot (F1 X F2) of a Principal Correspondence Analysis of the samples based on the physical and chemical variables. Green triangles represent the mineral springs studied and the spring number is presented in Table S1. The two groups of springs are presented.

Table 2. Results of the Kruskal–Wallis tests performed on the physical and chemical variables using the two groups created with the PCA. *p*-values in bold are statistically significant.

Variable	*p*-Value
Li^+	**<0.0001**
Na^+	**<0.0001**
NH_4^+	**0.000**
K^+	**<0.0001**
Mg^{2+}	0.106
Ca^{2+}	0.896

F^-	0.052
Cl^-	**<0.0001**
Br^-	**<0.0001**
NO_3^-	0.848
PO_4^{3-}	**0.009**
SO_4^{2-}	0.229
HCO^{3-}	**<0.0001**
Conductivity	**<0.0001**
pH	**<0.0001**
Water temperature	**<0.0001**
TDS	**<0.0001**

3.2. Diatom Composition and Diversity: Influence of the Environmental Variables

The species richness (S) (mean ± SD = 12.7 ± 10.6) ranged from one species at Tennis spring (number 1) and 67 at Bonnefont spring (number 71), the estimated richness (Chao1) (mean ± SD = 12.7 ± 10.8) from one (at Tennis spring) et 137.8 (at Bonnefont spring) underlining the differences between the studied springs (Table S1). It appeared that for many springs that have a man-made artificial construction around the emergence sites, specific richness and estimated richness (Chao1) was lower than for the natural springs (Table 3). The same result was obtained using the Shannon and Piélou indexes.

Table 3. Mean of the different diversity characteristics (mean ± SD) of the natural versus "artificial" springs.

Variables	Natural Springs	"Artificial" Springs
S	18.94 ± 12.66	8.60 ± 6.21
S.chao1	26.90 ± 24.02	10.73 ± 8.54
Shannon index	1.57 ± 0.76	0.91 ± 0.51
Piélou index	0.09 ± 0.03	0.12 ± 0.06

The Shannon–Wiener diversity index ranged from 0 (spring Tennis) to 3.53 (Tête de Lion spring (number 23)), and the Piélou index ranged from 0 (spring Tennis) to 0.269 (Croizat spring (number 15)). To test whether differences existed between the two groups of springs observed in the PCA, Kruskal–Wallis tests were also performed. The species richness ($p = 0.020$) and the Shannon–Wiener diversity index ($p = 0.043$) were statistically significant. Springs in group 2 had a higher biodiversity than those in group 1 (Figure 5).

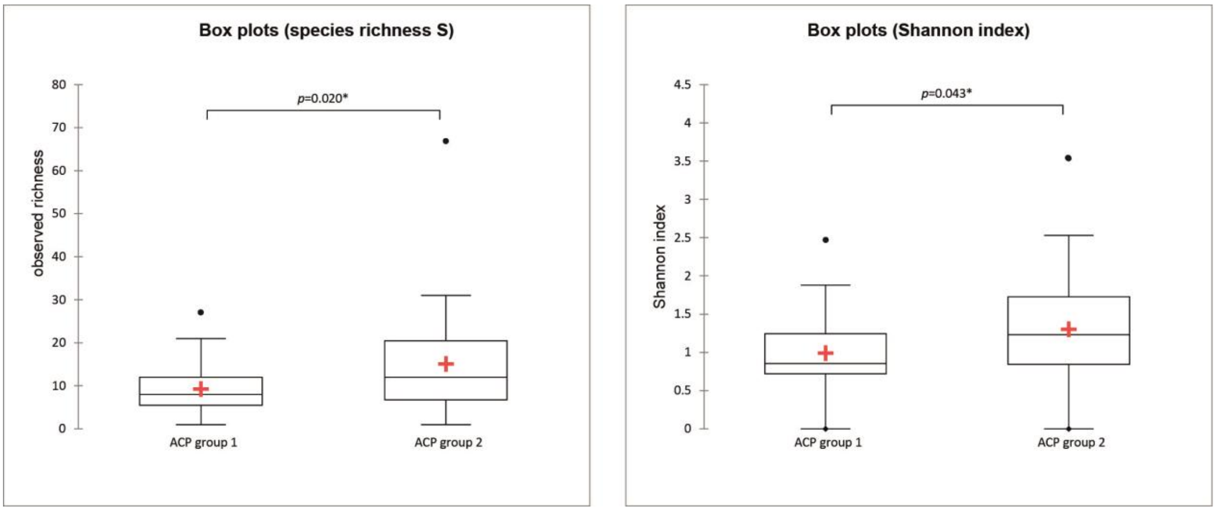

Figure 5. Box plots of the significant parameters (using Kruskal–Wallis tests) performed on the studied springs. The red crosses represent the mean of data and the black bullets, the outlier points. * represent the statistically significant *p*-value.

Overall, 207 different species were observed (Table S2). The species, with a relative abundance ≥ 1% of the community, encountered in the studied spring included: *Crenotia angustior* (Grunow) Wojtal [24] (Figure 6a), *C. thermalis* (Rabenhorst) Wojtal [24] (Figure 6b–d), *Chamaepinnularia salina* Beauger, C. E. Wetzel, Allain & Ector [38] (Figure 7g), *Fragilaria famelica* (Kützing) Lange-Bertalot [69] (Figure 8a,b), *Halamphora coffeaeformis* (Agardh) Levkov [57] (Figure 8c,d), *Navicula sanctamargaritae* Beauger [14] (Figure 8e), *Navicula veneta* Kützing [70] (Figure 8f), *Nitzschia communis* Rabenhorst [71] (Figure 9a,b), *N. valdecostata* Lange-Bertalot et Simonsen [72] (Figure 9c,d), *Pinnularia kuetzingii* Krammer [73] (Figure 7h), *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot [74] (Figure 7a–d), *Sellaphora labernardierei* Beauger, C. E. Wetzel et Ector [15] (Figure 6e,f), and *Surirella patella* Kützing [70] (Figure 9e,f). Among the species, with a relative abundance < 1% of the whole community, included: *Achnanthidium minutissimum* (Kützing) Czarnecki [75] and *Adlafia bryophila* (Petersen) Moser Lange-Bertalot & Metzeltin [76], *Fallacia pygmaea* (Kützing) Stickle et D. G. Mann [77], *Gomphonema lippertii* Reichardt & Lange-Bertalot [78], *Halamphora normanii* (Rabenhorst) Levkov [57], *Mastogloia elliptica* (C. A. Agardh) Cleve [79], *Navicula salinarum* Grunow in Cleve & Grunow [80], and *Pseudostaurosira cubonii* D. M. Williams & C. E. Wetzel [81].

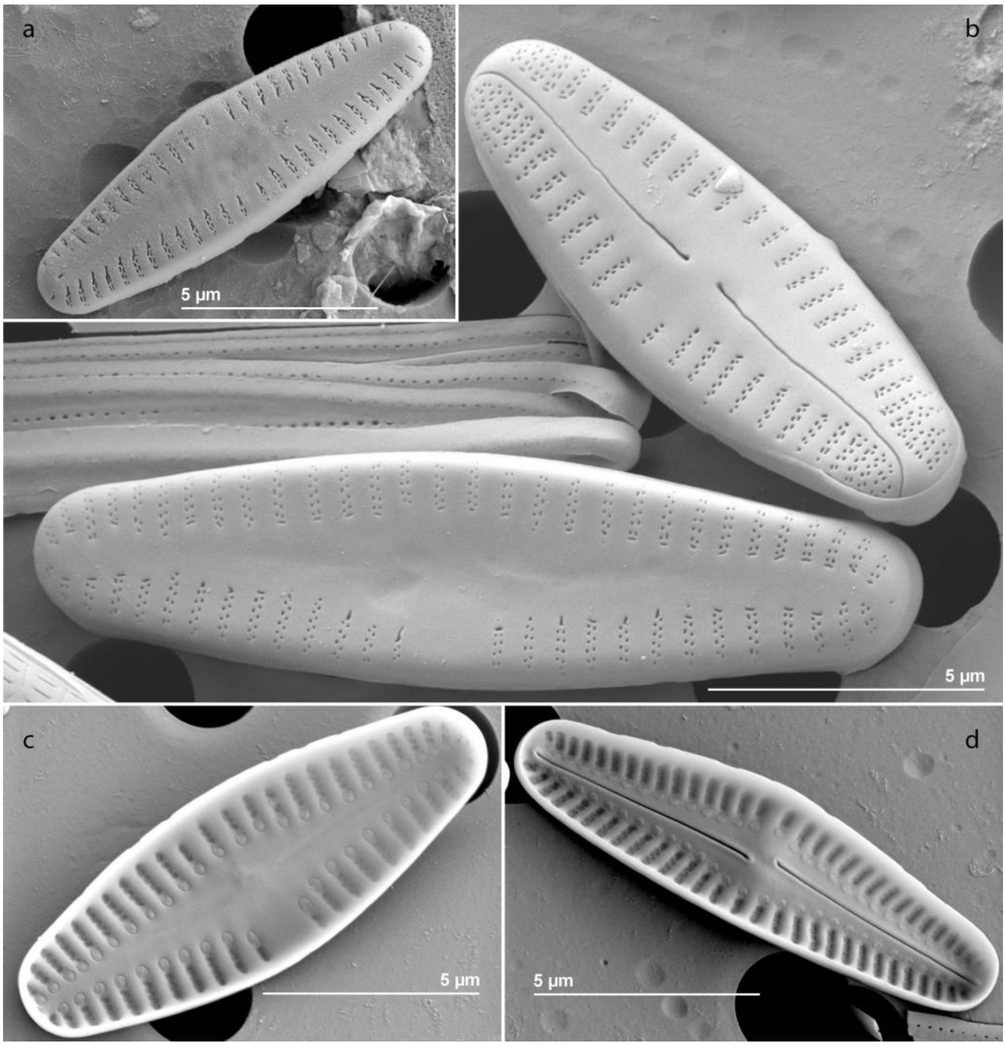

Figure 6. Scanning Electron Microscopy illustrations of some individuals observed in the studied springs. *Crenotia angustior* (**a**). *Crenotia thermalis* (**b**–**d**).

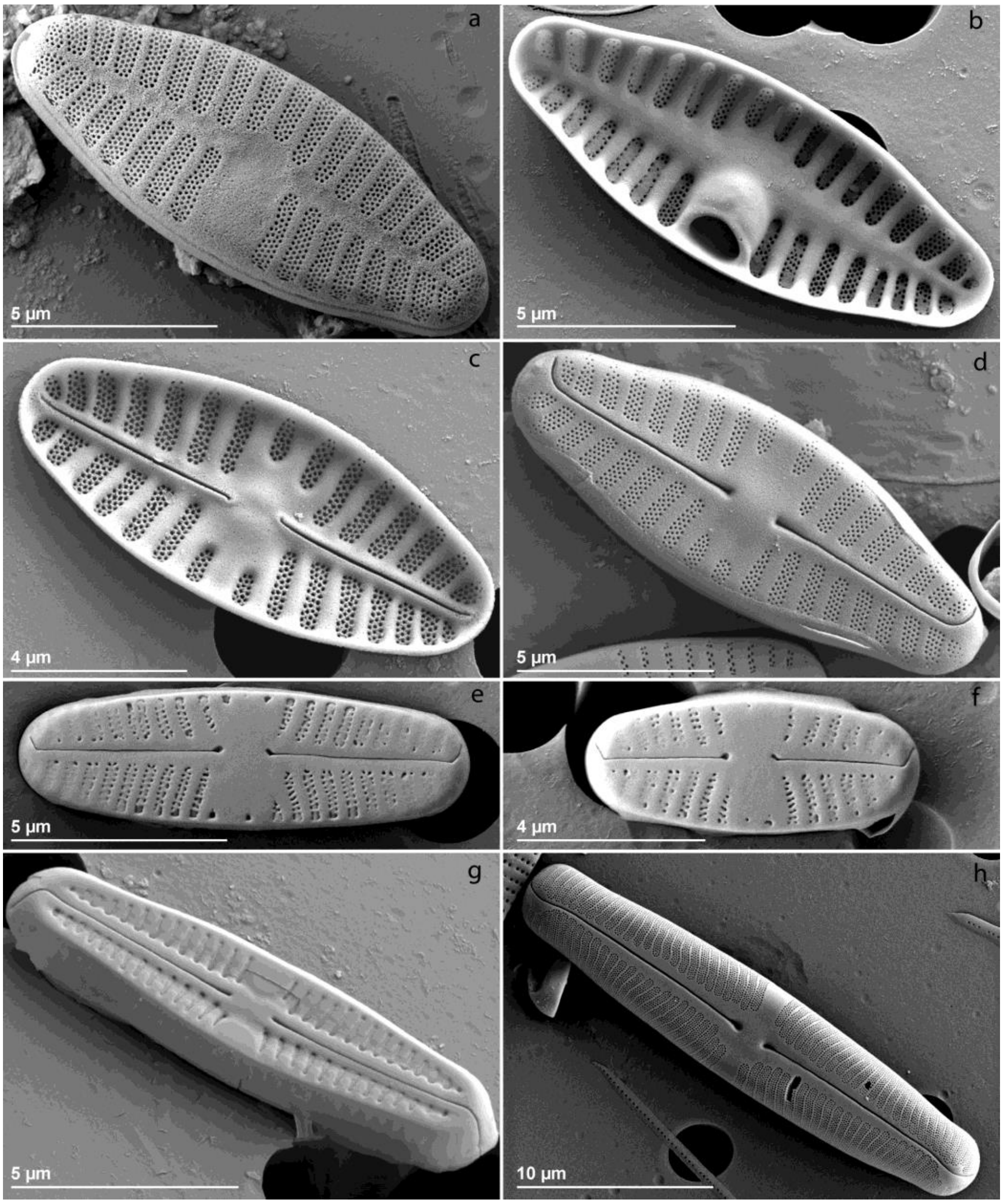

Figure 7. Scanning Electron Microscopy illustrations of some individuals observed in the studied springs. *Planothidium frequentissimum* (**a**–**d**). *Sellaphora labernardierei* (**e**,**f**). *Chamaepinnularia salina* (**g**). *Pinnularia kuetzingii* (**h**).

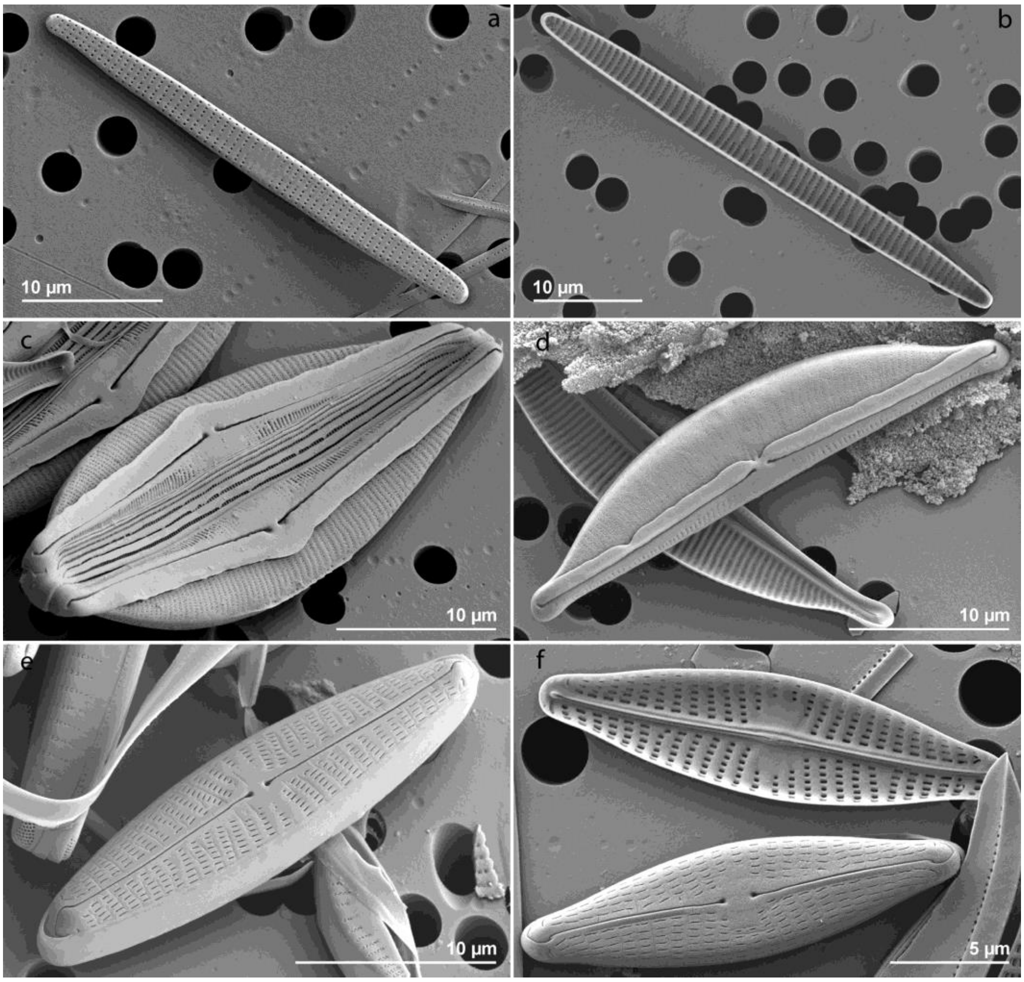

Figure 8. Scanning Electron Microscopy illustrations of some individuals observed in the studied springs. *Fragilaria famelica* (**a**,**b**). *Halamphora coffeaeformis* (**c**,**d**). *Navicula sanctamargaritae* (**e**). *Navicula veneta* (**f**).

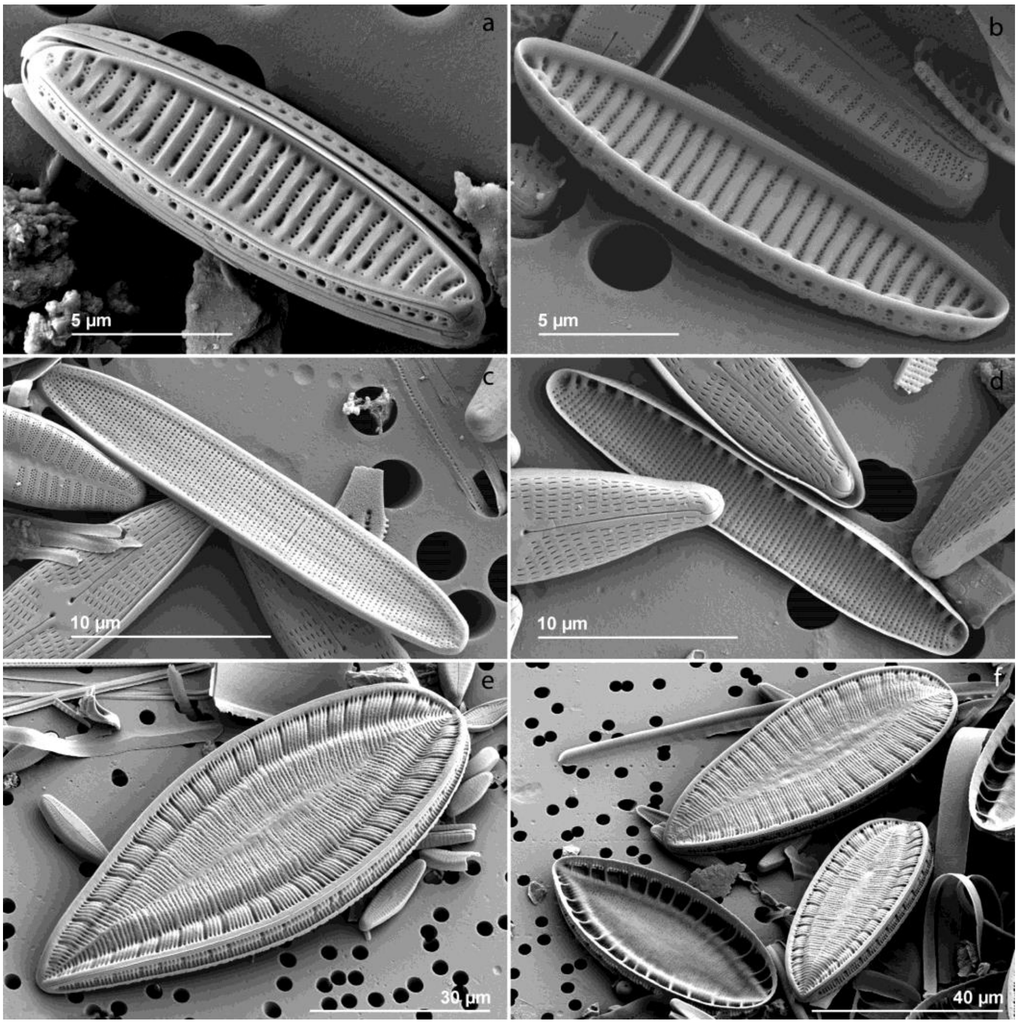

Figure 9. Scanning Electron Microscopy illustrations of some individuals observed in the studied springs. *Nitzschia valdecostata* (**a**,**b**). *Nitzschia communis* (**c**,**d**). *Surirella patella* (**e**,**f**).

The eigenvalues of the first CCA axis (0.257) and the second CCA axis (0.114) were both significant ($p = 0.01$ with Monte Carlo permutations test with 999 permutations), and the total explained variance in the species data was 18.5% (Figure 10). The diatom–environment correlations (Pearson) for CCA axis-1 (0.875) and CCA axis-2 (0.681) were high, indicating a relatively strong relationship between diatom species and measured environmental variables. *Pinnularia kuetzingii*, *Fragilaria famelica*, and *Navicula veneta* were associated with the highest values of pH, conductivity, and TDS, and also with the highest concentrations in lithium, potassium, chloride, and bromine ion concentrations. *Crenotia angustior*, *Navicula sanctamargaritae*, *Sellaphora labernardierei*, and *Surirella patella* were linked with the highest concentrations of magnesium and calcium ions, and *Crenotia thermalis* and *Nitzschia valdecostata* with the highest fluoride ion concentration.

The variables with a correlation above 0.5 on the axes of the CCA were lithium, sodium, potassium, calcium, chloride, bromine, fluoride, TDS, conductivity, and pH. Then, they were transformed in classes according to concentration (using a k-means-based discretization method) used in the Kruskal–Wallis and the MRPP analyses (Table 4).

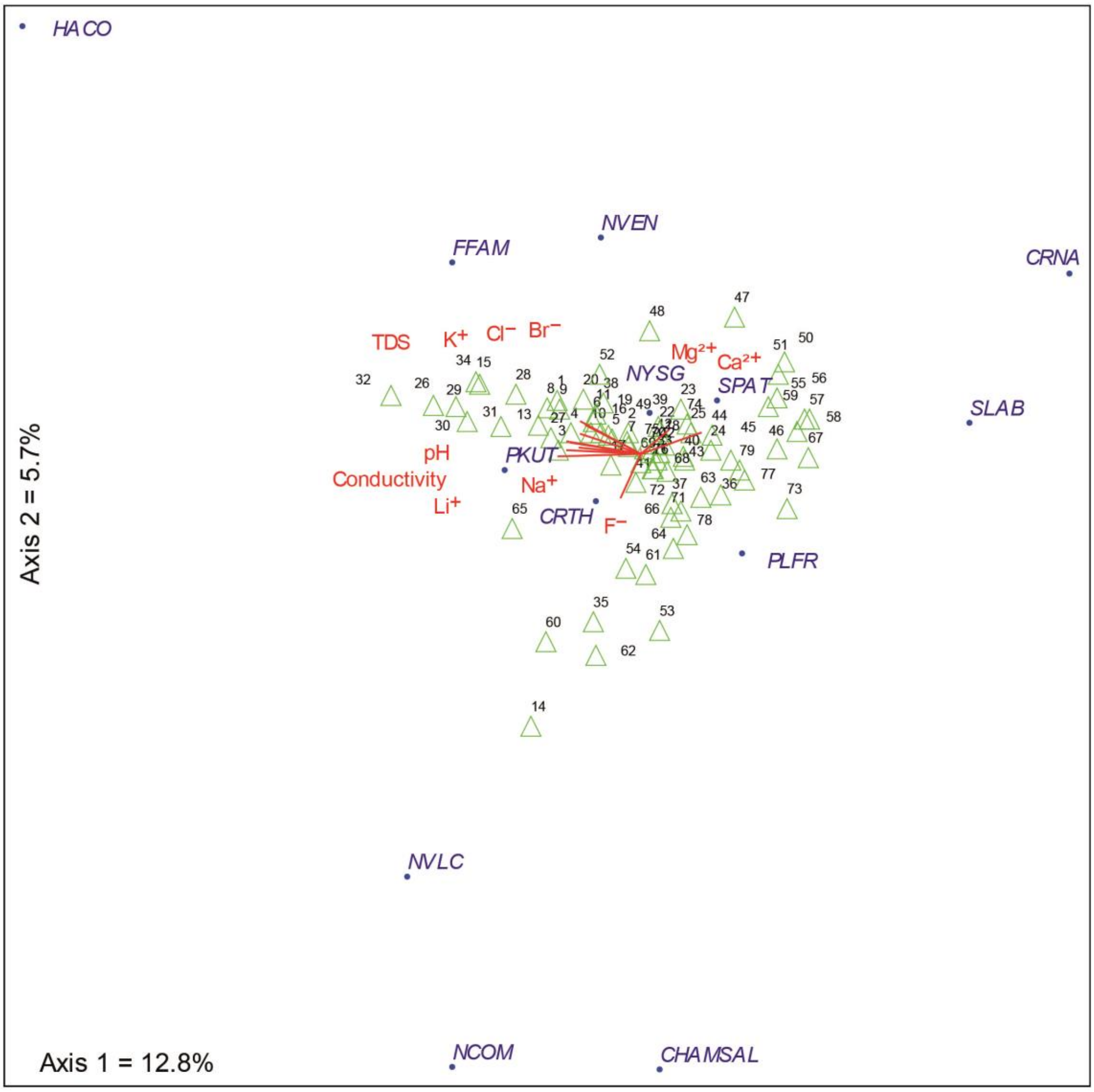

Figure 10. Canonical Correspondence Analysis (CCA) diagram showing the ordination of diatom species (in blue) and sampling site (i.e., springs, green triangles) along the first two axes and their correlation with environmental variables (in red). The spring numbers are presented in Table S1. TDS: Total Dissolved Solids. CRTH: *Crenotia thermalis*, CRNA: *Crenotia angustior*, CHAMSAL: *Chamaepinnularia salina*, FFAM: *Fragilaria famelica*, HACO: *Halamphora coffeaeformis*, NYSG: *Navicula sanctamargaritae*, NVEN: *Navicula veneta*, NCOM: *Nitzschia communis*, NVLC: *Nitzschia valdecostata*, PKUT: *Pinnularia kuetzingii*, PLFR: *Planothidium frequentissimum*, SLAB: *Sellaphora labernardierei* and SPAT: *Surirella patella*.

Table 4. Range of environmental variables for each class.

	Class 1	**Class 2**	**Class 3**
Li^+ (mg L^{-1})	[1.04–5.80)	[5.8–11.30)	≥11.3
Na^+ (mg L^{-1})	[185.77–1178.70)	[1178.7–1833.50)	≥1833.5
K^+ (mg L^{-1})	[10.46–94.07)	[94.07–165.44)	≥164.44
Ca^{2+} (mg L^{-1})	[11.58–207.50)	[207.5–410.38)	≥410.38
Cl^- (mg L^{-1})	[24.88–1118.89)	[1118.89–2066.06)	≥2066.06
Br^- (mg L^{-1})	[0–1.44)	[1.44–3.81)	≥3.81
F^- (mg L^{-1})	[0.092–3.49)	≥3.49	

TDS	[3018–5175)	[5175–7195)	≥7195
Conductivity (μS cm^{-1})	[3340–6350)	[6350–9220)	≥9220
pH	[6.12–6.82)	[6.82–7.56)	≥7.56

Differences in species richness (S) were statistically significant using Kruskal–Wallis tests between the different classes of lithium, sodium, TDS, and conductivity (Table 5). The highest richness was observed in class 1 of these four variables (Figure 11). Differences in estimated species richness were also statistically significant between the different classes of TDS. For the Shannon index, differences were observed only between the classes of lithium and conductivity. Finally, the Piélou index appears to be different for each of the classes considered.

Table 5. Results of the Kruskal–Wallis tests performed on the physical and chemical variables using the classes performed on the lithium, sodium, potassium, calcium, chloride, bromine, fluoride, TDS, conductivity, and pH. *p*-values in bold are statistically significant.

Variables	Species Richness (*p*-Value)	Estimated Richness (*p*-Value)	Shannon Index (*p*-Value)	Piélou Index (*p*-Value)
Li^{+}	**0.048**	0.118	**0.036**	**0.003**
Na^{+}	**0.019**	0.055	0.055	**0.010**
K^{+}	0.181	0.384	0.640	0.063
Ca^{2+}	0.578	0.525	0.335	0.617
Cl^{-}	0.160	0.265	0.309	0.171
Br^{-}	0.103	0.084	0.303	0.626
F^{-}	0.139	0.089	0.094	0.911
TDS	**0.001**	**0.003**	0.054	**0.035**
Conductivity	**0.020**	0.054	**0.045**	**0.005**
pH	0.172	0.394	0.061	0.159

MRPPs were used to test whether the lithium, sodium, potassium, calcium, chloride, bromine, fluoride, TDS, conductivity, and pH classes induced differences in the diatom communities. Diatom composition was significantly different between the three lithium classes ($T = -8.3$, $A = 0.05$, and $p = 0.00000062$); sodium classes ($T = -6.0$, $A = 0.04$, and $p = 0.00000807$), potassium classes ($T = -4.9$, $A = 0.03$, and $p = 0.00034706$), calcium classes ($T = -4.5$, $A = 0.03$, and $p = 0.00061100$), chloride ($T = -4.5$, $A = 0.02$, and $p = 0.00062031$), bromine ($T = -4.7$, $A = 0.03$, and $p = 0.00053149$), TDS classes ($T = -4.8$, $A = 0.03$, and $p = 0.00044247$), the three conductivity classes ($T = -6.3$, $A = 0.04$, and $p = 0.00002596$) and the three different pH classes ($T = -8.9$, $A = 0.05$, and $p = 0.00000015$). No differences were observed between the two classes of fluoride concentration.

Using Indicator Species Analysis, *Crenotia angustior* was associated with the lowest concentrations of lithium and sodium, the lowest conductivity and pH, but the highest concentration of calcium (such as *Sellaphora labernardierei*) (Table 6). *Planothidium frequentissimum* lived in waters with the lowest lithium, sodium, bromine ion concentrations (such as *Sellaphora labernardierei*) and the lowest TDS, conductivity, and pH and also in the medium concentration of chloride. In contrast, *Halamphora coffeaeformis* was associated with the highest concentrations of lithium ions (such as *Fragilaria famelica* and *Nitzschia communis*), sodium (such as *Nitzschia valdecostata*), potassium (such as *Navicula sanctamargaritae*), chloride, bromine, and the highest TDS, conductivity, and pH (such as *Navicula veneta*). *Surirella patella* lived in waters characterized by intermediate concentrations of bromine, and *Crenotia thermalis* in waters characterized by intermediate pH.

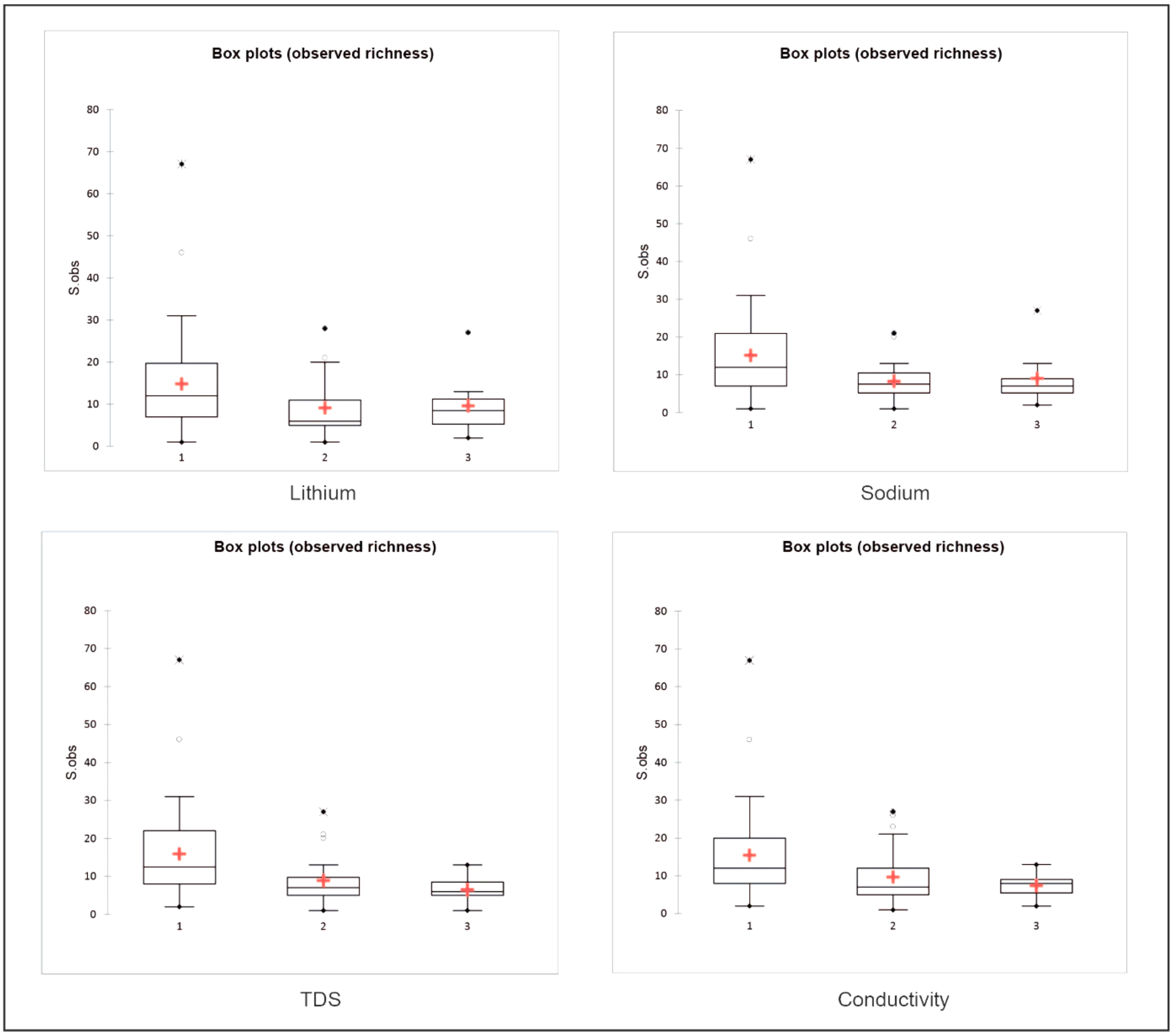

Figure 11. Box plots of the species richness made using the classes of the significant variables (using Kruskal–Wallis tests) of the studied springs. The red crosses represent the mean of data and the black bullets, the outlier points.

Table 6. List of indicator species for concentration classes with an observed indicator value (IV) greater than 20%, along with mean standard deviation and significant *p*-values (* < 0.05). Different diatoms were linked to each group.

Indicator Species	Observed Indicator Value (IV)	Mean	S.dev	*p* *
Lithium (mg L^{-1})				
[1.04–5.8)				
Crenotia angustior	32.2	14.8	5.63	0.0150
Planothidium frequentissimum	44.2	29.3	4.81	0.0067
≥11.3				
Fragilaria famelica	27.3	17.9	5.51	0.0701
Halamphora coffeaeformis	61.6	11.6	5.04	0.0017
Nitzschia communis	28.7	14.0	5.36	0.0234
Sodium (mg L^{-1})				
[185.77–1178.7)				
Crenotia angustior	35.3	14.8	5.55	0.0117
Planothidium frequentissimum	49.4	29.6	5.11	0.0050

≥1833.5				
Halamphora coffeaeformis	47.6	12.4	5.61	0.0017
Nitzschia valdecostata	25.4	13.8	5.49	0.0484
Potassium (mg L^{-1})				
≥164.44				
Halamphora coffeaeformis	36.4	10.6	3.81	0.0010
Navicula sanctamargaritae	41.5	31.7	3.07	0.0080
Calcium (mg L^{-1})				
≥410.38				
Crenotia angustior	45.3	15.3	6.01	0.0030
Sellaphora labernardierei	33.1	15.0	5.63	0.0140
Chloride (mg L^{-1})				
[1118.89–2066.06)				
Planothidium frequentissimum	44.1	29.6	4.79	0.0140
≥2066.06				
Halamphora coffeaeformis	42.4	12.1	5.23	0.0020
Bromine (mg L^{-1})				
[0–1.44)				
Planothidium frequentissimum	44.0	28.0	3.77	0.0010
Sellaphora labernardierei	30.5	13.7	4.13	0.0030
[1.44–3.81)				
Surirella patella	45.8	21.1	4.28	0.0010
≥3.81 mg L^{-1}				
Halamphora coffeaeformis	33.5	10.5	3.85	0.0020
TDS (mg L^{-1})				
[3018–5175)				
Planothidium frequentissimum	55.9	28.9	4.62	0.0010
≥7195				
Halamphora coffeaeformis	33.1	11.6	4.99	0.0050
Conductivity (µS cm^{-1})				
[3340–6350)				
Crenotia angustior	28.8	15.5	6.36	0.0451
Planothidium frequentissimum	55.1	30.0	5.47	0.0017
≥9220 µS cm^{-1}				
Halamphora coffeaeformis	59.5	12.6	6.46	0.0017
pH				
[6.12–6.82)				
Crenotia angustior	41.2	15.4	6.58	0.0117
Planothidium frequentissimum	44.0	30.1	5.70	0.0351
[6.82–7.56)				
Crenotia thermalis	44.8	33.4	4.03	0.0017
≥7.56				
Halamphora coffeaeformis	31.7	12.8	6.37	0.0250
Navicula veneta	56.1	25.5	6.63	0.0033

4. Discussion

4.1. Physical and Chemical Characteristics of the Saline Springs

Geothermal activity stimulates water penetration into the rocks, even though the Auvergne volcanoes have been extinct for thousands of years [82]. These superficial infiltrations and the characteristic volcanic imprint reflect and indeed are the cause of the high-water temperature, high mineralization, and water enriched with sodium and chloride observed.

Considering the 79 studied saline springs with a TDS between 3000 ppm and 10,000 ppm, numerous springs were bicarbonate-rich, with sodium and potassium and sodium and potassium chloride or sodium sulphate waters, confirming the saline character of the emergences. Some springs were found to be intermediate in these respects. Although all springs were characterized by high TDS, two groups of springs were observed with highly mineralized emergences (with a high concentration of carbonate, lithium, sodium, chloride, and bromine) with low nitrate and phosphate concentrations in contrast to the others, which were less mineralized. As diatom species are primary producers, they are more directly affected by physical and chemical factors. Thus, these differences in water characteristics could also induce differences in diatom composition. In addition, the Poix 3 spring was seen to differ from the other springs examined in the study and was associated with high concentrations of magnesium, calcium, nitrates, and phosphates due to the geological particularity of the site [83].

4.2. Diatom Structure and Composition

These abiotic characteristics are at the origin of a halophilic flora rarely encountered in the continental environment. Thus, organisms such as diatoms associated with springs are often unique [13], and these microalgae have been observed in all studied springs, including those characterized by high water temperature and high mineralization, which are factors generally considered to limit diatom growth (e.g., [84]). The species present are considered cosmopolitan elements, underlining easy dispersal mechanisms [85]. In contrast, some taxa—despite being widespread—are confined to specialized environments such as hot springs [86]. According to Owen et al. [33], these “subcosmopolitan taxa occur wherever suitable habitats are present and consequently develop a mosaic-like geographic distribution”. “Such phycogeographic variability has been reported for diatoms as well as many other phycological groups” [33,87,88].

A total of 207 different species were observed. Diatom communities from saline springs studied in the French Massif Central share many species with other communities from thermal springs in Europe, including Poland [24] and Italy [89,90], but also with other parts of the world, such as Chile [27] and Canada [32]. Because diatoms are known for their high dispersal ability [91], it is perhaps not that surprising for the species with a relative abundance ≥ 1% of the community to be found in different specific regions across Europe, or even the world, with the exception of *Sellaphora labernardierei*, which so far seems to be an endemic species of the Massif Central.

Some rare species were observed, such as *Pseudostaurosira cubonii*, described from a spring in Italia (Sicilia) [81]. It is the first occurrence of this species in France. Other species were also present in freshwater, such as *Achnanthidium minutissimum* and *Adlafia bryophila* [55]. New species were also observed, such as *Navicula* sp. and *Pseudostaurosira* sp., at the spring called “Saurier 3 grotte” and for which a taxonomic description is still in progress. Moreover, the richness was the lowest in the springs that present a man-made construction around the emergence sites, such as Croizat, Lefort, and Tennis springs. For the others, the richness varied between 10 and 137 (estimated richness). These results support those of other studies, such as Cantonati et al. [9] and Lai et al. [90], both of whom observed high species-richness values and suggest the influence of the ecotonal nature of springs and of different local abiotic factors. Differences in species richness were observed between the two groups of springs generated using PCA. The highest richness was associated with the lowest concentrations. This observation was confirmed by the differences observed between the classes of lithium, sodium, TDS, and conductivity, as the highest richness was associated with the lowest concentrations for each of them. Lai et al. [90] observed higher species richness in medium-mineral springs (conductivity 590–1193 µS cm^{-1}), whilst in this study it is associated with a higher conductivity range (3340–6349 µS cm^{-1}).

Among the species with a relative abundance ≥ 1% of the community, *Crenotia thermalis* was present in the mineral springs, except in 13 of them, such as Tennis, Giraudon, and Poix 2 and 3. This species is occasionally encountered in running waters and is characteristic of electrolyte-rich inland habitats, particularly thermal and mineral springs [24,55]. This was also observed, for example, in Italy [89], Poland [24], and Chile [27]. In the case of *C. angustior*, the species was observed in 18 studied springs. According to Wojtal [24], *C. angustior* was generally abundant in waters with very high conductivity, chlorides, sulphates, and other ion concentrations (such as Mg^{2+}, Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}), but low in nitrate and dissolved oxygen. Only Tennis, Croizat, Giraudon, Poix 2 and 3, and Camuse did not host one of the two *Crenotia* species.

Chamaepinnularia salina was present in seven of the springs studied, such as Sail (where it was described), Bard 2, Adrienne, and Vallée Couze d'Ardes. This species is mostly found in mineral springs of Na-K-HCO_3 water type [38].

Fragilaria famelica was encountered in 24 studied springs. This species, also encountered in Poland and Portugal, is typical of brackish waters [92–94] and, according to Wojtal [24], is associated with saline springs with a high concentration of chloride and sulphate, but very low dissolved oxygen and nitrate content. This was confirmed by the results of the CCA. Indeed, this species was associated with the highest concentration in chloride but also bromine and potassium.

Halamphora coffeaeformis was observed in 12 studied springs. It was mainly the springs in the area of Ste Marguerite (springs 1 to 13 on the map) and of the city of St-Nectaire (springs 26 to 34 on the map). Known to live in water naturally rich in salt and electrolytes [55], the species is here described for the first time in La Montagne spring (Châteldon) and is therefore a denizen of mineral springs [58]. *H. coffeaeformis* has also been sampled in Canada [33], Chile [27], and Tajikistan [95].

Navicula sanctamargaritae was present in almost all springs (14 springs excepted). This species is associated with high mineralization levels of water [14,89], as confirmed here by CCA. This species had previously only been encountered in Italy [89]. *Navicula veneta*, a more cosmopolitan species common in electrolyte-rich to brackish waters [51] was also well represented (in 39 studied springs). This species was hitherto observed in various countries, including Chile [27], Tajikistan [95], Poland [24], and Italy [89].

Regarding the two *Nitzschia* species that were often dominant, *N. communis* was present in 15 spring. It has been considered a significant element of the diatom assemblages of thermal springs in volcanic environments in São Miguel Island, Azores [94]. In addition, this species is found in calcium-rich waters of moderate–very high specific conductivity, very low dissolved oxygen, and very low nitrate concentration in Polish springs [24]. This species was associated with high fluoride concentration in the CCA. It has previously been encountered in springs from many countries around the world, including Kenya [33], Iceland [32], Poland [24], Greece [96], and Slovakia [97]. *N. valdecostata* is a saline species found in Chile [27] and also in a thermal spring in California (USA) [28].

Pinnularia kuetzingii was present in 27 studied springs. It is known to occur in waters with medium to higher electrolyte content, particularly in thermal springs [48], and has also been observed in Bosnia and Herzegovina and in petrifying *Cratoneurion* springs (petrifying springs with tufa formation) in Belgium [98]. In the CCA, this species was associated with high conductivity and TDS, and also high lithium, sodium, potassium, chloride, and bromine concentrations.

Planothidium frequentissimum was also present in most of the springs studied (with the exception of 27, such as Laplace and Ceyssat). This species has earlier been observed in other springs of Europe, such as Italy, except in those with higher temperature and conductivity [90]. This species is widespread and encountered in many springs from different countries around the world, e.g., Belgium [98], Chile [27], Italy [89], Poland [24], Spain [26], and Iran [99].

Sellaphora labernardierei was present in 18 mineral springs, such as Sail, Zagat, Etoile, Daguillon, and Saute Bedel, and it seems to be associated with the presence of nitrates [15,76]. In the CCA, it was associated with high calcium concentration.

Lastly, *Surirella patella*, observed in 34 springs studied, is also known to be a brackish water species [88] and was therefore associated with high calcium concentration in the CCA. Previously, it has been encountered in mineral springs in Georgia [100].

4.3. Environmental Variables Associated with Diatom Assemblages

Mineralization was an important factor in the separation of the saline mineral springs with high TDS and conductivity and high bromine, calcium, chloride, fluoride, lithium, potassium, and sodium ion concentrations. Our results confirmed those of previous studies carried out in other countries where diatom assemblage composition is clearly influenced by conductivity [27,29,30,33,101] and also by high concentrations of calcium, chloride, fluoride, lithium, potassium, and sodium ions [27,90]. In these studies, magnesium, carbonate, and sulphates are also significant variables. Using the MRPP and ISA analyses, *C. angustior* was associated with the lowest concentrations in lithium and sodium, the lowest conductivity and pH but the highest concentration of calcium, while *F. famelica* was linked with the highest lithium concentration. For *H. coffeaeformis*, TDS, conductivity, lithium, sodium, potassium, chloride, and bromine ions were the variables positively correlated with this species, showing it to be an indicator of highly mineralized water. On the contrary, *Planothidium frequentissimum* was found to live in waters with the lowest concentrations of lithium, sodium, and bromine ions; medium chloride ion concentration; and the lowest TDS and conductivity ([3340–6350) µS cm^{-1}). This species was seen to be an indicator of less mineralized water. Lai et al. [90] showed this species to be abundant in springs with conductivity ranging from 5270 to 6270 µS cm^{-1}.

Navicula sanctamargaritae was only associated with concentration of potassium ≥ 164.44 mg L^{-1}. Thus, it could be an indicator of the springs with a high concentration of this cation. *Nitzschia communis* was linked with the highest concentrations in lithium, whilst *Nitzschia valdecostata* was linked with the highest concentrations in sodium. According to Owen et al. [33], in New Zealand, *N. communis* is linked with high carbonate alkalinity. Regarding *Sellaphora labernardierei*, it was found by us to be linked to the highest calcium concentrations and the lowest bromine concentrations, whilst *Surirella patella* was associated with waters characterized by intermediate bromine concentration.

pH was also a significant variable, confirming the results obtained in other countries such as New Zealand, Thailand, and Italy [30,33,89]. In the studied saline springs, pH was acidic (mean ± SD: 6.87 ± 0.47). Using MRPP and ISA analyses, *C. angustior* and *P. frequentissimum* were associated with acidic waters, *C. thermalis* to waters with a pH that varied between 6.82 and 7.55, and *H. coffeaeformis* and *N. veneta* with basic pH.

Water temperature was not found to be an important driver of diatom community composition, contrary to other, previous studies [26,30,31,101].

5. Conclusions

The study evaluated and synthesized diatom biodiversity from seventy-nine (79) mineral saline springs located at the Massif Central region of France, along with the influence of environmental factors in the ecology of these communities. As springs are among the most threatened ecosystems on Earth [1,102], it is crucial to better understand their abiotic characteristics and the diatom communities that inhabit these extreme environments, both in order to protect as well as, if necessary, restore them. As found, diatom species richness was lowest in the springs presenting an artificial construction around the emergence sites, thereby underlining the need to restore such sites. In the other springs studied, the highest richness was associated with the lowest lithium, sodium, and TDS concentrations and conductivity. Mineralization and specific ions were the most critical driver of diatom community composition. Some species were typical of abiotic conditions, such as *N. sanctamargaritae*, which was associated with the highest potassium ion concentration; hence, this species would appear to be a good bio-indicator of such conditions. It will be interesting in future studies to investigate further the influence of the abiotic characteristics of all mineral springs analyzed, more especially those with TDS values < 3000 ppm.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15020283/s1, Table S1: List of the studied mineral springs and species richness (S), estimated richness (S.chao1), Shannon and Piélou indexes; Table S2: List of the diatom species encountered.

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