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## **Diatom DNA metabarcoding to assess the effect of natural radioactivity in mineral springs on ASV of benthic diatom communities**

Lory-Anne Baker, Aude Beauger, Sofia Kolovi, Olivier Voltaire, Elisabeth Allain, Vincent Breton, Patrick Chardon, Didier Miallier, Céline Bailly, Gilles F Montavon, et al.

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1 **Diatom DNA metabarcoding to assess the effect of natural**  
2 **radioactivity in mineral springs on ASV of benthic diatom**  
3 **communities.**

4  
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32  
33 **Abstract**

34 Little is still known about the low dose effects of radiation on the microbial communities in the  
35 environment. Mineral springs are ecosystems than can be affected by natural radioactivity.  
36 These extreme environments are, therefore, observatories for studying the influence of chronic  
37 radioactivity on the natural biota. In these ecosystems we find diatoms, unicellular microalgae,  
38 playing an essential role in the food chain. The present study aimed to investigate, using DNA  
39 metabarcoding, the effect of natural radioactivity in two environmental compartments (i.e.  
40 spring sediments and water) on the genetic richness, diversity and structure of diatom  
41 communities in 16 mineral springs in the Massif Central, France. Diatom biofilms were  
42 collected during October 2019, and a 312 bp region of the chloroplast gene *rbcL* (coding for  
43 the Ribulose Bisphosphate Carboxylase) used as a barcode for taxonomic assignation. A total  
44 of 565 amplicon sequence variants (ASV) were found. The dominant ASV were associated with  
45 *Navicula sanctamargaritae*, *Gedaniella* sp., *Planothidium frequentissimum*, *Navicula veneta*,  
46 *Diploneis vacillans*, *Amphora copulata*, *Pinnularia brebissonii*, *Halamphora coffeaeformis*,  
47 *Gomphonema saprophilum*, and *Nitzschia vitrea*, but some of the ASVs could not be assigned  
48 at the species level. Pearson correlation failed to show a correlation between ASV' richness and  
49 radioactivity parameters. Non-parametric MANOVA analysis based on ASVs occurrence or  
50 abundances revealed that geographical location was the main factor influencing ASVs  
51 distribution. Interestingly,  $^{238}\text{U}$  was the second factor that explained diatom ASV structure.  
52 Among the ASVs in the mineral springs monitored, ASV associated with one of the genetic  
53 variants of *Planothidium frequentissimum* was well represented in the springs and with higher  
54 levels of  $^{238}\text{U}$ , suggesting its high tolerance to this particular radionuclide. This diatom species  
55 may therefore represent a bio-indicator of high natural levels of uranium.

56

57 **Keywords:** diatom diversity, mineral springs, naturally occurring radioactivity, *rbcL*,  
58 metabarcoding, genetic variants

## 60 1. Introduction

61 Since the formation of the Earth some 4.5 billion years ago, background radiation levels  
62 have varied spatially and temporally in the habitats of the planet (Karam, 2005). For at least 3.5  
63 billion years, life has been evolving and adapting in ecosystems with different levels of natural  
64 radioactivity (Martell, 1992; Garzón, 2001). Currently, there are regions on the Earth where  
65 natural radioactivity can reach very high levels, such as India (Mohanty, 2004), Iran  
66 (Khodashenas, 2012) and Brazil (Oliveira, 1994). In Europe, median concentration of uranium  
67 in soils is estimated to be around 2 mg.kg<sup>-1</sup>, with a range of variation from less than 0.1 to more  
68 than 50 mg.kg<sup>-1</sup> (De Vos et al., 2006). In France, the highest concentrations are found in the  
69 Massif Central (a mountain range in the middle of France), where it can reach hundreds of  
70 mg.kg<sup>-1</sup> in granitic soil (Garnier-Laplace, 2010).

71 In the Massif Central, several mineral springs show significant levels of natural radioactivity  
72 (Jacquet, 1929; Boineau, 1972; Risler, 1974; Ielsch, 2017). This natural radioactivity comes  
73 from <sup>40</sup>K and the decay chains of three primordial radioelements <sup>235</sup>U, <sup>238</sup>U and <sup>232</sup>Th that are  
74 naturally present in the Earth's crust, especially in granitic rocks (Métévier, 2001). Because of  
75 its longer half-life (4,47 billion years), <sup>238</sup>U and its nuclear decay products (<sup>226</sup>Ra, <sup>222</sup>Rn, <sup>210</sup>Po)  
76 are today the most dominant radionuclides in mineral springs (Eróss et al., 2018; Rihs et al.,  
77 2000; Morais et al., 2022). They are  $\alpha$ -particle emitters, the most mutagen ionising radiation  
78 involved in radioactive stress mainly related to internal irradiation in cells. Thus, mineral  
79 springs in the French Massif Central are not only extreme environments due to their significant  
80 mineralisation levels, but also their high level of natural radioactivity.

81 Living organisms that inhabit these extreme environments have been evolving and  
82 adapting to these conditions. Such mineral springs are therefore interesting 'natural

83 laboratories' in which to study the influence of mineralisation and natural radioactivity on  
84 biodiversity. These ecosystems are characterised by short food webs which could be  
85 interesting to study the effects of natural radioactivity on primary producers. Diatoms are  
86 part of the phytobenthos, and one of the main groups of primary producers in aquatic  
87 ecosystems, including mineral springs. Algae are known to be good indicator for  
88 radionuclides in aquatic environments and show differences in radionuclides tolerances  
89 between species due to their ecological variations (Pradhan, 2019; Banerjee, 2022).

90 The influence of radioactivity on diatoms can be studied at three ecological organizational  
91 levels, *viz.* individual, population and community levels (Stevenson, 2014; Pandey, 2017).  
92 Gross and Koczy, (1946) (cited in Driver, 1994) reported a severe reduction of diatom survival  
93 in a radioactive and experimental medium. In addition, some surveys have already been  
94 conducted to study the impact of radioactivity on the communities of diatoms in tailing waters  
95 (Ruggles, 1979 cited in Driver, 1994), brackish waters (Snoeijs and Notter, 1993), and in rivers  
96 (Herlory, 2013) and mineral springs (Millan, 2019). High diatom species richness (>50) was  
97 found in springs with significant levels of radioactivity by Millan et al. (2019). This study also  
98 showed that high levels of  $^{222}\text{Rn}$  activities in mineral springs are correlated with higher rates of  
99 benthic diatom frustule deformations. All of these studies suggest that diatoms can resist high  
100 radioactivity levels but did not provide any information on the effect of radioactivity on the  
101 structure of diatom communities themselves.

102 In the present study, and in order to bring new insights at both population and community  
103 levels, DNA metabarcoding was employed to assess the effect of enhanced natural radioactivity  
104 of water and sediments on benthic diatom communities in several mineral springs of the Massif  
105 Central. To this end, a 312 bp region of the chloroplast gene *rbcL* (encoding for the Ribulose  
106 Bisphosphate Carboxylase Large) was used as a barcode for diatom species identification  
107 (Vasselon, 2017), a convenient marker for such discriminations (Mann, 2010; Kermarrec, 2014;

108 Bailet, 2020). Following on from earlier published results of Millan et al. (2019), where species  
109 richness was high even in radioactive mineral springs, our main working hypothesis was that  
110 radioactivity is not a major factor that influences diatom genetic richness. Furthermore, because  
111 microbial species have different levels of resistance to ionising radiation (Shuryak, 2019), our  
112 second hypothesis was that radioactivity can influence diatom community structure because  
113 ionising radiation can shape the microbial community. In this light, a series of studies were  
114 performed in order to test these ideas empirically.

## 115 **2. Material and methods**

### 116 ***2.1. Study sites and sampling***

117 The sampling campaign took place in autumn from the 15<sup>th</sup> to the 30<sup>th</sup> October 2019, where  
118 diatom samples were collected from a total of 16 mineral springs distributed in the Auvergne  
119 region of the French Massif Central (Fig. 1). The French Massif Central (over 85,000 km<sup>2</sup>) is  
120 influenced by oceanic, continental, and Mediterranean climates. It is composed of mountain  
121 reliefs, plateaus, and volcanoes (quiescent), where the Hercynian platform appears at the  
122 surface, surrounded by different sedimentary basins. Classically, the many faults in the massif  
123 allow the circulation of water in the rocks and the emergence of this having travelled some  
124 distance across the earth's crust. The study sites (mineral springs) present a diversity of  
125 physical, chemical, and radiological characteristics including a three orders of magnitude  
126 gradient of natural radioactivity.

### 127 ***2.2. Sampling of diatom communities***

128 Benthic diatom samples were collected in the 16 mineral springs (Fig. 1). Since it was earlier  
129 demonstrated that the substrate has little influence on benthic diatom composition in mineral  
130 springs (Baker, 2022), the biofilm was recovered and considered to be the dominant substrate,  
131 representative of each of the springs sampled. In some springs, biofilm was collected by

132 scrubbing the upper surface of rocks over which water flowed with a clean toothbrush. In others,  
133 biofilm was sampled by scraping the first millimetre or so of sediments from the spring basin.  
134 For each sample, the suspension in the was fixed with using  $\geq 70\%$  ethanol as required by  
135 European protocols for diatom metabarcoding procedures (CEN, 2018).

136

### 137 **2.3. *Physical and chemical analyses***

138 At the 16 spring sampling sites, water temperature ( $^{\circ}\text{C}$ ), conductivity ( $\mu\text{S}\cdot\text{cm}^{-1}$ ) and pH (pH  
139 unit – Sorenson scale) were measured with a multi-parameter WTW probe FC 340i (VWR  
140 International, Radnor, PA, USA) by submerging the probe in the water. The percentage of  
141 dissolved oxygen (DO) was obtained with an Ysi ProODO oxygen probe (Yellow Springs  
142 Instruments, OH, USA). For chemical analyses, 200 mL of water was initially filtered with  
143 Whatman GF/C filters. For cation and anion analysis, a Thermo Scientific Dionex ICS1100 and  
144 a Thermo Scientific Dionex Aquion systems were used, respectively, to measure concentrations  
145 of the following ions (expressed as  $\text{mg}\cdot\text{L}^{-1}$ ): lithium ( $\text{Li}^+$ ), sodium ( $\text{Na}^+$ ), ammonium ( $\text{NH}_4^+$ ),  
146 potassium ( $\text{K}^+$ ), magnesium ( $\text{Mg}^{2+}$ ), calcium ( $\text{Ca}^{2+}$ ), fluoride ( $\text{F}^-$ ), chloride ( $\text{Cl}^-$ ), nitrite ( $\text{NO}_2^-$ ),  
147 nitrate ( $\text{NO}_3^-$ ) and phosphate ( $\text{PO}_4^{3-}$ ).

### 148 **2.4. *Radiological and trace elements characterization***

149 The activity of dissolved  $^{222}\text{Rn}$  in water samples collected using 1 L Marinelli beakers was  
150 measured on a well-shaped Germanium detector (GCW3523, Canberra, 35% relative  
151 efficiency) operated at Clermont Physics Laboratory (University Clermont Auvergne, Aubière,  
152 France) upon returning from the field. For other radiological analyses, water, and sediment  
153 samples were collected in clean vials from the water-column either near the emergence or at its  
154 outlet. Samples were transported to the laboratory under cold conditions in refrigerated boxes  
155 protected from light and heat.

156

157 Analyses were conducted at Subatech (Nantes, France) and ICN (Nice, France) laboratories.  
158 After PTFE filtration (0.45 $\mu$ m) and acidification of water samples (HNO<sub>3</sub> or HCl for Po  
159 analysis), uranium was analysed by ICP-QMS (Xseries 2, Thermo Electron). Ultra-trace  
160 concentrations of <sup>226</sup>Ra were measured without chemical purification by HR-ICP-MS in low-  
161 resolution mode (Element XR, Thermo Scientific) with an APEX-Q high sensitivity desolvating  
162 sample solution introduction system. In order to remove major polyatomic interferences from  
163 the spectrum of <sup>226</sup>Ra in low-resolution mode thereby potentially leading to inaccurate  
164 assessment of concentrations of this the commonest isotope of this highly radioactive element,  
165 preliminary water sample solution analyses were also performed in medium resolution mode  
166 where major interferences can be resolved. The observed consistency of <sup>226</sup>Ra activity measured  
167 in both low and middle resolution modes showed no significant evidence of the production of  
168 major interferences (Verlinde, 2019). Preference for use of the low-resolution mode was  
169 characterised by a high sensitivity (4.7 $\times$ 10<sup>5</sup> CPS/Bq. mL<sup>-1</sup>) and a low instrumental detection  
170 limit (0.8 mBq.L<sup>-1</sup>) for <sup>226</sup>Ra. Pb and Bi-based polyatomic interferences unresolved in low and  
171 medium resolution modes were not considered in the present study due to low signal intensities  
172 measured for Pb (< 60,000 CPS) and Bi (< 1 000 CPS). Lastly, <sup>210</sup>Po was measured after  
173 spontaneous deposition onto a silver disc, by alpha spectrometry using dual alpha spectrometers  
174 EG&G Ortec 576A equipped with boron-implanted silicon detectors (Le, 2019).

175 Sediment samples were sieved to 2mm and dried at 105°C. <sup>226</sup>Ra and <sup>228</sup>Ac were analysed  
176 using a  $\gamma$ -Spectrometer with coaxial hyperpure Ge detector (GX4519, Mirion, >45% relative  
177 efficiency at 1.33 MeV between 10 keV to 10 MeV) according to NF EN ISO 18589-3.  
178 Measurements were conducted at least four weeks after the sealing of the sample container to  
179 ensure the secular equilibrium between <sup>226</sup>Ra, <sup>222</sup>Rn, <sup>214</sup>Pb and <sup>214</sup>Bi. The data were then  
180 processed via LabSOCS™ Calibration Software. <sup>238</sup>U, <sup>232</sup>Th and trace elements were measured



181 by ICP-QMS (Xseries 2, Thermo Electron) after microwave digestion (Ethos Easy Milestone)  
182 with concentrated HNO<sub>3</sub>, centrifugation at 2650g during 10 min, then PTFE filtration (0.45µm).

### 183 ***2.5. DNA extraction and PCR amplification for amplicons library preparation***

184 For each mineral spring, two sub-samples (duplicates) of 2 mL of biofilm were taken for  
185 DNA extraction. After homogenization by manually shaking of the raw material, samples were  
186 centrifuged at 13 000 rpm for 30 min. at 4°C to remove the supernatant (ethanol). Total genomic  
187 DNA was extracted from the pellet with the commercial DNA extraction kit Nucleospin for  
188 Soil (Macherey-Nagel, Düren, Germany). DNA quality of the samples was assessed by  
189 spectrophotometry using the NanoDrop ND-1000 (Nanodrop Technologies, Wilmington,  
190 Delaware). From each extraction duplicate, PCR amplifications targeting a 312 bp fragment of  
191 the marker gene *rbcL* were done on two groups of triplicates. Amplification was performed  
192 using the primers as described in Vasselon et al. (2017). Each PCR product group was pooled  
193 to provide a PCR duplicate per extraction replicate. In summary, four replicates per mineral  
194 spring were obtained. Library pools were sequenced on four Illumina MiSeq runs (2x250 bp  
195 paired-end V3) by GenoToul GeT-PlaGe platform (INRAE Genomics, Toulouse, France).

### 196 ***2.6. Analysis of diatom communities***

197 The sequencing platform performed the demultiplexing and provided two fastq files per  
198 mineral spring, one corresponding to forward reads (R1) and reverse reads (R2), respectively.  
199 The R package DADA2 was used for the amplicon sequence variant (ASV) from the  
200 demultiplexed Miseq following the method described by Callahan et al. (2016). An average of  
201 132,420 sequences per sample were obtained before using DADA2 to filter the sequences.  
202 Primers were removed from the R1 and R2 reads using CutAdap (Martin, 2011). Based on their  
203 quality profile, R1 and R2 reads were truncated to 200 and 170 bp, respectively. Reads with  
204 ambiguities and errors (maxEE) higher than 2 were eliminated. Then, chimeric sequences were

205 removed from the samples, resulting in an average of 120,364 sequences per sample. Amplicon  
206 sequence variants (ASV) detected were assigned to taxa using version 10 of the curated  
207 reference database for diatoms Diat.barcode (Rimet, 2021) available at  
208 <https://doi.org/10.15454/D7FO2T>. ASV's not classified as Bacillariophyta were removed from  
209 the analysis. A total of 565 ASVs was detected and assigned to the phylum of Bacillariophyta.  
210 Several species and genera were not assigned taxonomically to any known sequence, as is  
211 frequently the case in metabarcoding analyses (e.g. Rivera et al. 2020). Thus, an internal  
212 database of GEOLAB (that will be added to Diat.barcode) allowed the identification of some  
213 of the most abundant species belonging to the genus *Navicula* in springs, such as *N.*  
214 *sanctamargaritae* Beauger, using light microscopy.

215 The diversity data of all samples were exploited using the 'Phyloseq' package (McMurdie,  
216 2013) in R version 4.1.2. The files obtained from the DADA2 data processing: ASVs table,  
217 taxonomy table and a table with the corresponding spring for each replicate were implemented  
218 in R to create a phyloseq object and facilitate data manipulation. Sample  $\alpha$ -diversity indices  
219 (ASV richness and Shannon–Wiener's (Hill, 1973) diversity index) were calculated for each  
220 replicate of the biological samples using the function "estimate\_richness" (Fig. 2).

221 Replicates in metabarcoding allow better extrapolation of diatom diversity in mineral  
222 springs. To reduce the number of files to be analysed, replicates of the same spring were merged  
223 using the function "merge\_sample." This function allows all ASVs present in the different  
224 replicates to be combined as effectively representative of one sample per spring. Relative  
225 abundances of species were calculated in each sample, and only species with an abundance >>  
226 1% and representative of all the studied springs, were retained. The plot of the species  
227 composition for all samples was built with the ggplot2 package (Fig. 3). The ecology of the  
228 diatom species was determined using several diatom atlases, research papers and books (Cleve-  
229 Euler, 1953; Carter and Bailey-Watts, 1981; Hartley et al, 1986; Krammer et al, 1997a, 1997b,

230 2000a, 2000b, 2004; Krammer, 2000, 2002; Potatova and Hamilton, 2007; Lange-Bertalot  
231 1979, 2001; Lange-Bertalot et al, 2011, 2017; Bey and Ector, 2013a, 2013b; Beauger et al,  
232 2015; Levkov et al, 2016; Li et al, 2018).

## 233 ***2.7. Statistical analysis***

234 Prior to performing statistical analyses, radiological measures were modified in order to obtain  
235 the contribution of the spring. The limits of detection (LD) are very low and below the  
236 environmental background noise. For the statistical study, samples below the LD were set to  
237 "0". LD values were subtracted from all results.

238 Different statistical analyses were performed to test the effect of physical, chemical and  
239 radiological variables on the biotic data. As abiotic data (table S1, S2) did not follow a normal  
240 distribution, they were standardised using z-score normalisation  $((x-\text{mean})/\text{standard deviation})$ .  
241 This transformation also allows comparison of variables that initially had different units. Forty-  
242 nine (49) abiotic variables were available from measures in water and sediments of the mineral  
243 springs monitored (Table S1, S2). To increase statistical robustness, collinearity tests were  
244 performed in parallel with principal component analyses (PCA), and thus the final dataset was  
245 reduced to 16 variables. These analyses were performed on the concentrations of major ions,  
246 trace elements and radionuclides. Variables with a cosine of less than 0.4 on both axes of the  
247 PCA were not retained. When two variables had a correlation coefficient  $\gg 0.9$ , the variable  
248 with the higher cosine was retained as presented in Table 1. Thus, in the springs, some  
249 radiologic parameters were more important and showed more variation in the spring water or  
250 the sediment. Dissolved oxygen, conductivity, pH, and water temperature were added to the  
251 dataset due to their ecological importance for diatoms (Patrick, 1977). A final PCA was  
252 performed on the main environmental parameters selected in each spring.

253 The correlation between ASV richness and physico-chemical and radiological variables  
254 was tested by calculating Pearson correlation coefficient with XLSTAT 2020 version 5.1, to  
255 determine if these elements measured in our study influenced the number of diatom species in  
256 mineral springs (Table 2).

257 In order to extract further information from our analyses, the different diatom  
258 communities of mineral springs were compared. A matrix of dissimilarities based on Jaccard  
259 dissimilarity index (Jaccard 1901) was generated with the function `vegdist` of the R package  
260 “Vegan” (Oksanen et al., 2013, version 2.5–7). This index only takes into account the  
261 absence/presence of species, so a second matrix with the abundance-based Jaccard dissimilarity  
262 index “`ab.Jaccard`” (or `chao.jaccard`) described in Chao et al. (2006) was also generated. The  
263 function “`dist.ldc`” of the package “Adespatial” version 0.3–14 (Dray et al. 2012) was used to  
264 generate a dissimilarity matrix with this second index. The individual influence of five  
265 environmental and radiological factors such as “Geographical location”, “ $^{238}\text{U}$ ”, “ $^{222}\text{Ra}$ ”,  
266 “ $^{210}\text{Po}$ ”, “ $^{228}\text{Ac}$ ” activities level on diatom community diversity in springs were determined  
267 (Table S3) with a non-parametric MANOVA analysis [i.e. ADONIS (Permutational  
268 MANOVA)] based on Jaccard and “`ab.Jaccard`” dissimilarity indices using the function `Adonis`  
269 of the `vegan` R package. The “geographical location” represents not only springs that belong to  
270 the same geographical area but also to similar hydrogeological contexts linked to the fault  
271 system and the geological layers associated. The radioactivity classes for four radionuclide  
272 variables (i.e.  $^{222}\text{Rn}$ ,  $^{238}\text{U}$ ,  $^{226}\text{Ra}$ ,  $^{210}\text{Po}$  level,  $^{228}\text{Ac}$ ) were determined from the orders of  
273 magnitude observed for these variables in the sediments of the sampled springs (Table 1). For  
274  $^{238}\text{U}$  and  $^{226}\text{Ra}$  activities, three classes were determined by: Low: activity  $< 0.01 \text{ Bq.L}^{-1}$ ;  
275 Medium:  $0.01 \text{ Bq.L}^{-1} \leq \text{activity} \leq 0.1 \text{ Bq.L}^{-1}$ ; High: activity  $> 0.1 \text{ Bq.L}^{-1}$  and Low: activity  $<$   
276  $0.1 \text{ Bq.g}^{-1}$ ; Medium:  $0.1 \text{ Bq.g}^{-1} \leq \text{activity} \leq 1 \text{ Bq.g}^{-1}$ ; High: activity  $> 1 \text{ Bq.g}^{-1}$  respectively.  
277 Similarly,  $^{210}\text{Po}$  activity levels were Low: activity  $< 0.00001 \text{ Bq.L}^{-1}$ ; Medium:  $0.00001 \text{ Bq.L}^{-1}$

278  $\leq \text{activity} \leq 0.0001 \text{ Bq.L}^{-1}$ ; High: activity  $> 0.0001 \text{ Bq.L}^{-1}$ . For  $^{228}\text{Ac}$  activity levels, the classes  
279 were: Low: activity  $< 1 \text{ Bq.g}^{-1}$ ; Medium:  $1 \text{ Bq.g}^{-1} \leq \text{activity} \leq 10 \text{ Bq.g}^{-1}$ ; High: activity  $> 10$   
280  $\text{Bq.g}^{-1}$ .

281

282 Further correlations between the 14 most abundant species and physico-chemical and  
283 radiological variables were computed with XLSTAT 2020 version 5.1. The difference of ASV  
284 structure between radioactivity classes (Table S3) was estimated using the Multi-Response  
285 Permutation Procedure (MRPP) (Zimmerman, 1985, Mielke, 1991). MRPP is non-parametric,  
286 multivariate approach that tests the null hypothesis that two or more predefined groups are not  
287 different in species composition (here, predefined groups are geographical location and  $^{238}\text{U}$ ,  
288  $^{226}\text{Ra}$ ,  $^{210}\text{Po}$ ,  $^{228}\text{Ac}$  activities). The computed MRPP statistic delta ( $\delta$ ) represents the overall  
289 weighted mean of within-group means of pairwise dissimilarities among plots. In addition, the  
290 chance-corrected within-group agreement A statistic was computed (A = 0 means that in-group  
291 and out-group heterogeneities are equal; A = 1 means that all members of each group are  
292 identical and different from the members of other groups). This analysis was complemented  
293 with an Indicator Species Analysis (ISA) (Dufrêne and Legendre's, 1997; McGeoch and  
294 Chown, 1998) again using the vegan R package.

### 295 **3. Results**

#### 296 *3.1. Environmental variables in mineral springs*

297 The physical, chemical, and radiological characteristics of the studied mineral spring sites are  
298 reported in Table 1. Conductivity of the mineral springs varied between 3090 and 9680  $\mu\text{S.cm}^{-1}$   
299 (mean of  $5682.56 \pm 1693.30 \mu\text{S.cm}^{-1}$ ). pH did not display significant variations among the  
300 mineral springs (mean  $\pm$  SD:  $6.9 \pm 0.3$ ). The mean water temperature of the studied mineral  
301 springs was  $16.6 \pm 3.6$  °C. Mineral springs were not well oxygenated with a mean value of 22.3

302  $\pm 3.6\%$ . Concentrations of  $\text{Na}^+$  and  $\text{HCO}_3^-$  are high in mineral springs with an average of  $939 \pm$   
303  $361 \text{ mg.L}^{-1}$  and  $2318 \pm 516 \text{ mg.L}^{-1}$  respectively. In the water,  $^{238}\text{U}$  activity varied between  
304 detection limit ( $2.5 \text{ mBq.L}^{-1}$ ) and  $0.193 \text{ Bq.L}^{-1}$  and  $^{210}\text{Po}$  varied between 9 and  $392 \mu\text{Bq.L}^{-1}$ . In  
305 the sediments,  $^{226}\text{Ra}$  activity variation extended on almost two orders of magnitude, from 0.56  
306 to  $52.1 \text{ Bq.g}^{-1}$ . Lastly, , considering all the springs,  $^{228}\text{Ac}$  varied between detection limit ( $45$   
307  $\text{mBq.L}^{-1}$ ) and  $28.8 \text{ Bq.g}^{-1}$ .

308         Analysis of the data sets using PCA (Fig. 2) shows the distribution of springs based on  
309 their physical, chemical and radiological parameters. Axis 1 and 2 explained, respectively,  
310  $41.53\%$  and  $24.34\%$  of the data variability. Overall, PCA allows identification of four groups.  
311 Thus, in the top quadrant, the springs for which the  $^{226}\text{Ra}$  activity is significant, include  
312 Daguillon, Graviers, Ours, Tennis and Tête de lion. Also, Chemin, Graviers, Ours, Salut,  
313 Saulcée, Tennis and Tête de lion are represented by higher  $^{228}\text{Ac}$  activity. Bard, Saladis,  
314 Giraudon and Tennis springs show higher  $\text{Ca}^{2+}$ ,  $\text{HCO}_3^-$  and  $\text{K}^+$  concentrations. In the lower left  
315 quadrant, Estreys, Sail, Salins, Saulcée and Trois sauts show high  $^{210}\text{Po}$ ,  $^{238}\text{U}$  activities and  
316  $\text{Mn}^{2+}$  and  $\text{PO}_4^{3-}$  concentrations. In the lower right quadrant, Giraudon, Rocs bleus, Saladis are  
317 associated with higher temperature, conductivity, pH and DO and also with high  $\text{Br}^-$  and  $\text{Na}^+$   
318 concentrations.

### 319 *3.2. Influence of physical, chemical and radiological factors on ASV richness*

320  
321 A total of 565 ASV were detected. Figure 3 shows the number of ASVs observed and Shannon's  
322 diversity index obtained for each mineral spring using the four technical replicates. The number  
323 of ASVs ranged from 6 and 74 with a mean  $29 \pm 17$ . The mineral spring with the most of ASVs  
324 was "Salut" (i.e.  $74 \pm 7$ ) and the lowest "Salins" (i.e.  $8 \pm 1$ ). In terms of Shannon's diversity  
325 index, the richest spring was "Chemin" (i.e.  $2.38 \pm 0.04$ ), the poorest "Tennis" (i.e.  $0.18 \pm 0.09$ ).

326 There was no significant correlation between ASV richness and physico-chemical or  
327 radiological ( $^{238}\text{U}$ ,  $^{226}\text{Ra}$ ,  $^{210}\text{Po}$ ,  $^{228}\text{Ac}$  activities) parameters.

### 328 *3.3. Influence of environmental and radiological factors on diatom communities' structure*

329 The contribution of several environmental factors, including radiological parameters, to the  
330 variability of diatom communities in mineral springs was determined using an Adonis test  
331 (Table 3). These contributions were calculated by taking into account the ASVs shared between  
332 samples (Jaccard) and the abundance of these ASVs (ab.Jaccard). The geographical location of  
333 the mineral springs explains, respectively, 47.0 % (Jaccard) and 53.5% (ab.Jaccard) of the ASV  
334 variability. For the radiological parameters,  $^{238}\text{U}$  activities in the spring water explains 17.2 %  
335 (Jaccard) and 38.6 % \* (ab.Jaccard) of the variability while its descendant  $^{226}\text{Ra}$  activity in the  
336 spring sediments explains 10.7 % (Jaccard) and 7.7 % (ab.Jaccard).  $^{210}\text{Po}$  activities in the  
337 springs' water contributes to 5.6 % (Jaccard) and 0 % (ab.Jaccard) of the variability while  $^{228}\text{Ac}$   
338 activities in the springs sediments explains 13.3 % (Jaccard) and 3.5 % (ab.Jaccard) of this  
339 variability.

### 340 *3.4. Taxonomic composition of diatom communities in mineral springs*

341 Sixty percent of the ASVs were assigned to a diatom genus, forty-two percent to a species.  
342 Fifty-two genera were determined of which only 22 were associated to ASVs with read  
343 abundance > 10 % in all samples. The dominant genera were *Achnantheidium*, *Amphora*,  
344 *Anomoeoneis*, *Caloneis*, *Craticula*, *Cyclotella*, *Diatoma*, *Diploneis*, *Epithemia*, *Fallacia*,  
345 *Fistulifera*, *Fragilaria*, *Gedaniella*, *Gomphonema*, *Halamphora*, *Mayamaea*, *Navicula*,  
346 *Nitzschia*, *Pinnularia*, *Planothidium*, *Sellaphora*, *Surirella*. The most dominant ASV (relative  
347 abundance above 1% in at least on sample) were assigned to 32 diatom species (Figure 4): 69%  
348 of the diatom species are freshwater species, 17% found in both freshwater and marine  
349 ecosystems, 7% in brackish water, and 7% in marine habitats. Among the 20 dominant ASVs,

350 some could not be assigned to a species and belong to the genera *Gomphonema*, *Navicula*,  
351 *Pinnularia* and *Surirella*. The other dominant assigned species are *Navicula sanctamargaritae*,  
352 *Gedaniella* sp., *Planothidium frequentissimum*, *Navicula veneta*, *Diploneis vacillans*, *Amphora*  
353 *copulata*, *Pinnularia brebissonii*, *Halamphora coffeaeformis*, *Gomphonema saprophilum*, and  
354 *Nitzschia vitrea*. Different ASVs were assigned to the same diatom species suggesting the  
355 presence of genetic variants.

356 Pearson correlations between the abundance of the 14 dominant ASVs and the physical,  
357 chemical and radiological parameters were determined (Table S4). All significant correlations  
358 were positive. Na<sup>+</sup> was correlated with *Pinnularia* sp2 (R<sup>2</sup>=0.60), which also correlated with  
359 several parameters as PO<sub>4</sub><sup>3-</sup> (R<sup>2</sup>=0.60), conductivity (R<sup>2</sup>=0.52), pH (R<sup>2</sup>=0.51), DO (R<sup>2</sup>=0.54),  
360 temperature (R<sup>2</sup>=0.71). *Navicula veneta* correlated with PO<sub>4</sub><sup>3-</sup> (R=0.68) and temperature  
361 (R<sup>2</sup>=0.76). *Pinnularia brebissonii* with pH (R<sup>2</sup>=0.65) and DO (R<sup>2</sup>=0.83). The genetic variant A  
362 of *Planothidium frequentissimum* correlated with <sup>238</sup>U activity in water (R<sup>2</sup>=0.91) and <sup>210</sup>Po  
363 activity in water (R<sup>2</sup>=0.91), but no correlation was found for the genetic variant B. Additional  
364 analyses (MRPP; Table 4) showed that there is a significant difference in ASV composition  
365 between samples with different location (with Jaccard matrix: A=0.146, p=0.002) and different  
366 <sup>238</sup>U activity levels (in water) (with ab.Jaccard matrix: A=0.230, p=0.004). No significant  
367 differences were found between ASV composition for the different levels of the other  
368 radionuclides. The Indicator Species Analysis revealed that the ASV of the genetic variant A  
369 of *Planothidium frequentissimum* was associated with high level of <sup>238</sup>U (in water) (R<sup>2</sup>=0.998,  
370 p=0.01).

#### 371 **4. Discussion**

372 This study provides the first analysis of diatom communities living in mineral springs using  
373 DNA metabarcoding to assess the influence of enhanced natural radioactivity on these  
374 assemblages. These extreme aquatic environments in effect comprise a field laboratory in which



375 to assess the contribution of natural radioactivity through radionuclides' activity on diatom  
376 community structure. From this, two hypotheses were posited: (1) that radioactivity is not a  
377 factor that particularly influences diatom genetic richness; and (2), that radioactivity is a factor  
378 that can influence diatom community structure.

#### 379 *4.1. Natural radioactivity did not impact ASV richness*

380 A first parameter to describe the  $\alpha$ -diversity of communities with metabarcoding is ASV  
381 richness. Generally, diatom ASV richness was high in the springs monitored and varied greatly  
382 between mineral springs with a factor of about 11. No significant correlations were observed  
383 between ASV richness and the various radiological parameters, viz.  $^{238}\text{U}$ ,  $^{226}\text{Ra}$ ,  $^{210}\text{Po}$ ,  $^{228}\text{Ac}$   
384 activity. These results validate the first hypothesis: radioactivity does not influence diatom  
385 community genetic ASV richness. In the literature and to our knowledge, no other study besides  
386 the present one has yet been conducted on the effect of radioactivity on ASV richness of  
387 microbial communities. Even so, a negative impact of radioactivity on the species richness of  
388 macroorganisms in terrestrial ecosystems are a reduction of species richness, for instance forest  
389 birds in an area with high radiation doses around Chernobyl (Moller, 2007; Mousseau 2016).  
390 For microorganisms, Shuryak et al. (2018) suggest that diatom richness in some radioactive  
391 waterbodies in Russia can be greatly reduced by high radiation levels: in this particular study,  
392 a negative correlation of -0.81 was measured between diatoms species richness and the level of  
393 radioactive contamination. The result obtained in our study is significantly higher than that  
394 observed in the monitored mineral springs because radioactive levels are elevated by a factor  
395 of eight in the radioactive reservoirs in Russia. In another study, comparison of bacterial  
396 communities in geographically-separated soils affected by caesium releases showed that  
397 species richness was lower in the most contaminated soils (Ihara, 2021). However, in aquatic  
398 environments, different results are reported: for example, Herlory et al. (2013), revealed that  
399 diatom species richness was not affected by the treated uranium mining effluents; Videvall et

400 al. (2022) that radioactivity had no effect on prokaryote richness in Chernobyl wetlands, whilst  
401 Murphy et al. (2011) also showed that radioactivity had no effect on macroinvertebrate  
402 communities from lakes exposed to Chernobyl-derived ionising radiation. The latter results  
403 were obtained in the context of anthropogenic contamination by the addition of radionuclides  
404 to the environment, which implies stress on the surrounding organisms due to the introduction  
405 of these elements. In the case of radioactive mineral springs, organisms grow in the presence  
406 of radioactivity for long periods. Thus, we assume that organisms are adapted to radioactivity  
407 in mineral springs where the radioactive level is higher.

#### 408 *4.2. Uranium activity explained part of diatom community structure*

409 Geographical location was found to be the main factor explaining diatom community structure  
410 in mineral springs considering ASV occurrence. This result is similar to those of Potapova and  
411 Charles (2002), Virtanen and Soininen (2012), Soininen et al. (2016) and Baker et al. (2021)  
412 that studied benthic diatom assemblages in rivers, streams and springs.  $^{238}\text{U}$  activity was the  
413 second parameter that explained the variability of the diatom communities, whereas the  
414 variability of diatom community structure was more poorly explained by  $^{226}\text{Ra}$ ,  $^{210}\text{Po}$  and  $^{228}\text{Ac}$   
415 activities.

416 In the literature, study of the effects of uranium on communities of various macro- and  
417 microorganisms is quite common in the context of anthropogenic contamination or laboratory  
418 experiments. Several studies have demonstrated that uranium in water courses and static bodies  
419 of water influences the taxonomic composition of microbial communities (Reardon, 2004;  
420 Islam, 2011; Brzoska, 2016; Sutcliff, 2017; Suriya, 2017; Zeng, 2018; Chuiyun, 2021; Videvall,  
421 2022). For example, Suriya et al. (2017) showed that increased concentration of uranium in  
422 sediments of the Cauvery River in India favour the presence of several Proteobacteria and  
423 Gammaproteobacteria species. Exposure of the bacterial community found in anaerobic  
424 granular sludge to uranium leads to the modification of the composition of the community

425 (Zeng, 2018). However, there do not seem to be any studies on the effect of naturally occurring  
426 uranium in the environment. This study is therefore a pioneer study on diatom community  
427 structure in natural radioactive areas. Moreover, uranium contamination (from mining, nuclear  
428 accidents, or the release of nuclear waste into the environment) is a relatively recent event, as  
429 it is linked to the growing demand for uranium by the domestic and military industries since  
430 the Second World War. In the context of mineral springs, microorganisms have been naturally  
431 exposed to uranium in a continuous and prolonged manner, and uranium activity seems to have  
432 a significant influence on the structure of the communities, especially considering the genetic  
433 variants present.

434         This last view validates the second hypothesis: radioactivity is a factor that can influence  
435 diatom community structure. Furthermore, bioaccumulation of  $^{226}\text{Ra}$  by several organisms such  
436 as plants, algae, bacteria, plankton and zooplankton is also documented to occur (Iyengar, 1990;  
437 Zakeri, 2012; Carvalho, 2014; Heidari, 2017).  $^{226}\text{Ra}$  accumulates in a similar way to calcium in  
438 human and animal body tissues, by accumulating on the surface of bones, whilst also leading  
439 to the cancers of various kinds (Gad, 2005; Gad, 2014). Diatoms can also absorb and use  
440 calcium in their metabolic processes calcium and therefore it is also likely that  $^{226}\text{Ra}$  is  
441 accumulated by diatoms and thereby involved in their metabolism too.  $^{210}\text{Po}$  bioaccumulation  
442 is well documented in marine environments (Bennett, 1979; Carvalho, 2018; Uddin, 2018),  
443 marine phytoplankton (Horowitz, 2020), and invertebrates from the Baltic sea (Skwarze and  
444 Falkowski, 1988). All these radionuclides can be accumulated in cells of living organisms.  
445 Brown et al. (2004) observed that  $^{210}\text{Po}$  and  $^{226}\text{Ra}$  are the main contributors of absorbed dose  
446 rates when they measured the internal concentration of several radionuclides within marine and  
447 freshwater organisms (e.g., phytoplankton, macrophytes). However, in our study,  $^{226}\text{Ra}$  and  
448  $^{210}\text{Po}$  are the two radionuclides that contribute the least to explanation of the community  
449 structure of diatoms frequenting natural bodies of water.

450 4.3. Taxonomic composition of diatom communities

451 The main challenges and limitations of the metabarcoding method as here described are the  
452 reference barcoding libraries themselves. Indeed, these reference libraries are often incomplete,  
453 and difficult to produce and maintain (e.g. Weigand et al., 2019). Morphological discrimination  
454 under a powerful microscope is still found to be necessary for species identification and can in  
455 turn be complemented by metabarcoding. The lack of genus and/or species identification could  
456 also be explained by the presence of new cryptic taxa due to the insularity of mineral springs.  
457 Indeed, springs are biotopes that can be marked by insularity via physical or physico-chemical  
458 barriers. Such barriers are characterised by high levels of physical stress related for example to  
459 temperature water availability and radioactivity (Cantonati, 2012; Cartwright, 2019). Thus, they  
460 present specific biotopes distinct from surrounding ecosystems and as such, may contain novel  
461 species due to potential allopatric speciation events.

462 A large proportion of the taxa herein observed by us could not be identified down to  
463 species level, but the general ecology of those that could be so identified demonstrated that  
464 most of those colonizing springs are present in various types of environments (freshwater,  
465 marine species and brackish water). Furthermore, their distributions are related to some  
466 chemical similarities between mineral springs and marine or brackish environment such as high  
467 conductivity, high ions contents among others. Also, some diatoms species have large  
468 ecological range and inhabited environments with different ecological statue like freshwater,  
469 springs and brackish water. Only *Navicula sanctamargaritae* is typical to mineral springs  
470 (Beauger et al., 2020). Thus, the observations made in the mineral springs show that some  
471 species are well distributed in the mineral springs, but their abundance varies according to the  
472 local environmental conditions.

473 The correlations between the species associated to the 14 most abundant ASVs and  
474 physical, chemical and radiological parameters of the springs monitored clearly revealed that

475 one genetic variant of *Planothidium frequentissimum* is particularly abundant in mineral springs  
476 with high natural concentrations of the isotope  $^{238}\text{U}$ . This result was confirmed with MRPP and  
477 ISA, which revealed interestingly that this species is associated with high  $^{238}\text{U}$  activity. Hence,  
478 this finding means that there are clearly genetic variants of the same diatom species that do not  
479 have the same distribution and ecological tolerances. This finding agrees with those of Pérez-  
480 Burillo et al. (2021), working on the distribution and ecological preferences of four diatom  
481 species, who concluded that ecological tolerance of some diatom species resulted from  
482 overlapping preferences among genetic variants. These genetic variants individually showed  
483 much more restricted preferences and distributions. In a previous study, a higher frequency of  
484 deformations in the most radioactive springs had been observed for *P. frequentissimum* (Millan,  
485 2019).

486 Mineral springs with high levels of radioactivity (i.e.  $^{238}\text{U}$  activity) are habitats in which  
487 environmental pressure is more important than springs with low levels of radioactivity. If so, it  
488 questions the ability of this species to tolerate intracellular accumulation of radionuclides.  
489 Indeed, bioaccumulation of radionuclides depends on several environmental factors including  
490 external concentrations of these radionuclides in the water. In this context, Snoeijs and Notter  
491 (1993), demonstrated that external concentrations of radionuclides and benthic diatom  
492 concentrations are related: the higher the radionuclides concentrations, the higher the  
493 intracellular concentrations in the diatoms. This thus strongly suggests that in environments  
494 with high concentrations of radionuclides, diatom species are able to tolerate high radionuclide  
495 concentrations. If this in turn is so, the genetic variant A of *P. frequentissimum* is a potentially  
496 a good bio-indicator species for high natural uranium levels in spring water. Lastly, diatoms are  
497 the main producers in aquatic environments and the possible high accumulation of  
498 radionuclides within the cells implies – importantly – that diatoms are themselves potential  
499 vectors of radionuclides in the high levels of the food webs.

## 500 **Conclusions**

501 Mineral springs are extreme environments that may have naturally high levels of radioactivity.  
502 Such environments are good field ‘laboratories’ to observe and understand the adaptation of  
503 organisms in the face of higher levels of natural and chronic background radioactivity. Our  
504 present study, the first on diatom community structure in natural radioactive areas, reveals that  
505 DNA metabarcoding proves that diatom species are very abundant in the mineral springs of the  
506 Massif Central. Radioactivity in the springs has no apparent effect on the diatom ASV richness  
507 in these mineral springs. Geographical location is the first factor explaining the structure of  
508 diatom communities in the mineral springs, and  $^{238}\text{U}$  the second factor. This radionuclide is  
509 known to influence microbial community composition. Very interestingly, one of the genetic  
510 variants of *Planothidium frequentissimum* was detected as a bio-indicator of high level of  
511 uranium in natural environment (i.e., mineral springs). Lastly, our results show that some  
512 diatom species are well distributed in the mineral springs, but their abundance varies according  
513 to the environmental conditions found locally. Thus, some species are particularly abundant in  
514 mineral springs with a high enhanced level of natural radioactivity. This exemplifies the fact  
515 that diatoms are organisms that have a great capacity for tolerance to radionuclides.

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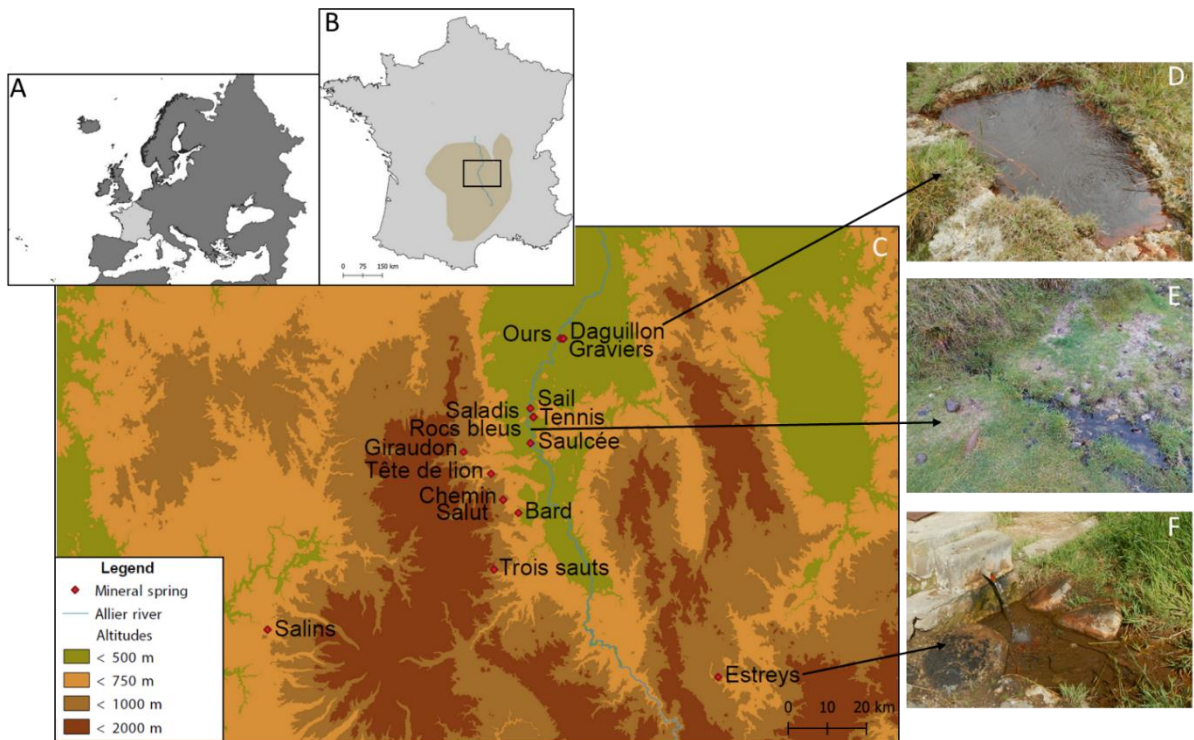
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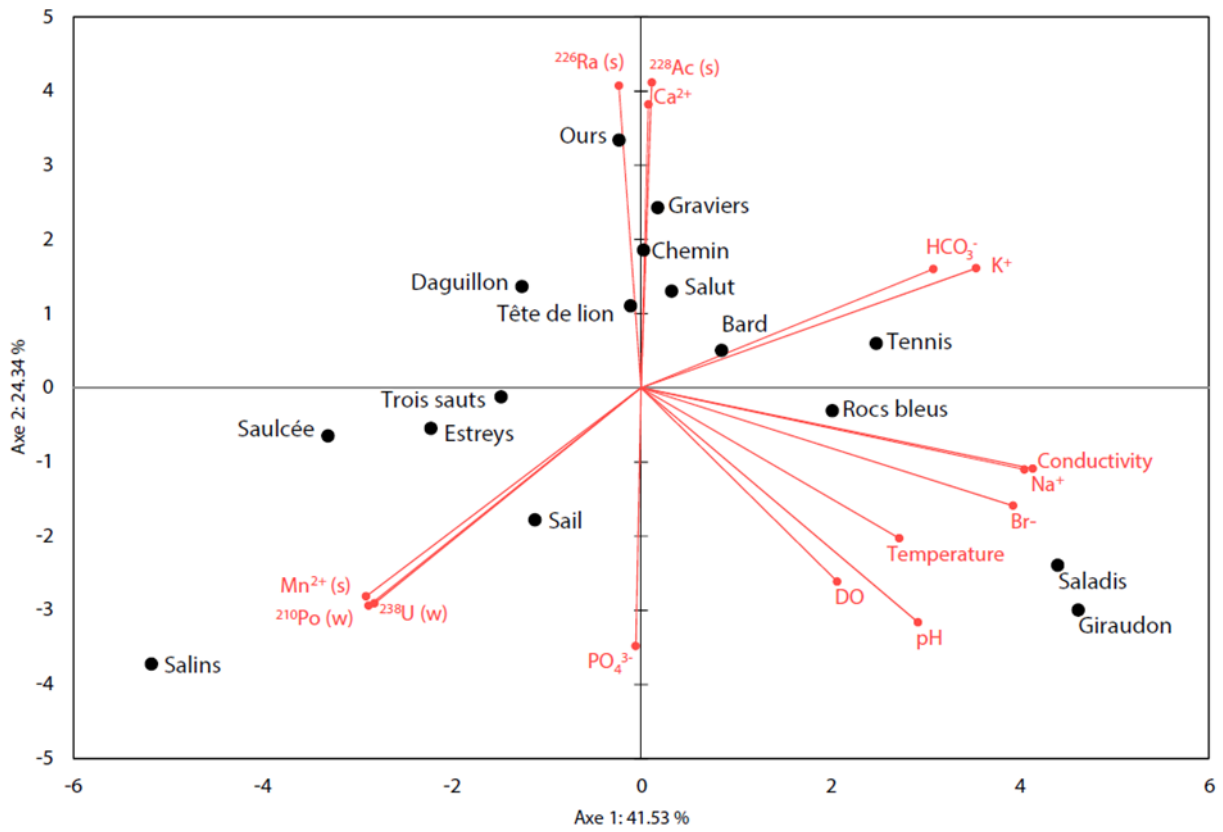
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 985 **Figure 1:** Location map of the study sites. (A) Map of Europe, (B) Map of the Massif Central in France, (C) Map  
 986 of the 16 mineral springs monitored in the Massif Central; photographs of some mineral springs (D, E, F).  
 987 monitored.

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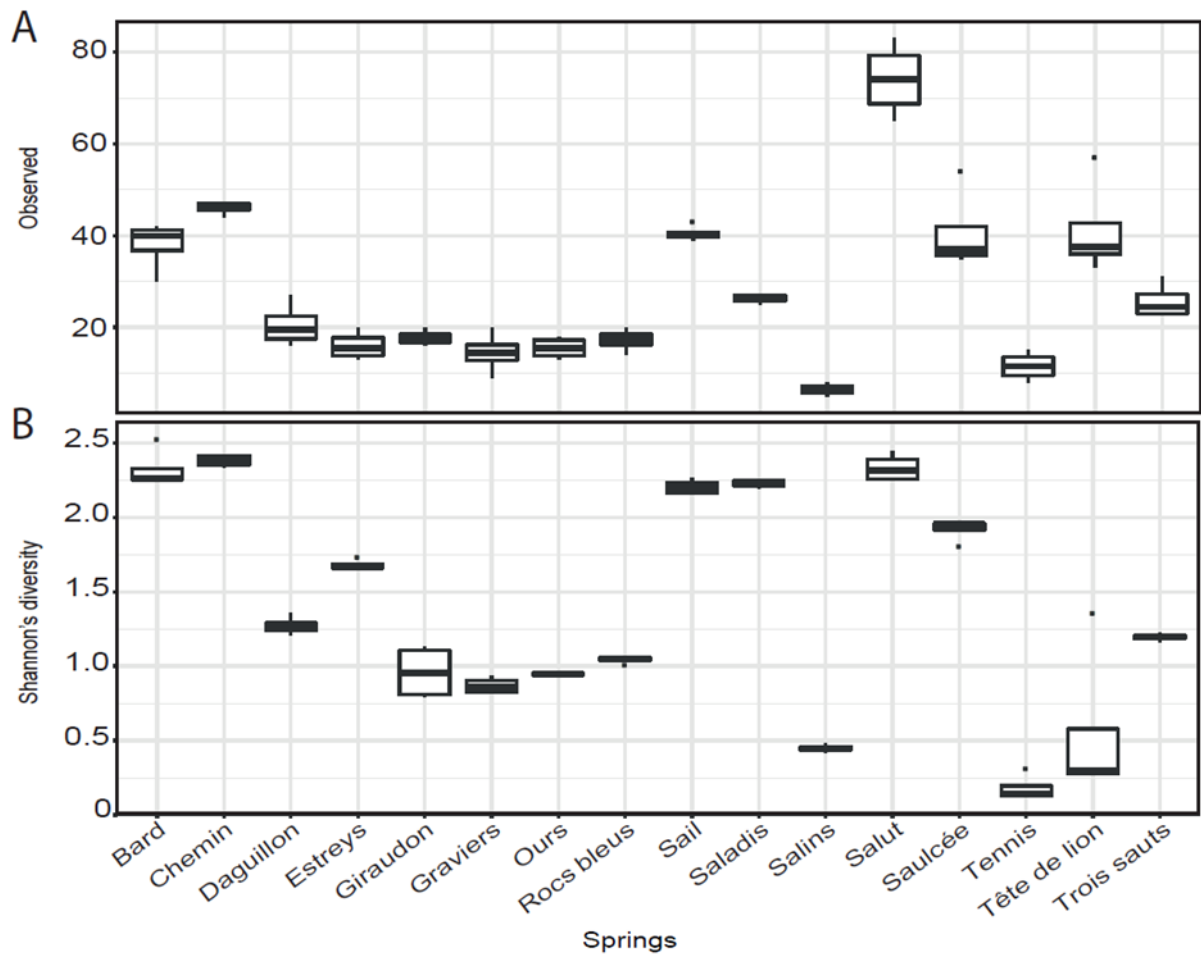




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990 **Figure 2:** Principal component analysis (PCA) shows the springs' distribution based on physical, chemical and  
 991 radiological variables measured in water (w) or sediments (s).

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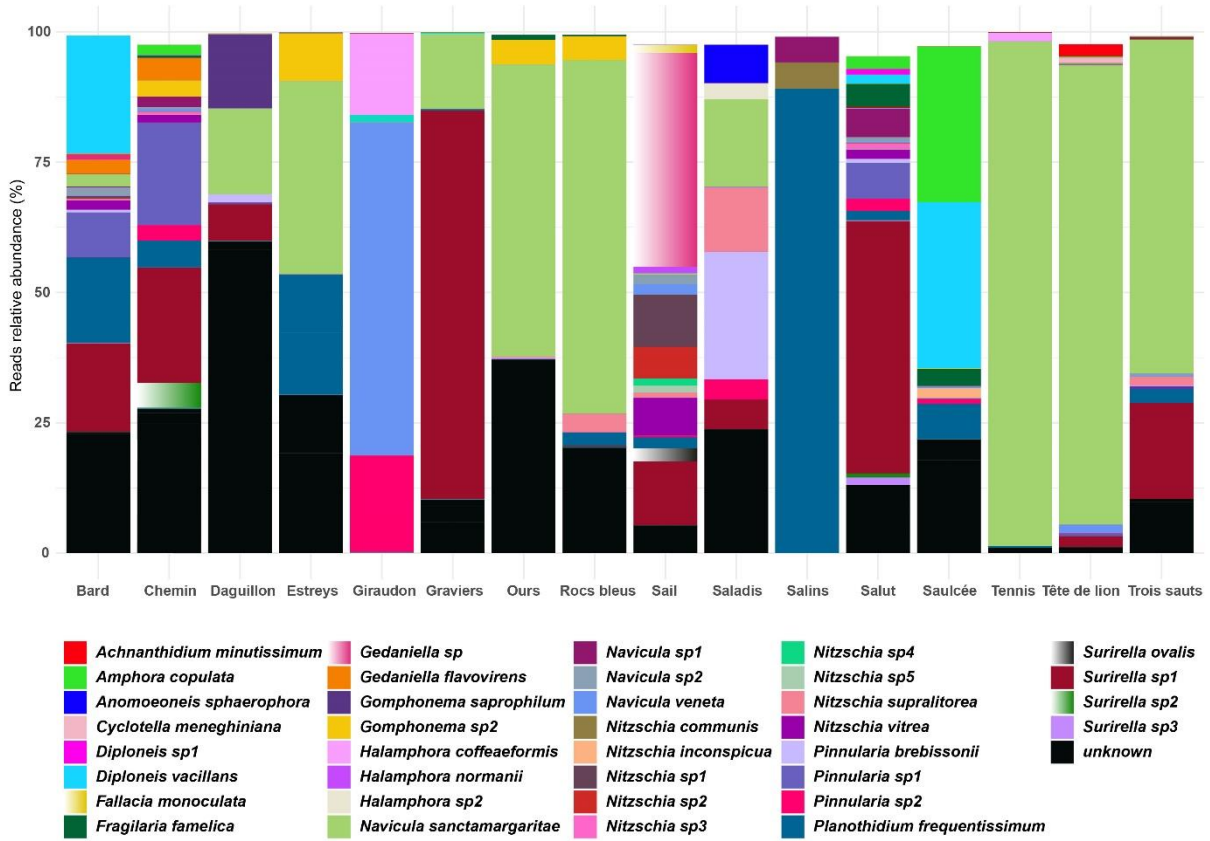
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994 **Figure 3:** Boxplot showing the mean of (A) ASV richness corresponding to benthic diatom species richness and

995 (B) Shannon's diversity for each technical replicate (N=4) of the mineral springs monitored in the Massif Central

996 (France).

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1000 **Figure 4:** Taxonomic composition of diatom communities for the mineral springs monitored in the Massif Central  
 1001 based on DNA metabarcoding results for the *rbcL* chloroplast gene. Only species with reads relative abundances  
 1002 above 1% are represented.

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**Table 1:** Physical, chemical and radiological variables measured at the mineral springs monitored in the Massif Central (France) after the selection of variables with stepwise followed by the subtraction of detection limits (DL). Cond: conductivity; DO: dissolved oxygen; Temp: Temperature.  $^{238}\text{U}$ ,  $^{210}\text{Po}$   $^{226}\text{Ra}$ ;  $^{228}\text{Ac}$  expressed radionuclides activity in spring water (w) or sediments (s).

Springs	Na <sup>+</sup> (mg.L <sup>-1</sup> )	K <sup>+</sup> (mg.L <sup>-1</sup> )	Ca <sup>2+</sup> (mg.L <sup>-1</sup> )	Br <sup>-</sup> (mg.L <sup>-1</sup> )	PO <sub>4</sub> <sup>3-</sup> (mg.L <sup>-1</sup> )	HCO <sub>3</sub> <sup>-</sup> (mg.L <sup>-1</sup> )	Cond (μS.cm <sup>-1</sup> )	pH	DO (%)	Temp (°C)	<sup>238</sup> U (w) (Bq.L <sup>-1</sup> )	<sup>226</sup> Ra (s) (Bq.g <sup>-1</sup> )	<sup>210</sup> Po (w) (μBq.L <sup>-1</sup> )	<sup>228</sup> Ac (s) (Bq.g <sup>-1</sup> )	Mn <sup>2+</sup> (s) (mg.g <sup>-1</sup> )
<b>Bard</b>	1,028.88	185.88	233.76	1.88	0.12	3,012	6,380	6.81	3.1	14.7	0	2.2	0	0.6	0.33
<b>Chemin</b>	990.42	179.01	202.55	1.72	0.09	2,515	4,860	6.41	15.3	14.2	0.002	10.8	0	26.1	0.24
<b>Daguillon</b>	503.16	112.22	297.06	0.56	0.13	2,142	4,030	6.6	29	13.9	0	23.0	0	4.6	0.30
<b>Estreys</b>	504.61	45.10	128.21	1.65	0.11	1,185	3,480	6.38	16.4	16	0.012	1.9	17	0.0	0.40
<b>Giraudon</b>	1,787.50	189.50	151.28	4.91	0.70	2,814	9,201	7.51	54	32.1	0	4.2	0	5.9	0.24
<b>Graviers</b>	823.10	159.59	381.87	1.66	0.13	2,730	5,430	6.69	19.5	14.4	0	26.7	0	17.9	0.39
<b>Ours</b>	713.30	146.24	313.27	1.20	0.16	2,648	5,490	6.72	13.8	13.7	0	52.1	0	28.7	0.11
<b>Rocs bleus</b>	1,396.77	144.23	255.14	3.68	0.06	2,696	8,150	7	9.3	19.2	0	0.6	0	0.1	0.16
<b>Sail</b>	686.62	83.46	85.67	1.13	0.54	1,150	5,390	7.02	9.2	16.6	0.004	0.8	0	0.0	0.32
<b>Saladis</b>	1,813.91	197.52	112.55	4.09	0.05	2,928	9,680	7.82	88.6	16.1	0	1.2	0	0.1	0.17
<b>Salins</b>	291.27	53.52	204.16	0.23	0.53	1,948	3,090	6.83	20.1	13.3	0.193	0.8	382	0.4	9.32
<b>Salut</b>	1,041.30	189.46	179.92	1.67	0.09	2,740	5,850	6.46	11.7	12.1	0	9.5	0	12.8	0.10
<b>Saulcée</b>	464.48	67.75	173.14	0.95	0.05	1,412	3,420	6.66	26	14.4	0.082	19.3	158	11.7	5.35
<b>Tennis</b>	1,408.88	154.92	281.91	3.34	0.09	2,778	8,380	7.05	8.9	27.1	0	21.6	0	11.9	0.11
<b>Tête de lion</b>	768.30	147.04	239.54	1.62	0.08	2,454	4,200	6.99	18.9	14.4	0	15.9	0	12.9	0.41
<b>Trois sauts</b>	803.89	69.23	135.35	0.89	0.06	1,940	3,890	6.66	12.8	13.1	0.004	2.1	0	1.1	0.53

**Table 2:** Pearson correlation coefficient (R) between ASV richness of benthic diatom communities and the physical, chemical and radiological variables measured in studied mineral springs of the Massif Central (N=16). ASV: Amplicon sequence variant richness, Cond: Conductivity, DO: Dissolved oxygen, Temp: Temperature, <sup>238</sup>U; <sup>210</sup>Po <sup>226</sup>Ra; <sup>228</sup>Ac expressed radionuclides activity in spring water (w) or sediments (s). Values in bold are significant p-value ( $\alpha = 0.05$ ).

	ASV	Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	Br <sup>-</sup>	PO <sub>4</sub> <sup>3-</sup>	HCO <sub>3</sub> <sup>-</sup>	Cond	pH	DO	Temp	<sup>238</sup> U (w)	<sup>226</sup> Ra (s)	<sup>210</sup> Po (w)	<sup>228</sup> Ac (s)	Mn <sup>2+</sup> (s)
ASV	<b>1</b>	-0.01	0.29	-0.32	-0.15	-0.23	0.01	-0.11	-0.26	-0.15	-0.36	-0.25	-0.15	-0.25	0.18	-0.21
Na <sup>+</sup>	-0.01	<b>1</b>	<b>0.75</b>	-0.13	<b>0.95</b>	0.05	<b>0.68</b>	<b>0.96</b>	<b>0.73</b>	<b>0.53</b>	<b>0.66</b>	-0.49	-0.23	-0.48	-0.08	<b>-0.50</b>
K <sup>+</sup>	0.29	0.75	<b>1</b>	0.27	<b>0.63</b>	-0.12	<b>0.90</b>	<b>0.73</b>	0.40	0.30	0.29	<b>-0.54</b>	0.13	-0.52	0.35	<b>-0.55</b>
Ca <sup>2+</sup>	-0.32	-0.13	0.27	<b>1</b>	-0.12	-0.29	0.48	-0.03	-0.25	-0.32	-0.07	-0.10	<b>0.70</b>	-0.08	<b>0.53</b>	-0.09
Br <sup>-</sup>	-0.15	<b>0.95</b>	<b>0.63</b>	-0.12	<b>1</b>	0.13	<b>0.56</b>	<b>0.93</b>	<b>0.73</b>	<b>0.52</b>	<b>0.78</b>	-0.42	-0.24	-0.41	-0.14	-0.43
PO <sub>4</sub> <sup>3-</sup>	-0.23	0.05	-0.12	-0.29	0.13	<b>1</b>	-0.17	0.11	0.35	0.15	0.47	0.35	-0.25	0.34	-0.24	0.30
HCO <sub>3</sub> <sup>-</sup>	0.01	<b>0.68</b>	<b>0.90</b>	0.48	<b>0.56</b>	-0.17	<b>1</b>	0.67	0.37	0.22	0.26	-0.35	0.19	-0.33	0.30	-0.36
Cond	-0.11	<b>0.96</b>	<b>0.75</b>	-0.03	<b>0.93</b>	0.11	<b>0.67</b>	<b>1</b>	<b>0.77</b>	0.48	<b>0.68</b>	-0.45	-0.13	-0.43	-0.11	-0.46
pH	-0.26	<b>0.73</b>	0.40	-0.25	<b>0.73</b>	0.35	0.37	<b>0.77</b>	<b>1</b>	<b>0.73</b>	<b>0.56</b>	-0.09	-0.24	-0.08	-0.34	-0.09
DO	-0.15	<b>0.53</b>	0.30	-0.32	<b>0.52</b>	0.15	0.22	0.48	<b>0.73</b>	<b>1</b>	0.26	-0.02	-0.16	-0.01	-0.19	-0.01
Temp	-0.36	<b>0.66</b>	0.29	-0.07	<b>0.78</b>	0.47	0.26	<b>0.68</b>	<b>0.56</b>	0.26	<b>1</b>	-0.20	-0.12	-0.20	-0.13	-0.21
<sup>238</sup> U (w)	-0.25	-0.49	<b>-0.54</b>	-0.10	-0.42	0.35	-0.35	-0.45	-0.09	-0.02	-0.20	<b>1</b>	-0.17	<b>1</b>	-0.20	<b>0.99</b>
<sup>210</sup> Po (w)	-0.25	-0.48	<b>-0.52</b>	-0.08	-0.41	0.34	-0.33	-0.43	-0.08	-0.01	-0.20	<b>1</b>	-0.16	<b>1</b>	-0.19	<b>0.99</b>
<sup>226</sup> Ra (s)	-0.15	-0.23	0.13	<b>0.70</b>	-0.24	-0.25	0.19	-0.13	-0.24	-0.16	-0.12	-0.17	<b>1</b>	-0.16	<b>0.79</b>	-0.14
<sup>228</sup> Ac (s)	0.18	-0.08	0.35	0.53	-0.14	-0.24	0.30	-0.11	-0.34	-0.19	-0.13	-0.20	0.79	-0.19	<b>1</b>	-0.17
Mn <sup>2+</sup> (s)	-0.21	<b>-0.50</b>	<b>-0.55</b>	-0.09	-0.43	0.30	-0.36	-0.46	-0.09	-0.01	-0.21	<b>0.99</b>	-0.14	<b>0.99</b>	-0.17	<b>1</b>

**Table 3:** Non-parametric Multivariate Analysis of Variances (NPMANOVA or Adonis) results for unifactorial analysis of location,  $^{238}\text{U}$ ,  $^{226}\text{Ra}$ ,  $^{210}\text{Po}$  and  $^{228}\text{Ac}$  activity levels in spring water (w) or sediments (s), made with Jaccard (J) and ab.Jaccard (ab.J) distances performed on ASV matrix with relative abundances. The matrix contains only ASV with a relative abundance of more than 2% in at least one sample of the mineral springs monitored in the Massif Central (N=16). Significant p-value is indicated with (\*).

<b>Factors</b>	<b>R<sup>2</sup> (J)</b>	<b>R<sup>2</sup> (ab.J)</b>
<b>Location</b>	47.01 %	53.53 %
$^{238}\text{U}$ activity level (w)	17.18 %	38.59 % *
$^{226}\text{Ra}$ activity level (s)	10.69 %	7.74 %
$^{210}\text{Po}$ activity level (w)	5.64 %	0
$^{228}\text{Ac}$ activity level (s)	13.34 %	3.45 %

**Table 4:** Multiple Response Permutation Procedure (MRPP) results for analysis of differences of community structure between sample group of location, <sup>238</sup>U, <sup>226</sup>Ra, <sup>210</sup>Po and <sup>228</sup>Ac activity levels in spring water (w) or sediments (s) made with Jaccard (J) and ab.Jaccard (ab.J) distances performed on ASV matrix with relative abundances. The matrix contains only ASV with a relative abundance of more than 2% in at least one sample of the mineral springs monitored in the Massif Central (N=16). MRPP is based on 999 permutations. ASV: Amplicon sequence variant richness, Cond: Conductivity, DO: Dissolved oxygen, Temp: Temperature.

Distance index		Geographic location	<sup>238</sup> U Activity	<sup>226</sup> Ra Activity	<sup>210</sup> Po Activity	<sup>228</sup> Ac Activity
(J)	Chance-corrected within-group A	0.146	0.026	-0.049	-0.010	0.005
	Observed $\delta$	0.674	0.768	0.455	0.797	0.785
	Expected $\delta$	0.789	0.789	0.434	0.789	0.789
	Significance of $\delta$	p=0.002	p>0.05	p>0.05	p>0.05	p>0.05
(ab.J)	Chance-corrected within-group A	0.368	0.230	-0.011	-0.040	0.005
	Observed $\delta$	0.274	0.334	0.797	0.451	0.785
	Expected $\delta$	0.434	0.434	0.789	0.434	0.789
	Significance of $\delta$	p=0.11	p=0.004	p>0.05	p>0.05	p>0.05

**Supplementary Table S1:** Physical and chemical variables measured in the mineral springs monitored in the Massif Central (France). N=16

<b>Springs</b>	<b>Na<sup>+</sup> (mg.L<sup>-1</sup>)</b>	<b>K<sup>+</sup> (mg.L<sup>-1</sup>)</b>	<b>Ca<sup>2+</sup> (mg.L<sup>-1</sup>)</b>	<b>Br<sup>-</sup> (mg.L<sup>-1</sup>)</b>	<b>PO<sub>4</sub><sup>3-</sup> (mg.L<sup>-1</sup>)</b>	<b>HCO<sub>3</sub><sup>-</sup> (mg.L<sup>-1</sup>)</b>	<b>Cond (μS.cm<sup>-1</sup>)</b>	<b>pH</b>	<b>DO (%)</b>	<b>Temp (°C)</b>
<b>Bard</b>	1028.88	185.88	233.76	1.88	0.12	3012	6380	6.81	3.1	14.7
<b>Chemin</b>	990.42	179.01	202.55	1.72	0.09	2515	4860	6.41	15.3	14.2
<b>Daguillon</b>	503.16	112.22	297.06	0.56	0.13	2142	4030	6.6	29	13.9
<b>Estreys</b>	504.61	45.10	128.21	1.65	0.11	1185	3480	6.38	16.4	16
<b>Giraudon</b>	1787.50	189.50	151.28	4.91	0.70	2814	9201	7.51	54	32.1
<b>Graviers</b>	823.10	159.59	381.87	1.66	0.13	2730	5430	6.69	19.5	14.4
<b>Ours</b>	713.30	146.24	313.27	1.20	0.16	2648	5490	6.72	13.8	13.7
<b>Rocs bleus</b>	1396.77	144.23	255.14	3.68	0.06	2696	8150	7	9.3	19.2
<b>Sail</b>	686.62	83.46	85.67	1.13	0.54	1150	5390	7.02	9.2	16.6
<b>Saladis</b>	1813.91	197.52	112.55	4.09	0.05	2928	9680	7.82	88.6	16.1
<b>Salins</b>	291.27	53.52	204.16	0.23	0.53	1948	3090	6.83	20.1	13.3
<b>Salut</b>	1041.30	189.46	179.92	1.67	0.09	2740	5850	6.460	11.7	12.1
<b>Saulcée</b>	464.48	67.75	173.14	0.95	0.05	1412	3420	6.66	26	14.4
<b>Tennis</b>	1408.88	154.92	281.91	3.34	0.09	2778	8380	7.05	8.9	27.1
<b>Tête de lion</b>	768.30	147.04	239.54	1.62	0.08	2454	4200	6.99	18.9	14.4
<b>Trois sauts</b>	803.89	69.23	135.35	0.89	0.06	1940	3890	6.66	12.8	13.1



**Supplementary Table 2:** Raw data of the radiological parameters and trace elements measured in the mineral springs monitored in the Massif Central (France). NA in the column “ $^{226}\text{Ra}$  (Bq.L $^{-1}$ )” specified that this variable could not be measured for the sample.

Springs	Water		Sediments		
	$^{238}\text{U}$ (Bq.L $^{-1}$ )	$^{226}\text{Ra}$ (Bq.L $^{-1}$ )	$^{210}\text{Po}$ ( $\mu\text{Bq.L}^{-1}$ )	$^{228}\text{Ac}$ (Bq.g $^{-1}$ )	$\text{Mn}^{2+}$ (mg.g $^{-1}$ )
<b>Bard</b>		0.922	<10	0.62	0.333
<b>Chemin</b>	0.004428	1.15	<10	26.11	0.237
<b>Daguillon</b>	<2.5E-3	3.221	<10	4.60	0.301
<b>Estreys</b>	<2.5E-3	1.204	27	0	0.400
<b>Giraudon</b>	<2.5E-3	NA	<10	5.91	0.235
<b>Graviers</b>	0.014514	2.331	<10	17.92	0.391
<b>Ours</b>	<2.5E-3	2.621	<10	28.72	0.105
<b>Rocs bleus</b>	<2.5E-3	NA	<10	0.05	0.158
<b>Sail</b>	0.006765	0.472	<10	0	0.321
<b>Saladis</b>	<2.5E-3	2.013	<10	0.07	0.170
<b>Salins</b>	0.195693	0.736	392	0.41	9.323
<b>Salut</b>	<2.5E-3	1.548	<10	12.78	0.097
<b>Saulcée</b>	0.084255	1.438	168	11.65	5.351
<b>Tennis</b>	0.002706	3.338	<10	11.88	0.112
<b>Tête de lion</b>	0.002583	NA	<10	12.87	0.413
<b>Trois sauts</b>	0.00615	<1.4E-1	<10	1.09	0.530

**Supplementary Table 3:** Qualitative variables of the mineral springs monitored in the Massif Central indicating geographical location, and activity levels of  $^{238}\text{U}$ ,  $^{210}\text{Po}$ ,  $^{226}\text{Ra}$  and  $^{228}\text{Ac}$  in water (w) or in sediment (s).

<b>Springs</b>	<b>Geographical location</b>	$^{238}\text{U}$ level (w)	$^{226}\text{Ra}$ level (s)	$^{210}\text{Po}$ level (w)	$^{228}\text{Ac}$ level (s)
<b>Bard</b>	Boudes	low	medium	low	low
<b>Chemin</b>	Ternant les eaux	medium	high	low	high
<b>Daguillon</b>	Joze	low	high	low	medium
<b>Estreys</b>	Polignac	low	medium	medium	low
<b>Giraudon</b>	Saint-Nectaire	low	medium	low	medium
<b>Graviers</b>	Joze	low	high	low	high
<b>Ours</b>	Joze	low	high	low	high
<b>Rocs bleus</b>	Martes-de-Vevre	low	low	low	low
<b>Sail</b>	Mirefleurs	low	low	low	low
<b>Saladis</b>	Martes-de-Vevre	low	medium	low	low
<b>Salins</b>	Apcher	high	low	high	low
<b>Salut</b>	Ternant les eaux	low	medium	low	high
<b>Saulcée</b>	Coudes	medium	high	high	high
<b>Tennis</b>	Sainte-Marguerite	low	high	low	high
<b>Tête de lion</b>	Saint-Floret	low	high	low	high
<b>Trois sauts</b>	Leyvaux	low	medium	low	medium

**Supplementary table 4:** Pearson correlations between the abundance of the 14 dominant species and the radiological parameters  $^{238}\text{U}$ ,  $^{226}\text{Ra}$ ,  $^{210}\text{Po}$ ,  $^{228}\text{Ac}$ , activities and trace element concentration ( $\text{Mn}^{2+}$ ) in the springs monitored in the Massif Central (N=16). Significant p-value are in bold ( $p < 0.05$ ). Species are classified with decreasing their gradient of relative abundances in the mineral. Cond: Conductivity, DO: Dissolved oxygen, Temp: Temperature.

	$\text{Na}^+$	$\text{K}^+$	$\text{Ca}^{2+}$	$\text{Br}^-$	$\text{PO}_4^{3-}$	$\text{HCO}_3^-$	Cond	pH	DO	Temp	$^{238}\text{U}$ (w)	$^{210}\text{Po}$ (w)	$^{226}\text{Ra}$ (s)	$^{228}\text{Ac}$ (s)	$\text{Mn}^{2+}$ (s)
<i>Navicula sanctamargaritae</i>	0.13	-0.04	0.30	0.16	-0.41	0.15	0.12	0.07	-0.24	0.18	-0.30	-0.30	0.26	0.09	-0.31
<i>Surirella</i> sp1	-0.03	0.31	0.34	-0.16	-0.18	0.25	-0.07	-0.32	-0.17	-0.33	-0.23	-0.23	0.11	0.29	-0.22
<i>Unknown species1</i>	-0.18	-0.12	0.31	-0.17	-0.26	-0.03	-0.10	-0.13	0.12	-0.21	-0.21	-0.21	0.42	0.00	-0.22
<i>Planothidium frequentissimum</i> genetic variant A	-0.41	-0.43	-0.06	-0.36	0.41	-0.19	-0.36	-0.07	-0.07	-0.18	0.91	0.91	-0.26	-0.27	<b>0.85</b>
<i>Navicula veneta</i>	0.49	0.28	-0.21	<b>0.58</b>	<b>0.68</b>	0.20	0.44	0.46	0.39	0.76	-0.10	-0.10	-0.16	-0.07	-0.10
<i>Diploneis vacillans</i>	-0.20	-0.10	-0.06	-0.18	-0.22	-0.14	-0.19	-0.14	-0.11	-0.16	0.22	0.22	0.00	-0.05	0.32
<i>Gedaniella</i> sp.	-0.15	-0.24	-0.41	-0.16	0.45	<b>-0.50</b>	-0.03	0.12	-0.17	0.00	-0.08	-0.10	-0.22	-0.24	-0.09
<i>Pinnularia</i> sp1	0.07	0.41	-0.03	-0.07	-0.20	0.25	-0.06	-0.38	-0.22	-0.22	-0.16	-0.15	-0.12	0.40	-0.16
<i>Amphora copulata</i>	-0.27	-0.29	-0.13	-0.21	-0.21	-0.37	-0.29	-0.18	0.03	-0.14	0.32	0.31	0.13	0.13	0.43
<i>Planothidium frequentissimum</i> genetic variant B	-0.30	-0.40	-0.34	-0.16	-0.25	<b>-0.51</b>	-0.34	-0.46	-0.27	-0.17	-0.00	-0.02	-0.30	-0.24	0.01
<i>Unknown species2</i>	-0.14	-0.01	0.08	-0.06	-0.16	0.00	-0.11	-0.26	-0.25	-0.12	-0.13	-0.13	-0.17	-0.23	-0.15
<i>Pinnularia</i> sp2	<b>0.60</b>	0.41	-0.29	<b>0.65</b>	0.60	0.28	<b>0.52</b>	<b>0.51</b>	<b>0.54</b>	<b>0.71</b>	-0.13	-0.13	-0.20	-0.02	-0.13
<i>Pinnularia brebissonii</i>	<b>0.50</b>	0.33	-0.30	0.41	-0.19	0.27	0.49	<b>0.65</b>	<b>0.83</b>	-0.04	-0.11	-0.10	-0.19	-0.24	-0.12
<i>Gomphonema</i> sp1	-0.15	-0.26	-0.05	0.01	-0.22	-0.26	-0.15	-0.38	-0.22	-0.07	-0.15	-0.16	0.06	0.09	-0.20