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Virus-to-prokaryote ratio in spring waters along a gradient of natural radioactivity

Accepted Version - Hydrobiologia

Lory-Anne Baker^{1,2}, David G. Biron^{† 1,2}, Aude Beauger^{2,3}, Sofia Kolovi^{2,4}, Jonathan Colombet^{1,2}, Elisabeth Allain^{2,3}, Olivier Voldoire^{2,3}, Vincent Breton^{2,4}, Patrick Chardon^{2,4}, Téléphore Sime-Ngando¹, Karine David^{2,5}, Gilles Montavon^{2,5}, Hervé Michel^{2,6}, and Angia Sriram Pradeep Ram^{1*}

¹ Université Clermont Auvergne, CNRS, LMGE, F-63000 Clermont-Ferrand, France

² LTSER “Zone Atelier Territoires Uranifères”, F-63000 Clermont-Ferrand, 63178 Aubière Cedex, France

³ Université Clermont Auvergne, CNRS, GEOLAB, F-63000 Clermont-Ferrand, France

⁴ Université Clermont Auvergne, CNRS/IN2P3, Laboratoire de Physique de Clermont (LPC), UMR 6533, F-63178 Aubière Cedex, France

⁵ Laboratoire SUBATECH, UMR 6457, IN2P3/CNRS/IMT Atlantique/Université de Nantes, 4, rue Alfred Kastler, BP 20722, 44307 Nantes Cedex 3, France

⁶ Institut de Chimie de Nice (ICN), UMR 7272, Université Côte d’Azur, 28 Avenue Valrose, 06108 Nice Cedex 2, France

[†] deceased

Statements and Declarations

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Conceptualization, Biron D.G. and Pradeep Ram A.S.; methodology, Pradeep Ram A.S., Beauger A., Allain E., Voldoire O., Colombet J., Kolovi S., Chardon P., Breton V., Karine D., Montavon G, and Michel H ; formal analysis, Baker L.-A. with the inputs from the other authors.; data curation, Baker L.-A.; writing—original draft preparation, Baker L.-A.; writing—review and editing, Baker L.-A, Pradeep Ram A.S., Sime-Ngando T with the inputs from the other authors ; supervision, Biron D.G., Pradeep Ram A.S. and Beauger A; project administration, Biron D.G. and Pradeep Ram A.S.; funding acquisition, Beauger A. and Biron D.G. All authors have read and agreed to the published version of the manuscript.

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38

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41

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47

48 **ORCID :**

49 Lory-Anne Baker <https://orcid.org/0000-0002-8346-8578>

50 David G. Biron <https://orcid.org/0000-0002-6531-4647>

51 Aude Beauger <https://orcid.org/0000-0002-0911-0500>

52 Sofia Kolovi <https://orcid.org/0000-0003-4778-2823>

53 Jonathan Colombet <https://orcid.org/0000-0003-3763-6566>

54 Elisabeth Allain <https://orcid.org/0000-0002-6411-5873>

55 Olivier Voltaire <https://orcid.org/0000-0003-1306-3054>

56 Vincent Breton <https://orcid.org/0000-0001-8197-7080>

57 Telesphore Sime-Ngando <https://orcid.org/0000-0002-7240-5803>

58 Karine David <https://orcid.org/0000-0003-0977-5905>

59 Gilles Montavon <https://orcid.org/0000-0002-3049-0106>

60 Angia Sriram Pradeep Ram <https://orcid.org/0000-0002-5469-0452>

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66 *Corresponding author:

67 Angia Sriram Pradeep Ram,

68 Laboratoire Microorganismes : Génome et Environnement,

69 UMR CNRS 6023,

70 Université Clermont-Auvergne,

71 1 Impasse Amélie Murat,

72 63178, Aubière Cedex,

73 France

74 Email : pradeep_ram.angia_sriram@uca.fr

75 Telephone: +33 473407463

Fax: +33 473407670

76 **Abstract** Although a strong link between viruses and prokaryotes is commonly known to
77 exist in aquatic systems, few studies have investigated their relationship in spring waters. In
78 the French Massif Central, certain springs are known to exhibit varying levels of naturally
79 occurring radioactivity. Therefore, the aim of this study was to examine the standing stock of
80 viruses together with prokaryotes, and determine the potential environmental factors
81 influencing them in springs characterized by contrasted radioactivity gradient. Among the
82 investigated 15 spring habitats, flow cytometry analyses indicated that both viral (VA) and
83 prokaryotic abundances (PA) varied by an order of magnitude accompanied by virus-to-
84 prokaryote ratio ranging between 8 and 144.2. Significant differences in VA was evident
85 among springs where low abundances (mean \pm SD: $21.3 \pm 7.0 \times 10^7$ VLP l⁻¹) corresponded to
86 high (mean \pm SD: 1911 ± 814.0 nGy.h⁻¹) gamma (γ) dose radiation (²²²Rn) and vice versa.
87 Adverse effect of water radioactivity (²²²Rn) on VA could perhaps corroborate our hypothesis
88 of drastic effect of ionizing radiations on viruses, which might explain for lack of significant
89 relationship between PA and VA. In such a scenario of prevailing low VA, the adopted phage
90 life strategies could define their existence in these specialized ecosystems.

91
92 **Keywords:** viruses; prokaryotes; virus to prokaryote ratio, mineral springs; naturally
93 enhanced radioactivity; extreme environment.

94

95 **Introduction**

96 Viruses are found wherever life occurs, and current estimates suggest that they are the most
97 abundant and diversified biotic agents in aquatic systems (Weinbauer, 2004; Breitbart, 2012;
98 Schweichhart, 2021). Their omnipresence in wide variety of aquatic habitats is undoubtedly
99 through the existence of two major pathways, namely lytic and lysogeny to confront
100 environmental challenges (Suttle, 2007; Sime-Ngando, 2014). Viruses not only play an
101 important role in microbial dynamics and evolution but also influence nutrients and carbon
102 cycles through the release of dissolved organic matter during lysis which is referred to as
103 “viral shunt” (Whitman et al., 1998). This dissolved organic matter is directly available for
104 non-targeted prokaryotic population which stimulates their growth and activity (Wilhelm &
105 Suttle, 1999). In aquatic systems, prokaryotes serve as principle hosts for viruses
106 (bacteriophages) owing to their numerical dominance. As actors of cellular lysis, viruses can
107 directly affect prokaryotic abundances as well as their structure, which probably depends on
108 the contact rates between them (Parikka et al. 2017). Since viruses are entirely dependent on
109 the host cells for their proliferation, a positive correlation between viruses and prokaryotes
110 abundances can be expected. Accordingly, several studies have highlighted the existence of
111 such a significant relationship between them in marine (Bongiorni et al., 2005; Bettarel et al.,
112 2008), freshwater (Peduzzi & Schiemers, 2004; Brum et al., 2005) and glacier environments
113 (Anesio et al., 2007; Rassner et al., 2016). However, some exceptional studies have reported
114 the absence of any relationship between viruses and prokaryotes abundances (Leff et al.,
115 1999; De Corte et al., 2010; Kyle & Ferris, 2013).

116 Although viruses are generally accepted as key players in aquatic food web, relatively little
117 is known on the factors (abiotic and biotic) influencing them in spring habitats. A lack of
118 opportunity to document viral ecology in such specialised systems has resulted in paucity of
119 knowledge about these environments. Freshwater springs are small water bodies that act as
120 crucial interfaces within the Earth’s Critical Zone. They serve as multiple three-way ecotones
121 that link terrestrial and aquatic habitats, ground and surface waters (Reiss & Chiffard, 2017).
122 To our knowledge virus-prokaryote interactions have been investigated in high temperature
123 environments such as hot springs (Breitbart et al., 2004; Kepner, 2015) and deep-sea
124 hydrothermal vent systems (Williamson et al., 2008) focussing principally on microbial
125 compositions and dynamics. A study carried out in the terrestrial hot fluid system situated in
126 cold environment of Southern hemisphere (French Southern and Antarctic lands) have

127 reported a significant positive relation between viruses and prokaryotes accompanied by a low
128 virus-to-prokaryote ratio (Parikka et al., 2018).

129 The Auvergne region of the French Massif Central harbours a wide variety of mineral
130 springs that are characterised by diverse groups of microbial communities (Lai et al., 2019,
131 Millan et al., 2019). Due to the presence of high levels of uranium in their granitic ground,
132 some of the mineral springs exhibit enhanced levels of natural radioactivity together with
133 varying physico-chemical conditions. Microbes inhabiting in these environments are highly
134 adapted and known to survive in mineralized and extreme ionizing conditions that are
135 eventually seen as chronic stress for their survival (Shukla et al., 2017). Lethal effects of
136 radioactivity on organisms are real due to their detrimental capability to alter their DNA
137 structure (Ravanat & Douki, 2016). Some micro-organisms, notably prokaryotes have
138 developed biological mechanisms to resist and tolerate high concentrations of radionuclides
139 through DNA repair mechanisms. Genome size is also a parameter implicated in resisting
140 high levels of radiations (Shuryak, 2019). Prokaryotes can accumulate radionuclides either in
141 their intracellular compartment (bioaccumulation) or on their external membrane
142 (biosorption) (Tabak et al., 2005). Henceforth, ionising radiation can induce radiobiological
143 effects that lead to the inactivation of cells or viruses (Lea, 1946; Sullivan et al., 1971). The
144 direct destructive effect of these radiation can produce simple or double strand breaks in
145 nucleic acid chains (Ravanat & Douki, 2016), whereas the indirect consequence results in the
146 production of reactive oxygen species (ROS) through water radiolysis. A correlation between
147 the size of the virus and inactivation dose was made. The smaller the virus, higher the dose is
148 required to inactivate it (Lea, 1946). Apart from laboratory studies, no effort has been made to
149 determine the abundance and dynamics of naturally occurring environmental phage
150 communities in freshwater springs. Henceforth, we hypothesize viral abundances to decrease
151 with increasing radioactivity due to the effects of ionizing radiation, even if this radiation is of
152 natural origin. The purpose of this study was to gain insight into (1) viral and prokaryotic
153 abundances in a set of mineral springs along a gradient of naturally enhanced radioactivity
154 and (2) to determine the influence of environmental factors, especially γ -ray radiation and
155 activities of radionuclides belonging to the uranium decay chain, on viral and prokaryote
156 abundances. The present study is one of the few which assess virus-prokaryote relation in
157 mineral springs which are characterised with variable levels of naturally occurring
158 radioactivity.

159

160 **Materials and methods**

161

162 ***Site description and sampling***

163 The sampling campaign was carried out in autumn from October to November 2019, where
164 water samples were collected from a total of 15 mineral springs distributed in the Auvergne
165 region of the French Massif Central (Fig. 1). At each site, water samples were collected in
166 triplicates (i.e. from three independent sampling procedures). The study sites were chosen
167 based on their physical, chemical and radiological characteristics in order to include an
168 increasing gradient of natural radioactivity. The radioactivity comes from the decay chains of
169 three radioelements ^{235}U , ^{238}U and ^{232}Th that are naturally present in the earth crust, especially
170 in granite rocks. The disintegration of these radioelements and their daughters result in the
171 emission of ionizing particles including γ -particles that can be detected at spring water
172 surfaces. The sampling at 13 out of 15 sites was conducted in October, whereas for Montagne
173 1 and Montagne 2 sites it was done in November (Supplementary Table 1).

174

175 ***Physical and chemical analyses***

176 At the 15 sampling sites, water temperature, conductivity and pH were measured with a multi-
177 parameter WTW probe FC 340i (VWR International, Radnor, PA, USA) by submerging the
178 probe in the spring water. Percentage saturation of dissolved oxygen was obtained with a Ysi
179 ProODO oxygen probe (Yellow Springs Instruments, OH, USA). Bicarbonate (HCO_3^-)
180 concentrations were determined using Hach Carbonate AL-DT kit (Hach, Loveland, CO,
181 USA). For chemical analyses, 200 ml of water were initially filtered with Whatman GF/C
182 filters. The filtered samples were then analyzed by high pressure ion chromatography technic,
183 using a Thermo Scientific Dionex ICS1100 system (Thermo Fisher Scientific, Courtaboeuf,
184 France) for cations [sodium (Na^+), magnesium (Mg^{2+}) and ammonium (NH_4^+)] and a Thermo
185 Scientific Dionex Aquion system (Thermo Fisher Scientific, Courtaboeuf, France) for anions
186 (chloride (Cl^-) and nitrate (NO_3^-)).

187

188 ***Radiological characterization***

189 Gamma (γ -ray) dosimetry measurements were conducted just above the water surface near to
190 the emergence of each spring using a Colibri radiometer (Mirion Technology, CA, USA), as a
191 qualitative indicator of water radioactivity. Dose radiation (DR) measurements were
192 performed for 2 min. and repeated for at least two times in each mineral spring to reduce the
193 statistical uncertainties.

194 For the measurements of dissolved ^{222}Rn activity in each spring, Marinelli beakers (1 L)
195 were used to sample water from the mineral springs. A well-shaped Germanium detector
196 (Canberra) operated at Clermont Physics Laboratory (Université Clermont Auvergne,
197 Aubière, France) was used to measure the ^{222}Rn activity. For other radiological analyses,
198 water samples were collected in clean recipients from the water-column either near the
199 emergence of some springs or at its outlet. Since the distance of each mineral spring to the
200 laboratory was different, utmost care was taken to transport the water samples in refrigerated
201 boxes to the laboratory, where they were processed immediately.

202 In addition to characterizing the ambient radiation dose and the ^{222}Rn activity in water, it is
203 also important to determine the possible contribution of radioactivity in relation to dissolved
204 radionuclides. Among those present, we focused on the most abundant long-lived isotope
205 (^{238}U) as well as the descendants of this chain which are known to be highly radiotoxic (alpha
206 particle emitters), i.e. ^{226}Ra and ^{210}Po . The analyses were conducted at Subatech (Nantes,
207 France) and ICN (Nice, France) laboratories after PTFE filtration ($0.45\mu\text{m}$) and acidification.
208 Uranium was analyzed by ICP-QMS (Xseries 2, Thermo Electron). Ultra-trace concentrations
209 of ^{226}Ra were measured without chemical purification by HR-ICP-MS in low resolution mode
210 (Element XR, Thermo Scientific) with an APEX-Q high sensitivity desolvating sample
211 solution introduction system. In order to remove major polyatomic interferences from ^{226}Ra
212 spectrum in low resolution mode, preliminary analyses were performed in medium resolution
213 mode, where major interferences are resolved. The good consistency of ^{226}Ra concentration
214 measured in both low and medium resolution modes showed evidence of no significant
215 production rate of major interferences (Verlinde et al., 2019). The preference to use low
216 resolution mode for ^{226}Ra was characterized by a high sensitivity (4.7×10^5 CPS/Bq. ml^{-1}) and
217 a low instrumental detection limit (0.8 mBq. l^{-1}). Pb and Bi-based polyatomic interferences
218 unresolved in low and medium resolution modes were not considered in the present study due
219 to low signal intensities measured for Pb ($< 60,000$ CPS) and Bi ($< 1\ 000$ CPS). Finally, ^{210}Po
220 was measured, after spontaneous deposition onto a silver disc, by alpha spectrometry using
221 dual alpha spectrometers EG&G Ortec 576A equipped with boron-implanted silicon detectors
222 (Le et al., 2019).

223

224 ***Abundances of viruses and prokaryotes***

225 For enumeration of viruses (expressed as viral like particles, VLP) and prokaryotes by flow
226 cytometry, 2 ml of water samples were fixed with paraformaldehyde at 0.5% final
227 concentration and transported to the laboratory in quick time (< 2 hours) on ice and in the

228 dark for immediate analysis. The optimized flow cytometry protocol (different sample
229 dilutions, threshold levels, flow rates, etc.) for the enumeration of prokaryotes and viruses
230 from our previous investigations in French Massif Central lakes was adopted (Pradeep Ram et
231 al. 2014). Prior to analysis, samples were diluted with 0.02 μm filtered TE buffer (10 mM
232 Tris-HCl and 1 mM EDTA, pH 8) and stained with nucleic acid-specific green fluorescent
233 dye SYBR Green I (Molecular Probes, Oregon, USA) at a final concentration of 0.5×10^{-4}
234 (for viruses) and 1×10^{-4} (for prokaryotes) dilution of the commercial stock. For viral
235 samples, staining was carried out in the dark at 80 °C for 10 min, whereas for prokaryotic
236 samples it was done at room temperature for 15 minutes. Subsequently, the viral samples
237 were left to cool at room temperature for 5 minutes before analysis. Counts were performed
238 on a FACS Aria Fusion SORP cytometer (BD Sciences, San Jose, CA) equipped with an air-
239 cooled laser providing 50 mW at 488 nm with 502 long pass, and 530/30 band pass filter set-
240 up. Groups were identified and discriminated based on their 488 nm excited right angle light
241 scatter (SSC) and green fluorescence (530 nm wavelength) following the protocol optimized
242 by Brussaard et al. (2010). Flow cytometric data were analyzed using CellQuest Pro software
243 (BD Biosciences, San Jose, CA, USA; version 4.0). Control blanks consisting of TE buffer
244 with autoclaved 0.2 μm filtered sample at same dilution factor as the natural samples were
245 used before proceeding with sample analysis. Very low coincidence and background
246 fluorescent levels were detected. Identification of viral and prokaryote population via flow
247 cytometry in a mineral spring sample together with a control blank are shown in
248 Supplemental Figure 1.

249

250 *Statistical analysis*

251 Firstly, all environmental and microbial data (averaged from replicates from each sampled
252 site) which do not follow normal distribution were standardized using the z-score
253 normalization [(x-mean)/standard deviation)]. This transformation allows to compare
254 variables that initially had different units. Then, a Hierarchical clustering also called
255 ascending hierarchical classification (AHC) based on Pearson correlation was performed to
256 identify the groups of springs that showed environmental similarities. The groups were
257 merged with simple link or nearest neighbor grouping. Secondly, microbial abundances (i.e.
258 prokaryotes and viruses) were log transformed and relationships between environmental and
259 microbial variables were tested using Pearson correlation. We ordinated the relation of
260 microbial variables with abiotic variables through a non-metric multidimensional scaling

261 plots (NMDS) scaling using the ‘metaMDS’ function with R Package Vegan in R version 2.6-
262 2. Euclidian distance was used to measure distribution between samples.

263 Finally, the mineral springs were classified according to their radioactivity levels to test the
264 difference in microbial abundances between classes by using the Kruskal-Wallis test. Gamma
265 (γ) dose radiation (DR) categories were created according to the mean background DR in
266 these sites which ranged between 70 and 250 nGy.h⁻¹. The radioactivity classes for four
267 radionuclides variables (i.e. ²²²Rn, ²³⁸U, ²¹⁰Po and ²²⁶Ra) were determined from the orders of
268 magnitude observed for these variables in the sampled springs (Supplementary Table 2).

269

270 **Results**

271 *Environmental variables*

272 The environmental characteristics of the studied spring sites are shown in supplementary
273 material (Supplementary Table 1). The mean \pm SD conductivity of the springs was 1369 \pm
274 2816 μ S.cm⁻¹ with a highest value (11,830 μ S.cm⁻¹) recorded in the most mineralized spring
275 (Croizat). pH did not vary among the springs (Mean \pm SD = 6.6 \pm 0.1). Water temperature
276 varied between 9.9 °C and 36.4 °C (Mean \pm SD = 14.7 \pm 6.2 °C). Gamma radiation which
277 presented a natural radioactivity gradient in radiation dose ranged from 120 nGy.h⁻¹ (Bard 2)
278 to 1491 nGy.h⁻¹ (Montagne 1) with a mean value of 396 \pm 347 nGy.h⁻¹. For the radionuclides
279 activities, ²³⁸U ranged from 0.003 to 0.196 Bq. l⁻¹ (Mean \pm SD = 0.03 \pm 0.05 Bq. l⁻¹). ²²²Rn
280 activity varied between 5.6 and 3,110 Bq. l⁻¹ (Mean \pm SD = 552.6 \pm 897.4 Bq. l⁻¹). ²²⁶Ra had
281 values between 0.14 and 3.2 Bq. l⁻¹ (Mean \pm SD = 1.4 \pm 0.9 Bq. l⁻¹). And finally, ²¹⁰Po showed
282 values ranging from 10 to 392 μ Bq. l⁻¹ (Mean \pm SD = 57.1 \pm 101.6 μ Bq. l⁻¹).

283 *Viral and prokaryotic abundances in mineral springs with variable levels of radioactivity*

284 Both viral (VA) and prokaryotic abundances (PA) were found to vary by an order of
285 magnitude among the mineral springs (Fig. 2, Supplementary Table 3). VA and PA ranged
286 from 4.2 x 10⁷ to 88.5 x 10⁷ VLP l⁻¹ (mean \pm SD = 21.1 \pm 21.1 x 10⁷ VLP l⁻¹) and 3.12 x 10⁷
287 to 20 x 10⁷ cells l⁻¹ (mean \pm SD = 3.1 \pm 5.3 x 10⁷ cells l⁻¹), respectively. The maxima of VA
288 and PA were observed in different springs (Supplementary Table 3). The virus to prokaryote
289 ratio (VPR) varied between 1.8 (Salut) and 144.2 (Estreys) with a mean \pm SD of 30.5 \pm 37.9.
290 Interestingly VA showed a significant negative correlation with ²²²Rn activity (R= - 0.56,
291 p<0.05) (Table 1). VA and PA were not correlated with the other radionuclides activities
292 (²³⁸U, ²¹⁰Po, ²²⁶Ra) (Table 1).

293 ***Relationship of viral and prokaryotic abundances with abiotic variables***

294 The NMDS ordination allowed the samples to be distinguished into two separate groups (Fig.
295 3). PA and VA was clustered by abiotic variables such as conductivity, temperature, DR, pH,
296 Na, Cl, No, HCO and ²²²Rn whereas VPR was clustered by abiotic variables such as DO, Mg,
297 ²¹⁰Po, U, ²²⁶Ra. However, correlation of microbial abundances with physico-chemical
298 parameters were less robust. VA was weakly correlated ($p < 0.05$) with nitrates (NO_3^-) (0.58,
299 $p > 0.05$), DR (0.52, $p > 0.05$) and ²²²Rn (0.59, $p > 0.05$) (Table 1). Among the sampled mineral
300 springs, correlation between VA and PA was non-significant ($r = 0.12$, $p > 0.05$) (Table 1).

301 Five clusters which were created by similarity between the samples (Pearson) (Fig. 4)
302 was grouped into two major ones. The first cluster (containing the blue and black clusters)
303 was associated with five samples exhibiting high doses of gamma radiation (radiation dose \geq
304 500 nGy.h^{-1}) and ²²²Rn (activity $> 400 \text{ Bq.l}^{-1}$), whereas the second (containing the green, grey
305 and blue clusters) was predominantly associated with samples that represented lower levels of
306 radioactivity.

307 Additionally, Kruskal-Wallis test showed significant differences ($p < 0.001$) between
308 in VA between springs with low (mean \pm SD = $16.0 \pm 7.5 \times 10^7 \text{ VLP.l}^{-1}$) and medium (mean \pm
309 SD = $41.5 \pm 27.8 \times 10^7 \text{ VLP.l}^{-1}$) DR (Fig. 5A). Low VA were measured in springs with high
310 DR but no significant difference was determined by the test. Also, Kruskal-Wallis showed
311 significant differences ($p < 0.03$) in VA between springs with low (mean \pm SD = $21.3 \pm 7.0 \times$
312 10^7 VLP.l^{-1}) and high (mean \pm SD = $6.9 \pm 3.4 \times 10^7 \text{ VLP.l}^{-1}$) level of ²²²Rn activity (Fig. 5B).
313 Such variations or differences were not observed for PA (Supplementary Fig. 2), and VPR.

314

315 **Discussion**

316 The spring waters collected across the study site provided a compelling opportunity to test
317 and evaluate the impact of natural radioactivity through the gamma radiation dose and
318 radionuclides activities on virus-prokaryote relationship.

319 Flow cytometry enumeration of viral abundances in the mineral springs fell within the
320 lower end of values (10^7 - $10^{10} \text{ VLP.l}^{-1}$) as previously reported from environments like aquifers
321 (Roudnew et al., 2013) and hypersaline lakes (Jiang et al., 2004) springs. Large variability in
322 viral and prokaryote abundances by one and two orders of magnitude respectively could be
323 attributed to the prevailing conditions in different springs. Differences and variability in
324 conductivity and temperature are mainly related to the depth at which groundwater has
325 infiltrated and then, to its speed to reach the surface (Boineau & Maisonneuve, 1972). In fact,

326 the adaptability and flexibility of viruses to survive in the most mineralized spring (Croizat)
327 with a conductivity of $11,830 \mu\text{S}\cdot\text{cm}^{-1}$ suggest that phages are an important component of
328 crenic communities. Studies investigating freshwater viromes have attributed to large
329 differences in viral diversity to salinity (Tseng et al. 2013) and conductivity gradients (Roux
330 et al. 2012). Significant relationship of nitrate to viruses rather than to prokaryotes in our
331 study were similar to a report from a freshwater reservoir (Peduzzi and Schiemer, 2004), but
332 however the role of nitrate in influencing viral abundance is unclear and less discussed in
333 literature.

334 Viruses are host specific and largely rely on the availability of susceptible host cells
335 (mainly prokaryotes) for their proliferation. However, in contrast to reports from other
336 freshwaters systems (Peduzzi and Schiemer 2004, Pradeep Ram et al. 2014), viral and
337 prokaryote abundances obtained in our study were not significantly correlated. Since mineral
338 springs are restrictive and oligotrophic environments (Reiss and Chiffard 2017), it is likely
339 that the nature and dynamics of viral strategy could influence viral and prokaryote counts in
340 such harsh or stressful conditions. The fact that prokaryotes serve as dominant host for viruses
341 always remains a difficult hypothesis to test. The application of recent molecular tools would
342 help to identify specific host–virus relationships and assess their interactions.

343 Virus-to-prokaryote ratio (VPR) has often been a descriptor of relationship between
344 viruses and prokaryotes in a given environment (Parikka et al. 2017). VPR is the result of a
345 comprehensive balance of factors, such as viral production, transport of viruses through
346 sinking particles, decay rates and life strategies (Wigington et al., 2016). Large variation of
347 VPR by two orders of magnitude among the springs (1.4 to 144) is much higher than reports
348 from hot springs located in Southern Hemisphere (0.1 to 6.0, Parikka et al. 2018), but similar
349 to the reported range in near surface oceans (1.4 to 160, Wigington et al. 2016). The broad
350 distribution of VPR together with a lack of significant relationship between VA and PA in the
351 springs imply that the increase in the former is a poor qualitative predictor of latter. The
352 observed increase in PA along with VPR has been attributed to direct dependence of viral
353 production on PA, thus imposing additionally a possible selective pressure that can lead to a
354 reduced volume of host cells (Hara et al. 1996). Low VPR observed in some springs could
355 either be as a result of host cell resistance or specific phage adsorption to host when their
356 diversity is low (Maranger et al. 1994, Tuomi et al. 1995). In our study, none of the abiotic
357 factors were found to explain for the variation in VPR which was probably due to the
358 differences in the prevailing environmental parameters among the study habitats. It is more
359 likely that the factor(s) influencing VPR could be specific to each spring.

360 In the mineral springs, ^{222}Rn accounted for the highest nuclear activity (number of
361 disintegrations per time unit) compared to others radionuclides. Adverse effect of water
362 radioactivity on viral abundance, as seen by a significant negative correlation between VA
363 and ^{222}Rn activity could perhaps corroborate our hypothesis on the drastic effect of ionizing
364 radiation on viruses. However, the correlation between VA and the other radionuclides
365 activities (^{238}U , ^{226}Ra and ^{210}Po) was not evident. VA varied between springs with low and
366 medium DR. The low mean value of VA in springs with high ^{222}Rn activity suggests that viral
367 decay could be more important in these habitats.

368 Ionizing radiation or exposure to alpha particles from radon is known to generate and
369 induce DNA mutations, chromosomes aberrations and other cellular abnormalities such as
370 protein modification (Robertson et al., 2013; Reisz et al., 2014). Also, viral inactivation has
371 been reported in the context of intense γ -ray irradiation (Sullivan et al., 1971). Irradiation of
372 lambda phage induces DNA strand breakage and lead to the inactivation of phages (Bertram,
373 1988). The findings from laboratory investigations could perhaps extend support to aquatic
374 microbes, where the presence of high levels of radon might affect viral capsid integrity and
375 prokaryotes membranes. However, phages too can appear to be more resistant to ionising
376 radiations (Lea, 1946). In the case of prokaryotes, their membrane that enables nutrient
377 exchange between the cell and the environment can absorb radionuclides and be subjected to
378 external and internal irradiation. Although it has been previously hypothesized that micro-
379 organisms are known to have colonised the spring ecosystems billions of years ago (Djokic et
380 al. 2017), radioactivity arising from natural origin could be a stressor for them and affect the
381 relationship between these two protagonists. In such a scenario it could be interesting to
382 identify the adaptive strategy of viruses (lytic, lysogeny or pseudolysogeny) under
383 challenging environmental conditions. Therefore, investigations pertaining to viral infection
384 in mineral springs can provide important information on virus-prokaryote interaction and their
385 mediated biogeochemical processes.

386 In conclusion, no significant correlation was found between viruses and prokaryotes in
387 the analyzed spring waters. Among different parameters considered for natural radioactivity
388 (radon activity, radiation dose, dissolved radionuclide activity), radon activity appears to be
389 the most influential parameter. Low viral abundances in springs amidst of high levels of ^{222}Rn
390 activity and vice versa are suggestive of drastic effect of ionizing radiation on viruses which
391 could possibly be linked to viral decay due to DNA damage.

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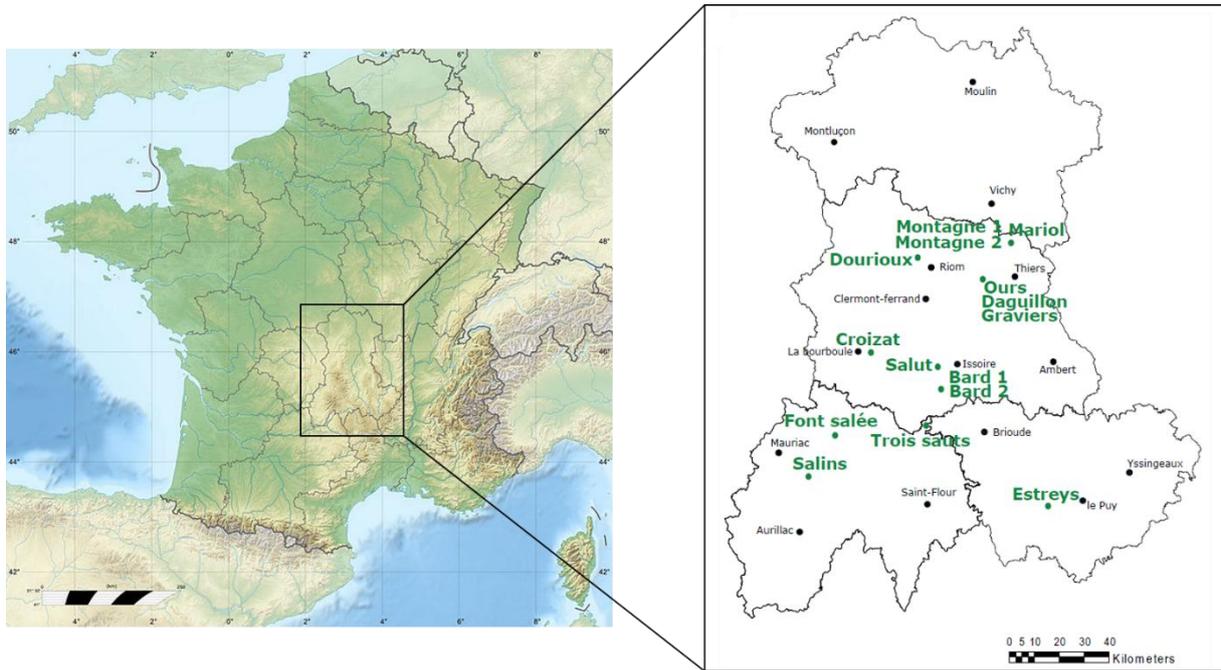
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550 **Fig. 1** Map showing the location of 15 mineral springs (green) in the Auvergne region of
551 French Massif Central

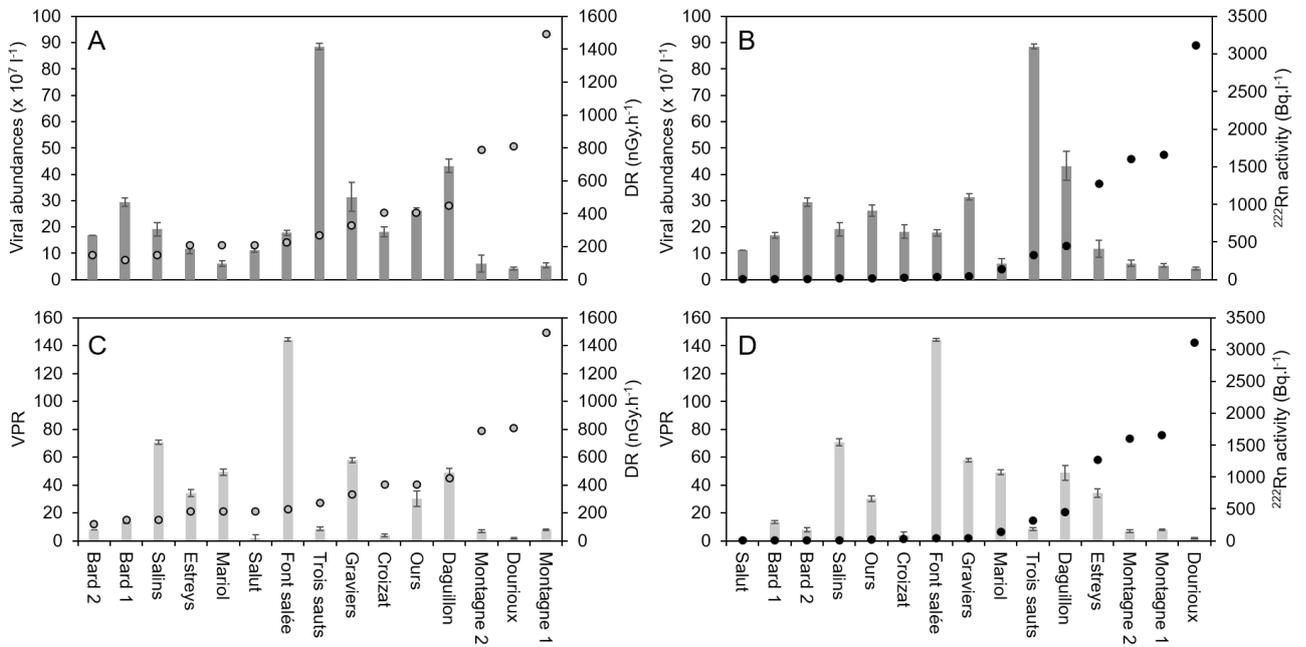


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555 **Fig. 2** Histograms showing viral abundances (A) together with dosage radiation, DR and
 556 (B) ^{222}Rn activity and, VPR (virus to prokaryote ratio) with (C) dosage radiation, DR and
 557 (D) ^{222}Rn activity for each spring. On each histogram, the mineral springs are classified with
 558 an increasing radioactivity gradient (DR and ^{222}Rn represented in grey and black circles
 559 respectively). Data represent mean \pm SD ($n = 3$) for each sampled spring.



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Figure 3: Non-metric multidimensional scaling (NMDS, stress value = 1) illustrating similarity between samples. Relations between biotic and abiotic variables are represented. HCO: HCO_3^- ; Na: Na^+ ; Cl: Cl^- ; Mg: Mg^{2+} ; NO: NO_3^- ; NH: NH_4 ; Cond: conductivity; Temp: temperature; DO: dissolved oxygen; U^{238} : U activity; Ra: ^{226}Ra ; Rn: ^{222}Rn ; Po: ^{210}Po ; DR: dose radiation; VA: viral abundance, PA: prokaryote abundance; VPR: viral-prokaryote ratio.

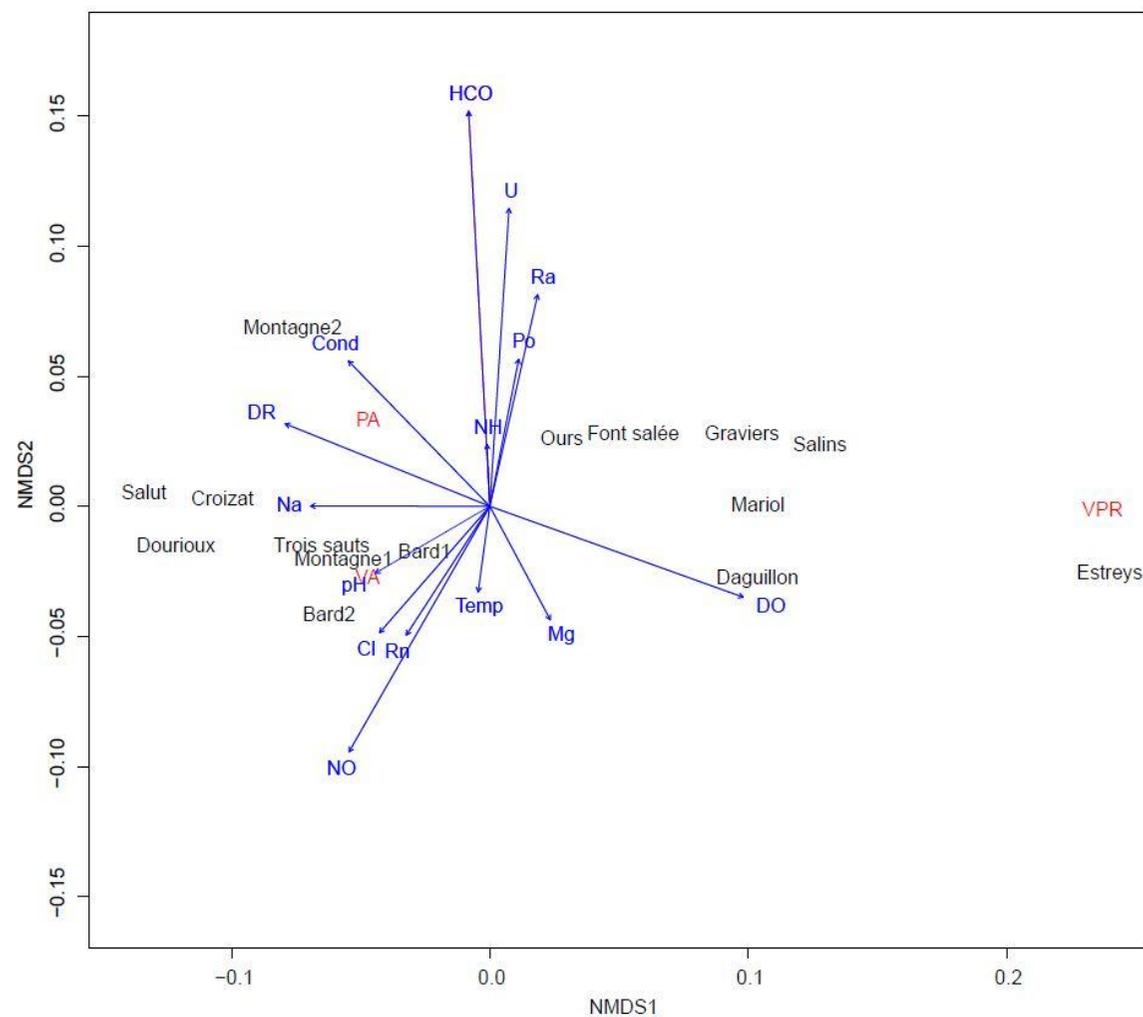
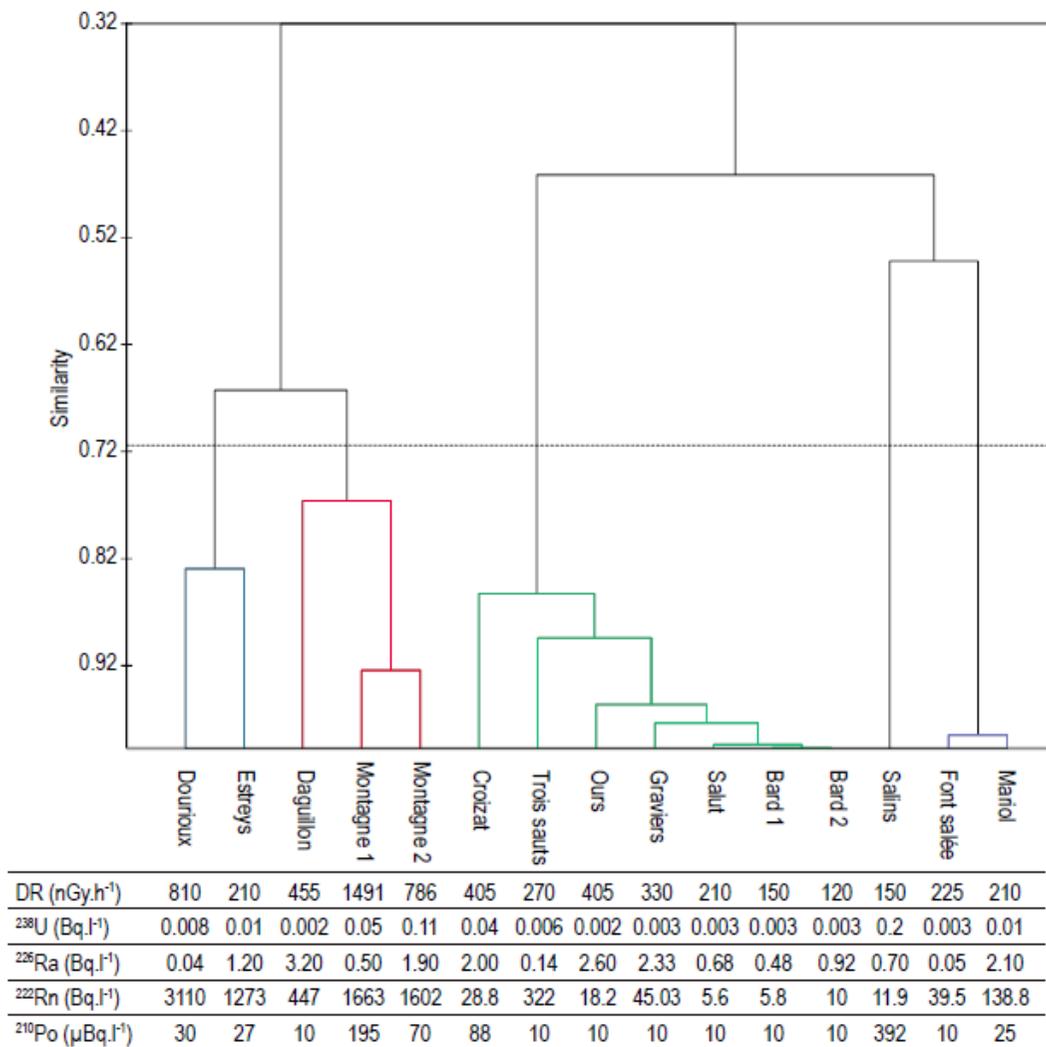


Fig. 4 Dendrogram based on hierarchical ascendant classification analysis (HAC) of springs performed on physical, chemical and radioactive parameters measured. DR: dose radiation, ^{222}Rn : ^{222}Rn activity, ^{238}U : ^{238}U activity, ^{210}Po : ^{210}Po activity, ^{226}Ra : ^{226}Ra activity.



Values of radioactive parameters measured in each springs

Figure 5: Kruskal-Wallis tests on differences in the mean viral abundances in mineral springs classified by (A) gamma (γ) dose radiation (DR) (3 classes: Low: $DR < 250 \text{ nGy.h}^{-1}$ (7 sites); Medium: $250 \text{ nGy.h}^{-1} \geq DR \leq 500 \text{ nGy.h}^{-1}$ (5 sites); High: $DR > 500 \text{ nGy.h}^{-1}$ (3 sites) and (B) ^{222}Rn activity levels (3 classes: Low: activity $< 100 \text{ Bq.l}^{-1}$ (8 sites); Medium: $100 \text{ Bq.l}^{-1} \geq \text{activity} \leq 1000 \text{ Bq.l}^{-1}$ (3 sites); High: activity $> 1000 \text{ Bq.l}^{-1}$ (4 sites). Red crosses represent the mean value with SD calculated for each level based on the number of sites. Star indicates significant p value ($p < 0.05$).

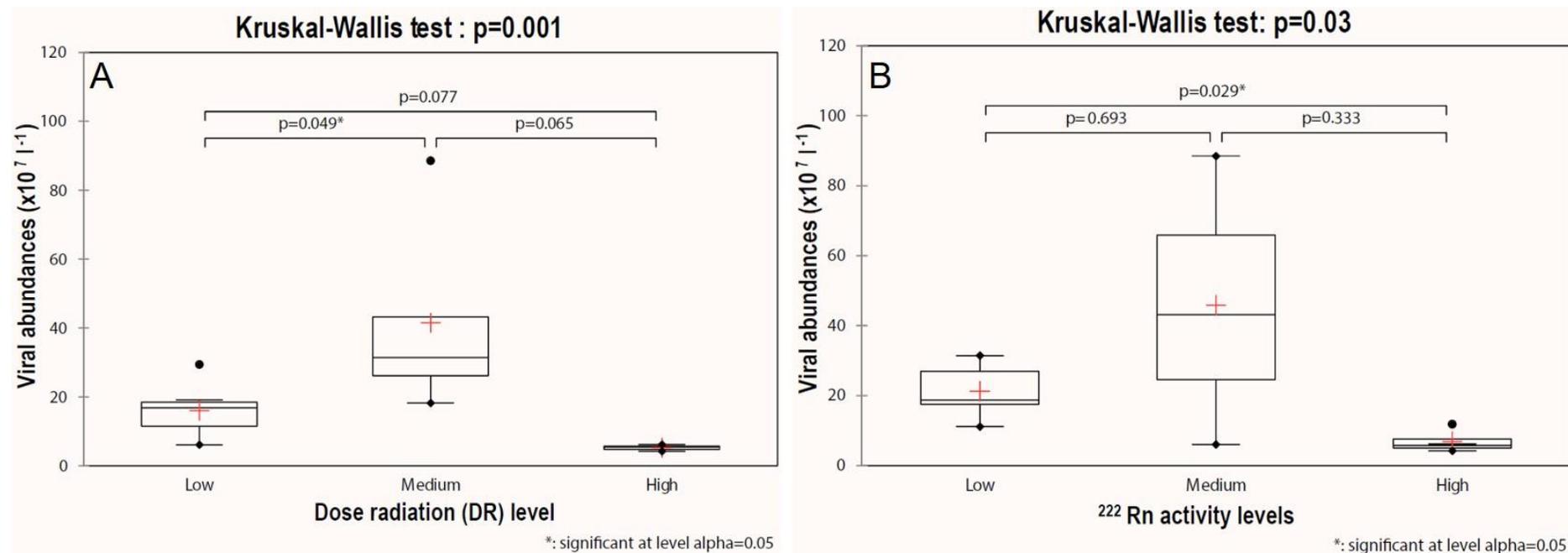


Table 1 Pearson correlation coefficient (r) between microbial and abiotic parameters (n=15).

	VA	PA	VPR	pH	DO	Temp	Cond	Na ⁺	Mg ²⁺	Cl ⁻	NO ₃ ⁻	NH ₄ ⁺	HCO ₃ ⁻	²³⁸ U	²²⁶ Ra	²²² Rn	²¹⁰ Po
VA	1.00	0.12	0.17	0.39	0.51	0.17	-0.02	0.08	0.46	0.20	-0.58	0.38	-0.40	-0.18	0.09	-0.59	-0.17
PA	0.12	1.00	-0.03	0.18	0.12	-0.09	0.05	0.01	0.11	-0.03	-0.36	0.25	0.47	0.41	0.27	-0.30	0.16
VPR	0.17	-0.03	1.00	-0.29	0.45	-0.01	-0.25	-0.21	0.17	-0.16	-0.31	-0.05	-0.25	0.13	0.15	-0.07	0.14
pH	0.39	0.18	-0.29	1.00	0.05	-0.21	-0.26	-0.20	0.60	-0.13	0.05	0.28	-0.22	0.20	-0.22	-0.12	0.17
DO	0.51	0.12	0.45	0.05	1.00	0.15	0.02	0.10	0.25	0.10	-0.12	0.33	-0.52	0.15	0.51	-0.11	0.19
Temp	0.17	-0.09	-0.01	-0.21	0.15	1.00	0.90	0.94	-0.08	0.94	-0.28	0.31	-0.33	-0.02	0.26	-0.32	0.00
Cond	-0.02	0.05	-0.25	-0.26	0.02	0.90	1.00	0.99	-0.27	0.92	-0.11	0.17	-0.06	0.09	0.17	-0.15	0.08
Na ⁺	0.08	0.01	-0.21	-0.20	0.10	0.94	0.99	1.00	-0.17	0.96	-0.14	0.26	-0.21	0.04	0.18	-0.20	0.05
Mg ²⁺	0.46	0.11	0.17	0.60	0.25	-0.08	-0.27	-0.17	1.00	0.00	-0.45	0.74	-0.33	0.19	-0.06	-0.56	0.21
Cl ⁻	0.20	-0.03	-0.16	-0.13	0.10	0.94	0.92	0.96	0.00	1.00	-0.24	0.41	-0.30	-0.09	0.18	-0.29	-0.07
NO ₃ ⁻	-0.58	-0.36	-0.31	0.05	-0.12	-0.28	-0.11	-0.14	-0.45	-0.24	1.00	-0.49	-0.15	-0.02	-0.22	0.89	0.10
NH ₄ ⁺	0.38	0.25	-0.05	0.28	0.33	0.31	0.17	0.26	0.74	0.41	-0.49	1.00	-0.31	-0.06	0.37	-0.69	-0.02
HCO ₃ ⁻	-0.40	0.47	-0.25	-0.22	-0.52	-0.33	-0.06	-0.21	-0.33	-0.30	-0.15	-0.31	1.00	0.20	0.02	0.01	-0.04
²³⁸ U	-0.18	0.41	0.13	0.20	0.15	-0.02	0.09	0.04	0.19	-0.09	-0.02	-0.06	0.20	1.00	-0.16	0.06	0.89
²²⁶ Ra	0.09	0.27	0.15	-0.22	0.51	0.26	0.17	0.18	-0.06	0.18	-0.22	0.37	0.02	-0.16	1.00	-0.16	-0.28
²²² Rn	-0.59	-0.30	-0.07	-0.12	-0.11	-0.32	-0.15	-0.20	-0.56	-0.29	0.89	-0.69	0.01	0.06	-0.16	1.00	0.04
²¹⁰ Po	-0.17	0.16	0.14	0.17	0.19	0.00	0.08	0.05	0.21	-0.07	0.10	-0.02	-0.04	0.89	-0.28	0.04	1.00

VA: viral abundances, PA: Prokaryotes abundances, Na: Sodium, Mg: Magnesium, Cl: Chlorides, NO₃: Nitrates, HCO₃: Carbonates, Cond: Conductivity, DO: Percentage of dissolved oxygen, Temp: Temperature, DR: Gamma (γ) dose radiation, ²²²Rn: ²²²Rn activity, ²³⁸U: ²³⁸U activity, ²¹⁰Po: ²¹⁰Po activity, ²²⁶Ra: ²²⁶Ra activity. Values in bold are significant at p value (alpha=0.05).