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

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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], Télesphore Sime-Ngando [1], 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

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ORCID :
Lory-Anne Baker https://orcid.org/0000-0002-8346-8578
David G. Biron https://orcid.org/0000-0002-6531-4647
Aude Beauger https://orcid.org/0000-0002-0911-0500
Sofia Kolovi https://orcid.org/0000-0003-4778-2823
Jonathan Colombet https://orcid.org/0000-0003-3763-6566
Elisabeth Allain https://orcid.org/0000-0002-6411-5873
Olivier Voldoire https://orcid.org/0000-0003-1306-3054
Vincent Breton https://orcid.org/0000-0001-8197-7080
Telesphore Sime-Ngando https://orcid.org/0000-0002-7240-5803
Karine David https://orcid.org/0000-0003-0977-5905
Gilles Montavon https://orcid.org/0000-0002-3049-0106
Angia Sriram Pradeep Ram https://orcid.org/0000-0002-5469-0452

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*Corresponding author:
Angia Sriram Pradeep Ram,
Laboratoire Microorganismes : Génome et Environnement,
UMR CNRS 6023,
Université Clermont-Auvergne,
1 Impasse Amélie Murat,
63178, Aubière Cedex,
France
Email : pradeep_ram.angia_sriram@uca.fr
Telephone: +33 473407463 Fax: +33 473407670

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

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

Introduction

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

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

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

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

Materials and methods

Site description and sampling

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

Physical and chemical analyses

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

Radiological characterization

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

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

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

Abundances of viruses and prokaryotes

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

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

Statistical analysis

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

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

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

Results

Environmental variables

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

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

Both viral (VA) and prokaryotic abundances (PA) were found to vary by an order of magnitude among the mineral springs (Fig. 2, Supplementary Table 3). VA and PA ranged from 4.2 x 10^7 to 88.5 x 10^7 VLP l^{-1} (mean ± SD = 21.1 ± 21.1 x 10^7 VLP l^{-1}) and 3.12 x 10^7 to 20 x 10^7cells l^{-1} (mean ± SD = 3.1 ± 5.3 x 10^7 cells l^{-1}), respectively. The maxima of VA and PA were observed in different springs (Supplementary Table 3). The virus to prokaryote ratio (VPR) varied between 1.8 (Salut) and 144.2 (Estreys) with a mean ± SD of 30.5 ± 37.9. Interestingly VA showed a significant negative correlation with ^{222}Rn activity ($R = -0.56$, $p<0.05$) (Table 1). VA and PA were not correlated with the other radionuclides activities (^{238}U, ^{210}Po, ^{226}Ra) (Table 1).

Relationship of viral and prokaryotic abundances with abiotic variables

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

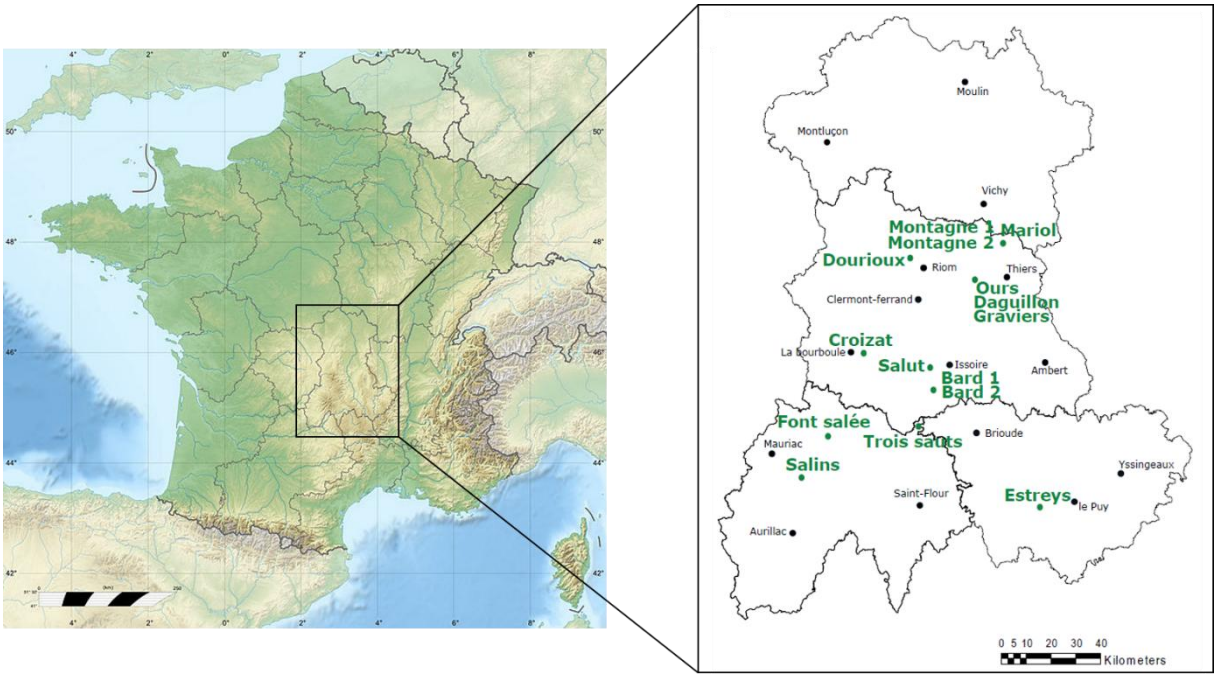


Fig. 2 Histograms showing viral abundances (A) together with dosage radiation, DR and (B) ^{222}Rn activity and, VPR (virus to prokaryote ratio) with (C) dosage radiation, DR and (D) ^{222}Rn activity for each spring. On each histogram, the mineral springs are classified with an increasing radioactivity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

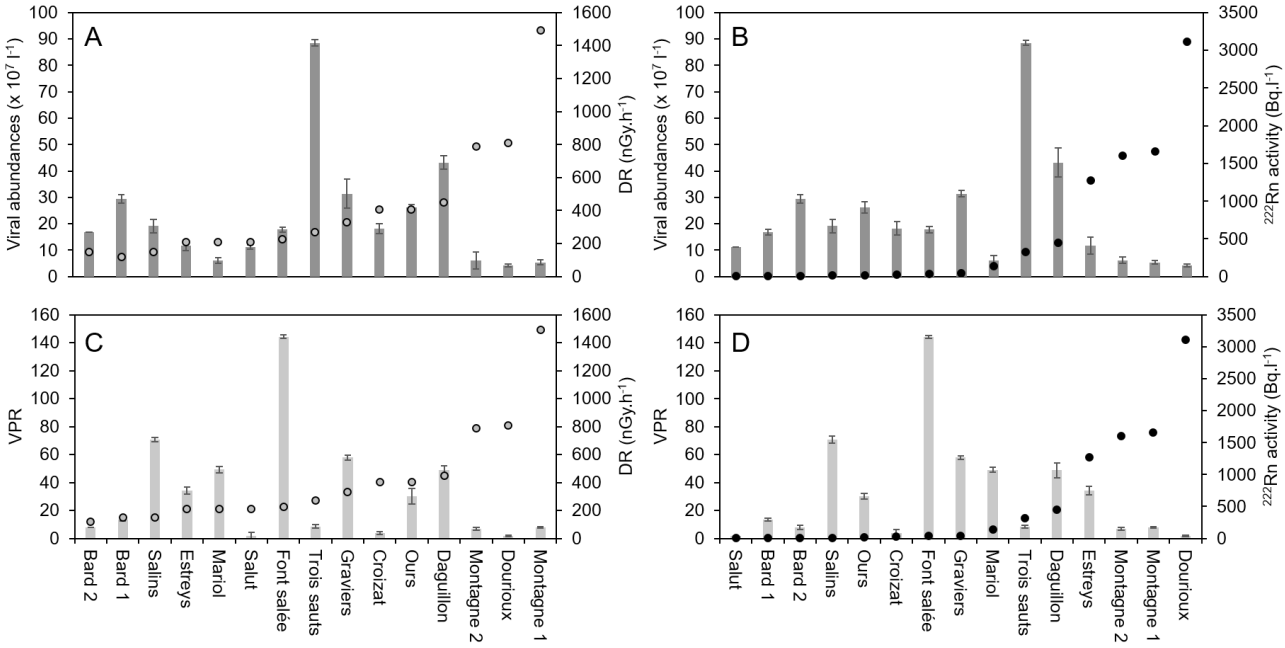


Figure 3: Non-metric multidimensional scaling (NMDS, stress value = 1)) illustrating similarity between samples. Relations between biotic and abiotic variables are represented. HCO: HCO_3^-; Na: Na^+; Cl: Cl^-; Mg: Mg^{2+}; NO: NO_3^-; NH: NH_4; Cond: conductivity; Temp: temperature; DO: dissolved oxygen; 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.

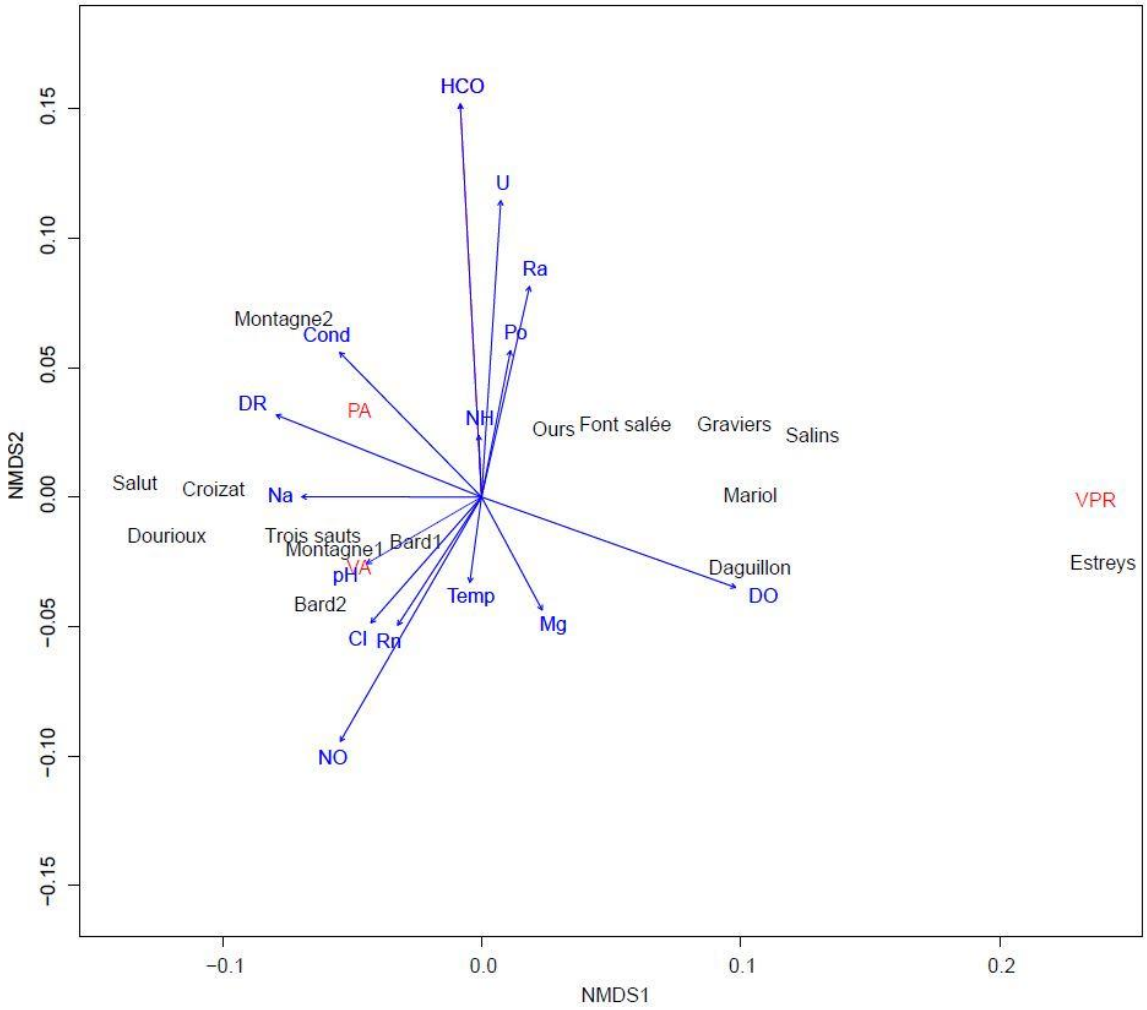
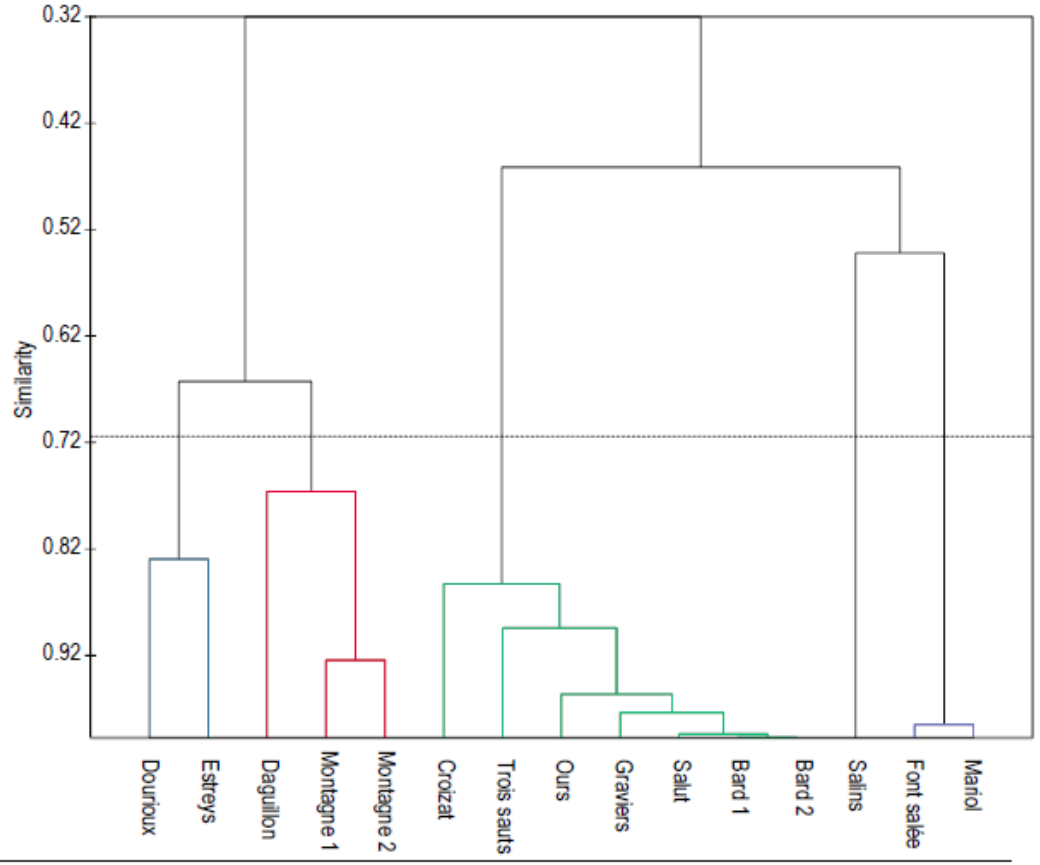


Fig. 4 Dendrogram based on hierarchical ascendant classification analysis (HAC) of springs performed on physical, chemical and radioactive parameters measured. DR: dose radiation, ^{222}Rn: ^{222}Rn activity, ^{238}U: ^{238}U activity, 2^{10}Po: ^{210}Po activity, ^{226}Ra: ^{226}Ra activity.



	Dourioux	Estreys	Daguillon	Montagne 1	Montagne 2	Croizat	Trois sauts	Ours	Graviers	Salut	Bard 1	Bard 2	Salins	Font salée	Mañol
DR (nGy.h^{-1})	810	210	455	1491	786	405	270	405	330	210	150	120	150	225	210
^{238}U (Bq.l^{-1})	0.008	0.01	0.002	0.05	0.11	0.04	0.006	0.002	0.003	0.003	0.003	0.003	0.2	0.003	0.01
^{226}Ra (Bq.l^{-1})	0.04	1.20	3.20	0.50	1.90	2.00	0.14	2.60	2.33	0.68	0.48	0.92	0.70	0.05	2.10
^{222}Rn (Bq.l^{-1})	3110	1273	447	1663	1602	28.8	322	18.2	45.03	5.6	5.8	10	11.9	39.5	138.8
^{210}Po (µBq.l^{-1})	30	27	10	195	70	88	10	10	10	10	10	10	392	10	25

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 nGy.h^{-1} (7 sites); Medium: 250 nGy.h^{-1}≤ DR ≤ 500 nGy.h^{-1} (5 sites); High: DR > 500 nGy.h^{-1} (3 sites) and (B) ^{222}Rn activity levels (3 classes: Low: activity < 100 Bq.l^{-1} (8 sites); Medium: 100 Bq.l^{-1} ≤ activity ≤ 1000 Bq.l^{-1} (3 sites); High: activity > 1000 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).

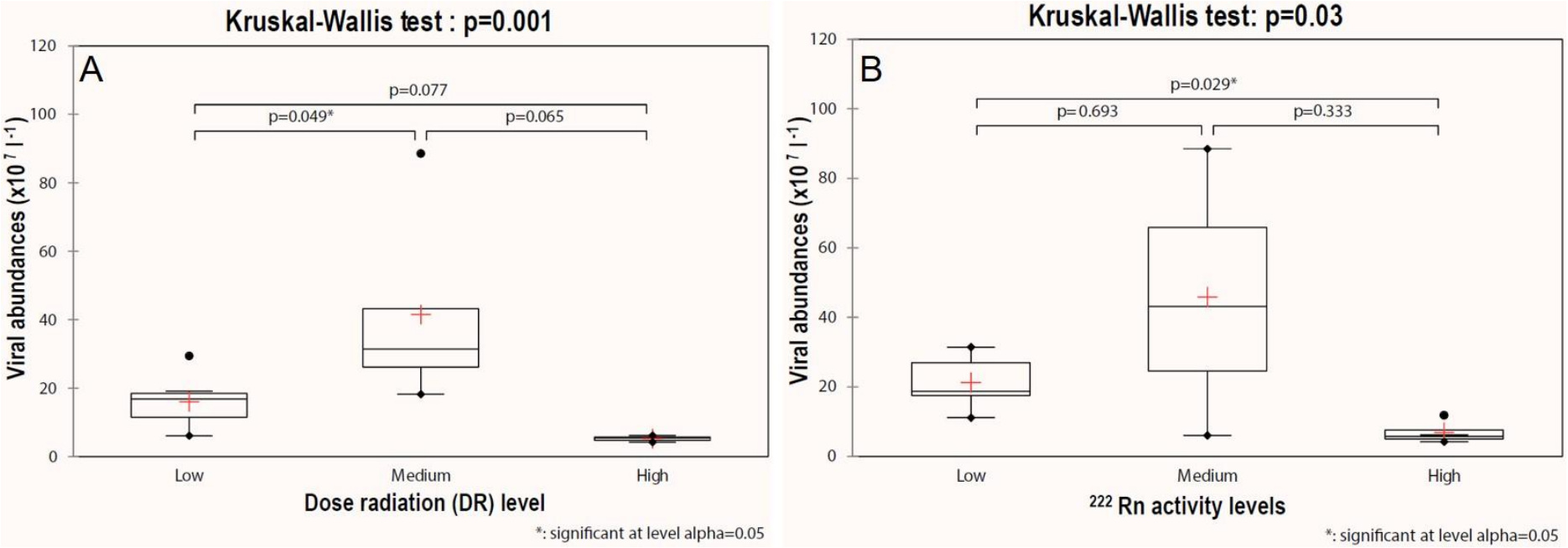


Table 1 Pearson correlation coefficient (r) between microbial and abiotic parameters (n=15).

	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, NO_3: Nitrates, HCO_3: Carbonates, Cond: Conductivity, DO: Percentage of dissolved oxygen, Temp: Temperature, DR: Gamma (γ) dose radiation, ^{222}Rn: ^{222}Rn activity, ^{238}U: ^{238}U activity, ^{210}Po: ^{210}Po activity, ^{226}Ra: ^{226}Ra activity. Values in bold are significant at p value (alpha=0.05).