Radiation exposure of microorganisms living in radioactive mineral springs

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Abstract. The TIRAMISU collaboration gathers expertise from biologists, physicists, radiochemists and geologists within the Zone-Atelier Territoires Uranifères (ZATU) in France to analyze the radiation exposure of microorganisms living in naturally radioactive mineral springs. These springs are small waterbodies that are extremely stable over geological time scales and display different physicochemical and radiological parameters compared to their surroundings. Water and sediment samples collected in 27 mineral springs of the volcanic Auvergne region (Massif Central, France) have been studied for their microbial biodiversity and their radionuclide content. Among the microorganisms present, microalgae (diatoms), widely used as environmental indicators of water quality, have shown to display an exceptional abundance of teratogenic forms in the most radioactive springs studied (radon activity up to 3700 Bq/L). The current work presents a first assessment of the dose received by the diatoms inhabiting these ecosystems. According to ERICA tool, microorganisms living in most of the sampled mineral springs were exposed to dose rates above 10 µGy/h due to the large concentration of radium in the sediments (up to 50 Bq/g). Radiological analyses of water and sediments were used as inputs to Monte Carlo simulations at micro- (GATE) and nano- (Geant4-DNA) scale in order to assess the direct and indirect damages on the diatom DNA.

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1 Introduction

Naturally radioactive mineral springs can be described as isolated ecosystems of various physicochemical properties with elevated levels of radionuclide content in comparison to their surroundings in which minerals and radioisotopes of the three natural decays series (^{238}U, ^{232}Th and ^{235}U) are abundant [1,2]. Specifically, ^{226}Ra ($t_{1/2}$ = 1600 y) and its gaseous descendant ^{222}Rn ($t_{1/2}$ = 3.82 d), both emitting alpha particles of maximum energy 4.8 MeV and 5.5 MeV respectively [3], are found in high concentrations in the sediment and the water of the mineral springs. Recent studies concerning organisms that live in naturally radioactive environments have already shown a correlation between mutation rates and radioactivity [4].

Auvergne, situated in Massif Central of France, is a volcanic region rich in mineral springs and high uranium content [5]. Diatoms, a species of unicellular microalgae, thrive in these peculiar environments [6]. It has been recently observed that they display an exceptional abundance of teratogenic forms in the most radioactive springs in Auvergne [8].

Diatoms are eukaryotic, photosynthetic microorganisms, accounting for a great part of the carbon dioxide fixation in the aquatic environments. Their dimensions are of the order of a few micrometres and they are uniquely characterized by their frustule, a silicate membrane serving as a skeleton [9] which remains as fossil after their death [10]. In addition to being used as water quality bio-indicators due to their sensitivity and direct response to environmental stresses [11], diatoms are now studied for their response to natural radioactivity in the frame of TIRAMISU project.

The TIRAMISU (biodiversiTy in the RAdioactive Mineral Springs in Auvergne) project is dedicated to the study of the radiological and radiotoxic effects of natural radioactivity on microorganisms living in naturally radioactive mineral springs within ZATU [12]. In the current study, we are focusing on the radiological content of these ecosystems and the simulation of the radiation exposure on diatoms.

In order to characterize the ecosystems, campaigns of sampling were launched including the collection of water, diatoms, sediment, and travertine cores, in 27 locations. The radiological content of the water and sediment samples was analysed using γ -spectroscopy. The study and calculation of the radiation effects on diatoms has been feasible due to Monte Carlo Simulation Codes (MCSC). Especially at the nanoscale, the stochastic nature of the interactions responsible for the DNA damage due to ionizing radiation, make the use of MCSC an essential part of radiological studies [13].

In the current study, an evaluation of the dose rate based on the measured activity values for the biota of freshwater ecosystems is initially performed using the ERICA tool. Microdosimetric calculations follow using GATE, while nanodosimetry is covered by the use of Geant4-DNA.

2 Materials and Methods

2.1 Sampling and Analysis by γ - Spectrometry

Water samples have been collected from 27 mineral springs in Auvergne, Massif Central, France (Figure 1). A High-Purity Germanium (HPGe) well-type detector was used for the spectroscopic analyses at LPC (Clermont Ferrand, France). For the determination of the ^{222}Rn activity, measurements were conducted at least 4 hours post sampling in order to use the 352 keV γ-ray line of ^{214}Pb ($t_{1/2}$ = 27.06 min) under the assumption of radioactive equilibrium.

Sediment samples were also collected from the different sites. The measurements for the determination of ^{226}Ra activity were conducted at least four weeks after the sealing, allowing for the radioactive equilibrium among ^{226}Ra, ^{222}Rn, ^{214}Pb and ^{214}Bi to be reached. These γ- spectroscopic analyses took place at Subatech (Nantes, France) using a coaxial HPGe detector. The data were then used as an input to the simulations described in the next section.
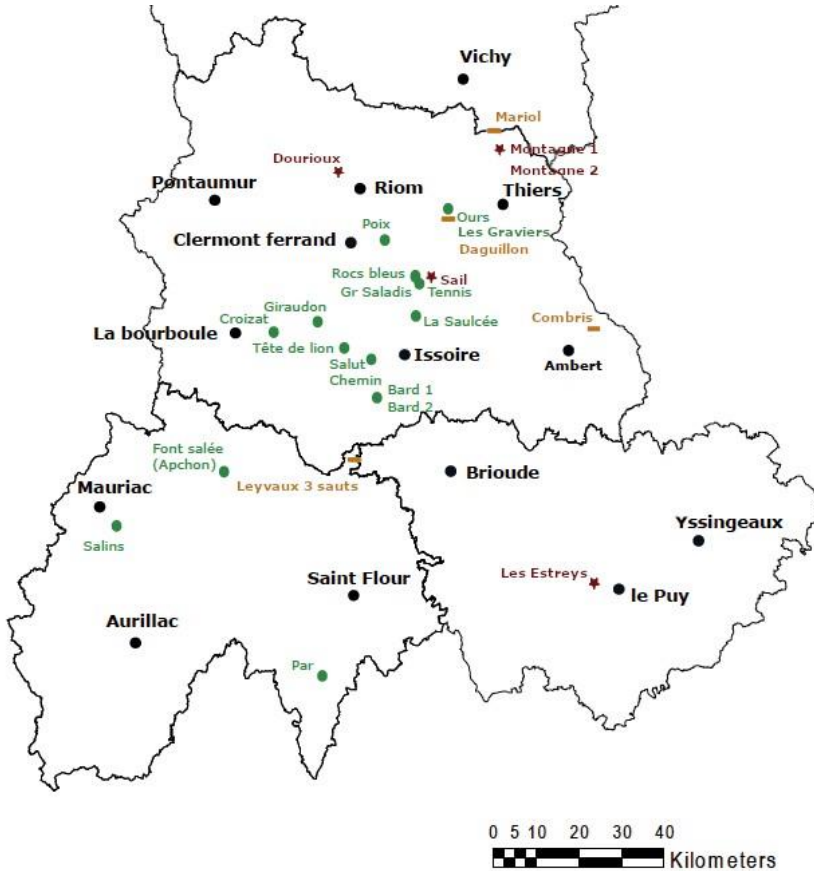

Fig. 1. Map of the sampled mineral springs in Auvergne illustrated according to the radon activity levels in water: green circle – low activity, orange line – medium activity, red star – high activity.

2.2 Simulation

2.2.1 ERICA

ERICA tool v.1.3 [14] has been developed to evaluate dose rates to biota from internal and external distributions of radionuclides. The evaluations are performed using site specific, measured concentrations of radionuclides which are coupled with distribution coefficients for terrestrial and aquatic environments and a database of radiation effects for the model organisms [15].

In the current study, a dose rate assessment was performed for phytoplankton in a freshwater aquatic ecosystem for each individual mineral spring using the measured sediment activity concentrations (in Bq/kg) for [226]Ra, [238]U and [210]Pb. The [222]Rn water activity concentrations were excluded due to absence of noble gazes from the radiological factor database of ERICA.

The basic steps followed were the estimation of the total weighted absorbed dose rates (DR in µGy/h) for the phytoplankton in each mineral spring and then, their comparison to the default dose rate screening value for the protection of the ecosystems (SDR= 10 µGy/h) [16]. The risk quotient (RQ) was then assessed as:

$$RQ = \frac{DR}{SDR} \qquad (1)$$

For phytoplankton, the concept of risk should be interpreted as the risk of a negative effect on the growth ability with a morbidity endpoint.

In our case, the constraints imposed by ERICA, including the absence of [222]Rn in dose rate computations, as well as the need of a complete evaluation of the radiation exposure, highlighted the necessity to use Monte Carlo Simulation Codes.

2.2.2 GATE

Geant4 is an open-source simulation toolkit for the tracking of particles through matter [17] which is well established for modelling and simulating from nanoscale up to macroscale [18]. GATE, an opensource platform based on Geant4 libraries initially dedicated to medical physics applications [19,20], has been employed for the simulation of the absorbed dose to diatoms due to the natural radioactivity present in the mineral springs.

In the current work, we modelled the diatom as a 20 µm radius water sphere surrounded by 100 µm radius water and/or sediment sphere serving as the surrounding environment. When considering sediments, a mixture consisting of a user-defined "dry sediments" material and "G4_WATER" was used, characterized by its porosity values (in %) as described in eq. 2:

$$Porosity = \frac{Volume_{water}}{Volume_{sediments}} * 100 \qquad (2)$$

Focusing on the two most abundant radionuclides measured in the water and the sediment of the mineral springs, the alpha spectra of [222]Rn and [226]Ra were introduced as volumetric, isotropic sources surrounding the diatom and irradiating it either externally and/or internally and the total dose deposited in the diatom (D in Gy) was collected.

For the needs of the Geant4-DNA simulation, information on the type, kinetic energy and direction of the particles entering the diatom were also recorded in a file using the dedicated GATE "PhaseSpace" (PhS) actor.

2.2.3 Geant4-DNA

Geant4-DNA is an open-source Monte Carlo Track Structure (MCTS) code dedicated to micro and nanodosimetry allowing the tracking of particles down to eV energies [21–24] Geant4-DNA allows also the simulation of the indirect DNA damage due to water

radiolysis [25] as well as the evaluation of Single and Double Strand Breaks (SSBs and DSBs respectively) due to the direct energy deposition of the ionizing particles through clustering algorithms.

In this study, the assessments of SSBs and DSBs in diatoms is performed using the clustering algorithm DBSCAN [26]. The diatom is represented by a water sphere of 10 µm radius with a spherical nucleus of 0.5 µm. Considering that approximately 10 DNA base pairs (bp) make up one full twist of DNA helix, we are following the modelling suggested by Charlton et al. [27]. The DNA molecules are modelled as nanometric cylinders randomly positioned in the nucleus. Two sizes are considered: 10 nm diameter and 5 nm height representing a nucleosome and 2 nm diameter and 2 nm height representing 10 bp. The source simulated is the PhS file containing only the alpha particles which was collected from the GATE simulation described in section 2.2.2. The specific energy (z in Gy), corresponding to the dose deposited in the cylinders, is then collected.

According to the DBSCAN clustering algorithm, the formation of SSBs is considered a function of the energy deposition following a probability distribution function. For deposited energy (e_{dep}) < 5 eV the damage probability is considered zero while it increases linearly up to 37.5 eV. For $e_{dep} \geq 37.5$ eV the algorithm considers that all the events can cause SSBs. The minimum number of SSBs to form a DSB is set to 2 within a radius of 3.3 nm, representing roughly the distance between 10 DNA base pairs.

3 Results and Discussion

3.1 γ- Spectrometry

The ^{222}Rn concentration activity (A_{Rn} in Bq/L) values in the waters of the mineral springs range between 1.3 and 4000 Bq/L. The mineral springs were classified according to the values of ^{222}Rn activities in three categories of low (A_{Rn} < 100 Bq/L), medium ($100 \leq A_{Rn} <$ 1000 Bq/L) and high activity ($A_{Rn} \geq 1000$ Bq/L) levels. In overall, 65% of the sampled locations belong to the low activity level class while 19% belong to the high activity level class. Among all the mineral springs, La Montagne is presenting the highest values of ^{222}Rn activity concentration in the water, with a maximum of 4000 Bq/L so far.

According to the analyses, ^{210}Pb is present in the sediments in 80% of the mineral springs with a mean specific activity value of 1.4 Bq/g. ^{238}U and ^{226}Ra are present in the sediments of all the mineral springs with specific activities ranging between 7.6 10^{-3} and 0.5 Bq/g, and 0.028 to 52 Bq/g respectively.

3.2 Theoretical simulations

Using the activity values measured by γ-spectrometry, the dose rate assessment for the phytoplankton in the mineral springs indicated that the contribution of ^{238}U and ^{210}Pb to the dose rate is very small. On the other hand, it was found that the dose rate to phytoplankton exceeded the threshold limit (10 µGy/h) in 71% of the sampled spots due to the presence of ^{226}Ra in the sediments. In Figure 2, the bar plot shows the total estimated dose rate (in µGy/h) corresponding to the ^{226}Ra specific activity (Bq/kg) of each spring. The solid line represents the respective values of the ^{226}Ra specific activity, while the dashed line indicates the dose rate threshold limit (10 µGy/h) for ERICA risk assessment in the ecosystems.

The dose rate values range from 0.17 µGy/h for Poix up to 125 µGy/h for Daguillon. A clear correlation between the ^{226}Ra specific activity and the dose rate is also observed.
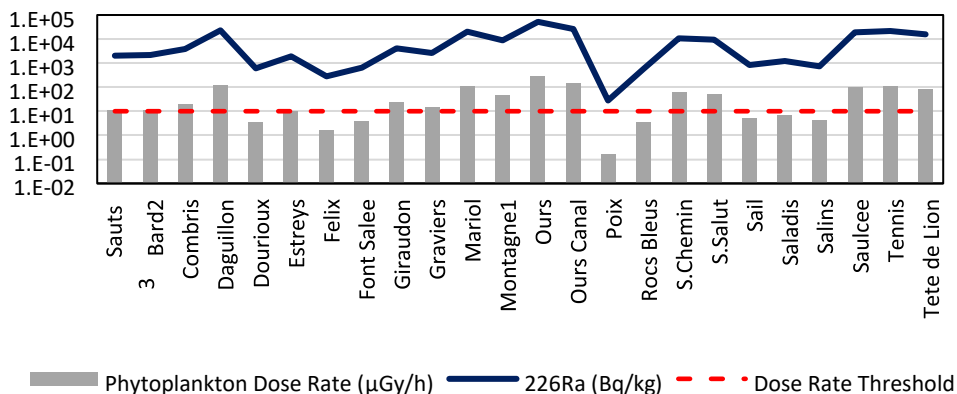
Fig. 2. Radium specific activity in sediments and dose rate for phytoplankton in each spring.

The dose rate (in μGy/h) received by the diatoms as a function of the porosity of the sediments is presented in Figure 3. The specific activities and activity concentrations here represent mean real values that have been measured from the collected samples. The porosity values at the two edges of the x-axis correspond to a totally dry sediments composition (0% of water) in the case of 0% porosity, while 100% porosity refers to a composition of 100% water.
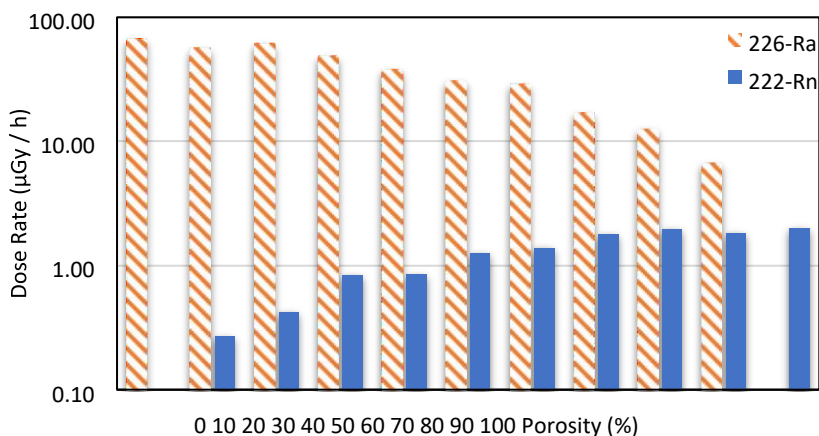

Fig. 3. Dose rate to the diatoms as a function of porosity in the sediments scaled for 30 Bq/gr of ^{226}Ra and 1000 Bq/L of ^{222}Rn.

The observed trend is that the dose rate received by the diatoms decreases as a function of the porosity. That indicates the higher contribution of the ^{226}Ra (present in the sediments) to the dose received by the microorganisms living in mineral springs compared to the dose imposed by the presence of ^{222}Rn in the water.

The impact of the internal and/or external exposure to the dose rate received by the diatoms was also evaluated. An increase of the dose rate is observed in the case of the combined internal/external exposure for both the mean measured values of ^{222}Rn in the water (1000 Bq/L) and ^{226}Ra the sediments (30 Bq/g). The result can be interpreted by the

fact that both radionuclides are alpha emitters of relatively small energies (5.5 MeV and 4.8 MeV respectively) which deposit their energy in a few tens of micrometers. In water, their energy range is well known [28], while in the case of the sediments the simulation offers a first evaluation of the range of the alpha particles in this specific medium.

The nanodosimetric simulations for the case of a nucleosome surrounded by water with 1000 Bq/L ^{222}Rn activity concentration assessed a specific energy rate of 0.023 μGy/h. The respective simulation for 10 base pairs, resulted in a specific energy rate of $8.4 \cdot 10^{-4}$ μGy/h. The observed 2-orders-of-magnitude difference can be attributed to the different sizes of the modelled molecules.

For the case of the simulation of the direct DNA damage using the clustering algorithm, 131 SSBs per particle and 57 DSBs per particle were predicted. These preliminary results can only offer an order of magnitude for the DNA damages expected in the diatom.

4 Conclusions

In this study, we simulated the effects of natural radioactivity on microorganisms living in radioactive mineral springs. This work contributes to the part of the understanding and the evaluation of the doses received to diatoms in the mineral waters in Auvergne region in France.

In total, 27 mineral springs have been sampled and analyzed. The highest ^{222}Rn activity concentration measured in water was 4000 Bq/L, while the highest ^{226}Ra specific activity in the sediments was 52 Bq/g.

Considering the measured activity values, a dose rate estimate for aquatic freshwater ecosystems has been performed using the ERICA tool. The results indicate dose rates above ERICA risk threshold imposed to the phytoplankton in 71% of the mineral springs observed as a result of the high ^{226}Ra concentration in the sediments.

Microdosimetric simulations have been conducted using the GATE open-source Monte Carlo platform. The results have shown among others that the highest contribution to the dose received by the diatoms is due to the alpha particles present in the natural decay series. From this study, it was also evaluated that the most important contributor to the dose is the ^{226}Ra which is present in the sediments of the mineral spring ecosystem. The investigation of the case of only external or a combination of internal and external radiation exposure has highlighted the necessity of dedicated studies. These studies are currently investigating the capacity of the radionuclides to penetrate the silicate frustule of the diatoms.

The simulated impact of the sediment composition on the dose received by the diatoms has also provoked the launch of measurements for the complete characterization of the chemical contents in the sediments.

Nanodosimetric simulations using Geant4-DNA have given an order of magnitude for the specific energy rate on the DNA of the diatoms due to the presence of ^{222}Rn in the water, while simulations are currently performed ^{226}Ra in the sediments, too. The values range between 0.023 μGy/h and $8.4 \cdot 10^{-4}$ μGy/h as a function of the size of the modelled molecule for a ^{222}Rn source isotropically distributed in water.

SSBs and DSBs were calculated for ^{222}Rn in water, showing a trend of two times more single than double strand breaks. These preliminary results will be supplemented by the respective ones for ^{226}Ra in sediments. In order to be used for a complete DNA damage evaluation, these results need to be consolidated with additional indirect damage caused by chemical species after radiolysis, a work which is currently in progress.

Finally, the response of the diatoms to external alpha and X-ray beams is currently being investigated in an effort to couple the predicted DNA damage results stemming from the simulation to observables from experiments.

The studies have showed so far that there is a great potential for the evaluation of the link between natural radioactivity and the diatom teratogenic forms observed in natural radioactive mineral springs.

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