

Grant Proposal

Monitoring and risk assessment for groundwater sources in rural communities of Romania (GROUNDWATERISK)

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Abstract

In the past 100 years, a decreasing rainfall trend has been recorded on Romanian territory, a trend that continues today. Therefore, realistic estimation of the groundwater resources is crucial, especially for the rural communities lacking the economic power to use alternative sources of drinking water. The groundwater sources used by rural communities in Romania generally originate directly from caves, wells or springs with no proper evaluation of the water quality. Groundwater is exposed to different pollutants, as bats' guano in caves, fertilizers in agricultural areas or livestock (cattle, sheep, goats, etc.) farms on the surface. On the other hand, the water extracted directly from inside the caves is affecting groundwater ecosystems, highly vulnerable to any human impact and neglected by European legislation so far. The project aims to monitor, during two consecutive years, groundwater sources with different degrees of above- and underground pollution, from different regions of Romania. To achieve the goals of the project, a multidisciplinary monitoring strategy that will include measurements of hydrological, physico-chemical and biological (microbiology and aquatic invertebrates' assessment) parameters alongside the quantification of radon and stable isotopes, rainfall or possible inflows of water. The

specific outcomes of this project are: i) to test, develop and validate a new, more rapid and efficient method for monitoring and risk assessment of groundwater sources – and not only – by using molecular techniques, and propose this method to the water agencies in Romania; ii) to propose for Romanian authorities to implement a harmonized coherent methodology to measure radon concentration in water, as a consequence of EURATOM Directive; and iii) to educate local communities that are using groundwater as source for drinking water and raise young people's awareness on the benefits of ecosystem services provided by the groundwater.

Keywords

groundwater, springs, microbiology, chemistry, stable isotopes, radon, risk assessment, ecosystem services, rural communities, Romania, Norway

Overall aim of the project

The research aims of the **GROUNDWATERISK project** (Fig. 1) are to improve the methods of groundwater monitoring for a better quality of life in rural communities that use local sources of water and to raise the awareness of groundwater users on potential health risks and the need to protect vulnerable groundwater ecosystems in connection to the surface land use.



Figure 1. [doi](#)
The project logo.

The proposed **OBJECTIVES** are:

1. Interdisciplinary evaluation of groundwater ecosystems used as sources of drinking water in rural communities
2. Finding the most feasible method for groundwater microbiological monitoring to ensure water quality for human use and to better protect against possible outbreaks of pathogenic bacteria caused by contaminated drinking water
3. Risk assessment for the groundwater sources used by rural communities
4. Assessment of the groundwater ecosystem services to enhance conservation practices in rural communities.

The **target group** of our research are the rural communities that are not connected to a public water supply. According to Eurostat 2019, in Romania in 2017, only 67.5% of the population is connected to a public water supply, a number which is continuously increasing. It means that the rest of more than 32.5% of the population, mostly rural inhabitants, has to use local sources of water. These sources are represented by water taken directly from caves, wells or springs. The quality of these water sources are not monitored on a regular basis by the water agencies or by agencies for public health. To the danger represented by the lack of water quality monitoring adds, in rural areas, the agricultural practices and the lack of waste management. Agriculture and household wastes accumulation on the surface are two main groundwater polluting factors. Moreover, the waters taken directly from caves can be polluted by the inhabiting bats that adds to the inherent lack of mechanical filtering of karst waters.

Key targets to be achieved in the project are related to the proposed objectives:

- An evaluation of the quality and sustainability of groundwater sources used by the rural communities across Romania;
- Establishing the most feasible method for microbiological monitoring and radon concentration measurements of drinking water to be applied by responsible agencies;
- To assess the environmental and human-induced risks for groundwater sources in different regions of Romania;
- To educate people for the protection of their local source of water and its sustainable use.

State of the art

Groundwater is defined in the Water Framework Directive (WFD), Directive 2000/20/EC, as “all water which is below the surface of the ground in the saturation zone and in direct contact with the ground or subsoil”. Groundwater is the largest supply of water for human consumption with 97% of all freshwater on the Globe being underground (Gibert et al. 1994). It is therefore a vital resource, which requires constant protection. The Groundwater Directive 2006/118/EC, a derived directive of the WFD, is protecting groundwater in terms of quality (chemical composition) and quantity, while attention is paid to groundwater as

ecosystem only in association with surface systems through the Groundwater Directive 2014/80/EU and the Groundwater Directive 2006/118/EU.

Groundwater harbors a unique and vulnerable ecosystem characterized by lack of light and primary producers, relatively stable physico-chemical conditions and poor nutrients content - unless human-induced changes are interfering. The poor food resources originate from the surface as particulate organic carbon (POC) or dissolved organic carbon (DOC) and microbial activity, which is low per volume of water. Groundwater animals in Romania are invertebrates, mostly Crustaceans, which have particular adaptations to life underground: lack or reduction of eyes, depigmentation, elongated appendages, fine body shape, slow metabolism and high vulnerability to high variation of their physico-chemical environment. They are used in ecological studies as an indicators of water quality, their presence and diversity indicating the ecological state of the environment.

Waterborne diseases are a global burden which is estimated to cause more than 2.2 million deaths/year and an even higher number of recorded and unrecorded illnesses (Bitton 2014, World Health Organization 2015) contribute to a high cost for prevention and treatment. Bacterial indicators such as total coliforms, faecal coliforms, *E. coli*, faecal streptococci, and many others are widely used for the assessment of water quality in Europe, including Romania. They are used for detection of anthropogenic impacts such as faecal pollution of water, mainly caused by raw and treated sewage and diffuse impacts from farmlands and pastures.

For the **monitoring of the quality of water** used for drinking, irrigation and bathing along the physico-chemical parameters, the examination of the microbiological standard parameters is mandatory: EU-Surface & Drinking Water Directive 75/440/EEC and EU-Bathing Water Directive 76/160/EEC. Nevertheless, the methods applied in the microbiological monitoring of waters are outdated and underestimate the level of microbial pathogens (e.g. the multiple-tubes method is used as a standard for Romanian and other European countries water estimation of pathogen bacteria such as *E. coli*) while groundwater microbiological monitoring is not performed at all. Although molecular techniques might improve the identification and abundance estimation of these pathogens, several disadvantages such as the lack of standardization of protocols and sample processing are still a challenge (Ramírez-Castillo et al. 2015).

Radon (^{222}Rn), found in soil, rocks and water all over the Earth, is listed by the World Health Organization as the second leading cause of lung cancer after cigarette smoking. Areal variations of radon levels in houses depend on numerous factors, such as geological features, environmental parameters or occupational patterns. Most of the cancer risks from radon in drinking water arise from the transfer of radon into indoor air, and the exposure through inhalation (World Health Organization 2009). The latter resulting from tap water utilized by households. Since radon is soluble in water, its degassing is added to the indoor exposure (Kendall and Smith 2002). Special attention is needed when groundwater is used for drinking water, as radon can become a risk factor for users if the radon concentration in the aquifer is high (Committee on Risk Assessment of Exposure to Radon in Drinking Water 1999). Generally, groundwater contains, potentially, much higher concentrations of

radon than surface water (Cosma et al. 2008). The radon activity concentrations in surface waters is low, usually below 1 Bq/L, while in groundwater vary from 1 to 50 Bq/L for rock aquifers in sedimentary rocks, 10 to 300 Bq/L for wells dug in soil, and 100 to 50 000 Bq/L in crystalline rocks. The highest concentrations are usually associated with high uranium concentrations in the bedrock. A characteristic of radon concentrations in rock aquifers is their variability; within a region with fairly uniform rock types, some wells having concentrations far above the average for that region (European Commission 2001). On the basis of Articles 35-36 of the Euratom Treaty, The European Commission's Drinking Water Directive (98/83/EC) established a maximum effective dose of 0.1 mSv/y for ingestion of water supplied by the public network (Council Directive 98/83/EC 1998, Euratom 2010). Radon and its decay products were excepted from the calculation of this maximum effective dose. Commission Recommendation 2001/928/Euratom (European Commission 2001) comes to meet this omission by establishing maximum concentration values for radon (100 Bq/L) and its long-lived decay products (^{210}Po : 0.1 Bq/L and ^{210}Pb : 0.2 Bq/L). The latest European Union 2013/51/Euratom (Council Directive 2013/51/Euratom 2013) states that Member States may set a level for radon which is judged inappropriate to be exceeded and below which optimization of protection should be continued, without compromising water supply on a national or regional scale. The level set by a Member State may be higher than 100 Bq/L but lower than 1,000 Bq/L. In order to simplify national legislation, Member States may choose to adjust the parametric value to this level. Remedial action is deemed to be justified on radiological protection grounds, without further consideration, where radon concentrations exceed 1,000 Bq/L. Recently the Romanian government has adopted the Law no. 301/2015 (Official Journal, Monitorul Oficial 2015) regarding the requirements for the protection of the health of the general public with regard to radioactive substances in drinking water, which transposes Directive 2013/51/Euratom laying down requirements for the protection of public health in radioactive substances in water intended for human consumption.

Stable isotopes used for environmental studies. Due to the direct relationship between air temperature and $\delta^{18}\text{O}$ and $\delta^2\text{H}$ in rainfall and spring water, we can establish the moment when karst aquifers recharge occurs and the delay between the moment of surface rainfall and runoff and underground recharge (e.g., by determining the time difference between the moment of winter precipitation with very low $\delta^{18}\text{O}$ and $\delta^2\text{H}$ and the moment these low values are registered in the underground streams). The hypothesis is that, for hydrokarstic systems with diffuse feeding, there is a several months interval between rainfall and the moment when water reaches the subsurface karst. For karst systems fed directly through ponors and caves, the rainfall (including the potential contaminants) reaches the subsurface within days.

Metabarcoding of water samples and detection of pathogens. By next-generation sequencing methods, high diversity of prokaryotes pertaining to both Archaea and Bacteria has been detected in groundwater, including members of Crenarchaeota, Euryarchaeota, Proteobacteria, Planctomycetes, Actinobacteria, Chloroflexi, Chlorobi, Bacteroidetes, Firmicutes and Cyanobacteria phyla (Schwab et al. 2017, Smith et al. 2012, Korbel et al. 2017).

In areas with high population density and/or intensive land use, groundwater is vulnerable to contamination, as various pathogenic microorganisms may enter groundwater due to septic systems, livestock manure, contaminated wells or recharge waters, etc. Groundwater contaminants detected through DNA-based studies include members of Xanthomonadales (known crop pathogens), Pseudomonadales (components of biofertilizers), and Burkholderiales (*Comamonadaceae*) used as biocontrol agents in agriculture (Korbel et al. 2017). Groundwater near animal farms may be a source of pathogenic *Campylobacter* sp., *E. coli*, *Yersinia* sp. (Pitkänen et al. 2011). Several human pathogens (*E. coli*, enterococci, *Pseudomonas aeruginosa*, *Salmonella*, *Bacillus*, *Shigella* and *Staphylococcus aureus*) have been detected in groundwater (Grisey et al. 2010), posing a serious risk in water resources, as these microbes drastically influence groundwater purity and availability of drinking water.

Risk analysis requires a holistic approach to assess stress and vulnerability of groundwater resources. Risk assessment of water supplies aims at:

1. identifying causes that may threaten the quality of the water supply;
2. assess whether the harm can be eliminated; and if not
3. suggest preventive or protective measures that can control and reduce the risk.

In the case of lacking (economic) resources, identification of highest risks is essential so that these risks can be handled first.

The management of groundwater contamination is a very difficult task due to the spatial heterogeneity of the aquifers and the natural processes in the soil and the unsaturated and saturated zones of the karst (Civita 2010). Once groundwater is contaminated, it is very costly to clean up and takes a very long time to recover. In addition, groundwater monitoring is time-consuming and too costly to adequately define the geographic extent of contamination at a regional scale. Thus, the appropriate way to manage groundwater contamination is to identify the spatial distribution of the areas with contamination risk or vulnerable to contamination (Lahr and Kooistra 2010, Mimi and Assi 2009). Groundwater risk is determined by combining the vulnerability and the hazard assessments. The highest groundwater contamination risk appears when hazards occur in a high vulnerability zone (Ravbar and Goldscheider 2007).

Groundwater in karst terrain is vulnerable to contamination due to the concentrated channel flow with low transit time and little self-purification within the karstic system. The European Approach to karst vulnerability and risk mapping of karst aquifers, by the COST Action 620, define two central terms: the intrinsic vulnerability of groundwater to contaminant which considers the geological, hydrological and hydrogeological characteristics of the karst area, and the specific vulnerability that accounts for the properties of the contaminant or group of contaminants (Zwahlen 2003). The methodology for assessing the intrinsic vulnerability of karst areas (Goldscheider et al. 2000, Vías et al. 2006), include evaluation of the overlying layer or protective cover, and the infiltration conditions like concentration of flow and precipitation. The European Approach also add the K-factor, the degree of karst and flow system development (Zwahlen 2003). A thick soil

cover with low permeability where dispersion, dilution and degradation may occur, have a protective function to the groundwater resource and may prevent contamination from the terrain surface to enter the karst system. Assessment of the quality and presence of a cover layer, and identification of swallow holes where the cover layer may be bypassed is an essential step in the risk assessment. Field survey and local information combined with remote sensing in GIS-analysis provide the basis for the intrinsic vulnerability maps for the ground surface.

Karst aquifers are unique in the way that enlarged fissures, conduits and caves provide habitats for macro and microorganisms and may give humans direct access to the water resource inside the aquifer. Biological contamination inside the karstic system may be an important threat to the water quality and safety. An evaluation of the degree of karstification and the flow system development as well as human and biological activity in accessible caves is a second approach in the risk assessment. Risk and vulnerability maps are useful tools for limited monitoring resources and in such areas a major effort is required to avoid or mitigate the impact of human activities on the environment (Almasri 2008, Thapinta and Hudak 2003). Risk estimate of groundwater contamination at a basin/sub-basin scale will help management plans and strategies to reduce the risk of surface and groundwater contamination and provide communities a better understanding of potential impacts to local water resources. A drinking water system is often described as an integrated chain of supply from source to consumer (World Health Organization 2008). When groundwater is the source for public water supply, understanding the impacts of land use and aquifer vulnerability are fundamental to groundwater protection (Somaratne et al. 2013). One of the important risks to groundwater is the adverse land use and therefore, it is important to identify which aquifer systems are at high risk in order to adopt appropriate risk management options. Several process-based approaches exist for assessing whether a contaminated site or surface applied chemicals constitutes a risk to groundwater.

Development of a microbial contamination susceptibility model for private groundwater sources has been carried out by assessing the presence of thermotolerant coliform (TTC) in groundwater (Hylands et al. 2012). Risk analysis in this study shows that source type, groundwater vulnerability, subsoil type, and set back distance from septic tanks are all important factors for the presence of TTC. However, risk assessment tools and risk management actions must be proactive rather than being a reactive response to the detection of coliform bacteria.

Ecosystem services. Ecosystem services are vital to human survival and wellbeing, and the judicious management of these systems being essential. Ecosystem service indicators are increasingly recognized as a key part of assessing whether ecosystem services are being managed appropriately and used sustainably (Brown et al. 2004). Several authors (Haines-Young and Potschin 2010, Rounsevell et al. 2010) have defined and assessed ecosystem services and highlighted key strengths and weaknesses within these frameworks (Reyers et al. 2014). It further resulted in the development of new frameworks which attempt to make the links between ecosystem structures, processes and human well-being much more explicit (e.g. Haines-Young and Potschin 2010, Potschin and Haines-Young 2011).

The important outcomes of the project

Project contribution beyond the state-of-the-art

The research we propose has several components that are new to science and others which were never applied in Romania, as follows:

- first overview of the quality of groundwater sources used as drinking water in Romania by using multidisciplinary indicators (physical, chemical, biological and microbiological);
- identifying a filter for microbiological sampling that will be effective not only in detecting the presence/absence of microbial pathogens but also in identifying the presence of pathogens at low concentrations;
- new protocol for groundwater microbiological monitoring and accurate identification based on molecular methods;
- first radon analysis on water and first derived map on radon risks in Romania;
- identification of groundwater invertebrates that can be used as indicators of water quality;
- identification of microorganisms possibly involved in water purification;
- first hydrological studies at a country level that will use stable isotopes as indicators of water origin and water residence time underground;
- first risk maps for groundwater sources in Romania and the first maps that will include microbial pathogens and radon in the analysis;
- first maps of groundwater ecosystem services in Romania.

Technical milestones and expected results

The milestones and the expected results are as follows:

- Compiling a database with all the results (physico-chemical, radon, stable isotopes, biological, microbiological) after the monitoring period. This database will also contain the identified invertebrate species, inferred pathogenic and nonpathogenic microorganisms in each of the studied sites and will provide the basis for the risk analysis and the ecosystem services approaches;
- Choosing both the optimal filter and filtration methods for retaining of pathogen's biomass and the optimal test for pathogens in groundwater in terms of costs, accuracy, efficiency and flexibility. The filter and the test will be used to validate a protocol for best practices in microbiological monitoring for groundwater sources of drinking water. The protocol will be published and distributed to water agencies;
- Risk analysis for all the studied sites. It will highlight the vulnerabilities of local groundwater ecosystems and the possible risks for the human populations;
- A GIS model with ecosystem services in Romania. A simplified model will be distributed in brochures and leaflets addressed to rural communities and local schools and for the training of the personnel of water and health agencies;
- Conferences held in local communities and schools on the need to protect groundwater sources and the importance of groundwater ecosystems;

- A guideline on measurement procedures for the determination of radon concentration in water;
- Publication of results in high impact factor journals and presentation of results at national and international conferences;
- Implication of students and young researchers in new fields of research and in interdisciplinary activities.

Methodologies to be used

A. Sites selection. Sites will be selected in different regions of Romania, with different surface use, different origin of the water (surface – short flow underground, surface – long flow underground, aquifer), various hydrology, various human impacts on the surface, etc. Samples will be taken seasonally during a 2-years period.

B. Sampling. Water will be collected in special bottles for chemical analysis, microbiology, stable isotopes and radon:

- Physico-chemical measurements. Electrical conductivity, temperature, pH, oxygen and flow speed will be measured in situ. For the other chemical parameters 0.5-1 L water sample will be collected in PTFE bottles. Samples will be stored and conserved using standardized protocols (metals by acidulating with concentrated nitric acid, organics by refrigeration).
- 25 ml aliquot of water will be sampled for stable isotope analyses. Every three months, samples will be collected from the stations and transported to the laboratory for analyses.
- Aquatic invertebrates will be sampled by using a 60 µm planktonic net directly from springs for a standardized quantity of water. All samples will be preserved in 95% ethanol.
- Microorganisms will be sampled on commercial films with growth media. At each sampling site, 1 mL of water will be directly applied, with a sterile plastic pipette, onto the growth medium surface, and then transported, on ice, to the laboratory for incubation.

C. Identification and molecular analysis of invertebrates. Samples will be sorted under the optical microscope and identified at least at fauna group level, except for the crustaceans that will be identified at species level. Specimens will be also sent to specialists for identification. Amphipods will be analyzed by molecular methods and a phylogeny with all the obtained sequences will be build-up.

D. Molecular identification of microorganisms and profiling of water-borne pathogens. We will attempt to explore the molecular diversity of putative pathogens in groundwater by sequentially and complementary using three different molecular biology approaches:

(a) Commercial films. The plates will be transported at constant temperature in a cooler bag, and placed in incubators. The plates will be analyzed at 24-hour intervals, the results

being expressed in the total readings after five days (Bercea et al. 2018). The visible colonies will be transferred from plates to agar for growth. After extraction, PCR products will be purified. DNA will be sequenced. The sequences obtained will be deposited in an online database.

(b) Metabarcoding. To rapidly screen for the putative diversity and abundances of bacteria (and Archaea altogether), the amplicon sequencing (or metabarcoding) technique targeting the highly conserved, taxonomic relevant 16S rRNA gene will be employed. Raw sequence data obtained by this approach will be analyzed. Several processing steps of joining pair-end reads, quality filtration, dereplication, singleton and chimera removal will provide good quality sequences for taxonomic assignment. Recently released DNA sequence databases are available and can be used for establishing taxonomic diversity (Silva 132, Greengenes '13-8', Ribosomal Database Project). The metabarcoding approach allows overpassing the limitations of culture-dependent techniques, being a cost-effective and fast assessment, providing data on the entire prokaryotic community including 'unculturable' or fastidious microbes. We expect that the metabarcoding approach will accurately resolve the microbial community composition down to family and genus level. Thus, the presence and abundances of bacterial families comprising pathogenic members (e.g., the Gram-negative *Enterobacteriaceae*, *Campylobacteriaceae*, *Aeromonadaceae*, the Gram-positive *Streptococcaceae*, *Staphylococcaceae*, etc.) will be quickly evidenced.

(c) Quantitative PCR (qPCR). If possible, presence of pathogens is inferred by method (a), the more sensitive qPCR assay will be performed targeting selected marker genes (Szekeres et al. 2018). The high sensitivity and detection speed of this method is facilitated by the simultaneous amplification and visualization of the newly formed DNA amplicons. With the appropriate primers and optimized qPCR conditions, the amplified DNA sequence can be unique to the level of genus, species, or even strain of microorganism (Schwab et al. 2017, Rochelle and Schwab 2011). Thus, qPCR allows a rapid screen and quantification of an array of pathogenic bacteria, which can be employed as tracers to understand groundwater vulnerability to microbial contamination. To appropriately target the microorganisms of interest in the qPCR assays, we will select specific primers designed for functional genes distinctive of the pathogens detected by method (a). For fast simultaneous microbial identification and profiling (i.e. detection of transcripts), dedicated kits can be used. However, as the drawbacks of the qPCR include false-positive results we will further proceed for the last, refining step that will clearly and most accurately evidence the cells and vectors (plasmids, phages) bearing pathogenic traits.

All molecular methods described above will be applied on the same samples collected from the same sites following the sub-splitting of membrane filters. The environmental DNA will be extracted from biomass retained on hydrophilic filter membranes with 0.22 µm pore size and large diameter (90 mm) under negative pressure (i.e., generated by vacuum pump). The filtered groundwater volumes (up to 15 L expected) will depend on how quick the filter membranes will be clogged. Each membrane will be then separated into slices needed for DNA extraction for further molecular analysis. The unused extracted DNA will be stored under freezing conditions.

E. Radon measurements. The radon measurements in water will use the Luk-VR system, which involves connecting a VR-scrubber to a radon detector. This method requires mixing of the dissolved radon from the water sample with the air above the water in the volume of the glass vessel. Following this procedure, the sample of air is transferred to the Luk 3P, and measured by the Lucas cell method.

The water samples will be collected in glass bottle of 0.5 L, fully filled and tightly sealed and transported to the laboratory for measurement purposes. The time interval between sampling and measurement is recommended to be of maximum 48 hours, in which case the half time must be considered and corrections are made accordingly

F. Stable isotopes. Precipitation will be collected continuously using specially designed collectors, constructed according to IAEA specification. A 3-L HDPE plastic canister is fitted with a funnel, prolonged with a plastic tube, channeling water to the bottom of the container. Excess air escapes the canister through to a narrow, 3 m long plastic tube, to minimize air exchange between the container and the outside environment. The funnel will be “sealed” with table tennis balls, to restrain insect access, but allow water collection. At the end of each month, the amount of water in the canister will be measured (to be compared with data provided by the Romanian National Meteorological Administration), and a 25 mL aliquot of water will be sampled for stable isotope analyses. The aliquot will be stored in HDPE scintillation vials at 4°C. In winter, snow samples will be collected after each event, allowed to melt at room temperature in closed vessels, and stored in similar manner to liquid samples. River water will be collected at the end of each month, from boats or bridges, from ca. 15 cm below water table, in 25 mL HDPE vials and refrigerated until analysis. Groundwater will be sampled from dug wells and deep wells (where available), as well as from springs.

Climate and hydrologic data will be provided by the respective national authorities. Where such data will not be available, we will install temperature data loggers, measuring air temperature with hourly resolution. An ongoing study has shown that there are no systematic differences between data from the national meteorological service and data provided by the loggers.

Stable isotope analyses will be performed in the laboratory. Prior to analysis, samples are filtered using 0.45 nm nylon microfilters. The results are calibrated against two internal standards (Greenland and Hawaii waters) and checked against a third one (Romanian water). Per laboratory internal regulations, an aliquot from each sample will be stored in 3 mL paraffin-sealed, screw-cap, glass vials.

G. Chemical analysis. Chemical parameters will be determined by standardized or alternative methods. Trace metals (Cd, Cu, Cr, Pb, Ni) will be determined by ICP-MS, mercury by atomic fluorescence spectrometry, major cations and P by ICP-OES and anions (nitrates, nitrites, phosphate, sulphates) by ion chromatography. Organic carbon and total nitrogen will be determined by a combustion analyzer with NDIR and respectively chemiluminescence detector. Chemical oxygen demand (COD) and alkalinity will be determined by volumetric, total dissolved solids by gravimetric, while phenol index,

ammonium and cyanides by spectrophotometric methods. Whenever applicable, standardized methods will be applied. For organics, water will be sampled in glass bottles, while for metals and anions in polyethylene bottles. Samples will be stored and conserved using standardized protocols (metals by acidulating with concentrated nitric acid, phenols by addition of phosphoric acid, organics by refrigeration). Where recommended, analysis (COD, DOC, alkalinity) will be carried out in the day of sampling. Quality control will be made by reference materials analysis and inter-laboratory trials.

H. Risk analysis. We will use an integrated method for assessing groundwater contamination risk, based on the interaction between natural conditions and human activities, and by using analytical and numerical tools within a GIS framework. Different factors along the contaminant pathway from source to groundwater will be incorporated in the GIS database and analytical and numerical tools in GIS software will be used. The spatial groundwater contamination will be classified into categories based on the degree of risk (very high, high, moderate, low and very low). This classification is performed by considering the factors that influence groundwater contamination and assigning relative weights to them. This process is performed in a GIS environment in which thematic maps are produced for every factor. The linear combination of the thematic maps and the selection of the weights yield the final map of groundwater contamination risk.

In addition to the 30 Romanian sites, the project will also benefit from monitoring and investigation of two sites in Northern Norway where karst springs serve as water supplies for small communities. These two sites will be used during the project as models for the Romanian sites and as school-sites for the Romanian students. At both sites, cave systems upstream have been surveyed and investigated.

I. Ecosystem services' evaluation. Identifying indicators takes a combination of scientific rigor and creative thinking. Creative thinking may be a surprising skill in this context, but the indicators with the greatest impact are often produced by combining different kinds of data. Scientific rigor is necessary to identify indicators that are conceptually valid and defensible for their purpose. In our case, the microbiological aspect will be important, because not only pathogens will be identified, but also microorganisms that could be of importance for water purification.

Data relevant for developing ecosystem service indicators will be available from our database. A wide range of models that exist for monitoring ecosystem services will be tested, as for example:

Co\$ting Nature that calculates the spatial distribution of ecosystem services for water, carbon, hazard mitigation and tourism and combines these with maps of conservation priority, threatened biodiversity and endemism to understand the spatial distribution of critical ecosystems (Mulligan 2015, Mulligan et al. 2010); *ARtificial Intelligence for Ecosystem Services (ARIES)* that maps and values ecosystem services and assesses the impacts of land use on them (Villa et al. 2014); *Lund-Potsdam-Jena Managed Land model (LPJmL)* is designed to simulate vegetation composition and distribution as well as stocks

and land-atmosphere exchange flows of carbon and water, for both natural and agricultural ecosystems (Schaphoff et al. 2018b, Schaphoff et al. 2018a).

J. Communication of the obtained results to local communities and water and health agencies is an important component of our project. The obtained results will be published and also presented to the general public on the project site. The editing of a brochure and a leaflet for the local communities and the children in the respective communities will be presented in a friendly manner. We will present not only the obtained results but also impact messages regarding the conservation of groundwater sources. Public conferences and training of people from water and health agencies are also part of our strategy for the improvement of groundwater monitoring and protection.

During our field work we will also try to establish contacts in the local communities and involve the children and young people in our monitoring activities.

Project structure

The project is structured in 4 Work Packages (WPs) distributed along the 48 months of the project (June 2019 – May 2023). The project is split in seasons as we will do the sampling seasonally and all the work will be organized, at least for the first 2 years, according to the sampling campaigns (see also Fig. 2 and Fig. 3).

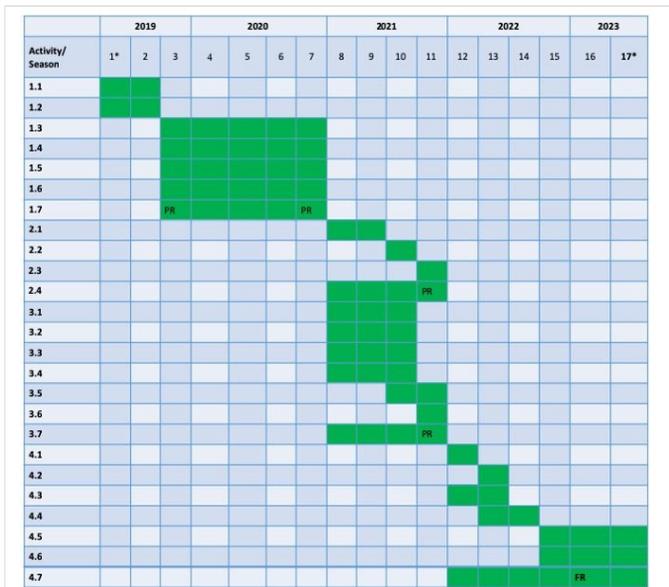


Figure 2. [doi](#)

Gantt Chart of the proposed activities during the project (June 2019 – May 2023). PR = Phase Report, FR = Final Report; *only 1 month (1) or 2 months (17).

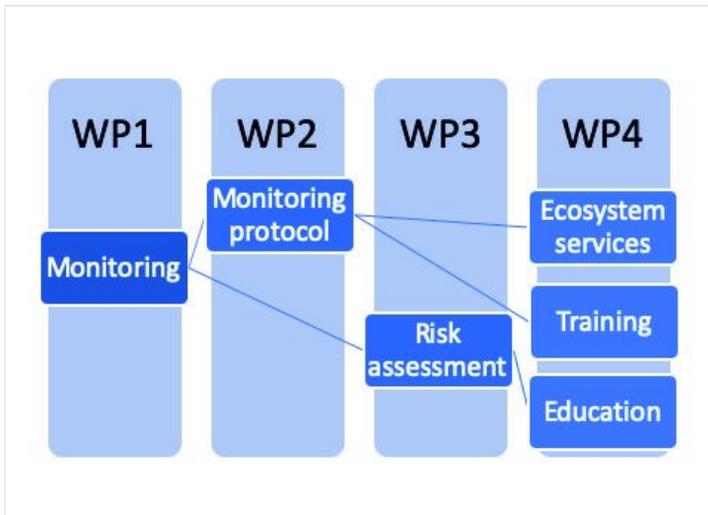


Figure 3. [doi](#)

The flow of the WPs and their relationship.

WP 1. Evaluating the groundwater ecosystems used as sources of drinking water

Objectives. Monitoring in different sites across Romania and Norway to ensure a multidisciplinary view on the groundwater quality used by rural communities as drinking water (wells, caves and springs).

Activities:

1. Selection of sites to be studied and preliminary sampling;
2. Testing of an efficient water filtering method for pathogens;
3. Continuous sampling of precipitation for isotopic analysis;
4. Seasonal sampling of microorganisms and invertebrate fauna in water for two successive years and on-site measurements;
5. Laboratory analysis;
6. Molecular identification of microorganisms and fauna identification in the laboratory by use of conventional morphological methods;
7. Project management.

Deliverables. Press conference, Stations established, Kick-off meeting, Best filter for water pathogens identified, Common field-work in Romania, Devices installed, Common field-work in Norway, Project site opened for the public access, First data introduced in the database, Workshop on methods in groundwater monitoring, Database completed, Results dissemination, Phase report.

WP 2. Improving the methods for groundwater microbiological monitoring to better protect against possible outbreaks of pathogen bacteria in the drinking water

Objectives. Testing and validating a method for groundwater microbiological monitoring for water sources.

Activities:

1. Laboratory testing of different methods for monitoring pathogenic bacteria;
2. Comparing the results of the three methods from all points of view;
3. Validating monitoring protocols on groundwaters of different qualities;
4. Project management.

Deliverables. Protocol for microbiological monitoring, Patent documentation, Results dissemination, Phase report.

WP 3. Risk assessment for the improvised groundwater sources used by rural communities

Objectives. Survey at the surface of areas and water basins where the monitored sites are located and production of GIS maps where the risks for each of the studied site will be highlighted.

Activities:

1. Assessment of the quality and presence of a cover layer;
2. Identification of swallow holes and inlets on the surface;
3. Assessment of the human and agricultural activities on the surface;
4. Evaluation of the radiological risk for groundwater;
5. Production of GIS-based vulnerability maps;
6. Risk reports for the study cases;
7. Project management.

Deliverables. Common field-trip in Romania, GIS vulnerability maps, Risk assessment reports, Report on radon, Results dissemination, Phase report.

WP 4. Assessment of the groundwater services in romania for enhancing environmental awareness and education

Objectives. Raise the interest of the rural communities for the ecosystem services provided by groundwater, including the important drinking water source service, and develop indicators and maps for these services by using the obtained results.

Activities:

1. Developing groundwater ecosystem services indicators;
2. Mapping the ecosystem services in the studied areas;
3. Developing a tool for good practices on measurement procedures for the determination of radon concentration in water;
4. Printing brochures and leaflets for local communities (for adults and for children);
5. Training for responsible water and health agencies for the best monitoring method and protection of groundwater sources;
6. Educating the local communities on the potential health impact associated with the pollution/radon from groundwater sources;
7. Project management.

Deliverables. Ecosystem services indicators, Inter-comparison report for radon, GIS model of ecosystem services, Tool for good practices for radon, End of project workshop, Training for the representatives of the water and health Romanian agencies, Leaflets/brochures edited and distributed in local communities, Conferences for local communities, Results dissemination, Final report.

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Babeş-Bolyai University of Cluj, Romania

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Author contributions

OTM wrote the proposal, ROS contributed to the risk assessment part, HLB contributed to the molecular biology methods, ADC contributed to the radon part, EAL contributed to the chemical methods, AP contributed to the stable isotopes method, SEL contributed to the Norwegian selection of sites. All authors corrected and approved the final manuscript.

Conflicts of interest

There is no conflict of interests.

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