Overview the whole process of the project

The research lasted from 01.03.2012 - 28. 02.2017. The progress of the project was in good agreement with the project proposal. The only deviation was the launching of the in-situ experiment. Anita Erőss was applied for the project as a postdoctoral fellow. However, she was substituted by Petra Bodor when Anita has gone to maternity leave. The in situ experiment could be started when Petra Bodor already had enough practice. Eleven senior researchers were involved into the project from different fields (ELTE) and many researchers joined it in more loose (ELTE and different institutions). Three PhD students worked directly on the project and three more connected to it partially. There were international experts involved into the project (John Molson and René Lefebvre from Canada, Jose Joel Carrillo-Rivera from Mexico) and researchers from BME, MTA.

23 student researchers participated, 3 BSc, 10 MSc and 10 Young Researcher theses were made connected to the project. The main researcher of the project is working on her thesis to be the doctor of HAS (Hungarian Academy of Sciences) based on the research with the title: Hydrogeological characterisation of the boundary of unconfined and confined carbonates in the frame of flow system approach (Mádl-Szőnyi 2017 under construction). The main researcher of microbiology, Andrea K Borsodi is planning to submit her thesis to the HAS in 2018 also from the results of the project. 12 SCI (IF: 21,766), 1 additional English and 5 Hungarian scientific papers were published, 2 have been accepted and 6 have already prepared for publication from the results of the project. 2 international book chapters were published and 1 has been accepted for publication connected to the recent research. 53 oral and poster presentations were given at international conferences, 17 at national conferences and 5 more will be presented in the next few months.

The open access to the publications of the project is guaranteed on ResearchGate (https://www.researchgate.net/project/Evaluation-of-hypogenic-karstification-focusing-on-the-microbially-mediated-processes). The project has also got some publicity soon after the beginning of the project, when the researchers of the project, Anita Erőss, Judit Mádl-Szőnyi and Andrea Borsodi were talking about biofilm communities (Erőss et al. 2012a) at the DELTA on MTV. In 2016, an interview was published with Judit Mádl-Szőnyi in Élet és Tudomány (Bajomi 2016) connected to the planned in-situ experiment of the project. There were not significant deviations from the planned budget of the project. There were IT investations to serve the very diverse subprojects of the research. The alpha spectrometer was not got because the measurements could be carried out with the help of Ra and U discs from Heinz Surbeck (Switzerland). The biggest investations were the devices necessary for the field experiment (electrodes, divers, Greisinger). The microbiological investigations required high amount supply.

The results of the project

discussed based on the original project proposal.

The preliminary hypothesis of the research was that hypogenic karstification processes in thick carbonate aquifers (caused by deep source fluids and gases) can be understood based on revealing of flow pattern, geometry and the geochemical characterization of involved fluids. The microbially mediated processes and the basic geochemical reactions causing hypogenic karstification were formerly known from international papers. However, the necessity of the involvement of flow system approach was just mentioned in the literature and was taken into consideration only in a preliminary research of the Buda Thermal Karst (BTK) (Erőss 2010; Erőss et al. 2010). This preliminary project focused on the discharge areas of the flow systems, therefore did not handled the flow field as a whole, in basin scale.

Therefore, this research project was the first not only in Hungary, but also internationally where the understanding of the whole flow field provides background for better understanding of location and character of those factors which can be responsible for different hypogenic karstification processes. (Due to this, the head of the research project was invited to write a chapter into the book about "Hypogene Karst Regions and Caves in the World" which will be published by Springer (Mádl-Szőnyi et al. 2017a). The basin scale hydrogeological approach (Tóth 2009), was adapted to apply for thick carbonate aquifers in the frame of this project. Therefore, hydrogeology denotes the main topic of the project complemented subordinately by some diagenetic and speleogenetic studies. The scale of these researches were regional and local. Microbially mediated hypogenic karstification processes are in the focus of interest nowadays and its significance was revealed formerly by Erőss (2010) in the BTK. Therefore, microbiology ensured the second priority of the research. The microbiological approach means so called interface or nano-scale studies. Biological precipitates are significant in the understanding of the role of microbes in hypogenic speleogenesis and in the radioactivity of springs, as well. Consequently, the nuclear physics had the third priority in the project. Examination of biofilms required special chemical analytics and mineralogical methods (namely ICP-MS, TXRF, XRD and Mössbauer Spectroscopy). The evaluation of the processes and products connected to the microbes required the use of scanning electron microscopic (SEM) technics.

Flow systems determining the controlling factors of different hypogenic karstification processes

Regional scale studies

The evaluation of the hydrogeological environment and connected flow regimes with the creation of well database for Budapest (Erhardt et al. 2017) and its broader environment included some parts of the Gödöllő Hills (Mádl-Szőnyi et al. 2015) and the Paleogene Basin (Mádl-Szőnyi et al. 2017b accepted to Marine and Petroleum Geology) were executed. Hydraulic, geothermal, water chemical, temperature and hydrostratigraphic data were collected from all water and hydrocarbon wells of the area. Compilation of pressure-elevation, hydraulic, waterchemical, and thermal maps, profiles, cross sections were done to determine main fluid driving forces and vertical-horizontal flow components of the area.

The quantitative understanding of the regional flow field required hydrostratigraphic evaluation and hydraulic conductivity and storativity data. In the first step the archive pumping test (dominantly recovery) data of the thermal wells for the carbonate and siliciclastic aquifers of the study area were collected and arranged into a data base. During evaluation, these data were reinterpreted by classical analytical Theis-Jacob method, the Visual Two-Zone (VTZ) numerical (Rathod and Rushton 1991) and the more complex WT pumping test evaluation model (Székely 2006). However, only short pumping tests were available. As a result, hydraulic conductivity (vertical and horizontal) and specific storage data were derived. It has to be mentioned that the derived data reflect only local hydraulic parameters from the surroundings of the wells. The scientific paper is before submission (MSc Thesis of Garamhegyi 2014; Garamhegyi et al. 2017 prepared for publication). These data were taken into consideration during numerical simulations.

The regional hydrostratigraphical classification of the formations of the BTK was carried out in the MSc Thesis of Martinecz (2014) for the broader environment of the BTK (included the Hungarian Paleogene Basin). The next step of regional studies was the preliminary numerical 2D test of the derived hydrostratigraphical categories in successive scenario simulations (MSc Thesis of Martinecz 2014; Martinecz et al. 2014).

Following the concept of the regional scale studies of the project, in the next step coupled density-dependent fluid flow and heat transport simulations were executed to better understand the basinal flow on geological time scale for the boundary of unconfined and confined carbonate systems. Applying the Heatflow-Smoker finite element model (Molson and Frind 2015) scenario modelling of three evolutionary steps of the BTK was carried out between the fully-confined carbonate stage to partly and completely unconfined stages. The results highlight the critical role of confining formations on flow patterns, and their effect on heat distribution and dissipation over geological time scales (Havril et al. 2016) (Fig.1). Not only the evolution of flow pattern on geological time scale is interesting, but also the transition from fresh to saline waters (Mádl-Szőnyi and Tóth 2015) because hypogenic karstification due to microbial activity was found to be active here (Gray and Engel 2013).

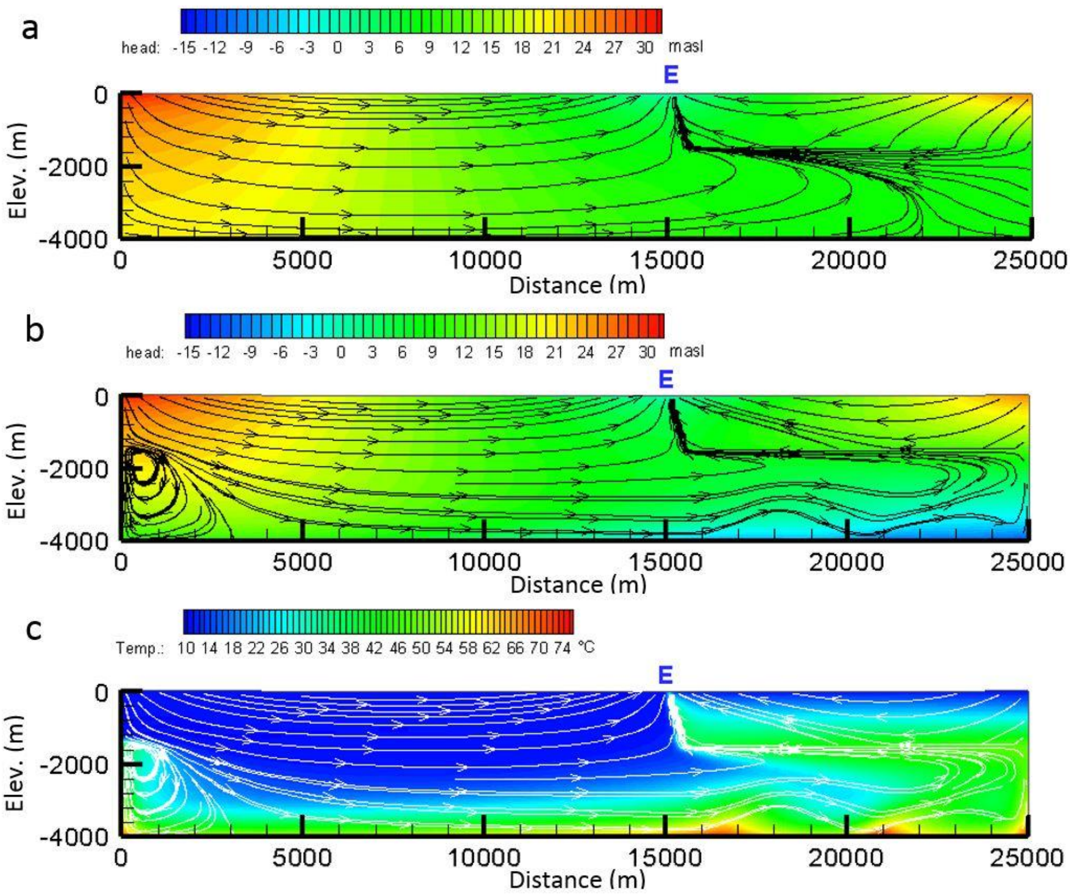

Fig. 1. Simulation Stage 3 (Erosion base (E), additional uplift of western block) showing a) the hydraulic head distribution and flow patterns generated by only the water table topography, and in b) and c) another wise identical simulation after 110 kyr, but considering both gravity and buoyancy, showing b) the quasi steady-state hydraulic heads and c) the temperature field. The (same) quasi-steady flow system after 110 kyr for the coupled case (b & c) is superimposed on both plots. (Havril et al. 2016)

Therefore, the hydrodynamic and salinity pattern of a confined basement carbonate region with its adjacent unconfined part and siliciclastic confining strata were interpreted for the surroundings of BTK (Mádl-Szőnyi et al. 2017b accepted to Marine and Petroleum Geology). Based on these studies the salinity boundaries could be delineated in the confined system. For the deep confined area, numerical simulations were carried out to better understand and interpret the role of gravity and heat as driving forces in this environment (Mádl-Szőnyi et al. 2016). The Gödöllő Hills, east of Budapest was evaluated in more detail to better understand its connection with the unconfined Buda area. The carbonate aquifers are covered by Paleogene and Neogene sequence here, and faulted by characteristic structural elements, such as the Szada normal fault zone, along which a 1000 m shift can be detected. The hydraulic evaluation has shown that the area is located primarily below a recharge regime and characterized by subhydrostatic or close to hydrostatic pressure patterns due to faults and uplifting of the Gödöllő Hills (Mádl-Szőnyi et al. 2015).
The flow field based on more detailed evaluation in the area of unconfined and semi-confined areas of Budapest shows strong correlation with the topographic conditions down to some hundred meter depth (Erhardt et al. 2017) (Fig. 2). The hydraulic study proved the existence of upward vertical flow in

those areas where hypogenic caves are found and developing recently (Leél-Őssy and Surányi 2003, Erőss et al. 2011). This can be recognized as potential plumes or mounds on hydraulic cross sections (Erhardt et al. 2017). The rising flow and associated morphological features are the most decisive characteristics of hypogenic speleogenesis (Klimchouk 2007). Barrier, as well as vertically conduit but transversally barrier faults, and aquitard units proved to be not perfect, but effective obstacles for lateral flows, and responsible for the differences in the discharge distribution (one and two-component). The role of faults was followed in the Gellért Tunnel based on water table differences in its two sides (Young Researcher Thesis of Priegl 2016). In case of one component thermal water discharge, the water table related geochemical (e.g. redox) changes govern the biogeochemical dissolutional processes. Based on evaluation of flow geometry for the broader area of Budapest, and the evaluation of fluid composition (Mádl-Szőnyi et al. 2017b accepted to Marine and Petroleum Geology), an underground interface could be hydraulically and chemically determined between flow pathes where mixing corrosion has to be active in karst corrosion.

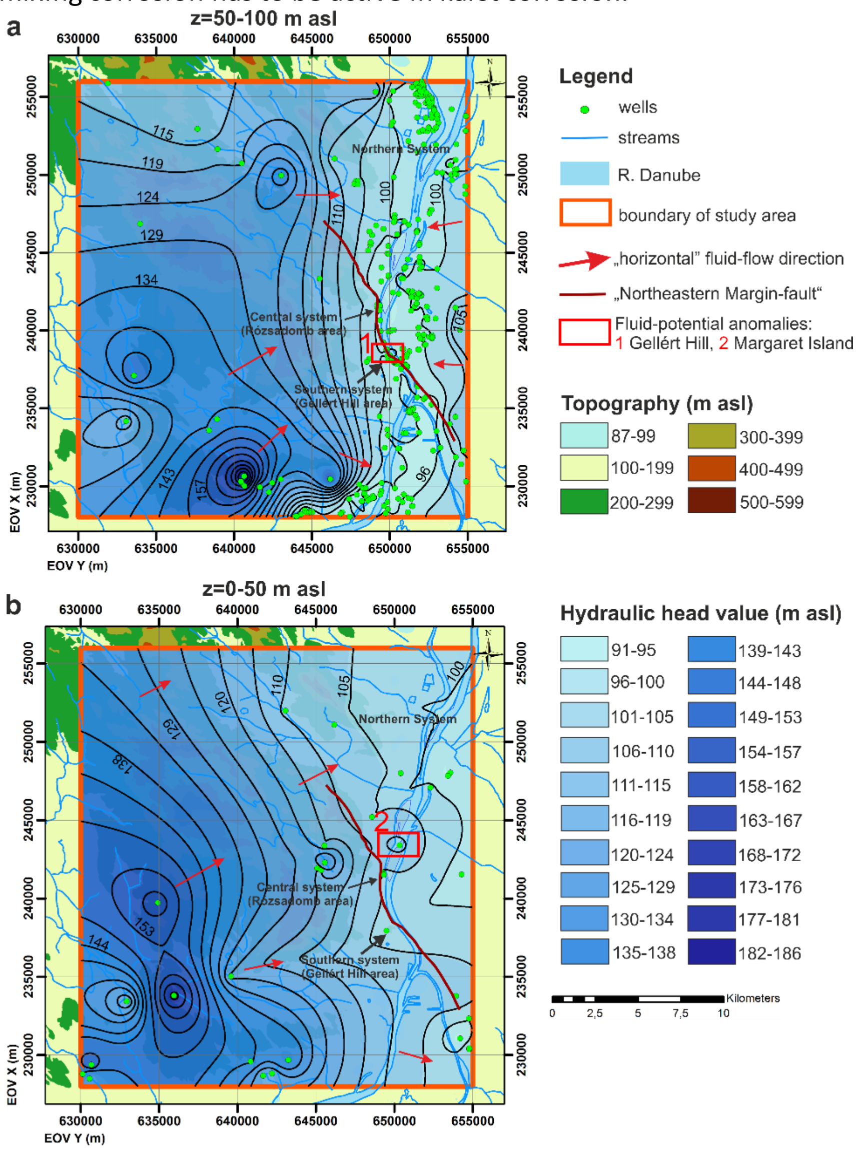

Fig. 2. a) Tomographic fluid potential map of z=50-100 m asl elevation interval, b) Tomographic fluid potential map of z=0-50 m asl elevation interval (Erhard et al. 2017)

The flow pattern indicates the zones of potential hypogenic karstification and these areas can be compared with the location of known cavities not only under the elevated Buda but also in the confined Pest area and in the deep confined system. The database of the project contains the data of thermal wells and these were used to evaluate the signals for underground cavities. The occurance of cavities in the wells extends from some meters to some hundred meters. Examples from the documentations evidence that pyrite-, iron oxide and calcite precipitates often accompany the cavities in the depth which are characteristic also in the well-known Buda caves (Young Researcher Thesis of Pável 2014; Pável et al. 2014).

Local scale research

During on local scale studies, water and biofilm samples were examined to understand their effects on karst corrosion. The springs and wells of the local study areas (Gellért Hill, Rózsadomb, Széchenyi Spa) were used for systematic analysis. The goals of the evaluation were to understand the composition of water and its relationship with biofilm; separation of organic and inorganic content of biofilm and understanding the chemical binding of inorganic elements by biofilm in addition to reveal the composition and role of microbial communities on karst corrosion. The in situ parameters (pH, temperature, electric conductivity, dissolved oxygen, redox potential) were measured. Chemical laboratory analyses of water and biofilm samples for major ions and basic chemical parameters were carried out. In case of trace elements ICP-MS for water and TXRF for biofilm were used. Total carbon (TC), total organic carbon (TOC), total inorganic carbon (TIC) and total nitrogen (TN) content were measured. The findings suggested that the samples contain high amounts of carbonates. The elemental composition shows that several elements (e.g. Ca, Fe, S, Cr, Mn) show significant concentration differences in different spring and well areas (Fig. 3). The enrichment factors (EF=concentration in biogeochemical sample/concentration in water) were calculated based on measurements. Unusually high EF values (10^7, 10^8) were found for certain elements (Fe, Sn), while S, which is responsible for the acidic corrosion, had relatively low EF values ($10^1 – 10^2$) at discharge areas.

Concentration ranges of elements detected in biogeochemical samples.

Concentration range (μg/g)	Elements
10,000–150,000	C, Ca, Fe
1000–10,000	N, S, P, K, Ti, Zn, Ms, As
100–1000	Ba, Cl, Cr, Cu, I, Pb, Sn, Sr, V
10–100	Ni, Co, Se, Br, Rb

Fig.3. Concentration ranges of elements detected in biogeochemical samples (Dobosy et al. 2016)

The results were published by Dobosy et al. (2016). The iron hydroxide composition of biofilm samples was decomposed into a sextet and two doublets and were assigned to goethite, hematite, ferrihydrite and siderite by ^{57}Fe Mössbauer spectroscopy (Kuzmann et al. 2014). The room temperature spectra indicated that goethite and/or hematite are in superparamagnetic state at room temperature. Additionally, isotope geochemical investigations were carried out for biofilm: δ^{34}S; and for water: δ^{34}S, ^{3}H, δ^{18}O. The sulphur isotope of biofilm and water samples were measured at Hertelendi Laboratory for Environmental Studies in Debrecen. The radioactivity measurement of water and biofilm was to acquire better understanding of the relationship between them. The springs of Rudas Spa (Gellért Hill) have the highest ^{222}Rn (600–970 Bq/L) and ^{226}Ra (220–500 mBq/L) concentrations. The biofilm from Török spring has the highest ^{226}Ra acitivity concentrations (1300– 2100 Bq/kg). Among precipitates of Török spring the biofilm has the highest ^{222}Rn exhalation (550 Bq/kg) (Freiler et al. 2013). The mineralogical composition of the radon emitter material is: calcite, aragonite, dolomite, goethite and ferrihydrite. Taking into consideration all the sampled locations, the specific activity of the ^{226}Ra isotope of the precipitates varies between 300– 2200 Bq/kg ± 15%, and the specific ^{222}Rn exhalation of the samples are 100–600 Bq/kg. The average ^{222}Rn activity of the water is 617±22 Bq/l, measured by liquid scintillation spectroscopy (Department of Nuclear Physics, ELTE). The ^{226}Ra activity of the water based on alpha spectroscopy however is low, 3 – 6 mBq/l, compared to the previous measurements (MSc Theses of Karlik 2013, Magyar 2014 and Kurcz 2014). These data confirmed that the source of the elevated ^{222}Rn content of the thermal water is not directly the ^{226}Ra activity of the discharging water. It can be explained only by the adsorption of the radium by the biogeochemical precipitate in agreement with Erőss et al. (2012b).

Therefore, the location of the precipitation in a phreatic underwater cave can indicate the mixing zones. Due to ^{226}Ra adsorption of this precipitates ^{222}Rn can be a good mixing indicator. The measurement of radon and electric conductivity in different parts of the underwater Molnár János cave was used to delineate the potential mixing zones of waters with different origin based on the elevated radon content of water (Csondor et al. 2016).

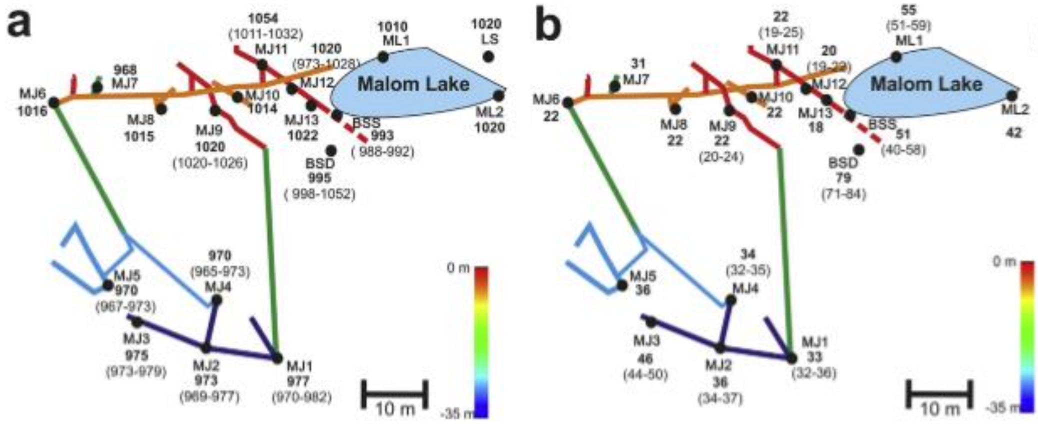

Fig.4. a) Distribution of the electrical conductivity values (mS/cm) in the Molnár János cave: average in bold, minimum and maximum in brackets. b) Distribution of the radon activity concentrations in the Molnár János cave (Bq/L): average in bold, minimum and maximum in brackets. The shallower and the deeper cave passages are marked by different color on the map (see the color scale of the depth on the right side of the figure), the red one is the shallowest region (5 m below surface) and the blue is the deepest (35 m below surface). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.) (Csondor et al. 2016)

Time scale investigations

Focusing on regional discharge zones (Molnár János cave– Boltív spring–Malom Lake– Danube at Rózsadomb), the main goal was the understanding of the role of direct precipitation and the effect of River Danube. The in situ parameters (temperature, electric conductivity, pH) were measured during 8 month period complemented by the measurement of dissolved CO_2, ^{222}Rn, δD and $\delta^{18}O$ and were compared by the level of the Danube and daily precipitation. The field and lab measurements were carried out in cooperation with BME and MTA CSFK colleagues. The first goal of the research was to complement our former knowledge (Alföldi et al. 1968; Fórizs 2011) regarding the time dependence of mixing rate at the discharge region. Correlation and cross-correlation methods were used during data evaluation. The time series and the statistical evaluation have already verified that the effect of direct precipitation is negligible due to the high storage capacity of the system which is indicated by huge discharge volume (Bodor et al. 2015). The effect of precipitation influences the water level in indirect way even in the recharge regions but only the integrated deviations of yearly precipitation were in correlation with the long-term water level changes of karst water observation wells (Bodor et al. 2015). In contrary, the effect of the Danube was revealed in more quantitative way than previously and could be detected not only in the water volume and temperature, but also in electric conductivity. Cross correlation examinations proved that the effect of the water level change of the Danube can be seen in some hours in the shallow observation wells but only after 3-4 days in the Molnár János cave– Boltív spring– Malom Lake system. It was found that the system's parameters are slightly variable but consequently there is limited change in the ratio of lukewarm and thermal components. The effects of human activities were also recognized in the parameters of Rózsadomb discharge area (Bodor et al. 2014).

Exploring the community structure and potential activity of microbes in spring caves and wells

Microbial communities of water and biofilm

A significant direction of the research was the examination of the relationship between microbial communities of water and biofilm in different springs and wells of BTK. The aim of the study was to examine the microbial communities developed in the form of biofilm on the cave walls, on calcite rafts and in the discharging water using molecular methods. From six sampling sites of BTK (GO, Gellért Ősforrás; RT, Rudas-Török spring; DH, Diana-Hygieia spring; RN, Rác Nagy spring;

MJ, Molnár János cave; VL, Városliget II. spring). The iron hydroxide precipitates developing on cell surfaces were studied using microscopic and spectroscopic methods. DGGE analysis of samples (17 water and biofilm) taken from the same sites showed a significant difference between water and biofilm communities, where biofilm samples proved to be more diverse. Altogether 21 molecular clone libraries were constructed. In the 8 bacterial and 7 archaeal biofilm, furthermore 4 bacterial and 2 archaeal water clone libraries almost 1100, 600, 500 and 200 molecular clones were analyzed, respectively (Fig. 5a). Based on the 16S rRNA gene sequences, members of 18 bacterial and 4 archaeal phyla, furthermore 9 candidate phyla were revealed from the biofilm samples which indicates a quite high taxonomic diversity. The 16S rRNA sequences of the representative clones showed the closest relation to uncultured clones from similar environmental sources (Anda et al. 2014, 2015, 2017a prepared for publication; Borsodi et al. 2017a prepared for publication). Molecular clones related to phyla Chloroflexi were characteristic to each biofilm sample but their distribution was different among the sampling sites. Members of phyla Nitrospirae and Proteobacteria (classes Beta- and Deltaproteobacteria) were present in all but one biofilm samples. The highest taxonomic diversity (with the presence of 14-10 different phyla) was found in the southern part of BTK where opposite trends could be observed in the distribution of molecular clones.

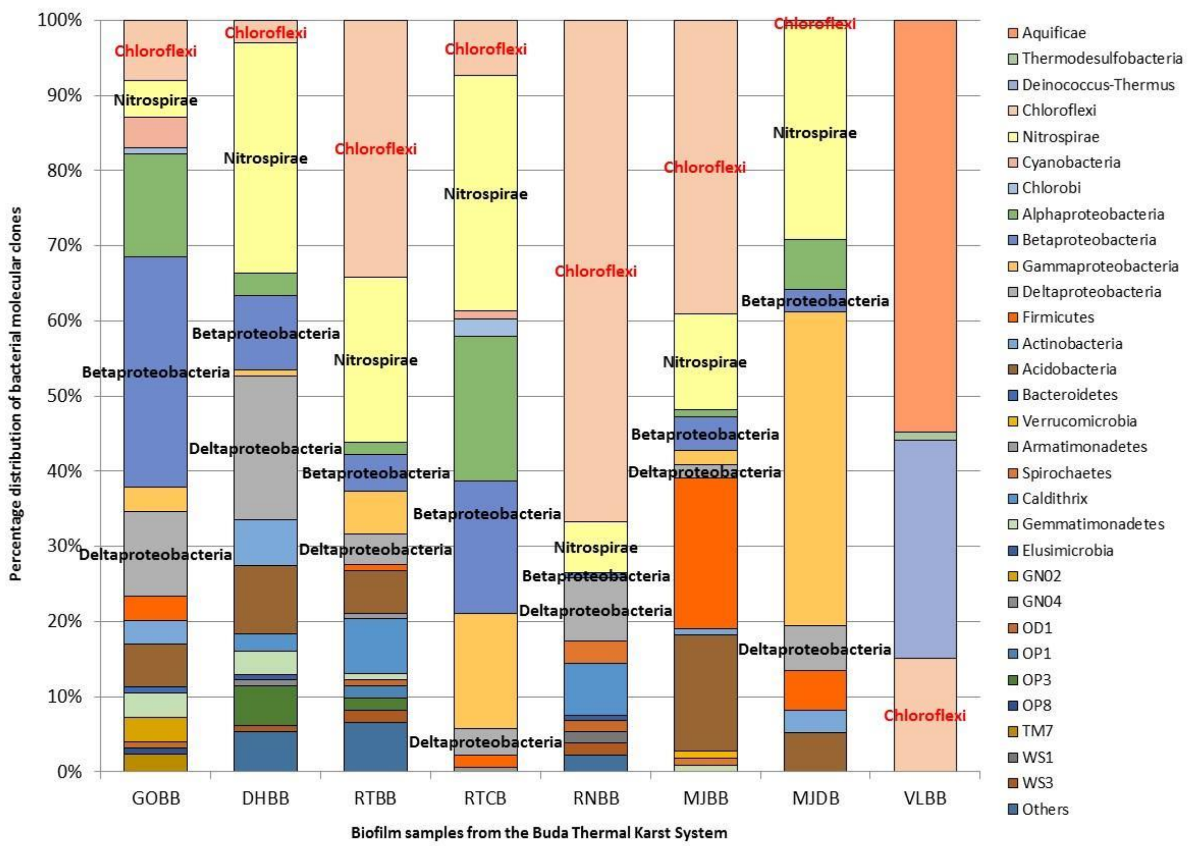

Fig. 5a Percentage distribution of bacterial molecular clones from the biofilm samples (Borsodi et al. 2017a prepared for publication)

In the MJ biofilm samples, molecular clones belonging to the class Gammaproteobacteria (black biogeochemical layer) while phyla Firmicutes and Acidobacteria (reddish-brown biofilm) were the most abundant besides Chloroflexi and Nitrospirae (Borsodi et al. 2017b; Anda et al. 2017a prepared for publication). The VL biofilm sample was dominated by molecular clones related to thermophilic bacteria of the phyla Aquificae and Deinococcus-Thermus (Anda et al. 2015). Bacterial clone libraries constructed from the water samples were much less diverse compared to the biofilm samples and dominated by *Thiobacillus* (Betaproteobacteria) in every case (Anda et al. 2014, 2015, 2016; Borsodi et al. 2017a prepared for publication). As regards the Archaea, all but one biofilm sample were predominated with phylotypes closely related to *Nitrososphaera* within the phylum Thaumarchaeota. Other phylotypes were members of Euryarchaeota and Crenarchaeota (Fig. 5b).

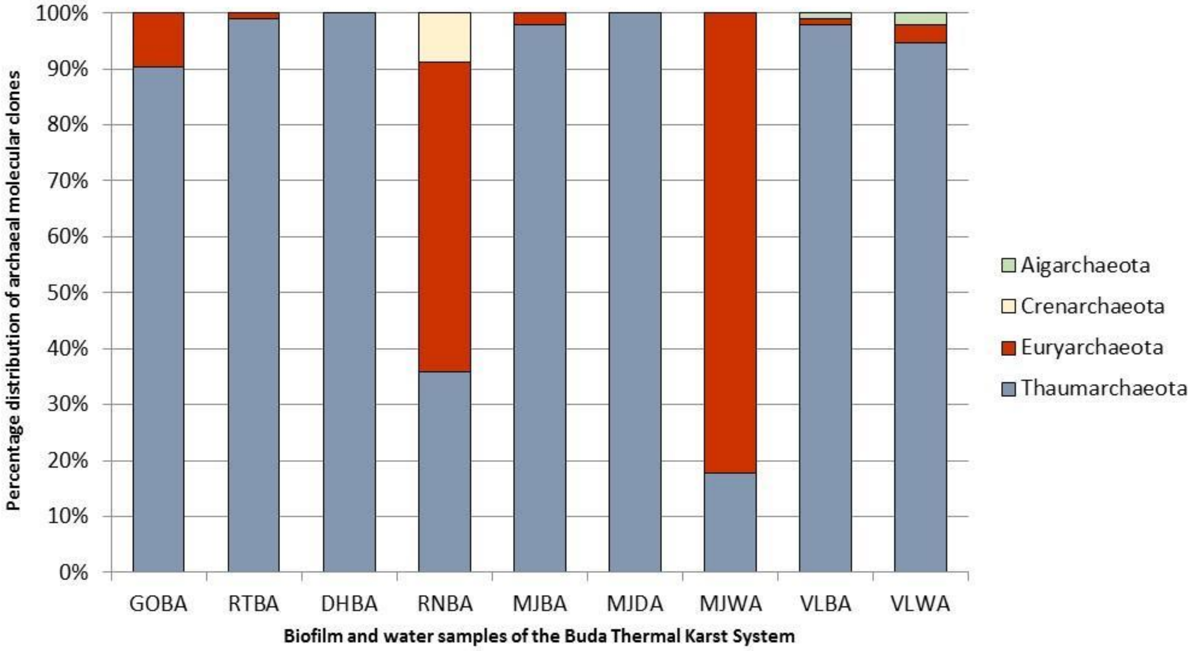

Fig.5b Percentage distribution of archaeal molecular clones from the water and biofilm samples (Borsodi et al. 2017a prepared for publication)

Based on the metabolic properties of bacterial species closely related to molecular clones, it can be assumed that members of biofilm communities may be involved mainly in the anaerobic sulfate and iron (III) reduction processes besides the anaerobic decomposition of organic materials. In contrast, water samples of all studied spring caves and wells was dominated by molecular clones related to aerobic and/or facultative anaerobic chemolithotrophic sulfur oxidizer bacteria which can be responsible for the sulfuric acid speleogenesis in the studied hypogenic cave system. In addition, the occurrence of phylotypes related to ammonia-oxidizing archaea and nitrite-oxidizing bacteria in all habitats indicates that oxidation of reduced nitrogen forms may also be an important microbial process beside the local sulfur and iron biogeochemical transformations (Anda et al. 2014, 2015, 2017a prepared for publication; Borsodi et al. 2012, 2017a prepared for publication).

Scanning electron microscopy (SEM) was used to examine the morphological structure and composition of mucilaginous, reddish-brown biofilms. The low magnification scanning electron microscope images (e. g. Városliget II. well, Rudas-Török spring) showed that different crystal structures of calcium carbonate minerals served as surface for colonization of bacterial cells and filaments (Anda et al. 2015). SEM micrographs (e.g. biofilm samples of Molnár János cave, Gellért Ősforrás and Rác Nagy spring) have revealed that multilayer network architecture structure in microbial mats formed by filamentous bacteria and other cells with mineral crystals and organic matter matrix. This is a common structural element that holds apart (separates) successive layers (Fig. 6A-D) (Anda et al. 2016).

Along with the assembly of filamentous bacteria of various thickness and appearance, numerous straight and curved rod-shaped, aggregate-forming cells of different size were visible. The samples contained numerous filamentous structures whose morphologies were similar to those produced by known iron-oxidizing bacterium (FeOB). The *Leptothrix*-type sheath-forming organisms were common in each sample (e. g. Diana-Hygieia thermal spring, Molnár János cave, Rudas-Török spring). *Leptothrix* species (Betaproteobacteria), one of the Fe/Mn-oxidizing bacteria, oxidize Fe(II). The screw-shaped bacteria were also found which is typical characteristic of Nitrospira. In the images taken in the backscattered electron mode (BSED), details showing the amorphous and globular shaped precipitates of the surfaces of filamentous bacteria and appearance were seen much brighter (white) compared to their environment which means that they carry parts containing elements of higher atomic number (e.g. heavy metals). The elements of greater atomic number indicate microbes accumulating heavy metal compounds (Erőss et al. 2017 prepared for publication).

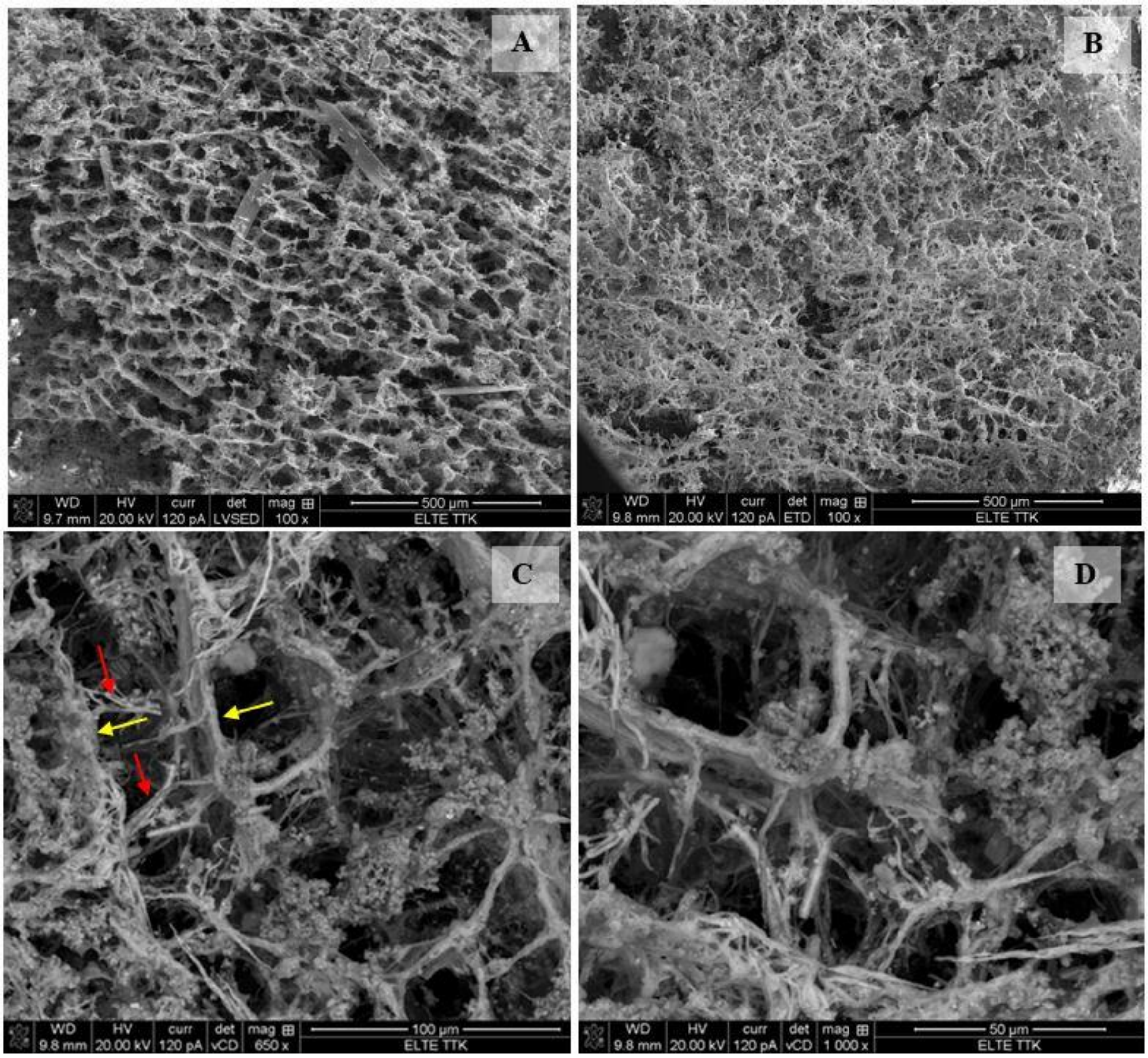

Fig. 6 SEM micrographs from biofilm samples of Ősforrás (A) and Molnár János cave (B-C). The SEM images show multilayer network structure that consists of intertwined filamentous bacteria [horizontal (yellow arrow) and vertical connections (red arrow)] that are held together of EPS

Connection and adaptation of microbes to the elevated radioactivity

This research program aimed to expose extremophilic bacteria adapted to the radioactive environment of BTK. Samples for this cultivation based studies were taken from submerged biofilm and calcite precipitation on the water table found in Rudas-Török spring (RT) and Diana-Hygieia (DH) sampling sites. Prior to the isolation of the ionizing radiation resistant bacterial strains (IRRBS), the biofilm samples were irradiated by gamma at various doses (5, 10, 15 kGy) using NORATOM equipment with a 60Co source. Altogether 196 and 227 pigment and/or endospore producing bacterial strains (115 and 152 from the irradiated as well as 81 and 75 from the non–irradiated biofilm samples) were isolated from the RT and DH samples using different media. Among the IRRBS representatives of phyla Firmicutes, Proteobacteria, Deinococcus-Thermus and Actinobacteria were identified. The irradiated biofilm samples proved to be more diverse than the non-irradiated samples in both sampling sites. Only some common species were found among the cultivated IRRBS from the non-irradiated and the irradiated biofilm samples. From the highest dose (15 kGy) irradiated biofilm samples members of the genera *Bacillus, Micrococcus, Paracoccus, Deinococcus, Brevibacterium* were identified. The applied gamma radiation method has proved to be successful for the detection of microbes belonging to the radioactive resistant bacterial group (Young Researcher Theses of Pál 2015 and Enyedi 2016; Enyedi et al. 2016; Enyedi et al. 2017 prepared for publication). Following the exposure of the biofilm sample from the RT spring cave to gamma radiation, a strain designated FeSTC15-38T was studied by polyphasic taxonomic methods and described as the type strain of a novel species, named *Deinococcus budaensis* sp. nov. (Makk et al. 2016).

Experimental studies for better understanding microbially mediated precipitation processes

Biofilms exist in many thermal spring caves and even in thermal water wells of the Buda Thermal Karst, Hungary and other karst systems worldwide. Though many properties are known about the existing biofilms, their evolution and their interaction with the water is less studied.

1stin situ experiment
The first in situ experimental examination started in September 2015 to study the biofilm development in the Rudas-Török spring cave. The applied in situ experimental model system offers optimal conditions for understanding the precipitation and dissolution processes in controlled spring environment. In the frame of this project the first one-year-long experiment was finished in September 2016. The main goal of the microbial-hydrogeological model system was to reveal the evolution of biological and mineral precipitates in time in a (quasi-stagnant spring with no measurable velocity) discharging thermal water. The experiment started with the set up of a complex monitoring system for field parameters (temperature, specific electric conductivity) in addition with the measurement of pH and dissolved oxygen content of water complemented by laboratory measurements (for major ions, ^{222}Rn, δ^{34}S, ^{226}Ra, $^{238+234}$U). In order to study the appearance and the formation of precipitate, sterile glass slides were put into the water into a tube rack. Slides were taken at regular intervals to examine them by light and scanning electron microscopy as well as using new generation sequencing method. The experiment resulted in an increase in the taxonomic diversity of bacterial communities which reached the maximum at phylum level in the third week. The members of phyla Chloroflexi, Parcubacteria, Plantomycetes, Proteobacteria, WCHB1-60 and Nitrospirae proved to be most abundant but their relative abundance changed at different rates during the studied period. The results of these analyses are under evaluation (Anda et al. 2017b, c, d prepared for publication). After one-year-long experimental phase the evolved precipitate was examined by chemical analytical (ICP-MS) and mineralogical (XRD) methods and Mössbauer and gamma spectroscopy, δ^{34}S measurements. In the further phase of the experiment (out of this NKFI period) a similar experiment is planned with emerged rock slices to follow not only precipitation but also dissolution processes. However, the complete evaluation of the recent phase is necessary to plan the further phase (MSc Thesis of Burkus 2017 before submission).

2nd in situ experiment

The second in situ experiment was executed in the so-called canal of the Gellért Tunnel (Fig. 7a). Here the main goals were the understanding of the formation of the biofilm from flowing thermal water.

The second question was the better understanding of the controlling factors influencing the transition between biofilms and calcium-carbonate precipitates. In this part of the study we had to face with many difficulties during the set up of the experiment: selecting and testing suitable devices for these extreme conditions, cleaning the canal of Gellért Tunnel from old (anthropogenic) precipitates, planning of the observed length of the tunnel and the sampling frequency. According to the planning circumstances and our preliminary observations, this second in situ experiment was divided into three parts.

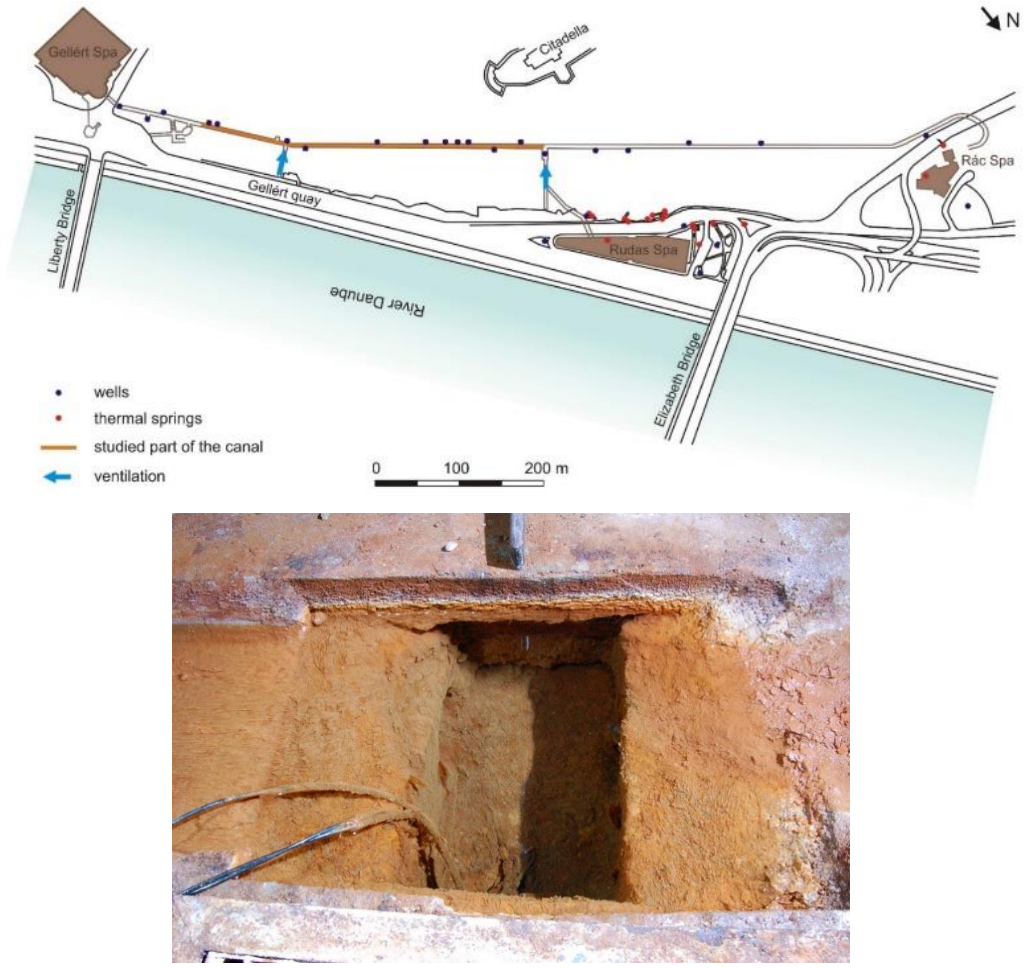

Fig. 7. a) Location of the in situ experiment in the Gellért Tunnel (Bodor and Mádl-Szőnyi 2017a); b The outflowing hole of the canal (photo: Hegedűs A.)

In the first phase the aim of our study was to follow the variation of physical-chemical parameters such as the temperature, specific electric conductivity, pH, dissolved oxygen content, redox potential, concentration of major ions, dissolved carbon dioxide content, concentration of ^{226}Ra, $^{234+238}$U and ^{222}Rn from the discharge location (Fig 7b) of thermal springs as long as they can be followed. Discharge volume and flow velocity of the flowing water were also measured. The experiment was conducted twice (March and April 2016) (Fig. 8) and the results were evaluated and compared by reactive transport modeling with PHREEQC. It is found in agreement with the literature that degassing of CO_2 is a key process controlling the water chemistry, including pH. Ingassing of oxygen also occurs and affects the redox state of the water along the canal. However, we can see deviations from the pure chemical processes which can be caused by microbiological activity close to the outflow of the thermal spring (Bodor and Mádl-Szőnyi 2017a; Bodor et al. 2017a; Bodor et al. 2017b prepared for publication). The conclusions of this study were used for the planning of further investigations to better understand the interaction of biological and chemical processes. During the two experimental period, the activity of ^{222}Rn was measured not only in the canal but also in the air and interpreted in the frame of geology (Young Researcher Thesis of Várkonyi and Tímár 2016).

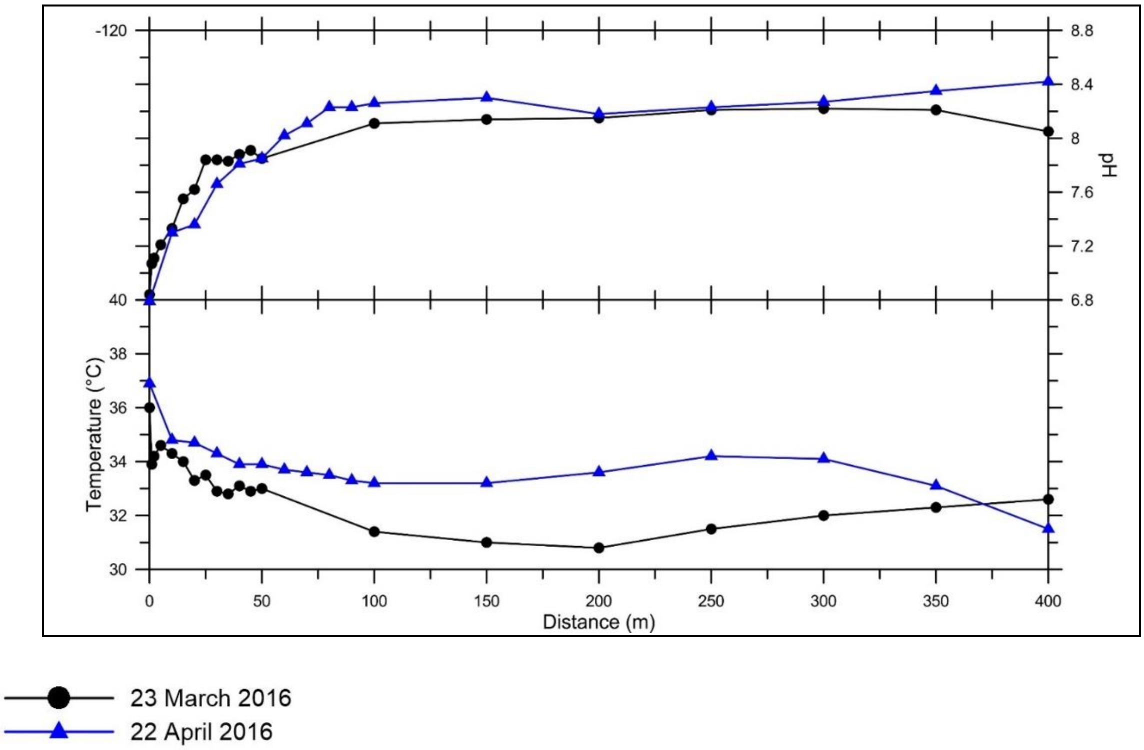

Fig. 8 The temperature and pH values in the 1st phase of the 2nd experiment (Bodor and Mádl-Szőnyi 2017a)

The research was continued in its second stage with the goal to follow the evolution of bacterial biofilms. During the experiment with the evolution of precipitates, concurrent monitoring of basic physical-chemical parameters (temperature, specific electric conductivity, dissolved oxygen content, pH) were carried out (Fig.9). So, a 12-week-long in situ experiment had been continued from December 2016 to February 2017. During the experiment the precipitates and the water were monitored in time and along the flow path (120 m long) in a controlled environment of the canal (Fig. 9). Temperature, pH, specific electric conductivity, dissolved oxygen content and volume discharge were monitored continuously at the beginning and at the end of the studied section of the canal. Other parameters (redox potential, concentration of major ions, dissolved carbon dioxide content, concentration of ^{226}Ra, $^{234+238}$U and ^{222}Rn, TOC and TN content, concentration of trace elements) were detected three times during the experiment (0, 6th, 12th weeks). The evolved precipitates were sampled twice (6th, 12th weeks) and were analyzed by XRD, SEM, ICP-MS, Mössbauer and gamma spectroscopy, and δ^{34}S measurements. The controlled environment helps to interpret the results and the influencing factors regarding the evolution of the precipitates (Bodor and Mádl-Szőnyi 2017b; Bodor et al. 2017c). The data are still under evaluation.

The comparison of the results of two in situ experiments with different flow dynamics can reveal the significance of flow velocity in the evolution of different precipitates in this special environment (Bodor et al. 2017d).

Fig. 9. a) The measuring devices in the inflow and outflow locations of the canal. b) The Gellért Tunnel with the canal (photos: Hegedűs A. and Csondor K.) (Bodor and Mádl-Szőnyi 2017b)

Adaptation of recent cave forming processes to the evaluation of palaeo caves

During the execution of the project we focused on the understanding of the relationship between geologically preserved and recent biofilms. The examination started with the sampling of the Aragonite cave (goethite) and it was examined by FEI Quanta 3D dual beam scanning electrone microscope (FIB-SEM). The samples show characteristic morphology for different tube-like forms. In greater resolution, we could see their different diameters with a hole in their center. It was also recognised that these filamentous bacteria accumulated minerals in different thickness on their surfaces (Anda et al. 2017e prepared for publication).

A research continued by the sampling of the "indicator" mineral paragenesis in caves of the local study areas of Rózsadomb (Molnár János cave) and Gellért Hill (Török, Rákóczi caves). The qualitative and quantitative analysis of distribution and morphology of mineral parageneses were achieved by binocular and petrography microscopes, XRD and SEM. It was found that gypsum is the dominant mineral phase on the walls above the water table dispite of different host rock (Rózsadomb, Buda Marl; Gellért Hill, Main Dolomite). However, the amount and appearance of gypsum is different in the different caves. Nevertheless, the abundance of gypsum is increasing in the vicinity of the water table. It was found that outgassing of H_2S appears on the water level which indicates strong correlation of cave formation and gypsum precipitation. Under the water level white Mg-calcite was found. In the Molnár János cave this mud is yellow, muddy and contains quartz, kaolinite and 10 Å-s stratasilicate. On the water level, calcite rafts can be found in the Török and Rákóczi caves while these are subordinate in the Molnár János cave. The black mineral cover above the water level in the Molnár János cave is characterized by quartz, kaolinite, illite and 10 Å stratasilicate, moreover S, Mn, Fe, Ca , Al and Si also could be detected (Young Researcher Thesis of Ambrus 2014).

To follow up the previous consideration the potential analogue of Aragonit cave (AC) (dry) (as we have seen in Young Researcher Theses of Pável (2014) and Ambrus (2014); Pável et al. (2014)) and the Rudas-Török spring cave (RT) were used. The sampling was taking place in November 2014 and in July 2015. The goals of the sampling were twofold: first of all, the mineral parageneses of Aragonit cave were sampled in more detail than ever previously. The sampling was carried out to represent the spatial and time differences in the geological evolution of this paragenezis in the Aragonit cave. The samples were collected not only for mineralogical, and nuclear physical, chemical examinations but also for microbiological purposes. The analyses were executed in the water by measurement of pH, electric conductivity, temperature, ^{222}Rn and in the rock and precipitate samples by field and microscopic (macro- and stereomicroscopic) study, XRD, Mössbauer spectroscopy, ICP-MS, SEM+EDS, stable isotope geochemistry, $\delta^{34}S$, etc. The Aragonite cave located ~15-20 m above the recent karst water level. Based on the investigations, four mineral parageneses were distinguished, indicating the changes in the palaeoenvironment. At the beginning, in phreatic circumstances, iron oxidizing bacteria resulted a layered, red deposit, containing mainly goethite. This was followed by a dry event when the palaeo-water table descended. Then an oversaturated thermal water (>30 °C) in respect of $CaCO_3$ took place in the cave, resulting a wide crust of "cave clouds" on the walls. As the water table started to decrease again, different sulphates (epsomite and gypsum) precipitated on the walls above the water table, due to H_2S oxidizing bacteria. In the last stage, the cave's circumstances become completely vadose and small aragonite needles and hydromagnesite started to precipitate on the surface of the "mammillary" calcite crust, alongside with the second generation of sulphates on the cave's wall (MSc Thesis of Pásztor 2016) (Fig. 10). The results of the evaluation of Török spring biofilm and water will be appeared in the MSc Thesis of Burkus (2017). The comparison of the recent and palaeo spring caves will be presented at the 44th IAH Congress in September 2017 in Dubrovnik, Croatia (Pásztor et al. 2017).
The Mössbauer study of the AC samples revealed superparamagnetic goethite as iron bearing component. Superparamagnetism indicated diffrent particle size distributions in samples of various origin. The biofilm samples from RT showed only paramagnetic spectrum components even at 78 K, indicating the smallest particle size found (Homonnay et al. 2016).

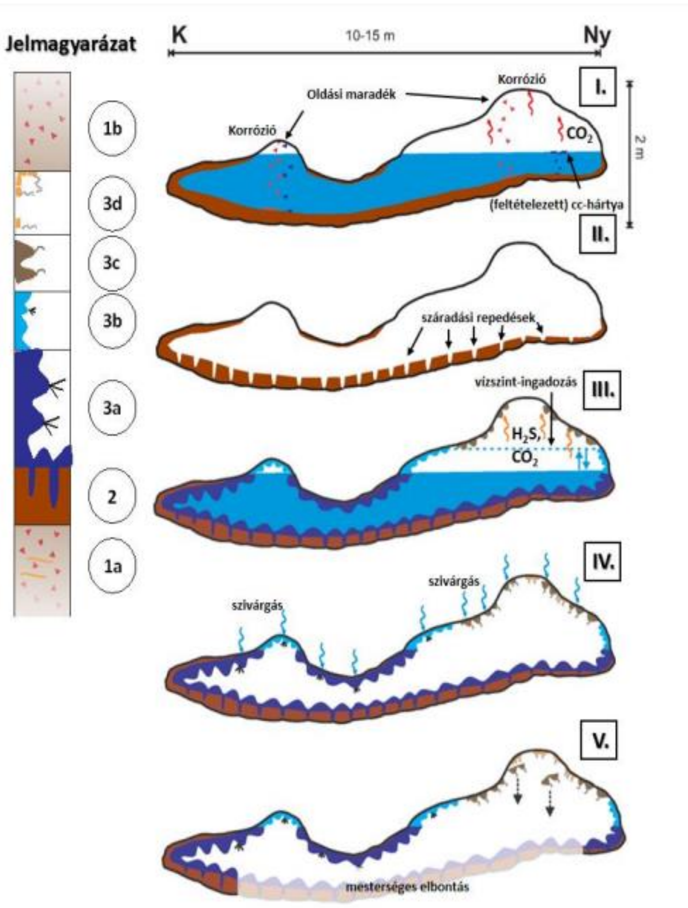

Fig. 10. a) Török spring: biofilm and calcite precipitate. b) Evolution phases of Aragonite cave (Pásztor 2016)

For the molecular studies, extraction of community DNA and polymerase chain reaction (PCR) for the amplification of the bacterial 16S RNA genes followed by next generation sequencing (NGS) were carried out. The bacterial diversity of cave wall associated communities in the dry Aragonit cave (ARA) was first examined by a molecular fingerprint method (DGGE) to compare the similarities of the different sample types, and then detailed phylogenetic diversity analyses on the representative samples was performed using NGS method. Bacterial communities of 8 out of the altogether 10 ARA samples formed one cluster, and separated from that of the Rudas - Török spring calcite (RTC) samples. Based on the DGGE results a goethite mineral sample with calcite veins (ARA 4), a spiked aragonite sample (ARA 6) and a basal rock sample (ARA 3) was submitted to NGS analysis (Fig. 11). From the three NGS analyzed ARA samples, a total of 10566 quality-filtered partial 16S rRNA gene sequences with an average length of 397 nucleotides were obtained. Nevertheless, high differences were found in the number of operational taxonomic units (OTUs) of the samples. From the ARA 3 and RT samples approximately 150-160 OTUs were revealed but only 70-80 OTUs from the ARA 4 and ARA 6 samples. The ARA samples showed less diverse bacterial community structures than the RT sample as twice as many representatives of different phyla were exposed from the RT than ARA samples.

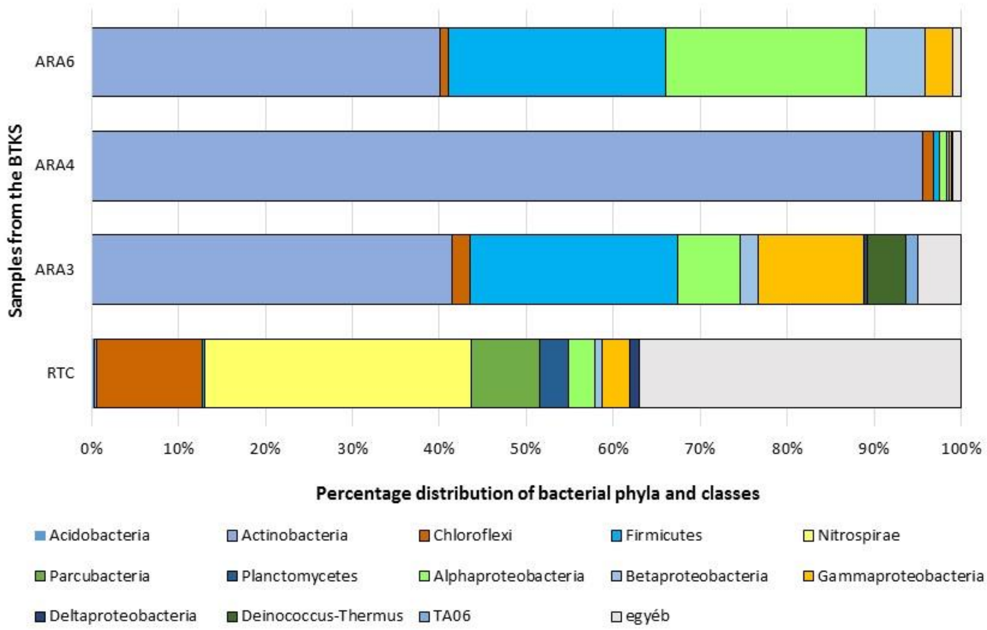

Fig. 11. Percentage distributions of amplicon sequences among the phyla and classes from the ARA samples (Borsodi et al. 2017 prepared for publication)

Sequences belonging to phyla Actinobacteria, Firmicutes, Chloroflexi and Proteobacteria were present in all samples. Contrary to RT sample, all the ARA samples were dominated by sequences of the phylum Actinobacteria which are frequently among the most abundant phylotypes of dry caves and known to be able to participate in biomineralization processes. It is interesting to note that molecular clones related to phylum Chloroflexi (*Anaerolinea*) were found to be present previously in every biofilm samples of the BTKS. Detailed data evaluation is still pending (Anda et al. 2017e prepared for publication).

Extension and generalization of the results for similar settings worldwide

We completed theoretical studies regarding the application of flow system approach for better understanding of hypogenic karst systems and we set up a literature database. It could be concluded that the hydrogeological environment is decisive not only in the development of groundwater flow regimes but also in hypogenic karstification and precipitation processes. The conceptual issues of basin-scale groundwater flow systems in an unconfined and confined carbonate region was discussed in the paper of Mádl-Szőnyi and Tóth (2015). The geological and fluid evolution of the system also play role (Havril et al. 2016). The springs are the most decisive indicators of groundwater flow systems. Therefore, their use for flow system interpretation was introduced in the form of a new method (clustering of springs based on gravity-driven flow related parameters: elevation of discharge, temperature, volume discharge, chloride content) and tested for the Transdanubian Range (Mádl-Szőnyi and Tóth 2015).

For the generalization of the findings from Buda Thermal Karst for thick unconfined-confined carbonate regions, a new model was worked out which has already explained not only the source of the karst water component in the system but also involved the basinal fluid component as well. Here the gravity is the main driving force and it is only modified by heat convection. The former models did not involve the vertical cross formational flow across low permeability confining layer as an influencing component of the BTK. The new model was derived from theoretical considerations, experiences and observations from mining-dewatering of Transdanubian Range, clustering of springs and 2D numerical flow and heat transport simulations by COMSOL Multiphysics (Fig. 12). The study has revealed the location of an underground interface under confined part of the BTK between basinal fluids and fresh karst water. Based on the new conceptional model the role of groundwater as a geologic agent could be explained (Mádl-Szőnyi and Tóth 2015). The decisive role of discharging fluid components in hypogenic karstification were found not only for the BTK but other analogues were also found (one component discharge for Bighorn Basin, Sheep Mts; two components discharge for Guadalupe Mt, Delaware Basin (USA) (BSc Thesis of Lovrity 2012). In addition to a comprehensive study, the applicability of the proposed conceptual approach for North China Plain (Chinan); South Dakota, Black Hills; Bükk Mountain and Karsts of Stuttgart were studied (BSc Thesis of Török 2016). The study found similarities and differences. The created conceptual model inspired international authors and it has been cited in case of other karst areas in Spain (Andreo et al. 2016) and in China (Wang et al. 2016; Yang et al. 2017). It has also been published as a chapter of EUROKARST 2016 Book (Mádl-Szőnyi & Tóth 2017). The theoretical findings were also used for the exploration of thermal water from deep carbonate systems.

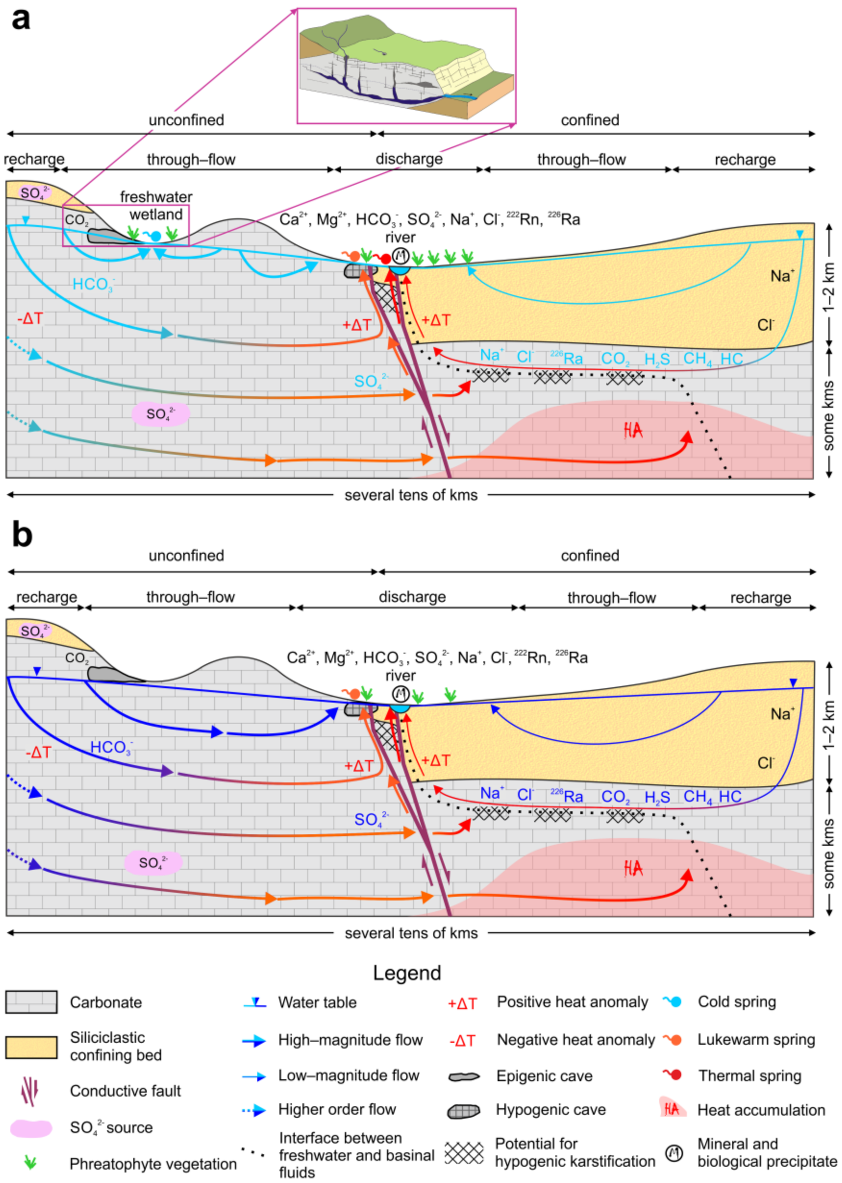

Fig. 12 a) Conceptual Tóth-type GDRGF pattern of thick carbonate systems and its consequences on flow-related manifestations; the shallow karst aquifer (modified after Goldscheider and Drew 2007) is embedded into the regional flow pattern as a local system. b) Change of flow pattern in the conceptual Tóth-type GDRGF for a thick carbonate basin, induced by water-table or hydraulic-head (pressure) decrease (Mádl-Szőnyi and Tóth 2015)

Additionally, a model was worked out for fully confined deep carbonate areas (east of the BTK in the Paleogene Basin, Gödöllő Hills) (Mádl-Szőnyi et al. 2017b accepted to Marine and Petroleum Geology). Here besides the gravity-driven flow the heat convection is also decisive. The results of this study can be significant for the understanding of hydrocarbon migration and geothermal exploration possibilities (Mádl-Szőnyi et al. 2015).

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