## FINAL REPORT

## Results

## Detailed results

During 2017-2021 two Hungarian occurrences of Jurassic age (Mecsek Mts, Réka Valley and Úrkút manganese deposit)(Polgári et al., 2017; Gyollai et al., 2018; Ruebsam et al., 2018) and three Chinese manganese carbonate ore deposits of Neoproterozoic, Carboniferous and Permian ages (Yu et al., 2019, 2021ab), and the Neoproterozoic Brazilian Urucum manganese oxide (Biondi et al., 2020) and iron oxide deposits (Polgári et al., 2021) and were studied, by high resolution methodology. The Triassic Bükkszentkereszt uranium-manganese-beryllium-phosphorous- rare earth element enrichment represent a unique microbial occurrence (Gál et al., 2020). Besides ore deposits and indications recent lake sediments were also studied (Karlik et al., 2018, 2021).

Based on thematic research on ore deposits and indications and lake sediments, general aspects of microbilally mediated metallogenesis were also summarized (Polgári et al., 2019; Gracheva et al., 2021, Polgári and Gyollai, 2021).

Concerning investigation of Jurassic manganese ore deposit at Úrkút, a summary book was published (Polgári et al., 2017).

About the biomats at Úrkút a journal paper was published (Gyollai et al., 2018). The mineralized biomat system of Úrkút is responsible for the high radon content which originated from the selectively enriched radionuclide content. The $^{220}Rn$ and $^{222}Rn$ concentration in the Úrkút Manganese Formation is anomalously high, and it causes health risk for the attendants of the Úrkút Manganese Mine. In this study we suggest a new model for the high ratio of different natural radionuclides by the new genetic model of the Úrkút Manganese Formation. Fossilized Fe-rich biomats were found in the whole section of the deposit. Based on these, the formation of different types of Mn-ores by one or two step microbial mediation can be assumed. The biomats represent large amounts of organic matter offering large surfaces with negative ligands, as well as poorly ordered mineral phases like ferrihydrite and clay minerals with large and reactive surfaces. These features offer high capability of cation binding, among them ions of radioactive elements, which could be fixed on the active or inactive organic matter, as well as on mineral surfaces. The decay of fixed nuclides could result high $^{220}Rn$ and $^{222}Rn$ concentration in the Úrkút Mine (Gyollai et al., 2018).

The results on the Mecsek Toarcian black shale was published in a high ranking journal (Gondwana Research). Environmental response to the early Toarcian carbon cycle and climate perturbations in the northeastern part of the West Tethys shelf. Early Toarcian (Early Jurassic; ~183 Ma) climate and carbon cycle perturbations were accompanied by widespread accumulation of bituminous sediments, formed during the Toarcian Oceanic Anoxic Event (T-OAE). On the northwestern Tethyan shelf, organic carbon accumulation was particularly widespread in hydrodynamically restricted regions of the European Epicontinental Basin System (EEBS) where it peaked during the formation of euxinicwaters extending into the photic zone. In the adjacent northeasternWest Tethys shelf (NE-WTS), an area proximal to the deeper and better ventilated NW Tethys, deposition of strata enriched in organic matter was restricted to depressions of limited spatial extent. Here, we investigated a stratigraphically well-constrained, exceptionally organic matter-rich sediment succession from the NE-WTS deposited during the T-OAE. Organic and isotope geochemical investigations provided insights into the evolution and dynamics of environmental conditions during the T-OAE in a setting proximal to the Tethys. The sedimentary rocks sampled for this study originated from a surface outcrop that experienced weathering related alteration of its mineral constituents. In particular, sulfides (mainly pyrite) were significantly altered, resulting in a near-quantitative loss of sulfur. In contrast, kerogen as well as bitumen fractions have not been significantly affected by weathering processes and carry a robust paleoenvironmental signal. The new data show that organic carbon accumulation occurred as a direct response to changing environmental conditions that also led to the early Toarcian carbon cycle perturbation. Increase in organic carbon burial occurred in a stepwise manner, following multiple injections of 12C-enriched carbon into the Earth's ocean-atmosphere system. High algal productivity and the subsequent organic matter decomposition by sulfate reducing bacteria led to an expansion of $H_2S$-rich waters into the photic zone, which promoted the growth of Chlorobiaceae. A steady increase in nitrogen isotope values testified to intensified denitrification in an expanded oxygen minimum zone. The resulting decline in the availability of bio-utilizable nitrogen in combination with harsh environmental conditions promoted the proliferation of cyanobacteria as indicated by increasing abundances of 2α methyl hopanes. Formation of euxinic waters in the NE-WTS was not associated with a freshwater driven salinity stratification. Organic geochemical salinity indicators (methylated chromanes) attest to normal marine salinity, supporting a strong influence of Tethyan water masses. This study enhances our knowledge on the environmental response towards the early Toarcian climate and carbon cycle perturbations in a setting proximal to the Tethys. We demonstrated that photic zone euxinia was a common and particularly widespread feature of the T-OAE and has not been confined to the hydrodynamically restricted EEBS (Ruebsam et al., 2018).

The P-Mn-U-Be-REE-enrichment in phosphatite at Bükkszentkereszt is also the result of microbial mediation. These results can be used in ore exploration on international level, ore processing and in the field of environmental protection. Strongly deformed phosphorite layers enriched with U-Be-Mn-REE occur in a weathered Triassic metarhyolite tuff near the town of Bükkszentkereszt, NE Hungary. The phosphorite is massive or earthy, has an especially fine-grained texture, and forms inhomogeneous bands with different Mn-oxide contents. High-resolution optical rock microscopy investigations helped to distinguish mineralized biosignatures, filamentous, vermiform structures, and coccoid-like aggregates. These microstructures encompass almost the whole phosphorite. Based on in situ FTIR and Raman spectroscopy, the phosphorites contain ferrihydrite and pyrite, and also different types of embedded organic matter. These structures can be interpreted as series of Fe-rich biomats, forming microbialites. On a larger scale, these microstructures can play a role in shaping the stratiform structure by evolving stromatolite-like bands. Results presented here propose a new interpretation of the origin of the P-Mn-U-Be-HREE-enrichment. The Bükkszentkereszt occurrence claim a mineralized microbially mediated deposit, and our results support this scenario based on the mineralized microbial structures, the embedded minerals, and the presence of organic matter (Gál et al., 2020).

Similar investigation of lake sediments supported the importance of microbial contribution. The detailed *in situ* detection and distribution of organic matter of the samples was going on by other sensitive methods like FTIR and Raman spectroscopy from 2018.

Karlik et al., 2018 Quaternary reported n alkane etc. on previously collected samples. Lake Bolătău-Feredeu is a small (surface: 0.3 ha; catchment area: 31 ha) landslide-dammed lake in

Bukovina (Eastern Carpathians, Romania). Elemental concentration, stable isotope composition of carbon and nitrogen, and n-alkane composition of the saturated hydrocarbon fraction of the organic material were analyzed along the sediment profile from Lake Bolătău-Feredeu covering the past ~700 years. The ranges of $\delta^{13}$C values (−30.5 to −26.6 ‰) and C/N atomic ratios (10.5 and 15.4) placed the organic material of the recent Bolătău-Feredeu sediment between the C3 Land Plants and the lacustrine algae. The n-alkane proxies provide better distinction between organic matter inputs. Detected major shifts in the n-alkane composition and the C and N concentrations along with the stable isotope compositions pointed towards three distinct stages in the environmental history of the lake-catchment system. The proxy information indicate a substantial landscape change characterized by deforestation and an increase in open pastureland with herbaceous vegetation from ~1820. The C/N, $\delta^{15}$N, and $\delta^{13}$C values showed the highest variability probably linked to a variable lacustrine environment and decreased productivity in the catchment between ~1640 and 1760. It can be assumed that the extended periods of lake ice cover during the cold decades experienced at the turn of the 17$^{th}$-18$^{th}$ centuries resulted in diminished biological productivity both in the lake and its catchment. Finally, afforestation and the decline in lake productivity have been reconstructed for the period of ~1470 – 1560 (Karlik et al., 2018).

On an earlier collected sample set detailed *in situ* detection and distribution of mineral composition and organic matter of the samples was made by FTIR and Raman spectroscopy, and the manuscript on these results was submitted to Carpathian Journal of Earth and Environmental Sciences (Karlik et al., 2021). The catchment (bedrock and soil) and sediments of lake Bolătău, Romania were studied by high resolution multi-methodological investigations to characterize paleoenvironmental and formation conditions. Particle size analyses, optical and cathodoluminescence microscopy, FTIR-ATR and Raman spectroscopy, X-ray powder diffraction, and XRF were applied for microtextural, chemical, micro-mineralogical and embedded organic material characterization and distribution of the sediments, especially concerning geochemical conditions, like pH and redox potential change. Our results support physical and chemical weathering in the process of soil formation with appearance of the new minerals appear (10Å sized phyllosilicates and clay minerals). Comparison of these studies offer possible differentiation of syn- and diagenetic mineralization, the clarification of debris contribution, microbial mediation and complex mineralization via decomposition of cell and extracellular polymeric substance. Based on the analyses on the abrasives, a suboxic environment prevailed in the depositional area and considerable microbial contribution is proposed via accumulation of lake sediments (Karlik et al., 2021).

In the frame of the project, the biomineralization of geological samples were studied, using methodological innovation elaborated by our research group, producing more international collaboration, like Neoproterozoic manganese ore from Datangpo area (China) and Urucum (Brazil). Within both collaboration our task was the identification of minerals and organic material by FTIR and Raman spectroscopy, interpretation of mineralogical and geochemical data for environmental reconstruction and diagenesis. The biosignatures were identified by high resolution optical microscopy.

At the first case, Chinese black shale-hosted Datangpo Mn carbonate deposit, as representative deposit of further ten similar large deposits of a Neoproterozoic belt was elaborated. On representative samples, high resolution microtextural, mineralogical and organic geochemical investigations (determination and distribution) were made by optical rock microscopy and cathodoluminescence microscopy (total 120 photos), AT-FTIR spectroscopy (55 spectra on 12 sample parts), electron-micropropе (EDS) study (26 back scattered electron images and 180 spectra), prelimiray XRF, and Raman spectroscopy (2500 spectra). The determination and characterization of the organic matter was made on the basis of in situ FTIR and Raman spectroscopy. Summarizing the results the genesis of the ore formation (two step microbially mediated ore formation model) was proposed as well as the environmental conditions during sedimentation and via diagenesis. The results were published in Precambrian Research in 2019 spring (Yu et al., 2019).

The Urucum district in Mato Grosso do Sul (Brazil), hosts the youngest and largest sedimentary Mn ore of Neoproterozoic age; units Mn-1, Mn-2, and Mn-3 are found in jaspilites and ironstones, and represent approximately 600 Mt of extractable rock with 27–44% Mn and 12–30% Fe. High-resolution optical- and cathodoluminescence microscopy, as well as Raman and FTIR spectroscopy. Here, we model the control of this ore mineralogy by homogeneous oxidation and microbial processes. Layers Mn-2 and Mn-3 contain kremydilite, as a characteristic ore structure, with 77–95 vol% cryptomelane, 0–23% hollandite, 9–19% braunite, 7–21% hematite, and 0–5% pores filled with clay minerals and and organic matter. These are present within a micro-nodule matrix composed of cryptomelane and hematite in varying proportions. The first syngenetic products of microbial enzymatic oxidation were, on the Fe side, ferrihydrite and lepidocrocite, and on the Mn side, vernadite, todorokite, birnessite, and manganite. These formed under obligatory oxic (Mn) and suboxic (Fe) conditions and close to neutral pH. We describe the genesis of Urucum via complex diagenetic processes, which include the decomposition and mineralization of cellular- and extracellular-polymeric substances from Fe and Mn bacteria and cyanobacteria. The kremydilite forms in successive stages of oxidation of organic matter mediated by microbes, which generate pores and produce methane and $CO_2$/$H_2$ bubbles. They are a unique type of diagenetic structure formed by heterotrophic cell colonies randomly activated in the microbialite milieu following burial in suboxic neutral/alkaline conditions, side-by-side with the lithification and stabilization of the mineral assemblages.

On the samples (14) high resolution microtextural, mineralogical and organic geochemical investigations (determination and distribution) were made by optical rock microscopy and cathodoluminescence microscopy (total 690+113 photos), AT-FTIR spectroscopy (415 spectra on 8 samples), and Raman spectroscopy (8 samples, 10,997 spectra). The determination and characterization of the organic matter was made on the basis of in situ FTIR and Raman spectroscopy, the occurrence of remnants of organic matter was scarce because of oxic conditions. Summarizing the results the genesis of the ore formation was proposed, a model was created as well as the environmental conditions during sedimentation and via diagenesis were characterized in detail. In both cases comparison with Úrkút deposit was made (Biondi et al., 2020).

The Neoproterozoic Urucum manganese deposit (Brazil) is a ~600 Mt microbially mediated sedimentary Mn ore. Proto-ore formation via sedimentation and diagenesis occurred under suboxic-oxic and semi-neutral pH conditions in the Ediacaran ocean, wherein microbial Mn(II) oxidation ensued from the fine-grained accumulation of Mn oxides and organic matter. Oxic conditions that facilitated enzymatic Mn oxidation and overwhelmed microbial Fe oxidation appears as a sharp contact between manganese and iron beds. The Urucum deposit arose from a complex suite of diagenetic processes, including decomposition and mineralization of microbially-derived organic matter involving extracellular polymeric substances. Kremydilite

– a new type of diagenetic concentric Mn mineral structure – formed by randomly activated heterotrophic cell colonies that generated pores in the microbialite sediment after burial, coincident with lithification.

The researh of Chinese black shale-hosted manganese deposits of variable geological ages continued, and after the Neoproterozoic occurrences (published: Yu et al. 2019, Precambrian Research), the Carboniferous Masi and the Permian Xinglong deposits were investigated.

The Chinese partner offered representative samples, on which high resolution microtextural, mineralogical and organic geochemical investigations (determination and distribution) were made by optical rock microscopy and cathodoluminescence microscopy (total 39 OM+48 CL photos – Masi and total 71 OM + 34 CL photos - Xinglong), AT-FTIR spectroscopy (105 spectra – Masi and 139 spectra – Xinglong on numerous sample parts), electron-microprobe (EDS) study (30 back scattered electron images and 172 spectra – Masi and 24 back scattered electron images and 355 spectra – Xinglong), preliminary XRF, and Raman spectroscopy (7677 spectra – Masi and 12,325 spectra - Xinglong), stable C and O isotope study (4 – Masi and 2 – Xinglong). The determination and characterization of the organic matter was made on the basis of in situ FTIR and Raman spectroscopy. Summarizing the results the genesis of the ore formation was proposed as well as the environmental conditions during sedimentation and via diagenesis (Yu et al., 2021ab).

The Brazilian research project continued on the Neoproterozoic Urucum iron deposit, which is an oxic type giant deposit. The Brazilian colleague offered samples (total 12). (This banded iron formation includes the manganese ore, which results were successfully published in 2020 in Precambrian Research as a first step, Biondi et al.) On the samples high resolution microtextural, mineralogical and organic geochemical investigations (determination and distribution) were made by optical rock microscopy and cathodoluminescence microscopy (total 690+549 photos), AT-FTIR spectroscopy (330 spectra on 8 samples), and Raman spectroscopy (8 samples, 6,045 spectra), electron-microprobe (EDS) study (30 back scattered electron images and 172 spectra), stable C and O isotope study (8 samples). The determination and characterization of the organic matter was made on the basis of in situ FTIR and Raman spectroscopy, the occurrence of remnants of organic matter was scarce because of oxic conditions. Summarizing the results the genesis of the ore formation was proposed, a model was created as well as the environmental conditions during sedimentation and via diagenesis were characterized in detail (Polgári et al., 2021).

**General results**

Investigation of sedimentary manganese ores of variable geological ages were carried on by high resolution optical rock microscopy, FTIR and Raman spectroscopy to characterize the micro-texture mineral and organic matter types and distributions. Based on data, the Chinese black shale-hosted manganese carbonate ore deposits (Neoproterozoic, Permian and Carboniferous) formed by microbial mediation, and later via diagenesis, the complex mineralization of cell organic matter and extracellular polimeric substance occurred. The manganese oxide ores of the Urucum deposit, Brazil also formed by microbial mediation, but in this case the system remained oxic via diagenesis. This is also the case in iron oxide ores of Urucum. The mineralization of cell and extracellular polymeric substance resulted complex mineralogy. These deposits are mineralized biomat systems, and their formation can be described by the two step microbially mediated formation model evaluated in Úrkút manganese deposit, Hungary. The trace element content of the deposits is determined by microbial processes.

Our recent results supported the validity of two step microbially mediated ore formation model developed in the Hungarian Úrkút manganese deposit. Further important scientific innovation is the determination of role of cell and extracellular polymeric substance in the element reservoir of ore forming systems, and the important contribution to the mass of ore deposits in the sence of quality (numerous mineral facies) and quantity (giant mineral mass) contribution. This asks for attention that not only the primary microbially mediated mineralization but also diagenetic processes represent considerable contribution to ore deposits.

Based on our experiences we found that the syngenetic situation is similar (double microbial ore forming system – suboxic in the case of Fe and oxic in the case of Mn – is similar, but the result is variable outlook of deposits concerning mineralogy (in Mn deposits Fe is in the shadow, in Fe deposits Mn is in the shadow). Naturally metal source is needed. Geodynamic situation refer to rifting (or failed rifting) zone, and distal hydrothermal discharge. What cause differences? What cause variable outlook of deposits concerning mineralogy? For example accumulation ratio differences (if the accumulation is slow, a part of the organic matter will be oxidized by atmospheric oxygen, and less amount of reactive organic matter will be burried), mass balance differences (accumulating organic matter (type, mass), metal oxide concentration and type, other forming minerals, etc.) We can surf from site to site through geological time, till now this was the experience. The oxygen supply can be the result of currents, cyanobacterial ventilation… And, these are starving basins… If there is a considerable debris contribution, microbial biomat system will be destroyed.

Microbially mediated processes – the role of cell metabolism and EPS – are very important in syngenetic mineralization, representing also a considerable element source reservoir in the form of bond cations and anions released via diagenesis, and are very effective factors influencing the syngenetic and diagenetic mineralization processes. These processes supply bioessential elements or toxic ones, depending on the pool of elements, the type of metabolism, microbial species, and the mineralogical characteristics. In fact, they facilitate the sedimentation of metals and other elements by adsorption from the water. Economically important selective element enrichments can be mentioned, as well as the role of microbes in industrial activity and in environmental protection. In summary, the mineralization of the cells and the EPS material contributes significantly to the material of the ore deposits, in terms of both quantity and a varied mineral composition. Microbial activity is highly effective in the mineralization and solid phase accumulation of metal ions dissolved in geofluids. It is also responsible for significant ion binding, apart from the metal enrichment processes. The organic materials of the cells and the EPS are similarly effective in the catalyzation of the processes and during the diagenetic mineralization after their degradation, which ultimately results in today's geological properties. The role of microorganisms is rarely considered to be significant in mineral or ore formation, as the biofilm they produce is only a millimeter thick. However, it should not be forgotten that these can cover large surfaces and thus dominate when abiotic processes occur (Polgári et al., 2019).

Methodological innovation was the usage of Mössbauer spectroscopy on the ore samples concerning the possibility of detection of microbial mediation in Fe minerals (Gracheva et al., 2021). A combination of various techniques was applied to investigate the mineralogy of the Neoproterozoic Urucum iron and manganese deposit (Brazil) and Carboniferous and Permian manganese carbonate deposits (China). The examined deposits exhibited signs of microbial mediation from Fe and Mn bacteria and cyanobacteria. The studied samples showed diversity in their composition and particle size. Probes from Urucum deposit revealed that the rocks consist mainly of hematite, showing Mn substitution which reflects the oxidation of Mn on the active surface of Fe-rich biomat. Nanominerals occurring in significant concentration also supported the microbial contribution to the formation of these ores. Representative samples of Neoproterozoic and Permian deposits showed considerable amount of mixed carbonates with variable composition. $^{57}$Fe Mössbauer spectroscopy analysis supported by X-ray diffraction and transmission electron microscopy data provided a detailed characterization of Fe-rich mineral phases of the samples, including metal ratio outlooks, particle size dimension and presence and type of impurities. Integrity and high resolution of the methods allowed to determine new features of the samples reflecting important signatures of microbial activity revealing the biogeochemistry of the biomat formation (Gracheva et al., 2021).

**The role of biogenicity in the mineral world is larger than many might assume**

Biological processes interact with physical and chemical processes at the Earth's surface and far below underground, leading to the formation, for instance, of banded iron formations and manganese deposits. Microbial mats can form giant sedimentary ore deposits, which also include enrichment of further elements. Microorganisms play a basic role in catalyzing geochemical processes of the Earth and in the control, regularization and leading of cycles of elements. Microbial mats and other biosignatures can be used as indicators for environmental reconstruction in geological samples. This article reviews the many ways that microbially controlled or mediated processes contribute to mineralization and examines some published case studies for clues of what might have been missed in the analysis of sedimentary rocks when biogenicity was not taken into account. Suggestions are made for tests and analyses that will allow the potential role of biomineralization to be investigated in order to obtain a more complete view of formation processes and their implications (Polgári and Gyollai, 2021).

**Suggestions for including a biomineralization dimension in studies**

The study of ore microbialites teaches us numerous connections and facts that can also be useful in the interpretation of non-ore systems or in other types of ore indication-bearing sedimentary systems. Among others we can mention the following examples, which are well known but still not routinely used in many cases: (i) lamination is not equal to anoxia; (ii) mass balance aspects; (iii) the importance of the suboxic diagenetic zone; (iv) the influence of microbial element enrichments (isotope effect) on element (isotope) ratio analyses; and (v) sources of elements.

To summarize, the four case studies reviewed in Polgári and Gyollai (2021), while quite thorough in many ways, did not include sufficient attention to the possibility of biomineralization. Mineralized microbial microtexture was not examined in any of them. In some studies detailed characterization of mineral phases was lacking, and consideration of the origin or sources of enrichment minerals was missing. The data collected (or reported) was not always sufficient to analyze the potential role of microbially mediated processes. Thus, the description of the rocks in the case studies is somewhat incomplete. The detailed description and interpretation of microbially mediated processes offer a more plausible coherent picture by including identification of formation conditions, syn- and diagenetic processes, and also element enrichments based on mineral assemblages.

If a through study of a geological site is planned, a complete interpretation can be made only if sufficient data is available, including evidence of potential microbial mediation. We recommend the following methods for collecting samples and data so that an analysis on microbially mediated formation can be carried out to inform comprehensive analysis and interpretation.

1. Dimension of investigations must fall into microbial size dimension (e.g. there is a need for high magnification microtextural observations as a first step to investigate the possible occurrence of microbial mediation)

2. Besides bulk analyses, *in situ* determination of mineral assemblage and embedded organic matter is needed.

3. If the possibility of microbial mediation can be raised in a formation (through microtextural evidence), the choice of appropriate methods is essential. For instance, XRD does not ”see” X-ray amorphous poorly crystallized microbially mediated minerals, furthermore, attention is needed concerning excitation energy, which can convert minerals like ferrihydrite to more stable hematite. Thus, along with Raman spectroscopy, FTIR spectroscopy at lower excitation energy is also suggested.

4. Debris character can be a “virtual” outlook (large minerals of authigenic processes), influencing textural features, which can be clarified easily by using cathodoluminescence microscopy.

5. Authigenic formation of minerals that are well known as being of a particular origin may actually appear as authigenic materials. One example is with magmatic or metamorphic condititions (quartz, feldspar-earlier known), but also, pyroxene as aegirine, amphibol like riebeckite, and others, like dickite as high T and p minerals also common as authigenic minerals. Thus further study may be needed concerning mineral type, and a complex approach is proposed to avoid a false interpretation.

6. Selective element enrichment (isotopes) resulting from microbial processes means that we have to use geochemical ratio methods with caution. This enrichment can indicate a microbially influenced element source, which often does not even appear as a possible interpretation, and also demonstrates the need for a multi-methodology approach and complex interpretation.

7. It is very important to distinguish whether clay-size dimensions or clay minerals occur. If size dimension is under discussion, what are the minerals in that fraction? If clay mineral, then what is the origin? Is is detrital, hydrothermal, or microbial? It can indeed be microbial, and often this is the case. So in the case of marls or claystones (other clay-bearing rocks) this must be studied. Why? Generally the interpretation of clay-rich rocks is simply assumed to be increased wet weathering and run off from terrestrial parts, but in many cases that may be a false interpretation.

8. It is important to distinguish between syngenetic anoxia and diagenetic, since it determines original formation conditions.

9. Mass balance considerations should not be ignored, because the ratio of accumulating metal oxides, organic matter, and other components will influence the diagenetic processes.

10. Identifying suboxic zones is important due to consumption of organic matter by metal oxides and hydroxides and forming diagenetic mineral types.

If these factors are overlooked, and therefore it is not recognized that a system is microbially mediated (or evidence of microbial mediation is studied but not convincingly excluded), the conclusions will be incomplete.

There is no doubt that the dimension of investigations is different when we consider also microbial evidence, and in many cases comparison between a case study's conclusions and microbial features cannot be made, because they are not comparable or data are missing. In most datasets the interpretation is made based on hydrothermal or debris contribution or sea level stands, while the enrichment factor is not mentioned, although microbial mediation is a vital factor in sedimentary environments. The outlook of the rocks will depend on complex factors, which can be described based on high-resolution multi-methodological characterization. In sedimentary systems the possiblity of microbial contribution must be considered and the appropriate data must be collected on the appropriate scale in order for this factor to be convincingly excluded or verified (Polgári and Gyollai, 2021).

It is important to consider the role of microbial life (in particular, bacteria and fungi) in the geological context of mineralization and mobilization processes, because the mechanisms governing such activities often supersede purely inorganic reactions. It is also useful to have an overview of the evolution of biomineralization as a global control factor of diluted chemical constituents in the oceans and in the solid phase.

Polgári and Gyollai (2021) offer a detailed summary on biogeochemical cycles, biomineralization, and sedimentary ore formations has been provided. Microbially mediated mineralization and biomineralization, including organic matter accumulation (cell and EPS offering element reservoir pools) and diagenetic processes, are important factors in the stabilization of ore minerals. Complex mineralization of cell material and EPS occur with the accumulated minerals acting also as bioindicator minerals. This complex mineralization results in the highly variable mineral assemblage of Mn- and Fe ore microbialites, in spite of their similar syngenetic formation processes. This paper reviewed not only the authors' own research results on ore microbialites but also studies of other formations. Besides a geochemical and mineralogical summary, high resolution methodology and interpretation were also summarized. Recent conditions (rock, ore) and knowledge of biomineralization offer paleoenvironmental estimations.

The case studies reconsidered in Polgári and Gyollai (2021) show that overlooking the role of biomineralization can lead to incomplete conclusions. It is clear that making use of the experience and knowledge gained from research on ore microbialites and the possibilities for high resolution multi-methodology data collection and complex interpretation offer a more plausible and comprehensive interpretation with fewer contradictions in given depositional environments.

The synthesis of well known knowledge on new aspects can provide basically new results in the identification of formation conditions, syngenetic and diagenetic processes, and also element enrichments based on mineral assemblages. The development of methods and instruments offers new horizons for investigation. Important analogies (Fe, Mn) for research of extraterrestrial life as robust biosignatures can also be proposed (Polgári and Gyollai, 2021).

## Comparison with the project research plan

## Aims

The aims of the recent project were the following, and the realization is compared with it:

1) Apply the multilevel hierarchical methodology in the case of sediments in which microbial proxies are expected which can serve as a key to understanding formation processes and to describing paleoenvironmental conditions;

*Sediments of Jurassic age of Mecsek Mts., Réka Valley were successfully published (Ruebasam et al., 2018).*

*Case studies on previously published papers on various sedimentary formations were made for comparison (Polgári and Gyollai, 2021)*

2) Extend the application of multilevel hierarchical methodology to recent(ly forming) sediments, like lake sediments;

*This point was elaborated on previously collected samples (Karlik et al., 2018, 2021).*

*Unfortunately the contact person of this field left the project and also the institute in 2019, and even the efforts by the new project member, the sampling did not happen. In case of Quaternary sediments, the planned sampling could not be realized because of the pandemic COVID situation.*

3) Introduce extended organic geochemical analysis (biological markers) into the methodology to compare and refine the results in the reconstruction of microbial assemblage and paleoenvironment.

*Concerning biomarker and other organic-geochemical measurements, the equipment at the institute is still under reconstruction, and it means that these planned measurements were not elaborated, further, the organic geochemist left the project and also the institute in 2019, and later the pandemic situation caused difficulties to find solution. In spite of this, 2 papers (Karlik et al., 2018 and and Ruebsam et al., 2018) include detailed organic geochemical approach, and investigation of ore deposits include determination of organic matter by FTIR and Raman spectroscopy.*

4) Apply the multilevel hierarchical methodology in the case of sediments in which microbial proxies are expected in the vicinity of hydrothermal discharge (U, Mn, Be, P mineral assemblage, Bükkszentkereszt; Mn ore assemblage, Úrkút, and China), and also metal isotope characterization (Fe and Mn isotope analyses), and also O isotope analyses from Mn-oxide minerals for temperature calculations of formation conditions.

*This point was elaborated in the case of metal enrichments and ore formations (10 papers, 1 book, 5 papers in the Special Issue "Ore Microbialites" of Ore Geology Reviews, published in 2021).*

*Concerning metal isotope characterization (Fe and Mn isotope analyses), and also O isotope analyses from Mn-oxide minerals for temperature calculations of formation conditions, PI sent* *samples for measurements, but the delay of start of the routine operation of the ICP MS equipment in ATOMKI did not solve this case. PI made efforts to analyse samples for O isotopes from Mn oxides abroad, but was not successful.*

Concerning the planned methodology ICP MS and XRF and metal isotope studies were not used, as well as TOC Rock Eval, organic geochemist left the project and the equipment was wrong. Instead, the organic matter was determined by high resolution FTIR and Raman spectroscopy.

Planned biological marker as an important novelty was not realized because of the above mentioned reasons.

We followed the working plan, preliminary results were presented on international conferences, and then all the manuscripts were successfully published.

Personal background has changed, as organic geochemist left the project and the institute, and a new project member was invited. All these were reported to the OTKA Office, who permitted it.

In the frame of project, students were also applied, and two of them took part in the scientific work, and they took part in publications as well (Péter Gál – Gál et al., 2020, and Henrietta Horváth is co author in Polgári et al., 2019.) Concerning the Bükkszentkereszt manganese-uranium-beryllium-phosphate research, the manuscript was successfully published in Ore Geology Reviews, 2020. The first author of this paper is Péter Gál, PhD student in the Doctor School of Earth Sciences, ELTE, who joined to the OTKA project as student participant in the first year. One of the co-authors is Henrietta Horváth, MSc student Szeged University, who joined to the OTKA project as student participant in the second year. Her thesis of MSc was written also in the frame of the OTKA research project.

**Comparison with expected results**

Four set of novel results were expected. (i) the detailed organic geochemical studies will corroborate or refine the former models of sedimentation and paleoenvironmental changes in the case of Hungarian geological sections presumably encompassing the TOAE, and/or preserving formerly not recognized microbial ore formation processes (in the vicinity of hydrothermal discharge zones).

*Jurassic occurrences like Úrkút manganese deposit (Polgári et al., 2017, Gyollai et al., 2018) and Mecsek Réka Valley belong to this point (Ruebsam et al., 2018). Results of Bükkszentkereszt ore indication were also published (Gál et al., 2020).*

(ii) the study of Quaternary sediments with multilevel hierarchical methodology combined with biological markers will provide expectedly very high resolution data on the climatic story of the last some thousands or hundreds of years.

*Recent lake sediments were studied on previously collected samples, and results were published on both purposes (Karlik et al., 2018, 2021).*

(iii) salinity, and lamination considerations on Jurassic sedimentary ores, *This point was planned but not realized because the pandemic situation slowed down the project work.*

(iv) metal isotope characterization in the frame of microbial mediation.

*This point was not realized because of the above mentioned reasons.*

**Other circumstances**

At the time of submission of the project proposal we did not know, that great international interest will occur in the subject of project, and scientific collaborations were organized on foreign manganese and iron ore deposits (Neoproterozoic Datangpo Mn dep., China, Urucum Fe and Mn ore dep., Brazil, Permian and Carboniferous Mn dep., China) (Yu et al., 2019, 2021ab; Biondi et al., 2020; Polgári et al., 2021).

Further, Mössbauer methodological research on Urucum deposit was made (Gracheva et al., 2021). Based on the results of investigations on ore microbialits let us to conceive general aspects (Polgári et al., 2019, Polgári and Gyollai, 2021).

**Details on the differences between budget plan and realization**

Line 1.2.1.: transgression was caused by the effect of increasing the salary of Ildikó L. Lukács from 1, January, 2018.
Lines 1.4. and 1.5.: In the 4th project period application of student fee was counted on line 1.4 because of the type of contract.
Line 1.6.: transgression was caused by the effect of increasing the salary of Ildikó L. Lukács from 1, January, 2018.
Line 3.4.: on lines 3.1. and 3.2. residuum was formed, because the planned visit of Russian scientist did not realize and further, the conference organized on Hawaii was finally an online conference because of pandemic situation. This residuum was used for microscopic investigations on line 3.6.
Line 3.6. sor: see details in Line 3.4.
Line 3.5.: in the 4th project period the current expences were balanced for the whole project time according to plan.
Line 3.: on lines 3.1. and 3.2. residuum was formed, because the planned visit of Russian scientist did not realize and further, the conference organized on Hawaii was finally an online conference because of pandemic situation. This residuum was used for microscopic investigations on line 3.6, and also for transgression caused by the effect of increasing the salary of Ildikó L. Lukács from 1, January, 2018.

**Reference list**


Biondi, J. C., Polgári, M., Gyollai, I., Fintor, K., Kovács, I., Fekete, J., & Mojzsis, S. J. (2020). Biogenesis of the Neoproterozoic kremydilite manganese ores from Urucum (Brazil)–A new manganese ore type. *Precambrian Research*, 340, 105624.

Gál, P., Polgári, M., Józsa, S., Gyollai, I., Kovács, I., Szabó, M., & Fintor, K. (2020). Contribution to the origin of Mn-U-Be-HREE-enrichment in phosphorite, near Bükkszentkereszt, NE Hungary. *Ore Geology Reviews*, 125, 103665.

Gracheva, M., Homonnay, Z., Kovács, K., Béres, K. A., Biondi, J. C., Gyollai, I., & Polgári, M. P. (2021). Mössbauer characterization of microbially mediated iron and manganese ores of variable geological ages. *Ore Geology Reviews*, 134, Paper 104124.

Gyollai, I., Polgari, M., Biro, L., Vigh, T., Kovacs, T. and Pal-Molnar, E. (2018). Fossilized biomats as the source of high natural radionuclide content at the Jurassic Urkut Manganese ore deposit, Hungary. *Carpathian Journal of the Earth and Environmental Sciences* 13(2), 477-487.

Karlik, M., Fekete, J., Mindrescu, M., Grădinaru, I., Bozsó, G., Bíró, L., & Kern, Z. (2018). Natural and anthropogenic changes in a lake-forest system in Bukovina (Romania) since 1340 AD documented by sedimentary organic geochemistry (C, N and n-alkanes). *Quaternary International*, 493, 166-175.

Karlik, M., Gyollai, I., Vancsik, A., Fintor, K., Szalai, Z., Mindrescu, M. Gradinaru, I., Vágási S., Bozsó G., Polgári M., & Pal-Molnar, E. (2021). High resolution mineralogical characterisation of sediments - lake Bolotau-Feredeu (Romania). *Carpathian Journal of Earth and Environmental Sciences*. 16(1), 199-210.

Polgári M., Szabó Z., Vigh T.: Mangánércbányászat Úrkúton (Manganese ore mining at Úrkút) (1917 - 2017) Geolitera, p. 214

Polgári, M., Biondi, J. C., Gyollai, I., Fintor, K., & Szabó, M. (2021). Origin of the Urucum Iron Formations (Neoproterozoic, Brazil): Textural and Mineralogical Evidence (Mato Grosso do Sul–Brazil). *Ore Geology Reviews,* 139, Paper 104456.

Polgári, M., Gyollai, I. (2021). Geochemical constraints on the element enrichments of microbially mediated manganese and iron ores – An overview. *Ore Geology Reviews,* 136, Paper: 104203.

Polgári, M., Gyollai, I., Fintor, K., Horváth, H., Pál-Molnár, E, Biondi, J.C. (2019). Microbially Mediated Ore-Forming Processes and Cell Mineralization. *Frontiers in Microbiology,* 10, Paper: 2731.

Ruebsam, W., Müller, T., Kovács, J., Pálfy, J. and Schwark, L., 2018. Environmental response to the early Toarcian carbon cycle and climate perturbations in the northeastern part of the West Tethys shelf. *Gondwana Research,* 59, 144-158.

Yu, W., Polgári, M., Fintor, K., Gyollai, I., Szabó, M., Velledits, F., Liu, Z., Du, Y. (2021). Contribution of microbial processes to the enrichment of Middle Permian manganese deposits in northern Guizhou, South China. *Ore Geology Reviews,* 136, Paper: 104259.

Yu, W., Polgári, M., Gyollai, I., Fintor, K., Huang, H., Szabó, M., Du, Y. (2021). Microbial metallogenesis of early Carboniferous manganese deposit in central Guangxi, South China. *Ore Geology Reviews,* 136, Paper: 104251.

Yu, W., Polgári, M., Gyollai, I., Fintor, K., Szabó, M., Kovács, I., Fekete, J. Du Y. & Zhou, Q. (2019). Microbial metallogenesis of Cryogenian manganese ore deposits in South China. *Precambrian Research*, 322, 122-135.