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Mössbauer study of biofilms formed at spring caves of Buda Karst, Hungary

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Abstract Biofilms formed at spring caves of Buda Karst, Hungary, were investigated by ^{57}Fe Mössbauer spectroscopy. 78 K Mössbauer spectra were decomposed into a sextet and two doublets. The subspectra were assigned to goethite, hematite, ferrihydrite and siderite, according to their known Mössbauer parameters. The room temperature spectra indicated that goethite and/or hematite are in the superparamagnetic state at room temperature. The results can be interpreted in terms of karstification of hypogenic caves by the role of biofilms via discharge features.

Keywords Biofilm · Spring caves · Buda Thermal Karst ·
^{57}Fe Mössbauer spectroscopy

1 Introduction

Europe's largest naturally flowing thermal water system can be found in Budapest. The springs and wells that supply the famous baths of Budapest discharge from a regional Triassic carbonate rock aquifer system (Fig. 1). As the result of the interaction of discharging waters and carbonate rocks, extensive cave systems have developed and are still developing today. These caves belong to the group of hypogenic caves, and their special morphology and peculiar minerals make Budapest, beside the city of spas, also "the capital of caves". According to recent developments

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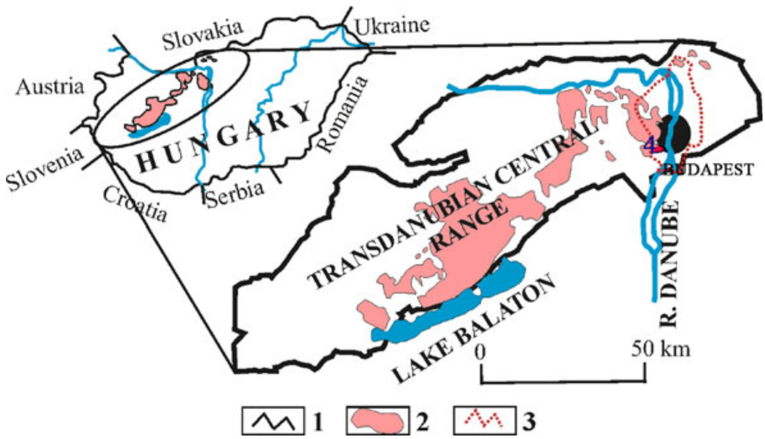

Fig. 1 Location of the Buda Thermal Karst in the Transdanubian Central Range: 1: Subsurface boundary of Mesozoic carbonates, 2: Uncovered Mesozoic carbonates, 3: Buda Thermal Karst, 4: location from which the samples originated (*red marked area*)

in the speleogenetic theories, hypogenic karsts and caves are viewed in a flow system context, and can thus be considered as the manifestations of flowing groundwater [1–3]. Biofilm bacterial communities are inhabiting the cave walls of the Buda Thermal Karst system [4].

The aim of the present work was to apply ^{57}Fe Mössbauer spectroscopy for phase analysis of the minerals [5] in biofilms formed in spring caves of Buda Thermal Karst.

2 Experimental

The biofilm samples formed in spring caves of Buda Thermal Karst (Fig. 1) were collected in glass tubes at springs of the Lukács, Rácz and Gellért spas and in the Molnár János cave. The samples were kept at 78 K till their Mössbauer measurements. Some of the samples were measured after lyophilisation (Table 1).

^{57}Fe Mössbauer spectra of samples were recorded in transmission geometry with conventional Mössbauer spectrometers (KFKI, WISSEL) working in constant acceleration mode. The γ-rays were provided by a 3×10^9 Bq ^{57}Co/Rh source. The measurements were performed at 78 K and at 300 K. Isomer shifts are given relative to α-iron. The Mössbauer spectra were analyzed by least-square fitting of Lorentzian lines by the help of the MOSSWINN code [6].

The samples were investigated by several other techniques (hydrogeochemical investigations, ICP-MS analysis, SEM, TEM, XRD, RAMAN, isotope analysis, etc.), however, the results of these measurements will be published separately, elsewhere.

3 Results and discussion

78 K Mössbauer spectra of biofilm samples originating from different spring caves of Buda Karst are shown in Figs. 2 and 3. All these Mössbauer spectra show an envelope

Table 1 Biofilm samples from spring caves of Buda Karst, Hungary

Source location	Sample name	No	Color
Lukács spa spring	LR I	1	Brown
Lukács spa spring	L LRI lyophilized	1L	
Rácz spa spring white	RBF	2	Yellow
Rácz spa spring white	RBF lyophilized	2L	
Molnár János cave	MJ BB	3	Red
Gellért spa spring	G OO	4	Brown
Rácz spa spring brown	RBB	5	Yellow brown

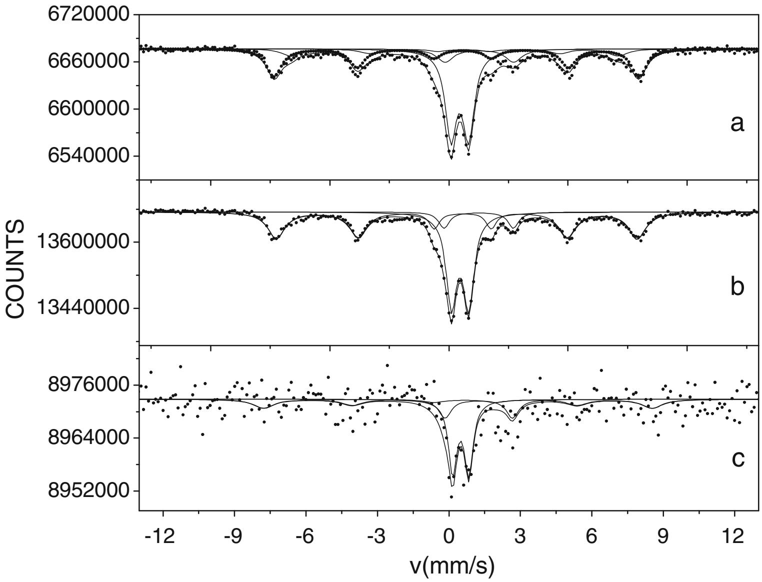

Fig. 2 78 K ^{57}Fe Mössbauer spectra of biofilms formed in spring caves of Buda Thermal Karst **a** Lukács source (sample No 1), **b** Lukács source (lyophilized sample No 1 L) and **c** Rácz source white (lyophilized sample No 2 L). A minor sextet ($\delta = 0.45$ mm/s, $\varepsilon = -0.24$ mm/s, $B = 43$ T) also appeared in this spectrum, which interpretation will be published elsewhere

superimposed from a sextet and a doublet. However, to achieve an acceptable fit we have to suppose one sextet and two doublets at least. The Mössbauer parameters obtained from these spectral fits are given in Table 2. The result of decomposition for more components was not conclusive.

The two doublets are well separated in all spectra according to their different isomer shifts. The isomer shift of the minor doublet (doublet 2) belongs to Fe^{II} while that of the major doublet (doublet 1) is characteristic of Fe^{III} microenvironments [7]. Both the isomer shift and the quadrupole splitting of doublet 2 correspond well to those of siderite [8, 9], therefore doublet 2 was assigned to siderite. According to the relative spectral area of doublet 2 indicated in Table 2, siderite is a minor phase in all biofilm samples.

The Mössbauer parameters of doublet 1 (Table 2) can be associated with ferrihydrite and also with common iron oxyhydroxides or iron oxides being superparamagnetic at 78 K [8–11]. We assigned doublet1 mainly to ferrihydrite taking into

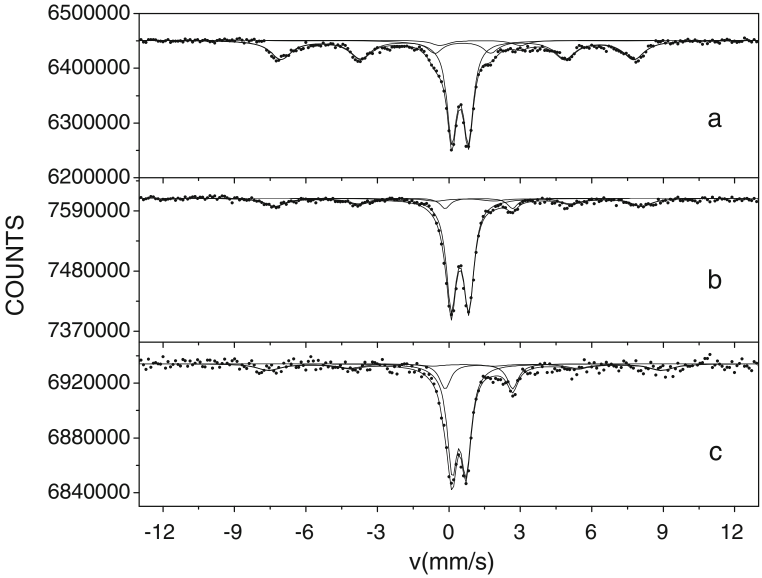

Fig. 3 78 K ^{57}Fe Mössbauer spectra of biofilms formed in spring caves of Buda Thermal Karst **a** Molnár János cave (sample No 3), **b** Gellért spa spring (sample No 4) and **c** Rácz spa spring brown (sample No 5)

Table 2 78 K Mössbauer parameters of biofilm samples originating from spring caves of Buda Karst, Hungary

Component/ sample	1	1L	2L	3	4	5
Sextet (1)						
A (%)	48	47	30	50	22	20
δ(mm/s)	0.46 ± 0.02	0.46 ± 0.02	0.49 ± 0.04	0.46 ± 0.02	0.47 ± 0.02	0.51 ± 0.02
ε (mm/s)	-0.25 ± 0.04	-0.24 ± 0.03	-0.26 ± 0.05	-0.22 ± 0.04	-0.25 ± 0.04	-0.18 ± 0.04
B (T)	47 ± 0.6	47 ± 0.6	49 ± 0.9	46 ± 0.6	48 ± 0.6	51 ± 0.5
Doublet (1)						
A (%)	44	44	50	47	71	62
δ (mm/s)	0.47 ± 0.02	0.47 ± 0.02	0.49 ± 0.02	0.47 ± 0.02	0.47 ± 0.02	0.45 ± 0.02
Δ (mm/s)	0.72 ± 0.03	0.73 ± 0.03	0.67 ± 0.04	0.69 ± 0.03	0.73 ± 0.03	0.59 ± 0.03
Doublet (2)						
A (%)	8	9	20	3	7	18
δ (mm/s)	1.24 ± 0.02	1.26 ± 0.02	1.25 ± 0.03	1.25 ± 0.02	1.25 ± 0.02	1.26 ± 0.02
Δ (mm/s)	2.90 ± 0.04	2.94 ± 0.04	2.85 ± 0.06	2.88 ± 0.04	2.85 ± 0.04	2.84 ± 0.04

consideration not only the isomer shift and quadrupole splitting data but also the large linewidth which is also characteristic of ferrihydrite [10, 11]. The occurrence of ferrihydrite was supported by the results of earlier studies in biofilms [12, 13] and also by the results of our measurements by additional techniques. The deviation of the Mössbauer parameters of doublet of sample No5 from those characteristic of the other samples indicates that an iron-bearing phase different from ferryhidrite may also be attributed to this doublet.

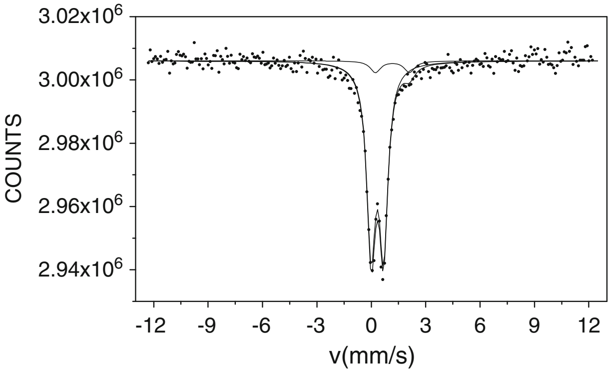

Fig. 4 Room temperature ^{57}Fe Mössbauer spectrum of biofilm formed in a spring cave of Buda Thermal Karst (Molnár János cave, sample No 3)

Table 3 Main phases of biofilms originated from spring caves of Buda Karst, Hungary

Biofilm sample	Phase1	Phase2	Phase3
Lukács spa spring	Goethite	Ferrihydrite	Siderite
Rácz spa spring white	N.A.	N.Á.	N.A.
Rácz spa spring white* lyophilized	Goethite	Ferrihydrite	Siderite
Rácz spa spring brown	Hematite	Ferrihydrite	Siderite
Molnár János cave	Goethite	Ferrihydrite	Siderite
Gellért spa spring source	Ferrihydrite	Goethite	Siderite

The Mössbauer parameters of the sextet with internal magnetic field not exceeding 49 T are characteristic of goethite [9–11], consequently we assigned the sextet to goethite in all samples, except sample No 5. The occurrence of goethite has already been shown in other biofilms [12]. We found goethite to be a major phase in most of the investigated biofilms.

The high hyperfine field observed in sample No 5 which originated from the spring of the Rácz spa ("brown") is typical of hematite [9–11] therefore this sextet was associated with hematite. Hematite has also been shown to be formed in the case of alteration of microbially precipitated iron oxides and hydroxides [13].

The room temperature Mössbauer spectra of all biofilm samples exhibit only paramagnetic spectral parts (which is the reason why the spectra were also measured at low temperature.). A typical Mössbauer spectrum is shown in Fig. 4. The sextets occurring in the 78 K spectra are not present at 300 K which clearly indicates that goethite and hematite are present in the samples in superparamagnetic form at room temperature [5, 10, 11].

The decomposition of RT spectra confirmed the assignment of the spectra we gave at 78 K. Furthermore, the presence of siderite was also confirmed in sample No3 by the RT spectrum when only a low occurrence was indicated in the spectrum at 78 K.

Table 2 shows that the parameters characteristic of the spectra of the samples which originated from the spring of the Lukács Spa (1 and 1 L) are practically the same before and after lyophilization. This means that lyophilization does not influence the phase composition of iron-bearing phases in these biofilms. Consequently, we can accept the results obtained for the lyophilized sample (2 L) to be characteristic of the original sample (2) which had not enough iron content to record a Mössbauer spectrum suitable for phase analysis.

The little variation of hyperfine parameters from sample to sample does not affect the result of qualitative phase analysis. However, the differences found in the relative spectral areas can reflect that different phase compositions are characteristic of biofilms formed at the different spring caves. The phases identified in the investigated biofilms are summarized in Table 3.

We have found that the main iron-bearing phases in the biofilms are ferryhidrite and goethite in the spring caves of Buda Thermal Karst (Table 3). This finding can be connected with the role of biofilms in the formation of hypogenic caves as discharge features [1, 3].

In the biomass, in the discharge zone, the bacterial communities are characterized by anaerobic ferri (Fe^{III}) or sulphate reducing or aerobic ferro (Fe^{II}) or sulphide oxidizing metabolic properties. These bacterial communities at a higher taxonomic level show similarity to those described in caves where microbially mediated sulphuric acid speleogenesis takes place [14]. The presence of ferryhidrite and goethite in the biofilms indicates a change from reducing to oxidizing realm. Our findings support the model developed for the dominant karstification processes of Buda Thermal Karst [1].

4 Conclusion

Goethite, ferrihydrite, hematite and siderite were identified in biofilms formed at spring caves of Buda Thermal Karst, Hungary, by the help of ^{57}Fe Mössbauer spectroscopy. Goethite and/or hematite were found in the superparamagnetic state at room temperature. The results can contribute to better understanding of the role of biofilms in the formation of hypogenic caves.

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