BACTERIAL INFLUENCE ON SPELEOTHEM OXYGEN ISOTOPE COMPOSITION: AN EXAMPLE BASED ON CAVE PISOIDS FROM PERLOVÁ CAVE (SLOVAKIA)

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Abstract: The origin of recently growing irregular cave pisoids in Perlová Cave (Veľká Fatra Mts, Slovakia) seems to be due to the activity of hydrogen-oxidizing bacteria. Several samples of water and freshly deposited calcite from cave pisoids were analysed for stable oxygen isotope ratios. The obtained values were checked using the O'Neil equation. Almost all the calcite samples display values more positive than their calculated counterparts. This phenomenon is ascribed to a fractionation process mediated by bacteria. The light isotope of oxygen is preferentially taken up by the hydrogen-oxidizing bacteria. It causes the relative enrichment in the heavy isotope in the bacterias' surroundings, which is recorded in the calcite precipitated around the bacterial cells.

Key words: Veľká Fatra Mts, stable oxygen isotopes, bacterial influence, speleothems.

Introduction

Carbonate speleothems which grow under conditions of isotopic equilibrium between calcite and ambient water are often used as a tool in paleoclimatic reconstruction (cf. Schwarcz 1986; Gascoyne 1992). In contrast, much less attention has been paid to the other speleothems of specific isotope compositions, since these are considered useless for paleoenvironmental studies.

The causes of disequilibrium in the C and O isotopes during the growth of speleothems were usually attributed to the rapid diffusion of CO_2 and/or to the evaporation effect (Hendy 1971). However, these processes of stable isotope fractionation have been only sporadically discussed so far. Study of recent cave pisoids from Perlová Cave in Slovakia offers the possibility of analysing and explaining a process of the stable oxygen isotope fractionation between ambient water and growing calcite speleothems.

Environmental setting

Perlová Cave (Perlová jaskyňa) lies in the Belanská Valley in the northern part of the Veľká Fatra Mountains (Great Fatra) in Slovakia (Fig. 1) (Mrázik 1987). Its entrance is situated at 910 m a.s.l. The average temperatures in the region range from 4 °C to 7 °C, depending on altitude (Droppa 1975).

Perlová Cave is developed in Guttenstein bedded limestone (Middle Triassic) of the Krížná Unit overthrusted over Mesozoic autochthonous cover of the crystalline core of the Veľká Fatra Mountains (Maheľ 1968). The 408 m-long cave is rich in speleothems (Mrázik 1987; Holúbek & Kleskeň 1993). The internal temperature ranges between 5.1–6.8 °C. The cave is devoid of any permanent or ephemeral watercourses. Water is
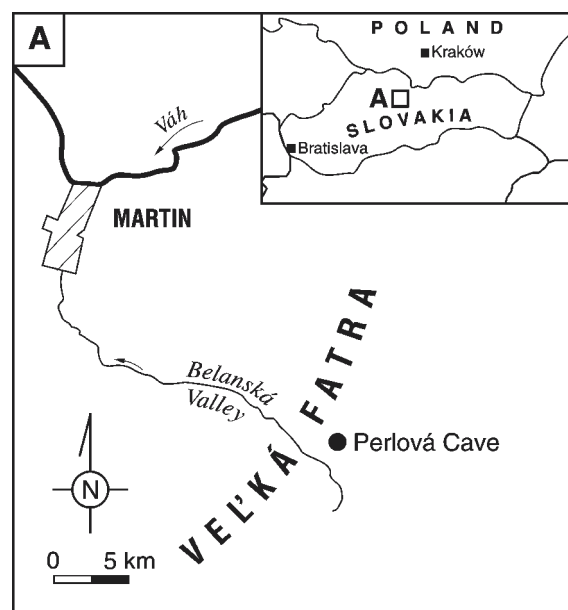

Fig. 1. Location of Perlová Cave.

pilled up only in small stepped rimstone pools (Fig. 2) located in two places: in the Pearls Passage (Perlová chodba) and in the Parliament Chamber (Parlament; Mrázik 1987). The depth of the pools ranges from 2 cm to 6 cm and the size of the biggest is 1×1.2 m. The water is of HCO_3–Ca–Mg type not differing from typical karst water in composition. Total dissolved solids (TDS) fluctuates between 302 and 432 mg/dm³. The saturation index (SI) indicates supersaturation both with respect to the aragonite and calcite (Gradziński 2001).

Individual pools host a dozen to several hundred pisoids of various sizes. Irregular rough surfaces, irregular subtle lamina-
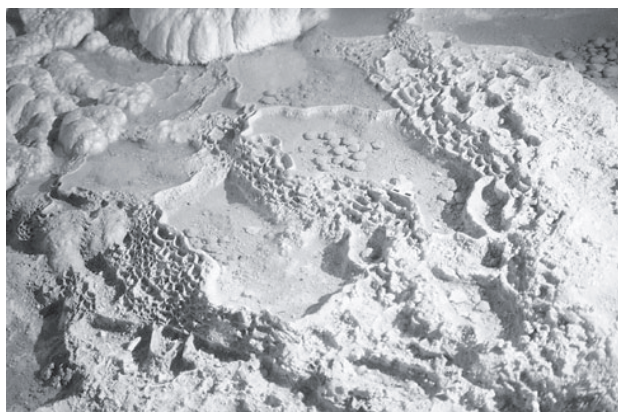
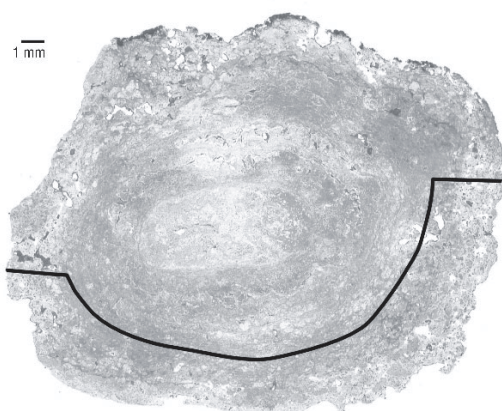**Fig. 2.** Stepped rimstone pools with pisoids, Pearls Passage, Perlová Cave.

Fig. 3. Cross-section of pisoid from Perlová Cave, location of sample taken for measurements of stable isotope composition is indicated.
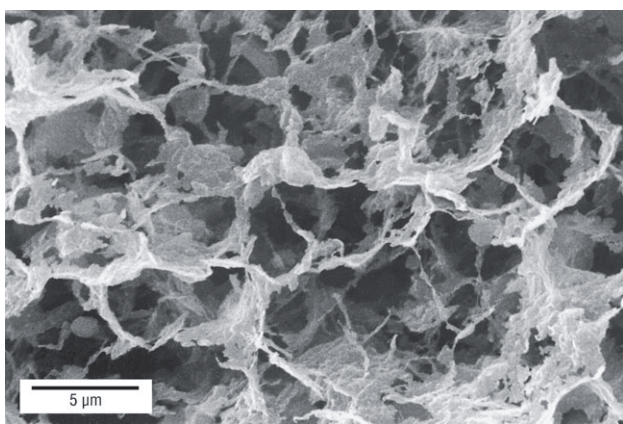

Fig. 4. Porous mucilaginous biofilm covering the surface of cave pisoid.

tion and lack of a nucleus characterizes the pisoids (Fig. 3). Low-Mg calcite is the only autochthonous carbonate phase that forms pisoids. The magnesium content in calcite does not exceed 5600 ppm (i.e., 1.47 mole % of $MgCO_3$). The pisoids grow due to physiological processes generated by chemo-lithoautotrophic hydrogen-oxidizing bacteria. The bacteria grow in the mucilaginous biofilm that occurs on the surface of

the pisoids (Fig. 4). Hydrogen-oxidizing bacteria actively up-take CO_2 from their surroundings (Aragno & Schlegel 1992) resulting in a disequilibrium state of the solution and, consequently, in precipitation of calcite upon the surface of pisoids. Thus, the growth of the pisoids is controlled by bacterial metabolic activity (Gradziński 2001).

Materials and methods

Six rimstone pools with pisoids were selected for isotopic examination. Three of these, P1, P5 and P6, were located in the Pearls Passage and the other three, P8, P9 and P10, came from the Parliament Chamber. Sampling of water for analysis of isotopic composition in selected rimstone pools was carried out twice: on November 11, 1998 and on May 19, 1999. Eleven pisoids were selected for the analysis of stable isotope composition. The diameters of the pisoids ranged from 1.2 to 2.5 cm.

Stable isotope ratios were analysed for the samples of water and pisoids. The analyses of the water samples were conducted in the Mass Spectrometry Laboratory of the Department of Environmental Physics, the Faculty of Physics and Nuclear Engineering, Academy of Mining and Metallurgy, Kraków. The procedure used was a standard procedure for the equilibration of a sample with gaseous CO_2 ($\delta^{18}O_w$) and reduction of water on metallic uranium at a temperature of 650 °C (δD). Oxygen and hydrogen isotope ratios were measured using a FINNI-GAN Delta S mass spectrometer. The analytical error for single measurements is ±0.1 ‰ for $\delta^{18}O_w$ and ±1 ‰ for δD. Stable isotope ratios in water are presented here vs. SMOW standard (cf. Hoefs 1997).

To define stable carbon and oxygen isotope ratios in the pisoids, samples of ca. 10 mg weight were taken with Dremel drilling machine. Where possible, the samples were taken from isochronous horizons. The measurements of carbonates $\delta^{13}C$ and $\delta^{18}O_c$ were conducted with a mass spectrometer SUMY in the Institute of Geological Sciences, the Academy of Sciences of Belarus in Minsk. The isotope ratios were measured in carbon dioxide obtained by way of reaction of the analysed samples with 100 % orthophosphoric acid. The carbon dioxide was subsequently trapped in liquid nitrogen and purified in a vacuum. The analytical error for single measurements is ±0.2 ‰. Stable carbon isotope ratios in the carbonate samples are presented here vs. PDB standard while stable oxygen isotope ratios vs. both PDB and SMOW standards (the latter values are given in brackets) to simplify the conducted calculations.

Some portions of each analysed calcite sample did not come directly from the surface, but from the subsurface part of the pisoids. It is due to the fact that, for technical reasons, the minimal mass of a calcite sample required for defining the content of ^{18}O is 10 mg. This resulted in contamination of the analysed portion with the portion of calcite which had been precipitated earlier (cf. Fig. 3). However, on the basis of insignificant year-to-year fluctuation in temperature (Wigley & Brown 1976) within caves as well as stable isotope composition in cave water (see Harmon 1979; Yonge et al. 1985), one can assume that it should not influence the measured stable isotope composition of the calcite.

Results

The proportion of stable isotopes in water (D, $^{18}O_w$) analysed in two series are quite uniform (Table 1). This is in accordance with the observations by Harmon (1979) and Yonge et al. (1985), who ascertained that the isotopic composition of percolating water is equal to the average annual isotopic composition of the rainwater in the catchment area. Admittedly, the δD and $\delta^{18}O_w$ values become more negative in the second series of samples in comparison with the first one. This is noticeable in pools P1 and P5. The shift is probably linked to so-called seasonal effects (cf. Rozanski & Dulinski 1988). The majority of the $\delta^{18}O_c$ values obtained from the analysed pisoids range from –8.3 ‰ (22.3 ‰) to –5.0 ‰ (25.8 ‰), with the exception of the $\delta^{18}O_c$ value from one pisoid (sample P6) which is 0.1 ‰ (31.0 ‰; Table 2).

Table 1: D and ^{18}O content in water from Perlová Cave, with measured temperature values.

Pool number	Sampling date	δD [‰ SMOW]	δ¹⁸O [‰ SMOW]	T [°C]	T [K]
1	11.11.1998	−69.9	−10.51	5.1	278.1
1	19.05.1999	−74.5	−10.87	5.8	278.8
5	11.11.1998	−72.0	−10.54	5.1	278.1
5	19.05.1999	−74.1	−10.85	5.9	278.9
6	11.11.1998	−70.9	−10.49	4.9	277.9
8	11.11.1998	−71.2	−10.53	5.0	278.0
8	19.05.1999	−70.7	−10.43	5.6	278.6
9	11.11.1998	−70.2	−10.41	4.9	277.9
9	19.05.1999	−70.3	−10.25	5.8	278.8
10	11.11.1998	−72.8	−10.70	5.1	278.1

Table 2: Stable isotope composition of water and calcite from studied pisoids and calculated values of fractionation factor (expressed as $10^3 \ln\alpha_{c-w}$).

Sample number	δ¹³C [‰ PDB]	δ¹⁸O_c [‰ SMOW]	δ¹⁸O_c [‰ PDB]	δ¹⁸O_w [‰ SMOW]	10³ln α_{c-w}
P 1/1	−5.3	25.2	−5.5	−10.51	35.45
P 1/1	−5.3	25.2	−5.5	−10.87	35.81
P 1/3	−3.9	23.8	−6.9	−10.51	34.09
P 1/3	−3.9	23.8	−6.9	−10.87	34.45
P 1/8	−6.3	22.3	−8.3	−10.51	32.62
P 1/8	−6.3	22.3	−8.3	−10.87	32.98
P 1/9	−6.7	23.4	−7.3	−10.51	33.70
P 1/9	−6.7	23.4	−7.3	−10.87	34.06
P 5/6	−7.5	22.6	−8.1	−10.54	32.94
P 5/6	−7.5	22.6	−8.1	−10.85	33.26
P 6/3	−5.0	24.0	−6.7	−10.49	34.26
P 6/5	−4.6	31.0	0.1	−10.49	41.07
P 8/2	−5.6	25.8	−5.0	−10.53	36.06
P 8/2	−5.6	25.8	−5.0	−10.43	35.96
P 9/1	−6.4	22.7	−8.0	−10.41	32.91
P 9/1	−6.4	22.7	−8.0	−10.25	32.76
P 9/2	−6.5	23.6	−7.1	−10.41	33.79
P 9/2	−6.5	23.6	−7.1	−10.25	33.63
P 9/4	−5.9	23.2	−7.5	−10.41	33.40
P 9/4	−5.9	23.2	−7.5	−10.25	33.23
P 10/2	−6.3	23.1	−7.6	−10.70	33.59

Assuming that the calcite crystallizes in isotope equilibrium, the content of ^{18}O in the calcite depends on the content of ^{18}O in the ambient water and on the temperature of crystallization. The knowledge of the temperature in the cave as well as the content of ^{18}O in the ambient water and the content of ^{18}O in the

calcite permitted a test of whether the process of crystallization proceeded under equilibrium conditions. The test was carried out using the dependence expressed by the equation formulated by O'Neil et al. (1969) and modified later by Friedman & O'Neil (1977):

$$10^3 \ln\alpha_{c-w} = 2.78 \ (10^6 \ T^{-2}) - 2.89 \qquad (1)$$

where T is the temperature of the process in Kelvin, and the parameter α_{c-w} is the calcite–water fractionation factor, which is expressed by equation as follows:

$$\alpha_{c-w} = \frac{1000 + \delta^{18}O_c}{1000 + \delta^{18}O_w} \qquad (2)$$

The values of expression $10^3 \ln\alpha_{c-w}$ for pisoids from Perlová Cave were calculated using the equation 2 and the measured values $\delta^{18}O_w$ and $\delta^{18}O_c$. The results of the calculations ($10^3 \ln\alpha_{c-w}$) are presented in Table 2. As the values $\delta^{18}O_w$ measured in two series differed slightly from each other the parallel calculations were made for both of them (in the case of the pisoids from pools P1, P5, P8 and P9). Since the temperature of crystallization is known, one can compare the expressions $10^3 \ln\alpha_{c-w}$ and $2.78 \ (10^6 \ T^{-2}) - 2.89$, which should be equal according to equation 1. However, the comparison reveals that these expressions are not equal. The above dependencies are presented in Fig. 5, where it is shown that the calculated values $10^3 \ln\alpha_{c-w}$ fall above the equilibrium line. This proves that the crystallizing calcite is enriched in the heavy oxygen isotope in relation to ambient water and that the majority of the analysed pisoids grew under conditions of disequilibrium between the water and calcite.

Stable oxygen isotope fractionation during the growth of pisoids in Perlová Cave

Several possible reasons for the disequilibrium conditions between water and calcite ought to be considered. The disequilibrium can be caused by: (i) rapid diffusion of CO_2 from solution to atmosphere; (ii) water evaporation; (iii) kinetic effects connected with the rate of calcite crystallization; (iv) incorporation of other (besides Ca^{2+}) cations into the calcite structure, and (v) the effects caused by metabolism of organisms.

The rapid diffusion of CO_2 from water to cave atmosphere preferentially removes isotopically lighter molecules ($C^{16}O_2$) from water (Usdowski et al. 1991). Thus, the above process should lead to the relative increase of $C^{18}O_2$ content in water. However, oxygen isotope equilibration between dissolved CO_2 and H_2O occurs simultaneously (Usdowski & Hoefs 1990), and can be expressed as follows:

$$C^{18}O_2 + H_2{}^{16}O \rightarrow C^{16}O_2 + H_2{}^{18}O \qquad (3)$$
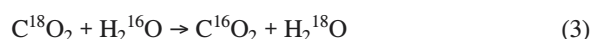
The systematic enrichment of dissolved CO_2 remaining in water in ^{16}O is the effect of the equilibration process operating during rapid diffusion of CO_2.

Thus, the above processes should result in the crystallization of calcite enriched in ^{16}O (Guo et al. 1996). The only ex-
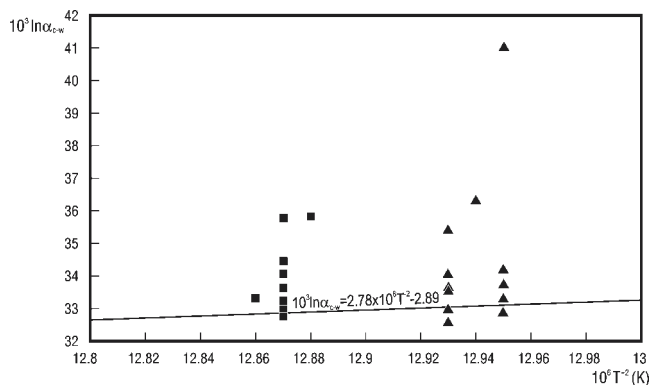
Fig. 5. Fractionation factor (expressed as $10^3 \ln\alpha_{c-w}$) vs. temperature (expressed as $10^6\,T^{-2}$); triangles — values calculated for temperatures of November, squares — values calculated for temperatures of May.

ception occurs when chemical reactions leading to the precipitation of the $CaCO_3$ proceed faster than the process of oxygen isotope equilibration between CO_2 and H_2O. Such a process occurs near springs fed by water overcharged with CO_2 (cf. Duliński et al. 1995; Guo et al. 1996), but not in a typical karst cave, such as Perlová Cave.

Evaporation should be regarded as the second process influencing the content of ^{18}O in calcite. Evaporation preferentially removes lighter molecules $H_2{}^{16}O$ from water (Epstein & Mayeda 1953). This leads to the isotope disequilibrium between water and dissolved CO_2 and HCO_3. Due to isotope re-equilibration the process expressed by equation (3) proceeds from right to left. The $CaCO_3$ crystallization under such conditions results in systematic enrichment in the heavy oxygen isotope of successively precipitated portions of $CaCO_3$. However, the precipitated $CaCO_3$ still reflects the changes of $^{18}O/^{16}O$ ratio in the water. Because $CaCO_3$ precipitates in isotopic equilibrium with the ambient water, the only exception is in conditions where crystallization proceeds faster than isotopic re-equilibration between dissolved CO_2, HCO_3 and H_2O. In such cases the precipitated calcite would be more enriched in the light isotope of oxygen than a carbonate system of the ambient water (see e.g., Usdowski et al. 1979; Dandurand et al. 1982; Turi 1986; Chafetz et al. 1991; Chafetz & Lawrence 1994). Therefore, the above mechanism cannot be applicable to Perlová Cave. It is noteworthy, that the relative humidity in deeper parts of single-entrance caves situated, like Perlová Cave, in the temperate climate zone is very high and reaches over 95 % in summer and even 100 % in other seasons (Wigley & Brown 1976). Thus evaporation in the studied cave seems not to proceed at all or proceeds on a negligible scale.

The rate of crystallization may influence the isotopic composition of calcium carbonate (Chafetz et al. 1991; Dickson 1991; Chafetz & Lawrence 1994). The more rapid the process of crystallization is, the more calcite is enriched in the light isotope of oxygen (cf. Hoefs 1997). This leads in the opposite direction to that detected in the studied pisoids. Therefore, the rate of crystallization can be excluded from the factors controlling isotope exchange between the ambient water and calcite. The influence of other cations apart from Ca^{2+} should also be excluded, because of the absence of their significant

admixture within the pisoids (cf. O'Neil et al. 1969; Mortimer & Coleman 1997).

Therefore another explanation is to be sought. The most plausible explanation deals with the physiology of the bacteria. Bacterial physiology may cause isotopic disequilibrium between water and calcite. Organisms show a metabolic preference for the light rather than the heavy isotope (Ehrlich 1996, 1998). The phenomenon is known mainly in relation to stable carbon isotopes (cf. Mertz 1992 and references quoted herein). Preferential use of the light oxygen isotope is decidedly less known. Such effects have been found in foraminiferal tests and coral skeletons (Grossman 1987; McConnaughey 1989). As for the foraminifers it is demonstrated in the enrichment of tests in the light isotope of oxygen (^{16}O) due to the incorporation of isotopically light metabolic oxygen molecules into the test (Grossman 1987). Recently, examples of fractionation of the oxygen isotopes caused by the metabolic processes of bacteria have also been reported. Mortimer & Coleman (1997) showed the influence of the bacteria *Geobacter metallireducens* on the $\delta^{18}O$ values of diagenetic siderite. Horita et al. (1998) also reported a similar process in the iron oxides of microbial origin.

As mentioned above, hydrogen-oxidizing bacteria occur in the biofilm covering the studied pisoids. The bacteria change the chemistry of their microenvironments by uptake of oxygen either in the form of O_2, which they use to obtain energy from reaction of the synthesis of the water, or in the form of CO_2 used to build up the organic matter (Aragno & Schlegel 1992).

Oxygen isotope disequilibrium can be caused by the preferential uptake of the $C^{16}O_2$ molecules and/or by the preferential uptake of $^{16}O_2$. It is not possible to determine which process is of crucial significance. Each can result in the relative depletion of $^{16}O_2$ in the bacterial microenvironment, that is within the biofilm. Since the biofilm is a zone isolated from the external environment (Decho 2000) this depletion is not sufficiently quickly re-equilibrated. Therefore, the calcite which grow around the bacterial cells within the biofilm is depleted in the light isotope of oxygen.

The $\delta^{13}C$ values of the studied pisoids fall in the range –6.7 to –3.9 (Table 2). It means that they do not differ from values typical of carbonate speleothems (cf. Schwarcz 1986; Baker et al. 1997). Unfortunately, it was impossible to carry out the study of carbon isotopic composition of carbonate molecules in the ambient solution. It was due to technical reasons (the relatively small volume of the studied pools and relatively big amount of water needed for analysis). Therefore, one cannot decipher the fractionation of the stable isotopes of carbon between the carbonate molecules in the ambient water and the growing pisoids.

The differences in $\delta^{18}O_c$ values between the pisoids growing in the single pool are another question which can be posed. They probably owe their origin to the variable intensity of bacterial physiological processes existing on a microenvironmental scale and their intensity can change over a very short distance (cf. Chafetz et al. 1991). Local mechanical degradation of a biofilm, which can cause faster re-equilibration of the stable isotope composition between the biofilm and external environment, seems to be another explanation.

Conclusions

This study suggests that the physiology of chemo-lithoautotrophic hydrogen-oxidizing bacteria accounts for exchange of stable oxygen isotope occurring during the growth of pisoids in Perlová Cave. This indicates that not only CO_2 diffusion and evaporation but also biological processes influence the oxygen isotope ratio within speleothems. It can be assumed that these processes also have an effect on other speleothems attributed to a microbial origin (Le Métayer-Levrel et al. 1997; Northup & Lavoie 2001; Jones 2001). This suggestion especially concerns moonmilk speleothems, which are also formed by mediation of hydrogen-oxidizing bacteria (Gradziński et al. 1997).

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