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Geomicrobiology and Redox Geochemistry of the Karstified Miocene Gypsum Aquifer, Western Ukraine: The Study from Zoloushka Cave

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The gypsum karst of the western Ukraine developed largely under artesian conditions. The Miocene aquifer is presently entrenched and dewatered over much of the territory, while it remains confined in the zone adjacent to the Carpathian Foredeep. The most prominent geochemical features of the Miocene aquifer system in the confined karst zone are: (1) the almost universal presence of a bioepigenetic calcite bed, enriched in the light carbon isotope, at the top of the gypsum (the "Ratynsky Limestone"), (2) the widespread sulfur mineralization associated with the above calcite bed (the region is one of the world's largest sulfur-bearing basins), and (3) high H_2S *and* CO_2 *in the groundwater. Intense microbial sulfate-reduction processes occur in the gypsum in this zone. Zoloushka Cave is the third longest (92 km) and the largest by volume (more than* 7×10^5 m^3*) gypsum cave in the world. It is a unique example of a young artesian cave that only during the Holocene became partly drained and during the last 50 years progressively dewatered due to a quarry operation. These rapid changes have induced a number of transitional geochemical processes, some of which appear to be bacterially mediated. Six groups of microorganisms have been identified in the cave. Our article discusses the aquifer geochemistry during the transitional stage in the light of the microbiological studies.*

Keywords cave microbiology, gypsum aquifer hydrogeochemistry, iron hydroxides, manganese hydroxides, Zoloushka Cave

The gypsum karst of the western Ukraine, developed in the Miocene (Badenian) gypsum (Figure 1), is a well-studied and world-renowned karst region. It has received particular attention due to exploration and study since the 1960s of the largest gypsum caves on the earth (Optimisticheskaya, 214 km; Ozernaya, 117 km; Zoloushka, 92 km; Mlynki, 27 km; Kristal'naya, 22 km). During the last 2 decades, the regional evolution of the geohydrologic settings has been described in detail (Andrejchuk 1988; Klimchouk 1996b, 1997a, 1997b;

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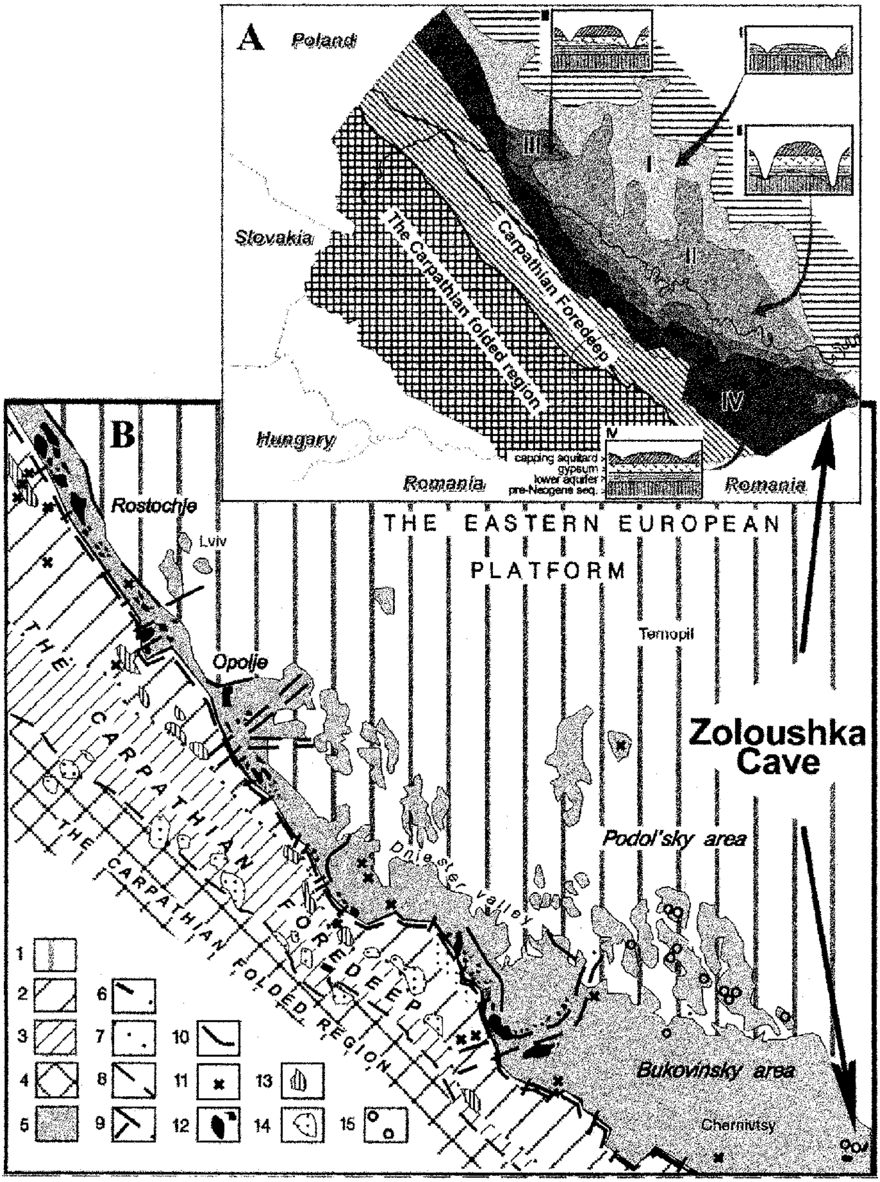

FIGURE 1 A—location and evolutionary types of the gypsum karst of the western Ukraine. Zones of different karst types are shown by Roman numerals: I = the gypsum is entirely denuded, II = entrenched karst, III = subjacent karst, IV = deep-seated (confined) karst. B—Distribution of the gypsum stratum, sulfur deposits, and large caves in the western Ukraine (modified from Klimchouk, 2000b). 1 = Eastern-European platform fringe. Carpathian Foredeep: 2 = outer zone, 3 = inner zone. 4 = Carpathian folded region; 5 = sulfate rocks on the platform. Tectonic boundaries include: 6 = platform/foredeep, 7 = outer/inner zone of the foredeep, 8 = foredeep/folded region. 9 = other major faults; 10 = flexures. 11 = sulfur mineralization; 12 = sulfur deposits; 13 = gas deposits; 14 = oil deposits; 15 = large maze caves in the gypsum.

Klimchouk, Aksem, Shestopalov, and Lisichenko 1985; Klimchouk and Andrejchuk 1988) and the region has been shown to be the world's foremost model of speleogenesis under artesian conditions (Klimchouk 1990, 1991, 1996a, 2000b). Most of the large maze caves are now relics, being located within the presently entrenched karst zone, although there is much evidence that active speleogenesis still takes place in the subjacent and deep-seated (confined) karst zones (Figure 1A). Zoloushka Cave is a unique example of a young artesian cave that only recently (during the Holocene) was brought into a partly inundated setting and has been progressively dewatered during the last 50 years by a quarry operation. These rapid changes gave rise to a number of transitional geochemical processes, some of which appear to show considerable microbial involvement. This article discusses the results of the preliminary geomicrobiological study of the cave in the context of the regional geochemistry of the Miocene aquifer.

Regional Geohydrological and Geochemical Context of Cave Development

The Miocene gypsum sequence is exposed along the southwestern edge of the eastern European platform, in the transition zone between the platform and the Carpathian Foredeep. Gypsum stretches from the northwest to the southeast for 340 km in a belt ranging from several kilometers to 40–80 km wide. It is the main component of the Miocene evaporite formation that girdles the Carpathian folded region to the northeast, from the Nida river basin in Poland across the Western Ukraine and Moldova to the Tazleu river basin in Romania (Figure 1A,B).

Most Miocene rocks along the platform margin overlie eroded Cretaceous strata, which include terrigenous and carbonate sediments, mostly marls and sandstones, together with detrital and argillaceous limestones. The Miocene succession comprises deposits of Badenian and Sarmatian age. The Lower Badenian unit, beneath the gypsum, includes mainly carbonaceous, argillaceous, and sandy beds (30–90 m thick) adjacent to the foredeep, and these grade into a calcareous biohermal and sandy facies (10–30 m thick) toward the platform interior.

The gypsum sequence, 10–40 m in thickness, is variable in structure and texture but almost everywhere occurs as a single bed. A layer of pelitic and cryptocrystalline limestone, ranging from several tens of centimeters to more than 25 m in thickness, overlies much of the gypsum. This limestone (locally called "Ratynsky") contains two genetic varieties that differ in carbon isotopic composition. The pelitic limestone has normal "evaporitic" $\delta^{13}C$ values (-3 to -8% $\delta^{13}C$). The other variety, which is crypto- and microcrystalline, formed bioepigenetically by replacement of the gypsum during sulfate reduction, which is evidenced by ^{12}C enrichment of calcite, with $\delta^{13}C$ signatures ranging from -32 to -65% (Lein, Ivanov, Rivkina, and Bonder 1977; Mamchur 1972). Where the limestone thickness exceeds 1 to 2 m, it consists of mainly epigenetic calcite, which locally replaces the gypsum stratum entirely. Together, this limestone and the gypsum comprise the Tyrassky Formation.

The Tyrassky Formation is overlain by the Upper Badenian unit, which begins with argillaceous and marly lithothamnion limestones and sandstone beds. Above this is a succession of clays and marls, with its lower part in the Upper Badenian (the Kosovsky Formation), and its upper part in the Lower Sarmatian, the total thickness ranging from 40–50 m in the Podol'sky area to 80–100 m adjacent to the foredeep. The clay cover thickens to several hundred meters close to regional faults that separate the platform edge from the foredeep.

The Miocene succession is overlain by late-Pliocene and Pleistocene glacio-fluvial sands and loams in the northwest section of the gypsum belt, and by sand and gravel alluvial terrace deposits left by the Dniester and Prut Rivers (late Pliocene—Pleistocene) in the Podol'sky and Bukovinsky areas. Many buried valleys, of early to mid-Pleistocene age, are entrenched 30 to 50 m into the Kosovsky and Sarmatian clays and, locally, into the upper part of the Tyrassky Formation.

The present distribution of the Miocene formations and the levels of their denudation vary in a regular way from the platform interior toward the foredeep. The Tyrassky Formation dips 1 to 3° in this direction and is disrupted by block faults in the transition zone. To the south and southwest of the Dniester Valley, large tectonic blocks drop down in a series of steps, the thickness of clay overburden increases, and the depth of erosional entrenchment decreases. Along the tectonic boundary with the foredeep, the Tyrassky Formation drops down to a depth of 1000 m and more. This variation, the result of differential neotectonic movement, played an important role in the hydrogeological evolution of the Miocene aquifer system and resulted in the differentiation of the platform edge into four zones (Figure 1A), three of which represent distinct types of gypsum karst: entrenched, subjacent, and deep-seated. The gypsum bed is largely drained in the entrenched karst zone, is partly inundated in the subjacent karst zone, and remains under artesian confinement in the deep-seated karst zone.

In hydrogeologic terms, the region represents the southwestern portion of the Volyno–Podolsky artesian basin (Shestopalov 1989). Sarmatian and Kosovsky clays and marls provide an upper confining sequence. The lower part of the Kosovsky Formation and the limestone bed of the Tyrassky Formation form the original upper aquifer (above the gypsum) and the Lower Badenian sandy carbonate beds, in places along with Carboniferous sediments, form the lower aquifer (below the gypsum), the latter being the major regional one. The hydrogeologic role of the gypsum unit has changed with time, from initially being an aquiclude, intervening between two aquifers, to a karstified aquifer with well-developed conduit permeability. Regional flow is from the platform interior, where confining clays and the gypsum are largely denuded, toward the large and deep Dniester Valley and the Carpathian Foredeep. However, in the entrenched karst zone (Podol'sky area) the deeply incised river valleys divide the Miocene sequence into a number of isolated interfluves. This is the area where most of the explored caves are located. To the south–southeast of the Dniester (Bukovinsky area) the gypsum remains largely intact and lies partly within the phreatic zone. Further in this direction, as the gypsum increases in thickness and entrenchment decreases, the Miocene aquifer system becomes confined. In this zone, the groundwater flow pattern includes a lateral component in the lower aquifer (and in the upper aquifer but to a lesser extent) and an upward component through the gypsum in areas of potentiometric lows.

This zone, represented by a dark tint on Figure 1A, is known for geomicrobiological processes within the Miocene aquifer, which are responsible for the formation of groundwater enriched in hydrogen sulfide and for large bioepigenetic sulfur deposits and their host calcite bed. Klimchouk (1997a) has suggested that artesian "ascending" speleogenesis in the gypsum layer played a fundamental role in regional geochemistry, particularly in the origin of sulfur deposits. This is not only because it provided large amounts of dissolved sulfates needed to fuel the large-scale sulfate reduction, but also because speleogenesis opened pathways for the flow of groundwater between the lower and upper aquifers through maze cave systems in the gypsum.

The lower aquifer, the major one in the system, has a predominantly Ca–HCO_3 composition with TDS as high as 1.0 g/L. In places, Cl–Na methane-bearing waters with TDS up to 7.5 g/L are found, rising along faults adjacent to the foredeep (Babinets and Tsapenko, 1960), an oil and gas bearing basin. Hydrocarbon gas shows have been observed at many sulfur deposits and other localities in this zone (Aleksenko 1967; Srebrodol'sky and Kachkovsky 1973). The methane content in released gases sometimes reaches 92%. Methane is believed

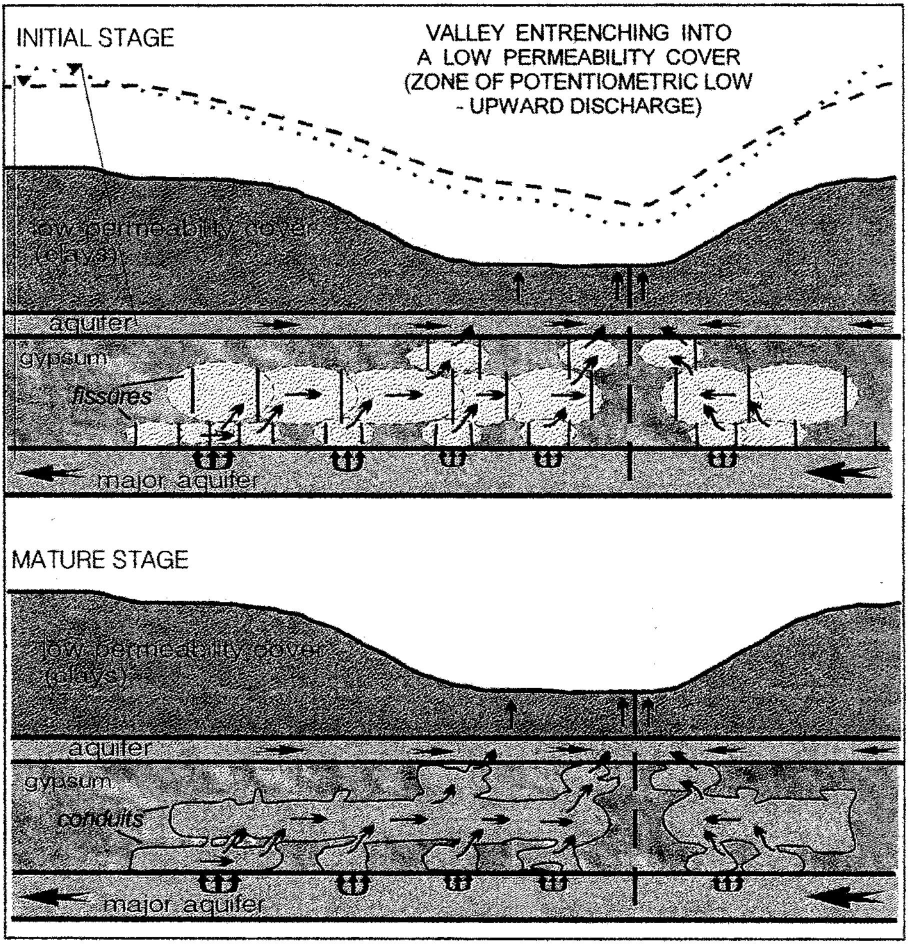

FIGURE 2 The conceptual model of speleogenesis and flow pattern in the Miocene aquifer in the western Ukraine (from Klimchouk, 1996b).

to be the primary source of organic carbon for sulfate reduction operating in the Miocene sequence.

Within areas of potentiometric lows, groundwater from the lower aquifer flows upward through the gypsum into the upper aquifer and then discharges locally through the zones where the confining properties of the capping clays are weakened by stratigraphic or tectonic discontinuities, or incising erosional valleys. Such flow patterns are shown to generate maze caves in the gypsum, the mechanism being described in Klimchouk (1994a, 1996a, 1997c, 2000a) and illustrated schematically in Figure 2. Circulating through the gypsum under sluggish flow conditions, the groundwater gains calcium sulfate. As the dissolved sulfate increases, oxygen and Eh decrease. A reducing environment is established within the gypsum and sulfate reduction processes are widespread, generating H_2S and replacing gypsum with epigenetic calcite at the top of the gypsum bed. Water in the upper aquifer commonly has TDS up to 3–5 g/L, SO_4^{2-} of 1.5–2.0 g/L, high H_2S (34–200 mg/L; up to 370 mg/L in places), and CO_2 (120–170 mg/L). Klimchouk (1994) suggested that sulfate reduction in the gypsum helps to maintain aggressiveness with respect to gypsum, hence contributing to speleogenesis.

Zoloushka Cave

In 1976, a few entrances to a vast labyrinthic cave were found in the face of an active gypsum quarry located in the southeast part of the region. The explored length of the cave is currently over 92 km (Figure 3). The cave area lies generally in the confined karst zone, although within uplifted tectonic blocks the gypsum was partially entrenched by the nearby Prut Valley during the Holocene, causing the water table to drop some 2–3 m below the top of the gypsum. The quarry operation and accompanying groundwater withdrawal since the 1950s has caused a further water-table drop to 17–19 m below the top of the gypsum and brought about various transformations in the aquifer. Studies in the cave system have allowed direct observation and investigation of many of these transitional processes, among which the geochemical and microbiological transformations were most pronounced.

Local Settings

The gypsum in the cave area is 23–25 m thick and the Ratynsky Limestone, up to 1 m thick, overlies it (Figure 3). The Kosovsky Formation (10 to 60 m thick depending on the local relief) overlies the cave area. It is comprised mainly of argillaceous sediments, with some minor sandstone and limestone beds in its lower part. Clays are grayish-blue due to dispersed hydroillite. At the base of the Kosovsky Formation, some beds are enriched in iron-manganese concretions with cores composed of crumbly manganese oxides. The gypsum rests on the sands and marls of the Lower Badenian (3–4 m), in turn overlying eroded Cretaceous limestones and sandstones. Together they form the present unconfined aquifer discharging to the Prut River through the terrace sediments. A cone of depression caused by water withdrawal from the quarry occurs in the cave area. The Quaternary aquifer is also present, perched on the Kosovsky clays. It is drained by local erosional valleys and by collapse structures in the clay succession.

Dewatering of the Cave

The quarry that opened the cave started operation at the end of the 1940s. Since then groundwater has been continuously pumped from the quarry, causing a cone of depression to develop around it. At first, the withdrawal rate was rather modest, about 20–50 m^3/hour. When the quarry reached 8–10 m in depth, the pumping rates increased to 100–500 m^3/hour. Since the middle 1960s, karst water inflow reached 700–800 m^3/hour, caused by excavating the third quarry terrace (to a depth of 18–22 m), and this is the pumping rate maintained today.

Prior to the quarry, the karst water table was 2–3 m below the top of the gypsum, some 1–2 m below the ceiling of Zoloushka's upper-story passages. The groundwater circulated slowly toward the Prut River and discharged through alluvium. It contained considerable H_2S and dissolved solids (3.0–4.5 g/L). With the start of the quarry operations, the quarry became the center of groundwater discharge. Within the drawdown cone, which expanded to several km in diameter, groundwater flow became radial. Flow rates increased and there was a decrease in TDS (to 1.9–2.6 g/L) and in the rate of H_2S degassing.

Lowering of the potentiometric surface and dewatering of the cave was most pronounced during the 1960s. During initial cave exploration (1976–1978), the passage floors were covered by "fresh" wet slippery clay, which progressively desiccated in the following years, leaving cracks in the sediment. Isolated pools were formed in the upper story, perched on clay fill, and they behave independently from one another. There are now about 50 such pools ranging from a few square meters to 1000 m^2 in area with depths ranging from 0.5 to 5 m. Small perched pools completely disappear with time due to evaporation and/or percolation losses. The lower story of the cave remains below the water table.

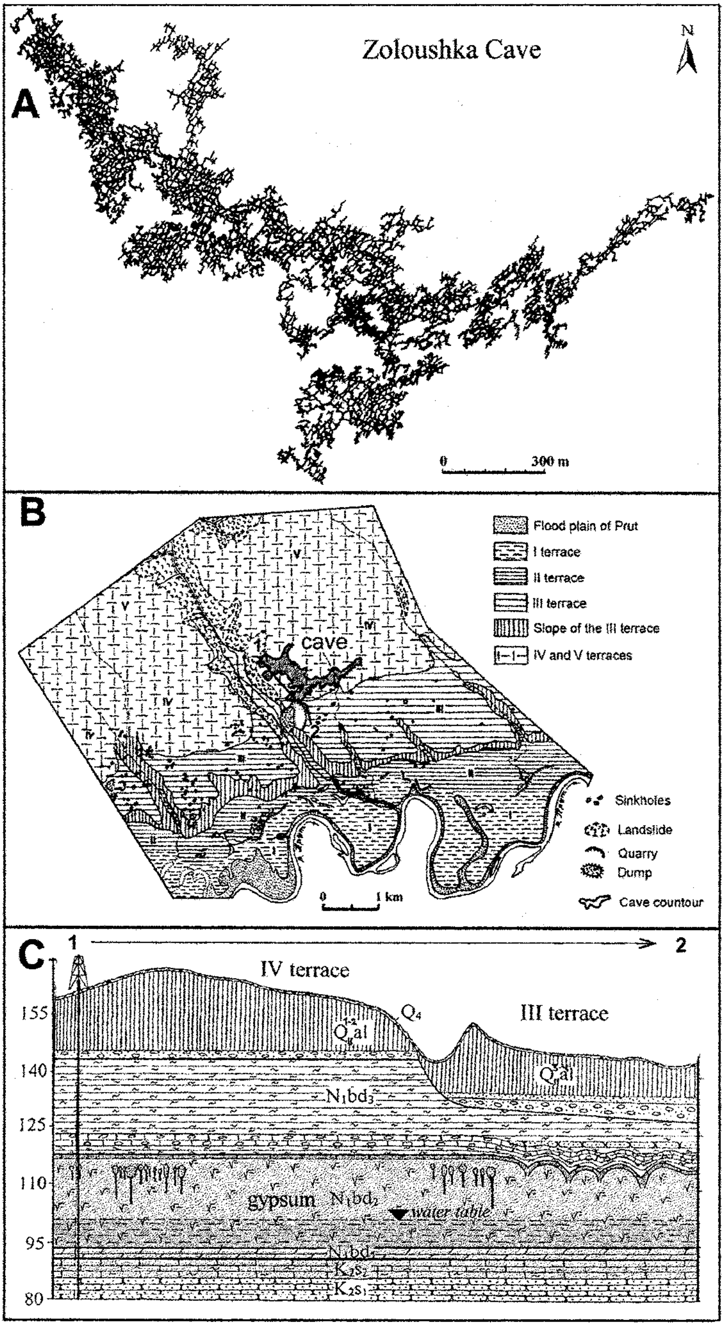

FIGURE 3 The map of Zoloushka Cave and geological profiles across the cave area. Key to cross-sections: 1 = soil; 2 = sandy-gravel sediments and loess, Plio-Quaternary; 3 = clays and marls, Kosovsky formation; 4 = gypsum with Rarynsky limestone bed on the top; 5 = sandstones and marles, Lower Badenian; 6 = sandstones and limestones, Cretaceous; 7 = karst cavities in the gypsum; 8 = groundwater level.

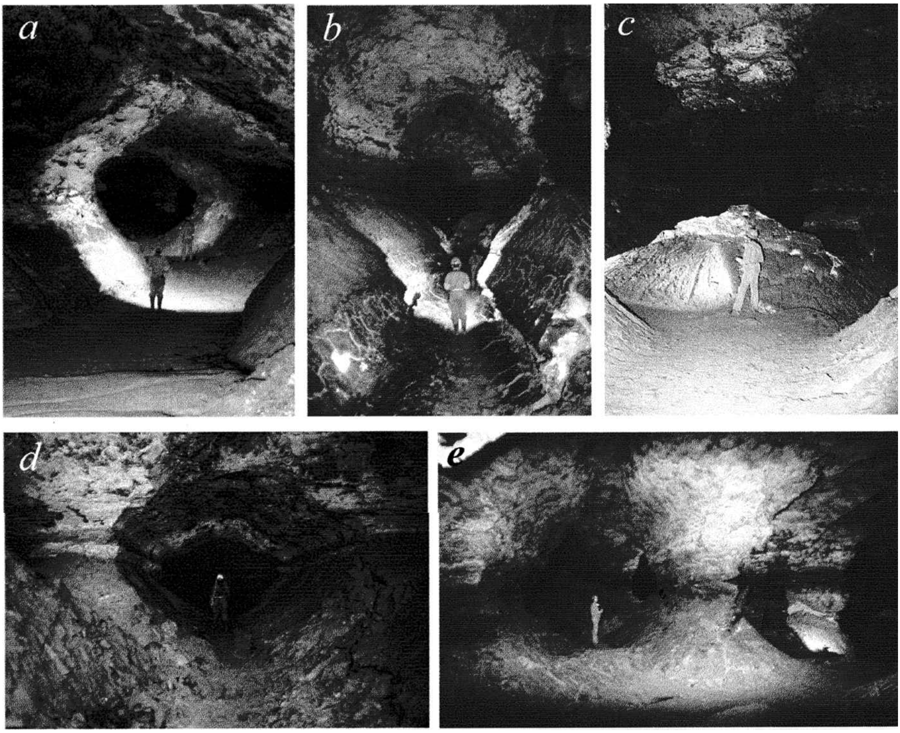

FIGURE 4 Typical passage morphologies of Zoloushka Cave. Note a rift-like extension in the passage bottom on photo (b), normally hidden by clay fill (a). Photo (d) shows horizontal notches on the walls developed due to preferential dissolution at the water-table level during the last period of the natural development. Such notches in densely spaced network may cut pillars between adjacent passages causing them to coalesce into large chambers (photo c and e). Photos by A. Klimchouk and V. Kisseljov.

Cave Morphology

Zoloushka Cave is a horizontal labyrinth of passages occurring in two stories. The upper story consists of large (1–6 m wide and 1–7 m high) passages (Figure 4) with ceilings located 1–3 m below the top of the gypsum. Passage cross-sections are oval, rhomb-like, or hemispherical. In some areas, passages coalesce laterally, forming large rooms (15,000–30,000 m^3) with only small pillars remaining in between passages (Figure 4c). In areas where the level of clay fill has lowered by subsidence, it is possible to observe 3–10 m deep rift-like extensions in the floors (Figure 4b) that are hidden under the fill. Thus entire cross-sections commonly have “keyhole” shapes, with the width of the rift being 0.3–3.0 m. The lower story of the cave, which is still inundated and only partly explored, lies along the bottom of the gypsum. It is connected with the upper level through pits, whose morphologies suggest “ascending” hydraulic communication during the formation period.

Cave Microclimate

As the cavities drained, they developed an internal microclimate. During the prequarrying stage they had been isolated from the open air. The opening of the quarry resulted in some ventilation strongly affecting the cave climate. The transitional microclimatic zone extends into the cave 30–60 m from the entrance and shows seasonal and daily variations similar to those of the outside atmosphere. In the stable zone the relative humidity is 100% and the temperature varies from 10.6 to 11.4°C. The water temperature is about 11.0°C. Air exchange with the outer atmosphere was rather sluggish due to the fact that the vast cave had only one entrance. It had been exposed in the quarry face at the upper story through the 1970s–1980s. Now the cave is connected to the surface by a concrete 20-m deep pit surrounded by a mass of quarry rubble. This slowed down air exchange between the cave and the open atmosphere.

The most pronounced characteristic of the cave microclimate is elevated CO_2 and nitrogen. CO_2 varies within 0.2–4.5 vol % and nitrogen varies within 79.6–83.1 vol %. In contrast, oxygen is low, varying from 13.7 to 19.5 vol %. There is some regularity in the spatial variation of gas compositions. Generally, CO_2 rises from the entrance toward distant parts of the labyrinth. In the distant parts, CO_2 is commonly 2.0 to 2.5%; in the western section it is considerably higher than in the eastern section. CO_2 concentrations are also higher in low spots such as rifts, pits, and lower passages. Concentrations up to 4.5 vol % are measured in such places. High CO_2 and low O_2 in the air have considerably complicated the exploration of the cave. During recent years, when the cave was connected to the surface only through the pit, CO_2 levels have probably risen even higher, as suggested by the recent death of two unprepared visitors in 1999 at the bottom of the entrance pit.

Sediments and Deposits—Fe–Mn Hydroxides

Grayish-blue fine-bedded clays are the most common cave fill. They formed by redeposition of collapsed material from the Kosovsky Formation during sluggish flow conditions. The cave clay fills passages to a variable extent (0.5 to 6 m), obscuring their actual shapes and sizes. Relatively fresh breakdown piles composed of the overburden material are also common.

A distinct feature of the cave is the widespread distribution of iron and manganese hydroxides throughout the labyrinth. Their deposition is related to microbial activity and presents one of the most interesting scientific problems of the cave studies. Previous works discussing the issue include Klimchouk (1994), Volkov, Andrejchuk, Janchak, and Smirnov (1987), Volkov (1990), and others.

Fe–Mn hydroxides occur everywhere in the cave (Figures 5 and 6). A visitor entering the Zoloushka Cave first notices the abundance of black, reddish, and orange colors on the passage walls and floors. The name of the cave owes its origin to these colors. On exiting the cave, female cavers covered with the reddish-black clay looked grimy, like Cinderella in the famous fairy tale (“Zoloushka” means Cinderella in Russian).

Hydroxides of Fe and Mn occur as films, powdery layers and masses, coatings, cluster and corn-kernel like aggregates, stalactites, and stalagmites (Figures 5 and 6). The most common occurrences are orange and yellow films up to 1 mm thick, which coat the walls and floors of passages. In many places, Fe-hydroxides form layers ranging from a few cm to 30 cm in thickness. While dehydrating, this material changes to friable masses with vestiges of fine bedding. From a bright red color it changes to red, reddish-brown, and finally to yellow when taken out to the surface (turning to oxides).

Also very common is a sooty-black powder of Mn-hydroxides, which occurs as 1-5-mm thick coatings or layers within the upper section of the clay fill. Local masses of this material reach 45 cm in thickness. Sometimes Mn-hydroxides form hemispherical, corn kernel-like and clustered aggregates and stalactites 5–8 cm in length. Such aggregates are very friable and fragile. A closer examination reveals that stalactites are composed of small bladed crystals that form inverted “fir-trees.” In one locality, thin (about 1 mm) filament-like formations were found. Among the unusual forms are hollow, tube-like stalagmites that are 2–20 cm in diameter and 10–50 cm high. Their walls are 0.5 to 2.0 cm

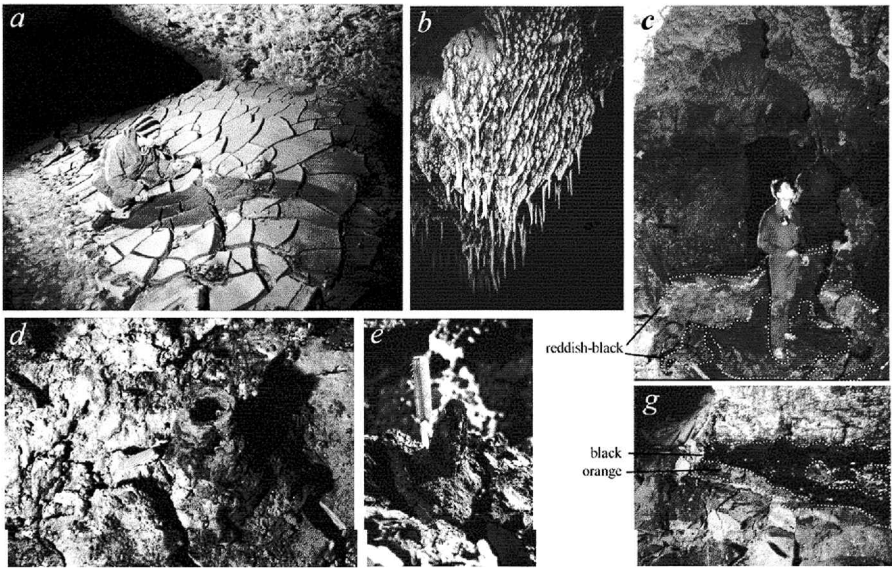

FIGURE 5 Iron-manganese hydroxide sediments and formations in Zoloushka Cave. Photos by A. Klimchouk and V. Kisseljov. a = dessication crack pattern on clay fill, with blocks covered by orange film of Fe-hydroxides; b = stalactites of Fe-hydroxides; c = blocks of Fe-hydroxides covering the floor and boulders; d–e = hollow stalagmites composed by Fe-hydroxides; g = sooty-black powdery masses of Mn-hydroxides and orange masses of Fe-hydroxides included into clay fill.

thick and have a distinct concentric structure. They are composed of Fe-hydroxides with minor interlayered Mn-hydroxides. The origin of such stalagmites is not clear.

Hydroxides of Fe and Mn are in various mineral forms and are characterized by a specific composition (Table 1). Hydroxides of Fe are represented by the amorphous phase of high sorption capability (oxyhydrate collector) such as $FeOOH \cdot nH_2O$, with an iron content

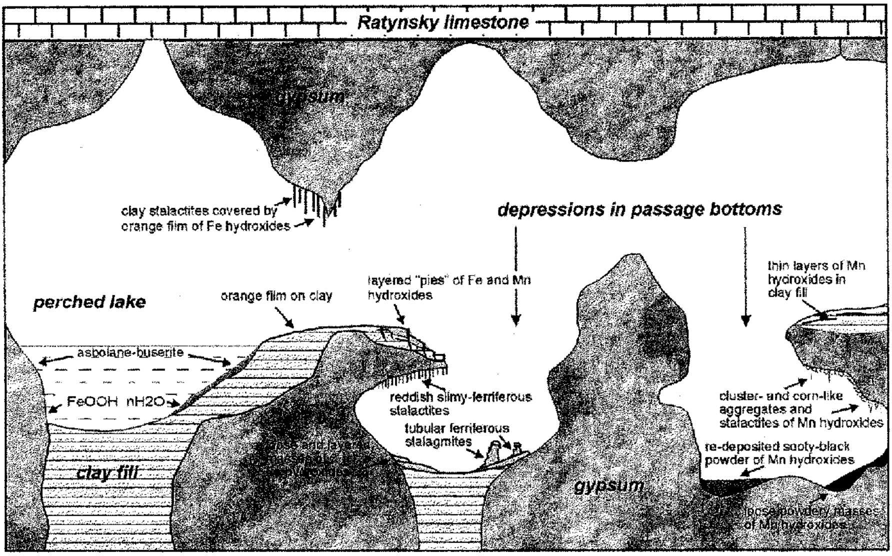

FIGURE 6 General scheme of occurrence of Fe–Mn hydroxides in Zoloushka Cave.

TABLE 1 Chemical composition (%) of Fe and Mn compounds in Zoloushka Cave (after Volkov 1990)

	Sample number and mineral phase of a sediment					
Component	1415 amorphous iron	1431 amorphous iron	1446 amorphous iron	1353 birnessite	3/23 birnessite	1083 mixture of asbolane-buserite and birnessite
SiO_2	4.520	2.70	4.98	2.14	*	2.920
TiO_2	0.020	0.01	0.02	0.07	0.08	0.030
Al_2O_3	0.300	0.27	0.05	0.07	1.66	0.610
FeO	0.140	1.00	0.84	0.10	0.10	0.100
Fe_2O_3	60.600	54.37	61.37	0.37	7.89	0.100
MnO	0.890	0.42	0.53	0.45	7.14	3.830
MnO_2	**	**	**	72.68	54.82	70.480
MgO	0.250	0.33	0.18	0.33	*	0.500
CaO	6.690	9.31	5.42	7.63	9.25	7.930
Na_2O	0.180	0.18	0.35	0.30	*	0.300
K_2O	0.190	0.10	0.18	0.23	0.42	0.670
H_2O^-	4.000	2.00	4.82	1.72	*	0.720
H_2O^+	*	*	*	*	13.10	*
P_2O_5	6.760	15.28	4.80	0.07	*	0.034
SO_3	0.650	0.41	0.44	0.30	–	0.200
П.п.п.	19.000	16.41	20.60	14.00	–	12.300
NiO	0.008	*	*	1.09	0.69	0.074
CoO	0.005	*	*	0.09	*	0.011
ZnO	0.010	*	*	0.45	*	0.100
Cu_2O	0.006	*	*	0.025	*	0.008
MoO_3	0.008	*	*	0.55	*	0.137
CO_2	**	1.55	**	*	5.81	*
Total, %	99.52	100.38	99.34	100.46	99.12	99.86

*Component was not found.

**Component was not determined.

of up to 50%. In its sorption capacity, Fe-oxyhydrate is second only to Mn-oxyhydrate. Fe-hydroxides contain a wide variety of absorbed microelements, among which a paragenetic association of P, Cr, As, Be, Ge, Ba, Pb, and Sr distinctly stands out. Hydroxides of Mn are represented by the two mineral species: asbolane–buserite [aggregate mixtures of asbolane: $(Co,Ni)_{1-y}$ $(Mn^{4+}O_2)_{2-x}$ $(OH)_{2-2y+2x} \cdot$ nH2O and buserite: γ-MnOOH] and birnessite [(Na,Ca) $Mn_7O_{14} \cdot 3H_2O$]. The latter is represented by a following variety: $Ca_{0.60}$ $K_{0.07}$ $Ni_{0.08}$ $Mn^{3+}{}_{1.43}Mn^{4+}{}_{5.57}O_{14} \cdot 5.9H_2O$. Zn, Co, Cu, and Mo are found in substantial amounts in this birnessite.

The Mn-hydroxide deposits in the cave commonly consist of a mixture of asbolane-buserite and birnessite. Asbolane-buserite is a primary mineral, which changes to birnessite due to partial dehydration. Fe- and Mn-hydroxides, particularly birnessite, contain a substantial amount of organic matter (Table 2). The iron–manganese deposits of Zoloushka Cave are similar in composition and structure to certain oceanic deposits. In the latter, for instance, mineral phases of Fe-hydroxides are also not found, and the Mn-hydroxides consist of asbolane–buserite and birnessite.

TABLE 2 Organics content in various types of cave sediments (after S. N. Volkov, 1990, with additions)

Type of sediments	Number of samples	Organic matter content, % average (range)			Presence of huminic acids
		Total, %	C_{org}, %	Bitumen, $n \cdot 10^{-4}$, %	
Clays of the Kosovsky Formation	3	0.88 (0.72–1.03)	0.72 (0.69–0.77)	8.3 (3.0–12.0)	Not found
Cave fill clays	8	1,97 (0.96–2.66)	1,47 (0.73–2.01)	2,2 (1.1–4.0)	In one sample—not found, in two samples—a little, in three samples—much, in three samples—very much
Fe–Mn formations	6			Not determined	Not determined
Fe-hydroxides	3	0.70 (0.36–0.96)	0.53 (0.27–0.72)		
Birnessite	2	3.19 (1.48–4.89)	2.39 (1.11–3.67)		
Mixture of asbolan–buserite and burnessite	1	2.36	1.77		
Argillo-carbonate (residual?) sediments at the base of the clay fill	8	0.76 (0.16–1.85)	0.56 (0.12–1.39)	2.3 (1.0–6.0)	In five samples—not found, in three samples—traces

There are two main questions concerning the Fe-Mn-hydroxides in the cave: (1) sources of Fe and Mn and (2) conditions and mechanisms for their formation. The most likely sources of Fe and Mn were the overlying clays of the Kosovsky Formation. Regional geochemical studies indicate that the average content of Mn in the Kosovsky clays is 0.15%, whereas it is only 0.03% in the gypsum. The question of the formation of hydroxides and their aggregates is discussed in the next sections.

Microbiological Studies in the Cave

Microbiological investigations in the cave were carried out in the middle 1980s to identify the microorganisms (Table 3) and their role in the formation of the Fe and Mn deposits and

TABLE 3 The relative chemical activity of different species of microorganisms in Zoloushka Cave

Environment	Bacteria species	Relative chemical activity*
Clay fill	***D. desulfuricans***	3
	P. denitrificans	3
	T. denitrificans	2
	T. thioparus	1
	Clostridium sp.	1
Surface of clay fill	***T. ferrooxidans***	3
Bottom silt of the cave pools	***P. denitrificans***	3
	Clostridium sp.	3
	Nonidentified fungus-like organisms	3
Surface of gypsum walls	***iron oxidizing***	3
	T. ferrooxidans	3
	T. thiooxidans	2
	P. denitrificans	1
Clay coating on the cave walls	*P. denitrificans*	1
	T. ferrooxidans	1
	T. thioparus	1
Accumulations of Fe-hydroxides	**Nonidentified fungus-like organisms**	3
	P. denitrificans	3
	Clostridium sp.	1
Accumulations of Fe- and Mn-hydroxides	**Nonidentified fungus-like organisms**	3
	iron oxidizing	3
	T. ferrooxidans	2
	P. denitrificans	2
	T. thioparus	1
Argillo-carbonate (residual?) sediments at the base of the clay fill	*P. desulfuricans*	1
	T. thiooxidans	1
The uppermost water layer on the cave pools	***iron oxidizing***	3
	P. denitrificans	2

*Numbers indicate the relative activity of bacteria: 3—highest, 2—medium, 1—lowest. Bacteria of the highest activity are also shown in bold characters.

speleothems so prominent in the cave. Samples were taken from typical cave microenvironments such as cave pool water, clay fills, residual clay coatings, and iron-manganese deposits. Materials at interfaces between such environments were also sampled, such as bottom sediments at the silt-water contact, uppermost clay layers at the silt-air interface, uppermost layers of water in pools at the air-water interface, and coatings on the gypsum cave walls. Samples were collected in sterile tubes.

The sampled materials were inoculated in a variety of culture media to investigate the following groups of bacteria: *Thiobacillus*, *Desulfovibrio*, *Clostridium*, *T. denitrificans*, *Pseudomonas dentrificans*, methane-producers, and *T. ferrooxidans*. *Clostridium* and *Thiobacillus* (*T. thiooxidans*, *T. thioparus*, *T. denitrificans*, *T. ferrooxidans*). These were investigated according to the methods of Kramarenko (1983). Laboratory studies were performed by A. N. Shul'ga in the laboratory of biotechnology of the Institute of Physical Chemistry of the National Academy of Sciences, Ukraine. *D. desulfuricans* were cultivated in the Tauson media, *T. ferroxidans* in the Liske media, and methane-producers in the Barker media. The thiobacilli and iron bacteria were cultured in aerobic conditions, but *D. desulfuricans*, *Clostridium*, *P. denitrificans*, and *M. formicicum* were cultured in anaerobic conditions. Sodium thiosulfate served as an energy source for *T. thiooxidans*, and the Moore salt $[Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O]$ was an energy source for *T. ferrooxidans*. For each medium, appropriate pH values were set up with the aid of a sterile solution of H_2SO_4 and a 10% alkali solution. The plates were incubated at 30°C for 20 days. The relative activity of bacteria (a nondimensional index indicating favorability of the cave media for microorganisms' propagation) was estimated according to Kramarenko (1983).

Microorganisms in the Cave

The preliminary studies identified six groups of microorganisms in the cave: the sulfate-reducers (*D. desulfuricans*), denitrificans (*P. denitrificans*), hydrogen-producing (*Clostridium*), thiobacilli *(T. ferrooxidans, T. thiooxidans, T. thioparus, T. denitrificans)*, iron bacteria, and nonidentified fungus-like organisms. Other groups may also be present even though they were not cultured.

Bacteria favor interface environments, such as the surface of the clay fill, gypsum walls (especially those coated with a wet clayey film), the uppermost layer of water in cave pools, as well as the lowermost water layer, together with silt deposits at the bottom (Figure 7). Notable quantities of microorganisms are also found in the wet cave clays and iron–manganese sediments and aggregates. The relative activity of these bacteria is characterized in Table 3.

In the clay fill the most abundant and active bacteria are *Desulfovibrio desulfuricans and Pseudomonas denitrificans*. The latter are also very active in the Fe- and Mn-hydroxide deposits, as well as in the silt in the bottom of cave pools. *Clostridium sp.* are also present in these environments, although they are less active.

Cave walls, either bare or coated with clay, have the most diversified bacterial composition. Here the thiobacilli and the iron-oxidizing bacteria are the most active, as well as (although to a lesser extent) *T. denitrificans and P. dentrificans*. The iron-oxidizing bacteria are active at the water surface in calcite rafts, but *Thiobacillus ferrooxidans* are active on the clay floors of passages. Fe–Mn deposits appear to be exceptionally rich in bacterial life, where a variety of specimens with a high chemical activity were identified. In such deposits almost all the microbial specimens found in the cave are present except *D. desulfuricans*. Iron-oxidizing, as well as unidentified fungus-like organisms, are the most active here and are present in large quantities.

Among the sampled environments, the clay-rich carbonate sediments found at the base of the clay fill (Figure 7) appear to contain the least abundant microorganisms.

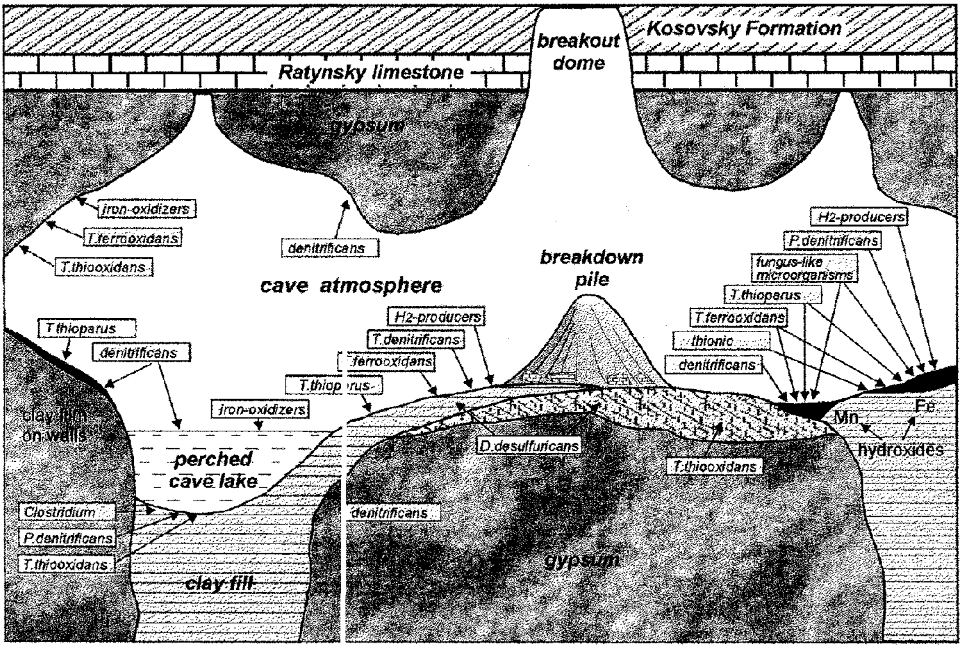

FIGURE 7 Distribution of microorganisms in Zoloushka cave.

Sulfate reducers and denitrificans are most common in environments enriched in organic matter (Tables 2 and 3), which they require for energy and as a carbon source. The cave sediments, the clay fill in particular, contain elevated amounts of organic matter (up to two times more than the clays in the overburden). Organic compounds are also present in the Fe–Mn deposits, especially in the birnessite accumulations (Table 2). The low amount of these bacteria in the (residual?) clay-rich carbonate sediments at the base of the clay fill may relate to the low amount of organic matter in them (see Table 2).

It can also be seen that the iron-oxidizing bacteria and *T. ferrooxidans* are abundant and active in all the contact environments: the surface of the clay fill, the gypsum walls, and the uppermost layer of water in the cave pools.

These preliminary data on the distribution and geochemical activity of microorganisms in Zoloushka Cave suggest that microbial activity was probably one of the most important factors that determined specific geochemical features of the cave. Moreover, these data and the peculiar features of the cave are compatible with regional geomicrobiological and geochemical studies and shed light on the processes operating in the Miocene aquifer system in the western Ukraine. These aspects are briefly discussed in the next section.

Discussion: the Role of Microbial Activity in the Formation of Geochemical Features in Zoloushka Cave and the Miocene Aquifer System

Regional Geochemical Features

The most prominent geochemical features of the Miocene aquifer system in the deep-seated (confined) karst zone are: (1) the almost universal presence of the bioepigenetic calcite bed, enriched in the light carbon isotope, at the top of the gypsum (the "Ratynsky Limestone"), (2) the widespread sulfur mineralization associated with this calcite bed (the region is one of the world's largest sulfur-bearing basins), and (3) high H_2S and CO_2 in the groundwater. It is well known that the Ratynsky Limestone and sulfur deposits in the region are bioepigenetic, formed by replacement of the original gypsum with calcite and sulfur. The replacement of calcium sulfate with calcite and sulfur occurs in a process of redox reactions resulting in reduction of sulfate to sulfide and oxidation of organic carbon to CO_2 and water. In low-temperature diagenetic environments, these reactions are microbially driven, as shown by microbiological and geochemical (particularly isotopic) studies by Davis and Kirkland (1970), Feely and Kulp (1957), Ivanov (1964, 1972), Kirkland and Evans (1980), Machel (1992), Ruckmic, Wimberly, and Edwards (1979), Vinogradov, Grinenko, and Ustinov (1961), and others.

The geochemical features described above occur mainly in the zone immediately adjacent to the Carpathian Foredeep (see Figure 1). This is because: (1) the Miocene aquifer system remains confined in this zone, whereas it is entrenched and drained in part or in whole in the internal sections of the outer platform, and (2) the neighboring foredeep, being a hydrocarbon-bearing basin, is a source of hydrocarbons (that rise at the edge of the platform along deep faults) needed to drive the sulfate reduction.

Sulfate Reduction

The most important geochemical process in the Miocene aquifer system is sulfate reduction driven by microbes, a heterogeneous assemblage of Desulfo-x. The process is summarized by the following reaction:

$$CaSO_4 + 2CH_2O \underset{\text{anaerobic bacteria}}{=======>} CaCO_3 + H_2S + CO_2 \tag{1}$$

where $2CH_2O$ represents a host of possible compounds that can be used as energy sources (including hydrocarbons).

In the confined Miocene aquifer, groundwater in the subgypsum layer is commonly oxidizing. Hydrocarbons (predominantly methane) enter the aquifer along faults from the adjacent foredeep and disperse along various flow paths from the points or lines of input. Near potentiometric lows (see Figure 2) groundwater ascends through fissures and cave systems in the gypsum and gains calcium sulfate. Here the environment becomes reducing, and conditions favorable for bacterially mediated sulfate reduction occur in the gypsum layer, especially in its upper part where replacement of gypsum by calcite takes place.

Sulfate-reducing bacteria are strictly anaerobic and require a reducing environment for growth ($Eh < -100$ mV), either throughout the environment, or in microenvironments that may be present or maintained by the bacteria themselves within an otherwise more oxidizing environment. A microbiological study by Ivanov (1962, 1972) showed that in the sulfur deposits the highest concentrations of sulfate-reducing bacteria (up to 10^5 per liter) were located in groundwater near the contact between the gypsum and the epigenetic calcite, whereas in the lower part of the gypsum the concentration of sulfate reducers was negligible or zero. This is in agreement with the hydrodynamic model employed in this article. The intensity of bacterial sulfate reduction was studied using radioactive sulfate, $^{35}SO_4^{2-}$ (Ivanov 1964). It was found that in areas of intense groundwater circulation and moderate H_2S content (about 50–80 mg/L), the rate of H_2S generation was 3 to 4 mg/L per day. In contrast, in areas with sluggish circulation and high H_2S (about 260 mg/L) its rate of generation was almost 200 times lower.

The above data indicate that for sulfate reduction to occur intensively and continuously it is necessary that H_2S be eliminated from the reaction zone, as its high accumulation makes the environment inappropriate for bacteria growth, and sulfate reduction ceases. In the accepted model, excess H_2S can be eliminated by ascending water flow or by oxidation to elemental sulfur within the upper aquifer. However, Kushnir (1988) hypothesized that anomalous sulfate reduction can be performed by bacteria if environmental conditions deteriorate; at which point sulfate is reduced to thiosulfate ($S_2O_3^{2-}$). In this way, sulfate-reducing bacteria can partially consume low-activity organics, including methane.

Consumption of sulfate in the course of sulfate reduction may serve to restore the solutional capacity of water with respect to gypsum in the upper part of the gypsum strata. Dissolution of gypsum at the top of the gypsum layer is concurrent with precipitation of calcite at the bottom of the overlying Ratynsky Limestone (hydrogenic replacement). The newly formed epigenetic calcite is incorporated into the upper limestone horizon (Klimchouk 1997a). If sulfate reduction proceeds long enough or at a sufficiently high rate, the replacement front propagates downward through the full thickness of the gypsum, removing it entirely.

The chemical environment in the upper aquifer can vary depending on the local relationship between the lateral (oxygen-rich waters coming from the marginal recharge areas) and vertical (waters ascending through the cave systems) flow components. Different processes can predominate in the upper aquifer to form either barren or sulfur–bearing epigenetic calcite (for further discussion of these aspects see Klimchouk 1997a; Kushnir 1988; Machel 1992).

Isotopically light carbon in the Ratynsky Limestone is one of the main points of evidence (along with the isotopic signatures of embedded sulfur) for its bioepigenetic origin. It inherits the isotopic composition of the initial organic compound. In the Pre-Carpathians, the isotopic composition of carbon in the epigenetic limestones of the Tyrassky Formation ($\delta^{13}C = -32$ to -65 %) clearly indicates that the initial organic compound involved in sulfate reduction was methane. This origin is strongly supported by finding light carbon ($\delta^{13}C = -32$ to -42 %) in atmospheric CO_2 in Zoloushka Cave after it had only recently been aerated (Klimchouk and Jablokova 1990; Klimchouk, Jablokova, and Ol'shytynsky 1984 et al.). However, some authors suggest that the sulfate reducers cannot metabolize hydrocarbons such as methane directly but require specific organic compounds, such as organic acids or aldehydes (Kushnir 1988; Machel 1992). If this is the case, methane may be transformed into more simple oxygen-bearing compounds in the aerobic environment of the subgypsum aquifer by fermenting bacteria that are widespread in nature. Moreover, Kushnir (1988) hypothesized that the transformation of organic matter can take place in anaerobic environments by methanogens that co-exist with the sulfate-reducers and provide nutrients for them. The latter hypothesis is supported by recent findings in marine envronment (Boetius et al. 2000, p. 623; Hinrichs, Hayes, Sylva, Brewer, and DeLong 1999, p. 802).

The high CO_2 in Zoloushka Cave and its light carbon isotopic signature strongly suggest that sulfate reduction still occurs in the cave environment, in the lower part of the gypsum, and within the wet clay fill. The range of the $\delta^{13}C$ values of the cave CO_2, measured in the present transitional (unconfined) stage, is somewhat heavier than the range of the epigenetic calcite. This can be explained by partial inclusion in sulfate reduction of organic matter recently introduced from the surface via collapse structures formed during the early unconfined stage. Moreover, CO_2 generated in the transitional stage by *T. denitrificans* and *P. denitrificans*, which utilize "surface" organics, can also contribute heavy carbon. This is also supported by the relative enrichment of the cave air by nitrogen (up to 79.6–83.1 vol%) that is one of the products of the biochemical reactions mediated by the previously noted bacteria:

$$\textit{T. denitrificans}: 5S + 6KNO_3 + 4NaHCO_3 = 3K_2SO_4 + 2Na_2SO_4 + 4CO_2 + 3N_2 + 2H_2O \quad (2)$$

$$\textit{P. denitrificans}: 4KNO_3 + 5CH_2O \rightarrow 2K_2CO_3 + 3CO_2 + 5H_2O + N_2 \quad (3)$$

However, the lightest $\delta^{13}C$ values suggest that some sulfate reduction still use extremely light carbon.

In the prequarrying period in the Zoloushka Cave area the concentration of H_2S in groundwater in the gypsum was around 90–150 mg/L. With progressive dewatering and aeration of the cave system, the level of H_2S decreased and reducing conditions were increasingly replaced by oxidizing conditions. At the end of the 1960s—beginning of the 1970s, when most of the cave system was drained, H_2S in the groundwater decreased to 4.2–10.2 mg/L. H_2S concentrations of 2.0–3.7 mg/L were measured in the degrading cave pools in the winter of 1981. Five years later, concentrations were 0.1–1.3 mg/L. In the beginning of the 1990s, H_2S was detected only in silt on the bottom of cave pools.

The Transitional Geochemical Environment and the Formation of Fe–Mn Hydroxides

It is apparent that the unusual deposits of Fe–Mn hydroxides owe their origin to the transitional (from the confined to the unconfined) stage of the cave's history, which was greatly accelerated by the quarry operations. The abundance of microorganisms in these deposits, particularly iron bacteria *T. ferrooxidans* and nonidentified fungus-like microorganisms, leads us to assume that their origin is closely related to microbial activity. The preliminary character of our study does not allow conclusive judgments about mechanisms and the degree of bacterial involvement. However, it can be suggested that the transitional geochemical evolution brought about a burst of geomicrobiological activity in the cave.

During the confined stage, a reducing environment prevailed in the gypsum aquifer, favoring intense sulfate reduction, dissolution of manganese, and alteration of active forms of iron to sulfides. It is likely that the formation of pyrite (FeS_2) and hydrotroillite ($FeHS \cdot nH_2O$) in the silty cave sediments took place during this stage. During the last phase of natural development (between 10,000 and 50 years ago) the aquifer in the Zoloushka Cave area was unconfined and the water table stood some 2–3 m below the top of the gypsum. Organics from the surface started to come into the cave with descending percolation via breakdown structures, although oxygen inflow was still low due to the absence of direct connections with the surface.

Dozens of boreholes drilled in the cave area during gypsum exploration have allowed oxygen into the cave atmosphere. This provoked abrupt activation of thiobacilli and oxidation of H_2S and sulfur:

$$\textit{T. thioparus}: 2H_2S + O_2 = 2H_2O + 2S^0 \tag{4}$$

$$\textit{T. thiooxidans}: S + H_2O + 1.5O_2 = H_2SO_4 \tag{5}$$

The activity of bacteria *T. ferrooxidans* contributed to oxidation of ferrous (II) iron to ferric (III) iron and accumulation of the latter in the groundwater:

$$4FeSO_4 + 2H_2SO_4 + O_2 \rightarrow 2Fe_2(SO_4)_3 + 2H_2O, SO_4^{2-}, Fe^{2+} \tag{6}$$

Oxidation of H_2S resulted in increased acidity and separation of the cave reservoir into two different redox zones: (1) the thinner upper zone, neutral or slightly alkaline, distinctly oxidizing, and (2) the thicker lower zone, more acidic, reducing to gley. Reducing conditions increasingly changed to gley conditions in this zone with increased H_2S consumption.

These two zones formed paragenetic environments for Fe–Mn lithogenesis. In the slightly acidic lower zone, Mn and Fe retained mobility and accumulated in the water. Conditions favorable for Fe and Mn deposition prevailed in the upper hydrogeochemical zone. Hydroxides were deposited here by either inorganic chemical mechanisms or with bacterial mediation. Aerobic neutral and slightly acidic conditions are favorable for biogenic oxidation of Fe and Mn. These processes, however, were not intense as most of the Fe and Mn remained in solution in the lower zone.

The opening of the cave system by the quarry and massive groundwater withdrawal have generally led to lowering of the phreatic zone and increased oxidation in an expanded upper zone leading to the eventual disappearance of the lower reducing zone. The gley environment and then the oxidizing environment have increasingly replaced the reducing environment, the latter being preserved only in the bottom layer of isolated cave pools and in fluid in the silt deposits. Neutralization of waters, occurring along with oxidation processes, has resulted in massive deposition of Fe and Mn (which were previously in solution due to acidity) in the form of hydroxides.

It is likely that autocatalytic Fe and Mn oxidation proceeded with active participation of manganese- and iron-precipitating bacteria. Besides all the necessary geochemical prerequisites, evidence for biological precipitation of hydroxides includes the abundance of various bacteria in iron–manganese deposits and enrichment of birnessite and asbolane–buserite with organic matter. The predominantly separate occurrence of Fe versus Mn hydroxides suggests that they were precipitated by geochemically specific species of microorganisms.

It is still difficult to judge the role of bacterial precipitation in the formation of various speleothem morphologies, especially stalagmites composed of iron hydroxides. It can be assumed, however, that this role was crucial, because "traditional" abiotic mechanisms do not produce these shapes.

The irregularity in the distribution of Fe- and Mn-hydroxides in the cave, and their localization in certain parts of the labyrinth, support a conclusion that the bulk of these sediments was "dumped" by groundwater during the transitional stage when the water table was lowered. In the course of fluctuations of the depressed piezometric surface due to seasonal variations or technological reasons (e.g., pump damage), sediments were repeatedly redeposited: they were transported as subcolloidal suspensions through the cave, precipitated as black or red wall deposits and eventually accumulated on the cave floor around points where water drained to lower levels.

Conclusions

During the last 50 years, Zoloushka Cave and the local Miocene aquifer have experienced a strong anthropogenic impact from the open quarrying and associated massive groundwater withdrawal. This stage is marked by the considerable role of microorganisms in the formation of the hydrogeochemistry of the cave environment, gas composition of the cave air, and some types of cave sediments and deposits. Preliminary studies have revealed the presence of six groups of microorganisms in the cave: the sulfate-reducers (*D. desulfuricans*), denitrificans (*P. denitrificans*), hydrogen-producing (*Clostridium*), thiobacilli (*T. ferrooxidans*, *T. thioxidans*, *T. thioparus*, *T. denitrificans*), iron bacteria, and unidentified fungus-like organisms.

During the confined stage a reducing environment prevailed in the gypsum aquifer, favoring sulfate-reduction (with intense H_2S generation and the formation of bioepigenetic calcite at the top of the gypsum), dissolution of manganese, and the conversion of active forms of iron to sulfides.

During the last phase of the natural development (10,000–50 years) the aquifer in the Zoloushka Cave area was unconfined and the water table was some 2–3 m below the top of gypsum. The present transitional hydrogeochemical stage was induced by the quarrying and associated groundwater abstraction. It is characterized by lowering of the water table, the general intensification of groundwater circulation, recharge from the perched (on the clay sequence) aquifer, as well as the surface recharge via breakdown structures, and inflow of surface organics and atmospheric oxygen. Reducing conditions increasingly changed through gley to oxidizing conditions, being preserved only in the lower section of the aquifer, the bottom layer of perched cave pools, and in moisture in the silt deposits. Sulfate-reduction processes still occur there, evidenced by the light carbon isotopic signatures in CO_2 of the present cave air and by the presence and relatively high activity of *D. desulfuricans*.

Neutralization of water during the anthropogenic transitional stage, as well as oxidation, has resulted in massive deposition of Fe- and Mn-hydroxides. Although Fe- and Mn-hydroxides in the cave may have a mixed inorganic/bacterial origin, we assume that bacterial precipitation has played a major role. Besides all the necessary geochemical prerequisites, evidence for this includes the abundance of various bacteria in iron–manganese deposits, enrichment of birnessite and asbolane–buserite with organic matter, and the specific distribution and morphology of the deposits.

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