doi:10.17746/1563-0110.2024.52.1.035-046

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The Origin of Biogenic Horizons in the Pleistocene Strata of Denisova Cave: Mineralogical and Geochemical Markers Help to Reconstruct the Sources of Matter

We outline the results of mineralogical and geochemical analyses of Middle Pleistocene sediments of layer 21 in the Main Chamber of Denisova Cave, Altai. The aim of the study was to reveal a set of mineralogical and trace element markers of the black-colored horizons or lenses and to distinguish them from other types of cave sediments. Results were matched with those relating to a similar set of markers of black-colored horizons in the Holocene part of the section in the East Chamber. Results indicate probable sources of organic and organogenic substances in layer 21. The preservation of geochemical marks was assessed for Pleistocene in comparison with Holocene strata, where those markers are distinct. Black-colored lenses in layer 21 resemble biogenic sediments from Holocene section of the East Chamber. Both layers are characterized by high contents of N-bearing organic matter, P, Zn, Cu, and Cd. In bulk samples from Holocene sediments, numerous fragments of chitin (insect exoskeletons) and patches of newly formed Ca and Ca-Mg phosphates were found. We conclude that these peculiar lenses consist mostly of guano from insectivorous bats, and had undergone deep biodegradation. All black-colored horizons and lenses studied in Denisova Cave have a similar set of geochemical markers and distinctly differ from the adjacent strata by their phase, macro- and trace element compositions.

Keywords: *Denisova Cave, Pleistocene, Holocene, mineralogical and geochemical markers, guano of insectivorous bats, biodegradation.*

Introduction

Paleolithic sites in rock shelters are among the most informative sources for studying ancient cultures of prehistoric man and his environment. As a rule, caves containing archaeological material are multi-layered and reveal complex stratigraphy of loose sediments. Artifacts and other evidence of anthropogenic activity are often embedded in the entire sedimentary sequence filling the karst cavity. The low rate of cave sedimentation promotes merging of different habitation horizons. Distribution of cave facies exhibits high variability, and stratigraphy is extra puzzled by the alternation and mutual penetration of different horizons. Sedimentation in various parts of a cave is controlled by the shape and size of the cavity and its location in the karst system. Post-depositional alteration of the sediments are under biological control. It is important to reveal stratigraphic markers for identification and correlation of cave sedimentary horizons.

Cave deposits bearing artifacts (cultural members) have been targeted by numerous studies realized by means of natural sciences' methods and approaches. This strategy provides good results in Denisova Cave (Altai), which is a key site in the studies of the prehistory of North and Central Asia.

Studies of culture-bearing cave deposits are a remarkable example of an interdisciplinary research. A new stage in the study of cave sedimentation and post-depositional alteration is connected with the discovery of diverse phosphate mineralization in the Holocene and Late Pleistocene sediments of the East Chamber and the reconstruction of their formation conditions (Shunkov et al., 2018; Sokol et al., 2022). Two main biogenic sources responsible for abnormally high phosphorus levels in the cave sediments (up to 33 wt% P_2O_5) have been identified: these are guano of insectivorous bats and bone remains. At certain stratigraphic levels of reference sections of Denisova Cave, phosphorus-rich sediments are accompanied by thin black or dark-brown horizons enriched in organic matter ($C_{org.}$ up to 32 wt%). Their specific appearance and composition allow one to use them as marker horizons for correlating cave sections. To prove the relevance of this approach, we should characterize these sediments, determine the ranges of the content of major and trace elements, and reconstruct the origins of each horizon in the excavated sections of Denisova Cave. Despite a similar appearance, these horizons could have different sources of organic matter (guano, charcoal, plant detritus, etc.).

The purpose of this study is to identify a set of characteristic mineralogical and geochemical features of the black-colored horizons, which distinguish them from all other types of sediments in Denisova Cave. Earlier, in the section of the East Chamber, the following strata were identified: those enriched with siliciclastic material (mainly clayey and sandy), proto-horizons of guano of insectivorous bats, chemogenic sediments that emerged in zones of intense phosphate leakage (caused by the biodegradation of organic component of guano), and layers with a notable amount of limestone' debris (Sokol et al., 2022). The mineralogical and geochemical characteristics of black-colored lenses from the base of layer 21.2 in the Main Chamber (Fig. 1) were established for the first time and compared with those of similar horizons in the Holocene part of the East Chamber section (Ibid.). This set of data allowed one to reconstruct depositional conditions and the most probable sources of organic and organogenic substances in the subunits of layer 21. The objectives of the work are also to estimate the states of preservation of geochemical marks in Pleistocene sediments and to compare them with Holocene ones, where these markers are obvious.

Materials and analytical techniques of studying cave sediments

The analyzed sedimentary column of the East Chamber is located in its entry zone, where the intensity and depth of penetration of phosphate solutions triggering the diagenetic alteration of primary sediments reach maximal values. Sediment samples were collected from layers 6–11.3, including two proto-horizons of insectivorous bat guano with relatively constant thickness—in layers 6 and 8. Cave deposits in the Main Chamber were collected from the column of layers 14–22.3.

The soil profile was sampled at the Ust-Karakol site, 1.8 km southeast of Denisova Cave. Six horizons of various colors in the section of the Late Pleistocene–Holocene sediments with a total thickness of 1.5 m were sampled. Samples were dried at 30 °C and then stored in air- and water-proof plastic bags.

The analytical work was carried out mainly at the Analytical Center for Multi-Elemental and Isotope Research (Sobolev Institute of Geology and Mineralogy (IGM), Novosibirsk, Russia). Identification and analysis of minerals, as well as recognition of organic matter, were carried out by the scanning electron microscopy (SEM) technique on a MIRA3-LMU scanning electron microscope (TESCAN ORSAY Holding) with an AZtec Energy Xmax-50+ microanalysis system (analysts M.V. Khlestov and V.A. Danilovskaya). The content of major elements was determined by the atomic emission technique on an atomic emission spectrometer with IRIS Advantage inductively coupled plasma (analyst N.G. Karmanova). Quantitative X-ray phase analysis of the sediments was carried out on a SHIMADZU XRD-6000 diffractometer (CuKα radiation with a graphite

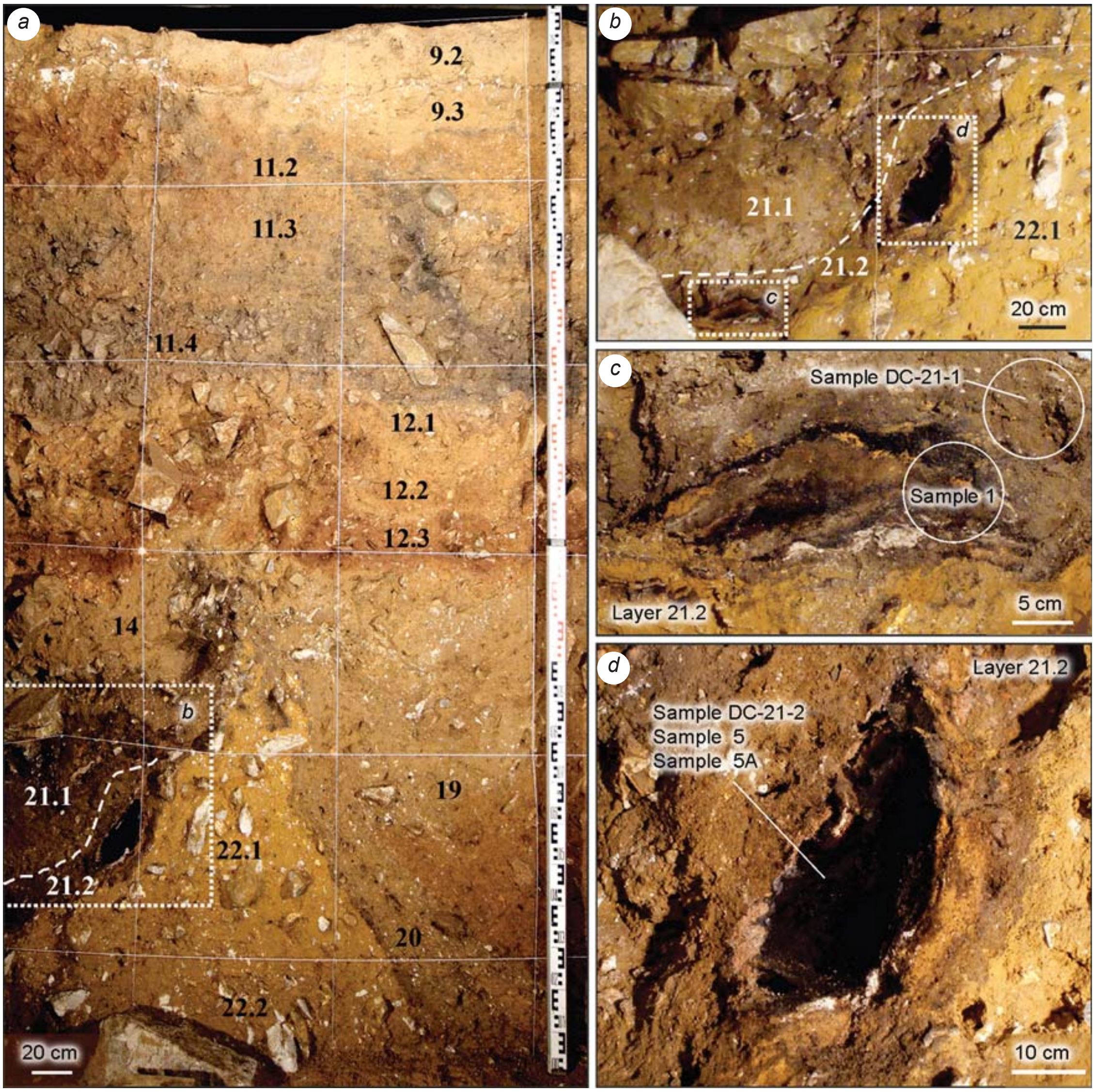


Fig. 1. Profile view of excavated Pleistocene sedimentary sequence (*a*) and black-colored lenses at the base of layer 21.2 with sampling locations (*b–d*) in the Main Chamber of Denisova Cave.

monochromator) at the South Ural Research Center of Mineralogy and Geoecology (SU FRC MG, Miass, Russia). SIROQUANT V.4 software was used to calculate the proportions of minerals.

The trace element composition of sediments was determined by inductively coupled plasma mass spectrometry (ICP-MS), using an Agilent 7700x spectrometer at the South Urals Federal Research Center of Mineralogy and Geoecology UB RAS, and a NexION 300S quadrupole inductively coupled plasma mass spectrometer (Perkin Elmer; analyst D.A. Kiseleva) at the Center for Collective Use "Geoanalyst" of the Institute of Geology and Geochemistry UB RAS. Elemental analysis (C, N, H, S) of organic matter was carried out at the Novosibirsk Institute of Organic Chemistry SB RAS using an EURO EA 3000 automatic CHNS analyzer according to the procedure (Fadeeva, Tikhova, Nikulicheva, 2008). For analytical details, see (Sokol et al., 2022).

Material characteristics of sediments of black-colored lenses from layer 21.2

In the East Chamber, thin black and brown horizons, which replaced highly degraded guano layers, are best preserved in the Holocene part of the section, on top of layers 6 and 8.

These horizons are contrast to adjacent layers in phase, macro- and microelement compositions: they are highly enriched in C, N, P, Zn, and Cu, depleted in silicate matter (Si, Ti, Al, Mg, K, Na), and contain numerous fragments of the chitin exoskeleton of insects (Ibid.). These features are best pronounced in the black-colored horizon on top of layer 8 (sample 10, which was used in this work as a reference one). In the Main Chamber, black-colored lenses of similar appearance are located at the base of the Pleistocene sequence—layer 21.2 (Fig. 1). High content of $C_{org.}$ was registered in these sediments by Nikolaev (1994), who assumed that the main source of organic matter was aerophilic lower plants.

The Pleistocene sequence in the Main Chamber consists of three units. The lower part of the section, including sublayers of layer 22 up to 2 m thick, is composed of heavy reddish-yellow to dull light yellow loams. The top of the layer has the OSL-date of 287 ± 41 ka BP (Jacobs et al., 2019). The medial part (layers 21–11 up to 2 m thick) is composed of multicolored loams with abundant fragments of limestone. These sediments were deposited after a prolonged hiatus. The medial layers are separated from layer 22 by a distinct horizon of dark-colored loam—layer 21 with a variable content of dispersed carbonaceous matter and black-colored lenses at the base, where the content of $C_{org.}$ reaches 32.3 wt% (absolute maximum for the sediments in Denisova Cave, Fig. 1, *b–d*, Table 1). The OSL-age of layer 21 is estimated as 250 ± 44 ka BP (Ibid.). The upper part of the section is layer 9 composed of light loess-like loams up to 0.5 m thick.

Layer 21.2, of varying thickness, is subdivided by color into brown and black horizons. According to XRD and SEM analyses, its composition is dominated by

Table 1. **Chemical composition of Pleistocene (layers 9–11.3) sediments in the East Chamber, Pleistocene (layers 14–22.3) and Holocene (layers 6–8) sediments in the Main Chamber in Devisova Cave, wt%**

Layer	Sample	SiO_2	TiO_2	Al_2O_3	Fe_2O_3	MnO	MgO	CaO	Na_2O	K_2O	P_2O_5	SO_3	LOI	TOTAL	$C_{org.}$	$N_{org.}$
East Chamber																
6	15*	4.34	0.03	0.87	0.39	0.09	0.44	29.22	0.07	0.51	32.31	1.77	29.93	99.97	7.68	2.17
7	12**	7.28	0.13	1.91	0.88	0.05	0.85	37.55	0.10	0.87	26.53	4.76	19.05	99.96	0.69	<0.3
7	11	15.83	0.22	4.38	1.67	0.05	0.72	34.23	0.32	1.37	33.27	1.14	6.76	99.96	0.81	0.33
8	10*	8.17	0.11	1.93	0.89	0.02	0.34	21.66	0.12	0.53	12.94	2.14	51.04	99.89	27.84	7.88
8	9**	41.45	0.59	9.82	4.07	0.15	0.39	12.12	0.72	2.53	10.92	0.55	16.59	99.91	4.77	0.95
9	7	50.74	0.64	12.12	4.81	0.03	0.16	4.38	0.72	5.28	10.89	0.15	10.19	100.10	1.09	<0.3
9	6	21.20	0.33	10.21	4.59	0.03	0.05	7.57	0.47	5.62	31.99	0.13	17.72	99.89	1.79	1.52
9	4	35.75	0.48	9.13	3.56	0.18	0.35	19.13	0.59	2.10	16.41	0.45	11.82	99.96	1.83	0.42
11.1	3	25.71	0.36	7.26	2.67	0.14	0.06	27.55	0.39	1.54	19.32	0.92	13.82	99.74	2.49	0.52
11.2	2	31.87	0.43	8.30	2.88	0.08	0.87	25.77	0.42	1.54	12.49	0.73	14.59	99.96	3.43	0.68
11.2	1	33.96	0.43	7.88	3.64	0.06	1.01	24.29	0.40	1.79	11.29	0.92	14.33	99.97	3.50	0.68
11.3	1	23.76	0.33	5.89	2.68	0.06	1.10	27.32	0.79	1.17	7.79	0.88	27.67	99.45	12.07	1.16
11.3	2	24.01	0.33	6.06	2.89	0.06	1.03	30.81	0.88	1.13	11.80	2.57	17.85	99.46	5.43	0.59
Main Chamber																
14	1	34.67	0.46	8.31	3.77	0.09	1.48	24.95	1.05	1.76	6.73	0.95	14.96	99.20	–	–
19	2	35.35	0.48	8.79	3.98	0.08	1.66	22.50	1.07	1.76	6.17	1.33	16.21	99.38	3.97	0.38
20	2	30.51	0.43	7.73	3.48	0.06	1.40	28.87	0.75	1.51	2..44	0.23	22.34	99.74	2.31	0.41
21.1	1	39.42	0.55	9.71	4.30	0.08	1.65	20.17	1.04	1.94	4.49	0.88	15.03	99.25	2.54	0.34
21.2	DC-21-1**	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
21.2	1**	20.04	0.25	4.59	2.18	0.08	1.15	29.31	0.80	1.27	17.17	1.56	21.42	99.82	8.29	1.80
21.2	DC-21-2*	6.91	0.08	1.44	0.78	0.10	0.73	23.85	0.59	0.49	12.31	1.18	50.03	98.47	32.27	6.34
21.2	5*	9.64	0.12	2.30	1.17	0.09	1.08	29.42	0.80	0.69	17.23	1.05	35.94	99.52	–	–
21.2	5A*	14.28	0.18	3.36	1.67	0.09	1.40	32.64	0.94	0.99	19.84	1.02	22.87	99.28	–	–
22.1	3	43.42	0.64	12.23	5.63	0.10	1.66	16.11	0.48	2.20	2.03	0.86	14.73	100.10	2.95	<0.3
22.2	3	52.34	0.81	15.03	6.94	0.13	1.85	7.34	0.25	2.70	1.85	0.65	9.33	99.23	1.95	<0.3
22.3	1	56.57	0.82	15.03	6.79	0.17	1.83	5.56	0.19	2.77	1.42	0.49	8.09	99.73	0.86	<0.3

*Black-colored horizons with high content of organic matter.

**Brown horizons containing organic matter.

dispersed organic matter and Ca phosphates of different degrees of crystallinity (the total content of X-ray amorphous matter reaches 40 %), while the amount of layered silicates and quartz does not exceed 7 % and 4 % of the total content of crystalline compounds, respectively.

In black lenses of layer 21.2, bulk contents of $C_{org.}$ and $N_{org.}$ reach 32.3 wt% and 6.3 wt%, respectively (sample 21-2). Small lenses of compressed organic matter, which are Zn- and N-rich (N – up to 4 wt% and Zn – 2000–3700 ppm), are disseminated in the finely dispersed organic matter (Fig. 2). Numerous fragments of insect exoskeletons were found in the black-colored lenses. Chitin particles are usually compressed, and their surface relief is smoothed or totally obliterated, in contrast to similar material from the black-colored horizons of the Holocene part of the section in the East Chamber (Fig. 2, *b–f*). However, in the older sediments, chitin also kept geochemical markers (enrichment in nitrogen and zinc) typical of the samples of younger cave horizons and modern insects (Forest insects…, 2010; Wurster et al., 2015). Plant detritus was not found via detailed SEM examination of the black-colored lens, which refutes the previously assumption that plants were the source of $C_{org.}$ in these sediments (Nikolaev, 1994).

The black-colored sediment of layer 21.2 mainly consists of organic and biogenic materials, with minor admixture of clayey and sandy components. In contrast to the Holocene sediments from the East Chamber section with abundant crystals of Ca, Mg, Fe, Al, K phosphates (Shunkov et al., 2018; Sokol et al., 2022), the sediments of layer 21.2 are dominated by Ca and Ca-Mg phosphates (crystalline and semi-amorphous) and partially preserved bone detritus. Newly formed Ca phosphates occurred as clots, flakes, and biomorphic forms (Fig. 3). The dominant carbonate-bearing apatite is the so-called dahllite ($Ca_5(PO_4,CO_3)_3(OH)$), whose amount exceeds 40 % and approaches its absolute maximum revealed in the black-colored horizon of the East Chamber (layer 8, sample 10, ~50 % dahllite). The low degree of crystallinity of such apatite is confirmed by IR spectroscopic and X-ray examination (Fig. 4). The surface of chitin fragments and plates of compressed organic matter is sometimes encrusted with tiny crystallites of apatite and whitlockite (up to 0.5–1.0 μm) (see Fig. 2, *a*, *d*). Bone detritus, often corroded, probably by organic acids was also revealed in black-colored lenses. Recrystallization of bone apatite has occasionally occurred (Fig. 5, *b*). The content of calcite in bulk samples of the sediments of layer 21.2 does not

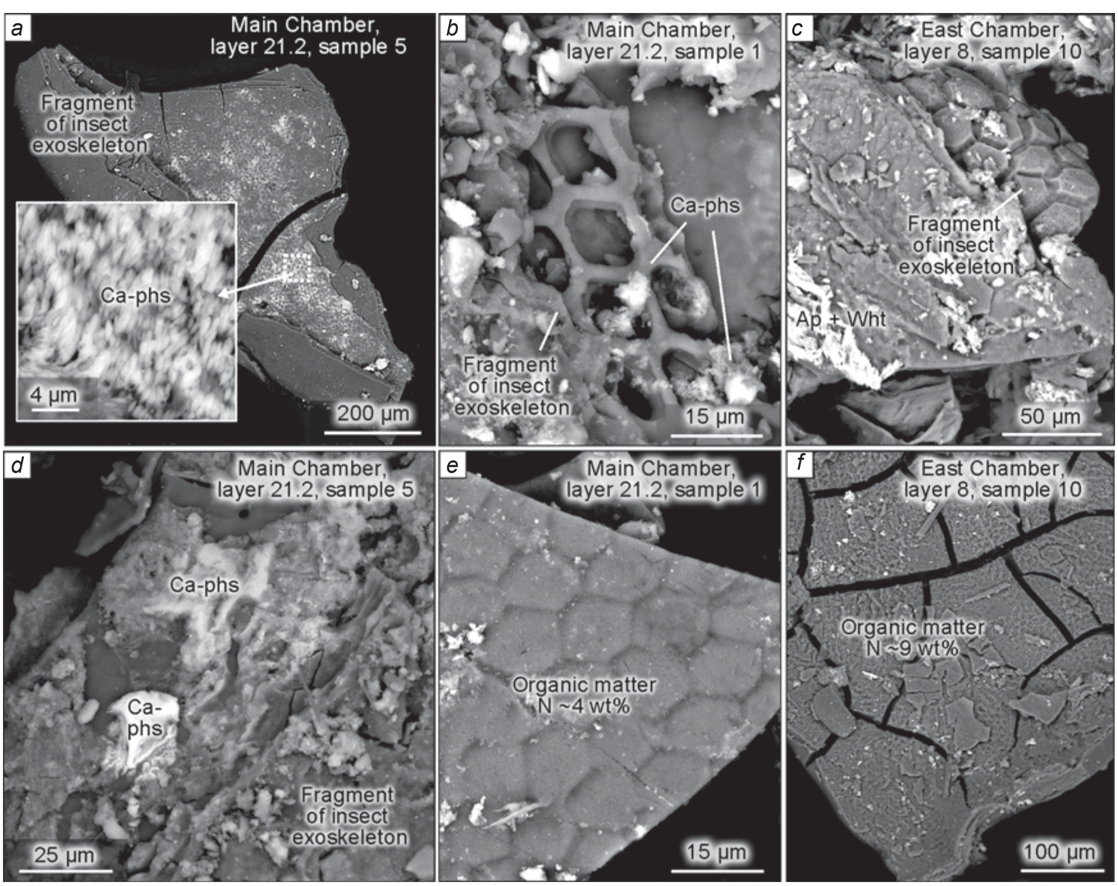


Fig. 2. Morphological diversity of organic remains from black-colored horizons of the sedimentary sequence of the Main Chamber (*a*, *b*, *d*, *e*) and the East Chamber (*c*, *f*) of Denisova Cave. Back-scattered electron (BSE) images.

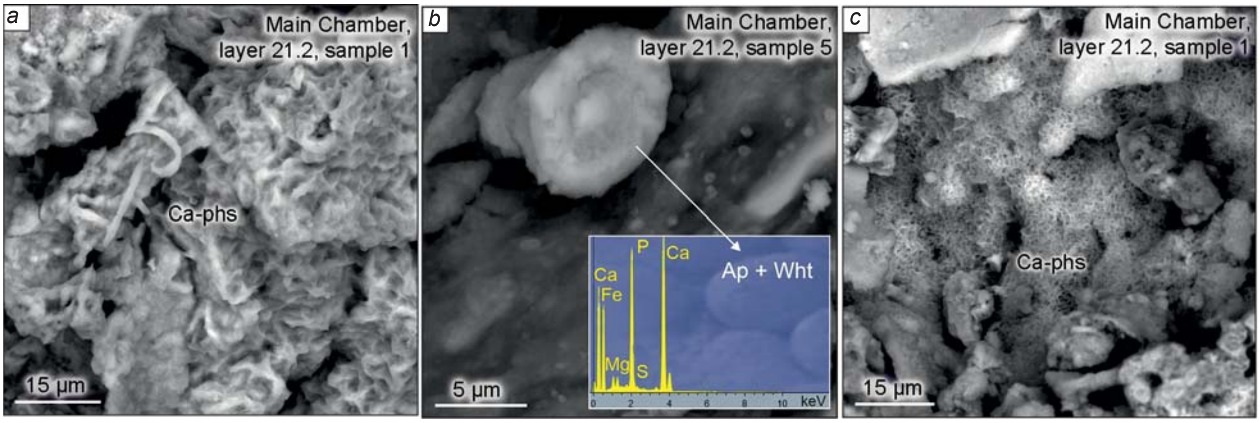
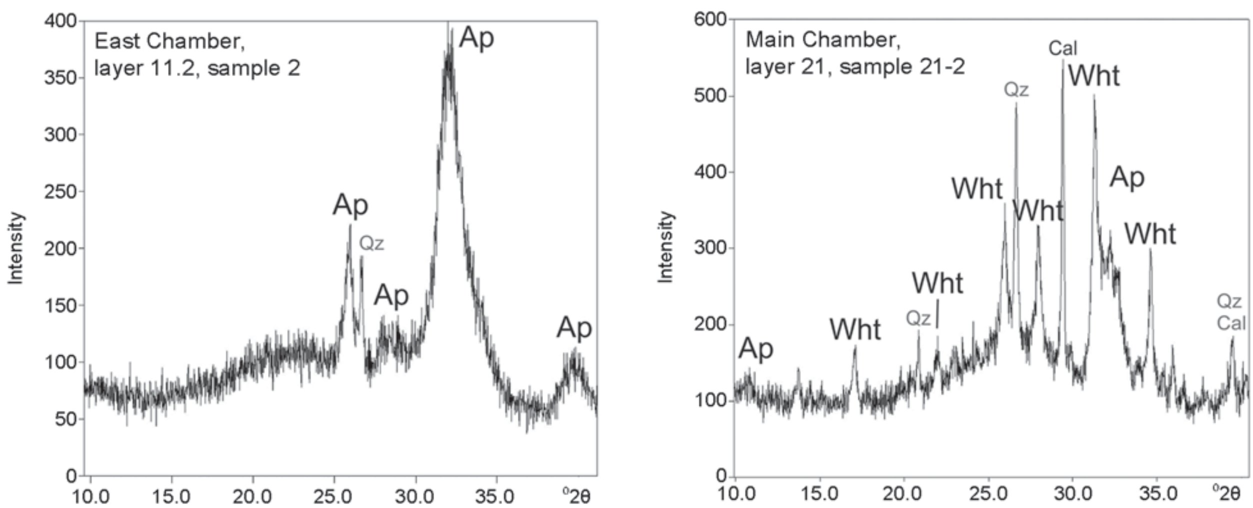


Fig. 3. Biomorphic segregation of Ca and Ca-Mg phosphates of low crystallinity (Ca-phs). Back-scattered electron (BSE) images. Ap – carbonate-bearing apatite – dahllite, Wht – whitlockite.



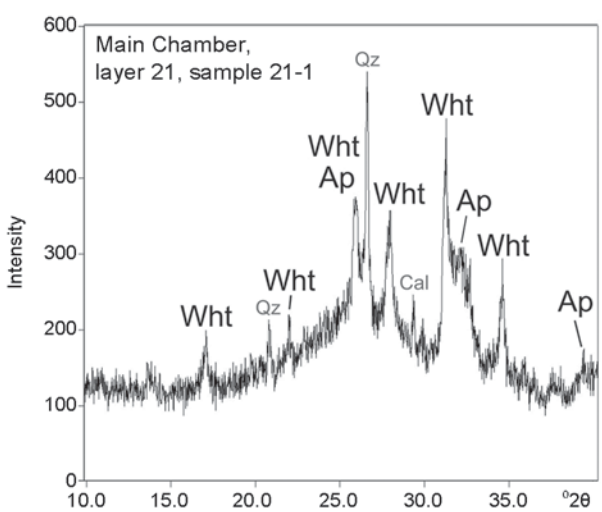


Fig. 4. Fragments of X-ray diffraction patterns of Ca and Ca-Mg phosphates of low crystallinity. The diffraction patterns show sharp peaks of well-crystallized whitlockite (Wht) and diffuse reflections of apatite (Ap). An intense halo indicates the predominance of the X-ray amorphous component. Cal – calcite, Qz – quartz.

exceed 6 %. The preserved small fragments of limestone are corroded and partially replaced by Ca phosphates.

Another horizon of layer 21.2 (sample 21-1) varies in thickness and consists of brown sandy loam with numerous whitish clots of Ca phosphates, and small fragments of partially replaced limestone and newly formed micritic calcite (Fig. 5, *c*). The sediment contains abundant bone detritus, which is largely avoided chemical etching and recrystallization. The surface of bone fragments are sometimes encrusted with dendrites of Mn (oxy)hydroxides. Infrared spectroscopy indicates that these sediments, as well as the material of the black lenses (sample 21-2), contain complex organic compounds, including nitrogen-bearing ones, which are most likely the products of destruction of the original protein compounds.

In the brown sediments of layer 21.2, single fragments of charcoal (up to 4 mm in size) with well-preserved tissue structures were found along with N-bearing structureless organic matter (Fig. 5, *a*). Owing to the fragility of charcoal under mechanical stress, it was most likely crushed to powder and dispersed in the host sediment;

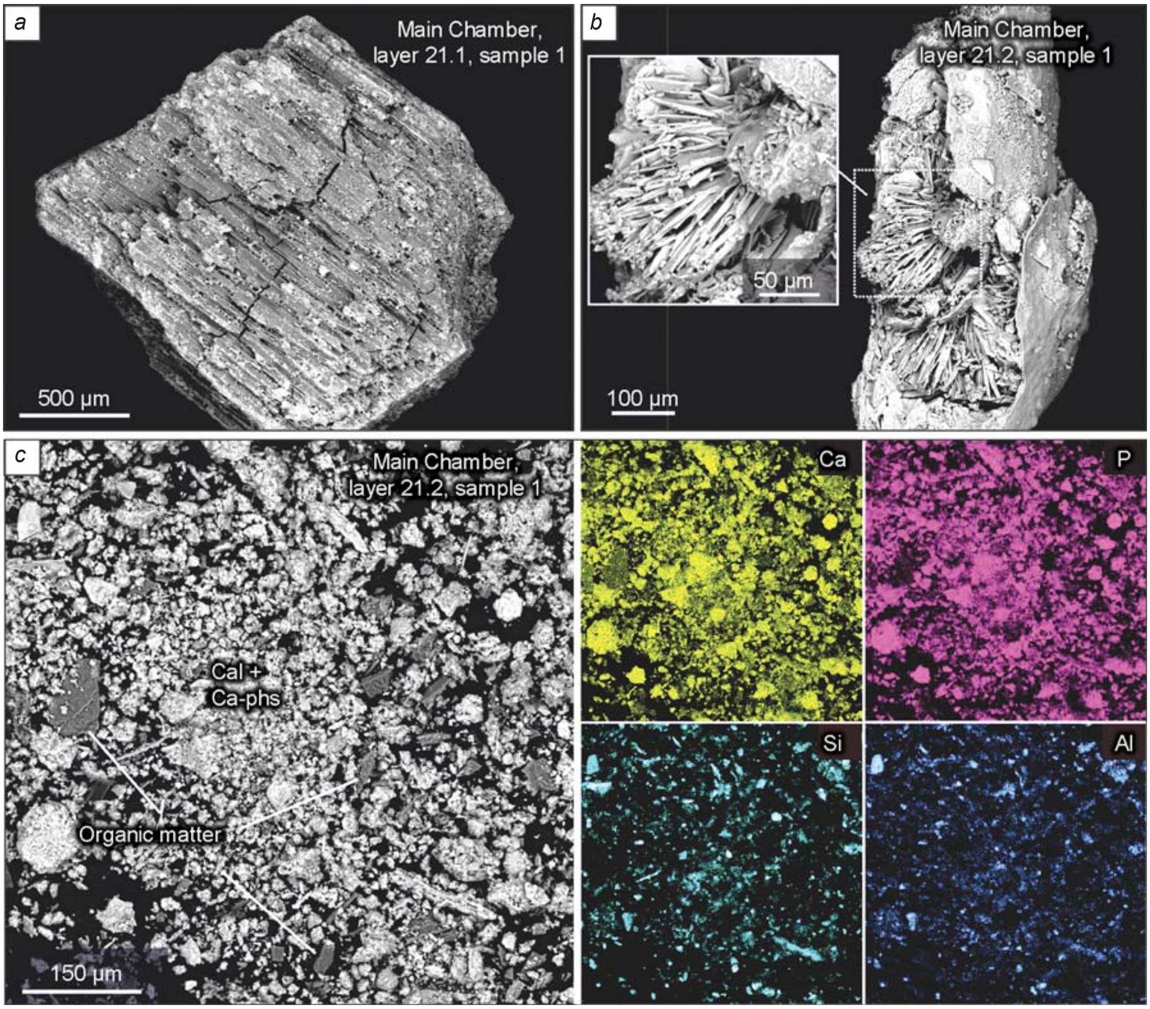


Fig. 5. Samples from layer 21 of the Main Chamber of Denisova Cave.
a – fragment of charcoal from layer 21.1; *b* – recrystallized bone fragment from layer 21.2; *c* – the ratio of organic matter, Ca phosphates, and siliciclastic material in the bulk sample of the brown horizon of layer 21.2. Images in back-scattered electrons (BSE) and element (Ca, P, Si, Al) maps.

therefore, its larger fragments have been rarely found. However, the contribution of this component to the total $C_{org.}$ budget of layer 21.2 is beyond doubt and should be taken into account in the future.

The overlying sediments of layer 21.1 (sample 1) contain ordinary concentrations of both $C_{org.}$ (2.5 wt%) and N (0.3 wt%), which are also typical of light-colored and organic-poor sediments of the Denisova Cave sedimentary sequence (Table 1).

Chemical composition of layer 21 in the Main Chamber and layer 8 in the East Chamber of Denisova Cave, comparatively

Comparison of major and trace element composition of the sediments of the upper part (layers 6–11.3) of the East Chamber sequence and lower and middle parts of the Main Chamber sequence (layers 14–22.3) (Table 1, 2) shows that reference sample 10 from layer 8 in the East Chamber, representing the bat-guano protohorizon, has unique chemical composition. It is characterized by the highest value of loss on ignition (LOI) – 50.5 wt%, which is mainly ensured by nitrogen-rich organic matter (in wt%: C – 27.9; N – 7.9; H – 1.9; S – 0.4); low content of all major elements connected with the sandy-clayey component of the sediment (SiO_2, TiO_2, Al_2O_3, Fe_2O_3, Na_2O, K_2O); moderate amount of phosphorus (~13 wt% P_2O_5); and abnormally high concentrations of essential trace elements (Zn, Cu, and Ni) involved in the metabolism of mammals.

The matter of black-colored lenses from layer 21.2 of the Main Chamber is generally similar to the one described above. The difference in the contents of major components (SiO_2, Al_2O_3, Fe_2O_3, K_2O, P_2O_5), as well as the LOI value in the compared sediments, does not exceed 20 rel%, and that of the concentrations of minor components (TiO_2, MnO, MgO, Na_2O, SO_3), 50 rel%. Contents of both $C_{org.}$ (27.8 and 32.3 wt%) and $N_{org.}$ (7.9 and 6.3 wt%) in the black-colored horizons and lenses are the largest and an order of magnitude higher than those in other types of sediments in Denisova Cave—sandy-clayey, calcareous, and phosphate (Sokol et al., 2022). The amounts of zinc and copper, the accumulation of which in cave sediments is connected with insect remains (proteins and chitin) (Wurster et al., 2015), reach their maximum values in these layers (~1000–3000 ppm Zn and ~200–1800 ppm Cu) (Table 2). On the contrary, concentrations of microelements connected

Table 2. **Concentrations of trace elements in bulk sediment samples from the Main Chamber and East Chamber of Denisova Cave, and from the soil profile at Ust-Karakol, ppm**

Layer	Sample	Zn	Cd	Cu	Ni	Co	Mo	U	Sc	Ga	Zr	Nb
Denisova East Chamber												
6	15*	822	1.43	235	28.3	7.40	5.76	1.36	7.57	6.33	45.8	5.43
7	12**	2553	1.68	507	7.43	1.94	11.5	0.37	2.48	2.34	15.7	1.78
7	11	3189	2.55	497	12.9	3.31	14.8	0.75	4.17	4.30	18.3	3.81
8	10*	3030	6.85	1837	204	29.5	6.54	1.64	2.05	2.22	8.64	1.61
8	9**	835	1.48	235	93.6	25.9	15.3	2.08	11.4	11.0	88.7	7.69
9	7	579	0.54	54.8	27.9	8.29	7.61	2.49	15.0	13.5	102	10.6
9	4	663	0.79	83.1	39.3	11.1	13.3	1.64	9.12	9.17	43.9	7.04
11.1	3	239	0.51	53.8	26.4	8.28	15.1	1.14	2.06	8.87	4.93	4.37
11.2	2	257	0.39	44.2	25.6	8.43	5.44	1.13	8.94	7.96	28.7	1.71
11.2	1	254	0.40	42.5	27.0	8.82	6.06	1.14	9.28	6.31	32.7	1.94
Denisova Main Chamber												
14	1	140	0.40	34.0	26.0	8.02	1.80	1.10	5.00	7.04	52.0	5.03
19	2	196	0.35	48.3	30.7	10.6	1.66	1.78	9.10	11.0	58.2	5.80
20	2	423	1.05	147	63.7	22.2	2.37	2.68	13.9	15.8	81.7	7.10
21.1	1	313	0.42	89.0	41.1	12.3	1.95	1.92	11.0	13.4	69.7	7.30
21.2	DC-21-1**	1230	0.88	108	13.0	3.47	2.12	0.82	1.03	1.20	1.93	0.20
21.2	1**	1210	1.39	408	48.4	18.3	7.03	1.39	3.60	4.65	7.20	2.00
21.2	DC-21-2*	2009	2.05	498	49.8	8.87	3.53	0.85	1.47	1.39	2.17	0.27
21.2	5*	800	1.04	310	27.0	9.04	3.20	1.10	0.23	0.90	2.63	1.01
21.2	5A*	900	1.33	270	30.2	7.99	7.00	1.02	1.30	2.30	10.0	2.00
22.1	3	182	0.37	65.3	49.8	15.3	1.36	2.10	13.1	14.9	80.8	5.30
22.2	3	216	0.43	70.8	56.5	18.0	2.17	2.36	16.6	18.5	101	5.60
22.3	1	168	0.47	61.8	52.4	22.5	3.39	2.60	15.3	17.2	100	8.60
Ust-Karakol												
UK-1	1	74.0	0.17	27.8	40.0	15.3	0.98	1.57	13.4	15.9	76.8	9.60
UK-2	2	78.0	0.22	29.0	45.2	16.5	1.02	1.59	13.9	17.3	77.3	10.0
UK-3	3	74.0	0.17	28.2	43.4	14.9	0.91	1.49	12.0	15.4	73.2	8.90
UK-4	4	74.0	0.20	30.9	43.8	15.0	0.99	1.55	12.2	15.8	75.9	9.10
UK-5	5	71.0	0.35	29.7	41.2	15.7	1.24	1.66	13.3	15.7	77.5	9.80
UK-6	6	73.0	0.20	27.8	44.6	15.3	1.22	1.82	12.2	15.8	87.6	10.2
	X_{avg}	74.2	0.22	29.1	42.7	15.5	1.03	1.57	13.0	16.0	76.1	9.48
	S	2.28	0.07	1.23	2.02	0.59	0.14	0.12	0.80	0.67	4.94	0.51

*Black-colored horizons with high content of organic matter.
**Brown horizons containing organic matter.
X_{avg} – average content of elements (n=6); S – standard deviation.

with sandy-clayey matter are the lowest (0.3–1.6 ppm Nb; 1.5–2.1 ppm Sc; 1.3–2.2 ppm Ga), which independently confirms the small contribution of the siliciclastic component to biogenic black sediments.

The normalization procedure is widely used to analyze the distribution of macro- and microcomponents in sedimentary rocks, reconstruct the sources of matter, and identify typical anomalies (Interpretatsiya…, 2001). In this case, the vertical axes of the graphs show the ratios between the absolute concentration values in the sample and in some reference composition (Fig. 6). To reveal trace element features of cave sediments, their compositions are commonly normalized on the corresponding concentration of elements in the soil of adjacent areas (the so-called background concentrations). In this study, the geochemically homogeneous soil profile at the site of Ust-Karakol was used as the background (Table 2).

The normalizing procedure for major components reveals a sharp enrichment of all black-colored and brown (biogenic) cave horizons with phosphorus (60–100-fold) and sulfur (up to 10-fold), with minimal input of sandy-clayey matter (coefficients for Ti, Al, Fe, Na, K are in the range of 0.1–0.5) (Fig. 6, *a*). The normalization also allows us to subdivide indicator trace elements into two groups with contrasting distribution in the studied cave sediments. Relative to the regional background (soil), all black-colored and brown biogenic cave horizons are consistently enriched in Zn (10–30-fold), Cu (4–20-fold), and Cd (4–10-fold), with a high positive correlation between Zn and Cd (R^2 = 0.83, n = 23) (Fig. 6, *a*). The level of Mo accumulation in these sediments reaches 2–7-fold. However, since other types of cave sediments show a similar level of Mo enrichment, this element was excluded from the list of reliable indicators of guano protohorizons. A similar conclusion was also made regarding other biophile elements, such as Ni and Co, which contents are comparable in different types of cave sediments. Uranium, which generally shows high accumulation levels in organic and bone materials (Tribovillard et al., 2006), in studied cave sediments is mainly connected with siliciclastic matter, and its content in black-colored horizons is lower than in soils (Table 2; Fig. 6, *b*). For these reasons, Ni, Co, and U were also excluded from the list of indicators of biogenic sedimentation in the cave.

The second group of microelements includes Sc, Zr, Nb, and Ga, which are typomorphic for sandy-clayey material. All black-colored and brown horizons show a steady depletion of these elements, and the relevant accumulation coefficients fall up to 0.02–0.3.

The accumulation trends of biophile elements (P, S, Zn, Cu, Cd, Mo) in loamy sediments (contaminated by limestone debris) from the lower part of the Main Chamber section coincide with those in black-colored horizons. However, the accumulation coefficients in the sediments

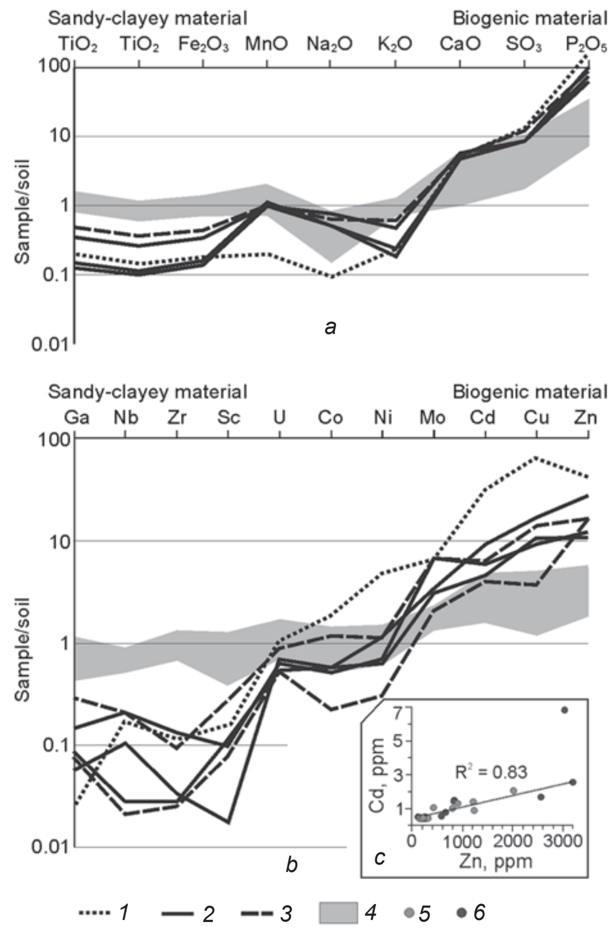


Fig. 6. Graphs of distribution of major (*a*) and some trace elements (*b*) in Pleistocene sediments of the Main Chamber of Denisova Cave (concentrations were normalized to the average composition of soils in the adjacent territory; the absolute elements concentration see in Tables 1 and 2). The inset shows the distribution of Zn and Cd in bulk sediment samples with high content of organic matter.

1 – material from layer 8 (sample 10) of the East Chamber (reference sample); *2* – black-colored lenses in layer 21.2 of the Main Chamber; *3* – brown sediments from layer 21.2; *4* – graph of other sediment types in the section of Main Chamber (layers 22.3–14); *5* – sediments from the section of the Main Chamber; *6* – sediments from the section of the East Chamber.

of the Main Chamber are notably lower (P 7–35-fold, S 2–10-fold, Zn 2–6-fold, Cu and Cd up to 5-fold), as also the content of organic matter (Table 2; Fig. 6, *a*, *b*).

In general, the protohorizons of guano of insectivorous bats in Denisova Cave reveal a common set of mineralogical and geochemical features:

abnormally high accumulation levels of biophile elements (C, N, P, S, Zn, Cu, Cd);

presence of chitin fragments, compressed N-bearing organic matter, and newly formed phosphates Ca (± Ca-Mg); and

predominance of organic and biogenic amorphous matter over the siliciclastic component.

Thus, a set of revealed features makes it possible to reliably identify this type of biogenic sedimentation in the sequence of Denisova Cave.

Discussion

Recently, biogeochemists and archaeologists have been jointly investigating chemical processes that change the composition of sediments, both exposed on the surface and buried at shallow depths (Birkeland, 1999; Bohn, Myer, O'Connor, 2002; Retallack, 2001; Shahack-Gross et al., 2004; Karkanas, 2010; Wurster et al., 2015). These studies are basically important for understanding soil-forming processes, influenced by environmental factors and microbial activity. When studying cave sedimentation, one should also take into account the factors of vital activity of birds and mammals, as well as human activities (Prirodnaya sreda…, 2003; Shahack-Gross et al., 2004; Karkanas et al., 2002; Karkanas, 2010; Wurster et al., 2015).

Biochemical (mainly bacterial) degradation of insectivorous bat guano is one of the principal factors of post-depositional alteration of archaeological caves' sediments. Large colonies of chiropterans use caves as shelters, and breed there only in the periods of human non-occupation (abandonment). Therefore, thick guano horizons are considered as indicators of the periods of absence or rare appearance of people in the caves (Shahack-Gross et al., 2004; Karkanas, 2010; Wurster et al., 2015).

When a colony of bats leaves a cave, the organic component of the guano degrades quickly—during tens of years maximum. The rate of degradation is the highest in warm climates and high humidity in a cave. The initial amount of P_2O_5 in guano horizons is very high (from 12–15 to 34–37.5 wt%) (Wurster et al., 2015). Solutions of organic acids (pH=3–5) that are formed during the biodegradation of guano, gradually wash out phosphorus from the remains of insects and decomposing soft tissues and bone material. Percolating down, these solutions interact with various components of sediments, and dissolve bone material, which is the second important source of phosphorus in cave sediments (Berna, Matthews, Weiner, 2004). As a result, in the sediment column below the degrading guano horizon, two coupled profiles are occurred: geochemical and mineralogical ones (both phosphate). They reflect the gradual neutralization of the initially acidic solutions (Onac et al., 2002; Shahack-Gross et al., 2004; Onac, Forti, 2011; Wurster et al., 2015). The Holocene section of the East Chamber of Denisova Cave is a striking example of a complete chemogenic-sedimentary sequence of this type (Shunkov et al., 2018; Sokol et al., 2022).

Based on new analytical data set (similarity of phase, macro- and trace element compositions, as well as the presence of insect chitin fragments) (see Tables 1, 2; Fig. 2, 6) and on analogy with the horizons earlier studied in the Holocene part of the East Chamber sequence (Ibid.), black-colored lenses from layer 21.2 in the Main Chamber can be identified as the remains of a proto-horizon of insectivorous bat guano. Notably, these biogenic sediments are characterized by a common set of geochemical markers, namely, abnormally high levels of Zn and Cu accumulation, and sharply reduced Sc, Zr, Nb, and Ga contents.

Because layer 21.2 was heavily damaged due to viscoplastic deformations, it impedes reasonable estimations of size of a bat colony or the duration of bat habitation in the Main Chamber. The mineral composition of the black-colored lenses indicates relatively small size of the bat colony populating this part of the cave during the time of accumulation of the layer. Coexistence of carbonate-hydroxyapatite with calcite in layer 21.2 are typical of the phosphate profile, which acid-generating potential was almost exhausted. The relatively good preservation of fine bone detritus also indicates the moderate alkalinity of the contacting solutions (Berna, Matthews, Weiner, 2004; Shahack-Gross et al., 2004). In the considered case, organic acids were gradually neutralized by the limited resource of limestone debris accumulated in layer 21.2. The mentioned facts point to the conclusion that the amount of organic acids, and therefore the guano that produced them, was insignificant.

In the sedimentation history of Denisova Cave, layer 21.1 is of particular importance; it marks the earliest time of regular human habitation of the cave, which affected the composition of the cave taphocenosis (Prirodnaya sreda…, 2003). From this very stratigraphic level, a sharp reduction of both the diversity of bat species and the number of bat remains began (from ~50 to ~15 % of the total amount of small vertebrates) (Ibid.). These data suggest that during the time interval corresponding to the boundary between layers 22 and 21, the habitat of cave-dwelling bats changed to unfavorable. The sharp decrease in the number of Chiroptera remains at this period is in agreement with general changes in the structure of the cave taphocenosis: the number of forest voles and arboreal forms of rodents noticeably decreased, and the proportion of steppe and meadow species increased, which was due to the total effect of climatic factors (Ibid.) and growing anthropogenic impact. Increased human activity in the Main Chamber during the period of the layer 21.1 accumulation is also supported by numerous signs of fire residue (fragments of charcoal and micritic calcite) that are evidences of regular fire use inside the cave.

Conclusions

Each type of sediment is characterized by individual chemical hallmarks, which are controlled by the similarity of matter sources and sedimentation regime (Interpretatsiya…, 2001). However, post-depositional processes (diagenesis, dissolution, and leaching) can modify some chemical features up to their total disappearing. For archaeological sites, reconstruction of the parent sediments, as well as estimation of its preservation degree, help to assess the extent to which different types of organic matter (bones, pollen, spores, plant remains, and charcoal) were preserved in a particular deposition environment. In the case of deep diagenetic transformations of sediment, it is of principal importance to reveal secondary indicators of organic materials survived in modified depositional environment (Karkanas, 2010).

It has been established that in the sedimentary strata of Denisova Cave, a number of ancient black-colored horizons and lenses reveal a common set of geochemical features, and contrast with adjacent layers in phase, macro- and trace element compositions. These biogenic sediments are mainly composed of X-ray amorphous matter dominated by nitrogen-bearing organics and Ca phosphates of low-crystallinity (up to 40 %). Markers of these sediments include: high C, N, P, Zn, and Cu contents; small amount of siliciclastic material (Si, Ti, Al, Mg, K, Na); and the presence of fragments of the chitin exoskeletons of insects. The combination of these features is typical of the insectivorous bat guano protohorizons.

Regardless of the age, this type of cave sediments is characterized by unique and reproducible geochemical marks (high enrichment in $C_{org.}$, P, N, Zn, Cu and strong depletion in Sc, Zr, Nb, and Ga), which survived even in Pleistocene deposits.

In the sedimentation records of the Altai cave sites, these horizons can be identified as markers and used for correlation of different sections in the same cave; in the future, these markers can be used for comparison of different sites. In each Altai intermountain valley, the Quaternary deposits have individual geochemical features; therefore, it is necessary to analyze not only cave sediments, but also open-air soil profiles, in order to compare the sections of different archaeological sites. Identification of trace element composition of cave sediments requires comparison with the local geochemical background (normalization procedure). This procedure allows one to identify local and regional geochemical markers of different cave sediments. A set of characteristics and methodological approaches tested in Denisova Cave can be used to categorize the deposits in other cave sites.

Acknowledgements

This study was supported by the Russian Science Foundation, Grant No. 22-28-00049, https://rscf.ru/project/22-28-00049/.

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Received April 4, 2023.
Received in revised form May 17, 2023.