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Supplement of

The mirabilite microbiocosm in a Carpathian contact cave

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Supplementary material

Table S1: The most abundant bacteria genera in Tausoare Cave samples. See Table 1 for abbreviations.

Genus	MTS1dec	MTS2dec	MOTSapr	STS1mar	STS2mar	STS1sep	STS2sep	STS3Asep	WTS4mar	WTS4sep	Lit_TS2mar	Lit_TS2sep	Lit_TS3Asep	Lit_TS3Bsep
Pseudomonas	0.778	0.324	0.555	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.074	0.004	0.157	0.115
Lysobacter	0.031	0.140	0.000	0.201	0.593	0.313	0.405	0.142	0.031	0.082	0.018	0.000	0.000	0.000
Rickettsia	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.280	0.172	0.010	0.389
NA-Longimicrobiaceae	0.021	0.016	0.000	0.440	0.002	0.327	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Paeniglutamicibacter	0.001	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.112	0.371	0.022
NA-Actinomarinales	0.057	0.028	0.000	0.020	0.020	0.064	0.076	0.026	0.000	0.000	0.000	0.000	0.000	0.000
Anseongella	0.000	0.014	0.000	0.006	0.004	0.061	0.093	0.109	0.000	0.000	0.000	0.000	0.000	0.000
Streptomyces	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.150	0.015	0.009
Pseudarthrobacter	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.091	0.057	0.017
Arthrobacter	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.019	0.140	0.013
Flavobacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.151	0.002	0.000	0.000	0.013
Acinetobacter	0.000	0.000	0.000	0.009	0.003	0.000	0.015	0.013	0.023	0.007	0.086	0.001	0.000	0.002
Sporosarcina	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.032	0.114	0.003
Candidatus_Arthromitus	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.126
Luteimonas	0.000	0.000	0.000	0.000	0.026	0.007	0.061	0.047	0.000	0.000	0.000	0.000	0.000	0.000
NA-Xanthomonadaceae	0.000	0.000	0.000	0.007	0.022	0.091	0.003	0.003	0.000	0.000	0.000	0.000	0.000	0.000
NA	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.100	0.002	0.000	0.000	0.000	0.000
Acidovorax	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.095	0.000	0.000	0.000	0.000
NA-Flavobacteriaceae	0.002	0.037	0.000	0.000	0.000	0.056	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NA-Saccharimonadales	0.000	0.000	0.040	0.007	0.004	0.000	0.031	0.000	0.013	0.001	0.000	0.000	0.000	0.000
Pelobium	0.000	0.000	0.000	0.000	0.001	0.000	0.038	0.055	0.000	0.000	0.000	0.000	0.000	0.000
Blastocatella	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.072	0.000	0.000	0.000	0.000	0.000	0.000
Sphingopyxis	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.000
Alkanindiges	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.062	0.000	0.000	0.000	0.000
Rhodoferax	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.074	0.000	0.000	0.000	0.000
Staphylococcus	0.000	0.000	0.000	0.010	0.003	0.000	0.003	0.004	0.008	0.003	0.041	0.000	0.000	0.001
Sphingomonas	0.000	0.000	0.000	0.012	0.028	0.002	0.010	0.008	0.000	0.000	0.011	0.000	0.000	0.002
Enterococcus	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.068	0.000	0.001
Sphingobacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.039	0.025	0.000
Serratia	0.000	0.000	0.000	0.000	0.035	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.001	0.020
Cutibacterium	0.000	0.000	0.000	0.004	0.003	0.001	0.001	0.014	0.000	0.000	0.033	0.000	0.000	0.001
Brevundimonas	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.057	0.000	0.000	0.000	0.000
NA-S0134_terrestrial_group	0.000	0.000	0.000	0.027	0.010	0.008	0.009	0.001	0.000	0.000	0.000	0.000	0.000	0.000
Rhodococcus	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.003	0.002	0.043
NA-Methyloligellaceae	0.007	0.008	0.000	0.005	0.001	0.002	0.007	0.001	0.022	0.001	0.000	0.000	0.000	0.000
NA-Vicinamibacterales	0.000	0.000	0.000	0.000	0.012	0.000	0.025	0.003	0.010	0.002	0.000	0.000	0.000	0.000
Lelliottia	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.048	0.000
Streptococcus	0.000	0.000	0.000	0.017	0.005	0.000	0.001	0.000	0.010	0.001	0.014	0.000	0.000	0.000
Hydrogenophaga	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.032	0.000	0.000	0.000	0.000
NA-Gitt-GS-136	0.000	0.000	0.000	0.000	0.001	0.000	0.021	0.022	0.000	0.000	0.000	0.000	0.000	0.000
Chryseobacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.000
Qipengyuania	0.000	0.000	0.000	0.000	0.007	0.000	0.018	0.016	0.000	0.000	0.000	0.000	0.000	0.000
NA-Saccharimonadales-WWH38	0.000	0.000	0.041	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Bacillus	0.000	0.000	0.000	0.025	0.003	0.000	0.000	0.000	0.010	0.001	0.000	0.000	0.000	0.000
Delftia	0.000	0.000	0.000	0.015	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
wb1-P19	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.006	0.005	0.000	0.001
Clostridium s.str.1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.007	0.002	0.025
Lachnoclostridium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.034	0.000	0.000
Enhydrobacter	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.011	0.017	0.000	0.000	0.000
Sphingorhabdus	0.000	0.000	0.011	0.001	0.003	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NA-Gammaproteobacteria-PLTA13	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.030	0.002	0.000	0.000	0.000	0.000
Carnobacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.002	0.027
Stenotrophomonas	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000
Microbulbifer	0.000	0.000	0.000	0.000	0.006	0.000	0.022	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Aquabacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.000	0.000
Subgroup_10	0.000	0.000	0.000	0.000	0.002	0.000	0.016	0.011	0.000	0.000	0.000	0.000	0.000	0.000
NA-Bacillaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.023	0.000	0.004
NA-Cand_Woesebacteria	0.000	0.000	0.028	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NA-Vermiphilaceae	0.000	0.000	0.028	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NA-Berkelbacteria	0.000	0.000	0.027	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Corynebacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.011	0.001	0.000	0.001
Lactococcus	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.001	0.001
NA-Streptosporangiaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.001	0.000	0.000
NA-Ruminococcaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000

Buttiauxella	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.003
MND1	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.008	0.002	0.000	0.000	0.000	0.000
NA-Moraxellaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.018	0.000	0.000	0.000	0.000
Undibacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.017	0.000	0.000	0.000	0.000
Fluviicola	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000
Gaiella	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.004	0.000	0.000	0.000	0.000
Nitrospira	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.006	0.000	0.000	0.000	0.000
Achromobacter	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000
NA-Saccharimonadales LWQ8	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NA-Microbacteriaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.002	0.013
NA-Actinobacteriota MB-A2-108	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.003	0.000	0.000	0.000	0.000
NA-Acidimicrobiia IMCC26256	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NA-Patescibacteria WWE3	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NA-cvE6	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Phaeocystidibacter	0.001	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Aquicella	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000
Raoultibacter	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000
Cand_Ovatusbacter	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Massilia	0.000	0.000	0.000	0.001	0.001	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Chlamydia	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000
Micrococcus	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000
Woeseia	0.001	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Parabacteroides	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000
NA-Intrasporangiaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.001
Frondihabitans	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.010
NA-Gaiellales	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.002	0.000	0.000	0.000	0.000
NA-Parcubacteria GWA2-38-13b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000

Table S2: The physicochemical characteristics of the analysed samples in Tausoare Cave. See Table 1 for abbreviations.

UM	Element	STS1mar	STS2mar	STS1sep	STS2sep	STS3Asep	STS3Bsep	MTS1dec	MTS2dec	MOTSmay	UM	WTS4mar	WTS4sep
	pH	8.1	8.3	8.4	8.4	8.2	8.7	8.6	9.8	8.6		7.8	9.0
uS/cm	**EC**	3260	1944	3111	1369	2052	6430	10356	59553	121	**uS/cm**	104.0	115
mg/kg	**S**	4217	2099	9887	1444	3315	84	8548	44399	216	**mg/L**	2.6	1.84
mg/kg	**Water soluble SO_4^{2-}**	12633	4188	22113	4314	9883	240.0	24578	126940	646	**NA**	NA	NA
mg/kg	**Water soluble S**	4211	1959	9371	1438	3294	80	8193	42313	215	**NA**	NA	NA
mg/kg	**Na**	1667	790	3660	933	913	480	14724	27162	77	**mg/L**	1.4	1.54
mg/kg	**Mg**	2305	2020	7390	6003	5553	6087	2028	1113	814	**mg/L**	1.1	1.09
mg/kg	**K**	3500	3029	2007	1973	2437	2697	3190	1209	512	**mg/L**	1.0	1.068
mg/kg	**Ca**	49833	35333	74567	73533	49267	49130	15630	17353	335400	**mg/L**	18.6	21.19
mg/kg	**Al**	10947	9833	8363	10223	11383	12140	13586	2491	2042	**mg/L**	0.0	0.06
mg/kg	**Fe**	8093	8107	14110	22053	22773	27687	26245	1732	730	**mg/L**	0.0	0
mg/kg	**P**	784.3	503.0	542.3	442.3	1181.0	283.6	393	117	326	**mg/L**	0.0	0
mg/kg	**V**	32.0	31.6	14.6	20.8	24.4	34.2	34.0	1.8	3.3	**mg/L**	1.1	1.5
mg/kg	**Cr**	34.3	28.3	12.1	17.4	19.0	23.8	37.7	3.1	7.2	**mg/L**	0.6	1.26
mg/kg	**Mn**	237.9	298.6	84.4	222.0	206.7	209.3	448.7	36.5	140.0	**mg/L**	0.0	0.00
mg/kg	**Co**	7.7	8.2	2.6	5.0	4.7	6.6	6.0	0.8	0.9	**mg/L**	0.0	0.10
mg/kg	**Ni**	24.8	24.1	14.7	17.7	18.0	19.1	27.8	3.4	19.6	**mg/L**	0.0	1.89
mg/kg	**Cu**	18.9	17.0	5.1	8.3	9.6	9.6	183.7	2.1	3.3	**mg/L**	0.0	4.27
mg/kg	**Zn**	84.0	76.9	11.4	21.1	38.4	34.7	50.9	8.6	5.4	**mg/L**	0.3	8.59
mg/kg	**Cd**	0.29	0.30	0.10	0.16	0.21	0.18	0.2	0.3	0.6	**mg/L**	0.2	0.22
mg/kg	**Pb**	6.4	8.6	2.17	5.20	7.33	8.07	6.9	0.8	1.5	**mg/L**	0.1	1.2

Table S3: Mineralogical composition of the samples based on XRD analysis.

Mineral group	Mineral species	Chemical formula	Mineral abundance*								
			STS1mar	STS2mar	STS1sep	STS2sep	STS3Asep	STS3Bsep	MTS1dec	MTS2dec	MOTSapr
Carbonates	Calcite	$CaCO_3$	+	+	+	+	+	±	±	±	++
Oxides	Quartz	SiO_2	++	++	++	++	++	++	++	+	-
Silicates	Albite	$NaAlSi_3O_8$	++	++	++	++	++	++	+	++	-
	Orthoclase	$KAlSi_3O_8$	+	+	++	+	-	±	-	+	-
Sulfates	Thenardite	Na_2SO_4	±	±	-	-	-	-	±	++	-
	Gypsum	$CaSO_4{\cdot}2H_2O$	-	-	-	-	-	-	±	-	-
	Bassanite	$CaSO_4{\cdot}0.5H_2O$	-	-	+	-	±	-	-	-	-
	Mirabilite	$Na_2SO_4{\cdot}10H_2O$	-	-	±	±	±	±	-	-	-

* + + Major species (20–50%); + Minor species (5–20%); ± Trace species (<5%).

FTIR and XRD analysis

The FTIR spectrum of the MTS2dec mirabilite sample (Figure S2a) was characterized by strong absorption bands at 1088 cm^{-1} and 608 cm^{-1}, corresponding to the asymmetric stretching and bending vibrations of SO_4^{2-}, respectively. In addition, low-intensity bands at ~1433 and ~870 cm^{-1}, attributed to CO_3^{2-}, indicated the presence of minor carbonate phases. These suggested a sulfate-dominated mineral accompanied by traces of carbonates. The FTIR data agreed with XRD data that identified thenardite as main phase, accompanied by silicates (albite, orthoclase), quartz and traces of calcite. The FTIR spectrum of MTS1dec mirabilite sample exhibited similar bands to those observed in MTS2dec, but the specific bands for sulphate were less intense, whereas those of carbonate were more intense. A slight shift in peaks positions was observed, along with a large band at 994 cm^{-1}, likely resulting from the overlap of SO_4^{2-} and CO_3^{2-} vibrations with Si–O–Si stretching modes, probably associated with quartz. The XRD data of MTS1dec confirmed quartz as the dominant phase accompanied by albite, calcite, gypsum and traces of thenardite. This is consistent with the lower concentration of soluble S measured in this sample.

The FTIR spectra of the sediments (Figure S2b) showed a similar mineralogical composition. All spectra were dominated by an intense band at ~1000 cm^{-1} attributed to overlapping of Si–O–Si asymmetric stretching vibrations probably in quartz (1080-1100 cm^{-1}) with that of carbonate and sulphate groups. The bands at ~1420 cm^{-1} attributed to asymmetric stretching (ν_3) and ~870 out-of-plane bending (ν_2) of CO_3^{2-} were more intense in sediments collected in STS1 and STS2 both in March and September compared to those from STS3, suggesting relatively higher calcite contents in STS1 and STS2. This finding was in line with XRD data that indicated that calcite occurs as a minor phase in all sediments. The XRD data further showed that quartz and silicate minerals were the predominant crystalline phase in the sediments. Calcite was a minor phase in all sediment samples. In both sediment samples collected in March (STS1mar and STS2mar) traces of thenardite were identified whereas in all samples collected in September traces of mirabilite were identified.

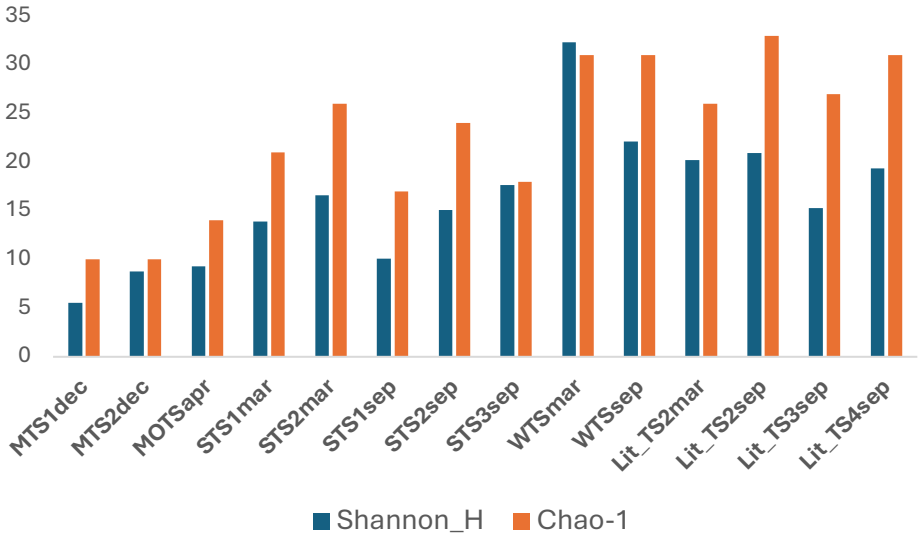

Figure S1: Diversity indices for the analysed samples. See Table 1 for abbreviations.

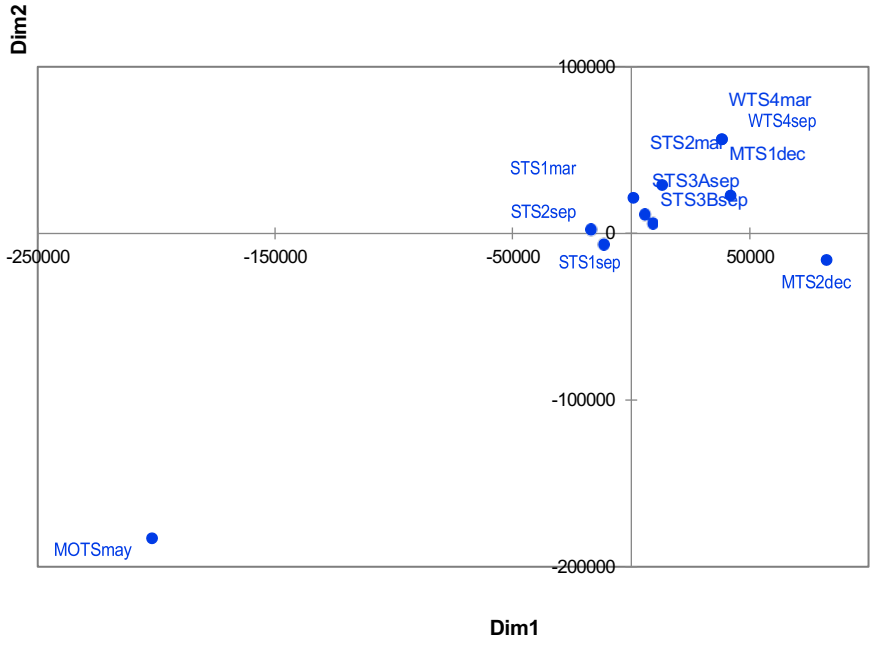

Figure S2: Two-dimensional MDS plot on the elemental concentration of mirabilite, moonmilk, water and sediment samples. Kruskal's stress (1) = 0.025 indicates the significance of dissimilarity between samples. See Table 1 for abbreviations and Table S2 for values.

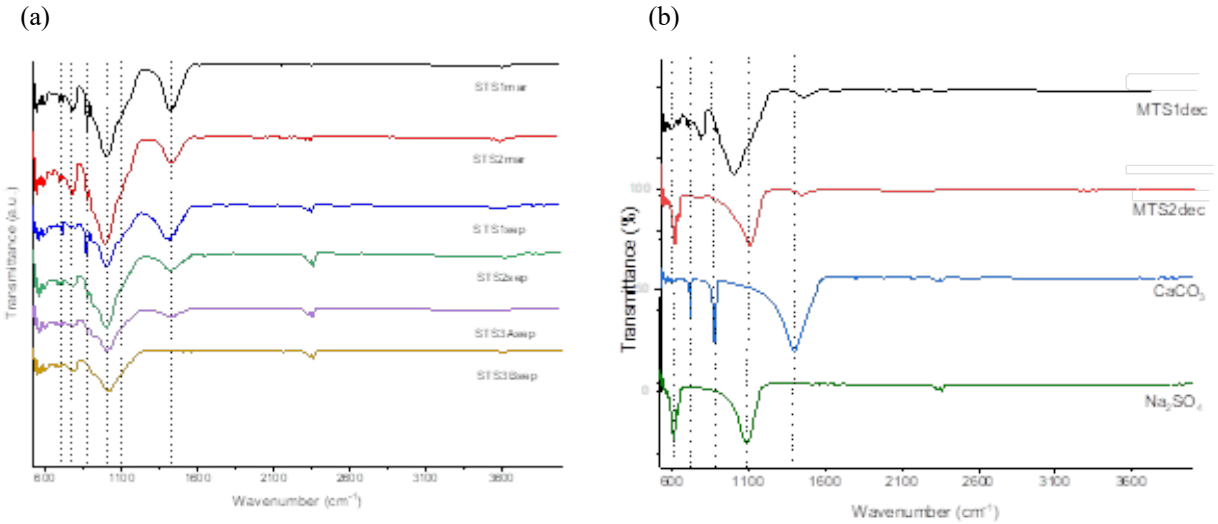

Figure S3: Fourier-transform infrared spectra of a) mirabilite, $CaCO_3$ and Na_2SO_4 and b) sediment samples.