

1 **Supplementary Materials**

2 Comprehensive mineralogical and physicochemical characterization of recent sapropels from
3 Romanian saline lakes for potential use in pelotherapy

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31 **Running Title:** Comprehensive characterization-of recent saline sapropels

32 **Table S1.** *In situ* measurements of physico-chemical parameters and water chemistry
 33 (average±standard deviation of three replicates) for Tekirghiol, Amara and Ursu lakes during
 34 October 2017.

Physico-chemical parameter/ Compound	Tekirghiol Lake	Amara Lake	Ursu Lake
Depth (m)	0.5	2	12
Temperature (°C)	15	15.79	21.6
pH	8.39	8.77	6.13
Dissolved oxygen, DO (mg L ⁻¹)	5.69	9.79	0.48
Redox potential, ORP (mV)	-48.1	189.6	-358.5
Electrical conductivity, EC (mS/cm)	105.7	18.3	350
Total dissolved solids, TDS (g L ⁻¹)	52.8	9.15	380
Na ⁺ (mg L ⁻¹)	41500	2270	180000
K ⁺ (mg L ⁻¹)	1160	83	134
Ca ²⁺ (mg L ⁻¹)	282	110	505
Mg ²⁺ (mg L ⁻¹)	2360	413	9.1
Cl ⁻ (mg L ⁻¹)	58500	3550	245000
Total Fe (mg L ⁻¹)	<0.05	<0.05	<0.05
Total Mn (mg L ⁻¹)	<0.01	<0.01	<0.01
SO ₄ ²⁻ (mg L ⁻¹)	8060	5325	2445
Estimated salinity as sum of ions (g L ⁻¹)	111.82	11.75	428.09
Total sulfides (S ²⁻) (mg L ⁻¹)	<0.02	<0.02	94.8
TP (mg L ⁻¹)	<0.05	<0.05	0.52

TN (mg L ⁻¹)	6.2±0.3	7.6±0.4	37±2
DTN (mg L ⁻¹)	4.8±0.2	5.5±0.3	34±2
NH ₄ -N (mg L ⁻¹)	1.75±0.10	1.36±0.08	35.95±1.70
ON (mg L ⁻¹)	4.45±0.20	6.24±0.28	1.05±0.05
TC (mg L ⁻¹)	144±6	80±5	189±8
TOC (mg L ⁻¹)	66±3	16±1	36±2
DC (mg L ⁻¹)	138±7	76±4	93±5
DOC (mg L ⁻¹)	64±3	14±1	20±1
δ ¹³ C (‰) ^a	-23.75	-29.07	-28.41
δ ¹³ C _{DIC} (‰) ^b	-2.08	-4.16	-17.48
δ ¹⁵ N (‰) ^a	11.83	7.72	6.51
δ ³⁴ S sulfides (‰) ^c	Nm	Nm	-6.88
δ ³⁴ S sulfates (‰) ^c	18.86	8.11	17.69
δ ¹⁸ O (‰) ^d	-1.58	0.24	-9.67
C1-hydrocarbon (CH ₄) (mg L ⁻¹)	0.0205	0.0034	0.1017

35 ^a from particulate matter retained onto 0.22 μm pore-sized membrane following filtration of
36 lake water; ^b – following water acidification; ^c- from lake water treated with Zn-acetate (for
37 sulfides recovery) or BaCl₂ (for sulfates recovery); ^d – from lake water distilled by azeotropic
38 distillation prior to analysis; Nm –not measured.

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43 **Table S2. Comparative contents of rare earth elements (lanthanides) in sediments of tested**
 44 **lakes and Darscho and Neusiedlersee lakes (Austria) [1], and Colour Lake (Canada) [2],**
 45 **respectively.**

Rare earth elements (mg/kg)	Tekirghi ol Lake	Amara Lake	Ursu Lake	Darscho Lake	Neusiedlersee Lake	Colour Lake
La	10.33	8.57	1.99	10.2	9.10	1.87
Ce	22.56	20.17	5.19	20.5	17.0	4.21
Pr	2.82	2.51	0.69	Nd	Nd	0.55
Nd	11.25	10.22	2.69	8.29	6.76	2.20
Sm	2.58	2.47	0.67	1.84	1.31	0.38
Eu	0.63	0.65	0.20	0.41	0.31	0.08
Gd	2.88	2.76	0.78	1.66	1.22	0.33
Tb	0.41	0.40	<0.33	0.26	0.17	0.05
Dy	1.86	1.81	0.54	Nd	Nd	0.29
Ho	0.83	0.37	0.15	Nd	Nd	0.06
Er	0.96	0.92	<0.33	Nd	Nd	0.17
Tm	<0,33	<0.33	<0.33	0.26	0.25	0.02
Yb	0.73	0.69	0.26	0.86	0.54	0.15
Lu	<0.33	<0.33	<0.33	Nd	Nd	0.02
Total	57.84	51.54	13.16	44.28	36.66	10.38

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47 **Note:** Detection limit was 0.33 mg kg⁻¹ sediment; Nd –not determined.

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49 **Table S3. GC-MS analysis of bacterial acid methyl esters extracted from water and**
50 **sediments of three Romanian salt lakes.**

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Peak No.	Fatty acid	<u>% from total fatty acids</u>						
		Tekirghiol Lake		Amara Lake		Ursu Lake		
		Water	Sed.	Water	Sed.	Water (3 m)	Water (11 m)	Sed.
1	(C8:0)	0.32	1.22	0	1.30	0	0	0.45
2	(C10:0)	0	0.66	0	1.15	0	0	0.28
3	(C12:0)	0	0.97	0	1.08	0.92	0.59	0.49
4	(C14:0)	2.34	3.58	3.46	5.56	4.93	4.34	5.74
5	(C15:0)	0	0.42	2.04	0.35	2.96	1.10	1.46
6	12-methyltetradecanoate (a C15:0)	0	0.38	0	0.47	0.76	0	0.30
7	14-methylpentadecanoate (i C16:0)	0	1.29	0	1.58	0.24	0.80	0.68
8	(C16:0)	37.52	34.20	31.29	33.34	43.99	38.82	34.47
9	(C16:1 n-9)	1.70	4.78	1.45	7.11	23.43	4.00	1.66
10	(C16:1 n-7)	2.21	3.10	4.80	2.44	2.57	8.16	9.37
11	(C18:0)	9.64	12.69	8.69	13.62	4.54	11.61	9.68
12	(C18:1 n-9)	23.50	17.60	7.07	14.84	5.95	18.50	13.58

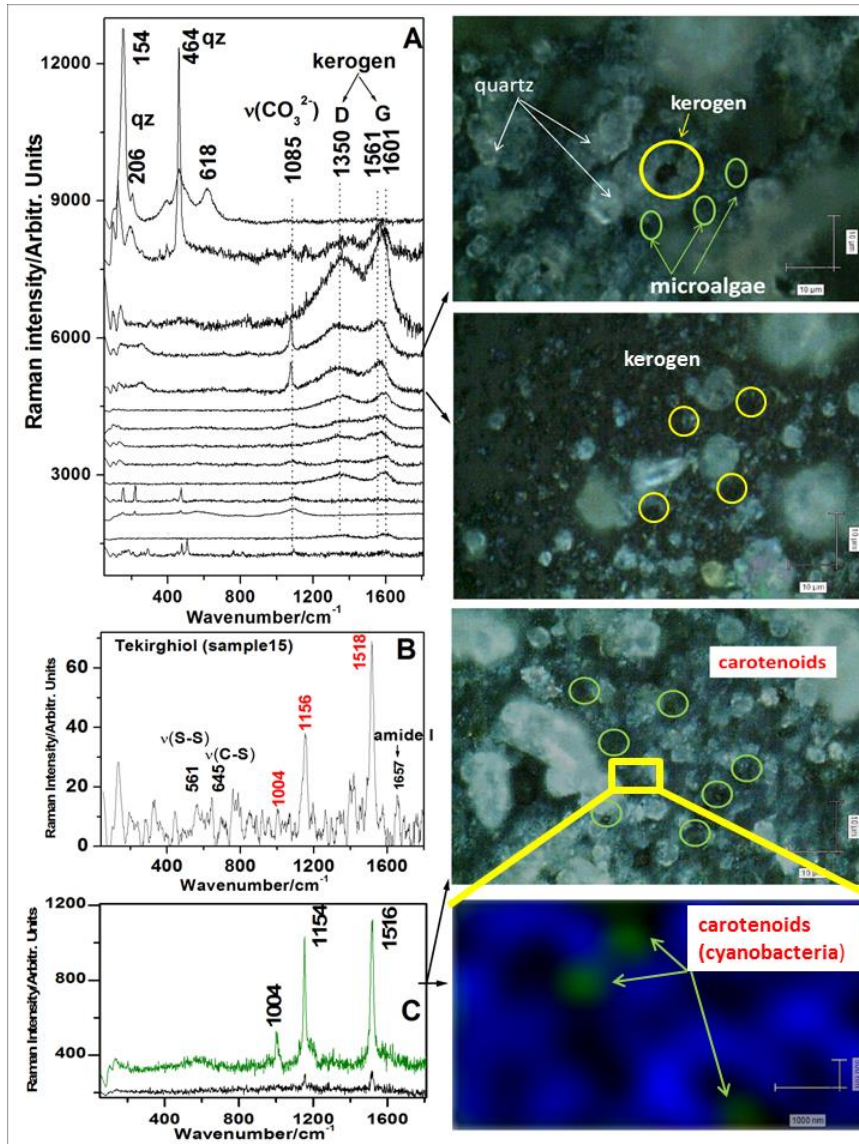
13	(trans-C18:1 n-9)	7.83	1.27	6.42	3.45	5.21	4.33	1.44
14	(C18:2 n-6)	4.67	12.23	10.59	10.47	3.03	4.98	8.40
14'	(C18:3 n-3)	10.28	1.54	24.21	0.92	1.47	1.05	5.31
15	(C20:0)	0	1.52	0	1.77	0	0.45	1.78
16	2-OH C16:0	0	2.56	0	0.55	0	1.28	4.90

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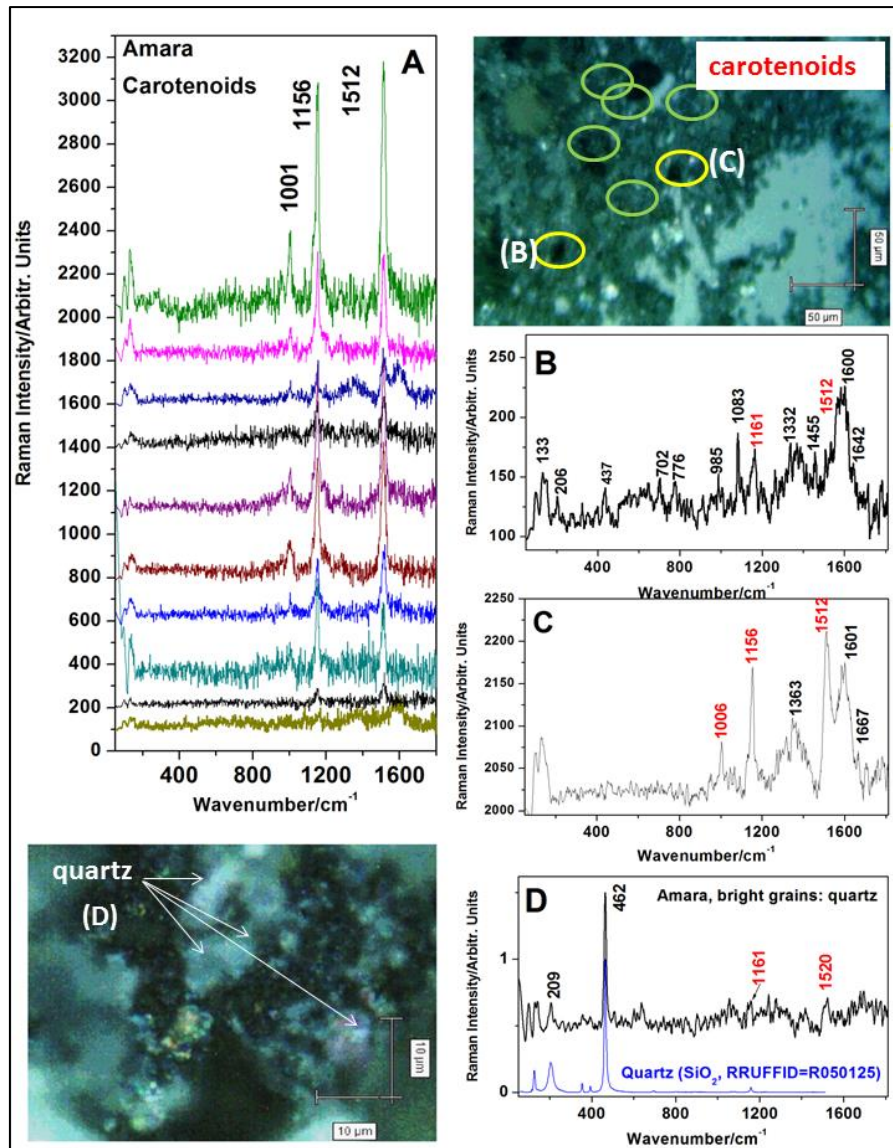


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57 **Figure S1. Raman spectroscopy briefing the dominant Tekirghiol sapropel composition.**

58 Series of spectra featuring kerogen A) along quartz and carbonate mineral; typical
 59 carotenoids signal accompanied by organic trace of proteins B); and typical, clear RR spectra
 60 of carotenoids C); Corresponding light microscopy images taken with Raman microscope
 61 during measurements, highlight the inorganic bright crystals, black spots of kerogen and
 62 dark green microorganisms signaling carotenoids. A small map (bottom, right) of 5 μm x 3
 63 μm illustrates quartz signal distribution (blue) embedding green spots (< 1 μm) of
 64 carotenoid signal, most probably from cyanobacteria. Excitation: 532 nm.

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68 **Figure S2. Typical micro-Raman spectra collected from raw mud from Amara. Series of**

69 background subtracted spectra showing mainly carotenoid bands (A) at 1512 (C=C mode) and

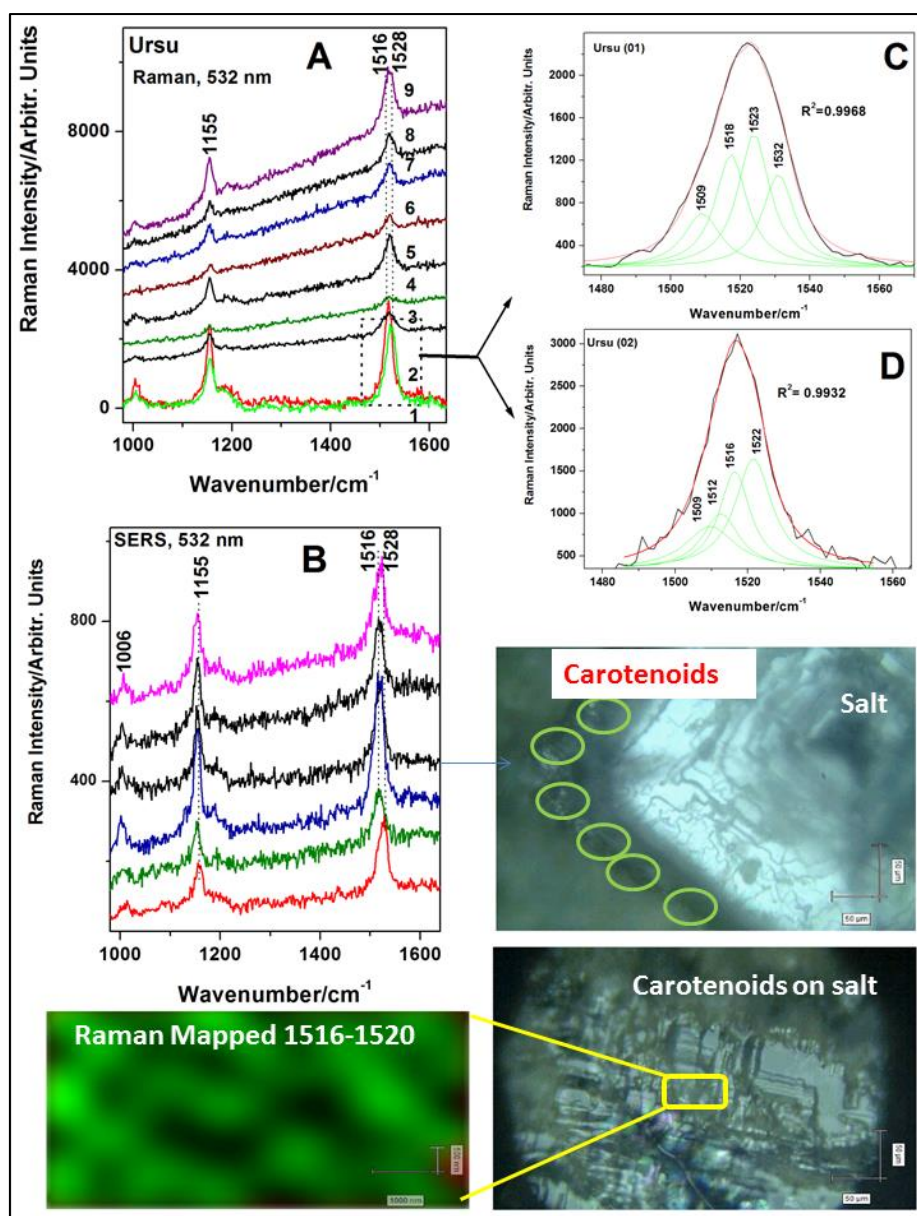
70 1156 cm^{-1} (C-C) mode and trace of organic (B, C) or inorganic (D) matter. The carotenoid bands

71 are plotted in red (B, C, D), while D shows a typical signal of quartz. Micrographs collected

72 from the single point spectra taken in B, C, D are showed in the right. Bright grains are

73 associated with quartz crystals while dark, spherical spots were typical for carotenoids.

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77 **Figure S3. Typical resonance Raman (RR) signal of carotenoids collected from Ursu point**

78 **samples numbered from 1 to 9. Two distinct signatures are highlighted in spectrum 1 and 2**

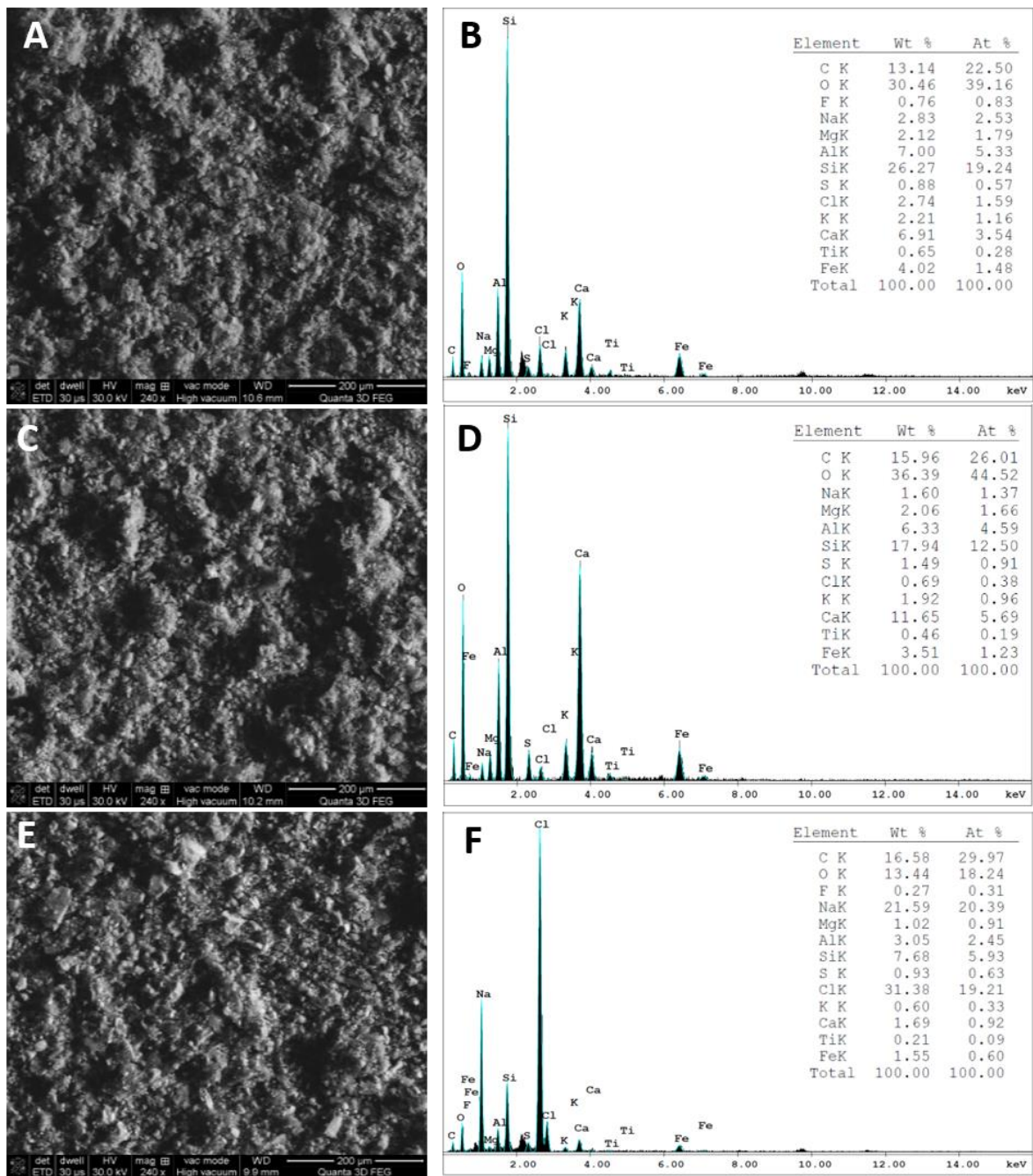
79 (A); SERS technique was applied for fluorescence quenching reason revealed similar bands

80 positions but with considerably lower background, decreased absolute intensity under similar

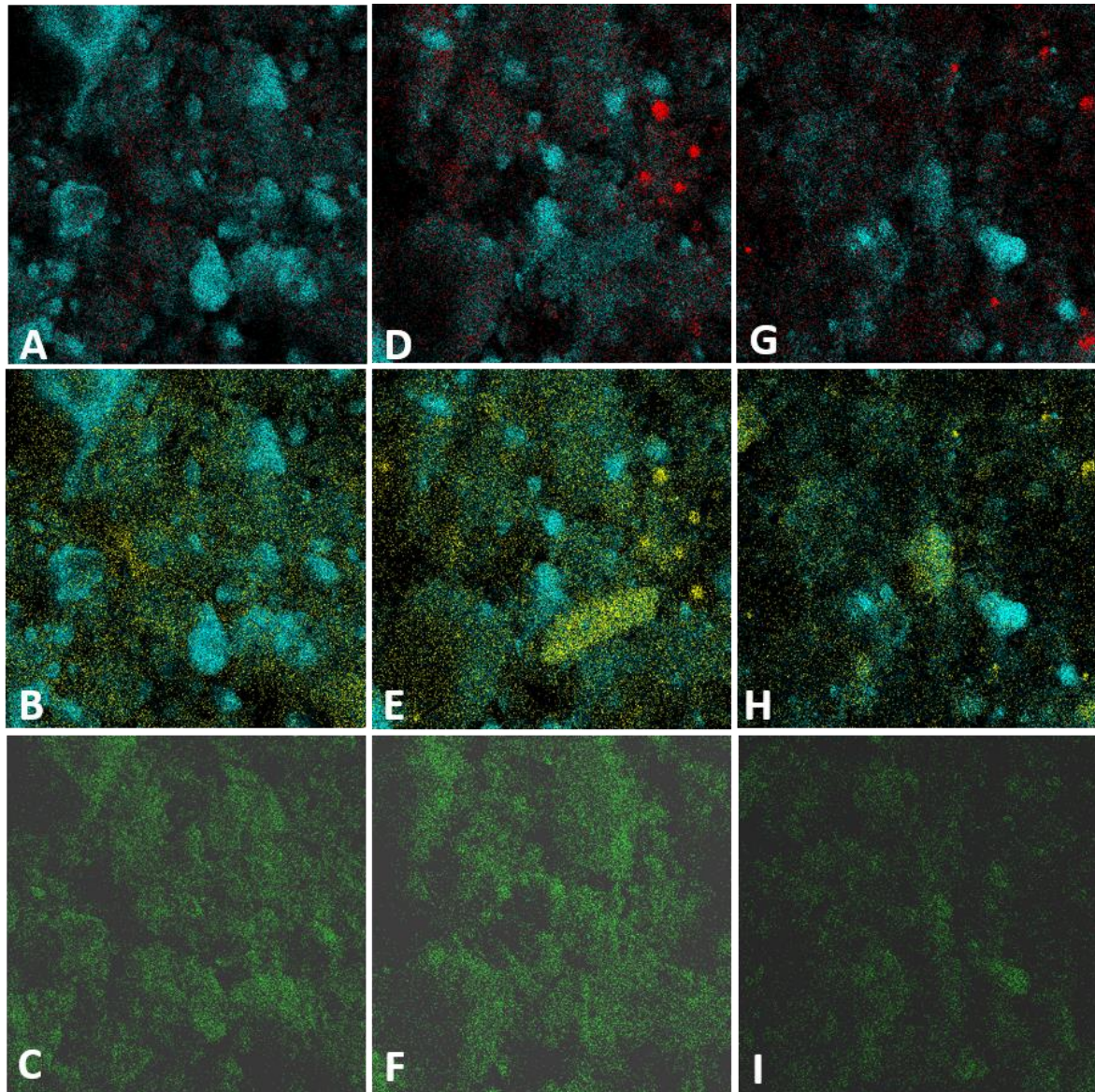
81 collection optics (B); Details of the Lorentzian deconvolution of the main C=C band are

82 showed in C) for spectrum 1 and D) for spectrum 2, respectively. Distinct bands components

83 determined with a correlation coefficient $R^2 > 0.99$ are presumably associated with distinct
 84 carotenoids species comprised in focused microorganisms. Excitation: 532 nm.
 85
 86
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88 **F**
 89 **Figure S4. SEM-EDX analysis of Tekirghiol (A, B), Amara (C, D), Ursu (E, F) sapropels.**



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91 **Figure S5. SEM elemental mapping for sulfur (red), iron (yellow), and oxygen (green) in**

92 **sapropels from Tekirghiol (A, B, C), Amara (D, E, F), and Ursu (G, H, I) lakes. The elemental**

93 **mapping was performed at x 1000 magnification.**

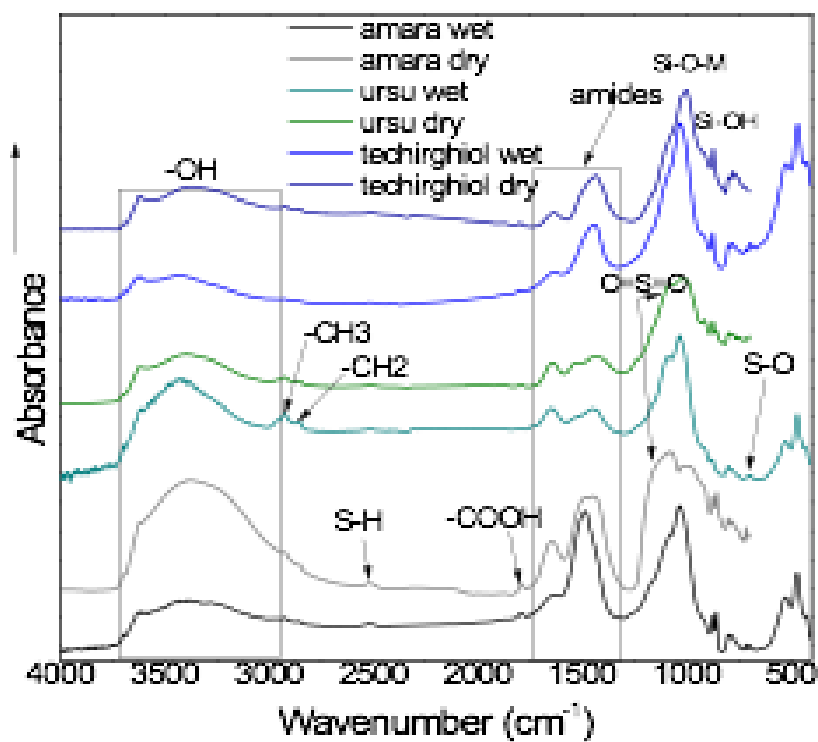
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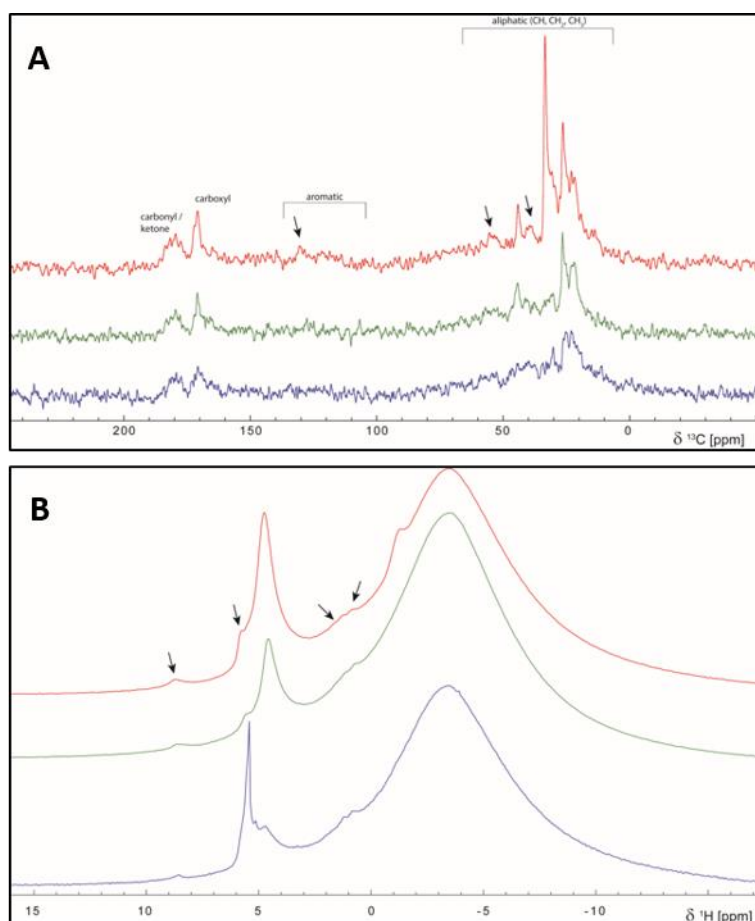
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100 **Figure S6.** Fourier transform infrared spectroscopy (FT-IR) spectra of sediments samples from
 101 Tekirghiol, Amara and Ursu lakes.

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105 **Figure S7. The ^{13}C CP-MAS ss-NMR (A) and ^1H ss-NMR spectra (B) recorded on the Tekirghiol**

106 **(red), Amara (green), and Ursu (blue) lyophilized sapropels.** The spectral windows where the

107 ^{13}C NMR lines of various chemical moieties can be typically found are highlighted. By arrows,

108 we mark the positions of very low intensity lines, close to the noise level. The differences

109 between the overall spectral intensities of the samples correlate well with the organic carbon

110 quantitative differences determined by the loss-on-ignition. The ^1H ss-NMR spectra are

111 dominated by intense NMR lines at -3.4 ppm and in the 4.5-5.5 ppm spectral region,

112 corresponding to inorganic components, -OH and water molecules in various binding

113 environments. By arrows we mark the positions of very low intensity ^1H NMR lines associated

114 with organic matter.

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116 **Supplementary Methods**

117 *FT-IR spectroscopy and SEM*

118 The FT-IR absorption spectra of the dried and homogenized sediments were recorded in
119 reflection configuration with a Jasco 6000 spectrometer (Jasco Co. Ltd, Japan), at room
120 temperature, in the range 400–4000 cm^{-1} and spectral resolution of 4 cm^{-1} using the KBr
121 pellet technique. The FT-IR spectra of the wet sediments were recorded using a Jasco IRT-
122 5000 FT-IR microscope (Jasco Co. Ltd, Japan) coupled to a Jasco FT-IR-6000 spectrometer in
123 reflection configuration in the range 4000–650 cm^{-1} , at 4 cm^{-1} resolution, using the $\times 32$
124 Cassegranian objective, imaging a sample area of $\sim 50 \times 50 \mu\text{m}$. The recorded spectra were
125 smoothed by 5-point Savitzky-Golay function. For the SEM investigations, a FEI Quanta 3D
126 FEG dual beam – in high vacuum mode operating at 30 KV, equipped with an ApolloX SDD
127 Energy Dispersive X-ray (EDX) detector was used. The dried and homogenized samples were
128 examined in a random order using scanning electron microscopy (SEM) at $\times 70$, $\times 240$, $\times 1000$
129 magnification. On the $\times 240$ images energy spectra were collected for 60 seconds and peak
130 intensities for all elements were recorded.

131

132 *Solid-state ^{13}C - and ^1H -NMR*

133 To assess organic matter fraction extractable by alkaline hydrolysis, the wet sapropel samples
134 were mixed 1:1 (v/v) with 2 M NaOH followed by stirring and subsequent supernatant
135 separation and lyophilization. Then solid-state (ss) ^{13}C (^1H) NMR spectra of the investigated
136 samples were recorded at 125.73 and 499.99 MHz ^{13}C and ^1H Larmor frequencies with an
137 AVANCE III 500 MHz spectrometer (Bruker Optik GmbH, Germany) operating at room
138 temperature. ^1H ss-NMR was performed at 14 kHz spinning frequency, using a simple one-pulse
139 experiment. Variable recycle delay values were used to determine the longitudinal relaxation
140 time of the organic vs inorganic component. The ^{13}C CP-MAS (Cross-Polarization under Magic
141 Angle Spinning) spectra were acquired at 14 kHz spinning frequency, 2 ms contact time, and
142 high-power proton decoupling (100 kHz) under TPPM, by averaging 180k transients with a
143 recycle delay of 3 s. All of the reported ^{13}C and ^1H ss-NMR spectra are calibrated relative to the
144 CH_3 line in TMS (tetramethylsilane), through an indirect procedure which uses adamantane as
145 an external reference.

146

147 *Pigment analysis, lipids extraction and GC-MS analysis of fatty acids*

148 Total photosynthetic pigments (chlorophyll a and total carotenoids) were determined by
149 spectrophotometric measurements of methanol extracts, as described by Wetzel and Likens
150 [3]. Total lipids (TLs) were extracted from water and sediment samples using a
151 chloroform/methanol mixture [4]. The fatty acid methyl esters (FAMES) of the total lipids were
152 prepared by an acid-catalyzed transesterification procedure using 1% sulphuric acid in
153 methanol [5]. The FAMES were analyzed by gas chromatography–mass spectrometry (GC-MS),
154 according to the procedure of [58]. Identification of FAMES was achieved by comparing their
155 retention times with those of known standards (Bacterial Acid Methyl Ester, BAME Mix Cat. No.
156 47080-U, Supelco, Sigma Aldrich) and the resulting mass spectra to those in our database (NIST
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