

# THE SALINE ORGANIC-RICH SEDIMENTS: A SHORT OVERVIEW

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**ABSTRACT.** The saline organic-rich sediments: A short overview. Organic-rich sediments (or ‘sapropels’) are complex organo-mineral matrices that form at the O<sub>2</sub>-depleted bottom of stagnant water basins. Besides geochemical transformations, slow rate microbially-driven processes underpin the early diagenesis of these sediments. The fossil sapropels found in various marine or lacustrine environments are known as proxies for past climate or hydrological fluctuations. Present-day organic-rich sediments have been described both in fresh (‘gyttja’) and brackish or saline waters mostly in the boreal zones. They are currently used for agricultural or therapeutic purposes. Few saline lakes with significant deposits of modern sapropels are known worldwide. On Romanian territory, saline lakes with substantial organic-rich sediments are found in the Transylvanian Basin (e.g. Ursu Lake, Sovata) and in the South-Eastern part (e.g. the inland, athalassohaline Amara, and the coastal, thalassohaline Techirghiol lakes). The exploration of microbial diversity inhabiting the saline sapropels will improve our current understanding on the processes leading to formation and mineralization of organic-rich sediments of economic value.

**Keywords:** microbial dark matter, mud therapy, saline, sapropels, Ursu Lake

## 1. DEFINITION, COMPOSITION AND GENESIS

Although disputed among marine geologists, sedimentologists, petrologists, and limnologists, the term sapropels (Gr., *sapros* - rotten, *pelos* – soil) (Stein, 2014) is generally assigned to the dark-colored, fine-grained (<0.1 mm), organic carbon-enriched (>2% by dry weight) discrete sediments deposited in stagnant, stratified water bodies (Emeis, 2009; Kidd et al., 1978; Rossignol-Strick et al., 1982; Schnurrenberger et al., 2003; Stein, 2014). Structurally, sapropels are multicomponent biogenic systems comprising a matrix of organics such as humic substances, lipids, heteropolysaccharides, and proteins, mostly derived from detrital phytoplankton, zooplankton (Leonova et al., 2011; Yermolaeva et al., 2016) or terrestrial plant biomass (Bulgăreanu et al., 1981; Bulgăreanu et al., 1989) combined with fine particles of sedimentary minerals (silicates, carbonates). Thus, sapropels appear as instable, soft sludge buried on lakes’ or sea floors.

According to most credited theories, the sapropel formation is triggered in stagnant and stratified water basins with anoxic (and reducing) conditions where consistent communities of primary producers (eukaryotic and prokaryotic phototrophs) that presumably support significant zooplankton population. Sapropel

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settling is seemingly favoured in double-stratified water bodies with lower-density surface strata and denser bottom-waters (Rossignol-Strick et al., 1982; Wegwerth et al., 2018). Most of the water bodies that develop permanent density stratification are saline and they include (semi-) enclosed seas (i.e. such as Mediterranean, Baltic or Black seas) (Rossignol-Strick et al., 1982; Conley et al., 2009; Wegwerth et al., 2018) and lakes (Strakhovenko et al., 2016). In such water system, the water circulation is impeded, thus preventing oxygenation that ultimately leads to anoxia (Kidd et al., 1978). Subsequently, bottom water anoxia supports slow-growing organisms such as microaerophiles, fermenters or anaerobic-respiring microbes that rely on a metabolism driven by energetically less favourable electron acceptors (i.e. with lower Gibbs free energy yields) (Lever, 2012). As a consequence, organic matter is degraded at a low rate and might be preserved for prolonged period (Emeis, 2009). Based on their site of formation, one can distinguish between marine, coastal and inland (lacustrine) sapropels, while on formation time, sapropels may be secluded into recent (contemporary) or fossil ones.

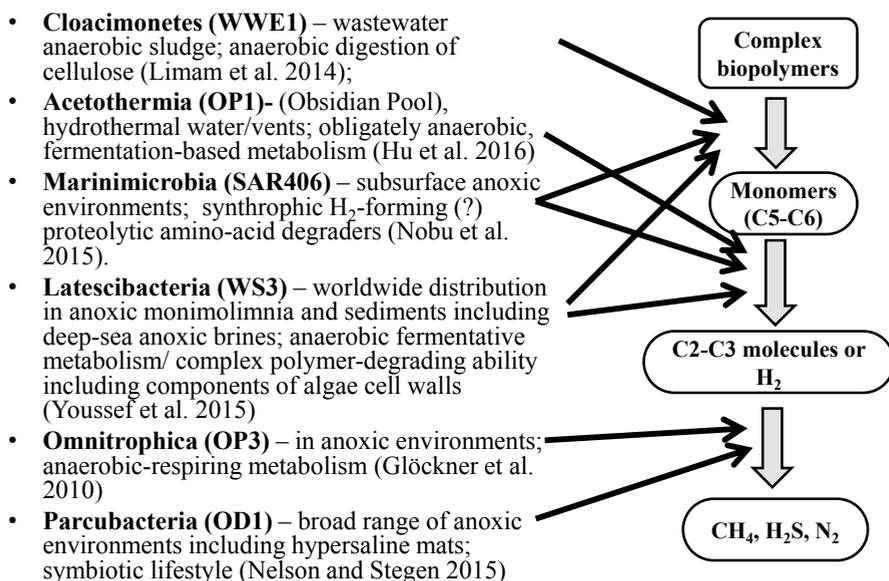
## 2. MICROBIAL DIVERSITY

Little is known on the structure and composition of microbial (including fungal) communities inhabiting sapropels and even less on their role in the biogeochemical cycling of major (C, O, N, P, S) and minor (Fe, Mn, Zn, etc.) elements leading to genesis of organic-rich muds. The overall observations point on the pivotal contribution of fungi (of Basidiomycota and Chytridiomycota) to the plant biomass decay in terrestrial and aquatic ecosystems (Bianchi, 2011). Active fungal communities dominated by Agaricomycetes (Basidiomycota) representatives were observed in anoxic and sulfidic sediments (thus resembling sapropels) associated with mangroves (Arfi et al., 2012). In the second line of decomposition lie prokaryotes that can be divided into primary degraders (that decompose recalcitrant animal and/or plant biopolymers such as chitins or hemicellulose into monomers) and secondary degraders that use monomers derived by first group into smaller molecules such as CH<sub>4</sub>, H<sub>2</sub>S, N<sub>2</sub>, etc. (Sorokin et al., 2015). A few attempts to reveal microbial diversity were conducted on ancient marine sapropels (Sass and Parkes, 2011) and fewer on recent, inland freshwater sapropels ('gyttja') (Stankevica et al., 2015).

One should distinguish between ancient marine sapropels that are assigned to subsurface biosphere, and inland, freshly-formed organic-rich sediments that are presently overlooked by systematic investigations. Eastern Mediterranean millenary-old sapropel samples harbored high cell counts ( $>10^7$  cells·cm<sup>-3</sup>), active culturable bacteria belonging to *Photobacterium*, *Brachybacterium*, *Halomonas* or *Alteromonas* genera, as well as DNA belonging to Chloroflexi phylum and candidate bacterial ('Atribacteria'/ JS1) and archaeal ('Bathyarchaeota'/ MCG, 'Lokiarchaeota'/ DSAG, 'Hadesarchaea'/ SAGMEG) lineages that are typical deep biosphere inhabitants (Parkes et al., 2014; Sass and Parkes, 2011). These 'deep' microbes are adapted to extreme nutrient (electron acceptor)-limitation suffering a 'substrate-accelerated death' when exposed to 'unnaturally' high (i.e. >milimolar)

substrate concentrations (Sass and Parkes, 2011). Members of yet-untapped microbial diversity were generically termed ‘microbial dark matter’ (abbrev. as MDM) (Rinke et al., 2013). It is speculated that the contribution of MDM to the global (and local) biogeochemical cycles of main and minor elements was largely underestimated (Rinke et al., 2013). As MDM is part of so-called ‘rare biosphere’, it escaped traditional tools employed for diversity investigation. Thus, by the aid of modern, high-throughput and/or highly sensitive techniques such as next-generation sequencing (NGS) their existence was only recently revealed. However, the systematic exploration of the abundant MDM in various environments is just at the beginning. By using small-subunit (SSU) ribosomal DNA metabarcoding approach, the diversity of microbial communities associated with sapropels in two hypersaline lakes from Transylvania (Central Romania) was revealed (Andrei et al., 2017). The two analogous sapropels collected from hypersaline Ursu and Fara Fund lakes at 3 and 2 m depth, respectively, appeared to harbor fifty-nine prokaryotic phyla with Proteobacteria, Bacteroidetes and Chloroflexi among the most abundant groups. Surprisingly, the SSU rDNA metabarcoding analysis of environmental samples further suggested that representatives of thirty-two candidate divisions and possibly undocumented prokaryotic lineages might be present in the hypersaline sapropels of both lakes. Among the most abundant MDM members detected were OP1 (‘Acetothermia’), OP3 (‘Omnitrophica’), OD1 (‘Parcubacteria’), WWE1 (‘Cloacimonetes’) and Parvarchaeota (Andrei et al., 2017). These microbes might be involved in the iron-cycling (Parvarchaeota, Chen et al., 2017), anaerobic digestion of refractory biopolymers such as cellulose (WWE1, Limam et al., 2014), fermentation of monomers (OP1, Hu et al., 2016) or might possess a symbiotic lifestyle (OD1, Nelson and Stegen, 2015) (Fig. 1). Overall, a more or less complete cycling of C, N and S seems to occur in these niches by an anaerobic microbial consortium that is turning over a plethora of carbon sources and electron donors. The large variety of carbon and energy sources might be the main factor driving unusual high diversity observed in these saline sapropels.

An unusual 14-m thick layer of contemporary organic-rich sediment was observed in the subtropical Mangrove Lake (Bermuda), a coastal, brackish (ca. 3‰ salinity) and polymictic lake fed by ocean water seepage (Hatcher et al., 1982). The sediment is topped by a 2-m water column. Phototrophic microbial communities were evidenced in a stratified pattern. The top 10 cm was dominated by cyanobacteria (*Microcystis* sp., *Aphanothece* sp., *Spirulina* sp.) seconded by sulfur-oxidizing bacteria (SOB), diatoms (*Navicula* sp.), purple (PSB) and green sulfur bacteria (GSB). In the next layer (10 to 30 cm depth), the PSB of *Chromatiaceae* family become more abundant alongside cyanobacteria and SOB, whereas at deeper layer, sulfate-reducing bacteria (SRB) prevailed (Stolz, 1991). Altogether, oxygenic phototrophic cyanobacteria as primary producers, SOB, PSB and GSB as reduced sulfur (i.e. H<sub>2</sub>S) oxidizers, and SRB alongside unidentified heterotrophs as anaerobic-respiring and fermenting bacteria, respectively, are apparently driving the full C and S cycling in these sediments (Stolz, 1991).



**Fig. 1. Putative metabolic capabilities of ‘microbial dark matter’ detected in hypersaline sapropels of Transylvanian salt lakes.**

### 3. BENEFITS

Whereas fossil sapropels are well-investigated as relevant palaeoceanographic, palaeoclimatic (Meyers, 2006), palaeohydrological (Oviatt, 2012) and palaeoecological (Principato et al., 2006) proxies, contemporary organic-rich sediments such as gyttja are exploited as fertilizers in farming or additives in livestock (e.g. in Baltic countries, Belorussia, Ukraine, and Russia; Stankevica et al., 2012; Stankevica et al., 2015). The Dead Sea “black mud” with low organic carbon contents (ca. 0.8%) is used in cosmetics and mud therapy (Abdel-Fattah and Pingitore, 2009) similarly to saline sapropels from Romanian lakes in Sovata, Amara or Techirghiol (Bulgăreanu, 1993). In addition, humic substances found in sapropels were shown to have anti-inflammatory, pro-angiogenic and antioxidant properties (Rensburg, 2015). When mud therapy (or ‘pelotherapy’; Gomes et al., 2013) is concerned, sapropels are renewable resource that may indirectly boost local economic prosperity through stimulating the healthcare and wellness tourism (Șerban and Alexe, 2006).

### 4. CONCLUSIONS AND PERSPECTIVES

In spite of their easy availability, contemporary sapropels are presently scarcely investigated. Assessment of diversity and functionality of microorganisms dwelling the O<sub>2</sub>-depleted organic-rich sediments of inland, stagnant and saline waters, is needed to: i) understand and model biogenic processes leading to sapropel formation in less documented lacustrine ecosystems; ii) reveal rare,

uncultured or novel microbial lineages with uncommon metabolic traits; iii) design strategies for durable preservation in areas where sapropels are valuable economic resources.

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