

Sapropel processing approaches towards high added-value products

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Abstract. Sapropel is an organic sediment from fresh water bodies that is widely distributed in the northern regions of the world. The distribution and unique properties of sapropel make it an important natural resource that can be used in agriculture, horticulture, forestry and farming directly as obtained. The aim of this study was to investigate the extraction possibilities of sapropel and potential applications of its extracts. Humic substances constitute an important ingredient of sapropel, and they can be extracted by mild alkaline extraction. Humic substances from peaty sapropel have significant differences in composition and properties, thus demonstrating the impact of precursor biological materials on the properties of humic substances formed in the humification process.

Key words: sapropel, extraction, agriculture, humic substances, biological activity.

INTRODUCTION

Sapropel (gyttja) is the bottom deposits of fresh water bodies. It contains more than 15% organic matter and, thus, is a partially renewable geological resource found in inland waters or under bogs. Sapropel is formed from the residues of algae, plankton and benthic organisms, although terrestrial vegetation can also contribute to its development (Niessen & Sturm, 1987). Depending on the composition and properties of sapropel and the availability of resources, it can be used in a number of areas, such as agriculture, medicine, veterinary medicine, construction, livestock farming, balneology and cosmetic applications.

Sapropel consists of three main components that interact with each other: organic and mineral elements as well as living organisms. The average elemental composition of the organic matter of sapropel (%) is: C 60.0, H 6.0, N 2.5, O 35.0 (Poznyak & Rakovskiy, 1962). The nitrogen content of sapropel is not directly related to its mineralisation degree (Braksh, 1971); in different types of sapropel, it ranges from 2.7% to 6% of organic substances and 0.5% to 4.0% of dry weight (Ponomareva, 2002).

Sapropel contains various groups of chemical compounds (Poznyak & Rakovskiy, 1962): a) bitumens, b) water-soluble substances, c) easily hydrolysable substances (including humic and fulvic acids), d) cellulose, e) non-hydrolysable substances.

Comparing the groups of chemical compounds in sapropel, it was established that the groups of substances (humic acids, non-hydrolysable substances, etc.), according to their chemical nature from different sites, are not identical and depend on the properties of sapropel-forming organisms (plankton, vascular plants, humic substances) and their transformation conditions (Braksh, 1971). Bitumens (lipids) are organic substances that are extracted from sapropel with a variety of organic solvents. The composition of bitumen is characterised by fatty acids, steroids, carotenoids, paraffin, wax and glycerol content (Orlov et al., 1996).

The bitumen components of sapropel attract particular attention, because they have a high bactericidal, bacteriostatic and antioxidant activity. Several studies have focused on the easy and efficient methods to obtain these substances from sapropel (Kireycheva & Khokhlova, 2000; Shinkarev et al, 2000). The bitumen content in sapropel is 2–7% of the organic mass (Lopotko et al., 1992). Moreover, when using a gasoline and alcohol-benzene mixture for extraction, even ~10% of bitumens were obtained (Poznyak & Rakovskiy, 1962).

Sapropel is characterised by low carbohydrate content, because active decomposition of carbohydrates to carbon dioxide and humification (formation of humic substances in the reactions of amino acid condensation) take place during the formation of sapropel. The average content of hemicellulose in the organic matter of sapropel is 6–25%, of cellulose – 1–8% (Pidoplichko & Grishchuk, 1962). The components of sapropel contain an average of 1–2% cellulose. The carbohydrate complex of sapropel, in turn, consists of $\geq 80\%$ of hemicellulose (Lopotko et al., 1992).

The water-soluble vitamins have also been found in sapropel, including ascorbic acid (C) and B group vitamins: thiamine (B1), riboflavin (B2), pantothenic acid (B5), pyridoxine (B6), folic acid (B9) and cyanocobalamin (B12). Large quantities of fat-soluble vitamins – tocopherol (E) and vitamins D and P – have also been found (Shtin, 2005).

The composition and properties of humic substances in sapropel are determined by its key features like biological activity, biochemical stability, binding ability, etc. They contain humic acids, fulvic acids and humine. The humic substances in sapropel differ from those in soil. The former have a higher carbon/hydrogen ratio and have no saturated aromatic rings (Orlov et al., 1996). They are also more reduced and possess a greater activity than the humic substances in soil.

Humic substances are commonly extracted from the minerals and organic compounds in sapropel following the classical scheme of Tyurin, which he used for soils (Orlov et al., 1996). Humic acids make the largest group of organic substances, and they are usually extracted from sediments with alkaline solvents and precipitated into an acid environment (pH 1–2).

Although this method is simple in use, it does not provide for a complete extraction of all low-molecular-weight components (carbohydrates, alcohols, amino acids); therefore, depending on the investigated material and purposes, this scheme is often modified (Karpukhin, 1998; Kireycheva & Khokhlova, 2000).

The aim of this study is to investigate the extraction possibilities of sapropel in respect to the potential applications of sapropel extracts.

MATERIALS AND METHODS

Sapropel samples were taken from Lake Pilvelis in Latgale region, Latvia. The samples were obtained with an Eckman drag and stored at 4 °C until analysis. Identification of the type sapropel and its biological analysis were done by a microscope (400–1,000 magnification), using known methods (Bellinger & Sigee, 2011). The loss-on-ignition was determined by heating sapropel samples at 550 °C and 950 °C (Heiri et al., 2001).

Preparation of the extracts of sapropel

A 5.00 g sample of dried (at 105 °C to constant weight) sapropel in a 120 mL flask was extracted for 24 hrs on a shaker (Bio San PSU-20, Latvia) with NaOH and KOH solutions at the concentrations of 0.1%, 0.5%, 1%, 2%, 4% and 8%. After treatment, the extracts were filtered, and sorption at 410 nm was measured (Hach Lange DR 2800). The extract was acidified with conc. HCl to pH 2 and filtered after 24 hrs to estimate the concentrations of humic acids (precipitate) and fulvic acids (soluble phase). A calibration graph was accordingly drawn, using the reference humic and fulvic acids. The ratio of the humic and fulvic acid yield was calculated as a dry weight (in mg) ratio. Treatment with ultrasound was done using 0.27 kW and 3 kW ultrasound baths, respectively.

Characterisation of sapropel extracts

The Fourier transform infrared spectra (FTIR) were obtained on a Nicolet AVATAR 330. The elemental composition (C, H, N, S) of humic substances in sapropel was determined using an Elemental Analyzer Model EA-11008. The concentration of total polyphenolics was determined by the Folin-Ciocalteu colorimetric method (Siriwoharn et al., 2004). The radical scavenging activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Aldrich). The mixture was incubated for 20 minutes in a dark place at room temperature. The absorption was measured in a quartz cuvette ($d=1$ cm) with a spectrophotometer (Hach-Lange DR 2800) at a 517 nm wavelength. Three parallel measurements were made. The radical scavenging activity was expressed in millimoles of Trolox equivalent in respect to 1 gram of dry extract mass (mM Trolox eq/g) (Siriwoharn et al., 2004). The metal-binding capacity of the sapropel extracts was estimated potentiometrically using a Cu^{2+} ion selective electrode (Shi et al., 2018). 5 mg samples of the humic substances of sapropel and a 20 mL solution containing 1–100 mg L^{-1} Cu^{2+} were added into a 50 mL centrifuge tube and shaken at 200 rpm overnight. The concentrations of Cu^{2+} in the solutions were measured by a ion-selective electrode, and the absorption capacities were calculated as differences between the initial and equilibrium concentrations of metal ions per gram of humic acid.

RESULTS AND DISCUSSION

According to the pollen analysis, the studied samples of sapropel are rich in *Betula* (up to 42%), *Pinus* (26%) and *Picea* pollens. The share of cultivated plants, such as *Secale cereale*, in the area reach about 1.5%. The dominant species of macrofossils include *Potamogeton natans*, *Nymphaea alba* and *Characeae*. *Daphnia* and *Bryozoa* occurred in small quantities. The organic matter of gyttja in Lake Pilvelis is characterised

by low content of carbon (45%) and high content of oxygen (48%), nitrogen (2.5%) and hydrogen (5.3%). According to sediment types, the studied samples belong to peaty sapropel. The loss-on-ignition analysis indicates that the organic matter in sapropel makes 82.86%, mineral matter – 15.94%, and carbonates – 1.18% (on dry matter basis). The pH of sapropel samples is nearly neutral – 7.19.

Humic substances belong to the group of substances that influence the application possibilities of sapropel extracts. Alkali metal hydroxides have traditionally been used for the extraction of these substances. As demonstrated in Fig. 1, extraction with KOH gives significantly higher yields in comparison with NaOH.

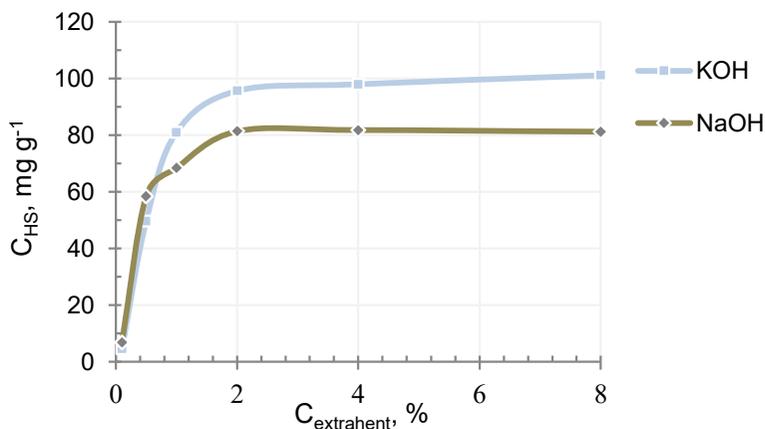


Figure 1. Yields of humic substances obtained from sapropel depending on the type and concentration of an extrahent.

An important parameter influencing the properties of sapropel is the relation (ratio) between humic and fulvic acids (Fig. 2). Humic acids dominate in the studied sapropel samples. However, when the extraction is done with concentrated alkali solutions, the HA/FA ratio is likely to decrease as a result of destruction of humic acids and reduction in their molecular mass (transformation of humic acids into fulvic acids).

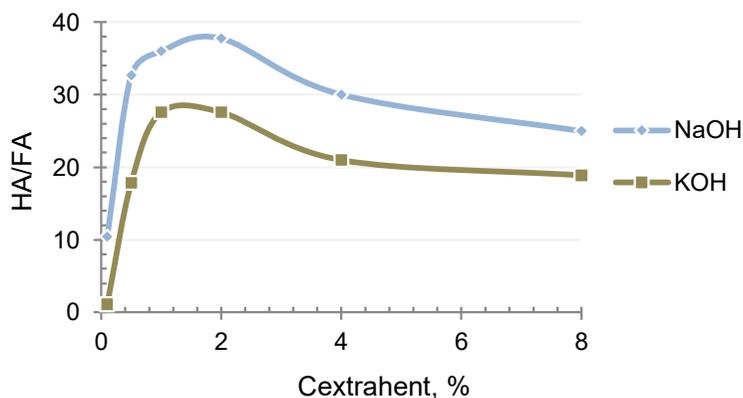


Figure 2. Changes in the humic acid/fulvic acid ratio depending on the extrahent concentration and type.

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A major factor influencing the extraction efficiency is the intensity of treatment during the extraction process. While maceration gives low yields of extracts, intensive treatment with ultrasound, especially at a high power and additional heating, gives high yields. Notably, in the latter case, a possibly major factor contributing to the solubilisation of sapropel is the chemical and mechanochemical destruction of its detrital particles (Fig. 3). At the same time, the intensive extraction methods provide for high yields of extracts for diverse applications.

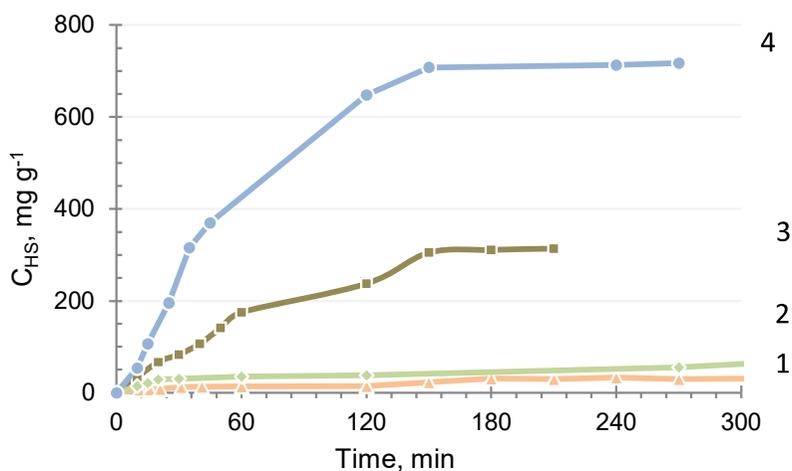


Figure 3. The impact of extraction methods on the yield of humic substances. 1 – maceration; 2 – shaking (140 rpm); 3 – treatment with ultrasound (0.27 kW); 4 – treatment with ultrasound (3 kW) and heating (80 °C).

The obtained extracts were characterised by their elemental composition: C 54.02–54.87%, N 2.01–4.07%, H 4.87–6.54%, O 38.10–42.48%, S traces. The loss-on-ignition analysis shows that the obtained sapropel extracts fully consist of organic matter (98.72%). The concentration of nitrogen in the sapropel extracts can be considered as high and might support the application of such extracts in agriculture.

The FTIR spectra of humic and fulvic acids in sapropel can be split by regions, depending on the informativity and the presence of important functional groups. The absorption bands in the spectral region of 3,600–2,800 cm^{-1} are very broad. The absorbance in this spectral region is determined by the presence of -OH groups. Sorption at the wavelengths of 2,950 and 2,850 cm^{-1} identifies the presence of CH_3 - and CH_2 -groups. Typical intensive sorption lines are common for the region around 1,700 cm^{-1} (1,725–1,700 cm^{-1}), which is characteristic for carbonyl groups in aldehydes, ketones and carbonic acids. The actual sorption maximum greatly depends on the conjugation degree, presence of substituents, and hydrogen bonding. In the spectral region of 1,690–1,500 cm^{-1} , it is possible to identify the sorption maximum of amide bonds (1,620–

1,640 cm^{-1} and 1,550–1,540 cm^{-1}). In the region of 1,625–1,610 cm^{-1} , the sorption indicates the presence of aromatic C = C and carbonyl groups, and hinones. At the wavelengths of 1,470–1,370 cm^{-1} , there are bands typical for C-H and O-H bonding and sorption maximums typical for C-O. For wavelengths below 1,000 cm^{-1} , fingerprint patterns are evident. The sorption in this spectral region provides information about a possible role of the share of carbohydrates in the structure of humic molecules. Sorption at 1,080 cm^{-1} shows the OH deformation or C-O stretch of phenol and alcohol OH groups, and 1,040 cm^{-1} indicates the C-O stretch of polysaccharide components. The major differences between humic and fulvic acids are visible at the signals of 1,590–1,700 cm^{-1} and 1,125–1,045 cm^{-1} and are related to the differences in the number of carboxylic groups and organic amides, as well as carbohydrates. The FTIR spectra demonstrate the presence of a high number of different oxygen-containing functional groups.

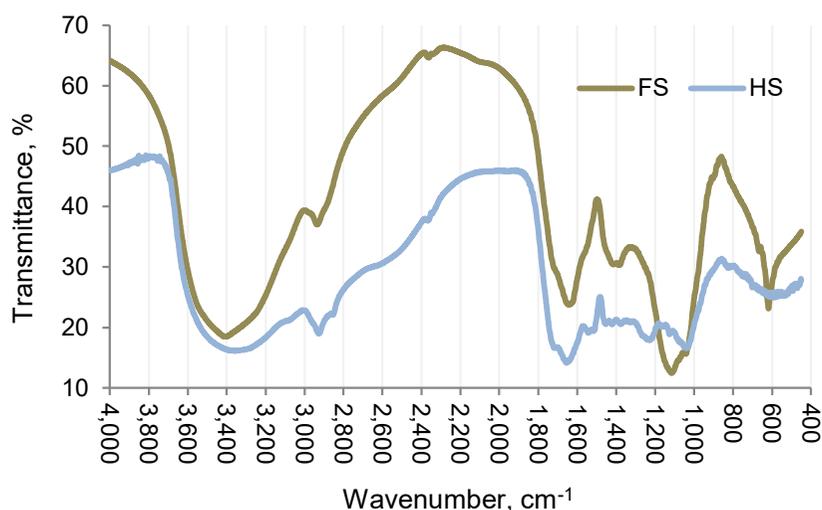


Figure 4. The Fourier transform infrared spectra of the humic (HS) and fulvic (FS) acids in sapropel.

An important characteristic of the extracts of sapropel is their ability to scavenge free radicals. For the analysis of the radical scavenging activity, we used the stable free radical DPPH, which has an intense purple colour that can be measured spectrophotometrically. In the presence of compounds that are capable of either transferring an electron, or donating hydrogen, DPPH will become discoloured. In this fashion, the change in DPPH absorbance after the addition of a test material is often used as an indicator of the antioxidant capacity of the material. The extracts of sapropel have a relatively high radical scavenging activity (7.17–13.20 mM Trolox eq g^{-1}). The detected radical scavenging activity of the extracts of sapropel can be compared with that of ascorbic acid (Vitamin C, a well-known antioxidant): 13.9 ± 0.7 7.17–13.20 mM Trolox eq g^{-1} ; cranberry extracts: 15.5 ± 0.8 7.17–13.20 mM Trolox eq g^{-1} . The radical scavenging activity of sapropel extracts is much higher than the activity of humic substances extracted from peat (0.2–3.1 mM Trolox eq g^{-1}) or soil

(1.8–2.2 mM Trolox eq g⁻¹), indicating a possible field of application of sapropel extracts as antioxidants in cosmetics and biopharmacy. The total concentration of polyphenolics in sapropel extracts is between 0.67 and 1.03 mg gallic acid equivalent/g of extract and so can be considered as high, at least compared to peat humic acids, where the concentration of polyphenolics is significantly lower (0.02 mg gallic acid equivalent g⁻¹).

An important parameter characterising humic substances is their metal-binding capacity (Evangelou et al., 2002; Shi et al., 2018). The maximum metal-binding capacity of humic substances in sapropel is 9.48 ± 0.12 ; and for fulvic acids – 12.21 ± 0.08 mM of Cu²⁺ g⁻¹ humic substances. The metal-binding capacity for humic substances in sapropel detected in our study is higher than the values found in the literature describing the humic substances of other origins. The studied metal-binding properties of humic acids in sapropel indicate their application potential for detoxification (metal binding). Other potential contributions could be for the use of sapropel extracts in agriculture (possibilities of use in metal-contaminated soils), cosmetics and biopharmacy (metal binding).

CONCLUSIONS

Sapropel is an organic sediment from fresh water bodies and is widely distributed in the northern regions of the world. The aim of the study was to investigate the extraction possibilities of sapropel and potential applications of its extracts. An important ingredient of sapropel is humic substances, and they can be extracted by mild alkaline extraction. The humic substances from peaty sapropel demonstrate significant differences in composition and properties from the humic substances of other origins (peat, soil, etc.), thus indicating the impact of precursor biological material on the properties of humic substances formed during the humification process. The alkali extracts of sapropel samples from a Latvian lake were studied. The solution of potassium hydroxide at a concentration not exceeding 2 wt% extracted the coloured humic substances more completely than the solution of sodium hydroxide. The maximum extraction was determined as 100 mg g⁻¹. Treatment with ultrasound (at 3 kW) and heating (at 80 °C) enhanced the extraction up to 700 mg/g. The scavenging activity of the prepared extracts was determined as the reaction of inhibition of the generation of radicals by DPPH. The humic substances in sapropel have a significant radical scavenging activity and a distinct metal-binding capacity. Therefore, the extraction of sapropel can contribute to the development of high added-value products with diverse application possibilities.

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REFERENCES

- Bellinger, E.G. & Sigee, D.C. 2011. *Freshwater Algae: Identification and Use as Bioindicators*. Chichester: Wiley, 265 pp.
- Braksh, N. 1971. *Sapropel sediments and application possibilities*. Zinatne, Riga, 282 pp. (in Russian).
- Evangelou, V.P., Marsi, M., Chappell, M.A. 2002. Potentiometric-spectroscopic evaluation of metal-ion complexes by humic fractions extracted from corn tissue. *Spectrochimica Acta PtA* **58**, 2159–2175.
- Heiri, O., Lotter, A.F. & Lemcke, G. 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. *Journal of Paleolimnology* **25**, 101–110.
- Karpukhin, A. 1998. Complex Compounds of Humic Substances with heavy Metals. *Eurasian Soil Science* **31**(7), 764–771.
- Kireycheva, L. & Khokhlova, O. 2000. The elemental composition of sapropel humic substances. *Soil Science* **9**, 1–376 (in Russian).
- Lopotko, M., Yevdokimova, G. & Kuzmitskiy, P. 1992. *Sapropelits in agriculture*. Science and Technology, Minsk, 1–215 (in Russian).
- Niessen, F. & Sturm, M. 1987. Die Sedimente des Baldeggersees (Schweiz): Ablagerungsraum und Eutrophierungsentwicklung der letzten 100 Jahre. *Archiv für Hydrobiologie* **108**, 365–378.
- Orlov, D., Biryukova, O. & Sukhanova, N. 1996. Soil organic matter of the Russian Federation. *Soil Science* **2**, 197–207 (in Russian).
- Pidoplichko, A.P. & Grishchuk, R.I. 1962. Certain Results of research of Belarussian SSR sapropel deposits. *Chemistry and genesis of peat and sapropel*, 258–274. (in Russian).
- Ponomareva, M.A. 2002. *Chemical composition and application of sapropel in Tatarstan*. Doctoral Thesis. Tula State Pedagogical University, Tula, 245 pp. (in Russian).
- Poznyak, V. & Rakovskiy, V. 1962. The chemical composition of the organic mass of Byelorussian SSR sapropel. *Chemistry and genesis of peat and sapropel*, 298–308. (in Russian).
- Shinkarev, A., Lyutakhina, N. & Gnevashov, S. 2000. Separation of the Groups of Humic Substances Upon Recurrent Treatment with Solvents. *Eurasian Soil Science* **33**(7), 709–712.
- Siriwoharn, T., Wrolstad, R.E., Finn, C.E. & Pereira, C.B. 2004. Influence of cultivar, maturity, and sampling on blackberry (*Rubus* L. Hybrids) anthocyanins, polyphenolics, and antioxidant properties. *Journal of Agricultural and Food Chemistry* **52**(26), 8021–8030.
- Shi, W., Lu, C., He, J., En, H., Gao, M., Zhao, B., Zhou, B., Zhou, H., Liu, H. & Zhang, Y. 2018. Nature differences of humic acids fractions induced by extracted sequence as explanatory factors for binding characteristics of heavy metals. *Ecotoxicology and Environmental Safety* **154**, 59–68
- Shtin, S.M. 2005. *Lake sapropel and their complex development*. MSMU, Moscow, 373 pp. (in Russian).