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Geochemical study of peat in the function of balneological characteristics of peloids

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Abstract: The *Dürnberger* Moor peat in the *Zirbitzkogel-Grebenzen* National Park area, located near the settlement of Sankt Lambrecht, Austria, was investigated. Organic geochemical approaches, which are most commonly used in studying the origin and geological history of various types of organic substances in sedimentary rocks, were applied to explain its potential peloidal properties, i.e. to assess its application in balneology. The content of total organic matter, and the content of fulvic and humic acids were first determined in the samples. The group composition (percentage content of saturated hydrocarbons, aromatic hydrocarbons and NSO, nitrogen, sulfur, oxygen compounds) was determined in the extracted bitumen. In the isolated saturated hydrocarbon fraction, *n*-alkanes, isoprenoids, terpanes and steranes were analyzed by GC-MS technique (SIM method). In addition to β-amyrin, an organic compound with proven therapeutic properties, the tested samples contain humic substances of the type fulvic and humic acids. Specific organic geochemical parameters showed a very low degree of thermal maturity of the organic substance and provided evidence that its humification had not yet reached the level of humin. It was concluded that the tested peat can be used as a geological material for the formation of peloids, and thus as a means in balneotherapy.

Keywords: natural materials; therapeutic properties; humic substances; biomarkers; maturation.

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INTRODUCTION

Peat is defined as coal of the lowest degree of carbonization. On that carbonization row, above it are lignite, then older brown coals, hard coals and finally, as the most mature, anthracite.[1,2] The organic substance of coal differs from oil in the first place according to the type of precursor biomass. Oil is mainly of marine origin, and coal contains an organic substance that is formed by the deposition and humification of a large amount of extinct autochthonous terrestrial plant material, products of the primary photosynthetic production of higher plants, coastal swamp, shrubby and woody vegetation, and possibly some algal remains, deposited spores and pollen and other resistant plant allochthonous material.[1,2]

Microbiological degradation of lignin and cellulose, as the dominant macromolecular compounds of higher plants, produces their monomers, or possibly oligomers. Glucose is produced from cellulose as a metabolite, and various phenolic compounds from lignin. The part of these geomonomers, or oligomers, that microorganisms do not further decompose to even smaller inorganic molecules and methane, polymerizes to fulvic- and humic acids. This is an organic substance that is soluble in alkaline aqueous solvents. Further insolubilization processes produce an organic molecule of a higher degree of macromolecularity, called humin. Due to its higher degree of macromolecularity, it is insoluble. This completes the process of carbonization at the peat level. Everything takes place at shallow depths in processes in which microorganisms play a dominant role. In organic geochemical literature, this phase is called early diagenesis. At the surface, under oxidative conditions, processes occur rapidly, and later, after being covered by new plant material and standing water, the environment becomes predominantly reducing. At the surface, the environment is neutral or slightly acidic, and the pH decreases, and thus the environment becomes increasingly acidic at increasing depth. Depending on the chemical conditions, different types of bacteria and fungi develop. Microbiological activity decreases with sediment depth, and the biomass composed of bacterial and fungal remains, together with their non-gaseous metabolites, becomes part of the overall organic material.[3]

Tectonic processes such as the formation of depressions, graben structures, or some other subsidence areas also contribute to the formation of peatlands. Depending on the type of precursor plant material, geological environment, water suply, water capacity, and degree of decomposition, peats are divided into high (ombrotrophic), low (minerotrophic) and transitional.[4,5]

Peat is light brown to dark brown in color. Freshly extracted peat contains over 70 % moisture, and air-dried peat contains 15-40 %. Ash varies within wide limits (1-20 %). The lower calorific value of completely dry peat is about 21000 kJ/kg, and air-dried peat up to 16000 kJ/kg. Peat is of local importance as a fuel. Only in some places in the world is it used as fuel in thermal power plants. It can

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also be used as an organic fertilizer, because it contains humic substances that have a stimulating effect on plant development.[2]

Recently, there has been increasing talk about the therapeutic effects of peat. Natural materials used for therapeutic purposes are called peloids, or "healing mud". They are obtained by mixing water with appropriate geological material, which leads to the so-called maturation process[8-10]. If peat is used as a geological material, "peloid peats" are obtained. They are also used in balneology.[6,7] They are most often of the transitional or low type[4]. They are characterized by a relatively high concentration of humic substances [6,11,12]. Thus, it is known that peloid peats with an increased amount of plant steroids and fatty acids improve skin permeability and hydration, making them effective in the treatment of skin diseases.[13,14]

In this paper, peat from an Austrian deposit was examined with the idea that organic geochemical approaches that are most commonly used in studying the origin and geological history of various types of organic substances in sedimentary rocks of the lithosphere can also be used to explain their potential peolidic properties, i.e. applications in balneology.

EXPERIMENTAL

Two peat samples were examined at the *Dürnberger* Moor peat site in the *Zirbitzkogel-Grebenzen* National Park area, located not far from the settlement of Sankt Lambrecht, Austria. The peat belongs to the type of High-moor peat. The thickness of the *Dürnberger* moor is 8.5 m and is one of the thickest peat deposits in Europe. The dominant vegetation in the peatland zone is peat moss, *Sphagnum mosses*, and marsh rosemary, cranberry and other marsh grasses can also be found. The peatland is characterized by anaerobic conditions and suitable hydrogeological parameters for the formation of this type of sediments.

In this paper, the samples are designated as T_1 and T_2. Sample T_1 was taken from a depth of 0.5 m, and sample T_2 from a depth of 1.5 m. They have a similar physical appearance and dark, brown-black color.

Natural and hygroscopic moisture was firstly determined from the samples. Afterwards, the content of total organic matter, and the content of fulvic- and humic acids was determined. The bitumen fraction was extracted, in which the group composition was determined (percentage content of saturated hydrocarbons, aromatic hydrocarbons and NSO, nitrogen, sulfur, oxygen - compounds). Finally, the isolated saturated hydrocarbon fraction was analyzed at the molecular level. Both samples were analyzed in duplicate for every parameter to ensure analytical precision.

Natural and hygroscopic moisture content

Natural moisture content was determined by drying the original samples at 105 °C for at least two hours until a constant mass was reached. The mass difference before and after drying represented the natural moisture content. Hygroscopic moisture content was determined in the same manner, using air-dried samples, where the change in mass indicated the hygroscopic moisture level.

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Total organic matter

To determine the total organic matter content, the hygroscopic moisture was first removed from the air-dried samples by drying at 105 °C until a constant mass was reached. The samples were then calcined at 440 °C. The difference in the mass of the dry sample and the resulting ash represented the mass of total organic matter.

Total fulvic- and humic acids

The content of fulvic and humic acids was determined in air-dried samples using the standard method ISO 5073-2021. The values obtained were then recalculated to the dry sample.

Approximately 0.2 g of the air-dried sample was weighed into an Erlenmeyer flask (measurement accuracy ± 0.2 mg). 150 cm^3 of alkaline pyrophosphate solution was added. The Erlenmeyer flask with the solution was heated in a water bath until the peat sample was completely "wetted". After cooling, the solution was transferred to a normal vessel. It was diluted with distilled water to a volume of 200 cm^3. It was then filtered. The first 10 cm^3 was discarded, and the remainder was used to determine the total content of humic acids. The filtered sample extracts are oxidized with a potassium dichromate solution (15 cm^3, concentration 0.4 mol/dm^3) and concentrated sulfuric acid while heating in a water bath. After cooling, the samples are diluted with distilled water to a volume of 100 cm^3. 1,10-phenanthroline is added as an indicator and the solution is titrated with ammonium iron(II) sulfate solution to a dark brown color. A blank test is also performed at the same time, consisting of a solution of sodium pyrophosphate, potassium dichromate and sulfuric acid. The total content of humic acids is determined from the following expression:

$$W_{HA} = [(V_0 - V_1) \times 0.003 \times C] / [0.59 \times m] \times [V_e/V_a] \times 100 \quad (1)$$

(*V_{HA} – mass content of total humic acids; V_0 – consumed volume of ammonium iron(II) sulfate in cm^3 for the blank test; V_1 – consumed volume of ammonium iron(II) sulfate in cm^3 for the sample; C – concentration of ammonium iron(II) sulfate in mol/dm^3; V_e – volume of extract; V_a – volume of aliquot taken for titration; m – mass of sample taken for analysis in grams*)

Bitumen fraction (extract)

From air-dried samples, which were then ground in an agate mortar, the soluble (bitumen) fraction was isolated by the Soxhlet method. Around 250 mL of an azeotropic mixture of methylene chloride and methanol (88:12, v/v) was used as the solvent. The extraction lasted 36 hours.

Group composition

In order to determine the group composition of the extract, i.e. to separate the organic extracts into three fractions of increasing polarity (saturated hydrocarbon fraction → aromatic hydrocarbon fraction → NSO fraction), column chromatography was performed. Silica gel, previously activated at 180 °C for 24 hours, was used as the stationary phase. Pentane, in portions of 3 × 1.8 cm^3, was used to elute the saturated hydrocarbon fraction, and a mixture of dichloromethane and pentane (7:3), in portions of 5 × 1.8 cm^3, was used for the aromatics. In this way, the percentage composition of these fractions in the bitumen extract was also determined. The content of the third, polar, NSO fraction was determined from the difference in the mass of bitumen applied to the column and the sum of the masses of saturated and aromatic fraction.

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Saturated hydrocarbons

The fractions of saturated hydrocarbons were analyzed by gas chromatography-mass spectrometry (GC-MS). An Agilent 7890A gas chromatograph with a 30 m × 0.25 mm HP5-MS capillary column and a 0.25 μm film thickness of the stationary phase, polydimethylsiloxane, was used. Helium was used as the carrier gas with a flow rate of 1.5 cm^3/min. An Agilent 5975C mass detector (70 eV, ion source temperature 230 °C, mass analyzer temperature 230 °C) was used for compound detection. The injector temperature was 250 °C. The column was heated from 80°C to 300°C at a rate of 2°C/min, followed by isothermal heating for 20 minutes at 300°C and one minute at 310°C. The Full Scan method and Single Ion method were used for peak identification. Fragmentograms *m/z* 71, *m/z* 191 and *m/z* 217 were obtained analyzing saturated hydrocarbon fractions of bitumen peat samples.

RESULTS AND DISCUSSION

Group parameters

The values of the "group" parameters (natural moisture content - NM, hygroscopic moisture - HM, total organic matter - TOM, ash content, total fulvic- and humic acid content - FA & HA, bitumen content and group composition of bitumen) for the two peat investigated samples (with indicated depths) are shown in Table I.

TABLE I. Values of "group" parameters for the investigated peat samples

Sample (with depths, m)	^NM (%)	#HM (%)	*TOM (%)	Ash content (%)	*FA & HA (%)	Bitumen (%)	Saturated fraction (% of bitumen)	Aromatic fraction (% of bitumen)	**NSO compounds (% of bitumen)
$T_{1\ (0.5)}$	85.41	6.42	87.82	12.18	18.57	3.28	1.83	3.67	94.50
$T_{2\ (1.5)}$	84.64	8.09	83.75	16.25	28.11	1.81	1.96	3.92	94.12

^Relative to original sample; #Relative to air dried samples; *Relative to dry sample; **Nitrogen, Sulfur, Oxygen - compounds are calculated from the difference

The content of total organic substances in both samples is within the theoretical range for peat.[1,2] In the deeper sample, T_2, it is slightly lower (83.75 % vs. 87.82 %, Table I) as a result of more intensive, i.e. longer, biodegradation of organic substances. Thus, in this sample, a larger part of the geomonomers was transformed into "small" molecules, including methane, and returned to the biosphere.

Both samples meet the classification requirements for peat as a peloid, based on their natural moisture content, ash content, and humic substance content. Humic substances, especially humic acids, are known for their antioxidant, anti-inflammatory, antibacterial, and antifungal effects. As biochemically active substances abundant in peat, they can help treat skin disorders, musculoskeletal and rheumatic conditions.[6,7]

Sample T_2, because it comes from a greater depth, is at a higher level of humification and as a result of this fact has a higher amount of humic substances,

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i.e. fulvic- and humic acids (28.11 % vs. 18.57 %, Table I). Within the total organic matter (83.75 and 87.82 %, Table I), the humic substances is relatively small. Based on these results alone, the theoretical question remains open as to whether the remaining, larger part of the organic matter is in the form of organic compounds that will yield fulvic- and humic acids during the humification process, or whether it is humin. In that case, the identified humic acids are those that lag behind in the polymerization process towards the formation of humin, as a form of organic matter of sedimentary rocks of a higher degree of macromolecularity. Molecular parameters (known in the organic-geochemical literature as "specific parameters")[1, 2], which are determined from the distribution and abundance of some biological markers of the saturated hydrocarbon fraction, should help answer this question.

This sample also has a lower content of bitumen, i.e. its part that can be extracted by a classical extraction method such as Soxhlet (1.81 % vs. 3.28 %, Table I). This is the so-called "free bitumen". In the deeper sample T_2, a larger amount of bitumen was incorporated into humic substances and thus became unavailable for extraction with an organic solvent.

The group composition of bitumen is almost identical for the investigated samples. The extremely low content of the saturated and aromatic hydrocarbon fractions, i.e. the distinct dominance of polar compounds (94.50 and 94.12 %, Table I) is logical, considering that the organic substance of peat, as a sedimentary rocks, is geologically extremely recent, and as such has a very low degree of thermal maturity.

Molecular (specific) parameters

Fig. 1 shows the fragmentograms of *n*-alkanes and isoprenoid alkanes of the saturated hydrocarbon fractions of bitumen from peat samples T_1 and T_2 (*m/z* 71). The appropriate parameters are given in Table II.

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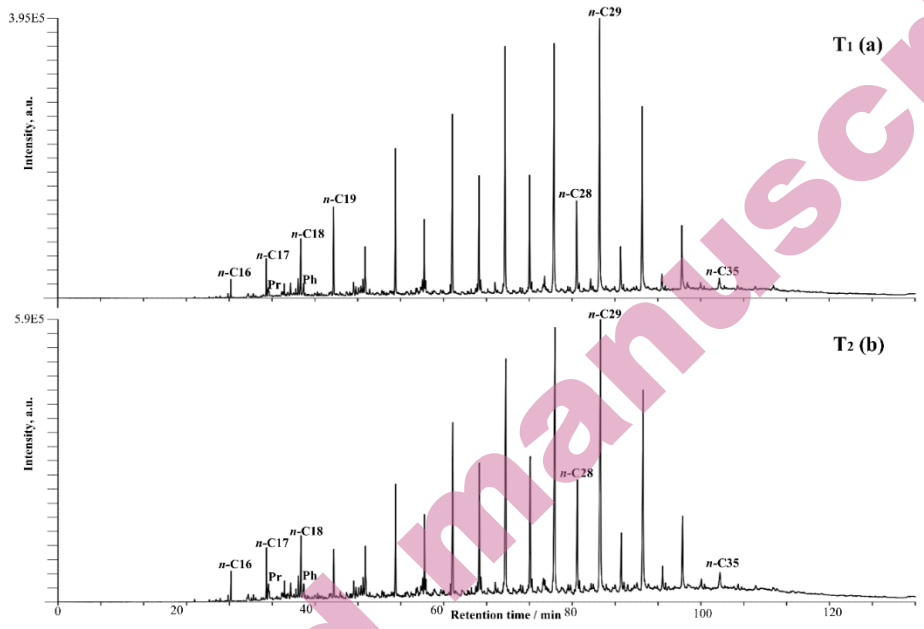

Fig. 1. Fragmentograms of *m/z* 71 ion obtained by GC-MS analysis of saturated hydrocarbon fraction of peat samples T_1 (a) and T_2 (b). *Pr – Pristane; Ph – Phytane*

TABLE II. Parameters calculated from the *m/z* 71 fragmentograms

Sample	Range of *n*-alkane	The most abundant *n*-alkane	*CPI	#Pr/*n*-C_{17}	^Ph/*n*-C_{18}
T1	C_{16}-C_{35}	C_{29}	4.30	0.20	0.18
T2	C_{16}-C_{35}	C_{29}	2.53	0.29	0.28

*Carbon Preference Index; #Pr/n-C_{17} – ratio of Pristane and normal hydrocarbon C_{17}; ^ Ph/n-C18 – ratio of Phytane and normal hydrocarbon C_{18}.

The Carbon Preference Index (CPI) and ratios of Pr/*n*-C_{17} and Ph/*n*-C_{18} are molecular parameters used to identify the depositional environment, source and thermal maturity of organic matter.[1,2] Both samples contain *n*-alkanes in the same ranges (C_{16}-C_{35}) and with the same most abundant member (C_{29}, Fig. 1, Table II). However, Sample T_2, which is from a greater depth, has a significantly lower value for CPI (2.53 vs. 4.30, Table II). This is a consequence of a higher concentration of even members of the homologous series of *n*-alkanes compared to the odd ones. This also means a higher degree of maturity of its bitumen compared to the bitumen of the sample from a smaller depth, T_1. The difference in depth of one meter between samples T_1 and T_2 in sedimentological conditions, when taking into account the entire diagenetic-catagenetic sequence of transformations of the organic part of the geosphere, is not significant [1, 2]. However, in the phase of early

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diagenesis, it is significant, and can contribute to maturation changes in bitumen that produce such significant changes in the distribution of *n*-alkanes.

The amount of isoprenoid alkanes pristane and phytane in the alkane fractions of both samples is very small (Pr and Ph, Fig. 2). It is known that they are mostly formed from phytol, an isoprenoid alcohol that is part of the chlorophyll molecule of autotrophic plants [1, 2]. In the early diagenetic phase, this process is just beginning, and therefore pristane and phytane occur only in traces in the examined alkane fractions. However, despite this, it is noticeable that their concentration in the deeper sample T_2 is somewhat higher (parameters Pr/*n*-C_{17} and Ph/*n*-C_{18} in Table II). This difference is also the result of the higher degree of maturity of T_2 compared to T_1, which contributed to the somewhat more intense transformation of phytol to these isoprenoid aliphatic hydrocarbons.

Fig. 2 shows the fragmentograms of terpane, *m/z* 191, and sterane, *m/z* 217, obtained by GC-MS analysis of saturated hydrocarbon fractions of samples T_1 and T_2 (SIM, Single Ion Monitoring method). In order to see the quantitative relationship between these polycyclic alkanes in the examined samples, overlayed fragmentograms are given.

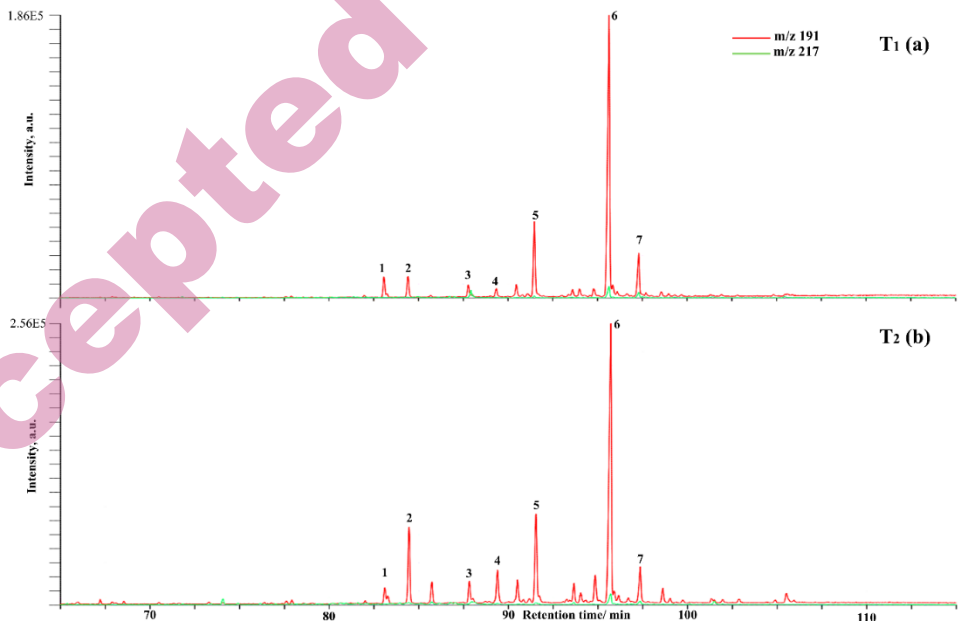

Fig. 2. Fragmentograms of *m/z* 191 (terpanes) and *m/z* 217 (steranes) ions, which were obtained by GC-MS analysis (SIM) of saturated hydrocarbon fractions of peat samples T_1 (a) and T_2 (b).

1 - C_{27} hopene 22,29,30-trisnorneohop-13(18)-ene; 2 – C_{27} 17β(H)-22,29,30-trisnorhopane; 3 – C_{29}-hopane, 28-nor-17α(H) hopane; 4 - C_{29} moretane, 17β(H)21α(H); 5 – C_{30} hopene, 17β(H)-hop-20(21)-ene; 6 – C_{30} diploptene, hop-22(29)-ene; 7 - β-amyrin

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he representation of terpanes in both samples is significantly higher than that of steranes (Fig. 2). It is a consequence of the bacterial origin of the organic substance settled in environments with low oxygen concentrations.[15-19]

In both samples, the terpane distribution is dominated by a peak of C_{30} hopene, diploptene (peak 6, Fig. 2). It originates from ferns, mosses, and lichens.[1,2] In addition to diploptene, C_{27} hopene, 22,29,30-trisnorneohop-13(18)-ene (peak 1, Fig. 2) was also identified in both samples. The presence of this isomer of hopene is evidence that the organic substance is in the stage of early diagenesis and intensive microbial decomposition. In the deeper sample T_2, peaks originating from C_{29}-hopane, 28-nor-17α(H) hopane and C_{29} moretane, 17β(H)21α(H) (peaks 3 and 4) were also identified and are more prominent. These are hopane isomers that are formed in sediments during maturation processes.[1,2] In this case, their presence is evidence that the sample at a greater depth is of a slightly higher degree of maturity than the T_1 sample at a shallower depth.

The presence of β-amyrin (oleanane structure, Fig. 3) in both samples (peak 7, Fig. 2) is evidence of residual plant material in the examined peats.[20] This isomer, as well as its relative α-amyrin, is known as a compound with therapeutic properties. They have anti-inflammatory effects, diabetes, atherosclerosis, Parkinson's disease. According to some authors, they also show antibacterial, anticancer and anti-HIV effects. They are often present in plants as glycosides and saponins. They have also been found in the surface layers of wax, on tomato and dandelion plants.[21-23]

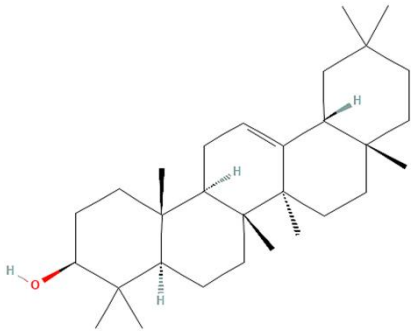

Fig. 3. Structure of β-amyrin identified in alkane bitumen fractions of samples T_1 and T_2.[24]

Based on the organic geochemical investigations of samples T_1 and T_2, it can be concluded that the examined peat can be used as a geological material for the formation of peloids, and thus as a means in balneotherapy. In addition to β-amyrin, an organic compound with proven therapeutic properties, it also contains humic substances of the type fulvic- and humic acids. Specific organic

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geochemical parameters determined from the distribution and abundance of biological markers, such as *n*-alkanes, isoprenoid aliphatic alkanes and polycyclic alkanes, terpanes, showed a very low degree of thermal maturity of the organic substance and provided evidence that its humification has not yet reached the level of humin.

CONCLUSION

The content of total organic matter in both investigated samples is within the theoretical range for peat. In the deeper sample, T_2, organic matter is slightly smaller (83.75 % vs. 87.82 %) as a consequence of a slightly more intense, i.e. longer, biodegradation of the organic substance.

Sample T_2 is also at a higher degree of humification with a slightly higher amount of fulvic- and humic acids (28.11 % vs. 18.57 %). The presence of these bioactive compounds gives us insight into peat's potential to be used as a peloid.

Sample T_2 contains less bitumen (1.81 % vs. 3.28 %). In it, a larger amount of bitumen was incorporated into humic substances and thus became unavailable for extraction with an organic solvent.

The extremely low content of alkane and aromatic fractions in the bitumens of both samples, i.e., the distinct dominance of polar compounds (94.50 and 94.12 %) is logical, since the organic substance of peat is geologically recent, and as such has a very low degree of thermal maturity.

The *n*-alkane distribution of sample T_2 is characterized by a significantly lower value for CPI compared to T_1 (2.53 vs. 4.30). This is a consequence of the higher degree of maturity and evidence that in conditions of diagenesis, a depth of even one meter (the difference in depths between T_1 and T_2) is of geochemical significance.

The amount of isoprenoid alkanes pristane and phytane in the saturated hydrocarbon fractions of both samples is very small. This is logical for peats where the transformation of phytol to these isoprenoids has just begun. In T_2, the concentration is slightly higher as a result of slightly higher maturity.

The presence of terpanes in both samples is significantly more pronounced than steranes, as a consequence of the bacterial origin of the organic substance settled in environments with low oxygen concentrations.

In both samples, the distribution of terpanes is dominated by the peak of the C_{30} hopene, diploptene originating from ferns, mosses and lichens. C_{27} hopene, 22,29,30-trisnorneohop-13(18)-ene was also identified in both samples as evidence that the organic substance is in the phase of early diagenesis and intensive microbiological decomposition. In sample T_2, peaks originating from C_{29}-hopane, 28-nor-17α(H) hopane and C_{29} moretane, 17β(H)21α(H) were more prominent as an evidence that it is a deeper sample and at a slightly higher degree of maturity.

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The presence of β-amyrin in both samples indicates residual plant material in the studied peats. This isomer and its relative, α-amyrin, possess known therapeutic properties. In geochemical contexts, their preserved structures serve as highly specific terrestrial biomarkers. These markers reflect historic higher-plant organic matter input and distinct environmental conditions during peat accumulation.

Based on the organic geochemical investigation of samples T_1 and T_2, it can be concluded that the examined peat from the *Dürnberger* Moor peat site in the *Zirbitzkogel-Grebenzen* National Park area, located not far from the settlement of Sankt Lambrecht, Austria, has geochemical value and the potential for balneological use. Along with β-amyrin, an organic compound known for its therapeutic benefits, the samples also contain humic substances, including fulvic and humic acids. From an organic-geochemical perspective, these complex macromolecules function as definitive indicators of early-stage organic transformation and localized redox conditions. This study suggests that peat could serve as a peloid, pending additional research in molecular bioactivity, toxicology, and clinical testing to confirm its suitability. Specific organic geochemical parameters showed a very low degree of thermal maturity of the organic substance and provided evidence that its humification had not yet reached the level of humin. This case study serves as groundwork for peat characterization, providing fundamental conclusions and explicit scientific contributions for future standardized method development for peloid characterization. In doing so, this research acts as a model for further studies to connect geochemistry with future interdisciplinary fields.

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И З В О Д

ГЕОХЕМИЈСКО ПРОУЧАВАЊЕ ТРЕСЕТА У ФУНКЦИЈИ БАЛНЕОЛОШКИХ КАРАКТЕРИСТИКА ПЕЛОИДА

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Испитиван је тресет из налазишта Дирнбергер Мор (*Dürnberger Moor*), у подручју Националног парка Цирбицкогел-Гребенцен (*Zirbitzkogel-Grebenzen*), који се налази у близини насеља Санкт Ламбрехт у Аустрији. Органскогеохемијски приступи, који се најчешће користе у проучавању порекла и геолошке историје различитих врста органских

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супстанци у седиментним стенама, примењени су да би се објаснила његова потенцијална пелоидна својства, односно да би се проценила његова примена у балнеологији. У узорцима је прво одређен садржај укупне органске материје, као и садржај фулвинских и хуминских киселина. У екстрахованом битумену је одређен групни састав (процентуални садржај засићених угљоводоника, ароматичних угљоводоника и *NSO*, једињења азота, сумпора, кисеоника). У изолованој фракцији засићених угљоводоника, *n*-алкани, изопреноиди, терпани и стерани су анализирани *GC-MS* техником (*SIM* метода). Поред β-амирина, органског једињења са доказаним терапеутским својствима, тестирани узорци садрже хуминске супстанце типа фулвинских и хуминских киселина. Специфични органскогеохемијски параметри показали су веома низак степен термичке зрелости органске супстанце и пружили доказе да њена хумификација још није достигла ниво хумина. Закључено је да се испитани тресет може користити као геолошки материјал за формирање пелоида, а самим тим и као средство у балнеотерапији.

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