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Organic composition of Igalo bay peloid (Montenegro)

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Igalo peloid is known for a number of therapeutic properties (resort of healing, cosmetic or aesthetic application) and its closeness of the sea, which makes the peloid extremely valuable. So far, the organic composition of the Igalo peloid was not investigated or determined. Also, there are studies for its medical application and biological activity, which are directly related to its chemical composition. In this paper we analyzed the content of organic compounds (fatty acids, proteins, amino acids, sugars) and we also listed their main and well-known biological, pharmaceutical and medical roles and purposes. For the purpose of this study, different analytical techniques were applied to the collected peloid, including extraction, chromatographic, electrophoretic and NMR techniques.

Keywords: Fatty acids, Igalo, Peloid, Perspective, Proteins, Sugars

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Peloid, as a natural product that contains different components in its composition, has extensive use in treatment of many diseases. Organic compounds have special significance because many of them have biological activity, wherefore they can be applied in the treatment of various diseases. The therapeutic use of peloids is called pelotherapy.

Peloid is mud found at the coast in Igalo (Montenegro) with therapeutic and cosmetic frequently used in pharmacy, spas and aesthetic medicine[1]. Thus far, no studies on the chemical composition of the Igalo peloid have been carried out, nor have its potential medicinal properties been examined. According to the majority of publications, the chemical composition is the main factor upon which the final characteristics and hence the use of peloids depend (organic and inorganic substances constituting the peloid). Peloids are complex and variable matrices, and their chemical composition is affected by different factors such as the mineralogical composition of clay (geomaterials), organic matter, type of water and micro-organisms involved in the maturation process. Thus complete organic chemical characterization of peloids becomes a complex procedure in chemical analysis. During pelotherapy, specific types of chemical components penetrate the skin by diffusion and electrophoresis[2].

The Institute for Physical Medicine, Rehabilitation [illegible] Milošević", known for the use of peloid for medical and cosmetic purposes, is located on the shore of this part of the bay. The Igalo peloid is created in an ambient where saltwater and fresh water are mixed under the hydrodynamic impact of the water mass, resulting in the re-sedimentation of the carbonate flysch, rich in lime and silt. The peloid extends from the mouth of the Sutorina River to Njivice settlement, at 42 degrees and 28 min north latitude and 18 degrees and 32 min eastern longitude (as shown in Fig. 1).

According to the International Society of Medical Hydrology, peloid is a natural product consisting of a

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Fig. 1 — Sample Area of the Igalo Peloid.

mixture of sea, salt lake or minero-medicinal water (liquid phase), with organic and inorganic material (solid phase) produced by biological action and geological action. Apart from this, a new definition of peloid was proposed by Gomez and associates[3]: peloid is a maturated mud or muddy dispersion with healing and/or cosmetic properties, composed of a complex mixture of fine-grained natural materials of geologic and/or biologic origin, mineral water or seawater and commonly organic compounds from biological metabolic activity.

In this paper we examined the content of organic compounds in the Igalo peloid. It is considered that the role of some organic compounds in the peloid is very important for the application of pelotherapy. According to some authors, the contribution of organic matter for successful therapy is associated with the organic compounds with significant biological activity[4] and also its possible function as carriers of trace chemical elements to the organism[5].

It is considered that the role of some of the organic compounds in the peloid is very important for the application of pelotherapy[6]. Association of the organic compounds with biological activity and specificity of therapeutic applications of peloid relies on the penetration of these compounds through the skin[5]. Thus far, no studies on the chemical composition of Igalo peloid have been carried out, nor its potential medicinal properties have been examined. The aim of this paper was to determine physico-chemical properties and to characterize biologically active organic constituents of Igalo peloid.

Methodology

The analyzed sample of peloid was collected one kilometer away from the place where the Sutorina River flows into the Adriatic sea (Fig. 1), the supply site of the Igalo Institute. The peloid was extracted from a boat, using a special shovel designed for those purposes, in the shallow sea (2 m). The mud is deposited into special concrete basins where it is soaked with seawater and homogenized until a certain consistency is achieved. The samples of the peloid together with the accompanying water were collected from Igalo Beach. Deionized water and organic solvents were used for suspending, extraction and analysis of organic matter. Organic solvents were purified using standard laboratory procedures[7]. The peloid organic composition was investigated using chemical instrumental analytical techniques such as gas chromatography, HPLC, ^{1}H NMR spectrometers and electrophoresis techniques.

Materials and methods

Analysis of physico-chemical properties of peloid. An aqueous solution was made by suspending peloid in deionized water at 1% (w/v) concentration for determination of pH value and conductivity. After stirring the suspension for 20 min and 25°C it was left for sediment before the measurement. All the measurements were performed at a temperature of 25°C (Table 1). pH values were measured by means of pH-meter HI 221 (Hanna Instruments, Italy), while conductivity was determined by means of Digital conductometer (Hanna Instruments, Italy).

Determination of total organic matter (TOM) and total organic carbon (TOC). This is a very important part of any soil or sediment analysis and ecological parameters of the environment because its quantity can be a precise indicator of the content of organic components in the soil or sediment. In soil, forms of organic carbon are formed by the decomposition of plant and animal remains[8]. Total organic carbon can be measured directly (which is the approach that we have used in our work) or can be determined by the difference of the total carbon content and the inorganic carbon (carbonates, bicarbonates) that is present in the soil[9]. The concentration of total organic matter (TOM) was determined by using two different methods.

Method 1. The principle of the method is based on the combustion of organic and easily volatile materials at high temperatures. The measured amount of lyophilized peloid was heated at 800°C in the annealing furnace for one hour and the change in mass was measured. After the heating was complete, the pots were cooled in the desiccators for

Table 1 — Basic physical characteristics of the Igalo peloid

Physical parameter	Value
Temperature	37±0.5°C
pH-value	6.23 ±0.01
Density	1.63 ±0.01 g/cm^3
Density of dry peloid (after lyophilization)	0.73 ±0.01 g/cm^3
Conductivity	134.0 ±0.1 μS

Table 2 — TOM and TOC in sample of the Igalo peloid

Total organic matter (TOM) Experimentally obtained		Total organic carbon (TOC) Calculated	
Method 1	Method 2	Method 1	Method 2
8.8052%	8.5133%	5.1193%	4.9496%
8.8506%	8.8643%	5.1457%	5.1537%
8.8206%	8.7985%	5.1282%	5.1154%
Σ = 8.8254%	Σ = 8.7254%	Σ = 5.1311%	Σ = 5.0729%
Σ = 8.7754%		Σ = 5.1021%	

half an hour and the weight change was measured. The measurements were made on three samples (Table 2).

Method 2. This method is based on the oxidation of organic matter by hydrogen peroxide with slight heating up. Namely, 40.0 mL of the solution of hydrogen peroxide in water (1:3, v/v) was poured over the weighed mass of dry peloid (around 1.0 g), and heated to 60°C for over two hours. During heating, 20.0 mL of 30% hydrogen peroxide was added until the gas bubble was exhausted. The resulting mixture was placed to solidify the solid phase, the water was separated by decanting, and the solid was transferred to the porcelain cup and evaporated in the dryer to a constant weight. The measurements were made on three samples.

The concentration of total organic carbon (TOC) was determined using equation:

$$TOC=TOM/1.72$$

The water content was determined by drying up of sample to 80°C during 24 h, and then by gravimetric analysis techniques.

The percentage of total nitrogen was determined by Kjeldahl method (Instrument K-350 Distilation Unit, K-436 Speed digester, K-415 Duo scrub).

Determination of lipid content and fatty acids analysis. Fatty acids content analysis was performed on an Agilient 7890 A gas chromatography system, with CP-Sil 88 column, and FID detector. Column temperature was set to gradient 80-240°C. The first analysis on the topic of lipids concerned the determination of the total fat content in the dry peloid. For that purpose, the dry peloid sample was homogenized in a homogenizer. After measuring of 20 g, the sample was heated for 15 min in a mixture consisting of water and concentrated hydrochloric acid (100/60; v/v). The resulting mixture was allowed to wrap for 30 min. The solution was filtered, filter-paper was transferred into the socket of the Soxclet extractor. As a solvent for extraction, a petroleum-ether was used. The extraction took six hours. After completion the extraction, the solvent was evaporated on a vacuum evaporator, and the weight of the residual fats was measured with an accuracy of 0.01 g. On the same sample, two determinations were made.

The main constituents of the lipid moiety are fatty acids, which were analyzed by standard gas chromatographic methods. The method is based on esterification of the fat, previously extracted from the sample, using methanol in the base medium. Determination was made according to the following procedure: about 0.5 g of a peloid sample (with an accuracy of 0.01 g) was measured in the scaled Erlenmeyer flask. Hexane (3 mL) was added to the sample, followed by addition of 0.6 mL of 2 M solution of potassium hydroxide and the final solution was stirred for about a minute. The mixture was heated on a water bath at a temperature of 70°C for one min. After cooling, 1.2 mL of 1M hydrochloric acid in methanol and 3 mL of hexane were added to the mixture, after which we waited for the separation of layers. A hexane layer, which contained fatty acid esters, was separated from aqueous phase, transferred to a normal vessel and diluted with hexane to 5 mL. For the gas chromatographic analysis, 1 mL of this solution was measured.

Amino acids and protein analysis were performed on an Agilent 1260 Infinity liquid chromatography system, equipped with a μ-degasser (G1379B), 1260 binary pump, standard auto sampler, thermo stated column compartment and multiple wave length detector. The amino acid analysis was made according to a regulation available in the literature[10]. Sample of dry and lyophilized peloid (1.0 g) was finely ground to pass through a 0.5 mm sieve and after than reconstructed in 2 mL of 6 M HCl[11]. The samples were hydrolyzed for 24 h at 110°C. After the hydrolysis, the mixtures were evaporated to dryness under vacuum. The hydrolysates were reconstructed in 2 mL of 0.1 M HCl.

In order to determine the content and number of different protein fractions, as well as their molar

masses, gel electrophoresis was performed as follows: 0.5 g of peloid was extracted with 30 mM phosphate buffer saline (PBS), pH 7.4, for 2 h at room temperature after which it was centrifuged 10 min at 14.000 xg at 4°C. The supernatant was used for the experiments after determination of total protein concentration by using Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions[11].

For in-gel analysis of protein profile, extract was resolved under reducing conditions in a discontinuous buffer system using a vertical electrophoresis slab system (Hoefer, Holliston, USA) with a 4% (w/ v) stacking and a 12% (w/v) resolving gel. Each gel lane was loaded with 12.5 μg of total protein. Gels were stained with Coomassie Blue (Serva, Heidelberg, Germany).

Analysis of carbohydrates were perfomed on Shimadzu LC-20A, RID detector, LC-20AB pump. Separating was done on the column Interstil NH2, 250_4.6 mm. As a mobile phase, a mixture of acetonitrile/water (80:20) was used. The separation time was 25 min, at temperature 40°C. Preparation of the sample included mixing 5.0 g of lyophilized peloid sample with 20 mL of water and 10 mL of hydrochloric acid. The mixture was boiled in a water bath for half an hour and after cooling it was added 25 mL of methanol. After filtration through a microfilter of 45 μg, the sample was placed on a HPLC-column.

Nuclear magnetic resonance spectra of peloid sample were obtained using Brucker Avance III NMR spectrometer 500 MHz for ^{1}H NMR spectrum. Chemical shifts are presented in ppm (δ), using tetramethylsilane (TMS) as internal standard.

Statistical analysis

Statistical analysis was performed using Graph Pad Prism v5.03 for Windows (San Diego, California, USA). A significance level of $p \leq 0.05$ was used for analysis of variance, implemented using the Kruskal-Wallis test followed by the Tukey's post-hoc test ($p \leq 0.05$).

Results

Physico-chemical properties

Samples of Igalo peloid were examined directly or after resuspension in deionized water. The Igalo peloid has the smell of sulfides, it is dark gray in color and possesses elastic and adhesive characteristics. In Table 1 some important physical properties of the Igalo peloid are listed.

Given the fact that the pH value and the electrical conductivity could not be determined directly from the peloid sample, a 1% aqueous solution was made in order to measure these constants. Measurements were performed at a temperature of 25°C. As can be concluded from the measured data, the peloid is mildly acidic, with extremely poor conductivity.

Water content

After two measurements we calculated the mean value of water content - the Igalo peloid contents 39.69% of H_2O.

Total organic matter (TOM) and total organic carbon (TOC)

The results of these two series of experiments and the calculation of total organic carbon are shown in the Table 2. The total organic matter content in the peloid was 8.825±0.023% as determined by the first method, or 8.725±0.187 as determined by the second method. There is no statistically significant difference between the obtained TOM values ($p>0.05$). Total organic carbon was derived from TOM. Again, using two methods similar results were obtained: 5.131±0.013% and 5.073±0.108%, with no statistically significant difference between obtained values ($p>0.05$).

Determination of total nitrogen, amino acids and protein.

The amino acid analysis was made by gas chromatography after lyophilization and hydrolysis of peloid sample. Chromatogram of amino acid analysis is shown in Fig. 2. The results of the analysis are given in the Table 3. It is evident that the most abundant amino acid after hydrolysis is alanine, followed by aspartic acid, proline, leucine and histidine. Other protein amino acids are also present but they much less abundant in peloid hydrolysate. The amount of glycine is comparable to the amount of arginine, while phenylalanine is similar in concertation to isoleucine. We found tyrosine to be the most infrequent amino acid in Igalo peloid hydrolysate.

The protein content of Igalo peloid was determined after extraction of the material with phosphate buffer saline. Protein concentration determined was 3.71±0.01 μg/mL of wet mass of peloid.

Protein composition was further analyzed using SDS-PAGE. Upon staining of the gel several bands ranging from 35 kDa to over 116 kDa could be observed. There are two prominent bands visible: one around 40 kDa and another corresponding in weight to 66.2 kDa marker. Proteins with lower molecular weight than 35 kDa have not been observed.

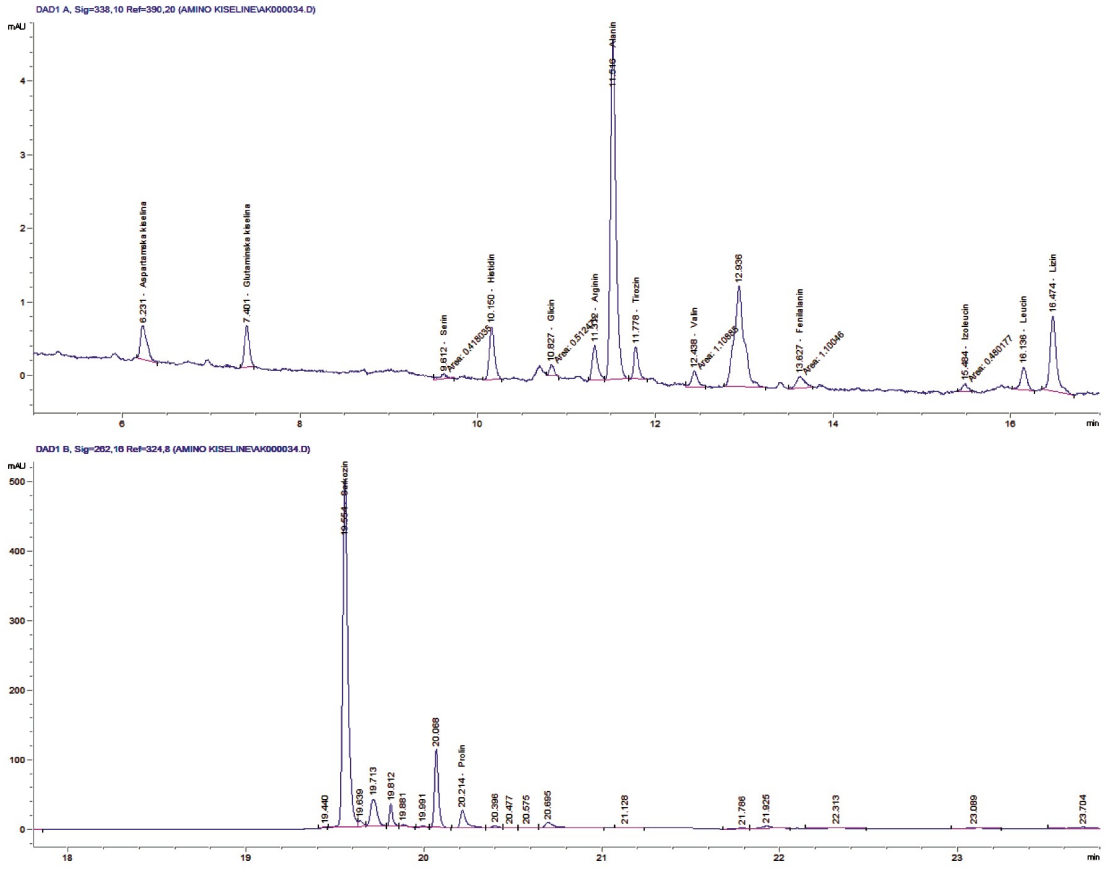


Fig. 2 — The Chromatogram of Amino acid Analysis in the Peloid Hydrolysate.

Table 3 — Content of amino acids in the Igalo peloid hydrolysate (essential amino acids are marked with an asterisk)

Amino acid	Content (nmol/mL)
Aspartic acid	60.82
Glutamic acid	38.84
Serine	36.45
Histidine*	44.66
Glycine	22.89
Arginine*	24.32
Alanine	131.99
Tyrosine	2.67
Valine*	18.13
Phenylalanine*	7.22
Isoleucine*	7.14
Leucine*	49.24
Lysine*	16.62
Proline	56.32

Resulting electrophoregram of protein fraction in peloid is shown in Fig. 3.

At the end of this step of investigation, we examined the content of nitrogen in peloid using Kjeldahl method and we founded that it is around 0.1%.

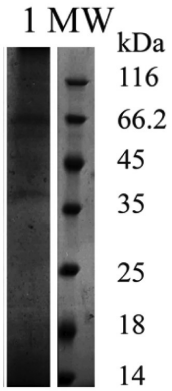


Fig. 3 — Electrophoretic Detection of Total Protein Content of Igalo peloid: 1) protein extract of peloid; MW) molecular weight markers.

Lipids

Dry peloid material contained 0.028±0.09% of fats. Total lipid content of the peloid was determined after lyophilization and extraction procedures. The main constituents of the lipid moiety, fatty acids, were analyzed by standard gas chromatographic methods. The most abundant fatty acid detected in peloid is

palmitic acid with 49.662±4.739%, followed by oleic and stearic acid, respectively. Chromatogram of fatty acids analysis is shown in Fig. 4. The results of the analysis are given in the Table 4 (two measurements have been made and their mean value is given in the table).

Carboxydrats

Using chromatographic analysis we were able to detect and determine only four monosaccharides, which are listed in the Table 5. The most abundant monosaccharide present in peloid was arabinose. However, no statically significant difference can be observed between arabinose and fructose ($p>0.05$). The least abundant monosaccharide detected in peloid was galactose, but the amount found is not statistically significant when compared to mannose ($p>0.05$).

Nuclear magnetic resonance technique

(NMR) is a valuable tool for the characterization of soil organic matter and humification processes

Table 4 — Content of fatty acids in the Igalo peloid

Fatty acid	Content (%)	Fatty acid	Content (%)
Hexanoic acid (caprinic acid)	1.74	Stearic acid	10.78
Dodecanoic acid (lauric acid)	0.91	Oleic acid	19.15
Tetradecanoic acid (myristic acid)	2.30	(9Z,12Z)-Octadeca-9, 12-dienoic acid (linoleic acid)	4.54
(E)-Tetradec-9-enoic acid (myristoleic acid)	2.02	Arachidonic acid	1.61
Pentadecanoic acid	2.05	Docosanoic acid	3.38
Palmitic acid	46.06	(Z)-Docos-13-enoic acid	3.71
(E)-Hexadec-9-enoic acid	2.01	(5Z,8Z,11Z,14Z)-Icosa-5,8,11,14-tetraenoic acid	2.17
Heptadecanoic acid	3.15	Tetracosanoic acid (lignoceric acid)	2.05

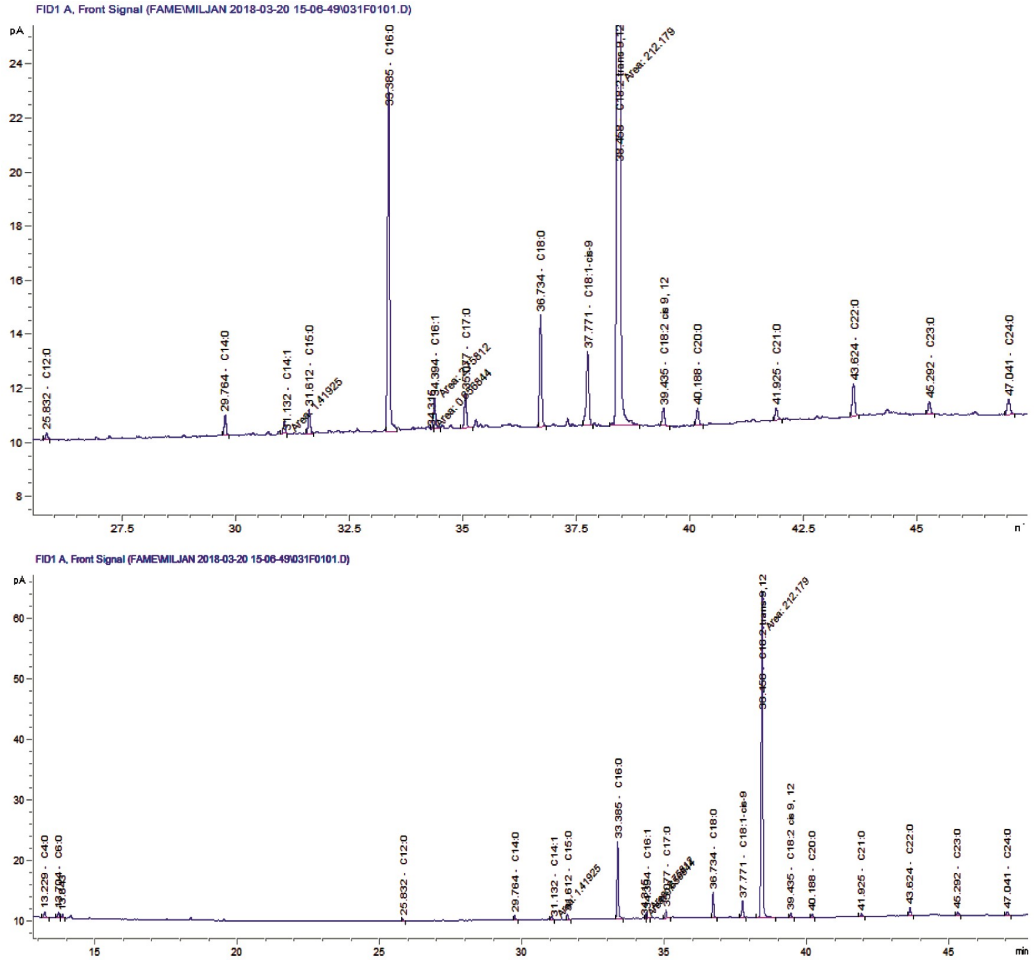
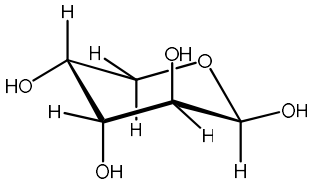
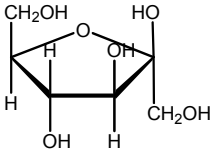
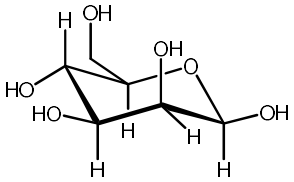
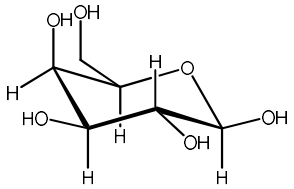


Fig. 4 — Chromatogram of Fatty Acids Analysis in the Igalo Peloid.

Table 5 — Monosaccharides content in the Igalo peloid sample

Monosaccharide	Structure	Content(mg/g)
Arabinose		1.0
Fructose		0.9
Mannose		0.6
Galactose		0.3

in soils[12]. A sample of dry and lyophilized peloid (15 mg) was suspended in deuterohloroform ($CDCl_3$); after separation of the insoluble part by filtration, the proton NMR spectrum from the resulting solution was recorded (500 MHz). As seen on the spectrum Fig. 5, the signals of the aliphatic part of the fatty acids were dominant (area 0.8-2.4 ppm). Signals of double bonds were noticed in the 4.6-5.4 ppm area, while the area around 7.25 ppm indicates that peloids contain some aromatic compounds (aromatic amino acids).

Discussion

Revising the literature, we concluded that the complete chemical characterization of peloids has been studied rarely, but there are some papers containing specific studies of different organic fractions of peloids[13-15]. Chemical composition of peloids depends on many different factors such as the mineralogical composition of clay, content of organic compounds, type of water and biological properties of environment (e.g. type of microorganisms involved in the maturation process). Natural peloids are three-phase systems. The solid phase is composed of solid inorganic, organic or mixed components, the liquid

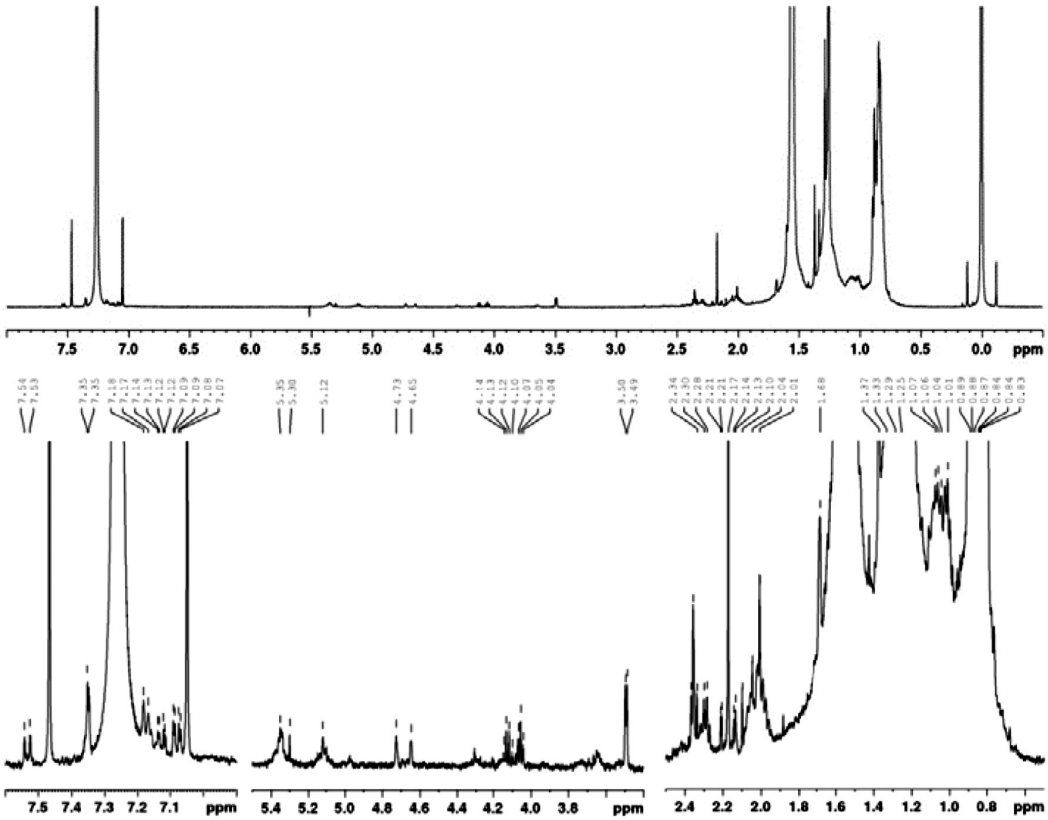


Fig. 5 — ^{1}H NMR Spectrum of the Peloid Sample.

phase is from the river, lake or sea water and the gas phase consists of gases that are often found as accompanying gases of peloids (H_2S, CO_2, NH_3, CH_4)[16].

Igalo peloid is mildly acidic (pH 6.23; Table 1.) with poor conductivity. Lower levels of acidity are beneficial since this hampers the absorption of heavy metals due to decreased mobility in peloid at neutral and slightly alkaline pH values. The lower pH value of a peloid is, the more metal can be found in the solution and thus more metal is mobilized[17].

Igalo peloid is a natural peloid matured in the natural sediment where it occurs. Maturation process of the peloid is important to its medicinal and cosmetic properties since new therapeutically active compounds could be formed during maturation by the action of the growth of living organisms including diatoms, cyanophyceae, bacteria, protozoa, and the organic compounds or ignited by their metabolic activity and degradation[18,19].

Depending on the overall chemical composition, natural peloids are divided into three categories: essentially inorganic, essentially organic and mixed inorganic/organic peloids[20]. Based on the content of organic matter, peloids can be classified into two groups: with high mineral composition and low organic matter (1-5%), and with higher content of the organic matter[21,22]. With close to 9% of total organic matter, Igalo peloid can be classified into the second class. Presence of organic matter is important for pelotherapy as organic compounds can exert biological activity[23]. They also function as carriers of trace elements into the organism enabling their passage through skin barrier[5].

Therapeutic mechanisms of pelotherapy are not yet fully understood; however they are most likely derived from the synergistic effect between organic compounds and inorganic components[24]. There is no consensus which compound (organic or inorganic) should be attributed to the healing properties of peloids at the moment. Nevertheless, presence of organic compounds with reported biological activity could provide strong evidence for observed therapeutic effects[25,26,27].

Presence of amino acids and proteins is due to biochemical transformations of natural organic matter, plant residues and animal waste during the humification process[28]. Amino acids are known to be a component of the skin natural moisturizing factor (NMF) which serves as a sponge, keeping water in the *stratum corneum* and imparting a subjective sensation of smooth skin. For this reason, amino acids have been widely applied in the cosmetic industry[29]. According to organic compounds found in the Igalo peloid sample we can conclude that it is rich in amino acids which are important for human health. Alanine is present in most proteins and in the most of them is represented in the share 2-7%; aspartic acid occurs in all animal proteins, primarily in albumins; proline is present in wheat proteins, gelatin and casein, and leucine is an essential amino acid, present in muscle tissue, cereal proteins and during alcoholic fermentation fusel oil is formed from leucine and isoleucine; histidine is present in blood proteins about 6% and it is essential amino acid[30]. Leucine, isoleucine and valine are known as branched-chain amino acids (BCAA) which are most notably known to increase protein synthesis through modulating protein translation[31]. Although the potential link and mechanism between BCAA and skin are still unknown, some recent studies showed that their deficiency can reduce Type I and Type III tropocolagen synthesis in skin by suppressing the action of mTOR (mammalian target of rapamycin)[32].

After the discovery that proline accelerates wound healing[33], it was published that exogenous proline stimulates Type I collagen and HIF-1α expression in human skin fibroblasts[34].

Glycine, another amino acid of importance in dermatologic therapy, is traditionally used for the treatment of scars and has beneficial effects on the skin reparation process and the overall rate of wound healing[35].

It is well-known that arginine stimulates fibroblast proliferation[36] but recent studies also described anti-apoptotic and immune-enhancing effects of this amino acid in culture of skin fibroblasts[37]. Also, studies proved that there was a significant decrease in the levels of histidine in the samples obtained from patients with psoriasis as compared to healthy subjects[38].

Carboxylic acids found in the Igalo peloid have antioxidant biological activity and have also shown immune response for some precursors[39]. Besides, fatty acids, next to antioxidant activity, have the role of membrane regulators[40]. On the other hand, it is known that different types of carboxylic acids (fatty acids, humic acids etc.) are complexing ligand of trace elements, so it is also possible to form complexes with amino acids, peptides, steroids or carbohydrates.

This characteristic can be responsible for eliminating potentially toxic elements, desmutagenic effects, or their antioxidant or anticoagulant activity[41]. Fatty acids are important in providing membrane fluidity and maintaining the cutaneous water permeability barrier. Fatty acids function as precursors of eicosanoids, which include prostaglandins and leukotrienes, influence cellular interactions, cellular proliferation, and inflammation[42]. As it can be seen in the Table 4, saturated fatty acids (palmitic and stearic) dominate in the lipid composition of peloids, while unsaturated fatty acids account for a significant percentage of oleic acid. It is known that palmitic acid salts have a strong antifungal effect on *Scedosporium Apiospermum*[43].

Olive oil, rich in oleic acid, is supposed to present modulator effects in a lot of physiological functions, while some studies also suggest a beneficial effect on cancer, autoimmune and inflammatory diseases, and have the potential role on the future establishment of novel therapeutic approaches for infections, inflammatory, immune, cardiovascular diseases or skin repair based on this fatty acid mainly found in the Mediterranean diet[44]. Conveyors for some antifungal drugs based on oleic acid were developed[45]. Here we have to mention that oleic acid is connected with pathogenesis of acne vulgaris in some research[46]. Dietary deficiency of linoleic acid, the major 18-carbon n-6 polyunsaturated fatty acid in normal epidermis, results in a characteristic scaly skin disorder and excessive epidermal water loss[47]. Its salts have an antibacterial effect against dermatopathogenic *Staphylococcus spp*[48], while topical application of a linoleic acid-ceramide containing moisturizer could be a valuable approach for the treatment and prevention of psoriasis[49].

Arachidonic acid stimulates human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) *in vivo* and its molecular mechanism *in vitro*[50].

Carbohydrates in peloid are the nourishment which enables numerous microorganisms to live and occupy the mud environment. Monosaccharide composition is a useful tool for identifying the sources of carbohydrates[51]. Monosaccharides identified in the peloid can be found in naturally occurring biopolymers and therefore were most probably derived from a mixture of phytoplankton, marine bacteria and terrestrial plants[52]. As is evident, arabinose and fructose were found in the largest amount of all monosaccharides in the peloid. Arabinose is aldopentose and is found in nature as a component of biopolymers such as hemicellulose and pectin. *D*-Arabinose is degraded by *Escherichia coli* B via some of the *L*-fucose pathway enzymes and a *D*-ribulokinase which is distinct from the *L*-fuculokinase of the *L*-fucose pathway. *L*-fucose and *D*-arabinose acted as the apparent inducers of the enzymes needed for their degradation[53]. Arabinose is an inhibitor of sucrase, the enzyme that breaks down sucrose into glucose and fructose in the small intestine[54]. It is also a potential prebiotic, because it cannot be absorbed by human intestine and could be utilized by probiotics[55]. Fructose is found in the peloid in approximately the same amount as arabinose. Fructose is ketohexose, which is naturally chemically bound in sucrose, as well as free in honey. It belongs to the sweetest natural sugars. Fructose is preferred in food and beverage manufacturing to replace sucrose and glucose due to the lower effect of fructose on blood glucose levels following a meal. Clinical research have provided no or only limited direct evidence that fructose itself is associated with elevated LDL cholesterol and triglycerides levels leading to metabolic syndrome[56].

Although there are a lot of discussions about negative effects of fructose on humans, some recent findings described protective effects of fructose - a short-term application of fructose induces a mild carbonyl/oxidative stress-stimulating cellular defensive mechanisms responsible for cell survival under lethal stress[57].

Mannose is an aldohexose and it is C2 epimer of glucose. Mannose is important in human metabolism, especially in the glycosylation of certain proteins – several congenital disorders of glycosylation are associated with mutations in enzymes involved in mannose metabolism[58]. The human immunodeficiency virus displays considerable amount of mannose residues due to the tight clustering of glycans in its viral spike[59]. Also, topical application of mannose and fructose accelerated epidermal permeability barrier recovery rate[60]. Galactose is an aldohexose and it is a C4 epimer of glucose. In nature, it is mostly chemically bound to glucose, whereby it forms disaccharides lactose - milk sugar. Some studies suggest that galactose may have a role in treatment of focal segmental glomerulosclerosis (a kidney disease resulting in kidney failure and proteinuria)[61]. In O- and A- antigens, there are two monomers of galactose on

the antigens, whereas in the B- antigens there are three monomers of galactose[62].

Conclusion

In this paper, we examined the organic composition of the Igalo peloid. This is the first study of this type in Montenegro. For the development of many biological processes, the presence of various organic and inorganic substances is necessary. The components of this natural resource have biological activity thanks to which they can be used for therapeutic purposes. The peloid activity will depend more on the chemistry of peloid than on its physical or physico-mechanical characteristics. Mechanisms involved in therapeutic effects are not clear and fully understandable. According to some authors, a synergistic effect between organic compounds and inorganic components of its composition is responsible for the therapeutic properties and biological activity of peloids. Peloid from Igalo in the larger part contains fatty acids (saturated and unsaturated) as well as essential amino acids, many of which have significant physiological, medical and pharmaceutical effects. The paper also presents some important biological, pharmaceutical and medical properties and applications of detected organic molecules.

The distribution and concentration of organic substances in Igalo peloid is a direct consequence of impact and contributions of marine plants and bacterial activity. Our results suggest that the presence of many biologically active compounds may be beneficial for the balneological value of Igalo peloid.

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