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IDENTIFICATION OF ORGANIC COMPOUNDS IN CAJÍO PELOID (CUBA)

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ABSTRACT

Thermal muds ("peloids") are a typical example of natural sediments used in the treatment of different pathologies. Nowadays, because of their use in therapeutics, relax and cosmetic issues, a proper management of peloids is of increasing importance. Peloid characterization and monitoring for quality control purposes are therefore the main aspects of peloid management. Organic chemical quality is directly related with the biological active compounds and the therapeutic properties of peloidsto be used in human health and with the determination suitable contamination.

This study was performed to characterize the organic fraction of Cajío peloid (Cuba) in order to identify the organic components with possible biological effects and establish its natural or anthropogenic origin. For this purpose, a procedure with chromatographic analysis and mass spectrometry detection has been performed.

More than 40 compounds were identified, in Cajío peloid, mainly of natural origin. Among them were found alkanes, steroids, fatty acids, alcohols and other heteroatoms compounds, some of them with reported biological activity in their isolated form as antioxidant, analgesic, anti-inflammatory, and others. The results provide evidence for medical interpretation of the therapeutic action of Cajío peloid in the treatment of inflammatory and dermatological diseases and contribute to the understanding of pelotherapy, giving some scientific basis for its future development.

Keywords: Peloids, Peotherapy, Organic Characterization, Sediment, Cajío

INTRODUCTION

Thermal muds ("peloids") are a typical example of natural sediments used in the treatment of different pathologies [1]. Nowadays, because of their use in therapeutics, relax and cosmetic, the correct management of peloids is increasing its importance [1]. This situation highlights the significance of scientific research on the chemical characterization of thermal muds, firstly, to find the organic and inorganic substrates of their therapeutic activity, which provides some support for their medical use, and secondly, to certify their quality to be used in medical treatments [1]. The characterization and monitoring of these sediments (quality control) are main aspects in the management of these mineral resources. Organic chemical quality is directly related with the biological active compounds and the therapeutic properties of peloids to be used in human health and with the determination suitable contamination.

Peloids are complex and variable matrixes, and their chemical composition depends on different factors such as: the mineralogical composition of clay (geomaterials), organic matter, type of water, and micro-organisms involved in the maturation process. Therefore, the total organic chemical characterization of peloids becomes a complex procedure in chemical analysis. That fact justifies that, although inorganic and quality controls are established, the requirements for organic composition are scarcely described [2]. Current literature reports only few and specific studies of different organic fractions of peloids (non-polar compounds such as long-chain hydrocarbons, carboxylic acids, lipids, vitamins and pigments, and of polar compounds such as amino acids and proteins) [1]-[5], but there is still lack of literature on general characterizations. The identification of organic compounds of biological relevance in peloids is this reports are done mainly using gas-liquid chromatography coupled with mass spectrometry.

Cajío Beach is a locality of the Güira de Melena municipality in the eastern part of Cuba, and contains one of the most important deposits of peloids of the region. This peloid have been used in an empiric way for the residents of the province to

improve inflammatory (osteoarthritis, rheumatoid arthritis, bursitis) and dermatological (psoriasis, acne, cutaneous seborrhea and mycosis) processes and as an analgesic for painful processes of osteomioarticular system. Although in Cuba there are different deposits of peloids in dissimilar stages of characterization and exploitation, no reports have been found of chemical characterization of Cajío peloid.

This study was performed to characterize the organic fraction of Cajío peloid (Cuba) in order to identify the organic components with possible biological effects and establish its natural or anthropogenic origin.

MATERIALS AND METHODS

Sampling and pretreatment procedures

Sample pretreatment of Cajío peloid sample was done following previously reported procedures used for a similar peloid [1].

Briefly, the thermal mud was collected from two sampling stations in Cajío Beach deposit, where different subsamples were obtained, and afterwards a composite sample was prepared *in situ*, sealed in clean polyethylene containers, placed in a cooler at 4ºC, and transported to the laboratory immediately for further analysis.

Total organic matter (TOM) was determined using the Walkey Black volumetric method [6], and the n-hexane removable substances were determined using the gravimetric method [6].

Clean-up procedures

Sulfur removal was done according to a previously reported procedure [1], based on solvent extraction of the peloid sample with solvents of different polarity. The clean-up procedures were followed by centrifugation and drying at room temperature to constant weight. The solids obtained were grounded and sifted to a particle size of 125 mm, constituting the solid phases for the separation procedures.

Separation procedures

After removal of sulfur interference, the solid phases were subjected to specific analytical procedures for separation and identification of non-polar and polar organic compounds. In the case of non-polar compounds, the solid obtained for non-polar compounds analysis was macerated with 100 mL of n-hexane for 14 h in a stirring machine at room temperature, at a frequency of 200 min^{-1}, followed by centrifugation for phase separation. The liquid phase (non-polar fraction) was then reduced by rotary evaporation down to 1.5 mL and injected in a reverse phase (RP18) column and eluted with 2 mL of eight solvents (n-hexane, cyclohexane, di-isopropyl ether, acetone, ethyl acetate, acetonitrile, ethanol, methanol). The resultant fractions were later analyzed by gas chromatography–mass spectrometry (GC-MS).

For the polar compounds, the solid obtained, after removal of sulfur, was macerated with methanol for 14 h at room temperature. The extract of this polar fraction was analyzed by GC-MS.

A blank study was also carried out, as reported by [2], to determine if some of the organic compounds could be present because of contamination during the analytical procedure.

Instrumental analysis

Separation by gas chromatography (GC) was achieved using a fused silica capillar column HP 5MS of intermediate polarity (5% phenyl) column (15.0 m x 250 μm x 0.1μm) with intermediate polarity in a Agilent GCMS-7890A-5975C equipment.

The GC operating conditions were the following: temperature is held at 100°C for 1 min, increased from 100 to 215°C at a rate of 20ºC min^{-1}, hold for 10 min, increasing again to 290°C at a rate of 20°C min^{-1}, with a final isothermal hold at 290°C for 20 min; the pressure was 72.3 kPa, and the flow of 1.0 mL min^{-1}, using helium as carrier gas. The samples were split injected (2μL) with the injector temperature at 250°C. The mass spectrometer was operated in electronic impact mode (EI) at 70 eV ionization energy at 200°C, and scanned from 40 to 900 Da. Identification of individual compounds was performed using the GC-MS Solution 1.10 software for the spectrum construction, and by comparison of mass spectra with library data or interpretation of mass spectrometric fragmentation patterns. Other chemical software and databases (MS Windows NIST Mass Spectral, AMDIS Search Program Version 1.7-2005, MS Windows NIST Mass Spectral Search Program, Version 1.5-1997, and the MSDB NIST Standard Reference Data Base Series 1a. Version 4.5-1994: NIST 21, NIST 98, Wiley 229) were also used in the analysis of the results. Match qualities of 90% or greater against NIST 107 and 21 (National Institute of Standards and Technology) libraries were assumed to give reliable identifications. Tentative identification refers to qualities between 70% and 89% against these libraries. Analytes yielding match qualities of 69% or less were assumed to be not identified (unknown).

RESULTS AND DISCUSSION

Total organic matter determination

The levels of organic matter in the sediment allows for the classification of peloids into two possible groups, one with low content of organic matter (1%–5%) and high mineral composition (e.g. mud or fangi, limus), and the other with high organic matter content (e.g. peat, bioglea, sapropel) [7]. On the basis of that Cajio peloid classify in the first group (TOM=1.42%). A further classification can be done according to the characteristics of the water used in the maturation process. In general, mineral peloids formed from hydrothermal waters are known as mud or fangi, while the limus type is associated with seawater. In the case of Cajío peloid, formed in the sea, it can be classified as limus.

On the other hand, the n-hexane removable substances value was 1.34%, representing 94% of the compounds present in the TOM, indicating the predominance of organic non-polar (e.g. alkanes, steroids) over the polar (e.g. carboxylic acids, alcohols, aldehydes) compounds (6%) in Cajío peloid.

Organic characterization

Table 1 shows that, in Cajío peloid, separated (S) and identified (I+TI) compounds are 52 and 46 compounds respectively, for 88% percentage of identification. All the fractions show high identification percentage, with the exception of acetonitrile. These results indicate that the chromatographic methodology employed was appropriate for the characterization of Cajío peloid. The majority of the compounds (65%) were separated in the n-hexane and cyclohexane fractions demonstrating the prevalence of non-polar compounds over the polar ones in the as indicated by the values of n-hexane removable substances. The blank study indicates that no organic compounds product of the contamination from sample manipulation during the analytic procedure was found.

Non-polar fraction

Figure 1a-h shows the chromatograms of the eight non-polar fractions separated in Cajío peloid, in general, with good resolution and low base line. The only affected fractions in resolution are n-hexane and cyclohexane, were the main non-polar compounds are extracted, however high percentages of identification (>90%) were still obtained. These chromatograms contain the alkane region that frequently is characterized by complex mixture of linear, branched and cyclic hydrocarbons of difficult resolution [1]. Also it is important to remark that no interference of sulfur can be observed in the base line of the obtained chromatograms, indicating that the clean-up procedure was effective.

In the n-hexane fraction (Table 2), the identified compounds were mainly paraffins (alkanes and other members of the homologous series such as alkenes, alcohols and aldehydes). In the cyclohexane and di-isopropyl ether fractions (Table 2), the organic compounds that were identified consisted mainly in linear or cyclic paraffins, members of the homologous series such as (alcohols and ketones, carboxylic acids) and the presence of isoprenoids and steroids as squalene and fucosterol. In the acetone fraction the compounds identified correspond to the homologous series of alkanes of short chain (alcohols, carboxylic acids). The most polar compounds in this non-polar fraction (Fig. 1e-h) show few separated compounds corresponding to long chain alcohols, the dodecanoic (lauric) acid and citrate (Table 2).

Some of the organic compounds found in Cajío peloid, such as alkanes, alkenes and monocyclic hydrocarbons, steroids, lactones, hydroxy acids, carboxylic acids, ketoacids, esters, aromatic compounds, fatty carboxylic acids and terpenoids, have been reported in sediments of different origin and few reports in peloids [1]-[5], [8]-[11].

Table 1 Resume of compounds separated in each fraction

Fraction	S	I	TI	U	% (I+TI)
Fraction of Non-Polar Compounds					
n-hexane	20	5	13	2	90
cyclohexane	14	3	10	1	93
di-isopropyl ether	4	2	1	1	100
acetone	4	1	3	0	100
ethyl-acetate	1	1	0	0	100
acetonitrile	2	1	0	1	50
ethanol	2	1	1	0	100
methanol	1	0	1	0	100
Fraction of Polar Compounds					
methanol	4	1	2	1	75
Total	**52**	**15**	**31**	**6**	

S-Separated compounds, I-Identified compounds, TI-Tentatively Identified compounds, U-Unknown compounds.

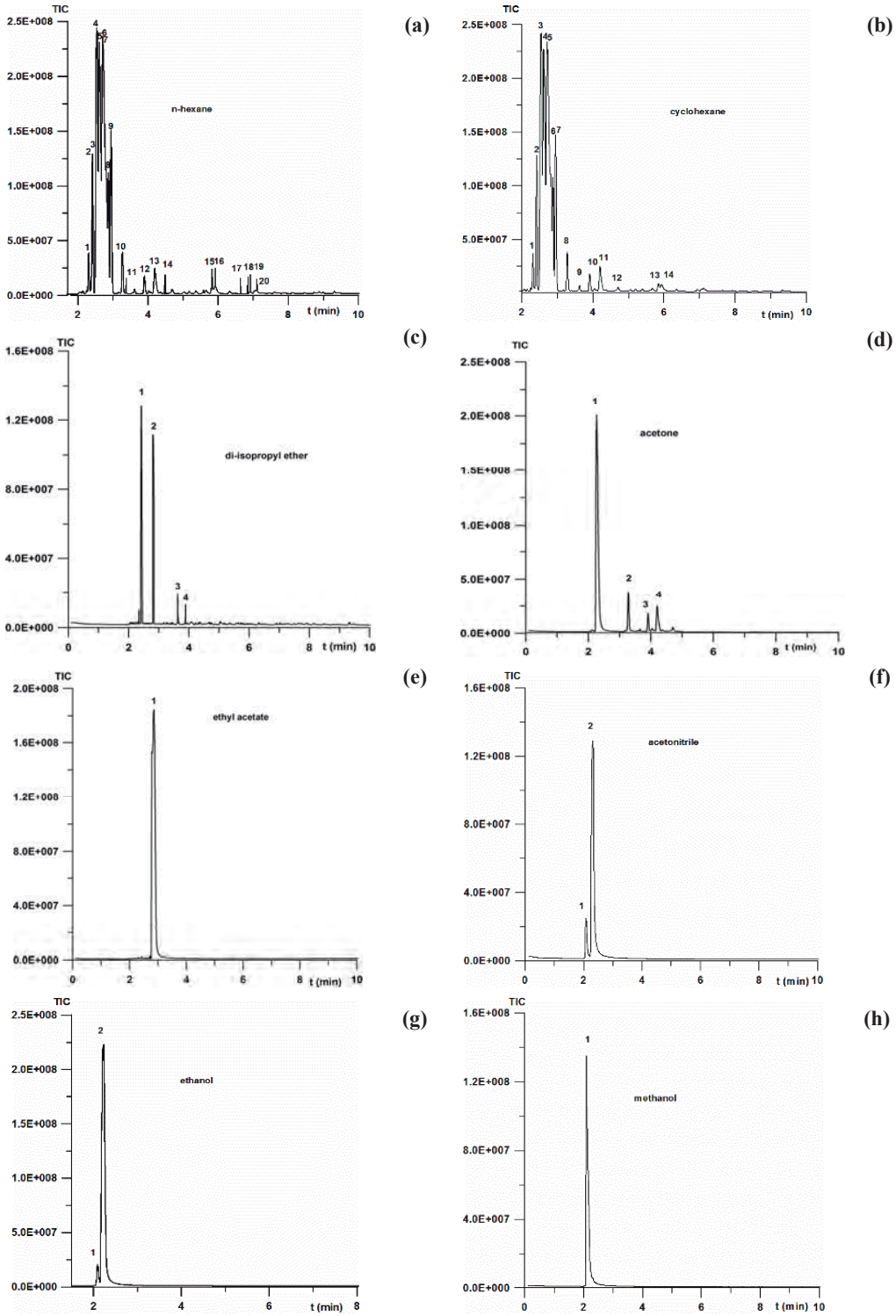

Fig. 1 Chromatographic profile of eluted non-polar fraction of Cajío peloid sample: a) n-hexane, b) cyclohexane, c) di-isopropyl ether, d) acetone, e) ethyl acetate, f) acetonitrile, g) ethanol and h) methanol

Polar fraction.

Good resolution and identification percentage (75%) was obtained for polar methanol fraction (Fig. 2). In this fraction only four compounds were separated, corresponding mainly to fatty carboxylic acid group. The poor compound separation is expected by the n-hexane removable substances analysis, but other factors can be influencing these results: probable decomposition of thermolabile and volatile polar organic compounds (presumably vitamins and proteins), during sample heating in the GC experimental procedure and/or the fact that higher molecular weight compounds (such as fulvic and humic acids), which contribute to the total organic matter, could not be determined by the procedure developed in the present work [11]. The polar fraction have been less investigated and only few reports have been found in sediments related to saccharides and some carotenoid derivatives [9].

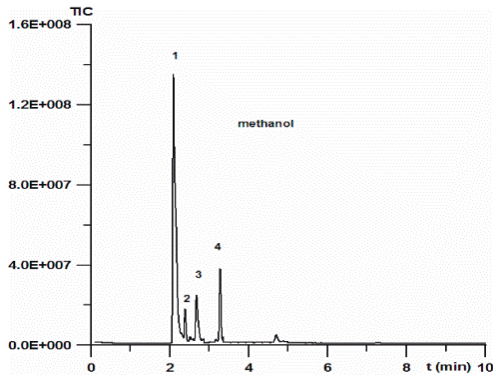

Fig. 2 Chromatographic profile of eluted methanol polar fraction of Cajío peloid sample

General organic composition of Cajio peloid

The main group of compounds identified (35%) in Cajío peloid is the hydrocarbons from C5 to C29 (straight, ramified or cyclic alkanes). These compounds are present in natural sediment as a result of plant biomolecule degradation and as microbiota byproducts [10].

As reported in previous works, paraffins (long, lineal or substituted alkanes) can strongly influence the physico-chemical properties of thermal therapeutic muds, related to heat retention, caloric capacity, humidity conservation, and toxins adsorption [1] and therefore the paraffin content in Cajío peloid could be related to its empirical reported thermotherapeutic and anti-inflammatory effects.

The next important fraction also represents approximately 35 % and it is dominated by the homologous compound series of n-alkanes (n-alkenes, n-alkanals, n-alkanols, n-alkanoic acids, n-alkanones and aliphatic carboxylic acids). These compounds reported to be mainly derived from the degradation of terrestrial plants, pheromones, essential oils and other biomolecules [10]. It has been not found reported the biological activity for these compounds.

Another 14.6 % of the total identified compounds is formed by various compounds of diverse nature, predominantly the nitrogenous compounds such as N-ethyl-N-methyl acetamide, the ubiquitous antioxidant BHT and compounds with oxygen. The origin of some of these compounds could not be assigned [10].

The fatty acids and terpenoids/steroids (phytosterols) represent approximately the remaining 12% of the total identified compounds. The fatty acids found in Cajio peloid were stearic acid (C18:0), palmitic (C16:0) and oleic (C18:1). Carboxylic acids are common in organisms and they are mainly originated from animal and plant internal lipid components such as essential oils of terrestrial plants, pheromones, and other biomolecules [10], [11]. These fatty carboxylic acids have also been reported to act as antioxidants, and membrane regulators in its isolated form and with significant anti-inflammatory activity [2]. Also these compounds were shown to play a protective roleagainst free radicals and UV radiations [2].

On the other hand, the terpenoids and steroids found in Cajío peloid are also important compounds identified. Squalene, particularly, has reportedly immune stimulant, antineoplastic and detoxicant action, and it is commonly used in pharmaceutical and cosmetic products because it acts protecting the unsaturated fatty acids of the lipid peroxidation, which is the cause of natural or premature aging [12]. In addition, the steroids (fucosterol) possess a well-studied and known biological activity in its isolated form, as hormones, analgesics and anti-inflammatory agents [13].

The absence of petroleum biomarkers (hopanes, pristane and others) suggests that the main sources of paraffins are typical plant wax alkanes [11] and no anthropogenic contamination.

These results provide strong evidence for medical interpretation of the therapeutic action of Cajío peloid in the treatment of inflammatory diseases and as analgesic.

Table 2 List of the identified compounds

Fraction	No.	Compound	Ident.	Fraction	No.	Compound	Ident.
Fraction of Non-Polar Compounds							
n-hexano	1	pentane	I	di-isopropyl ether	1	unknown	U
	2	unknown	U		2	octadecanoic acid (stearic acid)	I
	3	unknown	U		3	hexadecanoic acid (palmitic acid)	I
	4	1,2-dimethyl cyclopropene	TI		4	fucosterol	TI
	5	4-Pentenal	TI	acetone	1	2-butenoic acid	I
	6	cis 1-ethyl-2-methyl cyclopropane	TI		2	3-methyl-1-penten-3-ol	TI
	7	tetramethyloxirane	TI		3	3-methyl-1,3-pentadiene-5-ol	TI
	8	1-cyclopropyl-1-propanone	TI		4	3 hexen-2-ona	TI
	9	methyl benzene	TI	ethyl acetate	1	dodecanoic acid (lauric acid)	I
	10	butilatedhidroxytoluene (BHT)	TI	acetonitrile	1	2-octadeciloxy-ethanol	I
	11	hexadecane	I		2	unknown	U
	12	2,3-butanediol	TI	ethanol	1	2-hexadeciloxy-ethanol	I
	13	heptadecane	I		2	2-octadeciloxy-ethanol	TI
	14	3,4-dimethyl-2-pentene	TI	methanol	1	Citrate	TI
	15	eicosane	I	**Fraction of Polar Compounds**			
	16	heneicosane	TI	Methanol	1	Citrate	TI
	17	tetracosane	TI		2	hexadecanoic acid (palmitic acid)	I
	18	pentacosane	TI		3	9-ctadecenoic acid (oleic acid)	TI
	19	hexacosane	I		4	unknown	U
	20	2-methyl octacosane	TI				
Cyclo-hexane	1	unknown	U				
	2	pentiloxiran	TI				
	3	cis 1-ethyl-2-methyl-cyclopropane	TI				
	4	butilatedhidroxytoluene (BHT)	I				
	5	bencene	TI				
	6	3-ethyl-2,2-dimethyloxirano	TI				
	7	3-methyl-2-pentanona	TI				
	8	4-methyl 3-penten-2-ona	TI				
	9	3,4-dimethyl-2-pentene	TI				
	10	2,3-butanediol	I				
	11	methyl decanoate	TI				
	12	heptadecanoic acid	I				
	13	N-ethyl-N-methyl acetamide	TI				
	14	squalene	TI				

CONCLUSIONS

The analytical procedure developed in this work allowed the organic characterization of Cajío peloid permitting that 52 and 46 compounds were separated and identified respectively; most of them of natural origin. All the fractions show high identification percentage, and total percentage reached 88%. The majority of the compounds (65%) were separated in the n-hexane and cyclohexane fractions demonstrating the prevalence of non-polar compounds over the polar compounds as indicated by the values of n-hexane removable substances.

The main group of compounds identified in Cajío peloid is the hydrocarbons from C5 to C29 (straight, ramified or cyclic alkanes). Also were identified compounds of the series of n-alkanes (n-alkenes, n-alkanals, n-alkanols, n-alkanoic acids, n-alkanones and aliphatic carboxylic acids), fatty acids, terpenoids, steroids and various compounds of diverse nature. An important result derived from this research is the identification in this peloid of relevant organic compounds such as paraffins, squalene, fucosterol and fatty carboxylic acids, with reported biological activity in their isolated forms as antioxidants, hormones, analgesics and anti-inflammatory agents. These results provide strong evidence for medical interpretation of the therapeutic action of Cajío peloid in the treatment of inflammatory diseases and as an analgesic for painful processes of osteomioarticular system.

REFERENCES

[1] Suárez M, Domínguez R, González P, et al. “Identification of organic compounds in San Diego de los Baños peloid (Pinar del Río, Cuba)”. Alternative and Complementary Medicine, Vol. 17, 2011, pp. 1-11.

[2] Centini M, Tredici MR, Biondi N, et al. “Thermal mud maturation: organic matter and biological activity”. International Journal of Cosmetic Science, Vol. 37, 2015, pp. 339–347.

[3] Curri S, Gonzato, “Chromatographic analyses of peloide lipid and steroid fractions”. Biochimica e Biologia Sperimentale. Journal of the AbanoTerme, Italy, 2004.

[4] Odabasi H, Gul E, Macit M, et. al.“Lipophilic Components of Different Therapeutic Mud Species”. Journal of Alternative and Complementary Medicine, Vol. 13, 2007, pp. 1115-1118

[5] Tserenpil Sh, Dolmaa G, Voronkov MG. “Organic matters in healing muds from Mongolia”. Applied Clay Science, Vol. 49, 2010, pp. 55–63.

[6] Hiromi Sato J, Celio de Figueiredo C, Leandro Marchão R, et. al. “Methods of soil organic carbon determination in Brazilian savannah soils”. Scientia Agricola, Vol, 71, 2014, pp. 7-10.

[7] Oficina Nacional de Normalización. “Norma Cubana de Peloides (NC 6: 98). Especificaciones”. 1998. Habana, Cuba. 9 pp.

[8] Huang X, Zeng Z, Chen S, et. al. “Component characteristics of organic matter in hydrothermal barnacle shells from Southwest Indian Ridge”. Acta Oceanologica Sinica, Vol. 32, 2013, pp. 60-67.

[9] Al-Mutlaq KF, Stanley LJ, Simoneit BRT. “Composition and sources of extractable organic matter from a sediment core in Lake Kivu, East African rift valley”. Applied Geochemistry, Vol. 23, 2008, pp. 1023–1040.

[10] Oros DR, David N. “Identification and Evaluation of Unidentified Organic Contaminants in the San Francisco Estuary”. RMP Technical Report. Regional Monitoring Program for Trace Substances: San Francisco Estuary Institute, 2002. SFEI Contribution 45. 119 pp.

[11] Rushdi AI, Kassim T, Simoneit BRT. “Organic tracers in sediments from the coastal zone of Ras Abu el-Darag, Gulf of Suez”. Environ Geol, Vol. 58, 2009, pp. 1675–1687.

[12] Fox CB. “Squalene Emulsions for Parenteral Vaccine and Drug Delivery”. Molecules, Vol. 14, 2009, pp. 3286-3312.

[13] Ardiles AE, González-Rodríguez A, Núñez MJ, et. al. “Studies of naturally occurring friedelane triterpenoids as insulin sensitizers in the treatment of type 2 diabetes mellitus”. Phytochemistry, Vol. 84, 2012, pp. 116-128.