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ABSTRACT
Peloids are natural products that contain compounds with biological activity, permitting its therapeutic application. The control of these mineral resources is very important because they are used in human health and they are extracted in natural environments where they are suitable to microbiological, inorganic and organic contamination due to agricultural, port and fishing activities. The objective of this work is to determine the presence of anthropogenic organic compounds in San Diego de los Baños peloid, Pinar del Rio, Cuba. To identify the organic compounds, the samples were analyzed using gas chromatography and mass spectrometry. The results show the absence of common toxic contaminants. On the other hand, the anthropogenic compounds identified are mainly represented by different phthalates and butylated hydroxytoluene, which have several apparent pathways in waters and sediments. The degree of toxicity of these compounds is still under discussion in environmental samples.

Key words: peloid, organic, sediment, phthalate, butylated hydroxytoluene

INTRODUCTION
Thermal muds or peloids are basically sediments constituted by humus (organic substances) and minerals formed over a very long period of time by physical, chemical, biological and geological processes, and that are used in therapeutic applications. Nowadays, because of their use in therapeutics, relax and cosmetic, the correct management of peloids is increasing its importance. The control of peloids quality is one of the main aspects in the management of these mineral resources because they are extracted in natural environments and they are resources suitable to microbiological, inorganic and organic contamination due to agricultural, port and fishing activities. Also, chemical quality is directly related with the biological active compounds and the therapeutic properties of peloids. In general, inorganic and microbiological controls are well established in these environmental samples, but the determination of anthropogenic organic compounds is scarce due to its complexity. Organic anthropogenic compounds in muds can derive from different sources like the atmospheric deposition, effluent discharges, urban and agricultural activity, ground erosion, and port activities. The most common anthropogenic organic compounds are phenols (used as anti-rust and preservatives), polycyclic structures, aromatic hydrocarbons, phthalates (utilized as plasticizers), petroleum derivatives, fertilizers, nitrogenous compounds, and in the last years, different drugs from urban waste waters. One of the Cuban Thermal Centers characterized by the use of peloids, is San Diego de los Baños, located in Pinar del Río (Figure 1). This Center has great experience in the use of peloids in the treatment of different inflammatory and dermatological processes, as analgesic, in male and female infertility treatments, and as cosmetics.
The peloid used in San Diego de los Baños is a limous-clayed sediment, extracted from the estuary of the river of the same name and later enriched with inorganic, organic components and the microbiota of the calcium sulfate and the sulfurous thermal waters of San Diego de los Baños Termal Center (maturation process). In this maturation process the peloid exchanges with the water the elements that confer it its final quality.6

The objective of this work was to determine the presence of possible anthropogenic organic compounds in San Diego de los Baños peloid, Pinar del Río, Cuba, using Gas Chromatography and Mass Spectrometry (CG-MS).

MATERIALS AND METHODS

In the procedure for the extraction and chromatography analysis developed, the peloid was collected from the maturation pool in San Diego de los Baños Thermal Center. Afterwards, due to its water retention capacity,7,8 it was centrifuged at 5000 rpm during 5 minutes in nine successive centrifugations (total time: 45 minutes), to separate the watery phase from the solid phase. Afterwards, the solid phase is dried at room temperature until constant weight, homogenized, grounded to a fine powder in an agate mortar, and then sifted to a particle size of 125 µm.

The powder obtained (5 g) was washed with methanol for 24 hours at 200 min⁻¹ frequency, in a stirring machine; to remove the sulfur (main interference for the organic analysis) contained in the powder.9 The methanol extract is separated by centrifugation and the solid phase is then macerated with n-heptane for 14 hours to a 200 min⁻¹ frequency. The resultant n-heptane fraction (50 mL) is later concentrated by rotary evaporation up to 1.5 mL, and this concentrated extract is loaded into a silica column to obtain three separate fractions eluted using n-heptane, cyclohexane and di-isopropyl ether. The eluates obtained were concentrated by rotary evaporation up to 1.5 mL and then analyzed by Gas Chromatography - Mass Spectrometry (GC-MS) using a Shimatzu QP 5050A equipment. Separation was achieved on a fused silica capillary column coated
with phenyl at 5% (XTL5 30 m x 0.25 mm internal diameter x 0.25 μm film thickness). The Gas Chromatography operating conditions were the following: temperature hold at 100ºC for 1 minute, it increased from 100 to 290ºC at a rate of 20ºC per minute, with final isothermal hold at 290ºC for 3 minutes, pressure of 72.3 kPa and a flow of 1 mL/min. Helium was used as carrier gas. The samples were split injected (2 μL) with the injector temperature at 280ºC. The mass spectrometer was operated in electronic impact mode (EI) at 70 eV ionization energy, and scanned from 40 to 900 Da. Data were acquired and processed with GC-MS Solution 1.10 software. Individual compounds were identified by comparison of its mass spectra with literature and library data, and further interpretation of mass fragmentation patterns. In the analysis of the results, match qualities of 90% or greater against NIST 107 and 21 (National Institute of Standards and Technology) libraries are assumed to give reliable identifications. Tentative identification refers to qualities between 70% and 89% against the library. Analytes yielding match qualities of 69% or less were considered as unidentified.

RESULTS AND DISCUSSION

In the chromatograms of the eluted fractions (n-heptane, cyclohexane and di-isopropyl ether; Figures 2, 3 and 4, respectively), there are some resolved peaks that allow possible separation and identification of the majority of the organic compounds, under the selected experimental conditions.

In figures 2, 3 and 4 the significant chromatographic peaks observed, correspond mainly to saturated long chain hydrocarbons (alkanes) and alcohols. The origin of these saturated long chains hydrocarbons in peloids can be natural and/or anthropogenic. According to Philp and Mansuy (1997)\(^\text{10}\) anthropogenic hydrocarbons are represented by n-alkane petroleum signature and are commonly accompanied by other specific indicators (markers). These markers in sediments are isoprenoids hydrocarbons such as pristane (2,6,10,14 tetramethyl pentadecane) and phytane (2,6,10,14 tetraethyl hexadecane)\(^\text{4}\) and other compounds such as tricyclic terpanes, hopanes, stearanes, diasteranes, polycyclic aromatic hydrocarbons (PAH), and complex mixtures composed of highly branched and cyclic hydrocarbons\(^\text{11}\).

These markers were not found in the San Diego de los Baños peloid (Fig. 2, 3, 4), so alkanes present in this peloid, has a natural origin, related with the processes of degradation of the complex organic matter of animal and/or vegetable origin, to basic chains, in agreement with the reports by Oros and David (2002)\(^\text{4}\) and Labunksa et. al. (2000)\(^\text{5}\) in the analysis of different sediments by GC-MS.

The anthropogenic compounds in the n-heptane fraction are phthalates, such as di-n-butyl phthalate (DBPC), and the butylated hydroxytoluene (BHT), used as anti-rust, and both identified through their characteristic fragmentations in the mass spectra (Figure 2). Oros and David (2002)\(^\text{4}\) and Vondracek et. al. (2001)\(^\text{12}\) have reported the presence of these compounds accompanied by other plasticizers, such as the bis (2-ethyl hexyl) phthalate and the bis (2-ethyl hexyl) adipate in sediments of rivers and estuaries.
Figure 2. Chromatogram and fragmentations of the anthropogenic organic compounds identified in the n-heptane fraction of San Diego de los Baños peloid.

The anthropogenic compounds identified in the fraction eluted with cyclohexane (Figure 3), are the butylated hydroxytoluene (BHT), also present in the n-heptane fraction (Figure 2), and the bis (2-ethylhexyl) phthalate (DEHP), identified through their characteristic fragmentations in the mass spectra (Figure 3). BHT and DEHP are also eluted in the isopropyl ether fraction (Figure 4).

Figure 3. Chromatogram and fragmentations of the anthropogenic organic compounds identified in the cyclohexane fraction of San Diego de los Baños peloid.
Figure 4. Chromatogram and fragmentations of the anthropogenic organic compounds identified in the di-isopropyl ether fraction of San Diego de los Baños peloid.

Anthropogenic organic compounds may derive from a variety of sources (e.g., atmospheric deposition, sewage treatment plant effluent discharge, boating activities, agricultural and urban runoff, and soil erosion) and therefore can also been of different organic groups. The most common detected in the environmental samples are nitro and polycyclic musks used as fragrances (musk ketone, musk xylene), p-nonylphenol used in the production of non-ionic surfactants, phenols used as antioxidants and preservatives (butylated hydroxy toluene and butylated hydroxyl anisole), polybrominated diphenyl ethers (PBDEs) used as flame retardants, phthalates used as plasticizers in industrial polymers (di-n-butylphthalate, butylbenzyl phthalate, and bis(2-ethylhexyl)phthalate), triphenylphosphate used as a flame retardant and plasticizer, pesticides (benfluralin and trifluralin), petroleum (n-alkanes, biomarkers, and PAH), fertilizers, nitrogenous compounds and drugs. Only phthalates and butylated hydroxy toluene, were found in San Diego de los Baños peloid.

Phthalates are routinely identified in environmental samples and sample blanks during organic analyses because they are now ubiquitous synthetic contaminants in the environment, and they can appear in the analytic process as result of the use of plastic accessories. A blank study was carried out to determine the origin of the phthalates in San Diego de los Baños peloid. The blank study indicates that these anthropogenic organic compounds are not a product of the contamination from sample manipulation during the analytic procedure, and thus suggests that they are originally present in the peloid sample.

Several detailed reviews that address the toxicology and environmental fate of phthalates have been published. Data for the acute and chronic toxicities of butylbenzyl phthalate and di-n-butylphthalate on aquatic organisms such as microbes, algae,
invertebrates, and fish have also been reviewed in detail,\textsuperscript{16,17} but on the light of the current knowledge, uncertainty still remains on the effects of the phthalates in humans\textsuperscript{18} and there isn't a definitive approach on the possible toxicity of these compounds.

Ingestion is identified as the principal pathway for phthalates exposure in humans, followed by lower exposures from indoor air and drinking water.\textsuperscript{4,16} There are no reports of toxicity by skin contact of these compounds, supporting the safety of the peloid in topical therapy (Table 1).

On the other hand, butylated hydroxytoluene risk is reported by ingestion in high levels, and only the pure compound has been described with risk for eyes and skin irritation.\textsuperscript{19}

Table 1. Chemical structures, common uses, and toxicity of the anthropogenic organic compounds in San Diego de los Baños peloid (Oros, 2002).

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Chemical Structure</th>
<th>Common Uses</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>bis(2-ethylhexyl) phthalate</td>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>Plasticizer in flexible polyvinyl chloride products; replacement for PCBs in dielectric fluids for electric capacitors</td>
<td>By ingestion is considered a potential endocrine system disruptor</td>
</tr>
<tr>
<td>Formula: C_{24}H_{38}O_{4}</td>
<td>MW: 390</td>
<td></td>
<td></td>
</tr>
<tr>
<td>di-n-butylphthalate</td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>Plasticizer added to polyvinyl chloride to increase flexibility, adhesives, and coatings; lubricant for aerosol valves, antifoaming agent, skin emollient, plasticizer in personal care products and cosmetics</td>
<td>By ingestion is considered a potential endocrine system disruptor</td>
</tr>
<tr>
<td>Formula: C_{16}H_{22}O_{4}</td>
<td>MW: 278</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butylated hydroxytoluene</td>
<td><img src="image3" alt="Chemical Structure" /></td>
<td>Antioxidant for food, animal feed, petroleum products, synthetic rubbers, plastics, animal and vegetable oils, and soaps; anti-skinning agent in paints and inks</td>
<td>Unknown</td>
</tr>
<tr>
<td>Formula: C_{15}H_{24}O</td>
<td>MW: 220</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSIONS

The analytical procedure developed in this work allowed the identification, in the San Diego de los Baños peloid, of anthropogenic organic compounds such as butylated hydroxytoluene (BHT), di-n-butylphthalate and Bis(2-ethylhexyl) phthalate (DEHP). Nowadays these compounds are routinely identified in environmental samples and although the toxicity degree of these compounds is still under discussion, the prospect of...
probably riskier exhibition excludes the topical application. There are not other toxic anthropogenic compounds, considered and described in the literature as pollutants for muds. The results support the safety of the use San Diego de los Baños peloid in therapeutics, relax and cosmetic.

LITERATURE CITED


