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Sources and distribution of organic matter along the Ring of Cenotes, Yucatan, Mexico: Sterol markers and statistical approaches

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HIGHLIGHTS

- Sterol analysis and PCA used to characterize sources of organic matter.
- Marine, autotrophic, terrigenous, and anthropogenic sources along the Ring of Cenotes were assigned.
- Consistency between source assignment, land use context and season.

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ABSTRACT

The Yucatan Peninsula is a large low lying platform of limestone, dolomite and evaporite deposits, forming an extensive and mature karst aquifer with many sinkholes locally called cenotes. In Yucatan, the only source of drinking water is groundwater and its quality could be impaired by: (i) infiltration of contaminants and (ii) saltwater intrusion. To investigate the sources of organic matter in this aquifer, sediment samples (46) were collected from cenotes and analysed using gas chromatography–mass spectrometry. Sterol analysis, coupled with principal component analysis (PCA), allowed us to distinguish three sources of natural organic matter (e.g. marine, autotrophic and terrigenous) and to detect an anthropogenic input (e.g. fecal contamination). Good consistency was observed between the source assignment and the land use context (forest, agricultural, rural or urban areas) and the season, except for some of the samples where a direct correlation could not be made. The latter cases are most likely a result of the karstic character of the system.

1. Introduction

The Yucatan State is on the northwest side of the Yucatan peninsula, along the Gulf of Mexico. It is a tropical zone, characterized by two seasons (Gonzalez-Herrera et al., 2002): a rainy season (May to September) and a dry season (October to April). Annual rainfall ranges between 500 and 1500 mm, increasing from the coast to the interior (INEGI, 1992). The average temperature remains nearly constant throughout the year, ranging from 23 °C in January to 28 °C in May. The area is a large low lying platform and consists of limestone, dolomite and evaporite deposits (Perry et al., 1995), forming an extensive and mature karstic aquifer (Marin and Perry, 1994; Escolero et al., 2000; Bauer-Gottwein et al., 2011). Due to the geology of the peninsula, many sinkholes (locally called cenotes) have developed in rocks of Tertiary age (Perry et al., 1995). Particularly in the Yucatan State, the sinkholes form a rough semicircle, called the Ring of Cenotes. It extends southeast from Celestun to Cuzama and northeast from Cuzama to Dzilam de Bravo (Pérez-Ceballos et al., 2012). Along the ring, the spacing between cenotes varies widely, from a few meters to several kilometers (Pope and Duller, 1989; Perry et al., 1995).

This karst is characterized by a complex hydrological system with high permeability and porosity, so it is vulnerable to contamination (Marin and Perry, 1994). Because groundwater drawn from the aquifer is the only source of drinking water, it is important to investigate potential contamination. In the area, two phenomena can impair groundwater quality: (i) infiltration of contaminants from anthropogenic activity, particularly discharge of domestic, agricultural, industrial and medical wastes (Marin and Perry, 1994; Marin et al., 2000) and (ii) saltwater intrusion (Marin and Perry, 1994; Escolero et al., 2000). Importantly, there are no municipal treatment plants in the cities, and only some domestic septic tanks (Marin et al., 2000). Infiltration of untreated sewage into the aquifer constitutes a real threat of biological

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contamination (e.g. fecal contamination) leading to plausible public human health troubles. Finally, few studies have been undertaken to assess the potential impact of domestic discharge on the system.

Sterols are classically used as molecular markers to characterize the sources of natural organic matter (OM; e.g. marine and terrigenous) (Volkman, 1986; Yunker et al., 1995) and to identify anthropogenic inputs (e.g. fecal contamination) (Leeming et al., 1996; Derrien et al., 2012; Martins et al., 2014). Campesterol, stigmasterol and sitosterol (C_{28} and C_{29}) have been commonly used as markers of terrigenous OM, because of their predominance in terrestrial higher plants (Wen-Yen and Meinschein, 1976; Bouloubassi et al., 1997; Volkman, 2005; Martins et al., 2011). The main marine sterol marker is cholesterol, because it is the dominant sterol in invertebrates and marine zooplankton. Brassicasterol and dinosterol can be included in the marine marker group, being more specific for marine phytoplankton and dinoflagellates, respectively (Volkman, 1986; Wakeham and Canuel, 1988; Harvey, 1994; Bouloubassi et al., 1997). Anthropogenic input can be characterized via the 5β-stanol C_{27} sterols (e.g., coprostanol and epicoprostanol) (Grimalt et al., 1990; Leeming et al., 1996). These compounds are byproducts of cholesterol, via intestinal anaerobic microbial reduction in the digestive systems of higher animals (Leeming et al., 1996). They are commonly associated with domestic/animal sewage contamination (Bull et al., 2002; Isobe et al., 2002; Jardé et al., 2007; Shah et al., 2007; Derrien et al., 2012).

However, sterol analysis generates a large amount of data, often difficult to process and analyze. Multivariate statistical methods have demonstrated their effectiveness in this type of study. For example, several authors have used them in lipid marker distributions (fatty acids and sterols) to identify sources of OM (Yunker et al., 1995; Mudge and Duce, 2005; Martins et al., 2011; Derrien et al., 2011, 2012; Abreu-Mota et al., 2014). In this study, we applied principal component analysis (PCA) with a sterol marker approach in order to characterize the sources of OM, and potential anthropogenic inputs to sediments from the Ring of Cenotes.

2. Material and methods

2.1. Sampling and sample preparation

The sediment samples were collected from 23 selected sites along the Ring of Cenotes in the Yucatan state, Mexico. The sampling locations were chosen on the basis of land use patterns (e.g., local water extraction, dumping, tourism, in cattle farming, etc.), but also some rarely visited cenotes were included. The sediments were collected from cenotes with different morphometric characteristics: submarine springs, sinkholes (open or semi-caves, minor cavities and caverns) and waterholes (basin); during two seasons: the rainy season (September 2011) and the dry season (May 2012). Table 1 summarizes this information. The aim was not to observe a seasonal variation but rather to get an integrative view of organic matter inputs in a context where sediments are naturally (e.g. movement of material by biological, wind, or tidal forcing) or anthropogenically (e.g. recreational use or extraction of water) disturbed and where ways and mechanisms of transport are still relatively unknown.

Samples were collected from the first 5 cm of the sediment column, using a stainless steel grab. About 2 kg of sediment was collected for each sample. After homogenization, about 0.2 kg of each was frozen for 2 days, freeze dried for 3 days and sieved to obtain the <500 μm fraction that were used for extraction and steroid analysis.

TOC was determined following Daesslé et al. (2009), and the analyses were carried out with a LECO CHNS 932 elemental analyzer.

2.2. Reagent and chemicals

Solvents were HPLC grade. Dichloromethane (DCM), MeOH, and hexane were from Sigma-Aldrich. The mixture (99:1, v:v) of N,O-bis(trimethylsilyl)trifuoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) was from Supelco. Coprostanol (5β-cholestan-3β-ol), cholesterol (cholest-5-en-3β-ol), cholestanol (5α-cholestan-3β-ol), stigmasterol (24-ethylcholesta-5,22-dien-3β-ol), sitosterol (24-ethylcholest-5-en-3β-ol), sitostanol (24-ethylcholest-5α-en-3β-ol), 5α-cholestane, and cholesterol-2,2,3,4,4,6-d_6 were from Sigma-Aldrich.

Table 1
Description of sampling areas along the Ring of Cenotes, Yucatan, Mexico. Land use pattern as reported by Arcega-Cabrera et al. (2014).

Sample	Type	Land use pattern
Dzilam de Bravo		
(N 21° 20′ 21.3″–21° 24′ 24.5″/W 88° 34′ 31.3″–88° 53′ 55.7″)		
1	Xbuya submarine spring	Urban area
2	Submarine spring	Urban area
3	Elepeten sinkhole	Urban and touristic area
4	Submarine spring	Urban area
5	Submarine spring	Urban area
6	Sinkhole	Urban area
7	Waterhole	Cattle farming area
8	Waterhole	Cattle farming area
Cuzama		
(N 20° 33′ 07.3″–20° 44′ 29.5″/W 89° 16′ 05.3″–89° 28′ 52.4″)		
9	Telchaquillo sinkhole	Forested and touristic area
10	Nahyah sinkhole	Agricultural and touristic area
11	Noh-Mozon sinkhole	Forested and touristic area
12	Kalcuch sinkhole	Forested and touristic area
13	Tanimax sinkhole	Forested and touristic area
14	Santa Maria cave	Touristic area
15	Yaxpakaltun sinkhole	Agricultural and touristic area
Celestun		
(N 20° 40′ 25.1″–21° 08′ 54.2″/W 89° 43′ 55.4″–90° 14′ 08.0″)		
16	Yaxcopoil sinkhole	Forested and touristic area
17	X'batun sinkhole	Forested and touristic area
18	San Ignacio sinkhole	Touristic area
19	Sinkhole	Forested area
20	Doña Lucy sinkhole	Urban area
21	Ba'as submarine spring	/
22	Waterhole	Crop area
23	Waterhole	Crop area

2.3. Extraction and fractionation

The extraction protocol was modified from Isobe et al. (2002). Extraction was performed using a Cole-Palmer Ultrasonic Processor, 500 and 750 W, Model CV. The freeze dried sample (2 g) was placed in a 40 mL glass tube, spiked with deuterated cholesterol (cholesterol-2,2,3,4,4,6-d_6) standard in DCM (2 μg) as recovery standard and ultrasonically extracted with MeOH, MeOH/DCM (1:1, v:v), and DCM, consecutively, using 30 mL of each. Extractions were carried out with 40% of the sonicator amplitude during 7 min, with 1 min switch on and 2 min switch off for each solvent. After sonication, the suspension was filtered through a glass filter (Whatman GF/C, 1.2 μm). The extracts were combined and concentrated just to dryness using a rotary evaporator at 60 °C and 337 mbar.

The combined extract was re-dissolved in a fixed volume of hexane (10 mL) and the extract or an aliquot fractionated using column chromatography on silica, previously washed with 200 mL hexane and DCM and activated at 100 °C for 24 h. Three fractions (aliphatic, aromatic, and polar) were eluted successively with hexane, followed by hexane/DCM (65:35, v/v) and MeOH/DCM (1:1, v/v). The polar fraction was the focus of the study.

2.4. Gas chromatography–mass spectrometry (GC–MS)

For analysis, 50 μL of the polar fraction was dried under a gentle flux of N_2 and then 50 μL of BSTFA + TMCS (N,O-bis(trimethylsilyl) trifluoroacetamide and trimethylchlorosilane) were added. The derivatization was performed at 70 °C for 30 min. 1 μL of the derivatized sample was injected into an Agilent 5975B VL MSD GC–MS system (Agilent