ISOTOPIC EVIDENCE FOR THE MIGRATION OF THERMOGENIC METHANE INTO A SULFIDIC CAVE, CUEVA DE VILLA LUZ, TABASCO, MEXICO

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Abstract: Methane (CH4) is an economic resource and a greenhouse gas, but its migration through rocks is not immediately associated with speleogenesis. Sulfuric-acid speleogenesis is a cave-forming mechanism that has produced a variety of economically important oil fields and aquifers, and is theorized to be related to the oxidation of CH4 and hydrocarbons. Despite hypotheses that the oxidation of CH4 may provide a basis for the generation of sulfides during sulfuric-acid speleogenesis, evidence from active systems has not yet been obtained. In this study, we address how CH4 influences the development of sulfidic cave systems by sampling the CH4, H2S, and CO2 concentrations, as well as δ13CCH4, δ34SH2S, and δ13CCO2 values, in a cave currently forming by sulfuric-acid speleogenesis, Cueva de Villa Luz. CH4, H2S, and CO2 concentrations were highest directly above springs in the cave, showing that all three gases enter by means of the spring water. The δ13CCH4 and δ34SH2S in the air of CVL ranged from −47.92 ± 0.15 to −35.47 ± 0.12 % (VPDB) and −117 to −83 % (VSMOW), respectively. Keeling plots suggest that CH4 with δ13CCH4 = −24 ± 3 % and δ34SH2S = −40 ± 50 % was outgassing from spring water. This stable-isotope signature does not fall within traditional published δ13CCH4 versus δ34SH2S fields. Our data suggest that the CH4 entering Cueva de Villa Luz is the remnant of a larger thermogenic CH4 flux that is incompletely oxidized in the subsurface as it travels to Cueva de Villa Luz. Our data support links between the processes forming Cueva de Villa Luz and the proposed mechanisms for other caves associated with sulfuric acid.

INTRODUCTION

The migration of subsurface methane (CH4) through rock units toward the earth’s surface creates natural gas deposits, contributes to the atmospheric CH4 burden, and supports microbial communities, but is rarely thought to alter the encountered rock units. However, CH4 and other hydrocarbons may enhance the porosity and permeability of limestones by fostering sulfuric-acid speleogenesis (Davis, 1980; Egemeier, 1981; Kirkland and Evans, 1976; Hill, 1990, 1995). As CH4-rich fluids migrate within the subsurface and interact with evaporites, such as anhydrite or gypsum, they obtain sulfate (SO4^2-) that may react with CH4 to form hydrogen sulfide (H2S):

\[
\text{Ca}^{2+} + 2\text{SO}_4^{2-} + 2\text{CH}_4 + 2\text{H}^+ \rightarrow 2\text{H}_2\text{S} + \text{CaCO}_3 + 3\text{H}_2\text{O} + \text{CO}_2
\]

(1)

The introduction of sulfide to oxygen in near-surface environments results in the formation of sulfuric acid (H2SO4), a strong acid capable of rapidly dissolving limestone. With some of the world’s largest hydrocarbon deposits and aquifers hosted in sulfuric-acid dissolution-influenced limestones (Hill, 1995), a better understanding of how these caves form is important for finding new oil fields and drinking water. The suggestion that sulfuric-acid speleogenesis is associated with hydrocarbons originated from studies of Lower Kane Cave in Wyoming (Davis, 1980; Egemeier, 1981). Later, the morphologies and mineral assemblages of caves in the Guadalupe Mountains spurred hypotheses that these caves formed through sulfuric-acid speleogenesis (Hill, 1990, 1995; Peryt et al., 2012). The negative δ34S values of hydrogen sulfide from springs and sulfide minerals in the caves suggested that microorganisms were responsible for the generation of the sulfides. Additionally, δ13C CO2 values from carbonates in these and other settings suggested that CH4 and hydrocarbons were oxidized in tandem with SO4^2- reduction. Although the migration of CH4 is hypothesized to be important for the formation of sulfidic caves, to our knowledge no studies of these caves have measured CH4 concentrations in multiple locations from their entrances to their interiors, nor have measurements of both δ13CCH4 and δ34SH2S values been made in a cave currently undergoing sulfuric-acid speleogenesis.

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Stable-isotope ratios of carbon and hydrogen in CH₄ provide a tool for understanding the origins and reactions of CH₄ in the environment. For example, atmospheric CH₄ has δ¹³CCH₄ and δ²HCH₄ values that are roughly −47.5 ‰ (VPDB) and −100 ‰ (VSMOW), respectively (Thom et al., 1993). Thermogenic CH₄, which forms from the breakdown of organic matter by heat, has δ¹³CCH₄ and δ²HCH₄ values that generally range from −56 to −20 ‰ and from −300 to −70 ‰, respectively (Schoell, 1988; Whiticar, 1999). The original δ¹³CCH₄ and δ²HCH₄ values are altered during both biotic and abiotic CH₄ oxidation; the former causes an increase of 8.5 ‰ in δ²HCH₄ for each % increase in δ¹³CCH₄ (Feisthauer et al., 2011). Thermochemical sulfate reduction, a form of abiotic methane oxidation, shows δ¹³CCH₄ and δ²HCH₄ values of roughly −30 and −120 ‰ respectively (Liu et al., 2014). Furthermore, the mixing of CH₄ from different sources can create CH₄ with unusual isotopic ratios. For example, the Homorod mud volcano of Romania exhibits variations in δ²HCH₄ values from −25 to +30 ‰ that result from mixing between microbially produced CH₄ and CH₄ with a δ²HCH₄ of +124 ‰ generated from incomplete abiotic oxidation (Etiope et al., 2011).

CH₄ has been measured from only two caves undergoing sulfuric-acid speleogenesis. The caves showed different characteristics. In Movile Cave in Romania, the CH₄ in an isolated pocket of air between the cave’s roof and a water body had a concentration of 10,000 ppmv and a δ¹³CCH₄ value of −60 ‰, indicating the presence of microbially generated CH₄ (Sarbu et al., 1996; Hutchens et al., 2004). CH₄ concentrations in Frasassi Cave in Italy ranged from 1.9 to 2.2 ppmv at a single collection site (Jones et al., 2012), although isotopic compositions were not reported. The current CH₄ data set from sulfidic caves must be augmented in order to rigorously test current theories about hydrocarbon-driven sulfuric-acid speleogenesis.

We tested the hypothesis that hydrocarbons are fundamental in sulfuric-acid speleogenesis in the active Cueva de Villa Luz (CVL) system by measuring CH₄, H₂S, and CO₂ concentrations and δ¹³CCH₄, δ²HCH₄, and δ¹²C CO₂ values. If CH₄ oxidation is responsible for the generation of H₂S in sulfidic caves, then abundances of CH₄, H₂S, and CO₂ should be correlated in cave air as the gases follow the same pathway into the cave unless there are additional destruction or production mechanisms along the way. Additionally, the δ¹³CCH₄ and δ²HCH₄ values should show signs of oxidation, and δ¹²C CO₂ values should be relatable to oxidized CH₄. This study presents the first observations of CH₄ concentrations along gradients from cave entrance to interior and of the stable-isotope ratios of hydrogen in CH₄ in the air of an actively forming sulfidic cave.

**Geological Setting**

**Regional Geological Setting**

CVL is a shallow cave, has several natural openings, and is located in the Sierra de Chiapas 2 km south of Tapijulapa, Tabasco, Mexico. It is hosted within a carbonate shelf of the middle Cretaceous Sierra Madre Formation (Rosales Lagarde et al., 2006; Fig. 1). The sedimentary record of the region spans from the Jurassic to the Tertiary and includes hydrocarbon formation (Aguilar Rodriguez, 2007; Clara Valdée et al., 2009). Regional hydrocarbons, such as oils located about 50 km north of CVL, are presumably derived from Cretaceous marine limestones (Guzman-Vega and Mello, 1999).

Several potential sources of hydrogen sulfide (H₂S) are present in the CVL region. Possible sources of H₂S for CVL include the volcano El Chichón located 50 km west of CVL; petroleum basins located 50 km north of CVL such as the Macuspana and the Comalcalco basins; petroleum deposits within the Sierra de Chiapas; and economic sulfide deposits 25 km west of CVL (Pantoja-Alor, 1989; Hose et al., 2000; Rosales Lagarde et al., 2006, 2014; Fig. 1). However, sulfur-isotope ratios of dissolved sulfide in the H₂S-rich brackish springs of CVL range from −24 to −6 ‰ Vienna Canyon Diablo Troilite (VCDT) and indicate that biological sulfate reduction of anhydrite-derived sulfate is the main mechanism producing the hydrogen sulfide in the groundwater column feeding the cave (Rosales Lagarde, 2013).

The regional tectonic history includes periods of extension and compression resulting in large scale faulting, fracturing, and folding (Meneses-Rocha, 2001; Alcántara García et al., 2004; García Palomo et al., 2006). The main passage of CVL follows the strike of east-northeast bedding planes of Cretaceous limestone. This limestone is folded into a northwest plunging anticline and cut by a thrust fault (Rosales Lagarde, 2013; Fig. 1). Faulting and fracturing seem to facilitate the upward migration of deeper fluids. Additionally, hydrocarbon seeps in the region are common (Aguilar Rodriguez, 2007; PEMEX, personal communication).

**Geomorphology and Geochemistry of Cueva de Villa Luz**

The cave’s main entrance and twenty-four skylights connect the air of CVL with the atmosphere (Fig. 2) (Hose and Pisarowicz, 1999). CVL is a weakly hydrothermal system and has a slightly elevated mean annual cave-air temperature of 28 °C compared to the regional mean annual atmospheric temperature of 27 °C. Sulfide-rich springs release H₂S into the cave air, where it ranges in concentration from 1 to 40 ppmv depending on location (Hose and Pisarowicz, 1999). CVL is known to experience strong H₂S and carbon monoxide outgassing events when the H₂S concentration may increase from < 12 ppmv to > 240 ppmv in less than 3 minutes (Kleina, and Hose, pers. comm.; Boston, unpublished results). The cause of these outgassing events is unknown.

CVL’s twenty-six known internal springs broadly fall into two categories: (i) springs with < 4.3 mg L⁻¹ dissolved O₂ and < 0.1 mg L⁻¹ dissolved H₂S and (ii) springs with < 0.1 mg L⁻¹ dissolved O₂ and 300 to 500 mg L⁻¹ dissolved H₂S (Hose et al., 2000). Non-sulfidic springs have a higher temperature than
those with sulfide (Rosales Lagarde, 2013). All springs are brackish, and the water chemistry is influenced by dissolution of limestone, dolomite, halite, and gypsum (Rosales Lagarde et al., 2014; Hose et al., 2000, Spilde et al., 2004).

METHODS

AIR SAMPLING

The geochemistry of cave air is affected by atmospheric conditions, which may dominate near entrances or skylights, and internal cave processes, which may dominate the chemistry in cave interiors (Peyraube et al., 2016). We sampled air in CVL using in-situ and discrete techniques. We used in-situ sampling to measure H2S concentrations and obtained discrete air samples in 1 L Tedlar bags for later analysis of CH4 and CO2. We selected sampling locations based on their proximity to springs and entrances and measured the distances from each sampling location to the nearest entrance or spring based on the map of Hose and Pisarowicz (1999). Samples were defined as “near a spring” if they were obtained < 2 m from a spring and samples were defined as “near an entrance” if they were < 10 m from an entrance.

We measured H2S concentrations with a GasBadge Pro H2S instrument (Industrial Scientific, Pasadena, Texas) and a Ventis MX4 device (Industrial Scientific, Pasadena, Texas) from nine locations in CVL (Fig. 2) (Table 1). We measured H2S concentrations at several elevations above the stream to assess the extent to which exsolution of H2S from spring waters altered the concentration of H2S in cave air. When data were measured with both the GasBadge and the Ventis (Table 1), we took the average of the H2S concentrations (average difference, Ventis – Gasbadge = −2 ppmv). At locations 1 through 12 we obtained discrete air samples in the cave, and we also obtained a discrete air sample from the local atmosphere 20 m from the main entrance of CVL using
standard air-sampling techniques (Fig. 2) (Webster et al., 2016). All discrete cave-air samples from locations near springs were obtained 30 cm above the springs.

LABORATORY ANALYSES

We measured CH4 and CO2 concentrations from discrete samples with a Varian 450 gas chromatograph (GC) (see Webster et al., 2016). CH4 and CO2 calibration curves for concentration and uncertainty estimates were calculated following the methods of Webster et al. (2016).

We measured the δ13CCH4, δ2HCH4 and δ13CCO2 values to help determine the sources of CH4 and CO2 entering CVL. δ13CCH4, δ2HCH4 and δ13CCO2 values were measured on a Thermo Finnigan Delta Plus XP isotope-ratio mass-spectrometer (IRMS). δ13CCH4 and δ2HCH4 values were measured in continuous-flow mode using a gas chromatography-oxidation/pyrolysis-IRMS (GC-ox/pyr-IRMS) interface following previously published methods (Miller et al., 2002; Webster et al., 2016). δ13CCO2 was measured with a Thermo Finnigan GasBench II inlet connected to the IRMS.

Raw 13C/12C and 2H/H isotope ratios measured by the mass spectrometer were converted to conventional δ-values using two-point calibrations. The in-house CH4 reference materials were calibrated against international measurement standards NBS 19, LSVEC, VSMOW, and SLAP. Reference materials had δ13CCH4 values of −38.25 ± 0.06 and −58.64 ± 0.15 %o and δ2HCH4 values of −41 ± 3 and −160 ± 4 %o, respectively. Analytical uncertainties of the δ13CCH4 and δ2HCH4 values of the CH4 reference materials ranged from ± 0.07 to ± 0.15 %o (2 SD), and ± 8 to ± 12 %o (2 SD), respectively, depending on the day of measurement, and these were assigned to data from air samples that were measured on the same day.

Raw 13C/12C ratios of CO2 were converted to δ13CCO2 values on the VPDB scale using a single standard with
QUANTITATIVE ANALYSES

Relationships between CH4, CO2, and H2S concentrations and their distances to a spring were assessed through two-sample t-tests assuming unequal variance. Additionally, correlations between CH4 and CO2 concentrations in the air of CVL were examined through regression analysis. The isotopic composition of the source of CH4 from cave springs was assessed using Keeling plots, as described in Thom et al. (1993). This approach assumes that gas samples represent varying mixtures of two gas end-members with contrasting 13CCH4 and 12HCH4 values of CH4 entering from springs were measured ten times. Sample uncertainties were assessed using the methods of Webster et al. (2016).

RESULTS

H2S concentrations in the air of CVL ranged from 0.9 ± 0.8 to 23.05 ± 0.05 ppmv (Table 1) and were highest closest to springs (two-sample t-test unequal variance, t14 = 3.02, p = 0.005; Fig. 3). CH4 concentrations ranged from 1.88 ± 0.10 ppmv to 3.67 ± 0.18 ppmv (n = 12), and CO2 concentrations ranged from 400 ± 20 ppmv to 920 ± 50 ppmv (n = 12; Fig. 4; Table 2). The concentrations of CH4 and CO2 in the local atmosphere were 1.96 ± 0.10 ppmv and 430 ± 20 ppmv, respectively. CH4 and CO2 concentrations were positively correlated in the atmosphere of CVL (r = 0.95, n = 12, p < 0.01; Fig. 4). CH4 and CO2 concentrations were higher at locations close to springs than at other locations (CH4: two-sample t-test unequal variance, t5 stat = 3.57, p = 0.008; CO2: two-sample t-test unequal variance, t6 stat = 3.05, p = 0.01; Fig. 5A). CH4 and CO2 concentrations were not related to the distance from an entrance (CH4: two-sample t-test unequal variance, t5 stat = −1.16, p = 0.15; CO2: two-sample t-test unequal variance, t3 stat = −0.75, p = 0.25; Fig. 5B).

δ13C CO2 values ranged from −12.0 ± 0.2 to −8.7 ± 0.4 ‰ (Table 3). Air samples near cave entrances showed the lowest concentrations of CO2 and had δ13C CO2 values around −9.6 to −9.4 ‰. The δ13C of atmospheric CO2, −10.8 ± 0.6 ‰, did not match the δ13C CO2 values of other samples with near atmospheric CO2 concentrations, nor samples of atmospheric CO2 from other studies (Peyraube et al., 2013; Fernandez-Cortes et al., 2015), and was excluded from further analyses. As CO2 concentrations increased, their δ13C CO2 values diverged from the δ13C of low-CO2 samples (Table 3). The slope of δ13C CO2, data in a Keeling plot was not different from zero (r2 = 0.23, n = 12, p = 0.11).

The relationships between CH4 concentration and δ13C CH4, and δ2H CH4 values in CVL air fit a two-end-member mixing model in Keeling plots (Fig. 6). δ13C CH4 ranged from −47.92 ± 0.15 to −35.47 ± 0.12 ‰ and was linearly related to the inverse CH4 concentration (δ13C CH4 = [−45 ± 7] × [CH4]−1 + [−24 ± 3], r2 = 0.98, n = 9, p < 0.01; Fig. 6A). δ2H CH4 ranged from −117 ± 9 to −65 ± 12 ‰ and was linearly related to the inverse CH4 concentration (δ2H CH4 = [−140 ± 80] × [CH4]−1 +[50 ± 4], r2 = 0.96, n = 12, p < 0.01; Fig. 6B).

Figure 3. H2S concentration in air 20 cm above the cave stream versus distance to the nearest spring in Cueva de Villa Luz. The elevated H2S concentration at a distance of 28 m from a spring may be related to turbulent water entering from an adjacent room.

δ13C CO2 = −12.0 ± 0.2 ‰. Samples and standards were measured ten times. Sample uncertainties were assessed using the methods of Webster et al. (2016).

Figure 4. CH4 versus CO2 concentrations in Cueva de Villa Luz (CVL) showing a strong positive correlation (r = 0.95, n = 12, p < 0.01). Open circles represent CVL air samples, whereas the black circle represents the open atmosphere outside of the cave.
Air samples from locations nearest to entrances define one end-member of CH$_4$ entering the cave with a $\delta^{13}$C$_{\text{CH}_4}$ of $-47.6 \pm 0.8\%$ and a $\delta^{2}$H$_{\text{CH}_4}$ of $-112 \pm 14\%$. Samples obtained near springs suggest a second source of CH$_4$ entering CVL with a $\delta^{13}$C$_{\text{CH}_4}$ of $-24 \pm 3\%$ and a $\delta^{2}$H$_{\text{CH}_4}$ of $-40 \pm 50\%$ (Figs. 6, 7).

### DISCUSSION

**Hydrogen Sulfide, Methane, and Carbon Dioxide Enter CVL from Springs**

The observed concentrations in CVL of H$_2$S, CH$_4$, and CO$_2$, and the stable-isotope compositions of CH$_4$ and CO$_2$ result from a combination of airflow through the cave,

### Table 2. Sample collection dates and measurement dates for the concentrations of CH$_4$ and CO$_2$. Dates are listed as mm/dd/yyyy.

| Site     | Collection Date | CH$_4$ concentration | CO$_2$ Concentration |
|----------|-----------------|-----------------------|-----------------------|
|          | Analysis Date   | Conc. (ppmv)          | e (2SD)$^a$           | Analysis Date   | Conc. (ppmv) | e (2SD)$^a$ |
| Site 1   | 12/17/2013      | 12/31/2013            | 1.89                  | 0.09             | 12/31/2013    | 460          | 30          |
| Site 2   | 12/17/2013      | 12/31/2013            | 2.14                  | 0.11             | 12/31/2013    | 520          | 30          |
| Site 3   | 12/17/2013      | 12/31/2013            | 2.12                  | 0.11             | 12/31/2013    | 470          | 30          |
| Site 4   | 12/17/2013      | 12/31/2013            | 2.03                  | 0.10             | 12/31/2013    | 450          | 30          |
| Site 5   | 12/16/2013      | 12/31/2013            | 1.98                  | 0.10             | 12/31/2013    | 490          | 30          |
| Site 6   | 12/17/2013      | 12/31/2013            | 1.97                  | 0.10             | 12/31/2013    | 470          | 30          |
| Site 7   | 12/17/2013      | 12/31/2013            | 1.88                  | 0.09             | 12/31/2013    | 400          | 20          |
| Site 8   | 12/17/2013      | 12/31/2013            | 3.16                  | 0.16             | 12/31/2013    | 860          | 50          |
| Site 9   | 12/16/2013      | 12/31/2013            | 3.03                  | 0.15             | 12/31/2013    | 860          | 50          |
| Site 10  | 12/16/2013      | 12/31/2013            | 2.63                  | 0.13             | 12/31/2013    | 590          | 40          |
| Site 11  | 12/17/2013      | 12/31/2013            | 2.33                  | 0.12             | 12/31/2013    | 570          | 30          |
| Site 12  | 12/17/2013      | 12/31/2013            | 3.67                  | 0.18             | 12/31/2013    | 850          | 50          |
| Outside Cave | 12/17/2013 | 12/31/2013 | 1.96                  | 0.10             | 12/31/2013    | 430          | 30          |

$^a$ SD = Standard deviation.

### Table 3. Sample stable isotopic compositions of CH$_4$ and CO$_2$. Dates are listed as mm/dd/yyyy.

| Site     | Collection Date | $\delta^{13}$C$_{\text{CH}_4}$ (VPDB) | $\delta^{2}$H$_{\text{CH}_4}$ (VPDB) | $\delta^{13}$C$_{\text{CO}_2}$ (VPDB) |
|----------|-----------------|---------------------------------------|---------------------------------------|---------------------------------------|
|          | Analysis Dates  | % (VPDB) e (2SD)$^a$                   | % (VPDB) e (2SD)$^a$                   | % (VPDB) e (2SD)$^a$                   |
| Site 1   | 12/17/2013      | 4/9/2014                              | -47.26                               | 0.12                                  | LSV$^b$                                |
| Site 2   | 12/17/2013      | 4/3/2014                              | -45.71                               | 0.15                                  | 3/18/2014                              | -96         | 10          | 1/9/2014    | -9.4       | 0.2        |
| Site 3   | 12/17/2013      | 4/3/2014                              | -46.51                               | 0.15                                  | 3/19/2014                              | -97         | 12          | 1/9/2014    | -9.6       | 0.3        |
| Site 4   | 12/17/2013      | SL$^c$                                | ...                                  | ...                                   | LSV$^b$                                | ...         | ...         | 1/9/2014    | -9.6       | 0.3        |
| Site 5   | 12/16/2013      | 4/4/2014                              | -45.73                               | 0.07                                  | 3/18/2014                              | -116        | 10          | 1/9/2014    | -9.6       | 0.7        |
| Site 6   | 12/16/2013      | 4/3/2014                              | -47.92                               | 0.15                                  | 3/21/2014                              | -117        | 9           | 1/9/2014    | -9.5       | 0.3        |
| Site 7   | 12/16/2013      | 4/9/2014                              | -38.28                               | 0.12                                  | 3/18/2014                              | -104        | 10          | 1/9/2014    | -12        | 0.2        |
| Site 8   | 12/16/2013      | 4/3/2014                              | -42.45                               | 0.15                                  | 3/19/2014                              | -87         | 12          | 1/9/2014    | -10.6      | 0.2        |
| Site 9   | 12/16/2013      | 4/9/2014                              | -35.47                               | 0.12                                  | 3/19/2014                              | -65         | 12          | 1/9/2014    | -10.4      | 0.2        |
| Site 10  | 12/16/2013      | 10/9/2014                             | -39                                  | 3                                     | ...                                   | ...         | ...         | 1/9/2014    | ...        | ...        |
| Site 11  | 12/16/2013      | 4/3/2014                              | -40.63                               | 0.15                                  | 3/21/2014                              | -83         | 8           | 1/9/2014    | -9.7       | 0.3        |
| Site 12  | 12/17/2013      | SL$^c$                                | ...                                  | ...                                   | LSV$^b$                                | ...         | ...         | 1/9/2014    | -9.1       | 0.3        |
| Outside Cave | 12/17/2013 | 4/3/2014                              | -47.34                               | 0.15                                  | 3/18/2014                              | -107        | 10          | 1/9/2014    | -8.7       | 0.4        |

$^a$ SD = Standard deviation.

$^b$ Sample was not measured due to low sample volume (LSV = low sample volume).

$^c$ The sample had leaked at the time of measurement (SL = sample leakage).

$^d$ This measurement did not agree with other measurements of low CO$_2$ concentration air.
biogeochemical processes operating within the cave, and
biogeochemical processes in the waters that feed the cave
springs. The temperature of the open atmosphere at the time of
sampling was lower than the temperature inside CVL, and
cool air was felt entering from the skylights. This type of
airflow should result in cave air composition near skylights
that resembles the open atmosphere, as was observed in CVL
at the time of sampling. Additionally, high rates of
atmospheric exchange are indicated by roughly atmospheric
CH$_4$ and CO$_2$ concentrations at sample sites that were neither
close to an entrance nor immediately adjacent to springs.

Elevated CH$_4$ and CO$_2$ concentrations at springs imply that
CH$_4$ and CO$_2$ are exsolving from spring water (Fig. 4). The
only site located close to a spring that did not show elevated

CH$_4$ and CO$_2$ concentrations was Midway Spring (Site 4),
which is located 9 m from a skylight (Fig. 4). Elevated CH$_4$
concentrations in cave air near springs have not been reported
from other caves. The CH$_4$ concentrations in CVL that were
not located by springs (1.9 to 2.2 ppmv) are more similar to
those measured in the sulfidic Frasassi Cave system (1.9 to 2.2
ppmv) in Italy (Jones et al., 2012) than they are to the sulfidic
Movile Cave (10,000 ppmv) in Romania (Hutchens et al.,
2004). This difference may be due to the lack of atmospheric
connectivity at the site in Movile Cave, which is mostly water-
filled. We did not observe CH$_4$ concentrations below 1.5
ppmv, as have been observed in many epigenic, non-sulfidic
caves in Gibraltar, Australia, the United States, and Spain
(Mattey et al., 2013; Fernandez-Cortes et al., 2015; McDo-
nough et al., 2016; Webster et al., 2016; Lennon et al., 2016).
Our results demonstrate that if in-situ CH$_4$ oxidation processes
were operating in CVL, they were not strong enough to react
all of the CH$_4$ in the collected samples. We can therefore

Figure 5. A) CH$_4$ concentrations in cave air at different
distances from springs. B) CH$_4$ concentrations in cave air
at different distances from an entrance. Dashed lines
indicate the global atmospheric average CH$_4$
concentration (Adushkin and Kudryavtsev, 2013).

Figure 6. Keeling plot of $\delta^{13}$C$_{\text{CH}_4}$ from cave air (A, $\delta^{13}$C$_{\text{CH}_4}$
$\approx [-45 \pm 7] \times [\text{CH}_4]^{-1} + [-24 \pm 3], r^2 = 0.98, n = 9, p <$
0.01) and $\delta^2$H$_{\text{CH}_4}$ (B, $\delta^2$H$_{\text{CH}_4} = [-140 \pm 80] \times [\text{CH}_4]^{-1} +$
$[-40 \pm 50], r^2 = 0.62, n = 8, p = 0.02$. The error bars are
smaller than the data points in A. The star marks the y-
intercept that indicates the $\delta^{13}$C$_{\text{CH}_4}$ and $\delta^2$H$_{\text{CH}_4}$ of the
dissolved CH$_4$ in the spring water. The open circles
represent Cueva de Villa Luz air samples, and black
circles represent the outside atmosphere as measured
during the study.

The star marks the y-intercept that indicates the $\delta^{13}$C$_{\text{CH}_4}$ and $\delta^2$H$_{\text{CH}_4}$ of the
dissolved CH$_4$ in the spring water. The open circles
represent Cueva de Villa Luz air samples, and black
circles represent the outside atmosphere as measured
during the study.

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conclude that two most likely processes affecting CH₄ in CVL are atmospheric circulation and spring-water outgassing.

**Stable Isotopic Composition and Sources of Methane**

The CH₄ concentrations and values of δ¹³CCH₄ and δ²HCH₄ observed in CVL are consistent with mixing between an atmospheric CH₄ source and a CH₄ source entering from the springs. The lowest concentrations of CH₄ in the air of CVL were measured at locations that were both close to entrances and far from springs. The δ¹³CCH₄ and δ²HCH₄ values from these locations plot near the respective values of atmospheric CH₄ (Figs. 6, 7). The concentrations and isotopic composition of CH₄ from the local atmosphere and cave locations near skylights agree with other measurements of atmospheric CH₄ (Mikaloff Fletcher et al., 2004). The CH₄ concentrations in CVL suggest that a secondary source of CH₄ with a δ¹³CCH₄ of −24 ± 3 ‰ and a δ²HCH₄ of −40 ± 50 ‰ enters CVL dissolved in spring water. CH₄ with this stable isotopic composition does not closely match known isotopic compositions generated by primary biotic or abiotic processes, and is driven largely by relatively positive δ²HCH₄ values (Fig. 7).

The origin of the CH₄ entering CVL at springs can be constrained in three ways, by comparing the CH₄ isotopically to other CH₄ sources in the region, by taking into account the regional thermal history, and by compensating for shifts in CH₄ isotopic composition caused by oxidation. A possible explanation for δ¹³CCH₄ values in cave air is that the CH₄ entering CVL from the springs has an original isotopic composition with a δ¹³CCH₄ value of −24 ± 3 ‰ and a δ²HCH₄ value of −40 ± 50 ‰, based on the Keeling analysis. However, this δ¹³CCH₄ of CH₄ from CVL spring water is incompatible with CH₄ from neighboring sedimentary basins, which have δ¹³CCH₄ values of −32 to −70 ‰ in the Macuspana Basin; −38 to −45 ‰ in the Papaloapan Basin; −39 to −55 ‰ in the Cuichapa Basin; and −40 to −52 ‰ in the Reforma Basin (Clara Valdés et al., 2009). The most similar δ¹³CCH₄ value from a regional natural gas well is about −32 ‰ from the Macuspana Basin (Talukdar et al., 2002; Clara Valdés et al., 2009). Additionally, the proposed δ²HCH₄ value of CH₄ entering CVL from springs is very positive compared to values from the literature (Schoell, 1980; Etiope and Sherwood Lollar, 2013; Etiope and Sherwood Lollar et al., 2013). The evidence implies that a parental CH₄ with the proposed δ¹³CCH₄ and δ²HCH₄ values is unlikely, and supports the idea that CH₄ entering CVL in springs is the remnant of a larger CH₄ flux at depth that has been mostly oxidized to CO₂ on the way to the cave.

The thermal history of the CVL region provides a way to help determine whether abiotic or biotic oxidation is most likely to have altered the CH₄ entering CVL, because the temperature ranges at which they occur do not overlap. The minimum temperature for the onset of abiotic CH₄ oxidation has been estimated to range from 100 to 140 °C, whereas microbial CH₄ oxidation (methanotrophy) generally takes place from 0 to 80 °C (Machel, 1998, 2001; Worden et al., 1995; Holler et al., 2011). Vitritine reflectance (Rₒ) data from source rocks in the region range from 0.5 to 0.8 % (Guzman-Vega and Mello, 1999) and correspond to maximum

Figure 7. Isotopic characteristics of methane in air samples from Cueva de Villa Luz (CVL) plotted on published occurrence envelopes for microbial, thermogenic (T), and abiotic methanes (Etiope and Sherwood Lollar, 2013). Solid grey lines represent observed isotopic boundaries of methane from terrestrial and marine source rocks (Schoell, 1980). Data from CVL air samples (squares) plot along a bidirectional mixing line between an atmospheric source of CH₄ and a methane entering CVL with a stable isotopic signature of δ¹³CCH₄ = −24 ± 3 ‰, δ²HCH₄ = −40 ± 50 ‰.
subsurface temperatures of 60 to 90 °C (Bjørlykke, 1989). Additionally, modern water temperatures from oil wells in the region (30 to about 60 °C) and dissolved silica geothermometers from ground water in the region do not suggest that volcanic heat flow is affecting groundwater temperature (Nencetti et al., 2005). These observations suggest that methanotrophy is responsible for consuming CH4 in the subsurface before entering CVL.

Correcting for the expected isotopic shifts associated with partial methanotrophic loss of CH4 can hindcast the original isotopic composition and reveal the origin of CH4 entering CVL. Microbial oxidation imparts an increase of about 8.5 % in the δ13CCO2 value for every increase of 1 % in the δ13CCH4 value (Feisthauer et al., 2011). If methanotrophy is assumed to be occurring in the subsurface, then the original stable isotopic composition of the CH4 entering CVL is hindcast to match a thermogenic source (Fig. 7).

Possible Electron Acceptors for Methane Oxidation

The co-occurrence of CH4 and H2S supports our claim that sulfate is the most likely electron acceptor, but what about other potential acceptors? Alternative electron acceptors such as O2, and nitrate (NO3−) are known to be metabolically coupled to CH4 oxidation in other environments (Hu et al., 2011; Mattey et al., 2013). However, O2 and NO3− concentrations in the anoxic springs of CVL were below detection in previous studies, where the concentration of each was < 0.1 mg L−1 (Hose et al., 2000; Rosales Lagarde et al., 2014). Additionally, organoclastic sulfate reduction competes with anaerobic CH4 oxidation only when organic matter is abundant (~ 1 % by sediment weight) (Malinverno and Pohlmans, 2011; Pohlman et al., 2013), and the organic carbon concentration in CVL spring water is comparatively low, ranging from 0.5 to 3.5 mg L−1 (Rosales Lagarde, 2013).

Stable Isotopic Composition and Sources of Carbon Dioxide

The δ13CCO2 values near springs suggest that oxidized CH4 is not the primary contributor to dissolved CO2 in the spring water. The lowest CO2 concentration in CVL (Site 6, 1 m from an entrance) had a δ13CCO2 value of −9.5 ± 0.3 %o which matches known atmospheric CO2 concentrations (400 ± 20 ppmv) and δ13CCO2 values (−10 %o) (Peyraube et al., 2013; Fernandez-Cortes et al., 2015). The two highest CO2 concentrations in CVL at Site 11 (Sulfur Spring) and Site 7 (Slot Spring) were associated with the second-most-positive δ13CCO2 value of −9.1 ± 0.3 %o at Site 11 and the most negative δ13CCO2 value of −12.0 ± 0.2 %o at Site 7. Processes that may cause diverging δ13CCO2 values of CO2 near springs include the oxidation of CH4 and other organic compounds, which are typically 13C-depleted, and the dissolution of marine carbonates, like those that host CVL, that have δ13C values from 1 to 4 %o (Keith and Weber, 1964; Knauth and Kennedy, 2009). δ13CCO2 values of the air above these springs are similar to the δ13C values of −9 to −12 %o for dissolved CO2 measured in sulfidic springs 45 km northwest of CVL (Nencetti et al. 2005). The similarity of δ13CCO2 ranges from both areas demonstrate that processes causing diverging δ13CCO2 values at high CO2 concentrations are regional rather than specific to CVL.

Comparison with Other Sulfidic Cave Systems

Our proposed model of CH4 migration into CVL from petroleum basins not only provides sufficient CH4, but also agrees with proposed pathways of sulfur migration into CVL (Rosales Lagarde et al., 2014). The entrance of thermogenic CH4 into CVL also agrees with observations of CH4 and longer-chain hydrocarbons emanating from “Group II” springs of the Sierra de Chiapas region northwest of CVL (Nencetti et al., 2005). The CH4/ethane molecular ratios from these springs generally match the CH4/ethane ratios from petroleum basins when the ratios from springs are corrected for the differential solubility of CH4 and ethane in water (Clara Valdés et al., 2009; Dai et al., 2014). More broadly, a thermogenic source of CH4 suggests that the processes forming CVL are analogous to those that have formed Lower Kane Cave and the caves of the Guadalupe Mountains (Kirkland and Evans, 1976; Davis, 1980; Hill, 1995).

Conclusions

High CH4, H2S, and CO2 concentrations occur in cave air near springs in CVL, demonstrating that the three gases entered in solution via spring water. The poor match of the proposed stable isotopic signature of CH4 entering CVL from springs (δ13CCH4 = −24 ± 3 %o, δ13CCO2 = −40 ± 50 %o) with known δ13CCH4 values from other studied systems and the thermal history of the region implies that the CH4 is of a secondary origin, with subterranean anaerobic methanotrophy as the most likely explanation. If methanotrophy is altering the CH4 as it traveled to CVL, the original source of the CH4 is mathematically predicted to have a thermogenic origin. The incomplete oxidation of CH4 in the presence of sulfate appears to be generating the H2S that enters CVL with spring water. This evidence for CH4 migrating from regional petroleum basins while being oxidized in the presence of sulfates connects the geochemical processes that are forming CVL to other caves, like those of the Guadalupe Mountains and Lower Kane Cave, whose origins have been attributed to sulfuric acid speleogenesis.

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REFERENCES CITED

Adushkin, V.V., and Kudryavtsev, V.P., 2013, Estimating the global flux of methane into the atmosphere and its seasonal variations: Izvestiya, Atmospheric and Oceanic Physics, v. 49, no. 2, p. 128–136. https://doi.org/10.1134/S0014383103020023.

Aguilar Rodriguez, A., 2007, Presence of hidrocarburos en el municipio de Tacotalpa, Tabasco: Horizonte Epleroratorio, v. July-September 2007, p. 5–9.

Alcántara García, J.R., Ham Wong, J.M., Medina Flores, U., Hernández Peñaloza, J.J., de la Cruz Rivera, V.M., Peña Ramirez, H., Namson, J., and Spaw, J.M., 2004, Identification and evaluation of plays in the Simojovel Project, southeastern Mexico: American Association of Petroleum Geologists International Conference October 24–27, 2004, Cancun, Mexico: abstract a90217, 6 p.

Björlykke, K., 1989, Sedimentology and Petroleum Geology. Berlin

References
of CH4 and its 13C/12C isotopic ratios: 1. Inverse modeling of source processes: Global Biogeochemical Cycles, v. 18 (GB4004). https://doi.org/10.1029/2004GB002223.

Miller, J.B., Mack, K.A., Dissly, R., White, J.W.C., Dlugokencky, E.J., and Tans, P.P., 2002, Development of analytical methods and measurements of 13C/12C in atmospheric CH4 from the NOAA Climate Monitoring and Diagnostics Laboratory Global Air Sampling Network: Journal of Geophysical Research, v. 107, no. D13, p. ACH 11-1–ACH 11-15. https://doi.org/10.1029/2001JD000630.

Nencetti, A., Tassi, F., Vasielli, O., Macias, J.L., Magro, G., Capaccioni, B., Minissale, A., and Mora, J.C., 2005, Chemical and isotopic study of thermal springs and gas discharges from Sierra de Chipas, Mexico: Geofísica Internacional, v. 44, no. 1, p. 39–48.

Pantoja-Alor, J., 1989, Informe geológico minero de la Mina de Sta. Fe, Chis, in Salas, G.P., ed., Geología Económica de México, p. 413–420.

Peryt, T.M., Durakiewicz, T., Peryt, D., and Pobrezhsky, A., 2012, Carbon and oxygen isotopic composition of the Middle Miocene Badenian gypsum-associated limestones of West Ukraine, Geologica Acta, v. 10, no. 4, p. 319–332. https://doi.org/10.1034/j.1600-0889.2012.00175.x.

Peyraube, N., Lastenren, R., Denis, A., and Malaurent, P., 2013, Estimation of epikarst air P CO2 using measurements of water 13C/12C, cave air P CO2, and 13C/12C: Geochimica et Cosmochimica Acta, v. 118, p. 1–17. https://doi.org/10.1016/j.gca.2013.03.046.

Peyraube, N., Lastenren, R., Denila, J., Villanueva, J.D., Houillon, N., Malaurent, P., and Denis, A., 2016, Effect of diurnal and seasonal temperature variation on Cusac cave ventilation using CO2 assessment: Theoretical and Applied Climatology 2016. 14 p. https://doi.org/10.1007/s00704-016-1824-8.

Pohnman, J.W., Riedel, M., Bauer, J.E., Canuel, E.A., Paull, C.K., Lapham, L., Grabowski, K.S., Coffin, R.B., and Spence, G.D., 2013, Anaerobic methane oxidation in low-organic content methane seeps: Geochimica et Cosmochimica Acta, v. 108, p. 184–201. https://doi.org/10.1016/j.gca.2013.01.022.

Rey, A., Etioge, G., Beelli-Marchesini, L., Papale, D., and Valentini, R., 2012, Geological carbon sources may confound ecosystem carbon balance estimates: Evidence from a semi-arid steppe in the southeast of Spain. Journal of Geophysical Research Biogeosciences 117 (G03034), https://doi.org/10.1029/2012JG001991.

Rosales Lagarde, L., 2013, Sulfidic Karst Springs and Speleogenesis in the Sierra de Chipas. Austin, Association for Mexican Cave Studies bulletin 24, 79 p.

Rosales Lagarde, L., Boston, P.J., Campbell, A.R., Hose, L.D., Axen, G., and Stafford, K.W., 2014, Hydrogeology of northern Sierra de Chipas, Mexico: a conceptual model based on a geochemical characterization of sulfide-rich karst brackish springs: Hydrogeology Journal, v. 22, no. 6, p. 1447–1467. https://doi.org/10.1007/s10040-014-1135-z.

Rosales Lagarde, L., Boston, P.J., Campbell, A., and Stafford, K.W., 2006, Possible structural connection between Chichón Volcano and the sulfur-rich springs of Villa Luz Cave (a.k.a. Cueva de las Sardinas), Southern Mexico, in Ramón Espinasa-Pereña and John Pint, eds., Proceedings of the X, XI, and XII International Symposium on Vulcanospeleology. Austin, Association for Mexican Cave Studies bulletin 19, p. 177–184.

Sarbu, S.M., Kane, T.C., and Kinkle, B.K., 1996, A chemotaxotropically based cave ecosystem: Science, v. 272, no. 5270, p. 1953–1955. https://doi.org/10.1126/science.272.5270.1953.

Schoell, M., 1980, The hydrogen and carbon isotopic composition of methane from natural gasses of various origins: Geochimica et Cosmochimica Acta, v. 44, no. 5, p. 649–661. https://doi.org/10.1016/0016-7037(80)90155-6.

Schoell, M., 1988, Multiple origins of methane in the Earth, in Schoell, M., ed., Origins of Methane in the Earth: Chemical Geology, v. 71, p. 1–10. https://doi.org/10.1016/0009-2541(88)90010-5.

Spilde, M.N., Fischer, T.P., Northup, D.E., Turin, H.J., and Boston, P.J., 2004, Water, gas, and phylogenetic analyses from sulfur springs in Cueva de Villa Luz, Tabasco, Mexico (abst.): Geological Society of America Abstracts with Programs, v. 3, no. 5, paper 106-11.

Talukdar, S.C., Guevara, E.H., Jones, R.H., Galindo, A., Romero, M.A., Wawrzyniec, T.F., Villanueva, L., Khaled, F., Ambrose, W.A., Jennette, D.C., Dunlap, D.B., Meneses-Rocha, J.J., Sánchez-Barreda, L., and Lugo, J.E., 2002., Hydrocarbon source and charge in the Neogene in the Macuspana and Veracruz Basins, Mexico: Gulf Coast Association of Geological Societies Transactions, v. 52, p. 925–934.

Thom, M., Böisinger, R., Schmidt, M., and Levin, I., 1993, The regional budget of atmospheric methane of a highly populated area: Chemosphere, v. 26, p. 142–160. https://doi.org/10.1016/0045-6535(93)90418-5.

U.S. Geological Survey World Petroleum Assessment 2000: U.S. Geological Survey Digital Data Series DDS, 60, 4 CDROMs.

Webster, K.D., Mirza, A., Deli, J.M., Sauer, P.E., and Schimmelmann, A., 2016. Consumption of atmospheric methane in a limestone cave in Indiana, USA: Chemical Geology, v. 443, p. 1–9. https://doi.org/10.1016/j.chemgeo.2016.09.020.

Whitecar, M.J., 1999, Carbon and hydrogen isotopic systematics of bacterial formation and oxidation of methane: Chemical Geology, v. 161, p. 291–314. https://doi.org/10.1016/S0045-6535(99)00092-3.

Worden, R.H., Smalley, P.C., and Oxtoby, N.H., 1995, Gas sourcing by thermochemical sulfate reduction at 140 °C: AAPG Bulletin, v. 79, no. 6, p. 854–863.

ISOTOPIC EVIDENCE FOR THE MIGRATION OF THERMOGENIC METHANE INTO A SULFIDIC CAVE, CUEVA DE VILLA LUZ, TABASCO, MEXICO