

Microbial impact on the isotope composition of methane in both thermal and hyperalkaline waters of central Greece

D'Alessandro W.¹, Gagliano A.L.¹, Daskalopoulou K.², Calabrese S.^{1,3}, Li Vigni L.¹

¹*Istituto Nazionale di Geofisica e Vulcanologia, Sezione di Palermo, Italy*

²*GFZ, German Research Centre for Geosciences, Potsdam, Germany*

³*Università degli Studi di Palermo, Dipartimento delle Scienze della Terra e del Mare (DiSTeM), Palermo, Italy*

Corresponding Author walter.dalessandro@ingv.it

Introduction

The different origins of methane can be subdivided in biogenic (either directly produced by microbial activity or deriving by decay of organic matter at $T > 150^{\circ}\text{C}$) and abiogenic (from pure inorganic reactions). Among the latter, one of the most debated origins comes from serpentinization processes of ultramafic rocks in ophiolitic sequences at low temperatures ($T < 80^{\circ}\text{C}$). Moreover, further secondary processes (diffusion, inorganic or microbial oxidation, etc.) may also contribute and thus mask the original chemical and/or isotope composition. Primary and secondary processes acting on CH_4 can be recognised mainly through its isotope ($\delta^{13}\text{C}$ and $\delta^2\text{H}$) composition and the ratio between CH_4 and C_2+C_3 light hydrocarbons [Bernard et al. 1978; Schoell 1980].

Microorganisms may be involved in the methane cycle not only as active producers but also as consumers. Methane oxidizing bacteria (or methanotrophs) are microorganisms with the ability to use methane as the only source of carbon for energy and biomass production. Methanotrophs are ubiquitous and play an important role in the global carbon cycle, acting as a natural filter between the subsoil and the atmosphere. They were isolated from several environments such as soils, wetlands, freshwater, marine sediments, water columns, groundwater, rice paddies, and peat bogs [Murrell and Jetten, 2009]. Some species were adapted also at extreme environments characterized by high temperature (up to 81.6°C), extremely low or high pHs (1.5-11) or even anaerobic conditions. Due to the fact that methanotrophs metabolize preferentially light isotopes, biologic methane oxidation brings sometimes to extremely positive $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values [Cadieux et al., 2016].

The Greek territory belongs to the geodynamically active Alpine-Himalayan orogenic belt. As such, it shows intense seismic activity, active volcanic systems and areas of enhanced geothermal fluxes. One of these areas is the Sperchios Basin and the northern part of Euboea Island in central Greece, where thermal manifestations are widespread [D'Alessandro et al., 2014]. The complex geology of Greece includes also two important parallel running ophiolitic belts, with the Othrys Massif (central Greece) belonging to the westernmost of them. In and around this wide ophiolite outcrop, some cold hyperalkaline and some hypothermal ($T < 30^{\circ}\text{C}$) alkaline waters are present.

In the present paper we discuss data about chemistry and methane isotope composition of bubbling or dissolved gases in both thermal springs and hyperalkaline springs of Central Greece.

Sampling and Analytical Methods

Free bubbling gas samples were taken using an inverted funnel. All free gas samples were stored in Pyrex bottles with two vacuum stopcocks. Samples for dissolved gas analyses were collected in glass vials sealed underwater. In the laboratory, the chemical analyses were carried out by gas-chromatography (Agilent 7890B GC System) using Ar as the carrier gas. Dissolved gases were

extracted after equilibrium was reached at constant temperature with a host-gas (high-purity argon) injected in the sample bottle. The measurement precision was better than $\pm 5\%$ for common gases and $\pm 10\%$ for trace gases such as the alkanes. The chemical composition of the dissolved gas phase was obtained from the gas-chromatographic analyses taking into account the solubility coefficients (Bunsen coefficient " β ", $\text{cc}_{\text{gas}}/\text{ml}_{\text{water}}$ STP) of each gas specie, the volume of gas extracted and the volume of the water sample (details in Capasso and Inguaggiato, [1998] and Liotta and Martelli, [2012]). Starting from the total amount of dissolved gases (ccSTP/L) we calculated the relative abundances for every single gas species in equilibrium with the dissolved gas phase and expressed the analytical results in $\mu\text{mol/mol}$ of gas at atmospheric pressure, allowing the comparison of dissolved gases with free gases.

Carbon and hydrogen isotope compositions of CH_4 were measured using a Thermo TRACE GC and a Thermo GC/C III interfaced to a Delta Plus XP gas source mass spectrometer. $^{13}\text{C}/^{12}\text{C}$ ratios are reported here as $\delta^{13}\text{C}$ values ($\pm 0.1\text{‰}$) with respect to the V-PDB standard. $^1\text{H}/^2\text{H}$ ratios are reported here as $\delta^2\text{H}$ values ($\pm 2\text{‰}$) with respect to the V-SMOW standard.

The oxygen and hydrogen isotopic compositions of water were analysed on unfiltered samples with the use of Analytical Precision AP 2003 and FinniganMAT Delta Plus IRMS devices, respectively. The isotope ratios are expressed as the deviation per mil ($\delta\text{‰}$) from the reference V-SMOW. The uncertainties ($\pm 1\text{‰}$) were $\pm 0.1\text{‰}$ for $\delta^{18}\text{O}$ and $\pm 1\text{‰}$ for $\delta^2\text{H}$.

Results

Five thermal springs, with temperatures from 33 to 80°C, were sampled in the study area. All show elevated fluxes of bubbling gases whose prevailing species are either CO_2 or N_2 . Methane concentrations range from 27 to 4000 $\mu\text{mol/mol}$, whilst the isotope composition of CH_4 covers a wide range with $\delta^{13}\text{C}$ values ranging from -21.7 to +16.9‰ and $\delta^2\text{H}$ values ranging from -124 to +370‰.

Seven alkaline hypothermal waters were collected in five areas (Amplas, Platystomo, Kaisa, Smokovo and Soulanta) while 10 hyperalkaline waters in two areas (Arhani and Ekkara); all samples were collected from different springs and wells and some of the sites presented bubbling. All samples present low concentrations of H_2 (from <2 to 2500 $\mu\text{mol/mol}$), CO_2 (up to 26,000 but generally below 1000 $\mu\text{mol/mol}$) and O_2 (up to 16,000 but generally below 3000 $\mu\text{mol/mol}$). Gases in alkaline waters (pH <10) are in their majority dominated by CH_4 (from 128,000 to 915,000 $\mu\text{mol/mol}$). Hyperalkaline (pH > 11) waters are N_2 dominated (from 727,000 to 977,000 $\mu\text{mol/mol}$) and have CH_4 concentrations from 11,500 to 279,000 $\mu\text{mol/mol}$. Also all these samples display a wide range of isotope compositions of CH_4 ($\delta^{13}\text{C}$ from -74.5 to -14.5‰ and $\delta^2\text{H}$ from -343 to -62‰).

Discussion

Thermal springs

Methane in most of the bubbling gases found in the thermal waters of Greece display a small range in isotope composition close to -21‰ for carbon and to -130‰ for hydrogen [Daskalopoulou et al., 2018] and plot in the middle of the field of volcanic and geothermal systems (Figure 1). In the study area, only the hottest (Edipsos) of the thermal manifestations displays similar values. All the remaining samples fit a methane oxidation trend reaching extremely positive values (Figure 1).

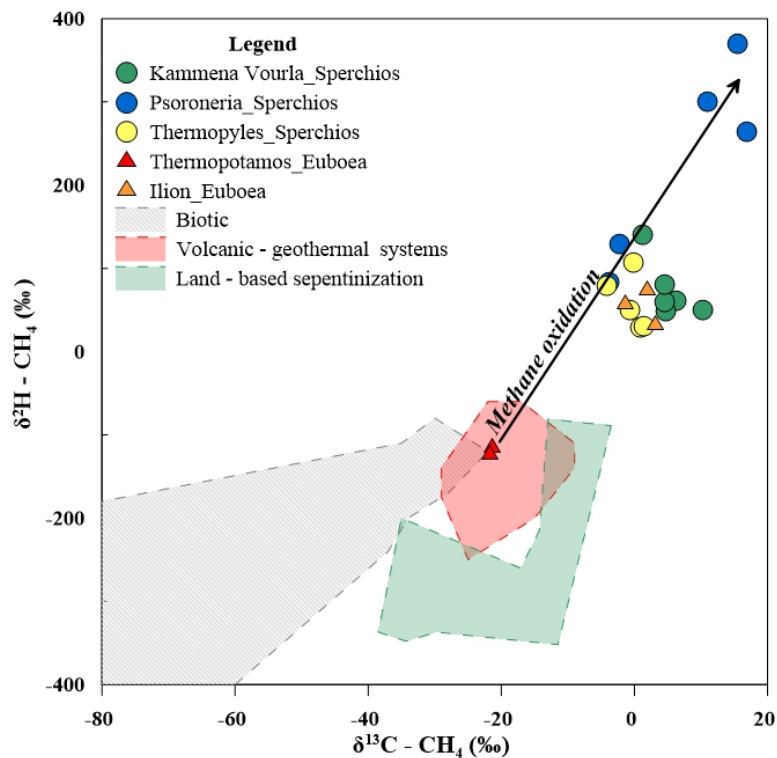


Figure 1 $\delta^{13}\text{C}$ vs. $\delta^2\text{H}$ of methane in the gases collected in the thermal springs of Sperchios Basin and northern Euboia.

If we consider the lowest values as the deep hydrothermal marker the obtained $\Delta\text{H}/\Delta\text{C}$ values range between 5 and 13 which are close to those typical of microbially driven oxidation [Coleman et al., 1981].

Although the outlet temperature of the hottest manifestations is at the upper limit for methanotrophic microorganisms [Sharp et al., 2014], we can hypothesize that environmental conditions are not favourable for their survival at this site. On the contrary, methanotrophs can thrive in the sites characterized by lower temperatures (33-65 °C), strongly consuming methane. The most positive values were measured at Psoroneria and indicate a very high consumption fraction. Considering again the values of Edipsos as the deep hydrothermal marker, a Rayleigh fractionation modelling in a closed system and kinetic fractionation factors for microbial oxidation [Coleman et al., 1981] we estimate a consumption of more than the 75% of the initial CH_4 .

Alkaline and hyperalkaline waters

Alkaline waters present mostly isotope values for CH_4 compatible with a biogenic origin ($\delta^{13}\text{C}$ from -62.0 to -37.5 ‰ and $\delta^2\text{H}$ from -247 to -154 ‰). Only the sample of Kaitsa falls above the biogenic field, indicating possible fractionation due to CH_4 oxidation (Figure 2). Most of the hyperalkaline waters have CH_4 isotope values compatible with an abiogenic origin through serpentinization processes (Figure 2). But some of the CH_4 collected in the hyperalkaline waters show values falling in the biogenic field, with at points, very negative $\delta^{13}\text{C}$ values (< -70‰). Methanogens were found also in other hyperalkaline waters taking advantage of the presence of sometimes very high hydrogen concentrations [Woycheese et al., 2015; Miller et al., 2018]. Also methanotrophs were rarely found in hyperalkaline waters [Woycheese et al., 2015; Miller et al., 2018] and their presence may justify the most positive values found in the study area (Figure 2).

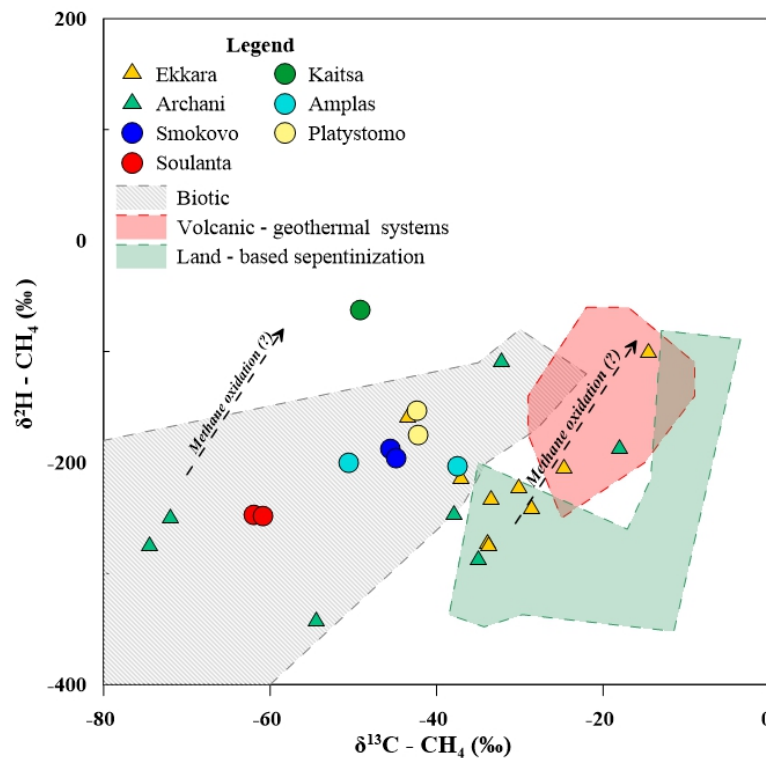


Figure 2 $\delta^{13}\text{C}$ vs. $\delta^2\text{H}$ of methane in the gases collected in alkaline and hyperalkaline waters of the Othys massive.

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