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Study of opportunities for assessment of tightness of fault systems in Northwest Bulgaria through a combination of gas chromatography and molecular-genetic methods

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Abstract. The object of the study is the Eastern part of the Western Fore-Balkan (Northwestern Bulgaria). In previous gas chromatography (GC) studies abnormalities in the content of C2-C5 alkanes in the surface layers of soils were found. In the present study, GC methods were combined with microbiological analyses of ten sample points. Both, GC and microbiological methods prove the presence of hydrocarbons in the soils of the study area. The concentration of C1 was between 19 and 98 ppm; 8-115 ppm of C2; 1-15 ppm of C3; 1-8 ppm of *n*C4; and, 1-3 ppm of *n*C5. The numbers of C2-, C3- and, *n*C4- soil oxidizing bacteria have been determined using a standard microbiological method. In the study area, the hydrocarbon oxidizing bacterial count ranged between 105 and 107 CFU/g of soil. By 16 S rDNA sequencing molecular genetic identification of dominant microbial strains, oxidizing C2-C4 alkanes from five sample points with abnormalities was performed. The presence of typical alkane-degrading bacteria belonging to the genera *Arthrobacter, Flavobacterium* and *Pseudomonas* was established. In the area of faults, higher values of hydrocarbons are not observed, compared to the entire study area. Compared to geological data, the role of major faults in providing pathways for gas migration to the surface is not proven. The developed methodology can be applied both in the assessment of hydrocarbon deposits and in the assessment of the tightness of underground gas storage which is the main important rule to have long exploitation of the facility.

Key words: hydrocarbon deposit storage, fault tightness, gas-chromatography, alkane-oxidizing bacteria, molecular genetic methods.

Introduction

Tectonically, the Eastern part of the Western Fore-Balkan is a system of faults of different types and characters (Fig. 1), which complicate the southern flank of an extensive anticlinorium (Bokov & Vladov, 1980; Monov, 1991, 1992). The established commercial and semi-commercial hydrocarbon accumulations are related to structures with complicated tectonic settings. Tectonic preconditions are an important factor in preserving the hermeticity of hydrocarbon accumulations located near and associated with established faults. This is an area that implies analysis and study, by obtaining new data that will allow a thorough assessment of the possible risks of leakage. The assessment of the amount and origin

of hydrocarbon gases in the surface (soil) layers, the presence of which can be associated with active deep migration and/or technological activities, is one of the methods that give results with sufficient reliability.

Hydrocarbon (HC) gases from the homologous series of alkanes (methane, C1; ethane, C2; propane, C3, and *n*-butane, *n*C4) can be registered at a depth of 70 - 100 cm (Deshev, 1991; Hunt, 1996; Peeva, 2012). It should be noted that for boundary values, according to the content of gaseous HCs on the surface, different criteria are used. They are determined by the specific geological and technological features (degree of study of the researched area, tectonic conditions on or near the surface layer, density of wells, etc.) of the studied territory. According to some authors (Deshev, 1991) values of about 0.004% (40 ppm) can be assumed for the clarke content of HC gases at the surface. Other researchers, Likholatnikov (1999), Stolp et al. (2006) and Glagolev (2014), indicate values in the range of up to and around 100 ppm. Regionally, the concentrations of C1 in the environment vary widely, depending on the geographical, geomorphological, geological, anthropogenic conditions. For example, for the northern latitudes (Alaska, Canada, Norway, the arctic parts of Russia) near gas fields many authors (Likholatnikov, 1999; Sabrekov et al., 2016; Thonat et al., 2019) note surface C1 isotope emission rates in the order of 2.4×10^4 to 7.6×10^4 ppm. The average concentration of dissolved C1 in the region of Elba River (Bussman et al., 2022) is 2.03 ppm and varies from 1.82 to 2.79 ppm. The C1 content in the surface layer, with values from 5.5×10^4 up to 7.0×10^4 ppm, is mainly associated with the biogenic decomposition of the organic material (Thonat et al., 2019). According to the same authors, isotopic signatures with levels from 5.5×10^4 ppm to 2.5×10^4 ppm are an indicator of the thermogenic origin of C1, and concentrations from 2.5×10^4 ppm to 1.3×10^4 ppm - pyrogenic origin.

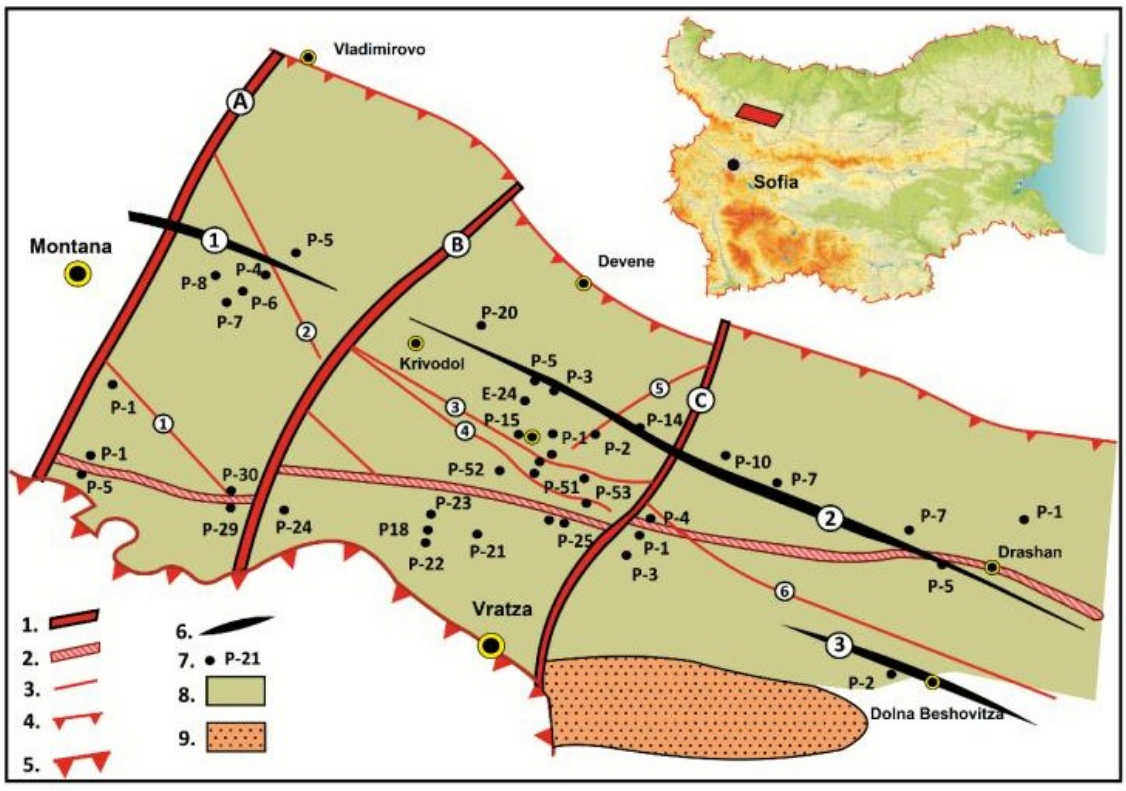

Fig. 1. Tectonic scheme of the Eastern part of Western Fore-Balkan
Legend: 1 – faults (A –Ogostenski; B – Kotliano-Krivodolski; C- Mramorenski); 2 – Drashan-Glavashka fault zone; 3 – normal faults (1 – Blagovski; 2 – Pastrinishki; 3 – Ponorski; 4 – Liliashki; 5 – Milinkamakski; 6 –Beshovishki); 4 – Forebalkan thrust-belt fault; 5 – Staroplaninska line; 6 – anticline's axis (1 – Mihaylovgradska; 2 – Mramorenska; 3 – Beshovishka); 7 – well; 8 – Western Forebalkan; 9 – Mezdenska syncline (according to Monov (1992), with modifications).

C1 is the main hydrocarbon present in the atmosphere, with an average concentration of 1.7 ppm. Variations between the northern and southern hemispheres are average 0.14 ppm and show a seasonal variation of about 0.03 ppm. Two forms of C1 oxidation in soils are recognized - 'high-affinity oxidation' occurring at C1 concentrations close to atmospheric (< 12 ppm) and 'low-affinity oxidation' occurring at > 40 ppm (Mer & Roger, 2001). The C1 content in the surface layer, with values up to 100 ppm, is mainly associated with the biogenic decomposition of organic material. The presence of C2, C3, and *n*C4 is a possible sign of migration with depth origin.

Gas chromatography (GC) is one of the applied chemical methods for evaluating the quantities and composition of HC gases in soil layers. Additional, reliable indicators for establishing the genesis of HCs are microbiological markers (Davis, 1967; Wagner et al., 2002; Ding et al., 2017), which can be combined with the analysis of soil gas component data.

Biological systems for the aerobic oxidation of alkanes also differ depending on the number of C-atoms in the molecule: C1, C2-C5 (gaseous), and C6-Cn (liquid). In recent years, special attention has been paid to the synthesis of C2, C3 and, *n*C4. It is suggested that the presence of these HCs in the environment may indicate natural emission from underlying HC deposits or are contamination due to HC use or manufacturing activities.

The microorganisms able to metabolize C1 are known as methanotrophs and they are of 2 types: type I - *Methylococcus*, *Methylomicrobium*, *Methylobacter* and *Methylomonas* and type II - *Methylosinus* and *Methylocystis*. C1-oxidizing bacteria are extremely widespread in all types of environments (Redmond et al., 2010). Microorganisms able to grow by using gaseous alkanes (C2-C4) are present in the environment and are thought to be important indicators of the presence of oil and gas in the subsurface. Some of the strains able to grow on C3 and/or *n*C4 presence have been isolated. Alkanes can be oxidized to form either primary or secondary alcohols, or both, depending on the type of bacteria (de Ferra, 2007). The aerobic alkane degradation path-way is performed by oxidation or incorporating molecular oxygen in the HC by a membrane-bound alkane monooxygenase and two soluble enzymes, rubredoxin and rubredoxin reductase. They act as electrons carriers between NADH and the hydroxylase for conversion of alkane to alcohol (van Beilen et al., 2003).

Worldwide a relationship between the microbial population and the HC concentration in the soils in various producing reservoirs has been observed (Wagner et al., 2002; Galitskaya et al., 2021). Different types of microorganisms such as *Brevibacterium, Corynebacterium, Flavobacterium, Mycobacterium, Nocardia, Pseudomonas, Rhodococcus etc.* utilize the short-chain HCs (C2, C3, and *n*C4) (Rasheed et al., 2013). Primary degradation of HCs in the environment was carried out by bacteria and fungi. The most active bacteria in soil HCs degradation are *Pseudomonas, Arthrobacter, Alcaligenes, Corynebacterium, Flavobacterium, Achromobacter, Micrococcus, Mycobacterium, Acinetobacter, Rhodococcus, Bacillus* and *Nocardia* (Frick et al., 1999; Vasudevan et al., 2007), whereas *Cellulomonas, Clavibacter, Curtobacterium, Pseudomonas* and *Microbacterium* have been suggested as the most promising endophytic bacteria (Ryan et al., 2008). The microbial prospecting method has been used to prioritize the drilling locations and to evaluate the HC prospects of an area, thus reducing risks and achieving a higher success ratio in petroleum exploration (Pareja, 1994; Rasheed et al., 2013).

Geological, geophysical and GC methods have been successfully integrated with microbiological analyzes for HCs surface exploration to assess subsurface petroleum and gas accumulation and fault tightness (Chikere et al., 2011; Rasheed, 2013; Bougi et al., 2019).

The present research aims to evaluate the tightness of fault systems in Western Fore-Balkan (Northwestern Bulgaria) using a combination of GC and molecular genetic methods. The results can help in the assessment of environmental pollution in the region, in the search for the pollution source, in appraisal of the tightness of fault systems, etc. The information is indispensable in finding a proper way for HC storage, monitoring and preventing emergency situations.

Materials and Methods

Samples preparation and GC analysis

Gas and soil samples were taken from representative areas that are close to faults or other zones, located in a profile grid, adapted to the morphology of the terrain. In the areas of pro-

bable faults, the sampling net is of close order spaced, which is consistent with the main purpose of the study (Fig. 2). Sampling is carried out at a predetermined sampling depth - of up to 200 cm. Based on the GC data, ten representative sample points were selected, conventionally marked with T and serial number for microbiological analyses. The containers used for the gas samples are hermetic vacutainer type with a volume of not less than 10 ml, and those for soil samples are sterile with a volume of not less than 100 ml. The methodology and equipment of the research and laboratory activities are described in detail and published in previous paper (Zaneva-Dobranova et al., 2019).

To establish and track the gas background over a selected territory characterized by oil and gas potential, a methodical approach is applied to allow monitoring observations on the surface geochemical situation. It was developed by the authors as a combination of methods: field research, sample pre-treatment, gas chromatographic and microbiological research and interpretation of results (Hristov et al., 2020).

Microbiological analyses

Enumeration of C2-, C3- and *n*C4 oxidizing bacteria for soil samples was carried out by C2-, C3- and *n*C4 oxidizing Standard Plate Count (SPC) method. The soil sample is suspended in sterilized water to prepare ten-fold serial dilutions (10^{-1} to 10^{-6}). To detach cells from the soil sample the suspension was incubated for 2 h on a rotary shaker at 200 rpm.

From each dilution, inoculation is made on Mineral Salts Medium (MSM) petri plates containing 1.0 g of $MgSO_4.7H_2O$, 0.7 g of K_2HPO_4, 0.54 g of KH_2PO_4, 0.5 g of NH_4Cl, 0.2 g of $CaCl_2.2H_2O$, 4.0 mg of $FeSO_4.7H_2O$, 0.3 mg of H_3BO_4, 0.2 mg of $CoCl_2.6H_2O$, 0.1 mg of $ZnSO_4.7H_2O$, 0.06 mg of $Na_2MoO_4.2H_2O$, 0.03 mg of $MnCl_2.4H2O$, 0.02 mg of $NiCl_2.6H_2O$, 0.01 mg of $CuCl_2.2H_2O$ and 20 g agarose in 1000 mL of distilled water, at pH 7.0 (Rasheed et al., 2013). For each dilution, three replicates were made. The plates were placed in a glass desiccator, filled with hydrocarbon gas and zero air in a ratio of 1:1 v/v. For isolation of C2-, C3- and *n*C4 oxidizing bacteria, the desiccators were filled with either C2/C3/*n*C4 gas with zero air, respectively. The desiccators were kept in bacteriological incubators at 28 ± 2°C. The developed bacterial colonies of C2-, C3- and *n*C4-oxidizing bacteria were counted after 10 days.

Molecular genetic analyses

The taxonomic identification of C2-to C4-oxidizing microorganisms is done through the use of molecular biological methods. The isolation of bacteria is based on the morphology of the predominant colonies on the plate. Twenty-three dominant types of single colonies are selected and used for microbial identification.

The microbial DNA from soil samples is extracted with QIAGEN's DNeasy^R Power Soil^R Pro Kit. The prepared DNA is used for PCR analyses. The amplification is performed on a personal T100 thermocycler (Bio-Rad, USA). The universal primers for 16S rDNA (27F - AGAGTTTGATCCTGGCTCAG, 1492R - TACGGYTACCTTGTTACGACTT) are used through the experiments for molecular taxonomic identification. PCR conditions are as follows: the first cycle of 3 min at 95°C; followed by 35 cycles at 95°C for 30 s, 60°C for 40 s and 72°C for 90 s; a final cycle at 72°C for 5 min. The PCR reaction mixture has a final volume of 40 µL, containing: 20 µL of PCR iProof™ HF Master Mix (Bio-Rad, USA), 2 µL of each primer, 6 µL distilled water and 10 µL of DNA sample. The obtained PCR products are identified by 2% agarose electrophoresis (Mini-Sub Cell GT Cell, Bio-Rad, USA). The resulting PCR products are visualized in the gel using a transilluminator. The isolated DNA is sent for purification and sequencing in Microsynth Seqlab GmbH, Germany. DNA sequences are formatted in a form suitable for comparison by the BLAST analysis database of NCBI (National Center for Biotechnology Information). The pair and multiple sequence alignment are performed using Sequence scanner AB.

Results and discussion

Gas-chromatographic analysis

The overall results, constituting individual values of HC gases, are integrated into specialized geological software, allowing the creating of maps with distribution anomalies and over-clarke content of HC components in the surface layers (Fig. 2).

Data on the composition of the gas mixture in the ten sampling points (T1-T10) are presented in Table 1. The highest C1 content was obtained in sample T1. In the remaining samples, the C1 values vary from 19 (T9) to 98 ppm (T1).

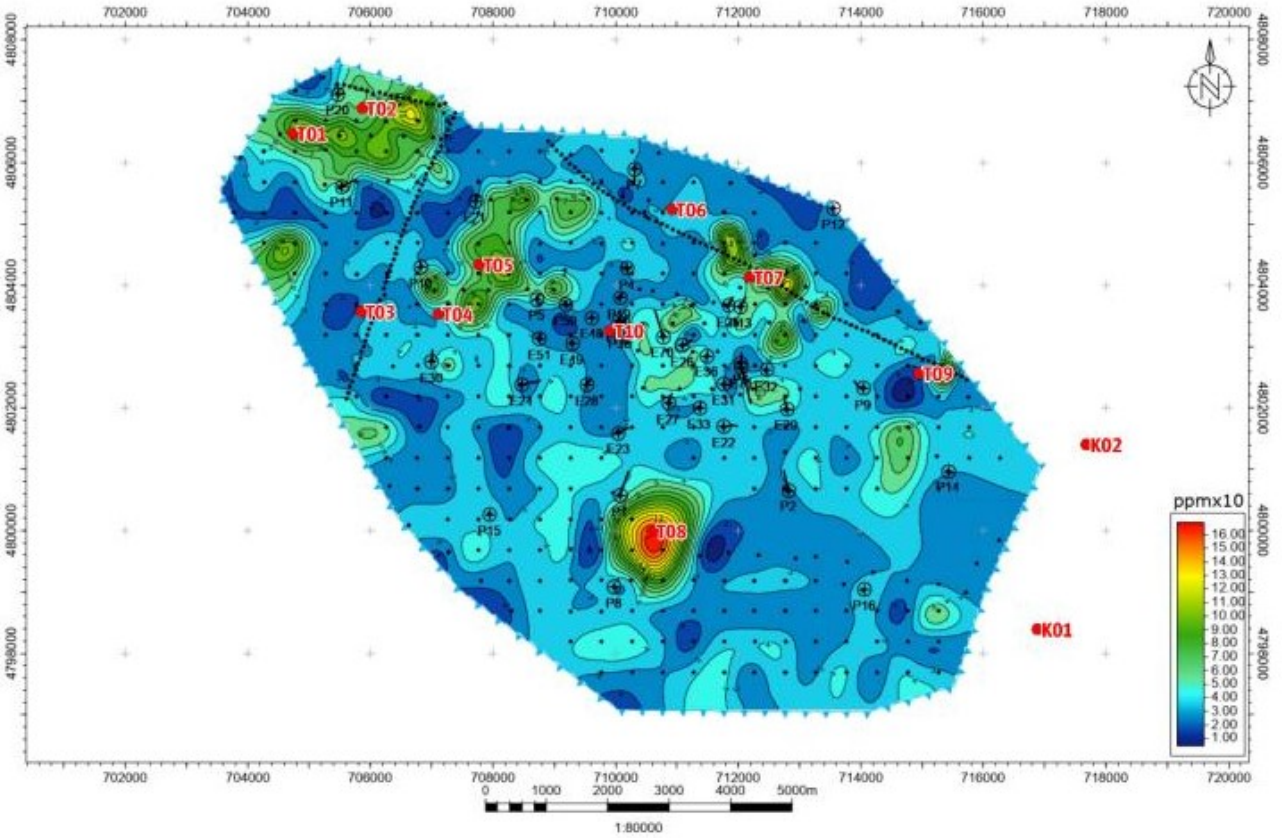

Fig. 1. Map of C1-C5 hydrocarbon content from analysis of gas samples and marked characteristic sampling points for microbiological analysis.

Table 1. Component composition of the gas mixture at the selected points

Sample point	Hydrocarbon content, ppm					Total hydrocarbon, ppm
	C1	C2	C3	*n*C4	*n*C5	
T1	98	21	2	1	1	136
T2	32	38	5	1	2	81
T3	33	10	8	2	1	58
T4	43	11	1	3	1	61
T5	58	18	15	8	3	112
T6	36	9	4	2	2	58
T7	39	28	5	3	2	81
T8	43	115	2	1	1	169
T9	19	8	4	1	1	39
T10	28	9	3	2	1	46

Regionally, the higher contents are in the northwestern part of the studied area, and the low ones are in the east. In the rest of the central part, the intermediate values are located. The second highly abundant component is determined – C2, from 8 (T9) to 115 ppm (T8). Its territorial distribution is as follows: lower values in the east; higher in the south and intermediate in the rest. Regarding the C3 content, the highest contents were determined in a limited section of the central part of the study area (T5 and T3), while for the rest the values change in a narrow range - from 1 to 5 ppm. For *n*C4, the territorial distribution of relatively high and low values is similar to that of *n*C5 – higher in a limited section of the central part of the area (T5, T4 and T7) and

insignificant in the rest. The total HC from C1 to C5 homologs (Fig. 2) shows increased content in the west (T1 and T5) and south (T8) and lower values (from 39 to 81 ppm) in the rest of the study area. Regarding fault systems (apparent and suspected) the total content of C1 and homologs (C1-C5) varies from 81 ppm (T2 and T7), 58 ppm (T3 and T6) to 39 ppm (T9). These values are lower than the minimal concentrations recommended by Deshev (1991) and Peeva (2012).

The gas concentration map (Fig. 2) interpretation admits the presence of microseepage of gaseous HCs in the area studied.

The anomalous concentrations of C1-C5 in the soil layer are used to estimate subsurface petroleum and gas accumulation. According to Baklouti et al. (2017) the concentrations of C1-C5 alkanes in soil samples from the El Hajeb field in the Eastern part of Sfax, Tunisia, vary in similar limits - up to 134.3 ppm. The same authors found that the concentration of C1 was between 0 and 134.31 ppm; of C2 from 0 to 38.66 ppm; of C3 from 0 to 19.52 ppm; of *n*C4 from 0 to 6.48 ppm; and, of *n*C5 from 0 to 2.06 ppm.

Microbiological analyses

HC-utilizing bacteria are usually present in the soil and they did not relate exclusively to the presence of alkane in it. In the study area of the eastern part of the Western Fore-Balkans, the HC oxidizing bacterial counts ranged between 105 and 107 CFU/g of soil, which amount is significant and thereby admits the seepage of lighter HCs accumulations from the tightness of underground gas storage (Table 2). With few exceptions, C2-oxidizing bacteria are higher abundant than C3- and *n*C4-utilizing bacteria.

Of all the sample points analyzed T3, T7 and T9 fall into the fault zone, but no significant difference in the number of alkane-oxidizing bacteria was observed compared to the other points.

A positive correlation between the low concentrations of alkanes in the areas with anomalies and the number of hydrocarbon-oxidizing bacteria was found (Beghtel et al., 1987; Rasheed et al., 2011). According to Rasheed et al. (2012), there is a good correlation between HC-utilizing bacteria and concentrations of adsorbed soil C1-C5 gases in the range of 1 to 977 ppb. In the same study, data from microbiological analyzes showed the presence of a smaller number of C2, C3, and *n*C4-oxidizing bacteria: 8.50×10^5 CFU/g, 6.86×10^5 CFU/g and 5.70×10^5 CFU/g, respectively.

In the present study, the data for the high number of HC-utilizing bacteria corresponds to the higher HC content based on the data from the GC analysis.

Table 2. Number of HC-utilizing microorganisms from the studied areas, in CFU/g soil.

Sample point	C2-oxidizing bacteria	C3-oxidizing bacteria	*n*C4-oxidizing bacteria
T1	6.2×10^7	7.5×10^6	1.2×10^7
T2	5.1×10^6	3.0×10^6	2.0×10^6
T3	1.1×10^7	3.2×10^6	2.2×10^6
T4	4.3×10^7	9.7×10^6	3.1×10^6
T5	2.1×10^7	7.0×10^6	2.4×10^7
T6	2.1×10^7	6.0×10^6	2.5×10^7
T7	7.0×10^7	9.0×10^7	3.0×10^7
T8	3.2×10^6	1.8×10^5	1.5×10^7
T9	1.5×10^7	4.0×10^7	3.5×10^7
T10	1.3×10^7	7.2×10^6	1.1×10^7

Molecular genetic analyses

Molecular genetic identification of dominant strains of bacteria was performed on isolates from five points – T1, T3, T4, T8 and T10. The points were selected to identify the dominant species of bacteria in the microbial community when combining high, medium or low HC content with high or low numbers of HC-utilizing microflora.

Data on the isolated dominant strains, when cultivated with C2, C3 and *n*C4 as a carbon and energy source, are presented in Tables 3, 4 and 5.

The isolated dominant strains, when cultivated with C2 as a carbon and energy source (Table 3), belong to genera *Arthrobacter, Bosea, Mucilaginibacter, Paenibacillus, Bradyrhizobium, Mesorhizobium* and *Neorhizobium*. Of the nine strains iden-

tified, three (T1E1, T10E1 and T10E2) belong to the genus *Arthrobacter*. Another three dominant strains (T4E1, T4E5 and T8E1) belong to the rhizobia group (Shahrajabian et al., 2021). The bacteria that were found in petri dishes with C3 (Table 4) as a C-source were from the genera *Flavobacterium, Pseudarthrobacter, Ideonella, Arthrobacter, Pseudomonas, Phyllobacterium, Pseudonocardia* and *Paenarthrobacter*. Molecular genetic identification of the dominant strains of bacteria using *n*C4 as a carbon source (Table 5) indicates the presence of the genera *Flavobacterium, Ideonella, Pseudarthrobacter, Dyadobacter* and *Caulobacter*.

From the data presented in Tables 3-5, it can be seen that representatives of the typical HC-utilizing genera *Arthrobacter* (T1E1, T10E1, T10E2, T4P1 and T8P1), *Pseudomonas* (T8P2) and *Flavobacterium* (T1P1 and T1B2) are abundant in the studied area. Established genera *Pseudarthrobacter* (T1P2 and T4B1) and *Paenarthrobacter* (T10P3) are phylogenetically close to the genus *Arthrobacter* (Gupta et al., 2020).

According to Rasheed et al. (2013), the short-chain HCs, i.e., C1-*n*C4, can be degraded by *Mycobacterium, Flavobacterium, Nocardia*, and *Pseudomonas*. Some authors (Anthony, 2000; de Ferra, 2007) have also found that the most frequently isolated HC-oxidizing bacterial genera are part of the genera *Arthrobacter, Pseudomonas*, *Flavobacterium, Mycobacteria, Nocardia, Clavibacter* and *Corynebacterium*. Additionally, McLee et al. (1972) have isolated *Brevibacter* and *Arthrobacter* which utilized C2, C3, and *n*C4 as a source of carbon and energy.

Bacterial degradation of alkanes requires the participation of specific enzymes synthesized by the microorganisms. According to Olowomofe et al. (2019), the degradation of alkanes by bacteria *Pseudomonas aeruginosa, Bacillus cereus* and *Dyadobacter koreensis* proceeds by the alkane monooxygenase, encoded by the *alkB* gene. Cello et al. (1997) isolated the species *Acinetobacter venetianus* that can use alkanes (C10, C14, and C20) as the sole carbon source from the Venice lagoon and observed that strains contained an alkB gene.

Table 3. Molecular genetic identification of C2-oxidizing bacteria.

Sample point	Isolate	Genus	Suggestion of GenBank	Length bp	Similarity	Identities
T1	T1E1	*Arthrobacter*	*Arthrobacter globiformis strain JCM 1332*	1476	99.36%	1081/1088(99%)
			Arthrobacter globiformis strain DSM 20124	1464	99.36%	1081/1088(99%)
	T1E2	*Bosea*	*Bosea vaviloviae strain Vaf-18*	1405	100.00%	394/394(100%)
T3	T3E1	*Mucilaginibacter*	*Mucilaginibacter dorajii strain DR-f4*	1444	97.90%	1025/1047(98%)
	T3E2	*Paenibacillus*	*Paenibacillus alginolyticus strain DSM 5050*	1497	99.81%	1073/1075(99%)
			Paenibacillus alginolyticus strain NBRC 15375	1467	99.72%	1072/1075(99%)
T4	T4E4	*Bradyrhizobium*	*Bradyrhizobium embrapense strain SEMIA 6208*	1450	99.45%	1076/1082(99%)
			Bradyrhizobium mercantei strain SEMIA 6399	1468	99.45%	1076/1082(99%)
	T4E5	*Mesorhizobium*	*Mesorhizobium mediterraneum strain LMG 17148*	1426	99.51%	1019/1024(99%)
			Mesorhizobium tamadayense strain Ala-3	1477	99.51%	1019/1024(99%)
T8	T8E1	*Neorhizobium*	*Neorhizobium galegae strain NBRC 14965*	1406	99.24%	1047/1055(99%)
			Neorhizobium galegae strain LMG 6214	1433	99.24%	1047/1055(99%)
T10	T10E1	*Arthrobacter*	*Arthrobacter sp. strain gene*	1389	97.99%	974/994(98%)
			Arthrobacter pascens strain LMR740	1434	97.89%	975/996(98%)
	T10E2	*Arthrobacter*	*Arthrobacter sp. strain DK7*	1428	99.45%	1094/1100(99%)
			Arthrobacter globiformis strain IARI-CRK	1432	99.27%	1093/1101(99%)

Table 4. Molecular genetic identification of C3-oxidizing bacteria

Sample point	Isolate	Genus	Suggestion of GenBank	Length bp	Similarity	Identities
T1	T1P1	*Flavobacterium*	*Flavobacterium collinsii strain 983-08*	1437	98.95%	1039/1050(99%)
			Flavobacterium plurextorum strain 1126-1H-08	1457	98.95%	1039/1050(99%)
	T1P2	*Pseudarthrobacter*	*Pseudarthrobacter siccitolerans strain 4J27*	1420	99.35%	1064/1071(99%)
			Pseudarthrobacter psychrotolerans strain YJ56	1378	98.69%	1052/1066(99%)
T3	T3P1	*Ideonella*	*Ideonella paludis strain KBP-31*	1414	95.82%	963/1005(96%)
T4	T4P1	*Pseudarthrobacter/ Arthrobacter*	*Pseudarthrobacter enclensis strain NIO-1008*	1415	97.90%	1026/1048(98%)
			Arthrobacter pokkalii strain P3B162	1424	97.90%	1025/1047(98%)
T8	T8P1	*Arthrobacter*	*Arthrobacter oryzae strain KV-651*	1465	99.63%	1081/1085(99%)
			Arthrobacter humicola strain KV-653	1463	99.17%	1078/1087(99%)
	T8P2	*Pseudomonas*	*Pseudomonas corrugata*	1442	99.23%	129/130(99%)
			Pseudomonas kilonensis strain 520-20	1528	99.23%	129/130(99%)
			Pseudomonas thivervalensis strain SBK26	1430	99.23%	129/130(99%)
			Pseudomonas brassicacearum strain DBK11	1473	99.23%	129/130(99%)
T10	T10P1	*Phyllobacterium*	*Phyllobacterium bourgognense strain STM 201*	1401	98.83%	1012/1024(99%)
			Phyllobacterium loti strain S658	1562	97.75%	998/1021(98%)
	T10P2	*Pseudonocardia*	*Pseudonocardia kujensis strain A4038*	1465	98.05%	151/154(98%)
	T10P3	*Paenarthrobacter*	*Paenarthrobacter nicotinovorans strain DSM 420*	1468	99.32%	1015/1022(99%)
			Paenarthrobacter histidinolo-vorans strain DSM 20115	1480	98.92%	1010/1021(99%)

Table 5. Molecular genetic identification of *n*C4-oxidizing bacteria

Sample point	Isolate	Genus	Suggestion of GenBank	Length bp	Similarity	Identities
T1	T1B2	*Flavobacterium*	*Flavobacterium piscis strain 412R-09*	1459	98.53%	1070/1086(99%)
			Flavobacterium saccharophilum strain NBRC 15944	1439	98.43%	1067/1084(98%)
T3	T3B1	*Ideonella*	*Ideonella paludis strain KBP-31*	1414	96.92%	1040/1073(97%)
T4	T4B1	*Pseudarthrobacter*	*Pseudarthrobacter defluvii strain 4C1-a*	1463	99.61%	1014/1018(99%)
			Pseudarthrobacter equi strain IMMIB L-1606	1487	99.41%	1011/1017(99%)
T8	T8B1	*Dyadobacter*	*Dyadobacter psychrophilus strain BZ26*	1501	99.24%	1051/1059(99%)
T10	T10B2	*Caulobacter*	*Caulobacter soli strain Ji-3-8*	1356	99.01%	997/1007(99%)
			Caulobacter henricii strain ATCC 15253	1283	98.81%	995/1007(99%)

For the first time, Sun et al. (2014) detected the ability to degrade alkanes and the alkB/alkM gene in *Rhizobium, Rhodobacter, Trichococcus, Micrococcus, Enterococcus* and *Bavariicoccus* strains, and the alkM gene in *Firmicutes* strains. The same authors also proved that *Rhizobium* strains typi-

cally inhabit soils and plant rhizosphere because of a special alkane monooxygenase gene that could efficiently degrade *n*C16. In the present study, dominant representatives in the microbial community belong to rhizobia (T4E4, T4E5 and T8E1) were also established.

The molecular genetic identification data presented in Tables 3-5 give evidence both for similarities and differences in the determined dominant strains in the five study sites. Probably could be explained by differences in the physico-chemical and chemical composition of the soil. For example, Deng et al. (2016) have proven that bacterial community structures in the soil above oil and gas fields depend mainly on environmental parameters.

In future, our efforts will be focused on the metagenomics analyses of the studied sample and the structure of the microbial communities.

Conclusions

In our thorough study of HCs in soils of the Eastern part of the Western Fore-Balkans by GC and microbiological methods we have determined the following ranges: - C1 amounts vary from 19 to 98 ppm; C2 - from 8 to 115 ppm; C3 - from 1 to 15 ppm; *n*C4 - from 1 to 8 ppm; and, *n*C5 - from 1 to 3 ppm. The high number of HC-utilizing bacteria (mostly often about 107 CFU/g) was explained by the increased HCs abundance in soils under study.

On the territory of the studied area, there are real gas deposits and storage that are in the operational stage. It is supposed that in the process of development of these sites, technological pollution of the environment is possible as a result of technologically imperfect drilling and equipment. They can be regarded as potential sources of gaseous alkanes.

In comparison to the entire study area in the area of faults, higher values of HCs are not detected. Therefore, based on the obtained data, the role of major faults in providing pathways for gas migration to the surface is not proven.

The combination of GC technique and microbiological research makes it possible to assess the tightness of fault systems of different orders and their adjacent territories. The methodology has the potential to be applied for monitoring the HC seepage in the region and to prevent emergency situations by timely announcements.

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