ORGANIC-GEOCHEMICAL CHARACTERISTICS OF THE MUD FROM THE TECHIRGHIOL LAKE, ROMANIA

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ABSTRACT

The Techirghiol Lake, located on the Romanian coast of the Black Sea, is a hypersaline lake. In this environment, a unique ecosystem in Europe has developed with the specific capacity of producing mud by the decomposition of flora and fauna under the microbial activity (saprogenic sludge), which is used for therapeutic purposes.

In this study, hydrocarbon compositions of the Techirghiol Lake mud are investigated in order to determine the origin and type of organic matter (OM). All samples are characterized by a high abundance of *n*-alkanes, short and high chain alkenes, pimarane, phyllocladane, indicating that OM was mainly derived from phytoplankton, filamentous green alga *Cladophora vagabunda*, Cyanobacteria, submerged/floating macrophytes, terrestrial and emergent plants. The terrestrial origin of organic matter is probably related to a significant input by wind or rivers. Typical oil distributions of terpanes and steranes biomarkers clearly indicate that the muds of Techirghiol Lake, in addition to native organic matter, contain a relatively low contribution of petroleum type pollutants, as well as PAH compounds of pyrogenic origin.

The relatively high abundance of 1-chloroalkenes, which in some samples represent the most abundant compounds in the total distribution of hydrocarbons, together with isoprenoid thiophenes indicate the local presence of significant amounts of chloride and sulphur species in the water column or surface sediment.

KEYWORDS
Techirghiol Lake, hypersaline environment, mud, biomarkers, petroleum hydrocarbons

INTRODUCTION

Hydrocarbons make up only a small percentage of bulk organic matter (OM) [1], but they are widely used in geochemical studies of lake sediments [2, 3, 4]. Since certain hydrocarbons degrade very slowly or are transformed into more stable chemical structures, they can be used as biomarkers in the investigation of sources and fate of organic matter in sediments [1, 5]. The qualitative and quantitative composition of hydrocarbons presents a balance between external inputs and in-lake processes [6, 7]. Sources of autochthonous hydrocarbons typically originate from algae and macrophytes [8], while sources of allochthonous hydrocarbons are, in part, the product of atmospheric dust and gases, and leaf and root litter, as well as primary and secondary metabolites of microorganisms [9].

This study is carried out on muds of the Techirghiol Lake, Romania. In this hypersaline environment, a unique ecosystem in Europe has developed with the specific capacity of producing mud from organic matter (saprogenic sludge), which is used for therapeutic purposes [10, 11].

The Techirghiol Lake is located in the Dobrudja area, southeast of the city Constanta, east of Romania (Figure 1). The lake is separated from Black Sea by a sand-belt, 100 m wide and 3 km long. It is the largest salt lake in Romania, covering an area of 10.68 km^2 and with a maximum depth of 9 m [11, 12]. The unique biodiversity of this area led to including the Techirghiol Lake in the European network Natura2000 in order to ensure the protection and support for the conservation status of this specific area [11, 13].

As a result of low annual precipitation and high evaporation, the lake water has become hypersaline. During the last decade, the salinity of the water varies between 55 $gdm^{-3}$and 66 gdm^{-3} [11]. The most representative anions for the Techirghiol Lake are Cl^- and SO_4^{2-}, the concentration of the chlorine ion varies between the interval 30.77 gdm^{-3}- 34.38 gdm^{-3} [14]. The hypersalinity, as the defining feature of Lake Techirghiol, is also influenced by the freshwater input. Such a change entails the imbalance of the whole system, with repercussions on the genesis and

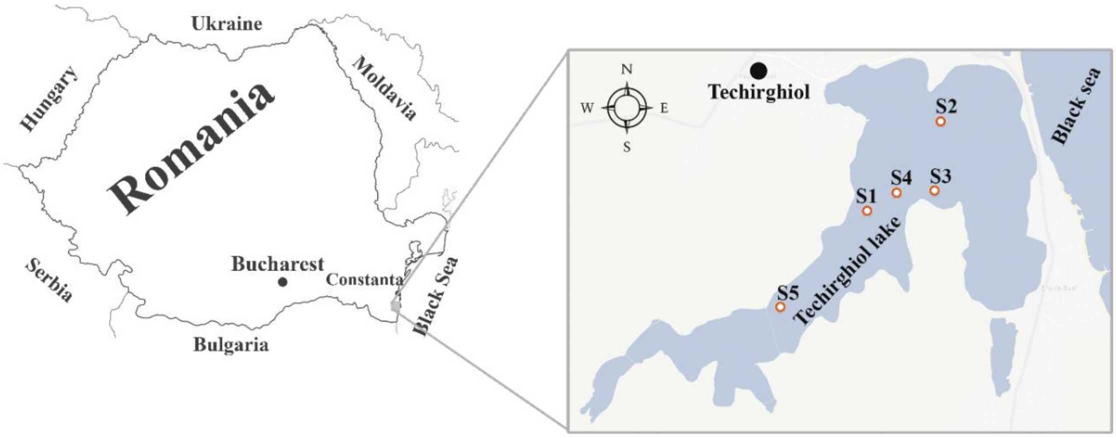

FIGURE 1
Map of Romania and location of investigated samples in the Techirghiol Lake.

TABLE 1
Coordinates of microlocations in Techirghiol lake

Samples	**Coordinate**	
	Longitude	Latitude
S1	44° 02' 13.5"	28° 36' 43.0"
S2	44° 03' 00.4"	28° 37' 35.1"
S3	44° 02' 23.9"	28° 37' 25.6"
S4	44° 02' 22.5"	28° 36' 58.4"
S5	44° 01' 25.5"	28° 35' 38.8"

the quality of water and sludge. This external contribution may be the result of the combined action of precipitation waters, drainage waters in enclosed valleys, subterranean waterfalls through springs and infiltrations through protective dams. The ecological balance of the lake can also be affected by anthropogenic influence in the lake area.

The Techirghiol Lake has multiple organic matter sources including *in situ* primary production by organisms such as phytoplankton, macroalgae or aquatic and benthic plants, as well as allochthonous input from terrigenous plant debris and soil OM, which enter the system mainly through riverine inflow [11]. Halophilic microorganisms are present in a wide range of diverse saline environments and they have been successfully used in bioremediation of polluted environments [15]. A number of 107 taxonomic units have been identified in the phytoplankton. Most of the algae species belong to the diatoms group, followed in descending order by representatives of *Chlorophyceae*, *Dinophyceae*, *Euglenophyceae*, *Chrysophyceae*, *Cryptophyceae* and *Xantophyceae* [14]. Diatoms and Cyanobacteria were the groups with the highest diversity from the phytoplankton.

Considering the importance of Techirghiol Lake and the diversity of organic matter inputs, this study aims to characterize the organic matter from mud samples. The Techirghiol Lake is relatively well known in terms of biological communities, biodiversity [14, 16] and of salinity regime, being quite well investigated for many years either from relatively high importance of therapeutic properties of the medicinal (sapropelic) mud present in the lake [10], but a detailed investigation of origin and type of organic matter in the mud of the Techirghiol Lake was not conducted so far.

MATERIALS AND METHODS

Sampling. Sediment samples were collected with a stainless steel Van Veen grab sampler from five sites in the Techirghiol Lake (Figure 1, Table 1). The material in the grab was immediately transferred into a bucket and transported to the laboratory. Five samples of mud were investigated in this study. A list of samples with their geographic coordinates is presented in Table 1.

Analytical methods. Sediment samples were air-dried, finely ground and homogenized in an agate mill. Dry sediment samples (~ 10 g) were extracted with a mixture an azeotrope mixture of methylene chloride and methanol (88:12 volume ratio) using Extraction System Buchi B811. The saturated, aromatic, and NSO-fractions (polar fraction, which contains nitrogen, sulfur, and oxygen compounds) were isolated from bitumen using column chromatography (adsorbents: SiO_2 and Al_2O_3). The saturated hydrocarbons were eluted with *n*-hexane, aromatics with benzene, whereas the NSO-fraction was eluted with a mixture of methanol and chloroform 1:1, *v*/*v*. Elemental sulfur was removed by the method suggested by Blumer [17].

Saturated and aromatic hydrocarbons were analyzed by gas chromatography-mass spectrometry (GC-MS). The GC-MS analyses were performed using an Agilent 7890A gas chromatograph (H5-MS capillary column 30 m x 0.25 mm, He carrier gas 1.5 cm^3/min, FID) coupled to an Agilent 5975C mass selective detector (70 eV). The column was heated from 80 to 310ºC, at a rate of 2 °C/min and the final temperature of 310 ºC was maintained for an additional 25 minutes. The individual peaks were identified from the total ion current (TIC) by the comparison of mass spectra and retention times with literature data [6, 18, 19] and based on the total mass spectra (library: NIST5a). Detailed analysis of the target compounds from saturated hydrocarbon fraction was conducted using the following ion chromatograms: *m/z* 69 (*n*-alkenes), *m/z* 71 (*n*-alkanes and isoprenoids), *m/z* 217 (steranes) and *m/z* 191 (terpanes). The ions monitored for polycyclic aromatic hydrocarbons (PAH) were: *m/z* 128 (naphthalene), *m/z* 152 (acenaphthalene), *m/z* 154 (acenaphthene), *m/z* 166 (fluorene), *m/z* 178 (phenanthrene and anthracene), *m/z* 202 (fluoranthene and pyrene), *m/z* 228 (benzo[a]anthracene and chrysene), *m/z* 252 (benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]fluoranthene), *m/z* 276 (indeno[1,2,3-c,d]pyrene and benzo[g,h,i]perylene and *m/z* 278 (dibenzo[a,h]anthracene). Biomarker and aromatics parameters were calculated from GC-MS chromatogram peak areas (GC-MS Data Analysis software).

RESULTS AND DISCUSSION

Group parameters. The contents of soluble organic matter (bitumen) in analyzed mud samples are relatively uniform (3969-4545 mg kg^{-1}, Table 2). The relatively high content of bitumen is characteristic for recent sediments [6]. Group bitumen composition, the content of saturated hydrocarbons, aromatics and NSO compounds in the analyzed mud samples are given in Table 2. The relative contents of saturated and aromatic hydrocarbons are low, which indicates an immature organic matter (Table 2). The composition of hydrocarbons is characterized by having an approximately equal value of aliphatic and aromatic hydrocarbons.

Specific parameters. The total ion current (TIC) chromatograms of the saturated fractions are shown in Figure 2. The TIC pattern of all investigated samples is characterized by the presence of *n*-alkanes, short and high chain alkenes, pimarane, 16α(H)-phyllocladane, isoprenoids pristane (Pr) and phytane (Ph), isoprenoids thiophenes.

The distributions of saturated hydrocarbons of samples S1 and S4 are characterized by the dominance of 1-chloroalkenes, followed by *n*-alkanes/*n*-alkenes with the domination of long chain odd *n*-alkanes/*n*-alkenes *n*-C_{27}/*n*-$C_{27:1}$ and *n*-C_{29}/*n*-$C_{29:1}$. In samples S2, S3 and S5 1-chloroalkenes are not identified, while in the distribution of saturated hydrocarbons *n*-alkanes/*n*-alkenes are the dominant compound with maximums at *n*-C_{27}/*n*-$C_{27:1}$ and *n*-C_{29}/*n*-$C_{29:1}$. Biomarker distributions shown in Figure 2 suggest differences between the sample S5 and other analyzed samples, since it contains a higher amount of short and mid-chain *n*-alkane homologues. The reason for this is probably caused by a different position of S5 sample related to other samples (Figure 1).

1-Chloroalkenes. As mentioned above, two samples, S1 and S4, are characterized by the dominance of long chain 1-chloroalkenes in TIC (Figure 2). The determination of 1-chloroalkene is based on their mass spectrum and position in TIC, concerning C_{30}-chloroalkene elute just before the C_{33}-alkanes, with mass 454 [18, 19]. Distributions of 1-chloroalkenes show these compounds' presence in the mass range of 454-510 (C_{30}-C_{32} 1-chloroalkenes). These chloroalkenes are characterized by terminal chlorine atoms and differ from previously reported naturally chlorinated hydrocarbons from sediments (<C_{15}, [20]; C_{28}-C_{34}, [19]). In these samples, the most abundant chloroalkene is $C_{32:1}$ chloroalkene, followed by $C_{30:1}$ and $C_{31:1}$ chloroalkene.

Presumed origins of these compounds are: 1) in situ laboratory forming from pyrolysis of geomacromolecules; 2) anthropogenic contamination; 3) specific environmental conditions that have led to the production of these unusual lipids and/or their precursors. Zhang *et al.*, [19] have excluded the first presumption, although they used the same solvents, but significantly more extreme conditions in the extraction. The second and third options remain possible in the case of mud from the Techirghiol Lake.

TABLE 2
Values of group organic geochemical parameters

Sample	Bitumen	Saturated hydrocarbons	Aromatic hydrocarbons	NSO-fraction	Residue
	mg/kg	%	%	%	%
S1	4077	3.15	3.44	40.40	53.01
S2	4300	4.23	3.17	48.59	44.01
S3	4545	4.05	3.04	39.19	53.72
S4	3969	3.23	3.55	35.16	58.06
S5	4169	2.98	3.40	37.87	55.74

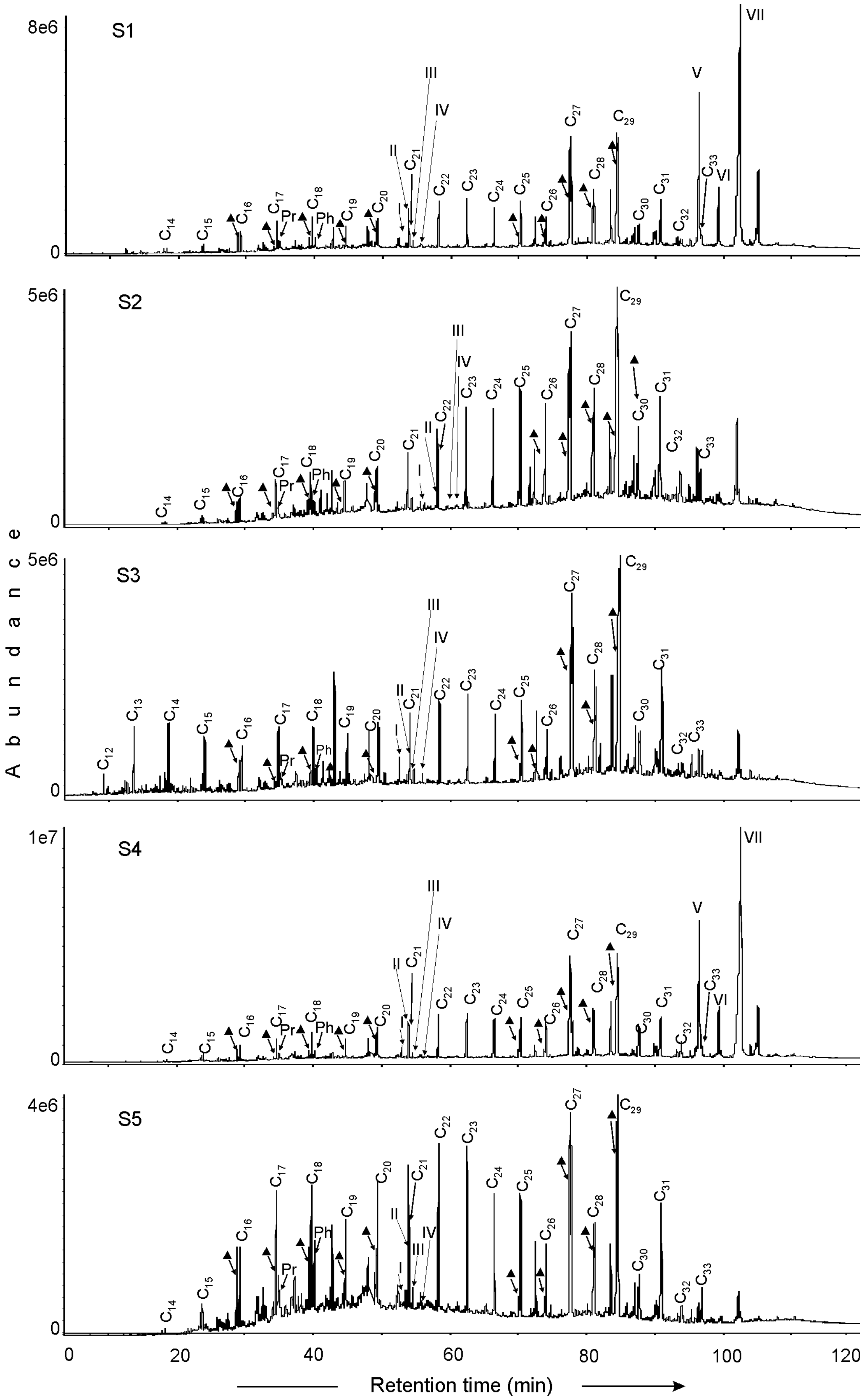

FIGURE 2

Total ion current (TIC) of investigated samples.

I - Pimarane; **II** -16(α)H-Phyllocladane ; **III** - 3-methyl-2-(3,7,11-trimethyldodecyl)thiophene; **IV** - 3-(4,8,12-trimethyl-tridecyl)thiophene; **V** – C_{30}–1-chloroalkene; **VI** - C_{31}–1-chloroalkene; **VII** - C_{32}–1-chloroalkene.;
Pr - pristane; Ph - phytane; ▲- *n*-alkenes**.**

TABLE 3
Values of parameters calculated from distributions and abundances of *n*-alkanes and isoprenoids.

	S1	S2	S3	S4	S5
***n*-Alkane range**	C_{14}-C_{33}	C_{14}-C_{33}	C_{12}-C_{33}	C_{13}-C_{33}	C_{14}-C_{33}
C_{max}	C_{29}	C_{29}	C_{29}	C_{29}	C_{29}
CPI (C_{14}-C_{33})	1.68	1.87	2.26	1.34	1.52
***n*-Alkene range**	C_{26}-C_{29}	C_{27}-C_{29}	C_{25}-C_{29}	C_{25}-C_{29}	C_{26}-C_{29}
TAR	3.83	6.76	4.19	4.17	2.19
Pr/Ph	0.71	0.72	0.79	0.77	0.60

CPI – Carbon Prefernce index;
CPI (C_{14}-C_{33}) = $\frac{1}{2}*[(\Sigma odd(n\text{-}C_{15}\text{-}n\text{-}C_{31}) / \Sigma even(n\text{-}C_{14}\text{-}n\text{-}C_{30}) + \Sigma odd(n\text{-}C_{15}\text{-}n\text{-}C_{31} / \Sigma even\ n\text{-}C_{16}\text{-}\ n\text{-}C_{32})]$
TAR = $(C_{27} + C_{29} + C_{31}) / (C_{23} + C_{25} + C_{29} + C_{31})$; Pr/Ph = Pristane/Phytane

The chloroalkenes in the Techirghiol Lake sediments are characterized by terminal chlorine atoms and thus bear some structural similarity to chloroalkanes in halophytes, characterized by an odd/even predominance in the C_{19}–C_{29} range [19, 21]. Long-chain alkanes in the lake sediments are characterized by odd/even predominance in the range C_{21}–C_{33} [22], indicating a higher plant origin [23], but are distinct from the pattern of even-numbered chloroalkenes, excluding higher plant alkanes as a precursor. The formation of naturally occurring long-chain chloroalkenes requires specific environmental conditions, i.e. significant amounts of chloride in the water column or surface sediment. In the literature, alkenols, diol and hydroxyl acids, and their similar chain length and even/odd predominance are considered diagenetic precursors of chloroalkenes in the environment rich with chlorine [19]. Since 1-chloroalkenes are not identified in all analyzed samples, it can be presumed that the uniform horizontal distribution of chloride and sulfur species was not present.

***n*-Alkanes and isoprenoids.** The *n*-alkane patterns of all analyzed samples are dominated by long-chain homologues (*n*-C_{27}-*n*-C_{31}) with a maximum at *n*-C_{29} and a marked odd over even predominance (CPI in range 1.34-2.26, Table 2, Figure 3), as well as high values of terrestrial to aquatic ratio (TAR>2, Table 3). A predominance in *n*-C_{27} to *n*-C_{31} range alkanes is usually related to the input of epicuticular waxes derived from higher plants, although long chain *n*-alkanes may also originate from seagrasses and diatoms [24-27].

Mid chain *n*-alkanes (*n*-C_{21} to *n*-C_{25}) are present in lower amounts than long-chain homologues in all samples. The most abundant alkane in this range is *n*-C_{23}. So far, the dominance of *n*-C_{23} alkane has been observed in *Sphagnum* [28, 29], submerged/ floating aquatic macrophytes [30], seagrass and associated sediments [31-33], as well as *Betula* leaves [27]. Mid-chain *n*-alkanes *n*-C_{23} and *n*-C_{25} can be mostly attributed to aquatic sources, terrestrial plants or emergent macrophytes [27, 30, 33].

The short-chain *n*-alkanes without strong odd-to-even carbon number preference were dominated by either *n*-C_{17} or *n*-C_{18}. *n*-Alkanes with maxima at *n*-C_{17}/*n*-C_{18} are derived from lipids synthesized by phytoplankton and benthic algae [34, 35]. The lower *n*-alkanes, C_{14}–C_{22}, showed a uniform distribution of odd and even homologues, resulting in CPI_1(C_{14}–C_{22}) values lower than 1.17 (Table 3). The relative abundance of short chain *n*-alkanes is higher in samples S1 and S5 compared to other samples, which is reflected in the lowest TAR values in the mentioned samples (Table 3). Relevant research showed that the *n*-alkanes derived from the oil, automobile exhaust and fossil fuel combustion are mainly low carbon aliphatic hydrocarbons, the dominant carbon number is C_{20} or C_{21} and the carbon number distribution displayed single peak distribution without odd-even predominance [36].

Taking all this into consideration, it can be concluded that the origins of the *n*-alkanes in the analyzed muds have both biogenic and anthropogenic origin. The predominance of odd *n*-alkane homologues in the range of C_{23}–C_{35} with the maximum at *n*-C_{27} or *n*-C_{29} implies that the mud of the Techirghiol Lake contains organic matter of terrestrial origin brought in by wind or rivers. The uniform distribution of lower *n*-alkanes, C_{14}–C_{22}, in all samples could be considered an indicator of the anthropogenic oil type pollutants or algal organic matter.

The isoprenoid hydrocarbons, pristane (2,6,10,14-tetramethylpentadecane, Pr) and phytane (2,6,10,14-tetramethylhexadecane, Ph) are present in all analyzed samples. The relatively uniform values of Pr/Ph (0.60-0.79) that are lower than 1 in all samples (Table 3) generally are characteristic for a reducing depositional environment [37]. However, a low Pr/Ph ratio (Table 3) was also reported in hypersaline environments [38]. Therefore, low values of Pr/Ph ratio can imply sedimentation under reducing conditions, which contributed to the good preservation of organic matter.

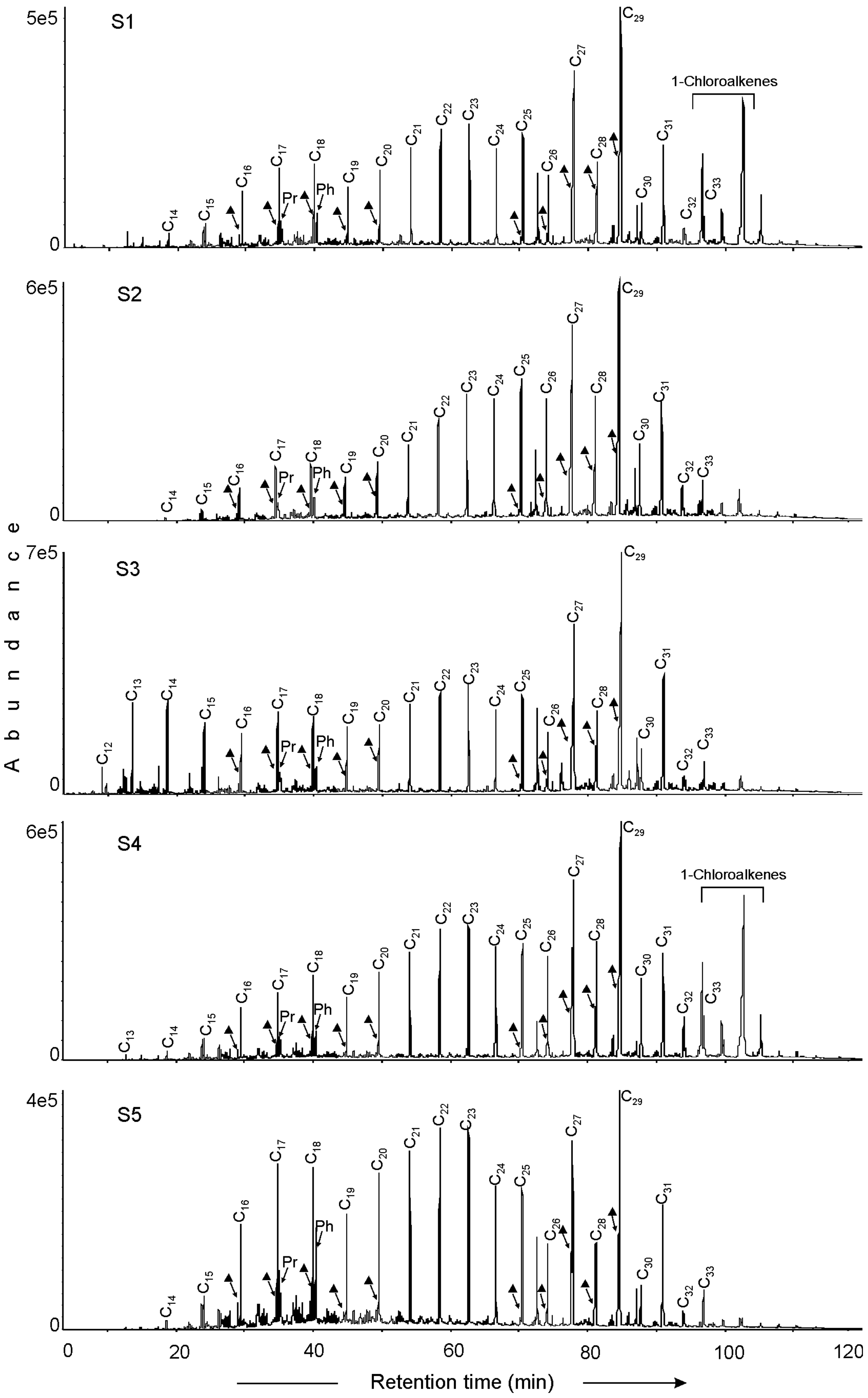

FIGURE 3

Fragmentograms of *m/z* 71 *n*-alkanes and isoprenoids.

(Pr - pristane; Ph - phytane; ▲- *n*-alkenes).

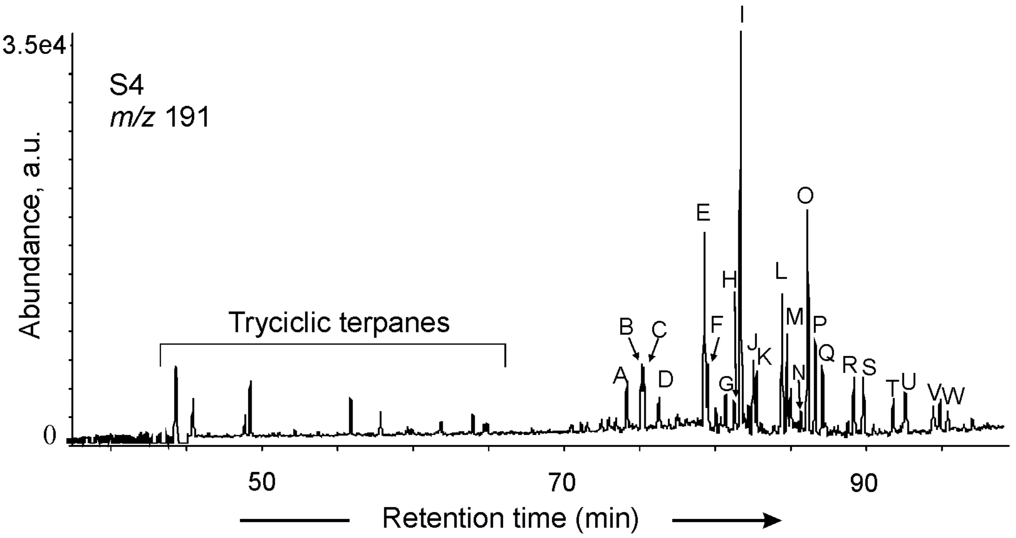

FIGURE 4

Typical GC–MS terpanes distribution.

Legend: **A** – Ts (C_{27}18α(H) 25,29,30 trisnorneohopane; **B** – C_{27}-hop5(6)-ene; **C** – Tm (C_{27}17α(H) 25,29,30 trisnorhopane); **D** - C_{27}17β(H)21β(H)-hopane; **E** – C_{29}17α(H)21β(H)-hopane; **F** - C_{29}Ts; **G** – C_{29}17β(H)21α(H)-moretane; **H** - Oleanane; **I** – C_{30}17α(H)21β(H)-hopane; **J** – C_{30}-hop17(21)-ene; **K** – C_{30}17β(H)21α(H)-moretane; **L** – C_{31}17α(H)21β(H)22(S) hopane; **M** - C_{31}17α(H)21β(H)(R) hopane; **N** – Gammacerane + C_{31}17β(H)21α(H)-moretane; **O** – Hop-22(29)-en; **P** - C_{32}17α(H)21β(H)22(S)-hopane; **Q** - C_{32}17α(H)21β(H)22(R)-hopane; **R** – C_{33}17α(H)21β(H)22(S)-hopane; **S** – C_{33}17α(H)21β(H)22(R)-hopane; **T** - C_{34}17α(H)21β(H)22(S)-hopane, **U** - C_{34}17α(H)21β(H)22(R)-hopane; **V** – C_{35}17α(H)21β(H)22(S)-hopane; **W** - C_{35}17α(H)21β(H)22(R)-hopane.

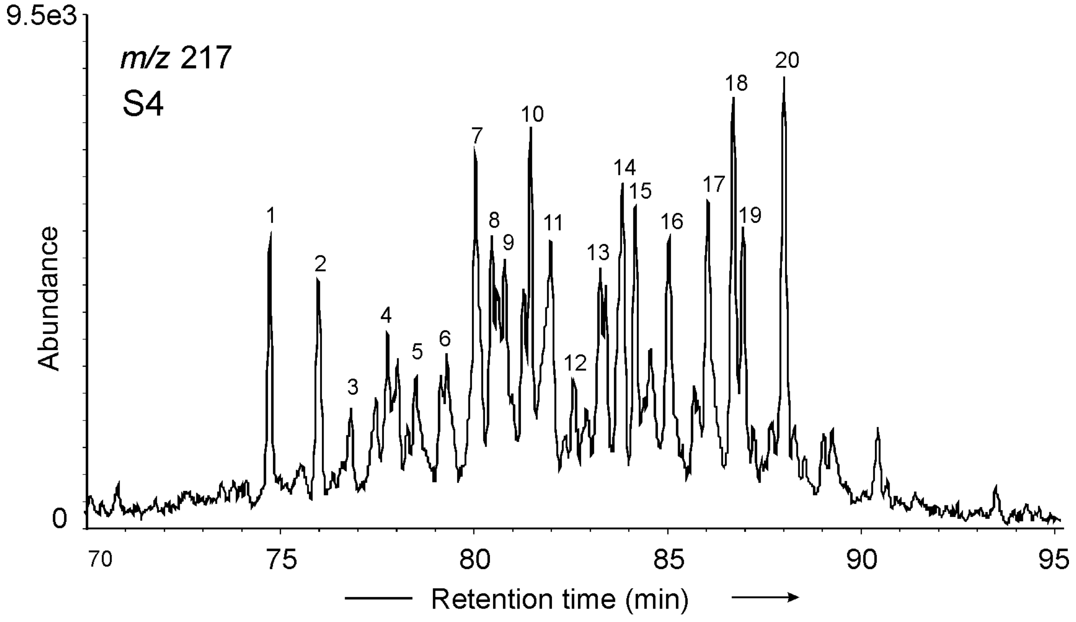

FIGURE 5

Typical GC–MS C_{27}-C_{29} steranes and diasteranes distribution.

Legend: **1** - C_{27}13β(H)17α(H)20(S)-diasterane; **2** - C_{27}13β(H)17α(H)20(R)-diasterane; **3** - C_{27}13α(H)17β(H)20(S)-diasterane; **4** - C_{27}13α(H)17β(H)20(R)-diasterane; **5** - C_{28}13β(H)17α(H)20(S)24(S+R)-diasterane; **6** - C_{28}13β(H)17α(H)20(R)24(S+R)-diasterane; **7** - C_{28}13α(H)17β(H)20(S)-diasterane + C_{27}14α(H)17α(H)20(S)-sterane; **8** - C_{29}13β(H)17α(H)20(S)-diasterane + C_{27}14β(H)17β(H)20(R)-sterane; **9** - C_{28}13α(H)17β(H)20(R)-diasterane + C_{27}14β(H)17β(H)20(S)-sterane; **10** - C_{27}14α(H)17α(H)20(R)-sterane; **11** - C_{29}13β(H)17α(H)20(R)-diasterane; **12** - C_{29}13α(H)17β(H)20(S)-diasterane; **13** - C_{28}14α(H)17α(H)20(S)-sterane; **14** - C_{29}13α(H)17β(H)20(R)-diasterane + C_{28}14β(H)17β(H)20(R)-sterane; **15** - C_{28}14β(H)17β(H)20(S)-sterane; **16** - C_{28}14α(H)17α(H)20(R)-sterane; **17** - C_{29}14α(H)17α(H)20(S)-sterane; **18** - C_{29}14β(H)17β(H)20(R)-sterane; **19** - C_{29}14β(H)17β(H)20(S)-sterane; **20** - C_{29}14α(H)17α(H)20(R)-sterane.

***n*-Alkenes.** *n*-Alkenes *n*-$C_{16:1}$ to *n*-$C_{20:1}$ and *n*-$C_{25:1}$ to *n*-$C_{29:1}$ are detected in the sediment of Techirghiol Lake. Although the exact position of the double bonds was not determined, these compounds eluted just before the corresponding *n*-alkanes [39]. Monounsaturated *n*-alkanes in lake sediment could originate from microbial transformation of the corresponding *n*-alkanes or direct inputs from organisms [40, 41]. The *n*-$C_{25:1}$ – *n*-$C_{30:1}$ range of *n*-alkanes shows strong odd carbon preference in abundances, with C_{max} at *n*-$C_{27:1}$ and *n*-$C_{29:1}$ in all samples. *n*-$C_{16:1}$ to *n*-$C_{20:1}$ range of *n*-alkenes shows even- over-odd carbon preference, which is sharply in contrast with the distribution pattern of *n*-C_{25} to *n*-C_{29} range *n*-alkenes. These alkenes could originate from at least two sources. A diagenetic reduction of monounsaturated fatty acids was suggested as the major sources of *n*-$C_{16:1}$ to *n*-$C_{20:1}$ [3, 42], Simultaneously, eustigmatophytes can be a possible contributor of the long-chain *n*-alkenes because these algae are common in the Techrghiol Lake, and they are known to biosynthesize *n*-C_{27}:1 alkene [14].

Based on the most abundant hydrocarbons, *n*-alkanes and *n*-alkenes in the muds, it can be considered that organic matter of the mud is a mixture of terrigenous and aquatic sources, with the domination of terrigenous sources. This is highlighted by the presence of the pimarane and 16α(H)-phyllocladane in relatively high abundance in all samples (Figure 2). The relatively high abundance of these diterpenoids suggests conifer (*Taxodiaceae*, *Pinaceae*, *Cupressaceae*) contribution to organic matter [43].

Isoprenoid thiophenes. Two isomers of C_{20} isoprenoid thiophenes (3-methyl-2-(3,7,11-trimethyldodecyl)thiophene and 3-(4,8,12-trimethyltridecyl)thiophene) are identified in all investigated samples, eluting between the *n*-C_{21} and *n*-C_{22} alkanes (Figure 2). These compounds have been identified in many marine [44, 45] and lakes sediments [18], during the early stages of diagenesis in the water column and they are formed surface sediments by the reaction of H_2S to phytol or phytadiene derived from chlorophyll [44, 46, 47].

***n*-Alkan-2-ones.** Based on mass fragmentogram, *m/z* 59 *n*-alkan-2-ones are identified in range C_{14} to C_{35}. *n*-Alkan-2-ones are ubiquitous in aquatic environments, and similar molecular distributions have been reported for sediments characterized by higher plant or microbial organic matter inputs [48]. Similar to *n*-alkane distribution, the *n*-alkan-2-ones patterns are dominated by odd long-chain homologues C_{25} to C_{31}. In all samples, *n*-alkanes have a maximum at C_{29}, followed by C_{27},

TABLE 4
Values of parameters calculated from distributions and abundances of terpanes and steranes

	S1	S2	S3	S4	S5
C_{31} S/(S+R) hopane	0.59	0.58	0.59	0.58	0.58
C_{31}-C_{34} S/(S+R) hopanes	0.55	0.59	0.59	0.54	0.59
Ts/(Ts+Tm)	0.45	0.48	0.56	0.50	0.51
C_{29}αα20S/(20S+20R) steranes	0.49	0.50	0.58	0.50	0.69
C_{29} ββ/(ββ+αα) steranes	0.57	0.56	0.56	0.59	0.60

C_{31}-C_{34} S – C_{31-34}17α(H)21β(H)22(S) hopane; **C_{31}-C_{34} R** – C_{31-34}17α(H)21β(H)22(R)hopane;
Ts–C_{27}18α(H) 25,29,30 trisnorneohopane; **Tm**–C_{27}17α(H) 25,29,30 trisnorhopane;
C_{29}ααS– C_{29} 4α(H)17α(H)20(S)-sterane; **C_{29}ααR**– C_{29} 14α(H)17α(H)20(R)-sterane;
C_{29}ββ– C_{29} 4β(H)17β(H)20(R)-sterane; **C_{29}αα**– C_{29} 4α(H)17α(H)20(R)-sterane.

TABLE 5
Values of diagnostic ratios and typically diagnostic ratios for processes

	ΣLMW/ ΣHMW	Fluo/(Fluo+Pyr)	IP/(IP+BghiP)	BaA/(BaA+Chr)	BaP/BghiP	MP/P
S1	0.41	0.42	0.48	0.35	0.59	0.77
S2	0.29	0.54	0.43	0.35	0.50	0.76
S3	0.32	0.56	0.46	0.37	0.59	0.96
S4	0.40	0.56	0.48	0.33	0.54	0.79
S5	0.29	0.58	0.48	0.35	0.48	0.92
Pyrogenic	< 1	> 0.4	> 0.5	> 0.35	< 0.6	< 1
Petrogenic	>1	< 0.4	< 0.2	< 0.2	> 0.6	>1
Reference	[63, 67]	[64]	[65]	[65]	[68]	[65, 69]

Legend: **LMW** - low molecular weight polyaromatic hydrocarbons; **HMW** - high molecular weight polyaromatic hydrocarbons; **Fluo** - fluoranthene; **Pyr** - pyrene ; **IP** - Indeno(123cd)piren; **BghiP** - benzo[g.h.i]perylene; **BaA** - benzo[a]anthracene; **Ch**r - chrysene; **BbF** - benzo[b]fluoranthene; **BkF** - benzo[k]fluoranthene; **BaP** - benzo[a]pyrene; **MP** - methylphenanthrene; **P** - phenanthrene.

whereas the most abundant *n*-alkan-2-one is C_{31}, followed by C_{29}. These dominant peaks in *n*-alkan-2-ones homologues range are characteristic for high plants [49]. In addition to the above stated, *n*-alkan-2-ones 6,10,14-trimethylpentadecan-2-one (TMP), an isoprenoidal ketone is also found in all samples. This compound can be related to filamentous green algae *Cladophora Vagabunda* (family *Cladophoraceae*), one of the most widespread algae in the Black Sea and the Techirghiol Lake [50]. The abundance of 6,10,14-trimethylpentadecan-2-one and *n*-alkan-2-ones is particularly pronounced in the samples S1 and S4, while samples S2, S3 and S5 contain relatively lower concentrations of these compounds.

Terpanes and steranes. The terpane distributions (*m/z* 191) are characterized by the dominance of pentacyclicterpanes, i.e. hopanes, and relatively low abundance of tricyclic (C_{23}-C_{26}) terpanes (Figure 4). The values of specific organic–geochemical parameters, calculated from the distributions of these biomarkers, are given in Table 4.

The hopane series C_{27}-C_{34} (except C_{28}) is found in all samples (Figure 4) and contains isomers typical for biogenic and matured organic matter. They were characterized by a predominance of the thermodynamically more stable isomers with $17\alpha(H)21\beta(H)$ and 22(S) configurations, which are typical for mature organic matter (fossil fuel hydrocarbons). Besides the presence of these compounds, the occurrence of C_{27}-hop-5(6)-ene, C_{27}-17β(H)-hopane, C_{30}-hop-22(29)-ene (diploptene) and hop-17(21)-ene confirms the contribution of recent OM (Figure 4). Diploptene is present in various types of bacteria and occurs in diverse environments [51-53]. Cyanobacteria *Chroococcusturgidus*, abundant in the Techirghiol Lake [14], can be considered a precursor of this compound. Hop-17(21)-ene is regioisomer of diploptene and it is considered as diagenetic product of diploptene [54] or bacteria [25, 55, 56].

Based on the distributions of terpanes in the saturated fractions of the muds and the values of the corresponding maturity parameters ($C_{31\text{-}34}$(S)/C_{31}-C_{34}(S+R)-homohopanes ratio, Ts/(Ts+Tm), Table 4), it could be concluded that, in addition to native, the analyzed samples also contain organic matter of anthropogenic origin, which could be related to the petroleum hydrocarbons.

The distribution of steranes (Figure 5) confirms anthropogenic organic matter in the samples of the Techirghiol Lake sediments. It is characterized by the predominance of structural and stereochemical isomers, typical for the oil as a most mature form of the organic substance in the geosphere [6, 57]. In the fragmentograms of steranes, in addition to the biolipid isomers C_{27}-C_{29} 14α(H), 17α(H), 20R, diasteranes, typical geoisomers and geolipid sterane isomers C_{27}-C_{29} 14β(H), 17β(H), 20S are also present (Figure 5). All of the investigated samples have $C_{29}\alpha\alpha\alpha$ 20S/(20S+20R) and C_{29} ββ/(ββ+αα) sterane ratios ranging from 0.46 to 0.69 and 0.49 to 0.60, respectively (Table 4) and values of these ratios confirm high maturity of the organic substances in the investigated samples.

However, it is necessary to emphasize that sterane and terpane "fingerprints" (Figures 4, 5) characteristic for crude oils, have low levels compared to the abundance of *n*-alkane. Bearing this in mind, it can be assumed that organic matter of oil type is present only at the level of contamination in the mud of the Techirghiol Lake.

Aromatic compounds. In order to further distinguish the probable sources of hydrocarbons in these sediments, other indicators of petroleum, such as the composition of aromatic hydrocarbons, were analyzed. Polyaromatic hydrocarbons (PAHs) are ubiquitous in sediments and originate from different emission sources, natural or anthropogenic [58]. Among the aromatic compounds, besides the naturally polyaromatic hydrocarbons, such as retene and perylene, the presence of polyaromatic hydrocarbons of anthropogenic origins is found. Perylene, a five-ringed PAHs, was distinctly separated from the other five-ringed PAHs. The sources for perylene are likely from biogenic, terrestrial precursors, and it is most probably produced in the reducing lake environments and from natural terrestrial and possibly aquatic plant precursors [59]. Similarly, retene is produced in reducing environments and from the biogenic conversion of terpenoid compounds in higher plants [59], however [60] notes that retene can be produced from the combustion of coniferous wood. These results confirm alochtones input of terrestrial organic substance to the lake.

In the analyzed samples the existence of the follow anthropogenic PAH compounds is revealed: acenaphtylene (Acy), acenaphtene (Ac), phenanthrene (P), fluoranthene (Fluo); pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF); benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), Indeno(123-cd)piren(IP), benzo[g.h.i]perylene (BghiP).

The PAHs patterns differ according to the sources and several PAHs concentration diagnostic ratios have been extensively used to identify and quantify the contribution of the source of contamination to the investigated environment [61, 62]. More significant PAH ratios were drawn from the correlation and calculated for the samples: LMW/HMW=(P+Ac+Py+Fluo)/(BaA+Chr+BbF+BkF), Fluo/[Fluo+Py], BbF/BaP, IP/(IP + BghiP), BaA/(BaA + Chr) (Table 5; [63-66]). The LMW/HMW ratio (sum of the low molecular weight PAH levels versus the sum of higher molecular weight concentration) is undertaken as a primary index in the investigation of PAH sources [67]. This ratio shows that PAHs of low molecular weight predominance in these samples indicate pyrogenic origin. The values of the LMW/HMW ratio in the investigated samples are lower than 1 (ranged between 0.29 and 0.41), indicating the pyrogenic origin of PAHs. The LMW/HMW ratio values in the investigated samples are lower than 1 (ranged between 0.29 and 0.41), indicating the pyrogenic origin of PAHs. This prevalence of combustion-derived PAHs was confirmed in the studied muds by isomer ratios IdP/(IdP + Bper) (>0.2), BaA/(BaA + Chr) (>0.2) and Fluo/(Fluo + Py) (>0.4) (Table 5). The metthylphenanthrenes to phenanthrene (MP/P) ratio is widely used for this purpose, too [68, 69]. The values of this parameter (MP/P<1) confirm the pyrogenic origin of PAHs.

Domination of pyrogenic PAHs (Table 5) in these samples implies that the sediments are contaminated by the incomplete combustion of organic matter, probably from surrounding agricultural activities (burning of plant residues on the field and wood), from low emission sources (home furnaces) and industrial wastes. The possible mean in which PAH can enter the surface waters is river catchments (runoff), atmospheric deposition, rain drainage and municipal or industrial wastewater.

CONCLUSION

In this study, the muds from the hypersaline Techirghiol Lake (Romania) are investigated. The contents of soluble organic matter are relatively high and similar in all samples with the dominance of the NSO-fraction.

Based on the distribution of most abundant hydrocarbons, *n*-alkanes and *n*-alkenes in the analyzed samples, it can be considered that organic matter in the analyzed muds are derived mainly from phytoplankton, filamentous green alga *Cladophora vagabunda*, Cyanobacteria, submerged/floating macrophytes, terrestrial and emergent plants, i.e. that is mixture of terrigenous and aquatic sources, with the domination of terrigenous sources. The terrestrial origin of organic matter is probably related to a significant input by wind or rivers.

Typical oil distributions of terpanes and steranes along with values of the corresponding maturity parameters clearly indicate that the muds of the Techirghiol Lake, in addition to immature organic matter, contain a relatively low contribution of petroleum type pollutants. The distribution of these biomarkers in all samples is very similar.

Domination of pyrogenic PAHs in investigated samples implies that they are contaminated by the incomplete combustion of organic matter, probably from low emission sources (home furnaces) and industrial wastes and entered primarily through atmospheric deposition.

Organic matter of the muds from the Techirghiol Lake is deposited in a hypersaline environment, which is favorable for its preservation to a great extent. The relatively high abundance of 1-chloroalkenes, which in some samples represents the most abundant compound in the total distribution of hydrocarbons, and the presence of isoprenoid thiophenes indicate the presence of significant amounts of chloride and sulfur species in the water column or surface sediment locally.

The obtained results may be useful for related environmental monitoring in the future.

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