Journal of the Serbian Chemical Society
version electronic
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS

J. Serb. Chem. Soc. 83 (10) 1167–1175 (2018)
JSCS–5141

UDC 686.862.6.000.57:665.008.68:631.4:
627.8.034(497.6)
Original scientific paper

Application of alkane biological markers in the assessment of the origin of oil pollutants in the soil and recent river sediments (River Vrbas, Bosnia and Herzegovina)

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(Received 1 May, revised 11 June, accepted 12 June 2018)

Abstract: In this paper, an attempt was made to contribute to the use of biological markers, *n*-alkanes, and polycyclic alkanes of the sterane and terpane type, in the assessment of the source of oil pollutants in the environment using the example of the correlation between recent river and coastal sediments,. Four samples of recent river sediments of the Vrbas River and four samples of adjacent bank sediments (soils), in the part of the River that belongs to the city of Banja Luka (Bosnia and Herzegovina) were analyzed. In the alkane fractions of isolated extracts, a bimodal distribution of the *n*-alkanes was observed. Lower homologues dominated in the recent river sediments with maximum at C_{15}, but higher *n*-alkanes dominated in the soil samples, with a maximum at n-C_{29} or at n-C_{31}. The higher concentration of steranes and terpanes with oil type distributions in the recent/fresh river sediments (compared to the bank sediments) represents evidence that lower *n*-alkanes originate from oil sources. The higher content of total hydrocarbons in the recent river sediments than in the bank sediments further confirms this conclusion. At the same time, these fundamental considerations provide evidence that oil pollution primarily occurred in the water flow and then the oil pollutants propagated towards the riverbank.

Keywords: oil type pollutant; recent river sediments; riverbank sediments correlation; native and anthropogenic organic substances; biomarkers.

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https://doi.org/10.2298/JSC180501061S

INTRODUCTION

The amount of native bitumen/oil type organic substances (soluble in organic solvents) in soil and recent sediments is not defined and, theoretically, can be in the range from 0.00 (recent sediments without any organic substance) to up to 100 % (sediment formations containing so-called "surface oil"). Within this wide range are the quantities found in different sediments, or, for example, in surface deposits of oil shales or coals of a different degree of carbonification.[1,2] For this reason, it is not possible to determine "maximal allowable quantity" for this type of organic pollutant in the soil and in recent sediments. In other words, the amount of organic extract in a particular sample is not a sufficient parameter to determine the presence of an oil type pollutant, because it is not possible to estimate the origin (native or anthropogenic) of the isolated organic substance based only on the amount of extract in a soil or in a recent sediment.

Previous studies of the origin and fate of oil pollutants in the environment have shown how native and the anthropogenic organic substance can be distinguished. The approach is based on the fact that oil represents a mature organic substance in the geosphere. Compared to the organic substance of recent sediments, oil contains a larger amount of carbon isotope ^{13}C. Furthermore, the most thermodynamically stabile structural and stereo isomers dominate in the biomarker distributions of alkane and aromatic fractions. In this sense, *n*-alkanes and isoprenoid aliphatic alkanes, as well as polycyclic alkanes of sterane and terpane type could be advantageous. The use of these biological markers and $\delta^{13}C_{PDB}$ for "forensic" purposes, *i.e.*, the distinction of native and anthropogenic organic substance, was demonstrated in previous papers.[3–9]

Moreover, the study of biological markers in oil-type pollution has proved beneficial in terms of assessing its fate in various recent sediment formations, including groundwater and surface waters. By monitoring the change in the abundance and distribution of the alkane and aromatic oil biomarkers under natural conditions, as well as under laboratory-simulated conditions (*n*-alkanes, isoprenoids, steranes, terpanes, phenanthrenes and their methyl derivatives), the intensity and kinetics of oil biodegradation in the environment,[10–13] and the mechanism of its migration through "water wet" sediments were estimated.[14]

It is well known that processes in the environment are very complex. Previous empirical studies set the transformation of oil pollutants into theoretical frameworks well. However, the specific features of each particular environment lead to specific phenomena and specific transformations of the oil, *i.e.*, to the transformation of its constituent compounds. This provides opportunities for establishing new theoretical regularities.

In this study, four samples of recent river sediments of the Vrbas River, as well as four samples of adjacent bank sediments (soils) in the part of the river flowing through the city of Banja Luka (Bosnia and Herzegovina) were analyzed.

Based on organic–geochemical knowledge of oil as a form of organic substance in the geosphere, and up-to-date findings of methods for the identification of anthropogenic organic matter of an oil type and its fate in the environment, on the example of the interaction of recent river and bank sediments, an attempt was made to contribute to the use of biological markers, *n*-alkanes and polycyclic alkanes of the sterane and terpane type, in assessing the source and fate of oil pollutants in the environment.

EXPERIMENTAL

Samples

The sampling details are given in Supplementary material to this paper.

Separation and instrumental techniques

The sediment samples were cleaned from plant and animal tissues and debris, air-dried and then homogenized. The soluble organic matter (extract) was obtained by the Soxhlet method during 36 h using chloroform. The content of soluble organic matter in the samples was determined from the ratio of the extract mass (dried to constant mass) and the initial mass of the sample.

The following method was employed to separate the extracts into fractions (Fig. 1). The extract was first saponified using a potassium hydroxide solution in methanol (5 %). The solution was then neutralized by the addition of hydrochloric acid (10 %). After that, the sample was dissolved in *n*-hexane and transferred to the top of the adsorption layer placed in the glass column. Aluminum oxide and silica gel were used as adsorbents. The saturated hydrocarbon fraction was eluted using hexane, the aromatic fraction using dichloromethane, while the alcohols were eluted using a mixture of dichloromethane and methanol (1:1). The fourth fraction, *i.e.*, fatty acid (in the form of their methyl esters) was eluted with a sulfuric acid solution in methanol (5 %). The solution of the fourth fraction was further treated with aqueous sodium chloride solution (20 %) and *n*-hexane.

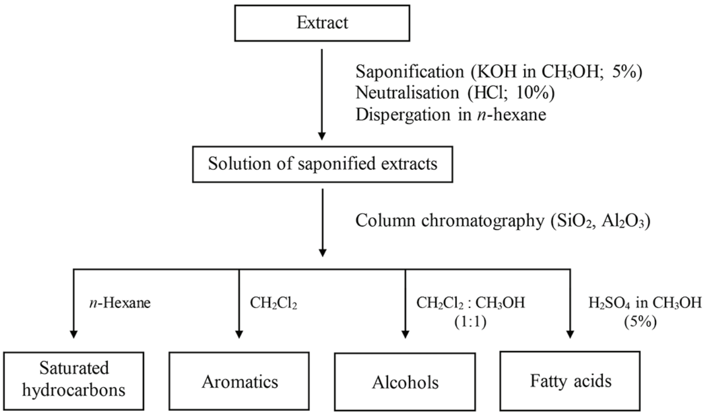

Fig. 1. Schematic representation of the extract fractionation.

The saturated hydrocarbons from the extracts were analyzed by gas chromatography–mass spectrometry (GC–MS). GC–MS was performed using an Agilent 7890A gas chromatograph (HP5-MS column, 30 m×0.25 mm, 0.25 μm film thickness, He carrier gas, 1.5 cm^3 min^{-1}) coupled to an Agilent 5975C mass selective detector (70 eV). The column was heated from 80 to 310 °C, at a rate of 2 °C min^{-1}, and the final temperature of 310 °C was maintained for additional 25 min. Detailed analyses of the target compounds were conducted using the following ion chromatograms: *m*/*z* 71 (*n*-alkanes), 217 (steranes) and 191 (terpanes). The individual peaks were identified by comparison with literature data[1,15] and based on their mass spectra (library: NIST11).

RESULTS AND DISCUSSION

Group parameters

The extractable amounts from the examined river sediments and soils, as well as the contents of the total hydrocarbons (*i.e.*, the sum of the saturated and aromatic hydrocarbons) and polar NSO compounds in the extracts are given in Table I.

TABLE I. The content of soluble organic matter (extract) in the investigated samples and content of total hydrocarbons and polar compounds in the extracts

Sample	Content of extract, %	Total hydrocarbons content in extract, %[a]	Content of polar compounds in extract, %[b]
RS_1	0.31	29.13	46.32
RS_2	0.39	11.05	40.23
RS_3	0.04	36.48	59.73
RS_4	0.06	42.36	54.41
S_1	0.09	12.54	83.45
S_2	0.05	14.28	65.09
S_3	0.19	6.61	59.02
S_4	0.06	10.89	85.10

[a]Total content of saturated and aromatic hydrocarbons; [b]polar compounds represent the sum of 3rd and 4th fractions (see Fig. 1)

The amount of the extracts in the samples RS_1 and RS_2 was slightly increased (0.31 and 0.39 %, respectively, Table I) in comparison to the other samples, which contained extractable amounts in a relatively narrow range of 0.04 to 0.19 % (Table I).

According to group composition, the extracts of the fresh river sediments were characterized by a slightly higher percentage of hydrocarbons, and a lower content of polar compounds compared to the soil samples (11.05–42.36 % *vs.* 6.61–14.28 % and 40.23–59.73 % *vs.* 59.02–83.45 %, respectively, Table I). Such a ratio of hydrocarbons and polar compounds might be indicative of a higher proportion of oil pollutants in the fresh river sediment samples (RS) compared to the soil samples (S).[1,2,15] In the fresh river sediments and in the soil samples, highly polar molecular compounds were present, which were not eluted in the described chromatographic procedure. Their percentage content represents the difference in the total of hydrocarbon and polar components (Table I) up to 100 %.

Specific parameters

The fragmentograms of *n*-alkanes (*m*/*z* 71), steranes (*m*/*z* 217) and terpanes (*m*/*z* 191) for the samples RS_1 and S_1, which are typical for all the investigated extracts of the river sediments and soils, are given in Fig. S-2 of the Supplementary material. In addition, the fragmentograms of the other samples are given in Fig. S-3 of the Supplementary material. The values of the parameters calculated from *n*-alkane distributions in the river sediments and soils are given in Table II.

TABLE II. Parameters of *n*-alkane distributions

Sample	*n*-Alkane range	The most abundant *n*-alkane	n-C_{15}/n-C_{29}	$CPI_{C_{22}\text{-}C_{36}}$ [a]
RS_1	C_{14}–C_{35}	C_{15}; C_{29}	5.31	1.74
RS_2	C_{14}–C_{34}	C_{15}; C_{29}	2.15	4.13
RS_3	C_{14}–C_{35}	C_{15}; C_{29}	3.62	1.52
RS_4	C_{14}–C_{36}	C_{15}; C_{29}	8.80	1.21
S_1	C_{15}–C_{36}	C_{16}; C_{31}	0.20	4.08
S_2	C_{15}–C_{36}	C_{16}; C_{29}	0.25	1.45
S_3	C_{15}–C_{36}	C_{15}; C_{29}	0.24	3.48
S_4	C_{14}–C_{38}	C_{16}; C_{31}	0.38	1.22

[a] Carbon preference index determined for the distribution of *n*-alkanes C_{22}–C_{36}, $CPI_{C_{22}\text{-}C_{36}} = 1/2\ [\Sigma\text{odd}(n\text{-}C_{23} - n\text{-}C_{35}) / \Sigma\text{even}(n\text{-}C_{22} - n\text{-}C_{34}) + \Sigma\text{odd}(n\text{-}C_{23} - n\text{-}C_{35}) / \Sigma\text{even}(n\text{-}C_{24} - n\text{-}C_{36})]$

The *n*-alkane distributions of the river sediment extracts are characterized by bimodal distribution with maximums at C_{15} and C_{29} (Fig. S-2a, Table II). The second part of bimodal distribution of *n*-alkanes is characterized by the prevalence of homologues with an odd number of C-atoms, whereby the carbon preference index values, $CPI_{C_{24}\text{-}C_{36}}$, are in the range of 1.21–4.13. Based on previous organic geochemical findings,[1,2,15] it could be concluded, with a great degree of reliability, that these *n*-alkanes belong to the organic substances of terrestrial origin. On the other hand, the first part of chromatogram with maximum at n-C_{15} could be indicative for native aquatic organic matter. However, these lower normal alkanes could also originate from oil.[1,2] The latter suggests that the studied extracts of the river sediments may contain a mixture of native organic substances and anthropogenic organic substances of an oil type. In all samples, n-C_{15} was considerably more abundant than n-C_{29} (n-C_{15}/n-C_{29} is in the range of 2.15–8.80, Table II), and this indicates that the proportion of native aquatic organic matter or anthropogenic organic substances of an oil type is higher than that of a naturally occurring terrestrial organic substances.

The identification of representative peaks from sterane and terpane fragmentograms is given in Table S-I of the Supplementary material. The distribution of steranes and terpanes, which is shown in Fig. S-2b and c, supports the assumption of the presence of anthropogenic organic matter of the oil type in the investigated samples of fresh river sediments RS_1–RS_4. These polycyclic hydrocarbons are characterized by structural and stereochemical isomers that are typical for the oil as a mature form of the organic substances in the geosphere.[1,2,15] For example, in the fragmentogram of steranes, in addition to the biolipid isomers 14α(H), 17α(H), 20R C_{27}–C_{29} (peaks 3, 5 and 7; Fig. S-2b, Table S-I), diasteranes, typical geoisomers, and thermodynamically more stable geolipid sterane isomers having hydrogen atoms in the β-position at C_{14} and C_{17} and *S*-configuration at C_{20} (for example, peaks 1, 2, 4 and 6; Fig. S-2b, Table S-I) are present.

Terpanes were also present in all the investigated samples of fresh river sediments, RS_1–RS_4, with distributions that are characteristic for oil (Fig. S-2c). In the fragmentograms of these samples, the peaks of thermodynamically stable isomers C_{29} 17α(H)21β(H)-hopane and C_{30} 17α(H)21β(H)-hopane (peaks 8 and 9, Table S-I) are dominant. Among the C_{31}–C_{35} homohopanes, the more stable 22*S* isomers dominate in comparison to 22*R* isomers (peaks 11–14).

It could be concluded that the identified steranes and terpanes in the recent river sediments, together with a part of lower *n*-alkanes (Fig. S-2a), originate from anthropogenic organic substances. Higher *n*-alkanes characterized by domination of odd homologues (*CPI* in the range 1.21–4.13) with a maximum at C_{29} (the second part of the bimodal distribution; Table II) are native and originate from higher terrestrial plants, *n*-alkanes in coastal sediments, *i.e.*, soils (S_1–S_4) also have bimodal distributions with maximums at C_{15} or C_{16} and C_{29} or C_{31} (*m*/*z* 71, Fig. S-2d, Table II). The second part of bimodal distribution of *n*-alkanes, similar to the fresh river sediments RS_1–RS_4, is characterized by distinct domination of homologues with an odd number of C-atoms ($CPI_{C24\text{-}C36}$ in the range 1.22–4.08). As has already been mentioned, it could be reliably claimed that these *n*-alkanes belong to the native organic substances of terrestrial origin. On the other hand, the lower *n*-alkanes with a maximum at C_{15} or C_{16} could also originate from anthropogenic oil sources. Thus, similarly to the RS_1–RS_4 river sediment samples, in the four investigated soil samples from nearby localities, S_1–S_4, there is a mixture of native organic matter and anthropogenic organic substance of an oil type. However, compared to the recent river sediments, the ratio of lower and higher *n*-alkanes is different. In the soil samples, higher *n*-alkanes dominate in comparison to the lower ones. In all four samples, the abundance of *n*-C_{29} is considerably higher than that of *n*-C_{15} (*n*-C_{15}/*n*-C_{29} is in the range of 0.20–0.38, Table II). Therefore, it could be concluded that in this case, the share of native organic matter is significantly greater than the proportion of anthropogenic organic substance.

The steranes and terpanes in the fragmentograms corresponding to the S_1–S_4 samples provide “fingerprints” that are characteristic for the oils (fragmentograms of steranes, *m/z* 217 and terpanes *m/z* 191 of the S_1 sample, typical for all the studied soil samples, are given in Fig. S-2e and f).[1,2,15] However, the intensity of the peaks is at the level of the electronic "noise" of the instrument, which indicates their extremely low concentrations. These low concentrations of the sterane- and terpane-type polycyclic alkanes correspond to the low concentrations of lower *n*-alkanes in the soil samples S_1–S_4 (Fig. S-2d).

The aforementioned values of the parameters, distributions and the relative proportions of *n*-alkanes, steranes and terpanes in the extracts of the fresh river sediments and the riverbank sediments (*i.e.*, soils) at the site could be indicative of pollution, as well as of the path of the dispersion of oil pollutants in the river system. The higher content of anthropogenic organic substance of an oil type in the recent river sediments compared to the soils (only a few meters away from the water flow) unambiguously suggests that the oil pollution occurred in the water flow. A small quantity of oil was then dispersed from the fresh river to the coastal sediments, *i.e.*, soil. This conclusion does not exclude factories and utility systems located near the sampling sites as potential sources of pollution.

CONCLUSIONS

The use of biological markers, *n*-alkanes and polycyclic alkanes of the sterane and terpane type in the assessment of the source and fate of oil pollutants in the environment has been demonstrated in the study of the fresh river and riverbank sediments of the Vrbas River in the part belonging to the city of Banja Luka (Bosnia and Herzegovina).

Based on the slightly higher proportion of hydrocarbons and the smaller content of polar compounds in the extracts of the fresh river sediments, RS_1–RS_4, in comparison with the soil samples, S_1–S_4, it was assumed that a higher amount of oil pollutant was present in the recent river sediments.

The *n*-alkanes in the extracts of the fresh river sediments are characterized by a bimodal distribution with the maximums at C_{15} and C_{29}. Among the higher *n*-alkanes, the apparent dominance of homologues with an odd number of C-atoms was evident. These *n*-alkanes belong to the native organic substance of terrestrial origin. The lower homologues with the peak maximum at *n*-C_{15}, and the identified steranes and terpanes confirm the presence of oil-type pollutants. Therefore, the extracts of the recent river sediments contain a mixture of native organic matter and anthropogenic organic matter of an oil type. Considering the fact that in these river sediments the lower *n*-alkanes are more abundant than the higher ones, it was concluded that the proportion of anthropogenic organic substance was greater than the proportion of native organic matter.

n-Alkanes of the banks sediments, *i.e.*, soils (S_1–S_4), also had bimodal distributions with maximums at C_{15} or C_{16} and C_{29} or C_{31}. The second part of bimodal distribution of *n*-alkanes was characterized by a distinct domination of homologues with an odd number of C-atoms, presenting strong evidence for native organic substances of terrestrial origin. However, based on the distributions and abundances of *n*-alkanes, steranes and terpanes, it was concluded, contrary to the fresh river sediments, that native organic matter notably prevailed over the anthropogenic organic substance in these samples.

The higher content of anthropogenic organic substance of an oil type in recent river sediments compared to adjacent land (which is a few meters away from the water flow) unambiguously suggested that oil pollution occurred in the water flow. After pollution, the small quantity of oil was probably dispersed from the river to the coastal sediments, *i.e.*, soil. However, this conclusion does not exclude factories and utility systems, located nearby sampling sites, as potential sources of pollution.

This study undoubtedly gives a fundamental contribution to the definition of the use of *n*-alkanes and polycyclic alkanes (steranes and terpanes) in the assessment of the origin and fate of oil type pollutants in river systems.

SUPPLEMENTARY MATERIAL

Additional information and data are available electronically at the pages of journal website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

Acknowledgement: This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No. 176006).

ИЗВОД

НОВИ ПРИСТУПИ У ПРИМЕНИ АЛКАНСКИХ БИОМАРКЕРА У ПРОЦЕНИ ПОРЕКЛА И СУДБИНЕ НАФТНИХ ПОЛУТАНАТА У РЕЧНИМ СЕДИМЕНТИМА (РЕКА ВРБАС, БОСНА И ХЕРЦЕГОВИНА)

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У овом раду је учињен покушај да се на примеру интеракције рецентни речни седимент–приобални седимент да допринос употреби биолошких маркера типа нормалних алкана, и полицикличних алкана типа стерана и терпана у процени извора нафтног полутанта у животној средини. Испитивани су свежи речни талози и приобални седименти блиских локалитета реке Врбас (по 4 узорка), у делу тока који припада граду Бања Лука (Босна и Херецеговина). У алканским фракцијама изолованих екстракта идентификована је бимодална расподела нормалних алкана, с тим што су у свежим речним седиментима доминирали нижи хомолози са максимумом на n-C_{15}, а код приобалних седимената, виши са максимумом на n-$C_{29,}$ односно n-$C_{31.}$ Виша концентрација стерана и терпана са нафтним расподелама у рецентним речним седиментима (у поређењу са приобалним седиментима) доказ су да нижи нормални алкани у њима потичу од нафте. Већа количина укупних угљоводоника у свежим речним седиментима у односу на приобалне, иде у прилог изнешеном закључку. У исто време ова фундаментална разматрања

пружају доказ да се нафтно загађење реке одиграло у самом току и да је нафтни полутант расејан у приобаље.

(Примљено 1. маја, ревидирано 11. јуна, прихваћено 12. јуна 2018)

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