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## The use of biological markers in the determination of the origin and type of organic matter in the sediments of the Tisza River

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**Abstract:** The objective of this study was to determine the origin and type of organic matter (OM) in recent sediments of the Tisza River, along a distance of 153 km through the territory of Serbia. For this purpose, group organic–geochemical parameters and biomarker compositions were used. All samples contained approximately same amount of OM, which was deposited under uniform, slightly reducing conditions. Based on the distribution of *n*-alkanes, the origin and type of OM could not be precisely estimated. However, the *n*-alkane patterns suggest the presence of immature singenetic organic matter of terrestrial origin. The distributions of terpanes and steranes and the values of the corresponding maturity parameters indicate that the Tisza River sediments, apart from immature singenetic organic matter, contain oil pollutants of anthropogenic origin. The identical compositions of these biomarkers in all samples confirmed that the recent sediments of the Tisza River, from Kanjiža Town to the confluence into the Danube River, contain the same type of oil pollutants. Based on the compositions of terpanes and steranes and the values of the biomarker parameters in Tisza sediments, it is supposed that the oil pollution generally could be related to heavy fuel oil from tankers, due to intense river transport and, to lower extent, to crude oils from the Elemir and Rusanda oil fields.

**Keywords:** sediments; Tisza River; organic matter; biomarkers; oil pollutants.

### INTRODUCTION

Biological markers (biomarkers) in bitumen (soluble organic matter extracted from sediments, coals, oil shales and source rocks) and oil are complex organic

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compounds with a strong resemblance in structure to their parent organic molecules in living organisms. In organic geochemistry, they are extensively used to estimate the origin of sedimentary organic matter (OM) and its geological evolution.<sup>1</sup> In other words, based on the presence, abundance and distribution of respective biomarkers in oils, the origin, depositional environment and maturity of the organic matter, as well as the relative length of migration pathways and the degree of biodegradation could be assessed.<sup>2</sup> For this purpose, numerous biomarkers, *n*-alkanes, isoprenoid aliphatic alkanes, polycyclic alkanes of the sterane and terpane types and alkylaromatics have been used. Recently, the application of biological markers in environmental chemistry has proved its self to be a promising tool for the determination and identification of petroleum pollutants in soils, recent sediments, ground and surface waters.<sup>3,4</sup> The content of singenic organic matter in recent sediments varies over a wide range from 0 to 80 %<sup>2</sup> and therefore can not be used as an indicator of the origin of organic matter. For this reason, biomarker fingerprints typical for crude oils, as highly mature organic matter, can be useful in evaluating whether the organic matter extracted from the environment, is singenic or it represents anthropogenic organic matter of petroleum origin. This primarily refers to *n*-alkanes. In crude oils, *n*-alkanes have uniform distribution of odd and even homologues (the Carbon Preference Index, *CPI*, is about 1), usually in the range of C<sub>10</sub>–C<sub>35</sub>. An *n*-alkane distribution with a strong predominance of lower homologues C<sub>12</sub>–C<sub>22</sub> indicates algal organic matter input and/or high maturity.<sup>1</sup> On the other hand, elevated concentrations of odd long-chain homologues (C<sub>25</sub>–C<sub>33</sub>) show that terrestrial OM also contributed to the precursor biomass.<sup>5,6</sup> It should be noticed that oil derivatives, such as gasoline and diesel, have different *n*-alkane distributions, which range from *n*-C<sub>6</sub> to *n*-C<sub>12</sub>, and *n*-C<sub>12</sub> to *n*-C<sub>25</sub>, respectively. Moreover, these derivatives do not contain polycyclic biomarkers of the sterane and terpane types.<sup>7,8</sup>

Similarly to *n*-alkanes, the distributions of polycyclic alkanes (C<sub>21</sub>–C<sub>22</sub> and C<sub>27</sub>–C<sub>30</sub> diasteranes and steranes, C<sub>19</sub>–C<sub>27</sub> tricyclic diterpenes and C<sub>27</sub>–C<sub>35</sub> pentacyclic triterpenes) in oils are distinctive and noticeably different from those observed in bitumen of recent sediments, due to their higher maturity.<sup>1,9</sup> However, different crude oils show unequal distributions of biomarkers. Each oil is characterized by a distinctive distribution of biomarkers as a kind of “fingerprint”, which depends on the origin, depositional environment, age, maturity, relative length of migration and mineral composition of the source and reservoir rocks.<sup>10,11</sup> This feature of oil can be very useful in environmental chemistry for the determination of the source of pollution. Consequently, analyses of this type obtain a forensic character.

In this paper, the surface sediments of the Tisza River, in the part of river flow from Kanjiža Town to its confluence into the Danube, were investigated. Considering the navigable character of the Tisa River, and thus its exposure to

anthropogenic impact, an investigation of the origin and type of organic matter in its sediments was the defined objective of this study. For this purpose, group organic–geochemical data and biomarker compositions were used.

#### EXPERIMENTAL

A total of 10 sediment samples were collected from 9 sites along the Tisza River on the territory of Serbia (Fig. 1; Table I). These sites are located near Kanjiža Town (entrance area of the River from Hungary to Serbia) up to its confluence into the Danube. The length of this river section is 153 km and it covers almost the entire length of the Tisza River through the territory of Serbia. The sediments consist of sand, silt and clay. Silt is predominant, with the exception of two samples (M22 and M9), in which sand is the most prominent (Table I).



Fig. 1. Map showing the sampling points.

TABLE I. List of the investigated samples

Sample	Distance from confluence into the Danube River, km	Depth interval cm	Lithology		
			Sand, %	Silt, %	Clay, %
M18	153	20	25	52	23
M21	136	0–10	29	50	21
M22	136	0–10	47	41	12
M25	123	0–10	20	55	25
M9	88	20	54	33	13
M7	60	20	28	53	19
M8	50	20	28	52	20
M6	12	0–5	3	65	32
M4	8	0–10	16	57	27
M5	6	20	36	41	23

Samples from the sediment top layer (0–20 cm) were collected using a plastic corer and transported to laboratory in polyethylene bags. The sediment samples were wet-sieved through a 63  $\mu\text{m}$  sieve and then air dried at room temperature. The dried samples were gently pounded in a porcelain mortar. Subsequently, the samples were split into smaller aliquots by coning and quartering.

Elemental analysis was performed to determine the contents of total organic carbon ( $C_{\text{org}}$ ), sulphur and nitrogen. The total organic carbon content was determined after removal of carbonates with 1:3 diluted hydrochloric acid. The measurements were realized using a Vario EL III, CHNS/O elemental analyzer, Elementar Analysensysteme GmbH.

Soluble organic matter (bitumen) was extracted (36 h) from pulverized samples using a Soxhlet apparatus with an azeotropic mixture of  $\text{CH}_2\text{Cl}_2$  and  $\text{CH}_3\text{OH}$  and quantified.

The bitumen extracts and crude oils were separated into saturated, aromatic and NSO fractions using column chromatography over silica gel. The saturated hydrocarbon fraction was eluted with *n*-hexane, aromatic hydrocarbons with mixture of *n*-hexane and dichloromethane (4:1 volume ratio) and the NSO fractions (polar fraction, which contains nitrogen, sulphur, and oxygen compounds) with mixture of dichloromethane and methanol (1:1 volume ratio).

The saturated hydrocarbons were analyzed by gas chromatography–mass spectrometry (GC–MS). A gas chromatograph Agilent 7890A GC (H5-MS capillary column, 30 m $\times$ 0.25 mm, He carrier gas 1.5 cm<sup>3</sup> min<sup>-1</sup>, FID) coupled to an Agilent 5975C mass selective detector (70 eV) was used. The column was heated from 80 to 310 °C, at a rate of 2 °C min<sup>-1</sup>, and the final temperature of 310 °C was maintained for an additional 25 min. Detailed analyses of the target compounds were conducted using the following ion chromatograms:  $m/z = 71$  (*n*-alkanes and isoprenoids),  $m/z = 191$  (terpanes) and  $m/z = 217$  (steranes). The individual peaks were identified by comparison with literature data<sup>12</sup> and based on the total mass spectra (library: NIST5a). The biomarker parameters were calculated from the GC–MS chromatogram peak areas (software GCMS Data Analysis).

## RESULTS AND DISCUSSION

### *Group organic–geochemical parameters*

The amount of the total organic carbon ( $C_{\text{org}}$ ) and soluble organic matter (bitumen) in the surface sediments of the Tisa River, from the most upstream analyzed point near Kanjiža Town down to its confluence into the Danube River are given in Table II.

The results indicate that in the relatively long part of the river flow (153 km), the contents of the total organic carbon ( $C_{\text{org}}$  0.85–1.66 %) and soluble organic matter (bitumen 0.07–0.14 %) in the surface sediments remained approximately uniform. Based on the predefined quantities, it could not be assessed whether it was singenetic organic matter, or polluting matter of anthropogenic origin. The soluble organic matter was mainly represented by polar, NSO compounds (87–93 %). The relative contents of saturated and aromatic hydrocarbons were low, which is in accordance with the low maturity of the surface sedimentary organic matter (Table II). The contents of nitrogen and sulphur were also relatively uniform. The content of sulphur did not exceed 0.1 %, indicating that pollution with sulphur compounds could be discounted.

TABLE II. Group organic–geochemical parameters; C<sub>org</sub> – total organic carbon content; db – dry basis; HC – hydrocarbons; NSO – polar compounds containing nitrogen, sulphur and oxygen

Sample	Content of						
	C <sub>org</sub> , % db	N, % db	S, % db	Bitumen, %	Saturated HC, %	Aromatic HC, %	NSO, %
M18	1.23	0.10	<0.1	0.08	5.9	5.1	89.0
M21	0.85	0.07	<0.1	0.07	5.3	2.7	92.0
M22	0.96	0.07	<0.1	0.14	6.6	7.6	85.8
M25	1.19	0.10	0.1	0.09	4.0	2.9	93.1
M9	1.3	0.11	<0.1	0.11	6.9	1.7	91.4
M7	1.18	0.09	<0.1	0.11	6.1	3.4	90.5
M8	1.13	0.08	<0.1	0.07	6.5	6.2	87.3
M6	1.29	0.10	<0.1	0.11	6.9	3.4	89.7
M4	1.66	0.13	<0.1	0.08	3.3	3.7	93.0
M5	1.36	0.09	<0.1	0.09	3.6	4.6	91.8

#### *Molecular composition of the organic matter*

*n*-Alkanes and isoprenoids. All samples showed very similar distributions of *n*-alkanes and isoprenoids. The *n*-alkanes were identified in the range C<sub>16</sub> to C<sub>35</sub>, with a maximum at *n*-C<sub>27</sub> or *n*-C<sub>29</sub> (Fig. 2).

The mass chromatograms (*m/z* 71) of the saturated fraction showed two distinct parts. The lower *n*-alkanes, C<sub>16</sub>–C<sub>22</sub>, showed a uniform distribution of odd and even homologues, resulting in *CPI* (C<sub>16</sub>–C<sub>22</sub>) values lower than 1.40 (Table III). The *n*-alkane patterns in range C<sub>23</sub>–C<sub>35</sub> were characterized by a marked odd over even predominance, with a maximum at *n*-C<sub>27</sub> or *n*-C<sub>29</sub>, which resulted in high *CPI* values for full distribution of *n*-alkanes (C<sub>16</sub>–C<sub>35</sub>) and high *CPI* values in the range C<sub>23</sub>–C<sub>35</sub> (Table III).

Based on these results, an explicit conclusion about the origin of the organic matter in the analyzed sediments could not be drawn. However, the *n*-alkane maximum at *n*-C<sub>27</sub> or *n*-C<sub>29</sub>, along with a sharp predominance of odd homologues in the range of C<sub>23</sub>–C<sub>35</sub> *n*-alkanes imply that the surface sediments of the Tisza River contain singenetic organic matter of terrestrial origin.<sup>13</sup> The uniform distribution of lower *n*-alkanes, C<sub>16</sub>–C<sub>22</sub>, in all samples could be considered as an indicator of the anthropogenic oil type pollutants.<sup>8,14</sup> However, such a distribution of lower *n*-alkanes could be attributed to algal singenetic organic matter as well.<sup>1,5</sup>

The pristane (Pr) to phytane (Ph) ratio is widely used as an indicator for the redox settings of the depositional environment.<sup>1,15</sup> The relatively uniform values of this parameter that were lower than 1 (with exception of sample M25; Table III) indicate that organic matter of all sediments was deposited under uniform, slightly reducing conditions. The concentrations of Pr and Ph were slightly lower than the concentrations of *n*-alkanes C<sub>17</sub> and C<sub>18</sub> (parameters Pr/*n*-C<sub>17</sub> and Ph/*n*-C<sub>18</sub>; Table III). However, this result is not useful, because it could be related to both singenetic organic matter and oil pollutants.<sup>14,16</sup>

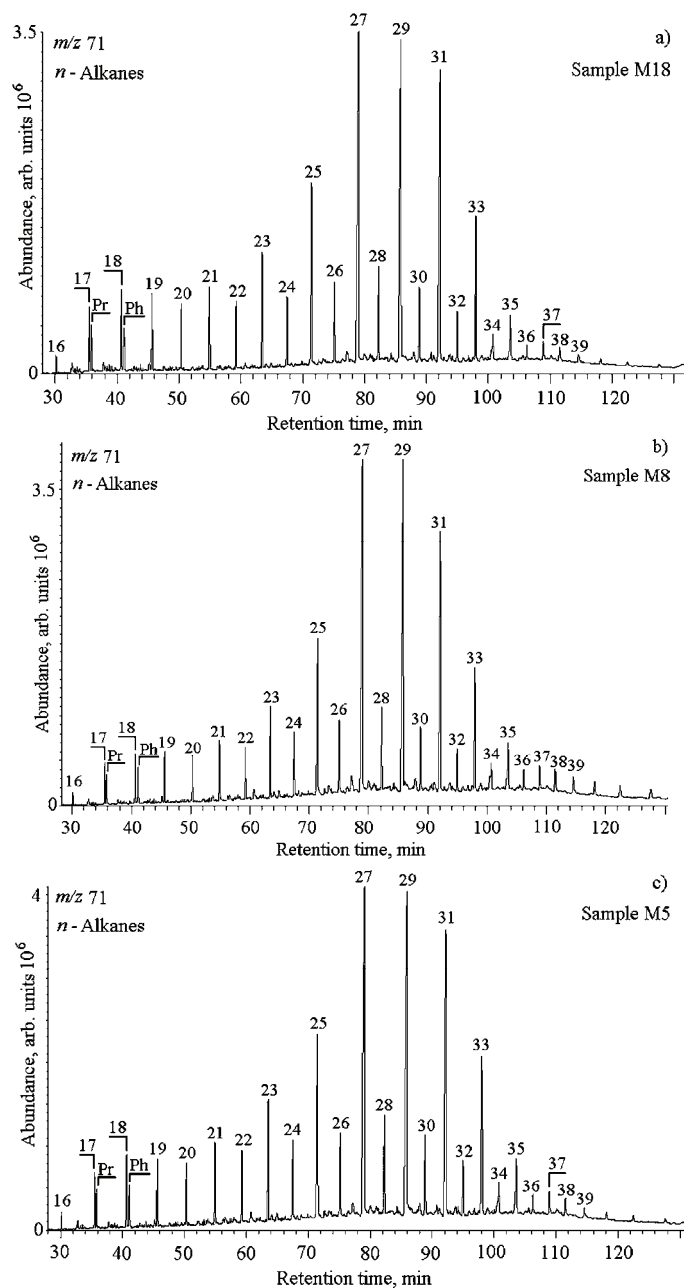


Fig. 2. GC–MS chromatograms of *n*-alkanes and isoprenoids,  $m/z$  71 of the saturated fraction isolated from the most upstream sample (a), a sample from the middle part of the analyzed river flow (b) and from the location close to the confluence into the Danube River (c). Peak assignments: *n*-alkanes are labelled according to their carbon number; Pr – pristane; Ph – phytane.

TABLE III. Parameters calculated from the distributions and abundances of *n*-alkanes and isoprenoids

Sample	<i>n</i> -Alkane range	<i>n</i> -Alkane maximum	<i>CPI</i> (C <sub>16</sub> –C <sub>35</sub> ) <sup>a</sup>	<i>CPI</i> (C <sub>16</sub> –C <sub>22</sub> ) <sup>b</sup>	<i>CPI</i> (C <sub>23</sub> –C <sub>35</sub> ) <sup>c</sup>	Pr/Ph <sup>d</sup>	Pr <i>n</i> -C <sub>17</sub>	Ph <i>n</i> -C <sub>18</sub>
M18	C <sub>16</sub> –C <sub>35</sub>	C <sub>27</sub> ; C <sub>29</sub>	3.40	1.25	5.64	0.95	0.90	0.71
M21	C <sub>16</sub> –C <sub>35</sub>	C <sub>27</sub> ; C <sub>29</sub>	4.00	1.26	5.10	0.83	0.70	0.64
M22	C <sub>16</sub> –C <sub>35</sub>	C <sub>27</sub> ; C <sub>29</sub>	3.98	1.32	5.14	0.88	0.63	0.69
M25	C <sub>16</sub> –C <sub>35</sub>	C <sub>27</sub> ; C <sub>29</sub>	4.77	1.36	6.18	1.12	0.81	0.78
M9	C <sub>16</sub> –C <sub>35</sub>	C <sub>27</sub> ; C <sub>29</sub>	5.07	1.27	6.01	0.75	0.78	0.70
M7	C <sub>16</sub> –C <sub>35</sub>	C <sub>27</sub> ; C <sub>29</sub>	5.17	1.32	6.24	0.90	0.97	0.83
M8	C <sub>16</sub> –C <sub>35</sub>	C <sub>27</sub> ; C <sub>29</sub>	4.39	1.26	5.24	0.76	1.00	0.94
M6	C <sub>16</sub> –C <sub>35</sub>	C <sub>27</sub> ; C <sub>29</sub>	5.50	1.32	6.66	0.89	0.96	0.89
M4	C <sub>16</sub> –C <sub>35</sub>	C <sub>27</sub> ; C <sub>29</sub>	4.68	1.13	5.28	0.54	0.65	0.50
M5	C <sub>16</sub> –C <sub>35</sub>	C <sub>27</sub> ; C <sub>29</sub>	4.39	1.23	5.38	0.82	0.93	0.77

<sup>a</sup>Carbon Preference Index (*CPI*) determined for the full distribution of *n*-alkanes C<sub>16</sub>–C<sub>35</sub> (mass chromatogram *m/z* 71),  $CPI(C_{16}-C_{35}) = 1/2 [\Sigma_{\text{odd}}(n-C_{17}-n-C_{35}) / \Sigma_{\text{even}}(n-C_{16}-n-C_{34}) + \Sigma_{\text{odd}}(n-C_{17}-n-C_{35}) / \Sigma_{\text{even}}(n-C_{18}-n-C_{36})]$ ; <sup>b</sup>*CPI* determined for the distribution of *n*-alkanes C<sub>16</sub>–C<sub>22</sub> (mass chromatogram *m/z* 71),  $CPI(C_{16}-C_{22}) = 1/2 [\Sigma_{\text{odd}}(n-C_{17}-n-C_{21}) / \Sigma_{\text{even}}(n-C_{16}-n-C_{20}) + \Sigma_{\text{odd}}(n-C_{17}-n-C_{21}) / \Sigma_{\text{even}}(n-C_{18}-n-C_{22})]$ ; <sup>c</sup>*CPI* determined for distribution of *n*-alkanes C<sub>23</sub>–C<sub>35</sub> (mass chromatogram *m/z* 71),  $CPI(C_{23}-C_{35}) = 1/2 [\Sigma_{\text{odd}}(n-C_{23}-n-C_{35}) / \Sigma_{\text{even}}(n-C_{22}-n-C_{34}) + \Sigma_{\text{odd}}(n-C_{23}-n-C_{35}) / \Sigma_{\text{even}}(n-C_{24}-n-C_{36})]$ ; <sup>d</sup>Pr/Ph = pristane/phytane

*Tricyclic and pentacyclic terpanes.* The distribution of tricyclic diterpanes and pentacyclic triterpanes (hopanes) in the saturated fractions of the sediments (ion chromatograms *m/z* = 191) for the most upstream sample, a sample from the middle part of the analyzed river flow and a sample from the part that is close to the confluence into the Danube river are shown in Fig. 3. The values of specific organic–geochemical parameters, calculated from the distributions of these biomarkers are given in Tables IV and V.

All the samples had almost identical distributions of tricyclic and pentacyclic terpanes (Fig. 3). The values of the terpane source parameters indicate the same origin of the organic matter in the sediments (Table IV). The terpane distributions in all samples were typical for oils. They were characterized by a predominance of the thermodynamically more stable isomers with 17 $\alpha$ (H)21 $\beta$ (H) and 22(S) configurations, as well as by the presence of typical geo-isomers, 18 $\alpha$ (H)-neohopanes (Fig. 3). These compounds have never been reported in biosphere and recent singenetic organic matter. The presence of singenetic immature organic matter in the sediments was confirmed by the identification of isomers with the biogenic 17 $\beta$ (H)21 $\beta$ (H) configuration in all samples (Fig. 3). The values of terpane maturity ratio (Table V) are in the range typical for crude oils.<sup>1</sup>

Based on distributions of terpanes in the saturated fractions of the sediments and the values of the corresponding maturity parameters, it could be concluded that, in addition to singenetic organic substance, the analyzed samples contained organic matter of anthropogenic origin, which could be related to crude oil. Furthermore, the identical distributions of these biological markers in all samples

represent strong evidence that the sediments of the Tisza River, in its flow from Kanjiža town to the confluence into the Danube River, contain the same type of petroleum pollutants.

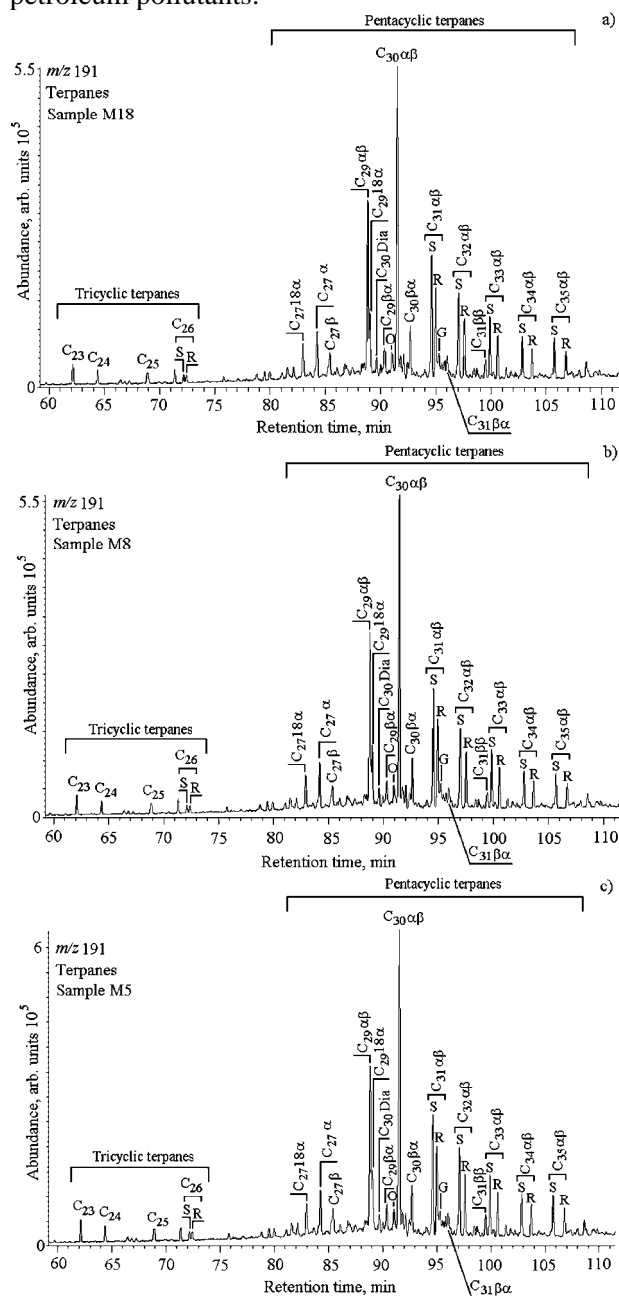


Fig. 3. GC-MS chromatograms of  $C_{23}$ – $C_{26}$  tricyclic terpanes and  $C_{27}$ ,  $C_{29}$ – $C_{35}$  pentacyclic terpanes,  $m/z$  191 of the saturated fraction isolated from the most upstream sample (a), a sample from the middle part of the analyzed river flow (b) and from the location close to the confluence into the Danube river (c). Peak assignments:  $\beta\beta$ ,  $\beta\alpha$  and  $\alpha\beta$  designate the configurations at  $C_{17}$  and  $C_{21}$  in the pentacyclic terpanes; (S) and (R) designate configuration at  $C_{22}$  in the tricyclic and pentacyclic terpanes; Dia – diahopane; O – oleanane; G – gammacerane.



TABLE IV. Source parameters calculated from distributions and abundances of tricyclic and pentacyclic terpanes; *OI* – oleanane index = oleanane×100/(oleanane+C<sub>30</sub>17α(H)21β(H)-hopane); *GI* – gammacerane index = gammacerane×100/(gammacerane+C<sub>30</sub>17α(H)21β(H)-hopane)

Sample	<i>OI</i>	<i>GI</i>	C <sub>26</sub> tricyclic terpane / C <sub>25</sub> tricyclic terpane	C <sub>29</sub> β/C <sub>30</sub> αβ <sup>a</sup>
M18	12.73	10.36	0.88	0.59
M21	13.91	9.68	0.97	0.60
M22	12.15	10.48	0.89	0.59
M25	12.56	9.07	0.95	0.60
M9	12.07	10.20	0.95	0.61
M7	11.63	10.99	0.88	0.60
M8	12.34	10.82	0.95	0.60
M6	13.45	10.29	0.99	0.60
M4	13.15	11.24	0.94	0.61
M5	13.66	10.41	0.85	0.60
Rusanda crude oil	12.59	9.68	0.85	0.51
Elemir crude oil	12.39	10.34	0.74	0.47

<sup>a</sup>C<sub>29</sub>17α(H)21β(H)-30-norhopane/C<sub>30</sub>17α(H)21β(H)-hopane; oleanane, gammacerane, tricyclic terpanes and pentacyclic terpanes (hopanes) were quantified from the mass chromatogram *m/z* 191

TABLE V. Maturity parameters calculated from the distributions and abundances of pentacyclic terpanes

Sample	C <sub>31</sub> αβ(S)/C <sub>31</sub> αβ(S+R) <sup>a</sup>	C <sub>30</sub> β/C <sub>30</sub> αβ <sup>b</sup>	C <sub>29</sub> Ts/C <sub>29</sub> αβ <sup>c</sup>	Ts/(Ts+Tm) <sup>d</sup>
M18	0.57	0.16	0.35	0.44
M21	0.56	0.17	0.34	0.40
M22	0.57	0.15	0.35	0.43
M25	0.56	0.16	0.34	0.44
M9	0.56	0.16	0.33	0.40
M7	0.57	0.16	0.34	0.41
M8	0.57	0.16	0.35	0.45
M6	0.56	0.16	0.35	0.41
M4	0.56	0.16	0.34	0.42
M5	0.56	0.16	0.39	0.46
Rusanda crude oil	0.60	0.11	0.27	0.44
Elemir crude oil	0.56	0.11	0.33	0.42

<sup>a</sup>C<sub>31</sub>17α(H)21β(H)22(S)-hopane/C<sub>31</sub>17α(H)21β(H)22(S+R)-hopanes; <sup>b</sup>C<sub>30</sub>17β(H)21α(H)-hopane/C<sub>30</sub>17α(H)-21β(H)-hopane; <sup>c</sup>C<sub>29</sub>18α(H)-30-norneohopane/C<sub>29</sub>17α(H)21β(H)-30-norhopane; <sup>d</sup>C<sub>27</sub>18α(H)-22,29,30-trisnorneohopane/(C<sub>27</sub>18α(H)-22,29,30-trisnorneohopane+C<sub>27</sub>17α(H)-22,29,30-trisnorhopane)

*Steranes and diasteranes.* The sterane biomarkers in all samples had almost identical distributions (Fig. 4). The uniform distributions of the C<sub>27</sub>–C<sub>29</sub> 14α(H)17α(H)20(R) regular steranes<sup>17</sup> (Table VI) in all samples confirmed that the organic matter in the sediments was of the same genetic type.

The sterane distributions in all samples were typical for oils, which corroborates the presence of oil-type pollutants in the sediments of the Tisza River. Apart from the regular steranes with a biogenic 14α(H)17α(H)20(R)-configur-

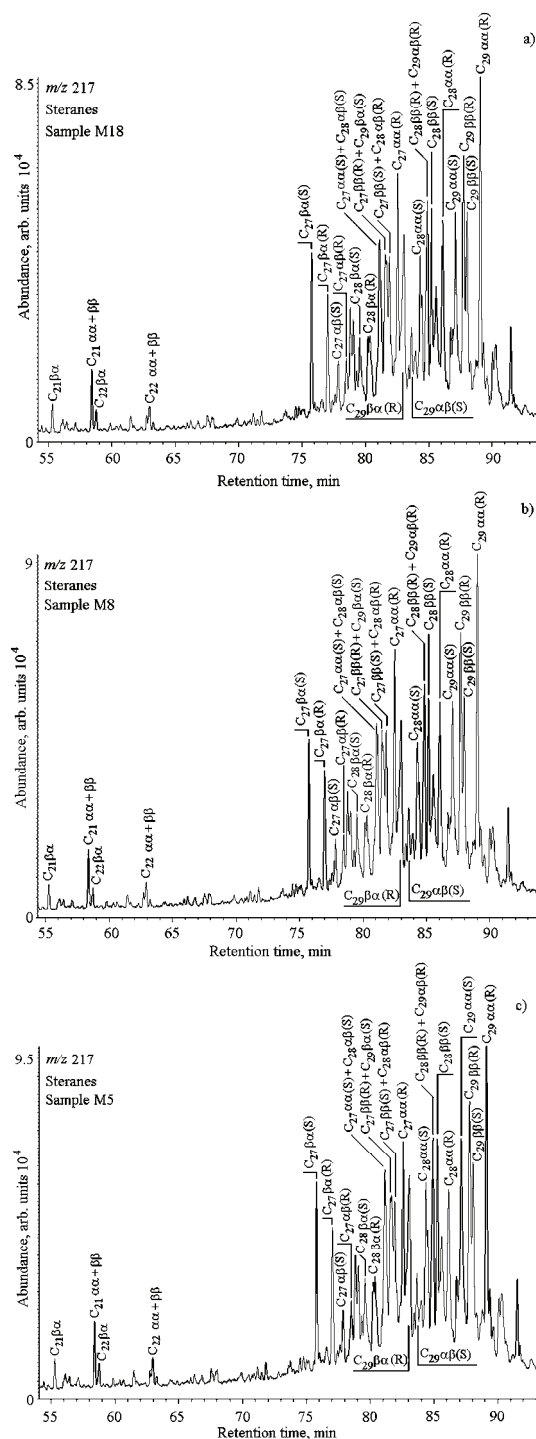


Fig. 4. GC-MS chromatograms of  $C_{21}$ – $C_{22}$  and  $C_{27}$ – $C_{29}$  steranes and diasteranes,  $m/z$  217 of the saturated fraction isolated from the most upstream sample (a), a sample from the middle part of the analyzed river flow (b) and from the location close to the confluence into the Danube river (c). Peak assignments:  $\alpha\alpha$  and  $\beta\beta$  designate the  $14\alpha(H)17\alpha(H)$  and  $14\beta(H)17\beta(H)$  configurations in the steranes;  $\alpha\beta$  and  $\beta\alpha$  designate the  $13\alpha(H)17\beta(H)$  and  $13\beta(H)17\alpha(H)$  configurations in the diasteranes; (S) and (R) designate configuration at  $C_{20}$  in both the steranes and diasteranes.

TABLE VI. Source and maturity parameters calculated from the distributions and abundances of steranes and diasteranes

Sample	% C <sub>27</sub> <sup>a</sup>	% C <sub>28</sub> <sup>b</sup>	% C <sub>29</sub> <sup>c</sup>	C <sub>29</sub> αα(S)/ C <sub>29</sub> αα(S+R) <sup>d</sup>	C <sub>29</sub> ββ(R)/ C <sub>29</sub> (ββ(R)+αα(R)) <sup>e</sup>	C <sub>27</sub> βα(S)/ C <sub>27</sub> (βα(S)+αα(R)) <sup>f</sup>
M18	33.2	29.4	37.4	0.49	0.49	0.30
M21	27.4	32.8	39.8	0.48	0.46	0.31
M22	31.8	30.9	37.3	0.49	0.50	0.29
M25	29.3	32.4	38.3	0.48	0.47	0.31
M9	32.2	31.0	36.8	0.52	0.49	0.28
M7	33.1	30.7	36.2	0.51	0.51	0.29
M8	28.6	31.6	39.8	0.48	0.48	0.34
M6	29.3	31.4	39.3	0.48	0.49	0.34
M4	27.5	33.0	39.5	0.51	0.48	0.32
M5	27.6	35.4	37.0	0.55	0.52	0.37
Rusanda crude oil	28.1	35.6	36.3	0.62	0.64	0.32
Elemir crude oil	27.0	36.8	36.2	0.57	0.59	0.22

<sup>a</sup>100×C<sub>27</sub>14α(H)17α(H)20(R)-sterane/Σ(C<sub>27</sub>–C<sub>29</sub>)14α(H)17α(H)20(R)-steranes; <sup>b</sup>100×C<sub>28</sub>14α(H)17α(H)20(R)-sterane/Σ(C<sub>27</sub>–C<sub>29</sub>)14α(H)17α(H)20(R)-steranes; <sup>c</sup>% C<sub>29</sub> = 100×C<sub>29</sub>14α(H)17α(H)20(R)-sterane/Σ(C<sub>27</sub>–C<sub>29</sub>)14α(H)17α(H)20(R)-steranes; <sup>d</sup>C<sub>29</sub>14α(H)17α(H)20(S)-sterane/C<sub>29</sub>14α(H)17α(H)20(S+R)-steranes; <sup>e</sup>C<sub>29</sub>14β(H)17β(H)20(R)-sterane/(C<sub>29</sub>14β(H)17β(H)20(R)-sterane + C<sub>29</sub>14α(H)17α(H)20(R)-sterane); <sup>f</sup>C<sub>27</sub>13β(H)17α(H)20(S)-diasterane/(C<sub>27</sub>13β(H)17α(H)20(S)-diasterane + C<sub>27</sub>14α(H)17α(H)20(R)-sterane); the steranes and diasteranes were quantified from the mass chromatogram *m/z* 217

ation, the short chain, C<sub>21</sub>–C<sub>22</sub>, and C<sub>27</sub>–C<sub>29</sub> isomers with the thermodynamically more stable 14α(H)17α(H)20(S)-, 14β(H)17β(H)20(R)-, and 14β(H)17β(H)-20(S)-configurations, as well as the typical geo-isomers, 13β(H)17α(H)- and 13α(H)17β(H)-diasteranes, were present (Fig. 4). The values of the sterane maturity ratios are in a range typical for crude oils (Table VI).<sup>1</sup> The slightly lower values of these parameters than in crude oils could be attributed to the influence of singenetic immature terrestrial organic matter (expressed through an elevated concentration of C<sub>29</sub>14α(H)17α(H)20(R)-sterane), the presence of which was confirmed by the distribution of the long-chain *n*-alkane homologues and identification of pentacyclic hopanes with the biogenic 17β(H)21β(H) configuration.

Therefore, distributions of sterane biomarkers clearly prove that in addition to the singenetic immature terrestrial organic matter, the analyzed samples also contain oil pollutants of anthropogenic origin. Moreover, the identical compositions of the steranes in all samples confirmed that the sediments of the Tisza River from Kanjiža Town to the confluence into the Danube River, along a distance of 153 km, contained the same type of oil pollutant.

#### Assessment of the oil pollutant source

Based on previous discussion, it was established that sediments of the Tisza River, in addition to some singenetic immature terrestrial organic matter, con-

tains an oil type pollutant. Considering navigable character of the Tisza River, the observed continuous oil pollution most probably resulted from persistent and extensive transport *via* river barges, which commonly use heavy fuel oil.

As mentioned above, the crude oils represent mature organic matter and are characterized by specific distributions of steranes and terpanes with a predominance of the thermodynamically more stable isomers, which are not found in biosphere and recent singenetic sedimentary organic matter. Consequently, most values of the maturity biomarker of the crude oils parameters, which represent the ratios of more and less stable isomers, vary in a relatively narrow range.<sup>1</sup> On the other hand, each crude oil sample has a characteristic distribution of biomarkers as a kind of “fingerprint”, which in addition to maturity, depends on the origin, depositional environment, age, relative length of migration and the mineral composition of the source and reservoir rocks. Therefore, crude oils can significantly differ in values of source parameters, such as distribution of C<sub>27</sub>–C<sub>29</sub> 14 $\alpha$ (H)17 $\alpha$ (H)20(R) regular steranes<sup>17</sup>, gammacerane and oleanane indices,<sup>18–20</sup> ratio of C<sub>26</sub> to C<sub>25</sub> tricyclic terpanes<sup>1</sup> and C<sub>29</sub>17 $\alpha$ (H)21 $\beta$ (H) to C<sub>30</sub>17 $\alpha$ (H)21 $\beta$ (H) hopane.<sup>1</sup> Comparison of the distributions of terpanes and steranes and the values of the corresponding biomarker parameters in the extracts of sediments of the Tisza River and previously investigated crude oils from oil deposits in the Serbian part of Pannonian Basin showed great similarity between extracts of investigated sediments and crude oils from Rusanda (well 5; depth interval 2665–2675 m) and Elemir (well 19; depth interval 1640–1644 m) oil fields (Figs. 3–8; Tables IV and V), which are located close to the investigated area

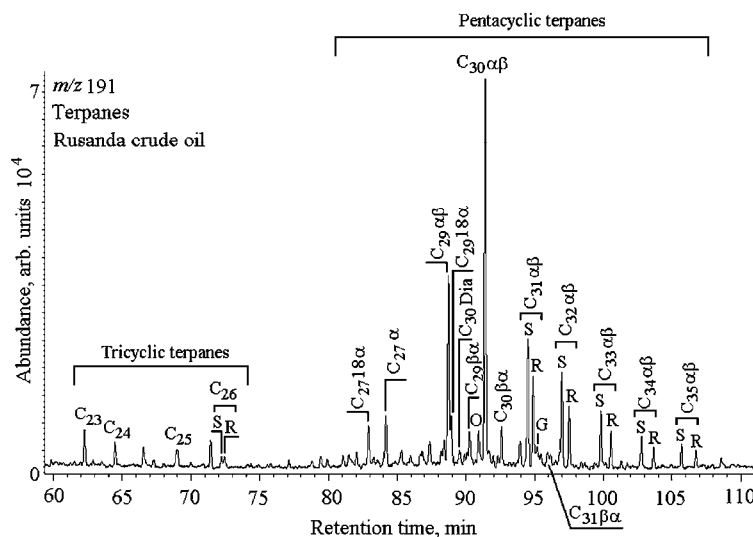


Fig. 5. GC–MS chromatograms of terpanes  $m/z$  191 of the saturated fraction isolated from Rusanda crude oils. For peak assignments, see the legend to Fig. 3.

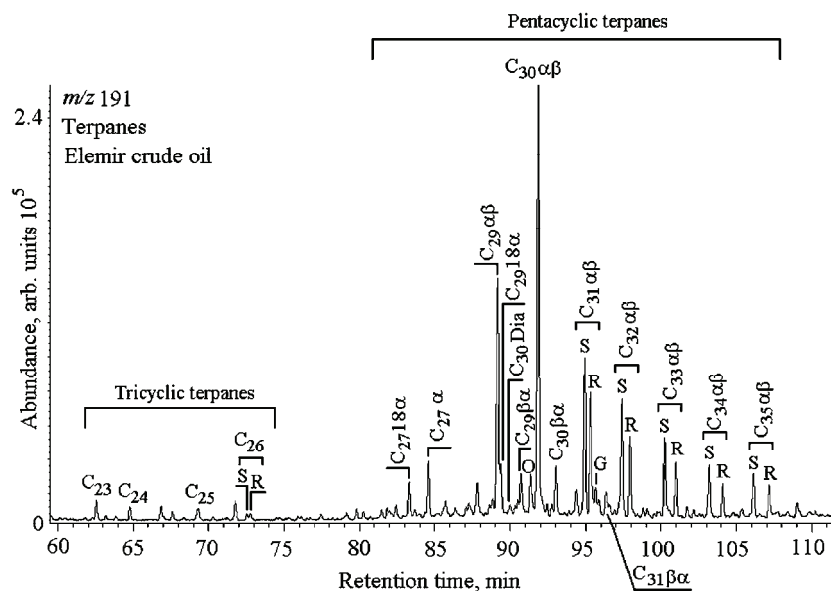


Fig. 6. GC-MS chromatograms of terpanes  $m/z$  191 of the saturated fraction isolated from Elemir crude oils. For peak assignments, see the legend to Fig. 3.

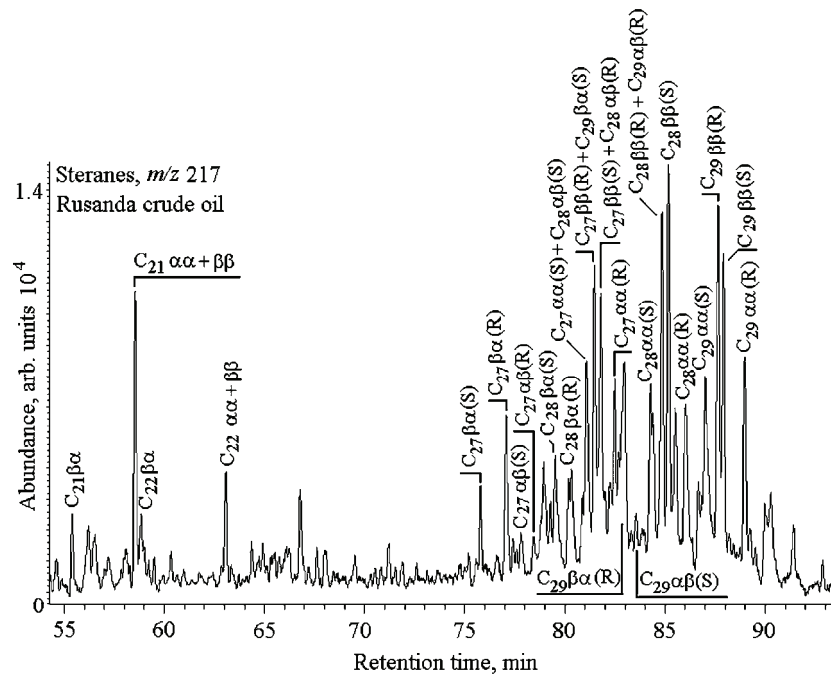


Fig. 7. GC-MS chromatograms of steranes  $m/z$  217 of the saturated fraction isolated from Rusanda crude oils. For peak assignments, see the legend to Fig. 4.

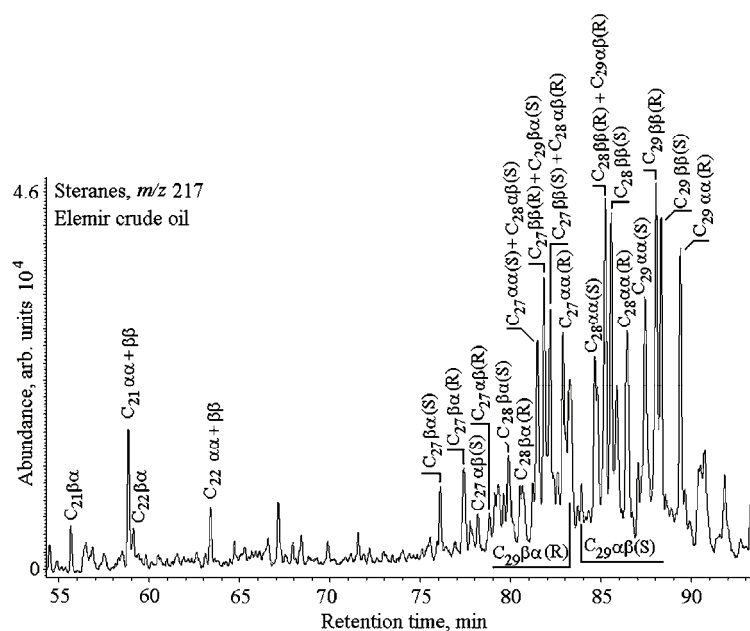


Fig. 8. GC-MS chromatograms of steranes,  $m/z$  217 of the saturated fraction isolated from Elemir crude oils. For peak assignments, see the legend to Fig. 4.

(Fig. 1). Therefore, the oil contamination observed in the Tisza sediments, apart from heavy fuel oil from barges, to a lower extent can probably be related to the Rusanda and Elemir crude oils. According to the geological setting of the terrain, there is no possibility of natural migration of oils from the oil fields to the Tisza River surface sediments. Therefore it could be assumed that observed oil pollution resulted from oil shipment at the station located on the river bank and further transport by barges.

#### CONCLUSIONS

The origin and type of organic matter of the Tisza sediments along its 153 km distance through the territory of Serbia were evaluated using group organic-geochemical data and the biomarker compositions.

The contents of the total organic carbon and soluble organic matter were similar in all samples. The organic matter was deposited under slightly reducing conditions. All the samples had almost identical distributions of *n*-alkanes, steranes, and tricyclic and pentacyclic terpanes, indicating the same origin of the organic matter.

Based on distribution of *n*-alkanes, the origin and type of organic matter could not be precisely estimated. However, the *n*-alkane maximum at *n*-C<sub>27</sub> or *n*-C<sub>29</sub> and the marked odd over even predominance in the range of *n*-C<sub>23</sub>-*n*-C<sub>35</sub> suggested the presence of immature singenetic organic matter of terrestrial origin.

Typical oil distributions of terpanes and steranes along with values of the corresponding maturity parameters clearly indicated that the sediments of the Tisza River, in addition to immature singenetic organic matter, contained oil pollutants of anthropogenic origin. The identical distributions of these biomarkers in all samples represent strong evidence that the recent sediments of the Tisza River, in its flow from Kanjiža Town to its confluence into the Danube River contained the same type of oil pollutants.

Based on the compositions of terpanes and steranes and the values of the biomarker parameters in the Tisza sediments, it is supposed that oil pollution generally could be related to heavy fuel oil from barges, due to intense river transport. Comparison of the distributions of terpanes and steranes and the values of corresponding biomarker parameters in the extracts of sediments of the Tisza River and previously investigated crude oils from oil deposits in the Serbian part of Pannonian Basin showed that the oil contaminants observed in Tisza sediments, apart from heavy fuel oil from tankers, to a lower extent, could be attributed to Rusanda and Elemir crude oils.

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## ИЗВОД

## ПРИМЕНА БИОЛОШКИХ МАРКЕРА У ОДРЕЂИВАЊУ ПОРЕКЛА И ТИПА ОРГАНСКЕ СУПСТАНЦЕ У СЕДИМЕТИМА РЕКЕ ТИСЕ

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Циљ рада је био да се утврди порекло и тип органске супстанце у рецентним седиментима реке Тисе, у делу тока који се налази на територији Србије, укупне дужине 153 километра. У ту сврху коришћени су групни органско–геохемијски параметри и састав биомаркера. Сви узорци садрже приближно исту количину органске супстанце таложене у благо редукционој средини. На основу расподеле *n*-алкана није било могуће прецизно утврдити порекло и тип органске супстанце. Међутим, *n*-алканске расподеле указале су на присуство незреле нативне органске супстанце сувоземног порекла. Расподеле терпана и стерана и вредности одговарајућих матурационих параметара указују да седименти реке Тисе, поред незреле нативне органске супстанце садрже нафтну загађујућу супстанцу антропогеног порекла. Идентичне расподеле ових биомаркера у свим узорцима потврђују да рецентни седименти реке Тисе, у делу тока од места Кањижа до ушћа у реку Дунав, садрже исти тип нафтног загађивача. На основу расподела терпана и стерана и вредности биомаркерских параметара претпостављено је да нафтно загађење

генерално потиче од мазута из танкера, услед интензивног речног транспорта и, у мањој мери, од сирових нафти из нафтних поља Елемир и Русанда.

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