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REVIEW

**The use of biological markers in organic geochemical investigations of the origin and geological history of crude oils (I) and in the assessment of oil pollution of rivers and river sediments of Serbia (II)**

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**Abstract:** Biological markers (BMs) are organic compounds in oils in which a precursor is known, and during the transformation of organic matter these compounds undergo certain structural and stereochemical changes. Based on the established precursors of BMs, the origin of the examined oils can be estimated, and based on the intensity and the type of changes and also geological history. It includes defining the deposition medium, the degree of maturation, the length of the oil migration path, the degree of biodegradation. The most studied and applied BMs are normal alkanes, isoprenoid aliphatic alkanes pristane and phytane, and polycyclic alkanes of the sterane and terpane type. On the other hand, in the environmental chemistry, these compounds can significantly contribute to the identification of petroleum pollutants, as well as to the assessment of the migration mechanism and the intensity of biodegradation. This review paper first presents the results related to the application of BMs in the organic geochemical correlations of oil in the south-eastern part of the Pannonian Basin (I). The second part provides an overview of those researches in which the same BMs were used in the identification of oil pollutants and in monitoring their changes during the migration and the biodegradation in rivers and river sediments of Serbia (II).

**Keywords:** organic matter; biomarkers; geochemical correlation; petroleum pollutants; biodegradation.

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## INTRODUCTION

The crude oil is a product of geochemical transformations of biogenic organic matter in the sedimentary rocks of the lithosphere. Geochemical processes have been taking place over the past 600 million years, since the Cambrian period until the present days. During these processes, the organic matter passes through four consecutive alteration stages, that are in organic geochemistry called diagenesis, catagenesis, metagenesis and metamorphism.<sup>1-7</sup>

After they accumulate in sedimentary rocks, the organic compounds from the biosphere undergo both structural and configurational changes in order to create thermodynamically more stable forms. During the early diagenesis, these changes are primarily governed by microorganisms. During the late diagenesis, catagenesis, and in the final stages methagenesis and metamorphism, heat, the pressure and the aluminosilicate minerals acting as catalysts, have a dominant role in the transformations of organic compounds. The most important factors in these changes are temperature and geologic time, which is measured in millions of years.<sup>1-6</sup>

In addition to graphite and gas, the final product of these changes in the sedimentary organic matter is crude oil. As a result of the aforementioned processes, crude oil consists mainly of thermodynamically stable compounds that usually differ significantly from their biological precursors.<sup>1-7</sup>

The first compounds of biogenic origin identified in crude oil were porphyrins.<sup>8</sup> That was in 1931. This discovery still has a great and historical significance. The identification of porphyrins in crude oil marked the the beginning of an era of biogenesis or biogenetic origin of petroleum. During the seventies and the eighties of the twentieth century, a large number of compounds were discovered in crude oils and their biological precursors were precisely determined.<sup>9-13</sup>

In organic geochemistry, these compounds are called molecular fossils, biological markers, or biomarkers.<sup>1-13</sup> So far, a large number of biomarkers in crude

oils have been identified. Even nowadays, identification of a new biomarker in oil, or its geochemically related form, represents a research challenge. The most studied biomarkers are *n*-alkanes, isoprenoid aliphatic alkanes (first of all C19, pristane and C20, phytane) and polycyclic alkanes of the terpane type (tricyclic and tetracyclic diterpanes and pentacyclic triterpanes) and the sterane type (including diasteranes and mono- and triaromatic steroids).<sup>1-7</sup> Today, it is possible to reliably determine the origin, *i.e.*, the precursor organic matter type of an individual oil. On the other hand, interpretation of the transformation pathways of these compounds from the unstable biolipid isomers to thermodynamically stable geolipid structural and configurational forms, allows us to examine the geologic history of a crude oil which includes the estimation of the characteristics of the depositional environment, degree of thermal maturity, length of the migration pathway from a source to the reservoir rock, are the extent of microbiological degradation, *etc.*<sup>1-17</sup>

The largest number of biological markers in oil is found in very low quantities. Practically only *n*-alkanes and the isoprenoids pristane and phytane can be successfully identified “only” by gas chromatographic (GC) analysis of the iphatic fraction of oil. For the identification of all other biomarker compounds, the application of more complex and sophisticated techniques is required. These analytical techniques include primarily mass spectrometry (MS). Because of that, it can be said that the development of organic-chemical investigations of biological markers was developing parallel with the development of instrumental techniques in organic chemistry, primarily gas chromatography - mass spectrometric (GC-MS) and gas chromatography-mass spectrometry-mass spectrometric (GC-MS-MS) systems.<sup>18</sup>

The use of oil started its rapid growth at the beginning of the twentieth century with the invention of the internal combustion engine. Organic geochemistry gave crucial importance to the prospective oil exploration, especially in the second half of the twentieth century.<sup>1-6</sup> With geological and geophysical surveys, organic geochemical methods have become irreplaceable in finding new oil deposits. Among them, the methods involving biological markers are considered the most important.

These same compounds have a completely different role in the environmental chemistry. When oil, or some of its derivatives, comes in the contact with the environment, its role completely changes its character, because from a very important fossil fuel, without which it is almost impossible to imagine life nowadays, it transforms into a very dangerous organic pollutant. This can be understood because oil is a complex mixture of iphatic, aromatic, and many other nitrogen, sulfur, and oxygen organic compounds. Many of them are toxic, carcinogenic, mutagenic, and even teratogenic. The biological markers in ecochem-

ical tests are invaluable for the identification of oil pollutants and for assessing their aim in the environment.<sup>19</sup>

The analysis of biological markers, *n*-alkanes, isoprenoid aliphatic alkanes, terpanes, and steranes, in oil as a form of the organic substance of the geosphere, *i.e.*, as a fossil fuel, and in oil as a pollutant in the environment is performed in an almost identical way.<sup>18</sup> However, the interpretation of the obtained results in organic geochemistry and in environmental chemistry goes in completely different directions. This review paper will first present the results, *i.e.*, the research related to the application of biological markers in the organic geochemical correlations of oil in the southeastern part of the Pannonian Basin in the territory of Serbia (I). The second part will provide an overview of those researches in which the same biological markers were used in the identification of oil pollutants and in monitoring their changes during migration and biodegradation in rivers and river sediments of Serbia (II).

## 2. BIOLOGICAL MARKERS IN ORGANIC GEOCHEMICAL CORRELATIONS OF OIL IN THE SOUTHEASTERN PART OF THE PANNONIAN BASIN ON THE TERRITORY OF SERBIA

The first geochemical explorations of oil in the southeastern part of the Pannonian Basin are dated from the early 1970s.<sup>20</sup> Based on the results of analyses of trace elements and physicochemical properties, they are classified into three genetic types. According to their origin, the oil fields of the Kikinda and Kikinda Varoš form the first group. The Mokrin and Palić oil fields are classified in the second, and the Velebit oil field in the third genetic group.

In most oils that have not been exposed to the effect of biodegradation, *n*-alkanes and isoprenoid aliphatic alkanes, pristane and phytane are the dominant hydrocarbons in alkane fractions. Therefore, they are relatively easily identified by GC analysis of the alkanes previously isolated from the oil maltene fraction by the column chromatography using petroleum ether as eluent. For this reason, these biological markers were first applied in organic geochemical studies in the southeastern part of the Pannonian Basin (Serbia). The results for the crude oils of the Banat, South Bačka, and Kostolac Depressions were announced at the II Yugoslav Symposium on Hydrocarbons, within the Conference of the Serbian Chemical Society in 1986.<sup>21</sup>

A detailed correlation study of these oils later included polycyclic alkanes of the steranes and terpanes type for the first time. They were identified from carbamide (urea) non-adducts of iphatic fractions by GC–MS technique, with application of single ion monitoring (SIM) method, and using ions *m/z* 217 (for steranes) and *m/z* 191 (for terpanes). The results were announced at the organic geochemical conference in Venice in 1987,<sup>22</sup> and published in the journal *Organic Geochemistry*, at that time the most important journal in this scientific field.<sup>23</sup>

Based on the distributions of *n*-alkanes, isoprenoids pristane, and phytane,  $14\alpha(H)17\alpha(H)20(R)$   $C_{27}$ – $C_{29}$  steranes, tricyclic  $C_{19}$ – $C_{26}$  diterpanes, pentacyclic triterpanes of the hopane type, gammacerane and oleanane, 19 oil samples from the above mentioned Pannonian depressions basins (labeled A–D and E in the publication) were classified into only two genetic types. Based on the steranes and terpanes migration parameters, it was concluded that they originate from different source rocks. Based on the maturation parameters  $22(S)/22(R)$   $C_{32}$  hopanes and  $14\alpha(H)17\alpha(H)20(S)/14\alpha(H)17\alpha(H)20(R)$   $C_{29}$  steranes, it was concluded that all tested oils were of relatively uniform degree of maturity. The abundances of *n*-alkanes and isoprenoids pristane and phytane, in alkane fractions, showed that only three of the total 19 tested oils were moderately biodegraded.<sup>22,23</sup> As an example, Fig. 1 shows the gas chromatograms of alkane fractions, typical for the non-biodegraded and moderately biodegraded oils of the southeastern part of the Pannonian Basin, and the fingerprints of steranes (*m/z* 217) and terpanes (*m/z* 191) obtained by GC–MS analysis of branched and cyclic alkanes, and using the SIM method. Peak identifications are given in Tables I and II.

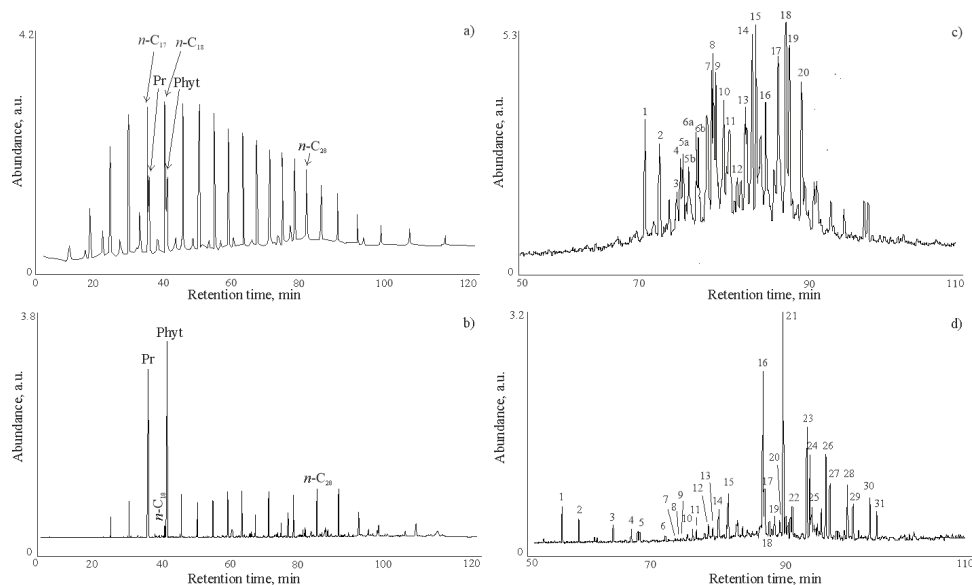


Fig. 1. Gas chromatograms of: a) iphatic fractions, typical for non-biodegraded and b) moderately biodegraded oils of the southeastern part of the Pannonian Basin and c) fingerprints of steranes (*m/z* 217) and d) terpanes (*m/z* 191) obtained from GC–MS analysis of branched and cyclic alkanes.<sup>23-27</sup>

The special attention in organic geochemical research was attracted by the naphthene-type oils from the oil field of the North Bačka Depression. Thirteen samples of the Velebit field marked “W” were analyzed in detail. All group and

specific correlation parameters, that were applied in similar studies worldwide in the late eighties of the twentieth century, were applied in these studies.<sup>24,25</sup>

TABLE I. Identification of the compounds in the Fig. 1c

Peak	Compound
1	C <sub>27</sub> 13β(H)17α(H)20(S)-diasterane
2	C <sub>27</sub> 13β(H)17α(H)20(R)-diasterane
3	C <sub>27</sub> 13α(H)17β(H)20(S)-diasterane
4	C <sub>27</sub> 13α(H)17β(H)20(R)-diasterane
5a	C <sub>28</sub> 13β(H)17α(H)20(S)24(S)-diasterane
5b	C <sub>28</sub> 13β(H)17α(H)20(S)24(R)-diasterane
6a	C <sub>28</sub> 13β(H)17α(H)20(R)24(S)-diasterane
6b	C <sub>28</sub> 13β(H)17α(H)20(R)24(R)-diasterane
7	C <sub>28</sub> 13α(H)17β(H)20(S)-diasterane + C <sub>27</sub> 14α(H)17α(H)20(S)-sterane
8	C <sub>29</sub> 13β(H)17α(H)20(S)-diasterane + C <sub>27</sub> 14β(H)17β(H)20(R)-sterane
9	C <sub>28</sub> 13α(H)17β(H)20(R)-diasterane + C <sub>27</sub> 14β(H)17β(H)20(S)-sterane
10	C <sub>27</sub> 14α(H)17α(H)20(R)-sterane
11	C <sub>29</sub> 13β(H)17α(H)20(R)-diasterane
12	C <sub>29</sub> 13α(H)17β(H)20(S)-diasterane
13	C <sub>28</sub> 14α(H)17α(H)20(S)-sterane
14	C <sub>29</sub> 13α(H)17β(H)20(R)-diasterane + C <sub>28</sub> 14β(H)17β(H)20(R)-sterane
15	C <sub>28</sub> 14β(H)17β(H)20(S)-sterane
16	C <sub>28</sub> 14α(H)17α(H)20(R)-sterane
17	C <sub>29</sub> 14α(H)17α(H)20(S)-sterane
18	C <sub>29</sub> 14β(H)17β(H)20(R)-sterane
19	C <sub>29</sub> 14β(H)17β(H)20(S)-sterane
20	C <sub>29</sub> 14α(H)17α(H)20(R)-sterane

Based on the absence of *n*-alkanes in all tested oils, it was confirmed that the oils were exposed to microbiological degradation in reservoir rocks (reservoir temperatures: 62–70 °C). In the aliphatic fractions of the three samples, neither pristane nor phytane were identified, due to a slightly higher degree of biodegradation. Specific steranes and terpanes source parameters showed that the tested field W oils were of mixed terrestrial-marine origin. Based on the parameters C<sub>30</sub> moretane/hopane and C<sub>29</sub> 14α(H)17α(H)20(S)/14α(H)17α(H)20(S) + 20(R) steranes, and also based on the presence of oleanane it was estimated that the oils originated from the source rocks of Pre-Tertiary age (Upper Cretaceous).

Many years later, the Velebit oil and gas field was examined in even more detail.<sup>26</sup> Twenty-five geologically very well-characterized samples were analyzed. Modern instrumental techniques have enabled very good identification of biological markers from the total aliphatic fractions. In other words, there was no need to separate the aliphatic fraction with urea into the *n*-alkane fraction (adduct) and the fraction of branched and cyclic alkanes (non-adduct). In this paper, due to the GC–MS–MS analysis of total aliphatic fractions, a number of very reliable specific correlation parameters were determined. The estimates of

previous research with a more precise definition of geological history have been confirmed. For example, it was determined that the maturity of the source rocks of the Velebit crude oil was at the level of vitrinite reflection  $R_o > 0.80$  %.

TABLE II. Identification of the compounds in the Fig. 1d

Peak	Compound
1	C <sub>19</sub> -tricyclic terpane
2	C <sub>20</sub> -tricyclic terpane
3	C <sub>21</sub> -tricyclic terpane
4	C <sub>23</sub> -tricyclic terpane
5	C <sub>24</sub> -tricyclic terpane
6	C <sub>25</sub> -tricyclic terpane
7	C <sub>24</sub> -tetracyclic terpane
8	C <sub>26</sub> 22( <i>S</i> )- tricyclic terpane
9	C <sub>26</sub> 22( <i>R</i> )- tricyclic terpane
10	C <sub>28</sub> 22( <i>S</i> )- tricyclic terpane
11	C <sub>28</sub> 22( <i>R</i> )- tricyclic terpane
12	C <sub>29</sub> 22( <i>S</i> )- tricyclic terpane
13	C <sub>29</sub> 22( <i>R</i> )- tricyclic terpane
14	C <sub>27</sub> 18 $\alpha$ (H),22,29,30-trisnorhopane, Ts
15	C <sub>27</sub> 17 $\alpha$ (H),22,29,30-trisnorhopane, Tm
16	C <sub>29</sub> 17 $\alpha$ (H)21 $\beta$ (H)-hopane
17	C <sub>29</sub> 18 $\alpha$ (H),30-norhopane
18	C <sub>30</sub> 17 $\alpha$ (H)-diahopane
19	C <sub>29</sub> 17 $\beta$ (H)21 $\alpha$ (H)-moretane
20	Oleanane
21	C <sub>30</sub> 17 $\alpha$ (H)21 $\beta$ (H)-hopane
22	C <sub>30</sub> 7 $\beta$ (H)21 $\alpha$ (H)-moretane
23	C <sub>31</sub> 17 $\alpha$ (H)21 $\beta$ (H)22( <i>S</i> )-hopane
24	C <sub>31</sub> 17 $\alpha$ (H)21 $\beta$ (H)22( <i>R</i> )-hopane
25	Gammacerane
26	C <sub>32</sub> 17 $\alpha$ (H)21 $\beta$ (H)22( <i>S</i> )-hopane
27	C <sub>32</sub> 17 $\alpha$ (H)21 $\beta$ (H)22( <i>R</i> )-hopane
28	C <sub>33</sub> 17 $\alpha$ (H)21 $\beta$ (H)22( <i>S</i> )-hopane
29	C <sub>33</sub> 17 $\alpha$ (H)21 $\beta$ (H)22( <i>R</i> )-hopane
30	C <sub>34</sub> 17 $\alpha$ (H)21 $\beta$ (H)22( <i>S</i> )-hopane
31	C <sub>34</sub> 17 $\alpha$ (H)21 $\beta$ (H)22( <i>R</i> )-hopane

In the most GC–MS or GC–MS–MS correlation studies, the analyses of aliphatic fractions of crude oils are used to determine numerous values of individual source, maturation, or migration parameters. However, in some cases it is possible to make a very reliable correlation of crude oils only on the basis of “fingerprints” of steranes ( $m/z$  217) and terpanes ( $m/z$  191) obtained using the SIM method (Fig. 1a and b). This is shown in the example of six crude oils from the Kikinda, Mokrin and Požarevac fields.<sup>27</sup> The derived conclusions were in agree-

ment with those drawn earlier on the basis of numerous values of the calculated specific correlation parameters.<sup>22</sup>

The period of the end of the eighties and the first half of the nineties of the last century was marked by the numerous organic geochemical correlations of crude oils from the southeastern part of the Pannonian Basin. The research from the mentioned period was summarized in a review paper from 1998.<sup>28</sup> This correlation study included 80 samples of crude oils from the Banat Depression (18 crude oil fields) and 8 samples from the Drmno Depression (4 fields; Fig. S-1 of the Supplementary material to this paper). The crude oils were correlated based on the number of group and specific correlation parameters (Table III).

TABLE III. Organic geochemical correlation parameters applied in crude oils study of the southeastern part of the Pannonian Basin on the territory of Serbia<sup>28</sup>

Bulk correlation parameter	Specific correlation parameters
<i>API</i> -density	<i>CPI</i> ; most abundant <i>n</i> -alkane
Sulphur	Pr/Phyt
Asphaltenes	Pr/ <i>n</i> -C <sub>17</sub> , Phyt/ <i>n</i> -C <sub>18</sub>
Bulk composition (alkanes, aromatics, NSO)	Regular sterane distribution
	Oleanane/gammacerane index
	Geolipid/biolipid sterane isomers
	Biolipid/geolipid hopane isomers

Most of the crude oils in this basin have not been exposed to biodegradation in reservoir rocks. The samples from the fields Janošik, Jermenovci, Lokve and Velika Greda-jug have a minimal degree of biodegradation, Kikinda Gornje and ten samples from the Velebit field have a medium degree, and three samples from the Velebit field and one from both Kelebija and Gaj fields are in the category of more intensive biodegradation. The source parameters confirmed the conclusions of previous studies on the existence of two genetic types of crude oil. In this review paper, the crude oil from the Subotica field is singled out as the third type, which has not been investigated in previous research. According to the maturation parameters, crude oils were classified in the category of low-maturity crude oils formed in the phase of early catagenesis.

During the eighties of the last century, the reservoir rocks of crude oils were also found in the localities of the Stiška valley near the town of Požarevac (Drmno depression; crude oil fields: Sirakovo, Bradarac, Maljurevac, Bubušina). These samples were also included in the first correlation studies of the Pannonian Basin crude oils on the territory of Serbia (Drmno Depression, E1–E4).<sup>22</sup> The paper presenting these results was published at 2001.<sup>29</sup>

The crude oils of the Drmno Depression are characterized by the dominance of *n*-alkanes with an odd number of C-atoms (carbon preference index, *CPI*, is slightly above unity). The pristane is more abundant than phytane, and in the dis-



tribution of regular steranes  $C_{27}$ – $C_{29}$ ,  $14\alpha(H)17\alpha(H)20(R)$ , the  $C_{29}$  isomer dominates. These three parameters unequivocally show that an organic matter of terrestrial origin also participated in the formation of these crude oils in a larger share than in the case of crude oils from reservoir rocks from Vojvodina. The maturation parameters of steranes and terpanes showed that crude oils are of a slightly lower degree of maturity, formed in the early stages of the catagenetic sequence of crude oil formation ( $Ro$  in range 0.70–0.80 %). The source rocks of Stig crude oil are of Tertiary age.<sup>29</sup> The special examination of sediments from boreholes from the Sirakovo, Bubušinac and Bradarac locations (organic carbon content, bitumen and hydrocarbon content, distribution of biological markers) also defined their source rocks.<sup>30</sup>

The crude oil samples from the fields in Serbia were often used for fundamental research. For example, in the early 1980's, there was a controversy in the organic-geochemical research community, when it came to the use of the ratios of relative concentrations of pristane and phytane in isoprenic oil fractions (Pr/Phyt) as a maturation parameter. According to some authors, the value of this ratio decreases with increasing maturity.<sup>31</sup> To shed light on this question, Pr/Phyt ratios were determined in 63 crude oil samples of the Banat and Drmno depressions of the Pannonian Basin, and the obtained values were correlated with the values of more reliable terpane maturity parameters, as well as with  $API$  densities and ratios Pr/ $n$ - $C_{17}$  and Phyt/ $n$ - $C_{18}$ .<sup>32</sup> The correlation analysis has unequivocally shown that the value of the parameter Pr/Phyt increases with the maturity. Also, in order to assess the impact of microbiological degradation in the reservoir rocks on sterane and terpane changes, the best known maturity parameters of sterane and terpane of 36 crude oil samples from the southeastern part of the Pannonian Basin, together with 8 samples of oil fields from the eastern Russia were correlated. The results showed that steranes were more resistant to biodegradation than terpanes.<sup>33</sup>

Finally, the significance of maturation parameters determined from the distribution and abundance of saturated biomarkers and alkylaromatics in crude oil correlation studies was evaluated using a new approach in factor and cluster analysis. For this fundamental research, 23 crude oils from the part of the Pannonian Basin that belongs to Serbia were used.<sup>34</sup>

The experiences gained by studying biological markers in crude oils belonging to the southeastern part of the Pannonian Basin on the territory of Serbia have been applied in similar studies regarding the crude oils of the most important oil fields in the world. For example, a detailed correlation study of crude oils from 9 oil fields of Sakhalin Island (Russia) enabled the definition of phenanthrene content in the aromatic fraction as a very reliable maturation parameter.<sup>35</sup> On the other hand, the approaches used to define the origin and geological history of crude oils from the basins from the territory of Serbia, have also been success-

fully applied to the organic geochemical correlation of crude oils in Libya's most important oil fields.<sup>36-38</sup>

### 3. THE USE OF BIOLOGICAL MARKERS IN OIL-TYPE POLLUTANTS IDENTIFICATION AND MONITORING OF THEIR FATE IN RIVERS AND RIVER SEDIMENTS OF SERBIA

#### 3.1. Identification

In recent sediment deposits, surface and groundwaters, and soils, the presence of oil-type pollutants cannot be determined only based on the amount of organic extract (bitumen). Namely, from the theoretical, organic geochemical aspect, the amount of native bitumen in the surface parts of the lithosphere can be in the range from zero to one hundred percent.<sup>1-7</sup> There are numerous examples of the surface sediments without any of organic substances. On the other hand, there is the phenomenon of "seeping crude oil" (natural oil lakes), which can be defined as sedimentary formations with 100 % native organic matter. Therefore, to determine the presence of oil-type pollutants in these segments of the environment, it is necessary to geochemically characterize the organic substance (organic extract) isolated from a sample.

This approach is based on the fact that crude oil is a highly mature organic matter. On the other hand, the organic substance of recent sediments is of a rather low degree of thermal maturity. As a consequence, the distributions of biomarkers differ as well. Crude oil contains an incomparably higher concentration of thermodynamically most stable structural and stereochemical biomarker isomers. For the first time this was shown on the example of *n*-alkane distributions in a total of 17 samples of alluvial sediments taken from the localities of Zrenjanin (Begej River, 13 samples), Pančevo (Danube River, 3 samples), and Kraljevo (Ibar River, 1 sample).<sup>39</sup> Based on the *n*-alkane distribution, the oil-type pollutant was found only in a sample belonging to the Ibar River. In some samples there was a mixture of native and anthropogenic oil substances, and in some the oil-type pollutant was not even identified. Conclusions were made based on the organic geochemical knowledge that in crude oils, as a consequence of a high degree of maturation, there is a uniform distribution of odd and even homologues (*CPI* is around 1). Fig. 2 shows examples with the distribution of *n*-alkanes belonging to a native organic matter, then an organic matter that is a mixture of native and anthropogenic, and *n*-alkanes from an oil-type organic pollutant.

Due to the high degree of maturation, oil has the highest concentration of the heavier, <sup>13</sup>C isotope, compared to the other forms of organic matter in the geosphere. The isotopic analysis was performed from the samples of Begej, Danube, and Ibar River sediments. Gas chromatography–mass spectrometry–isotope ratio (GC–MS–IR) technique determined the values for  $\delta^{13}CPDB$  for individual *n*-alkanes C<sub>25</sub>, C<sub>27</sub>, C<sub>29</sub> and C<sub>31</sub>. The highest concentrations of the heavier, <sup>13</sup>C, iso-

tope (least negative values for  $\delta^{13}CPDB$ ) in the sample belonging to the Ibar River, provided the additional confirmation of the presence of oil pollutants in this sample.<sup>39</sup>

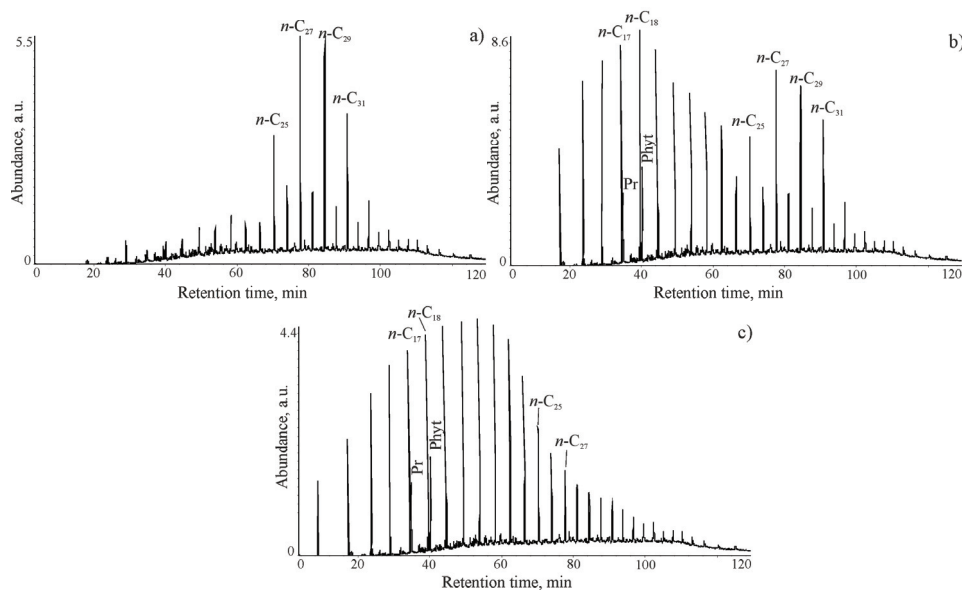


Fig. 2. Examples of the distribution of *n*-alkanes fitting to: a) the native organic matter, b) an organic matter that is a mixture of native and anthropogenic, and c) *n*-alkanes from the organic matter which represents the oil pollutant.<sup>39</sup>

*n*-Alkanes in the organic extracts of river sediments can also be a tool for determining the presence of the non-petroleum type organic pollutants. For example, 6 samples of sediments of the Lim River in Bjelo Polje (Montenegro) were investigated.<sup>40</sup> One sample was taken above the place of sewage waste discharge into the river, and five samples downstream from it. The dominance of odd homologues in the first sample was the proof that this sample contained a native organic substance. In all other samples, a pronounced dominance of even *n*-alkane homologues was identified (*CPI* in range 0.01–0.45). This was an unequivocal proof that these samples contained sewage, where microorganisms developed and produced even *n*-alkane homologues as their metabolites.

The polycyclic alkanes of sterane and triterpene type may serve as the most useful tools for the identifying of oil-type pollution in recent sediments (*m/z* 217 and *m/z* 191, Fig. 1c and d). In addition to regular, biolipid isomers  $C_{27}–C_{29} 14\alpha(H)17\alpha(H)20(R)$ , *n*-alkane fractions of crude oils contain high concentrations of geolipid isomers, such as diasteranes and  $C_{27}–C_{29}$  steranes with hydrogen atoms at  $C_{14}$  and  $C_{17}$   $\beta$  position and *S*-configuration at the  $C_{20}$  chiral center.

When it comes to triterpanes, in addition to biolipid moretane isomers, oleanane and gammacerane, the significant presence of homologous series of  $17\alpha(H)21\beta(H)$  ( $22S$  and  $22R$ ) hopanes in the range of  $C_{29}$ – $C_{35}$  are also characteristic of crude oil. Such distributions of steranes and terpenes in organic extracts of river sediments, soil, or some other recent sediments (examples are displayed in Fig. 1c and d), are a strong indication of the presence of oil-type pollutants.<sup>41–45</sup> These polycyclic alkanes can be considered as a very sensitive “forensic tool”. If oil-type pollutants are present in some samples and in very low concentrations, GC–MS analysis of steranes and terpanes will allow their identification. This was confirmed, for example, in the research of surface sediments of the Great War Island (Belgrade)<sup>46</sup> and sludge from the Techirghiol Lake (Romania).<sup>47</sup>

### 3.2. Migration

When an oil-type pollutant reaches the environment, it does not stay at the accident site. It migrates, either as a consequence of its fluidity or with the help of water. Based on the changes in the biomarker composition, during the migration of crude oil into the environment, the mechanism of this process can be assessed.

The migration of oil-type pollutants into the environment by water can be related to the migration of bitumen in natural geological conditions from the source to the reservoir rock. This migration is called secondary migration in the organic geochemical literature, and it takes place mainly by water. The assumed mechanisms are: 1) solutions, 2) colloidal solutions, 3) globules and droplets and 4) continuous phases mechanism.<sup>3,6</sup>

During the accident at the Ušće station near Kraljevo, a certain amount of oil was spilled. The accident happened in 1996<sup>48</sup> during rainy days, which is, from the research point of view, very important. The oil migrated through the crushed stone. In such a process of natural geochromatography, the stone had the role of the adsorbent and water the role of the eluent. It has been observed that with the migration the amount of nitrogen, sulfur, oxygen (NSO) compounds is increasing, that the amount of pristane and phytane also increases in the relation to *n*-alkanes  $C_{17}$  and  $C_{18}$ , and that the amount of higher homologues of *n*-alkanes increases in the relation to lower ones. Namely, there was a shift of the *n*-alkane maximum in the five tested samples from  $C_{19}$  to  $C_{26}$ . It was concluded that NSO compounds and water formed colloidal solutions, with the colloidal micelle channel having a diameter of 0.7 nm. The isoprenoid alkanes with a volume slightly smaller than 0.7 nm in diameter and *n*-alkanes with a volume diameter of 0.5 nm were incorporated into such a channel. Higher homologues made stronger inclusions and thus migrated to a greater extent than those with fewer C-atoms.

In the early organic geochemical studies, an interesting phenomenon related to the migration of bitumen through sedimentary rocks in natural geological con-

ditions was proven. Namely, those compounds that elute the fastest during chromatographic separation (analytical procedure in the laboratory), migrate the fastest in natural catagenetic conditions.<sup>49,50</sup> On the other hand, by examining the mechanism of oil pollutant migration in river sediments of the Vrbas River (Banja Luka) it was proven that the amount of lower *n*-alkanes during the migration increases relative to higher homologues.<sup>51</sup> In the case of polycyclic alkanes, the amount of C<sub>27</sub> 13β(H)17α(H)20(S) and C<sub>27</sub> 13β(H)17α(H)20(R) diasteranes increases relative to C<sub>27</sub> 14α(H)17α(H)20(R) sterane, and C<sub>28</sub> 14α(H)17α(H)20(S) relative to C<sub>28</sub> 14α(H)17α(H)20(R). These results, for the first time, proved that the migration phenomenon that occurs with the diasteranes oil pollutants in the environment is the same as with bitumen in catagenetic geological conditions.<sup>51</sup>

### 3.3. Biodegradation

Once it reaches the environment, the oil pollutant is exposed to a number of changes. Its composition is influenced by reactions of adsorption, photodegradation, evaporation, oxidation. However, the greatest changes in an oil pollutant occur as a result of microbiological degradation.

During the period from November 1997 to February 2000, five groundwater samples were taken from the same piezometer of the Pančevo Oil Refinery. The biological markers were analyzed in the isolated extracts by GC–MS technique (Fig. 3).<sup>52–56</sup> In these extracts a phenomenon, related to the fate of biological markers during oil biodegradation in groundwater of an alluvial sedimentary formation, was defined.

In the first sample, the relative concentrations of *n*-alkanes C<sub>17</sub> and C<sub>18</sub> were lower than the concentrations of pristane and phytane isoprenoids. Such a distribution of the *n*-alkane fraction peaks is typical for the oil in which the biodegradation process has already started.<sup>52</sup>

The process continued until the complete degradation of *n*-alkanes in the fourth sample taken in September 1999.<sup>53</sup> In the sampled groundwater a few months later, in February 2000, *n*-alkanes with an even number of C-atoms appeared.<sup>54,55</sup> The identification of even homologues of fatty acids (their methyl-esters) and even homologues of alcohols as well as of cholesterol, in this sample, is the evidence that even *n*-alkanes were synthesized by the single-celled, non-photosynthetic algae Pyrrophyta, known as “fire algae”, which are easily grown on an oil substrate.

The comprehensive understanding of the behaviour of biological markers in the process of oil biodegradation in natural geological conditions, as well as in the environment, has enabled their very efficient use in monitoring of the bioremediation process, as a procedure for purification of water, soil and sediment from oil type organic pollutants in *ex situ*<sup>56</sup> and *in situ* conditions (Fig. 4).<sup>57</sup>

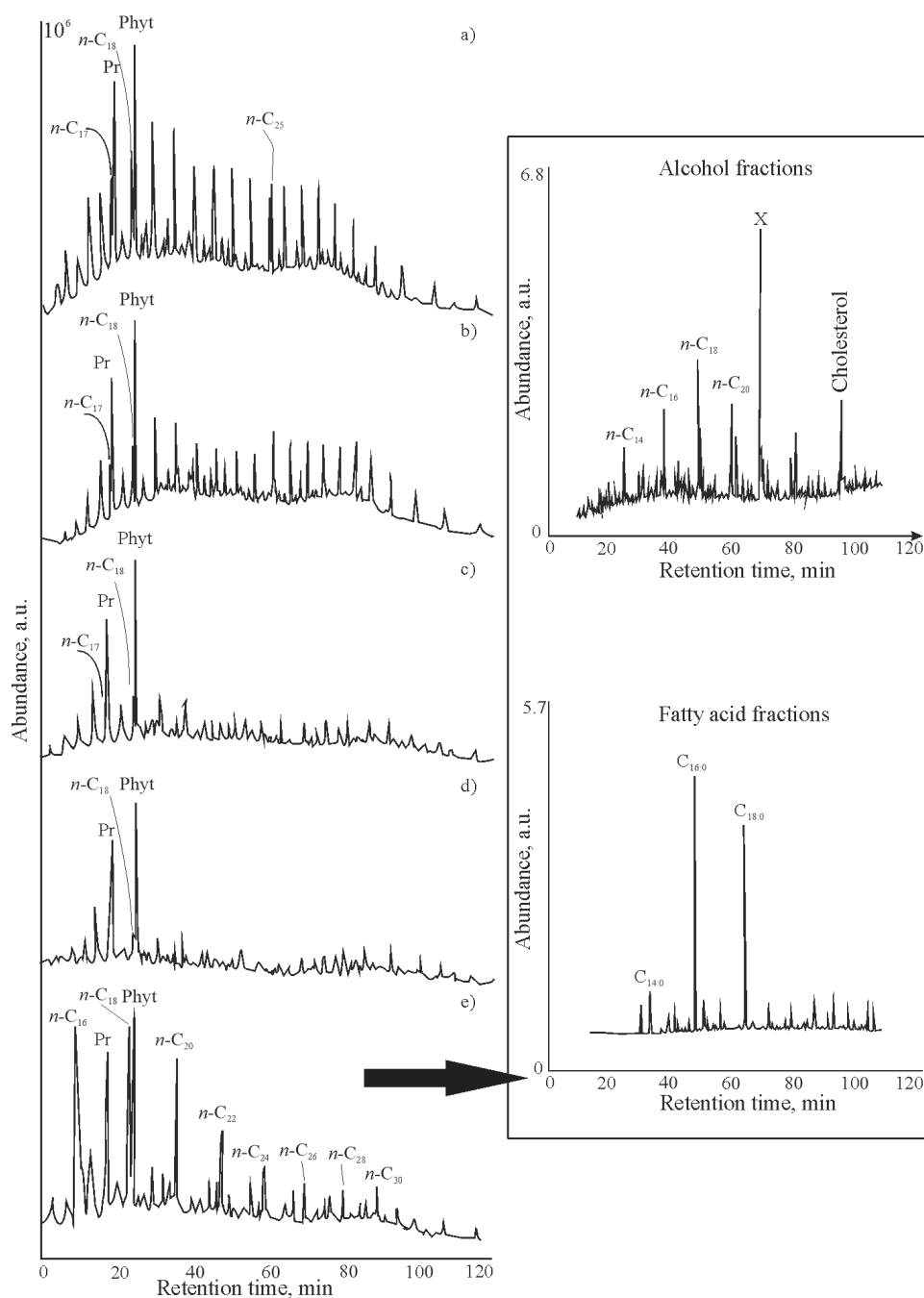


Fig. 3. Biological markers in the groundwater oil pollutant of the Pančevo Oil Refinery, samples of contaminated groundwater were taken from the same piezometer: a) November 1997, b) May 1998, c) September 1998, d) September 1999 and e) February 2000.<sup>52-56</sup>

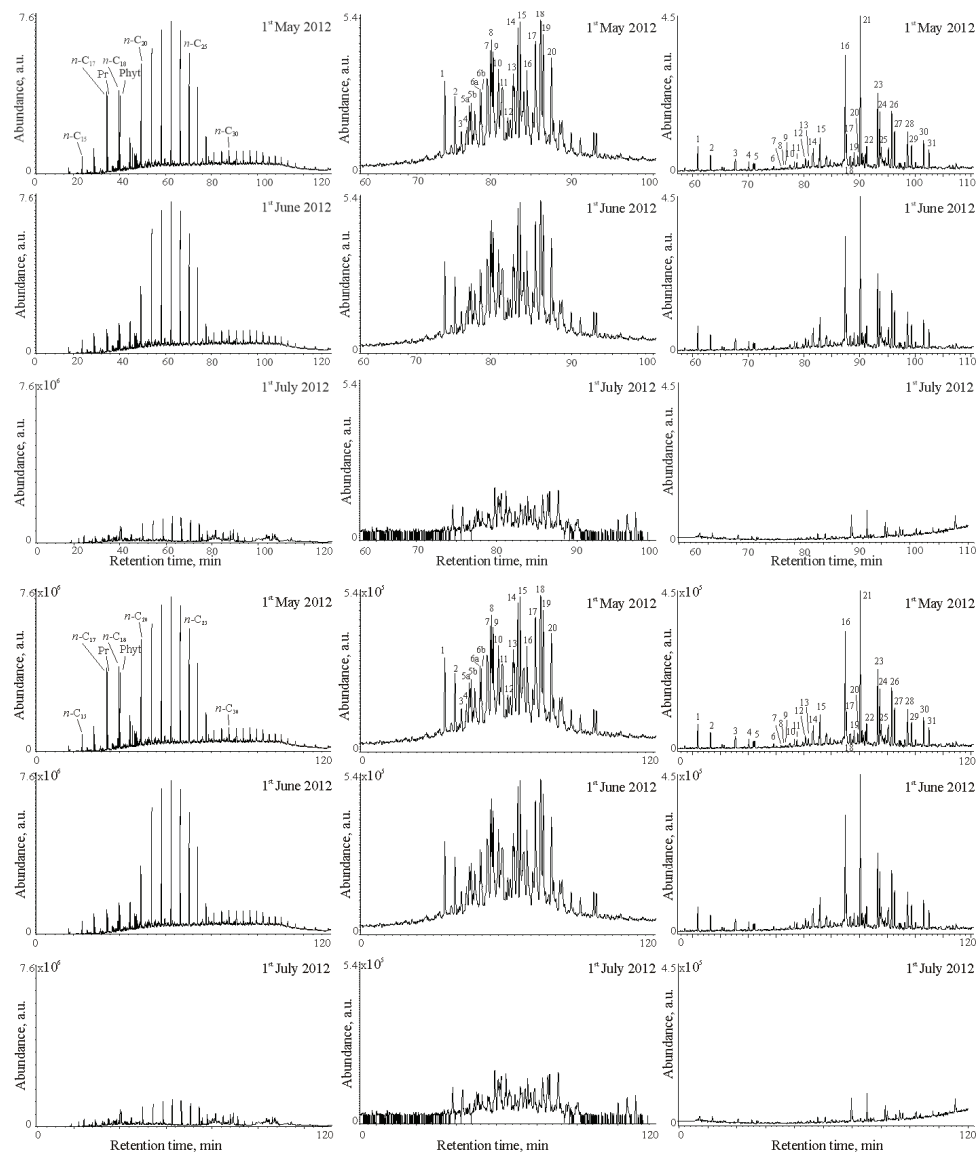


Fig. 4. An example of the degradation of biological markers of the *n*-alkane, sterane and triterpane type in groundwater petroleum pollutant during the in situ bioremediation process;<sup>57</sup> the identification of individual sterane and terpane isomers is given in Tables I and II.

#### 4. CONCLUSIONS

The organic geochemical research of oil in the Southeastern part of the Pannonian Basin in Serbia, as well as the process of identifying oil pollutants and monitoring its fate in rivers and river sediments in Serbia, in the last three dec-

ades, have shown which biological markers can be used as the geochemical, *i.e.*, chemical tool.

In the first case, *n*-alkanes, isoprenoid aliphatic alkanes, diasteranes, steranes, tricyclic and tetracyclic diterpanes and pentacyclic triterpanes enabled a very successful assessment of the type of oil biomass precursor and classified the tested oils from one or more oil fields. Furthermore, they allowed the assessment of the sedimentation environment, the degree of maturation, the length of the migration path from the source to the reservoir rock and the degree of biodegradation in the reservoir rocks.

In the environmental chemistry, these organic compounds are indispensable in the identification of oil pollutants and their sources, in different parts of the environment, in monitoring changes during its migration, as well as in defining the manner and the intensity of microbiological degradation. Additionally, they also enable the successful monitoring of the bioremediation process efficiency in the *ex situ* and *in situ* conditions.

#### SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/10916>, or from the corresponding author on request.

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#### ИЗВОД

#### УПОТРЕБА БИОЛОШКИХ МАРКЕРА У ОРГАНСКО-ГЕОХЕМИЈСКИМ ИСПИТИВАЊИМА ПОРЕКЛА И ГЕОЛОШКЕ ИСТОРИЈЕ СИРОВИХ НАФТИ (I) И У ПРОЦЕНИ НАФТНОГ ЗАГАЂЕЊА РЕКА И РЕЧНИХ СЕДИМЕНАТА СРБИЈЕ (II)

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Биолошки маркери у нафтама су једињења за које се зна прекурсор, у току трансформација органске супстанце ова једињења трпе извесне структурне и стереохемијске промене. На основу установљеног прекурсора појединачних биомаркера, процењује се порекло испитиваних нафти, а на основу интензитета и типа промена, геолошка историја. Она укључује дефинисање средине таложња, степена матурираности, дужине миграционог пута нафте, степена биодеградације. Највише изучавани и примењивани биолошки маркери су нормални алкани, изопреноидни алифатични алакани пристан и фитан, и полициклични алкани типа стерана и терпана. С друге стране, у хемији животне средине ова једињења у значајној мери могу да допринесу идентификацији нафтног полутанта, као и процени механизма миграције и интензитета биодеградације. У овом прегледном раду прво су приказани резултати који се односе на примену биолошких



маркера у органско геохемијским корелацијама нафти југоисточног дела Панонског басена (I). У другом делу дат је преглед оних радова у којима су исти биолошки маркери коришћени у идентификацији нафтних полутаната и у праћењу његових промена у току миграције и биодеградације у рекама и речним седиментима Србије (II).

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#### REFERENCES

1. G. Eglinton, M. T. J. Murphy, *Organic Geochemistry*, Springer-Verlag, Heidelberg, 1969, p. 20 (ISBN: 70-107318)
2. B.P. Tissot, D.H. Welte, *Petroleum Formation and Occurrence*, 2<sup>nd</sup> ed., Springer-verlag, Heidelberg, 1984, p. 3 (ISBN: 0-387-08698-6)
3. D. Waples, *Geochemistry in Petroleum Exploration*, International Human Resources Development Corporation, Boston, MA, 1985, p. 1 (ISBN: 90-277-208-8)
4. R. P. Philp, *Fossil Fuel Biomarkers, Applications and Spectra*, Elsevier, Amsterdam, 1985, p. 1 (ISBN: 0444424717)
5. K. E. Peters, C. C. Walters, J. M. Moldowan, *The biomarker Guide, Vol. 2: Biomarkers and Isotopes in Petroleum Exploration and Earth History*, Cambridge University Press, Cambridge, 2005, p. 475 (ISBN: 0-521-83763-4)
6. J. Schwarzbauer, B. Jovančičević, *Fundamentals in Organic Geochemistry – Fossil Matter in the Geosphere*, Springer, Heidelberg, 2015, p. 1 (ISBN: 978-3-319-11552-8)
7. J. Schwarzbauer, B. Jovančičević, *Fundamentals in Organic Geochemistry – From Biomolecules to Chemofossils*, Springer, Heidelberg, 2016, p. 1 (ISBN: 978-3-319-27241-2)
8. A. Triebs, *Ann. Chemie* **509** (1934) 103 (<https://doi.org/10.1002/ange.19360493803>)
9. D. H. Welte, D. Waples, *Sci. Nat.* **60** (1973) 516 (<https://doi.org/10.1007/BF00603253>)
10. J. M. Moldowan, W. K. Seifert, *Science* **204** (1979) 169 (<https://doi.org/10.1126/science.204.4389.169>)
11. W. K. Seifert, J.M. Moldowan, *Geochim. Cosmochim. Acta* **45** (1981) 783 ([https://doi.org/10.1016/0016-7037\(81\)90108-3](https://doi.org/10.1016/0016-7037(81)90108-3))
12. P. A. Albrecht, *Evaluation geochimique des polyterpanes at des sterols*, Prix Rousel, Paris, 1986, p. 1
13. H. L. Ten Haven, M. Rohmer, J. Rullkötter, P. Bisseret, *Geochim. Cosmochim. Acta* **53** (1989) 3073 ([https://doi.org/10.1016/0016-7037\(89\)90186-5](https://doi.org/10.1016/0016-7037(89)90186-5))
14. W. K. Seifert, R. M. K. Carlson, J. M. Moldowan, in *Advances in Organic Geochemistry 1981*, M. Bjorøy, P. Albrecht, C. Cornford, K. de Groot, G. Eglinton, E. Galimov, D. Leythaeuser, R. Pelet, J. Rullkötter, G. Speers., Eds., Wiley, Chichester, 1983, p. 710 (ISBN: 0-471-26229-3)
15. W. K. Seifert, J. M. Moldowan, G. J. Demaioson, *Org. Geochem.* **10** (1984) 633 ([https://doi.org/10.1016/0146-6380\(84\)90085-8](https://doi.org/10.1016/0146-6380(84)90085-8))
16. Z. Sofer, J. E. Zumberge, V. Lay, *Org. Geochem.* **10** (1986) 377 ([https://doi.org/10.1016/0146-6380\(86\)90037-9](https://doi.org/10.1016/0146-6380(86)90037-9))
17. J. Rullkötter, T. M. Peakman, H. L. ten Haven, *Org. Geochem.* **21** (1994) 215 ([https://doi.org/10.1016/0146-6380\(94\)90186-4](https://doi.org/10.1016/0146-6380(94)90186-4))
18. J. Schwarzbauer, B. Jovančičević, *Fundamentals in Organic Geochemistry – Introduction to Analytical Methods in Organic Geochemistry*, Springer, Heidelberg, 2020, p. 1 (ISBN: 978-3-030-38591-0)
19. J. Schwarzbauer, B. Jovančičević, *Fundamentals in Organic Geochemistry – Organic Pollutants in the Geosphere*, Springer, Heidelberg, 2018, p. 1 (ISBN: 978-3-319-68937-1)

20. M. Šarković, *Geochemical characteristics of layered fluids of oil and gas deposits in the southeastern part of the Pannonian Basin and their use in research*, Matica srpska, Novi Sad, 1973, p. 1 (in Serbian)
21. M. Šaban, B. Jovančičević, D. Vitorović, in *Proceedings of the II Yugoslav Symposium on Hydrocarbons*, 1986, Belgrade, Yugoslavia, II Yugoslav Symposium on Hydrocarbons, Serbian Chemical Society, Belgrade, 1986, p. 142 (in Serbian)
22. M. Šaban, B. S. Jovančičević, A. Hollerbach, D. Vitorović, in *Proceedings of 13<sup>th</sup> International Meeting on Organic Geochemistry*, 1987, Venezia, Italia, Organic Geochemistry in Petroleum Exploration, Pergamon Press, Frankfurt, 1987, p. 224
23. M. Šaban, B. S. Jovančičević, S. Saračević, A. Hollerbach, D. Vitorović, *Org. Geochem.* **13** (1988) 325 ([https://doi.org/10.1016/0146-6380\(88\)90052-6](https://doi.org/10.1016/0146-6380(88)90052-6))
24. M. M. Šaban, B. Jovančičević, T. Glumičić, S. Saračević, in *Abstracts of 14<sup>th</sup> International Meeting on Organic Geochemistry*, 1989, Paris, France, Organic Geochemistry in Petroleum Exploration, Pergamon Press, Paris, 1989, p. 197
25. M. M. Šaban, B. S. Jovančičević, T. Glumičić, S. Saračević, *Org. Geochem.* **16** (1990) 477 ([https://doi.org/10.1016/0146-6380\(90\)90063-6](https://doi.org/10.1016/0146-6380(90)90063-6))
26. T. Šolević, K. Stojanović, J. Bojesen-Koefoed, H. P. Nytoft, B. Jovančičević, D. Vitorović, *Org. Geochem.* **39** (2008) 118 (<https://doi.org/10.1016/j.orggeochem.2007.09.003>)
27. M. Šaban, B. S. Jovančičević, T. Glumičić, N. Dogović, *Rapid Commun. Mass Spectrom.* **4** (1990) 505 (<https://doi.org/10.1002/rcm.1290041207>)
28. B. Jovančičević, P. Polić, D. Vitorović, *J. Serb. Chem. Soc.* **63** (1998) 397 (<https://doi.org/10.2298/JSC161129022M>)
29. B. Jovančičević, H. Wehner, G. Scheeder, D. Plećaš, M. Ercegovac, D. Vitorović, *J. Serb. Chem. Soc.* **66** (2001) 297 (<https://doi.org/10.2298/JSC0105297J>)
30. B. Jovančičević, H. Wehner, G. Scheeder, K. Stojanović, A. Šainović, O. Cvetković, M. Ercegovac, D. Vitorović, *J. Serb. Chem. Soc.* **67** (2002) 553 (<https://doi.org/10.2298/JSC0209553J>)
31. B. Durand, in *Advances in Organic Geochemistry 1981*, M. Bjorøy, P. Albrecht, C. Cornford, K. de Groot, G. Eglinton, E. Galimov, D. Leythaeuser, R. Pelet, J. Rullkötter, G. Speers, Eds., Wiley, Chichester, 1983, p. 117 (ISBN: 0-471-26229-3)
32. B. Jovančičević, P. Polić, M. Šaban, D. Vitorović, *J. Serb. Chem. Soc.* **59** (1994) 983
33. B. Jovančičević, Lj. Tasić, P. Polić, J. Nedeljković, A. Golovko, D. Vitorović, *J. Serb. Chem. Soc.* **61** (1996) 817
34. K. Stojanović, B. Jovančičević, D. Vitorović, Y. Golovko, G. Pevneva, A. Golovko, *J. Serb. Chem. Soc.* **72** (2007) 1237 (<https://doi.org/10.2298/JSC0712237S>)
35. K. Stojanović, B. Jovančičević, G. S. Pevneva, J. A. Golovko, A. K. Golovko, P. Pfenndt, *Org. Geochem.* **32** (2001) 721 ([https://doi.org/10.1016/S0146-6380\(01\)00004-3](https://doi.org/10.1016/S0146-6380(01)00004-3))
36. M. A. M. Faraj, T. Šolević-Knudsen, H. P. Nytoft, B. Jovančičević, *J. Pet. Sci. Eng.* **147** (2016) 605 (<https://doi.org/10.1016/j.petrol.2016.09.030>)
37. M. A. M. Faraj, T. Šolević-Knudsen, K. Stojanović, S. Ivković-Pavlović, H. P. Nytoft, B. Jovančičević, *J. Serb. Chem. Soc.* **82** (2017) 1315 (<https://doi.org/10.2298/JSC170419075A>)
38. R. M. S. Saheed, T. Šolević-Knudsen, M. A. M. Faraj, Z. Nikolovski, H. P. Nytoft, B. Jovančičević, *J. Serb. Chem. Soc.* **85** (2020) 1489 (<https://doi.org/10.2298/JSC200501055S>)
39. B. Jovančičević, Lj. Tasić, H. Wehner, E. Faber, N. Šušić, P. Polić, *Fresenius Environ. Bull.* **6** (1997) 667 (<https://doi.org/10.2298/JSC200501055S>)

40. B. Jovančičević, Lj. Tasić, H. Wehner, D. Marković, P. Polić, *Fresenius Environ. Bull.* **7** (1998) 320
41. B. Jovančičević, P. Polić, B. Mikašinović, G. Scheeder, M. Teschner, H. Wehner, *Fresenius Environ. Bull.* **10** (2001) 527 ([https://www.prt-parlar.de/download\\_afs\\_2001/](https://www.prt-parlar.de/download_afs_2001/))
42. S. Grujić, B. Jovančičević, P. Polić, H. Wehner, *Fresenius Environ. Bull.* **12** (2003) 359 ([https://www.prt-parlar.de/download\\_feb\\_2003/](https://www.prt-parlar.de/download_feb_2003/))
43. I. Samelak, M. Balaban, N. Vidović, N. Koljančić, M. Antić, T. Šolević Knudsen, B. Jovančičević, *J. Serb. Chem. Soc.* **83** (2018) 1167 (<https://doi.org/10.2298/JSC180501061S>)
44. Z. Milićević, D. Marinović, G. Gajica, M. Kašanin-Grubin, V. Jovanović, B. Jovančičević, *J. Serb. Chem. Soc.* **82** (2014) 593 (<https://doi.org/10.2298/JSC161129022M>)
45. S. Štrbac, G. Gajica, A. Šajnović, N. Vasić, K. Stojanović, B. Jovančičević, *J. Serb. Chem. Soc.* **79** (2014) 597 (<https://doi.org/10.2298/JSC130614087S>)
46. M. Kašanin-Grubin, S. Štrbac, S. Antonijević, S. Djogo-Mračević, D. Randjelović, J. Orlić, A. Šajnović, *J. Environ. Manage.* **251** (2019) 109574 (<https://doi.org/10.1016/j.jenvman.2019.109574>)
47. S. Stojadinović, B. Jovančičević, A. Šajnović, M. Golumbeanu, R. Almasan, Dj. Jovanović, I. Brćeski, *Fresenius Environ. Bull.* **30** (2021) 1595 ([https://www.prt-parlar.de/download\\_feb\\_2021/](https://www.prt-parlar.de/download_feb_2021/))
48. B. Jovančičević, Lj. Tasić, S. Vujasinović, I. Matić, D. Malović, P. Pfendt, *J. Serb. Chem. Soc.* **61** (1996) 1025
49. A. Golovko, V. Ivanov, in *Proceedings of 19<sup>th</sup> International Meeting on Organic Geochemistry*, 1991, Istanbul, Turkey, Organic Geochemistry, TÜBITAK, Istanbul, 1999, p. 585
50. W. K. Seifert, J. M. Moldowan, *Geochim. Cosmochim. Acta* **42** (1978) 77 ([https://doi.org/10.1016/0016-7037\(79\)90051-6](https://doi.org/10.1016/0016-7037(79)90051-6))
51. I. Samelak, M. Balaban, M. Antić, T. Šolević-Knudes, B. Jovančičević, *Environ. Chem. Lett.* **18** (2020) 459 (<https://doi.org/10.1007/s10311-019-00937-2>)
52. B. Jovančičević, P. Polić, *Fresenius Environ. Bull.* **9** (2000) 232
53. B. Jovančičević, P. Polić, D. Vitorović, G. Scheeder, M. Teschner, H. Wehner, *Fresenius Environ. Bull.* **10** (2001) 178 ([https://www.prt-parlar.de/download\\_afs\\_2001/](https://www.prt-parlar.de/download_afs_2001/))
54. B. Jovančičević, P. Polić, M. Vrvic, G. Scheeder, M. Teschner, H. Wehner, *Environ. Chem. Lett.* **1** (2003) 73 (<https://doi.org/10.1007/s10311-002-0002-7>)
55. B. Jovančičević, M. Vrvic, J. Schwarzbauer, H. Wehner, G. Scheeder, D. Vitorović, *Water Air Soil Pollut.* **183** (2007) 225 (<https://doi.org/10.1007/s11270-007-9371-7>)
56. V. Beškoski, M. Takić, J. Milić, M. Ilić, G. Gojgić-Cvijović, B. Jovančičević, M. M. Vrvic, *J. Serb. Chem. Soc.* **75** (2010) 1605 (<https://doi.org/10.2298/JSC100505091B>)
57. V. Beškoski, S. Miletić, M. Ilić, G. Gojgić-Cvijović, P. Papić, M. Marić, T. Šolević-Knudsen, B. Jovančičević, T. Nakano, M. Vrvic, *CLEAN – Soil, Air, Water* **45** (2017) 1600023 (<https://doi.org/10.1002/clen.201600023>).