

SZENT ISTVÁN UNIVERSITY

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CHARACTERIZATION OF SOIL HUMIC SUBSTANCES IN HUNGARIAN AND IRANIAN SOILS

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1. INTRODUCTION

Organic materials are an important constituent of all natural environments. Vegetation significantly influence the quantity and quality of organic material in the soil. Different vegetation produce different soil properties and any changes in the ecosystem will upset the balance of the soil (Islam and Weil 2000; Lapola et al. 2010). One of the important fractions of soil organic matter are humic substances (HS) which determined as the dark colored amorphous polymers which are synthesized from biomass constituents or their metabolites biochemically and/or chemically in the environment (Baglieri et al. 2007). Füleky and Czinkota (1993) used a technique for extracting which called Hot Water Percolation (HWP) and uses heat energy and pressure together. Characterization of organic matter can be carried out using different method. Ultra Violet Visible (UV VIS) spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy and, etc. (Christensen et al. 1998).

Present study included two parts, in the first part we used HWP as a modern method to extract HSs of soil. The results of characterization of HSs which extracted with this method was one of the most important goals that could lead to an important evolution in soil organic matter extraction method. In the second part we have chosen a forest site which its native species (*Aluns subcordata*) replaced with different ratios of non native species (*Populus deltoids*). We used NMR spectroscopy to characterize the quantity and quality of organic components in soil sample of virgin and planted forests. Since the amount and type of organic component are differ in soil fractions, we fractionated soil samples physically and studied organic components in each fraction.

The most important aims of this study are:

- To study the optical properties of humic substances extracted with Hot Water Percolation method.
- The comparison of quantity and quality of soil humic substances in virgin and planted forests in the same climate and geographic area.
- To study the effect of different ratios of Alnus:Populus on soil organic matter.

To study the differences of organic component quantity and quality in different soil fractions.

2. LITERATURE REVIEW

1.1 Organic matter

Organic materials are an important constituent of all natural environments, organic molecules are found in both soluble and insoluble forms in a variety of environments such as, atmospheric aerosols, soils, sediments and aquatic systems (Thurman 1985). Organic matter affects the chemical, physical and biological properties of the soil such as: soil structure, moisture holding capacity, cation and anion exchange capacity, nutrient availability, diversity and activity of soil organisms, break up clay and compacted soils. Plant growth is influenced directly and indirectly by organic matter, they assists transferring micronutrients from the soil to the plant (Reeves 1997). One of the most important fractions of soil organic matter are humic substances (HS) determined as dark-colored amorphous polymers which are synthesized from biomass constituents or their metabolites biochemically and/or chemically in the environment (Baglieri et al. 2007).

1.2 Humic substances fractions

The classical method of fractionation of humic substances is based on differences in solubility in aqueous solutions at widely differing pH levels, (Schnitzer and Khan 1978). The fractions have been operationally defined as Fulvic acid, the fraction soluble in water under all pH conditions (Baglieri et al. 2007; Schnitzer and Khan 1978; Yates and von Wandruszka 1999). Fulvic acids are a mixture of weak aliphatic and aromatic organic acids. Their composition and shape is quite variable. Fulvic acids are light yellow to yellow-brown in color (Beznosikov and Lodygin 2009; Shin et al. 1996). Humic acid, the humic acid substances fraction are not soluble in water under pH < 2 conditions, but is soluble at higher pH (Baglieri et al. 2007; Yates and von Wandruszka 1999). Humic acid comprise a mixture of weak aliphatic (carbon chains) and aromatic (carbon rings) organic acids. Humic acid is the major extractable component of soil humic substances. It is dark brown to black in color. Humin, the fraction which is not soluble in water at any pH value and black in color (Baglieri et al. 2007; Schnitzer and Khan 1978; Yates and von Wandruszka 1999). The chemical and physical properties of humins are only partially understood. Humin present within the soil is the most resistant to decomposition (slow to breakdown) of all the humic substances (Baglieri et al. 2007; Schnitzer and Khan 1978).

1.3 Use of UV VIS and NMR in soil organic matter study

Characterization of humic substances can be carried out using UV VIS spectroscopy. Humic substances generally show strong absorbance in the UV–Vis range (from 190 to 800 nm), particularly in the UV region, because of the presence of aromatic chromophores and/or other organic compounds

(Schnitzer and Khan 1972). Soil scientists have used the ratio of optical densities or absorbance of dilute, aqueous humic and fulvic acid solutions at 465 and 665 nm (E_4/E_6) in order to characterize these materials (Chen et al. 1977; Helms et al. 2008; McDonald et al. 2004; Yang and Xing 2009). Higher molecular weight and higher degree of condensation of the aromatic rings will be indicated by lower E_4/E_6 ratio value. The UV Absorbance Ratio Index values (URI) (UVA₂₁₀/UVA₂₅₄) provides the information on the relative proportions between UV-absorbing functional groups and unsaturated compounds. Nuclear magnetic resonance (NMR) spectroscopy is a technique based on the magnetic properties of atomic nuclei. It is used to distinguish between different functional groups/compound classes present in organic compounds and can be applied to liquid as well as solid samples. NMR spectroscopy as an analytical chemistry technique used in quality control and research for determining the content and purity of a sample as well as its molecular structure.

1.4 Hot Water Percolation

Hot water extraction was introduced by Keeney (1966) to determine easily available N fraction. Hot water extraction also was used in determination of boron, when the soil-water suspension was boiled for a few minutes (Berger and Truog 1944). Hot Water Percolation (HWP) is an extraction method that uses heat energy and pressure together and inroduced by Fuleky and Czinkota (1993), this method has several advantages such as: being easy and fast to use and its ability to measure several parameters from the same solution.

1.5 Vegetation

Vegetation significantly influence the quantity and quality of organic material in the soil, different vegetation produce different soil properties and any changes in the ecosystem will upset the balance of the soil, the amount of carbon sequestration depends on forest management, yield class, trees type and regimes (Islam and Weil 2000; Lapola et al. 2010). Forests provide economical, ecological, social and other services to natural systems and humans, refuges for biodiversity, hydrologic cycle, landscape, provision of food, medicinal, wood and protection of soil resources are the most benefits of forests (Bonan 2008; Hassan et al. 2005; Lapola et al. 2010; Willis et al. 2003).

1.6 Reforestation

Reforestation is the natural restocking of existing forests and woodlands that have been depleted, usually through deforestation, reforestation can be used to improve the quality of human life by soaking up pollution and dust from the air, rebuild natural habitats and ecosystems, mitigate global warming since forests facilitate biosequestration of atmospheric carbon dioxide, and harvest for resources, particularly timber. A debatable issue in managed reforestation is whether or not the

succeeding forest will have the same biodiversity as the original forest. If the forest is replaced with only one species of tree and all other vegetation is prevented from growing back, a monoculture forest similar to agricultural crops would be the result. Another important factor is the natural regeneration of a wide variety of plant and animal species that can occur on a clear cut.

3. MATERIAL AND METHODS

16 soil samples were selected from soil bank of Szent Istvan University, Godollo, Hungary. In Iran, a composite sampling was done in Guilan province, Iran, from A horizon (0-20 cm) of a virgin forest and a 15 years old planted forest which was cultured with a mixture of Caucasian Alder (*Aluns subcordata*) and Eastern Cottonwood (*Populus deltoids*) with the rate of 100:0, 70:30, 50:50, 30:70 and 0:100 (Alnus:Populus) in three replication and completely randomized.

Extraction was done using following method and solutions:

- HWP: Hot Water Percolation method
- NaHCO₃: 20 ml 0.05 M NaHCO₃
- NaOH : 20 ml NaOH 0.5%
- NaF: 20 ml NaF 1%

DOC was measured in different fractions of HWP extracts. The kinetics of extracting of HWP–DOC was estimated using UV VIS spectroscopy method at 200-700 nm and following parameter were measured:

- E₂/E₃, the UV absorbance ratio of 254 to 365 nm.
- E₄/E₆, the UV absorbance ratio of 465 to 665 nm.
- URI, the UV absorbance ratio of 210 to 254 nm.
- SUVA was estimated : absorbance value at 254 nm *100)/ organic carbon (mg/L)
- K factor.

Physical and chemical properties including, soil texture, total organic carbon, nitrogen, EC, pH, of Iranian soil samples were measured and the samples were fractionated using sonication method to sand, silt and clay. Humic substances were extracted with 10mL 0.5M NaOH. Extracted humic substances were dried using dried freezing method. ¹H and ¹³C spectra were recorded using Bruker Avance 400 Ultrashield NMR spectrometer. Data was analyzed in randomized design, using SAS statistical software, proc GLM and LSD Test was used to separate the means (P < 0.05).

4. **RESULTS**

In Hungarian soils, the extraction of dissolved organic carbon using HWP method has increased with the time and in all of the samples the most increasing was at the first five minutes. The process followed from the second order kinetics equation which is:

 $Y=A_1(1-exp(-k_1x))+A_2(1-exp(-k_2x))+Q$

Where "Y" is the sum of DOC, "t" is the time, " A_1 " is the maximum amount of DOC, " A_2 " is the, "K1" is the constant rate, "K2" is the ...and Q is . The most amount of DOC was in the first fraction of extract. The amount of HWP dissolved organic carbon in the fractions was decreased with increasing the time of extraction process

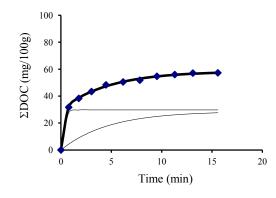


Figure 1: Diagrams of DOC kinetics in Magyaróvár sample.

UV-VIS spectroscopy

The absorbance values at 210, 254, 365, 465 and 665 nm in the first fraction of HWP extracts were shown in Figure 2. In all the samples the UV-VIS spectra followed the featureless, monotonic increase with decreasing wavelength. The spectra absorbance value has a positive relationship with the amount of organic compounds in the solutions (Fig. 2).

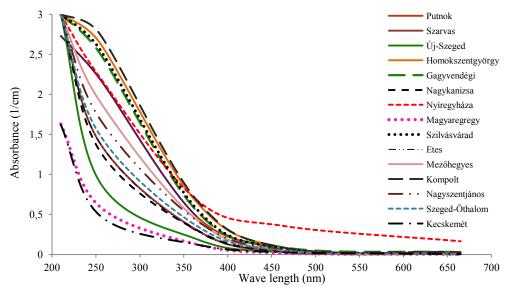


Figure 2: UV VIS absorbance of HWP extracts.

The absorbance value at 254 nm was found a measurement point of the amount of organic carbon in different fractions of HWP extracts (Fig. 3). There was a good correlation between the absorbance at 254 nm and the amount of HWP dissolved organic carbon in the fractions. The E_2/E_3 ratios in the samples were in a narrow range (3.29-4.84) and almost similar in different samples (Tab. 1).

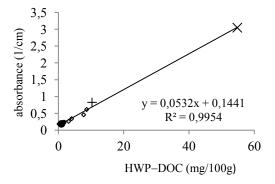


Figure 3: The correlation between HWP dissolved organic carbon (HWP–DOC) and absorbance value at 254 nm in different fractions (×: first fraction, +: second fraction and•: the last fraction) of Homokszentgyörgy sample.

The E_4/E_6 ratios of samples were in a wide range (2.14-13.5), Nyíregyháza, Putnok, Nagyszentjános, Gagyvendégi, Kompolt, Kecskemét had the lowest E_4/E_6 ratio, respectively (Tab. 1). present samples contain organic components with FA and NOM properties.

Samples	E_2/E_3	E_{4}/E_{6}	URI	SUVA
Magyaróvár	4.31	9.00	1.87	1.69
Szarvas	4.58	8.00	2.14	1.33
Új-Szeged	4.76	7.25	3.30	1.41
Homokszentgyörgy	4.70	8.60	0.98	1.85
Gagyvendégi	4.67	2.97	1.19	1.71
Nagykanizsa	4.28	4.43	2.31	1.19
Nyíregyháza	3.29	2.14	1.35	2.28
Magyaregregy	4.84	8.00	2.69	0.99
Szilvásvárad	4.56	12.17	1.05	1.90
Etes	4.82	13.5	2.14	2.34
Putnok	4.68	2.75	1.24	0.95
Mezőhegyes	4.45	6.25	1.57	1.23
Kompolt	4.09	3.33	1.05	1.43
Nagyszentjános	4.04	2.90	1.76	2.07
Szeged-Öthalom	4.23	5.87	2.00	1.30
Kecskemét	3.98	3.71	3.32	1.35

Table 1: UV VIS absorbance parameters of HWP extracts

Most of samples had the URI values in the range of humic acid (> 1.56) while Nagyszentjános and Magyaróvár samples were classified in the range of FA, the other samples which had the URI value more than 2, probably, contain lower aromaticity and more unsaturated compounds (Tab. 1). The

result of E_4/E_6 ratio of NaHCO₃ extracts indicated the presence of HA in the extracts of all the samples.

The result of K factor showed that most of samples were classified in third Hargitay group (3- Humic level of marsh, meadow–podzolic brown forest soil (10^{-2}) .

In the Iranian soil samples, soil texture for all samples was silty loam, there was a significant differences (P<0.05) in the percentage of total organic carbon, nitrogen and C:N ratio of different samples (Tab. 2).

Forest type*	Alnus:Populus%	TOC (%)	N (%)	C:N
VF		¹ a 3.28	a 0.127	b 26.18
	100:0	b 2.71	ab 0.1	b 27.43
PF	70:30	b 2.53	bc 0.073	b 30.64
	50:50	c 2.24	c 0.058	ab 39.08
	30:70	c 2.23	c 0.052	a 50.39
	0:100	d 1.92	c 0.054	ab 35.95

Table 2: The differences of TOC, N and C:N ratio in different samples

1: Values followed by the same letter are not significantly different at P < 0.05.

*: VF: virgin forest, PF: planted forest.

Virgin forest sample contained the maximum value of TOC, there was not a significant difference between 100:0 and 70:30 samples of planted forest. The 0:100 contained minimum value of TOC. There was a significant difference (p < 0.05) in the percentage of TOC, N and C:N ratio of soil fractions (Fig. 15). Clay and silt fractions contained the maximum and minimum values of TOC, respectively. The amount of N in the clay fraction was the highest and there was no significant difference between sand and silt fractions. Sand fraction had the maximum value of C:N ratio and no significant difference was found between silt and clay fractions in this parameter.

- NMR spectroscopy

¹H and ¹³C NMR spectroscopy analysis carried out on three soil fractions (sand, silt and clay). The result of ¹³C NMR showed different proportion of alkyl; O-alkyl; aromatic and olefin; carboxyl, carbonyl, ester and amid; aldehyd and ketone carbons in different samples (Tab. 3). This may be due to the fact that the soil organic matter in top soils is dominated by high proportions of plant residues with a relatively uniform composition. Organic compounds in sand particle size contained high proportion of oxygenated aliphatic groups (Tab. 3) that can be assigned methoxyl groups, O-alkyl groups and dioxygenated alkyl groups (Mao et al. 2001; Wilson 1987). The proportion of aromatic carbon in sand fraction of different samples was different and it was lower than aliphatic structure (except 50:50 sample) (Tab. 3). Zech et al (1992) indicated that the aromatic and olefin structures are found in lignin and tannins compounds. With humification progress, these compounds degrade to simple organic material with more aliphatic structures. The maximum proportions of aliphatic carbon in sand fraction of different samples were found in the 70:30 and 0:100 samples (Tab. 3). The highest

and lowest aromaticity in sand fraction were in the 50:50 and 100:0 samples, respectively. The result of ¹H NMR of sand fractions indicated the presence of different H forms linked to aliphatic groups, carbohydrate structure and aromatic molecules in the shifts 0.5-3, 3-4.2 and 6-8 ppm, respectively (Fig. 4, a). Virgin forest contains all three mentioned H forms in different proportions. Aliphatic H was the main organic group and aromatic the lowest. In the sample 100:0 the proportions of all aliphatic, carbohydrate and aromatic H are lower than virgin forest (Fig. 4, a). In the sample 70:30, the proportions of aliphatic and carbohydrate H are lower than virgin forest and higher than 100:0, but aromatic H is higher than both of them (Fig. 4, a). The samples 50:50 and 30:70 shows similar results while the peaks in some regions was higher in the sample 50:50. In the sample 0:100, high proportions of aliphatic and carbohydrate H are obvious, the aromatic H (6-8 ppm) is lower than two other H forms (Fig. 4, a). Overall the most important difference in sand fraction between virgin and planted forest was the aromatic H which was in virgin forest lower than planted forest.

		Chemical shifts					anamatiaita
Sample	Fraction	0-45 ^a	45–110 ^b	110-165 ^c	165-190 ^d	190- 220 ^e	aromaticity
		%					
	Sand	67.57	0.68	0.68	0.68	30.41	0.9
VF	Silt	16.92	29.27	20.81	27.24	5.75	31.06
	Clay	39.06	39.84	4.3	16.41	0.39	5.16
	Sand	0.07	59.11	0.07	40.68	0.07	0.11
100:0	Silt	68.03	0.68	2.72	0.68	27.89	3.81
	Clay	44.05	39.21	0.44	15.86	0.44	0.53
	Sand	38.17	40.08	8.01	13.36	0.38	9.29
70:30	Silt	33.33	22.67	0.33	43.33	0.33	0.59
	Clay	38.46	19.23	0.38	41.53	0.38	0.65
	Sand	0.18	6.13	63.75	0.18	29.77	90.99
50:50	Silt	21.88	16.63	0.22	61.05	0.22	0.56
	Clay	52.36	27.75	0.52	18.85	0.52	0.65
	Sand	15.7	31.55	22.13	27.47	3.14	31.9
30:70	Silt	0.94	22.21	26.50	37.87	12.48	53.39
	Clay	29.07	14.53	24.71	31.39	0.29	36.17
	Sand	26.45	45.50	15.34	12.43	0.26	17.58
0:100	Silt	24.27	34.22	15.05	22.813	3.64	20.46
	Clay	3.19	25.51	27.87	25.22	18.21	49.27

Table 3: The proportion of chemical shifts and different types of carbon in different fractions

a: Alkyl carbons (aliphatic –CH, –CH, and –CH groups); b: O-alkyl carbons (C of amino groups as well as of O–CH, C–O groups of carbohydrates, alcohols, and ethers); c: Aromatic and olefin carbons; d: Carboxyl, carbonyl, ester and amid carbon; e: Aldehyd and ketone carbon.

¹³ C NMR showed that organic compounds of silt fraction contained high proportion of carbonyl and carboxyl carbon in chemical shift of 165-190 ppm (Tab. 3). Zech et al. (1992) found the increasing of carboxyl groups from non humified to humified layer in the forests soil. In the 100:0 sample the highest proportion was found in chemical shifts of 0-45 ppm and alkyl carbon region (Tab. 3). The presence of high proportion of alkyl carbon in this sample is probably the result of O-alkyl compounds degradation. The most aromaticity in silt fraction was found in 30:70 sample (Tab. 3). ¹H NMR result of silt fractions indicated the presence of aliphatic, carbohydrate and aromatic H in the shifts 0.5-3, 3-

4.2 and 6-8 ppm in different proportion. All H forms in silt fraction of virgin forest sample have smaller peaks in comparison to the other sample (0:100 is exception) (Fig. 4). The result of ¹H NMR of silt fraction in planted forest showed high aliphatic, carbohydrate and aromatic H with a wide diversity in the samples 50:50 and 30:70 (Fig. 4, b). The proportions of all forms of H, aliphatic, carbohydrate and aromatic in planted forest sample were higher than virgin forest in this faction (Fig. 4, b).

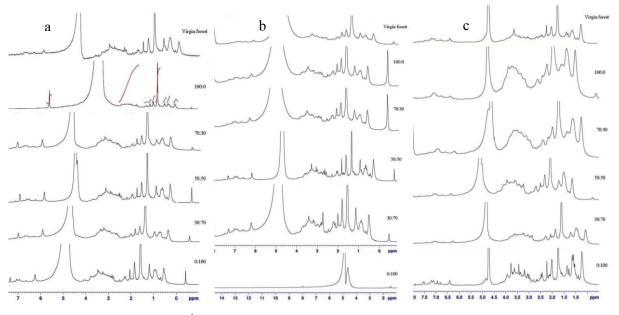


Figure 4: ¹H NMR of sand (a), silt (b) and clay (c) fractions of samples

The clay fraction mostly contained highest proportions of aliphatic which followed by oxygenated aliphatic groups in chemical shift of 0-45 and 45-110 ppm, respectively (Tab. 3). The fine fractions of soils are more different from the bulk soil in their organic matter composition than the coarse fractions. Clay-size fractions generally show a higher content of alkyl carbon than the whole soils, as revealed by ¹³C NMR spectroscopy (Mahieu et al. 1999). The clay fraction of 0:100 and 100:0 samples contained the highest and lowest proportion of aromaticity. All H forms in the clay fraction of the virgin forest sample showed higher and lower peaks in comparison to silt and sand fractions, respectively (Fig. 4,c). In the planted forest samples the proportions of aliphatic and carbohydrate H were higher than sand and silt (Fig. 4, c). The aromatic H in the clay fraction of 0:100 sample was higher than sand and silt, the result also showed more diversity in aliphatic and carbohydrate H in clay fraction of this sample.

5. CONCLUSION

Extracting process with the HWP method followed from second order kinetic equation. The highest values of DOC are extracted before 5 first minutes. There is a positive relationship between the absorbance value and DOC. In all the samples the UV-VIS spectra followed a decrease with increasing wavelength. It seems that HWP method is suitable to extract small and more soluble fraction of fulvic acids which has low degree of polymerization, low MW, less carbon and more oxygen content. The result of second part of study (Iranian soil) showed that virgin forest sample contained the maximum values of TOC and N. The C:N ratio of virgin forest sample was lower and indicated greater humification degree of soil organic matter in this sample. Clay fraction contained the maximum amounts of TOC, N and less C:N ratio. The organic carbon and nitrogen quantity of planted forests with the higher ratio of Alnus which was one of the native species in the sampling site was similar to virgin forest. Virgin forest and 100:0 of planted forest contained higher values of DOC in HWP extracts. Virgin forest had a wide range of aliphatic carbon that confirms more humification degree in this sample. Sample 0:100 of planted forest contained the most proportion of aromatic and olefin carbons which show less degree of humification in this sample.

- New scientific results
- The E₂/E₃, E₄/E₆ and URI parameters indicated that the most humic substances extracted with Hot Water Percolation method contain fulvic acid compounds.
- There is a good correlation between the value of HWP dissolved organic carbon and the absorbance at 254 nm.
- The organic carbon quantity of planted forest with the higher ratio of Alnus which was the most native species of the sampling site was more similar to virgin forest.
- In case of soil organic carbon quality and quantity, 70:30 was the best ratio of Alnus:Populus species.
- NMR study showed that the most of organic compounds in sand particle size contained high proportion of oxygenated aliphatic groups while in silt they were carbonyl and carboxyl carbons and for clay fraction both aliphatic, oxygenated aliphatic as well as carbonyl and carboxyl carboxs.

6. SUMMARY

In this study which included two parts, we selected 16 soil samples from Hungary. Soil samples were extracted with HWP method. The amount of HWP dissolved organic carbon in each fraction was measured. The absorbance of UV VIS spectra was measured at 200-700 nm, E_4/E_6 and E_2/E_3 ratios were determined, URI, SUVA was estimated in each sample. Soil samples were extracted with NaHCO₃ and their E_4/E_6 ratio was determined. In the second part soil sampling was carried out from both virgin and planted forests. Soil fractionation carried out using sonication method, and the percentage of total organic carbon and nitrogen and C:N ratio were measured in soil samples and fractions. Data were analyzed using SAS statistical software. Humic substances were extracted from soil fractions using NaOH. ¹H and ¹³C were analyzed using NMR spectrometer.

The extracting of HWP dissolved organic carbon followed from the second order kinetics equation which is: $Y=A_1(1-exp(-k_1x))+A_2(1-exp(-k_2x))+Q$

Where "Y" is the sum of DOC, "t" is the time, "A₁" is the maximum amount of DOC, "A₂" is the, "K₁" is the constant rate, "K₂" is the ...and Q is . There was a good correlation between the content of HWP dissolved organic carbon and the absorbance at 254 nm. URI, SUVA, E_2/E_3 and E_4/E_6 indicated that the most of HWP dissolved organic carbon of samples was fulvic acid components with low molecular weight. The soil sample of virgin forest contained the most total organic carbon, nitrogen and the minimum value of C:N ratio. Clay fraction showed the maximum amounts of total organic carbon and nitrogen. The most value of C:N ratio was estimated in sand fraction. The result of ¹H and¹³C NMR indicated a wide range of organic components with aliphatic and carbohydrate structure in different samples and the sample with non-native species showed lower degree of organic matter degradation and humification. However there was low and high proportions of alkyl and aromatic carbons in the sample with non-native species.

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8. RELATED PUBLISHMENTS

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