

COMPARISON OF CHEMICAL STRUCTURE OF ALGINITE HUMIC ACIDS ISOLATED WITH TWO DIFFERENT PROCEDURES WITH SOIL HUMIC ACIDS

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The different origin of alginite and soil organic matter may be the reason of differences in their humic acids (HA) chemical structure. One of the aims of this article is to compare the chemical composition of alginite HA and HA isolated from different soil types. Another aim of this article is to compare the chemical structure of humic acids of alginite isolated with two different procedures: modified IHSS (International Humic Substances Society) method and simplified extraction method. The modified IHSS method was applied for the isolation of alginite and soil HA. To obtain sufficient amount of alginite HA for biological experiments, simplified extraction method suited for large volumes of HA was applied. The differences in elemental analysis and ash proportion in HA extracted by modified IHSS method (C = 35.4, H = 43 atomic%, ash content = 0.08%) and simplified extraction method (C = 31, H = 31 atomic%, ash content = 7.42%) can be caused by different concentration of extraction solution and also differences in purification of HA. The differences in chemical structure between alginite HA and HA isolated from different soil types according to the data of elemental analysis (C content of alginite HA = 35.4 atomic%, C content in soils HA = 38.2–49.1 atomic%) and ^{13}C nuclear magnetic resonance (NMR) spectra (degree of aromaticity of alginite HA = 24.4% and soil HA = 35.9–53%) were found. Results of ^{13}C NMR show that the content of aromatic carbon was decreasing in the following order: Haplic Chernozem HA > Andic Cambisol HA > Haplic Cambisol HA > alginite HA. Based on the obtained results, it can be concluded that the differences in the chemical structure of alginite and soil HA can be explained by the difference in the origin of organic matter in alginite and soil samples. The source of organic matter in alginite is mainly type II kerogen from algae and that of soil is lignin and cellulose (type III kerogen) of higher plants.

Key words: alginite, ^{13}C NMR spectra, elemental analysis, humic acid, soil

Agricultural intensification that results in deterioration of soil structure and loss of organic matter is one of the main problems nowadays. The implementation of suitable natural materials that are non-toxic and their effect is almost in short time after application could be a chance for the future agriculture. One of such material would be alginite. The name *alginite* originally belonged to petrographic constituent of coal residues consisting of algae. However, in 1980s, Hungarian author Solti (1987) used this name for dark grey laminated rock with algae occurrence. Alginite is fossil algae created in the Pannonian lakes system. Three to four million years ago,

because of volcanic eruptions, algae together with eroded rocks formed continuous sediment (Kulich *et al.* 2001). Alginite is a natural organo-mineral substance, which in all respects meets the demands of clean and environmentally friendly revitalisation material. This neutral material with high amount of organic matter, balanced proportion of biogenic and trace elements, the absence of risk elements and high sorption capacity has prospective use in agricultural practice (Kulich *et al.* 2001). This non-toxic material occurs in the south part of Slovakia near Pinciná village and also in Gercei and Pula in Hungary (Kulich *et al.* 2001; Vass *et al.* 1997). Alginite

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applied to soil is able to improve the granular soil structure and helps in regeneration of soil organic matter. It is promising material that is capable of reclaiming the land without adverse effects. In agriculture, alginite is also able to improve the water and nutrients regime and increasing of soil colloids (Beláček 2006).

In addition to using alginite as a soil improver, we look for other options of its application in agriculture. Currently, we are working on a project to use alginite and humic acids (HA) isolated from alginite for stabilisation of beneficial micro-organisms and for the needs of the development of new forms of beneficial microorganisms as agents to stabilise and harmonise the microenvironment of animals. Alginite and HA isolated from alginite were used as stimulants for beneficial bacteria. Also the effect of alginite and HA isolated from alginite on the microenvironment of the intestine was studied.

Organic matter of alginite is a component of some types of kerogen with a predominance of type II alongside amorphous organic matter (Vass *et al.* 1997). Main precursors of soil humic substances are plant biopolymers (Tan 2014). Difference in origin of alginite organic matter and soil organic matter can be reason for the differences in chemical composition of one of the main fractions of humus substances – HA.

Generally, for the extraction of HA, various modifications of standard IHSS method are used (Hayes 1985). However, this method is not a very suitable method for the extraction of huge amount of alginite HA that were needed for testing of their biological effect. We tried to develop simplified extraction method suited for large volumes of alginite HA.

This article has two aims, one aim is to compare the chemical composition of alginite HA and HA isolated from different agriculture and forest soil types. Another aim is to compare chemical structure of HA of alginite isolated using two different procedures: modified IHSS method and simplified extraction method suited for large volumes.

MATERIAL AND METHODS

The alginite samples were taken from the supply, which is located near the Pinciná village

in the south part of Slovakia, $x = -374,479.89$, $y = -1,272,800.02$. GPS coordinates are in a coordinate system S-JTSK Krovak North East.

The main characteristics of alginite are given in Table 1, and detailed description of alginite supply and alginite samples can be found in our previous article (Litavec & Barančíková 2013).

For the isolation of soil HA, two soil samples were taken from agricultural soils and two samples from forest soils. The modified IHSS method (Barančíková *et al.* 2011) was applied for the isolation of alginite and soil HA (Method A).

Detail procedure of simplified extraction method suited for large volumes of alginite HA, method B, is as follow: 200 kg of preliminary drying alginite was mixed with 0.035 M KOH on the resulting pH=8. Subsequently, 2 kg of KOH was added. Concentration of KOH solution was increased to 0.1 M and the pH of solution was increased to 11.1. The resulting alkaline supernatant was set into big vessel. To this solution, concentrated HCl was added until pH dropped to 2.5. In this extremely acidic solution, HA were precipitated. The supernatant that remains after the precipitation of HA containing FA was added to next amount of 200 kg of preliminary drying alginite. Into this solution, 500 l of 0.035 M KOH was added. Subsequently, 2 kg of KOH was added and the concentration was increased to 0.01 M. This alkaline solution was added to precipitate HA. Subsequently, concentrated HCl was added, and the pH dropped to 2.24. Sedimentation of HA was accelerated by aeration and 200 l of distilled water was added. Precipitated HA was washed with distilled water.

Both the methods applied for the isolation of alginite HA are based on the same principle: the extraction of humic substances by alkaline solution of hydroxide. Dark brown solution of humic substances is precipitated by concentrated HCl to pH = 1–2. Despite the same principle of extraction of both methods, some differences exist between methods. The first difference is the extraction of humic substances by NaOH in case of modified IHSS method (method A) and by KOH in case of simplified method suited for large volumes (method B). This difference, however, does not cause the differences in quality but in the yield of HA. The yield of HA by the method A was higher in comparison with meth-

T a b l e 1

Organic carbon content [%] and basic biogenic elements [mg/kg] in alginite

	C _{org}	N	P	K	Ca	Mg	Mn
Alginite	7.5	3.090	440	1.550	6.889	7.157	448

C_{org} – organic carbon; N – total nitrogen; P – available phosphorus; K – kalium; Ca – calcium; Mg – magnesium; Mn – manganese

od B. The differences in elemental analysis and ash proportion in HA can be caused by different concentration of extraction solution and also differences in purification of HA. Isolated raw HA by method A is soluble in 2 M NaOH and subsequently is precipitated by concentrated HCl to pH=1–2. Precipitated HA is purified by 0.3% mixture of HCl–HF (170 ml of HCl–HF mixture per 100 g of alginite) and dialysed against distilled water until chloride free and then it is dried. The purification step (0.3% mixture of HCl–HF) is not included in the method B.

Elemental analysis of alginite and soil HA was made by elementary analyser CHN 2400 Perkin-Elmer. ¹³C NMR spectra were carried out on spectrometer Varian Inova 600 (frequency 150 MHz). As published by Malcolm (1990), Preston (1996), Enev (2014) and many others, ¹³C NMR spectroscopy is able to characterise the types of carbon and aromaticity degree of HA samples. Subdivision of the spectrum has been made by the commonly used scheme of Malcolm (1990). The degree of aromaticity of HA was calculated using the formula: $\alpha = C_{ar} / (C_{ar} + C_{aliph} \times 100$ [%]. Detailed procedures for the determination of organic carbon, total nitrogen, biogenic elements in alginite, elemental analysis of HA and ¹³C NMR parameters of HA can be found in the paper titled 'Methods of determining indicators of agrochemical soil properties' (Barančíková *et al.* 2011)

RESULTS AND DISCUSSION

Comparison of chemical structure of alginite HA isolated with two different procedures

As it can be seen in Table 2, the biggest differences between HA isolated by method A (HA A) and B (HA B) are in ash content. The ash in HA

represent inorganic substances, mainly silica (SiO₂) (Vass *et al.* 1997). Whilst HA A contains a minimal amount of ash (0.08%), the amount of ash in HA B is significantly higher (7.42%). We assume that this is mainly due to the absence of purification step in the method B.

Data of basic biogenic elements in HA A and HA B are substantially different (Table 3). The amount of organic carbon, hydrogen and nitrogen in HA A is significantly higher in comparison with HA B (Table 3).

Differences between HA A and HA B are also clear from elemental composition expressed in atomic percentages (Table 4). A number of scientists believe that elemental composition based on the weight percentages cannot be used to explain the molecular structure of humic substances. For the purpose of studying and devising structural formulas for humic substances, they suggest the use of atomic percentage (Tan 2014). Orlov (1985) and Steelink (1985) reported that atomic percentage and atomic ratios are useful as guiders in the identification of different types of humic acids. The greater C/H and C/O ratios indicate the higher role of the carbon atoms in the composition of molecular structure. Increase in atomic ratio indicates growth of benzenoid fragments and reduced level of aliphatic chains (Orlov & Grišina 1981). The values of C/H ratio less than 1, in HA A and HA B (Table 4), indicate a predominance of aliphatic structures in alginite HA. The low content of carbon and high value of hydrogen, as well as high value of H/C ratio in alginite HA, indicate predominance of aliphatic carbon and low humification degree of alginite HA because Debska *et al.* (2009) and Rossel *et al.* (1989) reported that the smaller the H/C ratio, the bigger will be degree of humification.

Significant predominance of aliphatic structures over aromatic condensed cores in alginite HA structure is clear also from ^{13}C NMR spectra (Figures 1 and 2). ^{13}C NMR spectra are significant contribution to deepening structure of HA (Barančíková 2008; Enev *et al.* 2014). ^{13}C NMR can quantitatively detect different carbon types (carbonyl, carboxyl, aromatic, olefinic, anomer, aliphatic carbon), and for this reason, this spectral technique is very useful to determinate HA chemical structure of different origin.

In ^{13}C NMR spectra of both HA A and HA B, sharp peaks can be seen in spectral region 43–87 ppm (Figures 1 and 2). This region is characteristic

for long $-\text{CH}_2-$ chains and also for methyl groups $-\text{CH}_3$ in spectral region 15–43 ppm (Figure 2). The sharp signal at 75 ppm is typical for carbohydrates (Preston 1996; Schnitzer *et al.* 2006) and relatively poor signal at 104 ppm is specific for di-O-alkyl carbon of anomeric carbons of polysaccharides (Preston 1996).

Aromatic region of alginite HA is less marked as aliphatic (Figures 1 and 2). Aromatic carbon is situated in 120–135 ppm (Schnitzer *et al.* 2006), and wide peak at 129 ppm is characteristic for H- and C-substituted aromats (Preston *et al.* 1994). Phenolic carbon of HA is in the 145–165 ppm and this area, in comparison with the C- and H-substituted

T a b l e 2

Basic parameters of alginite HA isolated using modified IHSS method (method A) and simplified procedure adjusted for large volumes (method B)

Alginite HA	Dry mater	Ash	Moisture	HA
	[%]			
HA A	90.57	0.08	9.43	90.50
HA B	90.57	7.42	9.43	83.16

HA A – alginite humic acids isolated by method A; HA B – alginite humic acids isolated by method B

T a b l e 3

Values of elemental analysis of alginite HA

Alginite HA	Real sample Weight [%]			100% ash free HA Weight [%]		
	C	H	N	C	H	N
HA A	47.20	5.85	2.96	52.16	5.29	3.27
HA B	30.86	3.68	1.62	37.11	3.15	1.94

HA A – alginite humic acids isolated by method A; HA B – alginite humic acids isolated by method B;
C – carbon; H – hydrogen; N – nitrogen

T a b l e 4

Elemental analysis [atomic%] and proportion of individual elements

Alginite HA	[Atomic%]								
	C	H	N	O	H/C	O/C	O/H	C/H	C/O
HA A	36.36	42.75	1.90	19.99	–	–	0.468	0.83	1.77
HA B	31.00	31.35	1.39	36.26	1.012	1.170	1.158	0.99	0.86

HA A – alginite humic acids isolated by method A; HA B – alginite humic acids isolated by method B;
C – carbon; H – hydrogen; N – nitrogen; O – oxygen

aromats, is often weak and broad and indicates the presence of the phenolic carbon of various origin (Gonzales-Perez *et al.* 2008). At 175 ppm, significant carbon peak characterising carboxyl, amides and esters (Preston *et al.* 1994; Schnitzer *et al.* 2006) can be found. Signal of carboxylic carbon is corresponding with carboxylic groups in aliphatic and benzenecarboxylic acids (Gonzales-Perez *et al.* 2008). The absence of sharp peaks in aromatic regions is evident in both alginite HA (Figures 1 and 2).

From the HA chemical structure point of view, the most important ^{13}C NMR parameters are the percentage of aliphatic and aromatic carbon and degree of aromaticity (α). In both HA isolates from alginite, percentage of aliphatic carbon is much higher as aromatic carbon (Table 5). The degree of aromaticity, which is the most common parameter to assess the degree of humification (Gonzales-Perez *et al.* 2008), is reported in most of the works that evaluated the chemical structure of HA based on the ^{13}C NMR spectroscopy (Malcolm 1990; Schnitzer *et al.* 2006;

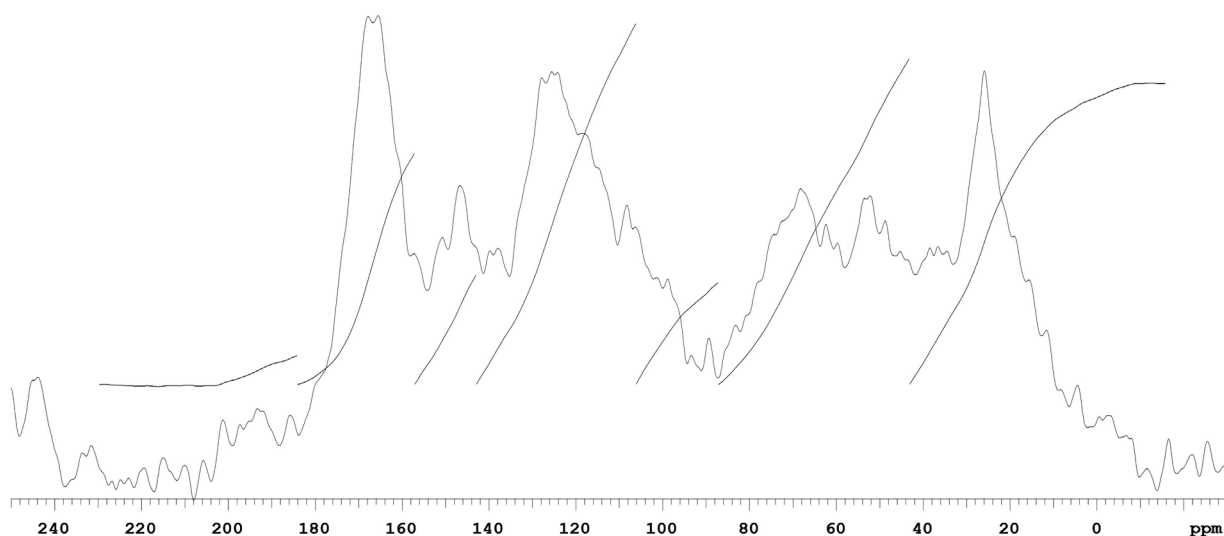


Figure 1. ^{13}C NMR spectra of alginite HA A

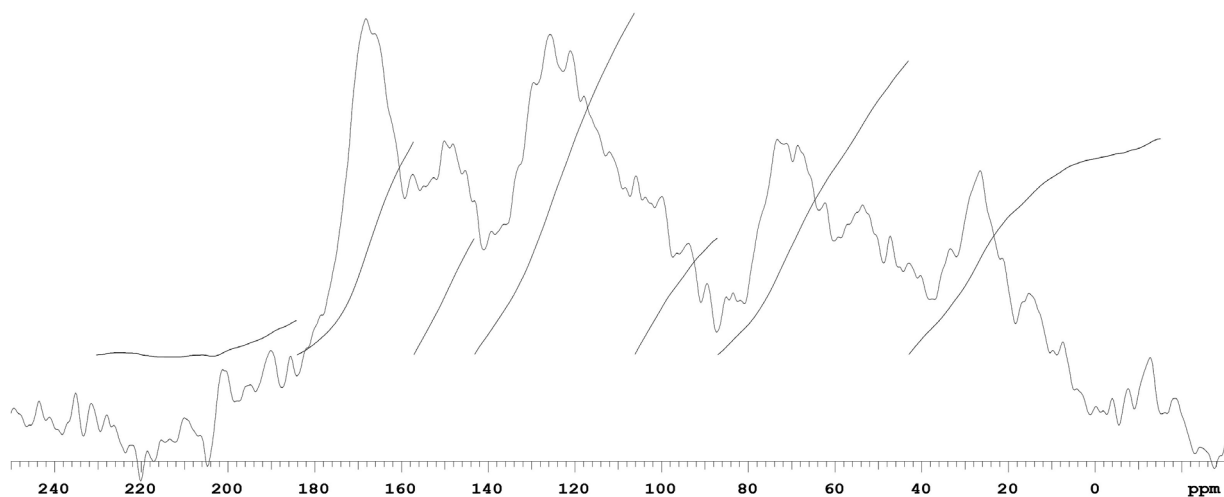


Figure 2. ^{13}C NMR spectra of alginite HA B

Ono *et al.* 2009). The higher values of α are characterised for more stable structure of HA. As it can be seen from Table 5, values of aromaticity degree are very low, the first of all in HA B.

Comparison of chemical structure of alginite HA and soil HA

Alginite HA A was taken for comparison of chemical structure of alginite and soil HA because for the

isolation of soil HA and alginite HA A, the same isolation method (modified international IHSS method) was applied. Soil HA were isolated from different soil types and different land use. Haplic Cambisol 1 and Andic Cambisol are forest soils, Haplic Chernozem is typical agriculture arable soil and Haplic Cambisol 2 is pasture. The lowest concentration of organic carbon was found in Haplic-Chernozem and

T a b l e 5

Relative integral intensities [ppm] and basic parameters calculated from ^{13}C NMR spectra of alginite HA

Alginite HA	230–184 [ppm]	184–157 [ppm]	157–143 [ppm]	143–106 [ppm]	108–87 [ppm]	87–43 [ppm]	43–15 [ppm]	C _{aliph} [%]	C _{ar} [%]	α [%]
HA A	1.95	8.12	3.96	17.88	9.27	39.27	18.95	67.49	21.84	24.5
HA B	2.24	8.79	1.15	10.11	11.79	50.81	15.11	77.71	11.26	12.7

HA A – alginite humic acids isolated by method A; HA B – alginite humic acids isolated by method B;
C_{aliph} – aliphatic carbon; C_{ar} – aromatic carbon; α – degree of aromaticity

T a b l e 6

Soil types, total organic carbon (TOC) in alginite and soils, amount of isolated HA [%], moisture and ash content of HA

Soil type/material	TOC [%]	HA	HA [%]	Moisture [%]	Ash [%]
Alginite Pinciná	7.3	Alginite HA	0.13	9.4	0.08
Haplic Cambisol 1	7.5	Soil HA 1	0.97	9.8	0.90
Andic Cambisol	18.5	Soil HA 2	0.92	11.0	0.54
Haplic Chernozem	2.1	Soil HA 3	0.41	11.7	0.78
Haplic Cambisol 2	5.2	Soil HA 4	1.03	9.7	1.06

TOC – total organic carbon; HA – humic acids

T a b l e 7

Elemental composition [atomic%] and atomic H/C and C/N ratios of HA

Samples	C	H	N	O	H/C	C/N
Alginite HA	35.4	42.8	1.9	20.0	1.21	18.63
Soil HA 1	40.6	38.7	3.2	17.6	0.94	12.69
Soil HA 2	42.1	35.9	2.8	19.2	0.85	15.04
Soil HA 3	49.1	28.1	3.8	18.6	0.58	12.92
Soil HA 4	38.2	40.7	3.5	17.5	1.07	10.91

C – carbon; H – hydrogen; N – nitrogen; O – oxygen

the highest concentration of organic carbon was detected in forest Andic Cambisol (Table 6).

Alginite has the concentration of organic carbon same as that of forest soil (Tables 1 and 6); however, amount of HA isolated from alginite (alginite HA A, Table 6) was more than seven times lower in comparison with HA isolated from Haplic Cambisol (soil HA1) (Table 6). Agriculture soils, mainly intensively arable Haplic Chernozem, has more than three times lower organic carbon content (Table 6) in comparison with alginite sample; however the

amount of HA isolated from this soil type (soil HA 3) was more than three times higher (Table 6).

The difference in the amount of HA in alginite and soil samples can be explained by the difference in the origin of organic matter in alginite and soil samples. Alginite is fossil biomass with high content of organic matter comes from mainly yellow-green algae *Botryococcus braunii* Kunz. Its high hydrocarbon potential and immature kerogen content correspond to the initial stage of the production of petroleum hydrocarbons (Vass *et al.* 1997)

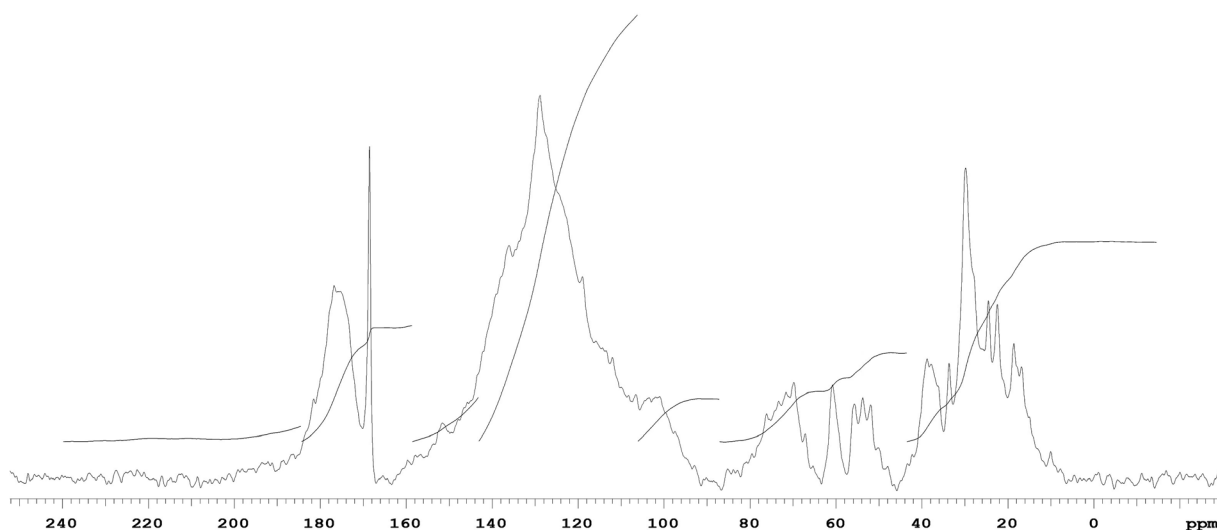


Figure 3. ^{13}C NMR spectra of soil HA 1

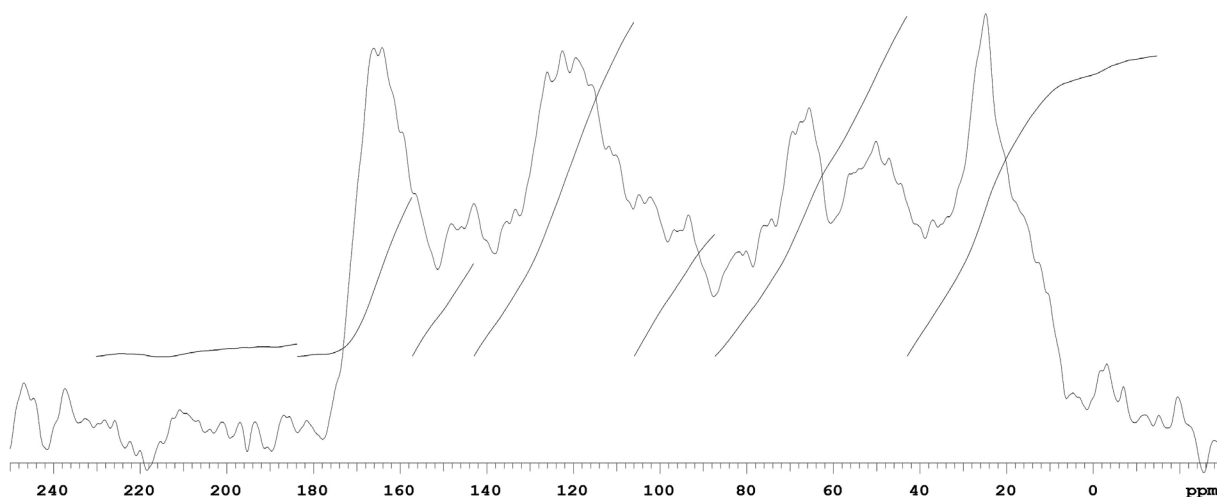


Figure 4. ^{13}C NMR spectra of soil HA 2

Source of soil organic matter is lignin and cellulose of higher plants (Madronova *et al.* 2011; Tan 2014). Moisture and ash content in all observed humic acids are relatively same, as they were isolated in the same way (Table 6).

Values of elemental composition of HAs are quite different (Table 7); however, they are typical for soil HA. As reported by Tan (2014), the amount of carbon in atomic percentage in soils is in the range 35–48%

and that of hydrogen is 30–43%. Similar range of C and H was found also in Slovak soils (Barančíková 2014). The highest concentration of organic carbon was found in HA isolated from Haplic Chernozem (soil HA 3), and the lowest concentration of C was detected in alginite HA; contrastingly, the highest H content was detected in alginite HA, and the lowest H content was detected in Chernozem HA (Table 7). In alginite HA, the lowest N content and the highest

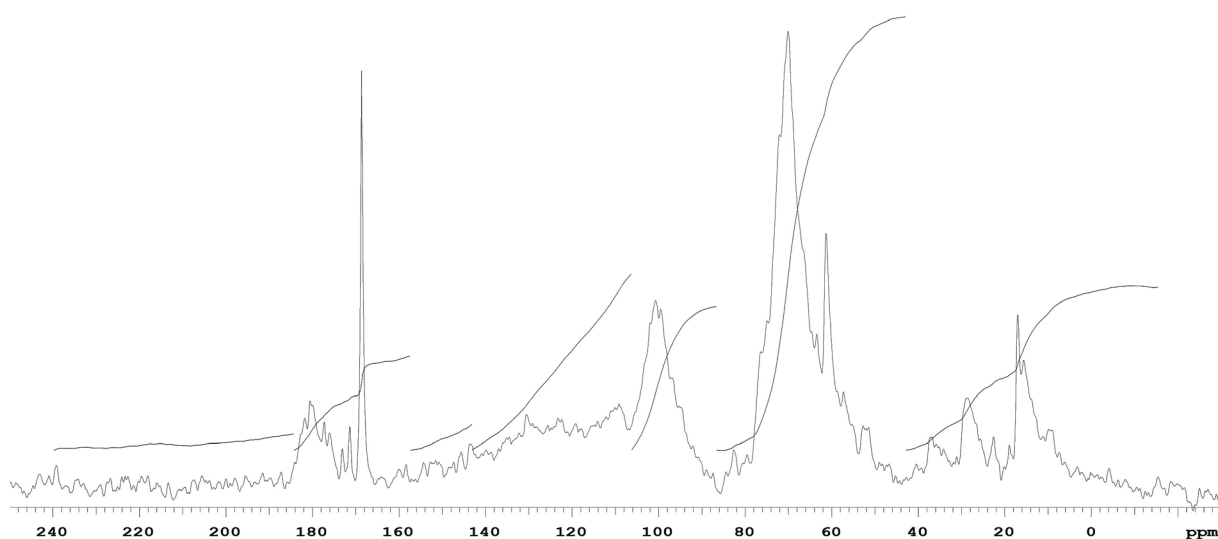


Figure 5. ^{13}C NMR spectra of soil HA 3

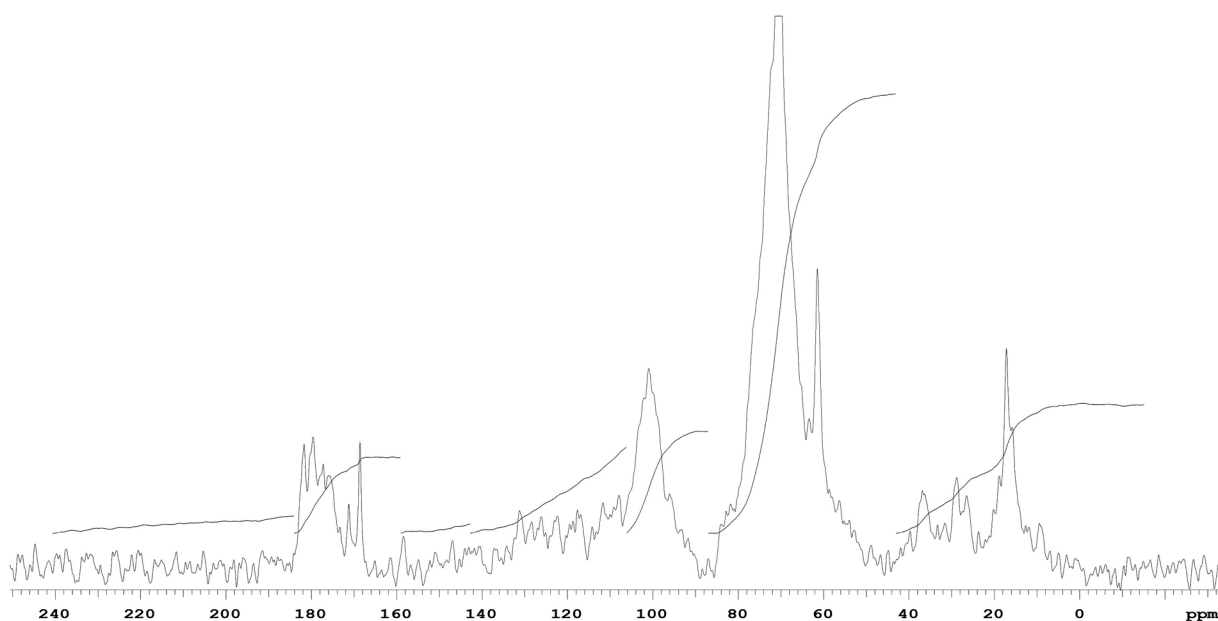


Figure 6. ^{13}C NMR spectra of soil HA 4

O content in comparison with soil HA was also detected (Table 7).

As it was mentioned earlier, atomic percentage and atomic ratios are useful as guider in the identification of different types of HA (Sterlink 1985). It is generally believed that the C/N ratio will decrease with increased rate of degree of humification, and C/N ratios between 10 and 15 are often considered to be the characteristic of well-developed HA (Tan 2014).

As can be seen in Table 7, all soil HA are well developed because their C/N ratios vary from 10 to 15; however, C/N ratio of alginite HA is greater than 18. Humification degree can also be judged according the H/C value. H/C atomic ratio is commonly used to indicate the maturity of humic substances (Sanchez *et al.* 2002). A low H/C ratio is characteristic of a complex humic substances structure with aromatic character as a consequence of polymerisation (humification) reactions. As it was mentioned earlier, Debska *et al.* (2009) and Rossell *et al.* (1989) reported that the smaller the H/C ratio, the bigger will be the degree of humification. According to this, amongst soil HA, Haplic Chernozem HA has the highest degree of humification and Cambisol HA on pasture the lowest (Table 7). Substantially higher H/C ratio in alginite HA than soil HAs (Table 7) indicates the lowest degree of humification of alginite humic organic matter compared to that of soil HA.

Considerable differences between humic acids isolated from different soil types and alginite HA can be seen also from ^{13}C NMR spectra. ^{13}C NMR technique allows to clarify in detail the chemical

structure of heterogeneous compounds. Spectral method of ^{13}C NMR can also detect the variation in chemical structure of HA of various soil types (Buurman *et al.* 2009), in one soil type at the change of soil management (Mao *et al.* 2008) or in different origin (Tan 2014). The analysis with ^{13}C NMR spectroscopy is capable of measuring the distribution of C in the various types of compounds, and this information can be used in structural analysis and in differentiating the different types of humic matter (Tan 2014). Significant predominance of aliphatic structures (C) over condensed aromatics cores (C_{ar}) in the alginite HA structure is evident also from ^{13}C NMR spectra (Figures 1 and 3–6, Table 8).

Strong signal at 75 ppm typical for carbohydrate carbon (Schnitzer *et al.* 2006) and much less marked aromatic regions (143–106 ppm) are the dominant features of alginite HA in ^{13}C NMR spectra (Figure 1). Higher content of aromatic carbon (C) and lower amount of aliphatic carbon (C) are characteristic of soil HA (Figures 3–6, Table 8), however, among them are significant differences.

Soil HA isolated from Haplic Chernozem (soil HA 3) has strong signal between 105 and 165 ppm chemical shift for aromatic carbon. In Haplic Cambisol HA (soil HA 1 and 6), aliphatic structures predominate, and carbon peak of methoxyl structure at 56 ppm and O-alkyl peak at 43–87 ppm can be recognised in ^{13}C NMR spectra (Gonzales-Perez *et al.* 2008). In Cambisol HA, peak at 75 ppm typical for carbohydrates (Preston *et al.* 1994; Schnitzer *et al.* 2006) (Figures 3 and 6) and peak at 104 ppm specific for di-O-alkyl carbon involving anomeric carbon of carbohydrates (Preston *et al.* 1994) can

T a b l e 8

Relative integral intensities [ppm] and basic parameters calculated from ^{13}C NMR spectra of alginite and soil HA

Sample	230–184 [ppm]	184–157 [ppm]	157–143 [ppm]	143–106 [ppm]	108–87 [ppm]	87–43 [ppm]	43–15 [ppm]	C_{aliph} [%]	C_{ar} [%]	α [%]
Alginite HA	1.95	8.12	3.96	17.88	9.27	39.27	18.95	67.5	21.8	24.4
Soil HA 1	2.00	15.85	7.52	24.73	6.97	22.32	20.61	49.9	32.3	39.3
Soil HA 2	2.59	16.01	8.75	25.66	8.76	22.04	16.19	47.0	34.4	42.3
Soil HA 3	2.90	11.80	6.20	39.10	4.80	12.50	22.80	40.1	45.3	53.0
Soil HA 4	0.90	11.71	6.86	24.54	8.96	24.99	22.04	56.0	31.4	35.9

C_{aliph} – aliphatic carbon; C_{ar} – aromatic carbon; α – degree of aromaticity

also be found (Figure 3). Results show that the content of aromatic carbon was decreasing in the following order: Haplic Chernozem HA > Andic Cambisol HA > Haplic Cambisol HA > alginite HA.

The dominant aromatic structure and the lowest amount of aliphatic carbon resulted in the highest degree of aromaticity (α) were found in soil HA 3 (Figure 4, Table 8), and it is typical for stable and mature organic matter of Haplic Chernozem. The lowest aromatic and the highest aliphatic carbon content were found in alginite HA (Figure 1, Table 8). As it was mentioned earlier, precursors of alginite HA are marine organic materials that come from algae (Vass *et al.* 1997) and not higher plants as in soil HA (Madronova *et al.* 2011).

CONCLUSIONS

Alginite is very useful in agricultural utilisation because of its positive influence on physical and chemical properties of soil. Because of the difference in origin of organic matter in alginite and soil, this article focuses in differences in chemical structure of alginite and soil HA. On the basis of obtained results, it can be concluded that the differences between HA isolated from soil and alginite HA are significant. Alginite HA isolated using two different procedures are characteristic of predominance of aliphatic carbon and very low degree of aromaticity mainly in alginite HA B isolated with simplified extraction method suited for large volumes of HA. ^{13}C NMR spectra also show differences between the soil HA isolated from different soil types; however, all soil HA have higher degree of aromaticity characterised by higher degree of humification and higher mature of soil organic matter compare to alginite HA. The differences in chemical structure of soil HA and alginite HA can be explained by the differences in origin of organic material in soils and alginite. In soil HA, type III kerogen originated from cellulose and lignin is dominant, and type II kerogen originated from algae is a characteristic of alginite HA.

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