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HUMIFICATION DEGREE OF RENDZINA SOIL HUMIC ACIDS INFLUENCED BY CARBONATE LEACHING AND LAND USE

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Abstract: The humification degrees of humic acids extracted from calcareous and decarbonated Rendzinas under different land use were characterized upon their optical properties, to evaluate the influence of carbonate leaching (decarbonation) and different land uses. Decarbonation influenced the humification degree of humic acids positively. Base leaching and acidification of decarbonated Rendzinas led to a decrease in humification degree of humic acids. In calcareous Rendzinas, the humification degree of humic acids was in descending order: grassland>arable land>forest land, and in decarbonated Rendzinas: arable land>grassland>forest land. The humification degree of humic acids was higher in the calcareous forest and grassland Rendzinas compared to decarbonated Rendzinas, analogously in decarbonated arable land compared to calcareous arable land Rendzinas. Differences in the humification degree of humic acids among various land uses that emerged between calcareous and decarbonated Rendzinas indicate the dominant influence of soil chemical characteristics (carbonate content and pH value) compared with the quantity and quality of the organic litter input.

Key words: VIS absorbance, E_4/E_6, $\Delta\log K$, *RF*, Serbia.

Introduction

According to soil classification by Škoric et al. (1985), Rendzinas are soils with mollic A horizon which gradually transits to loose C horizon. Rendzinas are formed on various calcareous parent materials, altitudes and relief forms, under various forest and grass species. They are primarily calcareous and evolve to decarbonated and brownized Rendzinas (Ćirić, 1991). According to our research, the greatest areas of Rendzinas in Serbia are calcareous, then decarbonated, whereas brownized areas are very rare. Rendzinas are mostly under forest and grass vegetation, and rarely arable.

The process of decarbonation (with a change of pH value and cation composition) and different land uses (with or without cultivation, variability in the

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quantity and quality of plant remains) can affect many soil characteristics (Martin et al., 1998; Novotny et al., 1999; You et al., 1999; Stevenson, 1994). The survey of Rendzinas in Serbia has verified previous claims: the process of decarbonation has not significantly influenced particle size distribution (Cupać et al., 2006c), and distribution and aggregate water stability (Cupać et al., 2006b), but it did affect chemical characteristics: pH value, C and N contents, while C/N ratio was not significantly altered (Cupać et al., 2006a). The content of mobile humus substances was significantly higher, while the type of humus has not significantly changed (Cupać et al., 2008). The particle size distribution is one of the factors that influence land use, so Rendzinas under forest have more rock fragments and less clay-size fraction, compared to Rendzinas under grass and arable Rendzinas (Cupać et al., 2006c). Soil cultivation influenced the structure of arable Rendzinas significantly, causing a decrease of the coefficient of soil structure and stability of structural aggregates (Cupać et al., 2006b). Rendzinas under grass and arable Rendzinas have decreased humus and nitrogen contents (Cupać et al., 2006a), and increased a portion of stable humus, but the same humus type (Cupać et al., 2007).

Previous results have shown changes in humus composition and content in Rendzinas, caused by the process of decarbonation and different land uses. Humic substances, including humic acids, are the most abundant fractions of soil organic matter and are the most reactive compounds in soil (Gerzabek and Ullah, 1989). Their composition and properties are predetermined by conditions of soil formation (Kononova, 1975), and they can be used as indicators of pedogenetic processes (Vishnyakova and Chimitdorzhieva, 2008), here by affecting pedogenesis and soil fertility (Martin et al., 1998). Humic acids are a good indicator of land use due to specific changes in their molecular characteristics that can be detected by spectroscopy (Aranda et al., 2011). Therefore, we investigated humic acids of Rendzinas in order to observe the consequences of decarbonation and different land uses of rendzinas in Serbia. Optical properties of humic acids were determined by spectroscopy in the visible part of the spectra (VIS), a method that is very informative and the most frequently used in characterization of humic acids (Shirshova et al., 2006).

Material and Methods

Optical properties of humic acids were examined in the A and AC horizons of 24 soil profiles of calcareous Rendzina (of which 9 were under forest, 9 under grass and 6 were arable) and 9 profiles of decarbonated Rendzina (4 under forest, 3 under grass and 2 were arable land). The A horizon was 14–30 cm deep, and the AC horizon was up to 20 cm thick, so the A horizon was sampled once or twice, and the C horizon only once. Soil profiles were located in eastern Serbia (Kosa, Vančina česma and Čubra), Vojvodina (southeast slopes of Fruška gora, Stari

Slankamen), western Serbia (Slovac, Jovanja and Rovni), southwestern Serbia (Pešter plateau, Brnjica), central Serbia – Šumadija (Oplenac, Mitrovčić and Banja), southeastern Serbia (Niš–Bela Palanka–Pirot: Ostrovica, Čifluk, Prebijen Del, Pirot).

Rendzinas for our study were on various altitudes (180–1.210 m). According to the annual average data for 1971–2010 period (Republički hidrometeorološki zavod, 2011), climatic conditions are fairly uniform, with 600–800 mm of precipitation and temperature of around 11ºC, except for Pešter plateau with 6.6ºC. In Rendzinas under forest, the deciduous forest of turkey oak (*Quercuscerris* L., *Quercuspubescens* Willd.) prevails, with some hawthorn (*Crataegus spp*.), hornbeam (*Carpinus spp*.) and ash trees (*Fraxinus spp.*). The exception is the park on Oplenac with planted black pine (*Pinusnigra* Arnold). In eastern and western Serbia, Šumadija and Niš area, oak forests are thick, with closed canopy and scarce grass vegetation, so the humus is formed entirely of tree remains, almost without any influence of herbaceous vegetation. In southeastern Serbia – Bela Palanka and Pirot, there is degraded forest with seldom oak, hawthorn and wild pyrus trees (*Pyruspyraster* Burgsd.), and the ground vegetation is scarcely present. For localities under grass, the most abundant cover is on Rendzinas on Pešter plateau, then in Šumadija. In eastern Serbia and southeastern Serbia (Niš – Pirot), grass vegetation is very scarce. Arable Rendzinas are mostly under small grains and corn.

A solution of humic acids for VIS spectroscopy was prepared according to Kononova's method (JDPZ, 1966). Light absorbance (A) was recorded at 726, 665, 619, 600, 574, 533, 496, 465 and 400 nm, on spectrophotometer UV–VIS RS 1166. The characterization of optical properties of humic acids was performed by optical indexes: ratio of absorbance at 465 and 665 nm (A_{465}/A_{665}), which is traditionally labelled as E_4/E_6, then $\Delta\log K$ ($\log A_{400}–\log A_{600}$) and *RF* ($15xA_{600}/c$, where c=mg C in 1 ml of humic acid solution). A decrease of E_4/E_6 and $\Delta\log K$, and an increase of *RF* index denoted the increase of the humification degree of humic acids, i.e. an increase of aromatic components and condensation of carbon rings and an increase of molecular weight (Plaza et al., 2007).

Results were processed using StatSoft, Inc. Statistica for Windows, Version 8. Correlation analysis was performed for optical properties of humic acids and basic characteristics of calcareous and decarbonated Rendzinas, and t-test was used to determine significance of differences between various Rendzinas.

Results and Discussion

Table 1 shows average values of general characteristics of Rendzinas. Significant differences between varieties have been commented in the introduction part.

Table 1. General properties of Rendzina soils (Cupać et al., 2006a, 2006c, 2007, 2008).

Soil properties			Calcareous		Decarbonated	
			Mean	*Std.Dev.*	*Mean*	*Std.Dev.*
clay (*%*)			30.39	9.85	32.77	5.78
$CaCO_3$ (*%*)			14.01	14.05	0	0
pH in H_2O			7.70	0.17	6.94	0.41
pH in KCl			7.04	0.25	6.02	0.54
H (*meq-100*$^{-1}$*soil*)			ND[+]	ND	3.04	1.83
V (*%*)			ND	ND	91.36	5.99
C (%)			3.09	1.43	2.40	1.21
N (%)			0.309	0.13	0.217	0.11
C:N			9.77	1.82	11.21	1.61
Ch:Cf			0.77	0.15	0.67	0.15
	Forest		Grassland		Arable land	
	Mean	*Std.Dev.*	*Mean*	*Std.Dev.*	*Mean*	*Std.Dev.*
clay (*%*)	28.16	8.96	32.69	9.32	33.58	7.34
pH in H_2O	7.44	0.52	7.62	0.24	7.51	0.40
pH in KCl	6.71	0.70	6.91	0.28	6.71	0.55
C (%)	3.62	1.43	3.04	1.09	1.63	0.54
N (%)	0.340	0.14	0.311	0.10	0.163	0.05
C:N	10.77	1.60	9.52	2.00	10.11	1.92
Ch:Cf	0.68	0.15	0.78	0.15	0.80	0.14

[+]ND–not detected.

Humic acids of calcareous Rendzinas in the A horizon had slightly higher absorbance (Figure 1), i.e. a higher degree of humification compared to decarbonated Rendzinas. Indexes E_4/E_6, $\Delta\log K$ and *RF* (Tables 2 and 3) also showed little difference in the humification degree of calcareous and decarbonated Rendzinas. In the AC horizon, the humification degree of humic acids increased in decarbonated and decreased in calcareous Rendzinas (not statistically significant), so in decarbonated Rendzinas, it was higher compared to calcareous Rendzinas. In calcareous Rendzinas, the absorbance and *RF* index significantly decreased with an increase of the carbonate content and an increase of pH in KCl (Table 4), while at the same time, there was an increase of E_4/E_6 indexes and $\Delta\log K$. Correlations between decarbonated Rendzinas were not statistically significant, but it is indicative that with an increase of pH there was an increase of the absorbance (at all wavelengths) and *RF* indexes, and a decrease of E_4/E_6 and $\Delta\log K$. At the same time, an increase of hydrolytic acidity and a decrease of base saturation led to a decrease of the absorbance and *RF* indexes, and to an increase of E_4/E_6 and $\Delta\log K$. The humification degree of humic acids was higher when clay content was high, in both calcareous and decarbonated Rendzinas.

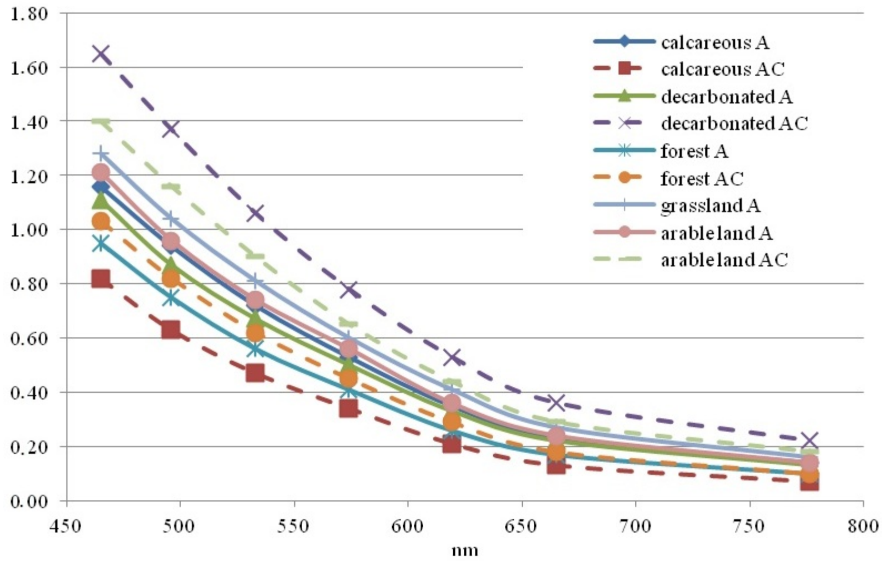

Figure 1. Light absorption of humic acids (mean values) in A and AC horizons of Rendzina soils.

Table 2. Optical indexes of humic acids in A and AC horizons.

Rendzina		$E_4/E6$		ΔlogK		RF	
	n	Mean	Std.Dev.	Mean	Std.Dev.	Mean	Std.Dev.
A horizon							
C^+	33	5.23	0.83	0.5650	0.09	66.2	30.74
D	14	5.54	1.04	0.5943	0.11	59.4	24.41
F	17	5.75	0.99	0.6273	0.10	54.3	33.66
G	17	4.90	0.52	0.5313	0.10	72.7	24.16
A	13	5.08	0.47	0.5591	0.07	65.8	19.72
CF	11	5.42	0.81	0.5988	0.10	61.3	42.83
CG	13	4.83	0.51	0.5242	0.10	74.8	24.95
CA	9	5.25	0.47	0.5824	0.07	59.8	18.80
DF	6	6.36	1.08	0.6794	0.10	41.5	18.12
DG	4	5.15	0.53	0.5542	0.11	66.1	23.34
DA	4	4.71	0.22	0.5068	0.05	79.5	15.93
AC horizon							
C	5	6.41	0.63	0.6720	0.06	39.3	8.87
D	3	4.50	0.19	0.4487	0.04	95.8	10.70
F	5	6.16	1.01	0.6303	0.11	49.1	23.97
A	3	4.93	0.90	0.5182	0.14	79.5	35.32

$^+$C–calcareous, D–decarbonated, F–forest, G–grassland, A–arable land.

Table 3. A significance level of differences in optical indexes in A and AC horizons, and between A and AC horizons (*p<0.05; **p<0.01).

Rendzina		E_4/E_6		logK		RF	
		t	p	t	p	t	p
A horizon							
C:D[+]		-0.825	0.42	-0.665	0.52	0.652	0.52
F:G		4.378**	0.00	3.939**	0.00	-2.314*	0.03
F:A		1.627	0.13	1.701	0.11	-0.694	0.50
G:A		-1.096	0.29	-0.884	0.39	0.862	0.40
C	F:G	3.005*	0.01	3.161*	0.01	-1.724	0,11
	F:A	0.436	0.67	0.359	0.73	0.416	0,69
	G:A	-3.538**	0.01	-4.227**	0.00	3.858**	0,00
D	F:G	2.341	0.10	1.774	0.17	-1.922	0,15
	F:A	3.899*	0.03	3.276*	0.05	-3.657*	0,03
	G:A	2.325	0.10	1.402	0.25	-1.721	0,18
C:D	F	-1.676	0.15	-1.813	0.13	1.283	0,25
	G	-2.072	0.13	-1.595	0.21	1.906	0,15
	A	1.767	0.17	0.889	0.44	-0.793	0,48
AC horizon							
C:D		3.440	0.07	3.036	0.09	-4.324*	0.05
F:A		1.645	0.24	1.177	0.36	-1.499	0.27
A:AC horizon							
C		-2.248	0.09	-2.780*	0.05	1.769	0.15
D		3.055	0.09	3.303	0.08	-4.390*	0.05
F		-1.340	0.25	-0.925	0.41	1.096	0.33
A		0.409	0.72	0.318	0.78	-0.361	0.75

[+]C–calcareous, D–decarbonated, F–forest, G–grassland, A–arable land.

Table 4. The correlation between optical properties of humic acids and main soil characteristics (*p<0.05; **p<0.01).

r	Absorbances at wavelength (nm)					$E_4:E_6$	Δlog*K*	*RF*
	496	533	574	619	726			
Calcareous								
clay	.56**	.55**	.56**	.57**	.54**	-.54**	-.55**	.48**
$CaCO_3$	-.61**	-.60**	-.60**	-.62**	-.63**	.65**	.58**	-.53**
pH in H_2O	-.17	-.17	-.19	-.18	-.16	-.34*	.25	-.11
pH in KCl	-.56**	-.55**	-.57**	-.58**	-.53**	.58**	.57**	-.50**
Decarbonated								
clay	.40	.41	.45	.40	.39	-.52*	-.46	.46
pH in H_2O	.29	.32	.28	.33	.35	-.42	-.42	.34
pH in KCl	.13	.14	.12	.15	.18	-.30	-.26	.16
H	-.09	-.11	-.07	-.13	-.16	.25	.20	-.13
V	.10	.12	.08	.14	.16	-.27	-.22	-.15

Humic acids had the highest humification degree, i.e. the most favourable ratio of C aromatic rings compared to C of lateral sequences in Rendzinas with neutral to slightly alkaline reaction. As soil reaction changed from neutral to acid or alkaline, the humification degree was decreasing. The carbonate content directly influences soil reaction, and microbiological activity. Namely, according to Kononova (1975), carbonates have an important role in transformation of soil organic matter due to Ca which, by means of soil pH, influences fresh organic matter decay. Soil reaction is of great importance, not only because of the effect upon the microbiological activity, but also on the hydrolysis of humic substances in acid soils. A decrease of the humification degree of humic acids in calcareous Rendzinas can be explained by quotes of Kužnicki and Sklodowski (1976), who claim that humic acids, in the presence of the great quantity of active carbonates, are very well neutralised by Ca ions, thus weakly polymerised. Decarbonated Rendzinas have lower pH values (average Rendzinas are neutral), but still have the high base saturation, so there are yet no conditions to show a statistically significant change of the humification degree.

Decarbonated Rendzinas have a slightly higher clay content, compared to calcareous Rendzinas (Cupać et al., 2006c), but since this difference was statistically insignificant, we can assume that clay could not notably affect organic matter stabilization, i.e. the microbiological activity of decarbonated Rendzinas.

Generally, in the A horizon of Rendzinas, the humification degree was as follows: grassland>arable land>forest land, and it was significantly higher in grassland compared to forest land. In the AC horizon, humic acids of arable Rendzinas had a higher humification degree, compared to Rendzinas under forest, but it was not statistically significant. Moreover, differences in land use were more prominent in the A horizon of calcareous Rendzinas than in decarbonated Rendzinas. In calcareous Rendzinas, the degree of humification was as follows: grassland>arable land >forest land, and it was significantly higher in grassland compared with arable land and forest land. In decarbonated Rendzinas, the humification degree was as follows: arable land>grassland>forest land, and the difference was significant between arable land and forest land. Humic acids of calcareous Rendzinas under forest and grass had a higher humification degree, compared with decarbonated Rendzinas under forest and grass, while under arable land it was the opposite, decarbonated Rendzinas had a higher humification degree.

According to literature (Stevenson, 1994), the content, quality and vertical distribution of organic matter differ in forest land, arable land and grassland. When humic acid properties are involved, results are very inconsistent. Some results showed a higher humification degree in arable land compared to grassland and forest land (Fasurová and Pospíšilová, 2011; Yonebayashi and Hattori, 1988), also in artificial forests compared with natural forests (Cunha et al., 2009), in a conventional olive grove compared to an organic olive grove and natural forest (Aranda et al., 2011). Reasons are various (Aranda et al., 2011; Cunha et al., 2009; Fasurová and Pospíšilová, 2011); in arable land under conventional agriculture there is a smaller input of organic remains in the soil, more pronounced degradation and less mineralization, so humic acids consist of more stable, aromatic compounds with a higher condensation degree. Soils under natural vegetation that have more organic inputs, greater microbiological mass and their metabolites have more pronounced mineralization and less degradation, and humic acids consist of aliphatic components.

On the contrary, according to Martin et al. (1998), cultivation causes a decline of the condensation level of aromatic rings in humic substances of arable land which favours mineralization of polysaccharides and lignin and decreases a molecular weight of humic substances. According to Novotny et al. (1999), cultivation leads to more expressed degradation of great humus components and synthesis of small humus components, caused by a greater inflow of fresh organic remains and soil fertilization. There are also authors who showed that cultivation had no impact on E_4/E_6 ratio of humic acids (Dormar, 1979).

Small differences between the humification degree of Rendzinas under grass and arable Rendzinas can be explained by weakly developed grass vegetation and a relatively scarce input of fresh organic matter in Rendzinas under grass, especially in southeastern Serbia. Differences in the humification degree among various land uses in calcareous and decarbonated Rendzinas pointed out a dominant influence of soil chemical properties on humic acids, compared to the quantity and quality of organic remains that enter the soil. Similar results were presented by Aranda et al. (2011), who point out that, next to the amount of organic matter, soil type and soil properties are also of great importance in the humification process. According to Kononova (1975), cultivation and organic and mineral fertilizer applications lead to essential changes of soil organic matter due to more intense syntheses and analyses of organic matter, but humus remains characteristic of the soil type.

Values of the absorbance of humic acids in the AC horizon compared to the A horizon were higher in decarbonated, forest and arable Rendzinas, and lower in all calcareous Rendzinas. According to optical indexes, the humification degree of humic acids of the AC horizon was higher in decarbonated (significantly) and arable, and lower in calcareous (significantly) and forest Rendzinas. The distribution of optical properties of Rendzina in soil profile is a result of the dominant influence of chemical characteristics (carbonate content and soil pH). A significant decrease of the humification degree of humic acids along the soil profile of calcareous Rendzinas, which deviates from the rule, is a consequence of an increase of carbonates and soil alkalinity. A further explanation of optical characteristics of studied Rendzinas would be possible with additional analysis of the molecular structure of humic acids.

Indexes E_4/E_6 and Δlog*K* are mutually highly positively correlated (r=0.90, p<0.01), and highly negatively correlated with *RF* index (r=0.81 and 0.92, p<0.01). A high correlation among E_4/E_6, Δlog*K* and *RF* has confirmed their applicability for monitoring the humification degree of soil organic matter.

Conclusion

The process of decarbonation has positively influenced the humification degree of humic acids in Rendzina soils. Base leaching and acidification of decarbonated Rendzinas had a negative influence, i.e. caused decreasing of the humification degree of humic acids.

The humification degree of humic acids in calcareous Rendzinas was in decreasing order: grassland>arable land>forest land, and in decarbonated Rendzinas: arable land>grassland>forest land. Humic acids of calcareous Rendzinas under forest and grass had a higher humification degree compared with decarbonated Rendzinas under forest and grass. The humification degree of humic acids was higher in decarbonated arable Rendzinas compared with calcareous arable Rendzinas. Differences between humification degrees of humic acids of calcareous and decarbonated Rendzinas under different land uses show the dominant impact of soil chemical properties compared with the quantity and quality of organic remains that get into the soil.

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UTICAJ PROCESA IZLUŽIVANJA I NAČINA KORIŠĆENJA RENDZINA NA STEPEN HUMIFICIRANOSTI HUMINSKIH KISELINA

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R e z i m e

Prema domaćoj klasifikaciji, rendzine su zemljišta sa moličnim A horizontom koji postepeno prelazi u rastresiti karbonatni C horizont. Primarno su karbonatna, i njihova evolucija teče u pravcu izluživanja pa zatim posmeđivanja. Najčešće su pod prirodnom šumskom i travnom vegetacijom, manji deo njih se obrađuje. U ovom radu su ispitivane optičke osobine huminskih kiselina zemljišta tipa rendzina, a istraživanja su imala za cilj praćenje posledica izluživanja i promene načina korišćenja ovog tipa zemljišta u Srbiji na stepen humificiranosti huminskih kiselina. Priprema rastvora huminskih kiselina za VIS spektroskopiju izvedena je metodom Kononove. Karakterizacija optičkih osobina huminskih kiselina je data preko optičkih indeksa: E_4/E_6, $\Delta\log K$ i *RF*. Rezultati su obrađeni u programu StatSoft, Inc. Statistica, Version 8. Proces izluživanja je pozitivno uticao na stepen humificiranosti huminskih kiselina u rendzinama (najveći je pri neutralnoj reakciji). Dalja debazifikacija i acidifikacija izluženih rendzina uticala je negativno, odnosno dovela do smanjenja stepena humificiranosti huminskih kiselina. Stepen humificiranosti huminskih kiselina u karbonatnim rendzinama se kretao travnjak>njiva>šuma, a u izluženim rendzinama njiva>travnjak>šuma. Huminske kiseline karbonatnih rendzina pod šumom i travnjakom su imale veći stepen humificiranosti u poređenju sa izluženim rendzinama pod šumom i travnjakom. U izluženim rendzinama pod njivom stepen humificiranosti huminskih kiselina je bio veći nego u karbonatnim rendzinama pod njivom. Razlike u stepenu humificiranosti huminskih kiselina prema načinu korišćenja koje su se pojavile između karbonatnih i izluženih rendzina ukazuju na dominantan uticaj hemijskih karakteristika zemljišta na osobine huminskih kiselina u poređenju sa količinom i kvalitetom organskih ostataka koji dospevaju u zemljište.

Ključne reči: VIS apsorpcija, E_4/E_6, $\Delta\log K$, *RF*, Srbija.

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