HUMIC ACIDS OPTICAL PROPERTIES OF RENDZINA SOILS IN DIVERSE ENVIRONMENTAL CONDITIONS OF SERBIA

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ABSTRACT

This study examined the impact of climate, altitude and landforms on humic acids (HA) optical properties (E₄/E₆, ΔlogK, RF indexes) in Serbian Rendzina soils. HA humification degree of calcareous and decarbonated Rendzinas under natural vegetation (forest and grassland, separately) decreased with altitude increasing. This particular rule was not manifested in arable Rendzinas. HA humification degree in Rendzinas on flat positions (hilltop and footslope) is significantly higher than in Rendzinas on slope sides. Humification degree of HA is the highest in Rendzinas in eastern Serbia (in the vicinity of Negotin), followed by central Serbia (Sumadija) and Srem, southeast and finally, southeast and southwest Serbia. With respect to the climate change foreseen for the end of this century, i.e. increase of precipitation and temperature variance among the regions in Serbia, also an increase in variance of quality of humic acids in Rendzinas on different altitudes and regions can be expected.

Key words: \( E₄/E₆, \Delta \log K, RF \), altitude, landform, region

INTRODUCTION

Humic substances, including humic acids (HA), are the most prevalent fraction of soil organic matter and are the most reactive compounds in soil [1]. They can be considered as indicators of pedogenetic processes [2] because their composition and properties are determined by conditions of soil genesis [3]. Difference in quantity and chemical composition of humic and fulvic acids depends on many variables, such as: parent material, altitude, slope gradient, climate, vegetation type and soil management [4,5,6]. Dominant effect of annual precipitation and temperature on the composition of humic acids was also reported by [7]. Due to climate change, impact of climate is becoming more important - the Intergovernmental Panel on Climate Change (IPCC) reported a global linear increase of 0.56–0.92°C during 1906–2005 [8]. For the last three decades there was a change in average temperature and annual rainfall in Serbia, with increasing difference between warmer and less humid northern and eastern parts of the country, and more humid and cooler western parts [9]. According to the estimations for the end of this century [10,11], annual temperature in our region will rise for 4°C, and some parts of Serbia could receive 20% less precipitation during summer.

According to soil classification by Škorić et al. [12], Rendzinas are soils with mollic A horizon which gradually transits to loose C horizon. Rendzinas are distributed all over Serbia, on various parent material (except on massive limestone and dolomites), altitudes and land forms, under various natural forest and grass species, or are turned to arable land. Hitherto research showed that processes of decarbonatization and base leaching, and also the change in land use influence optical properties of...
humic acids in Rendzinas of Serbia [13]. This paper examines the influence of environmental conditions, especially climate, altitude and landforms on optical properties of humic acids in Rendzinas in Serbia. Our data should bring some light onto the impact of these factors, and also could become basis for monitoring of influence of expected climate changes on characteristics of soil organic matter in Serbia.

MATERIAL AND METHODS

Optical properties of humic acids were determined in A horizon of 33 Rendzina soil profiles. Thickness of A horizon was 14-30 cm, so one or two samples were taken from each A horizon (total of 42 soil samples). Table 1 shows three ways of data grouping: according to altitude, landform (slope gradients and exposure) and regions. Most important environmental factors: altitude, parent material, annual precipitation and temperature, land use (forest, grass land, arable land) and basic characteristics of vegetation, are shown for each group.

| Table 1 General environmental parameters |
|-----------------------------------------|
| **GROUP** | **N.** | **Altitude (m a.s.l.)** | **Parent material** | **MS** | **Precipitation (mm)** | **Temperature (°C)** | **Land use/ Vegetation** |
|------------|--------|---------------------|-----------------|--------|-------------------|-------------------|------------------------|
| REGION     |        |                     |                 |        |                   |                   |                        |
| Srem       | 1      | 187-190             | sm              | 1      | 656.2             | 11.36             | G(2) lush              |
| West and southwest | 2 | 151-1210            | m, sl            | 2, 3   | 814.9-768.4      | 11.37-6.62         | F(2), G(2) lush; A(1) |
| Central    | 3      | 240-290             | sl              | 4      | 698.2             | 11.70             | F(2), G(1) lush; A(1) |
| East       | 4      | 199-250             | sl              | 5      | 652.6             | 11.71             | G(6) lush; F(3), A(3) |
| Southeast  | 5      | 335-720             | sm, cm, ml, sl,cg | 6, 7   | 592.9-605.8      | 11.39             | F(5), A(3), G(1) scare |
| ALTITUDE   |        |                     |                 |        |                   |                   |                        |
| Calcareous | 1      | <200                | m, sm, sl       | 1, 5, 2| 656.2-652.6      | 11.36-11.71        | G(7), F(39), A(1)     |
|            | 2      | 200-600             | cm, sm, sl, ml  | 2, 4, 5| 605.8-814.9      | 11.37-11.71        | F(5), A(4), G(1)     |
|            | 3      | >700                | ml, sl          | 3, 6   | 768.4-592.9      | 6.62-11.40         | F(1), G(1), A(1)     |
| Decarbonated| 1     | <200                | sl              | 5      | 652.6             | 11.71             | F(1)                  |
|            | 2      | 200-300             | sl              | 4, 5   | 652.6-698.2      | 11.71             | F(2), A(2), F(1)     |
|            | 3      | 400-700             | sl, cg          | 2, 7   | 605.8-814.9      | 11.37             | F(1), G(1)           |
| LANDFORM/exposure |        |                     |                 |        |                   |                   |                        |
| hill top, flat or very gentle slope |        |                     |                 |        |                   |                   |                        |
| 12 calcareous | 1 | 172-560             | sl, m, sm, cg   | 5, 2   | 605.8-814.9      | 11.36-11.71        | F(5), G(6), A(3)     |
| 3 decarbonated | 2 | 280-337             | sl              | 4, 7   | 605.8-698.2      | 11.37-11.70        | G(1), A(1)           |
| footslope, very gentle slope, south, southwest |        |                     |                 |        |                   |                   |                        |
| 1 calcareous | 1 | 151-438             | ml, sm, cm      | 7, 1   | 592.9-814.9      | 11.36-11.70        | F(4), G(2)           |
| 1 decarbonated | 2 | 240-1210            | sl, sm, ml      | 2, 6   | 592.9-814.9      | 6.62-11.70         | F(3), G(1), A(3)     |
| slope 20-60º, north, northwest, northeast |        |                     |                 |        |                   |                   |                        |
| 7 calcareous | 4 | 1210                | sl, sm, ml      | 2, 6   | 592.9-814.9      | 6.62-11.70         | F(3), G(1), A(3)     |
| 1 decarbonated | 1200 |                     |                 |        |                   |                   |                        |

* Meteorological data for stations: 1-Rimski šančevi, 2-Valjevo, 3-Sjenica, 4-Beograd, 5-Negotin, 6-Niš, 7-Pirot. b m-marl, cm-calcareous marl; sm-sandy marl, ml-marly limestone, sl-soft limestone, cg-calcareous gravel. F - forest; G - grassland; A – arable land. Thick forest, closed canopy, species: turkey oak (*Quercus cerris* L.), hawthorn (*Crataegus spp.*), hornbeam (*Carpinus spp.*) and ash tree (*Fraxinus spp.*); in Oplenac park, black pine (*Pinus nigra* Arnold). e on all arable land alternation of small grains and maize. Degraded forest and bush, species: oak, hawthorn and wild pyrus tree (*Pyrus pyraster* Burgsd.).
Particle size distribution was determined by pipette method with Na-pyrophosphate [14]; pH in water and in 1M KCl potentiometrically (soil:water/1M KCl ratio 1:2.5); solution of humic acids for VIS spectroscopy was prepared according to the method of Kononova [15]. Light absorbance (A) was recorded on wave length 665, 600, 465 and 400 nm on UV-VIS RS 1166 spectrophotometer. Characterization of optical properties was performed upon optical indexes: ratio of absorbance on wavelength 465 and 665 nm (A465/A665), which is traditionally labelled as E4/E6; then ΔlogK (logA600-logA665); and RF (15xA600/c, where c = mg of C in 1 ml of humic acid solution). Decrease of E4/E6 and ΔlogK, and increase of RF index indicate increase of humification degree of humic acids, i.e. increase of condensation degree of aromatic carbon chains [3], size and mass of humic acids molecules and content of condensed aromatic rings [16].

Statistical data analysis included descriptive statistics, correlation analysis and t-test (difference among altitudes, landforms and regions), and was performed using StatSoft, Inc. STATISTICA for Windows, version 8.

RESULTS AND DISCUSSION

Table 2 shows basic properties of Rendzinas, which, among other, determine humus characteristics and HA. Average clay content and pH value, do not differ significantly between calcareous Rendzinas on various altitudes, while the calcium carbonate content is increasing with altitude increase. Tophill Rendzinas have very heterogeneous clay and carbonate content, and pH values. Footslope Rendzinas have the highest clay and the lowest carbonate content. Rendzinas on northern slopes have the highest clay and carbonate content. Rendzinas on south slopes have the lowest clay content. Calcareous and decarbonated Rendzinas from different regions have various clay and carbonate content, while pH values are quite similar.

| Rendzina group | clay (%) | CaCO₃ (%) | pH in H₂O |
|----------------|----------|-----------|-----------|
|                | n        | average SD | average SD | average SD |
| Altitude (m)   |          |           |           |           |
| Calcareous     |<200      | 13        | 31.78 10.35 | 7.85 5.87 | 7.67 0.16 |
|                | 200-600  | 16        | 30.26 9.64  | 9.55 11.06 | 7.67 0.12 |
|                | >700     | 4         | 36.56 8.98  | 11.47 9.10 | 7.69 0.11 |
| Decarbonated   |<200      | 1         | 28.96 -     | 0 -       | 7.15 -     |
|                | 200-400  | 5         | 31.72 3.44  | 0 -       | 7.17 0.36  |
|                | 400-600  | 5         | 33.57 8.90  | 0 -       | 6.71 0.56  |
| Landform       |          |           |           |           |
| - hill top flat/very gentle slope | 25        | 31.58 8.58 | 8.10 10.30 | 7.45 0.46 |
| - fooslope, very gentle slope, south, southwest | 3         | 37.72 3.36 | 7.19 0.63 | 7.70 0.05 |
| - slope 45-80º, south, southeast, southwest | 5         | 28.85 5.14 | 8.73 6.84 | 7.60 0.16 |
| - slope 20-60º, north, northwest, northeast | 9         | 30.18 12.38 | 12.19 7.47 | 7.66 0.22 |
| Region         |          |           |           |           |
| Calcareous     | Srem     | 2         | 8.94 1.27  | 18.25 4.91 | 7.87 0.25 |
|                | West     | 4         | 24.92 10.11| 18.34 17.90| 7.68 0.09 |
|                | Central  | 2         | 41.26 4.95 | 5.35 4.95 | 7.63 0.20 |
|                | East     | 15        | 35.51 2.48 | 4.78 3.71 | 7.63 0.12 |
|                | Southeast| 10        | 31.08 10.56| 10.85 7.40 | 7.70 0.13 |
| Decarbonated   | Srem     | 0         | - -        | - -       | - -       |
|                | West     | 1         | 39.36 -     | 0 -       | 6.99 -     |
|                | Central  | 2         | 32.18 2.35 | 0 -       | 6.85 0.19 |
|                | East     | 4         | 30.80 3.90 | 0 -       | 7.00 0.23 |
|                | Southeast| 2         | 24.04 3.62 | 0 -       | 6.39 0.85 |
Table 3 suggests that increase in altitude of calcareous Rendzinas, influences increase of average values of $E_4/E_6$ and $\Delta \log K$, and decrease of $RF$, i.e. HA humification degree is decreasing, which can be attributed to increase of active carbonate content in soil, together with climatic factor. Actually, in the presence of large amounts of active carbonates, HA are rapidly neutralised by Ca ions and therefore weakly polymerised [17]. Decarbonated Rendzinas on various altitudes have very similar clay content and pH values. Altitude of decarbonated Rendzinas is not a significant parameter, considering the fact that the highest humification degree was present on medium altitude (200-600 m). However, in Rendzinas under natural vegetation (forest and grassland) HA humification degree is decreasing with altitude increase, in both calcareous and decarbonated varieties.

Table 3 Optical indexes of humic acids in Rendzina soils on different altitudes

| Rendzina   | Altitude (m) | $n$ | $E_4/E_6$ | $\Delta \log K$ | $RF$ |
|------------|--------------|-----|-----------|----------------|------|
|            |              |     | average   | SD             | average | SD |
| Calcareous | <200         | 13  | 4.68      | 0.41           | 0.5069  | 0.08|
|            | 200-600      | 16  | 5.47      | 0.67           | 0.5959  | 0.09|
|            | >700         | 4   | 5.38      | 0.32           | 0.6302  | 0.05|
| forest     | <200         | 4   | 4.75      | 0.61           | 0.5293  | 0.10|
|            | 200-600      | 6   | 5.80      | 0.73           | 0.6287  | 0.08|
|            | >700         | 7   | 5.82      | -              | 0.6977  | -   |
| grassland  | <200         | 9   | 4.64      | 0.33           | 0.4969  | 0.08|
|            | 200-600      | 3   | 5.22      | 0.79           | 0.5809  | 0.15|
|            | >700         | 1   | 5.32      | -              | 0.6000  | -   |
| arable land| 200-600      | 7   | 5.28      | 0.53           | 0.5741  | 0.08|
|            | >700         | 2   | 5.14      | 0.06           | 0.6116  | 0.04|
| Decarbonated | <200        | 1   | 5.61      | -              | 0.6086  | -   |
|            | 200-400      | 5   | 5.28      | 0.79           | 0.5643  | 0.08|
|            | 400-600      | 3   | 6.35      | 1.17           | 0.6937  | 0.10|
| forest     | <200         | 1   | 5.61      | -              | 0.6086  | -   |
|            | 200-400      | 1   | 6.65      | -              | 0.7058  | -   |
|            | 400-600      | 2   | 7.59      | 0.09           | 0.7936  | 0.03|
| grassland  | 200-400      | 2   | 5.06      | 0.24           | 0.5258  | 0.03|
|            | 400-600      | 1   | 5.85      | -              | 0.7067  | -   |
| arable land| 200-400      | 2   | 4.81      | 0.08           | 0.5320  | 0.04|

According to [7], $E_4/E_6$ ratio of humic acids under forest is significantly negatively correlated to temperature maximums; and $E_4/E_6$ ratio of fulvo acids is positively correlated with annual precipitation. Literature [18] report increase of $E_4/E_6$ ratio and decrease of aromatic character of humus substances with increase of precipitation in sub humid region. In fact, high precipitation and lower temperatures on higher altitudes cause decrease in lignin decomposition and forming of condensed aromatic structures of greater molecule mass, simultaneously emanating of humus substances of lower molecular weight rich in polysaccharides. On the other hand, higher temperatures at lower altitudes increase oxidation and humification degree of organic matter [7]. Difference in altitudes, precipitation and temperatures among three groups of Rendzinas are smaller compared to some literature data [7], but still big enough to cause differences in HA humification degree. Soil cultivation influence transformation (oxidation) of organic matter [7,6].

Influence of altitude in arable calcareous Rendzinas was not shown. Comparison within decarbonated Rendzinas was not possible because soil profiles were positioned on the same altitude. Generally, in Rendzinas (Figure 1) average $E_4/E_6$ index is the highest on medium altitudes. Correlation coefficient between $\Delta \log K$ and $RF$ is $r=-0.81$. According to classification of Kumada [16] HA in Rendzinas on all altitudes are mostly of P type (Figure 2), but on the very transition to A and B type. Only the smaller portion of HA in Rendzinas on 200-700 m belongs to less humified Rp type. Significantly lower HA humification degree (Table 4) is at medium compared to higher altitudes.
Average $E_4/E_6$ index (Figure 1) is the lowest and mutually alike at both flat positions (hilltop and foot slope), slightly greater on northern slopes, and the greatest on south slopes. According to $\Delta \log K$ and RF indexes (Figure 2) HA on hilltop are of P type (mostly at the transition to A, and to a smaller degree to B type); on foot slope P type on transition to B type; on both slope sides mostly of P type on transition to B type, and less of Rp type. As addition to Figure 2, Table 4 shows significantly lower HA humification degree on slope positions compared to flat positions. Number of calcareous and decarbonated profiles and types of land use of Rendzinas on all landforms do not differ significantly. Samples from both flat positions have higher average clay content compared to soils on both slope positions. Hydrothermal conditions on slopes with different exposures and inclination influence their vegetation and soil characteristics [19]. In this respect, lower HA humification degree in Rendzinas on slopes can be interpreted by adverse moisture conditions i.e. influence of drought in these permeable soils on steep positions, where water is additionally lost by surface runoff. Insufficient water could be the cause of low microbiological activity and weak humification of organic litter on slope positions compared to flat positions where the signs of drought are less expressed.

Table 4 Significant difference in Rendzinas soil humic acid optical indexes among different altitudes, landforms and regions in Serbia

|                | $E_4/E_6$     | $\Delta \log K$ | RF   |
|----------------|---------------|-----------------|------|
|                | $t$ $p$       | $t$ $p$         | $t$ $p$ |
| Altitude       |               |                 |      |
| 1 v.s. 2       | -2.96615      | 0.008657        | -2.56964 | 0.019889 | 2.71108 | 0.014828 |
| Lanform        |               |                 |      |
| 1 v.s. 3       | -4.61281      | 0.009935        |       |
| 1 v.s. 4       | -4.15535      | 0.003185        | -3.95194 | 0.004225 | 3.13234 | 0.013966 |
| 2 v.s. 3       |               |                 | 6.39199 | 0.023612 |
| Region         |               |                 |      |
| 1 v.s. 2       | -3.32085      | 0.045026        |       |
| 1 v.s. 5       | -4.67201      | 0.018518        |       |
| 2 v.s. 4       | 5.87000       | 0.004206        |       |
| 4 v.s. 5       | -4.23630      | 0.001398        | -4.57305 | 0.000799 | 3.92175 | 0.002386 |

Figure 1 Average humic acid $E_4/E_6$ index in Rendzina soil on different altitudes (m):
1 <200, 2 200-600, 3 >700; landforms (1-hill top, flat or very gentle slope; 2- footslope, very gentle slope, south, southwest; 3- side slope 45-80º, south, southeast, southwest; 4- side slope 20-60º, northern, northwest, northeast), and regions (1- Srem, 2- Western Serbia, 3- Central Serbia, 4- Eastern Serbia, 5- Southeastern Serbia)
Figure 1 shows average $E_4/E_6$ indexes in this order: southeastern > western > central > Srem > eastern Serbia. According to $\Delta \log K$ and RF indexes, HA from eastern Serbia have the highest humification degree and belong to P type on transition to A, or less to B type. There follow HA from central Serbia > Srem > western Serbia which belong to P type on transition to B type, followed by HA from southeastern Serbia, part of which belonging to Rp type. It should be noted that standard deviation of all three optical indexes is quite high in all analysed groups. Table 4 shows significantly higher HA humification degree in Rendzinas from eastern compared to southeastern Serbia.

The lowest HA humification degree was in southeast Serbia due to prevalence of Rendzinas under forest, and the highest HA humification degree was in eastern Serbia and Srem due to prevalence of Rendzinas under grass. Thus, we compared optical indexes of Rendzinas solely under forest, between 2,3,4 and 5 regions (results are not shown). Ratio between average values among regions, as well as significance of difference is the same as is in total Rendzina group, so reasons for expressed differences should be sought for in other environmental factors [7,20,21,22,23].

Eastern Serbia (as well as central (Sumadija) and Srem) are at the lowest altitudes, vegetation is lush and influx of organic remains is higher, with favourable temperature and water regimes. In west Serbia, especially on the southwest (Pester plateau), there is a variance of altitudes, but higher altitudes prevail, so the climate is more humid and cooler, vegetation is lush and influx of organic litter is high.

First of all, cooler and wetter climate contributes to weak transformation of organic matter and lower HA humification degree. Southeast Serbia has less precipitation, vegetation is scarce, so the influx of organic organic litter is slower, which causes weak microbiological transformation and lower HA humification degree. Besides, according to Kumada [16] the conditions of alternating water regime, periods of moistening alternating with periods of drying, favour mainly the formation of HA, creation of fairly condensed aromatic rings. Along with total annual precipitation and average temperature, dynamics of alternating periods of moistening and drying could be the cause of different HA humification degree in Serbian regions.
CONCLUSION

HA humification degree of calcareous and decarbonated Rendzina soils under natural vegetation (forest and grassland, separately) decreases with altitude increasing. This rule was not manifested in arable Rendzinas. HA humification degree in Rendzinas on flat positions (hill top and footslope) is significantly higher in Rendzinas in eastern Serbia (vicinity of Negotin) compared to Rendzinas on slope sides. HA humification degree was the highest in Rendzinas of eastern Serbia (vicinity of Negotin), followed by central Serbia (Sumadija) or Srem, southeast and, the lowest in west and southwest Serbia. Considering predicted climate change by the end of this century, i.e. increase in variation between water and temperature regimes in Serbian regions, it is to be expected that HA humification quality in Rendzinas among different altitudes and regions will vary all the same.

ACKNOWLEDGMENT

This study was supported by the Ministry of Education and Science of the Republic of Serbia (Project number: TR 37006).

(Received January 2018, accepted February 2018)

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