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SPECTROSCOPIC CHARACTERIZATION OF HUMIC SUBSTANCES OF ANTHROPOGENIC SOILS DERIVED FROM TERRA ROSSA

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ABSTRACT

The aim of the study was spectroscopic characterization of humic substances and evaluation of humus quality of anthropogenic soils derived from Terra Rossa. The study was conducted on 15 soil samples collected from top-soil horizon of olive groves in Middle Dalmatia (Croatia). Total organic carbon was determined according to Walkley-Black method (1934) and humic substances isolation following procedure given by Schnitzer (1982). Spectroscopic characterization of isolated humic substances was carried out by measuring absorbance in VIS spectral range 400-700 nm and optical indices ($Q_{4/6}$, $E_{4/6}$) were calculated. SOC content in top-soil samples varied from 1.45% to 4.21% with mean value of 2.98%. The absorption spectrum showed monotonous decrease of absorbance from 400 to 700 nm for all samples. The optical indices $E_{4/6}$ and $Q_{4/6}$ varied from 3.58 to 5.05 and from 3.91 to 5.04, respectively and indicated differences of humus quality. The optical index below 4, which implies a high quality of humus, was determined in six samples. The low humus quality (optical index above 4) was determined in other nine samples. The SOC content has significant positive correlation with optical indices $Q_{4/6}$ and $E_{4/6}$. Soils with higher organic carbon content have lower humus quality.

Key words: *VIS spectrum, $E_{4/6}$, humus quality, Dalmatia.*

INTRODUCTION

Humus defines key soil characteristics, its fertility and it is an indicator of the processes occurring in soil. Therefore, understanding of its content and quality is important for the sustainable land management, especially in Mediterranean karst region. Humic substances (HS) constitute a major fraction (60–70%) of soil organic matter and those are possibly the most abundant of naturally occurring organic macromolecules on the Earth (2–3 x 10^{10} t), (Jones and Bryan, 1998). Humic substances differ in molecular weight, elemental composition, acidity and cation exchange capacity and can be classified into three major groups according to their solubility; humic acid, fulvic acid and humins. The humic acids fraction consists of hydroxyphenols, hydroxybenzoic acids and other aromatic structures with linked peptides, amino compounds and fatty acids. Fulvic acids are typically composed of a variety of phenolic and benzene carboxylic acids that are held together by hydrogen bonds to form stabile polymer structures. The low molecular weight of fulvic acid has higher oxygen but lower carbon content than humic acid. There are also more acidic functional groups particularly -COOH in fulvic acid molecule (Schnitzer and Khan, 1978).

Non-destructive spectroscopic methods ensure valuable informations on molecular structure, chemical and functional properties of humic substances (Chen et al, 1977). Therefore, new approaches of spectrometry that include a wide variety of techniques (UV–VIS, DRIFT, SFS, and ^{13}C-NMR) have been successfully applied to the study of HS chemical composition and structure (Pospišilova et al, 2008; Milori et al., 2002; Sierra et al., 2005). Humic substances generally show strong absorbance in the UV–VIS range (from 190 to 700 nm) because of the presence of aromatic chromophores and other organic compounds (Rupiasih and Vidyasagar, 2007). Stevenson (1982) has shown that absorption of humic substances on wave length of 465 nm is equal to absorption of light form components linked to initial phases of humification (young humic substances – fulvo acid) and absortion of light on wave lengt of 665 nm refers to well humified components - humic acid. Optical index $E_{4/6}$ calculated as ratio of optical absorbance at 465 nm versus 665 nm for humic substances in solution is often used for evaluation of humus quality. Generally, lower molecular weight and lower degree of condensation of aromatic structures in humic substances show higher values of $E_{4/6}$ than humic substances with a high degree of humification (Orlov, 1985).

The objective of the study was spectroscopic characterization of humic substances and evaluation of humus quality of anthropogenic soils derived from Terra Rossa.

MATERIAL AND METHODS

The study was conducted on 15 soil samples collected in three olive groves area in Middle Dalmatia (Croatia) including: the island of Brač (43°22’00" N, 16°38’27" E), Marina (43°31’40" N, 16°09’31" E) and Primošten (43°34’47" N, 15°56’38" E). Within each selected area five average soil samples from the depth 0-25cm were collected. Investigated soils are anthropogenic soils derived from Terra rossa, shallow, skeletic on limestones and dolomites (Škorić et al., 1975). According to IUSS Working Group (WRB, 2014) studied soils can be identified as Chromic Leptic Skeletic Cambisol (Clayic Colluvic). Soil samples were prepared for analysis of physical and chemical properties according to HRN ISO 11464:2009. Soil pH was determined according to HRN ISO 10390:2005, soil organic carbon was determined according to Walkley-Black method (1934), available phosphorus and potassium were determined according to Egner et al. (1960) and particle size distribution was made by HRN ISO 11277:2009.

The isolation of soil humic substances (HS) was made by Schnitzer method (1982). 5 g of air dried soil sample was sieved at the mesh size of 1 mm and extracted with solution of 0.1 M NaOH + 0.1 M $Na_4P_2O_7$. The mixture was shaken mechanically for 24 h at room temperature. The supernatant solution was then separated from the residue by centrifugation at 4000 rpm for 20 min. The alkaline extract was acidified with concentrated H_2SO_4 to pH~1, allowed to stand for 24 h at room temperature to obtain the complete precipitation of humic acid (HA). The precipitated HA was separated from fulvic acid (FA) by repeating three times the following: centrifugation at 4000 rpm for 20 min, removal of the residue, washing the HA with 0.05 M H_2SO_4 solution. Finally, the centrifuged HA were dissolved in a minimal volume of 0.1 M NaOH and brought to dryness in a drying oven at 60°C. VIS spectra were measured by Shimadzu UV 1700 spectrometer in the range of 400-700 nm. Optical indices $E_{4/6}$ and $Q_{4/6}$ were determined as the absorbance ratio $A_{465}/_{665}$ and $A_{400}/_{600}$, respectively (Orlov, 1985; Szajdak et al., 2006).

RESULTS AND DISCUSSION

Descriptive statistics for basic soil properties given in Table 1. shows that investigated soils are weakly acid to neutral, non-calcareous to slightly calcareous and variably supplied with physiologically active nutrients. Soil supply with P_2O_5 varies from low to moderate, showing very high coefficient of variation (CV). Investigated soils are moderate to high supplied with K_2O and showing lower coefficient of variation. Soil organic carbon content varies from low to medium, in average medium (Table 1). These data are consistent with research of Miloš and Bensa (2012) which determined similar values of SOC content in anthropogenic soils derived from Terra Rossa (0.17-3.73%) in Dalmatia. Although, only less than a quarter (24.6%) of southern European top-soils contain medium to high (>2%) amounts of SOC (Zdruli et al, 2004). Higher level of SOC content in our research, compared to averages in European soils, can be related to land management and particularities of soils formed on the limestones and dolomites in Mediterranean region (high stoniness and rockiness and variable soil depth). Investigated soils are dominantly silty clay and silty clayey loam.

Table 1. Descriptive statistics for basic soil properties

Soil property	Mean	Median	Min.	Max.	Range	Std. dev.	#CV(%)
pH H_2O	7.62	7.65	6.53	8.02	1.49	0.40	5.25
pH KCl	6.67	6.87	5.36	7.18	1.82	0.51	7.63
$CaCO_3$ (%)	3.02	2.57	0.00	7.90	7.90	2.19	72.66
P_2O_5 mg/100 g	4.37	2.50	0,05	14.13	14.08	4.49	102.71
K_2O mg/100 g	35.89	34.20	18.00	69.38	51.38	12.01	33.46
SOC %	2.98	3.24	1.46	4.23	2.77	0.96	32.3
Coarse sand (%)	22.89	23.60	15.30	28.00	12.70	3.73	16.31
Fine sand (%)	6.64	7.00	0.30	15.10	14.80	4.48	67.42
Silt (%)	27.38	29.60	13.70	37.00	23.30	6.58	24.03
Clay (%)	43.09	43.00	31.00	61.80	30.80	9.04	20.99

#CV (%), coefficient of variation

The absorption spectrumes of humic substances (HS) extracted from the studied soils show monotonous decrease of absorbance in the range 400-700 nm, Figure 1. The steeper declines of curves imply domination of aromatic structures over apliphatics in humic substances (Pospišilova and Fasurova 2009; Fasurova and Pospišilova 2011; Milori et al. 2002). The steepest declines of curves were established in the VIS spectra of humic substances extracted from soils with the lowest SOC content. That means, soils with lower SOC content have more condensed aromatic structures than aliphatics in humic substances and higher humus quality.

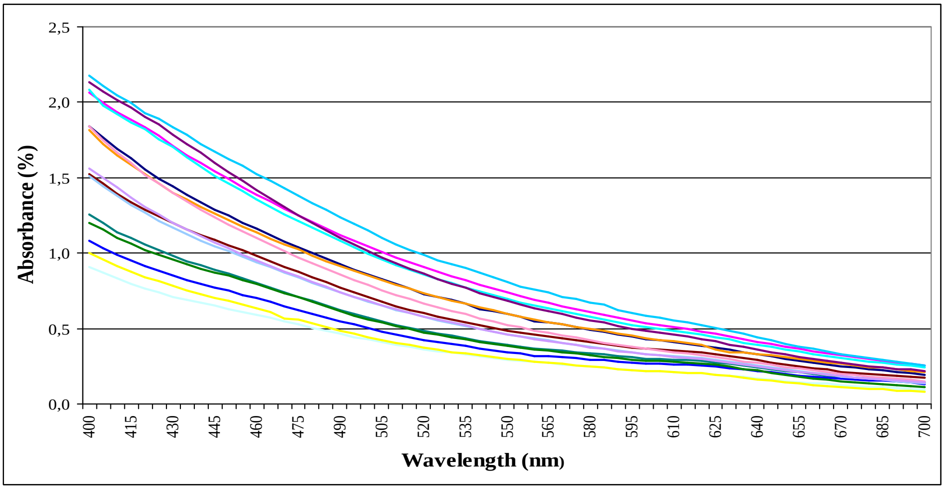

Figure 1. VIS spectra of extracted soil humic substances (HS) from top-soil horizon of investigated soils

Therefore the ratio between the absorbance at 465 nm and 665 nm ($E_{4/6}$) and 400 and 600 nm ($Q_{4/6}$) are frequently used to characterize composition of organic matter and evaluation of its humification degreee. The $E_{4/6}$ ratio decreases when the condensation and aromaticity of the humic substances rise and with increasing molecular weight (Stevenson, 1994; Fuentes et al., 2006), which is typical for more mature, more evolved organic materials, and is therefore useful as a humification indicator. The mean values of optical indices $E_{4/6}$ and $Q_{4/6}$ of humic substances isolated from investigated soils were 4.22 and 4.42 respectively, Table 2. The optical indices are characterized with short range and small coefficients of variation (CV %). These indices have lower coefficient of variation than SOC (Table 1), implying higher heterogenity of humus quantity than quality (Table 2).

Table 2. Descriptive statistics for optical indices

Optical index	Mean	Min.	Max.	Range	St. dev.	[#]CV(%)
$E_{4/6}$	4.22	3.58	5.05	1.47	0.50	11.74
$Q_{4/6}$	4.42	3.91	5.04	1.13	0.33	7.41

[#]CV (%), coefficient of variation

Generally, optical indices values above 4 indicate presence of more aliphatic and fewer aromatic compounds and lower humus quality. From a total of 15 samples, 6 have optical index below 4, which implies a high quality of humus. The other nine samples have optical index above 4 and low humus quality. The low humus quality were determined in soil samples with SOC content above 2%. These results shows that in anthropogenic soils developed from Terra Rossa under olive groves dominated low humus quality. This probably relates with specific soil management (addition organic matter), leading to increase of SOC content and higher proportion of aliphatic compounds in humic substances (higher E_4/E_6 ratio - lower humus quality).

These results are consistent with investigation (Čolak and Martinović, 1975.) which have found similar values of optical index of humic substances ($E_{4/6}$ 3.78-5.02) isolated from top-soil of Terra Rossa in Middle Dalmatia. Senesi et al (1999) also established high values of $E_{4/6}$ (4.6-4.9) in Terra rossa under olive groves in Mediterranean region.

Relations between SOC content and optical indices $Q_{4/6}$ and $E_{4/6}$ were fitted with linear type of equation: SOC % = -3.3522 – 0.5995*x + 2.1249*y, Figure 2.

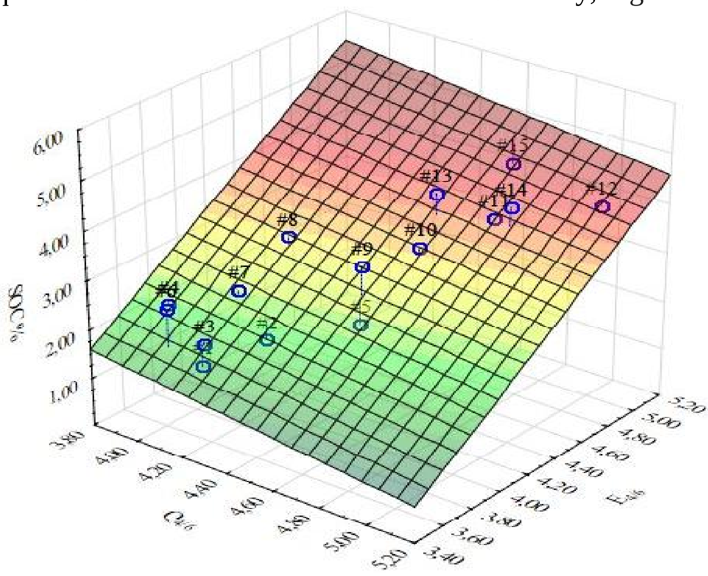

Figure 2. 3D surface plot of SOC against optical indices $Q_{4/6}$ and $E_{4/6}$

The 3D surface plot of SOC against optical indices $Q_{4/6}$ and $E_{4/6}$ (Figure 2) shows that increase of SOC content increases the value of the indices $Q_{4/6}$ and $E_{4/6.}$ The strength of these relations established with linear correlation shows that SOC content and the optical indices ($Q_{4/6}$ and $E_{4/6}$) are significantly positively correlated, Table 3. The optical index $E_{4/6}$ better correlates with SOC content than $Q_{4/6}$ due to possible inaccuaries in measurements when values of absorbance are up to 2 % (around 400 nm).

Table 3. Correlations of SOC content and optical indices $E_{4/6}$ and $Q_{4/6}$

	SOC %	$Q_{4/6}$	$E_{4/6}$
SOC %	1.00	0.76*	0.92*
$Q_{4/6}$		1.00	0.88*
$E_{4/6}$			1.00

* Marked correlations are significant at $p < 0{,}050$

CONCLUSION

Our research showed domination of low humus quality in top-soil horizon of anthropogenic soils developed from Terra Rossa under olive groves in Middle Dalmatia, Croatia. The humus quality indicators (optical indices $Q_{4/6}$ and $E_{4/6}$) significantly correlate with SOC content. The soils with higher SOC content have lower humus quality.

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