ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume LIX 41 Number 6, 2011

COMPARISON OF EXTRACTABLE SOIL CARBON AND DISSOLVED ORGANIC CARBON BY THEIR MOLECULAR CHARACTERISTICS

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Received: August 3, 2011

Abstract

ŠESTAUBEROVÁ, M., NOVÁK, F.: *Comparison of extractable soil carbon and dissolved organic carbon by their molecular characteristics.* Acta univ. agric. et silvic. Mendel. Brun., 2011, LIX, No. 6, pp. 337–342

The aim of this study was to compare the molecular characteristics of extractable soil carbon from biotopes of the Rašeliník watershed and dissolved organic carbon (DOC) from Rašeliník creek, by using the 0.1M pyrophosphate, 0.01M $CaCl_2$ and Britton-Robinson buffer as extraction agents. The molecular weight M_r and weight-average molecular weight $M_{w,}$ determined by the low pressure size exclusion chromatography, increased in the following sequence: humic substances (HS) in $CaCl_2$ < aquatic HS < HS in sodium pyrophosphate ≤ HS in a buffer. Elution curves of all humic substances were characteristic by two peaks with predominant low-molecular fraction. M_r and M_w values of aquatic humic substances were 5.9 and 7.9 kDa, respectively, and proportion of this low-molecular fraction reached 97%. This corresponds to the fact, that the main fraction of HS in surface waters constitute fulvic acids. Using soil extraction in $CaCl_2$ we obtained the fraction of organic carbon similar to the humic substances contain in DOC. Differences in quantity of humic substances extracted from soils among $CaCl_2$ (mean 0.42 ± 0.39), Britton-Robinson buffer (34.9 ± 11.2) and sodium pyrophosphate (293.2 ± 113.4) were statistically significant. The A_{465}/A_{665} ratio negatively correlated with molecular weight of humic substances.

A_{465}/A_{665} ratio, DOC, humic substances, molecular weight, size exclusion chromatography

Dissolved organic carbon (DOC) is a term for complex mixture of organic molecules of varied origin and composition that occur in surface waters. This mixture can be divided into humic and non-humic fractions. The humic fraction is represented mainly by fulvic acids (FA); the proportion of humic acids (HA) is usually small. As precursors of carcinogenic trihalogenmethans produced during chlorination of drinking water (Rook, 1974; Bellar *et al.*, 1974), humic substances (HS) are believed to be harmful to human health.

DOC plays a significant role in carbon flux in ecosystems (Remeš and Kulhavý, 2009; Fujii *et al.*, 2011). During the last 20 years, a widespread increase of the amount of DOC in surface waters has been reported in Europe and North America (Skjelkvale, 2003). Many studies have related this DOC increasing with warmer climate (Freeman *et al.*, 2001), changes in the amount of precipitation (Tranvik and Jansson, 2002) or decline in atmospheric acid deposition. Changes in acid deposition and decline surface water acidity (mediated by decreasing sulphur deposition) is being partially offset by increasing organic acidity. It is likely that DOC levels will continue to rise, with unpredictable consequences for the global carbon cycle (Monteith *et al.*, 2007).

Relationship between DOC and soil has been studied by many authors (Bárta *et al.*, 2010; Gungor and Bekbolet, 2010; Filep and Rekasi, 2011). DOC export into surface water is controlled by its content in the soil. Instead of difficult measurement of soil DOC, it is frequently determined the water extractable carbon (WEC), which is mixture of organic carbon compounds, obtained by extracting of soil sample by water or aqueous solution.

The aim of this work was (1) to compare humic substances isolated from the Rašeliník creek with extractable carbon from surrounding soil using molecular characteristics, and (2) to find an agent, extracting from peaty soil fraction of organic carbon similar to the humic substances contained in DOC.

MATERIAL AND METHODS

Sampling

Water for dissolved humic substances isolation (DHS) was sampled in the left-hand tributary of Rašeliník creek (Fláje dam catchment, Ore Mountains, North Bohemia) on July 7, 2010. Six soil samples were taken from the upper soil horizons (Table I) in representative biotopes of the Rašeliník watershed.

Extraction procedures

Soil extraction by Britton-Robinson and pyrophosphate buffers. 5 g of fresh soil sample were extracted in 100 ml of Britton-Robinson buffer (pH 9) or 100 ml 0.1M pyrophosphate agent (0.1M $Na_4P_2O_7$ + 0.1M NaOH, pH 12). At the beginning of extraction and after 20 hours, samples were shaken one hour on the horizontal shaker. The extract was separated by centrifugation and filtration.

Soil extraction by 0.01M $CaCl_2$ solution. 80 g of fresh soil sample were shaken with 400 ml of 0.01M $CaCl_2$ for 1 hour. The extract was centrifuged, filtered, concentrated in ultrafiltration cell Amicon (filter YM 1K) under dinitrogen, and freeze-dried in HETO 8.0 lyophilizer. The low concentration of divalent Ca^{2+} ions is used to minimize extraction of clay minerals and to ensure the constant ionic strength when the WEC from various soils is extracted.

Aquatic HS were isolated using a standard method (Malcolm, 1991). The pH of the sample was adjusted by HCl to 2.0, HS were then sorbed in the column filled with Amberlite XAD-8, washed with distilled water and eluted with 0.1M NaOH. Sodium fulvate was purified and concentrated by ultrafiltration, converted into H^+ form in column packed with the strong cation-exchange resin Dowex WX4, and freeze-dried.

Size exclusion chromatography

The column (485 × 18 mm) was filled with the Sephadex G-50. The mobile phase consisted of the Britton-Robinson buffer solution at pH 9.0. Calibration was made using Pharmacia standards. Before applying to the column, the SFP and SmM samples extracted in $CaCl_2$ were dissolved in 3 ml Britton-Robinson buffer, alkalized by NaOH to pH 10.5.

Relative concentrations of standards and humic substances in the eluate were measured by the UV/VIS spectrophotometer Libra S 22 at the wavelength of 280 nm in 0.01 m quartz cell. Every sample was analyzed twice, the relative error of MW was typically < 3%.

Average molecular weights were calculated by formula

$$\bar{M} = \frac{\sum_i N_i M_i^{r+1}}{\sum_i N_i M_i^{r}} = \frac{\sum_i W_i M_i^{r}}{\sum_i W_i M_i^{r-1}} ,$$

where M_i is the molecular weight of the fraction i, N_i *is* a number and *W*i is total weight of molecules with molecular weight M_i. The M_r value, also called *peak molecular weight* (Li *et al.*, 2008), is defined as M_i corresponded to the fraction of maximal absorption A_{280}. For $r = 0$ the resulting value M_n is called a number-average molecular weight, for $r = 1$ the resulting value M_w is called a weight-average molecular weight (Kudryavtsev *et al.*, 2000).

The A_{465}/A_{665} ratio was calculated as a ratio of absorbances at wavelengths 465 and 665 nm. Statistical calculations were made using Statistica 6.0 (Stat Soft).

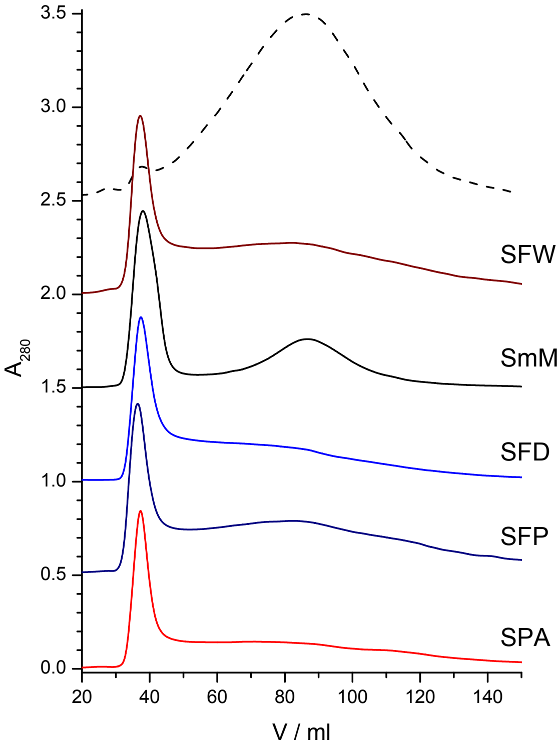
RESULTS AND DISCUSSION

Elution curves of all humic substances were characterized by two peaks with different ratio of individual fractions (Fig. 1, 2). The area of first peaks, representing high-molecular fraction of HS with M_w > 30 kDa, didn't exceed 45%. Molecular weights M_w and M_r of HS increased in the following sequence of extractants: 0.1M $CaCl_2$ < aquatic HS < pyrophosphate ≤ BR buffer (Tab. II). Low-molecular weight fraction represented by the

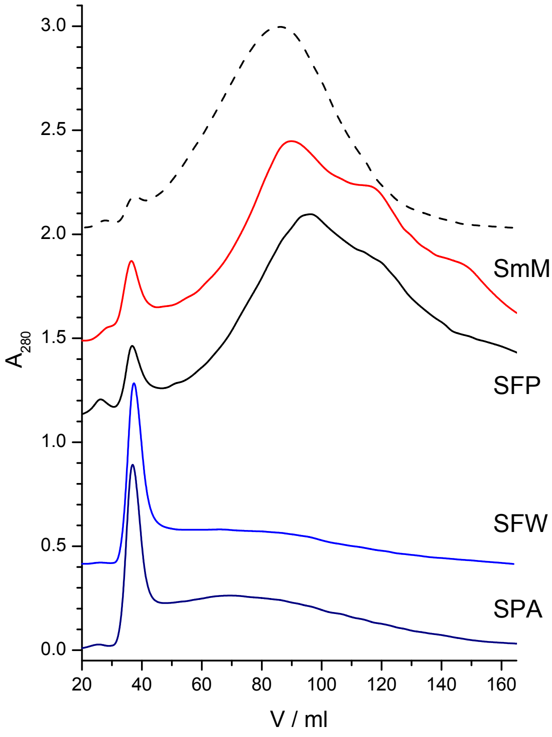
I: *Stand characteristics*

Acronym	Biotope / soil	Soil horizon	Association
SFW	waterlogged spruce forest /Podzol histic	O_m	*Mastigobryo-Piceetum* **and** *Equiseto-Piceetum*
SPA	sedge/peat ass. /Histosol mesic	T_m	*Caricion fuscae, Caricion lasiocarpae, Sphagno warnstorfiani-Tomenthypnion etc.*
SFP	spruce forest with peat /Podzol histic	O_h / O_m	Sphagno-Piceetum
SFD	spruce forest dry /Cryptopodzol	O_h	Calamagrostio villosae-Piceetum
SmM	submountain meadow /Cambisol dystric	A_d	alliance *Cynosurion*

second peak with M_w between 4.1–11.6 kDa, predominated in all samples. Occurrence of the low molecular weight substances in a soil sample was confirmed mainly in $CaCl_2$ extract, where the M_r and M_w approximate values were 4.5 kDa and the amount of this fraction was 95%, aquatic humic substances reached M_r and M_w values of 5.9 and 7.9 kDa, respectively, and the proportion of low-molecular fraction was similar (97%). As results obtained from most naturals waters show, the major fraction of dissolved organic matter closely resembles soil fulvic acids (Reuter and Perdue, 1977). The HA/FA ratio and molecular weight of HS differ depending on their origin and experimental conditions (extractants, degree of purification etc.). Published relative MWs of HS lie in a wide range from several hundreds to millions Da (Stevenson,



1: *Size exclusion chromatograms of organic carbon extracted from peat soils by pyrophosphate (full line) and DOC isolated from Rašeliník creek (dashed line). (See table I for abbreviations used).*



2: *Comparision of size exclusion chromatograms of DOC (dashed line) and organic carbon extracted from peat soils by 0.01M $CaCl_2$ (SmM, SFP) and by Britton-Robinson buffer (SFW, SPA). (See table I for abbreviations used).*

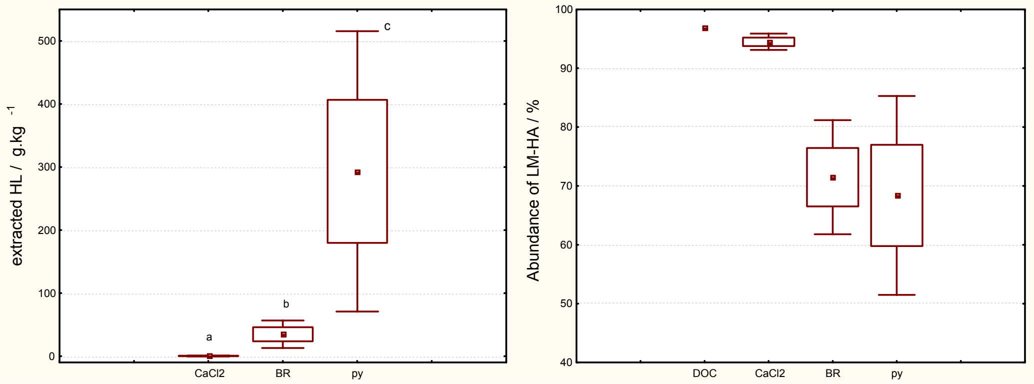
II: *Molecular weights and the A_{465}/A_{665} ratios of HS*

Sample	M_r of LM-HS	M_w of LM-HS	Rel. abundance of LM-HS	A_{465}/A_{665} extract	A_{465}/A_{665} HM-HS	A_{465}/A_{665} LM-HS
	kDa	kDa	%			
DHS	5.9	7.9	97	13.8*	4.0	16.7
SFW (Py)	6.9	9.9	76	5.2	4.9	6.7
SPA (Py)	10.3	10.6	67	5.1	4.7	6.2
SFP (Py)	6.9	9.1	76	5.1	4.8	6.6
SFD (Py)	9.0	11.6	68	6.6	4.9	10.6
SmM (Py)	5.8	8.0	55	8.2	6.6	9.6
SFP ($CaCl_2$)	4.0	4.1	95	n.d.	4.2	10.0
SmM ($CaCl_2$)	5.1	5.0	94	n.d.	3.4	9.8
SFW (BR)	12.6	11.1	68	7.7	4.7	5.0
SPA (BR)	11.0	10.4	75	7.5	4.6	5.0

M_r, molecular weight of the fraction with the highest concentration of HS; M_w, weight-average molecular weight; LM-HS, low-molecular humic substances fraction; HM-HS, high-molecular humic substances fraction; Py, sodium pyrophosphate; BR, Britton-Robinson buffer; * in 0.05M $NaHCO_3$; n.d., not determined.

III: *Extraction of soil HS by various agents (in g.kg^{-1} of dry soil)*

Sample	HS in 0.01M CaCl$_2$ extract	HS in B-R buffer extract	HS in pyrophosphate extract	pH
	g.kg^{-1}	**g.kg^{-1}**	**g.kg^{-1}**	**0.01M CaCl$_2$**
SFW	0.173	34.6	307	4.33
SPA	1.107	51.5	183	3.31
SPA	0.327	21.1	478	3.00
SFP	0.213	29.7	272	4.85
SFD	0.265	37.6	226	4.55
SmM	0.048	8.0	43	4.29



3: *a) Box and whisker diagram of organic carbon extracted from organic soils by various agents (graph on the left). Outlying value for agricultural meadow soil was excluded before calculation. Letters a, b, and c indicate ranges of significantly different values. b) Abundance of low-molecular humic substances in various extracts (on the right).*

1994). Perminova *et al.* (2003) mentioned values in narrower range of 4.7–30.4 kDa for samples isolated from different natural environment, whereas the maximum M_w values were measured for peat HS and the smallest M_w values for river water HS.

Extraction efficiency by selected extraction agents is illustrated by values in Tab. III. It is clear that quantity of humic substances extracted from soils was proportional to the pH value. Thus, pyrophosphate (pH 12) was the most active agent, extracted from soil samples up to 1770 times more HS (mean 1254 ± 387) than 0.01M CaCl$_2$, extracting at pH and ionic strength close to native soil conditions. The differences among tested extractants were statistically significant (Fig. 3a).

It is obvious that greater proportion of high-molecular humic substances was eluted using more efficient extraction agents (Tab. II, Fig. 3b), which was also reflected by lower value of the A_{465}/A_{665} ratio. On the other hand, the 0.01M CaCl$_2$ extracted fraction of soil organic matter with molecular characteristics close to DOC (94–95% of LM-HS *vs* 97%).

The A_{465}/A_{665} values of LM-HS (Tab. II) in a broad range were found in pyrophosphate extracts (mean 7.9). Extracts in a weak extraction agent (0.01M CaCl$_2$) had higher A_{465}/A_{665} ratios, close to values of HS from water of Rašeliník. According to Chen *et al.* (1977), high A_{465}/A_{665} values reflect relatively low molecular weight and degree of humification of soil organic matter, which corresponds to our results. The smallest A_{465}/A_{665} ratio was found after extraction in Britton-Robinson buffer in predominant low-molecular fraction.

The A_{465}/A_{665} ratio of FA between 16.6–18.4 was reported by Baes and Bloom (1990), Canellas and Façanha (2004) mentiones values of 4.0–7.0 and 7.2–9.7 for humic and fulvic acid fraction in ultisol, respectively.

On the basis of received results we can conclude, that the 0.01M solution of CaCl$_2$ is a suitable extraction agent for determination of soil organic matter (SOM) fraction readily leachable from peaty soils. Molecular characteristics of this fraction of SOM were similar to those of dissolved organic carbon.

CONCLUSION

Humic substances, representing a main fraction of dissolved organic carbon, have a great importance for carbon export into surface waters. Fulvic acids, due to their easy mobility, constitute the main fraction of aquatic humic substances. The determination of molecular weight, distribution of high and low-molecular humic substances and A_{465}/A_{665} ratio are suitable for the comparison of samples from different sources, and all these observed parameters can provide important information concerning the humic substances mobility in soil environment. From obtained results it's evident that molecular characteristics of HS are influenced not only by origin, but also by the extraction agents used for isolation procedure and that quantity of humic substances extracted from soil is proportional to the pH value.

SUMMARY

The main objective of this study was to compare the molecular characteristics of extractable soil carbon and dissolved organic carbon (DOC) by using the 0.01M $CaCl_2$, Britton-Robinson and sodium pyrophosphate buffers as extraction agents. Soil samples were taken from the upper soil horizons (O_h/A) of representative biotopes of the Rašeliník watershed, and water for dissolved humic substances isolation was sampled in the left-hand tributary of Rašeliník creek (Fláje dam catchment, Ore Mountains, North Bohemia). Molecular weight M_r and weight-average molecular weight M_w were determined by the low pressure size exclusion chromatography (SEC) on Sephadex G-50, the mobile phase consisted of the Britton-Robinson buffer solution at pH 9.0. Relative concentrations of standards and humic substances in the eluate were measured by the UV/VIS spectrophotometer Libra S 22 at the wavelength of 280 nm in 0.01 m quartz cell.

The molecular weight increased in the following sequence: HS in $CaCl_2$ < aquatic HS < HS in sodium pyrophosphate ≤ HS in a Britton-Robinson buffer. The low-molecular fraction with M_w ranged between 4.1–11.6 kDa predominanted in all extracts. Aquatic HS contained 97% of this fraction, which corresponds with the fact, that the major proportion in surface waters constitute low-molecular fulvic acids. The fraction of organic carbon similar to the HS in DOC we obtained by soil extraction in $CaCl_2$, where the M_r and M_w approximate values were 4.5 kDa and the amount of this low-molecular fraction was 95%. The pyrophosphate was the most active agent, extracted from soil samples 850–1770 times (mean 1254 ± 387) more humic substances than 0.01M $CaCl_2$. Differences in quantity of organic carbon extracted from soils among 0.01M $CaCl_2$ (mean 0.42 ± 0.39), Britton-Robinson buffer (34.9 ± 11.2) and pyrophosphate (293.2 ± 113.4) were statistically significant. The A_{465}/A_{665} values of LM-HS ranged between 5.0–10.6 and 16.7 by soil extracts and HS in DOC, respectively. The A_{465}/A_{665} ratio negatively correlated with molecular weight of humic substances.

It was verified that the extraction of peaty soils by 0.01M $CaCl_2$ provides determination of soil organic matter fraction with molecular characteristics close to DOC. Based on this finding, extensive soil screening in the watershed is carried out to identify biotopes with degrading soil, where ameliorative trees will be planted to reduce DOC leaching into surface water.

Acknowledgements

This work was supported by the project No. 100018967 Cíl 3 – ČR – Sasko „Investigation of possibilities of organic pollutants minimization in sources of drinking water in Ore Mts“.

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