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## The Proportion of Soil Aggregates in Dependence on the Fraction Composition of Humic Substances

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### Abstract

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Humic substances (HS) are an important stabilizing element in the formation of soil aggregates. The experiment included 6 soil types (Haplic Fluvisol, Haplic Chernozem, Cutanic Luvisol, Haplic Cambisol, Haplic Planosol, Rendzic Leptosol), each in four types of ecosystems (forest, meadow, urban, and agro-ecosystems). Soil macroaggregates were separated with the sieve (dry and wet sieve) to size fractions of net aggregates; humic acids (HA) and fulvic acids (FA) were divided into 3(4) fractions: free and bound with mobile  $R_2O_3$ , bound with  $Ca^{2+}$ , and bound with mineral components and stable  $R_2O_3$ . The influence of HA and FA fractions on the proportion of dry-sieved macroaggregates (DSA) and wet-sieved macroaggregates (WSA) was different. Mainly HA bound with polyvalent cations had a positive influence. In the case of HS, their influence depends also on the components with which HA and FA are bound. WSA of the 0.5–1 mm size fraction that is an important indicator of changes in ecosystems, had a higher proportion in the ecosystems influenced by man (agro-ecosystem > urban >) than in the ecosystems close to nature (> meadow > forest ecosystems). The influence of ecosystem was reflected in the proportion of those fractions of DSA and WSA on which the effect of soil type and HS was not shown, and vice versa.

**Keywords:** dry-sieved and water-resistant macroaggregates; ecosystem; fulvic acids; humic acids

Humic substances (HS) are the most widespread group of organic components in the natural environment and also belong to the most important fractions of organic matter (VERGNOUX *et al.* 2011). HS are considered as an important element in the formation of stable soil aggregates (TISDALL & OADES 1982; ŠARAPATKA *et al.* 2014), mainly long-term ones (SODHI *et al.* 2009), but their individual fractions participate in stabilisation to a different extent. Humified organic material can promote permanent improvement of aggregate stability (PICCOLO & MBAGWU

1999), but the functions of humic substances are closely related to their quantity and quality in soil and also exogenous organic materials (POSPISILOVÁ *et al.* 2017). An important role is played, as by the aggregating agents, by Al and Fe oxides (MBAGWU & SCHWERTMAN 2006), carbohydrates (WUDDIVIRA & CAMPS-ROACH 2007), and other soil components like fungi (WAGNER *et al.* 2007) or vegetation. The impact of the ecosystem on the proportion of soil aggregates is especially manifested through the vegetation cover either directly through the quantity and

quality of organic inputs (TOBIAŠOVÁ 2011; ZHAO *et al.* 2017), or indirectly, by the protection of soil against kinetic energy of water, thereby increasing their stability (PETLUŠOVÁ & PETLUŠ 2016). On the contrary, the formation of soil aggregates has a pronounced influence on the carbon stock and the stability of aggregates influences the dynamics of soil organic matter (SOM) (SIX *et al.* 2000). Often, the attention is focused only on water-resistant or wet-sieved macroaggregates (WSA), however, about the mechanisms of carbon stabilisation it is decided at the formation of dry-sieved macroaggregates (DSA). The aim of this research was to study the relations between humic substances and soil aggregates, which are applied in general; it means not only those that are typical of some soil or ecosystem, but general rules which play an important role in more soils and ecosystems. Besides that to find out if there is any relation between HS and DSA or WSA that are influenced only by soils or only by ecosystems. The concrete objectives of this study were: (i) to assess the influence of fractions of HS in the soil on the proportions of DSA and (ii) WSA, (iii) to determine the differences between the amount of DSA and WSA in soils and ecosystems in dependence on the proportions of HS in the soil.

## MATERIAL AND METHODS

**Characteristics of the territory and experimental details.** The studied areas are located in different parts of Slovakia, which are represented by different geology and climatic regions (Table 1). The experiment included 6 soil types: Haplic Fluvisol (HFl), Haplic Chernozem (HCh), Cutanic Luvisol (CLu), Haplic Cambisol (HCa), Haplic Planosol (HPl), Rendzic Leptosol (RLe) (IUSS Working Group WRB 2014), each in 4 types of ecosystems (forest, meadow, urban, and agro-ecosystems). These are the soils (Table 2) which cover the largest proportion in Slovakia and are intensively used in agriculture. The forest ecosystems are represented by more than 100-years-old managed forests; the meadow ecosystems are formed by the dense layer of grasses; the properties of the soils of both ecosystems are very close to soils of natural ecosystems. The urban ecosystems presented soils of urban landscape (permanent grasslands in towns influenced by human activities). The fields in agro-ecosystems were located on different farms and are used as arable soils.

**Soil samples and analytical methods used.** The soil samples were collected in 3 replications to a depth of 0.3 m, and dried at a constant laboratory

Table 1. Characteristics of the selected territory

Soil	Locality	Geology*	T (°C)**	R (mm)**
Haplic Fluvisol	Rybany (48°67'N, 18°26'E)	Danubian Upland; neogenic sediments (consist mainly of marls, conglomerates, sandstones, claystones and organogenic limestones)	9.0	629
Haplic Chernozem	Horná Kráľová (48°24'N, 17°91'E)	Danube Basin; neogene strata (consist mainly of claystones, sandstones and andesites, which are covered with younger quaternary rocks that are represented by different fluvial and aeolian sediments)	9.8	568
Cutanic Luvisol	Vráble (48°24'N, 18°31'E)	Danube Plain; neogene sediments (loess and loess loamy)	9.1	605
Haplic Cambisol	Spišská Belá (49°19'N, 20°46'E)	Poprad basin; core mountains of the central Western Carpathians (consist of mica schists to gneisses, locally phyllites of early paleozoic, which are covered with deluvial sediments)	8.6	673
Haplic Planosol	Vavrečka (49°38'N, 19°47'E)	Flysch Belt of Horná Orava (consist of clay slates, andstones and conglomerates in the layers with a various depth)	4.6	1010
Rendzic Leptosol	Beluša (49°07'N, 18°33'E)	Klippen Belt – the thickness in this region is the largest, more than 550 km; (consist of carbonate and paleogenic rocks of flysch development, marls, and marlstones, carbonate sandstones, limestones with ammonites)	8.2	740

\*ŠAJGALÍK *et al.* (1986); \*\*KOREC *et al.* (1997); T – average annual temperature; R – sum of annual precipitation

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Table 2. Average values of basic soil characteristics

	TOC (g/kg)	Humic acids	Fulvic acids	pH	Sand (%)	Silt	Clay
HFl	16.641 ± 7.871	42.30 ± 8.55	40.90 ± 4.10	7.14 ± 0.44	26.83 ± 15.27	55.09 ± 16.78	18.08 ± 5.74
HCh	18.514 ± 2.132	49.66 ± 6.60	28.76 ± 5.84	7.04 ± 0.75	37.33 ± 10.70	46.47 ± 9.54	16.19 ± 2.48
CLu	11.174 ± 4.464	45.18 ± 4.93	56.15 ± 7.23	5.93 ± 0.99	28.39 ± 6.42	56.71 ± 6.63	14.90 ± 1.97
HCa	30.138 ± 15.524	48.22 ± 2.28	28.24 ± 2.55	6.85 ± 0.33	41.12 ± 7.33	50.60 ± 4.68	8.27 ± 3.93
HPl	21.863 ± 6.902	26.34 ± 6.62	33.74 ± 1.63	5.43 ± 0.77	55.83 ± 11.01	23.81 ± 11.85	20.37 ± 4.95
RLe	25.823 ± 7.178	61.02 ± 12.41	44.40 ± 14.40	7.13 ± 0.35	38.55 ± 9.49	46.35 ± 6.15	15.11 ± 5.95

TOC – total organic carbon; HFl – Haplic Fluvisol; HCh – Haplic Chernozem; CLu – Cutanic Luvisol; HCa – Haplic Cambisol; HPl – Haplic Planosol; RLe – Rendzic Leptosol

temperature of  $25 \pm 2^\circ\text{C}$ . The soil samples for determination of chemical properties were ground. To determine the fractions of soil aggregates, the soil samples were separated with the sieve (dry and wet sieve) to size fractions of net aggregates (SARKAR & HALDAR 2005). The particle size distribution was determined after dissolution of  $\text{CaCO}_3$  with  $2 \text{ mol/dm}^3$  HCl and oxidation of the organic matter with 30%  $\text{H}_2\text{O}_2$ . After repeated washing, samples were dispersed using  $\text{Na}(\text{PO}_3)_6$ . Silt, sand, and clay fractions were determined according to the pipette method (VAN REEUWIJK 2002). In the soil, TOC by wet combustion (ORLOV & GRIŠINA 1981) and soil pH were determined. The pH of the soil was potentiometrically measured in a supernatant suspension of a 1 : 2.5 soil to liquid mixture. The liquid is  $1 \text{ mol/dm}^3$  KCl ( $\text{pH}_{\text{KCl}}$ ) (VAN REEUWIJK 2002). The fractions of HS were determined by the method of PONOMAREVA and PLOTNIKOVA (1975).

The obtained data were analysed using Statgraphic Plus statistical software. A multifactor ANOVA model was used for individual treatment comparisons at  $P < 0.05$ , with separation of the means by Tukey's multiple-range test. Correlation analysis was used to determine the relationships between HS in the soils and proportions of soil aggregates. Significant correlation coefficients were tested at  $P < 0.05$  and  $P < 0.01$ .

## RESULTS AND DISCUSSION

**Influence of humus substances on dry-sieved macroaggregates.** Fractions of HS had a more pronounced influence on DSA fractions than on WSA ones. Substantially lower proportions of larger DSA (3–7 mm) and simultaneously a higher proportion of smaller DSA (0.25–1 mm) were in HPl and just the

opposite situation was in HCh and RLe (Table 3). In the soils with a higher quantity and quality of SOM, the larger DSA accounted for a higher proportion, and vice versa, in the soil with its lower quality there were smaller DSA. An increased proportion of the smallest fractions of soil aggregates is a reflection of the deterioration of the soil structure condition (WHALEN & CHANG 2002). Less productive soils are more easily susceptible to deterioration of soil structure, but in the case of HPl, the pH/KCl value was also the lowest and FA in this soil had also a significantly higher proportion.  $\text{H}^+$  ions act destructively on clay minerals (CAMA & GANOR 2015), thereby many bonds in the aggregates are disrupted and consequently they are breaking down. In CLu, although the pH was higher only by about 0.5, the content of clay was lower by about 27% than in HPl. In HCh and RLe, the pH values were similar to those in HFl, but these are the soils where in the sorption complex  $\text{Ca}^{2+}$  cation is dominant, which has a positive influence on aggregation (WUDDIVIRA & CAMPS-ROACH 2007).

In the case of DSA (3–7 mm), a positive correlation was recorded with HA, especially bound with bivalent cations (HA2) and trivalent cations and stable  $\text{R}_2\text{O}_3$  (HA3), and vice versa, in the case of the smallest DSA (0.25–0.5 mm) a negative correlation was found (Table 3). In the case of FA, FA free and bound with mobile  $\text{R}_2\text{O}_3$  (FA1) were in a negative correlation with DSA (3–7 mm) and in a positive correlation with DSA (0.25–0.5 mm). The higher the proportion of HA (mainly those bound with polyvalent cations) in the soil, the higher was the proportion of larger DSA, and vice versa, the lower the proportion of these HA, the higher was the proportion of smaller aggregates. Among the cations, an important role in the aggregation is played not only by  $\text{Ca}^{2+}$ , but also by  $\text{Fe}^{3+}$  and  $\text{Al}^{3+}$  (WUDDIVIRA &

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Table 3. Correlation between humus substances and macroaggregates

	Dry sieved macro-aggregates						Water resistant macro-aggregates					
	> 7	5–7	3–5	1–3	0.5–1	0.25–0.5	> 5	3–5	2–3	1–3	0.5–1	0.25–0.5
HA1	-0.349*	ns	ns	0.312*	ns	ns	ns	ns	ns	ns	ns	ns
HA2	ns	0.462**	0.487**	ns	ns	-0.539**	-0.419**	ns	0.310*	0.305*	ns	ns
HA3	ns	0.414**	0.396**	ns	-0.401**	-0.653**	-0.355*	ns	0.444**	ns	ns	ns
ΣHA	ns	0.502**	0.563**	ns	ns	-0.600**	-0.453**	ns	0.436**	0.362*	ns	ns
FA1a	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
FA1	0.330*	-0.593**	-0.562**	ns	ns	0.414**	0.437**	ns	-0.376*	-0.393**	ns	ns
FA2	0.313*	ns	ns	ns	ns	-0.432**	ns	ns	ns	ns	ns	ns
FA3	ns	ns	0.333*	ns	-0.339*	-0.534**	ns	ns	ns	ns	ns	ns
ΣFA	0.403**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
C <sub>HA</sub> :C <sub>FA</sub>	-0.317*	0.405**	0.512**	0.336*	ns	ns	-0.322*	ns	0.376*	0.402**	ns	ns
C:N	-0.626**	ns	0.658**	0.555**	ns	ns	ns	ns	0.363*	0.332*	ns	ns

HA1 – free humic acids and humic acids bound with mobile R<sub>2</sub>O<sub>3</sub>; HA2 – humic acids bound with Ca<sup>2+</sup>; HA3 – humic acids bound with mineral components and stable R<sub>2</sub>O<sub>3</sub>; ΣHA – sum of humic acids; FA1a – free aggressive fulvic acids; FA1 – free fulvic acids and fulvic acids bound with mobile R<sub>2</sub>O<sub>3</sub>; FA2 – fulvic acids bound with Ca<sup>2+</sup>; FA3 – fulvic acids bound with stable R<sub>2</sub>O<sub>3</sub>; ΣFA – sum of fulvic acids; \*, \*\*P < 0.05, 0.01; ns – not significant

CAMPS-ROACH 2007; JAKŠÍK *et al.* 2015), mainly as sesquioxides that usually contribute to a large part of the soil specific surface area, which is important for soil shrinkage and cracking (GRAY & ALLBROOK 2002). In larger aggregates, an important role is played not only by HS, but also by cations themselves that enter into the mutual bonds between mineral and organic particles as cation bridges (VON LÜTZOW *et al.* 2006). In smaller aggregates, the mineral components participate more in their formation than the organic ones that are included in them in a smaller amount (SOHI *et al.* 2001), they act rather as the coats (KODEŠOVÁ *et al.* 2009), especially carboxylic carbon supports stabilization through the surface occlusion (VERCHOT *et al.* 2011). On the contrary, FA in comparison with HA, contain more functional

groups with acidic reaction (-COOH, -C=O, -OH) (STEVENSON 1982). Moreover, FA1 consist partly of free FA although for the most part they consist of salts and complex compounds with mobile hydrated sesquioxides, mainly with aluminium (KONONOVA 1966). Monovalent cations, mainly Na<sup>+</sup>, are a highly dispersive agent resulting directly in the breakup of aggregates (BATRA *et al.* 1997; WUDDIVIRA & CAMPS-ROACH 2007). Thus the result is that at a higher proportion of FA1 in the soil, a lower amount of larger (3–7 mm) and also a larger amount of smaller (0.25–0.5 mm) aggregates are presented in the soil. However, the largest fraction of DSA (> 7 mm) had a higher proportion in the case of a higher content of FA1 fraction. This could be explained by decreased pH values. In the area of lower pH values, the fungi

Table 4. Statistical evaluation of water-resistant macroaggregate fractions in different soil types

	> 5	3–5	2–3	1–3	0.5–1	0.25–0.5
	(mm)					
HFl	20.38 <sup>a</sup>	15.09 <sup>a</sup>	18.18 <sup>ab</sup>	17.59 <sup>ab</sup>	13.35 <sup>a</sup>	6.52 <sup>a</sup>
HCh	4.95 <sup>a</sup>	12.74 <sup>a</sup>	21.17 <sup>b</sup>	21.43 <sup>ab</sup>	17.51 <sup>a</sup>	12.81 <sup>a</sup>
CLu	13.17 <sup>a</sup>	10.80 <sup>a</sup>	13.70 <sup>ab</sup>	18.24 <sup>ab</sup>	18.31 <sup>a</sup>	11.84 <sup>a</sup>
HCa	6.93 <sup>a</sup>	12.19 <sup>a</sup>	17.36 <sup>ab</sup>	25.13 <sup>b</sup>	17.56 <sup>a</sup>	10.08 <sup>a</sup>
HPl	19.02 <sup>a</sup>	13.59 <sup>a</sup>	11.28 <sup>a</sup>	15.13 <sup>a</sup>	14.24 <sup>a</sup>	9.58 <sup>a</sup>
RLe	9.21 <sup>a</sup>	14.84 <sup>a</sup>	21.50 <sup>b</sup>	19.68 <sup>ab</sup>	13.67 <sup>a</sup>	8.84 <sup>a</sup>

HFl – Haplic Fluvisol; HCh – Haplic Chernozem; CLu – Cutanic Luvisol; HCa – Haplic Cambisol; HPl – Haplic Planosol; RLe – Rendzic Leptosol; <sup>a,b</sup>different letters between the factors show statistically significant differences (P < 0.05) – Tukey's test

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play an important role in the formation of larger aggregates (DAYNES *et al.* 2013) that become dominant. Fungi produce extracellular substances that have a cementing effect, but they also form a mycelium that holds soil particles together (TISDALL *et al.* 1997; DRIVER *et al.* 2005).

The wider ratio of  $C_{HA}$  to  $C_{FA}$  (Table 3) was connected with the presence of a higher proportion of larger DSA (3–7 mm), and simultaneously the proportion of the largest DSA was lower (> 7 mm). In the case of soils with the wider C:N ratio there was a lower proportion of the largest DSA (> 7 mm) and simultaneously a higher proportion of DSA (1–5 mm). DSA > 7 mm had a higher proportion at the narrower ratio of  $C_{HA}$  to  $C_{FA}$ , which is associated with the above-mentioned higher content of FA. A higher proportion of these aggregates was also at a narrower C:N ratio, which is associated with fungi, mainly arbuscular mycorrhizal fungi, as a significant fixator of nitrogen (LEIGH *et al.* 2009; HODGE & FITTER 2010). DSA (1–5 mm) had a higher proportion not only at a wider ratio of  $C_{HA}$  to  $C_{FA}$ , but also at a wider ratio of C:N. According to GREGORICH *et al.* (2003), the decomposition of organic nitrogen is faster than that of carbon, so it can be the result of a higher proportion of older organic substances, as well as recalcitrants, which are poor in carbon and support the formation of these aggregates.

**Influence of humus substances on water-resistant macroaggregates.** In the case of WSA, the soil type had an influence on the proportion of WSA 1–3 mm and > 5 mm, but it did not have any influence on the fractions of WSA 0.25–1 mm and 3–5 mm (Table 4). Just the opposite situation was in the ecosystem (Figure 1). WSA fractions that were not influenced by the ecosystem, were not influenced by HS either (Table 3). WSA, on which the influence of the ecosystem is reflected, but on the contrary, the influence of the soil type is not reflected, seem to be an important indicator of changes in the ecosystem. HS are stable components, the properties of which depend on their origin (PEÑA-MÉNDEZ *et al.* 2005), so on the soil type in this case.

Considering the effect of ecosystem in relation to the size fractions of aggregates, the order of ecosystems was opposite (Figure 1). Larger WSA (3–5 mm) had a higher proportion in the ecosystems that are close to nature (forest, meadow), which confirmed the results of EMADI *et al.* (2009), who found out more macroaggregates with diameter of 0.25–4.75 mm in these ecosystems. In the case of smaller aggregates,

the order of ecosystems was opposite, and their higher proportions, in particular of the above-mentioned fraction of WSA (0.5–1 mm), which seems to be an important indicator of deterioration of soil structure in ecosystems (TOBIAŠOVÁ *et al.* 2014), were in the urban and agro-ecosystems.

Agronomically the most valuable WSA (1–3 mm) were in a positive correlation with HA (Table 3), especially fractions of HA2 and HA3. Overall, the fractions of HA bound with polyvalent cations had a positive effect on the formation of the most valuable size fractions of DSA and WSA. Moreover, according to TISDALL and OADES (1982), the humus substances, together with polyvalent cations, participate in the formation of the most stable aggregates, and simultaneously according to MUNEEER and OADES (1989),

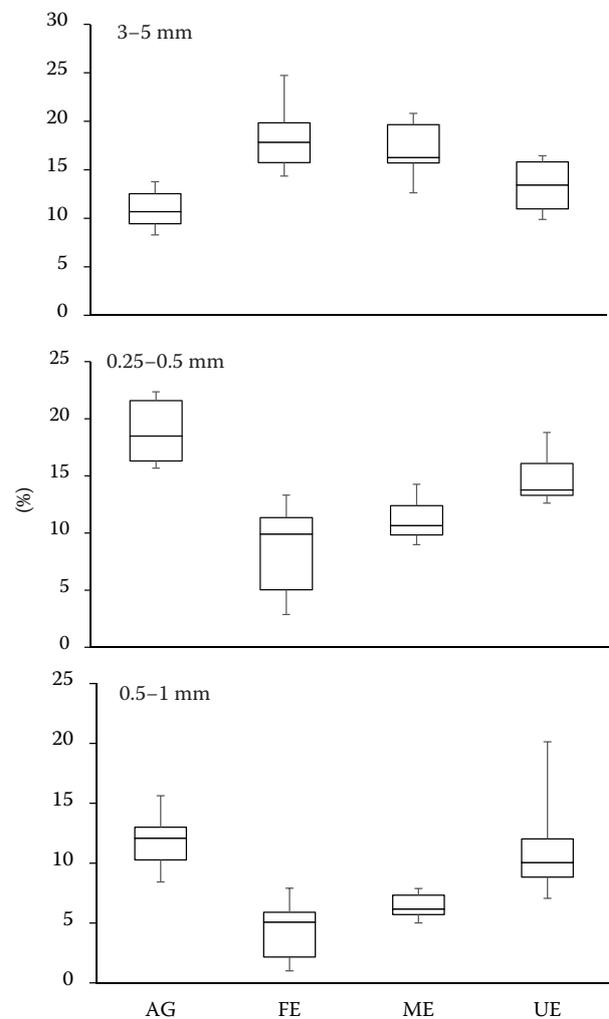


Figure 1. The percentage differences in water-resistant macroaggregates between ecosystems AG – agro-ecosystem; FE – forest ecosystem; ME – meadow ecosystem; UE – urban ecosystem

soil organic matter is also stabilized through the bridges of these polyvalent cations.

Considering the quality of HS, the same tendency was in the case of both DSA and WSA. It confirms the results of NOELMEYER *et al.* (2008), and according to them dry aggregates are found to be useful for studying the soil degradation, and they show similar trends as those indicated for WSA in the literature. In DSA, the beginning of carbon accumulation decides on the character of stabilization and subsequent carbon content in the WSA. Therefore, we can use DSA for prediction of the next tendency in WSA.

## CONCLUSIONS

HA, especially those bound with polyvalent cations, had a favourable influence on the formation of larger DSA (3–7 mm), and agronomically the most valuable WSA (1–3 mm).

In spite of the fact that FA also belong to HS, the effect of free FA and bound with mobile  $R_2O_3$  was opposite to HA.

In the case of HS, their influence depends not only on the fact if there is a predominance of HA or FA, but also on their speciation (type of bond with inorganic compounds and cations).

WSA fractions of the sizes 0.25–1 mm and 3–5 mm, which were influenced by ecosystem, but were not influenced by soil type, seem to be an important indicator of land use on the soil.

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