Potential effect of wetting agents added to agricultural sprays on the stability of soil aggregates

Antonín Kintl[1], Vítězslav Vlček[2], Martin Brtnický[2,3], Jan Nedělník[1], Jakub Elbl[1,4]

[1]Agricultural Research, Ltd., Zahradní 1, 664 41 Troubsko, Czech Republic
[2]Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, Brno 61300, Czech Republic
[3]Institute of Chemistry and Technology of Environmental Protection, Brno University of Technology, Faculty of Chemistry, Purkynova 118, 621 00 Brno, Czech Republic
[4]Department of Agrosystems and Bioclimatology, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

Corresponding author: Jakub Elbl (jakub.elbl@mendelu.cz)

Abstract. ~~**The presented research deals with the issue of the potential effect of adjuvants/wetting agents (WA) added to the spray mixture on the stability of soil aggregates (SAS) in agricultural soil.**~~ The potential effect of adjuvants/wetting agents added to the spray mixture on the water stability of soil aggregates (WSA) in agricultural soil was studied~~-~~. Nine sites~~localities~~ were chosen in the Czech Republic. Each site~~locality~~ was mapped using soil pits (depth min. 1.~~4~~ 3 m). A total of 54 mixed samples were collected from ~~the~~ topsoil horizons ~~i~~on the selected sites~~localities~~. The samples were exposed to the action of four different types of wetting agents (organosilicone wetting agent; methyl ester of rapeseed oil; mixture of methyl ester palmitic and oleic acids; Isodecyl alcohol ethoxylate). ~~-~~WSA~~S~~ was determined before and after the addition of wetting agents (WA)~~WA~~. Initial WSA values were at the same level in a majority of sampling points. Two sites were an exception, on which Haplic Luvisols and Relictistagnic Fluvisols occurred. These soil types featured the lowest WSA values. ~~-~~After the addition of WA across the sampling point, average WSA values ~~of SWWSAS after addition of WA across the sampling point~~ exhibited a demonstrable trend: ~~the~~ WSA ~~SAS value~~ of control sample (without the WA application) was at all times higher than in samples with the addition of WA (organosilicone wetting agent; methyl ester of rapeseed~~ssed~~; mixture of methyl ester palmitic and oleic acids; Isodecyl alcohol ethoxylate)~~, on average by more than 15 %~~. If the measured WSA~~S~~ values are compared in terms of overall means, it is obvious that the control variant always exhibited the highest WSA~~S~~ value (~~i~~on average ~~v průměru~~ 44.04~~ ~~%) and the variants with the application of WA showed always WSA~~S~~ values lower by min. 16~~ ~~%. The worst effect on WSA ~~had~~was that of wetting agents whose basic component was methyl ester of rapeseed~~,~~. These wetting agents caused a decrease in WSA by more than 50%. All soil samples were also analysed for basic soil parameters (glomalin, oxidizable carbon - Cox, pH, Na, P, Ca, K, Mg) in order to determine their potential influence on ~~S~~AS and to possibly eliminate the negative impact of WA. In this respect, only a significant influence of Cox content on WSA ~~SAS~~ was recorded, which positively correlated with ~~SW~~ the ~~water~~ stability of soil aggregates~~AS~~.

Introduction

A basic source for the assurance of human needs in the 21st century is agriculture which depends on the healthy and high-quality soil (Amundson et al., 2015). The main current threat to soil quality is global climate change and inappropriate arable land management, which reduces the resilience of the soil environment to fluctuations in meteorological phenomena (intensive rainfall, long periods of drought etc.). The consequence of these effects is water erosion, loss of nutrients from the soil and decreased content of soil organic matter (SOM) (Trnka et al., 2011; Panagos et al., 2015;~~Jaagus et al., 2021~~). The most readily influenceable of all mentioned impacts on the soil health and quality is management of arable land, which includes not only the mechanical processing of the soil but also the use of pesticides. Pesticides are most often applied in the form of sprays, and very frequently in a mixture of several substances (Mesnage & Antoniou, 2018). The mixture usually consists of water, active substance (pesticide) and improving substance (wetting agent, buffer solution etc.) the task of which is to enhance characteristics of the spray and to increase its efficiency (Hao et al., 2019; Mesnage & Antoniou, 2018). However, wetting agents can be used for example in irrigation, too (Lehrsch et al., 2012; Lehrsch, 2013).

Water is a universal solvent and the most important means for the preparation of agricultural sprays or spray mixtures. Active substances (pesticides) are dissolved in water either separately or in combination with nutrient preparations. However, due to its high surface tension, water exhibits low retention capacity when applied on targets with waxy and hydrophobic surfaces such as the cuticle~~sst~~ of plants (Castro et al., 2018). Therefore, substances are added to the spray mixture, which are called adjuvants or wetting agents. They serve to modify the spray viscosity (Slezak, 2015), to reduce the surface tension of the prepared fluid (Castro et al., 2018) and to enhance the capacity of spray mixture to cling to plant leaves. This also increases the efficiency of the used pesticide and reduces the amount that would have to be applied without the adjuvants (Hao et al., 2018; Castro et al., 2018). Apart from this, some pesticides (e.g. soil herbicides) can be applied together with the wetting agent directly into the soil where they gradually become decomposed and affect the whole soil environment (Hao et al., 2018; Baratella et al., 2018).

The addition of adjuvants to the spray mixture contributes to reduce the amount of used pesticides through the increased efficiency of their application. Accelerating the penetration, the adjuvants increase the permeability of cuticle and may alter the cuticular barrier to water loss (Räsch at al., 2018). In Europe, the first professional wetting agents were introduced in growing vegetables, namely species with a thick way layer on leaves. The main goal was to reduce surface tension of the liquid so that pesticides stay on the leaves. These adjuvants were simple surfactants. Later, higher alcohols and polymerizing substances started to be added to them in order to improve their resistance to wash out from plant leaves (by rain or irrigation). A breaking stage in the development of adjuvants was the use of silicon-based organic substances, which resulted in considerably reduced surface tension of the spray mixture at low doses of adjuvants (Räsch at al., 2018). General evaluation of the safety of using pesticides is nearly exclusively focused on active substances contained in them. Nevertheless, adjuvants which are included in the spray mixture and are added in order to reduce the consumption of pesticides, can be potentially dangerous by themselves as their negative impacts were observed both in humans and in the environment particularly in terms of their potential toxicity (Mesnage and Antoniou, 2018). Despite the existing knowledge about the negative impacts, adjuvants are not supervised and tested as for example pesticides are (Mesnage and Antoniou, 2018; Mesnage at al., 2013). By the principle of their action, adjuvants alter the surface tension of water as a solvent of pesticides. This is why an assumption exists that they could affect the wetting capacity of soil aggregates (SA) because the soil hydrophobicity increases the stability of SA (Mataix-Solera and Doerr, 2004). If the soil hydrophobicity is reduced due to changes in the surface tension of soil particles (reduced hydrophobicity of individual particles) due to the action of adjuvants, stability of SA might decrease through the impact on the hydrophobicity of soil particle bonds (Zheng et al., 2016; Mao et al., 2019). A stability of SA reduction due to the acting of spray mixture may occur only if the mixture reaches the soil surface. This may happen when the density of growth~~stand~~ to which it is applied is low and plant stems and leaves do not perfectly cover the soil surface. Thus, growth~~stand~~ density not only affects the direct contact between the soil aggregates and the spray mixture but also the soil resilience to erosion (Brant et al., 2017).

~~The frequency of arable soil erosion depends on agrotechnological methods of land use, which essentially affect the soil quality (Menšík et al., 2020; Borrelli et al., 2017). Factors affecting the occurrence of water erosion together with the intensity of soil management include also the intensity of rain precipitation, soil type and soil environment condition (content of SOM) which affects the stability of soil aggregates and topography (Karyda et al., 2014; Zhao et al., 2013).~~

~~Soil aggregates~~SA were defined as "naturally” occurring clumps or groups of soil particles, in which forces holding the particles together are much greater than forces between the neighbouring aggregates (Martin et al., 1955). Primary soil particles are held together by cohesion forces acting on clayey particles and soil organic matter, which is how the soil aggregates are formed (Papadopoulos, 2011). ~~Soil aggregate~~SA ~~consists of~~ comprise primary particles in an arrangement which allows the exchange of water and gases, biological activity and forms their stability. Soil aggregate stability (SAS) is a property of soil aggregatesSA to resist external forces acting on them at soil swelling, shrinkage and tillage. Soil aggregate stability SAS can be also understood as a capacity of associated soil particles to react to the presence of water in the soil, and to make possible its infiltration so that this association is not disrupted and the soil aggregates disintegrated (Papadopoulos, 2011; Angers, 1992). SASStability of SA is an important feature of the entire agro-ecosystem because it is strongly related to soil functions such as carbon storage, SOM stabilization, water management and soil resilience to erosion (Joshi et al., 2020; Vadas and Sims, 2014). In addition, there are specific soil substances such as glomalin (mixture of proteins, lipids and inorganic substances) that can stabilize the SA and thus enhance soil structure (Emran et al., 2012; Holátko et al., 2021). Combined with the occurrence of mycorrhizal fungi, the content of SOM (living and inanimate organic matter, proteins - glomalin) and the base saturation (Ca^{2+}, AlK^{3+}, Na^{+}, Fe^{2}Mg^{2+}) have an essential influence on the degree of soil aggregate stabilitySAS (Holátko et al., 2021; Wuddivira and Camps-Roach, 2007; Bronick and Lal, 2005).

The formation of soil aggregatesSA – aggregation is necessary for the development of an optimum soil structure which is one of primary prerequisites for soil fertility, i.e. production function of the soil. Aggregation directly relates to soil ↔ root interactions, hydrological soil characteristics and soil capability of providing non-production functions (Papadopoulos, 2011). Thus, the presence of soil aggregatesSA and the capacity of aggregation are indispensable for agricultural production (Brtnický et al., 2017) and applied agrotechnological methods should promote them (Zheng et al., 2018; Brtnický et al., 2017). Intensive tillage without using regenerative methods such as e.g. intermediate cropping and application of organic fertilizers results in the deterioration of soil structure and reduced Sstability of SA (Zheng et al., 2018). The most dramatic turning point in agriculture occurred in the second half of the 20th century thanks to the widespread use of pesticides, plant breeding, mineral fertilizers and modern agricultural machines (Dornbush and von Haden, 2017; Pingali, 2012). At that, iIt is exactlyWhereas the intensive soil tillage in combination with the excessive supply of mineral N into the soil that leads to reduced stability of SA SAS and hence to the degradation of soil structure (Tuo et al., 2017; Brtnický et al., 2017). Another potential problem is the application of pesticides, for example herbicides which are dissolved in water prior to the application, and solution properties are modified using further preparations. If applied outside the intended plant or at an inappropriate dose, such a solution can affect the surrounding environment by different ways (changes in soil chemism and biological activity) (Castro et al., 2018).

Water is a universal solvent and the most important means for the preparation of agricultural sprays or spray mixtures. Active substances (pesticides) are dissolved in water either separately or in combination with nutrient preparations. However, due to its high surface tension, water exhibits low retention capacity when applied on targets with waxy and hydrophobic surfaces such as cuticle of plants (Castro et al., 2018). Therefore, substances are added to the spray mixture, which are called adjuvants or wetting agents. They serve to modify the spray viscosity (Slezak, 2015), reduce the surface tension of prepared fluid (Castro et al., 2018) and enhance the capacity of spray mixture to cling to plant leaves. This also increases the efficiency of the used pesticide and reduces the amount that would have to be applied without the adjuvants (Hao et al., 2018; Castro et al., 2018).

The addition of adjuvants to the spray mixture contributes to reduce the amount of used pesticides through the increased efficiency of their application. It is a known fact that accelerating penetration, the adjuvants increase the permeability of cuticle and may alter the cuticular barrier to water loss (Räsch at al., 2018). General evaluation of the safety of using pesticides is nearly exclusively focused on active substances contained in them. Nevertheless, adjuvants which are included in the spray mixture and are added in order to reduce the consumption of pesticides, can be potentially dangerous by themselves as their negative impacts were observed both in humans and in the environment particularly in terms of their potential toxicity (Mesnage and Antoniou, 2018). Despite the existing knowledge about the negative impacts, adjuvants are not supervised and tested as for example pesticides are (Mesnage and Antoniou, 2018; Mesnage at al., 2013). By the principle of their action, adjuvants alter the surface tension of water as a solvent of pesticides. This is why an assumption exists that they could affect the wetting capacity of soil aggregates because the soil hydrophobicity increases SAS (Mataix-Solera and Doerr, 2004). If the soil hydrophobicity is reduced due to changes in the surface tension of soil particles (reduced hydrophobicity of individual particles) due to the action of adjuvants, SAS might decrease through the impact on the hydrophobicity of soil particle bonds (Zheng et al., 2016; Mao et al., 2019). A SAS reduction due to the acting of spray mixture may occur only if the mixture reaches the soil surface. This may happen when the density of stand to which it is applied is low and plant stems and leaves do not perfectly cover the soil surface. Thus, stand density not only affects the direct contact between the soil aggregates and the spray mixture but also the soil resilience to erosion (Brant et al., 2017; Kervroëdan at al., 2018).

The aim of the study: Our goal was to analyse the effect of wetting agents added to the spray mixture on the stability of soil aggregates. Specifically, we assessed how the recommended dose of conventionally used wetting agents (l/ha) for the preparation of agricultural sprays would affect the resilience of soil aggregates to disintegration upon a contact of the wetting agent with the soil. We also studied whether some soil properties can influence the effect in some way.

Material and methods

1.1 Soil sampling and characterization of sampling points

Soil was samplesd were taken atin three regions of the Czech Republic (Figure 1), withon three farms sampled in each of them, comprising a total of nine sites (Table 1)Soil sampling for the purposes of detecting the effect of the addition of wetting agents (WA) on the stability of soil aggregates (SAS) was done in three regions of the Czech Republic (Figure 1), in three agricultural enterprises, on 9 sites. Each region belongs in a different geomorphological unit, and sampling points were determined on each site (Table 1). All selected sites were subjected to a paedological survey – a total of 9 soil pits were excavated to a depth of of min. 1.43–1.5 m for the characterization of soil conditions on the given site on a specific agricultural plot. Each sitelocality was given a name after the village in the cadastral area of which it is situated. Six soil samples were then collected from the topsoil horizon at different sampling points (A–I) within the topsoil horizon ion each lsiteocality in accordance with the methodologymethodica for sampling of soil quality (ISO 10381-6: guidance on the collection, handling and storage of soil for subsequent testing under aerobic conditions in the laboratory2009). The sampling was made in 2019, at the end of the growing season, prior to the harvest of grown crops. As the measured WSA values did not differ at the individual sampling points (Annex a-1; Annex a-2), this designationbylo toto označení (A–I) was used in the text for individual variants. The marking of experimental variants (A–I) for further data processing was chosen due to the absence of significant differences (Annex a-1; Annex a-2) among individual samplings from the soil horizon within one locality/region in the selected parameters (e.g. SAS). Therefore, this way of sample distribution to individual sampling points was chosen and used to characterize the effect of WA addition on SAS.

Table 1 Sampling points

Sampling point	Region in the Czech RepublicCR	Climate characteristics	LocalityCadastral area	GPS coordinates	Number of collected samples
A	Českomoravská vrchovina (Bohemian-Moravian Highland)	Mean annual air temperature 6–7°C; mean annual precipitation amount 650–750 mm; sum of temperatures above 10°C = 2200–2400°C	Heroltice	N49.41297 E15.62965	6
B			Henčov	N49.43547 E15.61838	6
C			Rancířov	N49.35477 E15.61563	6
D	Haná/Olomouc Region	Mean annual air temperature 8–9°C; mean annual precipitation amount 550–650	Hulín	N49.30569 E17.48818	6
E			Bochoř	N49.42692 E17.43735	6

F		mm; sum of temperatures above 10°C = 2500–2800°C	Beňov	N49.40109 E17.50242	6
G	Slezsko (Silesia)	Mean annual air temperature 7.5–8.5°C; mean annual precipitation amount 700–900 mm; sum of temperatures above 10°C = 2500–2700°C	Suchdol nad Odrou	N49.63704 E17.93773	6
H			Prchalov	N49.64459 E18.12225	6
I			Kopřivnice	N49.60688 E18.12438	6

~~Detailed descriptions of sampling points are presented below; information on basic soil parameters and soil structure are presented in Annex b-3.~~

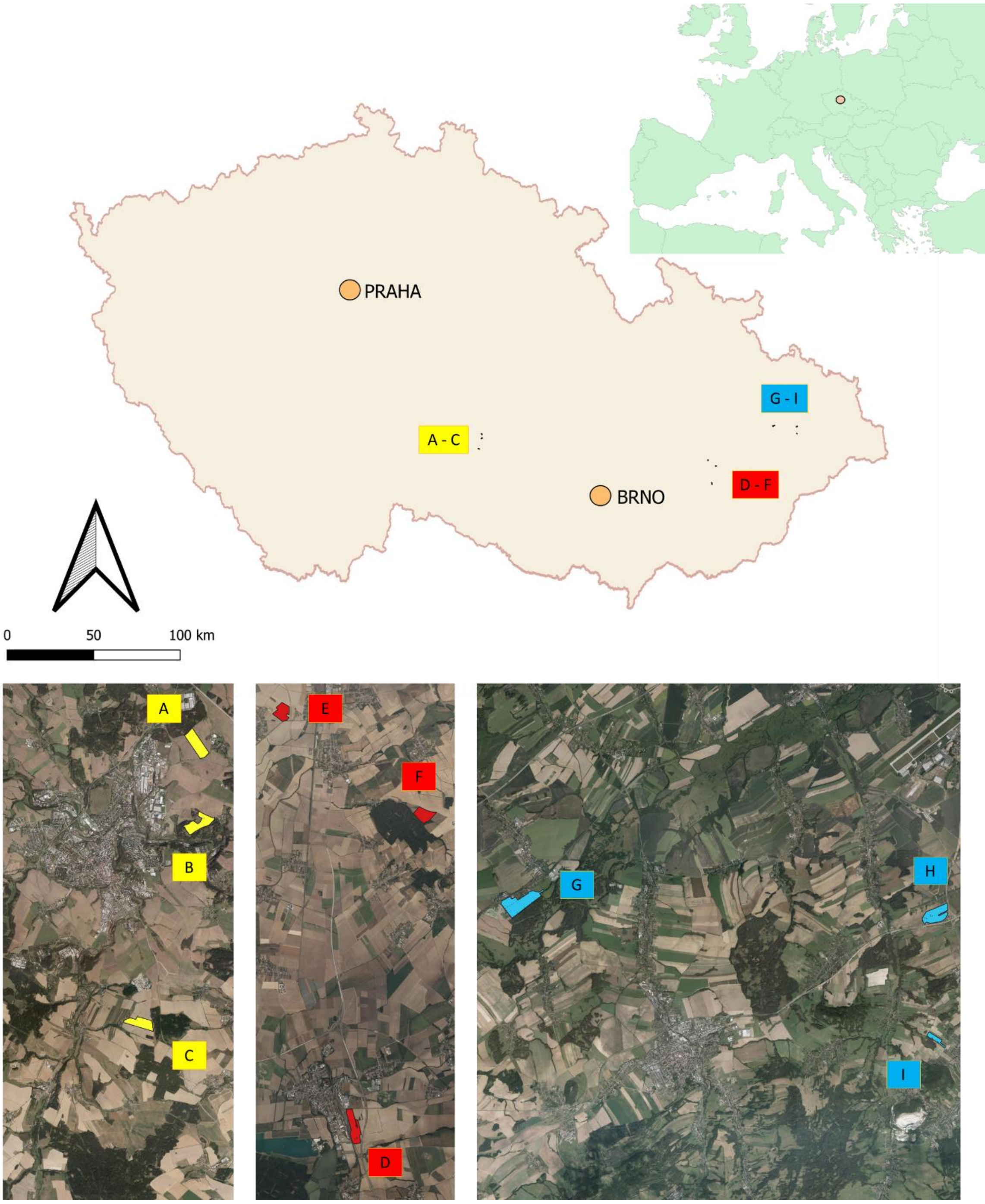


Figure 1 Location of sampling points and soil pits in the Czech Republic

Note to Figure 1: map was prepared in QGIS software (QGIS Development Team; license: GNU GPLv2) on the basis of data from the Czech Office for Surveying, Mapping and Cadast~~r~~er (CUZK). Spatial data belonging to the category of open data (including metadata) were used, this data was used free of charge under the Creative Commons CC-BY 4.0 license.

Detailed descriptions of sampling points are presented below; information on basic soil parameters and soil structure are presented in Annex b-3 and b-4. All sampling points were subjected to a pedological survey at which soil pits were excavated (Annex b-4) for the purpose of a detailed characterization of topsoil ~~and subsoil~~ horizons in the respective localities. Subsoil horizons are described in detail in Annex b-5.

Sampling point A – Henčov, Dystric Relictistagnic Regosols (Siltic, Aric, Densic) with the ~~:~~ ~~0.00–0.32 m t~~topsoil layer (qualifier Aric) ~~in~~at a ~~the~~ depth from 0.00 to 0.32 m: 7.5YR4/2 (w) brown; to 0.07 m granular structure, deeper sub-angular structure, texture class silt loam, small amount of coarse sand and small amount of Fe-Mn nodules. Sharp transition to the deeper horizon. The ~~F~~final soil pit depth ~~of soil pit~~ was 1.50 m.

- ~~0.32–(0.43–0.65) m mottled layer 1: combination of pinkish white 7.5YR8/2 and reddish yellow 7.5YR6/6; angular structure, texture class silt loam, the layer contains a small amount of coarse sand and a high amount of Fe-Mn nodules Ø5mm. Transition to the deeper horizon is undulated.~~

~~(0.43–0.65)–1.12 m mottled layer 2: combination of grey 5YR5/1 and yellowish red 5YR5/6; angular structure, texture class loam, admixture of coarse sand, a high amount of Fe-Mn nodules.~~

- ~~>1.12m transition layer to the parent rock material: alternation of colours grey 5YR6/1 and yellowish red 5YR4/6; without a clear structure, texture class loam, the content of soil skeleton (mica schist) very quickly growing with the soil depth.~~

~~Depth of soil pit 1.50 m.~~

Sampling point B – Heroltice, Skeletic Cambisols (Loamic, Aric) with the topsoil layer (qualifier Aric) ~~in~~at a ~~the~~ depth from 0.00 to 0.33 m:~~:~~

~~0.00–0.33 m topsoil layer (qualifier Aric):~~ brown7.5YR4/2 (w); granular structure, texture class sandy loam, approx. 20 % of soil skeleton, sharp transition to the deeper horizon. The ~~F~~final soil pit depth ~~of soil pit~~ was 1.30 m.

~~0.33–0.58 m cambic horizon: brown 7.5YR4/4; angular structure, texture class sandy loam, approx. 20% of soil skeleton. Clear transition to the deeper horizon.~~

~~>0.58m parent rock material: >90% of soil skeleton (stones), roots recognizable to 0.95m.~~

~~Depth of soil pit 1.30 m.~~

Sampling point C – Rancířov, Regosols (Loamic, Aric) with the topsoil layer (qualifier Aric) ~~in the~~at a depth from 0.00 to 0.28 m~~:~~

~~0.00–0.28 m topsoil layer (qualifier Aric)~~: dark yellowish brown 10YR3/4 (w); granular structure to 0.09 m~~, granular structure,~~ texture class sandy loam with approx. 25 % of soil skeleton. Sharp transition to the deeper horizon. The ~~F~~final soil pit depth ~~of soil pit~~ was 1.35 m.

~~0.28–0.60 m endopedon: colour brown 10YR4/3 to dark yellowish brown 10YR4/4, angular structure, texture class sandy loam, 25% of soil skeleton.~~

- ~~>0.60 m transition horizon to the parent rock material: yellowish brown 10YR5/6; without clear structure, texture class sandy class, the amount of soil skeleton growing with the depth from 30% to 100%, tight placement of weathered stones from a depth of 1.10 m.~~

~~Depth of soil pit 1.35 m.~~

Sampling point D – Hulín, Haplic Luvisols (Amphiloamic, Aric, Densic~~):~~) with the topsoil layer (qualifier Aric) ~~in the~~at a depth from 0.00 to 0.32 m:

~~0.00–0.32 m topsoil layer (qualifier Aric):~~ 10YR3/3 (w) dark brown; according to the soil structure, ~~we can divide~~ this layer can be divided into sublayer 1: 0.00–0.07 m with granular structure, very crumbly, and sublayer 2: 0.07–0.32 m with sub-angular blocky structure (qualifier Densic). Texture class silt loam. Sharp transition to the deeper horizon. The ~~F~~final soil pit depth ~~of soil pit~~ was 1.40 m.

~~0.32–0.60 m argic horizon (clay coats; clay ratio with surface horizon 1.8): angular blocky structure, surface of aggregates 10YR3/4 (w) dark yellowish brown, inside of aggregates 10YR4/6 (w) dark yellowish brown; Fe–Mn nodules. Texture class clay loam.~~

- ~~>0.60 m transition horizon to the parent rock material.~~

~~Depth of soil pit 1.40 m.~~

Sampling point E – Bochoř, Relictistagnic Fluvisols (Loamic, Aric, Densic) with the topsoil layer (qualifier Aric) in the depth from 0.00 to 0.29m:~~:~~

~~0.00–0.29 m topsoil layer (qualifier Aric):~~ 10YR4/1 (w), dark grey; loam, according to the soil structure we can divide this layer into sublayer 1: 0.00–0.13m with granular structure, very crumbly, and sublayer 2: 0.13–0.29 m with strong angular blocky structure (qualifier Densic). Texture class clay loam. Sharp transition to the deeper horizon. Final depth of soil pit was 1.40m.

~~0.29–0.62 m mottled layer 1 with stagnic properties: 70% 10YR4/2 (w) dark greyish brown and 30% 10YR5/6 (w) yellowish brown; small angular blocky structure, a small amount of Fe–Mn nodules. Texture class silty clay loam (0.35m) and clay loam (>0.50m) – qualifier Loamic.~~

- ~~> 0.62 m mottled layer 2 with stagnic properties (qualifier Relictistagnic): 50% 10YR5/2 (w), greyish brown and 50% 10YR4/6 (w), dark yellowish brown; a large amount of Fe–Mn nodules. 0.62–0.93m, small angular blocky structure, >0.93m, without structure,~~

~~Depth of soil pit 1.40 m.~~

Sampling point F – Beňov, Eutric Regosols (Siltic, Aric, Densic) with the topsoil layer (qualifier Aric) ~~in the~~at a depth from 0.00 to 0.33 m:~~:~~

~~0.00–0.33 m topsoil layer (qualifier Aric):~~ 10YR3/2 (w) very dark greyish brown; according to the soil structure~~, we can divide~~ this layer can be divided into sublayer 1: 0.00–0.08m with granular structure, and sublayer 2: 0.08–0.33m with sub-angular blocky structure (qualifier Densic). Texture class silt loam. Sharp transition to the deeper horizon. The ~~F~~final soil pit depth ~~of soil pit~~ was 1.50 m.

~~0.33–0.57 m: 10YR5/6 (w) yellowish brown and <10% 10YR4/1 (w) dark grey; small angular blocky structure. Texture class silt loam (qualifier Siltic).~~

- ~~0.57–0.93 m: 10YR4/3(w) brown; to 0.74m small angular blocky structure, from 0.74 to 0.93m structure prismatic; from 0.65m a small amount of Fe–Mn nodules. Texture class silty clay loam. Clay coats on aggregates surface, but do not meet criteria 2a) v. for argic horizon.~~
- ~~>0.93 m transition horizon to the parent rock material: 10YR4/6(w) dark yellowish brown; angular blocky structure, a weak amount of roots to a depth of 1.30 m.~~

~~Depth of soil pit 1.50 m.~~

Sampling point G – Suchdol nad Odrou, Fluvic Stagnic Phaeozems (Siltic, Aric) with the topsoil layer (qualifier Aric) ~~in the~~at a depth from 0.00 to 0.27 m:~~:0.00–0.27 m topsoil layer 1 (qualifier Aric):~~ 7.5YR2/2 (w), very dark brown/black; granular structure, texture class silt loam, a small admixture of stones, < 10 % of artefacts (pieces of bricks, polyethylene). Meets criteria for mollic horizon. Sharp transition to the deeper horizon. The ~~F~~final soil pit depth ~~of soil pit~~ was 1.50 m, depth of groundwater was 1.70 m (by core drill).

- ~~0.27–0.43 m topsoil layer 2: 7.5YR2/2–3/2 (w), very dark brown/dark brown; granular structure, texture class silt loam; artefacts (pieces of bricks, polyethylene) are uncommonly in this layer (<5%). Sharp transition to the deeper horizon.~~
- ~~0.43–0.79 m layer 1: fluvic material with stagnic properties, <10% of surface with colour 5YR5/6 (w) yellowish red and > 90% of surface with 7.5YR from 4/1 to 5/1 (w) dark grey /grey; angular structure, texture class silt loam, a small amount of Fe-Mn nodules.~~
- ~~0.79–0.92 m layer 2: fluvic material with stagnic properties approx. 20% of surface with mottles 5YR4/8–5/8 (yellowish red), other space with 2.5YR3/2 dusky red; angular structure, texture class silty clay loam.~~
- ~~> 0.92 m layer 3: fluvic material with stagnic properties 60–70% of surface with mottles 2.5YR4/5 (reddish brown/red) and 5YR5/8 (red), other space 5Y6/2 (w) light olive grey; prismatic structure, silty clay loam, a small amount of Fe-Mn nodules, a small amount of roots to a depth of 1.00 m.~~

~~Depth of soil pit 1.50 m, depth of groundwater 1.70m (by core drill).~~

Sampling point H – Prchalov, Stagnic Umbrisols (Loamic, Aric, Densic), with the topsoil layer (qualifier Aric) at a ~~in the~~ depth from 0.00 to 0.30 m: ~~0.00–0.30 m topsoil layer (qualifier Aric):~~ 7.5YR3/2 (w) dark brown; granular structure, texture class clay loam. Clear transition to the deeper horizon. Meets criteria for umbric horizon. The ~~F~~final soil pit depth ~~of soil pit~~ was 1.30 m.

~~0.30–0.85 m mottled layer 1: 7.5YR4/1 (w) dark grey, 7.5YR6/8 (w) reddish yellow; prismatic structure, texture class silty clay loam, random dark coats on aggregates, a small amount of Fe-Mn nodules. Clear transition to the deeper horizon.~~

- ~~>0.85 m mottled layer 2: grey 7.5YR6/1, reddish yellow7.5YR6/8; without clear structure, texture class silty clay loam.~~

~~Depth of soil pit 1.30 m~~

Sampling point I – Kopřivnice, Stagnic Regosols (Loamic, Aric, Drainic) with the topsoil layer (qualifier Aric) at a~~in the~~ depth from 0.00 to 0.36 m:

~~0.00–0.36 m topsoil layer (qualifier Aric):~~ 10YR3/4 dark yellowish brown; granular structure, texture class loam, <10 % rounded soil skeleton, >0.22 m, a small amount of Fe-Mn nodules. Sharp transition to the deeper horizon. The ~~F~~final soil pit depth ~~of soil pit~~ was 1.35 m.

~~0.36–0.94 m mottled layer 1: > 90% of surface 10YR5/8 yellowish brown, partly 10YR6/1 grey; without clear structure, texture class clay loam, 15–20% rounded soil skeleton (gravel), a small amount of Fe-Mn nodules, randomly dark Mn-coats, roots to 0.72m, a drainage pipe in the depth 0.53 m. Clear transition to the deeper horizon.~~

- ~~>0.94 m mottled layer 2: > 90% of surface 7.5YR4/6 strong brown, partly 7.5YR7/1 light grey; without clear structure, texture class sandy loam, to 1.12m approx. 15% rounded soil skeleton (gravel), deeper <5% soil skeleton (predominantly coarse sand).~~

~~Depth of soil pit 1.35 m.~~

All selected plots (Table 2) were managed by conventional methods, i.e. crops on them were grown with the use of mineral fertilizers and plant protection preparations. Dominant crops in the rotation were cereals and oilseeds. Calcium fertilizers to adjust pH were usually applied on average once in five years. The CaO dose was calculated based on the actual pH value on individual plots. The applied calcium fertilizers included sugar factory sewage sludge and dolomitic limestone. Sugar factory sewage sludge (saturation sludge) is a waste from the processing of sugar beet and contains Ca^{2+} in the form of $CaCO_3$.

Table 2 Overview of grown crops and applied calcium fertilizers at the respective sampling points

Sampling point	Crop 2017	Crop 2018	Crop 2019	Application of calcium fertilizers (2015 – 2019)		
				TypeDruh	Dose of CaO (kg/ha)	Year of application
A	Winter wheat	Poppy	Spring barley	Saturation sludge	1,-850	2015
B	Spring barley	Potatoes	Winter wheat	Saturation sludge	1,-850	2015
C	Phacelia	Oil seed rape	Winter wheat	Saturation sludge	3,-107	2017
D	Corn for silage	Winter wheat	Oil seed rape	Saturation sludge	1,-540	2015
E	Oil seed rape	Winter wheat	Corn for silage	-	-	-
F	Oil seed rape	Winter wheat	Spring barley	Saturation sludge	629	2018
G	Winter wheat	Sugar beat	Spring barley	Dolomitic limestone	1,500	2015
H	Winter wheat	Oil seed rape	Winter wheat	Dolomitic limestone	1,-443	2016
I	Oil seed rape	Spring barley	Corn for silage	-	-	-

1.32 Determining the content of basic nutrients, glomalin, C_{ox} and Na in the soil

In addition to SASWSA, other parameters determined in the collected soil samples were: contents of basic nutrients (P, K, Ca, Mg), oxidizable carbon (C_{ox}) and Na in the soil. Exchange soil reaction (pH) was determined, too.
The soil contents of P, K, Ca, and Mg were established according to Schroder et al. (2009); the individual elements were extracted using the Mehlich III reagent and then analysed using atomic emission spectroscopy (The Agilant55B AA, Agilent, CA, USA). The content of C_{ox} (oxidizable carbon) was established according to Nelson and Sommers (1996) using wet oxidation of chromic acid. C_{ox} contained in the soil sample was oxidized by potassium dichromate (0.167 M) in– the concentratedthe concentrated sulphuric acid (a so-called chrome-sulphate mixture). The content of Cox (wt%) Cox in the soil sample was calculated based on the consumption of titrant.
Glomalin was established according to the extraction method by Wright and Upadhyaya (1996): 1 g of soil sample +8 ml of 20 mM of sodium citrate solution. The mixture was homogenized for 30 minutes on the GFL3015 shaker. Then the sample was autoclaved (60 minutes at 121°C). After cooling, it was centrifuged for 15 minutes at 3,900 rpm. Until the time of measurement, the supernatant was kept frozen at -18°C. Easy extracted glomalin (EG) was determined as EE-BRSP (easy extracted Bradford reactive soil protein) using the method by Bradford (1976). The measurement was at all times repeated three times for each sample. Poorly extractable glomalin (total glomalin – TG) was extracted in a similar way using 50 mM of potassium citrate solution instead of 20mM.

1.23 Determining the effect of wetting agent application on the stability of soil aggregates

The collected soil samples were transported to the laboratory where they were analysed. All samples from each site were divided into five parts of identical weight for the establishment of water stability of soil aggregatessoil aggregates stability

(WSA~~SAS~~). Based on studies published by Kandeler and Murer (1993), Kandeler (1996), Bartlová et al. (2015), we selected the following procedure: Soil aggregates sized 1-2 mm were separated from the soil sample after the soil had been dried at a laboratory temperature. Then they were washed for 5 minutes in 100 ml of distilled water on the sieve washer (Adolf Herzog GmbH, Viena, Austria) with the washing speed being 42 strokes/min. Upon the end of washing, the samples were immediately transferred to evaporation dishes and dried at a temperature of 105°C in the drier (HS 32 A, Chirana Ltd., CZ) to constant weight. The dried and cooled samples (in desiccator) were added~~complemented with~~ 50 ml of pyrophosphate solution and the mixture was manually rubbed up. After 120 minutes, the samples were washed again on the same sieve washer for 5 minutes. The reason for this repeated washing was to wash out clay particles so that only sand would remain on the bottom of washer sieves, which was rinsed into an evaporation dish and dried to constant weight at 105°C. After cooling in the desiccator, the dried-up material was weighed again, and the percentage of aggregates unwashed down from the total sample weight was determined according to the following equation (1).

$$\text{Calculation of } \% \text{ \sout{SAS} WSA} = ((M_2 - M_3) / \; W - (M_3 - M_1)) \times 100 \qquad (1)$$

% W~~S~~SA~~S~~	percentage of stable soil aggregates
M_1	weight of dish (g)
M_2	weight of dish, stable aggregates and sand (g)
M_3	weight of dish and sand (g)
$(M_2 - M_3)$	weight of stable soil aggregates (g)
$(M_3 - M_1)$	weight of sand (g)
W	weight of sample (4 g)

WSA~~S~~ was always measured five times: 1) without the WA addition – control value; 2) – 5) after adding a specific wetting agent (WA) to the solution used for the measurement of WSA ~~SAS~~. The individual WA were applied directly into the liquid that was used as a solution for the dispersion of soil particles (Table 3). All chosen wetting agents are freely available on the EU market and are used for the application directly into the spray mixture or as a component of soil herbicides. The reason was to test conventionally used products which can be and are applied directly onto the soil surface (in spray mixture) or into the soil environment (with soil herbicides). There were altogether four wetting agents used (Table 3); their description is based on data provided by manufacturers on the labels or package leaflets of given products:

- WA 1 – Organosilicone wetting agent is a non-ion excipient for the enhancement of the degree of coverage of plant parts treated with the application fluid. It improves the wetting power and adhesive capacity of the fluid and allows better distribution also onto plant parts that are not directly reached by the application. As it significantly reduces the surface tension of liquids, high quality treatment can be achieved on plants whose surface does not allow an even adhesion of the application fluid. It increases resistance to washing with rain, enhances efficiency of pesticides and allows to reduce the amount of application fluid per 1 hectare. It features reduced foaming and low point of congelation.
- WA 2 – Methyl ester of rapeseed oil (MERO) is an adjuvant used together with preparations for plant protection including herbicides based on sulphonyl urea MaisTer, Atlantis WG, Chevalier and Husar, the effect of which it increases and stabilizes. By itself it has no herbicide effect. MERO reduces the surface tension of applied pesticide liquids by which it improves their contact with the surface of plants as well as the secondary distribution of active substances on the surface of plants, thus accelerating their entry into plant tissues.
- WA 3 – Represents a wetting agent which, when added to the spray mixture, increases the wetting power and adhesive capacity of preparations for plant protection as well as the resistance to washing with rain, and slows down the

evaporation of application fluid. By this, it prolongs and increases the effectiveness of herbicides permitted in the Czech Republic. The wetting agent features a dominant representation of methyl ester palmitic and oleic acids.

- WA 4 – Isodecyl alcohol ethoxylate; the addition of this wetting agent into the application fluid (spray mixture) increases the wetting power of the latter, thus facilitating adhesion and penetration of used preparations for plant protection.

The dosing of adjuvants to the soil samples in the ~~SAS~~ WSA determination followed the information on recommended dosage from the package leaflets (Table ~~2~~3). The dos~~es~~ages of wetting agents w~~ere~~as converted to 100 ml of distilled water used for SAS measurements.

Table ~~2~~3 List of used wetting agents

Wetting agent	Active substance	Type of wetting agent	Dosage l/ha (recommended by manufacturer~~-~~)
WA 1	Polyalkylene oxid heptamethyl trisiloxane 80 % Allyloxypolyethylene glycol 20 %	Organo-silicone	0.1 ~~0.01-0.15 % (max. 0.3 l/ha, usually 0.1 l/ha)~~
WA 2	Methyl ester of rapeseed oil 733 g/l	Oils	~~1-2 l/ha~~ 1
WA 3	Methyl ester of palmitic and oleic acids 37.5 % (350 g/l) Polyalkoxy ester of phosphoric acid 22.5% (210 g/l) oleic acid 5% (46 g/l)	Ionic	1 ~~0.5-2 l/ha (according to the area of use)~~
WA 4	Isodecyl alcohol ethoxylate 90 %	Nonionic	0.3 ~~0.05 – 0.1% (according to crop)~~

Note to Table 3: The respective wetting agents were applied into 100 ml of distilled water, which was used in the measurement of WSA. Dosing of the wetting agent was calculated according to manufacturer recommending dosage per 1 ha for 300 l of spray mixture. The applied amounts of wetting agents in the experiment were as follows: WA 1: 0.033 ml/100 ml of distilled water; WA 2: 0.33 ml/100 ml; WA 3: 0.33 ml; WA 4: 0.1 ml.

~~**1.3 Determining the content of basic nutrients, glomalin, C_{ox} and Na in the soil**~~

~~In addition to SAS, other parameters determined in the collected soil samples were: contents of basic nutrients (P, K, Ca, Mg), C_{ox} and Na in the soil. Exchange soil reaction (pH) was determined, too.~~

~~The soil contents of P, K, Ca, and Mg were established according to Schroder et al. (2009); the individual elements were extracted using the Mehlich III reagent and then analysed using atomic emission spectroscopy (The Agilant55B AA, Agilent, CA, USA). The content of C_{ox} (oxidable carbon) was established according to Nelson and Sommers (1996) using wet oxidation of chromic acid. C_{ox} contained in the soil sample was oxidized by potassium dichromate (0.167 M) in concentrated sulphuric acid (a so-called chrome-sulphate mixture). The content of C_{ox} (%) Cox in the soil sample was calculated based on the consumption of titrant.~~

~~Glomalin was established according to the extraction method by Wright and Upadhyaya (1996): 1g of soil sample +8ml 20mM of sodium citrate solution. The mixture was homogenized for 30 minutes on the GFL3015 shaker. Then the sample was autoclaved (60 minutes at 121°C). After cooling, it was centrifuged for 15 minutes at 3,900 rpm. Until the time of measurement, the supernatant was kept frozen at -18°C. Easy extracted glomalin (EG) was determined as EE-BRSP (easy extracted Bradford reactive soil protein) using the method by Bradford (1976). The measurement was at all times repeated three times for each sample. Poorly extractable glomalin (total glomalin – TG) was extracted in a similar way using 50mM of potassium citrate solution instead of 20mM.~~

1.4 Statistical data processing

~~The samples (n = 6) collected from each site were designated for further purposes of evaluation as individual groups or sampling points and marked as A – I (n = 54).~~ First, all data were subjected to an input exploratory data analysis ~~(EDTA)~~ in order to establish ~~symmetry, sharpness, local concentration of measured values,~~ the presence of extreme points and data normality. Then, one-way analysis of variance (ANOVA) was used in combination with the Tukey´s HSD test to determine significant differences in WSA~~AS~~ among the respective sampling points and to compare mean ~~S~~WSAA~~S~~ values before and after the addition of WA. Further on, a pair t-test was used to confirm the difference in WSA~~S~~ before and after the application of WA. Finally, a ~~personal~~ principal component analysis (PCA) was used to analyse the relationship between individual soil parameters and values of WSA~~S~~. Program Statistica 12 (Dell Software, Round Rock, TX, USA) was used for the implementation of the analyses and for the graphical data processing. The level of significance selected for all analyses was P < 0.05.

Results

2.1 Soil aggregates stability – Initial condition and condition after the addition of wetting agents

WSA was ascertained before and after the addition of WA in a total of 54 soil samples from 9 sampling points (A – I; Figure 1) across the Czech Republic. The development of WSA in the control samples from the respective sites~~localities~~ is very interesting. The control samples demonstrably differed in dependence on the sampling point (Annex a-1, c-6 and c-7). The highest value was measured in the sampling point A and the lowest one was measured in the sampling points D and E, which was significant as compared with the other variants (sampling points). Comparing the measured WSA values in terms of total means (Table 4), we can see that the control variant exhibited the highest WSA value (44.04%) while the variants with the applied WA showed lower WSA values at all times. The WSA value was changing in the following order: WSA – control > WSA WA1 > WSA WA4 > WSA WA2 > WSA WA 3- with the measured difference being demonstrable after the application of WA2, WA3 and WA4. Thus, the measured values clearly show the influence of WA application on the decreased WSA values. Furthermore, WA 1 apparently exhibited the least negative influence on WSA whereas WA 3 exhibited the worst influence on WSA.

Table 4 Results of post-hoc Tukey´s HSD test (P<0.05) – Comparison of average WSA values before and after the addition of WA

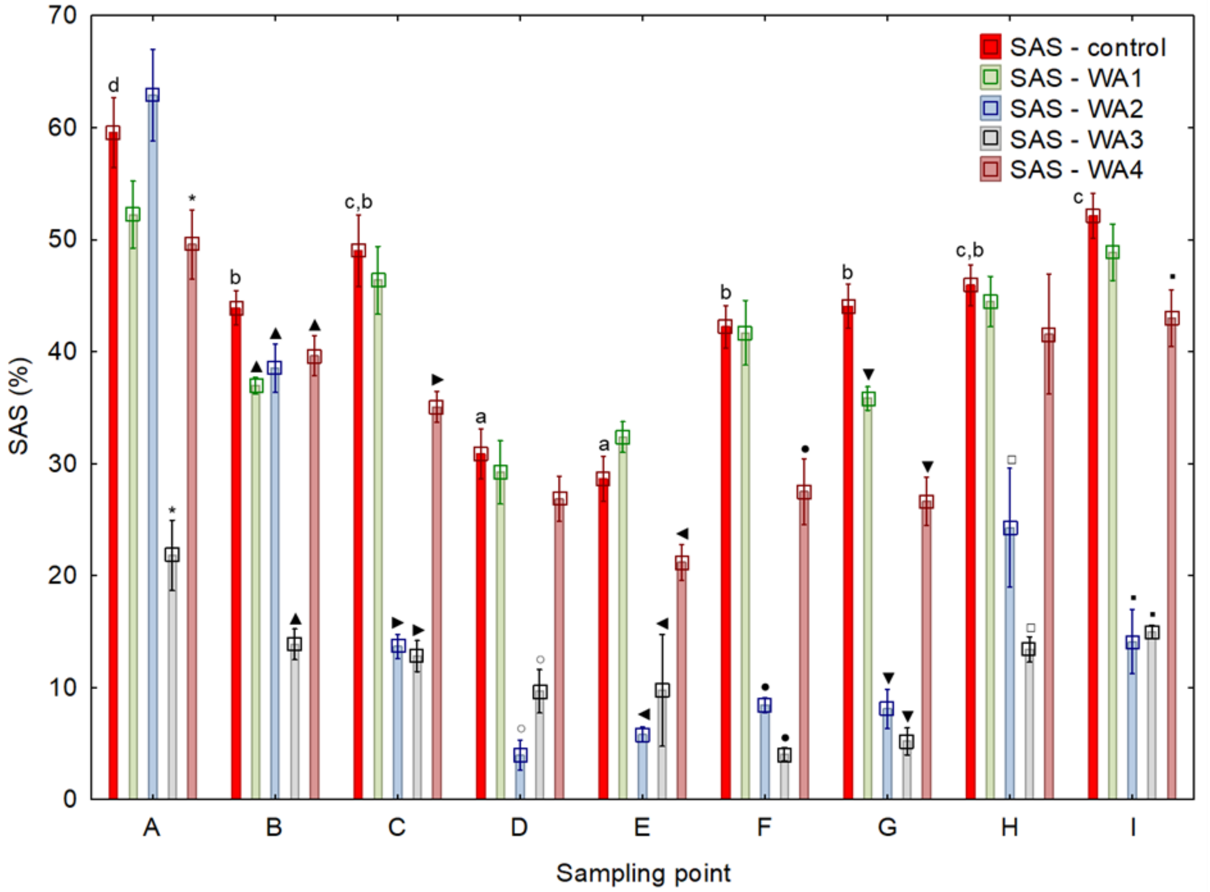
	WSA – control 44.04 %	**WSA - WA1 40.89 %**	**WSA - WA2 19.98 %**	**WSA - WA3 11.74 %**	**WSA - WA4 34.55 %**
WSA – control 44.04 %		0.664777	**0.000017**	**0.000017**	**0.000517**
WSA - WA1 40.89 %	0.664777		**0.000017**	**0.000017**	0.053832
WSA - WA2 19.98 %	**0.000017**	**0.000017**		**0.004041**	**0.000017**
WSA - WA3 11.74 %	**0.000017**	**0.000017**	**0.004041**		**0.000017**
WSA - WA4 34.55 %	**0.000517**	0.053832	**0.000017**	**0.000017**	

Note to Table 4: Statistically significant differences (P<0.05) are in bold

Above all, average values of WSA across the sampling points exhibited a clear trend: the value of WSA in the control sample (WSA – control) was at all times higher than in the samples with added WA1, WA3 and WA4 by more than 15 % at all sites (Figure 2). In addition, in the case of WA1 application, a significant decrease in WSA was found in the soil samples from sampling points B and G as compared with the control variant on average by 12 %. In the samples from the other sampling points, the level of WSA was identical as in the control sample.

In the case of WA2 application, significant differences were observed in WSA, which were negative as compared with the control samples in all variants with the exception of variant A (B – I) with the differences being from 10 % in samples from site B, over 50 % in samples from site H up to more than 65 % in samples from site E. The fact is very interesting as it shows that WA2 had the most different effect in dependence on the soil sampling point. Another specific is a difference in WSA between the individual sampling points, which is obvious across all variants (A – I). The greatest WSA fluctuations were recorded at sampling points D and E where the WSA values were always lower than in all other variants, sometimes even by more than 50 %. These differences were observed both in the soil samples with the addition of WA, and in the soil samples without it. The addition of WA at all times amplified (P<0.05) the WSA decrease. Site A exhibited the demonstrably highest values of WSA as compared with all other variants (Annex c-6 a c-7). However, the addition of WA always decreased WSA on the given site, the only exception being only the wetting agent WA2 which did not have a negative influence on the decrease in WAS as compared with the control variant.

WA3 was observed to have the most negative influence on WSA of all wetting agents. Compared with the control variant, the decrease was at all times significant, and the average decrease of WSA was by more than 73 %. On the other hand, although the application of WA4 had a significantly negative influence on WSA across all sites~~localities~~, too, the decrease compared to the control variant was demonstrably lower than after the addition of WA3 (on average by 22 %).



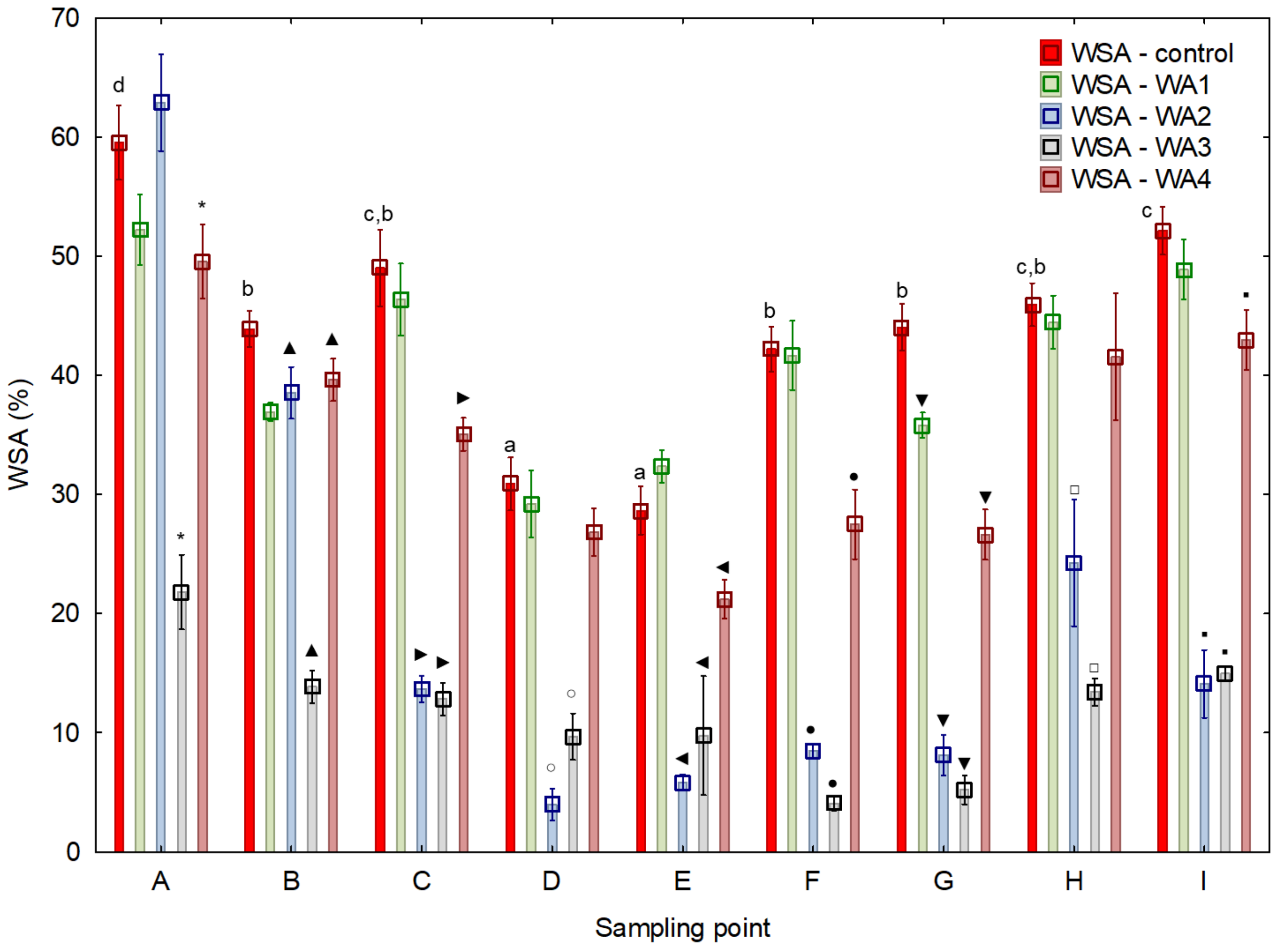


Figure 2 Water stability of soil aggregates (WSA) – Initial values on the respective sites and values after the addition of different wetting agents (WA).

*Note to Figure 2: Average WSA values (n = 6) from the individual sampling points are illustrated before and after the application of respective WA (1 – 4). Different symbols were chosen for each sampling point (A: *; B: ▲; C: ►; D: ○; E: ◄; F: ●; G: ▼; H: □; I: ▪). Their presence at the WSA value indicates a demonstrable difference between the particular variant (with the addition of WA) and the control (WSA – control) at a level of significance of P < 0.05 in one specific sampling point. Different lowercase letters indicate differences in WSA among the individual sampling points within the control collections of samples without the addition of WA.*

To obtain a further confirmation of the negative influence of WA application on WSA in the soil samples collected from the experimental sites, the individual values were compared using the pair t-test (P<0.05). We always compared WSA values from one site~~locality~~ – the control sample and the sample to which a wetting agent was added within the WSA measurement (Table 5). Differences among the individual experimental variants are obvious both from the result of the pair t-test, and from the box charts (Annex c-8) with median and mean values. The most conspicuous effect was that of WA2 and WA3 additions as the values of WSA median were always lower in these variants ~~if they were~~as compared with the WSA median of the control variant. Moreover, total differences between the control variant and variants with the addition of WA (2 and 3) across all sampling points were demonstrably significant with the average WSA value being at all times markedly lower in those variants. Other significant differences were found after the application of WA4 where the clearly negative influence on WSA after the application of the wetting agent was exhibited namely in the soil samples from sampling points E – I. The measured values indicated clearly that the application of WA decreased the average WSA value as well as the WSA median (Annex c-9).

Table 5 T-test results (P<0.05) – Comparison of differences among the average WSA values

	WSA - control	**WSA - WA1**	**WSA - WA2**	**WSA - WA3**	**WSA - WA4**

WAS - control	0.00	3,15	**24.06**	**32.31**	**9.49**
WAS - WA1	-3.15	0.00	**20.91**	**29.16**	**6.34**
WAS - WA2	**-24.06**	**-20.91**	0.00	**8.25**	**-14.57**
WAS - WA3	**-32.31**	**-29.16**	**-8.25**	0.00	**-22.82**
WAS - WA4	**-9.49**	**-6.34**	**14.57**	**22.82**	0.00

Note to Table 5: The comparison includes average WAS values from all sampling points. T-test results are shown – analysis of significant differences between the respective variants. The average WAS in controls was compared with the average WAS of all other variants from all sampling points. Statistically significant differences (P<0.05) are in bold.

2.2~~1~~ Basic soil parameters of sampling points

~~The effect of the application of wetting agents (WA) on the stability of soil aggregates (SAS) was studied on a total of 54 soil samples collected from 9 agricultural sites in the Czech Republic (three agricultural enterprises). The sampling points differed in soil textures and types as mentioned in Material and methods.~~ Prior to the establishment of WSA ~~SAS~~ in the individual soil samples before and after the addition of ~~wetting agents~~WA, basic parameters were determined that can indicate the soil environment condition and resistance to external effects – the contents of glomalin, C_{ox}, ~~Na~~ sodium and basic nutrients available to plants in particular (Table ~~3~~6 ~~and~~, ~~Table 4~~Annex c-10, c-11 and d-12).

We determined two basic forms of EG and TG glomalin. Mean values of their contents (mg/kg) were 0.9 for EG and 1.4 for TG (Table ~~3~~6). The contents of both glomalin forms exhibited increased variability across the sampling points, ranging from min. 0.4 to max. 1.6 for EG and from 0.6 to 2.3 for TG (Annex c-~~5~~10). On the other hand, it is possible to claim that the variability did not indicate a data anomaly, which was confirmed also by the analysis of data using the Shapiro-Wilk test of normality. The distribution of measured values was graphically illustrated by using a probability graph (Annex c-~~6~~11). Further, significant differences were found among the individual sampling points (Table ~~3~~6). The demonstrably highest values of glomalin EG were recorded in sampling points A, B, C, and G, and the highest values of glomalin TG were recorded in sampling points A, B, and H.

Another monitored parameter was C_{ox} in the soil, whose values ranged from 1.1 to 3.3 wt% with the mean content being 2.2 wt%. Similarly as glomalin (EG, TG), the measured values of Cox exhibited some variability among the sampling points. Significant differences among the respective sampling points (Table ~~3~~6) copied ~~-~~the trend of the development of glomalin content in the soil. The demonstrably highest Cox content was recorded in sampling points A, B and H ~~B~~, where the highest content of glomalin was measured, too. The correlation was corroborated also by the regression and factor analyses (Table 7) described below. The lowest values of Cox in the soil (< 1.62 wt%) were found at sampling points D, E and F. The values copied the trend of glomalin content in the soil only partly and only at sampling points E and F.

Apart from the above parameters, we monitored also the soil content of Na and contents of basic nutrients available to plants, i.e. P, K, Ca, and Mg (~~Table 4~~Annex d-12). The Na content was the most balanced of all parameters. Its values ranged from 223 to 369 mg/kg, with an average value of Na content in the soil being 273 mg/kg across the sampling points (Annex d~~c~~-~~5~~12). The low variability of values is also documented by the presence of merely two significant differences between sites G, H and all other sites. As to the content of available nutrients, differences were apparent between the groups of sampling points A, B, C – D, E, F – G, H, I (~~Annex a-1;~~ Annex ~~a~~c-~~2~~10). Values of Ca content in the soil were very variable with the minimum and maximum values being 1,259 mg/kg and 4,743 mg/kg, respectively (Annex c-10~~5~~). The highest values (> 3,000 mg Ca/kg) were measured in sampling points E and G. The lowest values (< 2,030 mg Ca/kg) were recorded in soil samples from sampling points A, B, C and I. The contents of remaining nutrients available to plants (P, Mg and K) were more balanced, with a lower variance of values ~~(~~(Annex d-12) ~~Table 3)~~. The lowest content of P in the soil was recorded in sampling points E, F, G and I, where its value was lower than 100 mg/kg. The highest contents were measured on sites C and H. As to the content of Mg, the lowest and highest concentrations in the soil were recorded on site I and on sites G and H (> 200 mg/kg), respectively. The content of K in the soil exhibited the second lowest variability of values (after Na) of all measured parameters. Sampling points

B, D, G and H showed the highest contents (> 279 mg/kg) as compared with the remaining sampling points (A, C, E, F, I) where the average content of K in the soil ranged from 172 to 243 mg/kg.

Table 6~~3~~ Contents of glomalin forms and oxidizable carbon in the soil, exchange soil reaction

Sampling point	Glomalin EG		Glomalin TG		C_{ox}		pH	
	mg/g ± SE	HSD	mg/g ± SE	HSD	wt% ± SE	HSD	±SE	HSD
A	1.10 ± 0.08	c	1.86 ± 0.04	d	2.99 ± 0.07	d	6.45 ± 0.03	cd
B	1.17 ± 0.02	c	1.73 ± 0.04	cd	2.84 ± 0.05	d	6.37 ± 0.03	cd
C	1.07 ± 0.08	c	1.49 ± 0.03	c	2.52 ± 0.14	c	5.05 ± 0.10	a
D	0.77 ± 0.04	b	1.44 ± 0.09	c	1.48 ± 0.07	a	5.83 ± 0.06	cb
E	0.60 ± 0.03	ab	0.92 ± 0.03	ab	1.62 ± 0.03	a	6.87 ± 0.02	d
F	0.62 ± 0.03	ab	0.77 ± 0.05	a	1.54 ± 0.03	a	6.20 ± 0.04	cd
G	1.19 ± 0.15	c	1.48 ± 0.09	c	2.08 ± 0.08	b	5.97 ± 0.45	c
H	1.24 ± 0.06	d	1.81 ± 0.16	d	2.88 ± 0.07	d	6.25 ± 0.02	cd
I	0.89 ± 0.06	cb	1.20 ± 0.04	bc	1.98 ± 0.08	b	5.47 ± 0.11	b

Note to Table ~~3~~6: different small letters indicate significant differences (P<0.05).

~~**Table 4** Contents of sodium and soil nutrients available to plants~~

Sampling point	Na		Ca		P		Mg		K	
	mg/kg ± SE	HSD	mg/kg ± SE	HSD	mg/kg ± SE	HSD	mg/kg ± SE	HSD	mg/kg ± SE	HSD
A	235 ± 3.2	a	2,029 ± 11.2	a.d	135 ± 5.5	b	127 ± 2.7	b	209 ± 4.8	b
B	253 ± 4.9	a	1,765 ± 39.6	a.b	174 ± 9.1	d	144 ± 14.5	b	305 ± 6.2	c
C	241 ± 9.3	a	1,411 ± 50.7	a	110 ± 21.9	c	171 ± 4.4	c	173 ± 7.0	a.b
D	230 ± 12.9	a	2,103 ± 83.6	b.d	137 ± 6.5	b	140 ± 4.0	b	340 ± 6.8	c
E	268 ± 4.8	a	3,366 ± 77.3	e	92 ± 4.2	a	150 ± 9.1	b	243 ± 17.0	b
F	283 ± 21.1	a	2,526 ± 118	d	68 ± 7.7	a	154 ± 6.7	b	227 ± 6.8	b
G	356 ± 10.2	b	3,240 ± 267	e	69 ± 10.2	a	220 ± 3.5	d	279 ± 15.3	c
H	369 ± 5.2	b	4,049 ± 225	f	156 ± 18.2	c.d	238 ± 6.9	d	355 ± 20.4	c
I	223 ± 26.3	a	1,792 ± 125	a.b	90 ± 14.6	a	82 ± 8.3	a	172 ± 20.6	a.b

~~Note to Table 4: different small letters indicate significant differences (P<0.05).~~

~~2.2 Soil aggregates stability – Initial condition and condition after the addition of wetting agents~~

~~SAS was ascertained before and after the addition of WA in a total of 54 soil samples from 9 sampling points (A – I; Figure 1) across the Czech Republic. Average values of SAS across the sampling points (Figure 3) exhibited a clear trend: the value of SAS in the control sample (SAS – control) was at all times higher than in the samples with added WA1, WA3 and WA4 by more than 15% at all sites (Figure 2).~~

~~In addition, in the case of WA1 application, a significant decrease in SAS was found in soil samples from sampling points B and G as compared with the control variant on average by 12%. In samples from the other sampling points, the level of SAS was identical as in the control sample. In the case of WA2 application, significant differences were observed in SAS, which were negative as compared with the control samples in all variants with the exception of variant A (B – I) with the differences being from 10% in samples from site B, over 50% in samples from site H up to more than 65% in samples from site E. WA3 was observed to have the most negative influence on SAS of all wetting agents. Compared with the control variant, the decrease was at all times significant, and the average decrease of SAS was by more than 73%. On the other hand, although the~~

application of WA4 had a significantly negative influence on SAS across all localities, too, the decrease compared to the control variant was demonstrably lower than after the addition of WA3 (on average by 22%).

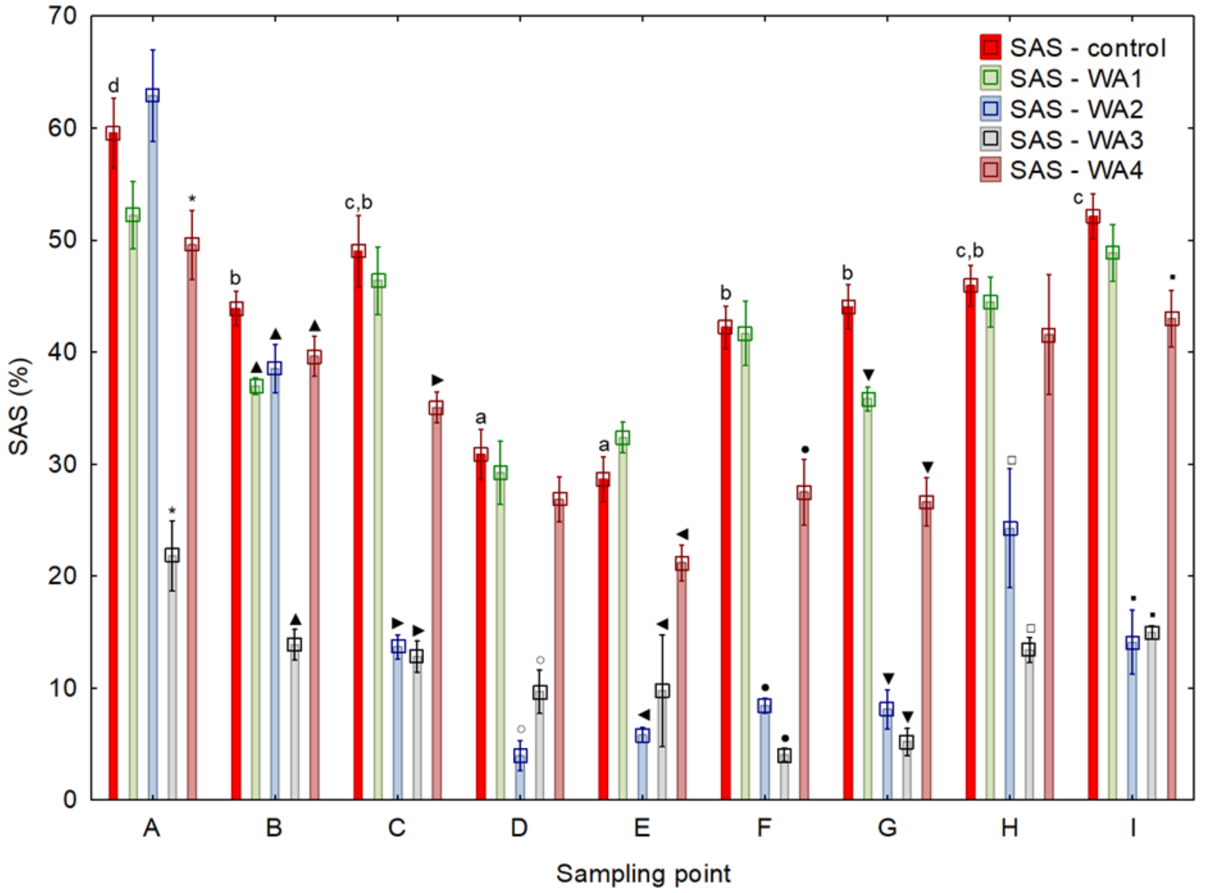


Figure 2 Stability of soil aggregates (SAS) – Initial values on the respective sites and values after the addition of different wetting agents (WA)

*Note to Figure 2: Average SAS values (n = 6) from the individual sampling points are illustrated before and after the application of respective WA (1 – 4). Different symbols were chosen for each sampling point (A: *; B: ▲; C: ▶; D: ○; E: ◀; F: ●; G: ▼; H: □; I: ▪). Their presence at the SAS value indicates a demonstrable difference between the particular variant (with the addition of WA) and the control (SAS – control) at a level of significance of P < 0.05 in one specific sampling point. Different lowercase letters indicate differences in SAS among the individual sampling points within the control collections of samples without the addition of WA.*

The development of SAS in the control samples from the respective localities is interesting too. The control samples demonstrably differed in dependence on the sampling point (Annex a-1). The highest value was measured in the sampling point A and the lowest one was measured in the sampling points D and E, which was significant as compared with the other variants (sampling points).

Comparing the measured SAS values in terms of total means (Table 5), we can see that the control variant exhibited the highest SAS value (44.04%) while the variants with the applied WA showed lower SAS values at all times. The SAS value was changing in the following order: SAS – control > SAS WA1 > SAS WA4 > SAS WA2 > SAS WA 3- with the measured difference being demonstrable after the application of WA2, WA3 and WA4. Thus, the measured values clearly show the influence of WA application on the decreased SAS values.

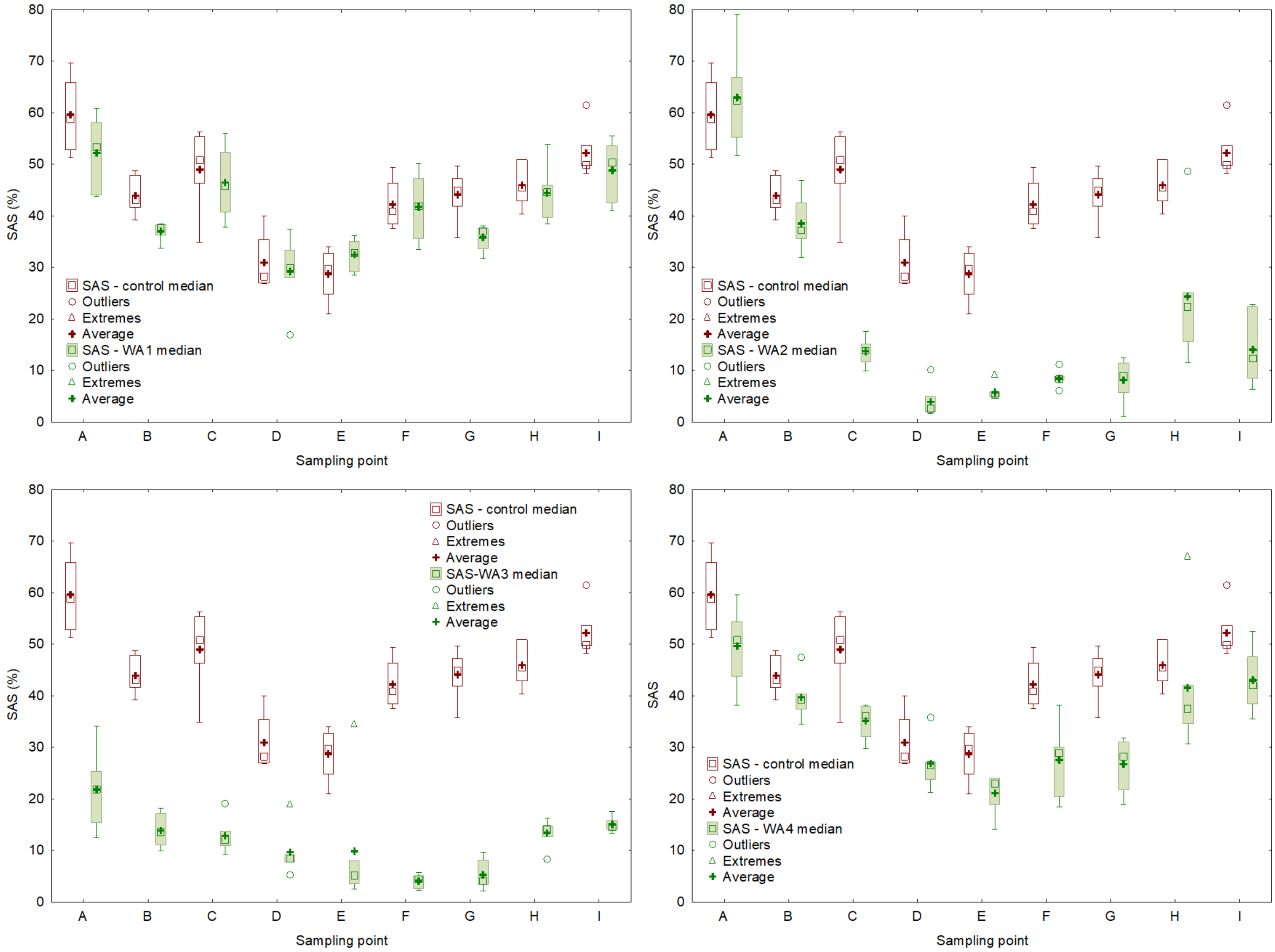
Table 5 Results of post-hoc Tukey´s HSD test (P<0.05) – Comparison of average SAS values before and after the addition of WA

SAS – control	SAS - WA1	SAS - WA2	SAS - WA3	SAS - WA4
44.04 %	40.89 %	19.98 %	11.74 %	34.55 %

SAS – control 44.04 %		0.664777	0.000017	0.000017	0.000517
SAS - WA1 40.89 %	0.664777		0.000017	0.000017	0.053832
SAS - WA2 19.98 %	0.000017	0.000017		0.004041	0.000017
SAS - WA3 11.74 %	0.000017	0.000017	0.004041		0.000017
SAS - WA4 34.55 %	0.000517	0.053832	0.000017	0.000017	

Note to Table 5: Statistically significant differences ($P<0.05$) are in red colour

To obtain a further confirmation of the negative influence of WA application on SAS in the soil samples collected from the experimental sites, the individual values were compared using the pair t-test ($P<0.05$). We always compared SAS values from one locality – the control sample and the sample to which a wetting agent was added within the SAS measurement (Table 6). Differences among the individual experimental variants are obvious both from the result of the pair t-test, and from the box charts (Figure 3) with median and mean values. The most conspicuous effect was that of WA2 and WA3 additions as the values of SAS median were always lower in these variants if they were compared with the SAS median of the control variant. Moreover, total differences between the control variant and variants with the addition of WA (2 and 3) across all sampling points were demonstrably significant with the average SAS value being at all times markedly lower in those variants. Other significant differences were found after the application of WA4 where the clearly negative influence on SAS after the application of the wetting agent was exhibited namely in the soil samples from sampling points E – I. The measured values indicated clearly that the application of WA decreased the average SAS value as well as the SAS median (Annex c-7).



~~**Figure 3** Comparison of initial soil aggregates stability (SAS) and effect of the application of individual wetting agents (WA).~~

~~*Note to Figure 3: SAS values are expressed by box plots. Each graph consists of upper (75th percentile) and lower (25th percentile) quartiles; each graph is added an information about the maximum (upper whisker) and minimum (lower whisker)*~~

~~**Table 6** T-test results (P<0.05) – Comparison of differences among the average SAS values~~

	~~SAS - control~~	~~SAS - WA1~~	~~SAS - WA2~~	~~SAS - WA3~~	~~SAS - WA4~~
~~SAS - control~~	~~0.00~~	~~3,15~~	~~24.06~~	~~32.31~~	~~9.49~~
~~SAS - WA1~~	~~-3.15~~	~~0.00~~	~~20.91~~	~~29.16~~	~~6.34~~
~~SAS - WA2~~	~~-24.06~~	~~-20.91~~	~~0.00~~	~~8.25~~	~~-14.57~~
~~SAS - WA3~~	~~-32.31~~	~~-29.16~~	~~-8.25~~	~~0.00~~	~~-22.82~~
~~SAS - WA4~~	~~-9.49~~	~~-6.34~~	~~14.57~~	~~22.82~~	~~0.00~~

~~*Note to Table 6: The comparison includes average SAS values from all sampling points. T-test results are shown – analysis of significant differences between the respective variants. The average SAS in controls was compared with the average SAS of all other variants from all sampling points. Statistically significant differences (P<0.05) are in red colour*~~

2.3 Analysis of the potential influence of basic soil parameters on the water stability of soil aggregates

Relations between the individual soil parameters and WSA ~~SAS~~ values before and after the application of WA were subject to the regression and PCA analyses. The correlation matrix is presented in Table 7. The presented R values show that the contents of basic nutrients in the soil (P, K, Ca, Mg) had no influence on WSA ~~SAS~~ before the application of WA (control variant) as the R values ranged from -0.11 to -0.38. Similar values were recorded when comparing WSA ~~SAS~~ after the addition of WA with the initial values of soil nutrient contents.

~~An analysis of the relation of SAS with the soil reaction (pH) and Na content in the soil did not reveal any dependence either, not even between SAS in the control variant without the addition of WA. With only one exception, the R values ranged within negative numbers from min. -0.06 to max. 0.24.~~

~~Significant dependences between the parameters were found only in the comparison of individual SAS values before and after the addition of WA together with the values of C_{ox} content in the soil and glomalin (EG and TG). In this case, the R value reached 0.7 and this is why it can be stated that the content of C_{ox} positively affected SAS.~~

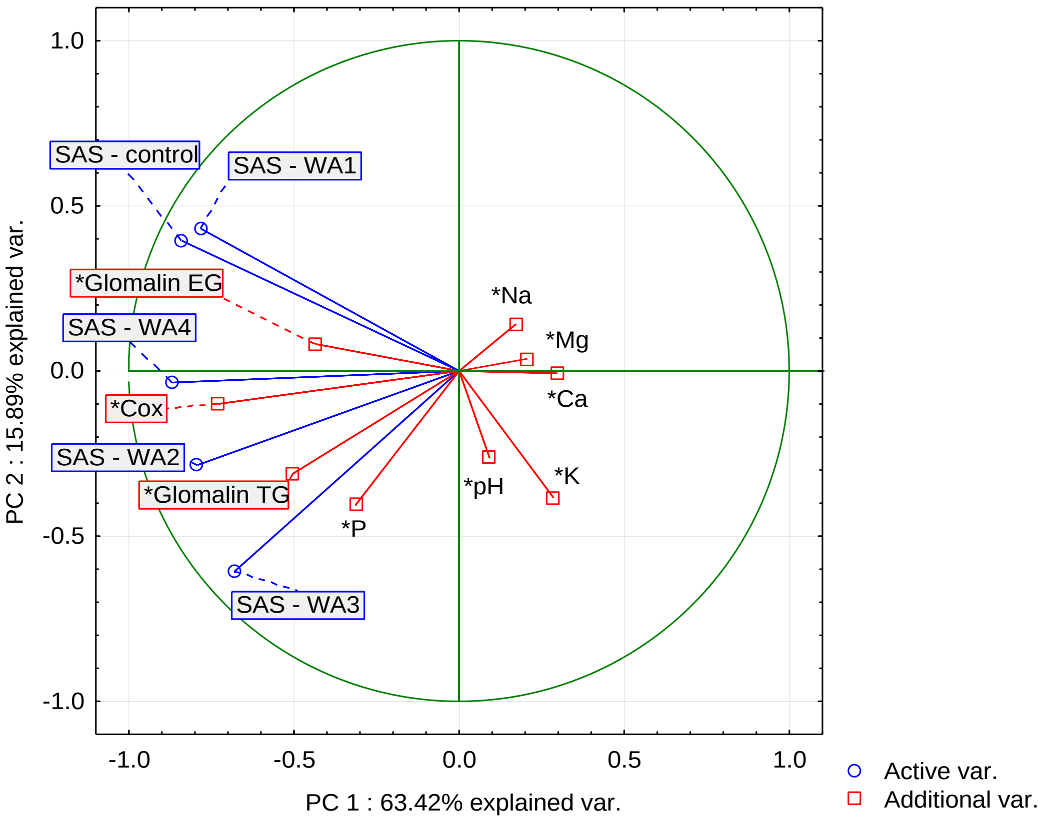


Figure 4 PCA biplot graph

An analysis of the relation of WSA with the soil reaction (pH) and Na content in the soil did not reveal any dependence either, not even between WSA in the control variant without the addition of WA. With only one exception, the R values ranged within negative numbers from min. -0.06 to max. 0.24.

Significant dependences between the parameters were found only in the comparison of individual WSA values before and after the addition of WA together with the values of C_{ox} content in the soil and glomalin (EG and TG). In this case, the R value reached 0.7 and this is why it can be stated that the content of C_{ox} positively affected WSA.

Another possibility for how to characterize the relation of individual values and explain their variability is a biplot graph which illustrates the projection of variables into the factor level (Figure ~~5~~4). The highest own number (Annex ~~ed 8~~13) explains 63.42% of the variability of measured values and the second number covers 15.89% of data variability. The graph of component weights (Figure 4) for the first two factors (components) shows correlations among WSA~~SAS~~, C_{ox} and glomalin (EG, TG) value levels. At the same time, these variables exhibit a very weak positive correlation with the P values and a negative correlation with the values of Ca, Mg and K.

Table 77 Correlation matrix

	WSA SAS - control	WSA SAS - WA1	WSA SAS - WA2	WSA SAS - WA3	WSA SAS - WA4	*Glomalin EG	*Glomalin TG	*Cox	*pH	*Na	*P	*Ca	*K	*Mg
WSA SAS - control	1.00	**0.72**	0.56	0.34	**0.68**	0.45	0.37	**0.60**	-0.23	-0.13	0.11	-0.29	-0.38	-0.14
WSA SAS - WA1	**0.72**	1.00	0.43	0.38	0.57	0.29	0.16	0.47	-0.18	-0.06	0.01	-0.21	-0.48	-0.18
WSA SAS - WA2	0.56	0.43	1.00	0.54	**0.63**	0.43	**0.61**	**0.73**	0.24	-0.15	0.45	-0.23	-0.04	-0.14
WSA SAS - WA3	0.34	0.38	0.54	1.00	0.54	0.17	0.45	0.47	-0.04	-0.23	0.38	-0.20	-0.06	-0.19
WSA SAS - WA4	**0.68**	0.57	**0.63**	0.54	1.00	0.36	0.43	**0.64**	-0.14	-0.12	0.32	-0.25	-0.16	-0.18
***Glomalin EG**	0.45	0.29	0.43	0.17	0.36	1.00	**0.70**	**0.70**	-0.04	0.24	0.27	-0.02	0.13	0.34
***Glomalin TG**	0.37	0.16	**0.61**	0.45	0.43	**0.70**	1.00	**0.71**	-0.02	0.12	**0.64**	-0.09	0.37	0.24
***Cox**	**0.60**	0.47	**0.73**	0.47	**0.64**	**0.70**	**0.71**	1.00	0.03	0.12	0.50	-0.01	0.08	0.25
***pH**	-0.23	-0.18	0.24	-0.04	-0.14	-0.04	-0.02	0.03	1.00	0.17	0.12	0.40	0.25	0.07
***Na**	-0.13	-0.06	-0.15	-0.23	-0.12	0.24	0.12	0.12	0.17	1.00	-0.10	**0.70**	0.36	**0.78**
***P**	0.11	0.01	0.45	0.38	0.32	0.27	**0.64**	0.50	0.12	-0.10	1.00	-0.11	0.51	0.04
***Ca**	-0.29	-0.21	-0.23	-0.20	-0.25	-0.02	-0.09	-0.01	0.40	**0.70**	-0.11	1.00	0.49	**0.68**
***K**	-0.38	-0.48	-0.04	-0.06	-0.16	0.13	0.37	0.08	0.25	0.36	0.51	0.49	1.00	0.52
***Mg**	-0.14	-0.18	-0.14	-0.19	-0.18	0.34	0.24	0.25	0.07	**0.78**	0.04	**0.68**	0.52	1.00

Note to Table 7: Spearman coefficients are presented. Values in red colour indicate a statistical dependence ($P < 0.05$) between two quantities. The correlation matrix was calculated as a part of the factor analysis.

Discussion

A majority of the tested WA had a demonstrably negative influence on WSA with the effect of individual WA being very likely depending on the soil type and method of arable land management. Namely the soil type can have a great influence on the resistance of soil particles to disintegration when these are exposed to some external forces (Papadopoulos, 2011; Lerch et al., 2012). Stability of soil aggregates was demonstrably affected by the addition of WA to the analysed soil samples with all wetting agents causing decreased WSA at least in one soil sample across the sampling points (A – I). Thus, the measured values confirmed that certain changes in water resistance of aggregates occur regardless of climate, soil type or crop rotation if the natural soil properties (e.g. soil aggregates stability) are affected by an abiotic factor. In our experiment, wetting agents were such a factor. Values measured in the control variant without the addition of adjuvants amounted on average to 44% while the mean WSA values for variants with the addition of adjuvants dropped below 40 %, even to 11.74%. According to Bartlova et al. (2015), WSA values ranging from 34.1 to 50% indicate the medium quality of soil structure. Almajmaie et al. (2017) favour a similar evaluation, considering the WSA values around 50% as average but depending on the chosen method of determination and concrete soil conditions. WSA values below 34.1% then indicate the low and very low soil structure quality. WSA is most frequently affected by the soil type and by soil management practices (Emerson and Greenland, 1990; Šimanský et al., 2015); in our experiment, however, the WSA value was clearly affected also by the addition of adjuvants. Although there were differences between the sampling points, it was impossible to determine in the submitted study whether these differences (Annex c-610 and 711) were caused only by different soil types or whether there were some other factors which affected the results, for example the already mentionedP management practices. Relative a few studies exist that would deal with the influence of soil surface active substances on WSA, Lehrsch et al. (2012) and Lehrsch (2013) are exceptions. In their studies, these authors claim that aggregate tensile strength differs primarily in dependence on the soil structure and depth rather than on the type of surfactants which the soil particles come into contact with (e.g. during irrigation or application of spray mixture). This was corroborated in our study only partly because WSA was at the same level on most of the sites (before the addition of WA), only the sites D and E exhibited relatively low values. One of possible reasons to the low water resistance on sites D and E, certainly not the only one though, could have been the impact of water. Site D with Haplic Luvisols was affected by the process of illimerization or depletion of the surface horizon of colloidal particles due to mildly acidic soil reaction. Site E with Relictistagnic Fluvisols developed through the activity of alluvial sediments, further affected by water (stagnic properties). The following addition of WA (type WA2 – 4) resulted in the demonstrable decrease of WSA at all sites. Further, a majority of sampling points were limed in the last 5 years, with a CaO application ranging from 600 to 3,100 kg/ha. No direct dependence was however found between the content of Ca^{2+} in the soil and the WSA values (either before or after the WA application). Although this is in contradiction with some scientific studies (Wuddivira and Camps-Roach, 2007) claiming that the application of Ca^{2+} into arable land has a positive effect on WAS, it should be pointed out that the content of Ca^{2+} did not show deficit values for the given soil types at any of the sampling points because the plots were regularly limed in the past.

We tested four types of WA which differed in their composition but the principle of action on the spray mixture was at all times the same. WA2 - oleic and WA3 - iontic types of wetting agents had the most negative influence on WSA. The basic substance of these WA types is methyl ester; methyl ester of rapeseed oil in the case of WA2 (733 g/l) and methyl esters of palmitic and oleic acids with polyalkoxy ester of phosphoric acid in the case of WA 3. The type of WA 3 wetting agent is interesting as it contains both oleic and ionic components. This could explain why its potential influence on the decrease of SA stability was the highest of all studied WA.

Methyl esters are substances derived from esters which are functional derivatives of carboxylic acids. They are prepared by carboxylic acids reacting with alcohols or phenols. Methyl ester of rapeseed oil (Fatty acid methyl ester – FAME) that was the main substance in WA2 is produced by the trans-esterification of triacylglycerols with methyl alcohol (Canoira at al., 2010). The other wetting agent (WA3) contained palmitic acid methyl ester (PAME) and oleic acid methyl ester (OAME). Similarly, as FAME, they are esters in chemical terms, namely methyl esters of vegetable oils and their production is similar, too (Canoira et al., 2010; Martínez et al., 2014). Nevertheless, a difference between the substances consists in their structure, which is obvious from their molecular formulas: $C_{17}H_{34}O_2$ (PAME) and $C_2H_3O_2$ (FAME). These substances have typically similar characteristics, density lower (< 900 kg/m^3) than water and hydrophilous effect which depends on the number of carboxyl groups and atoms of carbon in the chain of the given substance. Solubility of these substances increases with the increasing number of carboxyl groups and with the lower amount of carbon (Hazen, 2000; Simsek et al., 2015). In general, esters can be both hydrophobic and hydrophilous and this is why they are very often used as detergents (Miyake and Yamashita, 2017). Thus, it can be assumed that the addition of these wetting agents (WA2 and WA3) in the solution used for testing WSA affected the hydrophobicity of soil particles and hence their capability to hold together much more than wetting agents WA1 and WA4, the reason being exactly the chemical composition and physical properties of methyl esters which exhibit a stronger detergent effect as compared with substances contained in WA1 and WA4 (substances based on organic silicones and fatty alcohols) (Hazen, 2000). This effect was then responsible for the disruption of bonds between the soil particles.

Furthermore, ~~T~~the basic soil parameters measured on the individual sampling sites did not exhibit any extremes, and their values were presumably affected primarily by the method of management and by the soil type in the given region. Potential contents of glomalin and OM in the soil were markedly affected by the soil texture and type (Rilling et al., 2001). This partly explains the fluctuation of values measured across the sampling points. As to the content of nutrients available to plants, the most conspicuous differences were found in P and Ca. Together with N, these nutrients represent biogenic substances significantly affecting the growth of plants as well as the soil fertility (Rodriguez-Moreno et al., 2014). Thus, it can be assumed that the fluctuation of their contents across the sampling points resulted from the grown crops (crop rotation) because each of the crops (winter wheat, winter rape, sugar beet, spring barley etc.) had different requirements for these nutrients (Lošák et al., 2010; Hanlirova et al., 2017). Sampling points D – I were situated in the region where sugar beet is grown very often. The technology of growing sugar beet includes the application of high-quality organic matter (bovine dung) and the application of lime (dolomitic limestone; Table 2), which are necessary for optimum yield and sugar content in the bulb (Hlisnikovský et al., 2021). The fertilization certainly mirrored also in the soil contents of K, Mg and Na, and apart from the beneficial influence on the yield and quality of bulbs or soil characteristics, it also caused worse correlability of these elements with WSA ~~SAS~~ because all calcium supplied "in addition" above the threshold of colloidal coagulation worsens the correlation with WSA~~SAS~~, too. However, the threshold of coagulation depends on other soil properties such as C_{ox}, texture etc. The other sampling points (A – C) were situated in regions with the increased representation of cereals and oilseeds in the crop rotation, i.e. with the crops that are considerable consumers of P and K (Sun et al., 2021). This is why the contents of these nutrients were lower ~~in~~at the experimental sites~~localities~~. Moreover, soils in those regions exhibit lower potential fertility and hence also ~~a~~ naturally lower contents of nutrients (Gebeltova et al. 2020). The above facts are presumably further exacerbated by differences in the particle-size distribution (and hence by differences in sorption capacity) or by altitudes with higher mean annual precipitation amounts (see Table 1).

Interesting was the absence of correlation between the two forms of glomalin (EG and TG) and WSA; the only exception was the WSA – WA2 variant where the WSA value demonstrably increased on the site even after the addition of the wetting agent. According to Kaczorek et al.~~,~~ (2013), this was caused by the content of hydrophobic compounds in FAMEs (it can generally be caused by oils) which were a significant component of WA2. FAMEs could have contributed to the hydrophobic nature of the surface of aggregates and increased their water resistance. Causation between WSA in the respective variants (with or without WA) and the contents of Ca^{2+} and Na^+ ions in the collected soil samples was not demonstrated. This is rather interesting as there are studies (Emerson and Smith, 1970; Rengasamy and Marchuk, 2011; Bronick and Lal, 2005) which confirm the negative effect of the presence of Na^+ on WAS due to the effect of monovalent cations of sodium (Na) or potassium (K) as these may induce development of dispersion and clay swelling, which results in soil structure degradation (Rengasamy et al., 2016). According to Smiles (2006), K^+ can be considered as an Na^+ equivalent. Arienzo et al. (2012) recorded a higher stability of soil aggregates in the presence of K^+ compared with Na^+. On the other hand, there are long-term experiments (Almajmaie et al., 2017; Rengasamy and Marchuk, 2011) which confirm that Ca^{2+} ions are essential for the coagulation of soil particles and hence for the development of fixed connections between individual particles.

It should be added, however, that all types of wetting agents had a negative effect on WSA at least in one case compared with the control variant. If WSA depends on the presence of hydrophobic bonds between the soil particles (Mao et al., 2019), then the wetting agents have to cause its decrease by the principle of their action on the spray mixture. It follows out from the very essence of all wetting agents, the main goal of which is to increase the wetting ability of spray (capacity of liquid to adhere to the plant surface = decrease is hydrophobicity), which consists of water and active substance of pesticide (Pacanoski, 2015). The surface of soil aggregates is covered with clay and organoclay coatings which may affect the preferential flow of water in individual aggregates (Gerke and Köhne, 2002). Soil aggregates can be also understood as independent units whose hydraulic properties may affect the flow of water between the pores and the inside of aggregates and hence their stability. A change ~~of~~in surface tension can alter the hydraulic properties of water in relation to the hydrophobicity of soil aggregates (Zheng et al., 2016). Thus, there is a presumption that if a spray fluid with the addition of wetting agent enters such an environment, it has a potential to affect the hydrophobicity of soil particles, which is subsequently manifested in WSA changes. Another potential risk consists in the organo-mineral sorption complex of the soil based on SOM as hydrophobic substances (e.g. organic pollutants) can be adsorbed on the surface of soil particles when interacting with SOM components and create a complex affecting other soil properties (Ahmed et al., 2015).

~~According to Wuddivira and Camps-Roach (2007), the bridging effect of calcium ions and flocculation capacity of clays and organic substances takes place thanks to cations, which are decisive for the development and stability of soil aggregates. The reason for the absence of this finding could have been the lower content of Ca^{2+} ions in some soil samples, which could have affected the regression analysis. With only some exceptions, the soil samples contained average or slightly above average amounts of Ca^{2+} depending on the locality. Stability of soil aggregates was demonstrably affected by the addition of WA to the analysed soil samples with all wetting agents causing decreased SAS at least in one soil sample across the sampling points (A – I). Lze tedy předpokládat, že k větším či menším změnám ve vodostálosti agregátů dochází bez ohledu na klima, půdní typ nebo osevní postup. Values measured in the control variant without the addition of adjuvants amounted on average to 44% while the mean SAS values for variants with the addition of adjuvants dropped below 40%, even to 11.74%. According to Bartlova et al. (2015), SAS values ranging from 34.1 to 50.0% indicate the medium quality of soil structure. Almajmaie et al. (2017) favour a similar evaluation, considering the SAS values around 50% as average but depending on the chosen method of determination and concrete soil conditions. SAS values below 34.1 then indicate the low and very low soil structure quality. SAS is most frequently affected by the soil type and by soil management practices (Emerson and Greenland, 1990; Šimanský et al., 2015); in our experiment, however, the SAS value was clearly affected also by the addition of adjuvants.~~

~~If we take into account that SAS is conditioned by the presence of OM in the soil and by its linkage to soil particles at which a hydrophobic structure develops, which is resistant to decomposition (Volikov et al., 2016), then we have a presumption of negative effect of WA on SAS stability, the reason being the very nature of wetting agents as substances directly affecting the surface tension of water and its viscosity (McMullan, 2000; Hazen, 2000; Aliverdi and Ahmadvand, 2018). Thus, these substances have a potential, if in contact with soil particles, to affect their hydrophobicity and hence also the capacity to create soil aggregates or to disturb the stability of this association upon a contact with the already associated particles (Mao et al., 2019). We tested four types of WA which differed in their composition but the principle of action on the spray mixture was at all times the same. WA2 and WA3 types of wetting agents had the most negative influence on SAS. The basic substance of these WA types is methyl ester; methyl ester of rapeseed oil in the case of WA2 (733 g/l) and methyl esters of palmitic and oleic acids with polyalkoxy ester of phosphoric acid in the case of WA 3.~~

Methyl esters are substances derived from esters which are functional derivatives of carboxylic acids. They are prepared by carboxylic acids reacting with alcohols or phenols. Methyl ester of rapeseed oil (Fatty acid methyl ester – FAME) that was the main substance in WA2 is produced by the trans-esterification of triacylglycerols with methyl alcohol (Canoira at al., 2010). The other wetting agent (WA3) contained palmitic acid methyl ester (PAME) and oleic acid methyl ester (OAME). Similarly as FAME, they are esters in chemical terms, namely methyl esters of vegetable oils and their production is similar, too (Canoira et al., 2010; Martínez et al., 2014). Nevertheless, a difference between the substances consists in their structure, which is obvious from their molecular formulas: $C_{17}H_{34}O_2$ (PAME) and $C_2H_3O_2$ (FAME). These substances have typically similar characteristics, density lower (< 900 kg/m^3) than water and hydrophilous effect which depends on the number of carboxyl groups and atoms of carbon in the chain of the given substance. Solubility of these substances increases with the increasing number of carboxyl groups and with the lower amount of carbon (Hazen, 2000; Simsek et al., 2015). In general, esters can be both hydrophobic and hydrophilous and this is why they are very often used as detergents (Miyake and Yamashita, 2017). Thus, it can be assumed that the addition of these wetting agents (WA2 and WA3) in the solution used for testing SAS affected the hydrophobicity of soil particles and hence their capability to hold together much more than wetting agents WA1 and WA4, the reason being exactly the chemical composition and physical properties of methyl esters which exhibit a stronger detergent effect as compared with substances contained in WA1 and WA4 (substances based on organic silicones and fatty alcohols) (Hazen, 2000). This effect was then responsible for the disruption of bonds between the soil particles.

It should be added, however, that all types of wetting agents had a negative effect on SAS at least in one case compared with the control variant. If SAS depends on the presence of hydrophobic bonds between the soil particles (Mao et al., 2019), then the wetting agents have to cause its decrease by the principle of their action on the spray mixture. It follows out from the very essence of all wetting agents, the main goal of which is to increase the wetting ability of spray (capacity of liquid to adhere to the plant surface = decrease is hydrophobicity), which consists of water and active substance of pesticide (Pacanoski, 2015). The surface of soil aggregates is covered with clay and organoclay coatings which may affect the preferential flow of water in individual aggregates (Gerke and Köhne, 2002). Soil aggregates can be also understood as independent units whose hydraulic properties may affect the flow of water between the pores and the inside of aggregates and hence their stability. A change of surface tension can alter the hydraulic properties of water in relation to the hydrophobicity of soil aggregates (Zheng et al., 2016). Thus, there is a presumption that if a spray fluid with the addition of wetting agent enters such an environment, it has a potential to affect the hydrophobicity of soil particles, which is subsequently manifested in SAS changes. Another potential risk consists in the organo-mineral sorption complex of the soil based on SOM as hydrophobic substances (e.g. organic pollutants) can be adsorbed on the surface of soil particles when interacting with SOM components and create a complex affecting other soil properties (Ahmed et al., 2015).

There are scientific studies which deal with the significance of wetting agents in agriculture (Pacanoski, 2015; Baratella and Trinchera, 2018) and warn at the same time about potential negative effects of their application on the environment (Mesnage and Antoniou, 2018; Mesnage at al., 2013). There are however no detailed studies that would describe their potential impacts on the soil environment with respect to WSA SAS, mineralization of SOM or quantity and quality of microbial biomass. Therefore, a follow-up research will be necessary. It is known that appropriate and targeted application of spray mixture with the addition of adjuvants increases the efficiency of used pesticides (their active substances) and suppresses their potential adverse effect on the environment because the applied concentrations of pesticides can be reduced (Pacanoski, 2015; Mirgorodskaya et al., 2020). It should not be forgotten, however, that key factors responsible for the effectiveness of herbicides are not only the structure and concentration of substances active on the surface but also the treatment time, wetting effect of spray mixture and air temperature during the spray application on the crop standgrowth (Mirgorodskaya et al., 2020). Thus, it follows that if the spray is applied in a targeted manner and using technologies of precision agriculture, it should reach only parts of the plot with the plant biomass; then a greater part of the applied wetting agents should affect only the leaves of plants. The presumed negative impact of wetting agents is thus conditioned by their contact with the soil environment. A question is at what amount and concentration – this should be a subject of the further research. The above data show that wetting agents can reduce WSASAS even at a recommended dosage if they are applied inappropriately on the bare soil without the cover of plants (low leaf area index). Another important aspect explored was the influence of some soil parameters on WSA SAS both in the absence of adjuvants (control) and with their application (WA1 – 4). It was found out in our experiment that the Cox content in the soil positively correlated with WSA SAS in most variants (control, WA1, WA2 and WA4). Thus, it can be expected that if the content of SOM increases in the soil, WSA SAS would increase too. This was corroborated also by Haynes and Swift (1990) and Zhao et al. (2017) who describe and confirm a direct connection between SOM and SWSAS. SOM and organic matter in the soil are in general necessary for the development of a functional soil sorption complex with aggregated particles (Six et al., 2002; Ahmed et al., 2015) with interactions between hydrophobic and hydrophilous substances in the soil (within SOM) depending primarily on the SOM chemical composition (Ahmed et al., 2015). Interesting was the absence of correlation between the two forms of glomalin (EG and TG) and SAS; the only exception was the SAS – WA2 variant where the SAS value demonstrably increased on the site even after the addition of the wetting agent. According to Kaczorek et al., (2013), this was caused by the content of hydrophobic compounds in FAMEs (it can generally be caused by oils) which were a significant component of WA2. FAMEs could have contributed to the hydrophobic nature of the surface of aggregates and increased their water resistance. Causation between SAS in the respective variants (with or without WA) and the contents of Ca^{2+} and Na^{+} ions in the collected soil samples was not demonstrated. This is rather interesting as there are studies (Emerson and Smith, 1970; Rengasamy and Marchuk, 2011; Bronick and Lal, 2005) which confirm the negative effect of the presence of Na^{+} on SAS due to the effect of monovalent cations of sodium (Na) or potassium (K) as these may induce development of dispersion and clay swelling, which results in soil structure degradation (Rengasamy et al., 2016). According to Smiles (2006), K^{+} can be considered as an Na^{+} equivalent. Arienzo et al. (2012) recorded a higher stability of soil aggregates in the presence of K^{+} compared with Na^{+}. On the other hand, there are long-term experiments (Almajmaie et al., 2017; Rengasamy and Marchuk, 2011) which confirm that Ca^{2+} ions are essential for the coagulation of soil particles and hence for the development of fixed connections between individual particles. According to Wuddivira and Camps-Roach (2007), the bridging effect of calcium ions and flocculation capacity of clays and organic substances takes place thanks to cations, which are decisive for the development and stability of soil aggregates. The reason for the absence of this finding could have been the lower content of Ca^{2+} ions in some soil samples, which could have affected the regression analysis. With only some exceptions, the soil samples contained average or slightly above-average amounts of Ca^{2+} depending on the locality.

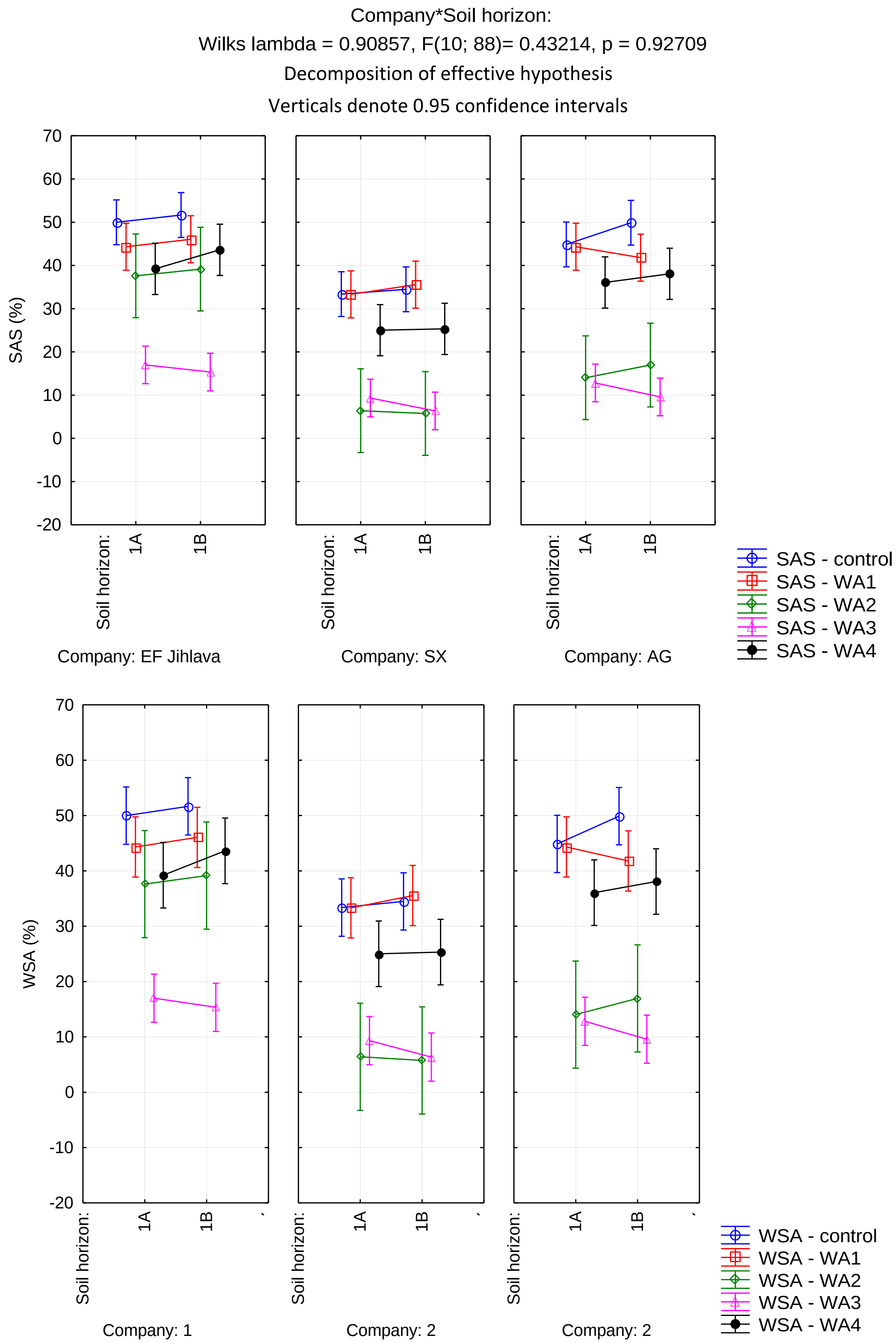
Another important aspect which should be taken into account when discussing the research results is that the experiment took place in the laboratory. Recommended doses of wetting agents were applied in laboratory conditions on the soil samples in which WSA SASwas then monitored. Song et al. (2019) inform for example that the application of wetting agents can affect soil water repellency and microbial community in the soil but that this effect significantly depends on the soil moisture content which is directly influenced by meteorological conditions. Important is also the amount of WA coming into contact with the soil, duration of its action (effect of meteorological conditions again), frequency of application in the region (Barton and Colmer, 2011; Song et al., 2019) and the way of how WA get into contact with the soil (Barton and Colmer, 2011). Whereas a fundamental difference in the (intensity) action on the soil environment exists between the WA which gets into the soil with the pre-emergency application e.g. of herbicides, and the WA which is applied on the plants together with pesticides and has to reach the soil environment through the topsoil layer (Tominack and Tominack, 2000; Song et al., 2019). At that, tThe presence of WA in the soil environment subsequently affects soil hydrophobicity and hence infiltration of water into the soil environment (Leighton-Boyce et al., 2007). The laboratory results point to the influence of WA on WSA SAS, and hence to the disintegration of soil aggregates. In natural conditions, aggregation of soil particles is a complex process controlled by abiotic factors (soil texture, climatic conditions) and mediated by the action of plants and other biotic factors (SOM, activity of microorganisms) (Rilling et al., 2014). Based on the above facts, it can be deduced that the effect of WA on WSASAS in field conditions can be influenced by the initial condition of the soil, e.g. by the amount of SOM, or by the growth of plants on the site as these factors affect soil aggregation in a complex way (Six et al., 2004; Rilling, 2014). Thus, it can be presumed that WA can act negatively on WSA SAS and affect other soil properties but the degree of this action will depend: on their chemical composition (Castro et al., 2018; Song et al., 2019), weather conditions (Song et al., 2019), application method and frequency (Barton and ColmerSong et al., 2019), factors affecting the process of aggregation and hence also resistance of soil particles to their disintegration (Rillig et al., 2014).

Conclusion

Based on the measured data, discussion and examples from literature, it is possible to state that the application of adjuvants (spray mixture) has a negative effect on SAS if the spray mixture gets into contact with the soil particles. In the laboratory experiment, a negative influence of wetting agents added to the soil samples on the stability of soil aggregates was recorded. Thus, a further research should be conducted to analyse the probability of spray mixture reaching the soil without the plant cover. Exactly such an application of spray mixture with the content of pesticides appears to be the most risky with respect to WSA SAS because in a majority of cases, the individual types of adjuvants exhibited a negative effect on WSA SAS as compared with the control variant. This adverse effect was however observed upon the direct contact of adjuvants with the soil aggregates, this is why a further research is needed. In addition to this impact, potential differences were recorded in the action of individual adjuvant types in dependence on their composition. If they contained hydrophobic substances (partly at least), their negative action was less severe. To have detailed and exact conclusions about the action of adjuvants on WSA SAS and other soil properties, it will be necessary to thoroughly analyse their chemical nature. This is however very difficult as the exact composition of adjuvants is rarely available and a detailed action of their individual components on the environment is not tackled either. Another important finding is a possibility to mitigate the adverse effect of adjuvants on WSA SAS through the increased SOM content. The presence of organic matter in the soil appears to be crucial, and in the case of studied localities, it was more significant than the presence of Ca^{2+} ions in the soil sorption complex.

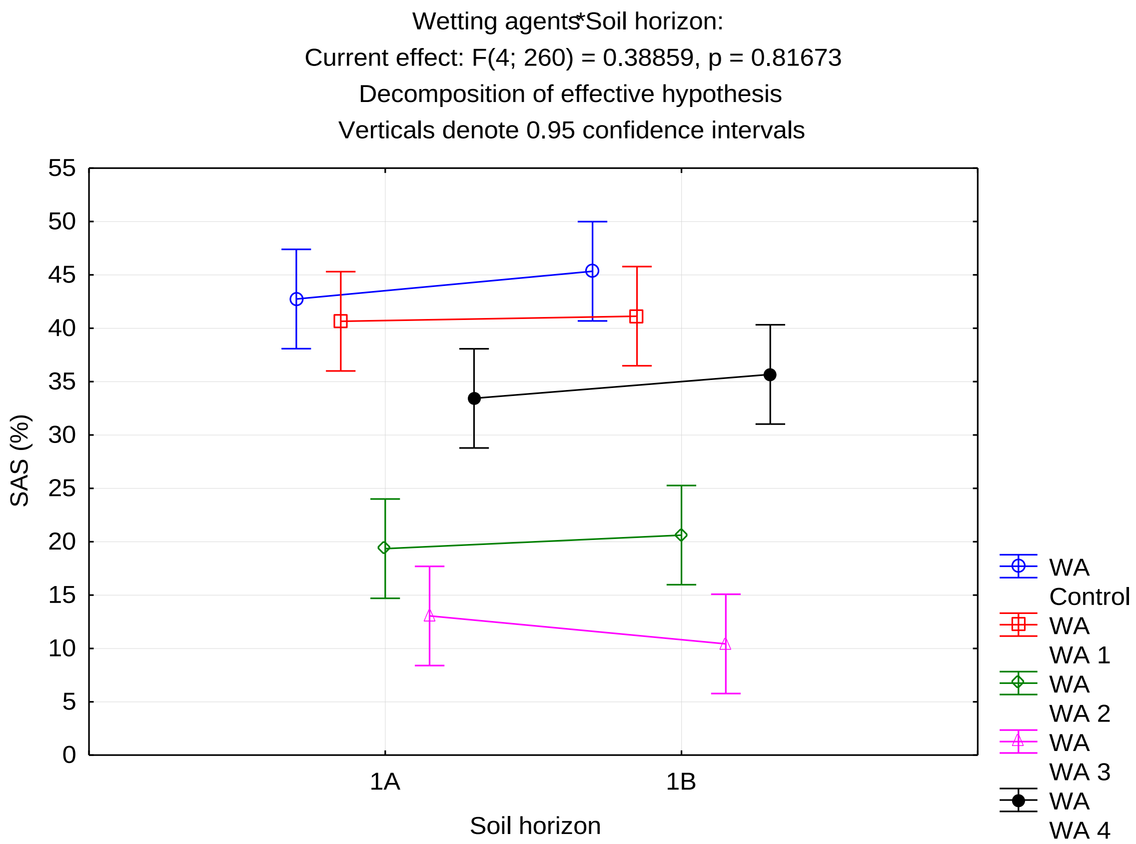
Annex

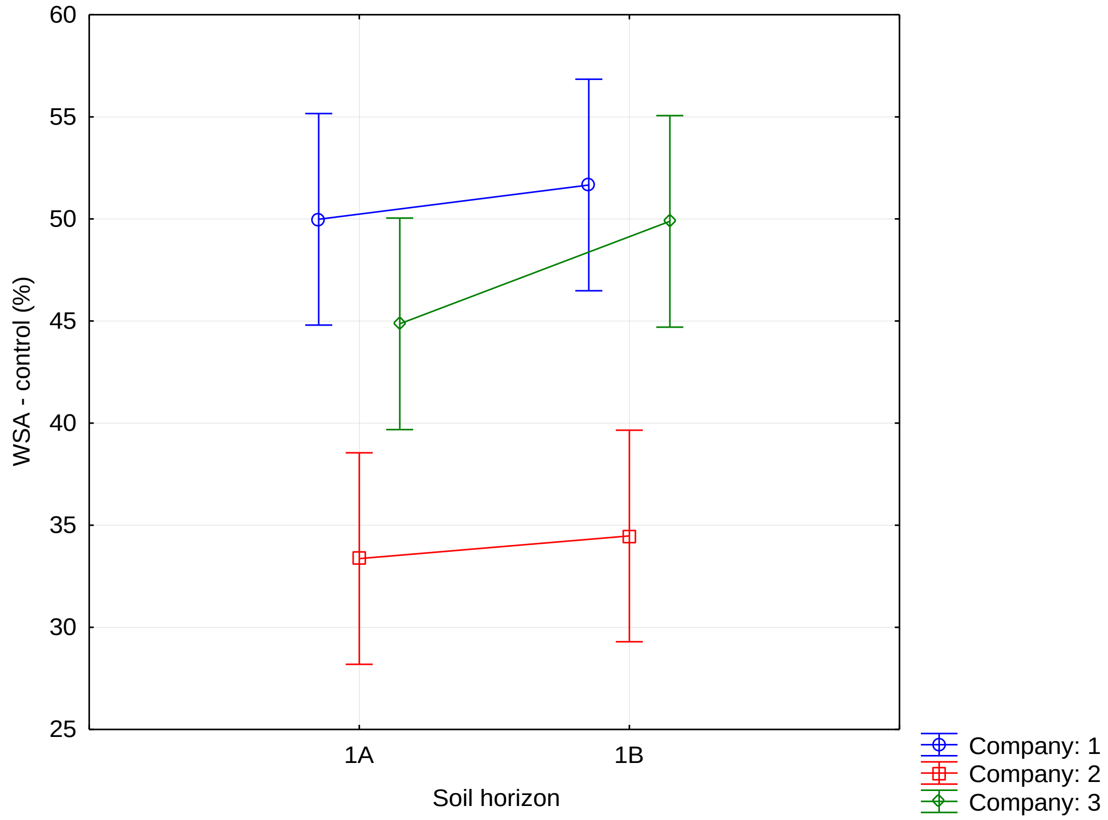
Annex A Testing the effect of sampling point



Annex a-1 Effect of sampling point on ~~SAS~~ WSA in the respective variants.

Note to Figure a-1: Average WSA values (n = 18) from the individual sampling points of topsoil layer within company 1 - 3 are illustrated before (WSA – control) and after the application of respective WA (1 – 4). Verticals denote 0.95 confidence intervals.





Annex a-2 Effect of sampling point on WSA SAS in the respectivecontrol variants.

Note to Figure a-1: Average WSA values (n = 54) from the individual sampling points of topsoil layer within company 1 - 3 are illustrated before (WSA – control) the application of respective WA (1 – 4). Verticals denote 0.95 confidence intervals.

Annex B Information about soils

Annex b-3 Basic information about soils in the respective sampling points

Sampling point	Depth (m)	pH in H2O	pH in 1MKCl	Cox (wt%)	Texture class*
A	0.10–0.20	6.39	5.05	1.36	SiL
	0.35–0.45	5.60	3.83	0.15	SiL
	0.60–0.70	5.05	3.64	0.11	L
	0.90–1.00	5.16	3.78	0.10	L
B	0.05–0.15	6.91	5.97	1.77	SL
	0.35–0.45	7.01	5.73	0.37	SL
C	0.05–0.15	6.90	6.21	2.09	SL
	0.35–0.45	7.09	5.29	0.39	SL
D	0.05–0.10	6.44	5.76	1.26	SiL
	0.20–0.25	6.49	5.95	1.08	SiL
	0.35–0.40	6.48	5.87	0.55	L
	0.50–0.55	6.66	5.79	0.34	CL
E	0.05–0.10	6.80	6.02	1.22	CL
	0.20–0.25	6.92	6.07	0.97	CL
	0.35–0.40	7.03	6.15	0.48	SiCL
	0.50–0.55	7.06	6.08	0.32	CL
F	0.05–0.10	6.40	5.70	1.11	SiL
	0.20–0.25	6.44	5.62	0.74	SiCL
	0.35–0.40	6.69	5.53	0.26	SiCL
	0.50–0.55	6.90	6.09	0.22	SiCL
G	0.10–0.20	7.21	6.51	4.32	SiL
	0.30–0.40	7.31	6.70	4.29	SiL
	0.50–0.60	7.16	6.66	1.40	SiL
	0.80–0.90	7.04	6.51	n/a	SiCL
	0.95–1.05	7.01	6.46	n/a	SiCL
H	0.05–0.15	6.75	5.81	1.76	CL
	0.45–0.55	6.40	5.34	0.43	SiCL
	0.90–1.00	6.42	4.98	n/a	SiCL
I	0.05–0.15	6.87	5.92	1.40	L
	0.45–0.55	7.03	5.99	0.24	CL
	0.95–1.05	6.48	3.53	n/a	SL

*SiL (Silt loam), SiCL (Silty clay loam), L (Loam), CL (Clay loam), SL (Sandy loam),

A – B – C

D – E – F

G – H – I

Annex b-4 Soil profiles in the individual sampling points

Annex b-5: A detailed characterization of other soil horizons:

Sampling point A:

- 0.32–(0.43–0.65) m mottled layer 1: combination of pinkish white 7.5YR8/2 (w) and reddish yellow 7.5YR6/6 (w); angular structure, texture class silt loam, the layer contains a small amount of coarse sand and a high amount of Fe-Mn nodules Ø5 mm. Transition to the deeper horizon is undulated.
- (0.43–0.65)–1.12 m mottled layer 2: combination of grey 5YR5/1(w) and yellowish red 5YR5/6 (w); angular structure, texture class loam, admixture of coarse sand, a high amount of Fe-Mn nodules.
- >1.12 m (to 1.50 m) transition layer to the parent rock material: alternation of colours grey 5YR6/1 (w) and yellowish red 5YR4/6 (w); without a clear structure, texture class loam, the content of soil skeleton (mica schist) very quickly growing with the soil depth.

Sampling point B:

- 0.33–0.58 m cambic horizon: brown 7.5YR4/4 (w); angular structure, texture class sandy loam, approx. 20 % of soil skeleton. Clear transition to the deeper horizon.
- >0.58 m (to the 1.30 m) parent (rock) material: >90 % of soil skeleton (stones), roots recognizable to 0.95 m.

Sampling point C:

- 0.28–0.60 m endopedon: colour brown 10YR4/3 (w) to dark yellowish brown 10YR4/4 (w), angular structure, texture class sandy loam, 25 % of soil skeleton.
- >0.60 m transition horizon to the parent rock material: yellowish brown 10YR5/6 (w); without clear structure, texture class sandy class, the amount of soil skeleton growing with the depth from 30 % to 100 %, tight placement of weathered stones from a depth of 1.10 m (to the final depth of soil pit 1.35 m).

Sampling point D:

- 0.32–0.60 m argic horizon (clay coats; clay ratio with surface horizon 1.8): angular blocky structure, surface of aggregates 10YR3/4 (w) dark yellowish brown, inside of aggregates 10YR4/6 (w) dark yellowish brown; Fe-Mn nodules. Texture class clay loam.
- >0.60 m (to the final depth 1.40 m) transition horizon to the parent rock material.

Sampling point E:

- 0.29–0.62 m mottled layer 1 with stagnic properties: 70% 10YR4/2 (w) dark greyish brown and 30% 10YR5/6 (w) yellowish brown; small angular blocky structure, a small amount of Fe-Mn nodules. Texture class silty clay loam (0.35 m) and clay loam (>0.50 m) – qualifier Loamic.
- > 0.62 m mottled layer 2 with stagnic properties (qualifier Relictistagnic): 50 % 10YR5/2 (w), greyish brown and 50 % 10YR4/6 (w), dark yellowish brown; a large amount of Fe-Mn nodules. 0.62–0.93 m, small angular blocky structure, >0.93 m (to the final depth 1.40 m), without structure,

Sampling point F:

- 0.33–0.57 m: 10YR5/6 (w) yellowish brown and <10 % 10YR4/1 (w) dark grey; small angular blocky structure. Texture class silt loam (qualifier Siltic).
- 0.57–0.93 m: 10YR4/3(w) brown; to 0.74m small angular blocky structure, from 0.74 to 0.93 m structure prismatic; from 0.65 m a small amount of Fe-Mn nodules. Texture class silty clay loam. Clay coats on aggregates surface, but do not meet criteria 2a) v. for argic horizon.
- >0.93 m (to the final depth 1.50 m) transition horizon to the parent rock material: 10YR4/6(w) dark yellowish brown; angular blocky structure, a weak amount of roots to a depth of 1.30 m.

Sampling point G:

- 0.27–0.43 m topsoil layer 2: 7.5YR2/2–3/2 (w), very dark brown/dark brown; granular structure, texture class silt loam; artefacts (pieces of bricks, polyethylene) are uncommonly in this layer (<5 %). Sharp transition to the deeper horizon.
- 0.43–0.79 m layer 1: fluvic material with stagnic properties, <10 % of surface with colour 5YR5/6 (w) yellowish red and > 90 % of surface with 7.5YR from 4/1 to 5/1 (w) dark grey /grey; angular structure, texture class silt loam, a small amount of Fe-Mn nodules.
- 0.79–0.92 m layer 2: fluvic material with stagnic properties approx. 20 % of surface with mottles 5YR4/8-5/8 (yellowish red), other space with 2.5YR3/2 dusky red; angular structure, texture class silty clay loam.
- > 0.92 m layer 3 (to the final depth 1.50 m): fluvic material with stagnic properties 60–70 % of surface with mottles 2.5YR4/5 (reddish brown/red) and 5YR5/8 (w) (red), other space 5Y6/2 (w) light olive grey; prismatic structure, silty clay loam, a small amount of Fe-Mn nodules, a small amount of roots to a depth of 1.00 m.

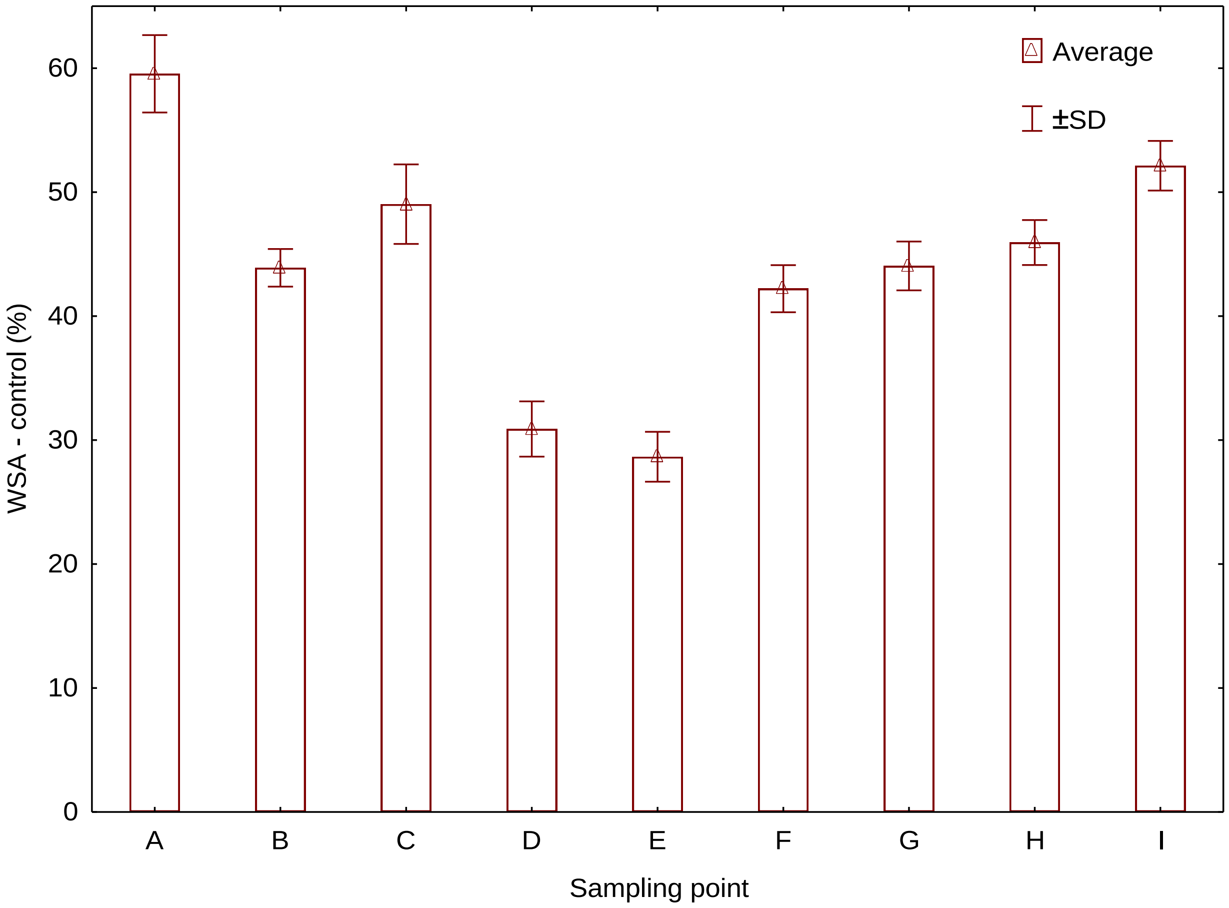
Sampling point H:

- 0.30–0.85 m mottled layer 1: 7.5YR4/1 (w) dark grey, 7.5YR6/8 (w) reddish yellow; prismatic structure, texture class silty clay loam, random dark coats on aggregates, a small amount of Fe-Mn nodules. Clear transition to the deeper horizon.
- >0.85 m (to the final depth 1.30 m) mottled layer 2: grey 7.5YR6/1, reddish yellow7.5YR6/8; without clear structure, texture class silty clay loam.

Sampling point I:

- 0.36–0.94 m mottled layer 1: > 90 % of surface 10YR5/8 yellowish brown, partly 10YR6/1 grey; without clear structure, texture class clay loam, 15–20 % rounded soil skeleton (gravel), a small amount of Fe-Mn nodules, randomly dark Mn-coats, roots to 0.72 m, a drainage pipe in the depth 0.53 m. Clear transition to the deeper horizon.
- >0.94 m mottled layer 2 (to the final depth 1.35 m): > 90 % of surface 7.5YR4/6 strong brown, partly 7.5YR7/1 light grey; without clear structure, texture class sandy loam, to 1.12 m approx. 15 % rounded soil skeleton (gravel), deeper <5% soil skeleton (predominantly coarse sand).

Annex C Descriptive statistics and tests of statistical significance

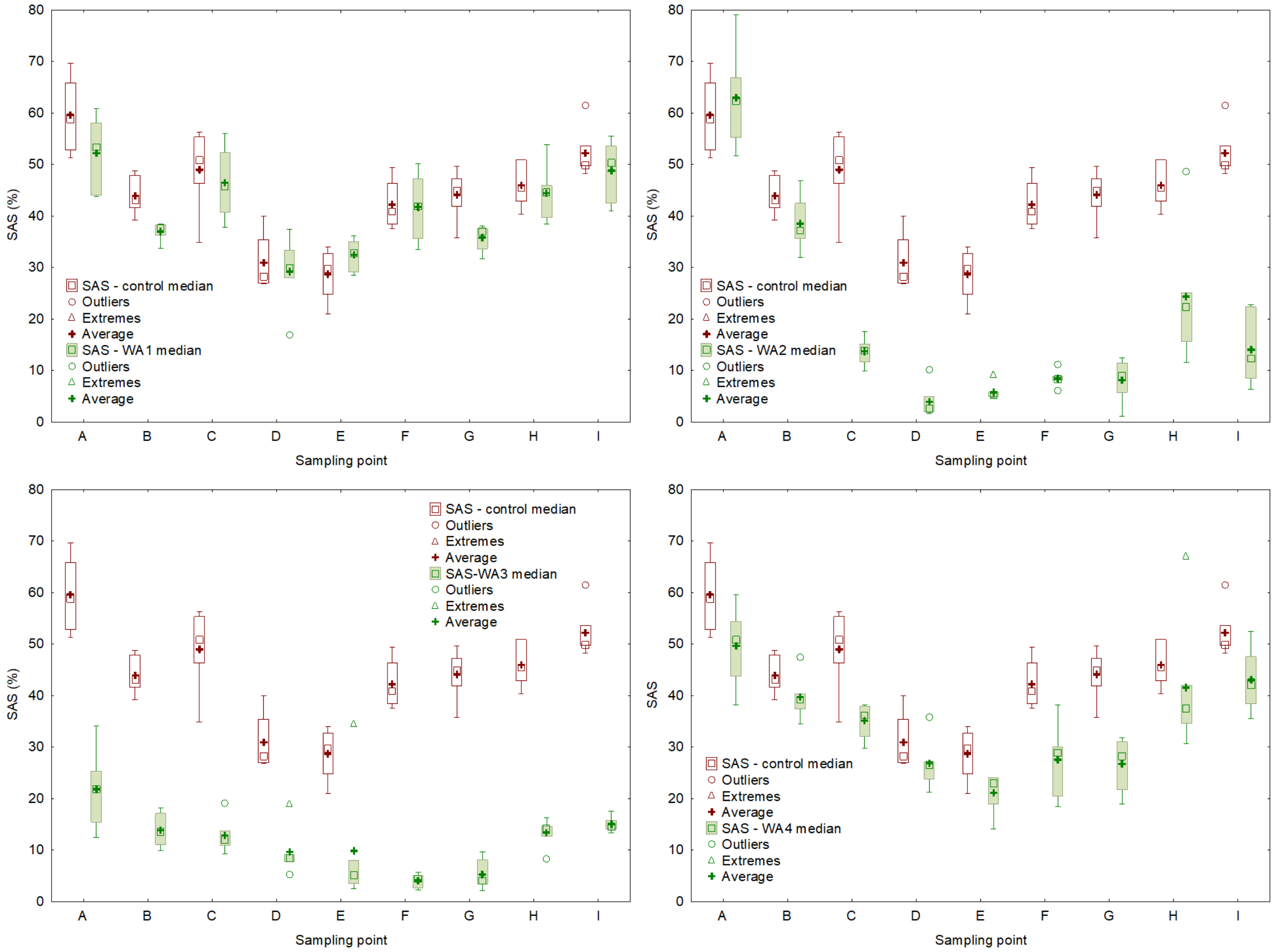


Annex c-6 Comparison of initial soil aggregates stability (WSA) at individual sampling points.

Annex c-7 Comparison of initial soil aggregates stability (WSA) at individual sampling points – Results of Tukey´s post-hoc HSD test.

WSA~~WAS~~	A	B	C	D	E	F	G	H	I
A		**0.000546**	**0.047044**	**0.000143**	**0.000143**	**0.000206**	**0.000616**	**0.003248**	0.356081
B	**0.000546**		0.798540	**0.005649**	**0.000755**	0.999833	1.000000	0.999296	0.227633
C	**0.047044**	0.798540		**0.000168**	**0.000144**	0.468757	0.823657	0.987292	0.986938
D	**0.000143**	**0.005649**	**0.000168**		0.998591	**0.024620**	**0.004908**	**0.000893**	**0.000143**
E	**0.000143**	**0.000755**	**0.000144**	0.998591		**0.003389**	**0.000666**	**0.000210**	**0.000143**
F	**0.000206**	0.999833	0.468757	**0.024620**	**0.003389**		0.999677	0.960245	0.074101
G	**0.000616**	1.000000	0.823657	**0.004908**	**0.000666**	0.999677		0.999604	0.248989
H	**0.003248**	0.999296	0.987292	**0.000893**	**0.000210**	0.960245	0.999604		0.595464
I	0.356081	0.227633	0.986938	**0.000143**	**0.000143**	0.074101	0.248989	0.595464	

Note to Table c-7: Statistically significant differences (P<0.05) are in bold.



Annex c-8 Comparison of initial soil aggregates stability (WSA) and effect of the application of individual wetting agents (WA).

Note to Figure c-8: WSA values are expressed by box plots. Each graph consists of upper (75th percentile) and lower (25th percentile) quartiles; each graph is added an information about the maximum (upper whisker) and minimum (lower whisker)

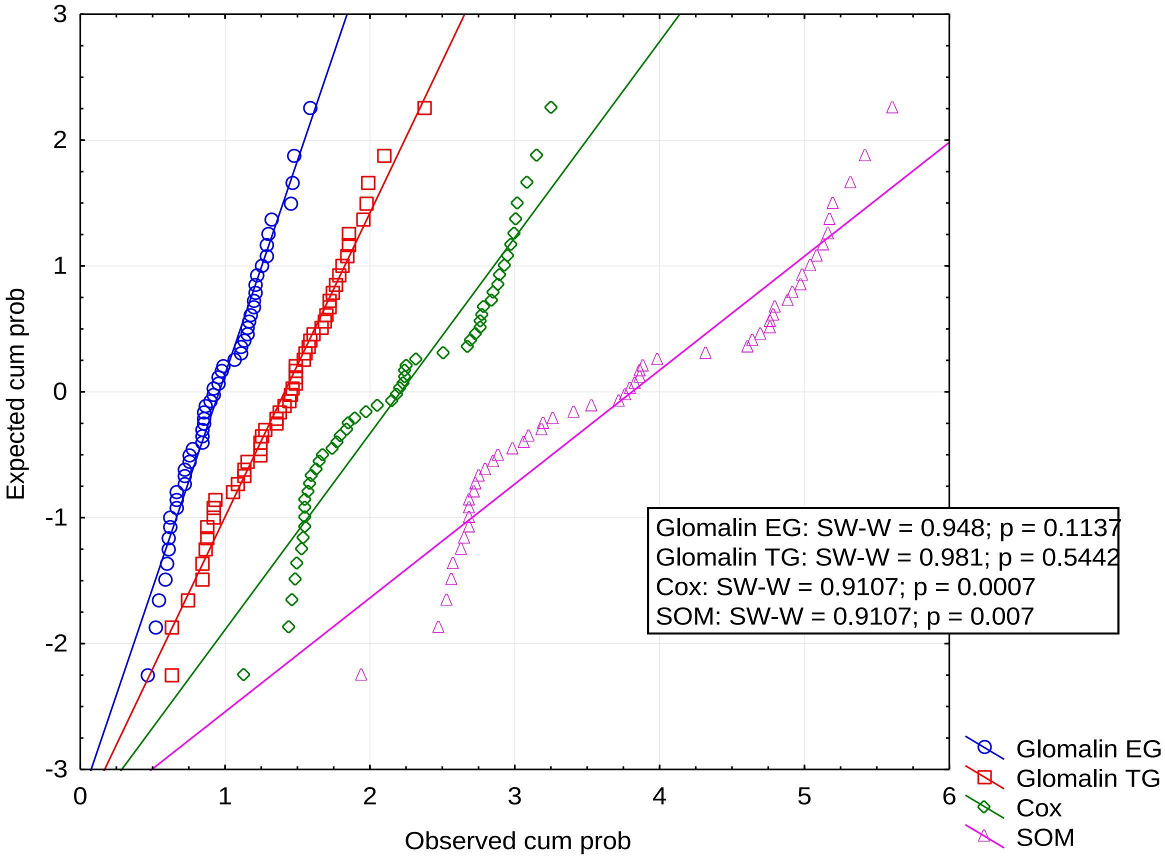
Annex c-9~~7~~ Descriptive statistics for the stability of soil aggregates.

Parameter	N valid	Average	Median	Min	Max	SD
WSA~~SAS~~ - control	54	44.041	44.955	20.94	69.63	10.52
WSA~~SAS~~ – WA1	54	40.891	38.325	16.82	60.89	9.10
WSA~~SAS~~ – WSA~~WA2~~	54	19.984	11.510	1.05	79.1	19.42
WSA~~SAS~~ – WA3	54	11.735	11.540	2.16	34.4	7.21
WSA~~SAS~~ – WA4	54	34.552	35.080	14.13	66.99	10.99

Annex c-10~~5~~ Descriptive statistics for basic soil parameters.

Parameter	N valid	Average	Median	Min	Max	SD
Glomalin EG	54	0.959	0.92	0.47	1.59	0.28
Glomalin TG	54	1.412	1.46	0.63	2.38	0.41

Cox	54	2.212	2.21	1,13	3.26	0.61
pH	54	6.05	6.2	3.8	6.9	0.64
Na	54	273.241	256.000	168	397	59.65
P	54	114.370	112.500	37	209	45.69
Ca	54	2,475.519	2,094.500	1,259	4,743	899.46
K	54	255.889	244.000	123	410	71.17
Mg	54	158.315	149.500	60	261	48.13



Annex c-116 Normal P-P plot of Glomalin, Cox and SOM content in the soil samples

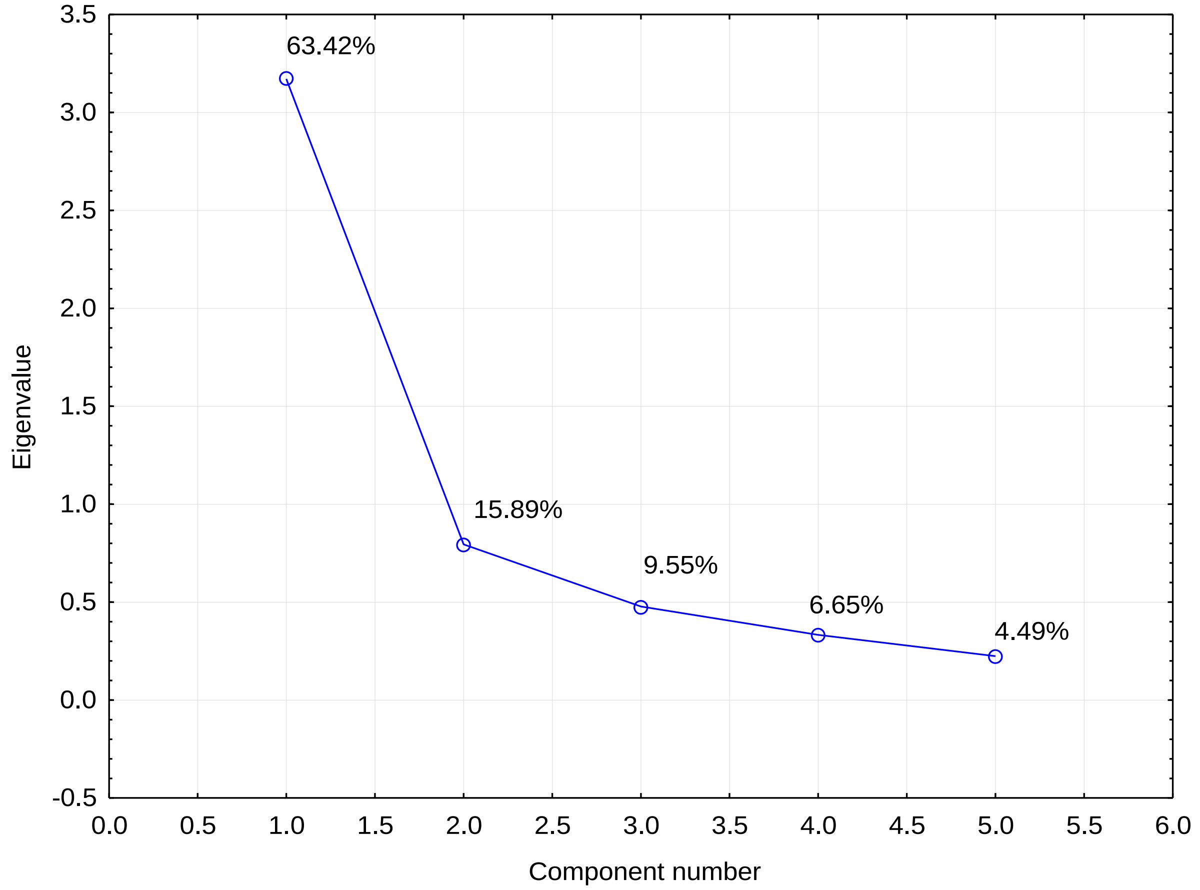
Annex D Basic soil parameters

Annex d-12 Contents of sodium and soil nutrients available to plants.

Sampling point	Na		Ca		P		Mg		K	
	mg/kg ± SE	HSD	mg/kg ± SE	HSD	mg/kg ± SE	HSD	mg/kg ± SE	HSD	mg/kg ± SE	HSD
A	235 ± 3.2	a	2,029 ± 11.2	a.d	135 ± 5.5	b	127 ± 2.7	b	209 ± 4.8	b
B	253 ± 4.9	a	1,765 ± 39.6	a.b	174 ± 9.1	d	144 ± 14.5	b	305 ± 6.2	c
C	241 ± 9.3	a	1,411 ± 50.7	a	110 ± 21.9	c	171 ± 4.4	c	173 ± 7.0	a.b
D	230 ± 12.9	a	2,103 ± 83.6	b.d	137 ± 6.5	b	140 ± 4.0	b	340 ± 6.8	c
E	268 ± 4.8	a	3,366 ± 77.3	e	92 ± 4.2	a	150 ± 9.1	b	243 ± 17.0	b
F	283 ± 21.1	a	2,526 ± 118	d	68 ± 7.7	a	154 ± 6.7	b	227 ± 6.8	b
G	356 ± 10.2	b	3,240 ± 267	e	69 ± 10.2	a	220 ± 3.5	d	279 ± 15.3	c
H	369 ± 5.2	b	4,049 ± 225	f	156 ± 18.2	c.d	238 ± 6.9	d	355 ± 20.4	c
I	223 ± 26.3	a	1,792 ± 125	a.b	90 ± 14.6	a	82 ± 8.3	a	172 ± 20.6	a.b

Note to Table d-12: different small letters indicate significant differences ($P<0.05$).

Annex ~~D~~E PCA analysis



Annex e~~d~~-13~~8~~ PCA scree plot – graph of own numbers (variances) of all factors.

Note to Figure e-13: The diagram serves to determine the number of significant main components. If the own number value is greater than 1, the given component explains more dispersion of total dispersion than one original variable. The first two components (1.0 and 2.0) explain nearly 80 % of the total dispersion of original data.

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