Spectroscopic Properties of Humic Substances in Permanent Grassland Soil

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Abstract

UV-VIS and infrared spectroscopy (FTIR) were used for humic substances quality evaluation. Both methods are widely applied because of their availability, sensitivity, rapidity, and affordability. The calculated spectral indexes assess the stability, hydrophobicity, and content of different functional groups in humic substances' molecules. Humic substances and humic acids were isolated from Gleyic Fluvisol (locality Jaroměřice, Czech Republic). The soil is under permanent grassland, with intensive management (4 cuts/year) and fertilisation with high-quality farmyard manure (FYM – the cattle loading 2 DJ. ha^{-1}). Soil samples were taken from a depth of 0–0.15 m from control and FYM variants during 2023–2024. Results were statistically evaluated (EDA, ANOVA /Tuckey's test/). UV-VIS spectra indicated a higher total humic substances content after amending soil with FYM. FTIR spectra showed more aliphatic and hydrophilic components after FYM amendments. Linear correlation was found between humic acid content and organic carbon content.

Keywords: *humic substances, grassland, farmyard manure, UV-VIS and FTIR spectroscopy*

1 Introduction

Soil organic matter (SOM) is very heterogeneous and is defined as the mixture of organic matter remaining after the decomposition of biomass (advanced stage), which includes a variety of organic compounds (Kim *et al.*, 2023). The original material and its transformation process directly influence the chemical composition, structure and properties of SOM (Uska-Jaruga *et al.*, 2023). It represents the main carbon reservoir in the biosphere (Park and Kim, 2015). Several methods can be used to assess the quality of humic substances. These are based on different analytical principles and analytical methods (e.g. micromorphology, spectroscopy, and others). Nowadays, more advanced and non-destructive spectral methods such as UV-VIS and FTIR spectroscopy are widely applied (Michalska *et al.*, 2023). Praus *et al.* (2015) stated that both methods are used for the analysis of inorganic and organic soil components.

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Humic substances (HS) are characterized by their high absorbance in the ultraviolet and visible spectral range and the absorbance ratio A465 nm/ A665 nm (E4/E6, $Q_{4/6}$) can them well characterized. The $Q_{4/6}$ value decreases with increasing molecular weight and humification degree of humic substances. (Fasurová and Pospíšilová, 2011; Feszterová *et al.*, 2024).

FTIR spectroscopy indicates the structural composition and various functional groups content in the HS molecule. The advantage of this method is rapidity and sensitivity, and samples can be reused or archived. Calculated indexes from infrared spectral region can help to assess hydrophobicity, stability and degradability of humic substances (Demyan *et al.*, 2012; Thabit *et al.*, 2024). It is also possible to assess humic substances reactivity and content of proteins or carbohydrate (Novák *et al.*, 2017).

This research aims to give the spectral characterization (UV-VIS and FTIR) of HS in grassland soils after amending soil with farmyard manure. Besides spectral parameters and indexes, the content of humic substances, humic and fulvic acids, and the HA/FA ratio were determined. The obtained results were statistically evaluated by Statistica (EDA, ANOVA /Tuckey's test/).

2 Material and Methods

The studied area is in the Boskovice Furrow unit and the sub-unit Mala Haná, district Jevíčsko – Lowlands. It is formed from the Permo-Carbonate deposits (Bína and Demek, 2012). The long-term experiment is situated near Jaroměřice (Svitavy district) – see Figure 1. The precise small plot was 1.25 m × 8 m, i.e. 10 m^2 of harvesting area with 0.25 m (width) path. Each variant is always in four replications. The experiment was established in 2004, at an altitude of 342 m above sea level (location: 49.6282881N, 16.7317036E). The soil was classified as Gleyic Fluvisol (see Figure 1) according to Němeček *et al.* (2011). Formed from non-calcareous

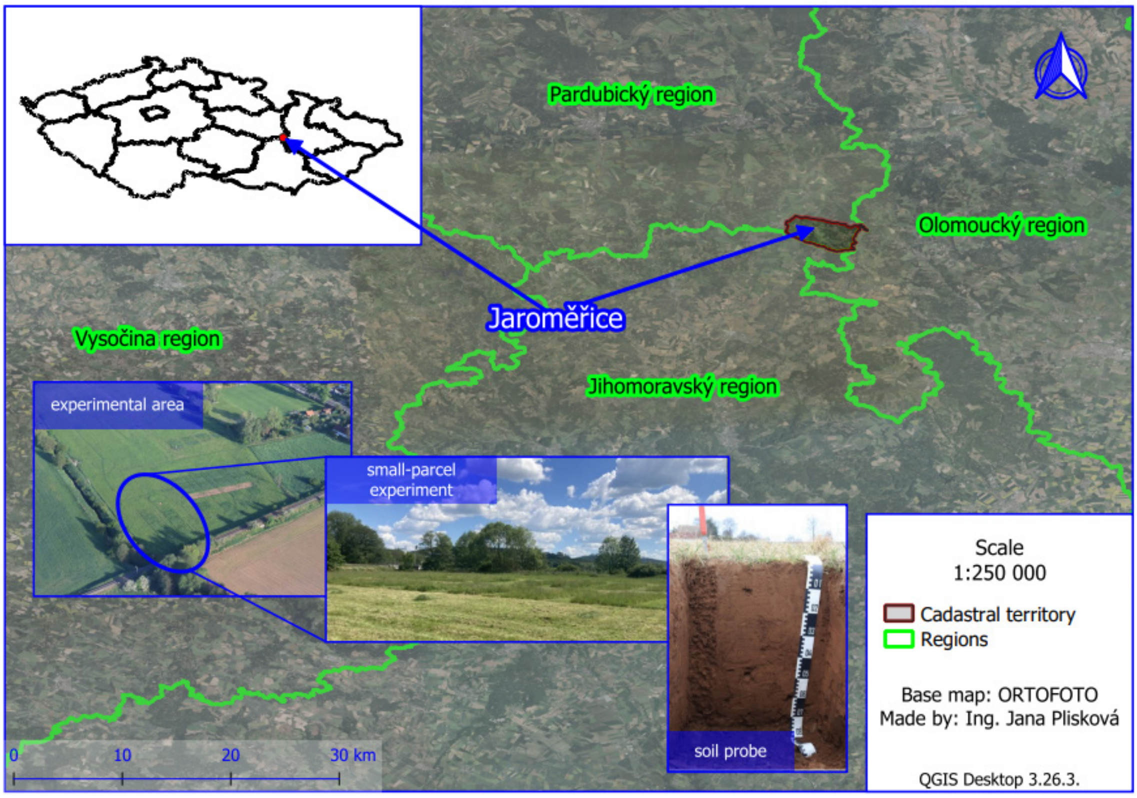

Fig. 1: *Studied area – locality Jaroměřice (Czech Republic)*

alluvial deposits. The basic soil properties were as follows: exchangeable soil reaction / pH/KCl / was 6.46, carbon content /Cox/ was 1.93%, and available nutrient content was: P – 37 $mg.kg^{-1}$, K – 68 $mg.kg^{-1}$, Mg – 130 $mg.kg^{-1}$). The botanical composition of grassland was mainly oatgrass (*Arrhenatherum elatius*). There was intensive management (4 cuts per year). Fertilizing followed the cattle loading (1 DJ = 60 kg $N.ha^{-1}$): intensive (4 mowings/year) = 2.0 $DJ.ha^{-1}$. The experimental variants were: /1/ control without fertiliser /Control/, /2/ farmyard manure + slurry /FYM/. The modelled experiment was designed according to the methodology of HBLFA Raumberg-Gumpenstein and VÚRV, v.v.i. (Menšík *et al.*, 2019).

Soil samples were collected from a depth of 0–0.15 m with a probe. An average sample was composed of 20–30 individual punctures. Humic substances (humic acids, fulvic acids) were extracted by a mixture solution of 0.1 M NaOH and 0.1 M Na4P2O7 (1:1, w/w). The quality of the humic substances was evaluated using the HA/FA ratio. In addition, UV-VIS and FTIR spectroscopy was applied for HS quality evaluation. UV-VIS spectrometer Varian Cary 50 probe (Varian Mulgrave, Victoria, Australia), with an optical fibre (within the range 300–700 nm) was used. A Thermo Nicolet Avatar 320 FTIR spectrometer (Nicolet, Madison, WI, USA), equipped with a Smart Diffuse Reflectance accessory (a range of 4000–500 cm^{-1}) was used. The colour index /$Q_{4/6}$/ ($A4_{46}/A_{665}$), humification degree and content of aromatic and aliphatic groups were determined according to Kumada (1987), Ellerbrock *et al.* (2005), Margenot et al. (2015), and Demyan *et al.* (2012). Colour index /$Q_{4/6}$/ was calculated as a absorbance ratio A_{446}/A_{665} nm. The results were statistically evaluated (ANOVA /Tuckey's test/).

3 Results

Humic substances (HS) fractional composition showed that amending soil with FYM led to an increase in HA content. Average content of HS represents 0.79%, HA 0.45%, and FA 0.34% after FYM amendments. On the control site, the average HS content represents 0.60%, HA 0.33%, and FA 0.23%. Differences were statistically significant. Linear correlation between HA and SOC content was determined (see Figure 2). HA/FA ratio was higher than one. A positive effect of FYM amendment was documented by higher absorbance in the UV-VIS spectral range – see Figure 3. Calculated colour index /$Q_{4/6}$/ was less than 6 after amending soil with FYM and more than 6 on the control site. Humification degree was medium 20–25%. DRIFT spectroscopy identifies the increase of aliphatic hydrophilic groups after amending soil with FYM. This indicates a higher amount of humic substances – see Figure 4. FYM variant consistently demonstrated this trend throughout the entire study period 2019–2021. It was concluded that labile and degradable carbon forms were more prevalent in the FYM variant, which is increasing the available nutrients content and generally improving microbial conditions in the soil.

4 Discussion

The status of humic substances, and consequently soil quality, might be affected by amending soil with exogenous organic materials This enhance the microbial activity of soil biota and improve the plant growth condition. Similarly, Deru *et al.* (2023), He *et al.* (2018), and Qi et al. (2023) stated that amending grassland soil with exogenous organic and mineral materials positively affects grasslands. Mainly organic fertilizers (farmyard manure and slurry) resulted in the accumulation of HS. This was also confirmed by our study. Kidd *et al.* (2017) and Rambaut *et al.* (2022) demonstrated, that not only is nutrient status improved but also more favourable soil pH is achieved. High quality grasslands are important in the ecosystems because they fulfil important ecosystem services such as feed production, impact on water and nutrient cycles, or they can be used in a future as arable.

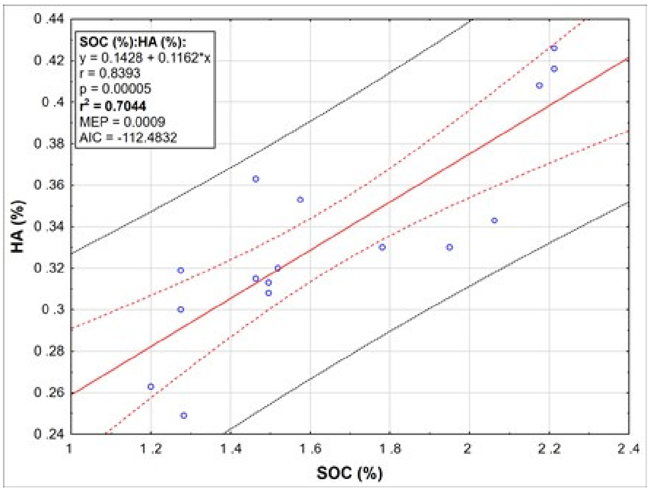

Fig. 4: *Linear correlation between humic acids (HA) and organic carbon content (SOC)*

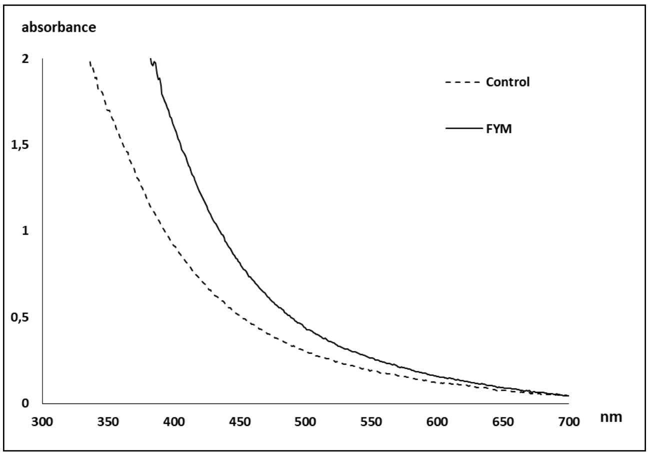

Fig. 3: *UV-VIS absorbance of humic substances in studied variants*

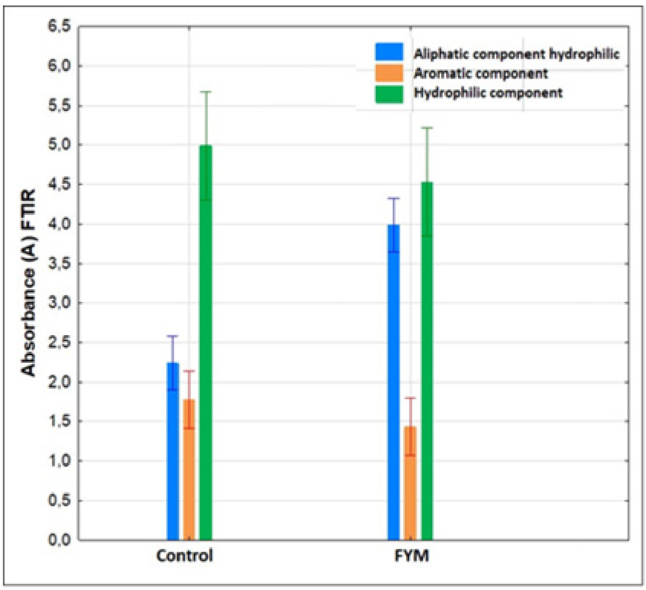

Fig. 2: *Composition of humic substances according to FTIR measurements*

5 Summary

Permanent grasslands are becoming highly topic today because they are used for feed production, as a high-quality substrate for biogas stations, or they represent the future arable soils. Attention is therefore being paid to the soil quality. Grasslands in the various types of fertilization and mowing management are widely studied. The paper aims to assess the results of a field experiment at the locality Jaroměřice (Malá Haná region, Czech Republic). The soil is under intensive management (4 cuts/year) and farmyard manure and slurry (FYM) were applied. The quantity and quality of humic substances were evaluated using a multicriteria ANOVA test. It was confirmed that the application of farmyard manure (FYM) significantly improved SOM content and quality. A linear correlation humic acids and organic carbon content was found. The UV-VIS spectra showed increasing in humic substances absorbance after amending soil with FYM. A higher proportion of aliphatic hydrophilic groups in the manure variant compared to the control was documented by FTIR spectroscopy..

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