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Assessment of the degree of soil biologization based on multispectral spectrum

***Abstract**. Spectroscopy in soil research is of particular interest because it addresses many of the soil's chemical properties and can become one of the proximity measurement methods, complementing conventional methods by which a field-scale picture of the spatial variability of soil fertility cannot be obtained with adequate resolution. The aim of the research is to qualitatively parameterize soil biologization (microbial life) in a way that makes it possible to realize the identification in question in real time. It was noted that within the mean values of solar radiation, observable variation was identified in the wavelength range from 500 nm to 650 nm, between soils in which the optical density of microorganisms was extremely different, namely 0.5 McF and 1.5 McF. There was also variation within the soil sample space, which, however, did not exceed 25%.*

***Streszczenie.** Spektroskopia w badaniach gleby jest przedmiotem szczególnego zainteresowania, ponieważ odnosi się do wielu jej właściwości chemicznych i może stać się jedną ze zbliżeniowych metod pomiaru, uzupełniając metody konwencjonalne, za pomocą których nie można w skali pola uzyskać obrazu przestrzennej zmienności żyzności gleby z odpowiednią rozdzielczością. Celem badań jest parametryzacja jakościowa biologizacji gleby (życia mikrobiologicznego) w sposób umożliwiający realizację przedmiotowej identyfikacji w czasie rzeczywistym. Odnotowano, że w obrębie wartości średnich promieniowania słonecznego obserwowalne zróżnicowanie zidentyfikowano w zakresie długości fali od 500 nm do 650 nm, miedzy glebą w której gęstość optyczna mikroorganizmów była skrajnie różna, czyli 0,5 McF i 1,5 McF. Odnotowano również zróżnicowanie w obrębie przestrzeni próbki gleby, które jednak nie przekraczało 25%. (**Ocena stopnia biologizacji gleby na podstawie widma multispektralnego**)*

Słowa kluczowe: spektrofotometr, gleba, mikroorganizmy, fala elektromagnetyczna
Keywords: spectrophotometer, soil, microorganisms, electromagnetic wave

Introduction

Diffuse Reflectance Spectroscopy (DRS) is fast, non-destructive and does not require soil sample preparation and does not consume environmentally hazardous chemicals, making it superior to also very promising electrochemical methods [1]. In the case of electrical and electromagnetic sensors, optical measurements are also influenced by a combination of soil property parameters. However, the influence of different properties on the result varies from one spectral band to another, making it possible to isolate some influences in measurements with a single sensor. Spectroscopy in soil research is of particular interest because it addresses many of the soil's chemical properties and can become one of the proximity measurement methods, complementing conventional methods, by which a field-scale picture of the spatial variability of soil fertility cannot be obtained with adequate resolution. Spatial variability of such fertility-related soil properties as organic matter, phosphorus, potassium, pH. In most cases, it is very expensive to apply laboratory methods to the number of samples that would guarantee a snapshot of spatial variability [2]. Since the 1990s, a whole range of optical sensors have been developed for proximity measurements, using one wavelength range or more, with different purposes [3,4]. The literature on the issues of using vis-NIR and mid-NIR spectroscopy for soil analysis is abundant and these techniques are increasingly being used [5,6,7]. Optical sensors use the visible light (vis: 400-700 nm), near-infrared (NIR: 700-2500 nm) and/or mid-infrared (mid-IR: 2500-25000 nm) bands for measurements [8]. Both vis-NIR and mid-IR are techniques that are highly sensitive to organic and inorganic soil constituents and are therefore potentially useful tools for assessing and monitoring soil and its quality and function. Basically, the NIR band absorption is shown by C-H, H-O-H, O-H, N-H and C-O functional groups, so these spectra contain important information on soil components such as organic matter, soil moisture, carbonates and clay minerals, nitrogen, potassium and magnesium. The mid-IR band contains much more information about soil mineral and organic components than the vis-NIR band, and mid-IR soil spectroscopy is particularly well suited for analyzing organic matter and mineral content, because the associated absorption bands are easily identifiable. In the vis-NIR technique, the absorption bands are broad and tend to overlap and, compared to mid-IR, are more difficult to interpret [9,10]. The cited studies show that the benefits of higher accuracy obtained in the mid-IR band than in vis-NIR do not offset the higher costs incurred with mid-IR. Optical methods have been used to determine many soil properties. Sternberg et al. [11] state that methods for measuring plant nutrient elements N, P, K, Fe, Ca, Na, Mg, are of great interest in agriculture. In precision agriculture technology for variable fertilization, data on the available form of phosphorus and potassium are needed to obtain high-resolution maps. Much attention is paid to estimating organic matter and organic carbon content using vis-NIR and mid-NIR [12]. Sternberg et al. [11] cite dozens of studies in this area, stating that of the studies done on the use of vis-NIR to predict soil properties, most related to the determination of organic carbon and clay content. Soil reflected and scattered light spectra are largely non-specific due to interference from overlapping spectra of soil components, which are themselves variable and interrelated. The lack of selectivity can be influenced by the operation of the instrument itself (noise, measurement errors). All these factors add up to a complex absorption pattern that must be mathematically emerged from the spectrum to correlate with soil properties. Spectral analysis of dispersive spectroscopy requires chemometric techniques and multivariate calibrations. Advanced statistical and mathematical methods, such as Principal Components Regression (PCR) analysis, Partial Least Squares Regression (PLSR), artificial neural networks (for estimating organic matter, phosphorus and potassium content from vis-NIR spectra), decision trees also from vis-NIR spectra and other. The huge diversity of soils can cause differences in the resulting spectra and also results in reduced prediction accuracy. Thomasson et al. [13] report that in the fields they studied, correlations of the reflected spectrum with soil properties varied, as well as wavelengths for the best-fit models differed between fields. Therefore, large spectral libraries can be helpful in revealing the hidden diversity between different classes of soils and enable wider application of the spectroscopic technique. Despite the many works, very few address the identification of the degree of soil biologization, which is becoming the basis of modern conservation-oriented agriculture including the soil environment.

Purpose, scope and methodology of the study

The aim of the study was to evaluate the light spectrum variation of soils with a diverse microbial flora. The scope of the study concerned spectral characteristics in the wavelength range from 190 nm to 1100 nm. The relative humidity of the soil samples was about 9%. The study included two types of microorganisms that are most commonly found in soil, namely fungi and vegetative bacteria. The research was carried out on production soil, which was properly prepared for this purpose by creating samples that were homogeneous in terms of a particular microorganism. Preparation of homogeneous samples required complete sterilization of the soil, which eliminated its biological life completely. This was followed by multiplying strains of specific organisms and applying a suspension of a specific optical density to the soil, thus differentiating the degree of biologization of the analyzed soil samples. Three concentrations of bacteria were used for the study, i.e. 0.5 McF, 1.0 McF, 1.5 McF, the level of which corresponds to the most common level of microbial life in the soil. Soil samples that were devoid of 0 McF microbial life were used as a reference sample. Figure 1 shows a view of the soil samples prepared for spectral testing.

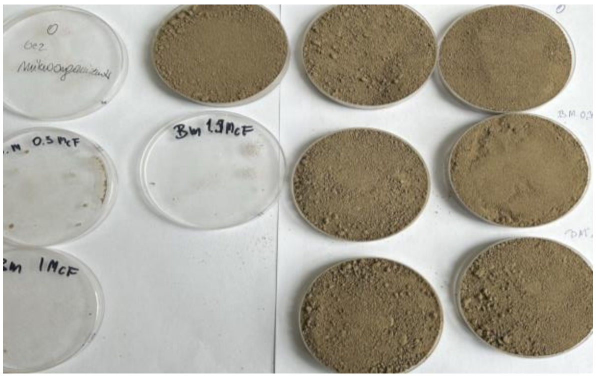

Fig. 1. Soil samples prepared for spectral studies

Spectral characterization was performed with an Exemplar spectrometer with a wavelength range of 190 nm to 1100 nm and a resolution of less than 0.1 nm to 10.0 nm. BWSpec software, whose dialog box is shown in Figure 2, was used to analyze the measurement results. The tests were carried out under actual daylight illumination of 35,000 lux. The spectroscope was calibrated before measurements.

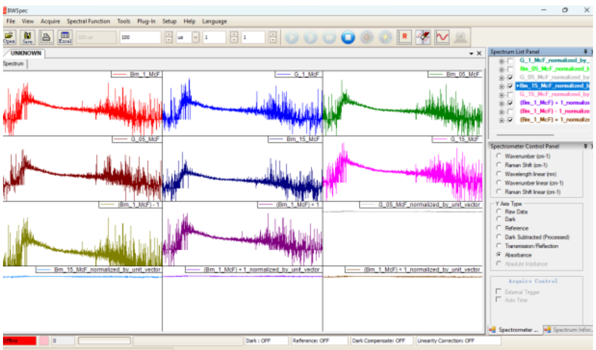

Fig. 2. View of the BWSpec spectral data analysis program window

Results

Figure 3 shows the spectral characteristics of the soil sample in which there was no biological life, constituting the reference sample. It was noted that in the case of the sterilized soil sample, the highest intensity of electromagnetic radiation was characterized by the wavelength range from about 500 nm to 540 nm with the exception of the wavelength of about 520 nm, where a temporary decrease in the intensity of electromagnetic radiation was noted.

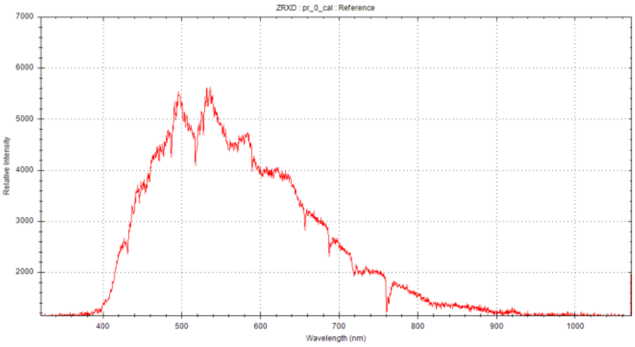

Fig. 3. Spectral characteristics of soil with zero microbial content

It was found that for the control sample, the identifiable electromagnetic radiation was in the wavelength range from 400 nm to 900 nm. Figure 4 shows the spectral characteristics of soil samples with varying numbers of Bacillus mycoides bacteria. The curve in blue characterizes soil samples where a bacterial suspension with an optical density of 0.5 McF was used. The red color indicates the spectral characteristics of soil where a suspension with an optical density of 1 McF was used. In contrast, the spectral characteristics of the soil sample, where a bacterial suspension with an optical density of 1.5 McF was used, are marked in green. It was observed that the shape of the spectral characteristics was very similar, while the intensity of the radiation allowed qualitative identification of the degree of saturation of the soil with bacteria. This observation is particularly true for the differences in the intensity of electromagnetic radiation between the soil samples where a suspension with an optical density of 0.5 McF was used and the other soil samples where suspensions with an optical density of 1 McF and 1.5 McF were used. It should be noted that the spectral characteristics of the soil samples where bacterial suspensions were used (regardless of their unit content in the soil) were clearly different from the spectral characteristics of the sterilized soil samples.

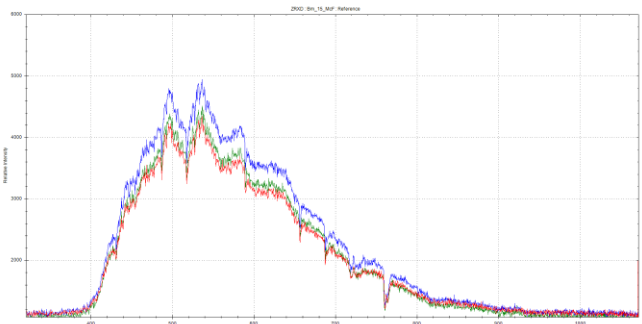

Fig. 4. Spectral characteristics of the soil, where a bacterial suspension with optical density was used: 0.5 McF (blue color), 1.0 McF (red color), 1.5 McF (green color)

Figure 5 shows the spectral characteristics of soil samples where fungal (Aspergillus flavus) microbial suspensions were used with optical densities of 0.5 McF (blue color), 1.0 McF (red color), 1.5 McF (green color).

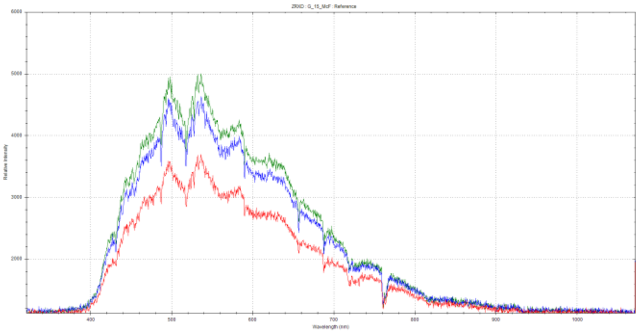

Fig. 5. Spectral characteristics of the soil, where a suspension of microorganisms in the form of fungi with optical density was used: 0.5 McF (blue color), 1.0 McF (red color), 1.5 McF (green color)

As in the case of soil samples with bacteria, differences in radiation intensity were also observed here. However, it should be noted in the case of soil where a suspension with Aspergillus flavus was used, the greatest variation was observed between soil samples where a suspension with an optical density of 1 McF and 1.5 McF was used. On the other hand, analyzing the spectral characteristics of soil samples where a suspension with an optical density of 0.5 McF was used, it was found that the intensity of electromagnetic radiation differed significantly from that recorded for the spectral characteristics of soil samples where a suspension with an optical density of 1 McF was used. Much less variation was found in the case of the spectral characteristics of the soil samples, where a suspension with an optical density of 1.5 McF was used. Comparing the spectral characteristics of soil samples where a suspension with the same optical density of 0.5 McF but different microorganisms was used, a significant similarity was observed (Fig. 6). There was minimal variation in the intensity of electromagnetic radiation, which was higher for soil where a suspension with bacteria was used.

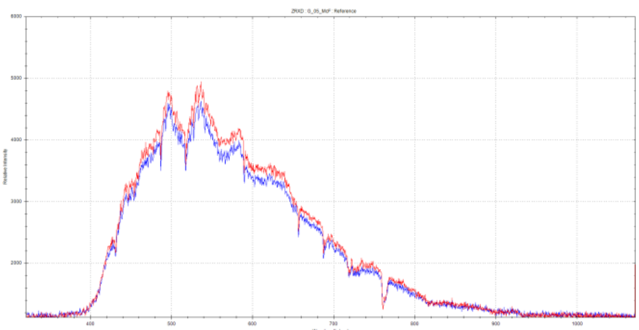

Fig. 6. Spectral characteristics of soil where a suspension with an optical density of 0.5 McF was used with microorganisms in the form of bacteria (red) fungi (blue)

Figure 7 shows the spectral characteristics where a suspension with an optical density of 1.0 McF was used, but with different microorganisms. It was noted that the intensity of electromagnetic radiation of soil samples where the suspension was bacteria was significantly higher compared to that intensity recorded for soil samples where the suspension was fungi. The highest variation was found for wavelengths of about 500 nm and 540 nm.

Comparing the spectral characteristics of soil samples where a suspension with the same optical density of 1.5 McF was used, but with a different composition of microorganisms, it was found that the spectral characteristics are similar to each other, but the intensity of electromagnetic radiation is different (Fig. 8).

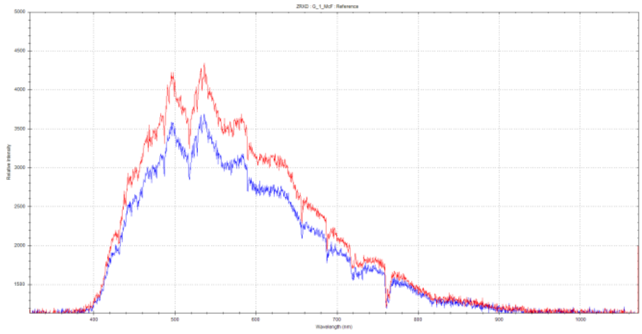

Fig. 7. Spectral characteristics of soil where a suspension with an optical density of 1.0 McF was used with microorganisms in the form of bacteria (red) fungi (blue)

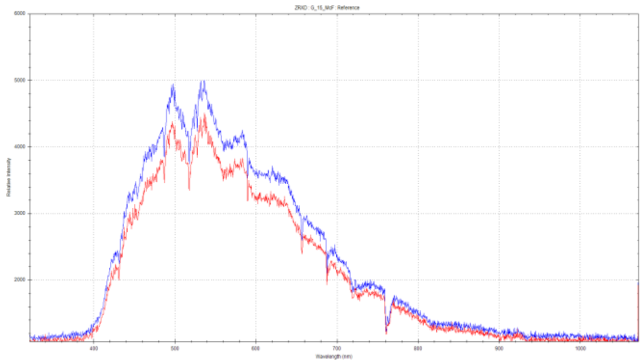

Fig. 8. Spectral characteristics of soil where a suspension with an optical density of 1.5 McF of microorganisms in the form of bacteria (red) fungi (blue) was used

In the case analyzed, soil samples where a suspension of microorganisms in the form of fungi was used had a higher intensity of electromagnetic radiation, while soil samples where a suspension with bacteria was used had a slightly lower intensity of electromagnetic radiation. Figure 9 shows all the spectral characteristics of the analyzed soil samples.

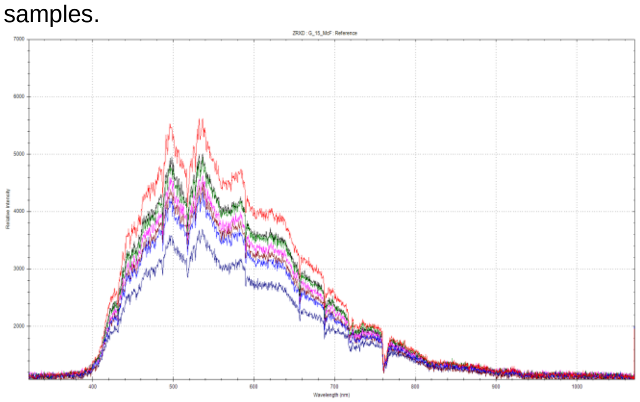

Fig. 9. Spectral characteristics of the soils involved in the experiment

It should be noted the high range of variation in the intensity of electromagnetic radiation, which can be an identifying marker of the degree of soil biologization. However, quite sublime differences in spectral characteristics at the current stage, further studies are required.

Conclusion

It was noted that it is possible to identify soil variation in terms of microorganism content using spectral methods under field conditions. It was found that the spectral characteristics for individual soil samples and the soil sample without microorganisms (the reference sample) had a similar pattern within the analyzed electromagnetic wavelengths. It was observed that it was possible to distinguish the type of microorganisms contained in the soil, provided that the appropriate amount of microorganisms is present, and in the case of the experiment analyzed, the optical density of the suspension should exceed 1 McF.

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REFERENCES

[1] Kim H.J., Sudduth K. a., Hummel J. W. 2009. Soil macronutrient sensing for precision agriculture. J. Environ. Monit. 11, 1810-1824.
[2] Schirman M., Gebbers R., Kramer E. 2013. Performance of automated near-infrared reflectance spec-trometry for continuous in situ mapping soil fertility at field scale. abstract, copyright Soil Science Society of America
[3] Adamchuk V.I., Viscarra Rossel R.A., Sudduth K.A., Schulze Lammers P. 2011. Sensor fusion for precision agriculture. W: Sensor Fusion - Foundation and Applications, Ed. Ciza Thomas, 28 – 40. http://www.intechopen.com/books/sensor-fusion-foundation-and-applications. Dostęp 19.07.2023.
[4] Korzeniewska E., Szczesny A., Lipinski P., Dróżdż T., Kiełbasa P., Miernik A., Politowski K. Textronics Interdigitate Electrodes for Staphylococcus Aureus bacteria detecting. 2021 *J. Phys.: Conf. Ser.* **1782** 012015
[5] Christy C., D. 2008. Real-time measurement of soil attributes using on-the-go near infrared reflectance spectroscopy. Computers and Electronics in Agriculture 61, 10-19.
[6] Reeves J.B., McCarty G.W., Hively W.D. 2010. Mid- versus near infrared spectroscopy for on-site analysis of soil. W: Proximal soil sensing, Springer Verlag, NY, 133-142.
[7] Viscarra Rossel R.A., Cattle S., Ortega A., Fouad Y. 2009. Using a digital camera to measure soil organic carbon and iron contents. Biosyst. Eng. 100, 149-159.
[8] Adamchuk V.I., Viscarra Rossel R.A., Sudduth K.A., Schulze Lammers P. 2011b. Sensor fusion for precision agriculture. W: Sensor Fusion - Foundation and Applications, Ed. Ciza Thomas, 28 – 40. http://www.intechopen.com/books/sensor-fusion-foundation-and-applications Dostęp 19.07.2016.
[9] Viscara Rossel R.A., McBratney A.B. 2008a. Diffuse reflectance spectroscopy as a tool for digital soil mapping. W: Hartemink i in. (Ed.) Digital Soil Mapping with Limited Data, 165- 172.
[10] Dhawale N.M., Adamchuk V.I., Prasher O., Viscarra Rossel R.A. 2015. Proximal soil sensing of soil texture and organic matter with a prototype portable mid-infrared spectrometer. European Journal of Soil Science 66, 661-669.
[11] Stenberg B., Viscarra Rossel R.A., Mouazen A.M., Wetterlind J. 2010. Visible and near infrared spectroscopy in soil science. W: Donald L. Sparks (Ed.); Advances in Agronomy 107, 163-215.
[12] Janik L.J., Skjemstad O., Shepherd K.D, Spouncer L.R. 2007. The prediction of soil carbon fractions using mid-infrared partial least square analysis. Aust. J. Soil Res. 45, 73-81.
[13] Thomasson J.A., Sui R., Cox M.S., Al-Rajehy A. 2001. Soil reflectance sensing for determining soil properties in precision agriculture. Transactions of the ASAE 44, 6, 1445-1453.