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Saud HAMIDOVIĆ[1], Amra SOFTIĆ[1],
Fahrudin TOPČIĆ[1], Mirza TVICA[1],
Blažo LALEVIĆ*[2], Monika STOJANOVA[3]

IMPACT OF SOIL MANAGEMENT PRACTICE ON THE ABUNDANCE OF MICROBIAL POPULATIONS

SUMMARY

Microorganisms in the soil have a very important role because they participate in numerous processes. Intensive and/or inadequate use of the soil leads to disturbance of the plant - microbial interactions, a decline in productivity, and degradation. The abundance and microbiological activity of a certain ecosystem are considered indicators of soil fertility. In this paper, surface (0-20 cm) and subsurface (20-40 cm) samples of grassland, agricultural soil, forest soil and coal-mine-affected soil at the Banovići municipality (Tuzla Canton, Bosnia and Herzegovina) taken in October 2021 and April 2022 were used for chemical and microbiological characterization. Chemical analyses were performed using the standard methodology, while the microbial count was determined using the agar plate method. Enzyme production was expressed through dehydrogenase activity. The lowest pH value was recorded in forest soil, while the highest in the grassland. In all samples, microbial abundance decreased with increasing soil depth. The lowest microbial activity was observed in coal mine-affected soil. The highest value of the total number of bacteria and ammonifiers was recorded in forest soil. Oligonitrophiles were most abundant in agricultural soil, while the number of actinomycetes was highest in grassland. Dehydrogenase activity was highest in forest and agricultural soil. In most of samples, microbial abundance was higher in spring, while dehydrogenase activity was higher in autumn. This research confirms the impact of land use on microbial abundance as parameter of soil quality.

Keywords: soil, chemical characterization, microbial abundance, soil dehydrogenase activity

[1]Saud Hamidović, Amra Softić, Fahrudin Topčić, Mirza Tvica, University of Sarajevo, Faculty of agriculture and food science, Zmaja od Bosne 8, Sarajevo, BOSNIA AND HERZEGOVINA;
[2]Blažo Lalević* (corresponding author email: blazol@agrif.bg.ac.rs), University of Belgrade, Faculty of Agriculture, Nemanjina 6, Belgrade-Zemun, SERBIA;
[3]Monika Stojanova, Open Science, Association for scientific research, educational and cultural activities, ul. Jane Sandanski 61, 6000 Ohrid, NORTH MACEDONIA
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INTRODUCTION

Soil is a three-phase system that provides the location for the life of many organisms (Nielsen *et al.* 2015) and supports their development through an accumulation of essential nutrients (Brevik and Sauer 2015). For more than 12000 years, the human population has managed soils through agricultural practice (Zeder 2011), which, due to the dramatic increase of Earth's population, became intensive over several last decades. This intensification led to an increase in fertilizer application in plant production; however, soil amendments rarely may replace the nutrient depletion during plant development, which contributes to agroecosystem degradation (Tan *et al*. 2005; Vitousek *et al*. 2009).

Vitousek *et al.* (2010) and Walker and Syers (1976) found low phosphorus availability in many soils, while Du *et al.* (2020) reported limitation in nitrogen content, which suggests the shifts in soil quality recommended for plant production (Silver *et al.* 2021). Thus, the reduction of land degradation and restoration of degraded soils are recommended to maintain the agroecosystem, increase plant productivity and provide food production (Bouma and Montanarella 2016; Keesstra *et al.* 2016). These postulates are integrated in the „2030 Agenda for Sustainable Development", which defines goals associated with soil management and long-term soil restoration (Keesstra *et al.* 2016).

According to Muñoz-Royas (2018), soil quality indicators can be divided into three groups: physical, chemical, and biological. The appropriate selection and application of soil indicators are very important for the realization of Sustainable Development goals and defining of ecosystem services (Costantini *et al.* 2016), such as nutrient cycling (Anaya-Romero *et al*. 2016; Pereira *et al.* 2018). Bender *et al.* (2016) and Wagg *et al*. (2014) described the role of microorganisms in nutrient cycling, ecosystem functions, and organic matter decomposition. Nannipieri *et al*. (2003) suggest that microbiological and enzymatic indicators are often used in the estimation of soil quality.

Although the microbial populations in soil are abundant and diverse, research studies are often focused on the impact of land use on bacterial and fungal count (Banerjee *et al.* 2019; Estendorfer *et al*. 2017; Tardy *et al*. 2015). An abundance of microbial populations has been frequently studied in grassland and forest soils (Lauber *et al*. 2013; Rasche *et al*. 2011; Stres *et al.* 2008), as well as in coal mine fields (Zhang *et al*. 2022). In addition, agricultural practices; fertilization, pesticide application, and irrigation affect the microbial diversity of soil and ecosystem functions (Ding *et al*. 2013). Furthermore, land use is one of the crucial parameters of the physical, chemical, and biological characteristics of soil (Fedele *et al*. 2018). Thus, the estimation of microbial indicators should be associated with the determination of the chemical properties of soil (He *et al.* 2021).

The objective of this paper was to determine the impact of land use shifts on the microbial count and some chemical parameters of soil.

MATERIAL AND METHODS

Samples of grassland (G), agricultural soil (A), forest soil (F), and coal-mine-affected soil (C) at the Banovići municipality (Tuzla Canton, Bosnia, and Herzegovina) were used for chemical and microbiological characterization. Soil sampling (0-20 and 20-40 cm) was performed in October 2021 and April 2022. Chemical analyses were performed at the start of experiment using the following methodology:

i. pH value in water and 1M KCl was measured according to ISO 10390:2005 standard;
ii. The humus content was determined using Mineev *et al.* (2001) method and;
iii. Available P and K content by Egner et al. (1960) method.

A microbial abundance of soil samples was determined using the agar plate method. The total number of bacteria (TNB) was determined using 0.1xTSA (tryptic soy agar), the number of ammonifiers (AM) was determined on nutrient agar (Torlak, Serbia), oligonitrophiles (ON) on Fyodorov`s agar, and actinomycetes (ACT) on starch ammonia agar. Incubation was carried out in an incubator (Binder, Germany). The incubation period for bacteria was five days at 30°C, while for actinomycetes 12 days at 30°C. The microbial number was expressed as CFU (colony forming units) per gram of dry soil (after drying in the oven at 105°C for 2 hours).

Estimation of dehydrogenase activity (DHA) was performed using Kasida *et al*. (1964) method and expressed as μg of triphenylformazan (TPF)/g/h.

The obtained results were statistically processed using the software package SPSS 20. To determine the statistical significant differences of the obtained values T-test ($p<0.05$) was performed.

RESULTS AND DISCUSSION

The results of this study showed variations in the chemical properties of samples, depending on management practices and soil depth (Table 1). In FS, the lowest pH value and available P content compared with other samples were noticed. On the other hand, the highest humus content was detected in the same sample. According to Wilpert (2022), forest soil has higher humus content than arable soils. Rozek *et al*. (2020) suggest that forest plots were characterized by lower pH values compared with other plots, probably due to organic matter degradation and the presence of root exudates (Schawe *et al*. 2007).

The highest pH value was detected in G. Schnoor *et al*. (2015) also found a high pH value in grassland, which is in agreement with our results. Organic matter content is lower in coal mine-affected soils compared with unmined soils (Guo *et al.* 2018); on the other hand, the same authors found a low potassium content in mined soils, which is opposite to our findings. However, Essandoh *et al.* (2021) found higher levels of K, Mg, and Na in mined sites.

Table 1. Chemical characterization of soil samples

sample	depth (cm)	pH H_2O	pH 1M KCl	humus (%)	P_2O_5 (mg/100g)	K_2O (mg/100g)
G	0-20	8.10	7.00	5.22	12.9	4.0
	20-40	7.60	6.90	3.75	7.5	2.3
A	0-20	7.95	6.01	3.85	27.4	12.0
	20-40	7.80	5.87	2.96	10.1	7.0
F	0-20	5.15	3.75	9.50	9.8	13.0
	20-40	4.90	3.59	5.70	3.2	6.0
C	0-20	7.96	6.20	1.66	22.6	19.0
	20-40	7.14	6.07	0.92	10.4	10.0

Legend: G – grassland, A – arable soil, F – forest soil, C - coal-mine-affected soil

Table 2. Bacterial abundance (x 105 CFU/g) in soil samples

S	D	ON 2021	ON 2022	TNB 2021	TNB 2022	AM 2021	AM 2022
G	0	4.52 ± 0.10^{aA}	5.41 ± 0.73^{aB}	6.20 ± 0.31^{aA}	6.41 ± 0.56^{aA}	5.40 ± 0.29^{aA}	5.59 ± 0.91^{aB}
	20	2.10 ± 0.22^{aA}	3.28 ± 1.32^{aB}	0.30 ± 0.29^{aA}	4.11 ± 2.20^{aB}	2.60 ± 0.33^{aA}	3.26 ± 0.57^{aB}
A	0	15.70 ± 3.61^{bA}	18.27 ± 1.87^{bB}	11.30 ± 3.35^{bA}	15.90 ± 1.73^{bB}	11.70 ± 2.45^{bA}	17.57 ± 3.25^{bB}
	20	7.60 ± 1.51^{bA}	9.40 ± 1.76^{bB}	5.50 ± 0.64^{bA}	6.03 ± 1.22^{bB}	5.70 ± 1.45^{bA}	9.10 ± 2.30^{bB}
F	0	8.30 ± 1.40^{cA}	13.30 ± 1.34^{cB}	23.50 ± 5.01^{cA}	35.57 ± 6.00^{cB}	23.70 ± 5.35^{cA}	35.40 ± 5.20^{cB}
	20	4.20 ± 0.43^{cA}	4.05 ± 1.37^{cA}	11.50 ± 2.01^{cA}	5.33 ± 0.66^{cB}	11.90 ± 1.91^{cA}	5.24 ± 1.21^{cB}
C	0	3.30 ± 0.55^{dA}	5.97 ± 1.61^{dB}	2.80 ± 0.79^{dA}	6.40 ± 0.36^{dB}	3.90 ± 0.71^{dA}	5.13 ± 1.15^{dB}
	20	1.80 ± 0.55^{dA}	1.83 ± 0.94^{dA}	1.30 ± 0.37^{dA}	3.30 ± 0.36^{dB}	1.70 ± 0.45^{dA}	2.13 ± 0.45^{dB}

Legend: S – sample, D – depth (cm), 0 – 0-20 cm, 20 – 20-40 cm, G – grassland, A – arable soil, F – forest soil, C - coal-mine-affected soil, ON – oligonitrophiles, TNB – total number of bacteria, AM – ammonifiers; a, b, c, d – values from the different sample at same depth for the same parameter and year marked with different letters are significantly ($p<0.05$) different, ANOVA post hoc Tuckey's test. A, B – values from the same sample at same depth for the same parameter and different year marked with different letters are significantly ($p<0.05$) different, T-test.

An abundance of microbes and enzyme activity showed variation depending on management practice, time and depth of sampling (Tables 2 and 3). In C, the significantly lowest value of bacterial number was registered in both years. Upadhyay *et al*. (2016) reported the poor microbial activity of coal mining sites in northern India. Ma *et al*. (2019) showed that coal mine-affected soil characteristics and processes have a strong impact on the soil microbial count. In both years, bacterial abundance were significantly lower in G compared with A and F (Table 2).

Although some studies suggest that low-intensity practices stimulate microbial diversity (Sünnemann *et al*. 2021) in grasslands compared to agricultural soils (Kamgan Nkuekam *et al*. 2018), Romdhane *et al*. (2022) reported the lowest microbial diversity in perennial grasslands. TNB and AM were significantly highest in the F sample, which may be associated with high humus and available potassium content in this sample. Galieva *et al*. (2018) found a high microbial abundance in organic matter-rich soils, which is corroborated by our results. In most of the samples, statistically higher bacterial abundance was observed in 2022. Mencel *et al.* (2022) mentioned that the highest microbial prevalence occured during vegetation period, which may be associated with the intensive root development and stimulation of microbial growth. In addition, Fierer *et al*. (2003) showed the decrease of microbial prevalence with increasing soil depth, which is confirmed in our study.

Table 3. Actinomycetal abundance and dehydrogenase activity in soil samples

S	D	ACT year 2021 (x 10^4 CFU/g)	ACT 2022	DHA 2021 (x10^5 µg TPF/g/h)	DHA 2022
G	0	19.0 ± 5.56^{aA}	26.2 ± 8.15^{aB}	1.76 ± 0.18^{aA}	1.28 ± 0.24^{aB}
	20	10.0 ± 4.35^{aA}	14.4 ± 5.78^{aB}	1.43 ± 0.22^{aA}	0.98 ± 0.16^{aB}
A	0	15.0 ± 5.95^{bA}	19.5 ± 1.92^{bB}	2.99 ± 0.52^{bA}	2.27 ± 0.36^{bB}
	20	7.0 ± 3.60^{bA}	10.5 ± 2.26^{bB}	1.84 ± 0.28^{bA}	1.06 ± 0.25^{bB}
F	0	10.0 ± 2.64^{cA}	14.5 ± 3.30^{cB}	2.66 ± 0.40^{cA}	1.22 ± 0.30^{cB}
	20	6.0 ± 1.73^{cA}	6.6 ± 1.84^{cA}	1.78 ± 0.20^{bA}	1.06 ± 0.25^{bB}
C	0	3.0 ± 0.57^{dA}	2.8 ± 0.70^{dA}	0.69 ± 0.19^{dA}	0.73 ± 0.16^{dB}
	20	1.2 ± 0.25^{dA}	1.1 ± 0.17^{dB}	0.33 ± 0.21^{cA}	0.34 ± 0.11^{cB}

Legend: S – sample, D – depth (cm), 0 – 0-20 cm, 20 – 20-40 cm, G – grassland, A – arable soil, F – forest soil, C - coal-mine-affected soil, ACT – actinomycetes, DHA – dehydrogenase activity; a, b, c, d – values from the different sample at same depth for the same parameter and year marked with different letters are significantly ($p<0.05$) different, ANOVA post hoc Tuckey's test. A, B – values from the same sample at same depth for the same parameter and different year marked with different letters are significantly ($p<0.05$) different, T-test.

The number of ACT was significantly highest in surface layer of G and A in both years (Table 3). In these samples, higher pH value was detected compared with other samples. Selianin *et al.* (2005) found the higher ACT presence in alkaline soils. The highest value of DHA was detected in A in both years. However, statistically significant decrease of DHA values in 2022 compared with 2021 was observed. Wolinska *et al*. (2015) revealed a positive correlation between dehydrogenase activity and TNB, which differs from our findings. Nevertheless, Kumar *et al.* (2013) pointed out that numerous factors, such are incubation procedure and time of incubation, temperature, soil aeration and moisture, disturbance, presence of pollutants, and management may affect the DHA in natural and mined soils.

CONCLUSIONS

Presented results showed that management practices have had an impact on the chemical properties and microbial abundance of soil. The lowest microbial and enzyme activity was recorded in coal mine-affected soil.

Although the forest soil was characterized by the lowest pH value and available P content, highest humus content compared with other samples stimulated the development of most bacterial groups and DHA. Further research will be focused on the determination of bacterial and fungal taxa in soil samples influenced by various management practices.

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