INTERNATIONAL
Agrophysics
www.international-agrophysics.org

Int. Agrophys., *2022*, *36*, *131-138*
doi: 10.31545/intagr/148101

Effect of mineral and organic additions on soil microbial composition**

Katarzyna Wolny-Koładka[1,2]*, *Renata Jarosz*[2], *Lidia Marcińska-Mazur*[2], *Tomáš Lošák*[3], *and Monika Mierzwa-Hersztek*[4,2]

[1]Department of Microbiology and Biomonitoring, University of Agriculture in Kraków, al. Mickiewicza 24/28, 30-059 Kraków, Poland
[2]Department of Mineralogy, Petrography and Geochemistry, AGH University of Science and Technology, al. Mickiewicza 30, 30-059 Kraków, Poland
[3]Department of Environmentalistics and Natural Resources, Mendel University in Brno, Zemědělská 1/1665, 613 00 Brno, Czech Republic
[4]Department of Agricultural and Environmental Chemistry, University of Agriculture in Kraków, Mickiewicza 21, 31-120 Kraków, Poland

Received February 15, 2022; accepted April 6, 2022

Abstract. The aim of the study was to evaluate the effect of different mineral-organic mixtures on changes in soil microbial composition and chemical properties. The design of the pot experiment included 6 treatments: soil without fertilization – C, soil fertilized with mineral NPK fertilizers – MF, soil with NPK + 3 or 6% lignite and 3% zeolite-vermiculite composite (MF+CW3%, MF+CW6%), soil with NPK + 3 or 6% leonardite and 3% zeolite-vermiculite composite (MF+CL3%, MF+CL6%). The test plants were spring oilseed rape and spring wheat. The highest number of microorganisms was observed: for oilseed rape – in the soil of the MF+CW3% and MF+CW6% treatments, and for wheat – in the soil of the MF+CL3% and MF+CL6% treatments. The maximum percentage increase in the number of analysed microorganisms, for spring rape and spring wheat, respectively, was: bacteria 190% (MF+CW3%) and 1198% (MF+CL3%), mould fungi 221% (MF+CW3%) and 1601% (MF+CL3%), *Azotobacter* spp. 248% (MF+CW6%) and 251% (MF+CL3%), actinomycetes 116% (MF+CW3%) and 251% (MF+CL3%). The beneficial effect of the applied mineral-organic mixtures on soil biological activity is closely related to the effect of these materials on soil chemical properties, such as pH or electrical conductivity, which was confirmed by the calculated correlation coefficients.

Keywords: microorganisms, soil, fertilization, spring wheat, spring oilseed rape

*Corresponding authors e-mail: katarzyna.wolny@urk.edu.pl
**This work was supported by the project "Fly ashes as precursors of functionalized materials for applications in environmental engineering, civil engineering and agriculture" – the project is carried out within the TEAM-NET programme of the Foundation for Polish Science POIR.04.04.00-00-14E6/18-00. (2020-2023).

INTRODUCTION

Soil microorganisms, their number and biodiversity have a significant effect on soil quality in terms of its use and fertility. Soil microbes are also indicators of soil quality given their participation in many biochemical processes that are essential for the environment and also for the ecological functions of soil (Mierzwa-Hersztek *et al*. 2020). Soil fertilization also influences the soil microbiome. The addition of external organic matter to the soil results in both qualitative and quantitative changes in microbial populations, as well as an increase or decrease in their activity (Tian *et al*., 2016). Biological activity is one of the characteristics of soil and microorganisms, these include bacteria and mould fungi and are one of the soil-forming factors involved in shaping soil fertility and providing nutrients to plants, as well as detoxifying harmful chemical compounds (Mierzwa-Hersztek *et al*., 2020). Atmospheric nitrogen-fixing bacteria, *Azotobacter* spp. and actinomycetes are considered to be saprophytes, they also play an important role in the soil environment. *Azotobacter* spp. are Gram-negative, free-living, aerobic soil bacteria (Mirzakhani *et al*., 2009). One of the most interesting features of these bacteria is that they have a beneficial effect on the growth of many plant species due to their ability to fix atmospheric nitrogen by converting it to ammonia, thereby producing plant-growth-promoting substances such as gibberellins, auxins and cytokinins (Martinez-Toledo *et al.*, 1989), vitamins and siderophores (Martyniuk and Martyniuk, 2003). They are also antagonistic to plant pathogens (Kizilkaya, 2009). These unique characteristics make *Azotobacter* spp. one of the most important free-living bacteria that fix atmospheric nitrogen, and their presence influences soil fertility and thus contributes to plant growth (Palleroni, 1984). Actinomycetes belong to the group of Gram-positive bacteria. They are commonly found in soil, composts, water and bottom sediments, causing the degradation of plant and animal residues, as well as barely degradable compounds, *e.g.* chitin, lignin, cellulose, higher fatty acids. Given their ability to decompose various chemical compounds, they play a key role in the circulation of elements in nature. They also participate in the formation of humic compounds in the soil (Bereza-Boruta, 2002). Actinomycetes have the ability to synthesize many enzymes, *e.g.* nitrogenase, as well as substances with an antibiotic activity, *e.g.* erythromycin. They are very numerous in the humus layer of soil. However, their presence in the soil environment is closely dependent, inter alia, on the chemical, physical, and physicochemical properties of the soil (Alvarez *et al*., 2012). Soil chemical properties are vital for microbial populations, this includes their enzymatic activity. In the opinion of many authors, enzymatic activity is one of the most rapidly manifested and also one of the most sensitive indicators of soil changes (Gul *et al*., 2015; Liu *et al*., 2017; Mierzwa-Hersztek *et al*., 2019). Soil enzymes are measures of the activity of microorganisms involved in biochemical processes such as mineralization and the synthesis of organic substances, as well as the nutrient cycle (Gul *et al*., 2015; Tian *et al*., 2016). Additionally, many studies have shown that changes in soil physical and chemical properties interact closely with enzymatic activity and that quantitative and qualitative changes in microbial populations have a significant effect on soil functional integrity (Khadem and Raiesi, 2017; Beheshti *et al*., 2018). In recent years, more and more attention has been paid to the restoration of soil organic matter resources through the use of mineral-organic fertilizers. Such a solution, on the one hand, allows for the provision of plants with the nutrients necessary for their proper growth and development, and on the other hand, contributes to increasing the organic carbon content in the soil. Lignite is one of the organic additives to mineral fertilizers which is gaining more and more attention (Dębska *et al*., 2002; Kwiatkowska *et al*., 2008). A very important component of this feedstock are fatty acids – a source of organic matter. Organic matter derived from lignite undergoes slow mineralization, due to which organometallic chelates are stable and do not release the metals bound by them. Humic substances (particularly fulvic and humic acids) formed from organic matter supplied to the soil, by forming complexes, immobilize heavy metals much more effectively and permanently. Humic acids improve soil chemical properties and biologically stimulate plant growth (Mikos-Szymańska *et al*., 2019). Górska *et al*. (2006) reported the positive effect of lignite on the functioning of soil microflora. The second organic additive used in the experiment was leonardite – a fossil that is an intermediate form between peat and lignite, it is very rich in humic acids (ranging from 50 to 75%) (Akinremi *et al*., 2000; Sanli *et al*., 2013). These acids show great potential in improving the biochemical properties of the soil, as a consequence plant growth and yield are enhanced. In addition, leonardite, as a soil additive, increases the available nitrogen, phosphorus and potassium in the soil (Sanli *et al*., 2013; Ratanaprommanee *et al*., 2016).

Inorganic additives are also used in agriculture, either natural or synthetic, as tools to maintain crop productivity, improve soil properties, increase carbon sequestration in the soil, and reduce inputs associated with the use of mineral fertilizers. Examples of such materials include synthetic zeolites or the zeolite-vermiculite composites produced from them (Szerement *et al*., 2021a, b; Jarosz *et al*., 2022). Zeolites are porous materials with a cation exchange capacity. Their function is to drive plant growth by increasing nutrient availability, soil conditioning, and soil moisture-holding capacity, thereby ensuring higher yields and better crop quality. Nutrient adsorption capacity is important in agricultural soils, especially in those that are intensively fertilized with nitrogen, as zeolite can regulate NH_4^+ availability and, at the same time, nitrification, thereby affecting N uptake and loss by crops (Moreno *et al*., 2017; Tzanakakis *et al*., 2021). Given the alkaline pH of the synthetic zeolites, the content of dissolved organic matter (DOM) may increase in the soil environment after their application. This is important in the context of metal-DOM complexes that can be degraded. The study of Oste *et al*. (2002) indicated that the DOM content increased significantly after the use of synthetic zeolite.

Considering the low content of organic matter in Polish soils, it is necessary to search for solutions to restore its resources. Taking the above into consideration, a study was carried out to evaluate the effectiveness of innovative mineral-organic mixtures containing a zeolite-vermiculite composite (sorbent function and stabilizing effect on the soil structure) and lignite or leonardite (source of OM) on changes in the microbiological composition and enzymatic activity of the soil. The effects of these mixtures on the soil have not been reported in the literature to date. The mixtures were applied in combination with mineral fertilizers, assuming that their specific properties would significantly improve the effectiveness of the fertilizer components, including microelements, and would also allow for a successive release of yield-forming components adapted to the changing needs of the plant during its growth and development, thus allowing for it to be fully exploited to its potential.

MATERIALS AND METHODS

The pot experiment was conducted in 2020 in the vegetation hall of the Faculty of Agriculture and Economics of the University of Agriculture, located in Kraków-Mydlniki.

Table 1. Selected soil properties prior to the pot experiment

Parameter	Value
Granulometric composition (%)	
2-0.05 mm	47
0.05-0.002 mm	50
<0.002 mm	3
pH_{H_2O}	5.84
pH_{KCl}	5.30
EC (μS cm^{-1})	149
N_{total} (g kg^{-1} D.M.)	0.98
C_{total} (g kg^{-1} D.M.)	10.40
S_{total} (g kg^{-1} D.M.)	0.120
$S_{available}$ (mg kg^{-1} D.M.)	26.65
$P_{available}$ (mg kg^{-1} D.M.)	138.18
$K_{available}$ (mg kg^{-1} D.M.)	112.14
$Mg_{available}$ (mg kg^{-1} D.M.)	56.26
Cd $CaCl_2$ (mg kg^{-1} D.M.)	0.14
Cr $CaCl_2$ (mg kg^{-1} D.M.)	0.02
Cu $CaCl_2$ (mg kg^{-1} D.M.)	0.20
Fe $CaCl_2$ (mg kg^{-1} D.M.)	0.60
Mn $CaCl_2$ (mg kg^{-1} D.M.)	26.88
Ni $CaCl_2$ (mg kg^{-1} D.M.)	0.09
Pb $CaCl_2$ (mg kg^{-1} D.M.)	0.08
Zn $CaCl_2$ (mg kg^{-1} D.M.)	2.42

The experiment was established on a soil with a sandy silt granulometric composition. The properties of the soil used in the experiment are presented in Table 1. The experiments were set up in PVC pots containing 8 kg of air-dried soil mass. The test plants were Tybalt spring wheat and Menthal spring oilseed rape.

For each plant species, the design of the pot experiment included 6 treatments carried out in 4 replications: C – soil without fertilization, MF – soil with NPK mineral fertilizers, soil with NPK + 3 or 6% lignite and 3% zeolite-vermiculite composite (NaX-Ver) (MF+CW3%, MF+CW6%), soil with NPK + 3 or 6% leonardite and 3% zeolite-vermiculite composite (MF+CL3%, MF+CL6%). The control treatment was soil without fertilization (C), and treatments with mineral fertilization were fertilized with chemically pure salts (N – NH_4NO_3; P – $Ca(H_2PO_4)_2 \cdot H_2O$; K – KCl) at an NPK ratio of: 1:0.5:1.25 (MF). The total doses of nutrients introduced into the soils of the fertilized treatments before the plants were sown are given in Table 2. During plant growth, in the presence of nitrogen deficiency symptoms, supplementary doses of this nutrient in the form of NH_4NO_3 solution were applied (top dressing). The additional nitrogen dose was 0.015 g N for spring wheat and 0.02 g N kg^{-1} D.M. for spring rape.

After applying mineral-organic mixtures and mineral salts and mixing them with soil, seeds of spring wheat and spring oilseed rape were sown. The plant density for spring wheat was 25 plants, while for spring oilseed rape it was 15 plants. Plant density in the pot was determined so as to provide optimal conditions for growth and development, including equal access to light, water, and nutrients.

Table 2. N, P and K doses applied to the soil in the form of prototype fertilizers and mineral salts before plant sowing

Plant species	N	P	K
	(g kg^{-1} D.M. soil)		
Spring wheat	0.15	0.08	0.20
Spring oilseed rape	0.20	0.10	0.25

The soil moisture content during plant growth was maintained at 40 to 60% of the maximum water capacity of the soil (depending on weather conditions and plant development stage).

The soil for the analyses was collected 5 months after the application of mineral-organic mixtures and mineral salts and stored at 4°C for 24 h for microbiological analyses and at 25°C for physicochemical and chemical analyses. The following parameters were determined in the air-dried and 1 mm sieved initial soil samples: granulometric composition – according to PN-R-04032 (The Polish…,1998), pH – potentiometrically in a suspension of soil and water as well as soil and a 1 mol dm^{-3} solution of KCl (soil : solution = 1: 2.5) and electrical conductivity (EC) – conductometrically. Total contents of carbon, nitrogen and sulphur were determined with a CNS analyser (Vario MAX Cube, Elementar) (Elementar Analysensysteme 2013). The total contents of phosphorus, potassium, calcium, magnesium and sodium were determined after ashing the sample in a chamber furnace at 450°C for 12 h and mineralizing its residues in a mixture of concentrated nitric and perchloric acids (3:2) (v/v). The contents of the investigated elements were determined in the obtained solutions by inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer Optima 7300 DV) (Oleszczuk *et al*., 2007). The available forms of heavy metals were extracted from the soil with a 0.01 M solution of calcium chloride ($CaCl_2$) (soil: solution = 1:10) for 2 h (Houba *et al*., 2000).

In order to evaluate the effect of mineral-organic mixtures on the number of selected soil microorganisms, soil samples with a natural moisture content were 1 mm sieved. By weighing 10 g from each soil sample and using the serial dilution method developed by Koch (Kopeć *et al*., 2020; Mierzwa-Hersztek *et al*., 2020) with a number of microbiological substrates, the following microorganisms were determined: total bacteria (Trypticasein Soy Lab Agar, BTL, Poland, grown at 37°C for 24 h), mould fungi (Malt Extract Agar, BTL, Poland, grown at 28°C for 5 days), actinomycetes (Actinomycete Isolation Lab Agar, Biocorp, Poland, grown at 28°C for 7 days), and *Azotobacter* spp. (Ashby`s Mannitol Agar (Atlas and Parks, 1997), grown at 28°C for 7 days). From an agricultural point of view, changes to the number of specific microbial groups are important for assessing fertilizer efficiency and they indirectly provide information about soil fertility and quality (Wolny-Koładka and Żukowski, 2019). The number of colony forming units (CFU) of microorganisms was determined using the dilution culture method, and the result was converted into 1 g D.M. of soil.

The dehydrogenase activity in the soil was determined using Thalmann's method (1968) based on the estimation of the TTC reduction rate to TPF after 24 h incubation of the soil samples at 37°C.

Differences between the applied mineral-organic mixtures were evaluated using one-way analysis of variance (ANOVA, Duncan's test, $p \leq 0.05$). The variability within each mixture was determined by calculating the standard deviation (± SD). The correlation coefficients between the chemical and microbial properties of the soil were calculated using Spearman's non-parametric test (Table 3). All statistical analyses were performed using Statistica PL 13 software (StatSoft Inc.).

RESULTS AND DISCUSSION

In their study, Aciego Pietri and Brookes (2008) showed that changes in soil pH have a pronounced effect on biomass and microbial activity in the soil. As reported by various authors, the optimal pH values affecting microbial activity are between 5.5 and 8.8 (Aciego Pietri and Brookes, 2008; Neina, 2019). Santoso *et al.* (2022) used zeolite, manure and their combination as soil additions in ramie crop (plant rhizomes were planted). Their results revealed that the application of zeolite did not significantly affect plant growth, while there was an increase in pH value and N-total and C-org contents by 6.26, 0.15, and 1.13%, respectively. Rusyn *et al.* (2020) investigated the effect of applying fertilizer capsules with zeolite and polyethylene terephthalate on the microbial composition and pH of the soil, as well as plant germination. Their results indicated an increase in the total number of microorganisms in the soil and a slight decrease in soil pH.

Table 3. Spearman's correlation coefficients between the selected soil chemical and microbiological properties (n=4)

Parameter	Bacteria	Mould fungi	Actinomycetes	*Azotobacter* spp.
		Spring wheat		
pH H_2O	0.45*	0.49*	0.17	0.15
pH KCl	0.27	0.20	0.07	0.09
EC	0.61**	0.61**	0.47*	0.67***
C_{total}	–0.07	–0.07	–0.21	–0.03
N_{total}	–0.33	–0.36	–0.33	–0.19
DHA	–0.44*	–0.33	–0.04	–0.34
		Spring oilseed rape		
pH H_2O	–0.60**	–0.56**	–0.33	–0.67***
pH KCl	–0.33	–0.25	0.02	–0.41*
EC	–0.11	–0.19	–0.46*	–0.14
C_{TOTAL}	–0.58*	–0.42*	–0.49*	0.09
N_{TOTAL}	0.40	0.41*	0.09	–0.78*
DHA	–0.85*	0.81*	0.75	–0.39

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ levels.

The soil used in the experiment was characterized by a slightly acidic pH (pH H_2O = 5.84) and relatively high contents of C_{total} (10.40 g kg^{-1} D.M.) and N_{total} (0.98 g kg^{-1} D.M.). In the spring wheat crop, a significant ($p \leq 0.05$) increase in soil pH (in H_2O) and electrical conductivity was found in all treatments with mineral-organic mixture addition, as compared to the control treatment (C) (Table 4). The opposite situation was observed for spring oilseed rape: the pH of the treatments with the addition of mineral-organic mixtures was significantly lower as compared to the control soil. These differences can probably be attributed to various mineralization rates of mineral-organic mixtures and the diverse release rates of alkaline and/or acidic substances. Root secretions of both plant species (wheat: the bundle system, rape: the pile system) may have an indirect impact on the rate of transformation of compounds in the soil. Root secretions are an excellent source of food for microorganisms living in the rhizosphere; therefore, an extensive root system can provide microorganisms with perfect conditions for growth and development. That thesis is also confirmed by our results – the number of individual groups of microorganisms in the treatments with wheat was significantly higher than in the soil sampled from rape cultivation. According to Natywa *et al.* (2010), agrotechnical treatments such as fertilization

Table 4. Selected soil properties after 5 months of the pot experiment

Treatment	pH H_2O	pH KCl	EC (µS cm^{-1})	C_{total} (g kg^{-1} D.M.)	N_{total} (g kg^{-1} D.M.)
			Spring wheat		
C	6.41a±0.02	5.62a±0.01	93.1a±2.59	10.4a±1.00	0.99c ±0.05
MF	6.68bc±0.04	5.64a±0.01	160d±4.89	10.7ab±0.87	0.91ab±0.03
MF+CW3%	6.73c±0.10	5.70a±0.04	135b±9.63	10.6ab±0.49	0.96bc±0.06
MF+CW6%	6.58b±0.07	5.61a±0.03	145c±2.72	10.2ab±0.50	0.91ab±0.01
MF+CL3%	6.65b±0.04	5.65a±0.04	162d±2.17	10.2ab±0.48	0.88a±0.05
MF+Cl6%	6.60b±0.05	5.66a±0.07	181e±8.94	11.1bc±0.49	0.94bc±0.05
			Spring oilseed rape		
C	6.31a±0.04	5.52a±0.02	87.3a±3.35	10.6a±0.05	0.91a±0.01
MF	6.02c±0.02	5.30cd±0.01	124b±5.78	10.0bc±0.05	0.91a±0.01
MF+CW3%	6.06c±0.02	5.40c±0.05	121b±7.33	10.2cd±0.24	0.98bc±0.05
MF+Cw6%	6.02c±0.04	5.28d±0.05	113b±4.22	9.93d±0.11	0.93ab±0.05
MF+CL3%	6.14b±0.03	5.35bc±0.07	138c±4.28	10.2cd±0.01	0.91a±0.01
MF+Cl6%	6.04c±0.04	5.31bc±0.04	122b±2.49	10.3cd±0.13	1.01c±0.08

Each value represents the mean of four replicates ± standard deviation; mean values marked with the same letters in the column do not differ significantly according to Duncan's test at $p \leq 0.05$, factor: fertilization.

(including organic and mineral nitrogen fertilization) have a significant impact on the activity of soil microorganisms. In addition to pH, the factors influencing the formation of soil microbial communities can also include the contents of organic matter and individual elements, temperature, as well as cultivation and crop protection practices.

For spring wheat, a significant increase in the C_{total} content was only found in the soil of the MF+CL6% treatment. In the case of the spring oilseed rape treatments, a substantial decrease in carbon content was discovered in each treatment amended with mineral-organic mixtures. A significant increase in nitrogen content for spring oilseed rape was only found in treatments MF+CW3% and MF+CL6%. The calculated values of Spearman's correlation coefficients indicated that electrical conductivity was positively correlated with the number of bacteria, fungi, actinomycetes and *Azotobacter* spp. in the spring wheat crop and with *Azotobacter* spp. in the spring oilseed rape crop (Table 3). On the other hand, the C_{total} content was negatively correlated with the number of bacteria, fungi and actinomycetes in the soil of the spring oilseed rape treatments. For spring oilseed rape, the soil pH was negatively correlated with the count of 3 groups of microorganisms (Table 3). The nitrogen content was positively correlated with the number of fungi and negatively correlated with the number of *Azotobacter* spp.

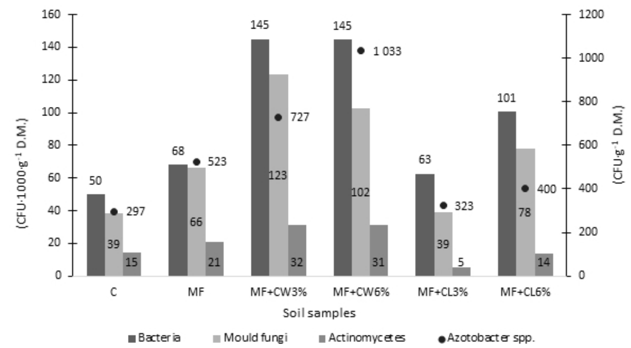

Fig. 1. Average number (CFU g^{-1} D.M.) of microorganisms in the soil samples – test plant: spring oilseed rape.

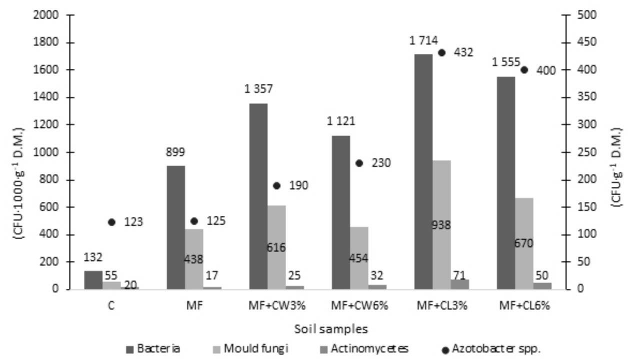

Fig. 2. Average number (CFU g^{-1} D.M.) of microorganisms in the soil samples – test plant: spring wheat.

The number of soil microorganisms was highly differentiated and changed depending on the type and amount of applied fertilization as well as the plant species cultivated; however, some close and very interesting relationships were noted (Figs 1 and 2). For both spring oilseed rape and spring wheat, it was found that the applied mineral NPK fertilization and the addition of mineral-organic mixtures containing lignite and leonardite increased the number of microorganisms, as compared to the control (soil without fertilization). Therefore, it may be concluded that the total number of bacteria, moulds, actinomycetes and *Azotobacter* spp. increased in treatments where soil fertilization was applied. Thus, the positive effect of the applied materials on selected groups of soil microorganisms was proven. Additionally, the obtained results clearly indicated that the highest number of microorganisms was found in the soils of treatments MF+CW3% and MF+CW6% (spring oilseed rape) and also in the soils of treatments MF+CL3% and MF+CL6% (spring wheat). Therefore, it may be concluded that the profiling of fertilization (using either lignite or leonardite) for a specific plant species is absolutely justified. In general, the most numerous group of microorganisms were the total bacteria, the number of which increased from 50 000 (soil without fertilization) to 145 000 (CFU g^{-1} D.M.) (MF+CW3%) in the case of spring oilseed rape, and from 132 117 (soil without fertilization) to 1 714 333 (CFU g^{-1} D.M.) (MF+CL3%) in the case of spring wheat. The second most abundant group were mould fungi, ranging from 38 500 (soil without fertilization) to 123 400 (CFU g^{-1} D.M.) (MF+CW3%) for spring oilseed rape and from 55 167 (soil without fertilization) to 938 330 (CFU g^{-1} D.M.) (MF+CL3%) for spring wheat (Figs 1 and 2). The growing number of total bacteria and mould fungi indicates favourable soil conditions for these microorganisms, the richness in nutrients and the optimal pH for them (Malinowski *et al.*, 2019). From studies conducted by Rousk *et al.* (2009) bacteria grow best at pH 4.0 to 8.0, and fungi grow best at pH 4.5 to 8.3. Therefore, the pH of the studied soils should be considered beneficial for both microbial groups. Bacteria grow best at a pH of H_2O from 6.5 to 7.5, and fungi grow best at a pH of H_2O from 4.5 to 8.3 (Rousk *et al.*, 2009); therefore, the pH of H_2O of the studied soils (Table 4) should be considered favourable for mould fungi and close to optimal for bacteria. The presence of *Azotobacter* spp. in soils is correlated with various soil properties (*e.g.* pH, organic matter content, soil moisture), this was also confirmed by the results of our study. The population of these bacteria ranges from negligible, sometimes zero, to 10^4 g^{-1} soil and depends on the physicochemical and microbiological properties (*i.e.* microbial interactions) of the soil (Kizilkaya, 2009). These bacteria are most common in neutral and slightly alkaline soils, while they are absent or only present in very low numbers in acidic soils (Martyniuk and Martyniuk, 2003). In our study, the number of *Azotobacter* spp. varied for spring oilseed rape from 297 (soil without fertilization) to 1 033 (CFU g^{-1} D.M.)

(MF+CW6%), and for spring wheat from 123 (soil without fertilization) to 432 (CFU g^{-1} D.M.) (MF+CL3%). On the other hand, the number of actinomycetes for spring oilseed rape ranged from 14 583 (soil without fertilization) to 31 517 (CFU g^{-1} D.M.) (MF+CW3%), and for spring wheat from 20 083 (soil without fertilization) to 70 500 (CFU g^{-1} D.M.) (MF+CL3%) (Figs 1 and 2). Thus, it may be concluded that fertilization based on mineral and organic additions in the form of lignite and leonardite combined with a zeolite-vermiculite composite effectively increased the number of the studied microbial groups.

Dehydrogenase activity is an indicator of the overall microbial oxidative metabolism showing the growth and activity of microbial populations (Burns *et al.*, 2013). As reported by Wolińska *et al.* (2016), when determining dehydrogenase activity in the soil, more than 90% of the amount of dehydrogenase determined is of biological origin; therefore, the test is believed to provide reliable information concerning the biological properties of soil. Our results have shown that the activity of dehydrogenases (DHA) in the soils analysed with the use of TTC as a substrate ranged from 1.90 to 2.56 μg TPF g^{-1} h^{-1} for the samples in rape cultivation (Fig. 3). On the other hand, in the wheat cultivation soil, dehydrogenase activity ranged from 2.62 to 3.72 μg TPF g^{-1} h^{-1} (Fig. 4). The highest level of dehydrogenase activity in rape cultivation was determined in soil from the control treatment, while statistically significant differences were only observed in MF, MF+CW6% and MF+CL6%. The determination of dehydrogenase activity resulting from wheat cultivation indicated a significant decrease in this parameter for treatments amended with fertilizer composites as compared to the control. An increase in soil dehydrogenase activity after the application of 5 and 10 t ha^{-1} zeolite doses was found in the study by Doni *et al.* (2020). These authors showed an increase in soil dehydrogenase activity in grapevine cultivation from 8 to 28% for zeolite dose of 5 t ha^{-1} and from 22 to 110% for zeolite dose of 10 t ha^{-1} (Doni *et al.*, 2020). On the other hand, in a study by Shivakumar *et al.* (2019), zeolite application in a dose of 50 kg ha^{-1} along with 100 and 125% of the recommended doses of fertilizers showed higher dehydrogenase, urease, and phosphatase activity as compared to other treatments. Natywa *et al.* (2010) observed a decrease in DHA activity with increasing doses of nitrogen. As a result of using high doses of nitrogen fertilizers, the qualitative composition of biocenoses is modified: there is a recession of *Arthrobacter, Azotobacter* and *Streptomyces* bacteria, the previous dominants from the group of autochthonous microflora, and the dominance of microbiocenoses is superseded by other species, *i.e.* mainly *Deuteromycetes* fungi.

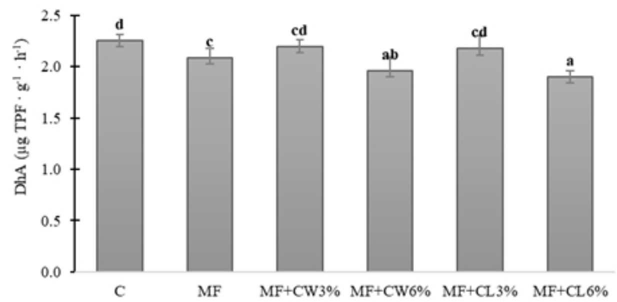

Fig. 3. Dehydrogenase activity in soil from spring oilseed rape. Each value represents the mean of four replicates. The different letters indicate a significant difference at p≤0.05 according to Duncan's ANOVA, factors: treatment × analysis data.

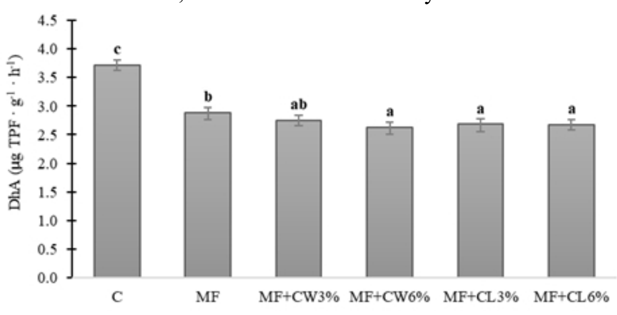

Fig. 4. Dehydrogenase activity in soil from spring wheat. Each value represents the mean of four replicates. The different letters indicate a significant difference at p≤0.05 according to Duncan's ANOVA, factors: treatment × analysis data.

The results above indicated that the interaction of many factors had a great effect on the chemical and microbiological properties of the soil. The addition of mineral-organic mixtures produces changes in the chemical properties of the soil and hence affects its microbial characteristics. With reference to the applied organic-mineral mixtures, the number of individual groups of microorganisms have a significant impact on the assessment of fertilization efficiency.

FUTURE PERSPECTIVES

In order to be able to evaluate the effect of using mineral-organic mixtures that contain functionalized and organic materials as additives to agricultural soils with particular reference to changes in chemical and biological properties, it is necessary to assess their long-term effects. It is also necessary to understand the effect of these fertilizers under different climatic and environmental conditions and in various plant crops. Long-term research that shows the advantages and disadvantages of using fertilizers made with the addition of zeolites will be particularly valuable. According to the authors, future research should also take into account ecotoxicological as well as nitrifying bacteria activity tests, which are considered to be very sensitive indicators of soil quality. This will allow for the verification of the gradual release of nutrients in the soil, and thus facilitate a better understanding of their activity (Eslami *et al.*, 2019). From an ecological point of view, it is also important to monitor the effects of applying such mixtures on the number and activity of soil microbes. Future studies should also include the verification of the effect of different doses of mineral-organic mixtures on various soil types. The obtained results are applicative and may constitute a compendium of knowledge for farmers.

CONCLUSIONS

1. Both NPK mineral fertilization and the addition of mineral-organic mixtures (zeolite-vermiculite composite, lignite, or leonardite) increased the number of microorganisms as compared to the control (soil without fertilization).

2. The type of fertilization applied should take into account the plant species grown and its nutritional requirements.

3. Mineral-organic fertilization produces changes to the chemical properties of the soil, which in turn significantly influences the quantitative and qualitative composition of the soil microbiome.

4. Only in the case of leonardite and spring oilseed rape, doubling the addition of organic materials (from 3 to 6%) increased the number of microorganisms; in other cases, such an effect was not observed and the opposite tendency was even found.

Conflict of interest: The authors declare that they have no conflict of interest.

REFERENCES

Aciego Pietri J.C. and Brookes P.C., 2008. Relationships between soil pH and microbial properties in a UK arable soil. Soil Biol. Biochem., 40(7), 1856-1861, https://doi.org/10.1016/j.soilbio.2008.03.020

Akinremi O.O., Janzen H.H., Lemke R.L., and Larney F.J., 2000. Response of canola, wheat and green beans to leonardite additions. Can. J. Soil Sci., 80(3), 437-443, https://doi.org/10.4141/S99-058

Alvarez A., Catalanos A., and Amorosom J., 2012. Heavy metal resistant strains are widespread along *Streptomyces* phylogeny. Mol. Phylogenet. Evol., 66, 1083-1088, https://doi.org/10.1016/j.ympev.2012.11.025

Atlas R.M. and Parks L.C., 1997. Handbook of Microbiological Media. CRC Press, Boca Raton, FL, USA.

Beheshti M., Etesami H., and Alikhani H.A., 2018. Effect of different biochars amendment on soil biological indicators in a calcareous soil. Environ. Sci. Pollution Res., 1-10, https://doi.org/10.1007/s11356-018-1682-2

Bereza-Boruta B., 2002. Selected enzymatic activities of actinomycetes of *Streptomyces* genus isolated from potato plantation (in Polish). Agricultura, 1, 27-36.

Burns R.G., De Forest J.L., Marxsen J., Sinsabaugh R.L., Stromberger M.E., Wallenstein M.D., Weintraub M., and Zoppini A., 2013. Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biol. Biochem., 58, 216-234, https://doi.org/10.1016/j.soilbio.2012.11.009

Dębska B., Maciejewska A., and Kwiatkowska J., 2002. The effect of fertilization with brown coal on Haplic Luvisol humic acids. Rostl. Vyroba, 48(1), 33-39, https://doi.org/10.17221/4203-PSE

Doni S., Gispert M., Peruzzi E., Macci C., Mattii G.B., Manzi D., Masini C.M., and Grazia M., 2020. Impact of natural zeolite on chemical and biochemical properties of vineyard soils. Soil Use Manag., 0-2, https://doi.org/10.1111/sum.12665

Eslami M., Khorassani R., Fotovat A., and Halajnia A., 2020. NH_4^+-K^+ co-loaded clinoptilolite as a binary fertilizer. Arch. Agron. Soil Sci., 66, 33-45, https://doi.org/10.1080/03650340.2019.1591617

Górska E.B., Maciejewska A., Jakubiak I., and Russel S., 2006. Effect of farm-yard manure, peat, brown coal and rekulter on occurrence of actinomycetes in soil (in Polish). Zeszyty Naukowe Uniwersytetu Przyrodniczego we Wrocławiu, 546, Rolnictwo, 89, 79-86.

Gul S., Whalen J.K., Thomas B.W., Sachdeva V., and Deng H.Y., 2015. Physico-chemical properties and microbial responses in biochar-amended soils: mechanisms and future directions. Agric. Ecosyst. Environ., 206, 46-59, https://doi.org/10.1016/j.agee.2015.03.015

Houba V.J.G., Temminghoff E.J.M., Gaikhorst G.A., and van Vark W., 2000. Soil analysis procedures using 0.01 M calcium chloride as extraction reagent. Commun. Soil Sci. Plant Anal., 31 (9-10), 1299-1396, https://doi.org/10.1080/00103620009370514

Jarosz R., Szerement J., Gondek K., and Mierzwa-Hersztek M., 2022. The use of zeolites as an addition to fertilizers - a review. Catena, 213, 106125, https://doi.org/10.1016/j.catena.2022.106125

Khadem A. and Raiesi F., 2017. Responses of microbial performance and community to corn biochar in calcareous sandy and clayey soils. Appl. Soil Ecol., 114, 16-27, https://doi.org/10.1016/j.apsoil.2017.02.018

Kizilkaya R., 2009. Nitrogen fixation capacity of *Azotobacter* spp. strains isolated from soils in different ecosystems and relationship between them and the microbiological properties of soils. J. Environ. Biol., 30, 73-82.

Kopeć M., Mierzwa-Hersztek M., Gondek K., Wolny-Koładka K., Zdaniewicz M., and Jarosz R., 2020. Biological activity of composts obtained from hop waste generated during the brewing. Biomass Conv. Bioref., 12, 1271-1279, https://doi.org/10.1007/s13399-020-00746-6

Kwiatkowska J., Provenzano M.R., and Senesi N., 2008. Long term effects of a brown coal-based amendment on the properties of soil humic acids. Geoderma, 148, 200-205, https://doi.org/10.1016/j.geoderma.2008.10.001

Liu X., Guo K., Huang L., Ji Z., Jiang H., Li H., and Zhang J., 2017. Responses of absolute and specific enzyme activity to consecutive application of composted sewage sludge in a Fluventic Ustochrept. PLoS ONE, 12(5), e0177796, https://doi.org/10.1371/journal.pone.0177796

Malinowski M., Wolny-Koładka K., and Vaverková M.D., 2019. Effect of biochar addition on the OFMSW composting process under real conditions. Waste Management, 84, 364-372, https://doi.org/10.1016/j.wasman.2018.12.011

Martinez-Toledo M.V., Moreno J., De la Rubia T., and Gonzalez-Lopez J., 1989. Root exudates of *Zea mays* and production of auxins, gibberellins and cytokinins by *Azotobacter chroococcum*. Plant Soil, 110, 149-152, https://doi.org/10.1007/BF02143553

Martyniuk M. and Martyniuk S., 2003. Occurrence of *Azotobacter* spp. in some Polish soils. Pol. J. Environ. Stud., 12, 371-374.

Mikos-Szymańska M., Schab S., Rusek P., Borowik K., Bogusz P., and Wyzińska M., 2019. Preliminary study of a method for obtaining brown coal and biochar based granular compound fertilizer. Waste and Biomass Valorization, 10, 3673-3685, https://doi.org/10.1007/s12649-019-00655-4

Mierzwa-Hersztek M., Gondek K. Klimkowicz-Pawlas A., Chmiel M.J., Dziedzic K., and Taras H., 2019. Assessment of soil quality after biochar application based on enzymatic activity and microbial composition. Int. Agrophys., 33, 331-336, https://doi.org/10.31545/intagr/110807

Mierzwa-Hersztek M., Wolny-Koładka K., Gondek K., Gałązka A., and Gawryjołek K., 2020. Effect of coapplication of biochar and nutrients on microbiocenotic composition, dehydrogenase activity index and chemical properties of sandy soil. Waste and Biomass Valorization, 11, 3911-3923, https://doi.org/10.1007/s12649-019-00757-z

Mirzakhani M., Ardakani M.R., Aeene Band A., Rejali F., and Shirani Rad A.H., 2009. Response of spring safflower to co-inoculation with *Azotobacter chroococum* and *Glomus intraradices* under different levels of nitrogen and phosphorus. Am. J. Agricult. Biol. Sci., 4, 255-261, https://doi.org/10.3844/ajabssp.2009.255.261

Moreno J.L., Ondoño S., Torres I., and Bastida F., 2017. Compost, leonardite, and zeolite impacts on soil microbial community under barley crops. J. Soil Sci. Plant Nutr., 17(1), 214-230,https://doi.org/10.4067/S0718-95162017005000017

Natywa M., Sawicka A., and Wolna-Maruwka A., 2010. Microbial and enzymatic activity in the soil under maize crop in relation to differentiated nitrogen fertilisation. Water-Environment-Rural Areas, 10, 2(30), 111-120.

Neina Dora, 2019. The Role of soil pH in plant nutrition and soil remediation. App. Environ. Soil Sci., 1-9, https://doi.org/10.1155/2019/5794869

Oleszczuk N., Castro J.T., da Silva M.M., Korn M., Welz B., and Vale M.G., 2007. Method development for the determination of manganese, cobalt and copper in green coffee comparing direct solid sampling electrothermal atomic absorption spectrometry and inductively coupled plasma optical emission spectrometry. Talanta, 73(5), 862-869, https://doi.org/10.1016/j.talanta.2007.05.005

Oste L.A., Lexmond T.M., and Van Riemsdijk W.H., 2002. Metal immobilization in soils using synthetic zeolites. J. Environ. Qual., 31(3), 813-821, https://doi.org/10.2134/jeq2002.8130

Palleroni N.J., 1984. Gram negative aerobic rods and cocci. In: bergey's manual of systematic bacteriology (Ed. N.R. Krieg), Williams &Wilkins, Baltimore, 140-199.

Ratanaprommanee C., Chinachanta K., Chaiwan F., and Shutsrirung A., 2016. Chemical characterization of leonardite and its potential use as soil conditioner and plant growth enhancement. Asia Pac. J. Sci. Technol., 22(4),1-10.

Rousk J., Brookes P.C., and Bååth E., 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Appl. Environ. Microbiol., 75(6), 1589-1596, https://doi.org/10.1128/AEM.02775-08

Rusyn I., Malovanyy M., Tymchuk I., and Synelnikov S., 2020. Effect of mineral fertilizer encapsulated with zeolite and polyethylene terephthalate on the soil microbiota, pH and plant germination. Ecol. Quest., 32, 1-12, https://doi.org/10.12775/EQ.2021.007

Sanli A., Karadogan T., and Tonguc M., 2013. Effects of leonardite applications on yield and some quality parameters of potatoes (*Solanum tuberosum* L.). Turkish J. Field Crops., 18(1), 20-26.

Santoso B., Cholid M., and Wijayanto R.A., 2022. Effect of zeolite and cow manure application on soil nitrogen content in ramie (*Boehmeria nivea*) plant growth. IOP Conf. Ser. Earth Environ. Sci., 974, https://doi.org/10.1088/1755-1315/974/1/012073

Shivakumara M.N., Krishna Murthy R., Subbarayappa C.T., Chamegowda T.C., Thimmegowda M.N., and Muthuraju R., 2019. Effect of zeolite and fertilizer application on soil microbial biomass and enzyme activity in finger millet. Int. J. Curr. Microbiol. App. Sci., 8, 1939-1957, https://doi.org/10.20546/ijcmas.2019.811.228

Szerement J., Szatanik-Kloc A., Jarosz R., Bajda T., Mierzwa-Hersztek M., 2021a. Contemporary applications of natural and synthetic zeolites from fly ash in agriculture and environmental protection. J. Clean. Prod., 311, 127461, https://doi.org/10.1016/j.jclepro.2021.127461

Szerement J., Szatanik-Kloc A., Mokrzycki J., and Mierzwa-Hersztek M., 2021b. The beneficial effects of agronomic biofortification with Se, Zn, and Fe on nutritional quality and stress defense of crops – a review. J. Soil Sci. Plant Nutr., 22, 1129-1159, https://doi.org/10.1007/s42729-021-00719-2

Thalmann A., 1968. Methods of dehydrogenase activity determination with triphenyl tetrazolium chloride (TTC) (in German). Landwirtsch. Forsch, 21, 249-258.

The Polish Committee for Standardization, 1998. Soils and mineral deposits – Sampling and determination of the grain size composition. PN-R-04032:1998 – (in Polish).

Tian J., Wang J., Dippold M., Gao Y., Blagodatskaya E., and Kuzyakov Y., 2016. Biochar affects soil organic matter cycling and microbial functions but does not alter microbial community structure in a paddy soil. Sci. Total Environ., 556, 89-97, https://doi.org/10.1016/j.scitotenv.2016.03.010

Tzanakakis V.A., Monokrousos N., and Chatzistathis T., 2021. Effects of clinoptilolite zeolite and vermiculite on nitrification and nitrogen and phosphorus acquiring enzymes in a nitrogen applied agricultural soil. J Soil Sci Plant Nutr., 21, 2791-2802, https://doi.org/10.1007/s42729-021-00566-1

Wolińska A., Zapasek M., and Stępniewska Z., 2016. The optimal TTC dose and its chemical reduction level. Acta Agroph., 23, 303-314.

Wolny-Koładka K. and Żukowski W., 2019. Mixed municipal solid waste hygienisation for refuse-derived fuel production by ozonation in the novel configuration using fluidized bed and horizontal reactor. Waste and Biomass Valorization, 10(3), 575-583, https://doi.org/10.1007/s12649-017-0087-7