Nr 37

INŻYNIERIA ŚRODOWISKA

2015

MONIKA JAKUBUS^{*} ARE EFFECTIVE MICROORGANISMS (EMTM) EFFECTIVE IN ENHANCEMENT OF SOIL FERTILITY?

Summary

The term Effective Microorganisms (EM^{TM}) refers to a microbial preparation promoting plant growth and health as well as improving soil fertility. In the study the effect of AEM^{TM} preparation (applied at a rate corresponding to 100 L per hectare) in combination with different organic substances (wheat straw, manure, sewage sludge, compost) on soil fertility and liberation of available nutrients was investigated in a 9-month incubation process. The soil samples were collected at the beginning and at the end of the incubation process. In the sampled soil material both main physicochemical properties and amounts of nutrients available for plants were analysed. On the basis of the results a positive role of the AEM^{TM} preparation may hardly be demonstrated, because its effect on most analysed properties was very weak and often could not be proven statistically. The interaction of experimental factors (incubation time and used organic substance) had a significant effect on quantitative changes in investigated nutrients. A special role of applied organic materials in the formation of available nutrients needs to be stressed, which was evident in the case of manure and compost.

Key words: effective microorganisms, decomposition, organic substances, soil properties, available nutrients

INTRODUCTION

In recent years management strategies in contemporary agriculture focus on reducing the inputs of chemical fertilizers and substitute them with natural substances. Natural fertilizers, also called biofertilizers [Mayer et al. 2010], are incomparably less harmful to the environment and as such they receive a lot of attention. Among different microbiological products, the EMTM (Effective Microorganisms) preparation is widely discussed and evaluated [Jakubus et al. 2010, Jakubus et al. 2012, Zydlik and Zydlik 2013]. The EM preparation was

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developed by Teruo Higa and has been described as a combination of over 80 genetically unchanged coexisting beneficial microorganisms [Higa 1994]. EM is reported to include a population of lactic acid bacteria and yeasts and smaller numbers of phototrophic bacteria, fungi and Actinomyces. According to Higa [1994], Woodwad [2003], Kaczmarek et al. [2008] and Sheng et al. [2008] such a selection of microorganisms in practice guarantees its high and advantageous impact on the environment exerted by maximizing conversion of organic matter into soil humus, increasing beneficial native microbiological populations and limiting the development of pathogenic organisms. Some studies reported that crop growth and yield were increased as a result of EM application [Khaliq et al. 2006, Javaid and Bajwa 2011, Hu and Qi 2013]. However, the reliability of these results is frequently questioned due to the short duration of these trials as well as their local nature [Condor Golec et al. 2006]. There is a certain group of authors who found no positive effects of EM on plants in general [Pyriadi et al. 2005, Van Vilet et al. 2006]. Despite these facts, studies dedicated to EM are being continued in many countries, including Poland. Polish researchers primarily focus on the determination of the effect of EM on the health and yields of crop plants [Boliglowa and Gleń 2008, Zydlik and Zydlik, 2013]. The reports concerning the role of EM in organic matter decomposition and improving soil quality in general are highly controversial and contradictory. Among different research projects one may notice some examples which confirmed such an influence [Formowitz et al. 2007, Valarini et al. 2003, Javaid and Bajwa 2011] and some which did not [Schenck zu Schweinsberg-Mickan and Müller 2009]. In the light of agricultural and environmental significance of organic matter, the effect of EM on its humification, mineralization and liberation of nutrients appears very important. The content of organic matter undergoes rapid changes and it is a natural process, especially in the case of intensive soil cultivation. Unfortunately, rapid organic matter decomposition leads to depletion in nutrients and creates a negative balance of organic substances in soil. Presently farmers can complement the organic matter using different fertilizers and wastes, which are very rich in both organic substances and nutrients. In Poland organic fertilizers such as manure and composts, as well as waste materials, e.g. sewage sludge and straw, are commonly used in practice. Despite their different chemical composition and the resulting diverse value, these substances are characterized by a common feature, i.e. slow release of nutrients in forms available for plants. According to the pioneer of EM technology, EM impact manifests itself in a faster rate of mineralisation-humification transformations of organic matter, which frequently results in increased quantities of organic carbon and improved soil fertility. Moreover, a combined application of EM with manure or organic substances is recommended to improve EM efficiency [Mayer et al. 2010].

Taking into account the above assumptions, an experiment was undertaken, which aims were:

- i. evaluation of EM efficiency in decomposition of different organic substances and
- ii. assessment of EM effects on enhancement of soil fertility, manifested by the increased amounts of available nutrients.

MATERIAL AND METHODS

Materials and experimental scheme

The assumed research objective was realised using soil samples from a 9month incubation experiment conducted under controlled conditions of temperature ($\pm 25^{\circ}$ C) and humidity ($\pm 60\%$). Soil used in experiment was collected from the humus horizon. Soil was classified according to WRB [IUSS Working Group WRB 2007] as Luvisols. The soil was incubated with wheat straw, manure, sewage sludge, compost and AEMTM preparation (Activated Effective Microorganisms). The organic material was applied into the soil at a rate corresponding to 4 tons of wheat straw per hectare (i.e. 1.4 g/kg of soil), 40 tons of manure per hectare (i.e.13.4 g/kg of soil), 10 tons of sewage sludge per hectare (i.e.3.3 g/kg of soil) and 30 tons of compost per hectare (i.e.10 g/kg of soil). Soil was mixed very carefully, sprayed with AEMTM at a rate corresponding to 100 L per hectare and then placed in 20 kg plastic containers of 32 cm in diameter and 42 cm in height. No additional mineral fertilization was applied during the experiment. The experimental design comprised the following treatments:

- T1 Luvisols (control)
- T2 Luvisols incubated with wheat straw
- T3 Luvisols incubated with wheat straw and AEM
- T4 Luvisols incubated with manure
- T5 Luvisols incubated with manure and AEM
- T6 Luvisols incubated with sewage sludge
- T7 Luvisols incubated with sewage sludge and AEM
- T8 Luvisols incubated with compost
- T9 Luvisols incubated with compost and AEM.

Each treatment was represented by 2 replications. The soil samples were collected at the first day of the experiment (initial stage - IS) and after 9 months of incubation (end stage - ES). Using hand auger individual samples were collected from 4 separated places into each container and then they were thoroughly mixed to obtain representative bulk soil samples.

Soil analyses

The following physicochemical properties were investigated in the soil samples collected from the combinations presented above: soil reaction (pH), hydrolytic acidity (Hh), total basic cations (Base Saturation - BS) and cation exchange capacity (CEC). CEC was calculated on the basis of Hh and BS values using the following formula: CEC=Hh+BS. The following chemical soil parameters were determined: contents of total carbon (C_{tot}) and total nitrogen (N_{tot}) , amounts of available macroelements (mineral N, P, K, Mg and S-SO₄) and microelements (Cu, Mn, Zn, Fe). The above properties were determined using methods commonly applied in soil science analyses; their detailed descriptions can be found in [Jakubus 2013]. Briefly: soil reaction in 1 mol·dm⁻³ KCl was measured by potentiometry, hydrolytic acidity was determined with 1mol·dm⁻³ calcium acetate (2 h, 1:2,5, w/v), while total base content was determined with 0.1 mol·dm⁻³ HCl (1 h, 1:5, w/v). Total carbon and total nitrogen levels were determined using a Vario Max CNS apparatus. Mineral nitrogen (sum of NH4⁺ and NO₃) content was determined after extraction in 2 mol dm^{-3} KCl (2 h, 1:20, w/v). Available forms of macronutrients in soil samples were assayed after extraction with a common extractor 0.04 mol·dm⁻³ Ca(CH₃CHOHCOO)₂·5H₂O (1.5 h, 1:50, w/v) for potassium and phosphorus; $0.0125 \text{ mol} \cdot \text{dm}^{-3} \text{ CaCl}_2$ (1.0 h, 1:10, w/v) for magnesium and 2% CH₃COOH (1.0 h, 1:5, w/v) sulphur. Available contents of metals in soil samples were determined by single extraction using 1 mol·dm⁻³ HCl. The soil:solution ratio was 1:2 Concentrations of microelements in extracts were determined by flame atomic absorption spectrometry (FAAS) using a Varian Spectra AA 220 FS analyser.

Data presented in this study are means of results from three independent laboratories three laboratory analyses. The results were subjected to formal evaluation using the analysis of variance for double -factorial experiments (A – time of incubation and B – treatments) applying the F test at the level of significance p=0.95. The least significant differences were calculated using Tukey's test at the level of significance of $\alpha=0.05$ and then uniform groups within the factor level were established.

RESULTS AND DISCUSSION

As it is evident from data in table 1, basic physicochemical properties of soil represented by CEC, Hh and pH did not undergo significant changes under the influence of the applied AEM. The values of CEC, BS and Hh varied only between the applied organic substances. The effect was particularly significant in the case of sewage sludge and composts. The highest values of CEC (255.5 mmol·kg⁻¹), Hh (12.0 mmol·kg⁻¹) and BS (243 mmol·kg⁻¹) were found in T8, being 40 - 60% higher than in the control soil, where the lowest values of CEC

(180.0 mmol·kg⁻¹), Hh (6.0 mmol·kg⁻¹) and BS (174.3 mmol·kg⁻¹) was recorded (tab. 1). Incubation time and AEM had no significant effect on physicochemical properties. Soil supplementation with a sewage sludge dose (T6) and a compost dose (T8) also resulted in the highest values of N_{tot} and C_{tot}. Irrespective of the treatment, as a result of incubation the amounts of total nitrogen in soil increased by 30 to 40% and at the same time the total carbon content decreased (tab. 1). However, the effect of experimental factors was confirmed only in the case of total nitrogen content.

These results appear to contradict literature data, which indicate a positive effect of AEM on reducing soil acidification (Hh) and increased soil sorption capacity (cation exchange capacity - CEC) [Valarini et al. 2003]. It is assumed that the objective of EM introduction to the soil is, among others, to stimulate quantitative changes of humus. Such a phenomenon was also confirmed by Valarini et al. [2003], who found a significant increase in the contents of C_{tot} (2.5 – 3.0 fold) and N_{tot} (by 45-65%) in loamy soil following EM application. However, the reports of the above-mentioned researchers were not corroborated by the results of our investigations, since neither the C_{tot} nor N_{tot} content changed in a meaningful manner after the addition of AEM.

Tab. 1. Basic physicochemical properties of soil and total nitrogen and organic carbon content

Treatment-T Kombinacja	Hh* BS		S	CEC		N _{tot}		C _{org}		
	mmol·kg ⁻¹						g·kg ⁻¹			
	IS*	ES	IS	ES	IS	ES	IS	ES	IS	ES
1	6.0	6.0	174.3	176.1	180.3	182.1	1.24	1.43	11.17	10.9
2	7.5	7.5	234.0	220.2	241.5	227.5	1.05	1.49	13.44	12.04
3	7.5	7.5	222.8	214.5	230.3	222.0	1.06	1.45	13.14	12.43
4	10.5	9.0	224.2	176.8	234.7	185.8	1.25	1.71	13.14	12.29
5	10.5	9.0	214.7	176.3	225.2	185.3	1.24	1.73	13.05	12.01
6	12.0	9.6	235.3	231.7	247.3	241.3	1.42	1.84	14.9	13.9
7	10.1	8.3	227.5	230.6	235.8	240.7	1.43	1.83	14.3	13.5
8	12.0	8.5	243.5	240.6	255.5	249.1	1.41	1.87	14.9	13.9
9	9.0	8.7	239.7	230.9	247.7	239.6	1.40	1.86	14.8	13.4
LSD for A	n.s	- n.i.	n.s	– n.i.	n.s	– n.i.	0.07		n.s. – n.i.	
LSD for B	n.s n	ı.i.	n.s 1	n.i.	n.s	n.i.	0.15	5	n.s n.i.	
LSD for AxB	n.s n	ı.i.	n.s 1	n.i.	n.s	n.i.	0.22	2	n.s n.i.	

Tab. 1. Podstawowe właściwości fizykochemiczne gleby oraz zawartość azotu ogółem i wegla organicznego

*Descriptions see at Material and Methods n.s.- not significant - n.i. - nieistotne

Organic matter is a huge store of nutrients, which are present in strong bonds with organic compounds and as a result they are unavailable for plants. The possibility of nutrient release from the organic matrix is dependent on its decomposition. From the agronomic point of view, the most important are these nutrients, which are found in forms available for plants. The rate of organic matter decomposition strongly affects the amounts of nutrients in mineral, ionic forms, which may be directly absorbed by plant roots. For this reason this study focused on the available, mineral forms of essential macro- and micronutrients, which are of key importance for plant nutrition. According to Khaliq et al. [2006], Mupondi et al. [2006] and Muthaura et al. [2010], EM play an important role in the initiation of organic matter decomposition, leading to the liberation of nutrients. Data in Tables 2 and 3 show that the interaction of experimental factors significantly influenced amounts of analysed nutrients. The incubation process resulted in increased contents of nitrogen, phosphorus, sulphur, magnesium and potassium. Although this phenomenon was statistically confirmed (tab. 2), the rise in the amounts of available K, P and S was very slight about 13-16%. Despite such a common trend, it may be observed that in soil enriched with manure amounts of sulphur were higher by 20% (T4) or by 27% (T5), while it was by 35% (T5) in the case of potassium. On the other hand, the application of sewage sludge without EM (T6), and compost with and without EM (T8 and 9) resulted in an increase in the level of available phosphorus by 20 -25% (tab. 2). From among the analysed macroelements, quantitative changes in the examined soil were considerable in the case of nitrogen and magnesium. Contents of the other nutrients underwent greater changes under the influence of the experimental factor. Irrespective of the experimental combination, the quantitative level of nitrogen ranged from 15.48 (IS) to 23.03 mg·kg⁻¹ (ES), while that of magnesium ranged between 85.45 (IS) and 115.67 mg·kg⁻¹ (ES) (tab. 2). These quantitative changes are presented in detail in Table 2. Regardless of the type of organic substance added to the soil, amounts of available nitrogen during incubation increased, being from 22% (T9) to 2.0 times (T2 -T5) higher in relation to the content at the beginning of the process. Following soil incubation with an addition of organic substances and biofertilizer to the soil, magnesium levels were also found to grow. This phenomenon was particularly marked in the soil enriched with manure and sewage sludge, with and without AEM preparation. Regardless of the above mentioned treatment, the contents of Mg were higher by 1.5 (T6 and T7) and 2.0 (T5 and T6) times at the end of incubation in relation to those initially found in soil (tab. 2). At the same time it should be mentioned that the effect of EM on the level of mineral nitrogen and magnesium was negligible.

Tab. 2. Influence of interaction of experimental factors on amounts of macronutrients available for plants $(mg \cdot kg^{-1})$

Tab. 2. Interakcyjny wpływ czynników doświadczenia na zawartość przyswajalnych makroskładników dla roślin

Treatment Kombinacja	Nitrogen Azot		Phosphorous Fosfor		Sulphur Siarka		Potassium Potas		Magnesium Magnez	
5	IS*	ES	IS	ES	IS	ES	IS	ES	IS	ES
1	10.06	10.90	287.90	283.94	105.66	112.99	124.80	122.70	40.59	45.73
2	11.52	19.40	309.87	347.62	116.92	121.00	133.83	145.83	43.59	49.66
3	10.87	23.77	306.62	350.83	114.47	122.98	124.60	144.10	47.52	47.87
4	20.10	35.44	377.0	412.68	106.79	135.55	160.38	164.70	81.98	148.59
5	19.25	35.77	385.91	411.19	108.18	130.05	159.53	167.50	84.13	135.71
6	15.25	19.94	323.33	404.28	165.59	185.27	241.05	277.90	91.31	129.29
7	15.93	17.50	324.91	376.37	168.82	179.06	243.40	268.55	91.91	132.14
8	16.19	20.04	319.43	383.41	179.45	194.12	317.58	378.15	142.86	173.08
9	20.13	24.50	321.94	387.33	179.45	197.81	310.48	382.13	145.21	179.0
mean średnia	15.48	23.03	328.54	373.07	130.98	153.20	201.74	227.95	85.45	115.67
LSD for A	1.1	16	7.979		3.500		5.130		2.531	
LSD for AxB	3.348		23.941		10.500		15.390		7.592	

Descriptions see at Material and Methods

There was no statistical confirmation for the effect of incubation time on levels of mineral zinc and manganese forms in soil. In general the available amounts of iron and copper decreased during the incubation process (Tab. 3). After 9 months of incubation soil amended with manure alone (T4) and with EM (T5) was characterized by 22-29% lower amounts of copper. Simultaneously, soil incubation with a sewage sludge dose corresponding to 10 tons per hectare (T6) and together with AEM preparation (T7) showed revealed contents of iron lower by 33 and 31%, respectively (Tab. 3).

In view of the fact that the effect of incubation time on the release of nutrients in available forms was inconclusive in relation to the investigated elements, it was decided in this study to analyse their variability in terms of means for collection dates (Fig. 1). Such an interpretation of the data facilitates a comprehensive assessment of the effect of applied organic additives and EM. Generally the biofertilizer was found to have a slight effect. Irrespectively of the applied organic additions, the effect of EM was either statistically non-significant or it caused a decrease in the amounts available nutrients. As it is evident from data presented in figure 1, in comparison with the quantities of Fe, K, P and S in the soil supplemented with organic substances without AEM addition, their quantities were the same. With respect to mineral nitrogen, magnesium, copper, zinc and manganese, the effect of AEM was statistically verified; unfortunately, the quantitative differences were very small and ranged between 10 to 20% (Fig. 1). Primarily the mean values of macro- and microelements for different incubation times were strongly determined by the applied organic materials, with the lowest contents determined in the control soil and the highest amounts found in soil fertilized with manure (N, P, Zn and Cu) and compost (S, K, Mg and Mn). The differences between contents of elements found in the control soil and soil amended with organic substances were 1.5-4.0 fold for macroelements. Low variability needs to be in microelement contents in the examined experimental soil samples, with mean contents ranging from 27% (Cu) to 45% (Mn) (Fig. 1).

Tab. 3. Influence of interaction of experimental factors on amounts of micronutrients available for plants $(mg \cdot kg^{-1})$

Treatment Kombinacia	Copper Miedź		Zi Cy	nc mk	Mang Mar	anese 1gan	Iron Żelazo	
Komonaeja	IS	ES	IS	ES	IS	ES	IS	ES
1	3.33	3.05	13.65	15.05	100.79	114.99	740.00	771.71
2	3.05	2.98	16.45	16.32	98.43	100.79	923.88	894.97
3	3.05	2.20	18.25	16.75	100.79	112.63	945.62	902.14
4	3.92	2.77	16.05	15.45	110.26	105.53	967.36	917.36
5	3.54	2.77	19.00	18.95	110.73	114.73	989.10	880.40
6	3.23	2.97	17.60	14.43	137.52	125.02	1210.00	820.00
7	3.23	3.23	13.55	15.02	135.44	135.44	1200.00	830.00
8	3.22	3.50	13.25	16.19	156.27	154.18	930.00	920.00
9	3.23	2.63	14.43	14.43	145.85	131.27	915.00	934.75
mean średnia	3.31	2.90	15.80	15.85	121.79	121.62	980.11	874.59
LSD for A	0.2	207	n.sn.i.*		n.sn.i.		27.558	
LSD for AxB	0.622		2.931		10.076		82.675	

Tab. 3. Interakcyjny wpływ czynników doświadczenia na zawartość przyswajalnych mikroskładników dla roślin

* Descriptions see at Material and Methods n.s.- not significant - n.i. - nieistotne

There is no agreement in the literature on the effect of AEM on the amounts of available nutrients. Hu and Qi [2013] reported that application of EM significantly increased the uptake efficiency of organic nutrient sources. On the other hand, Mayer et al. [2010] found that EM did not improve soil quality. Javaid and Bajwa [2011] maintained that the lack of distinct changes in soil chemical properties after EM application is related to its single application. In view of the obtained data this statement may explain the results unfavorable for AEM recorded in this study. However, Mayer et al. [2010] cited above revealed no positive effects after 4 years of EM application in a field experiment. For this reason it is hard to prove the validity of practical use of Effective Microorganisms in soil fertility enhancement; what is more, soil fertility is usually combined with high levels of organic matter and available nutrients, which was not observed in the presented investigations.







Fig. 1. Mean amounts of nutrients in soil for experimental treatments Rys. 1 Ilości składników w glebie w uśrednieniu dla zastosowanych dodatków

CONCLUSIONS

1. The AEM preparation had no effect on the rate of decomposition of the applied organic substance during the incubation experiment, which showed its non-significant effect on total organic carbon as well as the basic physicochemical properties of soil represented by CEC, Hh and pH.

2. The use of AEM did not cause any increase in contents of available potassium, phosphorus, iron and zinc, while AEM had an effect on the amounts of available nitrogen, sulphur, magnesium and manganese.

3. The application of organic substances had a very strong, statistically confirmed effect on the content of available nutrients, which was observed especially in the case of manure and compost.

4. The higher amounts of available nitrogen, potassium, phosphorus, sulphur and magnesium found in soil samples after incubation were caused by the interaction of experimental factors.

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CZY EFEKTYWNE MIROORGANIZMY (EMTM) EFEKTYWNIE PODNOSZĄ ŻYZNOŚĆ GLEB?

Streszczenie

Preparatowi Efektywnych Mikroorganizmów (EM^{TM}) przypisuje się szerokie spektrum działania w produkcji roślinnej, co szczególnie wyrażone jest poprawą zdrowotności roślin oraz żyzności gleby. W niniejszej pracy w 9 miesięcznym doświadczeniu inkubacyjnym oceniano wpływ preparatu $EM-A^{TM}$ (zastosowanego w dawce odpowiadającej 100 L na hektar w kombinacji) z różnymi substancjami organicznymi (słoma pszenna, obornik, osad ściekowy, kompost) na żyzność gleby oraz uwalnianie się przyswajalnych składników pokarmowych dla roślin. Próbki glebowe do badań pobrano na początku oraz końcu procesu inkubacji. W zgromadzonym materiale glebowym zostały określone właściwości fizykochemiczne gleb oraz ilości składników przyswajalnych dla roślin. Na podstawie uzyskanych danych trudno w jednoznaczny sposób wykazać pozytywna rolę zastosowanego preparatu EM-ATM, ponieważ jego wpływ był słaby i nie został potwierdzony statystycznie. Czynniki doświadczenia (czas inkubacji oraz zastosowane substancje organiczne) istotnie oddziaływały na zmiany ilościowe badanych składników. W tym kontekście należy podkreślić szczególną rolę wprowadzonego obornika oraz kompostu

Słowa kluczowe: efektywne mikroorganizmy, rozkład, substancje organiczne, właściwości glebowe, przyswajalne składniki