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THE EFFECT AUTO-INOCULATION OF THE SOIL ON THE CHANGE IN THE AMOUNT OF LIVE MICROBIAL BIOMASS

WPŁYW AUTOSZCZEPIENIA GLEBY NA ZMIANY ILOŚCI BIOMASY ŻYWYCH MIKROORGANIZMÓW

Abstract: Out of three light soils of various parameters were isolated bacteria's, actinomycetes and fungi. Using those, autovaccines were prepared, individually for each of them. Then, in laboratory soil-conditions were given autovaccines and during incubation, samples were being taken, in which the content of biomass of living microorganisms were determined. The largest amount of biomass of living microorganisms was found in the soil from Stuchowo, the lowest in the soil from Swierzno. During the incubation of soils in the laboratory, the amount of biomass of living microorganisms decreased in soils from Stuchowo and Swierzno, while it increased in the soil from Kepica. Bioaugmentation resulted in a statistically significant increase in the amount of biomass of living microorganisms in all soils tested, reaching up to 30 % compared to non-vaccinated soil. The increase was the highest in the soil from Stuchowo and then in the decreasing order in the soils from Kepica and Swierzno.

Keywords: soil, microorganism biomass, auto-inoculation, bioaugmentation

Introduction

Auto-inoculation of soil with microorganisms is one of the ways of their reclamation or significant improvement of their biological properties. There are theoretical grounds as well as various practical applications of this method of improving the soil fertility or removing contaminants such as pesticides. These treatments are carried out using various types of microorganisms adapted to the intended purpose [1]. They are characterized by different effectiveness [2]. Usually, bioaugmentation with micro-organisms is used to remove petroleum substances from the soil [3, 4], stimulating plant growth and yield [5], and some biopreparations [6], however, ambiguous effects are

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obtained due to the application of microorganisms known as effective microorganisms – EM [7–12].

The auto-inoculation method, involving the introduction of microorganisms originating from the environment or the organism being bioaugmented, i.e. own microorganisms, is known and used in various cases, especially in medicine or veterinary medicine. There are numerous data proving its effectiveness [13–17].

Many researchers consider the soil abundant in microbiota o be analogous to a living organism. Indeed, it has many qualities that can attest to such similarity. So this is soil metabolism, consisting of numerous cycles of transformation and circulation of elements. Has the ability to grow or soil formation and finally knows cases of disease – that is, in the case of soil its degradation [18]. Therefore, the question arises whether also in the case of the soil environment, the bioaugmentation can be a method that allows stimulation of biological activity and development of microflora, i.e. factors on which the soil fertility depends [19–24].

In this work, an attempt was made to verify the usefulness of the soil auto-inoculation method with parent microflora to stimulate the development of soil microflora, the activity of which was a determined by measuring the amount of live microbial biomass.

Material and methods

Three light soils were used for the tests; their parameters are presented in Table 1. All soils belong to the agronomic category of light soils. They have a neutral or alkaline pH and are generally characterized by high or medium content of available nutrients.

Native microorganisms were isolated from each of the tested soils by the method of soil dilution culture. MPA medium was used for bacteria, Cyganov medium for actinomycetes, and Rose bengal agar for fungi. Then the dilution dishes were selected, in which the number of colonies was between 30 and 300 per one dish. Each of them was flooded with 10 cm^3 of MRD (maximum recovery diluent). After a few minutes, the dishes were washed, transferring the washed solution to sterile flasks. Each of them contained contents washed from 6 dishes. Then 20 cm^3 sterile aliquots were transferred from each flask to 200 cm^3 medium and incubated in an incubator at 25–28 ^oC for 72 hours. BW medium – enriched broth – with the addition of $Tween_{20}$ was applied for bacteria, Cyganov medium with the addition of $Tween_{20}$ for actinomycetes and MEB medium – broth with wort – and addition of $Tween_{20}$ for fungi was used for the culture.

The density was determined by densitometry by bacterial density in the inoculum was 10^7 CFU, fungi 10^5 CFU and actinomycetes 10^5 CFU. The inoculum prepared in this way (auto-inoculation, bioaugmentation) was applied to 1000 g samples of tested soils in doses of 1, 5 and 10 % by weight, respectively. Soil moisture was adjusted to 60 % MWC (maximum water capacity). Soil samples were then incubated in the laboratory for 56 days at 20 ^oC, correcting their humidity if necessary by controlling water loss by weighing the samples. On days 1, 7, 14, 28 and 56, soil was collected from each soil sample to determine the amount of live microbial biomass.

The amount of live microbial biomass in the soil was determined by the physiological method SIR (Substrate Induced Respiration) developed by Anderson and

Table 1

Characteristics of soils used in the study

Location	Soil agronomic category	pH_{KCL}	Content [mg · 100 g^{-1} d.m. soil]						Content [mg · 100 g^{-1} d.m. soil]							
			Phosphorus		Potassium		Magnesium		Manganese		Copper		Zinc		Iron	
			P_2O_5	Rating	K_2O	Rating	Mg	Rating	Mn	Rating	Cu	Rating	Zn	Rating	Fe	Rating
Kepica	light	6.8	18.5	high	14.2	average	9.6	very high	223.0	average	5.2	high	17.8	high	1420	average
Stuchowo	light	7.1	24.1	very high	21.2	very high	8.6	very high	177.7	average	5.6	high	18.1	high	1304	average
Swierzno	light	7.7	30.4	very high	6.5	low	4.5	average	119.2	average	3.3	high	12.3	high	1018	average

Domsch. A 10 g soil sample was thoroughly mixed with glucose diluted with talc at 1 : 5 ratio. The amount of this mixture needed was previously determined for each soil by determining the maximum initial respiration. The measurement was carried out using an Ultragas U4S gas analyzer, recording the amount of carbon dioxide evolved in the third hour of measurement. The amount of live microbial biomass in soil was calculated from the formula [25]:

$$X = 40.4 \cdot Y + 0.37 \quad (1)$$

where: X – biomass of live microbial biomass [mg · 100 g^{-1} d.m. soil];
Y – maximal initial respiration, as CO_2 produced by 100 g d.m. soil

Statistical interpretation of results was performed using the Statistica 12 software.

Results and discussion

The obtained results were subjected to multifactorial analysis of variance (multifactorial ANOVA), the summary of which is presented in Table 2. As the data in this table indicate, highly significant differences were recorded for both the type of soil (location) as well as the applied dose of the bioaugmentation, as well as for the length of incubation period soil in the laboratory, i.e. for all experimental factors. The interaction of soil type (location) and incubation time proved to be highly statistically significant, which means that changes in the amount of live microbial biomass took place differently in individual soils (locations).

Table 2

Analysis of variance for the obtained experimental results
(statistically significant values are marked in bold)

Effect	F-Statistic	*p*-Value
Total	**14 892.04**	**0.000**
Soil	**212.22**	**0.000**
Dose	**19.39**	**0.000**
Day	**21.96**	**0.000**
Soil · Dose	0.78	0.585
Soil · Day	26.42	0.000
Dose · Day	0.96	0.492
Soil · Dose · Day	0.84	0.677

The highest amount of live microbial biomass was found in soil from Stuchowo (Fig. 1), which is probably due to the highest content of minerals in this soil [23]. At the beginning of incubation, it contained about 2000 mg · 100 g^{-1} d.m. soil while at the end of this period – about 1600 mg · 100 g^{-1} d.m. soil. In other soils, the amount of live microbial biomass at the beginning of incubation was about 1400 mg · 100 g^{-1} d.m. soil. and at the end about 800 mg · 100 g^{-1} d.m. soil. in the soil from Swierzno and just over

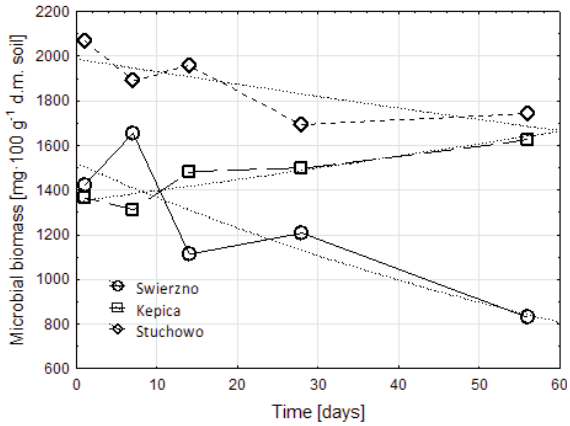

Fig. 1. Changes in the amount of live microbial biomass in tested control soils during incubation in laboratory conditions

1600 mg · 100 g^{-1} d.m. soil. in the soil from Kepica. On average, for the entire experiment period, the amount of live microbial biomass in control soils compared to the soil from Stuchowo was slightly more than 60 % in other soils. The content of live

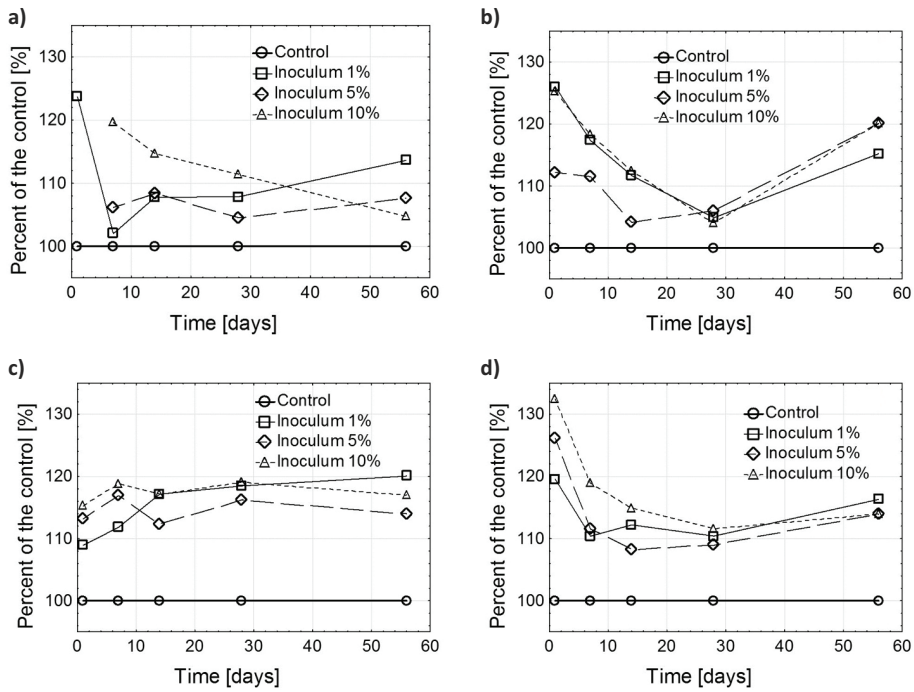

Fig. 2. Impact of soil incubation after inoculation on the content of live microbial biomass a) Swierzno, b) Kepica, c) Stuchowo, d) average

microbial biomass in soil originating in Swierzno and Stuchowo gradually decreased during incubation in the laboratory. This phenomenon is typical for soils incubated in the laboratory, which during the establishment of experiment were subject to mixing, moisturizing and other operations [24]. However, in the soil originating from Kepica, the amount of live microbial biomass increased over time. Such a phenomenon is observed in soil containing larger amount of organic substrate for microorganisms, e.g. from crop residues [22].

Introduction of the inoculum into the soil caused an increase in the amount of live microbial biomass. This phenomenon occurs most strongly in soil from Stuchowo, especially in the initial incubation period. Inoculum doses of 1 % and 10 % in this soil had the strongest effect. In the soil from Kepica and Swierzno, larger amounts of live microbial biomass were also observed after bioaugmentation with 1 % and 5 % inoculate doses being the strongest in the case of the latter. The average values for all soils clearly indicate an increase in the amount of live microbial biomass in the first incubation period, to about 10–15 days within 20–30 % compared to the control, and then within 10–15 %. The effect of a 1 % inoculum dose increases markedly at the end of soil incubation.

The average for individual inoculate doses calculated for all dates and locations indicates a clear increase in the amount of live microbial biomass after the bioaugmentation procedure (Fig. 3). Similar effects of 1 % and 5 % inoculum doses were observed, which increased the amount of live microbial biomass in soil by 13.77 % and 13.75 %, respectively. A larger increase occurred after the application of 10 % inoculum dose – it was 18.32 % compared to the control combination. As indicated by the cluster analysis (Fig. 4), nature of the data obtained for the control combination differs significantly from that obtained for soils subjected inoculation, which constitute a separate cluster. This additionally confirms the significance of changes in the amount of live microbial biomass in the soil after the treatment in question.

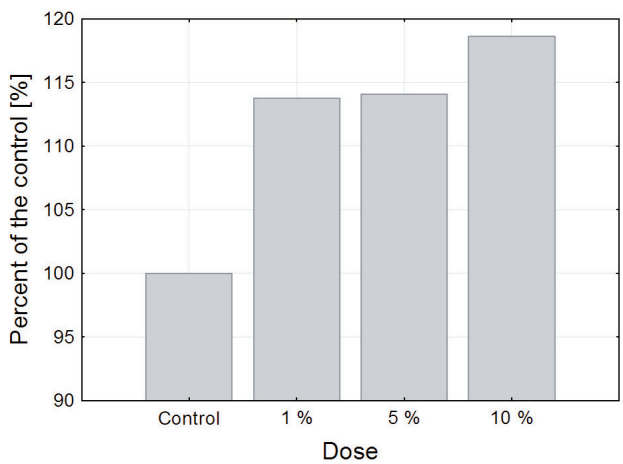

Fig. 3. Impact of soil incubation on the content of live microbial biomass expressed as a percentage in relation to the control

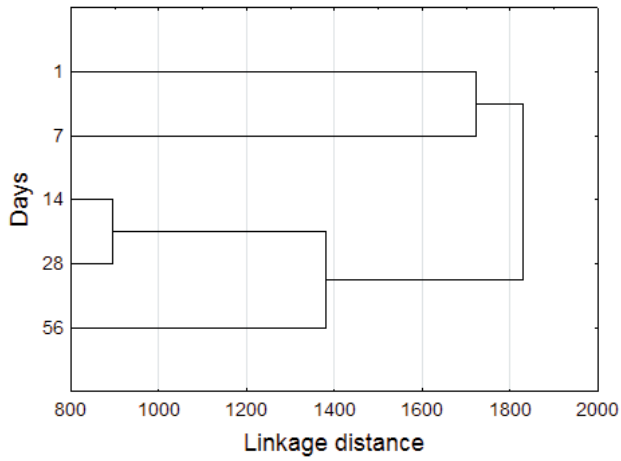

Fig. 4. Cluster analysis results based on the content of live microbial biomass found for various inoculants

The average amount of live microbial biomass in the inoculation soil, calculated for all applied inoculum and soil doses (locations), decreased gradually during the soil incubation in the laboratory, reaching at the end of this period a value of slightly over 80 % compared to the values found at the beginning of this period (Fig. 5).

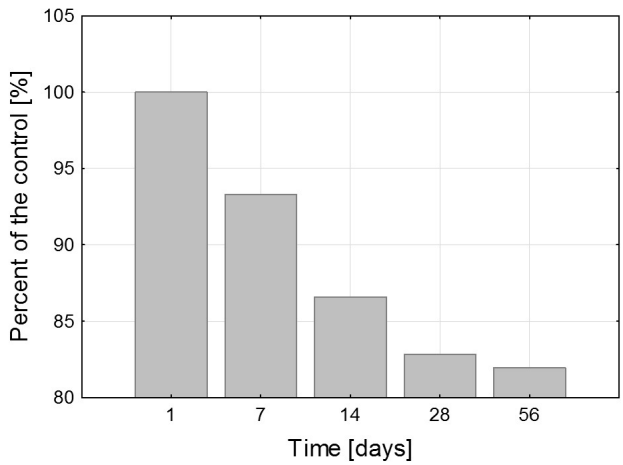

Fig. 5. Impact of soil incubation on the content of live microbial biomass expressed as a percentage in relation to the first day of incubation

Individual soils used in the studies after inoculation were characterized by diverse content of live microbial biomass (Fig. 6). The smallest amounts were found in soil from Swierzno. In the soil from Stuchowo, this amount was almost 50 % higher. The soil from Kepica occupied an intermediate position containing about 15 % more live

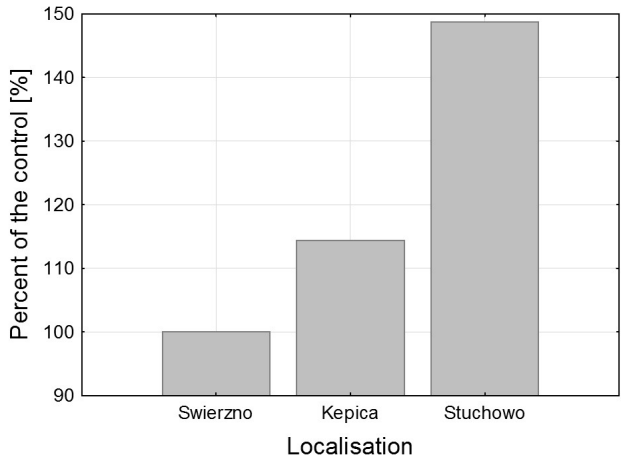

Fig. 6. The impact of soil incubation on the content of live microbial biomass expressed as a percentage in relation to the Swierzno location

microbial biomass than the soil from Swierzno. Analysis of the increase in the amount of live microbial biomass occurring as a result inoculation indicates similarity in the reaction of microflora in all soils. This increase amounts to 116.15 %, 113.95 % and 115.75 % for the soil from Swierzno, Kepica and Stuchowo, respectively, compared to the control combination.

The research conducted upon the reaction of soil inoculation with parent microflora allowed to determine the increase in the amount of live microbial biomass in the soil. It seems that this type of treatment leads to an increase in soil biological activity and may cause an increase in soil fertility and productivity, which should be the subject of further research.

Conclusion

1. During the incubation of soil in the laboratory, the amount of live microbial biomass changed. In the case of soil from Stuchowo and Swierzno, this quantity has been decreasing over time, while in the soil from Kepica, the content of live microbial biomass increases during incubation. The highest amounts of live microbial biomass were found in the soil from Stuchowo, the smallest in the soil from Swierzno.

2. Bioaugmentation causes statistically highly significant increase in the amount of live microbial biomass in all soils tested. This increase changed during the soil incubation and ranged from 20–30 % at the beginning to 10–15 % at the end for the tested soils.

3. Bioaugmentation resulted in a significant increase in the amount of live microbial biomass in the soil. It was close to 14 % for 1 % and 5 % inoculate doses, while the 10% dose resulted in an increase in the amount of live microbial biomass by nearly 20 %.

4. The smallest increase in the amount of live microbial biomass after bio-augmentation was found in soil from Swierzno, and then in growing order in soils from Kepica and Stuchowo.

References

[1] Kosicka D, Wolna-Maruwka A, Trzeciak M. Kosmos. 2015;64(2):327-35. Available from: http://kosmos.icm.edu.pl/PDF/2015/327.pdf.

[2] Mayer J, Scheid S, Widmer F, Fließbach A, Oberholzer H-R. Appl Soil Ecol. 2010;46(2):230-9. DOI: 10.1016/j.apsoil.2010.08.007.

[3] Bento FM, Camaargo FAO, William CO, Frankenberger T. Bioresource Technol. 2005;96(9):1049-55. DOI: 10.1016/j.biortech.2004.09.008.

[4] Hamdi H, Benzarti S, Manusadžianas L, Aoyama I, Jedidi N. Soil Biol Biochem. 2007;39(8):1926-35. DOI: 10.1016/j.soilbio.2007.02.008.

[5] Martyniuk S. J Res Appl Agricult Eng. 2010;55:420-23. Available from: http://yadda.icm.edu.pl/baztech/element/bwmeta1.element.baztech-article- BAR9-0009-0004/c/httpwww_pimr_poznan_plbiul20104sm.pdf.

[6] Wrońska I, Onyszko M, Cybulska K, Telesiński A, Mahdi-Oraibi S. Proc ECOpole. 2015;9(2):795-801. DOI: 10.2429/proc.2015.9(2)090.

[7] Martyniuk S, Księżak J. Polish J Agron. 2011;6:27-33. Available from: http://www.iung.pulawy.pl/PJA/wydane/6/PJA6_4.pdf.

[8] Janas R. Problemy Inż Rolniczej. 2009;3:111-8. Available from: http://yadda.icm.edu.pl/baztech/element/bwmeta1.element.baztech-article- BAR0-0046-0051/c/httpwww_ibmer_waw_plpir2009pelne3janasmozliwoscip.pdf.

[9] Kaczmarek Z, Wolna-Maruwka A, Jakubus M. J Res Appl Agricult Eng. 2008;53:122-8. Available from: https://www.researchgate.net/profile/monika_jakubus/publication/266180383_ changes_ of_the_number_of_selected_microorganism_groups_and_enzymatic_activity_ in_the_soil_ inoculated_with_effective_microorganisms_em/links/5504344c0cf24cee39fe1c8f/ changes-of-the-number-of-selected-microorganism-groups-and-enzymatic-activity-in-the-soil-inoculated-with-effective-microorganisms-em.pdf.

[10] Gałązka A, Kocoń A. Studia i Raporty IUNG-PIB. 2015;45(19):127-42. Available from: https://www.researchgate.net/publication/319465131_wplyw_preparatow_ z_mikroorganizmami_pozytecznymi_na_liczebnosc_i_biomase_mikroorganizmow_glebowych

[11] Avis TJ, Gravel V, Antoun H, Tweddell RJ. Soil Biol Biochem. 2008;40(7):1733-40. DOI: 10.1016/j.soilbio.2008.02.013.

[12] Górski R, Kleiber T. Ecol Chem Eng S. 2010;17(4):505-13. Available from: https://docplayer.net/104537527-Effect-of-effective-microorganisms-em-on-nutrient-contents-in-substrate-and-development- and-yielding-of-rose.html.

[13] Gliński Z, Chełmiński A. Życie Weterynaryjne. 2014;85:499-504. Available from: http://yadda.icm.edu.pl/yadda/element/bwmeta1.element.agro-c12e4e96-69db-451b-b06a-cb7d8ee7c7de/c/ZW-2014-06-07.pdf.

[14] Lal NR, Sil A, Gayen T, Bandyopadhyay D, Das NK. Indian J Dermatol Venereol Leprol. 2014;80:515-20. DOI: 10.4103/0378-6323.144146.

[15] Sonthalia S, Arora R, Sarkar R. Indian J Dermatol Venereol Leprol. 2014;80:361-2. DOI: 10.4103/0378-6323.136933.

[16] Valentine DL, Mezić I, Maćešić S, Črnjarić-Žic N, Ivić S, Hogan PJ, et al. Proc National Acad Sci. 2012;109(50):20286-91. DOI: 10.1073/pnas.1108820109.

[17] Witkowski L, Kaba J, Rzewuska M, Kita J. Życie Weterynaryjne. 2008;83(5):365-70. Available from: http://vetpol.org.pl/dmdocuments/2008_05_04.pdf.

[18] Mazur T. Zesz Probl Post Nauk Roln. 1995;422:9-19. Available from: https://www.infona.pl/resource/bwmeta1.element.agro-article-baf446cd-2d2e-4957-8a19-cf56cbb3e339/tab/summary?fbclid=IwAR28eZS7F0wBq1NurJKafKkb5RBKivtRsMGuXbqg1-A4QphNqfazpH0BTfE.

[19] Brookes P. Microbes Environ. 2001;16:131-40. DOI: 10.1264/jsme2.2001.131.

[20] Feng-Min L, Qiu-Hua S, Jjemba PK, Yuan-Chun S. Soil Biol Biochem. 2004;36(11):1893-902. DOI: 10.1016/j.soilbio.2004.04.040.
[21] Johns C. Living soils: The role of microorganisms in soil health. Future Directions Int. 2017. Available from: https://www.futuredirections.org.au/wp-content/uploads/2017/06/Living-Soils-the-Role-of-Microorganisms-in-Soil-Health.pdf.
[22] Li L, Xu M, Ali ME, Zhang W, Duan Y, Li D. PloS ONE. 2018;13(9):e0203812. DOI: 10.1371/journal.pone.0203812.
[23] Talwar HK, Anshu S. Int J Adv Res. 2018;6:1502-20. DOI: 10.21474/IJAR01/7960.
[24] Khan SU, Hooda PS, Blackwell MSA, Busquests R. Frontiers Environ Sci. 2019;7:1-9. DOI: 0.3389/fenvs.2019.00133.
[25] Anderson PE, Domsch KH. Soil Biol Biochem. 1978;10:215-21. DOI: 10.1016/0038-0717(78)90099-8.