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INFLUENCE OF PESTICIDES ON MICROBIAL ACTIVITY IN SELECTED SOIL TYPES OF SLOVAKIA

WPŁYW PESTYCYDÓW NA AKTWYNOŚĆ MIKROORGANIZMÓW W WYBRANYCH TYPACH GLEBY NA SŁOWACJI

Abstract: The aim of our work was to determine influence of pesticides on the soil respiration and the numbers of microorganisms (bacteria and their spores utilizing organic and inorganic nitrogen, actinomycetes, myxobacteria, *Azotobacter chroococcum,* microscopic fungi) in the three soil types (Haplic Chernozem, Haplic Luvisol, Cambisol).

Cumulative values of basal CO_2 production for 21 days represented from 595.62 mg · kg^{-1} to 1045.79 mg · kg^{-1} d.m. soil in tested samples of Haplic Chernozems and from 424.6 mg · kg^{-1} to 540.28 mg · kg^{-1} d.m. in tested samples of Haplic Luvisols and from 1789.84 mg · kg^{-1} to 2103.81 mg · kg^{-1} d.m. in tested samples of Cambisols. Potential CO_2 production was higher (statistical significantly, $p < 0.01$) in all variants (with addition of glucose, PVAL, herbicide and fungicide) than basal one. Stimulating effect of glucose addition was more expressive in Haplic Luvisol than in Haplic Chernozem and Cambisol. Pesticides addition did not significantly affect on the decrease of numbers of bacterial vegetative forms in the soil types Haplic Chernozem and Cambisol. The insignificantly decrease was observed in the numbers of bacterial spores in the soil type Cambisol and in the numbers of microscopic fungi only in the soil type Haplic Chernozem.

Keywords: soil respiration, pesticides, physiological groups of microorganisms, soil type

Millions of tons of xenobiotic compounds are applied globally as pesticides in agricultural production in soil each year. Soil, natural water in rivers, lakes, and aquifers has been contaminated with trace amounts of pesticide residues. Microorganisms and their enzymes play an essential role in the bioconversion and total breakdown of pesticides and other xenobiotics in the environment. The principal enzymes responsible for the bioconversion are various lyases and oxydoreductases, specifically hydrolyses, oxygenases and various enzymes capable of dehalogenation [1]. Metabolic pathway diversity depends on the chemical structure of the xenobiotic compound, the organism evironmental conditions, metabolic factors, and the regulating expression of these biochemical pathways [2]. The important role played by microorganisms in the degradation of pesticide residues is well recognized. Pieces of information were

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published about microbial strains (bacteria and fungi) capable of degrading such recalcitrant pesticides as 2,4 D or Dicamba [3], Dichlobenil [4], triazine herbicide Simazine [5], Folicur or Amistar [6], Topogard [7] etc. The xenobiotics affect soil properties in many different ways. Microbial and biochemical properties of soils are evidently more sensitive to stress factors including pollutants than the chemical and physical properties of soils [8]. Mineralization of organic compounds is characteristic for growth-linked biodegradation, in which the organisms converts the substrate to CO_2, cell components, and products typical of the usual catabolic pathways [9]. Soil respiration reacts differently to variant and cultivation methods and has been used most frequently for the assessment on the side effects of chemicals such as pesticides and heavy metals, etc. [10].

The investigations were conducted to microbial respirations and microbial numbers after application of pesticides in the different soil types in Slovakia (Haplic Chernozem, Haplic Luvisol and Cambisol). The influence of pesticide on the observed microbial characteristics was evaluated by statistical method in contrast with variants without additive or with additives such as glucose (easily available source of carbon) or polyvinyl alcohol (difficultly available source of carbon).

Material and methods

Selected chemical and microbial parameters were studied during the years 2001–2007 in the different soil types (from Haplic Chernozem – arable soils, Haplic Luvisol – arable soils to Cambisol – mountain soil, meadow and pasture). The Haplic Chernozem (HCh) and Haplic Luvisol (HL) are in the most fertile south-western part of Slovakia with altitude from 121 to 190 meters above the sea level (m a.s.l.). This part of Slovakia has own abnormal, warm, climatic conditions, the average year temperature of 9.7 ^{o}C and the sum of precipitation amounting to 561 mm. Haplic Chernozems were sampled from 7 localities of Drazovce (138 m a.s.l.), Sladkovicovo (121 m a.s.l.), Voderady (137 m a.s.l.), Stefanovicova (130 m a.s.l.), Kalna nad Hronom (160 m a.s.l.), Svatoplukovo (140 m a.s.l.) and Borovce (160 m a.s.l). Haplic Luvisols were sampled from 7 localities of Golianovo (149 m a.s.l.), Kolinany (190 m a.s.l.), Plave Vozokany (185 m a.s.l.), Nove Sady (168 m a.s.l.), Malanta (180 m a.s.l.), Risnovce (161 m a.s.l.) and Velke Ripnany (174 m a.s.l.). Cambisol (C) were sampled from 4 localities of "Pod Ploskou" 1.240 m a.s.l. (National park Velka Fatra), "Strungovy prislop" 1.150 m a.s.l. (National park Mala Fatra) and "Pod Keckou" 1.100 m a.s.l. (National park Nizke Tatry) and "Diel" – 920 m a.s.l. (sierra Stolicke vrchy). The Cambisols are situated in the region with very cold and wet climate, the average year temperature of 4 ^{o}C and the sum of precipitation amounting to 1.250 mm.

Soil samples (average of 4 probes) from arable soils were taken in spring (April) period after pre-sowing preparation and mountain soils in summer (July), from the layer (0–0.3 m). The samples were mixed; stones, plant and animal residues were eliminated and sieved through the 2 mm sieve and subjected to the samply chemical analyses. Proportion of the soil samples was stored (pre-incubated) during 8 weeks at temperature 4±1 ^{o}C [11, 12], for analyzed microbial parameters and activities. The respiration

activity (basal – Variant 1 and potential soil respiration – Variant 2–5) of microorganisms was observed through production of CO_2 by titration method with HCl in four replications for 9 times during 21 days incubation at 28 °C. Potential respiration of microorganisms was measured in the presence of glucose – G (2 g · kg^{-1} of soil) – Variant 2, polyvinyl-alcohol – PVAL (2 g · kg^{-1} of soil) – Variant 3, herbicide – Gesagard 80 (0.6 g · kg^{-1} of soil) – Variant 4 and fungicide-Fundazol 50 WP (0.24 g · kg^{-1} of soil) – Variant 5.

The following parameters were determined in the fresh samples:

– dry matter (d.m.) of samples,

– actual (pH/H_2O) and exchangable (pH/KCl) soil reaction-potentiometrically in 1 mol · dm^{-3} KCl solution,

– oxidizable carbon (C_{ox}) according to Tiurin method,

– total nitrogen (N_t) by distillation method according to Kjeldahl,

– NO_3^- – N colorimetrically (Interferometer 1100 Carl Zeiss, Jena, Germany) with phenoldisulphonic acid immediately after sampling and after 14 days of incubation at 28 °C, soil moisture was adjusted to 60 % full water holding capacity,

– NH_4^+ – N colorimetrically (Interferometer 1100 Carl Zeiss, Jena, Germany) with Nessler agent immediately after sampling and after 14 days incubation at 28 °C, soil moisture was adjusted to 60 % of full water holding capacity,

– biologically releasable nitrogen (N_{biol}) was calculated as a difference of inorganic nitrogen values before and after soil samples incubation (14 days),

– inorganic nitrogen (N_{in}) was calculated as the summation of ammonium and nitrate(V) form of nitrogen content,

– nitrification, which was calculated of the values NO_3^- – N as a difference of the values determined in non-incubated and incubated samples (14 days, 28 °C, 60 % of full water holding capacity).

After pre-incubation (8 weeks) the samples were moisturised to 60 % of full water holding capacity and further characteristics were determined:

– carbon of microbial biomass (C_{mic}) by fumigation extractive method [13],

– test of respiration, basal and potential (addition of glucose per kg of soil) production of CO_2 in model trial by titrimetric absorption method [14]. Respiration rates were determined after 24, 48, 72, 96, 120, 144, 168, 336 and 504 h by trapping CO_2 in 0.1 M NaOH. The residual NaOH was titrated with 0.1 M HCl after carbonates were precipitated with 0.5 M $BaCl_2$,

– hot water extractable carbon (C_{hwl}) [15],

– dehydrogenase activity (DHA) by triphenyltetrasolium chloride (TTC) [16],

– FDA hydrolysis by fluorescein diacetate [17],

– phosphatase activity [18]

– cellulose degradation in model experiment [19],

– microorganisms counts (after 21 days finishing of experiment). The classic dilution plating method was used for evaluation of microorganism counts using different agar media for their identification: bacteria and their spores utilizing organic nitrogen on meat-peptone agar (MPA), bacteria and their spores utilizing inorganic nitrogen on Thornton agar (TA), myxobacteria on champignon agar (ChA), microscopic fungi on

Czapek-Dox agar (Cz-DA) and malt extract agar (MEA), actinomycetes on Krainsky agar (KA) and Waksmann agar (WA) and *Azotobacter chroococcum* on Ashby's agar (AA). Numbers of colony-forming units (CFU) were expressed as logarithms per 1 g d.m. soil for statistical analysis.

All results are calculated on a soil dry matter (d.m.). Statistical processing of gained data were realized in Statgraphics 5.0 programme. Two-way univariate ANOVA was used for statistical analysis of the effects of variants and localities. In case of significant F-statistics Tukey test ($p \leq 0.01$) was selected to separate the means.

Results and discussion

The biological activity of microorganisms and their numbers were observed in the three different soil types after application of pesticide in laboratory conditions, for 21 days. These soil types were different not only in cultivation of soils (arable or meadow), but in the primary chemical and biological characteristics, too (Table 1).

Table 1

Average values (± standard deviation) of biological and chemical properties of observed soil types

Parameter	Unit	Soil type		
		Haplic Chernozem (n = 7)	Haplic Luvisol (n = 7)	Cambisol (n = 4)
C_{ox}	[%]	1.78 (0.31)	1.17 (0.16)	4.69 (1.90)
N_t	[%]	0.21 (0.02)	0.14 (0.02)	0.35 (0.12)
C : N	[-]	8.0 (1.11)	8.20 (0.94)	13.22 (1.92)
pH in H_2O	[-]	7.58 (0.43)	7.02 (0.66)	5.67 (0.50)
pH in KCl	[-]	6.62 (0.60)	5.92 (0.77)	5.14 (0.45)
N_{in}	[mg · kg^{-1} d.m.]	8.20 (3.09)	6.90 (6.16)	26.44 (7.50)
N_{biol}		8.75 (3.04)	10.18 (2.71)	36.67 (18.10
Nitrification		10.23 (2.95)	11.65 (4.46)	28.67 (7.90)
C_{hwe}		0.42 (0.08)	0.33 (0.08)	0.93 (0.29)
DHA	[mg · g^{-1} · h^{-1} d.m.]	9.64 (5.70)	4.87 (1.71)	16.24 (5.47)
FDA hydrolysis	[ΔA · g^{-1} · h^{-1} d.m.]	0.15 (0.05)	0.19 (0.07)	0.73 (0.17)
Phosphatase activity	[μg PNF · g^{-1} · h^{-1} d.m.]	12.43 (4.94)	20.66 (9.12)	64.45 (16.77)
C_{mic}	[mg · g^{-1} d.m.]	369.89 (206.34)	227.36 (188.03)	1294.60 (297.44)
Cellulose degradation	[%]	44.06 (17.28)	35.15 (21.43)	34.02 (24.54)

A – absorbation, PNF – triphenyltetrasolium chloride.

The Haplic Chernozem and Haplic Luvisol were arable soil with neutral soil reaction and similar chemical and biological properties. The weak acidity and acidity soil reaction and higher values of the all measured biological parameters (except for cellulose degradation) were characteristic for the soil type Cambisol (Table 1). The Cambisol had the

high carbon (C_{ox}) content and nitrogen organic and inorganic substances from pasture of cattle in these tested localities. The incidence of bacteria in soil samples was evidently dependent on the presence of fresh organic matter rather than the total carbon content in soil [20].

Course of cumulative values of basal CO_2 production for 21 days represented from 95.40 to 739.31 mg · kg^{-1} d.m. in tested samples of Haplic Chernozem, from 63.06 to 537.77 mg · kg^{-1} d.m. in tested samples of Haplic Luvisol and 104.7 to 1905.28 mg · kg^{-1} d.m. in tested samples of Cambisol (Fig. 1). Basal respiration represented mineralization of native organic substances in soil samples. The observed positive chemical characteristics of soil type Cambisol were confirmed with values from microbial respiration. The basal respiration of Cambisol was higher than basal respiration of Haplic Luvisol and Haplic Chernozem.

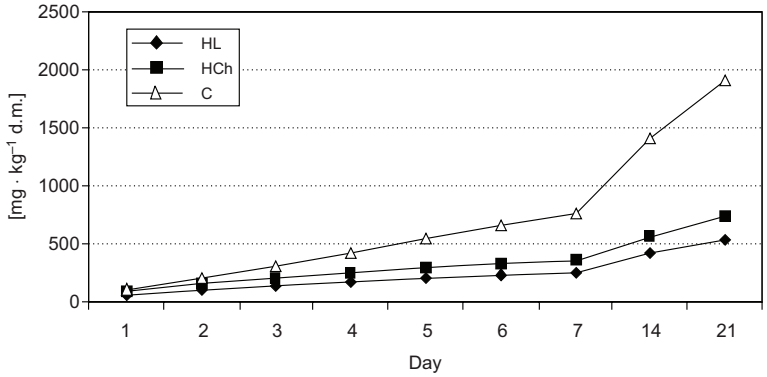

Fig. 1. Basal production (cumulative values) of CO_2 in tested soil types

Presence of active part of microflora in layer to 0.3 m of soil types was proved by determined CO_2 production values (Table 2).

Table 2

Basal and potential cumulative production of CO_2 [mg · kg^{-1} d.m.] in the observed soil types

Soil type	Variant				
	1	2	3	4	5
HCh	739.31 (218.24)	2503.99 (495.07)	1133.31 (261.21)	1053.55 (323.99)	999.28 (259.12)
HL	537.77 (115.28)	2562.58 (118.80)	877.58 (158.31)	699.08 (88.00)	781.42 (144.80)
C	1905.28 (345.32)	4435.61 (760.98)	2510.32 (406.11)	2381.61 (369.75)	2431.80 (445.42)

In brackets are values of standard deviation.

All of the additives including pesticides increased production of CO_2 in all the tested soil types during 21 days of incubation (Table 2). The negative influence (decrease) of pesticides on the microbial respiration was not confirmed as in the herbicide Topograd

application in acid soils [7]. Maximal value was measured in soil type Cambisol and the best stimulative effect (4.77 times) of glucose addition on the microbial respiration was determined in the Haplic Luvisol. It was consequence of deficiency of organic matter in this soil type. The course of respiration during 21 days respiration was similar between variants with PVAL and pesticides.

The biological activity of microorganisms in samples with pesticides in all soil types was comparable with samples which were amended with PVAL according to statistical evaluation of production of CO_2 (Table 4). Toxic influence of pesticides on the microbial respiration was not significant in the observed soil types. Fungicide was easier accessible source of carbon than herbicide for present soil microorganisms (Fig. 2). Glucose increased the production of CO_2 in comparison with values of the basal respiration. This stimulative effect was statistically significant ($p < 0.01$) in all soil types (Table 4).

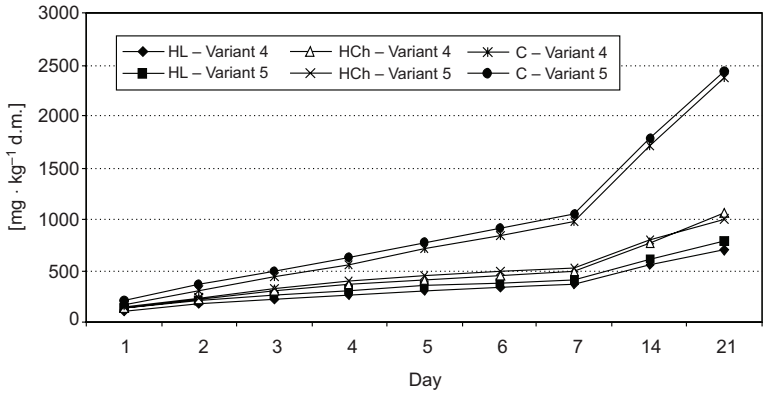

Fig. 2. Potential production (cumulative values) of CO_2 in observed soil types after addition of herbicide (Variant 4) and fungicide (Variant 5): HCh – Haplic Chernozem, HL – Haplic Luvisol, C – Cambisol

However, high CO_2 production is still not in agreement with numerous occurrences of microorganisms in samples. The very good correlation between bacterial numbers and the biodegradation of pesticides MCPP and IPU in sand soils presented Vinther et al [21]. However the high soil respiration can be a result of high activity of small soil microbial community as well as a result of low activity of large microbial community [22]. In a consequence of this it is important to know not only quantitative occurrence of microorganisms in soil, but also their specific composition and degrading capabilities.

The occurrence of microorganisms (Table 3, 4) corresponded with usual counts of microbes in arable soils [23] and soils of meadows and pastures [24]. The most numerous group from observed physiological groups in variant without additives (Variant 1) were bacterial vegetative forms and their spores utilizing organic nitrogen on meat-peptone agar (MPA) (Table 3). It was typicall for all observed physiological groups of microorganisms in comparison with soil types Haplic Chernozem and Haplic Luvisol. Preponderance of microorganisms remained multiplied after application of

pesticides in soil types Haplic Luvisol and Haplic Chernozem. The pesticides application inhibited the numbers of bacteria and their spores on the MPA and TA only in the soil type Cambisol (Table 3).

Table 3

Occurrence of bacteria and their spores in the 10^5 CFU · g^{-1} d.m. on the MPA and TA in Haplic Chernozem (n = 7), Haplic Luvisol (n = 8), Cambisol (n = 4)

Physiological group	Soil type	Variant				
		1	2	3	4	5
A	HCh	84.97 (91.52)	65.94 (68.00)	120.21 (124.32)	125.08 (119.24)	169.48 (148.74)
	HL	363.84 (716.84)	420.07 (763.12)	409.33 (757.60)	401.83 (809.69)	709.10 (1209.31)
	C	357.11 (247.05)	682.80 (664.12)	406.39 (416.93)	327.51 (361.66)	270.36 (208.48)
B	HCh	65.77 (96.80)	108.32 (155.97)	105.72 (155.46)	90.41 (141.75)	47.61 (44.32)
	HL	59.02 (63.64)	125.60 (183.92)	54.45 (56.69)	80.18 (97.23)	153.22 (204.05)
	C	704.85 (1110.76)	401.89 (367.12)	627.53 (1068.00)	150.06 (189.39)	145.81 (79.28)
C	HCh	542.33 (1186.44)	133.73 (897.63)	387.83 (736.39)	106.89 (116.48)	112.01 (113.52)
	HL	124.84 (185.11)	72.33 (64.37)	133.94 (135.35)	220.49 (343.75)	229.14 (209.95)
	C	238.79 (318.76)	401.98 (498.86)	244.52 (184.42)	209.50 (230.58)	118.41 (60.85)
D	HCh	51.24 (65.47)	72.27 (60.45)	68.67 (108.95)	54.76 (68.83)	82.72 (79.94)
	HL	80.35 (143.07)	86.62 (172.78)	96.12 (181.61)	661.96 (1457.89)	139.67 (250.95)
	C	99.14 (139.22)	16.58 (13.96)	40.24 (47.98)	63.27 (126.61)	36.14 (41.57)

A – bacteria utilizing organic nitrogen on (MPA, B – spores of bacteria utilizing organic nitrogen on MPA, C – bacteria utilizing inorganic nitrogen on TA, D – spores of bacteria utilizing inorganic nitrogen on TA, ND – non-deteremined, HCh – Haplic Chernozem, HL – Haplic Luvisol, C – Cambisol; in brackets are values of standard deviation.

Application the all of additives and pesticides had statistically insignificantly negative influence on the numbers observed physiological groups of microorganisms (Table 4). The Variant after glucose significantly stimulated the height numbers *Azotobacter chroococcum* in the Haplic Chernozem. Numbers of vegetative forms of bacteria (MPA and TA) and microscopic fungi decreased (statistically insignificantly) only in soil types: Haplic Chernozem and Cambisol. The occurrence of actinomycetes

was increased (statistically insignificantly) after application of pesticide in all the tested soil types. It is demonstration of very good adaptability of this group on the extraneously chemicals in the soil.

Table 4

Multiple range analysis for cumulative CO_2 production [mg · kg^{-1} d.m.] and total numbers of microorganism [log CFU · g^{-1} d.m.] in the soil types after 21 days of incubation (ANOVA, Tukey test, $p \leq 0.01$)

Respiration		Numbers of microorganism					
Variant	CO_2	Bacteria MPA + TA	Spore MPA + TA	Actino-mycetes	Myxo-bacteria	*Azotobacter chroococcum*	Microscopic fungi
Haplic Chernozem (n = 7)							
1	739.31^{a}	7.79^{a}	7.07^{a}	4.04^{ab}	3.24^{a}	3.22^{a}	4.31^{a}
2	2503.99^{c}	7.73^{a}	7.26^{a}	3.89^{a}	3.37^{ab}	3.77^{b}	4.28^{a}
3	1133.31^{b}	7.71^{a}	7.24^{a}	4.06^{ab}	3.33^{ab}	3.44^{a}	4.28^{a}
4	1053.55^{b}	7.37^{a}	7.16^{a}	4.23^{b}	3.42^{ab}	3.28^{a}	4.23^{a}
5	999.28^{ab}	7.45^{a}	7.12^{a}	4.14^{ab}	3.51^{b}	3.33^{a}	4.16^{a}
Haplic Luvisol (n = 7)							
1	537.77^{a}	7.72^{a}	7.18^{a}	4.18^{a}	3.48^{a}	3.40^{a}	4.07^{a}
2	2562.58^{d}	7.69^{a}	7.33^{a}	4.06^{a}	3.47^{a}	2.86^{a}	4.00^{a}
3	877.58^{c}	7.74^{ab}	7.18^{a}	4.67^{a}	3.76^{a}	3.31^{a}	4.11^{a}
4	699.079^{b}	7.79^{ab}	7.87^{a}	4.87^{a}	3.74^{a}	3.38^{a}	4.14^{a}
5	781.42^{bc}	7.97^{b}	7.47^{a}	4.87^{a}	3.88^{a}	3.45^{a}	4.10^{a}
Cambisol (n = 4)							
1	1905,27^{a}	7.78^{ab}	7.91^{a}	3.48^{a}	ND	ND	ND
2	4435.61^{c}	8.08^{b}	7.62^{a}	3.00^{a}	ND	ND	ND
3	2510.32^{b}	7.81^{ab}	7.82^{a}	3.78^{a}	ND	ND	ND
4	2381,61^{b}	7.73^{a}	7.33^{a}	3.97^{a}	ND	ND	ND
5	2431.80^{b}	7.59^{a}	7.26^{a}	3.42 ^{a}	ND	ND	ND

Differences between values (intra – columns) followed by the same common letter are not significant; ND – non-deteremined.

Conclusions

1. Potential CO_2 production was higher (statistically significantly, $p \leq 0.01$) in all variants (with addition of glucose, PVAL, herbicide and fungicide) than basal one.

2. Pesticides addition did not significantly affect decrease of numbers of bacterial vegetative forms in the soil types Haplic Chernozem and Cambisol. The insignificantly decrease was found only in the numbers of bacterial spores in the soil type Cambisol and in the numbers of microscopic fungi only in the soil type Haplic Chernozem.

3. Fungicide was easier accessible source of carbon than herbicide for present soil microorganisms.

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WPŁYW PESTYCYDÓW NA AKTWYNOŚĆ MIKROORGANIZMÓW W WYBRANYCH TYPACH GLEBY NA SŁOWACJI

Abstrakt: Celem naszych badań było określenie wpływu pestycydów na oddychanie gleby oraz liczebność mikroorganizmów (bakterii oraz ich spor zużywających organiczny i nieorganiczny azot, promieniowców, myxobakterii, *Azotobacter chroococcum*, mikroskopijnych grzybów) w trzech typach gleby (czarnoziemu, płowej, brunatnoziemnej). W glebie typu Haplic Chernozem kumulatywne wartości podstawowej produkcji CO_2 w ciągu 21 dni wynosiły od 595.62 do 1045.79 mg · kg^{-1} s.m. (suchej masy gleby). W glebach Haplic Luvisols wartości te wynosiły od 424.6 do 540.28 mg · kg^{-1} s.m., a w glebach typu Cambisol od 1789.84 do

2103.81 mg · kg^{-1} s.m. Wartości podstawowej produkcji CO_2 były mniejsze od potencjalnej produkcji CO_2 (różnice istotne statystycznie przy $p < 0,01$) we wszystkich wariantach eksperymentu (dodatki glukozy, PVAL, herbicydu, fungicydu). Stymulujący wpływ glukozy był bardziej wyraźny w glebie typu Haplic Luvisol niż w glebie Haplic Chernozem i Cambisol. Pestycydy nie wpłynęły w sposób istotny statystycznie na zmniejszenie liczebności wegetatywnych form bakterii w glebach typu Haplic Chernozem i Cambisol. Zaobserwowano nieistotny statystycznie spadek liczby spor bakteryjnych w glebie typu Cambisol oraz liczebności mikroskopijnych grzybów w glebie typu Haplic Chernozem.

Słowa kluczowe: oddychanie gleby, pestycydy, fizjologiczne grupy mikroorganizmów, typy gleby