DE GRUYTER OPEN

DOI: 10.2478/eces-2014-0037 ECOL CHEM ENG S. 2014;21(3):499-514

Alicja KSIĘŻOPOLSKA[1*] and Jolanta JONIEC[1]

ACTIVITY OF MICROORGANISMS PARTICIPATING IN ORGANIC MATTER TRANSFORMATION IN HAPLIC LUVISOL SOIL

AKTYWNOŚĆ MIKROORGANIZMÓW BIORĄCYCH UDZIAŁ W TRANSFORMACJI MATERII ORGANICZNEJ W GLEBIE PŁOWEJ

Abstract: The paper presents a study on the microbiological activity and the quality of organic matter of a Haplic Luvisol soil amended with selected organic and mineral materials (manure, clay, flotation lime, aluminium and iron oxides and a bark-keratin-urea preparation), during a three-year field experiment. In the soil under analysis (pH in 1M KCl pH_{KCl} - 4.46, organic carbon Corg. - 0.465%) the following parameters of microbiological activity were assayed: numbers of microorganisms, activity of dehydrogenases, and respiration. In addition, the quality of organic matter was analysed through the determination of its susceptibility to chemical (biological) oxidation (oxidable carbon and non oxidable carbon). The components applied to the soil had a varied effect on the studied parameters of microbiological activity during the whole period of the study. The application of manure and clay caused an increase in the numbers of bacteria, in the activity of dehydrogenases, and in the intensity of the process of soil respiration. Soil respiration was stimulated also by the application of manure and clay in combination with Fe_2O_3 at the dose of 6 kg. Moreover, a decrease in the number of bacteria in the soil was noted after the combined application of manure, clay and Fe_2O_3 6 kg. The materials applied in the study caused a variation in the susceptibility of organic matter of Haplic Luvisol soil to oxidation. Soils amended with manure and clay in combination with calcium, aluminium, iron, were characterised by limited susceptibility to organic matter oxidation, while the bark-keratin-urea preparation applied caused a considerable improvement of that susceptibility.

Keywords: numbers of bacteria, dehydrogenases activity, soil respiration, organic matter susceptibility to oxidation

Introduction

In the climate zone of Poland the processes of organic matter degradation in soils are characterised by a high intensity. This is the cause of minimal accumulation of humus, but also of increased emission of CO_2 from the soil to the atmosphere, which contributes to the

[1] Institute of Environmental Engineering, Off-Campus Faculty of Social Sciences in Stalowa Wola, The John Paul II Catholic University of Lublin, ul. Ofiar Katynia 6a, 37-450 Stalowa Wola, Poland, phone +48 15 642 25 42

[*] Corresponding author: alaksiezopolska2@kul.lublin.pl

greenhouse effect [1], as the greatest share in the global warming is attributed to increased concentration of CO_2 in the atmosphere [2]. Therefore, research is being undertaken on slowing down the processes of decomposition and mineralisation of organic matter through the addition of suitable components to the soil [3, 4]. The introduction of organic matter to the soil is accompanied by an increase of microbiological activity and acceleration of the rate of its mineralisation [5, 6].

The microbiological activity of soils is closely related with organic matter susceptibility to oxidation, as the processes of mineralisation and humification consist, in principle, in oxidation reactions proceeding with the participation of enzymes [7-9]. In this context, a number of biological and biochemical properties can be used as early and sensitive indicators of soil organic matter transformations and dynamics, nutrient cycling, and stress and recovery conditions in soil [10]. Numerous authors are of the opinion that the measurement of soil enzymes and soil respiration can be used as indicative of the biological activities and natural biochemical processes in soil [5, 11-14]. The numbers of microorganisms are also an indicator of changes taking place in the soil environment [15-18]. However, in the opinion of other authors that parameter does not fully reflect the true microbiological activity of soil. A large number of microorganisms found in soil are inactive or incapable of growth on artificial substrates [16, 19, 20]. The numbers and the activity of microorganisms are determined by a number of factors that may stimulate or inhibit microbial activity. Whereas, the activity of dehydrogenases is an indicator of the intensity of the respiratory metabolism of all populations of soil microorganisms [14]. Those enzymes are closely related with the microbiological activity of soils as they occur not only within living cells where they catalyse the oxidoreductive processes [14]. According to Winding et al [21], one of the biological parameters that should be taken into consideration in the estimation of the status of the soil environment is soil respiration measured by the amount of evolved CO_2. The main producers of CO_2 in the soil are heterotrophic microorganisms [11]. Therefore, measurement of the amount of evolved CO_2 provides information on the activity of soil microorganisms. A review of literature shows that the microbiological and biochemical indicators mentioned above have been used many times in studies on organic matter transformations in soil [4, 5, 13-15, 18, 19, 22].

In the study presented here we applied such indicators of the microbiological and biochemical activity as the numbers of microorganisms, activity of dehydrogenases, and the amount of evolved CO_2 for the estimation of the effect of selected organic and mineral components, applied in a field experiment, on organic matter transformations in soil. In addition, we assayed the content of organic carbon and the share of carbon fractions susceptible to chemical (biological) oxidation.

Materials and methods

Object of research and scheme of the experiment

The field experiment was set up in 2001, in Kolonia Boniewo near Lublin, eastern Poland, Europe, on Haplic Luvisol soil with an acid reaction (pH in water 5.19, in 1 M KCl 4.46) and with a low content of organic carbon C_{org} - 4.65 g kg^{-1} (Table 1).

Table 1

Basic characteristics of Haplic Luvisol soil in the field experiment

Horizon	Textural class [%]			pH		C [%]	N [%]	C/N index	K	P
	1-0.1	0.1-0.02	< 0.02	H_2O	[mol KCl dm^{-3}]				[mg kg^{-1}]	[mg kg^{-1}]
Ap	47	29	24	5.19	4.46	0.465	0.056	8.3	113	49.9
Eet	53	27	20	5.48	4.74	0.126	0.042	3.0	–	–
Bt/C	88	5	7	6.26	4.93	0.057	0.035	1.63	–	–
R	61	13	26	7.10	6.67	0.100	–	–	–	–

On that soil 10 micro-plots were established, each with surface area of 2 m^2. The surface horizon of the plots was enriched with selected organic and mineral materials (Table 2), and then sown with grass cv. *Dactylis glomerata*, commonly known as orchard grass or cock's foot grass.

Table 2

Fertility substance and their dose on the plot

Experimental plots	Fertility substance, dose
I	Control soil
II	Soil + manure (8 kg per plot)
III	Soil + manure (8 kg per plot) + clay (50 kg per plot)
IV	Soil + manure (8 kg per plot) + clay (50 kg per plot) + flotation lime (1.12 kg per plot)
V	Soil + manure (8 kg per plot) + clay (50 kg per plot) + iron in the form of Fe_2O_3 (6 kg per plot)
VI	Soil + manure (8 kg per plot) + clay (50 kg per plot) + aluminium in the form of Al_2O_3 (15 kg per plot)
VII	Soil + bark-keratin-urea preparation (BKU), (10 g per plot)
VIII	Soil + manure (8 kg per plot) + clay (50 kg per plot) + iron in the form of Fe_2O_3 (2 kg per plot)
IX	Soil + manure (8 kg per plot) + clay (50 kg per plot) + calcium in the form of calcium hydroxide (2 kg per plot)
X	Soil + manure (8 kg per plot) + clay (50 kg per plot) + flotation lime (2.24 kg per plot)

Characterisation of materials applied to the Haplic Luvisol soil

The materials used in the experiment included *cattle manure* from an agricultural farm, *clay* brought from the sulphur mine "Jeziorko", where it was used for the reclamation of post-mining lands, *flotation lime* - a waste product of the sulphur industry in the process of sulphur flotation, also obtained from the sulphur mine "Jeziorko" where it was used for reclamation of mining grounds. The composition of the flotation lime included calcite (65-75%), clay minerals, silica, celestine, and elemental sulphur (3.7%).

Such nutrients as iron and calcium were applied in the field experiment at two different doses. Calcium was applied in the form of flotation lime, at a dose conforming to the calculated hydrolytic acidity (Ap = 3 mmol (+) kg^{-1}), *ie* 1.12 kg on plot IV, and a double dose of 2.24 kg on plot X. Plot IX was amended with *hydrated lime* in the form of *calcium hydroxide*. Iron was applied in the form of Fe_2O_3, at doses of 6 kg on plot V and 2 kg on plot VIII. Aluminium was applied as *aluminium oxide*, in the form of ground white powder. The composition of the bark-keratin-urea (BKU) preparation used in the experiment, with humus-like properties, included 23.6% of modified keratin protein, 35.4% of urea, 31.5% of modified bark, and 9.5% of water; the elemental composition

included 0.054% P, 0.207% K, 0.178% Ca, and 0.011% Mg, the content of nitrogen being 28.3%, and carbon 33.3%.

Soil samples

The soil samples for the analyses of the basic properties of the soil, presented in Table 3, were taken in autumn 2002.

Table 3
Selected physical, chemical and physicochemical properties of Haplic Luvisol soil and enriched soils

Plots	Soil density [g cm^3]	Total porosity [%]	Macro-porosity [%]	pH_w	Moisture [%]	N [g kg^{-1}]	Surface area [m^2 g^{-1}]	Electrolitic conductivity [mS cm^{-1}]
I	1.59	39.7	3.1	4.9	16	0.07	5.9	$54 \cdot 10^{-3}$
II	1.61	38.9	3.3	5.6	15	0.08	5.2	$47.3 \cdot 10^{-3}$
III	1.44	45.0	11.0	7.1	19	0.07	5.3	0.240
IV	1.52	42.2	7.6	7.6	17	0.08	7.3	0.310
V	1.46	44.9	9.4	7.2	16	0.10	6.0	0.300
VI	1.58	40.3	4.9	7.4	19	0.08	6.2	0.265
VII	1.63	38.2	4.1	5.2	15	0.08	4.7	$39.9 \cdot 10^{-3}$
VIII	1.50	43.4	13.3	7.4	16	0.10	5.3	0.406
IX	1.56	41.3	7.3	7.9	15	0.08	4.2	0.375
X	1.64	37.8	3.4	7.6	14	0.11	5.4	0.330

The determination of the content of organic matter and its susceptibility to chemical (biological) oxidation was conducted in spring and autumn, *ie* after 10 and 14 months from the amendment of the soil with materials used in the experiment, and in spring of the following year. The microbiological analyses were made in spring, *ie* after 10 months from the beginning of the experiment, and in spring of the following year. The analyses were performed for soil taken from the surface horizon (0-10 cm). In addition, in the 2nd year of the study, assays concerning the microbial populations and soil respiration were performed also for the deeper soil horizon (10-25 cm).

Soil samples were screened through a sieve with 2 mm mesh and kept in a refrigerator. The assays performed for the soil material collected comprised the numbers of microorganisms, respiration activity, activity of dehydrogenases, content of organic carbon, and the fractions of carbon susceptible and resistant to chemical (biological) oxidation.

Microbiological, biochemical and chemical analyses

Number of bacteria

The numbers of oligotrophic bacteria were determined with the plate method, on a SEA medium with soil extract, containing 100 cm^3 of soil extract and 0.2 g K_2HPO_4, 1 g dextrose, 15 g agar, and 900 cm^3 tap water [23], after 10 days of incubation at temperature of 20ºC [24]. The assays were made in four replicates. The results were converted to 1 kg of dry matter of soil and presented in the form of colony forming units (cfu).

Soil respiration

The respiratory activity was determined with the Rühling and Tyler method [25] (in three replicates); the amount of evolved CO_2 was determined with the titration method and presented as mg C-CO_2 kg^{-1} d.m. soil d^{-1}.

Activity of dehydrogenases

Dehydrogenases activity was determined with the method of Casida et al [26]. The assays were made in 5-gram weighed portions of soil (in three replicates), incubated at temp. of 30°C for 24 hours. The method consists in soil incubation with colourless TTC (2,3,5-triphenyl tetrazolium chloride). The product of the enzymatic reaction was extracted from the soil with methyl alcohol and assayed colorimetrically (λ = 485 nm).

Chemical analyses

The content of organic carbon was determined using potassium dichromate with hydrochloric acid. The share of carbon fractions susceptible to and resistant to oxidation was determined with the method of model chemical oxidation of humus with solutions of $KMnO_4$ in a neutral medium, acc. to Loginow and Wisniewski [27]. For this reason in this paper we use the phrase "chemical (biological) oxidation of organic matter". The assays of those chemical parameters were made in three replicates.

Statistical analysis

The results were analysed statistically using analysis of variation (Statgraphics; 95% LSD) and regression (Excel).

Results

Number of bacteria

Under the conditions of the experiment, changes in the number of bacteria were observed in relation to the year of the study, depth in the soil profile (0-10 cm and 10-25 cm), and to the kind of organic and mineral materials applied (Table 4, Fig. 1).

Analysing the changes in the number of the studied microorganisms in the particular years it was found that in the first year of the study, *ie* in spring of 2002, their number in the horizon of 0-10 cm was lower compared to the year 2003 (Table 4, Fig. 2).

The lowest number of those microorganisms was noted within plot VIII (manure, clay, iron Fe_2O_3 at 2 kg), and the highest in plot III (manure, clay). In 2003 the number of the bacteria studied increased considerably and were the lowest in plot V (manure, clay, Fe_2O_3 6 kg) and the highest in plot III (manure, clay) (Table 4, Fig. 3).

In the 2nd year of the study changes in the number of bacteria were also observed in relation to depth (Table 4). At the depth of 10-25 cm they were notably lower than in the surface horizon. The organic and mineral components applied in the experiment had a certain effect on the number of the microorganisms under study (Table 4, Figs. 1, 3). Generally, the number of bacteria in the surface horizon (0-10 cm) in the particular periods of the study oscillated in most of the plots at a level similar to that observed in the control soil. Only the application of manure together with clay (plot III) caused a slight tendency towards stimulation of the growth of bacteria. That effect became observable already in the

spring of the 1st year of the study, and continued also in the 2nd year, though at a notably lower level. Whereas, the application of manure together with clay and iron at the dose of 6 kg (plots V), caused a slight tendency towards a reduction of the number of bacteria.

Table 4

Microbiological properties of soils

Plots	Layer [cm^{-1}]	Dehydrogenases activity [cm^3 H_2 kg^{-1} d^{-1}]		Number of bacteria [cfu 10^6 kg^{-1}d.m. soil]		Respiratory activity [mg C-CO_2 kg^{-1} d.m. soil d^{-1}]	
		2002 spring	2003 spring	2002 spring	2003 spring	2002 spring	2003 spring
I	0-10	0.571	0.584	10.51	15.93	18.05	15.59
	10-25				0.78		2.60
II	0-10	0.503	0.209	7.11	20.02	24.58	11.98
	10-25				1.45		8.95
III	0-10	0.945	0.330	20.37	24.55	62.75	22.36
	10-25				4.01		13.59
IV	0-10	0.514	0.208	10.54	14.27	11.30	30.15
	10-25				0.88		10.57
V	0-10	0.530	0.360	11.21	1.35	71.75	25.21
	10-25				1.11		4.06
VI	0-10	0.514	0.288	9.57	13.92	0.23	34.46
	10-25				1.00		3.16
VII	0-10	0.536	0.169	9.24	11.88	1.77	7.46
	10-25				1.51		3.00
VIII	0-10	0.464	0.169	4.77	21.01	0.23	24.98
	10-25				0.78		7.38
IX	0-10	0.530	0.413	8.83	18.50	2.35	32.40
	10-25				0.99		18.77
X	0-10	0.552	0.621	10.54	9.96	2.68	23.78
	10-25				1.99		9.07

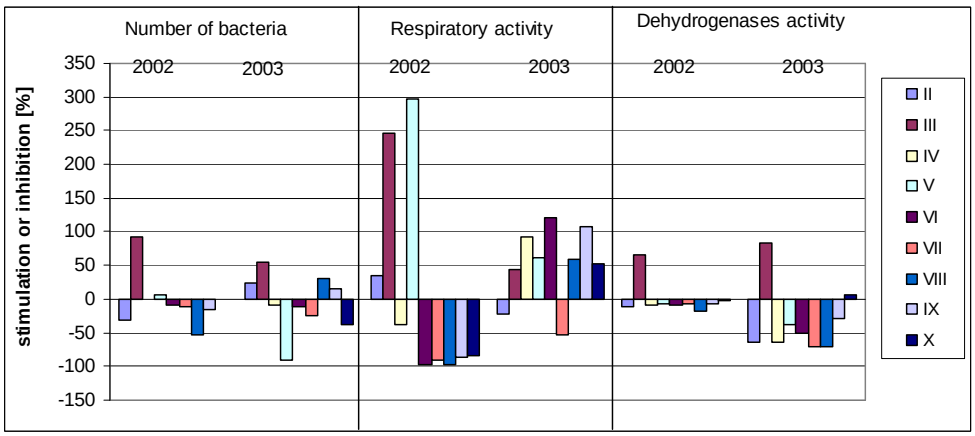

Fig. 1. Influence of components on the number of bacteria, respiratory activity and dehydrogenases activity in the soil

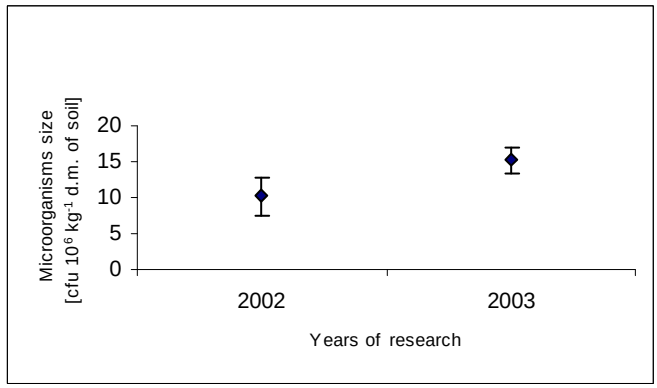

Fig. 2. Number of bacteria in soils experimental plots (years). Averages with 95% LSD intervals

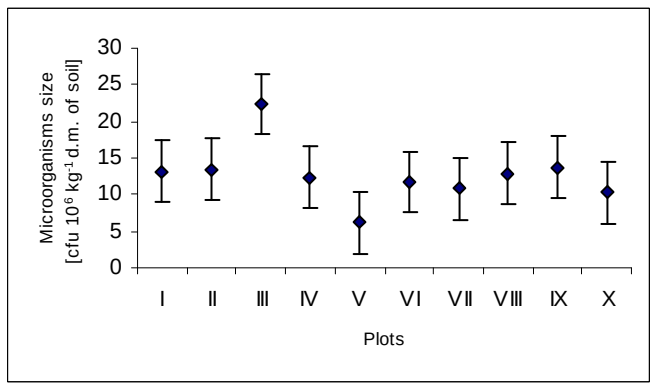

Fig. 3. Number of bacteria in soils experimental plots. Averages with 95% LSD intervals

In the deeper horizon of the soil (10-25 cm), analysed in the 2nd year of the study, a slight increasing tendency was observed in the number of the microorganisms under analysis, in the treatments with manure (plot II), the BKU preparation (plot VII), and manure in combination with clay and flotation lime at the dose of 2.24 kg (plot X), (Table 4). Whereas, a distinct increase in the numbers of bacteria was caused by the application of manure in combination with clay (plot III).

Statistical analysis of two-year mean values concerning the effect of the particular components on the number of bacteria under study confirmed significant changes in that parameter in treatments III (manure and clay), V (manure, clay, Fe_2O_3 6 kg), (Fig. 3). The application of manure and clay caused a stimulation of the numbers of the microorganisms studied. Whereas, the application of manure, clay and iron (6 kg), caused a decreasing trend in their numbers.

Moreover, significant differences were demonstrated between plots: III and all other, and V and I, II and III (Fig 3). In addition, the statistical analyses (test 95% LSD) revealed significant differences between the years of the study, *ie* between 2002 and 2003 (Fig. 2).

Activity of dehydrogenases

Data obtained on the activity of dehydrogenases show that it was subject to changes in relation to the year of the study and to the mineral and organic components applied (Table 4, Figs. 1, 4).

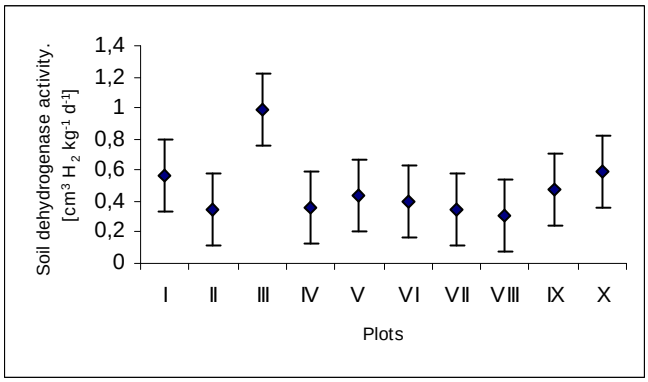

Fig. 4. Dehydrogenases activity (DHA) in soils experimental plots. Averages with 95% LSD intervals

Only in the control soil dehydrogenases activity remained at a fairly constant level throughout the period of the study. In the soil samples from the spring of 2002 the activity of dehydrogenases was generally decidedly higher compared to the year 2003. It was the lowest in plot VIII (manure, clay, Fe_2O_3, 2 kg), and the highest in the soil from plot III (manure, clay). In 2003 the lowest activity of dehydrogenases was noted in the soil from plot VII (BKU preparation) and VIII (manure, clay, Fe_2O_3, 2 kg), and the highest in the soil from plot III (manure, clay).

The analyses revealed that both in the 1st and the 2nd year of the study the activity of dehydrogenases was inhibited by almost all of the mineral and organic components applied (Table 4, Fig. 1). That effect was observed with varied intensity depending on the duration of the action of the components on the soil. In the 1st year of the study the negative effect of the components was rather small, but it intensified with the passage of time. The exception was the soil from plot III, amended with manure and clay, in which a distinct stimulation of dehydrogenases activity was observed in the 1st and 2nd year of the study. Moreover, a slight intensification of that enzymatic parameter was noted in the soil with an admixture of manure clay and lime at the dose of 2.24 kg (plot X), in the 2nd year of the study.

Statistical analysis based only on the two-year mean values did not reveal significance of those changes in all the cases (Fig. 4). In almost none of the treatments a significant effect of the components applied on that biochemical parameter was observed. Only the amendment of the soil with manure and clay (plot III) resulted in a significant increase in the activity of dehydrogenases.

The statistical analysis (test 95% LSD) did not reveal any significant differences between the years of the study. It did, however, show significant differences between the experimental treatments, *ie* between plot III and all other combinations (Fig. 4).

Intensity of soil respiration

Data presented in Table 4 and in Figure 1 indicate that during the two years of the study the soil respiratory activity varied in relation to the component applied. In addition, in the 2nd year of the study the intensity of that parameter depended also on the depth from which the soil was taken.

The amount of evolved CO_2 in soil samples taken in spring 2002 from the depth of 0-10 cm was the smallest in the soil from plot VI (manure, clay, aluminium oxide) and VIII (manure, clay, Fe_2O_3, 2 kg), while the highest values were noted for the soil from plot V (manure, clay, Fe_2O_3, 6 kg) and plot III (manure, clay).

In 2003 respiration of soil at the depth of 0-10 cm was the lowest in the soil from plot VII (BKU preparation), while high and similar intensities of respiration were noted in the soil from several plots, *ie* IV (manure, clay, flotation lime 1.12 kg), VI (manure, clay, Al_2O_3) and IX (manure, clay, $Ca(OH)_2$).

In the soil samples from 2003 the intensity of respiration was analysed for the soil horizons of 0-10 cm and 10- 25 cm (Table 4). Below 10 cm it was notably lower compared to that in the surface horizon of the soil. In the deeper soil horizon (10-25 cm) the lowest respiratory activity was noted on the control soil (I) and, as in the case of the soil from the surface horizon, after the addition of the BKU preparation (VII). Whereas, the highest values were observed in treatments III (manure, clay) and IX (manure, clay, $Ca(OH)_2$).

The data concerning the respiration in the surface horizon of the soil in the 1st year of the effect of the organic and mineral components (Table 4, Fig. 1) showed, in a majority of the treatments, a decrease in the intensity of that biochemical parameter (plots IV, VI, VII, VIII, IX, X). Only the application of manure (plot II), manure and clay (plot III), and manure with clay and iron at the dose of 6 kg (plot V) caused stimulation of the process of respiration, the application of manure alone causing the weakest effect on that parameter. Whereas, the application of manure in combination with clay, and with clay and iron, resulted in stimulation at a notably higher level.

In the second year of the study stimulation of the process of soil respiration was noted in almost all treatments (Table 4, Fig. 1). Only the application of manure (plot II) and of the BKU preparation (plot VII) caused a decrease in the activity of the parameter under analysis.

The results concerning the respiration in the soil from the horizon of 10-25 cm (Table 4) showed, in the 2nd year of the study, an increased stimulation of that parameter under the effect of all of the components applied.

Noteworthy is the fact that the stimulation observed was at a decidedly higher level than in the surface horizon of the soil.

The changes observed in this study in the intensity of soil respiration under the effect of the components applied had a varied character in the particular years (Table 4, Fig. 1). Therefore statistical analysis based only on the two-year mean values did not indicate significance of those changes in all the cases (Fig. 5).

Generally, no significant effect of the components on the soil respiration was observed. Only the application of manure and clay (III) and manure, clay and $Fe_2O_3$6 kg (plot V) caused a statistically significant stimulation of that process. Moreover, significant differences were demonstrated between plots III and all other combinations, and V and all other combinations (Fig. 5). The statistical analysis (test 95% LSD) did not reveal any significant differences between the years of the study.

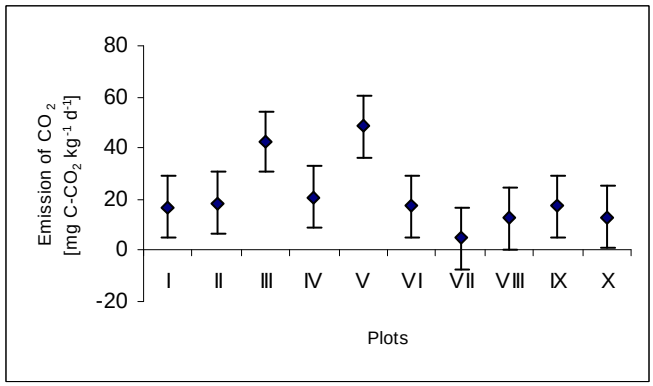

Fig. 5. Respiratory activity in soils experimental plots Averages with 95% LSD intervals

Content of organic carbon and shares of fractions of carbon susceptible to and resistant to chemical (biological) oxidation

The experiment demonstrated dynamism of qualitative and quantitative changes in organic matter in the particular treatments as well as in the periods of the study. In the successive years of the study the content of organic carbon (Table 5, Fig. 6) was varied within the plots, and only in the control treatment a stable level of carbon content was observed. In 2002, both in spring and in autumn an increase of the content of C_{org} was observed in all treatments amended with the components, compared to the control treatment. Whereas, in spring 2003 a notable decrease of C_{org} was observed in the particular treatments, with a continued stable level in the control treatment. As a result of organic matter mineralisation during the whole period of the study, it was found that in spring 2003 the content of C_{org} was the highest in treatment II, amended with manure alone, and the lowest in treatments IV and VII. Whereas, the soil from plots III, VI, VIII and IX was characterised by similar levels of C_{org}, comparable to the control treatment. Analysing the changes in the share of the fraction of carbon susceptible to chemical (biological) oxidation, a decrease of that parameter was noted in all the treatments amended with the components as compared to the control treatment (Table 5, Fig. 6). With the passage of time, an increase in the share of that carbon fraction was noted, the highest values being attained in 2003.

The highest share of that carbon fraction was noted in treatment VII (with the BKU preparation), *ie* 51.7% C_{org}, and the lowest in treatment VIII (with manure, clay and iron at the lower dose), *ie* 38.1% C_{org}. Whereas, analysing the changes in the share of carbon fraction resistant to chemical (biological) oxidation, an increase of that parameter under the effect of all of the components applied was demonstrated. That effect persisted throughout the period of the experiment, though it weakened with the passage of time. In 2003 the share of that carbon fraction was the lowest and oscillated within the range from 48.3% C_{org} in plot VII to 62.0% C_{org} in the soil from plot VIII.

The statistical analysis (95% LSD) revealed a lack of significant differences in the content of C_{org}, carbon fractions susceptible to and resistant to chemical (biological) oxidation, under the conditions of the research method applied, among the soils from the particular experimental treatments. Whereas, significant differences were found between the years of the

study, 2002 and 2003 (Figs. 7-9), and between the seasons of the year, *ie* between spring and autumn (Figs. 7-9). Therefore, the effect of the organic and mineral components applied to the soil was related with the season of the year and with the duration of that effect on the biochemical transformations.

Table 5

Content of organic carbon, oxidable and non oxidable carbon

Plots	Organic carbon C_{org} [g kg^{-1}]			Oxidable carbon [% C_{org}]			Non oxidable carbon [% C_{org}]		
	2002 spring	2002 autumn	2003 spring	2002 spring	2002 autumn	2003 spring	2002 spring	2002 autumn	2003 spring
I	5.8	6.0	5.7	31.3	35.0	44.7	68.8	65.0	55.3
II	8.2	7.5	6.7	14.7	20.2	40.5	85.3	79.8	59.5
III	7.5	8.2	5.9	20.2	18.4	42.9	79.8	81.6	57.1
IV	7.3	7.6	5.2	10.3	9.9	40.7	89.8	90.1	59.3
V	7.7	8.4	6.3	19.5	17.9	40.5	80.5	82.1	59.5
VI	7.2	8.4	5.7	16.7	17.9	39.5	83.3	82.1	60.5
VII	6.7	7.0	5.2	11.2	15.1	51.7	88.8	84.9	48.3
VIII	7.6	6.7	5.5	13.9	17.9	38.1	86.1	82.1	62.0
IX	7.2	7.8	5.8	10.4	9.6	41.2	89.6	90.4	58.8
X	6.8	8.1	6.3	8.8	9.3	43.3	91.2	90.7	56.7

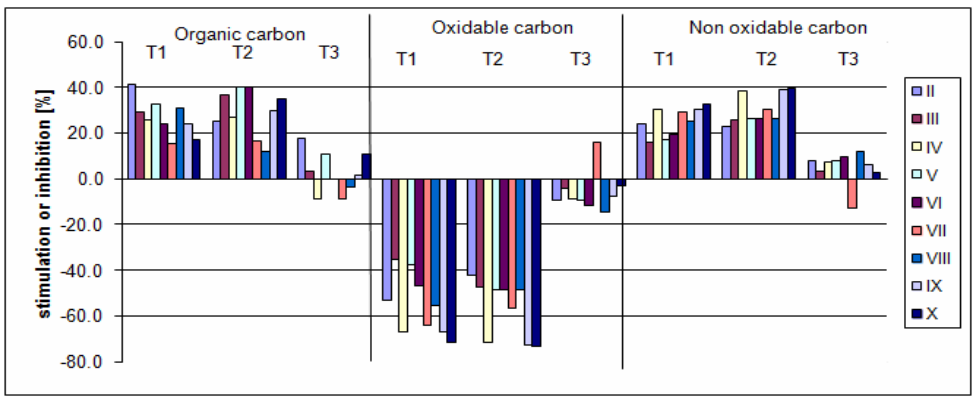

Fig. 6. Influence of components on the organic carbon, a oxidable carbon and non oxidable carbon in the soil (T1 - spring 2002; T2 - autumn 2002; T3 - spring 2003)

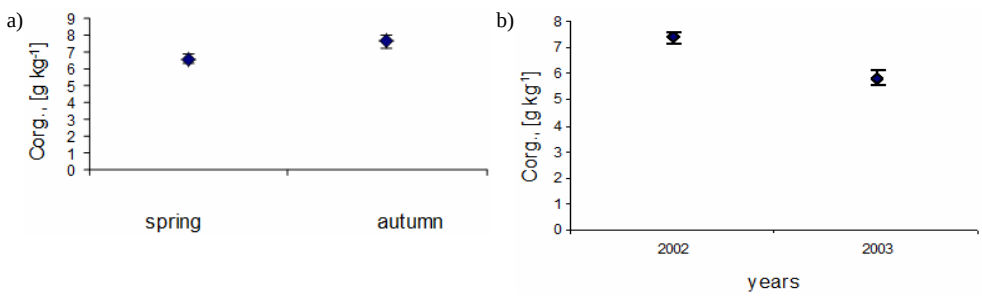

Fig. 7. Concentration of organic matter in soils experimental plots (a - spring, autumn 2002, 2003; b - years 2002, 2003). Averages with 95% LSD intervals

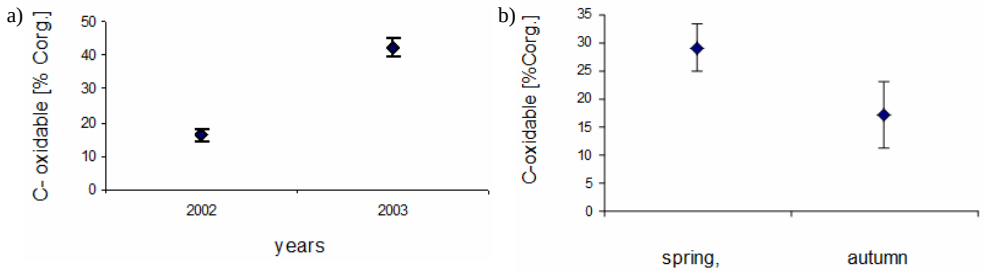

Fig. 8 Content of carbon fraction susceptible to chemical (biological) oxidation in soils experimental plots (a - spring, autumn; b - years 2002, 2003). Averages with 95% LSD intervals

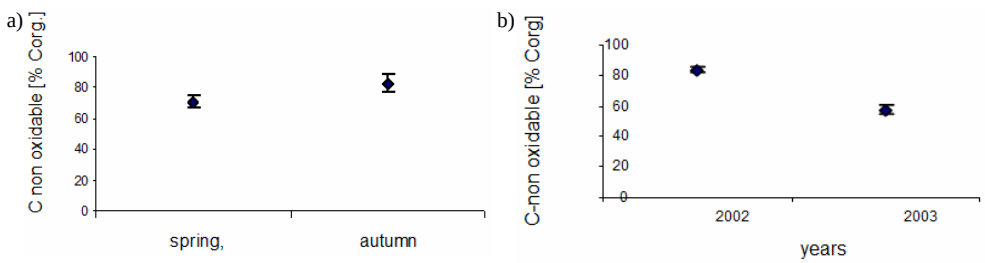

Fig. 9. Content of carbon fractions resistant to chemical (biological) oxidation in soils experimental plots (a - spring, autumn; b - years 2002, 2003). Averages with 95% LSD intervals

Discussion and conclusions

The increase noted in the number of the studied bacteria under the effect of the organic components, *ie* manure (plot II), manure and clay (plot III), and the BKU preparation (plot VII - in the soil from the horizon of 10-25 cm) (Table 4, Fig. 1), was most likely caused by the introduction of a certain amount of nutrients for those microorganisms into the soil. These observations are supported by research of other authors [17, 22, 28] which shows that oligotrophic bacteria can grow well also under conditions of an abundance of nutrients. Moreover, the increase of the number of microorganisms in the soil from plot III (manure, clay) was facilitated by the better physical and chemical properties of the soil, such as reduced density of the soil and increased volume of macroporous to 11%, increase of specific surface area and electrolytic conductivity or moisture (Table 3). In the treatment in which apart from manure and clay also flotation lime was applied at the dose of 2.24 kg (plot X in the soil from the horizon of 10-25 cm), the increase in the number of the studied microorganisms could have been caused by increase of reaction (Table 3). As it is commonly known, bacteria prefer a higher reaction of the environment. Studies by other authors indicate that the numbers of bacteria are positively correlated with soil moisture and reaction [15]. The least favourable for the growth of oligotrophic bacteria in the conditions under analysis, proved to be the addition of iron, at the dose of 6 kg (plot V), (Table 4, Fig. 1).

As the number of microorganisms does not provide information on their physiological activity, the authors determined also their enzymatic activity. Soil dehydrogenases activity

is considered to be an indicator of the overall microbiological activity of soils as they occur only within living cells where they catalyse the oxidoreductive processes [14]. Therefore the stimulation of microbial growth under the effect of manure and clay (plot III) was also reflected in an increase of the activity of dehydrogenases (Table 4, Fig. 1). The introduction of an additional source of nutrients for the microorganisms, in the form of organic matter, resulted therefore not only in a stimulation of their growth but also of their enzymatic activity. A stimulating effect of organic matter on the dehydrogenases activity of soils was noted also by Nicolas et al [5], Scherer et al [13] and Furczak and Joniec [22]. A certain effect on the stimulation of that activity could have also resulted from improvement of the living conditions of soil microorganisms producing those enzymes, ie. lowering of the soil density and increase in the volume of macroporous to 11%, increase of specific surface area, electrolytic conductivity or moisture (Table 3). A dependence of that parameter on various properties of the soil environment are also reported by Wolinska and Stepniewska [14].

The activity of dehydrogenases, as the number of microorganisms, was also inhibited when, apart from manure and clay, also iron (plots V), (Table 4, Fig. 1).

The above observations concerning the number of bacteria and the activity of dehydrogenases were supported by the analysis of regression which revealed significant correlations between dehydrogenases activity and the number of microorganisms and moisture (Table 6). Correlations between the number of microorganisms and dehydrogenases activity were noted also by Taylor et al [6] and by Furczak and Joniec [22]. Whereas, studies by other authors do not support those correlations [29].

Table 6

Values for the correlation coefficient obtained between dehydrogenases activity (DHA) in soil and microorganisms size, content of carbon fractions resistant to chemical (biological) oxidation, moisture soils and index C/N

Dependances	Equation	Correlation coefficient	Replication
DHA = f(L.d.)	$Y = 0.0317x+0.2402$	$R = 0.9393^{***}$	10
DHA = f(C odp.)	$Y = 0.0013x^{1.3483}$	$R = 0.5952^{**}$	20
DHA = f(C/N)	$Y = 0.2644e^{0.1076x}$	$R = 0.581^{*}$	10
DHA = f(W)	$Y = 5.7282x$	$R = 0.4724^{**}$	30

*** significant at P 0.001
** significant at P 0.01
* significant at P 0.05

In the opinion of Winding et al [21], one of the biological parameters that should be taken into account in the estimation of the status of the soil environment is respiration. Under the conditions of this experiment, the respiratory activity was subject to continual changes throughout the period of the study (Table 4, Fig. 1). In the particular treatments and years of the study, either stimulation or inhibition of soil respiration were observed. The effect of the components applied was rather without any distinct direction. However, it was observed that the addition of manure (plot II), manure and clay (plot III) and the BKU preparation (plot VII), caused, as in the case of the number of microorganisms (Table 4, Fig. 1), a stimulation of that biochemical parameter in the 2nd year of the study in the horizon of 10-25 cm, and of manure and clay also in the 1st year of the study (Table 4, Fig. 1). This was probably due to the introduction of respiration substrates for the

microorganisms together with the organic components, as heterotrophic microorganisms are considered to be the primary producers of CO_2 in the soil environment [11]. Literature review shows that organic matter introduced into soil contributes to an intensification of respiration processes [4, 5, 13, 22].

The results obtained demonstrated also that, as in the case of bacterial populations and dehydrogenases activity, the application of manure together with iron at the dose of 2 kg (plot VIII) and with lime in the hydroxide form (plot IX) caused also an inhibition of respiratory activity (Table 4, Fig. 1).

Components applied to soils, that supply large amounts of nutrients for microorganisms, stimulate their activity, thus accelerating the mineralisation of organic matter. This phenomenon is unfavourable from the viewpoint of plant nutrition as it contributes to C_{org} losses from the soil due to intensification of processes conducted by microorganisms [3]. Under the conditions of this experiment, such a component could have been manure applied in conjunction with clay which caused a violent increase in the activity of dehydrogenases, respiration, and also a certain tendency towards an increase in the number of microorganisms already in spring 2002 (Table 4, Fig. 1). However, with the passage of time that effect weakened considerably. These suppositions are supported by the results concerning the content of C_{org} which demonstrated that in the treatment with an addition of that component the value of that parameter initially increased, but with the passage of time it decreased notably (Table 5, Fig. 6).

It should be emphasised that the low humus content of the control soil, after its amendment with the organic and mineral components and sowing with grass cv. *Dactylis glomerata*, increased in all the treatments in 2002, but in the following year a considerable decrease was noted in all amended treatments compared to the year 2002 (Table 5, Fig. 6). This supports the conclusion that the transformations of organic matter in the soil are highly complex, have multidirectional relations and a varied effect on the soil environment. Those are typically biochemical processes that consist in the oxidation reactions with participation of catalytic systems. The rate of those processes is determined primarily by the quality of organic matter, ie. by its shares of carbon fractions susceptible and resistant to chemical (biological) oxidation [3]. In this experiment, the estimation of the susceptibility of organic matter to decomposition was made with the use of the method of model chemical oxidation of humus with potassium permanganate [27], in a neutral environment. For that purpose, organic matter from the soil of the experimental treatments was fractioned into carbon fractions susceptible to and resistant to oxidation. In the three-year observation of changes in the content of C_{org} and in the shares of those carbon fractions, noteworthy is the gradual decrease in the content of C_{org} in time, and its stability in the control treatment, which indicates that microorganisms inhabiting the control soil most actively utilised the native organic carbon (Table 5). Also noteworthy is the considerable decrease with time of the share of carbon fraction resistant to oxidation, and increase of carbon fraction susceptible to that process, in the spring of 2003, especially when compared to the initial period, ie. the spring of 2002 (Table 5, Fig. 6). Decrease of the share of carbon fraction resistant to oxidation by an average of 60% indicates transformations in the organic matter of the soils under analysis, and informs that the degradation of the organic matter was not uniform. With decrease in the share of carbon fraction resistant to oxidation, the share of carbon fraction susceptible to chemical (biological) oxidation increased. These observations indicate that soil microorganisms utilise first carbon compounds susceptible to biological

oxidation (*ie* easily degradable), which is reported also by other authors [4, 30]. Whereas, with the passage of time, in the soil under analysis there took place a selection of microorganisms capable of utilising the hard-degradable carbon fraction. This is indicated by the observed correlation between dehydrogenases activity and the share of carbon fraction resistant to chemical (biological) oxidation (Table 6). The above observations are supported also by the intensification of the rate of the process of respiration, observed in the soil only in the second year of the study. The conclusion from the above is that conducting studies on soil fertility with the use of biochemical parameters is justified in long-term (multi-year) experiments [10], as permanent biological changes in soil become manifested only after several years of application of specific cultivation treatments.

References

[1] Wang W, Guo J, Oikawa T. J Biosciences. 2007;32:375-384. www.ias.ac.in/jbiosci/.
[2] MacDonald NW, Randlett DL, Zak DR. Soil Sci Soc Am J. 1999;63:211-218. DOI: 10.2136/sssaj1999.03615995006300010030x.
[3] Fernandez JM, Plaza C, Hernandez D, Polo A. Geoderma. 2007;137:497-503. DOI: 10.1016/j.geoderma.2006.10.013.
[4] Księżopolska A, Włodarczyk T, Gliński J, Flis-Bujak M. Pol J Environ Stud. 2011;20:387-392. www.pjoes.com.
[5] Nicolas C, Hernandez T, Garcia C. Appl Soil Ecol. 2012;57:50-58. DOI: 10.1016/j.apsoil.2012.02.018.
[6] Taylor JP, Wilson B, Mills MS, Burns RG. Soil Biol Biochem. 2002;34:387-401. DOI: 10.1016/S0038-0717(01)00199-7.
[7] Chander K, Goyal S, Mundra MC, Kapoor KK. Biol Fert Soils. 1997;24:306-310. DOI: 10.1007/s003740050248.
[8] Frankenberger WT Jr, Dick WA. Soil Sci Soc Am J. 1983;47:945-951. DOI: 10.2136/sssaj1983.03615995004700050021x.
[9] Milne RM, Haynes RJ. Biol Fert Soils. 2004;39:172-178. DOI: 10.1007/s00374-003-0698-y.
[10] Dick WA, Tabatabai MA. Potential uses of soil enzymes. In: Metting B, editor. Soil Microbial Ecology. New York: Dekker; 1993.
[11] Kuzyakov Y. Soil Biol Biochem. 2006;38:425-448. DOI: 10.1016/j.soilbio.2005.08.020.
[12] Singh DK, Kumar S. Chemosphere. 2008;71:412-418. DOI: 10.1016/ j. chemosphere. 2007.11.005.
[13] Scherer HW, Mether DJ, Welp G. Plant Soil Environ. 2011;57:513-518. www.agriculturejournals.cz/web/pse.htm.
[14] Wolińska A, Stępniewska Z. Microorganisms abundance and dehydrogenase activity as a consequence of soil reoxidation process. In: Miransari M, editor. Soil Till Microb Activ. Singpost Research, India; 2011.
[15] Czaban J, Wróblewska B, Niedźwiecki J, Sułek A. Pol J Environ Stud. 2010;19:1171-1183. www.pjoes.com.
[16] Foght J, Aislabie J. Enumeration of Soil Microorganisms. In: Margesin R, Schinner F, editors. Manual of Soils Analysis. Part 5. Monitoring and Assessing Soil Bioremediation. Springer; 2005. books.google.pl/books?isbu=3540 28 9046.
[17] Hattori R, Hattori T. J Gen Appl Microbiol. 1980;26:1-14. DOI: org/10.2323/jgam.26.1.
[18] Whitelaw-Weckert MA, Rahman L, Hutton RJ, Coombes N. Appl Soil Ecol. 2007;36:224-232. DOI: 10.1016/ j.apsoil.2007.03.003.
[19] Dąbek-Szreniawska M, Hajnos M, Stotzky G, Collins Y, Malicki J. Int Agrophys. 2006;20:277-288. www.old.international-agrophysics.org/zeszyty.
[20] Hill GT, Mitkowski NA, Aldrich-Wolfe L, Emele LR, Jurkonie DD, Ficke A, et al. Appl Soil Ecol. 2000;15:25-34. DOI: 10.1016/S0.929-1393(00)00069-X.
[21] Winding A, Hund-Rinke K, Rutgers M. Ecotox Environ Safe. 2005;62:230-248. DOI: 10.1016/j.ecoenv.2005.03.026.
[22] Furczak J, Joniec J. Int Agrophis. 2007;21:39-48. www.old.international-agrophysics.org/zeszyty.
[23] Stotzky G, Broder MW, Doyle JD, Jones RA. Adv Appl Microbiol. 1993;38:1-98. DOI: 10.1016/S0065-2164(08)70214-4.

[24] Parkinson D. Filomentous fungi. In: Wearer et al, editors. Methods of Soil Analysis. Part 2. Soil Sci Soc Am J; 1994.
[25] Rühling A, Tyler G. Oikos. 1973;24:402-416. www.jstor.org/stable/3543816.
[26] Casida LE, Clein JD, Santero D. Soil Sci. 1963;98:371-376. DOI:10.1097/00010694-196412000-00004.
[27] Łoginow W, Wiśniewski W. Pol Ecol Stud. 1976;43:43-51.
[28] Fierer N, Bradford MA, Jackson RB. Ecology. 2007;88:1354-1364. DOI: 10.1890/05- 1839.
[29] Piotrowska-Cyplik A, Cyplik P, Czarnecki Z. J Res Appl Agr Engin. 2007;52:22-26. www.pimv.poznan.pl/wydawn.html.
[30] Włodarczyk T, Księżopolska A, Gliński J. Committee Prot Natural Environ. 2008;5A:153-163. www.pan-ol.lublin.pl/wydawnictwa/TOchr5a_2008.html.

AKTYWNOŚĆ MIKROORGANIZMÓW BIORĄCYCH UDZIAŁ W TRANSFORMACJI MATERII ORGANICZNEJ W GLEBIE PŁOWEJ

Instytut Inżynierii Środowiska, Katolicki Uniwersytet Lubelski im. Jana Pawła II

Abstrakt: W niniejszej pracy badano aktywność mikrobiologiczną oraz jakość substancji organicznej gleby płowej wzbogaconej wybranymi materiałami organicznymi i mineralnymi (obornik, ił, wapno poflotacyjne, tlenek glinu i żelaza oraz preparat koro-keratyno-mocznikowy) podczas trzyletniego doświadczenia polowego. W analizowanej glebie (pH_{KCl} - 4,46; C_{org}. 0,465%) określano następujące parametry aktywności mikrobiologicznej: liczebność drobnoustrojów, aktywność dehydrogenaz oraz oddychanie. Ponadto analizowano jakość substancji organicznej poprzez określenie jej podatności na chemiczne (biologiczne) utlenianie. Wprowadzone do gleby komponenty w różnym stopniu wpływały na badane parametry aktywności mikrobiologicznej w trakcie całego okresu badań. Zastosowanie obornika i iłu skutkowało wzrostem liczby bakterii, aktywności dehydrogenaz oraz nasileniem procesu oddychania. Oddychanie gleby było stymulowane również przez dodatek obornika i iłu (łącznie z Fe_2O_3 6 kg). Ponadto odnotowano spadek liczby bakterii w glebie po dodaniu obornika, iłu (łącznie z Fe_2O_3 6 kg). Zastosowane w doświadczeniu materiały wpłynęły na zróżnicowanie podatności na utlenianie substancji organicznej gleby płowej. Gleby wzbogacone obornikiem, iłem w połączeniu z wapnem, glinem, żelazem charakteryzowały się ograniczoną podatnością substancji organicznej na utlenianie, zaś zastosowany preparat koro-keratyno-mocznikowy przyczynił się do wzrostu tej podatności.

Słowa kluczowe: liczebność drobnoustrojów, aktywność dehydrogenaz, oddychanie gleby, podatność materii organicznej na utlenianie