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Soil Chemistry

BEATA KUZIEMSKA, STANISŁAW KALEMBASA,
ANDRZEJ WYSOKIŃSKI, DAWID JAREMKO*

ASSESSMENT OF IMPACT OF ORGANIC WASTE MATERIALS AND LIMING ON DEHYDROGENASE ACTIVITY, CONTENT OF ORGANIC CARBON, AND pH VALUE IN SOIL CONTAMINATED WITH NICKEL

Abstract. A four-year pot experiment was conducted at an experimental facility of the Siedlce University of Natural Sciences and Humanities; the experiment was set up in a random arrangement in three replications. The following factors were taken into account: I – organic fertilising (no organic fertiliser, sludge from the wastewater treatment plant in Siedlce, chicken litter and brown coal from the coal mine in Turów) at the dose of 2 g C kg^{-1} of soil; II - liming (no liming and liming at a dose calculated for 1 Hh of soil as $CaCO_3$) ; III – contamination of soil with nickel at different levels (no nickel, 100 and 200 mg Ni kg^{-1} of soil as aqueous solution of $NiCI_2$ $6H_2O$). Orchard grass (*Dactylis glomerata* L.) was used as the test crop and was harvested four times during each vegetation season. The soil was analysed in each year of the experiment after the fourth (last) harvest of the test crop. All the factors had a significant diversifying impact on dehydrogenase activity in the soil in the years of the experiment. The addition of organic waste and liming increased the activity of the enzymes in question, whereas increasing the soil contamination with nickel to 200 mg Ni kg^{-1} of soil had the opposite effect. The toxic impact of nickel was mitigated by the addition of organic fertilisation and liming.

Unlike other geological formations, soil shows what is referred to as biological activity, which is affected, among others, by soil microorganisms and the enzymes they secrete [14, 16]. Enzymes are natural mediators and catalysts of many important soil processes such as formation and decomposition of humus, releasing minerals and making them available to plants, bioreduction of molecular nitrogen, nitrification and denitrification.

* Prof. B. Kuziemska, DSc., Prof. S. Kalembasa, DSc., A. Wysokiński, DSc., D. Jaremko, DSc.; Soil Science and Plant Nutrition Department, Siedlce University of Natural Sciences and Humanities, B.Prusa 14, 08–110 Siedlce, Poland.

The level of enzymatic activity is a sensitive index of their fertility and it provides information about ecological changes in the soil environment [4, 5, 10, 11].

Dehydrogenases are a large group of oxidoreductases, located in cytosol or in special structures made up of cytoplasmatic membranes. They catalyse oxidation of organic compounds by making them lose electrons and protons. Regardless of the state of soil aeration, dehydrogenases are an element of respiratory metabolism which is closely connected with producing energy in a biologically available form (ATP). Dehydrogenase coenzymes (NAD and NADP) mediate the participation of protons lost by oxidised substrates in reactions of biosynthesis [6, 13].

The activity of dehydrogenases in the soil is associated with the action of many enzymes or enzyme systems, commonly occurring in soil microorganisms [15].

Many papers [1, 3, 11, 17] have described the close relationship between the activity of hydrogenases and organic matter content, soil fertility and the size of population of soil microorganisms, pH, water-air relationship, respiration (absorption of O_2, releasing CO_2), as well as heavy metal content. Enzymatic activity of soil is highly diverse during the vegetation period and depends upon several parameters. This variability is changed, among others, by the application of organic and mineral fertilizers as well as different compounds which have influence upon the contamination of soil.

On this background, the question arises of how often and at which time should the samples be taken for the determination of soil enzymatic activity. Searching the literature, we have decided that the soil samples for this experiment should be taken at the end of the vegetation period taking into consideration the most stable turnover of processes in the soil.

This paper aims to determine the impact of organic materials and liming on the activity of dehydrogenases in soil contaminated with nickel.

MATERIALS AND METHODS

Soil after four-year pot experiment was taken for this study; the pot experiment was conducted during the period from 2004 to 2007 at a facility of the University of Life Sciences and Humanities, Siedlce, in a totally random arrangement. The following factors were taken into account: I – organic fertilising (no organic fertiliser, sludge from the wastewater treatment plant in Siedlce, chicken litter and brown coal from the coal mine in Turów) at the dose of 2 g C kg^{-1} of soil; II - liming (no liming and liming at a dose calculated for 1 Hh of soil as $CaCO_3$); III – different levels of soil contamination with nickel (no nickel, 100 and 200 mg Ni kg^{-1} of soil applied as aqueous solution of $NiCI_2$ $6H_2O$). The chemical composition of the organic materials used is presented in Table 1. Orchard grass (*Dactylis glomerata* L.) was used as the experimental crop; it was harvested four times during each vegetation season. The soil formation used in the experiment was taken from the arable layer 0–20 cm of grey-brown podzolic soil with the granulometric composition of strong clayey sand. The following soil properties were determined before the experiment was set up: pH in 1 mol KCl dm^{-1} – 5,6; total nitrogen 0.98 g kg^{-1}; organic carbon 7.9 g kg^{-1} of soil. The content of available phosphorus and potassium was 69 and 75 mg kg^{-1} of soil, respectively. The content of nickel in the soil formation was 5.67 mg kg^{-1} of soil.

TABLE 1. CHEMICAL COMPOSITION OF ORGANIC MATERIALS USED IN POT EXPERIMENT

Component	Organic materials		
	Sludge from Siedlce	Brojlers droping	Brown coal
	(g kg^{-1}DM)		
C	371	399.1	541
N	60.5	16.8	4.0
P	31.17	23.6	0.11
K	4.28	20.0	0.84
Ca	39.6	39.19	5.18
Mg	8.42	6.96	2.33
(mg kg^{-1}DM)			
Zn	1276.8	295.6	17.16
Cd	1.99	15.2	0.07
Pb	50.5	5.00	3.71
Cu	137.7	54.1	10.12
Ni	20.57	39.32	5.10
Dry matter (g kg^{-1})	180	400	850

15 dm^3 pots were filled with 10 kg of soil each and humidity level of 60% of full water capacity was maintained during the vegetation season. Soil during each year of the experiment was analysed after the fourth (final) harvest of the test crop. Dehydrogenase activity was determined by the method developed by Casida *et al.* [7], using TTC. The method involves incubation of soil with colourless, water-soluble substrate, TTC (2,3,5-triphenyl tetrazolium chloride), which is reduced by enzymes to coloured, water-insoluble triphenylformazan (TPF) [9]. Organic carbon was determined by redox titration, and pH in 1 M KCl by the potentiometric method. The results were worked out statistically by the analysis of variance method using the Fisher-Snedecor and $LSD_{0.05}$ was calculated by the Tukey's test. The analysis of linear correlation was conducted in order to determine the relationship between the attributes in question.

RESULTS AND DISCUSSION

The average activity of dehydrogenase in the experiment ranged from 1.18 (no organic materials, after the first year of the grass cultivation) to 1.72 mmol TPF · kg^{-1} · h^{-1} (chicken litter, after the fourth year of grass cultivation) (table 2). Regardless of the time of taking a sample for analysis, the highest activity of dehydrogenases was found in the soil with chicken litter; it was lower by about 31% in the soil with no organic materials, with the lowest dehydrogenase activity. The application of organic materials increased the activity of the enzymes in the soil; it was proven to be statistically significant in the soil with chicken litter and brown coal in the first year; in the soil with chicken litter and sludge from the wastewater treatment in Siedlce in the second year and in the soil fertilised with all types of organic waste in the last two years of the experiment.

TABLE 2. DEHYDROGENASE ACTIVITY IN SOIL (mmol TPF kg^{-1} h^{-1})

Liming		0				Ca acc. 1 Hh				Means
Fertilization	Year	Doses of nickel (mg kg^{-1}soil)				Doses of nickel (mg kg^{-1}soil)				
		0	100	200	Means	0	100	200	Means	
Without organic fertilization	I	1.23	1.18	0.96	1.12	1.25	1.33	1.11	1.23	1.18
	II	1.28	1.20	1.06	1.18	1.31	1.39	1.22	1.31	1.24
	III	1.29	1.26	1.18	1.24	1.32	1.38	1.25	1.32	1.28
	IV	1.27	1.23	1.19	1.23	1.36	1.37	1.22	1.32	1.27
Means		1.27	1.22	1.10	1.19	1.31	1.37	1.20	1.29	1.24
Sludges from Siedlce	I	1.26	1.30	1.02	1.19	1.25	1.35	1.16	1.25	1.22
	II	1.32	1.31	1.19	1.27	1.37	1.41	1.26	1.35	1.31
	III	1.54	1.59	1.29	1.47	1.58	1.65	1.44	1.55	1.51
	IV	1.58	1.73	1.43	1.58	1.59	1.75	1.40	1.58	1.58
Means		1.47	1.48	1.23	1.38	1.45	1.54	1.31	1.43	1.40
Brojlers droping	I	1.47	1.53	1.38	1.46	1.48	1.53	1.42	1.48	1.47
	II	1.45	1.60	1.31	1.45	1.56	1.50	1.34	1.47	1.46
	III	1.64	1.75	1.50	1.63	1.70	1.64	1.63	1.66	1.64
	IV	1.73	1.78	1.57	1.69	1.75	1.88	1.65	1.76	1.72
Means		1.57	1.66	1.44	1.56	1.62	1.64	1.51	1.59	1.57
Brown coal	I	1.34	1.34	1.16	1.28	1.57	1.45	1.28	1.43	1.36
	II	1.24	1.20	1.18	1.21	1.28	1.34	1.23	1.28	1.25
	III	1.34	1.38	1.38	1.37	1.35	1.44	1.31	1.37	1.37
	IV	1.35	1.41	1.33	1.36	1.36	1.44	1.32	1.37	1.37
Means		1.32	1.33	1.26	1.30	1.39	1.42	1.29	1.36	1.33
Means of experiment		1.39	1.42	1.26	1.36	1.44	1.49	1.33	1.42	1.39

TABLE 2. CONTINUATION

	Years			
	I	II	III	IV
$LSD_{0.05}$ for:				
organic fertilization	0.058	0.070	0.080	0.067
liming	0.031	0.037	0.043	0.035
doses of nickel	0.045	0.055	0.063	0.052

The stimulating impact of organic material on dehydrogenase activity in the soil has been reported in earlier papers by Kalembasa and Kuziemska [11], and in reports by other authors [12]. Baran *et al.* [2] used mineral wool and sludge in the soil remediation, which caused an increase in their enzymatic activity as compared to a control treatment. The other factor examined in the experiment - liming - changed the attribute in all the years of the experiment, causing the dehydrogenase activity in the soil to increase considerably. The pH value has been found by Wyszkowska *et al.* [19] to affect the activity of enzymes: change of pH from 7.1 to 6.4 decreased the activity threefold.

The introduction of nickel as contamination to soil in the amount of 100 mg Ni kg^{-1} of soil did not significantly change the activity of dehydrogenases, whereas the dose of 200 mg Ni kg^{-1} of soil significantly decreased the activity of the enzymes in the soil, regardless of the time of taking the samples for analyses. Kalembasa and Kuziemska [11] and Wyszkowska and Wyszkowski [18] examined the impact of different levels of nickel in the soil on the activity of dehydrogenases and found it to be adverse when the element was present in the soil at a higher content. Both organic waste material and liming reduced the toxic impact in all the years of the experiment.

The pH value of the soil in successive years of the experiment, determined in 1 M KCl dm^{-3} solution, are displayed in Table 3.

This value ranged from 5.16 to 7.16 units and was changed by all the examined factors. The applied organic waste materials significantly increased the pH values in all the years of the experiment, except for year second, when an increasing tendency was also noticeable, but one which was not proven statistically. The pH of limed soil was higher than that of soil which was not limed, which is in line with the findings of other authors [8]. The greatest impact of the added organic materials and lime on the pH value was found in the first and the second year of the experiment, with the impact gradually disappearing in the third and the fourth year which could be attributed to the rate of calcium carbonate transformation in the soil. The impact of the different content of nickel in the soil on its pH manifested itself in the first and the last year of the experiment. The increased level of contamination with nickel was accompanied by a gradual decrease in the pH value. This may be related to the form of nickel introduced to soil ($NiCl_2$) and its transformations in the soil solution (salt hydrolysis with the pH value decrease).

TABLE 3. pH OF SOIL IN 1 M KCl

Liming		0			Ca acc. 1 Hh		
Fertilization	Years	Doses of nickel ($mg \cdot kg^{-1}$soil)			Doses of nickel ($mg \cdot kg^{-1}$soil)		
		0	100	200	0	100	200
Without organic fertilization	I II III IV	5.62 5.50 5.40 5.30	5.50 5.46 5.30 5.30	5.43 5.40 5.21 5.16	6.78 6.50 6.28 6.12	6.70 6.50 6.12 6.01	6.70 6.39 6.17 6.00
Sludges from Siedlce	I II III IV	5.90 5.92 5.84 5.70	5.84 5.86 5.70 5.58	5.81 5.79 5.71 5.56	6.94 7.00 6.82 6.35	6.80 6.96 6.80 6.28	6.76 6.82 6.78 6.20
Brojlers droping	I II III IV	5.94 5.99 5.88 5.70	5.92 5.90 5.86 5.60	5.90 5.84 5.86 5.60	6.88 6.92 6.84 6.28	6.90 6.84 6.80 6.23	6.84 6.80 6.60 6.10
Brown coal	I II III IV	6.41 6.52 5.42 6.14	6.38 6.50 6.30 6.08	6.40 6.48 6.40 6.05	7.16 7.12 7.00 6.40	7.08 7.14 7.02 6.20	7.10 7.09 7.03 6.20
				Years			
				I	II	III	IV
$LSD_{0.05}$ for: organic fertilization liming doses of nickel				 0.097 0.036 0.053	 n.s. 0.053 n.s.	 0.128 0.068 n.s.	 0.107 0.056 0.084

The content of organic carbon in the soil samples taken after successive years of cultivation of orchard grass ranged from 7.02 (no organic fertilisation) after the second year of cultivation to 9.59 g C kg^{-1} of soil (soil with brown coal; after the first year of cultivation of orchard grass - Table 4). Regardless of the time when samples were taken for analysis, the soil fertilised with organic waste material contained higher amounts compared to a control treatment. Carbon content was similar in every object where organic materials were used. This may have been caused by the fact that the amount of organic carbon introduced with them was the same (2 g kg^{-1} of soil). The analyses of the content of carbon in successive years of the experiment revealed gradual reduction of organic carbon content in the soil fertilised with organic materials which is caused by mineralisation processes.

The statistical analysis did not show any impact of liming or variable nickel content in the soil on the attribute in question, except in the second year of the experiment when liming significantly reduced the content of organic carbon in the soil.

The values of correlation coefficients calculated in different years of the experiment showed that significant relationships existed between dehydrogenase activity and organic carbon content in the first year of the experiment (r=0.45*) and between pH and organic carbon content in the third (r=0.56**) and fourth year (r=0.42*) of the experiment.

It can be claimed in conclusion of the four-year study that all organic waste material, i.e. sludge from the wastewater treatment plant in Siedlce, chicken litter and brown coal from the Turów mine, increased dehydrogenase activity, the pH value and organic carbon content in the soil.

Liming also increased the activity of the enzymes and the pH value, without differentiating to a statistically proven extent the organic carbon content in the soil. Increasing the level of soil contamination with nickel reduced gradually dehydrogenase activity and the pH value. The organic waste materials used in the experiment proved to be a good source of organic carbon in the soil and a factor increasing dehydrogenase activity in the soil under analysis.

CONCLUSIONS

1. Organic waste materials used in the experiment increased dehydrogenase activity and the pH value of the soil in all the years of the experiment.

2. Liming had a stimulating impact on dehydrogenase activity and the soil pH value.

3. Raising the level of soil contamination with nickel to 200 mg Ni kg^{-1} of soil decreased the activity of the enzymes.

4. Liming reduced the toxic impacts of high doses of nickel, especially in soils that waste organic materials were not used.

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BADANIE WPŁYWU ODPADOWYCH MATERIAŁÓW ORGANICZNYCH NA AKTYWNOŚĆ DEHYDROGENAZ W GLEBIE ZANIECZYSZCZONEJ NIKLEM

Czteroletnie doświadczenie wazonowe przeprowadzono w obiekcie doświadczalnym Uniwersytetu Przyrodniczo-Humanistycznego w Siedlcach, w układzie całkowicie losowym, w trzech powtórzeniach. W doświadczeniu uwzględniono następujące czynniki: I - nawożenie organiczne (bez nawożenia organicznego, osad ściekowy z oczyszczalni ścieków w Siedlcach, kurzeniec od brojlerów i węgiel brunatny z kopalni węgla brunatnego w Turowie) w dawce wprowadzającej do gleby 2 g C kg^{-1} gleby) ; II - wapnowanie (bez wapnowania i wapnowanie w dawce wyliczonej wg 1 Hh gleby w formie $CaCO_3$) ; III - zróżnicowane zanieczyszczenie gleby niklem (bez stosowania niklu, 100 i 200 mg Ni kg^{-1} gleby w formie wodnego roztworu $NiCl_2$ $6H_2O$). Rośliną testową była trawa - kupkówka pospolita (*Dactylis glomerata* L.), której w każdym sezonie wegetacyjnym zbierano po cztery pokosy. Analizie poddano glebę w każdym roku prowadzenia doświadczenia po czwartym, ostatnim pokosie rośliny testowej. Wszystkie czynniki w sposób istotny różnicowały aktywność dehydrogenaz w analizowanej glebie w latach prowadzenia badań. Zastosowane odpadowe materiały organiczne i wapnowanie spowodowały wzrost aktywności omawianych enzymów, natomiast zwiększenie stopnia zanieczyszczenia gleby niklem do 200 mg Ni kg^{-1} gleby, zmniejszenie ich aktywności. Toksyczne działanie niklu ograniczało nawożenie organiczne i wapnowanie.