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SEASONAL CHANGES OF SELENIUM AND SELECTED OXIDOREDUCTASES IN SOIL UNDER MANURE AND NITROGEN FERTILZATION

SEZONOWE ZMIANY ZAWARTOŚCI SELENU ORAZ AKTYWNOŚCI WYBRANYCH OKSYDOREDUKTAZ W GLEBIE W WARUNKACH NAWOŻENIA OBORNIKIEM I AZOTEM

Abstract: The aim of the study was to determine the changes of total and available Se concentrations in soil and some oxidoreductases activity in relation to applied doses of fertilizers over vegetation period. The experiment was conducted applying the following crop rotation system – potato – winter wheat with intercrop – spring barley+ undersown – red clover and grasses designed in a split-plot with four replications. The soil was fertilized with farmyard manure (FYM) at doses 0, 20, 40, 60 and 80 Mg · ha^{-1} (under potato) and with nitrogen at rates 0, 30 and 60 kgN · ha^{-1} under red clover and grasses. Total selenium content in soil under red clover and grasses cultivation ranged from 132 to 169 μg · kg^{-1}, what indicates that analysed soil is poor in this microelement. FYM fertilization significantly increased total selenium content in the soil with increasing doses of this fertilizer. The highest amounts of total selenium were found in soil at the beginning of the investigation period. The highest content of phytoavailable fractions and their share in the total selenium were observed in the case of fertilization with FYM at a dose of 40 Mg · ha^{-1} and then decreased with increasing doses of FYM. FYM fertilization as well as mineral nitrogen stimulated the activity of the investigated oxidoreductases, in comparison with non fertilized soil. The highest amounts of enzymes activity were obtained in July. The calculated correlation coefficients between total selenium and organic carbon and total nitrogen content in soil; enzymes activity and organic carbon and total nitrogen and between total selenium content and DHA activity, confirmed a close inter-relationship among these parameters.

Keywords: selenium, available fractions, dehydrogenases, catalase, farmyard manure, nitrogen

Introduction

Selenium is an essential nutrient for animals, microorganisms and some other eukaryotes. Although selenium has not been demonstrated to be essential in vascular

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plants, the ability of some plants to accumulate and transform selenium into bioactive compounds has important implications for human nutrition and health, and for the environment [1]. Selenium exists in four valence states, of which the 2-state predominates in organic Se compounds. Commonly occurring species, selenites (Se^{4+}) and selenates (Se^{6+}), do not form stable compounds in geochemical environments and are preferably adsorbed by minerals, particularly clay minerals, and Fe and Mn oxides as well as hydroxides [2].

Selenium is primarily taken up from the soil by plants as selenate or selenite. Selenate directly competes with sulfate for uptake by plants [1]. The factors which control Se mobility and availability in soils are pH, redox conditions and soil organic matter (SOM) content [2]. Redox conditions and pH determine the Se species in a soil environment, but in most conditions selenate and selenite are the main species. Selenate is predominant at near-neutral reaction and under aerobic conditions, whereas selenite is the main species at acid reaction and lower redox potential. Selenite is tightly bound to clay particles and Fe/Al oxides and thus only little available for plant uptake. Selenate is only weakly sorbed by soil particles, more mobile in the soil solution and therefore more plant-available [3].

According to Samuel et al [4], dehydrogenase activity of a soil is an indicator of biological redox-systems and can be considered as a measure of the intensity of microbial metabolism in soil. The catalase of aerobic organism splits the toxic H_2O_2 produced from the mitochondrial electron transport and from various hydroxylation and oxygenation reactions into water and oxygen [4]. Since aerobic organism predominates in non-compacted and non-waterlogged soils, catalase activity was used to characterize soil microbial activities.

The objective of this study was to evaluate the effects of organic and mineral fertilization on the available to plants forms of selenium and dynamics of dehydrogenases and catalase activity affecting the selenium status in soil.

Materials and methods

Soil samples were collected from the long-term static experiment established at the Agricultural Experimental Station at Grabow carried out since 1980 by the Department of Plant Nutrition of the Institute of Soil Science and Cultivation in Pulley (Poland). The experiment was conducted applying crop rotation "enriching in organic matter" (potato – winter wheat + intercrop – spring barley + undersown and red clover + grasses), designed in a split-plot with four replications (sub-plots). Organic fertilizer in a form of cattle manure (FYM) was applied under potato at the doses of 0, 20, 40, 60 and 80 $Mg \cdot ha^{-1}$ (factor I) and nitrogen in a form of ammonium nitrate at 0, 30 and 90 $kgN \cdot ha^{-1}$ under red clover and grasses (factor II). Soil samples were collected in March, May and July (factor III) 2004, from the 0–20 cm layer under red clover ('Jubilatka' cv.) and grasses. Soil samples were air-dried and sieved through a 2 mm screen.

Soil samples were analysed for granulometric composition according to Bouyoucoss--Cassagrande method, organic carbon (TOC) by wet oxidation with potassium di-

chromate, total nitrogen (TN) following by Kjeldahl method and pH potentiometrically in distilled water and 1 mol · dm^{-3} KCl solution [5].

Total selenium content in soils was determined by the method of Watkinson [6] using a Hitachi F-2000 spectrofluorometer. Samples were microwave digested with concentrated nitric and perchloric acids. The different forms of selenium in the samples were reduced by boiling with 10 % HCl. The selenium was complexed with 2,3-diaminonaphtalene (DAN) to give the fluorescent compound, which was extracted with cyclohexane and read on a spectrofluorometer at excitation and emission wave lengths of 376 and 519 nm, respectively. The analytical procedures gave satisfactory values for the standard reference material CRM024-050 Resource Technology Corporation (RTC), soil from Western US of a texture of loamy sand; 0.558 mgSe · kg^{-1} (certified value 0.540 mgSe · kg^{-1}). Available to plants forms of selenium were extracted from the soil by the part of sequential extraction method recommended by Chao and Sanzolone [7] with modification of Wang and Chen [8]. Firstly, 0.25 mol · dm^{-3} KCl solution was used to extract the soluble form of selenium (Se^{6+}). The exchangeable and specifically adsorptive forms of selenium (Se^{4+}) were extracted by 0.1 mol · dm^{-3} KH_2PO_4 solution. The final reaction solution of each extraction was adjusted with dilute HCl to a pH range 1.7–2.0. The selenite (Se^{4+}) was then chelated by adding 2,3-diaminonaphtalene to the solution and determined by fluorescence spectrophotometry [6].

Dehydrogenases activity (DHA) were assayed applying the method by Casida et al [9]. Soil DHA activity was estimated by reducing 2,3,5-triphenyltetrazolium chloride. Soil sample was mixed with $CaCO_3$ and 2,3,5-triphenyltetrazolium chloride (TTC) and incubated for 24 h at 37 °C. Dehydrogenase converts TTC to 2,3,5-triphenylformazan (TPF). The TPF formed was extracted with acetone, the extracts were filtered and absorption was measured at 485 nm spectrophotometrically. The enzyme activities were expressed as mg triphenyltetrazolium formazan (TPF) · g^{-1} · 24 h^{-1}.

Catalase activity (CAT) was measured using the method by Johnson and Temple [10]. Soil was incubated with hydrogen peroxide for 20 min at 20 °C. The remaining H_2O_2, not broken-down by catalase, was treated with potassium permanganate exposed to H_2SO_4. To eliminate a probable overestimation of enzyme activity due to chemical reduction of H_2O_2 added, a correction for autoclaved soil (0.1 MPa, 120 °C, 30 min) was made. The results were expressed in mg H_2O_2 consumed · g^{-1} · min^{-1}.

Three-way and two-way analysis of variance (ANOVA) was used to identify significant differences ($p < 0.05$) between TOC, TN, Se concentrations and enzymes activity in studied soils. Data analysis was carried out using Statistica 8.0 for Windows Stat.Soft. Inc.

Results and discussion

The soil, according to the FAO classification, was classified as Haplic Luvisols and demonstrated the texture of loamy sand and sandy loam. pH values measured in KCl and H_2O were in the ranges: 5.4–5.6 and 6.1–6.3, respectively (Table 1).

Table 1

General properties of the investigated soil

FYM dose [t · ha^{-1}]	N dose [kg · ha^{-1}]	Share of soil particles fraction		pH	
		< 0.02 mm	< 0.002 mm	in H_2O	in KCl
		[%]			
0	N0	18	6	6.1	5.5
	N1	17	6	6.3	5.5
	N2	19	8	6.3	5.4
20	N0	14	5	6.2	5.5
	N1	16	8	6.1	5.4
	N2	15	6	6.1	5.4
40	N0	19	8	6.2	5.5
	N1	17	6	6.2	5.4
	N2	13	5	6.2	5.4
60	N0	16	5	6.2	5.5
	N1	16	6	6.2	5.4
	N2	19	7	6.2	5.6
80	N0	15	4	6.2	5.5
	N1	16	6	6.3	5.6
	N2	15	5	6.3	5.4

The content of organic carbon (TOC) in the studied soil varied from 7.55 to 10.05 g · kg^{-1}, depending upon FYM doses and nitrogen fertilization (Table 2). The highest increase in TOC was observed for treatments with 60 and 80 Mg · ha^{-1} of FYM. Soil samples from the treatment without FYM contain 24 % less TOC than those from the treatment with the highest dose of FYM. Nitrogen fertilization affected TOC and TN. The content of organic carbon in the soil was the highest after applying the highest dose of nitrogen.

Table 2

Total organic carbon and total nitrogen content in the investigated soil

FYM dose [Mg · ha^{-1}] (Factor A)	N dose [kg · ha^{-1}] (Factor B)	TOC	TN
		[g · kg^{-1}]	
0	N0	7.55	0.679
	N1	7.60	0.683
	N2	7.14	0.711
20	N0	8.10	0.707
	N1	7.89	0.721
	N2	8.05	0.739

Table 2 contd.

FYM dose [Mg · ha^{-1}] (Factor A)	N dose [kg · ha^{-1}] (Factor B)	TOC	TN
		[g · kg^{-1}]	
40	N0	8.37	0.749
	N1	7.61	0.728
	N2	9.24	0.812
60	N0	9.67	0.854
	N1	9.87	0.826
	N2	10.05	0.861
80	N0	9.35	0.917
	N1	9.94	0.931
	N2	9.78	0.959
Mean for FYM doses (Factor A)	0	7.43	0.664
	20	8.01	0.725
	40	8.41	0.748
	60	9.66	0.814
	80	9.79	0.859
Mean for N doses (Factor B)	N0	7.81	0.739
	N1	7.78	0.750
	N2	8.05	0.771
$LSD_{0.05}$		A – 0.022; B – 0.018	A – 0.002; B – 0.001

Total selenium content in the soil samples ranged from 110 to 249 μg · kg^{-1} (Table 3).

We observed the increase of total selenium content almost 28 % in soil fertilized with the highest dose of FYM in comparison with soil from control plots. This may have been consequence by the amount of this microelement in farmyard manure, which was at the rate of 2.24 mg · kg^{-1}. Such low levels of selenium in soils indicated that plants growing on these soils are deficient in this microelement. According to Kabata-Pendias [2], the mean total selenium content in the soils worldwide is estimated as 0.44 mg · kg^{-1}, while its background contents in various soil groups range from 0.05 to 1.5 mg · kg^{-1}. The selenates and selenites content in the soil samples fluctuating between 16–35 μg · kg^{-1} and 15–43 μg · kg^{-1}, respectively (Table 3).

An increase in selenates and selenites was observed after application of FYM in comparison with control plots, but the highest contents of both of these ions were recorded in soil samples collected from plots fertilized with 40 Mg · ha^{-1} of FYM. The opposite tendency was noted in the case of a plot fertilized with the highest doses of FYM. The use of nitrogen fertilizer at 30 kgN · ha^{-1} caused an increase in selenates concentration in the studied soil. During vegetation period of red clover and grasses the total content of selenium and its phytoavailable fractions in the soil decreased.

Kabata-Pendias [2] was of the opinion that low mobility of selenium occurs in soils with high contents of hydroxides, clay granulometric fractions and soil organic matter,

which may act the reduction of selenate to selenite and thus reduce Se availability in soils.

Table 3

Total and phytoavailable fractions content of selenium in the studied soil under red clover [μg · kg^{-1}]

FYM dose [Mg · ha^{-1}] (Factor A)	N dose [kg · ha^{-1}] (Factor B)	Total Se			SeF1 (Se VI)			SeF2 (Se IV)		
		Date of sampling (Factor C)								
		March	May	July	March	May	July	March	May	July
0	N0	132	150	110	19	22	21	29	27	23
	N1	128	150	125	20	18	23	25	23	25
	N2	131	141	117	20	23	22	22	23	20
20	N0	165	132	144	18	16	13	19	17	16
	N1	121	142	135	21	23	26	21	23	24
	N2	145	132	140	23	25	23	22	24	20
40	N0	156	160	128	26	27	29	43	40	38
	N1	169	124	184	32	34	36	30	32	35
	N2	160	124	132	31	30	26	29	27	26
60	N0	164	180	123	27	25	23	22	19	16
	N1	156	174	116	29	26	23	23	22	19
	N2	178	187	134	22	19	16	22	18	15
80	N0	168	124	189	23	20	16	25	22	19
	N1	168	127	249	33	29	27	35	31	27
	N2	184	184	122	25	22	19	23	19	17
Mean for FYM doses (Factor A)										
	0	132			21			24		
	20	139			21			21		
	40	149			30			33		
	60	158			23			19		
	80	169			24			24		
Mean for N doses (Factor B)										
	0	149			22			25		
	N1	152			26			26		
	N2	148			23			22		
Mean for date of sampling (Factor C)										
	March	155			24			26		
	May	149			24			24		
	July	143			23			23		
	$LSD_{0.05}$	A – 3.11; B – 1.08; C – 1.23			A – 1.13; B – 1.21; C – 1.34			A – 1.14; B – 1.22; C – 1.28		

The activity of DHA over the vegetation period in the analysed soil fluctuated between 0.018 and 0.058 mg TPF · g^{-1} · 24 h^{-1} (Table 4). Fertilization with FYM and nitrogen significantly stimulated the activity of this enzyme, along with increasing doses of both fertilizers.

Table 4

Enzymatic activity in soil under study

FYM dose [Mg · ha^{-1}] (Factor A)	N dose [kg · ha^{-1}] (Factor B)	DHA activity [mg TPF · g^{-1} · $24h^{-1}$]			CAT activity [mg H_2O_2 · g^{-1} · min^{-1}]		
		Date of sampling (Factor C)					
		March	May	July	March	May	July
0	0	0.019	0.030	0.032	0.040	0.051	0.085
	N1	0.017	0.030	0.035	0.051	0.053	0.102
	N2	0.018	0028	0.030	0.053	0.047	0.102
20	0	0.023	0.040	0.030	0.051	0.060	0.102
	N1	0.025	0.033	0.037	0.047	0.055	0.104
	N2	0.031	0.035	0.032	0.060	0.055	0.109
40	0	0.032	0.034	0.033	0.053	0.057	0.109
	N1	0.029	0.036	0.039	0.055	0.060	0.115
	N2	0.032	0.038	0.046	0.045	0.057	0.113
60	0	0.032	0.046	0.050	0.045	0.057	0.119
	N1	0.030	0.044	0.054	0.051	0.066	0.113
	N2	0.036	0.048	0.056	0.047	0.060	0.113
80	0	0.041	0.047	0.057	0.047	0.060	0.119
	N1	0.043	0.046	0.056	0.051	0.057	0.115
	N2	0.038	0.042	0.058	0.053	0.055	0.115
Mean for FYM doses (Factor A)							
	0	0.027			0.064		
	20	0.032			0.071		
	40	0.036			0.075		
	60	0.044			0.075		
	80	0.048			0.075		
Mean for N doses (Factor B)							
	0	0.037			0.071		
	N1	0.037			0.073		
	N2	0.038			0.072		
Mean for date of sampling (Factor C)							
	March	0.030			0.050		
	May	0.038			0.057		
	July	0.044			0.109		
	$LSD_{0.05}$	A – 0.002; B – 0.001; C – 0.003			A – 0.004; B – 0.001; C – 0.002		

Manure application differentiated CAT activity in soil (Table 4). During vegetation period manure fertilization strongly stimulated soil catalase activity with increasing doses, especially after application of 80 Mg · ha^{-1}, in comparison with the control soil. The highest CAT activity was recorded after applying ammonium nitrate with the dose of 30 kg N · ha^{-1}, in comparison with a soil from plot not fertilized with nitrogen.

Enzymatic activity in studied soil demonstrated clear seasonal variations and considerable fluctuations depending on climatic conditions and availability of substrate. The highest amounts of DHA as well as CAT activities were observed in soil collected in July. Mocek-Plociniak [11] stated that adequately high moisture of soil is a fundamental condition of soil enzymes activity.

Significant correlations were found for the relationship between the total selenium content and organic carbon and total nitrogen. Positive Pearson indexes were also found for: dehydrogenases activity and organic carbon and total nitrogen; catalase activity and organic carbon and total nitrogen (Table 5). At the beginning of vegetation period (in soil samples collected in March) we found close inter-relationship between total selenium content and DHA activity (r = 0.77).

Table 5

Simple correlation coefficients (r) between selenium content and enzymatic activity and soil properties

Examined properties	Fraction < 0.002	pH_{H_2O}	pH_{KCl}	TOC	NT	Se_{tot}	Se VI	Se IV	DHA	CAT
Fraction < 0.02	0.62*	–0.21*	–0.23*	0.29*	0.30*	0.25*	0.03	0.07	0.22*	0.21*
Fraction < 0.002		0.00	0.12	0.39*	0.40*	0.25*	0.12	0.24	0.22*	0.30*
pH_{H_2O}			–0.03	–0.04	–0.07	–0.11	–0.12	–0.15	–0.04	0.05
pH_{KCl}				0.22*	0.21*	0.18	0.14	0.17	0.11	0.20*
TOC					0.88*	0.40*	0.38	0.33	0.92*	0.71*
NT						0.44*	0.37	0.31	0.94*	0.78*
Se_{tot}							0.21	0.15	0.31	0.35

* r significant at $\alpha = 0.05$.

Many authors [4, 12–14] reported that increase inputs of organic residue, plant or animal manures, increase biological activity. FYM treatment usually increases microbial biomass and soil enzyme activities compared to soils without any organic or inorganic amendments. Thus management practices that increase incorporation of organic residue typically increase biological activity. Use of inorganic fertilizer can increase the plant biomass production which in turn increases the amount of residue returned to the soil and stimulates biological activity.

Conclusions

Total selenium content in soil under red clover cultivation ranged from 110 to 249 μg · kg^{-1}, what indicate that the analysed soil is poor in this microelement. FYM

fertilization significantly increased total selenium content in the soil with increasing doses of this fertilizer. The application of the highest dose of FYM caused an increase of total selenium about 28 % compared with control. The highest amounts of total selenium were found in soil at the beginning of the investigation period. The highest content of phytoavailable fractions were observed in the case of fertilization with FYM at a dose of 40 Mg · ha^{-1} and then decreased with increasing FYM doses. FYM fertilization and applied nitrogen stimulated the activity of the investigated oxidoreductases, in comparison with control. The highest enzymes activity was observed in July. We found close inter-relationship between total selenium content and organic carbon and total nitrogen content in soil; enzymes activity and organic carbon and total nitrogen and between total selenium content and DHA activity.

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SEZONOWE ZMIANY ZAWARTOŚCI SELENU ORAZ AKTYWNOŚCI WYBRANYCH OKSYDOREDUKTAZ W GLEBIE W WARUNKACH NAWOŻENIA OBORNIKIEM I AZOTEM

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Abstrakt: Celem pracy było określenie zmian zawartości selenu przyswajalnego dla roślin oraz aktywności wybranych enzymów uczestniczących w przemianach oksydoredukcyjnych w glebie w warunkach zróżnicowanego nawożenia. Próbki gleby pochodziły z doświadczenia prowadzonego przez IUNG w Puławach na terenie RZD Grabów nad Wisłą, z wariantu wzbogacającego glebę w substancję organiczną, z następującym doborem roślin w zmianowaniu: ziemniaki – pszenica ozima + międzyplon – jęczmień jary z wsiewką – koniczyna z trawami. Próbki pobrano czterokrotnie (w marcu, maju, lipcu i wrześniu) w 2001 r. z obiektów, na których uprawiano koniczynę czerwoną. Nawożenie obornikiem zastosowano (jednorazowo w trakcie rotacji) pod ziemniaki w dawkach 0, 20, 40, 60 i 80 Mg · ha^{-1}, natomiast pod koniczynę czerwoną z trawami zastosowano azot w postaci saletry amonowej w ilości 0, 40 i 120 kgN · ha^{-1}. Fitoprzyswajalne formy selenu wyekstrahowano z gleby, wykorzystując część analizy specjacyjnej zgodnie z metodą Chao i Sanzolone (1989) w modyfikacji Wang i Chen (2003), a następnie oznaczono metodą Watkinsona (1966) przy użyciu

spektrofluorymetru F-2000 firmy Hitachi. Aktywność dehydrogenaz (DHA) oznaczono metodą Casida (1964), a katalazy (CAT) metodą Johnsona i Temple (1964). Ogólna zawartość selenu w glebie pod koniczynę czerwoną z trawami wahała się od 132 do 169 $\mu g \cdot kg^{-1}$, co oznacza glebę ubogą w ten mikroelement. Wykazano, że nawożenie obornikiem w glebie pod uprawą koniczyny czerwonej z trawami wpływało istotnie na zawartość selenu przyswajalnego dla roślin. Niezależnie od terminu pobierania próbek glebowych, zastosowanie największej dawki obornika spowodowało istotne zwiększenie zawartości tych frakcji selenu w glebie. Nie wykazano natomiast jednoznacznego wpływu azotu w tym zakresie. Zawartość oznaczonych fitoprzyswajalnych frakcji selenu w środowisku glebowym oraz aktywność wybranych enzymów podlegała stałym wahaniom i wykazywała zmienność sezonową. Nawożenie obornikiem wyraźnie stymulowało aktywność enzymatyczną gleby. Obliczone wartości współczynników korelacji wykazały istotne zależności między aktywnością badanych enzymów glebowych a zawartością węgla organicznego i ogólną zawartością azotu.

Słowa kluczowe: selen, frakcje przyswajalne, dehydrogenazy, katalaza, obornik, azot