Original Research

Dynamics of Changes of Selenium Content in Soil and Red Clover (*Trifolium pratense* L.) Affected by Long-Term Organic Fertilization on the Background of Selected Soil Oxidoreductases

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Abstract

The aim of the present research was to determine the effect of fertilization applying various doses of manure on the selenium content in soil and red clover on the background of the activity of selected soil oxidoreductases. The soil was affected by organic fertilization in a form of manure in the doses of 0, 20, 40, 60, and 80 $t \cdot ha^{-1}$. The content of total selenium in control soil was, on average, 0.101 $mg \cdot kg^{-1}$. A comparison of results reported in literature shows that the studied soil was poor in that element. The application of the highest manure dose resulted in a 1.7-fold increase in the content of Se in soil. Fertilization with manure resulted in an increase of dehydrogenases and catalase activities in soil with increasing doses of manure. The bioaccumulation and translocation coefficients of selenium demonstrated that above-ground red clover biomass absorbed and transported selenium more easily from soil without manure or treated with FYM at a dose of 20 $t \cdot ha^{-1}$.

Keywords: total selenium, oxidoreductases activity, organic fertilization, red clover

Introduction

Selenium (Se) has been demonstrated to be an essential trace element for animal and human health. The concentration of Se in agricultural products and animal feed depends on the content of Se in the soil and its bioavailability [1, 2]. In soils Se exhibits a broad range of oxidation states: +6 in selenates, +4 in selenites, 0 in elemental Se, and -2 in inorganic and organic selenides. Selenate, which is poorly adsorbed on oxide surfaces and thus the most mobile Se form, can be expected to occur under high oxidative conditions. At low redox potential it can be reduced to selenite, which has a much higher adsorption affinity. It is strongly retained by ligand exchange on oxide surfaces, especially at low pH, which reduces its bioavailability [1, 3]. The transformation of easily soluble selenates added to acidic or neutral soils into slightly soluble forms is relatively fast [1]. In soil the processes of decomposition and synthesis of mineral and organic matter occur all the time, and they are monitored and activated by a variety of enzymes. All these processes form soil metabolism, which is crucial for soil fertility maintenance and preservation [4]. Many authors have indicated a strong influence of Se on the activities of oxidoreductase enzymes, such as catalase, glutathione per-

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oxidase and superoxide dismutase in plants and animals [5-9]. However, there have been relatively few reports on the contribution of Se to biochemical processes in soil [10].

The selenium concentration in plants depends on the chemical form of Se, its concentration and bioavailability in soils, and on the accumulation capacity of the plant [1, 11]. Although all the plants are able to take up and metabolize selenium, the assumption about its necessity for plants has not yet been fully confirmed. Numerous studies have shown that at low concentrations Se exerts a beneficial effect promoting growth and increasing stress tolerance of plants by enhancing their antioxidative capacity, reducing lipid peroxidation and enhancing the accumulation of starch and sugars [12-15]. Higher plants vary in their capacity to accumulate and tolerate selenium and they are classified into non-accumulators, indicators, and accumulators [14, 15]. According to Whanger [9], the currently observed interest in selenium focuses on the health benefits of high-Se plants as a source of cancer-preventative Se compounds, for its unique role in recycling and delivering selenium from the soil to the food chain. The selenium concentration in agricultural products is very low in many areas throughout the world, and also in large areas of Central and Northern Europe, which is attributed to a poor supply of selenium from the soil, and ultimately the underlying geology. Stadlober [16] mentioned that selenium levels in crops can be enhanced either by adding organic supplements of high level selenium (sewage sludge, manure of selenium-supplemented farm animals) or by adding selenate containing mineral fertilizers.

The aim of the present research was to determine the effect of fertilization applying various doses of manure on the selenium content in soil and red clover with respect to the selected soil oxidoreductases.

Materials and Methods

Soil and plant samples were collected from the long-term static experiment established at the Agricultural Experimental Station at Grabów carried out since 1980 by the Department of Plant Nutrition of the Institute of Soil Science and Cultivation in Puławy. The experiment was conducted applying the following crop rotation system: 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 doses of 0, 20, 40, 60, and 80 $t \cdot ha^{-1}$. Soil samples were collected in the 24[th] year of the experiment, in July 2004, from the 0-20 cm layer under red clover. Soil samples were air-dried and sieved through a 2 mm screen. Red clover (cv. Jubilatka) was rinsed in deionized water to remove soil particles, separated into above-ground biomass and roots, and dried. Total selenium content was determined by the method of Watkinson [17] using a Hitachi F-2000 spectrofluorometer. Soil 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 the fluorescent compound, which was extracted with cyclohexane and read on the spectrofluorometer at excitation and emission wave lengths of 376 and 519 nm, respectively. The analytical procedures provided satisfactory values for the standard reference material CRM024-050 from the Resource Technology Corporation (RTC); determined value was 0.558 $mgSe \cdot kg^{-1}$ (certified value – 0.540 $mg \cdot kg^{-1}$). The certified reference material was included in each batch of samples for quality control. Dehydrogenases activity (DHA) was assayed applying the method by Casida et al. [18]. 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 37ºC. Dehydrogenase converts TTC to 2,3,5-triphenylformazan (TPF). The formed TPF was extracted with acetone, the extracts were filtered and absorption was measured at 485 nm spectrophotometrically. The enzyme activities were expressed as mg triphenyl tetrazolium formazan $(TPF) \cdot g^{-1} \cdot 24h^{-1}$. Catalase activity (CAT) was measured using the method by Johnson and Temple [19]. Soil was incubated with hydrogen peroxide for 20 min at 20ºC. 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 added H_2O_2, a correction for autoclaved soil (0.1 MPa, 120ºC, 30 min) was made. The results were expressed in mg H_2O_2 $consumed \cdot g^{-1} \cdot min^{-1}$. Peroxidases activity (PX) was assayed, applying the Zwiagincew method [20]. Soil sample was mixed with pyrogallol and H_2O_2, and incubated for 20 min at 30ºC. Peroxidases converts pyrogallol to purpurogallin, which was extracted with ethyl ether and absorption was measured at 430 nm spectrofotometrically. The results were expressed as mg $purpurogallin \cdot g^{-1}$ $d.w. \cdot h^{-1}$. The soil samples were analyzed for granulometric composition following the Bouyoucoss-Cassagrande method, organic carbon – applying wet oxidation with potassium dichromate, and pH in distilled water and 1 $mol \cdot dm^{-3}$ KCl potentiometrically. All the analyses were performed in triplicate. Mean values are presented in the tables.

Statistical analyses were performed using a single-factor analysis of variance ANOVA. Means were compared with the use of the least significant difference (LSD) test. A probability value of ≤ 0.05 was considered to be significant. The results were statistically verified in order to identify any correlations between the parameters, using Pearson's linear correlation method. The correlation coefficients were calculated and some relationships were presented as regression curves. All the computations were made using Statistica 8.0 for Windows Stat.Soft. Inc.

Results and Discussion

The general properties and total selenium content of the soil under study are given in Table 1. The soil, according to the FAO classification, was classified as Haplic Luvisols and demonstrated the texture of loamy sand and sandy loam.

Table 1. General properties of soil under study (mean values for sub-plots).

Dose of manure [t·ha^{-1}]	Soil particle size fraction [%]		pH in		Organic carbon [g·kg^{-1}]
	<0.02 [mm]	<0.002 [mm]	H_2O	KCl	
0	18	7	5.9	5.0	8.74±0.09 *a*
20	16	7	5.9	5.1	10.14±0.08 *a*
40	17	6	5.7	5.1	11.41±0.19 *a*
60	18	6	5.7	5.2	11.84±0.12 *a*
80	15	5	5.8	5.2	12.15±0.34 *a*

a Values are means of three determinations ±standard deviations.

The pH values of soil were in the slightly acidic range 5.0-5.9. The application of manure resulted in the highest contents of organic carbon in soil, especially in soil from the plots treated with FYM in the doses of 60 and 80 t·ha^{-1}. The average content of total selenium in soil from control plots, without FYM, was 0.101 mg·kg^{-1} (Table 2). Similar values (an average of 0.082 mg·kg^{-1}) were found in lessivè soils from the Pomorze and Kujawy region [21, 22], an important agricultural region of Poland. Such low levels of selenium in soils indicated that plants growing on these soils are deficient in this microelement. According to Kabata-Pendias [1], 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}, being the lowest in Podzols and the highest in Histosols. Aro and Alfthang [23] and Hartikainen [2] claim that soils containing less than 0.5 mgSe·kg^{-1} are likely to lead to crops and pastures with inadequate selenium concentrations (<0.05 mg·kg^{-1} d.w.). The total selenium content in soil under study was correlated with the organic carbon content (Table 3), which coincides with our earlier findings [21, 22] and those reported by other authors [1, 24, 25]. Another increase in FYM doses by 20 t·ha^{-1} significantly increased the total selenium content in soil, respectively, by 16, 27, 39, and 41%, as compared with the no-manure treatment (Table 2). According to literature reports [26-28], selenium is present in various manures in amounts varying from 0.32 to 2.4 mg·kg^{-1}. Thus, the increase in Se in FYM-treated soil could have been due to the amount of this microelement in FYM.

The effect of the FYM doses on dehydrogenases activity in soil is presented in Table 2. The organic fertilizer applied differentiated the activity of dehydrogenases in soil. Another increase in the manure doses by 20 t·ha^{-1} increased enzymatic activity by 56, 62, 62, and 71%, respectively, as compared with treatment without manure. The analysis of correlation demonstrated a significant dependence between DHA activity and organic carbon in soil (Table 3). Dehydrogenases play an essential role at the initial stages of oxidation of soil organic matter by transferring electrons or hydrogen from substrates to acceptors, and can serve as an indicator of microbiological redox systems in soils [29]. And as such, dehydrogenase activity is often used as a soil activity indicator since it is a marker of the destruction caused by pesticides, humidity excess, and faulty management. Zhang et al. [30] report on much different DHA activity being found in different soil types. DHA activity changed more intensively in light-textured than heavy-textured soil and different particle-size soil microaggregates showed different enzyme activity. In soil with a higher content of organic matter, the decomposition processes of organic compounds occur more easily; the organic compounds available as substrates for microbial metabolism can result in a stronger dehydrogenase catalytic ability.

The results obtained indicated that increasing FYM doses by 20 t·ha^{-1} significantly increased CAT activity by 7, 13, 16, and 16%, respectively, as compared with the treatment without manure (Table 2). A strict correlation was shown between CAT activity and organic carbon content (Table 3) in soil under study, which coincides with the report by Brzezinska et al. [31]. Catalase catalyses the decomposition of H_2O_2 to water and molecular oxygen. Hydrogen peroxide and other reactive oxygen species, formed during aerobic respiration as a by-product in a number of cellular systems, are the price aerobic organisms pay for the high efficiency of O_2-dependent respiration metabolism. Catalase, along with peroxidases and superoxide dismutase, serves as an efficient scavenger of reactive oxygen species [32]. Manure fertilization at the level of 20 or 40 t·ha^{-1} significantly increased the peroxidases activity in soil. The increase was, respectively, 14 and 6%, as compared with the treatment without manure (Table 2). However, following the highest-dose manure, treatment decreased peroxidases activity in soil. According to Sinsabaugh [33], peroxidases, through excretion or lysis, enter the environment where their aggregate activity mediates key ecosystem functions of lignin degradation, humification, carbon mineralization, and dissolved organic carbon export. Peroxidase catalyzes the oxidation of phenols and aromatic amines in the presence of hydrogen peroxide as an electron acceptor in the reactions.

Based on the analysis of the results, it could be expected that total selenium occurring in the amounts determined has a significant effect on the dehydrogenase and catalase activity. The correlation between total Se and DHA activity in soil is shown in Fig. 1 as a linear regression diagram; the determination coefficient (r^2) was 0.738. A strong linear relationship was demonstrated from the binding of total

Table 2. Total selenium content and enzyme activity in soil under study.

Description	Sub-plots	Doses of manure [t·ha^{-1}]				
		0	20	40	60	80
Total Se [mg·kg^{-1}]	1	0.105	0.155	0.142	0.156	0.168
	2	0.099	0.123	0.138	0.158	0.169
	3	0.100	0.133	0.161	0.150	0.168
	4	0.100	0.141	0.148	0.164	0.164
Mean		0.101	0.138	0.147	0.157	0.167
$LSD_{0.05}$ – 0.012						
DHA activity [mg TPF·g^{-1} d.w.·24h^{-1}]	1	0.015	0.025	0.019	0.031	0.032
	2	0.015	0.018	0.021	0.029	0.031
	3	0.013	0.020	0.023	0.033	0.027
	4	0.009	0.014	0.021	0.027	0.028
Mean		0.013	0.019	0.021	0.029	0.030
$LSD_{0.05}$ – 0.004						
CAT activity [mg H_2O_2·g^{-1} d.w.·min^{-1}]	1	0.085	0.102	0.109	0.119	0.119
	2	0.102	0.104	0.115	0.113	0.115
	3	0.102	0.109	0.113	0.113	0.115
	4	0.100	0.102	0.107	0.115	0.113
Mean		0.097	0.104	0.111	0.115	0.116
$LSD_{0.05}$ – 0.004						
PX activity [mg purpurogallin·g^{-1} d.w. h^{-1}]	1	0.056	0.077	0.086	0.055	0.047
	2	0.065	0.082	0.068	0.046	0.052
	3	0.060	0.062	0.052	0.053	0.052
	4	0.065	0.063	0.054	0.054	0.057
Mean		0.061	0.071	0.065	0.052	0.052
$LSD_{0.05}$ – 0.009						

selenium with catalase activity. The determination coefficient (r^2) for this relationship was 0.595 (Fig. 1). Many authors [5, 6, 31, 34] have suggested that soil enzymes play essential roles in soil processes such as nutrient cycling and energy transformation by catalyzing numerous chemical, physical, and biological reactions, and can be used as indices of soil fertility and soil health. Vegetation, soil type, land use history, and soil management strategy affect soil enzyme activity.

Navarro-Alarcon and Cabrera-Vique [35] report on selenium levels in soil generally being reflected in food and the Se levels in human populations. Se food content is influenced by the geographical location, seasonal changes, protein content, and food processing. In the present study the average selenium content in upper parts of red clover from control plots reached 0.122 mg·kg^{-1} d.w. (Table 3). There were observed the highest selenium concentrations in above-ground parts and roots of red clover from the plots treated with the dose of 20 t·ha^{-1}. Se content increased on average above 15% against the control. The application of manure in the doses of 60 or 80 t·ha^{-1} resulted in the decrease in selenium content in the investigated parts of plants (Table 4). Leguminous plants (clover and alfalfa) contain usually more selenium than grasses. The highest averages of leguminous plants were reported for India and the United States, 0.672 and 0.320 mg·kg^{-1}, respectively, while for other countries it varied from 0.015 in Canada to 0.090 mg·kg^{-1} in Germany [1]. The concentration of selenium in the investigated parts of red clover was positively correlated with the content of this microelement in soil and the activity of the soil oxidoreductases (Table 3). Kabata-Pendias [1] mentioned that there is a positive linear correlation between the Se in plants tissues and the Se content of soils, and Sippola [36] stated that the total soil selenium

Table 3. Correlation coefficients (r) between examined properties.

Examined properties	pH in KCl	pH in H_2O	Soil fraction <0.002 mm	Soil fraction <0.02 mm	C_{org}	PX	CAT	DHA	Se in red clover roots	Se in above-ground red clover parts
Soil Se	0.42	0.45*	-0.60*	-0.20	0.67*	0.69*	0.65*	0.70*	0.68*	0.66*
Se in above-ground red clover parts	0.64*	0.41	-0.90*	-0.52*	0.93*	0.91*	0.81*	0.92*	0.90*	
Se in red clover roots	0.66*	0.56*	-0.85*	-0.31	0.95*	0.90*	0.83*	0.93*		
DHA	0.66*	0.58*	-0.88*	-0.31	0.95*	0.96*	0.84*			
CAT	0.57*	0.71*	-0.77*	-0.18	0.81*	0.90*				
PX	0.68*	0.57*	-0.83*	-0.31	0.89*					
C_{org}	0.64*	0.53*	-0.49*	0.36						
Soil fraction <0.02 mm	-0.30	0.36	0.46*							
Soil fraction <0.002 mm	-0.54*	-0.49*								

r significant at α=0.05

Table 4. Selenium content in above-ground biomass and roots of red clover.

Se content [$mg \cdot kg^{-1}$]	Sub-plots	Doses of manure [$t \cdot ha^{-1}$]				
		0	20	40	60	80
Above-ground biomass	1	0.124	0.143	0.108	0.099	0.117
	2	0.121	0.163	0.113	0.094	0.105
	3	0.116	0.134	0.122	0.109	0.117
	4	0.129	0.122	0.114	0.122	0.127
Mean		0.122	0.140	0.114	0.106	0.116
$LSD_{0.05}$ – 0.024						
Roots	1	0.119	0.136	0.131	0.133	0.120
	2	0.117	0.143	0.126	0.124	0.128
	3	0.123	0.151	0.165	0.132	0.124
	4	0.127	0.131	0.142	0.125	0.133
Mean		0.122	0.140	0.141	0.129	0.126
$LSD_{0.05}$ – 0.020						

gives a better measure of plant response than do its soluble fractions, which contrast with other opinion [14].

The value of bioaccumulation coefficient (BC) reflects plant capacity for the nutrients uptake from soil and informs about the amount and the rate of the nutrient translocation from soil solution to above-ground plant parts [37, 38]. The parameter is a ratio of the element concentration in plant above-ground parts or roots to its amount in soil. The bioaccumulation coefficients (BC) of selenium demonstrated that above-ground parts and roots of red clover absorbed selenium more easily from soil of the control plots or from soil treated with FYM in the doses of 20 $t \cdot ha^{-1}$ (Fig. 2). The bioaccumulation coefficient (BC) decreased considerably as a result of the highest doses of manure. The distribution of selenium in various parts of the plant differs according to the species, its phase of development and its physiological conditions [14]. In Se-accumulators, Se is accumulated in young leaves during the early vegetative stage of growth, but at the reproductive stage high levels of selenium were found in seeds, while the Se content in leaves is reduced [14, 38]. Zayed et al. [39] report on the distribution of selenium in plants, also depending on the form and the concen-

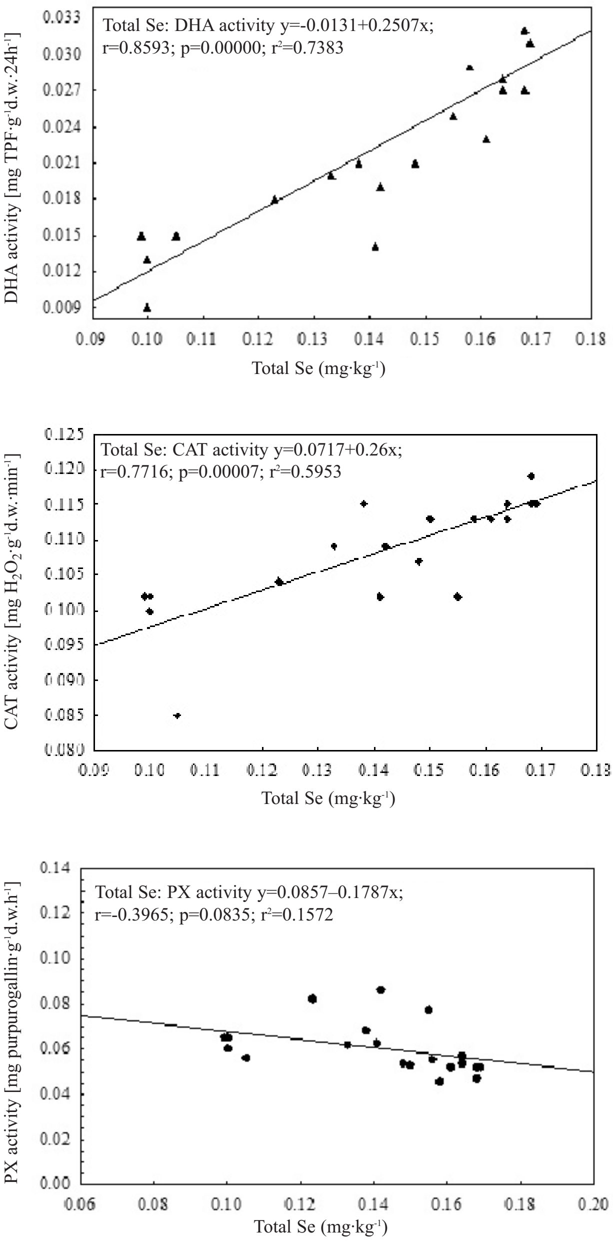

Fig. 1. Linear regression diagrams representing significant correlations between total selenium and enzymatic activity in the investigated soil.

tration of selenium supplies to the roots and on the nature and concentration of other ions, especially sulphates and on the degree of Se fixation in soils. Plants absorb Se easily from alkaline soils, where it often exists in water-soluble forms. Although acid soils may contain high selenium concentrations, plants assimilate only small amounts since Se is bound by insoluble iron compounds or by organic matter of soil [38]. According to Terry et al. [37], since selenium and sulphurs share similar properties, the Se compounds are absorbed and metabolized by mechanisms similar to those for S analogues. Soluble Se forms are likely to be taken up by plants – selenates and organic selenium are driven metabolically, but the uptake of selenites can be a result of passive mechanism. In general, organic Se is more readily absorbed by plants than inorganic forms. On the other hand, selenates are likely to be easily transported to above-ground parts than selenite or organic species (e.g. SeMet) [1].

The value of translocation coefficient (TC) informs about the amount and the rate of element translocation from roots to a plant's above-ground parts. The parameter is a ratio of the element concentration in above-ground plant parts to its amount in roots. Translocation coefficients (TC) demonstrated that selenium is more easily transported from roots to above-ground parts of plants on the control plots or on plots with the rate of FYM application of 20 $t \cdot ha^{-1}$ (Fig. 3). The translocation of Se from root to shoot depends on the form of Se supplied, selenate being transported much more easily than selenite [37]. Munier-Lamy et al. [38] observed the differences in transporting Se from roots to shoots between plant species, which could be related either to the root system, i.e. to soil exploration by roots and root exudation, or to the chemical conditions resulting from microbial activity in the rhizosphere. Indeed, some microorganisms may excrete organic compounds that increase bioavailability, and facilitate root absorption of essential metals such as Fe, as well as non-essential metals such as Cd. Soil microorganisms can also affect metal solubility directly by changing their chemical forms. When Se is supplied as selenate to plants, Zayed et al. [39] report on most of it being transported to the shoots unchanged with very little Se remaining in roots, which seems to suggest a reduction in selenate to selenium organic forms, which are less available in soil.

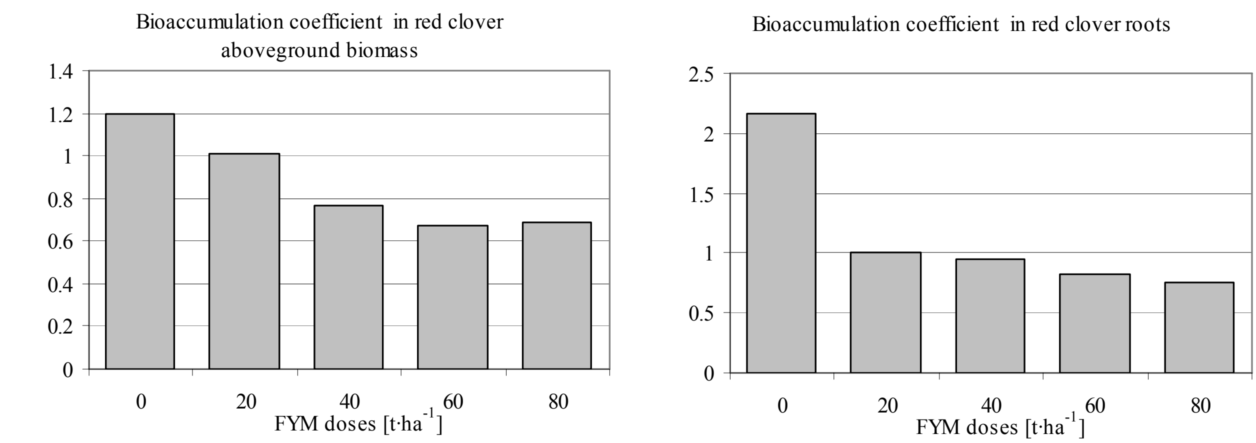

Fig. 2. Bioaccumulation of selenium in above-ground biomass and roots of red clover in relation to its content in soil (mean for sub-plots).

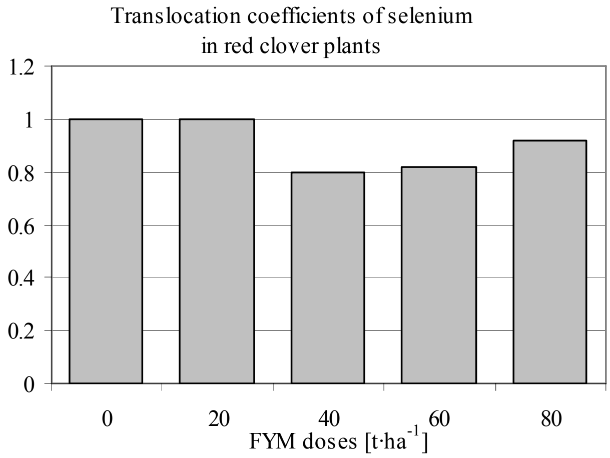

Fig. 3. Translocation of selenium from roots to above-ground parts of red clover (mean for sub-plots).

Conclusions

The content of total selenium in Luvisol not fertilized with manure was, on average, 0.101 mg·kg^{-1}. From the comparison of the results reported in literature one can observe that the studied soil was poor in selenium. The application of the highest manure dose resulted in a 1.7-fold increase in the content of total selenium in soil. For that reason the FYM application can be recommended as a source of selenium in Se-deficient soils. Fertilization with manure resulted in an increase of dehydrogenases and catalase activities in soil with increasing doses of manure. The present studies demonstrate a significant relationship between activity of soil enzymes and the organic matter content affecting the selenium status in soil and plants. We observed the highest selenium concentrations in the upper parts of red clover from the plots treated with 20 t·ha^{-1} of FYM, but the FYM application in the highest doses resulted in a decrease of the selenium content in above-ground parts and roots of plants. The bioaccumulation and translocation coefficients of selenium demonstrated that above-ground biomass of red clover absorbed and transported selenium more easily from soil without manure or treated with FYM at doses of 20 t·ha^{-1}. Both coefficients decreased considerably due to the highest doses of FYM.

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