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IMPACT OF FARMYARD MANURE AND DIFFERENT DOSES OF NITROGEN ON THE AVAILABILITY OF SELENIUM BY SPRING BARLEY (*Hordeum vulgare* L.)

WPŁYW NAWOŻENIA OBORNIKIEM I ZRÓŻNICOWANYMI DAWKAMI AZOTU NA PRZYSWAJALNOŚĆ SELENU PRZEZ JĘCZMIEŃ JARY (*Hordeum vulgare* L.)

Abstract: The objective of the study was to determine the total selenium content and its fractions available to spring barley plants affected by FYM and different doses of nitrogen. Comparison of the results of total soil Se concentration reported in this paper with other findings indicated that analysed soil had a very low selenium content. Such low levels of selenium in soils indicated that plants growing on these soils are deficient in this microelement. The application of manure and nitrogen resulted in the highest amounts of total selenium content in soil, which increased with increasing doses of both fertilizers. Fertilization with manure resulted in an increase of selenate and selenite in soil with increasing doses of manure, but the nitrogen treatment did not effect on the content of this fractions of selenium in soil. The share of Se-phytoavailable fractions in the total selenium content in soil under study ranged from 13.5 to 27.3 % and increased with increasing doses of FYM. Generally, the selenium concentrations in aboveground parts and roots of spring barley increased with increasing doses of FYM, but the application of nitrogen decreased the Se content in the investigated parts of plants.

Keywords: selenium, spring barley, farmyard manure, nitrogen fertilization

Selenium plays an important role in biological systems and the concentration range in which selenium is essential is narrow. As a natural constituent of soil minerals, selenium is normally present in soil at low contents ranging from 0.01 to 2 mg $\cdot$ kg^{-1}, but in most agricultural areas, soils contain so little available selenium that cultivated crops usually do not absorb more than traces of this element [1, 2]. The concentration and chemical forms of selenium in soils are governed by various chemical and physical

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factors, including pH, chemical and mineralogical composition, adsorbing surface, and oxidation and reduction status [2]. In Poland the dietary Se intake is on average 11–24 $\mu g \cdot d^{-1}$, what is about 4-times lower than the recommended level of 75 $\mu g \cdot d^{-1}$ for man and 60 $\mu g \cdot d^{-1}$ for woman set by World Health Organization (WHO) [3]. To minimize the occurrence of health problems, such as cancer and cardiovascular and viral diseases, which can be induced by selenium deficiency, increasing the selenium concentration in foods is an good way of Se supply to humans [4]. Cereals as the major foodstuff may offer the best opportunity to do so. According to Stadlober [5] and Seppanen et al [6], selenium levels in crops can be secured by agronomic biofortification *eg* 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 inorganic selenium compounds applied in fertilizers are taken up by plants and assimilated into valuable organic Se-compounds, which are safer and their retention in tissue is better. This paper presents the results of field investigations on total selenium and its phytoavailable fractions concentrations in soil long-term fertilized with farmyard manure and nitrogen. The availability of selenium by spring barley is also reported.

Materials and methods

Soil and plant 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 Pulawy. 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 in the doses of 0, 20, 40, 60 and 80 $Mg \cdot ha^{-1}$ (factor A) and nitrogen at the doses of 0, 40, 80 and 120 $kgN \cdot ha^{-1}$ was used under spring barley (factor B). Soil and plant samples were collected in the 23rd year of the experiment, in May 2003, from the 0–20 cm layer under spring barley. Soil samples were air-dried and sieved through a 2 mm screen. Spring barley was rinsed in deionised water to remove soil particles, separated into aboveground biomass and roots, and dried. The total selenium content was determined by the method of Watkinson [7] using a Hitachi F-2000 spectrofluorometer. Soil samples were microwave digested with concentrated nitric(V) and perchloric(VII) 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 wavelengths of λ = 376 nm 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. Available to plants forms of selenium were extracted from the soil by the part of sequential extraction method recommended by Chao and Sanzolone [8] with modification of Wang and Chen [2]. Firstly, 0.25 M KCl solution was used to extract the soluble form of selenium (Se(VI)). The exchangeable and specifically adsorptive forms of selenium (Se(IV)) were extracted by 0.1 M 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 cheleated by adding 2,3-diamino-naphtalene to the solution and determined by fluorescence spectrophotometry. The soil samples were analysed for granulometric composition according to Bouyoucos--Casagrande method, organic carbon by wet oxidation with potassium dichromate, total nitrogen following by Kjeldahl method and pH in distilled water and 1 M KCl potentiometrically.

Two-way analysis of variance (ANOVA) was used to identify significant differences ($p < 0.05$) between Se concentrations in parts of plants, corresponding soil and speciation. Data analysis was carried out using Statistica 8.0 for Windows Stat. Soft. Inc.

Results and discussion

The general properties of the soil under study are given in Table 1. The soil, according to the FAO classification, was classified as Haplic Luvisols and demonstrated

Table 1

General properties of soil under study

Sample symbol	Soil particle size fraction [%]		pH		C_{org} [$g \cdot kg^{-1}$]	N_{tot} [$g \cdot kg^{-1}$]
	< 0.02 [mm]	< 0.002 [mm]	H_2O	KCl		
0-N0	18	6	6.1	5.5	7.55	0.679
0-N1	17	6	6.3	5.5	7.60	0.683
0-N2	19	8	6.3	5.4	7.14	0.711
0-N3	17	7	6.1	5.2	7.29	0.728
20-N0	14	5	6.2	5.5	8.10	0.707
20-N1	16	8	6.1	5.4	7.89	0.721
20-N2	15	6	6.1	5.4	8.05	0.739
20-N3	18	7	6.1	5.4	7.73	0.760
40-N0	19	8	6.2	5.5	8.37	0.749
40-N1	17	6	6.2	5.4	7.61	0.728
40-N2	13	5	6.2	5.4	9.24	0.812
40-N3	19	6	6.2	5.2	9.48	0.851
60-N0	16	5	6.2	5.5	9.67	0.854
60-N1	16	6	6.2	5.4	9.87	0.826
60-N2	19	7	6.2	5.6	10.05	0.861
60-N3	20	6	6.2	5.5	9.96	0.865
80-N0	15	4	6.2	5.5	9.35	0.917
80-N1	16	6	6.3	5.6	9.94	0.931
80-N2	15	5	6.3	5.4	9.78	0.959
80-N3	14	5	6.2	5.1	10.23	0.949

the texture of loamy sand and sandy loam. The pH values of soil were in the slightly acidic range under which conditions Se exist predominantly as selenite in well-drained soils [4]. The application of manure resulted in the highest contents of organic carbon and total nitrogen in soil, especially treated with FYM at the doses of 60 and 80 Mg · ha^{-1}. The total soil Se concentration varied from 0.108 (control plots) to 0.170 mg · kg^{-1} from plots with the highest doses of farmyard manure (Table 2). 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 0.05–1.5 mg · kg^{-1} being the lowest in Podzols and the highest in Histosols. Total selenium content in soil under study was affected by organic and nitrogen fertilization. The application of manure resulted in the highest amounts of total selenium content in soil (Table 2), which increased with increasing doses of manure and nitrogen. The Se content in soil from plots fertilized with FYM at the dose of 80 kg · ha^{-1} rose in average above 30 % in comparison with control. Sager [9] and Broadley et al [10] reported that 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.

On account of the existence of different Se species in soils, total selenium concentrations does not necessarily reflect the extent to which growing plants take up selenium [5]. Additionally knowledge of different species of Se, particularly phyto-available, present in soil is required to understand the biological and environmental impact of this element. In the present study the concentration of soluble and exchangeable forms of selenium, which are presumably plant available, were in the range 0.015–0.048 mg · kg^{-1} (Table 2). Fertilization with manure resulted in an increase of selenate and selenite in soil with increasing doses of manure. However, following the nitrogen treatment did not effect on the content of this fractions of selenium in soil. The share of Se-phytoavailable fractions in the total selenium content in soil under study ranged from 13.5 to 27.3 % and increased with increasing doses of FYM. In soil under study we found the higher share of selenite(IV) than selenate(VI) in the total selenium content. 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, 11]. The transformation of easily soluble selenates added to acidic or neutral soils into slightly soluble forms is relatively fast [1]. Wang and Chen [2] pointed out that organic matter in soil affects bioavailability of selenium in soil; the content of soil Se is strongly influenced by leaching and hydrological transport processes; low selenium in soils have developed on the low selenium parent material with a very low flux of selenium between soil and plants.

In the present study the average selenium content in upper parts of spring barley from control plots reached 0.130 mg · kg^{-1} d.m. (Table 2). Generally, the selenium concentrations in aboveground parts and roots of spring barley increased with increasing doses of FYM. In upper parts of spring barley treated with the dose of 80 Mg · ha^{-1}, the

Table 2

Total and phytoavailable selenium content in soil and spring barley [mg · kg^{-1}]

Sample symbol	Se_{tot} in soil	Se(VI) in soil	Se(IV) in soil	Se in spring barley	
				aboveground parts	roots
0-N0	0.119	0.016	0.021	0.136	0.154
0-N1	0.108	0.017	0.024	0.136	0.153
0-N2	0.124	0.016	0.020	0.110	0.162
0-N3	0.123	0.016	0.017	0.137	0.128
20-N0	0.118	0.020	0.028	0.185	0.192
20-N1	0.127	0.020	0.030	0.159	0.163
20-N2	0.123	0.018	0.026	0.179	0.152
20-N3	0.122	0.015	0.020	0.155	0.153
40-N0	0.128	0.016	0.024	0.228	0.195
40-N1	0.122	0.017	0.030	0.169	0.190
40-N2	0.121	0.020	0.022	0.248	0.212
40-N3	0.128	0.017	0.024	0.266	0.216
60-N0	0.125	0.020	0.023	0.296	0.222
60-N1	0.129	0.019	0.024	0.313	0.253
60-N2	0.131	0.018	0.026	0.228	0.252
60-N3	0.152	0.021	0.025	0.260	0.222
80-N0	0.152	0.043	0.042	0.335	0.232
80-N1	0.147	0.041	0.038	0.273	0.189
80-N2	0.170	0.040	0.048	0.285	0.242
80-N3	0.161	0.048	0.043	0.264	0.261
Mean for FYM doses (Factor A)					
0	0.118	0.016	0.021	0.130	0.149
20	0.122	0.018	0.026	0.170	0.165
40	0.125	0.018	0.025	0.228	0.203
60	0.134	0.020	0.025	0.274	0.237
80	0.157	0.043	0.042	0.289	0.231
Mean for N doses (Factor B)					
0	0.128	0.023	0.028	0.236	0.199
N1	0.126	0.023	0.029	0.210	0.190
N2	0.134	0.022	0.030	0.210	0.204
N3	0.137	0.027	0.028	0.216	0.196
$LSD_{0.05}$	A – 0.004 B – 0.003 A/B – 0.008 B/A – 0.007	A – 0.008 B – n.s. A/B – 0.015 B/A – 0.014	A – 0.005 B – n.s. A/B – 0.009 B/A – 0.009	A – 0.030 B – 0.025 A/B – 0.060 B/A – 0.056	A – 0.006 B – 0.005 A/B – 0.012 B/A – 0.011

n.s. – not significant.

Se content increased on average above 2-times against the control. The application of nitrogen decreased the selenium content in the investigated parts of spring barley in comparison with plants from plots without nitrogen fertilization.

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 aboveground plant parts [12]. The parameter is a ratio of the element concentration in plant aboveground parts or roots to its amount in soil. The bioaccumulation coefficients (BC) ratio calculated for the investigated parts of spring barley increased with increasing doses of FYM, but the FYM application at the dose of 80 Mg · ha^{-1} caused that aboveground parts and roots absorbed selenium more difficultly (Fig. 1).

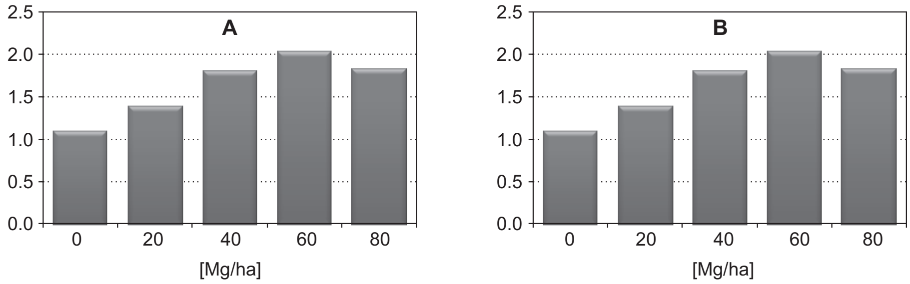

Fig. 1. Bioaccumulation coefficients (BC) for aboveground parts (A) and roots (B) of spring barley (mean for nitrogen doses)

The value of *translocation coefficient* (TC) informs about the amount and the rate of element translocation from roots to plant aboveground parts. The parameter is a ratio of the element concentration in aboveground plant parts to its amount in roots. The ratio values of TC increased with increasing doses of FYM (Fig. 2). According to Terry et al [13] the translocation of Se from root to shoot depends on the form of Se supplied, selenate being transported much more easily than selenite. Munier-Lamy et al [12] and Zhu et al [14] observed the differences in transporting Se from roots to shoots between plant species, which could be related either to the root system, *ie* to soil exploration by roots and root exudation, or to the chemical conditions resulting of microbial activity in

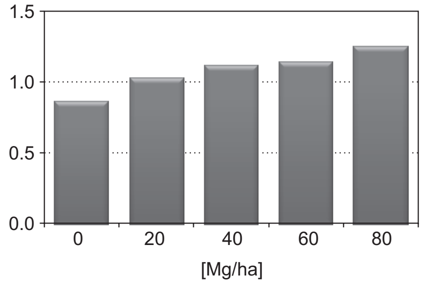

Fig. 2. Translocation coefficients (TC) for spring barley plants (mean for nitrogen doses)

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. In a pot experiment with barley [15] in which combined influence of N, P and S on the uptake of selenite was examined, a complex interaction between the three fertilizer anions and the plant uptake of selenite was revealed. Nitrogen application decreased the selenium concentration, which was to some extent only a dilution effect due to increase in yield. The decreasing effect of nitrogen fertilizer on selenium concentration was also found in pasture samples.

Conclusions

Comparison of the results of total soil Se concentration reported in this paper with other findings indicated that analysed soil had a very low selenium content. Such low levels of selenium in soils indicated that plants growing on these soils are deficient in this microelement. The application of manure and nitrogen resulted in the highest amounts of total selenium content in soil, which increased with increasing doses of both fertilizers. Fertilization with manure resulted in an increase of selenate(VI) and selenite(IV) in soil with increasing doses of manure, but the nitrogen treatment did not effect on the content of this fractions of selenium in soil. The share of Se-phytoavailable fractions in the total selenium content in soil under study ranged 13.5–27.3 % and increased with increasing doses of FYM. Generally, the selenium concentrations in aboveground parts and roots of spring barley increased with increasing doses of FYM, but the application of nitrogen decreased the Se content in the investigated parts of plants.

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WPŁYW NAWOŻENIA OBORNIKIEM I ZRÓŻNICOWANYMI DAWKAMI AZOTU NA PRZYSWAJALNOŚĆ SELENU PRZEZ JĘCZMIEŃ JARY (*Hordeum vulgare* L.)

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Abstrakt: Celem badań było określenie zawartości selenu i jego frakcji fitodostepnych w glebie oraz przyswajalności tego pierwiastka przez jęczmień jary w warunkach nawożenia organiczno-mineralnego. Próbki glebowe i roślinne pobrano w maju 2003 roku (6 rotacja) w trakcie wegetacji jęczmienia jarego z doświadczenia prowadzonego przez IUNG w Puławach na terenie RZD Grabów nad Wisłą, z wariantu z następującym doborem roślin w zmianowaniu: ziemniaki – pszenica + międzyplon (gorczyca biała) – jęczmień jary + wsiewka koniczyny – koniczyna + trawy. Zastosowano nawożenie obornikiem (jednorazowo w trakcie rotacji) pod ziemniaki w dawkach 0, 20, 40, 60, 80 Mg $\cdot$ ha^{-1} oraz azotem w ilości 0, 40, 80 i 120 kgN $\cdot$ ha^{-1}. Zawartość selenu ogółem w próbkach roślinnych i glebowych oznaczono metodą Watkinsona. Zawartość Se(VI) i Se(IV) oznaczono, wykorzystując część analizy specjacyjnej wg Chao i Sanzolone w modyfikacji Wang i Chen. Zawartość selenu ogółem w glebie wskazuje, że była ona uboga w ten pierwiastek. Zastosowanie obornika i azotu spowodowało istotny wzrost zawartości selenu ogółem w glebie wraz ze wzrostem ich dawek. Udział frakcji fitoprzyswajalnych w całkowitej puli tego pierwiastka w glebie kształtował się od 13,5 do 27,3 % i wzrastał wraz ze wzrostem dawek obornika. Na ogół zawartość selenu w częściach nadziemnych i korzeniach jęczmienia jarego wzrastała wraz ze zwiększaniem się dawek obornika, natomiast zastosowanie azotu obniżyło zawartość tego mikroelementu w badanych częściach roślin.

Słowa kluczowe: selen, jęczmień jary, obornik, nawożenie azotem