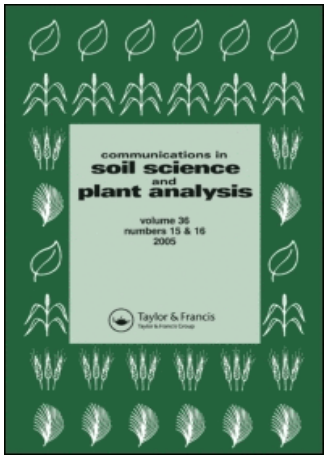

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Soil-Applied Selenium Effects on Tissue Selenium Concentrations in Cultivated and Adventitious Grassland and Pasture Plant Species

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Abstract: According to international nutritional standards, plant selenium (Se) concentrations in Belgium are too low. To correct this situation, adding Se in fertilizers for pastures and grasslands is suggested, similar to activities in Finland. However, there is a lack of data on meadow plant species' ability to absorb Se. Therefore, a pot experiment was initiated using 24 meadow plant species cultivated on a Belgian cambisol receiving standard fertilizer treatment, with or without the addition of 9 g Se ha^{-1} yr^{-1} as sodium selenate. Soil Se analysis confirmed the low Se status of the native soil. Mean foliar Se concentration in the control group was 0.05 mg kg^{-1}. Because plant deficiency may occur at levels less than 0.10 mg Se kg^{-1}, data provided further evidence for Se deficiency in Belgium plant production. When grown with Se, plant species showed wide variations for Se concentration, ranging from 0.08 to 0.49 mg Se kg^{-1}. All values were less than 2 mg Se kg^{-1}, the suggested threshold toxicity level for dairy cattle. There were two different types of plants in terms of response to Se fertilization. Most of the tested plants were known as nonaccumulators. There were also two probable secondary accumulators: *Sinapis arvensis* and *Melilotus albus*. Finally, one has to question the reliability of plant Se enhancement using this method when floristic composition is poorly controlled.

Keywords: Belgium, fertilizer, grassland, plants, selenium, soil

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INTRODUCTION

Selenium (Se) is an essential trace mineral for humans, animals, algae, and exclusively for plants endemic to seleniferous soils (Gupta and Gupta 2000; Rayman 2004; Tapiero, Townsend, and Tew 2003; Terry et al. 2000; Whanger 2004). Selenium mainly enters food chains through plants, after absorption from the soil. The average Se concentration in soils based on data collected from various countries can range up to 2 mg kg^{-1} (Kabata-Pendias and Pendias 1984). Nevertheless, soil Se is highly variable, and according to data reviewed by Gupta and Gupta (2000), ranges from less than 0.1 mg Se kg^{-1} in Finland podzols to as much as 80 mg Se kg^{-1} in seleniferous soils of the western United States.

On seleniferous soils (i.e., generally containing more than 5 mg Se kg^{-1}), specific floras of Se-accumulating plants grow, which could contain far more than 1000 mg Se kg^{-1}. A second category of plants, known as secondary Se accumulators, grow on soil of low to medium Se content and can accumulate up to 1000 mg Se kg^{-1} when Se is available (Brown and Shrift 1982; Terry et al. 2000). Nonaccumulator plant species generally do not accumulate more than 25 mg Se kg^{-1} when grown on seleniferous soils (Brown and Shrift 1982). Regions with chronic Se deficiency in animal or human populations generally have total soil Se concentrations lower than 0.6 mg kg^{-1} (Gupta and Gupta 2000). On these soils, most crop and forage plants contain less than 0.1 mg Se kg^{-1}, which is the minimal concentration that protects from mammalian deficiency disorders. The recommended Se concentration for forages fed to dairy cattle in the United States is 0.3 mg kg^{-1}, and the numbers drop slightly to 0.1 mg Se kg^{-1} for beef cattle (Buchanan-Smith et al. 2001). Finland presented the lowest Se concentrations in reference cereal crops (0.008 ± 0.003 mg Se kg^{-1}) among 32 countries of the Food and Agriculture Organization of the United Nations (FAO) (international value: 0.109 ± 0.259 mg Se kg^{-1}; Sillanpää and Jansson 1992). Since 1984, Se has been systematically added to fertilizers in Finland, which has resulted in an increase in the Se content in agricultural productions (Jukola et al. 1996).

In Belgium, soil-extractable Se concentrations are about double those of Finland (i.e., 0.010 mg Se kg^{-1}), and reference cereal crop Se concentrations were four times higher than those of Finland (i.e., 0.033 mg Se kg^{-1}) (Sillanpää and Jansson 1992). However, these concentrations are still too low to meet recommended Se requirements (Combs 2001; Rayman 2000, 2004; Robberecht and Deelstra 1994). For this reason, attempts at food-chain enrichment were undertaken in Belgium using Se-supplemented fertilizers. Selenium applications to winter barley (*Hordeum vulgare* subsp. *hexastichon*) and pastures used in suckler cow production increased the Se concentrations in feedstuffs to 0.2 mg kg^{-1}, which meets the recommended Se range. Consumption of Se-enriched feedstuffs also improved animal Se status (Dufrasne et al. 2004).

However, before suggesting systematic Se addition to fertilizers in Belgium, several questions have to be answered to guarantee safety in the food chain and in the environment. Currently there is a lack of knowledge on Se status and fate of Se in Belgian agricultural soils. Furthermore, little is known on the ability of western European plant material to accumulate Se because of the lack of seleniferous soils in the region. Pasture and grassland floristic composition changes yearly, as a function of time and management. In cases of better accumulators, crop Se content could be higher, because only a fraction of the applied Se (23 to 52% in Belgium trials) is absorbed (de Behr et al. 2003). In addition, the tolerable range for Se concentrations (from trace element requirement to lethality) is quite narrow (Terry et al. 2000). For mammals, the toxic dose is only 10 times higher than the recommended requirements (Wilber 2000; Yang and Zhou 1994). Further, Se toxicity in breeding animals could appear at a concentration of 2 mg Se kg^{-1}, which is also the maximum tolerable concentration for dairy cattle in the United States (Buchanan-Smith et al. 2001). The objective of this work was to determine the ability of several Belgian meadow adventitious plants species to accumulate Se from a native soil amended with sodium selenate fertilizers.

MATERIALS AND METHODS

The experiment was carried out on frequently encountered adventitious plant species of pastures and grasslands. A list of the most common and abundant species was drawn up from 123 phytosociological surveys (Braun-Blanquet 1965) conducted in 23 eastern Belgium meadows during 1998 and 1999 (Rouxhet and Walot 2002). Species were selected when they had at least a cover coefficient of 3 (covering 25% or more of reference area) in one relevé. Cultivated species were added to the list as internal controls. Seeds or cuttings were collected in the wild or obtained from European botanical gardens. During February to March 2005, 44 plant species were sowed (eight replicates) in 30-cm diameter × 26-cm high PVC containers with drain holes, filled with a meadow cambisol of the Entre-Vesdre-et-Meuse district (Baelen locality). Containers were installed outdoors in a trench to minimize solar lateral heating. The site was a meadow in the area of Liège (Belgium) at 150 m above sea level. Mean annual rainfall was 848 mm, and average temperature was 8.9°C (temperate climate with mixed oceanic and continental influences).

Formulas and applications of fertilizers followed standard meadow treatment. Selenium treatment group plants received a total of 9 g Se ha^{-1}, applied as sodium selenate, split over three separate monthly applications. The first application was made with a 15 nitrogen (N)–9 phosphorus pentoxide (P_2O_5)–18 potassium oxide (K_2O)+8 magnesium oxide (MgO) fertilizer (+8 mg Se kg^{-1} for the treatment groups) at a rate of 375 kg ha^{-1} on

April 27, 2005. Second and third applications took place on June 3 and July 1, 2005, respectively, with 25 N + 5 MgO fertilizer (+10 mg Se kg^{-1} for the treatment groups) at rates of 300 kg ha^{-1}. Fertilizers were applied as a water suspension for better homogeneity. Aerial plant tissues were collected on August 3 and 4, 2005, except *Sinapis arvensis*, which matured earlier and was cut just before the third fertilizer application on the June 3. Harvested plant tissues were dried for 2 weeks in paper bags in a herbarium drying room (40°C; 20% relative humidity) until subsampling. Plant subsamples and soil samples were dried to constant weight (60°C) before Se analysis.

Soil CEC (cation exchange capacity), pH, and exchangeable cations were estimated following standard procedures. Total soil Se content was determined on a 200-mg sample digested in a sealed Teflon® vessel placed in a microwave oven (Milestone: Ethos-Pro) with a mixture of concentrated hydrochloric acid (HCl), nitric acid (HNO_3), and hydrofluoric acid (HF) (1:1:1, v:v:v); the solution was then diluted to 50 mL with distilled water, and Se was determined using graphite furnace spectrophotometer (Perkin-Elmer AAnalyst 600). The detection limit was 0.30 mg kg^{-1}. Hot water-soluble Se (Se_{HW}) was prepared after the method described by Jump and Sabey (1989). A 10-g soil sample was placed in a flask, suspended in 50 mL of distilled water, and refluxed over a boiling water bath for 30 min. The soil suspension was then filtered, and Se in the filtrate was analyzed. Acetic acid–ammonium acetate–ethylenediaminetetraacetic acid (EDTA)–extractable Se (Se_{AAAc}) was prepared following Lakanen and Erviö (1971). A 25-g soil sample was suspended and shaken for 1 h in 250 mL of the extraction solution in which pH was 4.65, ammonium acetate and acetic acid were 0.5 M, and Na_2EDTA was 0.02 M. The soil suspension was then filtered, and Se in the filtrate was analyzed. Selenium was determined in plant samples and in both soil extracts by the high-pressure liquid chromatography (HPLC) fluorescence method of Hawkes and Kutnink (1996). Samples (1 mL for liquid sample or 400 mg for solid sample) were hot-ashed with a mixture of concentrated HNO_3 and perchloric acid ($HClO_4$) (5:1, v:v). Selenium (VI) in the form of oxyanions was reduced to Se (IV) by the addition of 1 mL of 4M HCl and heating to 160°C. Derivation with 2,3-diaminonaphthalene was realized after addition of glycine and Na_4EDTA at a pH adjusted to 1.75. The fluorophore was extracted in cyclohexane and injected in the HPLC system (Gilson Pump 307, Gilson Autoinjector 234) equipped with a Lichrosorb, 10 μm, 25-cm × 0.4-cm i.d. column (Merck). The mobile phase was 90% cyclohexane/10% ethyl acetate. Fluorescence was measured using excitation wavelength of 378 nm and emission wavelength of 530 nm with a Perkin-Elmer LS30 detector. Detection limits were 100 ng L^{-1} for soil extracts and 200 ng kg^{-1} for dry plant samples.

Plant Se concentrations were compared with the general linear model (GLM) procedure of SAS (1999) on log values in a variance analysis model including Se treatment, species, and interactions.

RESULTS AND DISCUSSION

Results of the soil analysis are given in Table 1. The total soil Se concentration was 0.38 mg kg^{-1}, a value lower than the threshold for chronic Se deficiency in mammalian populations (0.6 mg Se kg^{-1}; Gupta and Gupta 2000). The value for soil Se_{AAAc} (0.008 mg Se kg^{-1}) is close to the 0.010 mg Se kg^{-1} value determined by Sillanpää and Jansson (1992) for different Belgium agricultural soils. The value for soil Se_{HW} (0.022 mg kg^{-1}) is in the same range reported for Finnish soils in 1998 (Mäkelä-Kurtto and Sippola 2002). According to Sillanpää and Jansson (1992), four soil factors firmly modulate the plant Se availability: (1) increases with increasing soil pH, (2) increases with increasing soil electrical conductivity, (3) decreases with increasing humus content, and (4) changes with fluctuatible soil CEC. Conductivity and pH of the native soil utilized in this study were in the middle of the ranges reported by Sillanpää and Jansson (1992). However, soil humus content was definitely higher in the native soil. These soil characteristics, combined with the low exchangeable Se values, suggested that Se availability for the native soil used in this study may have been dramatically low.

Winter conditions extended up to March 10, which perturbed seedling establishment. Spring was characterized by abnormally low insulation with the consequence that several species were grazed by growing populations of slugs. Furthermore, water deficit occurred between May 21 and June 27, while maximum temperature rose up to 25°C for 5 days running and up to 30°C for 3 days running between June 18 and June 25; the plant growth was therefore slowed down. Because of these harsh climatic conditions, 20 of the 44 cultivated species (*Alchemilla xanthochlora, Anthriscus sylvestris, Bellis perennis, Centaurea jacea, Cynosorus cristatus, Geranium sylvaticum, Knautia arvensis, Luzula campestris, Medicago lupuluna, Meum athamanticum, Phleum pratense, Plantago major, Plantago lanceolata, Polygonum bistorta, Prunella vulgaris, Rumex conglomeratus, Rumex acetosa, Rumex acetosella, Stachys officinalis*, and *Thlaspi arvense*) did not produced

Table 1. Physical and chemical properties of a meadow cambisol soil collected from the Entre-Vesdre-et-Meuse district (Baelen locality)

Parameter	Value
Humus (%)	7.2
pH (KCl)	6.4
CEC (meq 100 g^{-1})	15.8
Conductivity (mS cm^{-1})	4.01
[Se] total (mg kg^{-1})	0.38
$[Se]_{AAAc}$ (mg kg^{-1})	0.008
$[Se]_{HW}$ (mg kg^{-1})	0.022

enough replicates or material for Se determination. Although there were drain holes, no roots grew out of the containers. To compare similar plant tissues, Se was measured for leaf samples only, although leaves were not the dominating tissue in all plants. Table 2 summarizes Se concentrations for harvested plants, grown with or without Se fertilizer. The observed values were within the ranges previously reported for fodders collected in various areas of eastern Belgium (Cabaraux et al. 2005). Selenium concentrations were rather low in all the control plants (mean value of 0.05 mg Se kg^{-1}) except for *Sinapis arvensis*. Such low values provide further evidence for the occurrence of possible Se deficiencies in Belgium plant production.

The addition of 9 $g \cdot ha^{-1}$ Se as sodium selenate to the fertilizers added to the cultures aimed to increase the Se forage content up to 0.2 $mg \cdot kg^{-1}$. The

Table 2. Mean leaf Se concentrations (mg kg^{-1}) for cultivated and adventitious pasture and grassland species grown on a meadow cambisol soil in control and Se treatment groups

		Treatment		
	Species	Control	Se	$P < F$
Cultivated				
Leguminosae	*Trifolium pratense*	0.018	0.133	0.0001
	Trifolium repens	0.034	0.188	0.0001
Poaceae	*Festuca pratensis*	0.051	0.259	0.0001
	Lolium perenne cv. Elgon	0.063	0.435	0.0001
	Lolium perenne cv. Ritz	0.047	0.267	0.0001
Adventices				
Asteraceae	*Achillea millefolium*	0.053	0.352	0.0001
	Crepis biennis	0.058	0.236	0.0001
	Hieracium pilosella	0.046	0.269	0.0001
	Leucanthemum vulgare	0.048	0.353	0.0001
	Taraxacum officinale	0.038	0.331	0.0001
Brassicaceae	*Sinapis arvensis*	0.121	0.493	0.0001
Chenopodiaceae	*Chenopodium album*	0.043	0.117	0.0031
Leguminosae	*Melilotus albus*	0.032	0.452	0.0001
	Trifolium dubium	0.037	0.201	0.0001
	Vicia cracca	0.037	0.242	0.0001
Poaceae	*Deschampsia cespitosa*	0.033	0.189	0.0001
	Elymus repens	0.042	0.274	0.0001
Polygonaceae	*Persicaria lapathifolia*	0.033	0.080	0.0014
	Polygonum aviculare	0.037	0.133	0.0002
	Rumex obtusifolius	0.046	0.280	0.0001
Ranunculaceae	*Ranunculus repens*	0.045	0.334	0.0001
	Ranunculus acris	0.055	0.197	0.0001
Rosaceae	*Sanguisorba officinalis*	0.026	0.301	0.0001
Rubiaceae	*Galium mollugo*	0.036	0.283	0.0001

response of all the plant species was significant (Table 2) but showed wide variations; the concentrations in the treated group varied between 0.080 and 0.493 mg $\cdot$ kg^{-1}. *Sinapis arvensis* Se concentrations reached the highest values both in the control and Se treatments, even though this species received Se only at 6 g $\cdot$ ha^{-1}. There were three plant species (*Melilotus albus*, *Lolium perenne cv.* Elgon, and *Sinapis arvensis*), in which the foliar Se concentration was more than 0.4 mg $\cdot$ kg^{-1}. Aerial plant tissues were partitioned in the two highest accumulated species, and Se concentrations were measured for both the control and Se treatments (Table 3). Significant increases ($P < 0.03$) in the Se concentrations were recorded for stem, fruit, and shoot tissues of *Sinapis arvensis* and for stem and shoot tissues of *Melilotus albus*. However, Se concentrations in aerial tissues other than leaves for both species remained less than the 0.2 mg Se kg^{-1} target.

The three cultivated grass species (*Poaceae*) accumulated Se concentrations of more than 0.2 mg kg^{-1} (Table 2). By contrast, Se concentrations were only 0.133 and 0.188 mg kg^{-1} for the two cultivated clover species (*Leguminosae*). In the adventitious group, there were only three plant species (*Chenopodium album, Persicaria lapathifolia, and Polygonum aviculare*) in which foliar Se concentrations were well less than the target value of 0.2 mg kg^{-1} after Se fertilization (Table 2). Data indicate enhancement success from Se fertilization strategies in pasture or conserved forage systems may be dependent on the proportions of *Leguminosae*/*Poaceae* and the proportions of adventices. Further, in meadow systems where the proportion of cultivated species is higher, it should be easier to maintain the 0.2 mg Se kg^{-1} target concentration because tissue Se variation among these species is lower than in the adventitious species.

Selenium accumulation is as a function of a species' sulfur requirement, protein content, and occurrence of sulfur-containing secondary metabolites; therefore Se accumulations can be expected to differ among plant species cultivated on low Se soils. Plant Se metabolism proceeds through normal sulfur

Table 3. Mean Se concentrations (mg kg^{-1}) in *Sinapis arvensis* and *Melilotus albus* aerial parts grown on a meadow cambisol soil in control and Se treatment groups

		Treatment		
Species	Part	Control	Se	$P < F$
Sinapis arvensis	Stem	0.029	0.119	0.03
	Fruit	0.042	0.160	0.03
	Leaf	0.121	0.493	0.0001
	Shoot	0.043	0.163	0.03
Melilotus albus	Stem	0.014	0.122	0.03
	Leaf	0.032	0.452	0.0001
	Shoot	0.018	0.190	0.03

metabolic pathways; in particular, selenate-Se follows normal sulfate metabolism (Terry et al. 2000). Sulfate absorption occurs in the roots and is highly dependent on nitrogen supply and protein composition (Leustek et al. 2000). Similarly, selenate is taken up by roots and translocated to the shoot, where it incorporated into Se-cysteine (Se-Cys) and Se-methionine (Se-Met). These seleno-amino acids are incorporated into proteins through the non-specific substitution of cysteine (Cys) and methionine (Met), respectively (Terry et al. 2000). Selenium hyperaccumulator plants metabolize Se-Cys as various nonprotein seleno-amino acids, such as Se-methylSe-Cys (Brown and Shrift 1982), Se-cystathione (Peterson and Robinson 1972), and the dipeptide γ-glutamyl-Se-methylSe-Cys (Nigam, Tu, and McConnell 1969). The incorporation of Se into secondary metabolites avoids potential plant Se toxicity by preferentially incorporating sulfur into protein amino acids (Zayed and Terry 1992). The atomic ratio of protein sulfur to protein nitrogen was found to range from 0.025 in *Leguminosae* to 0.032 in *Poaceae* and was relatively constant for a given species. For *Brassicaceae*, this ratio increased to 0.055, but there is no evidence of occurrence in such constant proportion under different conditions (Dijkshoorn and Van Wijk 1967). The higher ratio for *Brassicaceae* is associated with sulfur-containing secondary metabolites. Members of this plant family are well known to produce glucosinolates. These molecules are organic anions made of amino-acid derivatives receiving a thiol from a Cys, which is subsequently glucosilated. *Brassicas* typically contain 0.1% glucosinolates, on a dry-weight basis, but they can accumulate glucosinolates to as high as 10% in the seed tissues (Seigler 1995). At low Se soil concentrations, the sulfur-containing secondary metabolites are synthesized independently of Se soil availability and could be substituted by their Se equivalent. In our results, the better ability of *Sinapis arvensis* (*Brassicaceae*) and *Melilotus albus* (*Leguminosae*) to accumulate Se could be due to the synthesis of these molecules. Indeed, *Leguminosae* produce numerous nonprotein amino acids (Seigler 1995). When grown on Se-laden soil, two *Melilotus* species known to be Se secondary accumulators increased their synthesis of Se nonprotein amino acids, which might be the plant response to minimize Se toxicity (Wu, Guo, and Bañuelos 1997; Guo and Wu 1998).

Data from the current study demonstrate that the *Leguminosae* are effectively poor Se accumulators, excepted *Melilotus albus*, whereas the *Brassicaceae* and the *Poaceae* responded favorably to Se fertilization. Plant protein content is dramatically variable among species but also varies as a function of growth conditions and development; for example, low to high values were reported for *Festuca pratensis* (3.28% to 20.6%; Szyszkowska and Sowiński 2001; Jarrige 1980), *Lolium perenne* (7.6 to 17.3%; Jarrige 1980), *Trifolium pratense* (8.3 to 24.7%), and *Trifolium repens* (14.4 to 29.0%; Jarrige 1980; Duke 1981; Penkov, Pavlov, and Minovsky 2003). Mean values for protein content have been reported for the following species: *Taraxacum officinale*, 17.1%; *Achillea millefolium*, 17.0%; *Melilotus albus*,

15% (National Academy of Sciences 1971; Elliott and Flinders 1984; Bergen, Mayer, and Kozub 1990; Jarrige 1980); and *Chenopodium album*, 3.69% (Yildirim, Dursum, and Turan 2001). Although no protein determinations were carried out in the current study, *Chenopodium album*, characterized previously as a species with very low protein content, was the second worst Se accumulator (Table 2). By contrast, the *Poaceae*, characterized with elevated protein content in the reviewed data, are much better Se accumulators.

The higher Se foliar content obtained for *Melilotus albus* and *Sinapis arvensis* suggested that these species possess the accumulation mechanisms acting in the secondary accumulators. To avoid any risk to produce fodder with too high Se content, it is suggested to (1) test the Se accumulation ability of the highest accumulators revealed in this experiment with higher rates of sodium selenate, (2) determine the Se accumulation ability of other common adventitious meadow plants, and (3) map Se concentrations in Belgian agricultural soils to determine potential availability to meadow species. This last point also raises questions on the fate of Se in meadow ecosystems. What are the proportions of Se absorbed by the crop, assimilated by livestock, and excreted back to the meadow? Because a limited amount of added Se is absorbed by crops, it will also be important to measure leaching rates and concentrations in surface and groundwaters. A concentration of 1 μg Se l^{-1} could be reached in the leaching water, assessing 50% dissolution of 10 g Se ha^{-1} in an annual 0.5-m leaching water flux. This Se concentration is close to the 2 μg Se L^{-1} considered toxic for aquatic organisms (Crane et al. 1992). In contrast, if Se soil immobilization is effective, it is of interest to know the conditions and the risk of its release.

CONCLUSIONS

Giving the very limited extent of soil Se status knowledge in Belgium, the results of Se soil and plant species analysis in the control groups are important to confirm Se soil deficiency and the poor Se content of plants in this country. The addition of 9 g Se ha^{-1}, as sodium selenate, with standard fertilizers resulted in the accumulation of Se in treated plant species; however, tissue Se concentrations were consistently less than the 2 mg kg^{-1} toxicity threshold level for dairy cattle. In contrast, four plant species (*Trifolium pratense, Polygonum aviculare, Chenopodium album, and Persicaria lapathifolia*) appeared to be poor Se accumulators. In addition, higher proportions of adventices may increase tissue Se variability during Se enrichment of plant production systems. For these reasons, amending entire meadow ecosystems with Se fertilizers may not be the most reliable method to consistently increase plant Se levels when floristic composition is poorly controlled.

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