

Plant availability of soil selenate additions and selenium distribution within wheat and ryegrass

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Abstract Selenate fertilization is an effective way to secure selenium (Se) nutrition in Se-poor areas but the cycling of the added selenate in the soil-plant system requires further clarification. We examined the Se uptake efficiency of wheat and ryegrass and Se distribution within these plants in two pot experiments. The behaviour of added selenate in a sand soil under wheat was monitored by sequential extractions during a ten-week growing period. In addition, the relationship between Se uptake of ryegrass and the salt extractable and ligand exchangeable Se in a sand and silty clay soil were studied. The added selenate remained mainly salt soluble in the soil throughout the monitoring. Se uptake by wheat comprised 12% of the soluble Se pool in soil and extended over the whole period of growth. In wheat, over 50% of Se

accumulated in grains. The Se uptake of ryegrass comprised, on average, 40% of the soil salt soluble Se. In ryegrass, over 80% of the Se accumulated in roots. The distribution pattern of Se in plants can clearly have a major influence on both the Se cycle in soil and the nutritional efficiency of Se fertilization. The simple salt extraction showed fertilization-induced changes in the soluble soil Se pool, whereas the ligand exchangeable Se fraction reflected the difference in the nonlabile Se status between the two soils.

Keywords Selenium · Selenate · Fertilization · Nutrient uptake · Within-plant distribution · Soil extractions

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Introduction

Selenium (Se) has been shown to have beneficial effects on plant growth and quality due to its antioxidative effects (Hartikainen 2005 and references therein) but it has not been proven to be a plant nutrient. For humans and animals, however, Se is essential, and plants have a pivotal role in transferring Se from soil, the ultimate source of Se, into the food chain (Girling 1984; Combs 2001; Hawkesford and Zhao 2007). Cereals, meats and fish are the main food sources of Se (Combs 2001). In Finland, approximately 20% of the average daily Se intake comes directly from plant products and nearly 70% indirectly

via consumption of products of animal origin (Eurola et al. 2003).

Plants can take up Se from the soil solution as selenate (SeO_4^{2-}), selenite (SeO_3^{2-}), and as organic Se compounds (Gissel-Nielsen and Bisbjerg 1970; Abrams et al. 1990; Williams and Mayland 1992). The uptake mechanisms of various organic Se species are yet to be unravelled but permeases specific for S-containing amino acids also suggestively mediate the uptake of seleno-amino acids (Abrams et al. 1990 and references therein). The uptake of selenate occurs actively through high-affinity sulfate transporters (Terry et al. 2000; Sors et al. 2005). Selenite is also likely taken up actively but by the phosphate transport pathway (Li et al. 2008). Thus, sulfate competes with selenate and phosphate with selenite for plant uptake; sulfate being relatively stronger than phosphate in this competition (Hopper and Parker 1999). However, different plant species have different abilities to accumulate or discriminate against Se (Mayland 1994; Terry et al. 2000). Selenium accumulating plants even prefer selenate over sulphate and can absorb several grams of Se kg^{-1} dry weight, whereas nonaccumulators, including most forage and crop plants, rarely contain over 0.1 g Se kg^{-1} dry weight (Terry et al. 2000).

There are inconsistencies regarding the relative uptake rate of selenate and selenite in solution culture (Li et al. 2008). In soil, however, selenate is clearly more plant available (Bisbjerg and Gissel-Nielsen 1969; Ylärinta 1983b) because selenite tends to be sorbed on soil surfaces (Hingston et al. 1972; Parfitt 1978; Ylärinta 1983a). Selenate is soluble but persists only at high redox ($\text{pe} + \text{pH} > 15.0$) conditions (Elrashidi et al. 1987). The bioavailability of organic Se compounds has been studied much less than that of inorganic Se species, but selenomethionine and selenocystine have shown to be at least as plant available as selenite (Williams and Mayland 1992; Zayed et al. 1998).

Many researchers have studied the ability of various extractants to imitate plant Se uptake and thus estimate soil Se bioavailability (e.g. Williams and Thornton 1973; Sippola 1979; Wang and Sippola 1990; Goodson et al. 2003; Dhillon et al. 2005; Zhao et al. 2005). Their findings have, however, been inconsistent. Probably, as noted by Dhillon et al. (2005), the ability of a certain extractant in predicting the availability of soil Se to plants is related to the type and nature of the soil. Finnish soils are typically acidic and reducing, which favours Se sorption as

selenite and precipitation in elemental form (Koljonen 1975). A simple salt solution, which targets the soil labile Se pool very efficiently (Wright et al. 2003), might adequately indicate plant available soil Se reserves in Finnish soils. The salt soluble Se pool consists mainly of selenate but may also contain soluble organic Se compounds (Wright et al. 2003). Another potential method for estimating the bioavailable soil Se reserves is a phosphate buffer extraction, which is premised on the ligand exchange mechanism (Hingston et al. 1967). The aggressiveness of the phosphate buffer can be adjusted via pH and phosphate concentration (Balistreri and Chao 1987; Saha et al. 2005). In Finland, however, hot water extraction has traditionally been used in estimating soil Se bioavailability (Eurola et al. 2008). Hot water likely solubilizes some weakly adsorbed selenite or organic Se compounds besides the easily available selenate. Our previous study (Keskinen et al. 2009) showed hot water to be over five times more efficient in solubilizing Se than a salt solution in which the high ionic strength enhances sorption reaction (Ryden and Syers 1975). The hot water extractable Se levels in Finnish cultivated soils have remained constantly at a level of $0.01 \text{ mg Se l}^{-1}$ soil (Eurola et al. 2008).

Once taken up into the plant, inorganic Se is readily metabolized into various organic Se compounds exploiting the S-assimilation pathway, as well presented in the reviews by Terry et al. (2000) and Sors et al. (2005). Selenate is transported into leaves, where it is reduced to selenide and assimilated into selenocysteine (SeCys) and further to selenomethionine (SeMet). Selenite can be reduced into selenide non-enzymatically, wherefore it is mainly reduced and retained in the roots in organic compounds. Se absorbed in organic form likewise tends to accumulate in the roots. The transport, distribution and speciation of Se in plants thus greatly depends on the form of Se taken up from the soil, as well as on the plant species and the developmental stage of the plant (Bisbjerg and Gissel-Nielsen 1969; Williams and Mayland 1992; Terry et al. 2000; Whanger 2002). Selenate is the major inorganic Se species in crops, but the predominant form of Se in cereal grains and grassland legumes has shown to be selenomethionine (Whanger 2002). So far, Se containing proteins have not proven essential for plants (Sors et al. 2005) but Se has shown to have beneficial effects, especially on stressed plants, via enhancing the antioxidative capacity, particularly

the activity of glutathione peroxidase (Hartikainen et al. 2000; Xue et al. 2001; Seppänen et al. 2003).

In Finland, adequate Se nutrition is secured by sodium selenate fertilization due to the low Se availability of our soils. Since the margin of safe Se concentrations is very narrow, it is essential to comprehensively understand the behaviour and cycling of the added selenate in the soil-plant system. Therefore, information about the total uptake and Se distribution in our field crops is needed. In this study, we aimed to i) clarify the efficiency of fertilizer Se uptake by plants in mineral soils, ii) determine the proportions of plant Se removed from the field in the harvest and returned to the soil in the plant residues likely in organic form, and iii) examine the immediately bioavailable soil Se pool and its changes over the growing period. Thus, we carried out two individual pot experiments, in which we studied the Se uptake and transport in ryegrass and wheat on a whole plant level; monitored the short-term behaviour of added Se in soil by a single phosphate extraction and a sequential Se fractionation procedure; and examined the correspondence of the plant uptake to the amounts of soil salt extractable and ligand exchangeable Se.

Materials and methods

Wheat uptake and translocation of spiked-Se from soil

In the first pot experiment, the uptake and translocation of Se in spring wheat (*Triticum aestivum* L. cv. Manu) and the short term changes in soil Se fractions after an ample Se fertilization were studied. Wheat was grown in an experimental greenhouse in a sand soil collected from the 0–20 cm surface layer of an uphill field on the Viikki Research farm, Helsinki, Finland. The sand fraction (\varnothing 0.06–2 mm) constituted over 80% of this coarse soil with a pH of 5.9, 2.2% organic carbon and low Se-adsorbing oxide content (see Keskinen et al. 2009). The soil was homogenised by passing it through a 10 mm sieve and mixing thoroughly, after which 1510 g soil aliquots (as dry weight) were weighed in 47 1.5 l plastic pots. Soil in each pot was individually fertilized according to Table 1 by adding the macronutrients separately as solid compounds and micronutrients dissolved in one

Table 1 The nutrients added (mg l^{-1} of soil) in wheat and ryegrass pot experiments. The soil volume for wheat was 1.5 l (1510 g of soil), and for ryegrass 2 l (2484 g of sand and 1545 g of silty clay soil)

Nutrient	Amount (mg l^{-1})	Compound
N	150	NH_4NO_3 , KNO_3
P	50	$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$
K	125	KNO_3
Mg	25	$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$
S ^a	40	$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$
Mo	1	$\text{Na}_2\text{MoO}_4 \cdot \text{H}_2\text{O}$
Cu	2	$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$
Zn	5	ZnCl_2
Fe	5	$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$
Mn	5	$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$
B	1	H_3BO_3

^aNo S addition in the ryegrass experiment

combined solution. In addition, 0.15 mg of Se per pot (corresponding to 0.1 mg Se kg^{-1} of soil) was added as sodium selenate (Na_2SeO_4) solution. All nutrients were hand-mixed evenly into the soil volume. Twelve wheat grains were sown in each pot and after sprouting the wheat was thinned to a total 10 plants per pot. The pots were completely randomized and bounded with nurse pots to avoid the border effect. The temperature in the greenhouse was set to 20°C in the day and 16°C at night and the relative humidity was kept at 40%. Natural daylight was supplemented with high pressure sodium lamps from 6 a.m. to 10 p.m. at 300 W m^{-2} to maintain the photosynthetic photon flux density in the range of 250–300 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Excessive light intensity ($>500 \text{ W m}^{-2}$) was prevented with shading curtains. Deionised water (electrical conductivity $<1 \mu\text{S cm}^{-1}$) was added as needed to keep the pots moist. Exceeding the soil water holding capacity was avoided to prevent leaching of Se and nutrients.

Out of the 47 identical experimental pots, five chosen at random were harvested after one week, seven after two, three, four, six, and eight weeks, and the last seven pots after ten weeks of growth when the grain was ripened. At each harvest, the growth stage was specified on Zadoks' scale (Zadoks et al. 1974) and the above ground plant mass was separated into leaves, stems and spikes. At the end of the experiment, the ripen spikes were separated into grains and

head chaff. Roots were elutriated from three pots after one week of growth and from four pots at the following harvests using a root washer (Gillison's Variety Fabrication Inc. Model 13000). All plant material was frozen in liquid nitrogen immediately after harvesting. Frozen samples were stored in -20°C until lyophilized, weighed for dry mass and milled (Kika Labortechnik A10). The total Se concentration of plant samples was analysed as described below under Plant Se analyses. The pots from which roots were not collected were used for soil analyses after passing the soil through a 2 mm sieve. In the sieving, most roots were removed. Soil analyses were conducted directly after the harvest as described below under Soil Se analyses.

Ryegrass uptake and translocation of aged Se from soil

In the second experiment, Italian ryegrass (*Lolium multiflorum* L. cv. Meroa) was grown in a sand soil collected from the same location as the soil used in the wheat experiment described above, and in silty clay soil. The silty clay was collected from the 0–20 cm surface layer of a field on the Viikki Research farm, Helsinki, Finland. It contained 47% silt (Ø 0.002–0.06 mm) and 45% clay (Ø <0.002 mm), had a pH of 5.4, and an organic carbon content of 5.1%, and was high in oxides (see Keskinen et al. 2009). In the previous summer, the soils were used in a three week pot experiment with oilseed rape (*Brassica rapa*) as units of high sulphur (S) fertilization (200 mg S l^{-1} soil) with three levels of Se additions: Se0, Se1 and Se2, corresponding to 0, 0.0025, and $0.005 \text{ mg Se l}^{-1}$ of soil (Keskinen et al. 2007). Approximately 15% of the Se added had been removed from the soil in the above ground plant mass of rape. Presumably part of the Se added had been immobilized in the rape roots which were not collected, and possibly some Se was lost through volatilization. After the rape experiment, the soils had been stored over the winter in a cold greenhouse; soil from each pot was packed individually in a plastic bag. In July 2007, soil from each specified bag was weighed into a two-liter pot with a total set of 18 sand and 18 silty clay pots, both sets comprised of six replicates of each of the three residual Se fertilization levels. To fill the pot volume, 2484 g of sand and 1545 g of silty clay soil (as dry weight) was needed. The soil remaining in each bag after

filling the pots was passed through a 2 mm sieve and used for soil analyses as described below under Soil Se analyses.

The pots were individually fertilized as in the first experiment (Table 1) except no S or Se was added. A 1.3-g portion of ryegrass seeds was sown in each pot after which the pots were completely randomized. To minimize the border effect, the pots were rearranged once or twice a week. The greenhouse conditions and watering practices were the same as in the wheat experiment. Ryegrass was cut twice, four and six weeks after sowing. After the second cutting, roots were elutriated from three replicates of Se0 and Se2 treatments of both soils with a root washer (Gillison's Variety Fabrication Inc. Model 13000). Plant samples were frozen in liquid nitrogen and stored in -20°C until lyophilized, after which the samples were weighed for dry mass, milled (Cyclotec Sample Mill 1093) and analyzed for total Se concentration as described below.

An analysis of variance was conducted to test for differences in soil salt and phosphate buffer extractable Se ($\mu\text{g pot}^{-1}$); plant growth (drymass g pot^{-1}); plant Se concentration (mg Se kg^{-1} dry weight); and total plant Se uptake ($\mu\text{g pot}^{-1}$) between the residual Se treatments and the two soils. Individual means were compared using least significant difference (LSD).

Soil Se analyses

In the wheat experiment, both a single extraction with 1M phosphate ($\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$) buffer solution with a pH of 7.0 (soil to solution ratio 1:5, extraction time 4 h), and the first three steps of the sequential extraction procedure by Chang and Jackson (1957) were carried out in triplicate to monitor the fertilization and plant uptake-induced changes in soil Se status over the growing period. Phosphate buffer was used to solubilize adsorbed Se as phosphate displaces selenite due to greater sorption strength (Rajan and Watkinson 1976). In the sequential extractions, the objective was to examine the rate of reduction of the added selenate by following the subsequent adsorption on oxide surfaces. The Chang and Jackson (1957) procedure was chosen for this purpose because it has been proven to be efficient in extracting selenite added to soil (Nakamaru et al. 2005). Soil Se was fractionated into salt soluble, and aluminium oxide,

and iron oxide associated pools by successive 1M NH₄Cl, 0.5M NH₄F, (pH 8.5), and 0.1M NaOH extractions. A detailed description of the extraction procedures is presented in Keskinen et al. (2009).

In the ryegrass experiment, soil analyses focused on comparing the Se withdrawal by plants and soil extractants. The soil samples taken before planting were extracted in triplicate with 1M NH₄Cl as in the first step of the Chang and Jackson (1957) procedure, and with 0.1M phosphate buffer, pH 8.0 (soil to solution ratio 1:5, extraction time 4 h).

The Se concentration of the extracts was analysed from 5–15 ml aliquots by an electrothermal atomic absorption spectrometric method with graphite furnace AAS after an ammonium pyrrolidine dithiocarbamate-methyl isobutyl ketone-extraction system (Kumpulainen et al. 1983) as in Keskinen et al. (2009). In this method, selenite is the only measurable state of Se wherefore an oxidation-reduction procedure is needed to bring the total Se content of the extract into analysable form. In the wheat experiment, an attempt to separate selenite from other Se species was made by also analysing extracts without the oxidation-reduction step. In the NH₄F and NaOH extracts, which were rich in dissolved organic compounds, the separation of Se species was found to be unreliable because the organic substances changed the consistence of the MIBK phase of the extracts not subjected to oxidation-reduction step. Therefore, only the selenite concentrations of NH₄Cl and phosphate extracts can be reported.

Plant Se analyses

Plant Se analyses were conducted by the electrothermal atomic absorption spectrometric method for food samples (Kumpulainen et al. 1983; Ekholm 1996). The lyophilized and milled plant samples were dried at 70°C over night to remove any adsorbed moisture. Depending on the Se concentration of the sample, a 0.1–1.0 g aliquot was weighed into a wet digestion tube where 10 ml of an acid mixture (HNO₃-HClO₄-H₂SO₄ in a ratio of 2.5:1.5:1) was added. The sample was first allowed to stand in the acid mixture at room temperature overnight, after which it was heated in a wet digestion apparatus according to the following five-step temperature program: 1) 30 min in 70°C, 2) 3 h in 120°C, 3) 30 min in 160°C, 4) 30 min in 190°C, and 5) 5 h in 220°C. After the digestion, 10 ml of 4M HCl was added and the solution boiled for 20 min at

130°C to reduce Se, which after digestion was in selenate form, into selenite. The following ammonium pyrrolidine dithiocarbamate-methyl isobutyl ketone-extraction and determination of Se concentration with graphite furnace AAS was done similarly to the soil samples (for detailed description and instrumental parameters see Keskinen et al. 2009).

Plant samples were analyzed only from the pots from which roots were elutriated, that is ryegrass samples were done in triplicate and wheat samples in quadruple, except for the first sampling where only three wheat pots were collected for plant analyses. A standard reference material (Wheat flour 1567a, National Institute of Standards & Technology) with certified Se concentration of 1.1±0.2 mg kg⁻¹ dry weight and an in-house wheat flour control material with a known Se concentration of 0.040±0.008 mg kg⁻¹ dry weight were analysed as samples in all series to assess the accuracy of the plant analyses. For the standard wheat flour, a Se concentration of 1.1±0.3 mg kg⁻¹ (*n*=10) and for the in-house reference, a Se concentration of 0.043±0.003 mg kg⁻¹ (*n*=27) was attained.

Results

Wheat uptake and translocation of spiked-Se from soil

On the basis of the dry weight of the crop biomass as a whole, the wheat growth pattern followed a sigmoid curve, whereas the total Se content of the plant biomass increased linearly until the completion of growth approximately eight weeks after sowing (Fig. 1). At harvest, the total plant dry mass had decreased by 10% and the Se content by 15% from the highest values attained two weeks earlier.

The precise wheat development stages at the seven sampling times are described according to Zadoks' decimal code (Zadoks et al. 1974) in Table 2. The sown and germinated seeds decomposed during the first four weeks of growth (Fig. 2a). Root mass increased steadily until eight weeks after sowing, after which the mass began to decrease (Fig. 2b). The total leaf mass increased during the first three weeks only (Fig. 2c). At six weeks after sowing, the leaves were sorted into young and old ones. At that time, old leaves, specified as having over 50% of the leaf area withered, constituted 60% of the total leaf mass. In

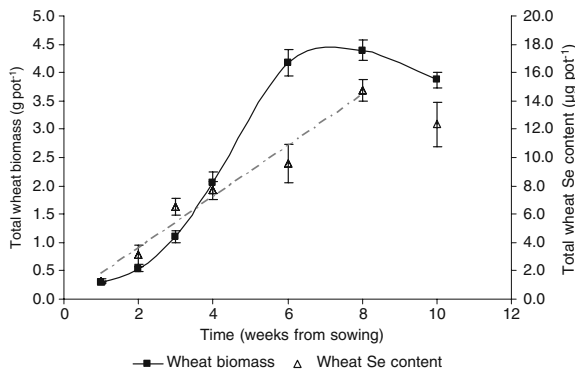


Fig. 1 Growth pattern for wheat as the total dry mass (g pot^{-1}) and the accumulation of plant Se content ($\mu\text{g pot}^{-1}$) in a sand soil fertilized with $0.15 \text{ mg Se pot}^{-1}$. Values are averages of four replications \pm standard deviation

the two last samplings, all leaves were defined as old. Stems emerged after three weeks of growth and grew for a three week period, after which the stem biomass began to decrease (Fig. 2d). Spikes began to develop after four weeks and were ready for harvest six weeks later (Fig. 2a). At harvest, 5% of the wheat biomass was in the roots, 15% in the leaves, 35% in the stems, and 45% in the spikes from which two-thirds were composed of grains.

The Se concentration in the vegetative organs (roots, leaves and stems) decreased as the plants aged, whereas in the spikes it increased towards maturity (Fig. 2a–d). The Se concentrations were highest in the young leaves and in the harvest ready grains, and lowest in the roots and stems of the mature plants. As for the Se content of wheat, the proportion of total Se in the roots was small over the whole growing period (Fig. 3). The average shoot/root

ratio of total Se increased during growth from three in the seedlings to over 30 in the mature plants. During the first month, Se accumulated in the leaves and emerging stems, but the amount of Se in these organs decreased rapidly as the spikes began to develop and accumulate Se. In the spikes, Se was found within the grains. In the harvest ready crop, on average 55% of the total Se content was in grains, 10% in the head chaff, 15% in stems, 15% in leaves and 5% in roots.

Before fertilization, no Se was detected in the salt soluble (NH_4Cl) fraction of the sand soil (Fig. 4). In the first soil samples, collected one week after the addition of selenate and the sowing of wheat, $84 \pm 8\%$ of the added Se was recovered in the salt extracts. No selenite was found in the salt extracts not subjected to the oxidation-reduction step at any of the sampling times. The total salt soluble Se concentration decreased slightly over the course of the growing period. Total wheat Se uptake accounted for 85% of this decrease. The wheat took up, on average, 10% of the fertilizer selenate addition (calculated from the maximum plant Se content at eight weeks after sowing), and 12% of the NH_4Cl -extractable soil Se (measured one week after fertilization).

In the NH_4F -extractable aluminium oxide associated Se concentration, an increase above the background level corresponding to $13 \pm 3\%$ of the selenate addition was detected in the first soil samples (Fig. 4). After this early fertilization-induced shift, the NH_4F -extractable Se concentration fluctuated slightly without a clear trend. The NaOH -extractable iron oxide associated soil Se concentration ranged between 5% and nearly 50% above the background level during the monitoring,

Table 2 Wheat growth stages on Zadoks' scale (Zadoks et al. 1974) at the seven different sampling times

Time (weeks from sowing)	Zadoks' growth stage codes	Codes description
1	12	2 leaves unfolded
2	12–13	2–3 leaves unfolded
3	14	4 leaves unfolded
4	15, 20, 33	5 leaves unfolded Main shoot only 3 rd node detectable
6	16,20,33,58,68	Emergence of inflorescence completed Anthesis complete
8	85	Soft dough
10	93	Caryopsis loosening in daytime

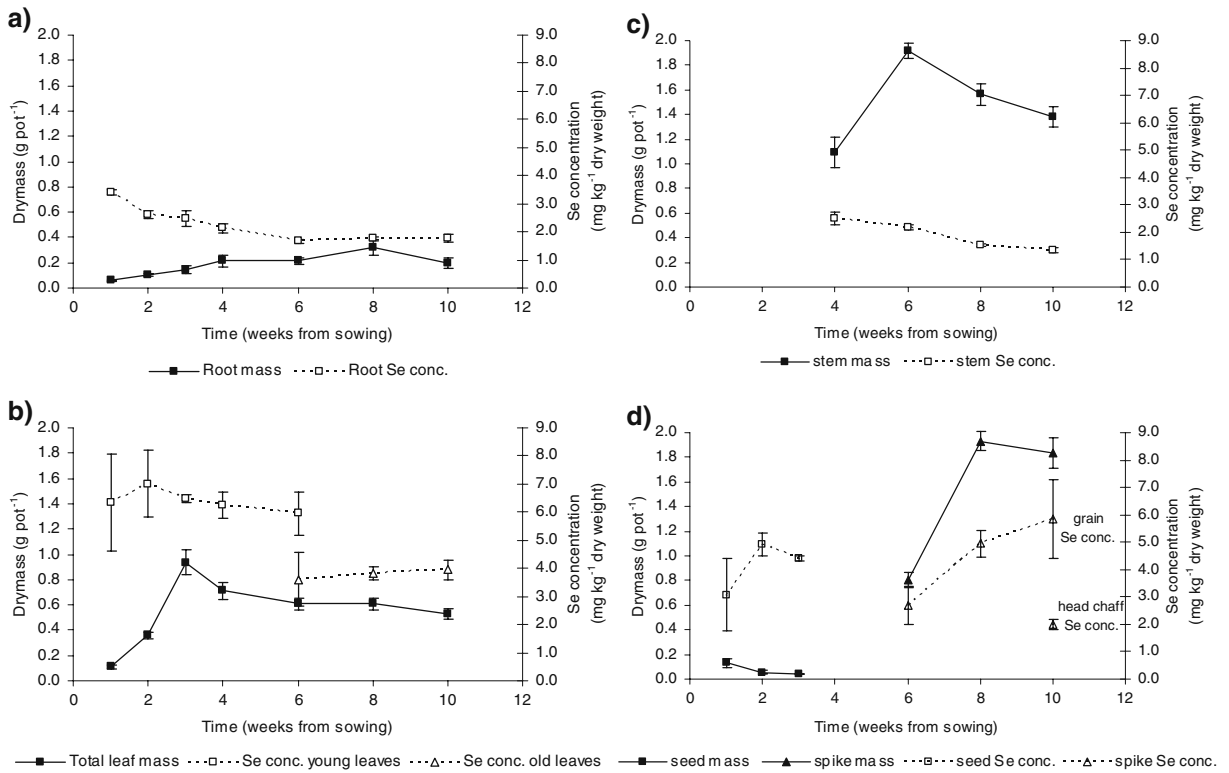


Fig. 2 a–d Dry masses (g pot⁻¹) and Se concentrations (mg kg⁻¹ dry weight) of wheat roots (a), leaves (b), stems (c), and germinated seeds and spikes (d) at seven samplings over a

ten-week growing period. Wheat was grown in selenate fertilized (0.1 mg Se kg⁻¹) sand soil. Values are averages of four replications±standard deviation

resulting in an average 10% increase, which corresponded, however, to only a few percent of the fertilizer selenate addition.

The initial phosphate extractable soil Se concentration was 0.021 mg kg⁻¹, from which 20% was

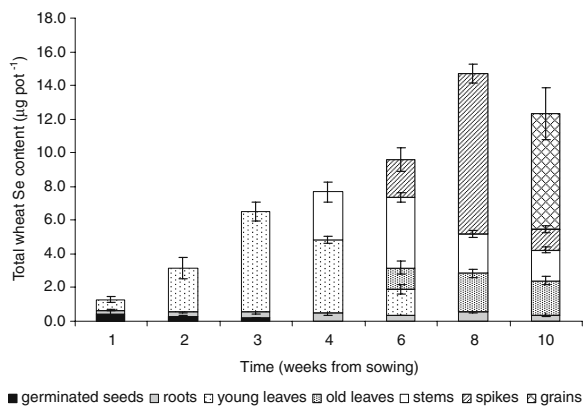


Fig. 3 Total wheat Se content (µg pot⁻¹) in different plant parts at seven sampling times over a ten-week growing period. On week 10, the portion of spikes represents the head chaff. Values are averages of four replications±standard deviation

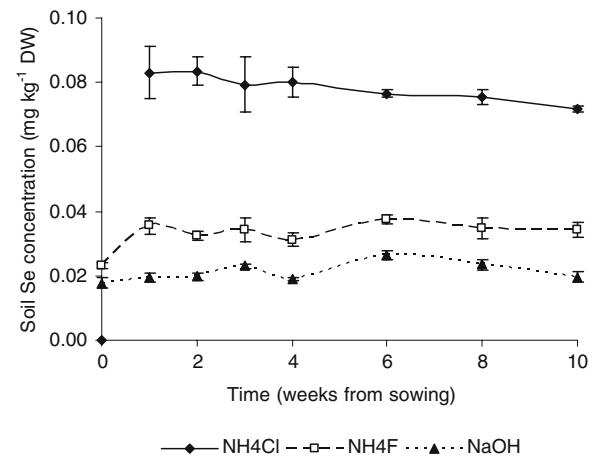


Fig. 4 Salt soluble (NH₄Cl), aluminium oxide-associated (NH₄F), and iron oxide-associated (NaOH) Se fractions of a sand soil under wheat over a ten-week monitoring period. The soil was fertilized with 0.1 mg selenate kg⁻¹ soil at sowing. The Se concentrations at 0 weeks from sowing represent the soil Se status before fertilization. Values are means of three replications±standard deviation

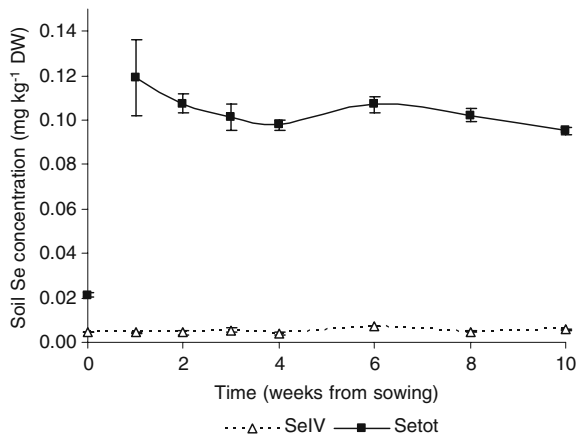


Fig. 5 Total phosphate buffer (1 M $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$, pH 7.0) extractable Se (Setot) and selenite (SeIV) concentrations of a sand soil under wheat over a ten-week monitoring period. The soil was fertilized with 0.1 mg selenate kg^{-1} soil at sowing. The Se concentrations at 0 weeks from sowing represent the soil Se status before fertilization. Values are means of three replications \pm standard deviation

selenite (Fig. 5). In the first soil samples, $99 \pm 17\%$ of the selenate addition was recovered in the phosphate extracts. The phosphate extractable selenite concentration remained at the same level throughout the experiment, but the total phosphate extractable Se

concentration decreased over the course of the growing period. Plant uptake accounted for only 40% of this decrease.

Ryegrass uptake and translocation of aged Se from soil

Ryegrass produced more leaf mass in the sand than in the clay soil but a similar soil type-dependent difference was not seen in the root masses (Table 3). The residual Se treatments had no effect on leaf growth but exerted a growth-promoting effect on roots in the sand soil. On average, 40% of the total ryegrass biomass was allocated in roots and a 30% proportion was harvested as leaf mass in each of the two cuttings.

The Se concentration and consequently the total Se content of the ryegrass leaves increased along with an increase in the amounts of previous Se fertilization (Table 3). In both soils, the two-fold Se treatment (Se2) doubled the Se concentration and total Se content of the leaves from the level attained in the lower Se addition (Se1). Due to an unfortunate contamination in the milling, the leaf samples of the non-Se-fertilized (Se0) plants of the clay soil were

Table 3 Drymass (g pot^{-1}), Se concentration (mg kg^{-1} dry weight) and total Se uptake ($\mu\text{g pot}^{-1}$) of two successive leaf cuttings and roots of ryegrass grown in sand and silty clay soil with three residual fertilizer Se levels (Se0–Se2), and the corresponding soil salt soluble (NH_4Cl) and ligand exchangeable ($\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$) Se contents ($\mu\text{g pot}^{-1}$) before sowing. Values are averages of three replications except for the dry masses of leaf cuttings which consist of six replications

	Sand			Silty clay			LSD _{0.05}
	Se0	Se1	Se2	Se0	Se1	Se2	
Drymass (g pot^{-1})							
1st Cutting	5.38	5.38	5.29	4.80	4.83	4.96	0.28
2nd Cutting	5.89	5.82	6.03	4.84	4.84	4.84	0.43
Roots	5.57	ND	7.65	6.82	ND	7.43	1.54
Total	16.7	ND	19.0	16.6	ND	17.1	1.51
Se conc. (mg kg^{-1} dry weight)							
1st Cutting	0.018	0.030	0.066	ND	0.029	0.057	0.009
2nd Cutting	0.019	0.026	0.053	ND	0.020	0.037	0.011
Roots	0.291	ND	0.365	0.322	ND	0.337	0.073
Total Se uptake ($\mu\text{g pot}^{-1}$)							
1st Cutting	0.096	0.164	0.344	ND	0.142	0.270	0.051
2nd Cutting	0.111	0.149	0.322	ND	0.095	0.188	0.067
Roots	1.63	ND	2.78	2.20	ND	2.55	0.92
Total	1.83	ND	3.45	ND	ND	3.01	1.07
Soil Se ($\mu\text{g pot}^{-1}$)							
NH_4Cl -extr.	3.71	4.57	7.71	4.12	5.21	8.55	1.81
$\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ -extr.	12.5	13.5	16.6	80.8	75.5	88.7	3.26

LSD_{0.05} Least significant difference at 5 % level within rows

ND No data

lost. In the sand soil, however, more Se was found in the leaves of the Se1 than of the Se0 treatment but this difference was significant only in the first cutting. In general, the residual effect of Se fertilization seemed to be less pronounced in the second than in the first leaf harvest. As for the concentration and total content of Se in the roots, the previous Se addition had no effect in the clay soil. In the sand, the residual fertilizer Se increased root Se but the effect was much smaller than in the leaves. The Se concentrations in the roots were, however, much higher than in the leaves. The shoot/root ratio of total Se content was on average 0.2 in the previously Se fertilized and 0.1 in non-Se-fertilized ryegrasses. Consequently, over 80% of the total Se uptake by ryegrass was accumulated in the roots. The total root Se content did not differ between the soil types. Therefore, no difference in the total Se uptake between the two soils was found, even though in the Se2 treatment more Se was found in the leaves of ryegrass grown in the sand soil than in the clay.

In both soils, the salt extractable Se contents, determined before sowing the ryegrass, were of the same order of magnitude and increased as the previous Se addition increased (Table 3). However, the clay soil was notably richer than the sand in the phosphate extractable soil Se. An increasing trend induced by the previous selenate addition was also seen in the phosphate-extractable soil Se contents, with the exception of the inconsistent response of the phosphate extractable Se to the Se1 treatment in the clay soil. In the sand soil, the ryegrass Se uptake corresponded to $47\pm 5\%$ of the soil salt soluble and $18\pm 4\%$ of the phosphate extractable Se content measured at the beginning of the experiment. In the clay, the corresponding proportions were $41\pm 11\%$ and $4\pm 1\%$.

Discussion

In the monitoring of Se uptake by wheat, a selenate addition 40-fold higher than the practical supplementation level was applied to ensure measurable Se concentrations of the different plant parts and various fractions in soil. No visual abnormalities were caused by the Se application, indicating that it was apparently not too high. In fact, the wheat growth followed a typical pattern for determinate cereals (Evans et al.

1975; Gourdiaan and van Laar 1994). According to Lyons et al. (2005a), wheat can tolerate selenate levels in the growth medium 20-fold higher than what we used. They reported the critical tissue concentrations of Se toxicity for wheat to be approximately $325 \text{ mg Se kg}^{-1}$ dry weight, whereas our highest tissue concentration, found in young leaves, was around 7 mg kg^{-1} dry weight.

The Se uptake by wheat continued throughout the whole period of growth (Fig. 1). However, the Se concentrations of the vegetative plant parts decreased over the course of the growing period. This decrease can be partly explained by biodilution but the Se translocation from leaves and stems to grains also contributed to the decrease. The absolute amount of Se in the leaves and stems decreased rapidly as the grains started developing (Fig. 3). The filling grains act as sinks for carbohydrates and proteins that are remobilized from leaves, stems, roots, and glumes (Evans et al. 1975; Simpson et al. 1983). McNeal et al. (1968) reported that over 65% of the total nitrogen in the top growth of spring wheat was translocated into grains. The Se containing amino acid, selenomethionine, is the major Se form in wheat grains (Stadlober et al. 2001; Whanger 2002). Lyons et al. (2005b) found that the endosperm and the aleurone layer contained over 95% of the total wheat grain Se content, although the Se concentration was highest in the embryo.

In the ryegrass experiment, the previous lower Se addition (Se1) approximated the level used in practical agriculture, which is adjusted to generate nutritionally adequate grain Se concentrations of around $0.1 \text{ mg Se kg}^{-1}$ dry weight (Eurola and Hietaniemi 2000). The residual effect of neither the Se1 nor the doubled Se2 treatment was sufficient to increase the Se concentrations of leaf yields to that target level, equivalent to the level recommended for cattle feed (MTT 2006), but the residual Se had an evident effect. The previous doubled Se addition approximately doubled the ryegrass Se concentration and total Se content in leaves. An increase in the Se concentration in the plant roughly proportional to the increase in the Se addition has also been found in previous studies (Tveitnes et al. 1996; Eich-Greatorex et al. 2007). The two successive ryegrass leaf cuttings showed a decreasing trend in the Se concentrations, indicating the depleting Se supply. The residual effect of Se fertilization on root Se was much slighter

than on leaf Se. In ryegrass, the root Se concentrations were, however, notably high even in the Se0 treatment.

The previous Se fertilization treatments had no effect on the leaf yield of ryegrass but increased the root biomass, in which most of the Se taken up was retained. In most studies, Se additions below toxic levels have not influenced plant yields (e.g. Ylänta 1983b; Tveitnes et al. 1996; Lyons et al. 2005a), but positive effects on plant growth have also been reported (Hartikainen et al. 2000; Peng et al. 2000; Djanaguiraman et al. 2005). In a recent study by Lyons et al. (2009), selenite addition increased root growth in wheat seedlings and seed production in Brassica.

The distribution of Se between the roots and the above ground plant parts differed greatly between the plant species; in wheat the Se shoot/root ratio was well above one throughout the growing period, whereas in ryegrass it was well below one. These two species may differ in their Se transport ability. Williams and Mayland (1992) found that an accumulator plant transported Se more readily to shoots than a nonaccumulator, and in studies by Zhang et al. (2006) the translocation rate of Se differed between two cultivars of rice. However, in a previous study by Ylänta (1983b) on average 40% of the selenate addition, corresponding roughly to the addition level used in our wheat experiment, was transported to ryegrass leaves. Therefore, rather than a radical dissimilarity in the inherent Se transport ability between wheat and ryegrass, dissimilar proportions of various Se species in soil likely explain the discrepancy in the Se translocation patterns in our two experiments. The translocation and distribution of Se within the plant is known to be governed by the species. Selenate is readily transported into leaves whereas selenite and organic Se species tend to accumulate in the roots (Smith and Watkinson 1984; Zayed et al. 1998; Terry et al. 2000; Li et al. 2008). When different Se species are available to the plant simultaneously, as they likely are in soils, the distribution pattern of Se may differ from the average of the separate Se sources as suggested by Li et al. (2008). They constructed a treatment in which both selenite and selenate were supplied and it was discovered that the translocation of selenate was suppressed in the presence of selenite. The distribution of Se within the crop clearly has a great effect on the efficiency of Se fertilization on the nutritional point of view as well as

on the cycling of the added Se in the soil-plant system. Therefore, in future studies, more attention should be given to the speciation spectrum of the available Se in different soils and to the corresponding Se translocation pattern in crops.

The amount of Se taken up by wheat and ryegrass on a whole plant level ranged from 12–47% of the NH₄Cl extractable soil Se. In the studies by Goodson et al. (2003), the cumulative Se withdrawal by plants was likewise less than 50% of the soil KCl-extractable Se. It thus seems that the plant Se uptake is restricted to this most easily soluble, salt solution extractable soil Se pool. Most likely, both the Se uptake capacity of the plant species and the concentration of competing anions, namely sulphate and phosphate, in the soil solution govern the ability of a plant to exploit the soluble Se reserves. In our study, ryegrass used the salt soluble Se relatively more efficiently than wheat but the prominent difference in the magnitude of the soluble Se fraction between the two experiments may have contributed to the difference. In the ryegrass experiment with increasing Se addition levels, the NH₄Cl extraction proved to be relatively sensitive to small fertilization-induced changes. Furthermore, it showed no clear difference between the sand and clay soil, thus corresponding to the results of plant uptake.

The phosphate buffer extraction releases selenite bound to soil surfaces by the ligand exchange mechanism in addition to the easily soluble Se species (Raján and Watkinson 1976). In the sand soil, the proportion of selenite in the phosphate buffer extractable (1M, pH 7.0) Se reserves determined before selenate addition was, however, only 20%, suggesting that a considerable portion of the adsorbed Se pool was organic. Contrary to the NH₄Cl extraction, the phosphate buffer extracts reflected the prominent difference between the two soils. Roughly five times more Se was solubilized by the phosphate buffer (0.1M, pH 8.0) from the silty clay soil high in adsorbing oxides and organic matter than from the sand soil that was notably poorer in both oxide and organic matter content. An equal difference was previously detected in the aqua regia extractable Se concentrations between these soils (Keskinen et al. 2009). Since the plant Se uptake was restricted to a portion of the salt soluble Se pool, the phosphate-buffer extraction was found to be considerably more powerful than ryegrass in removing Se. However, it proved to be useful in estimating and monitoring

changes in the potentially phytoavailable Se reserves. The soluble Se pool can be slowly replenished from nonlabile Se fractions through mineralization of organic Se compounds and desorption of adsorbed Se (Vuori et al. 1994). In the wheat experiment, however, the decrease in phosphate buffer extractable (1M, pH 7.0) Se clearly exceeded the removal through wheat uptake, indicating that in time Se tended rather to adsorb more strongly than to mobilize.

The sequential extractions conducted in the soil monitoring revealed that only a minor proportion of the selenate addition ended up in the NH_4F and NaOH extractable (aluminium and iron oxide-associated) Se fractions of the sand soil. Instead, the added selenate remained in the salt soluble Se pool, in which no selenite-Se could be detected. Therefore, reduction of selenate seemed to be unimportant during the 10-week experiment. The transformation rate of the added selenate could have been higher in a fine textured soil having predominantly fine pores and thus increased risk of restricted aeration (see Wall and Heiskanen 2003) or in a soil rich in easily degradable organic matter, which enhances reduction reactions via increasing microbial activity (Camps Arbostain 1998), than in the coarse soil studied. However, in experiments by Ylärinta (1983a) 60%, 80% and 90% of the selenate added to clay soil, fine sandy soil and *Carex* peat remained hot water-soluble throughout a three-month incubation at approximately field capacity. In Finnish field soils, selenate reduction is thus most likely to occur over the wettest autumn and spring seasons. In the ryegrass experiment, the Se fertilization of the previous year still significantly increased the salt soluble Se in soil. However, the pot experiment did not represent authentic field circumstances because of the short duration of the preceding rape experiment (3 weeks) and the storage of the soil samples as air dried and sealed. Ylärinta (1984) found that in field experiments part of the selenate addition may still remain available to plants in the following growing season. His selenate addition, corresponding to doses used in practical farming, slightly increased the Se concentrations of timothy grass on the first cut of the next growing season. The residual effect of an ample selenate addition was further seen in the second year after the application but the Se concentrations in grass diminished notably in every successive harvest.

Conclusions

Wheat Se uptake continued steadily throughout the period of growth and Se was transported very efficiently into the grains. In the wheat crop, over 50% of the plant Se was removed within the grains, and assuming that the straw yield would have been collected as well, only less than 20% of the plant Se would have cycled back into the soil. However, ryegrass grown on residual soil Se accumulated Se mostly in roots, in which case only a small proportion of the plant Se would have been withdrawn from the soil-plant-cycle. The difference between the plant species was likely caused by a difference in the Se species taken up, which effects the Se distribution within the plant and thus the nutritional efficiency of Se fertilization. Differences in the Se translocation pattern may cause false interpretations of the efficiency of plant Se uptake if all plant parts are not examined. In longterm experiments, roots have often been neglected but should receive more attention in the future to clarify both the possible positive growth response and the Se accumulation pattern.

The plant Se uptake was restricted to a portion of the most easily available soil Se pool, as the Se uptake of wheat and ryegrass ranged from 12–47% of the NH_4Cl -extractable soil Se. This simple salt solution extraction showed small changes induced by selenate additions and excluded the adsorbed Se fraction reflecting the nonlabile Se reserves. It thus appears that NH_4Cl extraction can be used to compare the amount of biologically available Se in different soils. However, the factors limiting the plant Se uptake from the salt soluble soil Se pool need to be determined. Selenate reduction and subsequent adsorption were shown to be unimportant in the sand soil over the 10-week experimental period.

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