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Doctoral Dissertation

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The acidity and low redox potential in soils in Finland render selenium (Se) scarcely soluble, thereby severely restricting its availability to plants. To achieve an adequate intake level of this microelement essential for human beings and animals, a national Se fertilization programme has been in operation in Finland since the 1985 growing season. Nutritionally, supplementing compound fertilizers with sodium selenate has been a success. The practice is, however, challenged by the unknown cycle of annual Se additions in the environment. In this study, several soil extractants were explored to find methods of sound basis for routine soil Se analyses and for a more detailed characterization of the soil Se status. To increase understanding of the behaviour of fertilizer Se in soil, both long- and short-term changes in soil Se reserves in response to Se additions were examined. The efficiency of plant uptake of fertilizer Se was also studied, emphasizing the influence of Se-adsorbing oxides and organic components on the retention of Se in soil. In addition, the uptake rhythm and translocation of Se within plants were determined.

Sequential extractions of selenate-fertilized organic and mineral soils with varying Se content revealed a characteristic pattern in the distribution of Se. A mere 1% of the soil Se reserves were extracted with a KCl solution, nearly 20% were recovered in adsorbed, 40% in organically associated, 15% in elemental and around 30% in recalcitrant fractions. The change in soil Se concentrations after a 13-year period of Se fertilization remained small. However, the data indicated that in mineral soils residual fertilizer Se had accumulated as adsorbed selenite and in the organically associated and recalcitrant pools. The minor fraction of salt-soluble Se reflected the instability of selenate in soils in Finland. Pot experiments, however, demonstrated that under moisture conditions drier than field capacity, easily soluble selenate may persist in acidic soils throughout the growing period.

Since Se accumulates in poorly soluble pools, very weak extraction methods are not suitable for describing changes in soil Se reserves. Of the single extractions tested, acid ammonium oxalate or phosphate buffer with high pH appeared to be more suitable extractants for estimating the soil Se status than the hot-water extraction used in national monitoring at present. However, salt solutions, which were less efficient than hot water in extracting Se, recovered more Se than what was found within plants. These weak extractants can thus be used in assessing the immediately bioavailable soil Se pool, although the association between the amounts of Se acquired by soil analyses and that absorbed by plants needs to be further clarified.

The Se uptake of wheat and ryegrass ranged between 5% and 50% of the amount of fertilizer selenate added in pot experiments. The Se uptake by wheat con-

tinued throughout the growth period and wheat transported over 50% of its Se content into the grains. Ryegrass grown in soil with residual Se, however, accumulated over 80% of its total Se uptake in the roots. The translocation pattern within the plant can thus have a marked influence on the apparent uptake efficiency of Se when only the harvested portion of the crop is considered. Since reduction of selenate to selenite was low during the growth period, the content of Se sorption components in the soil had minor influence on the plant availability of Se. As selenite, Se was retained very efficiently in both peat and in peat enriched with iron hydroxides. On artificial mineral surfaces, Se was adsorbed by ligand exchange, but in pure peat an unknown mechanism of retention was operating.

This study revealed that new approaches are needed in Se research to unravel the nature of the unfamiliar forms of organically bound Se that dominate Se reserves in soil. The results of the pot experiments suggest that processes other than rapid reduction and subsequent fixation of the added selenate govern the uptake of the annual fertilizer Se addition by plants. Factors limiting plant Se uptake from the soluble pool in soil need to be addressed in future studies.

Seleenilannoitus: otto kasviin ja jäännös maassa

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Maaperän happamuuden ja matalan hapetus-pelkistyspotentiaalin johdosta ihmisille ja eläimille välttämätön hivenravinne seleeni (Se) esiintyy suomalaisissa maissa pääosin kasveille käyttökelvottomissa, niukkaliukoisissa muodoissa. Suomalaisten riittävä seleeninsaanti onkin varmistettu vuodesta 1985 lähtien kansallisella Se-lannoitusohjelmalla. Ravitsemuksellisesti natriumselenaatin lisääminen lannoitteisiin on ollut menestyksekästä. Vuosittain toistuvien Se-lisäysten kiertoa ympäristössä ei kuitenkaan tunneta tarkasti, mikä varjostaa lannoituskäytäntöä. Tässä tutkimuksessa tarkasteltiin erilaisia maan uuttomenetelmiä pyrkimyksenä löytää rutiinianalyyseihin toiminnallisesti perusteltu, yksinkertainen uuttotapa sekä maan Se-varannon yksityiskohtaisempaan tutkimukseen sopiva menetelmä. Lannoiteseleenin maaperäkäyttäytymisen selvittämiseksi työssä tarkasteltiin Se-lisäysten pitkä- ja lyhytaikaisia vaikutuksia maan Se-varantoon. Kasvien lannoiteseleenin ottotehokkuutta tutkittiin painottaen Se-pidättävien oksidien ja orgaanisen aineksen merkitystä lisätyn Se:n käyttökelpoisuudelle. Lisäksi tarkasteltiin kasvien Se:n oton rytmiä ja Se:n kuljetusta kasvissa.

Kokonaisseleenipitoisuudeltaan toisistaan eroavilla Se-lannoitetuilla orgaanisilla ja kivennäismailla tehdyt perättäiset uutot osoittivat maan Se-varannolle tyypillisen jakauman. Vain 1 % maan Se:stä uuttui helppoliukoisena KCl-liuoksella, lähes 20 % oli adsorboituneena, 40 % orgaaniseen ainekseen sitoutuneena, 15 % alkuainemuodossa ja noin 30 % niukkaliukoisena. Seleenilannoituksen aikaan-saamat muutokset maan Se-pitoisuuksissa 13 vuoden tarkastelujaksolla olivat pieniä, mutta kivennäismaalla Se:n havaittiin kertyneen adsorboituneeseen, orgaaniseen sekä niukkaliukoiseen fraktioon. Helppoliukoisen Se:n pieni osuus kuvastaa selenaatin pysymättömyyttä suomalaisissa maissa. Astiakokeissa havaittiin kuitenkin, että kenttäkapasiteettia kuivemmissa olosuhteissa selenaatti voi säilyä maassa läpi kasvukauden.

Koska Se kertyy maassa vaikealiukoisiin muotoihin, heikot uuttomenetelmät eivät sovellu maaperän Se-varannon muutosten seurantaan. Työssä testatuista yksittäisuutoista hapan ammoniumoksalaatti ja fosfaattipuskuri korkeassa pH:ssa osoittautuivatkin Se-seurannassa käytettävää kuumavesiuuttoa lupaavammiksi menetelmiksi maan Se-varannon arviointiin. Tehokkuudeltaan kuumaa vettä heikommalla suolaliuoksella maasta uuttui kuitenkin huomattavasti kasvien ottamaa määrää enemmän Se:ä. Nämä heikot uuttomenetelmät ovat siten käyttökelpoisia välittömästi biosaatavissa olevan Se:n tarkasteluun, joskin maauuttojen ja kasvien Se:n ottotehokkuuden välistä yhteyttä tulee selvittää tarkemmin.

Vehnä- ja raiheinäkasvustot ottivat 5-50 % astiakokeissa lisätystä lannoiteselenaatista. Vehnän seleeninotto jatkui läpi kasvukauden. Yli 50 % vehnäkasvuston ottamasta Se:stä päätyi jyviin, kun taas edellisen vuoden selenaattilannoituksen jäännöksellä kasvaneen raiheinän ottamasta Se:stä 80 % kertyi juuriin. Seleenin kuljetuksella kasvissa voi siten olla merkittävä vaiku-

tus laskennalliseen Se:n ottotehokkuuteen, jos vain sadoksi korjattu osa kasvista tulee huomioiduksi. Astiakokeissa selenaatin pelkistyminen seleniitiksi oli vähäistä, joten maan Se:ä pidättävillä komponenteilla ei ollut juuri vaikutusta Se:n käyttökelpoisuuteen. Seleniittinä Se kuitenkin pidättyi tehokkaasti sekä puhtaaseen että rautaoksideilla rikastettuun rahkaturpeeseen. Keinotekoisilla mineraalipinnoilla Se:n pidättyminen tapahtui ligandinvaihtomekanismilla, mutta puhtaassa turpeessa pidättymistapa jäi tuntemattomaksi.

Tämä työ osoitti, että tulevaisuuden Se-tutkimuksessa tarvitaan uusia menetelmiä toistaiseksi tuntemattomien, mutta maassa hallitseviksi osoittautuneiden orgaanisten Se-muotojen tarkasteluun. Astiakokeiden tulosten perusteella muut tekijät kuin selenaatin nopea pelkistyminen ja sitä seuraava pidättyminen vaikuttavat määräävän kasvien lannoite-Se:n ottotehokkuuden. Maan helppoliukoisen Se:n kasvien ottoa rajoittavia tekijöitä onkin jatkossa syytä selvittää.

Avainsanat: *seleeni, selenaatti, seleniitti, esiintymismuoto, lannoitus, maauutto, adsorptio, ravinteiden otto*

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List of original publications

This thesis is based on the following publications, which are referred to by their Roman numerals:

I   Efficiency of different methods in extracting selenium from agricultural soils of Finland

Keskinen R, Ekholm P, Yli-Halla M and Hartikainen H

Geoderma 153 (2009) 87-93

II   Selenium fractions in selenate-fertilized field soils of Finland

Keskinen R, Räty M and Yli-Halla M

Nutrient Cycling in Agroecosystems 91 (2011) 17-29

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

Keskinen R, Turakainen M and Hartikainen H

Plant and Soil 333 (2010) 301-313

IV   Retention and uptake by plants of added selenium in peat soils

Keskinen R, Yli-Halla M and Hartikainen H

Manuscript.

Author's contribution to the articles

I   The experimental design, including the selection of soils and extraction methods to be tested, was compiled jointly by Helinä Hartikainen, Markku Yli-Halla and Riikka Keskinen. The laboratory work was conducted by Riikka Keskinen under the supervision of Päivi Ekholm. Riikka Keskinen calculated and interpreted the data and prepared the manuscript. All authors commented on the manuscript.

II   The experimental design was built on the idea of Helinä Hartikainen using a set of paired soil samples originally accomplished by Markku Yli-Halla. All authors contributed to the planning of the study. Riikka Keskinen conducted the laboratory work together with Mari Räty. The calculations, interpretation of the data and preparation of the manuscript was done by Riikka Keskinen. All authors commented on the manuscript.

III   All authors contributed to the planning of the experimental design. The pot experiments and laboratory analyses were carried out by Riikka Keskinen and Marja Turakainen together with technical assistance. Riikka Keskinen calculated and interpreted the data and prepared the manuscript. All authors commented on the manuscript.

IV   The experimental design was planned by Markku Yli-Halla and Riikka Keskinen. The pot experiments and laboratory analyses were carried out by Riikka Keskinen together with technical assistance. Riikka Keskinen calculated and interpreted the data and prepared the manuscript. All authors commented on the manuscript.

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Abbreviations

AAS   atomic absorption spectrometer

APDC   ammonium pyrrolidine dithiocarbamate

AR   aqua regia

DW   dry weight

EDTA   ethylenedinitrotetraacetate

MIBK   methyl isobutyl ketone

NMD   nutritional muscular degeneration

SeCys   selenocysteine

SeMet   selenomethionine

SEP   sequential extraction procedure

Contents

1   Introduction

Since its discovery in 1817, selenium (Se) was considered merely a toxic element causing a severe poisoning called alkali disease or blind staggers in livestock (Moxon and Rhian 1943). The first evidence of its essentiality was presented as late as the 1950s when Schwarz and Foltz (1957) found that Se is an integral part of Factor 3, an agent protective against necrotic degenerations. Currently, dozens of selenocysteine (SeCys)- containing proteins, i.e. selenoproteins, have been identified (Lopez Heras et al. 2011). Their vital functions in antioxidant protection, energy metabolism, redox regulation and gene expression, providing protection against cancer, heart diseases, muscle disorders and weakening of the immune system, are being increasingly studied (Combs 2001, Lopez Heras et al. 2011). Dietary recommendations show that an intake of 40–80 µg Se d^{-1} is adequate for healthy adults, but it is argued that a higher level of 200–300 µg Se d^{-1} would be more appropriate for the maintenance of health (Combs 2001, Schrauzer and Surai 2009). Combs (2001) estimated that hundreds of millions of people around the world are deficient in Se.

In Finland, cases of nutritional muscular degeneration (NMD) in cattle are known to have existed since the early 1900s, but not until studies initiated by exceptionally high incidences of the disease in the 1950s was it shown that the disease is caused by deficiency of Se in the forage (Oksanen 1965, Oksanen and Sandholm 1970). The low concentrations of Se in crops were soon associated with low amounts and low solubility of Se in soil (Koljonen 1974, 1975, Sippola 1979). Concern about the effects of low Se on public health increased after a survey of mineral elements in Finnish foods revealed that the average diet supplied merely 30 µg Se d^{-1} (Varo and Koivistoinen 1980). Yläranta (1985) con-

ducted extensive studies on increasing the Se content of crops and reported that small additions of selenate (SeO_4^{2-})-Se were efficient at elevating the Se concentration of cereal and grass crops. In 1983, the Ministry of Agriculture and Forestry set a working group to draft a proposal for supplementing compound fertilizers with Se and to develop a follow-up plan for monitoring fertilization-induced changes in soil, crops, feeds and foods and in the level of Se intake (Ministry of Agriculture and Forestry 1984). In early July 1984, national Se fertilization practice was initiated by a decision of the Council of State.

After over 20 years of Se fertilization accompanied by versatile monitoring, unanswered questions remain on the environmental aspects of the practice. The unknown fate of the residual Se in soil and considerable variation in the Se concentrations of crops have puzzled researchers (Yläranta 1990, Eurola et al. 2004, 2008). However, complexity of the Se species, trace amounts of additions in relation to the heterogeneity of soils and various interactions contributing to plant uptake continue to challenge the studies. In this thesis, the behaviour of the added selenate in soil is examined by characterizing the Se reserves and their changes in soils, using several extraction methods. In addition, the efficiency of Se fertilization is discussed, considering the Se uptake rhythm, translocation within plants and the influence of components retaining Se in soil.

1.1   Selenium in soil

Se is very unevenly distributed in the soils of the earth's surface (Oldfield 2002). Usually, soil Se concentrations range between 0.1 and 2.0 mg kg^{-1} but in seleniferous areas concentrations exceeding 1000 mg kg^{-1} are not uncommon, whereas low-Se soils

contain less than 0.04 mg Se kg^{-1} (Girling 1984, Oldfield 2002). In general, soils developed from igneous rock are low in Se, while soils originating from sedimentary rocks have higher Se contents (Girling 1984, Haygarth 1994).

Studies by Koljonen (1973a-c, 1974, 1975) showed that the young soils of Finland reflect the low content of Se in the underlying Precambrian bedrock. The lowest total Se concentrations are found in coarse mineral soils containing predominantly quartz and feldspars and having a low adsorption capacity, whereas the highest concentrations are found in clays, even though in Finland they are rich in illite with limited adsorption capacity (Koljonen 1975). Surveys preceding Se fertilization reported average total Se concentrations for coarse mineral soils of < 0.010 (Koljonen 1974), 0.142 (Sippola 1979) and 0.172 mg kg^{-1} (Yläranta 1983a). For clay soils the corresponding values were 0.320 mg kg^{-1} (Koljonen 1974), 0.329 mg kg^{-1} (Sippola 1979) and 0.290 mg kg^{-1} (Yläranta 1983a). As for organic soils, Koljonen (1974) found an average Se concentration of 0.180 mg kg^{-1} in mull soils and 0.120 mg kg^{-1} in peat soils, while noting that peats formed on lake shores were strikingly richer in Se than those not having received Se from the water flow. In Sippola's data (1979), mull soils contained on average of 0.228 mg Se kg^{-1} and peat soils 0.093 mg Se kg^{-1}. Yläranta (1983a) reported a somewhat higher average for organogenic soils, 0.464 mg Se kg^{-1}, but his data included an exceptional soil having Se concentrations as high as 1.281 mg kg^{-1}. In a more recent survey, Mäkelä-Kurtto et al. (2007) found a range of total Se concentrations between 0.03 and 5.4 mg kg^{-1} in cultivated soils of Finland, the average being 0.29 mg Se kg^{-1}. Yläranta (1983a) showed that the contents of clay, organic carbon and aluminium oxides explained nearly 60% of the variation in total Se of mineral soils in Finland. Knowledge of the total soil Se content is, however, of limited value in biological assessments, since the figure embodies Se fractions differing decisively in their bioaccessibility.

1.1.1 Selenium species and their characteristics

Being a redox-sensitive element, Se occurs at four oxidation states in soils. Depending on the pH and electron activity (pe) of the environment the predominant species can be fully oxidized selenate (VI), selenite (SeO$_3^{2-}$) (IV), elemental Se (Se^0) (0) or reduced selenide (Se^{2-}) (-II) (Geering et al., 1968, Elrashidi et al. 1987). In addition, Se exists in association with organic matter, either complexed or built into the molecular structures (Gissel-Nielsen et al. 1984, Abrams and Burau 1989). The different Se species have very different characteristics in terms of mobility and bioavailability, wherefore knowledge of the speciation is essential in environmental and nutritional contexts.

Selenate is the anion of the strong selenic acid (H$_2$SeO$_4$), which occurs in solution either as biselenate (HSeO$_4^-$) or SeO$_4^{2-}$, the pK$_{a2}$ for the dissociation of the proton being 1.9. Selenate exists only under high-redox conditions and becomes the predominant species when pe + pH exceeds 15.0 (Elrashidi et al. 1987). In its behaviour, selenate resembles sulphate (SO$_4^{2-}$); it is weakly bound and thus relatively mobile in soil (e.g. Alemi et al. 1988). Its adsorption on oxide surfaces is influenced by the properties of the adsorbing surface, pH and ionic strength of the soil solution, but seems to occur mainly via an outer-sphere surface complexation mechanism, although inner-sphere complex formation can occur, especially at low pH (Peak and Sparks 2002, Peak 2006). In soil, the high solubility makes selenate the most available species to plants (Gissel-Nielsen and Bisbjerg 1970, Eich-Greatorex et al. 2007), but on the other hand exposes it to leaching (Dhillon et al. 2008). In the laboratory experiments of Yläranta (1982), se-

lenate leached readily through columns of Carex peat, but in mineral soils its transport was slow.

Selenite is the anion of the weak selenous acid (H_2SeO_3) and, depending on the soil pH, it occurs as H_2SeO_3, biselenite ($HSeO_3^-$) or SeO_3^{2-}. The pK_a for dissociation of the first proton is 2.64 and that for the second 8.4. Selenite is the major species at medium redox range between pe + pH 7.5 and 15 (Elrashidi et al. 1987). It has a high affinity for soil surfaces and its sorption onto minerals and oxides has been studied very extensively (e.g. Hingston et al. 1974, Rājan and Watkinson 1976, Parfitt 1978, Rajan 1979, Balistrieri and Chao 1987, Saha et al. 2004). The bonding occurs either via bidentate, binuclear or mononuclear inner-sphere complexation mechanisms or through outer-sphere complexation, depending on the environment and the structure of the surface (Peak et al. 2006 and references therein). Mobility of selenite is enhanced by alkaline pH, high total selenite concentration and competition with more strongly adsorbing anions such as phosphate (PO_4^{3-}), arsenate (AsO_4^{3-}) or bicarbonate (HCO_3^-) (Balistrieri and Chao 1987).

Elemental Se occurs in low-redox soils as red crystalline or red or black amorphous granules that are scarcely soluble (Elrashidi et al. 1987, Haygarth 1994). Selenide likewise precipitates in reduced soil, forming insoluble metal Se^{2-} compounds (Elrashidi et al. 1987). However, Se^{2-}-Se also exists in organo-Se compounds produced via biological assimilation (Sors et al. 2005). In addition to the Se biotically incorporated into the organic structures, organically bound Se may occur as metal-humic complexes, Se^0 colloid-colloid associations and adsorbed onto oxides or clays fixed within the organic matrix (Bruggeman et al. 2007, Coppin et al. 2009). The behaviour of the organically associated Se can be assumed to vary according to the characteristics of the carrier compound. Coppin et al. (2006) found that Se associated with humic substances is linked rather with the fulvic acid than the humic acid fraction. Overall, organically associated Se has thus far been poorly characterized, even though it comprises a marked portion of the soil Se reserves (e.g. Gustafsson and Johnsson 1992, Coppin et al. 2006, Christophersen et al. 2012).

In soil, all the above-mentioned Se species can coexist, due to heterogenic micro-environments and relatively slow transformation rates from one species to another (Zawislanski and Zavarin 1996, Darcheville et al. 2008). Changes in the oxidation state are predominantly biologically mediated via bacterial oxidation of the reduced species into selenate or, in contrast, assimilatory reduction of the oxidized forms into organo-selenide compounds by plants and microorganisms or dissimilatory reduction of selenate via selenite into elemental Se by Se-respiring bacteria (Kulp and Pratt 2004, Stolz et al. 2006). However, abiological transformations through oxidative weathering and suboxic reduction occurring in the presence of reactive surfaces are also involved in the cycling of Se in the environment (Myneni et al. 1997, Chen et al. 2009).

1.1.2 Extraction of soil selenium

The distinct differences in solubility of the various Se species have enabled development of numerous single (e.g. Sharmasarkar and Vance 1995, Hagarová et al. 2005) and sequential extraction procedures (SEPs) (e.g. Chao and Sanzolone 1989, Tokunaga et al. 1991, Séby et al. 1997, Martens and Suarez 1997a, Mao and Xing 1999, Wright et al. 2003) targeting specific operationally defined soil and sediment Se fractions. The most easily soluble forms, namely selenate and dissolved organically associated Se, are commonly extracted with water or a simple salt solution, based on anion exchange and mass action (Wright et al. 2003). Selenite adsorbed onto soil surfaces is often acquired through ligand exchange (Hingston et al. 1967) by replacing selenite with more strongly binding phosphate anions (Rājan

and Watkinson 1976). The adsorbed forms of Se may also be attacked more aggressively by dissolving the adsorbing oxide surfaces, e.g. with acid ammonium oxalate (Jackson et al. 1986) or concentrated HCl (Chao and Sanzolone 1989). Organically associated Se is difficult to separate accurately from the inorganic Se species, but in SEPs dilute NaOH targets this fraction rather specifically (Wright et al. 2003). In general, extractions of organically bound or inorganically associated reduced selenide-Se rely on oxidizing agents, such as H_2O_2 (Zhang and Frankenberger 2003), $K_2S_2O_8$ (Zhang et al. 1999) or NaOCl (Zhang and Moore 1996). Elemental Se can be solubilized with CS_2 (Yamada et al. 1999) or by forming soluble selenosulphate in a reaction between Se^0 and Na_2SO_3 (Velinsky and Cutter 1990). When the total Se content of the soil is sought, a mixture of strong acids, such as $HCl-HNO_3$ or HNO_3-HClO_4-HF is used in dissolving the entire soil matrix (Sharmasarkar and Vance 1995).

The appropriate method for exploring soil Se is governed by the objective of the analysis, as well as the type and nature of the soil under study (Dhillon et al. 2005, Yli-Halla 2005). In Finland, hot-water extraction (Yläranta 1982) has been chosen for monitoring the fertilization-induced changes in soil Se status (Eurola et al. 2008). The method lacks a sound theoretical basis, but its boiling treatment likely solubilizes some weakly adsorbed selenite and organic Se compounds, in addition to the soluble selenate pool (Sharmasarkar and Vance 1997). Roughly 4% of the total Se reserves in soils of Finland are extractable by hot water (Yläranta 1983a). Accordingly, hot-water extraction serves in examining changes in the immediately biologically available Se fraction. However, the method does not extract the weakly soluble but the potentially available pool, in which Se assumably accumulates under acidic and semireducing conditions. Powerful acid digestions, in contrast, recover the majority of the soil Se, but substantial variation in the Se content

of the soil parent material easily overpowers small changes in the soil Se status (Yli-Halla 2005). Therefore, intermediate extractions targeting the accumulating Se pool would be ideal in monitoring the fertilization-induced changes in soil Se.

1.2   Selenium uptake by plants

In plants, unlike in humans and animals, essential functions have not been demonstrated for selenoproteins, wherefore Se is not considered a plant nutrient (Terry et al. 2000). However, Se-induced beneficial effects initiated by increased antioxidative capacity have been recorded, especially in stressed plants (Hartikainen et al. 2000, Xue et al. 2001, Seppänen et al. 2003, Lyons et al. 2009). Whether plants themselves profit from Se or not, they serve as an important link in transferring Se from soil into the food chain (Girling 1984, Combs 2001, Hawkesford and Zhao 2007).

Plant Se uptake occurs via several mechanisms, depending on the species absorbed. Selenate is taken up through high-affinity sulphate transporters (Terry et al. 2000, Sors et al. 2005), whereas selenite likely enters the plant via a phosphate transport pathway (Li et al. 2008). Due to the entrances shared, there is competition in the uptake between sulphate and selenate and phosphate and selenite (Hopper and Parker 1999). The uptake of organic Se compounds is not well known, but the permeases specific for S-containing amino acids may mediate the uptake of selenoamino acids as well (Abrams et al. 1990 and references therein).

The relative uptake rate of Se species has been addressed, both in hydroponic (e.g. Williams and Mayland 1992, Zayed et al. 1998) and soil studies (e.g. Gissel-Nielsen and Bisbjerg 1970, Eich-Greatorex et al. 2007). In the soil matrix, the dissimilar solubility of the species governs their availability (see section 1.1.1), but in solution culture the differences between the species seem less

pronounced. Li et al. (2008) found soluble selenite as available to plants as selenate, although they quote several studies showing inconsistent results, likely due to different experimental conditions. The organic forms selenomethionine (SeMet) and SeCys seem at least as available to plants as selenite when in solution (Williams and Mayland 1992, Zayed et al. 1998). All plant species likely access the same labile soil Se fraction, but some plants tend to accumulate Se, whereas others are able to discriminate against it (Mayland 1994, Terry et al. 2000, Goodson et al. 2003). Accumulator plants can contain several grams of Se kg^{-1} dry weight (DW), whereas the Se concentration of nonaccumulators rarely exceeds 0.1 g kg^{-1}, even when grown on seleniferous soils (Terry et al. 2000).

Within plants, selenate and selenite are readily metabolized into various organic Se compounds via the S-assimilation pathway (Terry et al. 2000, Sors et al. 2005). The oxidized species are first reduced into selenide and thereafter assimilated into SeCys and further to SeMet. These selenoamino acids may then be nonspecifically incorporated into proteins in place of Cys and Met, which can lead to alterations in protein structure and thus weakening of its functions. Plants accumulating Se can exclude this substitution, e.g. by metabolizing Se into nonprotein selenoamino acids, such as Se-methylselenocysteine, selenocystathionine, Se-methylselenomethionine and γ-glutamyl-Se-methylselenocysteine (Brown and Shrift 1981, Whanger 2002, Sors et al. 2005). In Se accumulators, these nonprotein selenocompounds can comprise the major portion of the total plant Se, whereas SeMet is the predominant form of Se in cereal grains, soybeans and grassland legumes (Stadlober et al. 2001, Whanger 2002). By methylation, plants can convert SeMet into volatile dimethylselenide, the main contributor to atmospheric distribution of Se (Chasteen 1998, Sors et al. 2005). Of the inorganic species, selenate predominates over selenite within

plants since reduction of selenate into selenite limits the biosynthesis rate of organic Se compounds (Ellis and Salt 2003).

The transport, distribution and speciation of Se within plants is ultimately governed by the plant species, its developmental stage and the species of Se absorbed (Bisbjerg and Gissel-Nielsen 1969, Williams and Mayland 1992, Terry et al. 2000, Whanger 2002). Selenate, which is highly mobile in plants, is transported into leaves to be reduced in the chloroplasts, whereas selenite can be reduced into selenide nonenzymatically in the roots, where it tends to accumulate as does Se absorbed in organic form (Terry et al. 2000).

1.3   Selenium fertilization

In vast areas of the world, food systems are too low in Se to support the maximal expression of the SeCys enzymes (Gissel-Nielsen et al. 1984, Combs 2001, Rayman 2002). Accordingly, increasing the intake of Se would have beneficial effects on health in most populations, even though actual Se deficiency diseases are rare. The use of Se medication in the form of high-Se-containing supplements (Rayman 2004), fortifying foods, such as table salt (Yu et al. 1997) or bread (Rayman 2002) with Se or supplementing animal feeds, thus increasing the level of Se in foods of animal origin (Aro et al. 1998), are possible remedies for Se undernourishment. However, probably the best way to extend the measure throughout the population in a safe way is to introduce Se generally into the food chain through fertilization (Broadley et al. 2006).

Feasible application techniques and sources of Se for field treatment of crops have been studied since the 1960s (summarized by Gissel-Nielsen et al. 1984, Gissel-Nielsen 1998). Most studies have focused on various selenate and selenite salts applied to soil as such or incorporated into compound fertilizers. In general, selenates have

proved to be more efficient than selenites in increasing plant Se content (e.g. Bisbjerg and Gissel-Nielsen 1969, Gissel-Nielsen and Bisbjerg 1970, Yläranta 1983a,b). Additions of elemental Se, thought to function as a slow-releasing fertilizer, and organic forms of Se have shown minor influence on the Se concentration of harvested crops (Gissel-Nielsen and Bisbjerg 1970, Eich-Greatorex et al. 2007). Fertilization via foliar spraying and coating of seeds with Se has also been explored. In seed treatment, Se amounts equal to those in direct soil application are needed to attain the desired Se concentration of crops, whereas with appropriately timed foliar spraying including the use of a surfactant, the plant Se concentration can be increased with a small Se supply (Yläranta 1984a–c, Gissel-Nielsen 1998). However, Yläranta (1985) concluded his extensive studies on Se fertilization by specifying soil application of Se-supplemented nitrogen-phosporus-potassium (NPK) fertilizer to be the most reliable and cost-effective method of increasing the Se content of all crops.

Encouraging results of Se fertilization trials (Gissel-Nielsen and Bisbjerg 1970, Yläranta 1985) persuaded the Finnish authorities to launch a national Se fertilization programme to improve the Se nutrition of the entire population (Ministry of Agriculture and Forestry 1984). The aim was to increase the Se concentrations of crops from the extremely low level of 0.01 mg Se kg^{-1} (DW) (Oksanen and Sandholm 1970, Sippola 1979, Yläranta 1990) up to 0.1 mg Se kg^{-1}, which is defined as adequate for animals and humans (Gissel-Nielsen et al. 1984). The first fertilizers supplemented with sodium selenate (Na$_2$SeO$_4$) were available for farmers for the third cut of grass in 1984. Se fertilization has been in general use since the 1985 growing season. At first, only compound fertilizers (NPK and NK) were supplemented with Se, but since 1996 N fertilizers have also been amended.

To control the safety of the Se fertilization practice, a group of experts has regularly monitored the average daily Se intake level and concentrations of Se in foods, feeds and human serum according to specific sampling policies (Ministry of Agriculture and Forestry 1985, 1990a). Based on follow-up data, the level of Se supplementation in fertilizers has been adjusted as follows:

1984 – 1990
6 mg Se kg^{-1} in fertilizers intended for grasslands
16 mg Se kg^{-1} in fertilizers intended for cereal crops

1990 – 1998
6 mg Se kg^{-1} in all fertilizers

1998 – 2007
10 mg Se kg^{-1} in all fertilizers

2007 onwards
15 mg Se kg^{-1} in all fertilizers, with the exception that those used in complementing manure may contain 25 mg Se kg^{-1}

The two initial supplementation levels were based on the studies of Yläranta (1985), showing the Se concentration of grasses increasing more efficiently than that of cereals. Doubts that Se fertilization causes increased algal growth in waterways together with detection of some high Se concentrations of crops led to the adoption of the lower supplementation level for all crops (Eurola et al. 2003). However, decreases in the Se intake level of people and livestock resulting mainly from a trend toward decrease in the amount of fertilizers used necessitated subsequent increases in fertilizer Se concentrations (Eurola et al. 2011). On a hectare basis, the annual Se addition is now typically around 7.5 g.

By means of Se fertilization, the average daily Se intake in Finland has increased from as low as 25–30 µg Se d^{-1} (Koivis-

toinen 1980, Varo and Koivistoinen 1981, Mutanen and Koivistoinen 1983, Eurola et al. 2008) to around 80 µg Se d^{-1} (Eurola et al. 2011). Nutritionally, the fertilization practice has thus proved successful. Yet, concern over health and environmental risks related to relatively small differences between Se essentiality and toxicity has thus far restricted implementation of the measure to Finland and low-Se areas of China, New Zealand and the UK, where Se fertilization is used in preventing Se deficiency of grazing livestock (Combs 2001, Broadley et al. 2006). Aquatic ecosystems are especially sensitive to elevated Se levels, wherefore leaching of fertilizer Se poses a particular threat to waterways (Maier et al. 1998). The efficiency of fertilizer Se uptake by plants is usually low, between 5% and 20% of the annual selenate application (Yläranta 1985), ranging, however, from below 1% to nearly 50% (Yläranta 1985, Tveitnes et al. 1996, Eich-Greatorex et al. 2007). In Finland, residual Se is assumed to be fixed in the soil (Yläranta 1985), in which case the computational fertilization-induced increase in the Se concentration of the plough layer of Finnish agricultural soils would be around 20% (Eurola et al. 2003). However, hot-water-extractable Se concentrations have not increased from the prefertilization level of 0.01 mg Se l^{-1} soil (Eurola et al. 2008). In acid digestion, deviation in the inherent Se concentration of the soil parent material overwhelms the small fertilization-induced changes (Yli-Halla 2005).

1.4 Aims of the present study

Due to the narrow margin of safe Se concentrations, impacts of Se fertilization on the Se content of foods, feeds and daily Se intake level have been regularly monitored in Finland since commencement of the supplementation practice in 1985 (Ministry of Agriculture and Forestry 1986a,b, 1987, 1988, 1989, 1990b, 1994, Eurola and Hietaniemi 2000, Eurola et al. 2003, 2008, 2011). Follow-up of the effects of added Se on soils and the environment has been less thorough and comprehensive understanding of the cycling of the added selenate in the soil-plant system is lacking. About 90% of the annually applied Se is assumed to be immobilized in the soil (Eurola et al. 2003), but the fate of this residual Se has not been verified by soil analyses.

The purpose of this thesis was to increase the level of understanding of the behaviour of fertilizer Se in the acidic soils of Finland. The specific aims were to:

Find suitable extraction methods of sound basis for both detailed examination of the Se reserves in soil and for routine analyses of the Se pool likely available to plants (I,III)

Characterize the distribution of soil Se fractions in Se-fertilized mineral and organic field soils in different parts of Finland and assess long-term changes in these fractions (II)

Examine plant availability of added Se in mineral and organic soils with emphasis on Se retention in soil (III, IV)

Gather information on the total uptake, rhythm of uptake and distribution of Se within crops (III, IV)

2 Material and methods

In this thesis, the results of two laboratory studies (I and II) and four pot experiments (III and IV) are presented. The laboratory studies consist of extractions of soil Se. First, various extractants were tested in two soils (I) and, thereafter, Se reserves of nine soil pairs from different parts of Finland were fractionated (II). In the first pot experiment, hereafter named EXP1, the uptake and translocation of Se in spring wheat (*Triticum aestivum* L. cv. Manu) was investigated concomitantly with changes in soil Se fractions (III). The second pot experiment, EXP2, aimed to clarify the Se uptake efficiency of Italian ryegrass (*Lolium multiflorum* Lam. cv. Meroa) and the congruence between plant Se uptake and soil Se extractions (III). The third and fourth pot experiments, EXP3 and EXP4, were conducted to determine the uptake of added Se by plants and its retention in peat soils, which are often low in adsorbing oxide components (IV).

2.1 Experimental designs

2.1.1 Soils used in the studies

The soils used in the experiments were mostly collected from fields in different parts of Finland (Figure 1, Table 1). Various Se extraction methods were compared in two mineral soils, sand and silty clay, collected from Viikki Research Farm, Helsinki. The same two soils were used in EXP2 and the sand soil also in EXP1. The sand was included as a control in EXP3, in which the uptake and fate of added Se was studied in two peat soils from Jaakkola Farm (Hausjärvi) and on a commercial sphagnum (*Sphagnum* L.) peat. EXP4 was conducted solely with a commercial sphagnum peat substrate manipulated chemically with iron (Fe). For characterizing the soil Se reserves, nine sample pairs from research stations of MTT Agrifood

Research Finland (Jokioinen, Maaninka, Mietoinen, Pälkäne, Rovaniemi, Ruukki and Ylistaro) were fractionated.

The field soils, except the peat soils of Hausjärvi, were collected from the plough layer, approximately 0–20 cm from the soil surface, using an auger or spade. The Hausjärvi topsoil peat was composed of the 30-cm surface layer, whereas the subsoil peat was collected from depths of 50–80 cm. The sand and silty clay soil were taken in May 2006 for soil extraction tests and for use in EXP2. For EXP1 and EXP3, peat soils and sand from the same location as in 2006 for EXP1 were collected in spring 2007. The first set of the paired MTT research station samples were taken in autumn 1992 (Urvas 1995) and
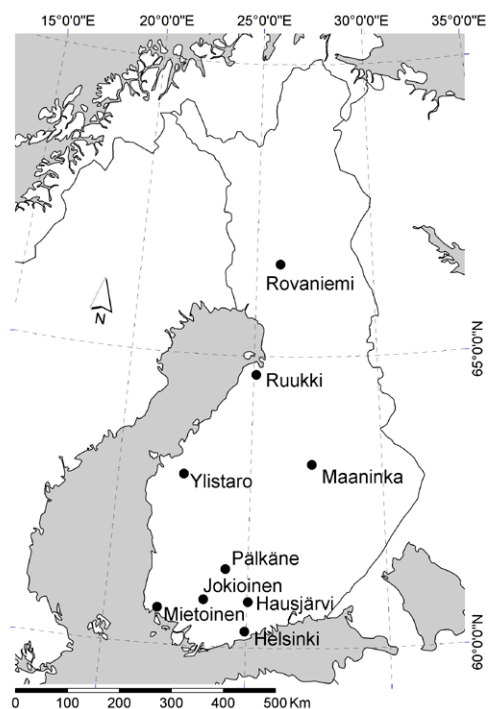
Figure 1. Sampling locations for the soils used in the study.

Table 1. Description of the experimental soils. For more detailed properties, see the appropriate articles.

Location	Identification[a]	Type[b]	pH[c]	Clay < 0.002 mm (%)[d]	C_{org} (%)[e]	Article
Helsinki	Sand	Sand soil from an uphill field	5.9	3	2.2	I,III,IV
Helsinki	Silty clay	Field soil composed of brackish water sediment of the former Baltic Sea	5.4	45	5.1	I, III
Jokioinen	JKA3	Clay soil	5.2	71	3.5	II
Jokioinen	JKA20	Organic field soil	4.7	72	17	II
Maaninka	PSA10	Organic field soil	4.9	24	11	II
Mietoinen	LOU5	Clay loam soil	5.3	39	2.2	II
Pälkäne	HÄM7	Sandy loam soil	5.0	10	2.7	II
Pälkäne	HÄM10	Organic field soil	4.6	40	22	II
Rovaniemi	LAP3	Silt loam soil	5.5	10	3.4	II
Ruukki	PPO2	Organic field soil	4.1	5	28	II
Ylistaro	EPO2	Silty clay loam soil	4.0	29	7.4	II
Hausjärvi	Topsoil peat	Peat from the surface layer of a moderately well humified field	5.2	na	30	IV
Hausjärvi	Subsoil peat	Raw peat from the subsoil layer of a peat field	4.4	na	53	IV
	Sphagnum peat	Commercial light-coloured sphagnum peat, Kekkilä A0	3.8	na	na	IV

na = not analysed
[a]Refers to the nomenclature used in the individual articles.
[b]The mineral soils are classified according to the USDA textural triangle.
[c]Measured in 0.01-M $CaCl_2$.
[d]The clay percentages are calculated from the inorganic matter of the soil.
[e]Analysed by dry combustion (LECO˙ CHN 900 analyser) except for Hausjärvi soils in which the percentage refers to loss of weight on ignition/1.724.

the same locations were resampled in autumn 2004 (Yli-Halla 2005). These soils were stored air-dried in cardboard boxes until taken to the fractionation analyses in summer 2009.

2.1.2 Examination of selenium in field soil samples

After a literature search, the performance of seven single extractants and two SEPs were chosen for testing on two dissimilar mineral soils (sand and silty clay). Of single extractants, aqua regia (AR), acid ammonium oxalate, hot water and phosphate buffer in four different concentrations and pH combinations were compared. The SEPs included were a four-step method developed by Chang and Jackson (1957) for soil P and a five-step procedure developed for Se by Zhang and Moore (1996)

and modified by Wright et al. (2003). For details of the methods, see section 2.2.

The SEP of Wright et al. (2003) was selected for further use in characterizing the Se reserves of field soils. Paired samples from research stations of MTT Agrifood Research Finland by Yli-Halla (2005) also enabled investigation of changes in the soil Se status between 1992 and 2004. Nine pairs of samples were carefully selected from the entire set of 48 locations to include both organic and mineral soils. In addition, the crop rotation and computational Se balances were considered. Grassland systems prone to uneven distribution of Se in soil due to topdressing of fertilizers and sparse tillage, and fields with Se balances below 30 g Se ha^{-1} were avoided. A further criterion was the accuracy of the sampling location, which was assessed in advance, based on volume weights. A considerable change in this variable reflecting the organic matter content of the soil would have been unlikely during the study period, wherefore an inequality in volume weight within the sample pair was assumed to signify differing sampling locations. The uniformity of the chosen pairs was confirmed with analyses of the organic carbon content and particle-size distribution of the inorganic matter. In one of the soils chosen (JKA20), these characteristics deviated considerably between the 1992 and 2004 samples. Therefore, this soil was omitted from pairwise comparisons.

2.1.3 Pot experiments

The batches of field soils collected for the pot experiments were first passed through a 10-mm sieve and mixed thoroughly. In EXP2, the residual effect of Se fertilization was exploited by reusing soils from a three-week pot experiment conducted with oilseed rape the previous year (results not shown). The soils were packed in plastic bags, each pot separately, and stored overwinter in a cold greenhouse.

These preprepared EXP2 soils and commercial sphagnum peat of EXP4 were not sieved, but mixed. After homogenization of the soils, plastic pots of 1.5 l in EXP1, 2 l in EXP2 and 3.5 l in EXP3 and EXP4 were filled with soil by weighing (Table 2). In EXP4, the Se sorption capacity of the peat was manipulated by iron hydroxide enrichment. This was done by mixing $FeCl_3$ solution and solid $Ca(OH)_2$ into the soil in the pots. Control pots (Fe0) received only $Ca(OH)_2$, whereas the moderately enriched pots (Fe1) received 2 g Fe kg^{-1} peat DW and the amply enriched pots (Fe2) 20 g Fe kg^{-1} peat DW.

In all experiments, the pots were individually fertilized by the same method (Table 3). In EXP2, however, no S was added, due to high S addition (200 mg l^{-1} soil) in the previous year's experiment. The macronutrients were applied separately as solid compounds and micronutrients dissolved in a single combined solution, after which all nutrients were hand-mixed evenly into the soil volume. The peat soils in EXP3 were limed with $Ca(OH)_2$ to elevate their pH to 5.5. In EXP4, enough $Ca(OH)_2$ was added with the $FeCl_3$ to increase the peat pH to 4.7 in the Fe0 and Fe1 treatments and to 4.5 in the Fe2 treatment. Se fertilization was conducted simultaneously with application of the nutrients according to Table 2. Se was applied as an Na_2SeO_4 solution. In EXP4, Se addition of 1 mg l^{-1} soil was applied also as a sodium selenite (Na_2SeO_3) solution.

In EXP1 and EXP3, spring wheat and in EXP2 and EXP4 Italian ryegrass were sown in the pots (Table 2). The EXP4 included plant-free pots for observation of the behaviour of Se in soil without the influence of plants. After sowing, the pots were completely randomized onto greenhouse tables in EXP1, EXP2 and EXP4. In EXP1, the border effect was avoided by surrounding the experimental pots with nurse pots, whereas in EXP2 and EXP4 the pots were rearranged periodically.

Table 2. Summary of the four pot experiments EXP1–EXP4 conducted in the study.

	EXP1	EXP2	EXP3	EXP4
Objectives	Defining the uptake rhythm and translocation of Se within wheat, and the short-term changes in soil Se fractions.	Comparing the efficiency of ryegrass and soil extractants in the withdrawal of Se and defining the distribution of Se in ryegrass.	Examining the efficiency of selenate fertilization in soils increasing in the content of organic matter.	Clarifying the effects of organic and inorganic components on the retention of Se in soil.
Pot volume (l)	1.5	2.0	3.5	3.5
Soil; g DW per pot	Sand; 1510	Sand; 2484 Silty clay; 1545	Sand; 3147 Topsoil peat; 920 Subsoil peat; 393 Sphagnum peat; 262	Sphagnum peat; 356 Fe0; Fe1; Fe2 enrichments
Se treatments (mg Se l^{-1} soil)	0.1	Residuals of 0; 0.0025; 0.005	0; 0.0025; 0.005; 0.1; 1	0; 0.005; 1
Replicates	47 pots in total	6	5	4 planted; 3 unplanted
Design	Completely randomized	Completely randomized	Randomized blocks	Completely randomized
Plant	Spring wheat (*Triticum aestivum* L. cv. Manu); 10 plants per pot	Italian ryegrass (*Lolium multiflorum* Lam. cv. Meroa); 1.3 g seeds per pot	Spring wheat (*Triticum aestivum* L. cv. Manu); 20 plants per pot	Italian ryegrass (*Lolium multiflorum* Lam. cv. Meroa); 2 g seeds per pot, plantless pots included
Sampling	Soil and plant samples after 1, 2, 3, 4, 6, 8 and 10 weeks of growth. Plant samples separated into leaves, stems, spikes, (grains) and roots.	Soil samples at the beginning of the experiment. Ryegrass leaf cuttings 4 and 6 weeks after sowing and roots after the second cut.	Soil and plant samples after ripening of the crop (10 weeks of growth). Plant samples separated into leaves, stems, grains and chaff.	Ryegrass leaf cuttings 3 and 6 weeks after sowing. Soil samples after the second cut.

EXP3 was arranged in a complete randomized block design and bordered with nurse pots.

The greenhouse settings were identical in all experiments. The temperature was set to 20 °C by day and 16 °C by night and

Table 3. Amounts of nutrients added (mg l^{-1} soil) in pot experiments EXP1–EXP4

Nutrient	Compound	Amount of nutrient added (mg l^{-1} soil)
N	NH_4NO_3, KNO_3	150
P	$Ca(H_2PO_4)_2 \times H_2O$	50
K	KNO_3	125
Mg	$MgCl_2 \times 6H_2O$	25
S[a]	$CaSO_4 \times 2H_2O$	40
Mo	$Na_2MoO_4 \times H_2O$	1
Cu	$CuCl_2 \times 2H_2O$	2
Zn	$ZnCl_2$	5
Fe[b]	$FeCl_3 \times 6H_2O$	5
Mn	$MnCl_2 \times 4H_2O$	5
B	H_3BO_3	1
Ca[c]	$Ca(H_2PO_4)_2 \times H_2O$, $CaSO_4 \times 2H_2O$, $Ca(OH)_2$	30–1000

[a]No S was added in EXP2.
[b]In EXP4, 0.2 g and 2 g Fe l^{-1} soil as $FeCl_3$ were introduced in Fe enrichment treatments.
[c]Within $Ca(H_2PO_4)_2 \times H_2O$ 32 mg Ca l^{-1}soil, and within $CaSO_4 \times 2H_2O$ 50 mg Ca l^{-1} soil were added. The peat soils of EXP3 and 4 received an additional 250 – 1000 mg Ca l^{-1} soil as $Ca(OH)_2$ to elevate the soil pH.

the relative humidity to 40%. Natural daylight was supplemented with high-pressure sodium lamps at 300 W m^{-2} from 6 a.m. to 10 p.m. to maintain the photosynthetic photon flux density in the range of 250–300 µmol m^{-2} s^{-1}. Excessive light intensity (> 500 W m^{-2}) was prevented by closing shading curtains. Deionized water (electrical conductivity < 1 µS cm^{-1}) was added as needed to keep the pots moist, yet avoiding leaching.

In EXP1, samples were collected after 1, 2, 3, 4, 6, 8 and 10 weeks of growth. Sampling was conducted by random selection of seven pots (five only in the first sampling), which were harvested after specifying the growth stage of wheat on Zadoks' scale (Zadoks et al. 1974). The aboveground plant mass was separated into leaves, stems and spikes, and in the last sampling of the ripend crop also into grains. From four pots (three in the first

sampling), roots were elutriated, using a root washer (Model 13000 Gillison's Variety Fabrication Inc.). In EXP3, wheat was harvested at maturity, separating leaves, stems and spikes that were further assorted into grains and chaff. The Italian ryegrass in EXP2 and EXP4 was cut twice. In EXP2, ryegrass was harvested 4 and 6 weeks after sowing, and in EXP4 3 and 6 weeks after sowing. In EXP2, the roots were elutriated after the second cutting from three replicates of two treatments (0 and 0.005 mg Se l^{-1}) of both soils with a root washer (Gillison).

All plant material, except the harvest-ripe wheat, was frozen in liquid nitrogen immediately after harvesting. The ripe wheat was dried directly at 60 °C. The frozen samples were stored at -20 °C until lyophilized. The dry mass of each plant sample was weighed and the samples milled (Cyclotec Sample Mill 1093, Kika Labortech-

nik A10). The dried samples were stored at room temperature until analysed for total Se concentration (see section 2.3.2).

In EXP1, the soil samples were collected at every sampling from the replicates not used for root elutriation and subjected directly to analyses. In EXP2, the soils were sampled at the beginning of the experiment from the batches remaining after filling of the pots. In EXP3 and EXP4, the soil samples were taken after the final harvest. For the soil extractions and Se analyses, see sections 2.2 and 2.3.1.

2.2 Soil selenium extractions

In comparing the extraction methods for soil Se (I), single extractants of varying aggressiveness (Table 4) and two SEPs frac-

tionating soil Se into separate operationally defined chemical pools (Table 5) were included. The strong acids in AR disintegrated the soil matrix nearly completely, thus producing semitotal Se concentrations. Acid ammonium oxalate extraction performed in the dark dissolves noncrystalline metal oxides (Jackson et al. 1986) and thereby releases Se, which is retained by poorly crystalline Al and Fe oxyhydroxides. Phosphate buffers recover ligand-exchangeable Se, since phosphate replaces selenite, due to its higher sorption strength (Rājan and Watkinson 1976). Hot-water extraction targets the easily soluble Se pool. The successive extraction steps of SEP1 (Chang and Jackson 1957) separated soil Se into four fractions: 1) the most easily soluble Se was extracted with a simple salt solution (NH_4Cl), removing Se in soil water and Se bound unspecifically by

Table 4. Summary of the single extraction methods used in the study.

Extractant	Description	Reference	Article
Aqua regia	A suspension of 3 g soil and 43 ml of a mixture of HCl and HNO_3 was allowed to stand at room temperature for 16 h, after which it was boiled under reflux for 2 h. The cooled extractant was filtered (Whatman Grade 589/2, White ribbon) into a 100-ml volumetric flask, which was filled to the mark with 0.5 M HNO_3.	ISO 11466	I, II
Acid ammonium oxalate	A suspension of 2.5 g soil and 50 ml of oxalate solution (0.18 M $(NH_4)_2(COO)_2$ + 0.1 M $(COOH)_2$, pH 3.3) was shaken in a reciprocating shaker for 2 h in the dark. Thereafter, the suspension was centrifuged (15 min, 3000 x g) and the supernatant filtered (Whatman Grade 589/3, Blue ribbon).		I
Phosphate buffer	A suspension of 2–10 g soil and 50 ml of either 0.1 or 1 M phosphate buffer solution (K_2HPO_4 -KH_2PO_4) at pH 4.4 or 7.0 was shaken in a reciprocating shaker for 4 h, then centrifuged (15 min, 3000 x g) and the supernatant filtered (Whatman Grade 589, Black ribbon).	e.g. Rājan and Watkinson (1976)	I, III
Hot water	A suspension of 25 g soil and 100 ml of milli-Q water was boiled for 30 min under reflux, then centrifuged (15 min, 3000 x g) and the supernatant filtered (Whatman Grade 589, Black ribbon).	Yläranta (1982)	I

Table 5. Summary of the sequential extraction procedures (SEPs) used in the study.

Extractant	Description	Reference	Article
SEP1	Step 1) A suspension of 10 g soil and 50 ml of 1-M NH_4Cl was shaken in a reciprocating shaker for 30 min, then centrifuged (15 min, 3000 x g) and the supernatant filtered (Whatman Grade 589, Black ribbon) and collected for analyses. Step 2) 50 ml of 0.5-M NH_4F, pH 8.5 was added to the residual soil and the suspension shaken in a reciprocating shaker for 1 h and thereafter centrifuged as before. The residual soil was rinsed with 25 ml of saturated NaCl solution, which was discarded. Step 3) 50 ml of 0.1-M NaOH was added to the rinsed soil and the suspension shaken in a reciprocating shaker overnight and then centrifuged and rinsed as before. Step 4) 50ml of 0.25-M H_2SO_4 was added to the residual soil, shaken in a reciprocating shaker for 1 h and thereafter centrifuged as before.	Chang and Jackson (1957)	I, III
SEP2	Step 1) A suspension of 10 g soil and 50 ml of 0.25 M-KCl was shaken in a reciprocating shaker for 2 h, and then centrifuged (15 min, 3000 x g). The supernatant was filtered (Whatman Grade 589, Black ribbon) and collected for analyses. Step 2) 50 ml of 0.1-M K_2HPO_4, pH 8.0 was added to the residual soil and the suspension shaken in a reciprocating shaker for 2 h, then centrifuged as before. The supernatant was collected and the extraction repeated. Thereafter, the residual soil was rinsed with 10 ml of 0.1-M KCl, which was combined with the extracts. Step 3) 50 ml of 0.1-M NaOH was added to the residual soil and the suspension shaken in a reciprocating shaker for 4 h, then centrifuged and rinsed as before. Step 4) 50 ml of 0.25 M-Na_2SO_3, pH 7.0, was added to the residual soil and the suspension disrupted with a 2-min sonication, then held in an ultrasonic bath for 4 h. The samples were centrifuged as before and thereafter the residual soil was rinsed twice with 20 ml of 0.25-M Na_2SO_3 and once with KCl as before. Step 5) 40 ml of 5% NaOCl, pH 9.5, was added to the residual soil and the suspension placed in a 90 ºC water bath for 30 min. The samples were centrifuged as before and thereafter the extraction was repeated.	Wright et al. (2003)	I, II

anion exchange and mass action, 2) Se associated with Al oxides was dissolved by NH_4F, assuming that fluoride complexes the Al selectively (Turner and Rice 1952), 3) Se bound by Fe oxides was obtained by NaOH via ligand exchange (Hingston et al. 1967) and 4) Se bound in acid-soluble form was dissolved with H_2SO_4. In SEP2 (Wright et al. 2003), five pools of soil Se were segregated: 1) salt-soluble Se

was recovered by KCl, 2) adsorbed Se was displaced with a phosphate buffer, 3) organically associated Se was extracted with NaOH, 4) elemental Se was released by oxidation with Na_2SO_3 and 5) recalcitrant forms of organic Se and metal selenide compounds were oxidized with NaOCl.

The SEP2 procedure was also used in fractionating the Se reserves of field soil samples (II). In EXP1, the hypothesized reduction of the fertilizer selenate to selenite and subsequent adsorption onto soil surfaces was followed closely throughout the growing period. The first three steps of the SEP1 shown to remove selenite added to soils very efficiently (Nakamaru et al. 2005) and a single 4-h phosphate buffer extraction (1 M KH_2PO_4/K_2HPO_4, pH 7.0) were carried out at 1-week intervals during the first month after sowing and thereafter at 2-week intervals until the wheat harvest. For the soils in EXP2, EXP3 and EXP4, salt solution extraction (step 1 of SEP1) and a phosphate buffer extraction (step 2 of SEP2 as one continuous 4-h extraction) were combined in a two-step sequential procedure. In EXP4, the concentration of the phosphate buffer was increased to 1 M. To standardize contrasting samples to correspond to the same soil volume, aliquots of 2 g sphagnum peat, 7 g humified peat and raw peat, and 10 g sand were weighed for the analyses.

Prior to the extractions, the soil samples were air-dried at room temperature and passed through a 2-mm sieve while removing and discarding visible plant roots. The only exception was EXP1, in which the soils were not dried but sieved and taken for analyses directly after sampling. The AR and hot-water extractions and step 5 of SEP2 were conducted in boiling flasks. All other extractions were carried out in plastic 100-ml tubes. The extractions were done in three replicates in all experiments, except EXP4, in which four replicates were analysed.

2.3   Analyses of selenium

The total Se concentration of the soil extracts and plant samples was analysed by a graphite furnace atomic absorption spectrometer (AAS) (PerkinElmer Zeeman 5100), using an electrothermal AAS method for food samples (Kumpulainen et al. 1983, Ekholm 1996). In the method, Se was concentrated in an organic solvent, methyl isobutyl ketone (MIBK) as an ammonium pyrrolidine dithiocarbamate (APDC) chelate. Selenite is the only chelateable species, wherefore an oxidation-reduction procedure was included for transforming all Se in the sample into an analysable form. In EXP1, an attempt to separate selenite from the other Se species in the extracts was made by also conducting the analysis without the oxidation-reduction step.

2.3.1 Soil extracts

Aliquots of 2–15 ml of the soil extracts were taken into the Se analyses. First, all Se in the samples was oxidized to selenate by adding 10 ml of concentrated HNO_3 and digesting the solutions for 45 min at 120 ºC. Thereafter, 10 ml of 4-M HCl were added and the digestion continued for 20 min at 130 °C to reduce the selenate to selenite. Subsequently, the samples were allowed to cool at room temperature, after which the pH of the solutions was adjusted, according to a bromphenol blue indicator, to a range between 4 and 5 with NH_3 or HNO_3. The pH was buffered to this range for optimal selenite chelation with 5 ml of 0.1-M ammonium citrate solution. To prevent competition for the chelating agent between Fe and selenite, Fe was first chelated by a 5-ml addition of 5% ethylenedinitrotetraacetate (EDTA), after which the selenite was bound with a 2-ml addition of 2% APDC. Next, the APDC-chelated Se was extracted to 2.5–10 ml of MIBK. After the MIBK addition, the samples were first shaken in a reciprocating shaker for 5 min and thereafter allowed to stand for 20 min to let the phas-

es separate. A sample from the upper-layer organic phase was then collected for analyses. For instrumental parameters, see I.

2.3.2 Plant samples

The dried and milled plant samples were further dried at 70 °C overnight prior to the Se analyses. After removal of the adsorbed moisture, aliquots of 0.1–1.0 g of each sample were weighed into wet-digestion tubes. Next, 10 ml of an acid mixture (HNO_3-$HClO_4$-H_2SO_4 in a ratio of 2.5:1.5:1) were added, after which the samples were allowed to stand at room temperature overnight. The acid digestion was completed according to a five-step temperature programme: 1) 30 min at 70 °C, 2) 3 h at 120 °C, 3) 30 min at 160 °C, 4) 30 min at 190 °C and 5) 5 h at 220 °C. Thereafter, the selenate was reduced to selenite with HCl, chelated with APDC and extracted with MIBK in the same way as for the soil samples.

In EXP1 and EXP2, the plant samples analysed for Se represented the replicates from which the roots were elutriated. Thus, the wheat samples were analysed in quadruplicate and the ryegrass samples in triplicate. In EXP3 and EXP4, the Se analyses of the plant samples were carried out in quadruplicate. In EXP4, the total Se concentration of the unplanted peat soil samples (0.5–1 g soil aliquots) were analysed in three replicates in a way similar to that of the plant samples.

2.3.3 Quality control

In the analyses of the soil extracts, blank samples were included in all series as a contamination control. Throughout the study, the Se concentration of the blanks remained below the detection limit of 1 µg Se l^{-1}, defined in the testing protocol (I) as the mean + 3 × standard deviation of 37 blank samples. Known Se additions were also used and the recovery of spiked Se was on average 97 ± 15% (n = 57). The extracts high in Fe needed dilution and an extra EDTA addition to avoid interference. For the soil Se analyses, an expanded uncertainty of 8% was defined by propagating uncertainties according to the International Organization for Standardization – Guide to the Expression of Uncertainty in Measurement (ISO-GUM).

In the plant analyses, a standard reference material (wheat flour 1567a, National Institute of Standards & Technology) and an in-house wheat flour control material were included in all sample series to assess the accuracy of the analyses. For the standard wheat flour with a certified Se concentration of 1.1 ± 0.2 mg kg^{-1} DW, an Se concentration of 1.2 ± 0.2 mg kg^{-1} DW (n = 40) was obtained. The in-house reference with a known Se content of 0.040 ± 0.008 mg kg^{-1} DW produced an average concentration of 0.045 ± 0.008 (n = 98).

3 Results and discussion

3.1 Extraction methods for assessing the selenium status in soils of Finland

3.1.1 Comparison of methods

In comparison to the standard method of AR extraction, which produces Se concentrations similar to the total amounts recovered by HNO_3-$HClO_4$-HF digestion (Yli-Halla 2005), the efficiency of the extractants tested in dissolving soil Se varied greatly (Figure 2, I). In Se acquisition, SEP2 was equally as efficient as AR. Due to this high efficiency and good reported specificity (Wright et al. 2003), the SEP2 method was considered suitable for characterization of the soil Se reserves (see 3.1.2). However, the five subsequent steps make it too laborious for use in routine analyses.

The next most efficient methods, SEP1 and acid ammonium oxalate, recovered roughly half of the amount of Se obtained with AR. Both of these methods target the oxide-associated Se pool, the oxalate by dis-

Relative efficiency of soil Se extractions

Method	Sand	Silty clay
Aqua regia	-1.16	-0.46
SEP2 total	-1.17	-0.42
SEP1 total	-1.38	-0.86
Acid ammonium oxalate	-1.47	-0.76
Phosphate buffer 1 M pH 7.0	-1.67	-1.01
Phosphate buffer 1 M pH 4.4	-1.82	-1.16
Phosphate buffer 0.1 M pH 7.0	-2.11	-1.44
Phosphate buffer 0.1 M pH 4.4	-2.16	-1.79
Hot water	-2.15	-1.88
Ammonium chloride (step 1 of SEP1)	nd	-2.65
Potassium chloride (step 1 of SEP2)	-3.05	-2.64

□ Sand □ Silty clay
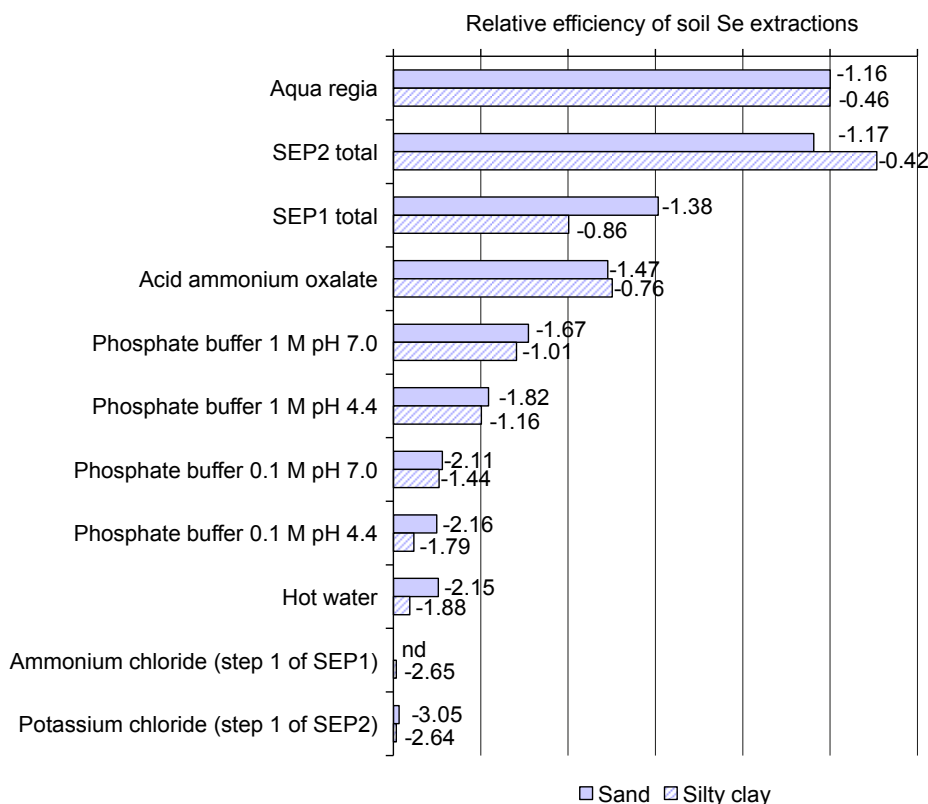
Figure 2. Relative efficiency of different soil Se extraction methods in comparison to aqua regia extraction in sand and silty clay soils. The bars are averages of three replicate extractions. The log-transformed means for the corresponding Se concentrations are reported at the end of each bar. Standard error for the log-transformed means is 0.03 and the least significant difference 0.08.

solution of the oxides and the NH_4F (step 2) and NaOH (step 3) of SEP1 by ligand exchange on the Al and Fe oxide surfaces, respectively. Most of the Se acquired with the SEP1 was recovered in these oxide-associated fractions. In clay soil, 60% of the total amount of Se extracted with this procedure was associated with Al oxides and 35% with Fe oxides. In the sand soil, the corresponding percentages were 50% and 50%. Accounting for the oxalate-extractable Fe/Al molar ratio of the soils, Fe oxides in the sand seemed to be preferred in Se sorption, but no such trend was seen in the clay. Yläranta (1983a) showed that Se was associated rather with acid ammonium oxalate-extractable Al than with Fe, but John et al. (1976) showed contrasting results.

All three extractants targeting the oxide-associated Se were particularly dark in colour, with dissolved organic matter suggesting the presence of organically associated Se. In preliminary tests, humic Se was precipitated from the NH_4F and NaOH extracts by acidifying them with H_2SO_4. This procedure reduced the Se concentration of the NH_4F extract by 40% in the sand and 30% in the clay soil. In the NaOH extract, the corresponding reductions were 50% and 40%. Séby et al. (1997) showed that the nonacid-soluble fraction may contain considerable amounts of selenite in addition to the humic acid-bound Se. However, organic Se seems to be preferentially associated with the fulvic acid fraction (Séby et al. 1997, Coppin et al. 2006).

The aggressiveness of the phosphate buffers in releasing soil Se was dependent on the phosphate concentration and pH of the buffer solution. The neutral concentrated buffer dissolved around 30% of the AR-extractable Se, whereas the corresponding recovery for the acidic and dilute buffer was merely 5–10%. The adsorption-desorption behaviour of selenite is dependent on the pH (Balistrieri and Chao 1987). Selenite adsorption is enhanced at low pH and de-

sorption is known to increase with increasing pH (Neal et al. 1987). An increase in the phosphate concentration of the desorbing solution increases competitive advantage for phosphate over selenite for the binding sites, resulting in increased acquisition of Se (Balistrieri and Chao 1987, Saha et al. 2005). These phenomena enable weakly adsorbed selenite to be distinguished from that bound to higher energy sites by adjusting the concentration and pH of the buffer extractant (Saha et al. 2005). However, in addition to the inorganic selenite, the phosphate buffer-extractable adsorbed Se fraction may contain organically associated Se, especially selenoproteins solubilized from plant material (Martens and Suarez 1997b, Wright et al. 2003). In the phosphate extracts of EXP1 soils, which were analysed both with and without the oxidation-reduction pretreatment to separate selenite from the other species, merely 20% of the total Se content of the extracts was recovered directly as selenite (II).

Hot water and salt solutions were placed at the weakest end in the efficiency of soil Se extractants (Fig. 2). The hot-water extraction dissolved over five times more Se than did the salt solutions, likely due either to the disruption caused by the boiling treatment or the low ionic strength in the water, which enhances desorption (Ryden and Syers 1975, Hartikainen and Yli-Halla 1982). Wright et al. (2003) found KCl extraction efficient at removing easily soluble selenate. The higher efficiency of the hot water in comparison to the KCl suggests that the method removes weakly bound selenite or organic Se in addition to the selenate. Sharmasarkar and Vance (1997) showed that hot-water extracts contain considerable proportions of organic Se in addition to the inorganic selenate.

Excluding the SEP2 and acid ammonium oxalate, the extractants tested were relatively more efficient in the sand than in the silty clay soil. This phenomenon result-

ed likely from the fact that in the clay soil the proportion of poorly soluble Se, possibly occluded within the mineral structures out of the reach of the extractants operating on the soil surfaces, was greater than in the sand soil. The absolute amount of Se extracted with AR from the silty clay (0.35 mg Se kg^{-1}) was fivefold higher than that acquired from the sand (0.07 mg Se kg^{-1}), an order of total Se contents typical between these soil types (Koljonen 1975).

Hot-water extraction has been used as the follow-up method in the Se monitoring programme in Finland (Eurola et al. 2008). It may be useful in assessing the readily bioavailable Se pool, but it is clearly too weak for observing the soil Se reserves. With the more aggressive methods, the accumulation of fertilizer Se could be observed before it is reflected in the easily soluble Se concentrations. Of the single extractions, AR reaches the majority of the Se reserves, but embodies fractions outside the biological cycles. The acid ammonium oxalate or strong phosphate buffers thus appear most promising for monitoring the changes in the potentially bioaccessible soil Se pool.

3.1.2 Selenium fractions of various field soils

In the laboratory studies, the Se reserves of 11 field soils were fractionated with the SEP2 method (I,II). The soils, including the sand and silty clay from Viikki Research Farm, Helsinki and the nine MTT Research Station samples, differed greatly in total Se, pH, organic matter content and texture, but produced noticeably similar Se distributions (Figure 3). The pattern of 1% salt-soluble, 20% adsorbed, 40% organically associated, 15% elemental and 30% recalcitrant organic Se or metal selenides can thus be considered characteristic for the agricultural soils of Finland. A rather similar Se distribution was reported for a silty clay loam soil from Rothamsted, UK (Coppin et al. 2006).

The small size of the salt-soluble Se fraction consisting of soluble selenate is in accordance with the thermodynamic equilibria of Se speciation showing selenite, elemental Se or metal selenides predominating under acidic and reducing conditions (Koljonen 1975, Elrashidi et al. 1987). The pool of
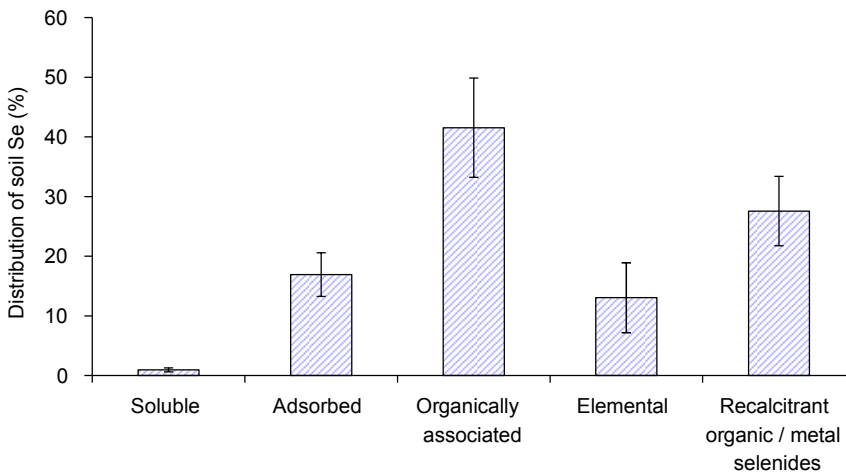

Figure 3. Distribution of the soil Se reserves in the field soils of Finland into soluble (KCl), adsorbed (K_2HPO_4), organically associated (NaOH), elemental (Na_2SO_3), and recalcitrant organic / metal selenides (NaOCl) according to the Wright et al. (2003) sequential extraction procedure. The bars are averages of 11 soils ± standard deviation.

adsorbed selenite recovered by phosphate buffer extraction remained low in comparison to the prominent role it has achieved in explaining the poor bioavailability of Se in soil. The NaOH-extractable organically associated Se, which in contrast has attracted very little attention, stood out as the major fraction. Overall, the predominance of the organically bound Se reserves over the inorganic pool was marked, even though there is some inaccuracy in the selectivity of the extraction steps. Mainly, the recalcitrant reduced inorganic and organic Se forms cannot be separated; the organically associated pool may contain some carry-over of the inorganic selenite, and on the other hand all of the inorganic fractions may contain some organically associated Se (Wright et al. 2003).

The forms of organically bound Se have thus far not been identified. The retention may occur through microbiological immobilization (Vuori et al. 1994, Stolz et al. 2006) or through the incorporation of Se into the molecular structures of various organic compounds by plants (Sors et al. 2005). Suggestively, Se may also be abiotically occluded or adsorbed onto organic particles (Hamdy and Gissel-Nielsen 1976, Bruggeman et al. 2007). Fulvic acids in particular have been linked to organically associated forms of Se (Coppin et al. 2006). However, the organic association of Se may alternatively occur indirectly via surface Fe oxides or clays (Coppin et al. 2009).

3.1.3 Estimating plant-available selenium

In EXP2, the applicability of soil extractions in estimating the Se uptake by plants was assessed by comparing the amounts of Se withdrawn from soils by a salt solution (1 M NH_4Cl), phosphate buffer (0.1 M K_2HPO_4, pH 8.0) and ryegrass (III). Ryegrass proved to be the weakest of the three in acquisition of Se (Fig. 4). The two subsequent leaf cuttings and roots had taken
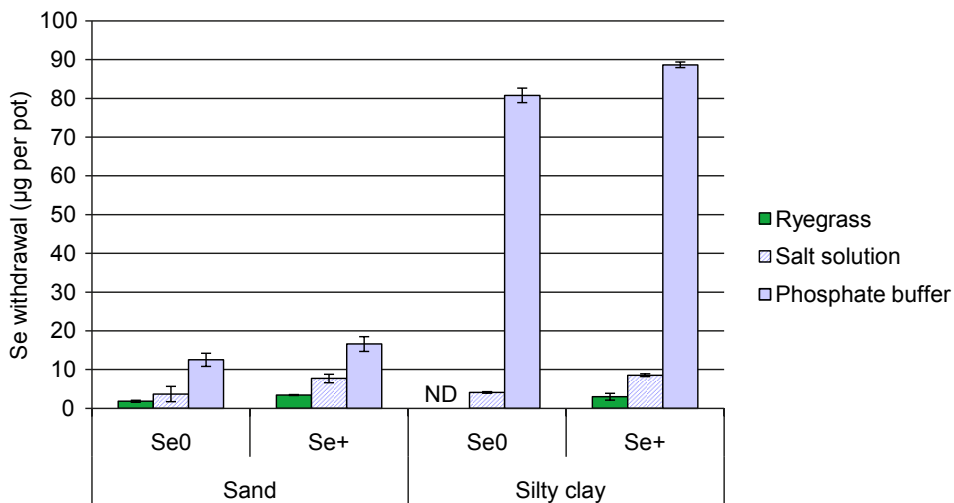
Figure 4. Withdrawal of Se (µg per pot) by two leaf cuttings and roots of ryegrass and the Se quantity extracted by a salt solution (NH_4Cl) and subsequent phosphate buffer (0.1 M K_2HPO_4, pH 8.0) extraction in sand and silty clay soils without added Se (Se0) or supplied with 0.0175 mg selenate-Se per pot (Se+) the previous year. The bars are averages of three replicates ± standard deviation. ND = no data, due to contamination of the leaf samples.

around 2 µg Se from those pots not fertilized with selenate the previous year and roughly 3 µg Se from pots supplied with 17.5 µg selenate-Se prior to the preceding 3-week oilseed rape experiment. The amount of Se recovered with the salt solution was on average 2.4 times that found in the plant. In the soil columns of Goodson et al. (2003), the cumulative Se withdrawal by plants was likewise less than 50% of the salt-soluble Se fraction. Within the Se treatments, no significant differences between the sand and silty clay soil were found in the total Se uptake by ryegrass or the recovery of Se by the salt solution. However, the residuals of supplemental selenate-Se increased significantly Se both in the plant and in the salt-soluble fraction in soil compared with the control not fertilized with Se. The simple salt extraction was thus relatively sensitive to small fertilization-induced changes and efficient enough to give a good estimation of the immediately bioavailable soil Se pool.

As for the phosphate buffer-extractable Se, the relationship with plant uptake was not as straightforward as with the salt solution, since the phosphate buffer dissolves non-labile adsorbed Se fractions. The clay soil was considerably richer in the adsorbed Se reserves than the sand. Thus, the plant uptake in the clay corresponded to merely 4 ± 1% of the amount of Se acquired with the phosphate buffer, whereas in the sand the corresponding proportion was 18 ± 4%. The phosphate buffer extraction thus appears less suitable for estimating the plant-available Se than the salt solution, but clearly more suitable in monitoring the potentially phytoavailable pool of Se.

In estimating the availability of an element to plants by soil extractions, a good correlation between the plant and soil analyses is more important than the quantitative difference between them (Sillanpää 1982). Since different soil features may have contrasting effects on the amounts of elements acquired by the plant and by

the extractant, the most applicable method would likely vary according to the soil properties (Sillanpää 1982, Dhillon et al. 2005). The ability of various extractants to imitate plant Se uptake has been studied, with inconsistent results (e.g. Williams and Thornton 1973; Sippola 1979; Wang and Sippola 1990; Goodson et al. 2003; Dhillon et al. 2005; Zhao et al. 2005). Factors affecting the availability of Se in the soluble pool, such as the amounts of anions competing for plant Se uptake (Hopper and Parker 1999) also need to be considered. Weng et al. (2011) found a major proportion of salt-soluble Se being incorporated in colloidal-sized organic matter, the mineralization of which seemed to control the availability of Se.

3.2 Behaviour of added selenium in soil

3.2.1 Monitoring short-term sorption

In EXP1, the distribution of the added Se between the salt-soluble and adsorbed pools in soil was closely followed throughout the 10-week growing period of wheat, using the first three steps of SEP1 (III). In EXP3 and EXP4, in contrast, sequential soil extractions with a salt solution and phosphate buffer were carried out only at the end of the experiments (IV). The outcomes of the soil analyses were, however, uniform in all studies, showing that most of the added selenate persisted in salt-soluble form in soil kept moist but drier than at field capacity (Fig. 5).

In EXP1, 84 ± 8% of the ample addition of 0.1 mg SeO_4^{2-}-Se kg^{-1} soil was recovered in the salt-soluble pool in the first sampling and 72 ± 1% in the final sampling. The decrease was mostly accounted for by the Se uptake of wheat. The Al oxide-associated Se fraction showed an increase corresponding to 13 ± 3% of the amount of Se added at the first sampling, after which no further increase was detected. The Se concentration of the Fe oxide-associated
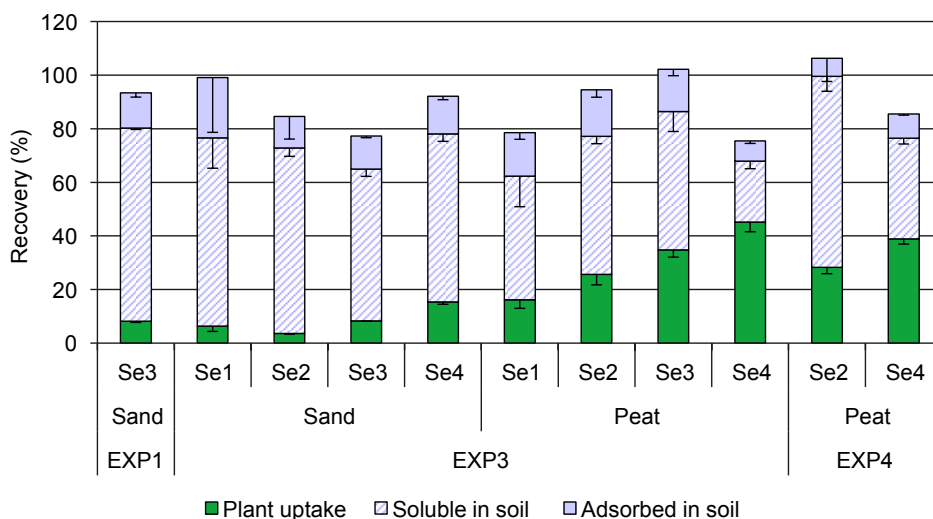
Figure 5. Recovery of the added selenate within the plant and as soluble and adsorbed in soil in three individual pot experiments (EXP1, EXP3 and EXP4). The Se addition levels correspond to 0.0025 mg Se l^{-1} soil (Se1), 0.005 mg Se l^{-1} soil (Se2), 0.1 mg Se l^{-1} soil (Se3) and 1 mg Se l^{-1} soil (Se4).

pool fluctuated during the monitoring, but even at its highest level the increase was less than 10% of the Se addition.

At the end of EXP3, 60–70% of the selenate added was recovered in salt-soluble form in the sand and on average 40% in the peat soils, where more Se was withdrawn by the wheat. Furthermore, in all soils the proportion traced as adsorbed was merely 14%. In the peat soils of EXP4, the same pattern was repeated; the average recoveries of the selenate added as salt-soluble were around 70% in the lower and 40% in the higher Se addition level, whereas the proportion recovered as adsorbed was roughly 10%.

The persistence of the added selenate in the acidic soils throughout the 1.5–2.5-month experiments contrasted with the hypothesis of rapid reduction of selenate to selenite and subsequent adsorption onto soil surfaces. However, Yläranta (1983a) found that selenate added to mineral and peat soils from Finland also remained hot-water-soluble throughout the 3-month incubation. In their studies, Vuori et al. (1994) identified four field soil groups differing in the sorption behaviour of selenate, but in most of their soils collected from different parts of Finland, over 50% of the amount of selenate added remained water-soluble after 75 days of incubation. Selenate reduction can, however, occur very rapidly under favourable conditions. Sposito et al. (1991) reported that without an oxygen supply, soluble selenate disappeared from a soil suspension amended with starch within 1 week. Since the reduction of selenate is predominantly a microbially mediated process, easily degradable organic matter enhances it markedly (Garbisu et al. 1996, Camps Arbestein 1998, Hunter and Manter 2008, Camps Arbestein and Rodríguez Arós 2001). The aeration status of the soil, i.e. the supply of oxygen, controls the redox conditions. In wet or waterlogged soil, the redox potential decreases below the stability limit of selenate (Koch-Steindl and Pröhl 2001). In the pot experiments, the soils were kept drier than field capaci-

ty, which facilitated the persistence of selenate. Under field conditions, heavy showers may well cause waterlogging during the growing period.

As long as the added selenate remains unaltered, its availability to plants is controlled by factors other than the Se sorption capacity of the soil. After reduction to selenite, however, sorption reactions emerge, as evidenced in EXP4, which included a treatment with selenite (IV). In peat soils fertilized with selenite, some of which contained artificially constructed Fe hydroxide surfaces, merely 2% of the selenite-Se addition was recovered in salt-soluble form after the 6-week study. In pure and moderately Fe- enriched peat, the recovery in the adsorbed fraction was not much higher, however, since it contained roughly 5% of the selenite addition. In peat soils amply enriched with Fe, in contrast, around 70% of the selenite addition was acquired as adsorbed. High recovery of the added selenite (92 ± 15%) in the total Se analyses of the unplanted soils revealed that the selenite added was retained by the soil in the pure and moderately Fe-enriched peat as well. It thus seems that in soils rich in oxide surfaces, adsorption occurs by ligand exchange, but evidently in organic soils an alternative, yet unknown, mechanism of retention is operating.

3.2.2 Fertilization-induced long-term changes in soil selenium fractions

The set of paired MTT Agrifood Research Station samples was used in examining the residual fertilizer Se accumulated in soil over a 13-year period (II). The paired sampling scheme and an additional verification of the accuracy of the resampling location by checking the physical similarity of the soils within a sample pair provided control over the heterogeneity of soil, thus rendering tracing of the small amount (27–67 g ha^{-1}) of residual Se meaningful. The drawback of the careful preselection was that it biased the data. The previous study by Yli-Halla (2005) evidenced, however, that spatial variability would have overpowered the small temporal changes induced by fertilization.

Selection of representative sample pairs from the organic soils proved to be difficult. Two of the four soils chosen for the study, JKA20 and PPO2, showed mismatched properties within the pair, JKA20 to the extent that it had to be omitted. Thus, the data on organic soils remained limited and showed no statistically significant changes in any of the soil Se fractions between 1992 and 2004. The set of five mineral soils, in contrast, was found satisfactory in terms of compatibility of the paired samples. At two locations, however, the Se accumulation obtained by the fractionation procedure was over two-fold higher than the expected value calculated by Yli-Halla (2005), based on detailed records of the crops grown and fertilizers used over the study period. This difference may, to some extent, be accounted for by inaccuracy in the transformation of soil Se concentrations (μg kg^{-1} DW) into a hectare-based balance and the roughness of the Se balances calculated, due to use of estimates of crop yields and Se concentrations. Most likely, the disparity between the measured and calculated balances was due to an uneven distribution of Se in the soil, as influenced by the use of placement fertilization, i.e. localized application of the fertilizer.

The data on mineral soils showed small but statistically significant increases from 1992 to 2004 in the Se concentrations of the adsorbed, organically associated and recalcitrant fractions, as well as in the total amount of Se acquired by the SEP2 method (Fig. 6). The changes in the salt-soluble and elemental fractions were not significant, but the soluble Se evidenced a clear trend to decrease. Accumulation of the fertilizer Se in the soluble pool would have been unexpected, since selenate is not

a stable species in the acidic and reducing soils of Finland (Koljonen 1975). It may, however, persist for lengthy periods, as Yläranta (1984c) found in field experiments. His ample selenate addition showed a residual effect in timothy grass in the second year after application. Sposito et al. (1991) demonstrated that the reduction of selenate follows the theoretical reduction sequence of nitrate (NO_3^-) > selenate > manganese oxide (MnO_2) at pH > 5, the reduction of selenate somewhat coinciding with denitrification. Several bacteria are known to be capable of reducing Se (Steinberg and Oremland 1990, Siddique et al. 2006, Hunter and Manter 2008). Weather conditions likely govern the reduction rate of the added selenate and thus the size of the soluble soil Se fraction.

The partitioning of the added Se into several species was found already by Cary et al. (1967). Darcheville et al. (2008) used a modification of the SEP2 method in examining the retention of added selenite in soil in a 6-d incubation and found that the Se retained was distributed mainly among the adsorbed and organically associated pools, but to a lesser extent also among the elemental and residual fractions. In the mineral soils of Finland, the greatest fertilization-evoked increase in Se was found in the organically associated pool, which was closely followed by the recalcitrant fraction embodying organic Se (Fig. 6). This finding indicates that much of the Se added is incorporated into the biological cycle. Organic Se compounds are returned to the soil in plant residues, but soil microorganisms may likewise produce organically associated Se (Stolz et al. 2006). Darcheville et al. (2008) found that microbial activity increased both the amount of Se retained by the soil and the strength of retention. In the studies of Gustafsson and Johnsson (1992), the selenite added was predomi-

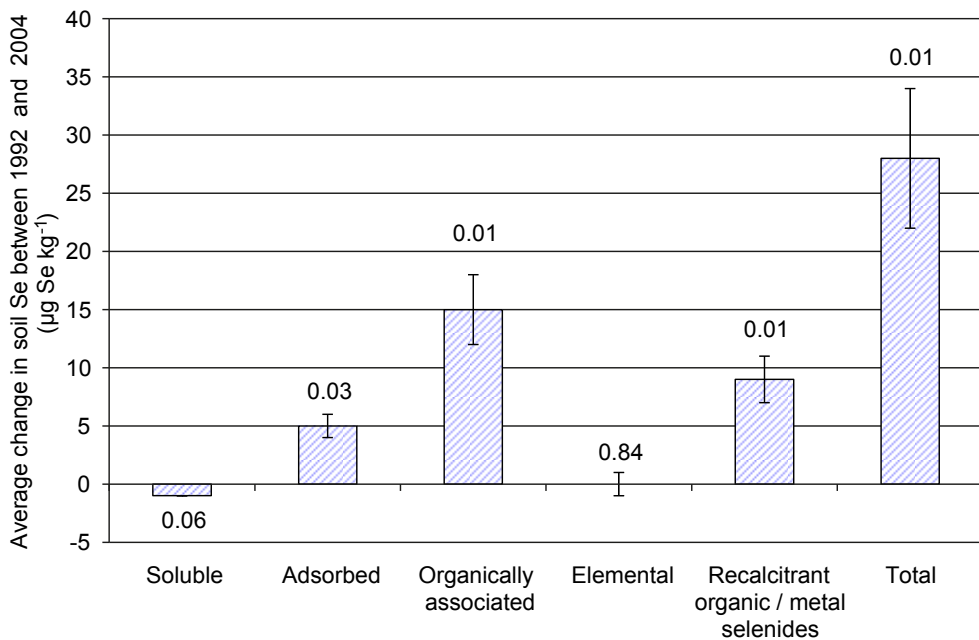Figure 6. Change in Se concentration (µg Se kg⁻¹ soil) between 1992 and 2004 in the soluble (KCl), adsorbed (K_2HPO_4), organically associated (NaOH), elemental (Na_2SO_3) and recalcitrant (NaOCl) fraction of selenate-fertilized mineral field soils. The bars are averages of five soil pairs ± standard error. At the end of each bar is given the P value of pairwise t test at the 0.05 significance level.

nantly fixed in organic matter by an un-known mechanism. Abiotic retention of Se by organic compounds via chelation or by mineral particles carried within the organic matrix cannot be excluded (Hamdy and Gissel-Nielsen 1976, Coppin et al. 2009).

3.3 Efficiency of plant selenium uptake

3.3.1 Selenium uptake rhythm and distribution of selenium within the plant

The 10-week follow-up in EXP1 showed that the Se uptake of wheat continued throughout the period of growth (III). The Se content of wheat was highest at 8 weeks after sowing and decreased by 15% during the last 2 weeks of ripening, when the plant dry mass decreased by 10% as well. In the Se concentrations of the vegetative organs (roots, leaves and stems), a trend to decrease over time was observed, whereas in the spikes a contrasting trend was shown. The highest Se concentrations, around 6 mg kg^{-1} DW, were attained in the young leaves and harvest-ready grains, whereas the lowest, around 2 mg kg^{-1} DW, were found in the roots and stems of the mature plants. The proportion of Se located in the roots remained low throughout the experiment. During the first half of the growth period, Se accumulated in the leaves and emerging stems and during the last half in the spikes. Clearly, Se was remobilized and translocated from the leaves and stems into the filling grains known to act as sinks for carbohydrates and proteins (Evans et al. 1975, Simpson et al. 1983). At harvest, 55% of the total Se content in the wheat was found in grains, 10% in the head chaff, 15% in the stems, 15% in the leaves and 5% in the roots (Fig. 7). In EXP3, the wheat roots were not collected, wherefore the Se distribution within the plant could be determined only for the aboveground plant mass (IV). However, the results demonstrated a similar high efficiency in the translocation of Se into the

grains, since roughly 50% of the plant Se was recovered in the grains, 35% in the leaves and 15% in the stems.

In EXP2, the distribution of Se between the roots and the leaf mass of ryegrass collected in two subsequent cuttings was defined (III). Of the total Se uptake of ryegrass, 5–10% was recovered in the first cutting, 5–10% in the second cutting and 80–90% in the roots (Fig. 7). The root/shoot ratio of plant Se thus differed considerably between wheat and ryegrass. Plant species are known to differ in their inherent Se transport ability (Williams and Mayland 1992), but usually the Se species taken up by the plant governs the distribution of Se. Selenate is readily transported into leaves, but selenite and organic Se species accumulate mostly in the roots (Smith and Watkinson 1984, Zayed et al. 1998, Terry et al. 2000, Li et al. 2008). In EXP2, the selenate fertilization was already ap-
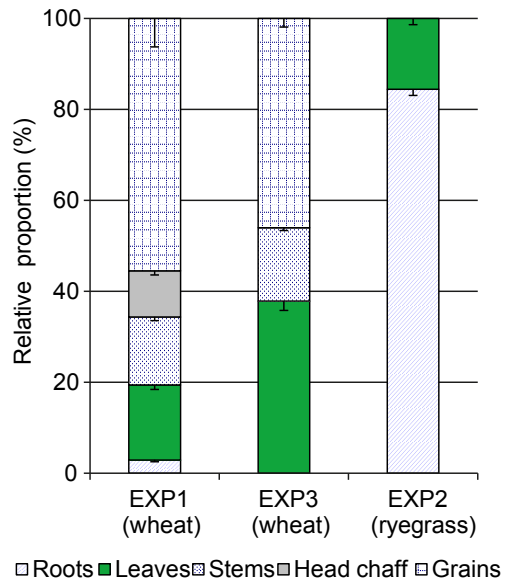

Figure 7. Distribution of Se among various plant organs in three individual pot experiments (EXP1–3). In EXP 3, the roots were not collected and the head chaff was not analysed for Se. The bars are averages of three (EXP2) and four (EXP1 and EXP3) replicates – standard error.

plied for the previous year's crop, thereby allowing for transformations in the soil. Li et al. (2008) discovered that the translocation of selenate was suppressed in the presence of selenite. Suggestively, the different Se translocation patterns between wheat and ryegrass were caused by dissimilar proportions of various Se species in the soil.

3.3.2 Selenium uptake in various soils

The average efficiency of fertilizer-SeO_4^{2-} uptake in EXP1, EXP3 and EXP4 ranged between 4% and 45% of the amount of Se added (Fig. 5, section 3.2.1, III, IV). Under field conditions, the utilization of selenate applied was reported to range between 5% and 35% (Yläranta 1990, Broadley et al. 2010) and in pot experiments between 10% and 28% (Eich-Greatorex et al. 2007). In general, the proportion of fertilizer Se taken up by the plants increased as the selenate addition increased. Broadley et al. (2010) observed a similar pattern in their field study. It may be explained by competitive advantage for selenate over sulphate, since these anions are known to enter the plant via the same transporters (Sors et al. 2005).

Overall, Se uptake was lower in the sand than in the peat soils. This difference could be attributed to plant growth being poorer in the sand, likely due to unfavoura-ble physical conditions and a lower rate of N mineralization. Other systematic differences in the efficiency of plant Se uptake between different soils were not observed. In the pot experiments, the focus was on examining the sorption of Se as a process restricting the availability. The factors limiting the uptake of Se from the soluble pool were not addressed. However, other studies have shown that plant species and even the cultivars of a certain species differ in their uptake capacity of Se (Mayland 1994, Munier-Lamy et al. 2007, Murphy et al. 2008), even though the Se uptake of all species is probably restricted to the same labile soil Se pool (Goodson et al. 2003). Yläranta (1985) found that grasses are able to utilize selenate fertilization more efficiently than cereals. The concentration of the competing sulphate (Hopper and Parker 1999) and phosphate ions (Eich-Greatorex et al. 2010) in the soil solution govern the uptake of Se. In addition, at least the yield level (Tveitnes et al. 1996), Se translocation within the plant (Williams and Mayland 1992) and soil pH (Eich-Greatorex et al. 2007) have been linked to the Se uptake efficiency of the crop. Stroud et al. (2010) found that the combination of KH_2PO_4-extractable soil S and AR and KH_2PO_4-extractable soil Se explained 86% of the variance in the grain Se concentrations of non-Se-fertilized UK wheat.

4 Conclusions

Under moisture conditions drier than field capacity, reduction of selenate in acidic mineral and organic soils was quite low during the 1.5–2.5-month pot experiments. The sorption reactions did thus not really restrict the availability of Se added as selenate. The plants took up 5–50% of the selenate supplied. In wheat it was very efficiently translocated into the grains, but in ryegrass grown in residual Se, a high accumulation of Se in the roots was observed, probably because much of the Se was taken up as selenite or organic Se forms. Even though this type of translocation pattern cannot be categorized as typical for ryegrass, clearly under certain conditions most of the Se taken up by the plant may not be removed from the field during harvest but may be returned to the soil-plant cycle, likely in organic form.

Different Se extractants varied in their ability to solubilize soil Se. Depending on the purpose, methods targeting merely the easily soluble Se pool or those dissolving more or less of the nonlabile fractions can be selected. Hot-water extraction or a simple salt solution can be used in assessing the easily available Se, but due to the tendency of Se to exist as scarcely soluble in the soils of Finland, more aggressive methods are needed to monitor the changes in soil Se status. Acquisition of total soil Se with acid digestion may not be desirable,

due to the inclusion of biologically inactive primary Se reserves. Acid ammonium oxalate or concentrated phosphate buffer with high pH appear to be more applicable extractants for exploring the potentially bioavailable Se pool. Including one of these two methods in the Finnish Se-monitoring programme in addition to hot-water extraction would strengthen follow-up of the residual fertilizer Se in soil.

Characterization of the Se reserves of selenate-fertilized field soils of Finland by the SEP of Wright et. al (2003) revealed that organically associated Se predominates over the inorganic Se pools. The distribution of Se among the five separate fractions was largely independent of the total Se, texture or organic matter content of the soil. Only 1% of the soil Se reserves were easily bioaccessible salt-soluble selenate, nearly 20% were recovered as adsorbed, around 40% were organically associated, 15% in elemental form and 30% as recalcitrant organic Se or metal selenides. Fertilizer selenate was accumulated as adsorbed selenite and in the organically associated and recalcitrant pools. Incorporation of Se into the biological cycle thus appears to play a prominent role in the behaviour of added Se in soil. Further research is needed on the speciation of organically associated Se.

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