



# Quantitative analysis of selenium species in the edible parts of cabbage, carrot, tomato and green pea treated with selenate-enriched irrigation water

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## Abstract

**Background and aims** Selenium (Se)-biofortified foods are able to compensate for Se deficient diets, but the proportion of different Se species varies among plant species, and the bioavailability of Se species also varies. This study aims to examine the Se species composition of four vegetables.

**Methods** The effect of Se-enriched irrigation water on the concentrations of Se species in the edible parts of cabbage, carrots, tomatoes and green peas grown on different soil types was investigated and quantified using HPLC-ICP-MS with either standard addition or isotope dilution.

**Results** Cabbage leaf and carrot root contained a high proportion (88 and 92%, respectively) of selenate (SeVI), while in tomato fruit and green pea seed

selenomethionine (SeMet) was found in the highest proportion (33% and 48%, respectively), but elemental Se was also detected. The elemental Se concentration of peas was significantly higher on sandy soil (2.29 mg kg<sup>-1</sup>) than on sandy silt (1.38 mg kg<sup>-1</sup>) or silt soil (1.34 mg kg<sup>-1</sup>). In 100 g of fresh edible parts, the largest amount of Se species was found in cabbage leaves, but organic Se in the form of SeMet was the most abundant in green peas, being significantly higher than in carrots or tomatoes. Tomatoes contained a nearly three-fold ratio of organic Se to inorganic Se, the latter being mostly comprised of the elemental form, which is unusually high in vegetables. The highest rate of unknown Se species exceeded 66% in the case of peas, while the highest identification rate (>95%) of Se could be achieved in tomato. **Conclusion** Tomatoes converted the absorbed Se into organic Se in the highest ratio, while green peas

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had the highest amount of organic Se in fresh biomass, so biotransformation was the most effective for these vegetables without any significant biomass reduction. These vegetables may be adapted to soils with a higher Se content.

**Keywords** Biofortification · Selenium speciation · Bioavailability · Vegetables · Elemental selenium · Isotope dilution

### Abbreviations

LOQ	Limit of quantification
MetSeCys	Methylselenocysteine
RSD	Relative standard deviation
SAX	Strong anion exchange
Se	Selenium
SeCys	Selenocysteine
SeMet	Selenomethionine
SeIV	Selenite
SeVI	Selenate

### Introduction

Greater emphasis should be placed on the appropriate intake of micronutrients, because for farmers nutritional value often takes a backseat compared with crop yield (Lončarić et al. 2021). Selenium (Se) is an essential trace element for both humans and animals. It maintains health via antioxidant effects and cardiovascular benefits, and also supports the immune system and the reproductive organs (Rayman 2012; Koszta et al. 2012; Tóth and Csapó 2018). Inadequate Se intake can lead to serious diseases, which in extreme cases may even be fatal (Hartikainen 2005).

The daily recommended dietary allowance of Se varies considerably across countries and regions. It ranges from 25 to 60  $\mu\text{g day}^{-1}$  for adult women and from 30 to 75  $\mu\text{g day}^{-1}$  for adult men, a value of 55  $\mu\text{g day}^{-1}$  being recommended in the USA and Canada (Fairweather-Tait et al. 2011). In the EU, 70  $\mu\text{g day}^{-1}$  is recommended for adults and 15  $\mu\text{g day}^{-1}$  for children aged one to three years, which increases to 70  $\mu\text{g day}^{-1}$  for adolescents aged 15–17 years. For lactating women an intake of 85  $\mu\text{g day}^{-1}$  is recommended to cover the amount of Se secreted in breast milk (EFSA NDA Panel, 2014). The tolerable upper intake level is 255  $\mu\text{g Se day}^{-1}$  for adult men and women (EFSA NDA Panel et al. 2023).

However, the daily intake falls short of these values in many areas of the world. The Se supply of soils may vary from very low to toxic levels even within countries, as reported in the USA, China, Canada, Venezuela, India, Japan and the UK. In North America, for example, the Pacific Northwest and the eastern US are low in Se, but the northern Great Plains and the prairie regions of Canada are rich in Se (McNeal and Balistrieri 1989). Se-deficient nutrition affects a larger area of the world than excessive selenium intake (Pyrzynska 2009). Se deficiency is characteristic of Australia, New Zealand and some areas of Africa, such as the Sub-Saharan region (Gissel-Nielsen et al. 1984; Long et al. 2018; Phiri et al. 2019; Muleya et al. 2021). In Europe, the soils of Spain are typically adequately or highly supplied (Moreno Rodriguez et al. 2005), while they are low in Se in Finland (Hartikainen 2005) and in the UK (Broadley et al. 2006). The Se supply of soils in Eastern European countries is also low (Galić et al. 2023), so the estimated daily intake is usually below the recommended dietary allowance (Rayman et al. 2008).

The primary source of human and animal Se intake is food, so the lack of Se can be effectively compensated for by the biofortification of food crops. Agromomic biofortification involves the enrichment of a deficient element in the crop by applying it to the soil or growing medium, or in the form of a foliar spray (Galić et al. 2021). In addition to the quantity, the ratio of different Se species is also an important aspect of intake. Organic forms of Se have greater bioavailability and are more easily absorbed by human and animal organisms than inorganic forms, so their biological role is greater (Fairweather-Tait et al. 2010; Thiry et al. 2012). Inorganic Se forms (SeVI, selenite - SeIV) and organic Se forms (SeMet and high-Se-yeast) are all able to increase selenoenzyme (glutathione peroxidase) activity, but organic forms raise blood Se concentrations more effectively (Rayman et al. 2008). SeMet can be metabolized directly to reactive forms of Se or stored in body proteins (Schrauzer 2000). MetSeCys has been shown to have anti-tumour effects (Ip et al. 2002). Regarding overdoses, inorganic forms of Se are more acutely toxic than organic forms, and SeIV is more toxic than SeVI (Rayman et al. 2008). Organic forms may represent a greater danger in the long term due to accumulation, although some experiments have refuted this theory (Rayman 2004; Reid et al. 2004). Since the chemical form of Se in food crops largely

determines the Se bioavailability in humans and animals, Se speciation is important for evaluating the effectiveness of biofortification (Kirby et al. 2008; Muleya et al. 2021).

Se is not an essential element for higher plants (White 2018). The Se species present in plant tissues are found in inorganic forms, typically as SeVI or in organic forms, as selenomethionine (SeMet) and methylselenocysteine (MetSeCys). One important aspect of biofortification is the Se tolerance of a given crop, that is, the amount of Se that can be taken up without having a toxic effect on the plant. SeVI is structurally similar to sulphate, so sulphate transporters in plant roots can absorb it from the soil (Terry et al. 2000; Hawkesford and Zhao 2007; Stroud et al. 2010). However, the majority of plants are unable to contain higher concentrations of Se, because Se is converted into the amino acids SeMet or selenocysteine (SeCys), which are incorporated into proteins (instead of methionine or cysteine) and inhibit their function (Gupta and Gupta 2017). This leads to toxic symptoms and, in more severe cases, to the destruction of the plant. At the same time, certain plants are able to accumulate Se in larger quantities without showing toxicity symptoms. Certain legumes species tend to accumulate more Se due to their high protein content (Poblaciones et al. 2014a), although the tolerance of some Se-accumulator *Astragalus* species is considerably greater than that of the sensitive species white clover (*Trifolium repens* L.) (Wu et al. 1994). Species in the *Brassicaceae* are able to incorporate Se into monomethylated amino acids, such as MetSeCys, which can accumulate without being incorporated into proteins (Terry et al. 2000; Ávila et al. 2014). They are also able to convert and store Se in the form of selenoglucosinolates (Wiesner-Reinhold et al. 2017). For example, cabbage may have a Se content as high as 100–1000 mg kg<sup>-1</sup> in dry mass without showing symptoms of toxicity (Gupta and Gupta 2017). In the case of high Se content, one of the defence mechanisms of plants is the synthesis of volatile Se compounds (Zayed et al. 1998). Non-Se-accumulator plant species tend to produce dimethyl selenide, whereas hyperaccumulators primarily synthesise dimethyl diselenide (Pilon-Smits and Le Duc 2009). Even non-Se-accumulator species may have a high enough Se content to provide reasonable Se supplementation, as confirmed in the case of tomatoes (Pezzarossa et al. 2014), carrots (Smoleń et al. 2016; Oliveira et al. 2018) and green peas (Poblaciones and Rengel 2018).

Green peas, tomatoes, cabbage and carrots are widely grown and consumed almost all over the world; in 2020, the total production was 19.9, 186.8, 70.9 and 40.9 million tonnes on 2.53, 5.05, 2.41 and 1.13 million hectares, respectively (FAO 2022). Green peas are very rich in protein and essential amino acids, tomatoes and cabbage are consumed for their vitamins and antioxidant-rich phytochemicals, while carrots are high in fibre, carotenoids and sugars, and all these vegetables play an important role in human nutrition due to their mineral content (Nithiyantham et al. 2012; Ali et al. 2021; Moreb et al. 2020; Smoleń and Sady 2009). Therefore, these crops may serve as good target plants for biofortification.

Se speciation (that is, the qualitative and quantitative distribution of different inorganic and organic forms of Se) may exhibit great diversity in different plant species (Fairweather-Tait et al. 2010). The ratio of organic to inorganic Se may also depend on the Se form with which the plant was supplemented. In leek, the inorganic form was present in a much higher ratio after supplementation with Na<sub>2</sub>SeO<sub>4</sub> (55%) than with Na<sub>2</sub>SeO<sub>3</sub> (21%), but the Na<sub>2</sub>SeO<sub>4</sub> treatment resulted in a multiple Se concentration in the plant. In both treatments, MetSeCys and SeMet were the dominant organic Se forms (Lavu et al. 2012). The Se content of chickpea was also much higher after Na<sub>2</sub>SeO<sub>4</sub> treatment and, depending on the inorganic Se treatment, 63–91% was converted into organic Se, the majority of which was SeMet (Poblaciones et al. 2014a). SeMet treatment resulted in SeMet dominance, and Na<sub>2</sub>SeO<sub>3</sub> treatment in MetSeCys dominance in hydroponically grown lettuce (Kowalska et al. 2020). Muleya et al. (2021) reported that more than 90% of the Se applied as SeVI was transformed into organic form after plant uptake, and 74% of that was bioaccessible. The main organic Se form was SeMet in maize, groundnut and cowpea. SeMet had the largest ratio in durum wheat (Poblaciones et al. 2014b), rice grain (Yuan et al. 2023) and alfalfa (Kovács et al. 2023). In general, non-accumulator crop species, such as cereal crops, contain organic Se mainly in the form of SeMet, while in Se-accumulator species, like species in the *Brassicaceae* family, higher molecular mass Se species such as selenohomolanthionine, selenocystathionine and selenolanthionine are also found in higher ratios (Schiavon et al. 2020; Schiavon and Pilon-Smits 2017; Ogra et al. 2007; Both et al. 2020).

Quantitative changes in Se accumulation have been widely studied, but Se speciation studies have been performed to a much more limited extent. To the best of our knowledge, the analysis of Se species in ripe tomato fruit has not been reported so far.

The aim of the experiment was to study the effect of Se-enriched irrigation water and soil texture on the yield and element composition of green peas, tomatoes, cabbage and carrot plants (Ragályi et al. 2021, 2022). This work presents the results of quantitative analysis on the non-volatile Se species that are accumulated in the edible parts of the plants and can be quantified using an LC-ICP-MS instrumental setup, that is, with the help of commercially available standards. Based on the results of previous research, it was hypothesised that the SeVI content of individual plants would vary depending on the soil type, reflecting changes in their total Se content, and that the organic Se content would be significantly higher in cabbage than in the other vegetables.

## Materials and methods

### Experiment setup

The effect of irrigation with Se-enriched water was investigated in a greenhouse experiment on the research site of the Institute for Soil Sciences, Centre for Agricultural Research (Órbottyán, Hungary). The test plants were grown in 10-litre pots with four holes ( $\varnothing$  0.5 cm) in the bottom, containing a 1 cm layer of washed gravel (4–8 mm) covered with a fine synthetic fibre fabric on which 10 kg of homogenised topsoil was placed (Dobosy et al. 2020). Three topsoils (0–20 cm) with different textures were investigated: sand (Mollic Umbrisol, Arenic, from Órbottyán, 47°40'N, 19°14'E), silty sand (Luvic Calcic Phaeozem from Gödöllő, 47°58'N, 19°38'E) and silt (Calcic Chernozem from Hatvan, 47°67'N, 19°64'E), the properties of which are shown in Table 1. The total number of pots was 36: 3 soils  $\times$  1 Se dose (500  $\mu\text{g}$  Se  $\text{L}^{-1}$  actual concentration  $561 \pm 49 \mu\text{g}$   $\text{L}^{-1}$ )  $\times$  4 plant species  $\times$  3 replications. The entire experiment was designed with Se contents of 0, 100 and 500  $\mu\text{g}$   $\text{L}^{-1}$  in the irrigation water. Se speciation analyses, however, were only performed for samples given the highest quantity to make species detection more reliable, since the speciation methods used in this study had

**Table 1** Selected properties of the soils

Properties	Sand	Silty sand	Silt
pH-H <sub>2</sub> O	7.96	6.83	7.34
OM (%)	0.91	1.24	2.12
CEC (Na meq 100 g <sup>-1</sup> )	9	17	37
CaCO <sub>3</sub> (%)	1.45	0.08	0.20
Total N (%)	0.064	0.092	0.135
AL-K (mg kg <sup>-1</sup> )	61.2	144	145
Total K (mg kg <sup>-1</sup> )	3164	7250	7639
AL-P (mg kg <sup>-1</sup> )	57.2	104	35.4
Total P (mg kg <sup>-1</sup> )	449	446	412
Water-soluble Se (mg kg <sup>-1</sup> )	< dl	< dl	< dl
LE-Se (mg kg <sup>-1</sup> )	0.009	0.016	0.010
Total Se (mg kg <sup>-1</sup> )	0.076	0.094	0.132
Total Fe (mg kg <sup>-1</sup> )	10,028	17,875	29,651
Total Mg (mg kg <sup>-1</sup> )	4035	3942	5399
Total Zn (mg kg <sup>-1</sup> )	22.9	49.1	62.1
Total Cu (mg kg <sup>-1</sup> )	5.00	13.8	20.1
Clay (<0.002 mm, %)	14	23	34
Silt (0.002–0.02 mm, %)	18	30	50
Sand (0.02–2 mm, %)	69	46	16

OM: organic matter content; CEC: cation exchange capacity; AL: ammonium-lactate soluble; Total: aqua regia-soluble; LE: ammonium-acetate + EDTA-soluble

relevant limit of quantification (LOQ) values between 0.5 and 4.0 mg kg<sup>-1</sup> in terms of dry mass. The test plants were tomato (*Solanum lycopersicum*, cv. Mano; ZKI) and cabbage (*Brassica oleracea* L. var. *capitata* cv. Zora; Réde) in 2018, and green pea (*Pisum sativum* L. var. Rajnai törpe) and carrot (*Daucus carota* L. var. *sativus* cv. Nantes-2) in 2019. Before the pot experiment, tomato, carrot and cabbage seeds were germinated and planted in propagation trays (1 seed per cell) filled with “VEGASCA Bio” (Florasca Hungary Ltd., Osli, Hungary) growing medium (a mixture of peat and grey cattle manure compost: organic matter > 50%; N > 0.3%; P<sub>2</sub>O<sub>5</sub> > 0.1%; K<sub>2</sub>O > 0.1%; pH of 6.8). The seedlings were cultivated in a growth chamber for 22 days under controlled conditions (day/night temperatures and photoperiods of 26/18°C and 16/8 h, respectively, with a photon flux density of 500  $\mu\text{mol}$  m<sup>-2</sup> s<sup>-1</sup> and relative humidity of 50–70%). The seedlings were irrigated with a total of 180 ml of tap water weekly, in 3  $\times$  60 ml doses every Monday, Wednesday and Friday. After a 6-day acclimatisation period in the greenhouse, the soil-free seedlings were transplanted into the experimental pots: 1 seedling

per pot for cabbage and tomato and 3 seedlings per pot for carrot. Germinated green pea seeds were sown directly (3 seeds per pot). In the case of both carrots and green peas, 3 plants per pot were grown during the whole vegetation period.

The plants were irrigated with an automated system using individual drip stakes placed in each pot. During the growing period, each pot was watered weekly with Hoagland solution (200 ml per pot). For the first three weeks after planting, tap water was applied without Se treatment. Irrigation with Se solution ( $\text{Na}_2\text{SeO}_4$  diluted with tap water) started three weeks after planting. The Se content of the irrigation water averaged  $0.287 \pm 0.044 \mu\text{g Se L}^{-1}$ . The solution in the tank was regularly monitored to ensure a constant Se concentration. The tap water used to prepare the Se solution was stored in  $0.5 \text{ m}^3$  tanks (separate tanks for each irrigation solution) before application to reduce the chlorine concentration. The volume of irrigation water was adjusted to the water requirements of the different plant species. Soil moisture content was monitored at a depth of 10 cm every hour (Decagon EC-5, ICT International, Armidale, Australia). The irrigation system delivered the preset amount of water at 7 a.m. each day. The details of growing periods, plant protection and irrigation are shown in Supplemental Materials (Table S1).

The experimental area received natural light. The greenhouse climate data (mean day and night temperature, air humidity and photon flux density of photosynthetically active radiation) were continuously monitored during the growing period (Table 2). Pesticides and fungicides were applied as necessary.

### Chemical analysis

The untreated composite soil samples used for the experiment were collected, dried, sieved through a 2 mm mesh and analysed. The soil pH was measured

in a 1:2.5 soil:water suspension after mixing for 12 h. The following analytical tests were performed: total N content with the Kjeldahl method (ISO 11261: 1995), organic matter (OM) content with the modified Walkley–Black method (FAO 2020),  $\text{CaCO}_3$  content with the Scheibler gas-volumetric method (ISO 10693, 1997), and cation exchange capacity (CEC) with the modified method of Mehlich (ISO 13536: 1995). The total Se concentrations were analysed after microwave-assisted digestion with aqua regia in a Teflon digestion vessel (ISO 12914: 2012). The plant-available P and K fractions were measured after extraction with ammonium acetate-lactate (AL-P and AL-K) (Egnér et al. 1960), and extractable Se (LE-Se) was measured using 0.5 M  $\text{NH}_4$ -acetate + 0.02 M EDTA extract (Lakanen and Erviö 1971).

After harvest, the plants were cleaned with deionized water, then the roots, shoots (aboveground vegetative parts) and fruit were separated, and the fresh weight of the plant parts was measured. The cabbage leaf samples were dried at  $40^\circ\text{C}$  for two days in a laboratory oven, while tomato fruit, green pea seed and carrot root samples were milled and freeze-dried at  $-70^\circ\text{C}$  in Christ Alpha 1 (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) equipment (200 Pa for 72 h) followed by determination of dry mass. All the samples were homogenised in a blending machine equipped with plastic housing and a stainless-steel blade. The dried, homogenised samples were kept in centrifuge tubes in a dark room at room temperature until analysis.

For total Se determination, the dried, homogenised samples were mineralised in a microwave-assisted acid digestion system (TopWave, Analytik Jena, Germany). Dried plant samples (400–500 mg) were digested in a mixture of  $7 \text{ cm}^3$  67%  $\text{HNO}_3$  and  $3 \text{ cm}^3$  30%  $\text{H}_2\text{O}_2$ . After digestion, the internal standards were added to the solutions, and the volume was made up to  $15 \text{ cm}^3$  with deionised water. The

**Table 2** Greenhouse parameters (daily average during the growing period)

Parameters	Cabbage	Carrot	Tomato	Pea
Mean day temperature ( $^\circ\text{C}$ )	$25.5 \pm 4.0$	$22.4 \pm 8.0$	$26.6 \pm 3.3$	$21.2 \pm 7.6$
Mean night temperature ( $^\circ\text{C}$ )	$18.2 \pm 3.4$	$14.6 \pm 6.6$	$19.1 \pm 2.3$	$13.7 \pm 6.3$
Photosynthetically active radiation ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )*	$703 \pm 197$	$155 \pm 93$	$982 \pm 419$	$149 \pm 91$
Air humidity (%)	$72.2 \pm 23.0$	$72.5 \pm 24.9$	$69.4 \pm 8.1$	$74.0 \pm 24.6$
Soil moisture (% v/v)	$22 \pm 6$	$23 \pm 2$	$22 \pm 3$	$23 \pm 1$

\*spectral band: 400–700 nm

concentrations of Se and other elements in the soil and plant samples were measured with an inductively coupled plasma mass spectrometer (ICP-MS, Plasma-Quant Elite, Analytik Jena, Germany) with a determination precision of 1–3% relative standard deviation (RSD) for Se. The total Se content and biomass production of plants have been published elsewhere (Ragályi et al. 2021, 2022).

Sample preparation for speciation analysis using strong anion exchange (SAX) HPLC-ICP-MS

For the sample preparation and quantification of SeMet, MetSeCys and SeVI, the methods published by Shao et al. (2014) and in the Annex of the Commission Implementing Regulation (EU 2020/2117) were taken and adapted. In short: dried and homogenised samples were further milled with a MM 200 mixer mill (Retsch GmbH, Haan, Germany). About 0.25 or 0.50 g of milled samples were weighed into centrifuge tubes for extraction. For the extraction of water-soluble compounds, 10 ml deionised water was added to the samples, which were sonicated in an ultrasonic bath for 15 min at room temperature. They were then centrifuged at 8000×g for 10 min. The supernatants were removed and filtered using a 0.22 µm pore size hydrophilic PTFE disposable syringe filter. In the case of peas, a starch deposit was observed in the clear supernatant, so filtration was done almost immediately before injection. The solid residues were enzymatically digested with Pronase E (from *Streptomyces griseus*, 4,000,000 PU g<sup>-1</sup>, Merck-Sigma, Darmstadt, Germany). About 40 mg enzyme per sample was dissolved in 9 ml 100 mM TRIS buffer (pH=9, adjusted with 1 M HCl) and mixed with the plant residues. The closed centrifuge tubes were shaken in a water bath at 37 °C overnight. An additional 40 mg Pronase enzyme per sample was dissolved in 1 ml TRIS buffer and added to the samples on the following day. After shaking for a further 6 h at 37 °C, the samples were centrifuged at 8000×g for 10 min. The supernatants were removed and filtered through a 0.22 µm pore size hydrophilic PTFE disposable syringe filter. Extracted samples were stored at +4 °C and analysed within 2 days. Before analysis, dithiothreitol (Merck-Sigma) was added to reach a 0.01% m/m concentration in the samples to prevent the formation of oxidised SeMet. Enzymatic extraction efficiency and method accuracy were

regularly checked and corrected for recovery with the help of the Se-enriched yeast (SELM-1) reference material (LGC Standards Ltd., Teddington, UK). One plant was sampled from each of the three replicates, and one extraction was carried out on each sample. In order to estimate the LOQ, three parallel injections were done from the 10, 20, 50 and 100 µg L<sup>-1</sup> standard solutions of the selenocompounds. Regarding the precision of the instrumental technique, RSD was calculated for the peak areas and the results were checked against the acceptance level (RSD ≤ 10%).

Se speciation by HPLC-ICP-MS

Species in water extract and enzymatic extract were separated by SAX chromatography and detected using ICP-MS. A Waters Alliance 2695 HPLC instrument (Waters Co., Milford, MA, USA) was coupled to an X-Series II ICP-MS (Thermo Scientific, Bremen, Germany). The injection volume was 10, 20, or 40 µl depending on the analyte concentrations. A Hamilton PRP-X100 (250×4.1 mm, 10 µm) SAX column (Hamilton Company, Reno, NV, USA) was used at a temperature of 25 °C. Gradient elution was done with ammonium acetate (pH=6.5, eluent A: 10 mM, eluent B: 250 mM) at a 2.0 ml min<sup>-1</sup> flow rate. The programme was as follows: 0–1 min: 0% B, 1–20 min: up to 100% B, 20–29 min: 100% B, 29–31 min: down to 0% B, 31–37 min: 0% B. If MetSeCys was found to exceed the LOQ in the samples, it was quantified in separate injections with a modified LC gradient (0–1 min: 0% B, 1–2 min: up to 5% B, 2–3 min: up to 100% B, 3–10 min: 100% B, 10–11 min: down to 0% B, 11–18 min: 0% B).

The ICP-MS parameters were as follows: forward power 1400 W, plasma gas flow rate 14.0 l min<sup>-1</sup>, nebuliser gas flow rate 0.86 l min<sup>-1</sup>, auxiliary gas flow rate 0.88 l min<sup>-1</sup>, pole bias -16.0 V, hexapole bias -10.0 V, dwell time 100 ms. H<sub>2</sub>-He (in 7:93%) collision cell gas was applied with a 6.0 ml min<sup>-1</sup> flow rate. A concentric nebuliser was used with a Peltier-cooled conical spray chamber (2 °C). The detected ions were <sup>78</sup>Se and <sup>80</sup>Se, but <sup>78</sup>Se was used for quantification. Standard addition was applied for the quantification of MetSeCys, SeMet and SeVI (Merck-Sigma Group) as shown in Supplemental Materials, Figs. S1–S3. Concentrations are expressed in dry mass.

### Post column isotope dilution analysis

A different method, post column isotope dilution analysis, was applied for the quantification of species in the enzymatic digestion of green pea samples due to high matrix interference.

An isotopically enriched spike solution was diluted with deionized water from a 10 mg  $^{82}\text{Se L}^{-1}$  stock solution (Inorganic Ventures, Christiansburg, VA, USA) to 20 ng  $\text{g}^{-1}$ . The spike solution was continuously dispensed into the effluent via a T-piece using a syringe pump at 0.1  $\text{g min}^{-1}$  and introduced into the ICP-MS. The HPLC and ICP-MS parameters were the same as those described for the HPLC(SAX)-ICP-MS analysis. The monitored isotopes were  $m/z$  76, 77, 78, 80, 81, 82 and 83. The intensity of hydride formation (1.5%) and the mass bias factor (-4.9% per mass unit) were determined based on the calculations of Hinojosa Reyes et al. (2003a). Intensity values of  $m/z$  77, 78, 80 and 82 were corrected for hydride formation and mass bias. The mass flow of Se in the effluent was determined using the natural isotope ratio of Se and the certified isotope ratios in the spike solution, corrected with a blank injection. The amount of Se species was quantified by summarising the mass flow in the retention window of the individual species in Microsoft Excel, taking into account the dwell times and the detector dead time. The concentration was determined from the amount of Se species and the injection volume (Hinojosa Reyes et al. 2003b; Ouerdane et al. 2020).

### Elemental Se analysis

Elemental Se was measured from tomato and green pea samples. After water extraction and enzymatic digestion, plant residues were extracted with  $\text{Na}_2\text{SO}_3$  extractant based on the method of Both et al. (2018) with minor modifications. Five millilitres of 1.0 M sodium sulphite (Merck-Sigma Group) was added to the residual plant materials. The samples were vortexed and shaken in a water bath at 37 °C overnight, and then centrifuged at 8000 $\times$ g for 10 min. After removing the supernatants, 4 ml of additional deionised water was pipetted to the residues. The samples were vortexed for one minute and were again centrifuged at 8000 $\times$ g for 10 min. The supernatants were merged and filled up to 10 ml with deionised water. Five ml of the  $\text{Na}_2\text{SO}_3$  extracts was pipetted

into PTFE vessels, then mixed with 7 ml nitric acid (65% w/w) and 1 ml hydrogen peroxide (30% w/w) from Scharlab S.L. (Sentmenat, Spain). The digestion was performed in a microwave-assisted digestion system (Milestone Start D, Sorisole, Italy) at 180 °C. Digested samples were filled up to 25 ml with deionised water. Before analysis, a 25 times dilution was applied, and an Rh internal standard was added. ICP-MS (X-Series II, Thermo Scientific, Bremen, Germany) was used for the quantification with the standard addition method. Instrument parameters were the same as those given for Se speciation with HPLC(SAX)-ICP-MS.

### Residual Se content in plant residues and in extracts

After water extraction, enzymatic digestion and sodium sulphite extraction, the solid plant residues of tomato and green pea were washed into PTFE vessels with 7 ml nitric acid (65% w/w), after which 1 ml hydrogen peroxide (30% w/w) was added. The digestion and analysis parameters were the same as those used for elemental Se analysis.

The water extract and the enzymatic extract of green pea samples were also digested to determine the total Se content. The extractions were repeated and 5.0 ml of the homogenised extracts was pipetted into PTFE vessels. The pipetted water extract contained a proportionate amount of the starch deposit that appeared shortly after the extraction. The digestion and analysis parameters were the same as those used for elemental Se analysis.

### Statistical analysis

The data were analysed for treatment effects using factorial analysis of variance and Tukey's post-hoc test. In the case of the Se species contents measured in fresh edible parts, the assumption of homogeneity of variances was not met based on Levene's test, so the Kruskal-Wallis test was used. The variances were calculated for soil types and vegetable species. Significant differences between the treatments were calculated at the  $p < 0.05$  level. Statistica v.13 (StatSoft Inc.) software was used for all the statistical evaluations. Data visualisation was implemented with MS Excel 2016 and R statistical software (R Core Team 2022) using the ggplot2 package (Wickham 2016).

## Results

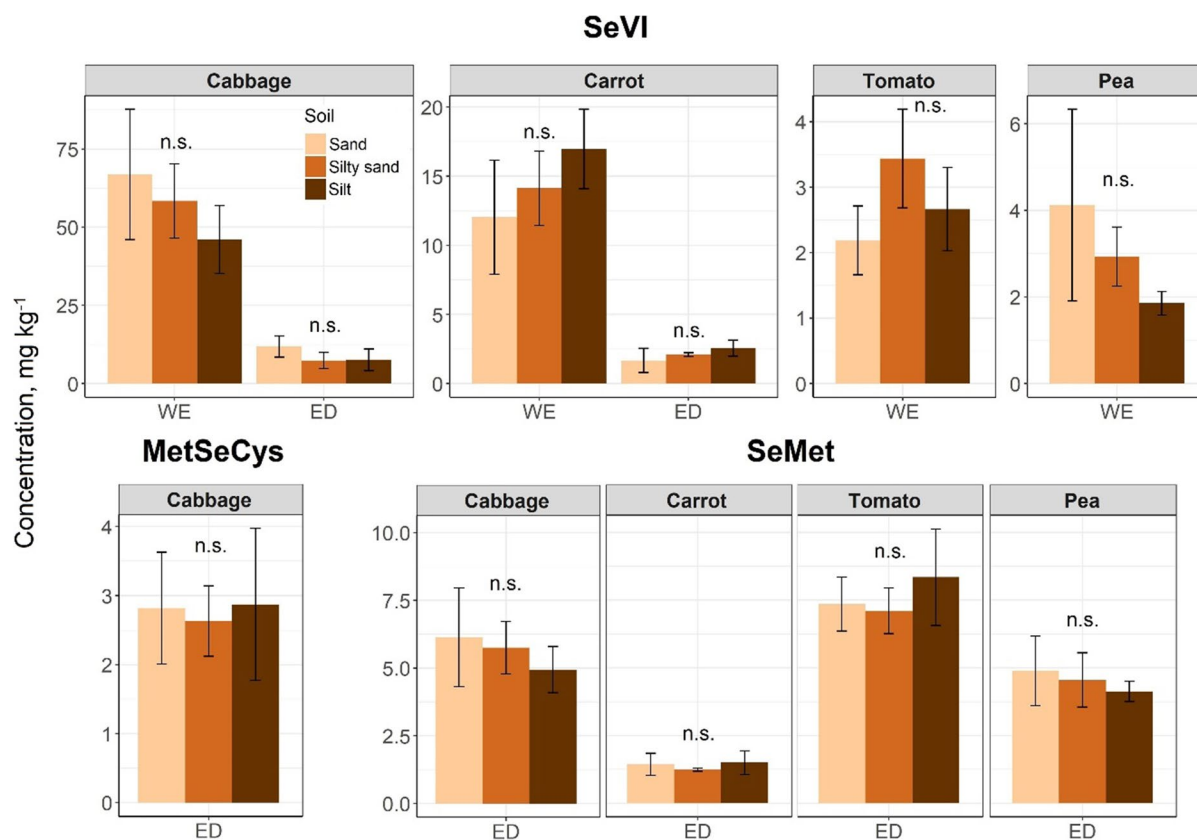
### Total Se content

The soil types used in the present experiment affected the total Se concentration measured after  $\text{HNO}_3\text{-H}_2\text{O}_2$  digestion. The Se concentration of cabbage leaf ( $150 \pm 6 \text{ mg kg}^{-1}$ ) and green pea seed ( $37.1 \pm 6.9 \text{ mg kg}^{-1}$ ) on sandy soil was significantly higher than on silty sand ( $116 \pm 5$  and  $29.0 \pm 3.9 \text{ mg kg}^{-1}$ , respectively) and silt soils ( $119 \pm 9$  and  $24.0 \pm 1.6 \text{ mg kg}^{-1}$ , respectively). The Se concentration of carrot root on silt soil ( $25.5 \pm 1.8 \text{ mg kg}^{-1}$ ) was significantly higher than that measured on sandy soil ( $17.6 \pm 3.2 \text{ mg kg}^{-1}$ ), whereas its concentration on silty sand

( $22.5 \pm 3.9 \text{ mg kg}^{-1}$ ) was not significantly different from that measured on the other two soil types. The Se concentration of tomato fruit was significantly higher on silty sand and silt soils ( $23.5 \pm 1.0$  and  $24.8 \pm 0.5 \text{ mg kg}^{-1}$ , respectively) than on sand ( $21.6 \pm 1.4 \text{ mg kg}^{-1}$ ) (Ragályi et al. 2021, 2022).

Concentrations of SeVI, MetSeCys and SeMet in water extract and enzymatic extract

The concentrations of water-extractable and enzymatic digestion-extractable Se species of vegetables are shown in Fig. 1. The soil type did not cause a significant difference for any of the Se species investigated. SeVI was found in both water extract



**Fig. 1** Water-extractable (WE) and enzymatic digestion-extractable (ED) SeVI, MetSeCys and SeMet species concentrations in vegetables grown on different soil types. In the missing cases, the specific Se species in the given extractant was below the LOQ. LOQ values were: WE-SeMet:  $0.5 \text{ mg kg}^{-1}$ , WE-MetSeCys:  $1.0 \text{ mg kg}^{-1}$  for cabbage; WE-SeMet:  $0.5 \text{ mg kg}^{-1}$ , WE-MetSeCys:  $1.0 \text{ mg kg}^{-1}$ , ED-MetSeCys:

$2.0 \text{ mg kg}^{-1}$  for carrot; WE-SeMet:  $0.5 \text{ mg kg}^{-1}$ , WE-MetSeCys:  $1.0 \text{ mg kg}^{-1}$ , ED-SeVI:  $0.8 \text{ mg kg}^{-1}$ , ED-MetSeCys:  $4.0 \text{ mg kg}^{-1}$  for tomato; and WE-SeMet:  $0.5 \text{ mg kg}^{-1}$ , WE-MetSeCys:  $1.0 \text{ mg kg}^{-1}$ , ED-SeVI:  $0.3 \text{ mg kg}^{-1}$ , ED-MetSeCys:  $1.5 \text{ mg kg}^{-1}$  for pea. Error bars represent the standard deviation of the means ( $n=3$ ). n.s.: no significant difference for different soil types

and enzymatic extract in cabbage and carrot, but only in water extract in tomato and pea. Water-extractable SeVI was found in the highest concentration in cabbage (between 46.0 and 66.9 mg kg<sup>-1</sup>). After enzymatic digestion, additional SeVI was extracted (between 7.35 and 11.8 mg kg<sup>-1</sup>). In the case of carrot roots, the water-extractable SeVI was 12.0–17.0 mg kg<sup>-1</sup>, and after enzymatic digestion, additional SeVI was detected, but only at a much lower concentration (1.67–2.57 mg kg<sup>-1</sup>). SeVI was found in the water extracts of tomatoes in the range of 2.19–3.44 mg kg<sup>-1</sup>, while in pea seeds it reached 1.86–4.12 mg kg<sup>-1</sup> in the water-soluble fraction.

MetSeCys only exceeded LOQ in the enzymatic digestion-extractable fraction of cabbage, with a concentration of between 2.63 and 2.87 mg kg<sup>-1</sup>. Figure 2 represents a typical chromatogram of a cabbage leaf extract after enzymatic digestion. MetSeCys is only retained to a low extent in SAX, so its presence must be identified by spiking. When a sample was spiked, the presence of MetSeCys was confirmed. The compound was then analysed by standard addition (Fig. 2b). MetSeCys also appeared in carrot root, tomato fruit and green pea seed, but was below LOQ. Further chromatograms for the water and enzymatic digestion extracts of the vegetables, with short descriptions, can be found in Supplemental Materials, Figs. S4–S8.

SeMet could not be detected in water extract, but exceeded the LOQ in the enzymatic digestion-extractable fraction of all four vegetables. Its concentration was 4.94–6.13 mg kg<sup>-1</sup> in cabbage,

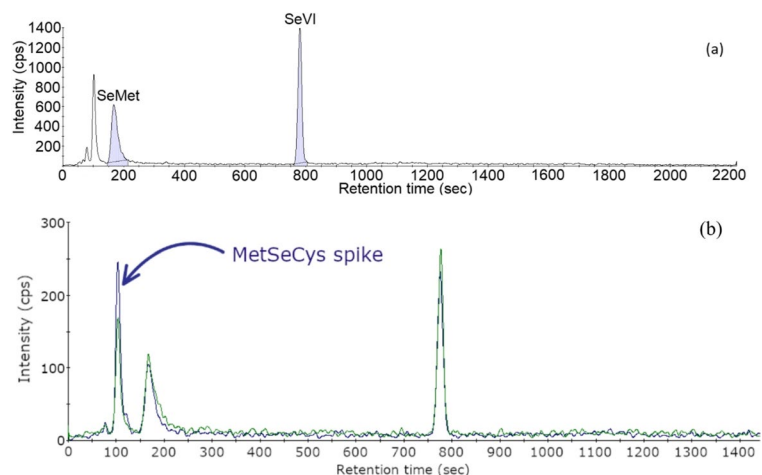
1.23–1.50 mg kg<sup>-1</sup> in carrot, 7.10–8.35 mg kg<sup>-1</sup> in tomato and 4.13–4.89 mg kg<sup>-1</sup> in pea (Fig. 1).

The enzymatic extracts of pea were analysed using post column isotope dilution analysis (HPLC-(IDA)-ICP-MS) instead of standard addition due to matrix (probably starch-based) interferences. A typical chromatogram for enzymatically digested pea is presented in Fig. 3a, while the mass flow of Se is illustrated in Fig. 3b for the same injection.

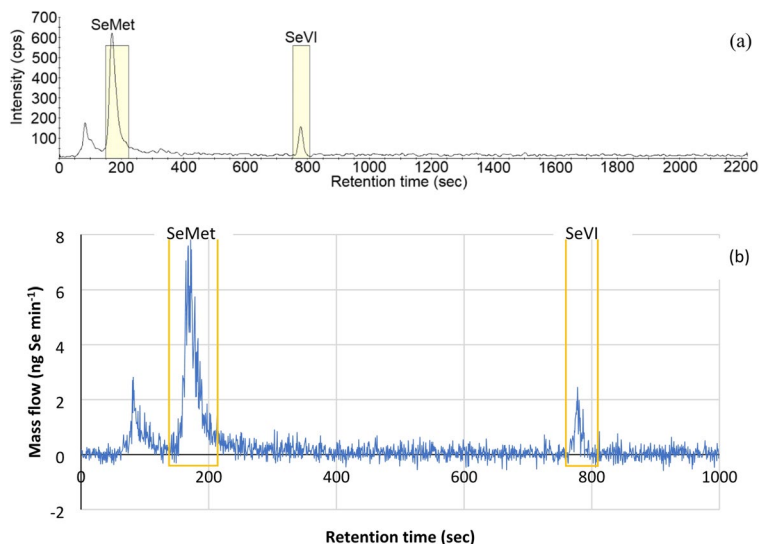
### Concentrations of elemental and residual Se

The combined concentrations of water-soluble and enzymatic digestion-extractable Se species in tomato fruits were 9.54–11.04 mg kg<sup>-1</sup>, about three quarters (67.6–77.3%) of which were SeMet and the remainder SeVI (22.7–32.4%). However, 55.1–55.9% of the total Se content was not detectable. Also, in peas, the concentration of quantified species was 5.98–10.07 mg kg<sup>-1</sup>, which was only 25.0–26.9% of the total Se content determined in samples digested with HNO<sub>3</sub>-H<sub>2</sub>O<sub>2</sub>. Therefore, the analyses were continued, and elemental Se, an often dismissed form, was quantified in plant residues after water extraction and enzymatic digestion. The 1.0 M Na<sub>2</sub>SO<sub>3</sub>-extractable Se was identified as elemental Se. A relatively high amount (5.37–6.17 mg kg<sup>-1</sup>) of elemental Se was found in tomato fruit, whereas in pea it was much lower, between 1.33 and 2.30 mg kg<sup>-1</sup>. However, in the case of pea, there was a significant difference between the soil types. The elemental Se concentration of pea grown on sand soil was significantly

**Fig. 2** A typical HPLC-ICP-MS chromatogram from the extract of a cabbage leaf after enzymatic digestion (injection volume: 20  $\mu$ L) (a), and zoomed to the peaks after spiking the extract with MetSeCys (injection volume: 5  $\mu$ L) (b), recorded on <sup>78</sup>Se isotope



**Fig. 3** A typical HPLC-ICP-MS chromatogram for the extract of pea after enzymatic digestion recorded on  $^{78}\text{Se}$  isotope (a) and the mass flow of Se determined by isotope dilution analysis (b). The concentrations of MetSeCys and SeVI did not exceed the LOQ level



higher than on silty sand and silt. The concentration of residual Se was between 5.73 and 8.67 mg kg<sup>-1</sup> in tomato, and between 0.433 and 0.733 mg kg<sup>-1</sup> in pea (Fig. 4).

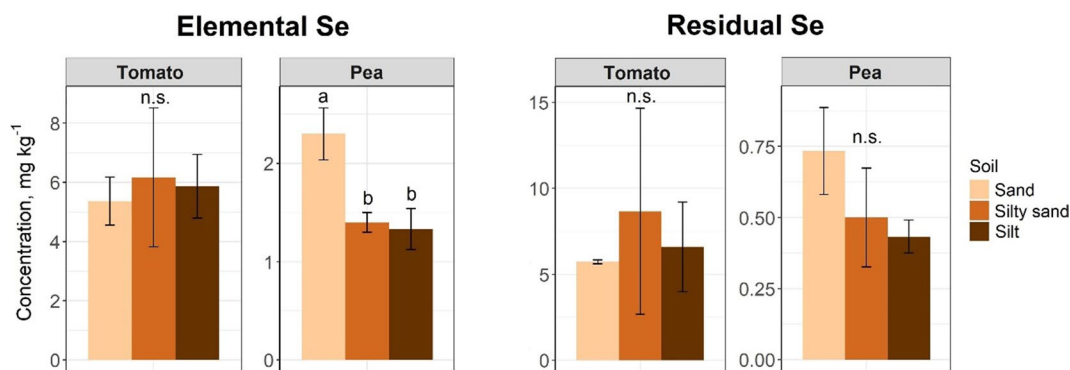
#### Ratios of Se species and concentration in fresh vegetables

After water extraction and enzymatic digestion, the combined concentration of soluble Se species in cabbage was 61.5–87.6 mg kg<sup>-1</sup>, depending on the soil type. This concentration range accounted for 51.6–63.7% of the total Se, whereas other fractions, which were not soluble using the methods applied,

accounted for 36.3–48.4% (Fig. 5). SeVI made up the largest part with 45.0–56.5%, while the organic SeMet and MetSeCys species together made up 5.98–7.21%.

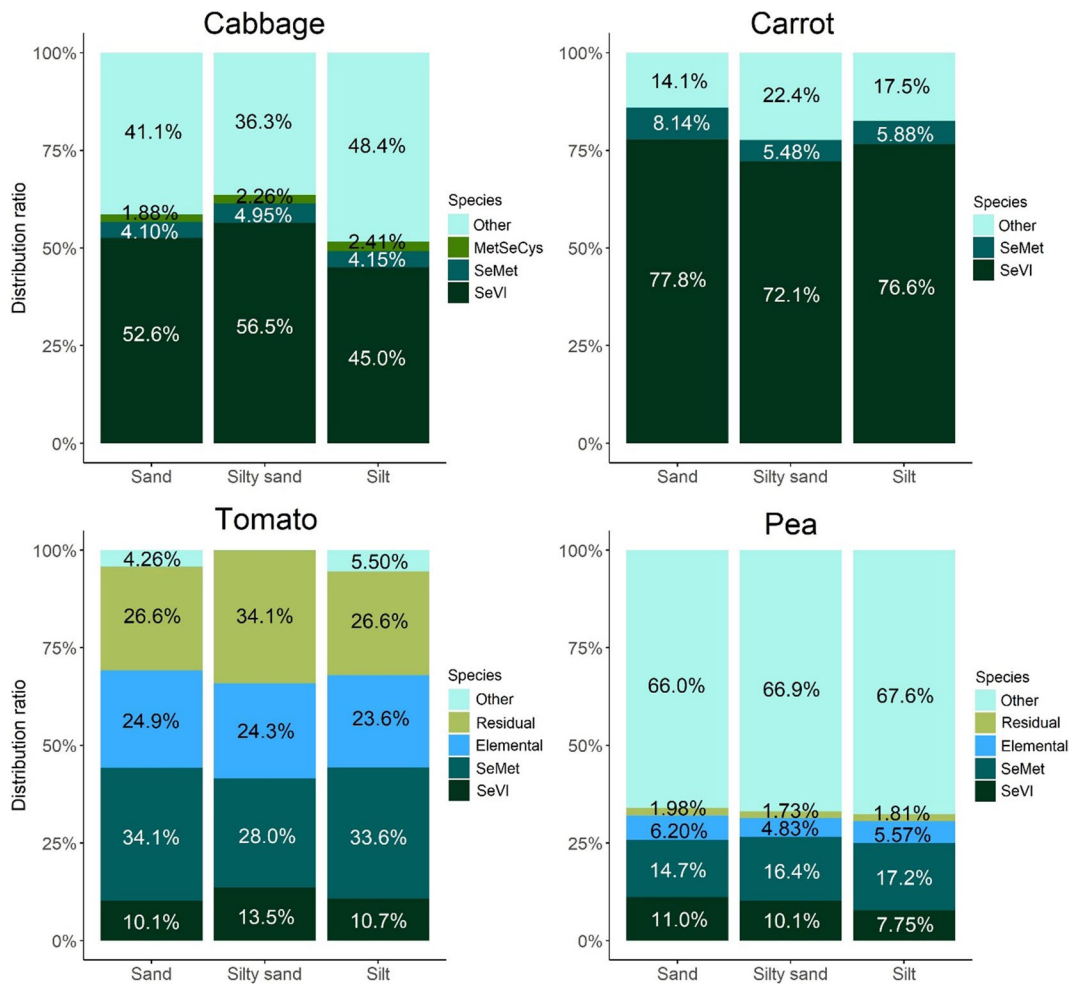
In carrot, the combined concentration of soluble Se species in the water extract and enzymatic extract was 13.7–19.5 mg kg<sup>-1</sup>, which was about three-quarters (77.9–84.0%) of the total Se content. The overwhelming majority, about 90.2–92.9%, of the extractable Se species was inorganic, namely SeVI, while SeMet accounted for the rest.

After the identification of the elemental and residual Se fractions in tomato, 94.5–100% recovery was reached, which was the highest for any of the vegetables studied. The proportions of SeMet and residual Se were in a similar range (28.0–34.1% and 26.6–34.1%, respectively,



**Fig. 4** Elemental and residual Se species concentrations of tomato fruit and pea grown on different soil types. Error bars represent the standard deviation of the means ( $n=3$ ). Different

lower case letters indicate significant differences for different soil types. n.s.: no significant difference between soil types



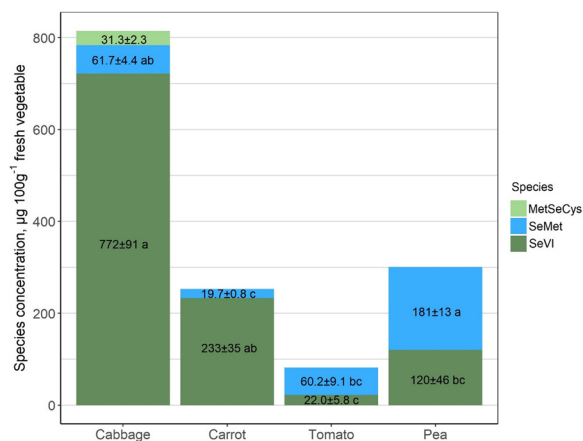
**Fig. 5** Species ratios compared to the total Se content of vegetables grown on different soil types

of the total Se content), and a relatively high proportion (23.6–24.9%) was found in elemental Se form in tomato fruit, while the SeVI ratio was lower (10.1–13.5%).

In peas, the mass balance was still not complete after analysing the water extract, enzymatic extract, elemental and residual fractions. The combined species concentrations obtained were only 32.4–34.0% of the total Se. Among the species identified, SeMet was dominant with 14.7–17.2% of total Se, followed by SeVI with 7.75–11.0%, elemental Se with 4.83–6.20% and residual Se with 1.73–1.98% (Fig. 5). It should be noted that after the centrifugation of the water extract, a starch deposit was observed in the clear supernatant. Speciation analysis was done on a filtered extract prepared almost immediately before injection. In contrast, digestion

was performed on a homogenised portion of the supernatant (containing the deposit).

The soil type had no significant effect on the amount of Se species found in 100 g fresh weight of the edible part of the plant for any of the species, so the data are presented as the average of the three soil types (Fig. 6). Differences between the vegetables, on the other hand, were significant. MetSeCys was only present in measurable amounts in cabbage. SeMet was found in the highest amount in fresh green peas, and this was significantly higher than in tomato fruit or carrot root. The second highest amount was found in cabbage, which was significantly higher than carrots, but not significantly different from tomatoes or peas. The amount of SeMet in tomatoes was similar to that in cabbage. The SeVI content in 100 g fresh



**Fig. 6** Concentrations of water-extractable (WE) and enzymatic digestion-extractable (ED) Se species in 100 g of fresh vegetables, averaged over soil types ( $n=9$ ). Different letters indicate significant differences between vegetables for each Se species separately. MetSeCys was only detected in cabbage

edible part of cabbage was significantly higher than that in tomatoes and peas. The second highest SeVI content was found in carrots, which was significantly higher than in tomatoes.

## Discussion

For several reasons, it is rather difficult to interpret the results of Se speciation analyses in the context of results from other experiments. On the one hand, there are relatively few reports of Se speciation studies, and even fewer discuss the analysis of the vegetables examined in this experiment. On the other hand, even if the same plant species is examined, several other factors also have an influence, for example, the form of Se (SeIV or SeVI) used for biofortification (Poblaciones et al. 2014a; Kápolna et al. 2009). The level of Se enrichment, that is, the Se concentration achieved in the biofortified plant, also has an influence on the ratio of Se species (Poblaciones et al. 2014a; Garousi et al. 2017). The extraction technique, extraction agent, the amount of enzyme (Mazej et al. 2006; Luo et al. 2022) and the incubation time (Cuderman et al. 2008) also largely determine the results (Montes-Bayón et al. 2006). Sánchez-Rodas et al. (2016) found considerable differences between the devices used for analysis. Different cultivation conditions (soil vs. hydroponics,

root uptake vs. leaf spraying) may also lead to different results (Maneetong et al. 2013).

In the present experiment, the water- and enzyme-soluble Se species accounted for 57.6%, 81.4%, 44.4% and 26.0% of all Se in the case of cabbage, carrots, tomatoes and green peas, respectively (not including the elemental and residual fractions of tomatoes and peas). A similar result was obtained for cabbage by Mechora et al. (2012), with 60% solubility after digestion with the enzyme protease XIV. Cuderman et al. (2008) found that soluble Se species accounted for 51–68% of the total Se content in potatoes. The higher proportion of the insoluble form might be the result of a detoxification mechanism, such as binding to phenolic substances (Vogrinčič et al. 2009). Water extraction extracted 44.2, 65.4, 11.8 and 10.7% of total Se from cabbage, carrots, tomatoes and green peas, respectively. Enzymatic digestion was therefore the most efficient in the case of tomatoes, where it was able to extract an additional 32.6%, while in the case of the other three plants, it only contributed 13.4–16.0% to the extraction efficiency. Stadlober et al. (2001) reported a greater increase: in the cereals investigated, water extracts could only identify 3–9% of the total Se content, while with enzymatic digestion this ratio increased to 80–95% due to the extraction of seleno-species from the protein matrix. In the case of peas, the proportion of soluble species after water extraction and enzymatic digestion was the lowest. One possible explanation would be the loss of late eluting species, but in the present case, an inherently long separation lasting 36 min was chosen. Compared to methods involving shorter gradients such as those described by Kowalska et al. (2020) and Luo et al. (2022), this gradient would have recovered late eluting analytes, even if long gradients might cause flattening of the peaks. Another possible explanation is the high starch content of the sample. The missing amount of Se could have been absorbed into the starch deposit and/or irreversibly retained (precipitated) on the column. Indeed, the complementary application of amylase enzymes would not generally provide adequate results because of either low recovery (<35% for SeMet, according to Cuderman et al. 2008) or their inherently high Se content, which biases the accuracy of the results (Cuderman and Stibilj 2009).

In the case of cabbage, SeVI was dominant both in water and enzymatic extracts (Fig. 1). Similar results

were obtained by Slekovec and Goessler (2005) after treatment with 20 mg Se m<sup>-2</sup> in the form of Na-selenate, which increased the Se content of cabbage leaves to 12 mg kg<sup>-1</sup>. The 9:1 methanol:water extraction resulted in an extraction efficiency of only 20%, after which approximately 90% of the total Se content was found in SeVI form. In contrast, Mechora et al. (2012) detected an increase in Se concentration to 4.77 mg kg<sup>-1</sup> in Na-selenate treated cabbage leaves, in which SeMet represented 13% of total Se, whereas SeVI and SeIV represented only 3.0 and 2.9%, respectively, after protease XIV extraction. The higher average SeVI rate of 88% in the present experiment was possibly due to the much higher (128 mg Se kg<sup>-1</sup>) Se content in cabbage leaf as a result of the treatment (Ragályi et al. 2021). In the current experiment, MetSeCys was practically only detected in cabbage, confirming that this Se species is typical of the *Brassicaceae* family. The presence of MetSeCys was confirmed after spiking, which is a very important step in species assignment when only element- (that is, Se-) specific detection is addressed, as retention times might shift because of matrix effects and column load (Kápolna et al. 2007). The need for enzymatic sample preparation to extract non-proteinaceous, that is, non-protein-bound methylated Se species, was previously reported for selenised bean and mushroom samples (Shao et al. 2014; Egressy-Molnár et al. 2016) and was proved here for cabbage as well; indeed, the decomposition of sample matrices is important to achieve adequate species recovery even for theoretically water-soluble compounds. Although SeVI treatment mostly increases the Se content of the plant more effectively than Se IV treatment, since SeVI is quickly translocated to the above-ground organs, most of the Se taken up in this way tends to remain in SeVI form, and it may happen that only a small proportion is converted into SeMet or other organic forms (Zayed et al. 1998). Generally, the relatively high SeVI concentration in plants can be explained by the fact that it is chemically similar to SO<sub>4</sub><sup>2-</sup>, so the sulphate transporter can mediate the uptake of both anions through the root plasma membrane. The reduction of SeVI in the plant by the enzyme ATP sulphurylase is the first step in the conversion to organic Se, but this is energy-demanding, and ATP sulphurylase is a rate-limiting factor (Pilon-Smits et al. 1999; Terry et al. 2000). Thus, in cases of high SeVI application, this species may be taken up

by the plant in large amounts, but little of it may be converted, so SeVI accumulates.

SeVI was also dominant in carrots, particularly in the case of water extraction, while enzymatic extraction released additional SeVI and an almost equal amount of SeMet (Fig. 1). Different results were obtained by Bañuelos et al. (2015), who found no significant increase in the recovery percentage of SeMet after the enzymatic digestion of carrot roots, in which the total Se content was 2.5 mg kg<sup>-1</sup>. Kápolna et al. (2009) applied SeVI to carrots in the form of a leaf spray. After digestion with proteolytic enzymes, Se mainly occurred in the form of SeMet and  $\gamma$ -glutamyl-selenomethyl-selenocysteine, and the SeVI concentration was only half that of SeMet. The different results may be due to the different mode of application (leaf spraying), since the reduction and conversion of SeVI into organic forms occur inside the chloroplasts in the shoots.

Although the Se biofortification of tomatoes has been investigated, few studies have been made on the Se speciation of ripe tomatoes. Asher et al. (1977) studied the transport of Se across the tomato root, Schiavon et al. (2013) reported the effect of Se on the chemical composition of tomato, and Brummell et al. (2011) investigated the effect of Se treatment on the MetSeCys production of transgenic tomatoes. Based on the results, the Se biofortification of tomatoes appears to have a positive effect, since in the present work, when four vegetable crops were treated under the same conditions, peas and tomatoes contained the highest ratios of organic Se (Fig. 5).

The water extract of green peas only contained SeVI, but the amount of SeMet extracted by enzymatic digestion was even higher (Fig. 1). A similar result was obtained by Garousi et al. (2017), who identified SeMet as the main component in green pea seed after enzymatic hydrolysis. Smrkolj et al. (2006) investigated plants grown from Se-enriched pea seeds. SeMet was the major Se species, but a higher SeMet ratio was reached using the lower Se treatment in the leaves and the higher Se treatment in the seeds. The composition of Se species was found to vary not only with the plant parts but also with plant maturity. In contrast, Yoshida et al. (2005) found the major part of Se in the form of SeVI in the pods and leaves of SeVI-treated green peas, while SeMet was only present in a minor proportion. The difference may be due to the fact that pea had a rather high total Se content

of 68 mg kg<sup>-1</sup>, while in the present experiment it was only 30 mg kg<sup>-1</sup> (Ragályi et al. 2022). Poblaciones et al. (2014a) applied SeVI as foliar spray in a field experiment. Interestingly, as the Se treatment level increased, the proportion of SeVI decreased and the proportion of SeMet increased in the chickpea test plant. About 84–91% of Se was found in the form of SeMet, but at the same time, the occurrence of SeVI (4–7%) was also significant. In the Se speciation analysis, the proportion of Se recovered was 57–67% of the total Se content.

The results of the present experiment show that the accumulation of SeVI is typical in the vegetative organs (cabbage leaf and carrot root) while that of SeMet is mainly observed in the reproductive organs (tomato fruit and green pea seed) (Fig. 5). This may be related to the transport and transformation of Se in plants. As mentioned earlier, the absorption of SeVI and its translocation in the plant takes place with the help of sulphate transporters, so the vegetative plant organs are able to accumulate SeVI. By following the S assimilation pathway, the SeVI can be further transformed into Se-amino acids, which predominantly takes place in the plastids in the leaves and result in the formation of SeMet (Pilon-Smits et al. 1999). Sulphur-containing methionine derivatives can be translocated through the phloem to the reproductive organs, where they are released for development and storage (Bourgis et al. 1999), as was also demonstrated in green peas (Tan et al. 2010). Assuming that the pathways of secondary Se metabolites in plants are analogous to their sulphur counterparts (White 2018), SeMet may also have followed this pathway in the present experiment, possibly explaining their higher ratio in the reproductive organs of tomatoes and green peas.

In 100 g of fresh edible parts, the largest amount of detectable Se species was found in cabbage leaves, but the highest amount of organic Se, in the form of SeMet, was found in green peas, a significantly higher amount than in carrots or tomatoes (Fig. 6). Tomatoes, on the other hand, contained an extremely high, nearly three-fold ratio of organic Se compared to inorganic Se. When evaluating the effectiveness of Se species in terms of increasing Se levels in humans or animals, several aspects must be taken into account. In general, selenium is absorbed efficiently, but organic Se forms are more efficient in increasing the Se content of the human or animal

body than inorganic forms (Steen et al. 2008). The uptake of inorganic forms also proved effective, especially in the case of SeVI, since 50 and 91% of SeIV and SeVI, respectively, was absorbed from a Se-fortified milk-based formula (Dael et al. 2001). At the same time, the organic forms of selenium, such as SeMet, are retained better in tissues, because they are incorporated nonspecifically into proteins, making them instrumental in Se storage (Huerta et al. 2004). MetSeCys has been investigated more widely for its inhibition of carcinogenesis (Whanger 2002), but this species has to be metabolised to be used for selenoprotein formation or excretion, so in the case of overload, this compound and its potential metabolites might cause cytotoxicity (Marschall et al. 2016). According to Fairweather-Tait et al. (2010), it is not possible to quantitatively evaluate the effectiveness of different forms of Se in terms of bioavailability and retention, because of the complexity of the foods containing them. The typical consumption method of the biofortified food might also be an important aspect. The cooking or salting of fish did not affect Se absorption or retention (Fox et al. 2004), and in wheat, only a small reduction in SeMet content was detected after processing and bread making (Hart et al. 2011). However, heat processing involving pressure-cooking or microwave cooking significantly reduced the bioaccessibility of Se in cereals (14.8–55% and 8–51%, respectively), pulses (7.6–35.1% and <43%, respectively) and green leafy vegetables (<64% and <52%, respectively) (Khanam and Platel 2016). These results justify the biofortification of tomatoes, which are widely consumed raw.

Elemental Se has different bioavailability compared to other inorganic forms (Kojouri et al. 2012), while its toxicity differs not only from that of other inorganic Se forms, but also from that of SeMet (Wang et al. 2007). The occurrence of elemental Se in living organisms can be linked to either bacterial activity (Tomei et al. 1995; Oremland et al. 1989; Kessi et al. 1999) or possible Se detoxification (Both et al. 2018; Pilon-Smits 2019), which may take place via several not yet elucidated pathways. According to Kim et al. (2003), who detected elemental Se in barley grain, SeIV is probably reduced by thioredoxin to selenide, which in turn is autoxidised to elemental Se. Van Hoewyk et al. (2005 and 2008) found that NifS-like proteins with selenocysteine lyase activity catalyse the breakdown of SeCys into alanine and

elemental Se, as demonstrated in *Arabidopsis thaliana*. Lindblom et al. (2013) reported that elemental Se was the second most abundant Se form in field-collected roots of *Astragalus bisulcatus* and *Stanleya pinnata*, which was attributed to the microbial activity of endosymbionts including nitrogen-fixing bacteria and endophytic fungi or bacteria. Based on the studies of Valdez Barillas et al. (2012) with *Astragalus bisulcatus*, elemental Se accounted for 11–31% of the Se species identified in the root, 34% in the stem, 7% in the flower and 20% in the cotyledons of the infected seed embryo. Both et al. (2018) found that in the Se hyperaccumulator plant *Cardamine violifolia* (*Brassicaceae*), inorganic Se was mostly present in the form of elemental Se. To the best of our knowledge, elemental Se has not yet been reported in the edible parts of vegetables biofortified with SeVI.

In order to evaluate the results and the success of biofortification, it is important to note that the fresh biomass of cabbage head and tomato fruit decreased non-significantly by 12 and 9%, respectively, in the Se treatment compared to the control (which were not subject to Se speciation analysis), but the dry matter content increased significantly in both plants, so dry biomass production changed only slightly. The yield of green peas exhibited practically no change, while the fresh biomass of carrot root decreased non-significantly by 10% and the dry biomass significantly by 20% (Ragályi et al. 2021, 2022). Based on these data, carrots are less able to tolerate higher Se content, while the cultivation of green peas is very promising on soils with higher Se supplies.

## Conclusions

This work aimed to present and evaluate the Se species composition of vegetable plants widely consumed by the human population and treated under identical conditions. As regards the total amount of Se species recovered, cabbage contained by far the most Se due to its inherent ability to accumulate this element as a member of the *Brassicaceae* family, so cabbage biofortification can be considered the most effective. At the same time, the concentration of the more bioavailable SeMet was the highest in the dry matter of tomatoes, though due to the higher dry matter content, green peas contained the highest amount of SeMet in 100 g of fresh edible plant parts, so the

biotransformation was the most effective for these two vegetables. The Se treatment only caused a significant decrease in biomass in the case of carrots, so the cultivation of cabbage, tomatoes and green peas could be well suited to soils with a high Se content. The different results obtained for Se species draw attention to the fact that the metabolism of Se differs in different plant species. Further research is needed to explore the effect of different doses, forms and sources of Se on the Se speciation in different vegetables in order to make the biofortification process more effective and controlled.

**Author contributions** P.R. contributed to the implementation of the experiment, prepared the manuscript and conducted the statistical data analyses and visualisation; T.T. contributed to the implementation of the experiment and the compilation of Materials and Methods; Á.S. contributed to the compilation of Materials and Methods and Results, and conducted speciation analyses and visualisation; B.K. conducted speciation analyses; M.D. contributed to the speciation analyses and proofread the manuscript; Z.L. contributed to the concept of the study; P.D. was the leader of the project and performed analytical work; G.Z. contributed to the concept of the study and proofread the manuscript; M.R. contributed to the design and implementation of the experiment and proofread the manuscript. All authors have read and approved the published version of the manuscript.

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**Data availability** The datasets analysed in the current study are available from the corresponding author on reasonable request.

## Declarations

**Competing interests** The authors have no relevant financial or non-financial interests to disclose.

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