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Effect of Se-Enriched Irrigation Water and Soil Texture on Biomass Production and Elemental Composition of Green Pea and Carrot and Their Contribution to Human Se Intake

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Abstract: Selenium (Se)-deficient diets are a problem in large areas of the world and can have serious health consequences, thus, the biofortification of foods with Se has been an important research field for several decades. The effect of Se-enriched irrigation water was investigated regarding the Se concentration in green peas and carrots. A pot experiment was set up in a greenhouse with irrigation water containing 0, 100, and 500 µg Se L^{-1} with sand, silty sand and silt soil types. Most of the treatments only slightly reduced the biomass, while the 500 µg Se L^{-1} treatment caused a significant decrease in the dry weight of carrot root. Treatment with irrigation water containing 100 µg Se L^{-1} increased the Se content in green peas and carrots 76 and 75 times, respectively, producing foodstuffs where 100 g of a fresh product covered 395% and 92% of the recommended dietary allowance, respectively, averaged over the three soil types. The Se concentration was higher for green peas in sand and carrots in silt. The treatments had little effect on the concentrations of other nutrients. The enrichment of irrigation water with Se may thus be a suitable method for the biofortification and production of functional food under certain conditions.

Keywords: irrigation; biofortification; selenium; recommended dietary allowance; element content

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1. Introduction

Selenium (Se) is an essential nutrient for humans and animals. Both Se deficiency and excessive Se can lead to the development of diseases. An adequate intake of Se promotes healthy growth and the normal function of the immune system, muscle activity, cardiovascular system and reproductive organs, while also contributing to defense against the spread of certain infections, the inflammatory response and carcinomas [1–3]. In areas with severe Se deficiency, multifocal myocarditis and joint diseases manifested in the form of shorter toes and fingers or dwarfism and may develop in humans, even causing death in extreme cases. Animals may also be affected, often in the form of metabolic diseases and liver damage [4]. In selenium-deficient regions where Kaschin–Beck or Keshan diseases appeared, there was a lower proportion of elderly people in the population, though this was primarily due not to the diseases themselves but to other health conditions caused by Se deficiency [5].

The most important source of Se for humans is food [6]. The Se content of the food crops produced in a given area is highly dependent on the Se supply in the soil. If Se intake is typically low, additional Se intake should be considered. One of the options for Se supplement is biofortification, i.e., the enrichment of crops grown for human consumption [7].

This practice has already been successfully applied on a larger scale in countries with low Se soils, such as Finland [4], the UK [8] and China [9]. The Eastern European region is also characterized by a low supply of Se, as also indicated by the low concentration of Se in the blood of the population, thus, biofortification may be justified in this area as well [10,11].

However, biofortification is influenced by a number of factors. The goal is to achieve an optimal Se concentration in the edible part of the plant. Se is mostly applied in inorganic form. Previous studies have shown that selenate is preferable to selenite because it translocates and accumulates more easily within the plant [12] and its uptake is easier whether applied through the soil [13,14] or as foliar spray [15–17]. When applied through the soil, the effectiveness of Se fertilizers is highly dependent on soil properties, especially aeration [18], pH [19], Fe and Al contents [20] and organic matter content [21], which affect its fate and uptake by the plant. However, the application of Se with irrigation water allows the plant to absorb Se directly, so the influence of the soil on Se availability may decrease. This needs to be confirmed in further investigations, however, because little is yet known about irrigation water enriched with Se for biofortification purposes.

Green peas and carrots are widely grown and consumed almost all over the world. In 2019, the total production was 21.8 and 44.8 million tons on 2.78 and 1.13 million hectares, respectively [22]. Green peas are very rich in protein and essential amino acids, while carrots are high in fiber, carotenoids and sugars, and both vegetables play an important role in human nutrition due to their mineral content [23,24]. Therefore, these vegetables may serve as good target plants for biofortification, especially considering the availability of processed, ready-to-consume products [25]. The effect of Se enrichment has been extensively studied for various plant species, either in hydroponic experiments or in a soil medium [7,26–28]. However, even though both peas and carrots are popular, only a limited number of experiments have focused on the biofortification of these vegetables with Se. Previous research has also covered the potential for enriching green peas and carrots with elements other than Se. Umaly and Poel [29] enriched green peas with iodine in nutrient solution culture, whereas Poblaciones and Rengel [30] studied the possibility of enriching green peas with zinc in a greenhouse pot experiment. The biofortification of carrot with iodine has been investigated in studies in field trials [13], in a greenhouse with a soilless system [31] or in a greenhouse with different soils [32].

Knowledge is particularly scant on the effect of biofortification with Se on the macro and microelement content of these vegetables. To the best of our knowledge, the effect of Se on the element content of carrots has only been reported so far by Oliveira et al. [14]. The present experiment also provides an opportunity to compare the results with the effect of Se-enriched irrigation water on green beans, cabbage, potato and tomato, which was previously studied under similar experimental conditions [33].

The aim of the present experiment was to investigate a possible method for the biofortification of the test plants—green peas and carrots with irrigation water containing Se. It was hypothesized that irrigation water enriched with Se would not affect biomass production but would increase the concentration of Se in the vegetables. Although the plant can absorb Se directly from the irrigation water, it was thought that the soil type might have an effect on the Se concentration in the plants: looser textured soil binds less Se, thus allowing a higher uptake. It was also assumed that Se would not cause a substantial change in the concentration of other nutrients in the plant.

2. Materials and Methods

2.1. Experimental Setup

An open greenhouse experiment was set up to study the effect of Se-enriched irrigation on the pea (*Pisum sativum* L. var. Rajnai törpe) and carrot (*Daucus carota* L. var. sativus cv. Nantes-2) at the Experimental Station of the Center for Agricultural Research in Őrbottyán, Hungary [32,34,35]. The experiment was performed using 10 L pots having four 0.5 cm diameter holes in the bottom to allow leached water to escape. A 1 cm layer of gravel with a diameter of 4–8 mm was placed at the bottom of the pots, which were covered with a fine synthetic fiber fabric to keep the gravel bed separate from the soil. Each pot was filled with 10 kg of soil. The soils were collected from the top 0–20 cm layer at three different locations in Hungary, having distinct properties: sand (Mollic Umbrisol, Arenic) from Őrbottyán, silty sand (Luvic Calcic Phaeozem) from Gödöllő, and silt (Calcic Chernozem) from Hatvan [36]. The basic characteristics of the soils are shown in Table 1.

Table 1. Characteristics of the soils.

Parameter	Sand	Silty Sand	Silt
pH-H_2O	7.96	6.83	7.34
OM (w/w%)	0.91	1.24	2.12
$CaCO_3$ (w/w%)	1.45	0.08	0.20
Total N (w/w%)	0.064	0.092	0.135
NH_4-N (mg kg^{-1})	1.4	2.3	3.9
NO_3-N (mg kg^{-1})	4.7	2.3	14.2
AL-K_2O (mg kg^{-1})	74	174	176
AL-P_2O_5 (mg kg^{-1})	131	238	81
CEC (Na meq/100 g)	9	17	37
Total Se (mg kg^{-1})	0.076	0.094	0.132
Extractable Se (mg kg^{-1})	0.009	0.016	0.010
Water-soluble Se (mg kg^{-1})	<dl	<dl	<dl
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, AL: ammonium-lactate-soluble, CEC: cation exchange capacity, Total: aqua regia-soluble, Extractable: extract according to Lakanen and Erviö, <dl: below the detection limit. Methods are described in Section 2.2. Sample preparation and analyses.

After germination, the carrot seeds were planted in propagation trays filled with a commercially available growth medium (Vegasca Bio soil mix; Florasca Hungary Ltd. (Osli, Hungary)—A mixture of peat and gray cattle manure compost: OM > 50%; N > 0.3%; P_2O_5 > 0.1%; K_2O > 0.1%; pH 6.8). The seedlings were grown for three weeks in a growth chamber under controlled climatic conditions (16/8 h photoperiod, 25–27/15–17 °C day/night temperature and 600 µmol/m^2/s photon flux density). After this period, the seedlings were acclimatized for an additional 6 days in the greenhouse and then transplanted into plastic pots. Green pea seeds were sown directly. For both carrots and green peas, three plants were grown per pot.

The young plants were irrigated with plain water for three weeks after transplantation to allow time for them to strengthen, and the Se-enriched irrigation water was only given to the plants after this period. The experiment included three treatment levels: Se-0: control, Se-1: 100 and Se-2: 500 µg Se L^{-1} in the form of Na_2SeO_4 in the irrigation water applied on three soil types: sand, silty sand and silt, in three replicates. It should be noted that the Se concentrations used for the treatments were well above the 20 µg Se L^{-1} value recommended for irrigation water by FAO [37], which served experimental purposes to remarkably increase the Se concentration of the test plants to allow the evaluation of the effect of provocative treatments on the studied parameters. The irrigation water was stored in 0.5 m^3 tanks, and irrigation was carried out with an automatic irrigation system. Individual drip stakes were placed in each pot, and the daily volume of irrigation water was adjusted according to the requirements of the plants. The irrigation system delivered the set amount of water every day at 7 a.m. Soil moisture content was monitored at a depth of 10 cm every hour with Decagon EC-5 sensors. The characteristics of the growth period and irrigation details are shown in Table 2.

Table 2. Growth period and irrigation parameters of the vegetables.

Parameter	Pea	Carrot
Growth period	7 May–20 June	11 April–4 July
Length of growth period (days)	45	85
Se solution (ml/pot)	8680	11,160
Se load in 100 µg L^{-1} treatment (mg/pot)	0.868	1.116
Se load in 500 µg L^{-1} treatment (mg/pot)	4.34	5.58

Climate data were measured and recorded throughout the growth period and are presented in Table 3. The plants received natural light in the greenhouse. The nutrient requirements of all the treated plants were fulfilled using 200 cm^3 of Hoagland solution per pot, applied weekly by hand during the whole vegetation period. Pesticides (Decis, Bayer, Leverkusen, Germany) were applied whenever necessary.

Table 3. Greenhouse parameters during the growth period of the vegetables.

Parameter	Pea	Carrot
Daytime average temperature (°C)	21.2 ± 7.6	22.4 ± 8.0
Nighttime average temperature (°C)	13.7 ± 6.3	14.6 ± 6.6
Photosynthetically active radiation (W/m^2)	149 ± 91	155 ± 93
Air humidity (%)	74.0 ± 24.6	72.5 ± 24.9
Soil moisture (% v/v)	23 ± 1	23 ± 2

2.2. Sample Preparation and Analyses

After harvest, the plants were washed with deionized water. Subsequently, the plant parts were separated (root, leafy shoot, fruit) and the fresh biomass was weighed. The roots and aerial parts of the plants were dried at 40 °C in a laboratory dryer for two days, except for carrot roots and pea grains, which were milled and freeze-dried at −70 °C, 200 Pa for 72 h in Christ Alpha 1 equipment (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harc, Germany), before measuring the dry mass of the plant organs. Dried samples were homogenized with a blending machine, equipped with plastic housing and a stainless-steel blade. The dried and homogenized plant samples were mineralized in microwave-assisted acid digestion equipment (TopWave, Analytik Jena, Jena, Germany). Plant samples weighing 400–500 mg were digested in a mixture of 7 mL 67% HNO_3 and 3 mL 30% H_2O_2 (VWR International, Radnor, PA, USA). After digestion, internal standards (Sc, Y, In) were added to the solutions with deionized water to make up the volume to 15 mL. The Se, macro and microelement concentrations (As, B, Cu, Fe, I, K, Mg, Mn, P and Zn) were measured in terms of the dry weight (DW) of the plant samples using an inductively coupled plasma mass spectrometer (ICP-MS) (PlasmaQuant Elite, Analytik Jena, Jena, Germany).

Untreated composite soil samples were collected from the soils used for the experiment in order to analyze their basic parameters. After harvesting the plants and removing plant residues, the Se concentrations of the soil samples from each pot were analyzed. The soil samples were dried and sieved through a 2 mm mesh. The soil pH was measured according to the Hungarian standard method [38] in a 1:2.5 soil:water suspension after mixing for 12 h, and the organic matter (OM) content was determined using the modified Walkley–Black method [39]. The Kjeldahl method [40] was used to measure the total N content, and the mineral N (NH_4-N and NO_3-N) concentrations were measured from KCl extracts according to the Hungarian standard [41]. The $CaCO_3$ content was determined using the Scheibler gas-volumetric method [38] and the cation exchange capacity (CEC) values with the modified method of Mehlich [42]. The "total" Se concentrations were determined from the samples using aqua regia in a microwave Teflon bomb [43]. The plant-available P and K fractions were measured after extraction with ammonium acetate-lactate (AL-P_2O_5 and AL-K_2O) [44]. The extractable Se and other macro and microelement contents were measured in 0.5 M NH_4-acetate + 0.02 M ethylenediaminetetraacetic acid (EDTA) extract according to Lakanen and Erviö [45] (referred to as "LE" or "extractable"). The element contents of the soil samples were analyzed using an ICP-MS instrument.

2.3. Statistical Analysis

The data were analyzed using a two-factor factorial analysis of variance, one factor being the Se dose and the other the soil type. The level of significance was set to a 95% confidence interval ($p < 0.05$). Significantly different groups were identified using Tukey's HSD post hoc test. All the statistical calculations were carried out with Statistica v.13 (StatSoft Inc., College Station, TX, USA) software. Data visualization was implemented with R statistical software [46] using the ggplot2 package [47].

3. Results

3.1. Total and Extractable Se Content of Soils

The total and extractable Se concentrations in the soils by the end of the experiment as a result of the Se treatments are shown in Figure 1. Both Se fractions showed a definite increase, which was proportional to the dose used. Regarding the original Se content in the control soils, the total Se content is roughly ten times the extractable content. The selenate applied in the treatments, on the other hand, is an easily extractable form, so 36% of the total Se was extractable in Se-1 treated soils and 80% in Se-2 treated soils.

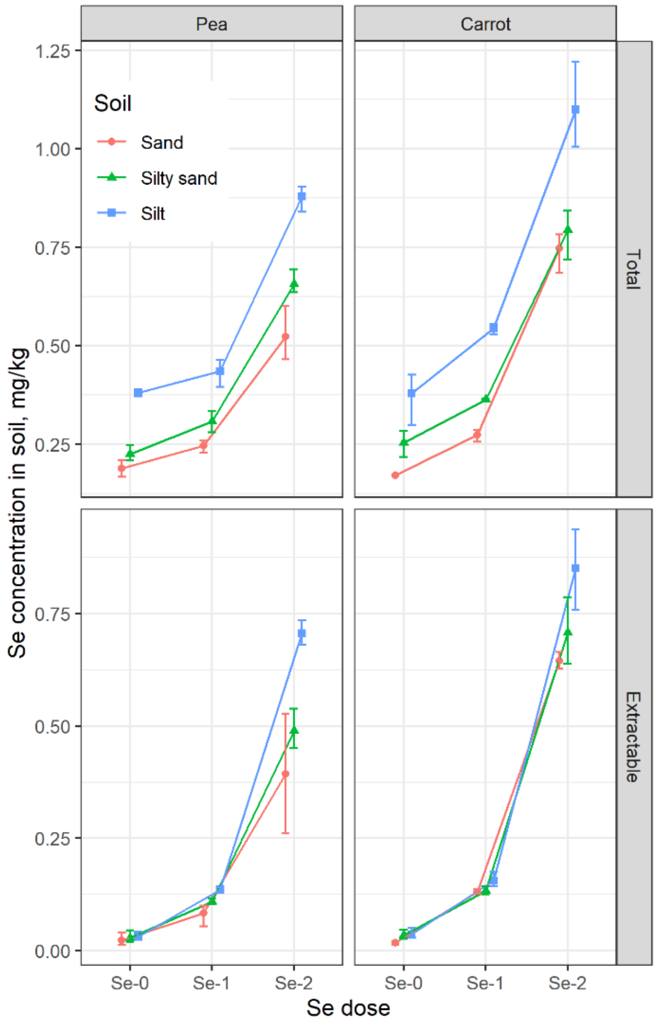

Figure 1. Changes in the total and extractable Se concentrations in the soil as a result of Se treatments. Error bars represent a 95% confidence interval.

3.2. Biomass Production

The effect of the Se treatments on the biomass of edible parts of vegetables grown in each soil type is shown in Table 4. Se treatments had no significant effect on the green pea grain biomass averaged over the soil types. The fresh grain yield on silty sand increased significantly in the Se-2 treatment and was significantly higher than that measured in the other two soil types. At the same time, a decreasing trend was observed for sand as the Se dose increased, while for silt, the Se-1 dose resulted in the highest fresh grain weight, though these changes were not significant. There were no significant differences in the dry weights either. For carrots, the Se-2 treatment had a significant negative effect on root dry weight compared to the control when averaged over the three soils, but this appeared to be mainly due to the low dry weight obtained in the Se-2 treatment on sand and silt soils. Comparing the soil types, both fresh and dry root weights were significantly higher for silty sand than silt soil at all Se levels, and higher than sand in the Se-0 and Se-2 treatments.

Table 4. Effect of Se treatments on the fresh and dry biomass production (g pot^{-1}) and dry matter content (%) of the edible parts of peas and carrots.

Plant	Parameter	Se Dose	Soil Type			Mean
			Sand	Silty Sand	Silt	
Green pea	Grain fresh weight (g)	Se-0	24.7 ± 0.2 aA	23.9 ± 5.0 aA	20.5 ± 4.7 aA	23.0 ± 3.9 A
		Se-1	21.5 ± 3.4 aA	24.5 ± 2.6 aAB	24.3 ± 4.1 aA	23.4 ± 3.3 A
		Se-2	17.4 ± 3.6 aA	29.1 ± 1.9 bB	18.9 ± 1.9 aA	21.8 ± 5.9 A
	Grain dry weight (g)	Se-0	9.60 ± 1.18 aA	10.6 ± 2.6 aA	8.75 ± 2.30 aA	9.65 ± 2.00 A
		Se-1	8.40 ± 2.00 aA	10.5 ± 2.1 aA	9.80 ± 1.30 aA	9.57 ± 1.82 A
		Se-2	6.78 ± 1.41 aA	11.7 ± 1.1 aA	7.66 ± 1.19 aA	8.72 ± 2.54 A
	Grain dry matter content (%)	Se-0	38.9 ± 4.9 aA	44.5 ± 7.2 aA	43.3 ± 10.4 aA	42.3 ± 7.3 A
		Se-1	38.7 ± 5.1 aA	43.1 ± 7.9 aA	40.5 ± 4.1 aA	40.8 ± 5.5 A
		Se-2	39.2 ± 5.5 aA	40.3 ± 1.4 aA	40.3 ± 2.2 aA	39.9 ± 3.1 A
Carrot	Root fresh weight (g)	Se-0	103 ± 23 aA	162 ± 16 bA	86.7 ± 8.1 aA	117 ± 37 A
		Se-1	115 ± 16 abA	141 ± 8 bA	90.8 ± 9.6 aA	116 ± 24 A
		Se-2	85.1 ± 8.4 aA	146 ± 22 bA	83.0 ± 6.7 aA	105 ± 33 A
	Root dry weight (g)	Se-0	17.0 ± 3.9 aA	25.5 ± 2.2 bA	14.3 ± 1.2 aA	18.9 ± 5.6 B
		Se-1	16.4 ± 2.4 abA	20.9 ± 2.8 bA	13.7 ± 1.3 aA	17.0 ± 3.7 AB
		Se-2	11.8 ± 1.1 aA	22.3 ± 3.1 bA	11.1 ± 1.6 aA	15.1 ± 5.7 A
	Root dry matter content (%)	Se-0	16.5 ± 0.9 aA	15.8 ± 1.2 aA	16.5 ± 1.5 aA	16.3 ± 1.1 B
		Se-1	14.3 ± 0.5 aA	14.8 ± 1.8 aA	15.1 ± 0.8 aA	14.7 ± 1.1 A
		Se-2	14.0 ± 1.8 aA	15.3 ± 0.2 aA	13.4 ± 1.5 aA	14.2 ± 1.4 A

Means ± std. dev., lower case letters indicate significant differences between columns (soil types) and capitals between rows (Se doses) (Tukey $HSD_{5\%}$).

3.3. Se Concentration in Plants

The Se concentrations in both species were markedly increased by Se treatments (Table 5). In the pea grain, a 46-fold increase in Se content was measured in the Se-1 treatment and a 254-fold increase in the Se-2 treatment compared to the control averaged over the three soil types. In the shoot, the increments were higher, 72-fold and 464-fold, respectively, but the highest Se concentration was found in the root, averaged over the three soils. Among the soil types, green peas had the highest Se content in sand; the Se-2 treatment resulted in significantly higher values in all three plant parts compared to silt, and in the root and grain compared to silty sand. In carrot root, the Se-1 treatment caused a 45-fold increase, and the Se-2 treatment, a 281-fold increase compared to the control, averaged over the three soil types. In the shoot, the degree of enrichment was practically the same (45-fold and 280-fold, respectively); however, the Se concentration was almost twice that of the root. Among the soil types, a significant difference was only found in the Se content of the root in the Se-2 treatment, where the value was higher for silt than for sand.

Table 5. Se concentrations in green peas and carrots as a function of Se treatments and soil types, mg·kg^{-1} DW.

Plant	Parameter	Se Dose	Soil Type			Mean
			Sand	Silty Sand	Silt	
Green pea	Grain	Se-0	0.101 ± 0.012 aA	0.146 ± 0.041 aA	0.108 ± 0.034 aA	0.118 ± 0.035 A
		Se-1	6.37 ± 0.61 aA	4.92 ± 0.55 aA	4.89 ± 0.61 aA	5.39 ± 0.90 B
		Se-2	37.1 ± 6.9 bB	29.0 ± 3.9 aB	24.0 ± 1.6 aB	30.0 ± 7.0 C
	Shoot	Se-0	0.118 ± 0.031 aA	0.130 ± 0.033 aA	0.068 ± 0.011 aA	0.106 ± 0.037 A
		Se-1	10.7 ± 3.0 aA	7.66 ± 1.48 aA	4.59 ± 0.92 aA	7.65 ± 3.17 B
		Se-2	58.9 ± 8.8 bB	60.0 ± 3.4 bB	28.9 ± 7.8 aB	49.2 ± 16.5 C
	Root	Se-0	0.889 ± 0.069 aA	0.456 ± 0.081 aA	0.375 ± 0.085 aA	0.574 ± 0.249 A
		Se-1	20.3 ± 3.0 aB	10.1 ± 1.7 aB	8.83 ± 7.17 aA	13.1 ± 6.8 B
		Se-2	98.9 ± 8.4 bC	49.7 ± 4.4 aC	48.1 ± 15.5 aB	65.5 ± 26.6 C
Carrot	Root	Se-0	0.062 ± 0.017 aA	0.110 ± 0.022 aA	0.061 ± 0.006 aA	0.078 ± 0.028 A
		Se-1	3.36 ± 0.17 aA	2.91 ± 2.39 aA	4.17 ± 0.49 aA	3.48 ± 1.34 B
		Se-2	17.6 ± 3.2 aB	22.5 ± 3.9 abB	25.5 ± 1.8 bB	21.9 ± 4.4 C
	Shoot	Se-0	0.086 ± 0.020 aA	0.171 ± 0.074 aA	0.153 ± 0.047 aA	0.136 ± 0.059 A
		Se-1	5.00 ± 1.06 aA	6.68 ± 1.00 aA	6.66 ± 0.73 aA	6.11 ± 1.17 B
		Se-2	37.5 ± 7.9 aB	39.9 ± 7.5 aB	37.0 ± 7.6 aB	38.1 ± 6.8 C

Means ± std. dev., lower case letters indicate significant differences between columns (soil types) and capitals between rows (Se doses) (Tukey $HSD_{5\%}$).

3.4. Se Content in Fresh Edible Parts in Relation to the Recommended and Toxic Se Intake

The amount of Se ingested with 100 g of fresh vegetables is shown in Table 6. The Se content in green pea grains was 43 times higher and in carrot roots 40 times higher in the Se-1 treatment than in the control, while the differences were 235 and 248-fold in the Se-2 treatment, respectively, averaged over the three soils. In green peas, however, the amount of Se was more than four times higher than that in carrot in the Se-1 treatment and almost four times higher in the Se-2 treatment.

Table 6. Se content in edible parts * of vegetables, µg 100 g^{-1} fresh weight.

Plant	Se Dose	Soil Type		
		Sand	Silty Sand	Silt
Green pea	Se-0	3.89 ± 0.19 aA	6.70 ± 2.78 aA	4.66 ± 1.68 aA
	Se-1	245 ± 13 aA	209 ± 22 aA	197 ± 26 aA
	Se-2	1451 ± 329 bB	1170 ± 171 abB	967 ± 118 aB
Carrot	Se-0	1.02 ± 0.24 aA	1.73 ± 0.38 aA	1.01 ± 0.19 aA
	Se-1	47.8 ± 1.9 aA	41.1 ± 33.0 aA	63.2 ± 8.8 aA
	Se-2	247 ± 59 aB	344 ± 55 bB	340 ± 30 bB

Means ± std. dev., lower case letters indicate significant differences between columns (Se doses) and capitals between rows (soil types) (Tukey $HSD_{5\%}$); * green pea grains, carrot roots.

The consumption of 100 g of fresh green pea grains treated with Se-1 would cover approximately four times the daily recommended dietary allowance (RDA), while this proportion was more than 20 times, averaged over the three soils, in the Se-2 treatment. In contrast, the Se content of 100 g carrot produced in the Se-1 treatment was around the recommended daily intake, while the higher dose gave values 4.5–6 times higher than RDA (Table 7).

Table 7. Amount of Se ingested with 100 g of fresh edible vegetable parts as a percentage (%) of the recommended dietary allowance (RDA) *.

Plant	Se Dose	Soil Type Sand	Silty Sand	Silt
Green pea	Se-0	7.07 ± 0.34	12.2 ± 5.0	8.48 ± 3.06
	Se-1	446 ± 23	381 ± 40	359 ± 47
	Se-2	2638 ± 598	2127 ± 311	1759 ± 214
Carrot	Se-0	1.85 ± 0.44	3.15 ± 0.70	1.85 ± 0.34
	Se-1	87.0 ± 3.5	74.8 ± 60.0	115 ± 16
	Se-2	449 ± 108	626 ± 101	619 ± 54

* Based on 55 µg Se RDA in the EU, USA and Canada [48].

Table 8 compares the Se content of 100 g of fresh vegetables with the tolerable upper intake level (UL), which is practically equivalent to the hazard quotient (HQ), expressed as a percentage. In the Se-1 treatment, green peas reached nearly half of the UL and carrots about 10%. In the Se-2 treatment, this ratio was more than two and a half times the UL for peas and 69% of the UL for carrots, averaged over the three soils.

Table 8. Amount of Se ingested per 100 g of fresh edible vegetable parts as a percentage (%) of the tolerable upper intake level (UL) *.

Plant	Se Dose	Soil Type Sand	Silty Sand	Silt
Green Pea	Se-0	0.864 ± 0.042	1.49 ± 0.62	1.04 ± 0.37
	Se-1	54.4 ± 2.8	46.5 ± 4.9	43.9 ± 5.7
	Se-2	322 ± 73	260 ± 38	215 ± 26
Carrot	Se-0	0.226 ± 0.054	0.385 ± 0.085	0.226 ± 0.042
	Se-1	10.6 ± 0.4	9.14 ± 7.34	14.0 ± 2.0
	Se-2	54.9 ± 13.2	76.5 ± 12.3	75.6 ± 6.6

* Based on 450 µg Se UL in the EU and UK [48].

3.5. Concentrations of Other Elements in Edible Parts

Figure 2 shows that in green pea grain, P and Fe tended to increase in parallel with the Se treatments, though only P increased significantly in silt and Fe in silty sand in the Se-2 treatment. K increased very slightly in most cases. In contrast, Zn decreased non-significantly in sand and silt soils. In carrot root, there was a significant increase in Zn for silty sand and silt in the Se-1 treatment, but most of the other elements, including Mg and Cu, only increased slightly in the Se-1 treatment. The As, B, I and Mn contents were not correlated with the treatments, so no detailed description of these elements is given. On average, the edible parts of green peas and carrots contained 0.003 and 0.014 mg kg^{-1} As; 11.1 and 13.9 mg kg^{-1} B; 0.015 and 0.013 mg kg^{-1} I; and 14.8 and 8.26 mg kg^{-1} Mn, respectively, in DW.

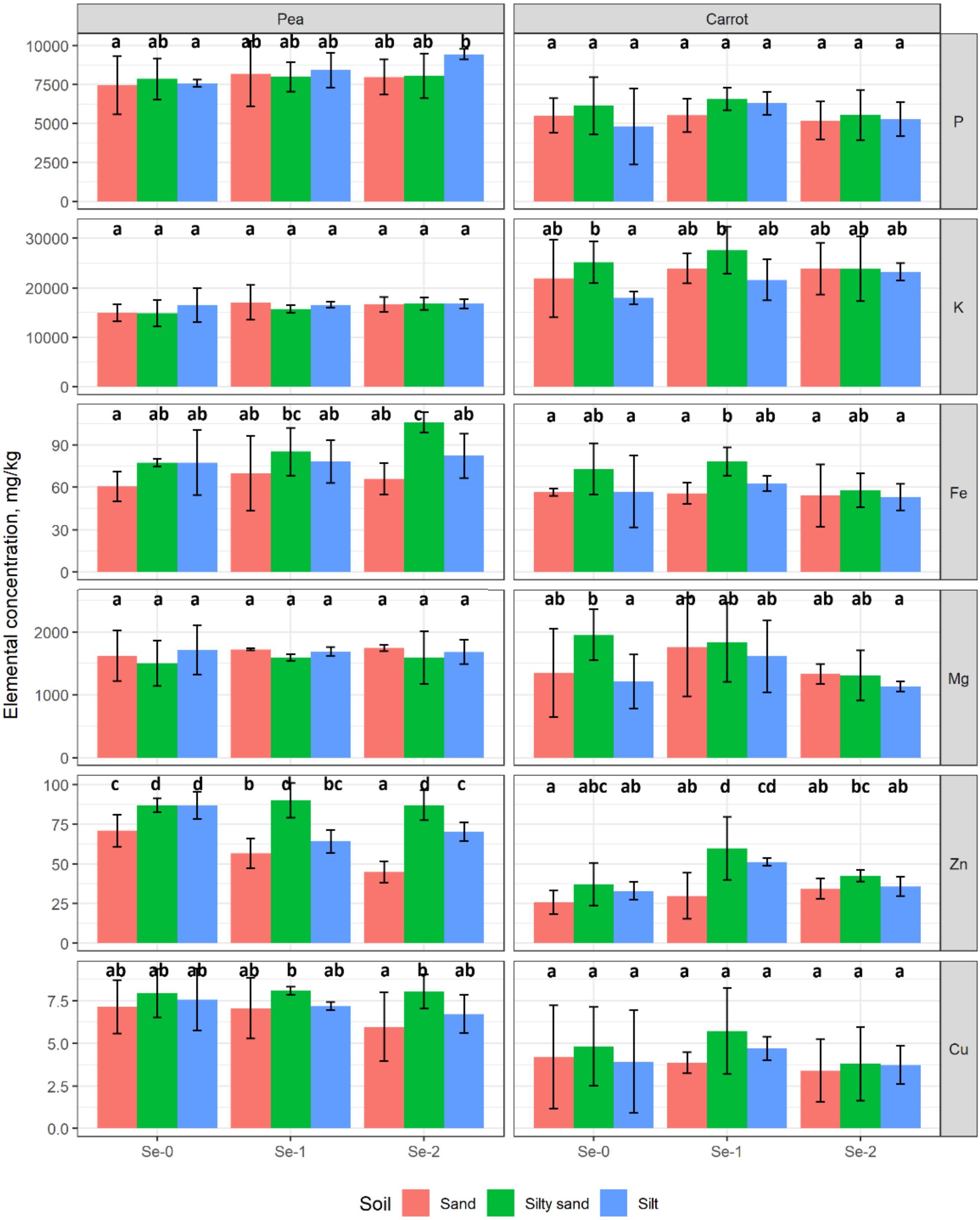

Figure 2. Macro and microelement concentrations of green peas and carrots, mg·kg^{-1} DW. Different letters indicate significant differences between treatments ($p < 0.05$). Error bars represent standard deviations.

4. Discussion

4.1. Se in Soils

As shown in Figure 1, the total Se content of silt soil was the highest among the control soils, followed by silty sand and sand. This is consistent with previous results showing that the total amount of Se in soils may increase with increasing clay content [49]. The ratio of the extractable to total fraction exhibits great variability, so it is important to examine both Se contents to get a complete picture. Poblaciones et al. [17] found that about 3.6% of total Se was plant-available in the soil used in their experiment, whereas Stroud et al. [50] found this ratio to be 1.1%–4.3% in soils from field trials in the UK. Another problem when comparing the total and extractable Se fractions is that a number of different methods are used to determine the extractable fraction. Zhao et al. [51] found that plant-available Se content was more strongly correlated with silt content than with clay content, but the results showed that biological availability was dominantly affected by the $CaCO_3$ content. In the present experiment, sandy soil had the highest $CaCO_3$ content (1.45%), followed by silt (0.20%) and silty sand (0.08%), but the extractable Se content was the lowest in the sand for both plant species and all treatments. This is probably due to the fact that the Se applied with irrigation water was adsorbed to the greatest extent on the surface of soil particles with the highest CEC value. However, the actual availability of Se is best indicated by plant Se uptake. Since green peas and carrots may have taken up Se directly from the irrigation water, the amount absorbed by the plants in the present experiment does not necessarily reflect the extractable Se fraction estimated by the extractant.

4.2. Enrichment of Se in Plants

In this present study, the Se concentration in untreated control pea grain was 118 μg kg^{-1} (DW), which was higher than in those grown under field conditions in Slovenia (11 μg Se kg^{-1} DW) [52] and in Spain (57 μg Se kg^{-1} DW) [17], and in greenhouses in Australia (38 μg Se kg^{-1} DW) [53]. In a comprehensive survey of 293 green pea samples in Canada, Gawalko et al. [54] found that the average Se content was 331 μg kg^{-1} (DW), while 56% of the samples tested had a Se content higher than 300 μg kg^{-1} due to the naturally Se-rich soils. In this work, in carrot root, 78 μg kg^{-1} (DW) Se was measured in the control soil. De Temmerman et al. [49] examined 121 carrot samples grown in Belgium and recorded an average Se content of 43.4 μg kg^{-1} (DW).

Some plant species, such as certain members of the Brassicaceae family, are capable of hyperaccumulation, i.e., they may have a very high Se content without any signs of toxicity [12]. However, carrots and green peas may exhibit severe toxicity as a result of high soil Se doses and, in extreme cases, when the Se content in carrots and green peas exceeds 63 and 176 mg kg^{-1} (DW), respectively, the crops may be completely eradicated [55–57]. This is because Se shows structural similarity to sulphur, making it easy for plants to take up. Subsequently, sulphur is replaced by Se in certain proteins, which consequently lose their function. Toxicity may also be caused by oxidative stress due to Se [58]. Several studies report that Se promotes plant development in low concentrations but inhibits it in high concentrations. Hegedűsová et al. [59] found that higher Se treatments reduced germination and root and shoot formation in seedlings, while low-dose Se increased root and shoot length by about 25%. Landberg and Greger [60] and Łukaszewicz et al. [61,62] reported a decrease in the root and shoot biomass in young pea plants grown in nutrient solution as a result of selenate treatments. Regarding the yield of mature green pea grain in the present experiment, no clear decreasing or increasing trend was observed in response to the Se doses, except for the increased fresh grain weight in silty sand (Table 4). Thus, it should be noted that no yield depression was observed even when a relatively high average concentration of 30 mg Se kg^{-1} developed in the pea grains in the Se-2 treatment (Table 5). All the other experiments described in the literature used foliar spray to enrich ripe green peas with Se. Nevertheless, these results are partly consistent with the results of the present experiment. Poblaciones and Rengel [63] found that foliar Se treatment increased the Se content of field-grown green pea grain to 1.415 mg Se kg^{-1} at the highest dose of

Na_2SeO_4 but had no effect on shoot biomass or grain yield, while the root weight and 100-grain weight increased. Hegedűsová et al. [64] applied 50 g and 100 g Se ha^{-1} sodium selenate during pea flowering by leaf spraying in a field experiment, which increased the Se content of the pea grain from 90–100 μg kg^{-1} to 1.16–1.30 mg kg^{-1} and 2.22–2.29 mg kg^{-1}, respectively, which is roughly half the Se content achieved in the Se-1 (100 μg L^{-1}) treatment used in the present experiment (Table 5). Nearly half of the Se content obtained in the Se-2 (500 μg L^{-1}) treatment was achieved by Poblaciones et al. [17], who increased the Se content of field-grown green pea grain to a maximum of 12.2 mg Se kg^{-1} and found no effect on the grain yield.

The carrot root FW showed a mostly decreasing trend, whereas Se-2 treatment had a significant negative effect on root DW compared to the control when averaged over the three soils (Table 4). This is partly in agreement with Smoleń et al. [13], who applied 0.5 kg Se ha^{-1} to the soil in the form of Na_2SeO_4 in a field experiment, and observed no changes in carrot root biomass yield, except for a minimal decrease when the Se content reached 25 mg kg^{-1} (DW) in the root, which is approximately comparable to the effect of the Se-2 treatment used in the present experiment (Table 5). Oliveira et al. [14] also applied sodium selenate to the soil, which had a very slight non-significant negative effect on the root weight while increasing the Se content of carrot roots to nearly 10 mg kg^{-1}. Bañuelos et al. [65,66] investigated the effect of mixing Se-rich *Stanleya pinnata* plant residues with the soil. The Se content of carrots reached a maximum concentration of 6.28 mg kg^{-1} as a result of the treatments without any decrease in biomass or any stress symptoms.

The results of this experiment confirmed that only a moderate non-significant decrease in yield if any can be expected for green peas and carrots with the high or higher Se contents achieved with the biofortification methods used so far. It should be noted that in a previous experiment with similar treatments, the fresh yield of green beans, tomato fruit, potato tubers and cabbage heads decreased slightly as a result of the Se treatment, but this effect was not significant. The Se content (DW) of green pea grain was quite similar to that of the green beans and tomato fruit, the lower Se content of carrot root was similar to or slightly higher than that of potato tuber, while the Se content of cabbage was much higher than that of the other vegetables studied [33].

The effect of the soil type on the Se enrichment of plants was controversial in this experiment. In the case of green peas, the Se content in the treated plants tended to be much higher for sand soil. This may be explained by the fact that sand is looser with lower CEC, so it binds less Se, and a higher proportion remains easily available for green peas. The same was observed in a previous similar experiment with green beans, cabbage and potato plants [33]. At the same time, in the case of carrots, the opposite trend was seen, though to a lesser extent, i.e., the highest Se contents in carrot roots were measured in silt soil. A possible explanation for this phenomenon is that pea roots are much denser, with more branching and a larger surface area than the taproot of carrot, enabling them to absorb the Se content of irrigation water much faster, while carrots might be more able to take up the Se content bound in the soil over a longer period of time. A similar conclusion was drawn by Bañuelos et al. [66], who compared the uptake of Se from the soil with broccoli with that of carrots. De Temmerman et al. [49] also found that on soils with similar Se concentrations, quite different Se concentrations may develop in different vegetable crops because the mode of uptake and accumulation is plant-specific.

4.3. Contribution of Se-Enriched Products to Human Se Intake

The daily RDA of Se may vary by country or region, but usually ranges from 25 to 60 μg day^{-1} for adult women and from 30 to 75 μg day^{-1} for adult men. A value of 55 μg day^{-1} is mostly recommended in the EU, USA and Canada. However, the daily UL of Se lies between 350 and 450 μg day^{-1} in different parts of the world, so the gap between deficient and toxic levels is relatively small [48]. In Eastern European countries, the estimated daily intake is usually between 30 and 40 μg Se day^{-1}, which is below the RDA [67].

Smoleń et al. [68] applied 0.25 kg Se ha^{-1} to the soil, which increased the Se content of carrot root from 2.21 to 10.97 mg kg^{-1} (DW). The consumption of 100 g of untreated carrot root would cover only 46% of the RDA while eating treated carrots would exceed RDA by 2.4 times. Poblaciones and Rengel [53] treated green peas with 0.03% or 0.06% (w/v) of $SeNaO_4$ leaf spray leading to Se contents of 67 and 95 µg Se, respectively, in 100 g of fresh grain weight, while Poblaciones et al. [17] treated green peas with 10 g Se ha^{-1} in the form of foliar application, resulting in 179 µg Se in 100 g of fresh grain weight, thus containing more than three times the RDA.

In the present experiment, the Se content of 100 g of fresh green peas grown in the Se-1 treatment exceeded the RDA value of 55 µg Se day^{-1} four times, but the average content of Se in carrots was 50.7 µg Se, so it was close to the RDA value (Tables 6 and 7). Irrigation water containing 100 µg Se L^{-1} can thus be considered a suitable method for the production of functional food from carrots in order to compensate for the low Se intake, but in the case of green peas, this concentration is too high. In a previous experiment, irrigation water containing 100 µg Se L^{-1} resulted in a Se content close to the RDA value in green beans and potatoes, while in tomatoes the content was only two-thirds of RDA, and in cabbage, it was four times higher. Thus, the degree of Se enrichment in green peas is similar to that of cabbage, which absorbs Se highly efficiently compared to the other tested vegetables. Irrigation water containing 100 µg Se L^{-1}, therefore, resulted in excessive Se levels, equal to half of the UL for both species. The effect of the Se-2 treatment was also similar for cabbage and green peas in terms of the UL (Table 8), because the Se contents were close to three-fold in both plants, while the enrichment of carrot was less than that of green bean and greater than that of potato, and about twice as much as that of tomato fruit [33]. The use of 100 µg Se L^{-1} concentration has already caused excessive Se enrichment in plants, so it is not recommended for biofortification.

The element content of raw vegetable products may change during the preparation of ready-to-eat food. For example, heat treatment reduces the Se content [69]. The bioavailable organic Se fractions may decrease due to protein denaturalization as a result of boiling, baking, microwaving or frying [70]. Cooking reduced the Se content of biofortified green peas by 7.4% [53] and in another study, by 12% [63].

4.4. Changes in Element Composition

Biofortification is a method to produce plant products rich in certain elements that are otherwise deficient, but the concentration of other nutrients important for human consumption should not be adversely affected. The antagonistic effect of Se on elements such as sulphur, mercury and molybdenum has been shown in previous studies [71–73]. However, for most elements, the results have been conflicting, often depending on whether Se was applied to soil or hydroponic growing medium (nutrient solution culture) [74] or as a leaf spray [75]. For example, the results of an in vitro experiment proved the synergistic effect of Se on sulphur in the case of wheat and rape seedlings [76]. These results underline the importance of investigating the effect of Se in irrigation water, as this application method differs from the methods commonly used. In a previous experiment, the concentrations of other elements (P, K, Fe, Mg, Zn, Cu) varied depending on the plant species or soil type or showed no substantial change (As, B, I, Mn), but no clear, consistent positive or negative effect of Se on the elements was observed [33].

In the present experiment, the increase in P, K and Fe and the decrease in Zn in green peas partly confirmed and partly contradicted previous results (Figure 2). Łukaszewicz et al. [62] found that Se applied in the form of selenate in hydroponic cultivation decreased P and Mg while increasing K content in green pea shoots when the first pair of leaves appeared. Reynolds-Marzal et al. [77] treated forage pea (*Pisum sativum* L.) with a dose of 10 g Se ha^{-1} in the form of Na_2SeO_4 in a field experiment, which increased the accumulation of Mg from 2.16 to 2.35 g kg^{-1} and that of Ca from 8.74 to 9.55 g kg^{-1} in relation to the control, but had no effect on Fe. According to Poblaciones and Rengel [53], Se treatment caused a non-significant increase in the Zn concentration of peas from 33 to 38 mg kg^{-1}, while it had no great effect on the Ca, Fe or Mg contents. However, in another similar study, it was found that the Mg concentration of green peas decreased from 1260 to 946 mg kg^{-1} in proportion to the increasing Se dose, while the Zn concentration increased from 43 to 47 mg kg^{-1}, and both changes were significant [63].

The concentration-dependent effect found for Se on most elements in carrots agrees with the results of Filek et al. [76], who recorded an increase in K, Mg, Mn, Zn and Fe compared to the control in a low Se treatment, but a decrease or a smaller increase due to a higher Se dose in rape and wheat seedlings. Oliveira et al. [14] applied 1.0 mg Se dm^{-3} to the soil in the form of Na_2SeO_4, which significantly reduced the K content of carrot shoots by 26% and non-significantly increased the Fe content by approx. 10%. Interestingly, both the Fe and Mn contents in the root were significantly reduced by the treatment.

5. Conclusions

From the point of view of Se biofortification, irrigation water with a concentration of 100 µg Se L^{-1}, referred to as treatment Se-1, can be recommended for the enrichment of Se in carrots, since this treatment increased the Se content by an average of 45 times, so consuming 100 g of the fresh root would approximately cover the 55 µg Se RDA per day. However, this concentration was too high for the biofortification of green peas, so a lower dose is recommended, the exact level of which needs to be determined in further research. It can be stated that only the Se-2 treatment, involving a concentration of 500 µg Se L^{-1}, caused a significant decrease in the dry weight of carrot, whereas the Se-1 treatment had a practically negligible negative effect on the biomass of the studied plants. The effect of Se treatment on the concentration of other nutrients is also minimal. The role of the soil type was controversial: green peas were able to absorb more Se in the looser sand soil, while carrots were able to take up more Se in the more compacted silt, presumably due to the different root systems of the plants. Based on previous and current results, the enrichment of irrigation water with Se could be a possible method for plant biofortification.

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