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Efficiency of selenium biofortification of spring wheat: the role of soil properties and organic matter amendment

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Abstract: The effect of soil selenate application to two different soils (Phaeozem and Cambisol) on biomass yield and selenium (Se) uptake by spring wheat (*Triticum aestivum* L.) was investigated in a pot experiment. Additionally, organic amendment (fugate, i.e. liquid by-product from the biogas plant) was applied to assess (i) the effect of organic matter on the bioavailability of Se and (ii) the fugate (containing 2.3 mg/kg of Se) as a potential source of Se for plants. Selenium was applied at two levels: 6.4 µg/kg (Se1) and 32 µg/kg (Se2) of soil. The efficiency of biofortification and the distribution of selenium within individual plant compartments were assessed in this case. The highest Se contents in the grain were achieved in the treatments receiving NPK fertiliser together with selenate, 455 µg/kg (Se1) and 2 721 µg/kg (Se2) when wheat was planted in Phaeozem. Fugate in co-application with selenate significantly reduced Se content in wheat plants as compared to treatments enriched solely with selenate. The lower Se contents in the wheat plants growing in Phaeozem were due to the biodilution effect, whereas in Cambisol, the decrease in wheat Se uptake was not clearly driven by a particular factor.

Keywords: microelement; nutrition; deficiency; selenisation effect; digestate; agronomic biofortification

Selenium (Se) is an essential microelement for human and animal nutrition. Low Se contents in the diet can lead to their deficiency in both human and animal populations (Fordyce 2013). The Se intake *via* the human diet is largely governed by Se contents in plants, which depends on the ability of crops to take up soil Se through the roots to the edible parts. Therefore, soil available Se is responsible for the Se content in plants and subsequently in food (Tan et al. 2002).

Selenium occurs in the soil in several inorganic forms, such as elemental selenium (Se^0), selenide (Se^{2-}), selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}). In a lesser extent, organic compounds of a wide range of molecular weights were reported in the soils (Elrashidi et al. 1987). Selenium also forms stable complexes with clay minerals, hydrated oxides and hydroxides in soil; these processes decrease the Se availability for plants (Abrams and Burau 1989). In acidic soils, the bioavailability of Se is generally low (Fernández-Martínez and Charlet 2009), growing with higher pH.

Biofortification to improve the nutritional value of crops belongs to reasonable agricultural strategies. They include agronomic biofortification, which is based on optimised crop fertilisation, either soil or foliar applications (Cakmak and Kutman 2017). Selenium fertiliser application has proved to be a safe, efficient and convenient way to produce Se-enriched wheat (Lyons 2010).

The effectivity of the biofortification depends substantially on the Se species and concentration present in the fertiliser (Luo et al. 2021). The most frequently investigated method is the application of selenate to the soil. Some researchers dealt with the effect of the co-application of common mineral fertilisers on the efficiency of Se biofortification. An inhibitory and/or competitive effect of anions (sulfate, phosphate) on the uptake of Se by roots was

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already previously described (Huang et al. 2008). However, as reported by Praus et al. (2019), under specific conditions, the application of phosporus (P) and sulphur (S) fertilisers can also increase the crop selenisation effect.

The fate of the organic substances applied to the soil is complex, and the Se organic matter interactions are not fully understood so far (Øgaard et al. 2006). The application of organic matter is also characterised by an indirect influence of organic carbon (C_{org}), which affects the microbial transformation of Se in soil (Neal and Sposito 1991). On the one hand, microorganisms can produce volatile Se compounds, leading to a loss of Se from the soil. On the contrary, microbial activity in the soil can promote Se incorporation into organic matter, making Se less sensitive to remobilisation (Darcheville et al. 2008).

Wheat (*Triticum aestivum* L.) belongs to the main food resources worldwide, and, therefore, sufficient Se content in wheat grains can help to ensure the well-balanced Se status of the world population. Selenium contents in wheat grain were determined in a wide range, typically 0.01–0.55 mg/kg Se (Hawkesford and Zhao 2007). The presence of selenomethionine, Se-methyl-selenocysteine, also inorganic SeO_4^{2-} or SeO_3^{2-} has been reported in wheat plants (López-Bellido et al. 2019).

As the number of biogas plants has increased, the issue of the use of by-products of anaerobic digestion on agricultural land has become more important (Tambone et al. 2010). Generally, digestate, the main semi-liquid residue from the biogas production (3–13% dry matter), is mechanically separated to the liquid phase (fugate) with up to 3% dry matter and solid phase (separate) with above 13% of dry matter (Kolář et al. 2010). The fugate contains a number of dissolved nutrients in a form available to plants, such as N, P, K, Ca, Mg, and S (Ditl et al. 2017). Previous studies investigated the effect of digestate or fugate on plant growth and development, soil edaphon (Tang et al. 2021) or assessed the risks associated with the introduction of hazardous substances into the soil (Koszel and Lorencowicz 2015). Total Se content in fugate usually ranges from 1 to 4 mg/kg in dry matter (DM); it strongly depends on the feedstock (Akhiar 2017). Because some regions, including Middle Europe and the Czech Republic, are characterised by low soil Se levels, often not exceeding 0.8 mg/kg of Se (Száková et al. 2015), fugate can be considered as a possible alternative source of Se in soil. However, the effect of fugate application on the Se content in field crops and on the efficiency of agronomic biofortification has not been addressed yet.

The objectives of this study were set to investigate whether: (i) the Se contents in the wheat plants will reflect the biofortification rate; (ii) selenium contained in the fugate can at least partially replace the soil application of the inorganic Se compounds, and (iii) there is any beneficial effect of the biofortification on yield of the wheat and Se distribution among the individual parts of the wheat plants.

MATERIAL AND METHODS

A pot experiment was established in the outdoor weather-controlled vegetation hall of the Czech University of Life Sciences in Prague. The altitude is approximately 280 m a.s.l., and the annual air temperature is around 9 °C. The average humidity and temperature between March and August were 58% and 14.7 °C. The soil for the experiment was collected in Doudleby nad Orlicí (DNO) and Krásná Hora nad Vltavou (KH), both in the Czech Republic. The DNO soil is characterised as Phaeozem (clay loam). The KH soil is Cambisol (sandy-loam). Table 1 shows the physicochemical properties of the soils. Fugate (Table 2) was collected at the agriculture biogas plant in Krásná Hora nad Vltavou and applied as an organic soil amendment and alternative source of Se.

Table 1. Physicochemical properties of the experimental soils

Soil site	Soil type	pH_{H_2O}	N_{min}**	P*	K*	Ca*	Mg*	S*	Se***
			(mg/kg DM)						
Doudleby nad Orlicí	Phaeozem	5.8 ± 0.0	4.39 ± 0.03	21 ± 2	184 ± 3	2 255 ± 23	182 ± 3	16 ± 1	0.03 ± 0.05
Krásná Hora nad Vltavou	Cambisol	6.9 ± 0.0	9.30 ± 0.02	254 ± 2	328 ± 10	3 215 ± 56	214 ± 2	28 ± 1	0.02 ± 0.02

*extraction by Mehlich 3; **extraction by $CaCl_2$ (0.01 mol/L); ***extraction by $(NH_4)_2HPO_4$ (0.1 mol/L); DM – dry matter

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Table 2. Physicochemical properties of fugate

	DM (%)	pH_{H_2O}	N	P	K	Ca	Mg	S	Se
			(mg/kg DM)						
Fugate	5.34 ± 0.10	9.1 ± 0.1	71 161 ± 282	13 840 ± 477	61 261 ± 1 769	36 569 ± 948	12 959 ± 445	7 726 ± 268	2.27 ± 0.06

DM – dry matter

After air drying, the soil was sieved with a mesh size of 5 mm. Then, 5 kg of dry homogenised soil was weighed, thoroughly mixed with NPK, and the source of selenium, selenate Na_2SeO_4 (Sigma Aldrich, Darmstadt, Germany), and/or the fugate, and put into individual 6-L pots. Selenium was applied at two levels, Se1 (6.4 μg/kg) and Se2 (32 μg/kg) of soil, with control treatment without Se addition (C) included, as well. The treatment F0.5-NPK0.5-Se1 means that half of Se (Se1 level) came from Na_2SeO_4, whereas the other half of Se originated from fugate. The treatment F-Se1 received all Se from fugate. The added N, P, and K amounts by NPK solution and fugate were kept constant throughout all treatments. A detailed description of the experimental design is presented in Table 3.

Spring wheat (*Triticum aestivum* L. cv. Scirocco) in the number of 30 seeds per pot was sown into the pots. After germination, it was thinned into 20 seedlings per pot. The vessels were then randomised. During the growing season, the plants were treated against diseases and pests and daily irrigated to 60–70% of maximum water saturation of soil pores. At harvest, grain, straw and roots were collected separately, weighted and dry at 35 °C. Dry samples were homogenised and ground on a grinder with a 1 mm sieve. The soil was collected before the experiment, dried at 20 °C, ground in a mortar, and passed through a 2-mm plastic sieve.

Plant samples were decomposed by a wet pressurised microwave-assisted digestion in a mixture of 65% HNO_3 (8 mL) and 30% H_2O_2 (2 mL) at 190 °C in Ethos 1 Advanced Microwave Digestion System (MLS GmbH, Leutkirch, Germany). The soil samples were extracted by the Mehlich 3 extraction procedure to determine available nutrients. For the determination of the available mineral nitrogen (N) proportions, the soil samples were extracted with $CaCl_2$ solution (0.01 mol/L) in a ratio of 1:10. The available proportions of Se in the soils were extracted with phosphate buffer.

Determination of the total Se content in the plant digests and soil phosphate extracts was performed by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x, Agilent Technologies Inc., Santa Clara, USA) operated in the collision cell (helium) mode. Available nutrient contents in the Mehlich 3 extracts were determined by atomic absorption spectrometry with flame atomisation on Varian 280FS (Varian, Mulgrave, Australia) in the case of potassium, and optical emission inductively coupled plasma spectrometry on Agilent 720 (Agilent Technologies Inc., Santa Clara, USA) for other nutrients. Mineral nitrogen in soils was determined using Skalar SAN Plus continuous-flow analyser (Skalar, Breda, Netherlands).

Statistical analysis. Statistical evaluation was performed using the Tukey *HSD* (honestly significant difference) test at a significant level of $P \leq 0.05$ by Statistica 12 software (Tulsa, USA).

Table 3. Design of the experiment

Treatment	Description	N	P	K	Se (μg/kg)	Fugate (g/kg DM)
		(g/kg)				
C	–	–	–	–	–	–
NPK	NPK solution	0.2	0.04	0.17	–	–
NPK-Se1	NPK solution + sodium selenate	0.2	0.04	0.17	6.4	–
F0.5-NPK0.5-Se1	fugate + NPK solution + sodium selenate	0.2	0.04	0.17	6.4*	26.4
F-Se1	fugate	0.2	0.04	0.17	6.4	52.6
NPK-Se2	NPK solution + sodium selenate	0.2	0.04	0.17	32	–
F-Se2	fugate + sodium selenate	0.2	0.04	0.17	32**	52.6

*half of the applied selenium (Se) comes from fugate; **one fifth of Se originates from fugate; DM – dry matter

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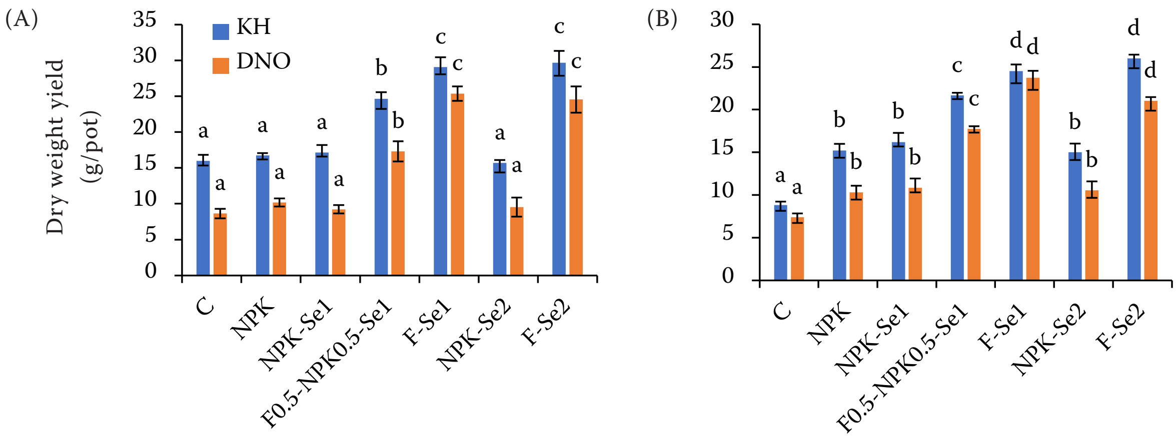

Figure 1. Dry weight yield of (A) wheat grain and (B) straw on Krásná Hora nad Vltavou (KH) and Doudleby nad Orlicí (DNO) soils. Different lowercase letters in the columns indicate a statistically significant difference among the treatments and within the individual parts of plants according to the one-way analysis of variance ($P < 0.05$), $n = 4$

RESULTS AND DISCUSSION

Biomass yield. Generally, lower grain production in DNO soil compared to KH was reported regardless of experimental treatment (Figure 1A). Moreover, grain yield in both DNO and KH soils did not respond to NPK fertiliser. These findings indicate that the supply of N, P, and K nutrients was not the factor limiting the grain yield within this experiment. The Mehlich 3 extraction (Table 1) confirmed a higher supply of macronutrients in KH soil compared to DNO, resulting in higher root, straw and grain yields in this soil (Figure 1; data for root yield are not shown, however, they were used for calculation of Se uptake by individual plant compartments; Figure 2). After fugate application, grain and straw yields significantly increased in all treatments as compared to the fugate untreated soils (Figure 1), reflecting the fugate rate applied to both soils. We suppose the growth-promoting effect may be attributed to the ameliorative capability of fugate with respect to soil physical properties in a pot experiment, although a minor effect of microelements and/or growth-stimulating compounds contained in fugate cannot be excluded. Similar findings were published by Abubaker et al. (2012); these authors evaluated wheat yield after organic fertilisation. Selenate application itself did not significantly ($P > 0.05$) affect the grain yield in any case (Ducsay et al. 2021).

Selenium content in wheat. The contents of Se in wheat grain, straw and roots harvested in KH soil

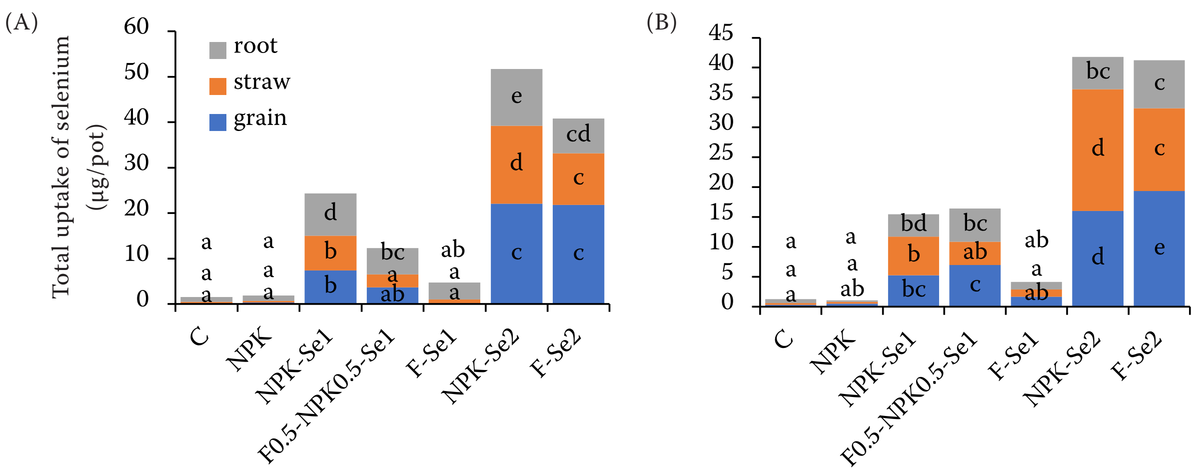

Figure 2. Total uptake of selenium by wheat parts planted at (A) Krásná Hora nad Vltavou soil and (B) Doudleby nad Orlicí soil. Different lowercase letters in the columns indicate a statistically significant difference among the treatments and within the individual parts of plants according to the one-way analysis of variance ($P < 0.05$), $n = 4$

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Table 4. Selenium (Se) content (µg/kg) in individual plant parts grown on the Krásná Hora nad Vltavou soil

Treatment	Grain	Straw	Root
C	10.7 ± 2.9[a]	23.8 ± 9.9[a]	34.8 ± 4.9[a]
NPK	15.2 ± 7.4[a]	16.3 ± 5.1[a]	33.5 ± 3.1[a]
NPK-Se1	381 ± 53[b]	346 ± 29[b]	231 ± 35[ab]
F0.5-NPK0.5-Se1	135 ± 13[ab]	105 ± 1[a]	197 ± 32[b]
F-Se1	30.2 ± 2.6[ab]	25.8 ± 3.4[a]	73.8 ± 5.6[a]
NPK-Se2	2 462 ± 206[d]	1 647 ± 91[d]	358 ± 53[d]
F-Se2	1 373 ± 221[c]	715 ± 62[c]	280 ± 32[c]

Different lowercase letters in the columns indicate a statistically significant difference between the treatments according to the one-way analysis of variance (P < 0.05), n = 4

are presented in Table 4. There was no significant (P < 0.05) difference in the grain Se contents between unamended control (11 µg/kg Se) and NPK (15 µg/kg Se) treatments. Expectably, selenium application at the level Se1 resulted in elevated Se contents in grain, as can be seen in NPK-Se1, F0.5-0.5NPK-Se1 and F-Se1 treatments. However, the individual treatments differed in their response to the applied form of Se and the presence of fugate. The highest Se content at the Se1 level was found in NPK-Se1 grain (381 µg/kg), which is in accordance with the highest plant availability of selenate among known soil Se species (Keskinen et al. 2010, Ducsay et al. 2020). The Se content in the F-Se1 grain decreased significantly (only 30 µg/kg), implying that Se contribution from fugate application was on the grain Se contents was negligible. Unfortunately, there is no available information concerning the Se species in fugate published so far. We can only speculate that organically-bound selenium (Se_{org}) and, to some extent, reduced inorganic Se species (Se^0, Se^{2-}) may dominate Se species distribution in fugate. Mobility and bioavailability of such Se species are considered to be generally low (Fernández-Martínez and Charlet 2009). The treatment F0.5-NPK0.5-Se1, where 50% of Se was added as selenate, showed an intermediate Se content in the grain (135 µg/kg), corresponding only to 35% of that Se content achieved when Se was applied exclusively in the form of selenate. At the level Se2, the highest Se content was determined in NPK-Se2 grain (2 463 µg/kg). When the ratio of Se originating from selenate salt to fugate was nearly 4:1 (F-Se2), the grain Se content decreased to 1 442 µg/kg, corresponding to 59% of Se content when Se was applied exclusively as selenate. In the straw and roots, the contents of Se in the individual treatments roughly followed the trends described in the case of grain.

Similar results were found for wheat grown on DNO soil (Table 5). No significant difference (P > 0.05) in Se contents in the grain, straw and roots was observed between C and NPK treatments. The control treatment on DNO soil had a higher content of Se (31 µg/kg) than the same treatment on KH soil (11 µg/kg), which is in accordance with the estimated available Se pools in the soils (Table 1). The highest content of Se in the grain at the Se1 level was found in NPK-Se1 (455 µg/kg), which is higher

Table 5. Selenium (Se) content (µg/kg) in individual plant parts grown on the Doudleby nad Orlicí soil

Treatment	Grain	Straw	Root
C	31.1 ± 3.4[a]	22.6 ± 7.2[a]	27.1 ± 3.2[a]
NPK	46.9 ± 6.9[a]	16.9 ± 5.5[a]	33.3 ± 5.3[a]
NPK-Se1	455 ± 31[b]	388 ± 58[b]	334 ± 30[b]
F0.5-NPK0.5-Se1	354 ± 35[b]	166 ± 8[a]	367 ± 15[b]
F-Se1	60.8 ± 11.1[a]	34.9 ± 9.6[a]	65.2 ± 4.9[a]
NPK-Se2	2 721 ± 156[d]	2 524 ± 233[e]	496 ± 29[c]
F-Se2	1 442 ± 143[c]	1 019 ± 74[c]	506 ± 49[c]

Different lowercase letters in the columns indicate a statistically significant difference between the treatments according to the one-way analysis of variance (P < 0.05), n = 4

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by 19% compared to KH soil in the same treatment. The lowest Se content among all fortified treatments was recorded in the F-Se1 grain (61 μg/kg), which is twice the content found in KH soil in the F-Se1. The treatment F0.5-NPK0.5-Se1 exhibited only a slightly lower Se content in the grain (354 μg/kg) compared to NPK-Se1. At the Se2 level, the highest Se content was found in the NPK-Se2 grain (2 721 μg/kg), which was 89% higher than in the F-Se2 treatment (1 442 μg/kg). Our results demonstrated clearly that the Se source is primarily responsible for different Se accumulation among individual treatments. Moreover, higher Se contents in wheat were always achieved in the DNO soil compared to KH. The fugate, when co-applied with selenate, acted as an inhibitor of the biofortification with Se, where this effect was more pronounced in KH soil compared to DNO. The immobilisation of exogenous Se by soil organic matter resulting in significantly reduced Se availability for various crops, including wheat, was already widely described (Sharma et al. 2011, Wang et al. 2016, Li et al. 2017). In this context, the importance of soil microorganisms in the fate of selenate applied to soil was highlighted (Hossain et al. 2021). There are several microbially driven pathways decreasing Se availability to plants, e.g. assimilatory and dissimilatory reduction and biomethylation (Wadgaonkar et al. 2018). The addition of an organic fertiliser reduced the availability of Se from exogenous SeO_4^{2-} to rapeseed plants (*Brassica napus* L.) as a result of stimulating the reductive microbial assimilation of SeO_4^{2-} (Ajwa et al. 1998, Dhillon et al. 2010). In this study, we proved that using Se in the form of selenate was more effective than the application of Se bound in organic soil amendments, such as fugate. Furthermore, according to Schiavon et al. (2020), Se biofortification is more effective in pH-neutral soils, where selenate accumulates in plants more easily than in acidic soils. However, in our case, we achieved higher Se contents in wheat on more acidic DNO soil. We believe that the explanation for different phytoavailability of exogenous Se should be derived from different statuses of microbial activity in KH and DNO soils. Similarly, Praus and Száková (2019) demonstrated how externally forced suppression (by gamma rays) and stimulation (by readily available C_{org} substrate) of soil microbial activity impacted a potentially plant-available Se pool after selenate addition into soils. Moreover, the authors showed that the suppression/stimulation potential depends on the soil type. We also suppose that Se volatilisation might occur, resulting in a reduction of Se uptake by wheat in the fugate-treated soils, as indicated by Shrestha et al. (2006) and Dhillon et al. (2010).

Selenium uptake by wheat. Because the individual treatments differed in the plant yields, the total Se uptake per pot is more informative than the Se content per kg for an assessment of the biofortification efficiency. The total Se uptake by wheat planted on KH and DNO soils is presented in Figure 2. At the level Se1, the highest total uptake of Se by whole plants was observed in NPK-Se1 treatment (24 μg/pot) in KH soil, whereas the F0.5-NPK0.5-Se1 showed only 50% of that uptake. Noteworthy, the total Se uptake in NPK-Se1 by whole plants was lower (15 μg/pot) in DNO soil, but it remained almost unchanged in F0.5-NPK0.5-Se1 treatment (16 μg/pot). This implies that the decrease in Se content in wheat grown in DNO soil (Table 5) after fugate application (F0.5-NPK0.5-Se1) can be assigned to a biodilution effect. In contrast, we can speculate that the decrease in Se content determined in the same treatment in KH soil (Table 4) may be predominantly due to an enhanced microbial immobilisation and higher volatilisation of Se induced by fugate if co-applied with selenate. The same observation can be made at the level Se2 when comparing Se uptake in NPK-Se2 and F-Se2 treatments between KH and DNO soil (Figure 2). The uptake of Se by whole plants in F-Se1 treatment in both KH and DNO soils was considerably low (5 and 4 μg/pot, respectively), indicating a poor plant availability of Se originating from the fugate. To assess biofortification efficiency, the percentage of applied Se fertiliser recovered by the whole plants was counted (Se content in control treatments was subtracted). The highest efficiencies (70 and 45%, respectively) were found in NPK-Se1 treatment for KH and DNO soil. At level Se2, the fortification efficiency decreased (down to 31% and 26%) in both soils. Figure 2 also documented that Se was accumulated more easily in straw and grain compared to roots. In the pot experiment presented by Ramkissoon et al. (2019), the recovery of soil-applied Se in the aboveground wheat biomass was commonly less than 50%; however, it reached even up to 100%. Lyons (2010) mentioned Se recovery in wheat grain to be 14% under field conditions. In our experiments, this index ranged by wheat grain from 0% to 22% across all the fortified treatments.

In conclusion, the fugate itself, despite its high total Se content, did not act as a source of readily available Se for wheat plants. Moreover, fugate reduced the Se

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content in wheat tissue if co-applied with selenate into the soil. Moreover, the important role of soil type and properties was proven in this experiment because the decrease in biofortification efficiency was observed in wheat grown in Cambisol soil after fugate application compared to the Phaeozem. On the contrary, the biofortification efficiency in Phaeozem was affected by the dilution effect due to the higher biomass yield, and the potential role of the fugate in decreasing the Se bioavailability cannot be excluded, as well. Thus, the use of fugate within agronomic biofortification strategies should be considered with caution.

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