Enrichment of Sunflower Seeds and Harvest Residues with Zn and Se Through Agronomic Biofortification

Obogaćivanje zrna i žetvenih ostataka suncokreta biofortifikacijom s Zn i Se.

Nemet, F., Perić, K., Zebec, V., Ivezić, V., Galić, L., Nikolin, I., Rékási, M., Ragályi, P., Lončarić, Z.

Poljoprivreda / Agriculture

ISSN: 1848-8080 (Online)
ISSN: 1330-7142 (Print)

https://doi.org/10.18047/poljo.32.1.3

Fakultet agrobiotehničkih znanosti Osijek, Poljoprivredni institut Osijek

Faculty of Agrobiotechnical Sciences Osijek, Agricultural Institute Osijek

ISSN 1330-7142
UDK=631.63.01+633.85:631.875
https://doi.org/10.18047/poljo.32.1.3

ENRICHMENT OF SUNFLOWER SEEDS AND HARVEST RESIDUES WITH Zn AND Se THROUGH AGRONOMIC BIOFORTIFICATION

Nemet, F., (1) Perić, K., (2) Zebec, V., (1) Ivezić, V., (1) Galić, L., (1) Nikolin, I., (1) Rékási, M., (3) Ragályi, P., (3) Lončarić, Z. (1)

Original scientific paper
Izvorni znanstveni članak

SUMMARY

This research was focused on the effectiveness of sunflower biofortification with Zn and Se towards enriched seeds, but also a side effect on the enrichment of crop residues, which can have a residual fertilizing effect. A two-year study of sunflower biofortification was conducted with foliar application of 10, 20, and 30 g/ha Se as Na-selenate or 3 and 6 kg/ha Zn as Zn sulphate in a randomized complete block design with three replications. Biofortification did not significantly affect either the seeds or the above-ground yields, but it increased the Se and Zn concentrations in seeds and vegetative mass. The Se in seeds increased 14-44 times, whereby 7.3 g/ha Se would be sufficient to achieve 300 μg/kg Se in seed. The Zn in the seed increased up to 72 mg/kg. The efficiency of biofortification in terms of Se accumulation in seeds was 15.4 – 19.3%, and Zn only 1.73 - 3.15%, which means that significant amounts of Se and Zn remain in crop residues whose incorporation into the soil has a residual effect of Zn and Se fertilization for subsequent crops.

Keywords: foliar application, microelements, residual effect, sodium selenate, zinc sulphate

INTRODUCTION

Agriculture as the basis of food production entails a whole series of challenges that can be summarized in several segments, above all, the production of a sufficient amount of food of appropriate quality and the sustainability of the activity in an environmental and economic sense. The productivity and fertility of the soil are closely related to the yield and quality of the food produced, and preserving the fertility of the soil is the safest and longest-lasting approach to sustainability.

In addition to insufficient food production, or more precisely, the inadequate pattern of required and produced food in certain parts of the world, malnutrition is a significant problem. Despite increasing access to sufficient food for all and significant achievements in reducing global hunger, micronutrient deficiencies, including zinc (Zn), iodine (I), and selenium (Se) continue to pose a global health problem (Biley et al., 2015). A concentration of Se in food and feed plants is a reflection of the concentration of Se in soil and its bioavailability (Manojlović and Lončarić, 2017). In many areas of the world, the concentration of Se in soil is low, which affects the level of Se in plants and, accordingly, animal and human health. Selenium deficiency is known as a problem particularly in southern China, eastern Canada, eastern and western USA, several European countries, e.g., England and Scotland, Finland, Poland, Norway, and Hungary (Fordyce, 2013; Gupta & Gupta, 2017), but also in Croatia, and in almost all Balkan region (Manojlović and Lončarić, 2017). Similarly, the lack of zinc in the human diet is a consequence of low availability in soils and low accumulation of Zn in plants. The largest areas with zinc deficiency are found throughout South, Central, and North Africa, the Eastern Mediterranean, Southeast Asia, and Central America (Galetti, 2019), often coinciding with selenium deficiency. Unlike selenium, zinc is an essential trace element for all plants (Dai et al., 2020). Approximately 50% of the world's agricultural land, where crops are grown, is considered deficient in zinc bioavailability (Cakmak and Kutman, 2018). One effective method to increase selenium concentration in plants is

---

(1) Franjo Nemet, M. Eng. Agr., Assoc. Prof. Vladimir Zebec, Assoc. Prof. Vladimir Ivezić, Lucija Galić, PhD, Iva Nikolin, M. Eng. Agr., Prof. Dr. Zdenko Lončarić (zdenko.loncaric@fazos.hr) – Josip Juraj Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, Vladimira Preloga 1, 31000 Osijek, Croatia, (2) Katarina Perić, M. Eng. Agr. – Agricultural Institute Osijek, Južno predgrađe 17, 31000 Osijek, Croatia, (3) Márk Rékási, PhD, Péter Ragályi, PhD – Institute for Soil Sciences, Centre for Agricultural Research, HUN-REN, Fehérvári Street 132-144, Budapest 1116, Hungary

agronomic biofortification, aimed at reducing selenium deficiency in humans and animals through the food chain (Galinha et al., 2015). Agronomic biofortification involves applying micronutrients to soil or through foliar application directly onto crop leaves to enhance their concentration in edible plant parts, thereby increasing the intake of the selected essential element in the human diet (Adu et al., 2018). Numerous results of successful biofortification with zinc and selenium have been published around the world (Joy et al., 2015; Velu et al., 2014; Zhang et al., 2017; Petković et al., 2022; Lončarić et al., 2021; Galić et al., 2021; Manojlović et al., 2019). The most common is the biofortification of cereals due to their dominant presence in the human diet, but to a certain extent also on other crops (Petković et al., 2019; Džomba et al., 2018; Novoselec et al., 2018). Significantly fewer authors published the results of selenium effect on sunflower (Habibi, 2017) or zinc (Poudel et al., 2023; Poudel et al., 2024), although sunflower, as a Se-hyperaccumulator with up to 1.8 g/kg in shoots (with no significant decrease in shoot biomass) can be a valuable plant in biofortification to improve animal and human nutrition (Garousi et al., 2018). Compared to the numerous authors who have researched the biofortification of crop grains, there is a significantly smaller number of studies on biofortification using crop residues (Khoshgoftarmanesh et al., 2017) or on the impact of biofortification on crop residues as a source of microelements for the next crop (Mathers et al., 2017).

Soil properties significantly affect the need for biofortification with Zn and Se and its effectiveness, and foliar biofortification is generally more effective than the application of Zn and Se to the soil because it results in higher concentrations of Zn and Se in the grain and other parts of the crop. However, the goal of biofortification of field crops is to produce grain with higher concentrations of zinc and/or selenium or other micronutrients, but at the same time, the concentration of Zn and Se in other parts of the plant, i.e. in the harvest residues, also increases. The goals of this research are to determine the effectiveness of agronomic biofortification on increasing Zn and Se concentrations in sunflower seeds, but also to assess the side effects on the enrichment of crop residues, which can have a residual fertilizing effect.

MATERIALS AND METHODS

Sunflower Growing

The field experiments with the cultivation and biofortification of sunflowers were conducted in 2020 and 2021 at the test site of the Faculty of Agrobiotechnical Sciences Osijek, locality Tenja, Croatia. The basic experimental plots of each individual treatment had an area of 151.2 m^2 with 6 rows of sunflowers 36 m long (4.2 m × 36 m = 151.2 m^2). Each treatment was set up in three repetitions, and the experiment was arranged as a randomized complete block design. The experiment was set up by sowing the sunflower hybrid Surimi CL on April 14, 2020, and April 12, 2021.

The soil of field experiment was neutral to slightly acidic reactions ($pH_{H2O} = 6.89$; $pH_{KCl} = 5.79$, according to ISO 10390), low to medium soil organic matter content (SOM = 2.03%, according to ISO 14235), poorly supplied with plant-available phosphorus (11.75 mg/100 g P_2O_5 according to AL method, Egnér et al., 1960), and good supplied with plant available potassium (20.88 mg/100 g K_2O according to AL method). Also, a moderate concentration of total Zn (49.96 mg/kg) and a low concentration of total Se (0.44 mg/kg) extracted by aqua regia (according to ISO 11466) were determined, as well as a medium availability of Zn (1.58 mg/kg) determined by EDTA method (Trieweiler and Lindsay, 1969).

Biofortification

Agronomic biofortification of sunflower was conducted by foliar application of Se (as sodium selenate solution) or Zn (as zinc sulphate solution) at the beginning of the sunflower flowering (second half of June). Foliar application (with a backpack accumulator sprayer) of 30 L of solution (water or an appropriate solution plus 30 mL of adjuvant) was applied to each plot of 151.2 m^2.

The following biofortification treatments in the experiment with Se were applied: 1. control (without Se or Zn solutions): water was applied; 2. Se1 treatment: 10 g/ha Se applied; 3. Se2 treatment: 20 g/ha Se; 4. Se3 treatment: 30 g/ha Se. The following biofortification treatments in the experiment with Zn were applied:

1. control (without Se or Zn solutions): water was applied; 2. Zn1 treatment: 3 kg/ha Zn applied; 2. Zn2 treatment: 6 kg/ha Zn. For both experiments (with Se and with Zn), the results of 3 same plots with control treatment (only water with adjuvant) were used.

Plant Material Sampling, Harvest, and Analysis

The plant material was sampled on the same day as the harvest (at the beginning of September). All the plants of two rows of sunflowers in a length of 5 m on each plot were collected, and the total above-ground mass was weighed. Five average plants from each plot were separated, and they were transferred to the laboratory where the aboveground organs of the sunflower were separated (stems, leaves, heads, and seeds). All samples were dried, and the mass of individual organs was weighed. The samples were prepared for chemical analysis. Harvesting of individual sunflower plots was done with a harvester, after which the seed from each plot was separated, and the yield was weighed.

All plant samples (stems, leaves, heads, and seeds) were prepared for chemical analysis by drying in a laboratory dryer to a constant mass at a temperature of 70°C. Dry samples were pulverized in an ultracentrifugal grinding mill without heavy metal residues (Retsch ZM 200, Haan, Germany). All stem, leaf, head, and seed samples were subjected to digestion in a laboratory microwave oven (CEM Mars 6, Charlotte, NC, USA) using the wet technique (Lončarić et al., 2021; Kingston and Jassie, 1986; Špoljarić et al., 2024). For digestion, 8 mL of 65% HNO_3 and 2 mL of 30% H_2O_2 were added to a certain mass of the plant sample in a Teflon cuvette, and after digestion, the solution was transferred through filter paper into a 50 mL volumetric flask and topped up to the mark with deionized water. The concentrations of Zn and Se (and other elements) were measured directly in the solutions using inductively coupled plasma mass spectrometry (ICP-MS, Agilent Technologies 7800 ICP-MS, Santa Clara, CA, USA). Zn concentrations are expressed as mg/kg dry matter, and Se as μg/kg. In each series of samples, measurement by mass spectrometry was performed with an internal pooled plasma control and with a certain reference material (Institute of Nuclear Chemistry and Technology INCT-SBF-4 and Institute for Reference Materials and Measurements BCR 129), prepared for analysis in the same way as all other samples.

The efficiency of each biofortification treatment was calculated as a percentage of the total added amount of Zn (3 or 6 kg/ha) or Se (10, 20 or 30 g/ha) found in the total above-ground mass of sunflower (or only sunflower seeds). In doing so, the amount of Zn or Se found in the plant mass of the control treatment (without biofortification) was subtracted from the amount of Zn or Se in each individual biofortification treatment.

Statistical Data Processing

Statistical analyses of data were performed with software packages *MS Excel* and *SAS for Windows* 9.1.3. (SAS Institute Inc., Cary, NC, USA). The experiment was designed with two factors (year and Se or Zn application), and factorial analysis of variance was performed. As part of statistical data analysis, Levene's test for homogeneity of variance was performed; afterwards, mean values were statistically processed by analysis of variance (ANOVA) and compared with Fisher's least significant difference (LSD) test. Differences significant at $p < 0.05$ were considered. *MS Excel* was used for regression modelling of Se or Zn concentration in sunflower seeds depending on the applied amount of Se and Zn.

RESULTS AND DISCUSSION

Yield of Sunflower Seeds and Vegetative Mass

The average yield of sunflower seeds in the two-year experiment of biofortification with Se was 4.07 t/ha, and in the experiment of biofortification with Zn, 4.03 t/ha. On average, higher yields were in 2020 (4.27 and 4.24 t/ha) than in 2021 (3.87 and 3.82 t/ha for Se and Zn experiments, respectively), but without a statistically significant effect of season or biofortification on seed yields (Figures 1 and 2). Also, no statistically significant interaction between seasons and Se or Zn concentrations in sunflower seeds was found.

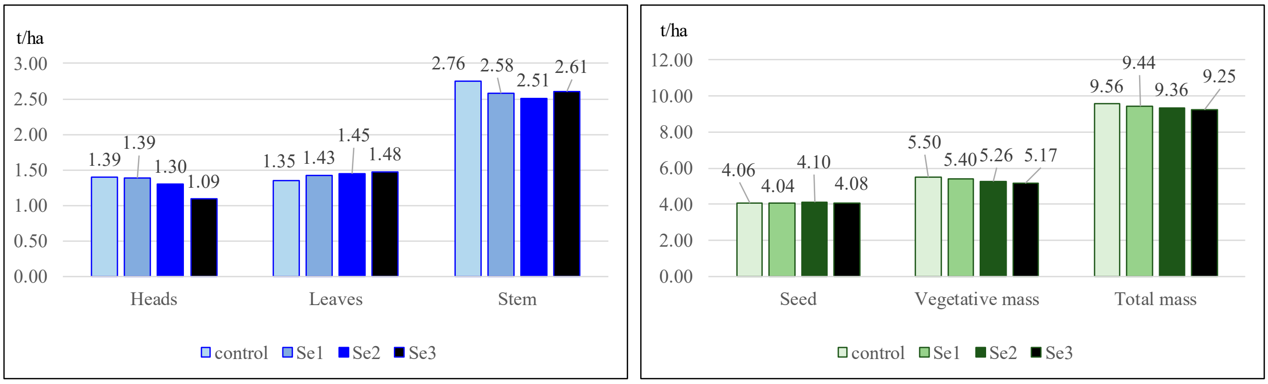

Figure 1. Mass of sunflower vegetative organs, seed, and total above-ground mass in Se biofortification experiment (mass in t/ha).

Grafikon 1. Masa vegetativnih organa suncokreta, zrna i ukupna nadzemna masa u pokusu sa Se (u t/ha).

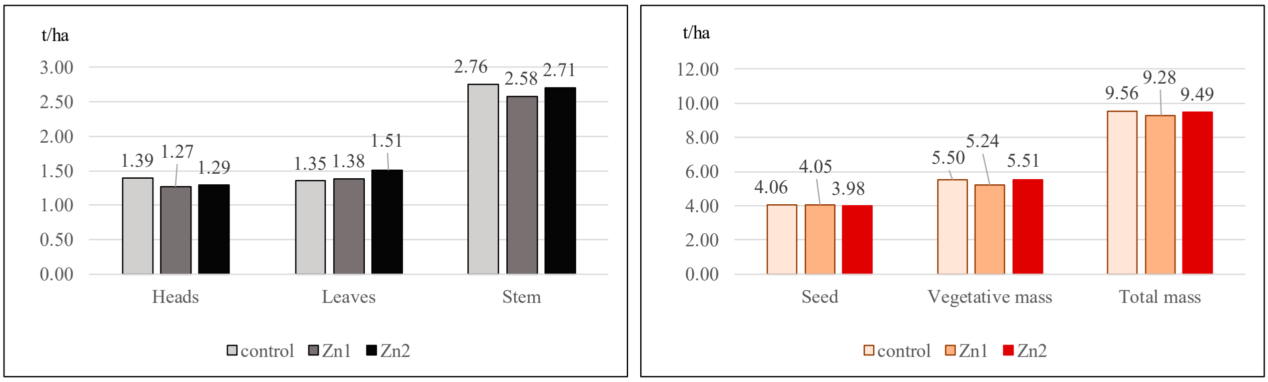

Figure 2. Mass of sunflower vegetative organs, seed, and total above-ground mass in Zn biofortification experiment (mass in t/ha).

Grafikon 2. Masa vegetativnih organa suncokreta, zrna i ukupna nadzemna masa u pokusu sa Zn (u t/ha).

The average total above-ground vegetative mass of sunflower in the two-season bio-fortification experiment with Se was 5.33 t/ha (5.42 and 5.24 in 2020 and 2021), and in the biofortification experiment with Zn, 5.41 t/ha (5.49 and 5.34 in 2020 and 2021), where no statistically significant influence of either the season or the biofortification treatment on the yield of sunflower above-ground vegetative mass was determined (Figure1 and Figure2).

On average, the total above-ground mass of sunflowers during two growing seasons was 9.40 t/ha (9.69 and 9.11 in 2020 and 2021) in the experiment with Se, and 9.44 t/ha (9.72 and 9.16 in 2020 and 2021) in the experiment with Zn. As in the case of seed yield and total vegetative above-ground mass, neither the biofortification treatment nor the season had a significant effect on the total yield of sunflower above-ground mass (Figures 1 and 2).

The average share of seeds in the total above-ground mass was 43.17%. (43.76% in 2020, 42.57% in 2021). In the Se experiment, and 42.62%. (43.40% in 2020, 41.84% in 2021) in the Zn experiment. Biofortification treatments of sunflower with Se or Zn did not affect the share of seed and vegetative above-ground mass of sunflower in the total above-ground mass.

Biofortification did not affect either the seed yield or the yield of the total above-ground mass of sunflower, as expected, considering the published results of other authors in the research of biofortification of field crops with zinc and selenium. In addition to the average high seed yield (4.05 t/ha), an even higher average yield of sunflower crop residues (5.37 t/ha) was achieved. The achieved average yields of sunflower seeds are comparable to the average yields in the same area in 2017, 2018, and 2019 (4.17, 4.15, and 4.27 t/ha, respectively) (Banaj et al., 2021). In research on biofortification with Se and Zn, many authors also determined the absence of influence of biofortification with Se on the yield of wheat (Lončarić et al., 2021; Manojlović et al., 2019; Lyons et al., 2004; Grant et al., 2007; Broadley et al., 2010), silage corn (Džomba et al., 2018) or alfalfa (Petković et al., 2019), as well as zinc biofortification on wheat yield (Lončarić et al., 2017) or alfalfa (Petković et al., 2019). However, other authors found a positive effect of biofortification with Se on the yield of wheat under stressful conditions (Yao et al., 2013; Nawaz et al., 2015), and on the yield of rye and potatoes (Hartikainen and Piironen, 2000; Yassen et al., 2011).

Concentrations of Se in Seed and Sunflower's Vegetative Organs

Selenium application had a statistically very significant effect on selenium concentrations in all above-ground parts of sunflower, both on average for two vegetations (Figure 3) and individually in each vegetation.

The highest selenium concentration was in sunflower leaves (average for all treatments 1,172.8 μg/kg), followed by seed (687.9), head (404.5), and stem (319.9 μg/kg). But more important is the fact that treatments with increasing amounts of applied Se (10, 20 and 30 g/ha) resulted in a significant increase in Se concentrations in sunflower seed compared to the control (14.4, 23.8 and 44.3 times), and also in the stem (3.6, 7.2 and 10.3 times), the head (7.4, 10.8 and 18.5 times), the leaf (13.6, 22.2 and 36.5 times) compared to the control (Figure 3). Thus, Se concentrations in sunflower seed reached from 473.7 μg/kg after biofortification with 10 g/ha Se (in average for both years) to 1,461.1 μg/kg after biofortification with 30 g/ha Se, while in the control treatment was 33.0 μg/kg Se (Figure 3).

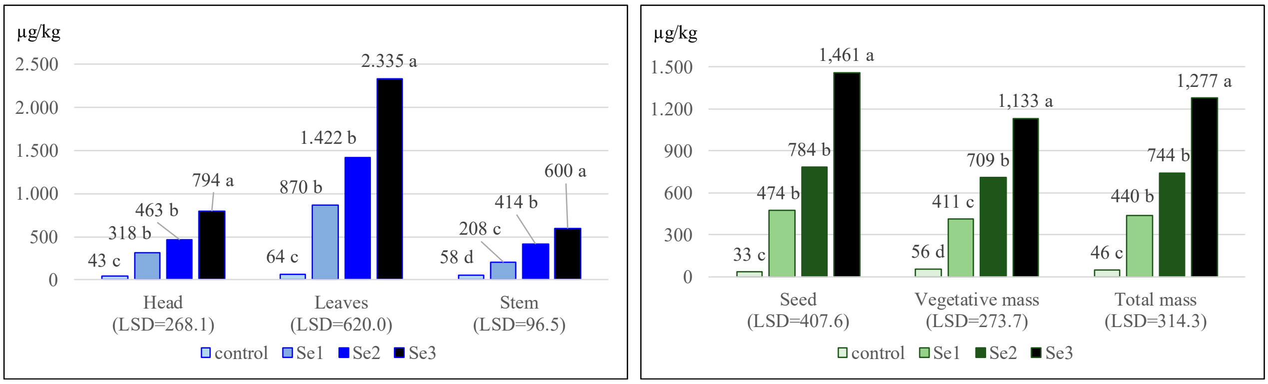

Figure 3. Increase in Se concentrations in all above-ground parts of sunflower after application of different amounts of Se.
Grafikon 3. Povećanje koncentracije Se u svim nadzemnim dijelovima suncokreta nakon aplikacija različitih količina Se.

Biofortification by foliar application of selenium resulted in almost linear regular increases in Se concentrations in all above-ground organs of sunflower. The highest concentrations of Se were determined in the leaf, where even the smallest dose of Se resulted in a 13.6 times higher concentration of Se in the leaf than in the control, and only in the seed with this dose was the relative increase even higher (14.4 times). In the control treatment without Se, the order of concentrations of seed<head<stem<leaves was established, but at all doses of Se biofortification, this was significantly changed to a constant order of stem<head<seed<leaves, which shows that the sunflower stem is the smallest and the seed and leaves are the largest accumulators of Se. The

increase in Se concentration was stated in accordance with the research of other authors who found an increase in wheat seeds (Manojlović et al., 2019; Lončarić et al., 2021; Broadley et al., 2010; Mao et al., 2014), but also in the above-ground mass of alfalfa (Petković et al., 2019) or silage corn (Džomba et al., 2018).

Biofortification of sunflower with only 10 g/ha of Se has already achieved a concentration of more than 300 μg/kg in the seed (411 μg/kg) as a general goal of biofortification. Using the created regression model of the influence of the applied amount of Se on the concentration of Se in sunflower seeds, it was determined that an average of 7.3 g/ha of Se would be sufficient to achieve the goal of biofortification. At the same time, biofortification with 30 g/ha Se produced about 4 t/ha of seed with a concentration of 1,133 μg/kg Se. Therefore, mixing with non-biofortified seeds for the food or livestock industry would practically produce about 15.4 t of sunflower seed with a concentration of 300 μg/kg. A significant increase in the concentration of Se in the above-ground mass above 300 μg/kg was also determined in the biofortification of alfalfa with 10 g/ha Se (Petković et al., 2019), where 680-770 μg/kg was determined, while in the above-ground mass of silage corn, 344 μg/kg was determined after biofortification with 20 g/ha Se (Džomba et al., 2018).

Concentrations of Zn in Seed and Sunflower's Vegetative Organs

Zinc application also had a statistically very significant effect on zinc concentrations in all above-ground parts of sunflower, both on average for two vegetations (Figure 4) and individually in each vegetation.

The highest two-year average zinc concentration was in sunflower leaves (average for all treatments 521.3 mg/kg), followed by seed (61.6), head (38.9), and stem (37.2 mg/kg). Also important is the fact that treatments with increasing amounts of applied zinc (3 and 6 kg/ha) resulted in a significant increase in Zn concentrations in sunflower seed compared to the control (51% and 60%), and also in the stem (2.2 and 3 times), the head (2 and 3.1 times) and the leaf (18.2 and 24.3 times) compared to the control (Figure 4). The Zn concentrations in sunflower seed were 68.3 mg/kg after biofortification with 3 kg/ha Zn (on average for both years) and 71.6 mg/kg after biofortification with 6 kg/ha Zn, while in the control treatment was 44.95 mg/kg Zn (Figure 4).

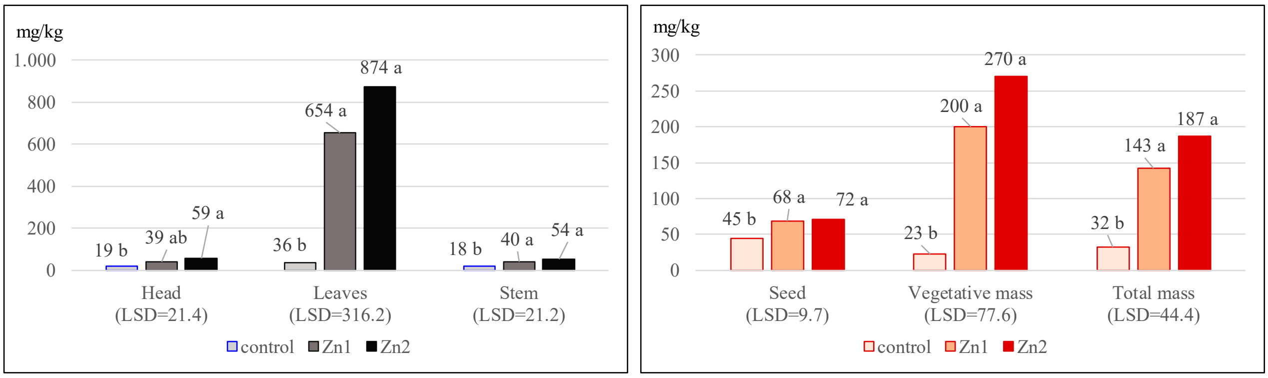

Figure 4. Increase in Zn concentrations in all above-ground parts of sunflower after application of different amounts of Zn.
Grafikon 4. Povećanje koncentracije Zn u svim nadzemnim dijelovima suncokreta kao rezultat biofortifikacije različitim količinama Zn.

Biofortification with zinc also resulted in a significant increase in Zn concentrations in all above-ground sunflower organs, except in the sunflower head with a lower dose of Zn (3 kg/ha). The order of Zn concentrations in the control treatment is stem<=head<leaves<seed, while after biofortification, the ratio of sunflower stem and head is the same, but there is significantly more Zn in the leaves than in the seed. It is evident that sunflower seed does not accumulate Zn as efficiently as Se compared to control treatments (although the highest seed Zn concentrations are 49 times higher than Se concentrations).

Successful increase of Zn concentration by biofortification was determined by research on wheat (Zhang et al., 2012; Velu et al., 2014; Lončarić et al., 2021), alfalfa (Petković et al., 2019), and other crops (Petković et al., 2022).

However, it is very significant that no statistically significant increase in Zn concentration was found in any organ by increasing the dose from 3 to 6 kg/ha of Zn, so we can conclude that an increase to a higher dose of Zn in foliar application is not necessary. Also, the concentration of Zn in the seed in the control treatment was 45 mg/kg, which is more than 40 mg/kg, as is the general goal of biofortification, so from that aspect, Zn biofortification is not even necessary. However, biofortification and the possibility of increasing the concentration of Zn in the seed are very significant for soils poorer in zinc (< 1.5 mg/kg of available Zn), but also for the preparation of mixtures for the food industry and for livestock feeding. For example, a sunflower seed produced on soils poorer in Zn may contain 30 mg/kg Zn, and biofortification with 3 kg/ha Zn produced about 4 t/ha seeds with a Zn concentration of 68 mg/kg, which would be enough to prepare 15.2 t of mixture seeds with an average of 40 mg/kg Zn.

Selenium and Zinc Removal by Seed and Possible Residual Effect of Biofortification

A statistically significant influence of all biofortification treatments with Se on the increase of removal (uptake) of Se by sunflower seed was determined. In the control treatment, 134 mg/ha Se was removed, and biofortifications with 10, 20, and 30 g/ha of Se significantly increased the amount of Se removed by seed 14.3 times (1.912 mg/ha), 24.0 times (3.221 mg/ha), and 44.3 times (5.929 mg/ha), respectively, compared to the control.

The efficiency of biofortification can be shown as an increase in concentration primarily in the seed. Thus, it was calculated that only 17.78%, 15.44%, and 19.32% of the Se added by biofortification (with 10, 20, and 30 g/ha) was contained in the total mass of seed (on average for two growing seasons), respectively. This means that the efficiency of biofortification with Se was 15.4 – 19.3% and consequently, after harvesting sunflower, 80.7-84.6% (8.2-24.2 g/ha) of the added Se remains for the next crop. The stated amounts also include Se in harvest residues of biofortified sunflower, assuming that they will be incorporated into the soil before the next growing season.

The total Zn removed by the seed yield was 180.8 g/ha on the control treatment, and by biofortification, it was increased by 52.4% and 57.6%, up to 275.3 and 284.6 g/ha. The efficiency of biofortification with Zn is rather lower than with Se, and in terms of Zn removal by seed is only 3.2% of 3 kg/ha Zn and 1.7% of 6 kg/ha Zn added by biofortification (on average for two growing seasons). But, on the other hand, it means that in the case of incorporating sunflower harvest residues into the soil, a total of 2,905 or 5,896 g Zn/ha will remain in the soil, which practically represents zinc fertilization for the next crop.

CONCLUSION

Biofortification of sunflower with Se or Zn did not significantly affect either the seed yield or the above-ground vegetative mass, but it significantly increased the concentration of Se and Zn in the seed and vegetative above-ground mass of sunflower. The concentration of Se in seed was increased 14-44 times by biofortification to a maximum of 1,461 μg/kg, whereby 7.3 g/ha of Se in foliar application would be sufficient to achieve a concentration of 300 μg/kg Se in seed. The concentration of Zn in sunflower seed increased up to 72 mg/kg, but biofortification with 3 kg/ha is sufficient because it results in a concentration of Zn in the seed of 68 mg/kg. The efficiency of Se biofortification was 15.4-19.3%, and Zn only 1.73-3.15%, which means that significant amounts of Se and Zn remain in the field after foliar application for the next crops, which practically represents fertilization with selenium or zinc for the next crop.

ACKNOWLEDGMENTS

The paper is the result of research within the project KK.01.1.1.07.0053 *Application of Innovative Bioagents in Sustainable Plant Production Technologies (InoBioTeh)*, funded by the European Union under the Operational Program Competitiveness and Cohesion 2014–2020, from the European Regional Development Fund.

REFERENCES

1. Adu, M., Asare, P., Yawson, D., Nyarko, M. & Osei-Agyeman, K. (2018). Agronomic biofortification of selected underutilised solanaceae vegetables for improved dietary intake of potassium (K) in Ghana. *Heliyon*, e00750. https://doi.org/10.1016/j.heliyon.2018.e00750
2. Bailey, R. L., West, K. Jr & Black, R. (2015). The epidemiology of global micronutrient deficiencies. *Annals of nutrition and metabolism*, 66 (2), 22-33. https://doi.org/10.1159/000371618
3. Banaj, A., Banaj, Đ., Petrović, D., Stipešević, B. & Tadić, V. (2021). Sowing systems as a factor of grain yield in sunflower production. *Poljoprivreda*, 27 (2), 84-90. https://doi.org/10.18047/poljo.27.2.11
4. Broadley, M., Alcock, J., Alford, J., Cartwright, P., Foot, I., Fairweather-Tait, S. & Zhao, F. (2010). Selenium biofortification of high-yielding winter wheat (*Triticum aestivum* L.) by liquid or granular Se fertilisation. *Plant Soil*, 332 (1), 5-18. https://doi.org/10.1007/s11104-009-0234-4
5. Cakmak, I. & Kutman, U. (2018). Agronomic Biofortification of Cereals with Zinc: A Review. *European Journal of Soil Sciences*, 69, 172-180. https://doi.org/10.1111/ejss.12437
6. Dai, H., Wei, S. & Twardowska, I. (2020). Biofortification of soybean (Glycine max L.) with Se and Zn, and enhancing its physiological functions by spiking these elements to soil during flowering phase. *Science of The Total Environment*, 740, 139648. https://doi: 10.1016/j.scitotenv.2020.139648
7. Džomba, E., Dikić, M., Gadžo, D., *Čengić-Dzombai*, S., Lončarić, Z. & Singh, B. R. (2018). Effect of different doses and application methods of sodium selenate on selenium status in maize for silage. *Agricultural and food science*, 27 (4), 255-263. DOI: 10.23986/afsci.69057
8. Egnér, H., Riehm, H. & Domingo, W. (1960). Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Böden. II. Chemische Extraktionsmethoden zur Phosphor- und Kaliumbestimmung. *K. Lantbr. Ann.*, 26, 199-215.
9. Fordyce, F. (2013). Selenium deficiency and toxicity in the environment. In O. Selinus (Ed.), Essentials of medical geology (rev ed., pp. 375-416), Dordrecht, Springer.
10. Galetti, V. (2019). Zinc deficiency and stunting. In R. P. Victor & B. P. Vinood (eds), Handbook of famine, starvation, and nutrient deprivation (pp. 1969-1991), Dodrecht, Springer.
11. Galić, L., Vinković, T., Ravnjak, B. & Lončarić, Z. (2021). Agronomic Biofortification of Significant Cereal Crops with Selenium-A Review. *Agronomy*, 11 (5), 1015. https://doi.org/10.3390/agronomy11051015
12. Galinha, C., Sánchez-Martínez, M., Pacheco, A., Freitas, M., Coutinho, J., Maç□s, B., Almeida, A., Pérez-Corona, M., Madrid, Y. & Wolterbeek, H. (2015). Characterization of selenium-enriched wheat by agronomic biofortification. *Journal of Food Science and Technology*, 52, 4236-45. doi: 10.1007/s13197-014-1503-7
13. Garousi, F., Kovács, B. & Veres, S. (2018). Sunflower seedlings hyperaccumulate selenium. *Acta biologica Hungarica*, 69 (2), 197-209. doi: 10.1556/018.69.2018.2.8.
14. Grant, C., Buckley, W. & Wu, R. (2007). Effect of selenium fertilizer source and rate on grain yield and selenium and cadmium concentration of durum wheat. *Can J Plant Sci.,* 87, 703-708. https://doi.org/10.4141/CJPS06060

15. Gupta, M. & Gupta, S. (2017). An Overview of Selenium Uptake, Metabolism, and Toxicity in Plants. *Front. Plant. Sci.*, 7, 2074. https://doi.org/10.3389/fpls.2016.02074
16. Habibi, G. (2017). Physiological, photochemical and ionic responses of sunflower seedlings to exogenous selenium supply under salt stress. *Acta Physiologiae Plantarum*, 39, 1-9. https://doi.org/10.1007/s11738-017-2517-3
17. Hartikainen, H., TL, X. & Piironen, V. (2000). Selenium as an antioxidant and pro-oxidant in ryegrass. *Plant Soil*, 225 (12),193–200. https://doi.org/10.1023/A:1026512921026
18. International Standard Organisation. (1994). Soil quality – Determination of pH. ISO 10390: 1994.
19. International Standard Organisation. (1998). Soil quality – Determination of organic carbon by sulfochromic oxidation. ISO 14235: 1998.
20. International Standard Organization. (1995). Soil quality – Extraction of Trace Elements Soluble in Aqua Regia. ISO 11466: 1995.
21. Joy, E. J., Stein, A. J., Young, S. D., Ander, E. L., Watts, M. J. & Broadley, M. R. (2015). Zinc-enriched fertilisers as a potential public health intervention in Africa. *Plant and Soil*, 389, 1-24. https://doi.org/10.1007/s11104-015-2430-8
22. Khoshgoftarmanesh, A. H., Norouzi, M., Afyuni, M. & Schulin, R. (2017). Zinc biofortification of wheat through preceding crop residue incorporation into the soil. *European Journal of Agronomy*, 89, 131-139. https://doi.org/10.1016/j.eja.2017.05.006.
23. Kingston, H. & Jassie, L. (1986). Microwave energy for acid decomposition at elevated temperatures and pressures using biological and botanical samples. *Anal. Chem.*, 58, 2534-2541. https://doi.org/10.1021/ac00125a038
24. Lončarić, Z., Ivezić, V., Kerovec, D. & Rebekić, A. (2021). Foliar Zinc-Selenium and Nitrogen Fertilization Affects Content of Zn, Fe, Se, P, and Cd in Wheat Grain. *Plants*, 10 (8), 1549. https://doi.org/10.3390/plants10081549
25. Lyons, G., Lewis, J., Lorimer, M., Holloway, R., Brace, D., Stangoulis, J. & Graham, R. (2004). High-selenium wheat: agronomic biofortification strategies to improve human nutrition. *J Food Agri Environ.*, 2, (1), 171-178.
26. Manojlović, M., Lončarić, Z., *Čabilovski*, R., Popović, B., Karalić, K., Ivezić, V., Ademi, A. & Singh, B. R. (2019). Biofortification of wheat cultivars with selenium. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science*. 69 (8), 715-724. https://doi.org/10.1080/09064710.2019.1645204
27. Manojlović, M. & Lončarić, Z. (2017). Selenium deficiency in regional soils affecting animal and human health in Balkan and other European countries. In B. R. Singh, M. J. McLaughlin & E. C. Brevik (Eds.), The Nexus of Soils, Plants, Animals and Human Health (pp. 87-98). Stuttgart, Germany: The Catena-Schweizerbart.
28. Mao, H., Wang, J., Wang, Z., Zan, Y., Lyons, G. & Zou, C. (2014). Using agronomic biofortification to boost zinc, selenium, and iodine concentrations of food crops grown on the loess plateau in China. *J Soil Sci Plant Nutr.*, 14, 459-470. doi: 10.4067/S0718-95162014005000036
29. Mathers, A. W., Young, S. D., McGrath, S. P., Zhao, F. J., Crout, N. M. J. & Bailey, E. H. (2017). Determining the fate of selenium in wheat biofortification: an isotopically labelled field trial study. *Plant and Soil*, 420, 61-77. doi 10.1007/s11104-017-3374-y
30. Nawaz, F., Ahmad, R., Ashraf, M., Waraich, E. & Khan, S. (2015). Effect of selenium foliar spray on physiological and biochemical processes and chemical constituents of wheat under drought stress, *Ecotoxicol Environ Saf.*, 113, 191-200. https://doi.org/10.1016/j.ecoenv.2014.12.003
31. Novoselec, J., Klir, Ž., Domaćinović, M., Lončarić, Z. & Antunović, Z. (2018). Biofortification of feedstuffs with microelements in animal nutrition. *Poljoprivreda*, 24 (1), 25-34. doi: 10.18047/poljo.24.1.4
32. Petković, K., Manojlović, M., *Čabilovski*, R., Krstić, D., Lončarić, Z. & Lombn-'s, P. (2019). Foliar Application of Selenium, Zinc and Copper in Alfalfa (*Medicago Sativa* L.) Biofortification. *Turkish journal of field crops*, 24 (1), 81-90. doi: 10.17557/tjfc.569363
33. Petković, K., Manojlović, M., *Čabilovski*, R., Lončarić, Z., Krstić, D., Kovačević, D. & Ilić, M. (2022). Nitrogen fertilisation affected zinc and selenium biofortification in silage maize. *Crop & Pasture Science*, 73 (8), 781-791. doi: 10.1071/CP21735
34. Poudel, P., Di Gioia, F., Lambert, J. D. & Connolly, E. L. (2023). Zinc biofortification through seed nutri-priming using alternative zinc sources and concentration levels in pea and sunflower microgreens. *Frontiers in Plant Science*, 14, 1177844. https://doi.org/10.3389/fpls.2023.1177844
35. Poudel, P., Connolly, E. L., Kwasniewski, M., Lambert, J. D. & Di Gioia, F. (2024). Zinc biofortification via fertigation using alternative zinc sources and concentration levels in pea, radish, and sunflower microgreens. *Scientia Horticulturae*, 331, 113098. https://doi.org/10.1016/j.scienta.2024.113098
36. *Špoljarić* Maronić, D., *Žuna* Pfeiffer, T., Bek, N., *Štolfa Čamagajevac*, I., Galir Balkić, A., Stević, F., Maksimović, I., Mihaljević, M. & Lončarić, Z. (2024). Distribution of selenium: A case study of the Drava, Danube and associated aquatic biotopes. *Chemosphere*, 354,141596. https://doi.org/10.1016/j.chemosphere.2024.141596
37. Trierweiler, F. & Lindsay, W. (1969). EDTA-ammonium carbonate soil test for Zn. *Proc Soil Sci Soc Am.*, 33, 49-54. https://doi.org/10.2136/sssaj1969.03615995003300010017x
38. Velu, G., Ortiz-Monasterio, I., Cakmak, I., Hao, Y. & Singh, R. A. (2014). Biofortification strategies to increase grain zinc and iron concentrations in wheat. *Journal of cereal science*, 59, (3), 365-372. doi: 10.1016/j.jcs.2013.09.001
39. Yao, X., Jianzhou, C., Xueli, H., Binbin, L., Jingmin, L. & Zhaowei, Y. (2013). Effects of selenium on agronomical characters of winter wheat exposed to enhanced ultraviolet-B. *Ecotoxicol Environ Saf.*, 92, 320-326. https://doi.org/10.1016/j.ecoenv.2013.03.024
40. Yassen, A., Safia, M. & Sahar, M. (2011). Impact of nitrogen fertilizer and foliar spray of selenium on growth, yield and chemi cal constituents of potato plants. *Aust J Basic & Appl Sci.*, 5 (11), 1296-1303.
41. Zhang, Y. Q., Sun, Y. X., Ye, Y. L., Karim, M. R., Xue, Y. F., Yan, P., Meng, Q., Cui, Z., Cakmak, I., Fu-Suo Zhang, F. & Zou, C. Q. (2012). Zinc biofortification of wheat through fertilizer applications in different locations of China. *Field Crops Research*, 125, 1-7. https://doi.org/10.1016/j.fcr.2011.08.003
42. Zhang, T., Sun, H., Lv, Z., Cui, L., Mao, H. & Kopittke, P. M. (2017). Using synchrotron-based approaches to examine the foliar application of ZnSO4 and ZnO nanoparticles for field-grown winter wheat. *Journal of agricultural and food chemistry*, 66 (11), 2572-2579. https://doi.org/10.1021/acs.jafc.7b04153

OBOGAĆIVANJE ZRNA I ŽETVENIH OSTATAKA SUNCOKRETA BIOFORTIFIKACIJOM S Zn I Se

SAŽETAK

Biofortifikacija suncokreta folijarnom aplikacijom Zn i Se provedena je s ciljem obogaćivanja zrna suncokreta, istraživanja efikasnosti biofortifikacije i obogaćivanja ostataka usjeva kao nuspojave koja može imati rezidualan gnojidbeni učinak. Dvogodišnje istraživanje provedeno je folijarnom primjenom 10, 20 i 30 g/ha Se u obliku otopine Na-selenata ili 3 i 6 kg/ha Zn u obliku otopine Zn sulfata u randomiziranom blok-dizajnu s trima ponavljanjima. Biofortifikacija nije značajno utjecala ni na prinos zrna niti na prinos nadzemne mase, ali je povećala koncentracije Se i Zn u zrnu i nadzemnoj vegetativnoj masi suncokreta. Koncentracija Se u zrnu povećana je 14-44 puta, pri čemu bi 7,3 g/ha Se bilo dovoljno za postizanje 300 µg/kg Se u zrnu. Koncentracija Zn u zrnu povećana je do 72 mg/kg. Učinkovitost biofortifikacije u smislu akumulacije Se u zrnu bila je 15,4 - 19,3%, a Zn samo 1,73 - 3,15 %, što znači da značajne količine Se i Zn ostaju u žetvenim ostatcima, čija inkorporacija u tlo ima rezidualan učinak gnojidbe cinkom i selenom za sljedeće usjeve.

Ključne riječi: folijarna primjena, mikroelementi, rezidualan učinak, natrijev selenat, cinkov sulfat

(**Received on April 20, 2026; accepted on May 23, 2026** – *Primljeno 20. travnja 2026.; prihvaćeno 23. svibnja 2026.*)