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ORIGINAL PAPER

Evaluation of the effect of sulphur and selenium applications on copper and zinc content in spelt wheat (*Triticum spelta* L.) and common wheat (*Triticum aestivum* L.)

Marzena S. Brodowska[1], Magdalena Kurzyna-Szklarek[1], Renata Czeczko[2]

[1] Departament of Agricultural and Environmental Chemistry
[2] Department of Chemistry
University of Life Sciences in Lublin

Abstract

Wheat is one of the most widely cultivated cereal crops in Poland and constitutes an important source of human nutrition. Therefore, the biofortification of wheat with selenium offers an opportunity to increase the intake of this nutrient in both human and animal diets. The aim of the study was to evaluate the effects of different sulphur and selenium doses, as well as the timing of their application, on the copper and zinc contents in wheat grain and straw. The experiment was conducted on Luvisol (silt loam), classified according to PTG (2019) and WRB (2022) systems. Three experimental factors were considered in the study. The first factor was the sulphur dose, applied at three levels (S_0, S_I, S_{II}), and the second was the selenium dose, also applied at three levels (Se_0, Se_I, Se_{II}). The third factor was the timing of selenium application, tested at two stages: tillering (BBCH 22-24) and stem elongation (BBCH 31-34). The field trials included 108 experimental plots, each measuring 3 × 6 m (18 m²). The test plants were winter spelt wheat (*Triticum spelta* L.) cv. Rokosz and winter common wheat (*Triticum aestivum* L.) cv. Astoria. The results demonstrated a beneficial effect of selenium application on zinc content in both spelt and common wheat grains. At lower doses, selenium may have increased the copper content in spelt wheat straw; however, overall, it reduced the copper content in common wheat grain. Sulphur fertilization increased the copper content in the grains of both wheat species, decreased the zinc content in common wheat grain, and increased the zinc content in the straw of both species.

Keywords: zinc, copper, sulphur fertilisation, selenium fertilisation, winter wheat

Renata Czeczko, Ph.D., Department of Chemistry, Faculty of Food Science and Biotechnology, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland, e-mail: renata.czeczko@up.lublin.pl
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INTRODUCTION

Wheat is a cornerstone of global food security, fulfilling human nutritional needs and serving as a fundamental component of livestock feed production. It provides not only carbohydrates, proteins, and vitamins but also micronutrients such as zinc (Zn) and copper (Cu), which are crucial for plant metabolic processes and constitute essential components of human and animal diets (White, Broadley 2009, Cakmak, Kutman 2017). Given the growing prevalence of Zn and other mineral deficiencies in global populations, wheat is increasingly regarded as a long-term vehicle for micronutrient biofortification, offering a promising solution. In plants, zinc functions as a cofactor for enzymes that are essential for photosynthesis, nitrogen metabolism, and DNA/RNA biosynthesis (Cakmak, Kutman 2017). Among other functions, it activates carboxylases and superoxide dismutase (SOD), stabilizes cell membrane structures, and regulates metabolic pathways (García-Latorre et al. 2024). Zinc deficiency results in shortened internodes, chlorotic leaves, and reduced yield and grain quality (Cakmak, Kutman 2017). Conversely, excess Zn disrupts enzyme activity, induces oxidative stress, and negatively affects the homeostasis of other micronutrients (e.g., Fe, Mn, Cu), leading to secondary deficiencies (Poblaciones, Rengel 2017). Copper is a component of cytochrome c oxidase and enzymes involved in cell wall lignification. It plays a key role in photosynthesis, contributes to nitrogen metabolism, and supports plant defence mechanisms against pathogens. Deficiency symptoms include leaf chlorosis, necrosis of shoot tips, and reduced protein content in grain, whereas excess Cu induces oxidative stress and decreases the uptake of other micronutrients (Lange et al. 2017, Kulczycki, Sacała 2020). Thus, inadequate Zn and Cu levels in wheat crops contribute to lower yields and reduced technological grain quality. The Zn and Cu content in wheat is primarily determined by the soil nutrient status, cultivar-specific genotypic traits, weather conditions, soil pH, organic matter content, and the rate and method of fertilizer application (Skwaryło-Bednarz et al. 2011, Hange, Awofolu 2017). Fertilization with sulphur and selenium is gaining importance owing to their involvement in plant metabolism and their potential influence on micronutrient availability and transport (Abdalla et al. 2024).

Fertilisation of wheat with selenium (Se) not only increases selenium levels in the grain but can also alter the contents of other micronutrients such as zinc (Zn) and copper (Cu). Studies indicate that foliar application of Se can reduce Zn and Cu concentrations, which is attributed to competitive interactions or alterations in ion transport. In contrast, soil application of Se often increases Zn and Cu levels, suggesting that the form and route of Se delivery are crucial for micronutrient interactions. These effects may depend on wheat genotype and environmental conditions. Therefore, during Se biofortification it is recommended to monitor Zn and Cu levels to avoid disturbances in the micronutrient balance of the grain (Liang et al. 2020, Reynolds-Marzal et al. 2021). When Zn and Se are applied simultaneously at moderate doses, a synergistic effect is observed, enhancing antioxidant enzyme activity and Zn accumulation while retaining Se; however, high Se doses may reduce Zn accumulation (Gu et al. 2022). With appropriate dosing, the combined application of Zn and Se avoids antagonistic effects and promotes micronutrient balance, including Cu stabilisation, in wheat grain (Ning et al. 2021). Sulphur (S) application increases Zn mobility but may bind Cu, leading to reduced Cu content in plant tissues. A study by Brodowska and Kurzyna-Szklarek (2017) showed that foliar application of sulphur (S) to spring wheat reduced Zn concentrations in both grain and straw. However, changes in Cu content were inconclusive and depended on the applied sulphur dose. In contrast, Barczak et al. (2017) analysed the effects of sulphur fertilisation on the micronutrient content of oat grain. They reported that sulphur application, particularly at doses of 20 and 40 kg S ha^{-1}, significantly increased Zn and Cu accumulation in the grain. At the same time, they observed a positive effect on plant yield, confirming the effectiveness of sulphur fertilisation in enhancing the nutritional value of cereals. Agronomic biofortification, defined as increasing the content of beneficial elements in crops through fertilisation, is a cost-effective and scalable strategy to improve the nutritional quality of staple foods. This approach is gaining increasing attention due to its practicality, particularly in low-income regions where access to nutritional supplements and fortified foods is limited. In contrast to genetic biofortification, agronomic methods enable relatively rapid improvements and provide flexibility across crop species and diverse environmental conditions (García-Latorre et al. 2024, Kong et al. 2024). Understanding the effects of sulphur and selenium fertilisation on Cu and Zn content in cereals will allow farmers to adjust fertiliser doses to avoid elemental competition or deficiencies. In this way, yields can be not only higher but also of improved technological and nutritional quality.

Research hypothesis: It was assumed that sulphur and selenium fertilisation, depending on the dose and timing of application, significantly affects the concentrations of zinc (Zn) and copper (Cu) in the grain and straw of spelt wheat (*Triticum spelta* L.) and common wheat (*Triticum aestivum* L.).

Research objective: The aim of the study was to determine the interactive effect of sulphur and selenium fertilisation on the accumulation of Zn and Cu in the grain and straw of two winter wheat species.

MATERIALS AND METHODS

The research was based on a three-year field experiment established at the Experimental Station of the University of Life Sciences in Lublin, located in Czesławice (51°18′23″N, 22°16′02″E). The experiment was conducted on soil classified, according to PTG (2019), as belonging to the order of brown earths, subtype lessive soil (Luvisol). In the WRB system (2022), it corresponds to the reference unit Luvisols (silt loam). Granulometric composition: sand fraction (2.0-0.05 mm) – 37.66% (including: 2.0-1.0 mm – 0.04%; 1.0-0.5 mm – 7.18%; 0.5-0.25 mm – 13.68%; 0.25-0.10 mm – 4.34%; 0.10-0.05 mm – 12.42%); dust fraction (0.05-0.002 mm) – 56.88% (including: 0.05-0.02 mm – 29.09%; 0.02-0.002 mm – 27.80%); clay fraction (<0.002 mm) – 5.46%. The soil showed high abundance of available phosphorus and magnesium, and medium abundance of sulphate sulphur and available potassium. It was characterised by a slightly acidic reaction (pH 6.5). The soil nitrogen content was 1.23 g N kg^{-1} d.m. The test plants in the experiment were winter spelt wheat (*Triticum spelta L.*) cv. Rokosz and winter common wheat (*Triticum aestivum L.*) cv. Astoria.

Experimental design

The experiment included three experimental factors. The first factor was the sulphur dose, applied at three levels (S_0, S_I, S_{II}). The second factor was the selenium dose, also applied at three levels (Se_0, Se_I, Se_{II}). The third factor was the timing of selenium application, at two levels: tillering stage (BBCH 22-24) and stem elongation stage (BBCH 31-34). The field trials included 108 experimental plots measuring 3 × 6 m (18 m^2). The field experiment was conducted using the split-plot design. Sowing of test plants in each year of the field experiment was performed in the first ten days of October, while harvesting was performed in the second ten days of August. Sulphur was applied pre-sowing in the form of ammonium sulphate $(NH_4)_2SO_4$ at three doses: S_0 – 0 kg S ha^{-1}, S_I – 15 kg S ha^{-1}, S_{II} – 30 kg S ha^{-1}. Selenium was applied in the form of sodium selenite Na_2SeO_3 at three doses: Se_0 – 0 g Se ha^{-1}, Se_I – 10 g Se ha^{-1}, Se_{II} – 20 g Se ha^{-1}. Selenium application was carried out at two growth stages: tillering (BBCH 22-24) and stem elongation (BBCH 31-34).

Pre-sowing nitrogen fertilisation was applied in the form of ammonium nitrate NH_4NO_3 at a dose of 20 kg N ha^{-1}. In spring, nitrogen was supplemented to a total dose of 80 kg N ha^{-1} at two dates: at the tillering stage (BBCH 22-24), when 40 kg N ha^{-1} was applied (including nitrogen introduced with sulphur fertilisation), and at the stem elongation stage (BBCH 31-34), when a final dose of 20 kg N ha^{-1} was added. Phosphorus and potassium were applied pre-sowing in doses of 60 kg P_2O_5 ha^{-1} and 80 kg K_2O ha^{-1}.

Plant protection against weeds, diseases, and pests was carried out according to the recommendations of the Institute of Plant Protection in Poznań for winter spelt and common wheat. Against fungal diseases, four fungicide treatments were applied: Yamato 303 SE (thiophanate-methyl + tetraconazole) at BBCH 30-31 at a dose of 1.5 dm^3 ha^{-1}; Optan 183 SE (pyraclostrobin + epoxiconazole) at BBCH 30-59 at 1.5 dm^3 ha^{-1}; Virtuoz 520 EC (prochloraz + tebuconazole + proquinazid) at BBCH 20-59 at 1.0 dm^3 ha^{-1}; and Tilt Turbo 575 EC (propiconazole + fenpropidin) at BBCH 30-59 at 0.9

dm³ ha⁻¹. At the BBCH 21-29 stage, herbicide treatment was applied using Chisel 75 WG (thifensulfuron-methyl + chlorosulfuron) at a dose of 60 g ha^{-1} with the adjuvant Trend 90 EC (ethoxylated isodecyl alcohol) at 0.1%. Pest protection consisted of applying Decis Mega 50 EW (deltamethrin) at a dose of 0.2 dm^3 ha^{-1}. Harvesting was carried out in a single stage, at full maturity, using a field harvester.

Meteorological conditions

Weather conditions during the field experiment were highly variable. The growing seasons differed considerably in the distribution and intensity of precipitation, as well as in average monthly air temperatures (Table 1).

Table 1

Meteorological conditions during the field experiment

Mean monthly precipitation totals (mm)													
Year/month	01	02	03	04	05	06	07	08	09	10	11	12	mean
1st	50.9	15.8	48.6	39.1	169.6	13.5	52.6	5.9	113	41.5	59.3	32.2	53.5
2nd	37.4	62.3	62.1	41.9	37.0	51.6	193.3	38.1	16.8	111.7	46.1	54.0	62.6
3rd	17.1	44.1	31.8	59.1	34.7	43.2	147.7	75.1	103.3	104.9	37.0	39.4	61.4
4th	20.7	12.9	16.0	27.1	59.1	74.8	91.2	55.5	54.7	41.3	15.2	51.9	43.3
Mean	31.5	33.7	39.6	41.8	75.1	45.7	121.2	43.6	71.9	74.8	39.4	44.3	55.2
Mean 1991-2020	38.9	34.3	40.5	39.2	67.9	74.2	93	71.1	62.4	50.9	43	41.6	54.8
Mean monthly air temperatures (°C)													
Year/month	01	02	03	04	05	06	07	08	09	10	11	12	mean
1st	0.9	-1.1	2.8	6.5	11.5	16.0	18.9	21.8	15.0	6.6	4.2	3.2	8.9
2nd	-5.2	2.7	2.9	8.1	13.8	18.0	18.7	17.7	15.1	7.1	2.9	-0.3	8.4
3rd	-5.9	-0.6	5.0	6.8	12.8	17.6	18.6	19.7	13.2	8.7	3.8	1.4	8.4
4th	-0.5	-4.2	-1.0	12.7	15.5	16.3	16.7	20.0	14,9	9.2	3.9	-0.1	8.6
Mean	-2.6	-0.8	2.4	8.5	13.4	16.9	18.2	19.8	14,5	7.9	3.7	1.0	8.6
Mean 1991-2020	-1.4	-0.4	2.8	8.3	12.9	16.4	18.4	18.1	13.4	8.5	3.8	0	8.4

The warmest year was the first year of the experiment, with an average annual temp. of 8.9°C, while the coldest was the third year, with an average annual temperature of 8.4°C. The coldest month during the experiment was January, with an average temperature of –5.5°C. The warmest month during the experiment was August. In the first year of the experiment, monthly temperatures were lower than the multi-year average during the periods of emergence and tillering, as well as stem elongation, heading, and plant maturation

The average rainfall during the experiment was 662.6 mm, which was slightly lower than the long-term average of 657 mm. Plants matured under highly variable precipitation conditions across the different years of the study. The harvested plant material (grain and straw) was mineralised in concentrated sulphuric acid with the addition of perhydrol. Copper and zinc contents were determined using atomic absorption spectrometry (AAS).

Statistical analysis

The results were statistically analysed using a three-factor analysis of variance (ANOVA) with the determination coefficient calculated in Statistica 13 software. The Tukey's HSD test was applied to determine the least significant difference (LSD) values and statistically homogeneous groups at a significance level of $p \leq 0.05$. Values reported in the tables for the interaction of factors A × B × C that share the same letters are not significantly different.

RESULTS AND DISCUSSION

In the experiment, the average zinc content in spelt wheat grain was 22.68 mg kg^{-1} d.m. This value was slightly lower than those reported in other studies (Rachoń, Szumiło 2009, Knapowski et al. 2016). The zinc content in common wheat grain (Table 2) and straw (Table 3) was lower than values reported by other authors (Gondek, Gondek 2010) and below levels considered optimal for fodder (Gorlach 1991, Symanowicz, Kalembasa 2012). Selenium application increased the zinc content of spelt wheat grain at most sites, with the lower dose associated with a 12% increase. The timing of selenium application did not significantly affect the zinc content of spelt wheat grain. At most sites, sulphur fertilisation decreased the zinc content of spelt grain. Application of the lower sulphur dose reduced average zinc content by 6% compared to unfertilised sites. In common wheat grain, zinc content increased under the higher selenium dose. Later selenium application was associated with an average 5% increase. Sulphur application increased zinc content in spelt wheat straw by 11% and 13%, respectively. Foliar feeding with the lower selenium dose resulted in a 10% increase. Plots with later selenium application showed 20% higher zinc content compared to those with earlier application. In common wheat straw, the higher sulphur dose increased zinc content by 18%. Foliar feeding with the lower selenium dose increased zinc content by 51%, while the higher dose increased it by 8%. Our results on zinc content in common wheat are consistent with the findings of Kozłowska-Strawska (2009), who reported a decrease under sulphur fertilisation, regardless of dose. In contrast, studies by Łukasiewicz et al. (2012), Barczak et al. (2019), Klikocka et al. (2018) found that sulphur fertilisation of spring wheat significantly increased grain zinc content.

However, no effect of mineral fertilisation on zinc content was reported by Stanisławska-Glubiak and Korzeniowska (2007) and by Bañuelos et al.

Table 2

Zinc content (mg kg^{-1} dm) in the grain of spelt wheat (*Triticum spelta* L.) and common wheat (*Triticum aestivum* L.)

Selenium dose (B)	Sulfur dose (A) (A)												Mean			
	S_0				S_I				S_{II}							
	Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear	
Spelt wheat (*Triticum spelta* L.)																
I selenium application date (C)																
Se_0	16.17	21.47	25.67	21.10[a]	16.17	27.93	25.43	23.18[a]	15.97	24.57	20.60	20.38[a]	16.10	24.66	23.90	21.55
Se_I	19.07	22.30	25.10	22.16[a]	18.87	20.03	45.53	28.14[a]	17.60	22.20	29.77	23.19[a]	18.51	21.51	33.47	24.50
Se_{II}	20.70	20.33	24.20	21.74[a]	22.47	21.30	16.51	20.09[a]	22.10	20.33	27.40	23.28[a]	21.76	20.65	22.70	21.70
	18.65	21.37	24.99	21.67	19.17	23.09	29.16	23.80	18.56	22.37	25.92	22.28	18.79	22.27	26.69	22.58
II selenium application date (C)																
Se_0	16.17	21.47	25.67	21.10[a]	16.17	27.93	25.43	23.18[a]	15.97	24.57	20.60	20.38[a]	16.10	24.66	23.90	21.55
Se_I	19.60	22.93	33.03	25.19[a]	18.87	20.47	27.33	22.22[a]	19.67	23.90	28.17	23.91[a]	19.38	22.43	29.51	23.77
Se_{II}	23.93	20.40	27.57	23.97[a]	20.40	20.17	23.47	21.35[a]	25.17	20.67	25.13	23.66[a]	23.17	20.41	25.39	22.99
	19.90	21.60	28.76	23.42	18.48	22.86	25.41	22.25	20.27	23.05	24.63	22.65	19.55	22.50	26.27	22.77
Mean																
Se_0	16.17	21.47	25.67	21.10	16.17	27.93	25.43	23.18	15.97	24.57	20.60	20.38	16.10	24.66	23.90	21.55
Se_I	19.34	22.62	29.07	23.68	18.87	20.25	36.43	25.18	18.64	23.05	28.97	23.55	18.95	21.97	31.49	24.14
Se_{II}	22.32	20.37	25.89	22.86	21.44	20.74	19.99	20.72	23.64	20.50	26.27	23.47	22.47	20.53	24.05	22.35
	19.28	21.49	26.88	22.55	18.83	22.98	27.29	23.03	19.42	22.71	25.28	22.47	19.17	22.39	26.48	22.68
$LSD_{0.05}$	A - n.s. B - n.s. C - n.s. AxB - n.s. AxC - n.s. BxC - n.s. AxBxC - n.s.															

cont. Table 2

Selenium dose (B)	Sulfur dose (A) (A)												Mean			
	S_0				S_I				S_{II}							
	Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear	
Common wheat (*Triticum aestivum* L.)																
I selenium application date (C)																
Se_0	17.43	21.37	19.40	19.40^{ab}	17.90	16.40	17.20	17.17^{ab}	16.00	23.57	18.03	19.20^{ab}	17.11	20.45	18.21	18.59
Se_I	17.73	20.57	19.77	19.36^{ab}	15.97	19.17	12.37	15.84^{a}	16.07	19.30	19.17	18.18^{ab}	16.59	19.68	17.10	17.79
Se_{II}	19.23	15.73	18.03	17.66^{ab}	18.63	16.03	19.90	18.19^{ab}	16.97	19.80	19.77	18.85^{ab}	18.28	17.19	19.23	18.23
	18.13	19.22	19.07	18.81	17.50	17.20	16.49	17.06	16.35	20.89	18.99	18.74	17.33	19.10	18.18	18.20
II selenium application date (C)																
Se_0	17.43	21.37	19.40	19.40^{ab}	17.90	16.40	17.20	17.17^{ab}	16.00	23.57	18.03	19.20^{ab}	17.11	20.45	18.21	18.59
Se_I	16.90	18.07	18.77	17.91^{ab}	16.57	22.20	21.67	20.15^{ab}	16.67	18.53	17.60	17.60^{ab}	16.71	19.60	19.35	18.55
Se_{II}	21.03	18.40	22.90	20.78^{b}	20.83	16.83	18.73	18.80^{ab}	19.07	20.30	21.63	20.33^{ab}	20.31	18.51	21.09	19.97
	18.45	19.28	20.36	19.36	18.43	18.48	19.20	18.70	17.25	20.80	19.09	19.04	18.04	19.52	19.55	19.04
Mean																
Se_0	17.43	21.37	19.40	19.40	17.90	16.40	17.20	17.17	16.00	23.57	18.03	19.20	17.11	20.45	18.21	18.59
Se_I	17.32	19.32	19.27	18.64	16.27	20.69	17.02	18.00	16.37	18.92	18.39	17.89	16.65	19.64	18.23	18.17
Se_{II}	20.13	17.07	20.47	19.22	19.73	16.43	19.32	18.50	18.02	20.05	20.70	19.59	19.30	17.85	20.16	19.10
	18.29	19.25	19.72	19.09	17.97	17.84	17.85	17.88	16.80	20.85	19.04	18.89	17.69	19.31	18.87	18.62
$LSD_{0.05}$	A - n.s. B - n.s. C - n.s. AxB - n.s. AxC - n.s. BxC - n.s. AxBxC - 4,86															

Values marked with the same letters do not differ significantly ($p \leq 0.05$). LSD for: A – sulphur dose, B – selenium dose, C – selenium application date, AxB, AxC, BxC, AxBxC – interactions; significant differences at p≤0.0,
S_0 – 0 kg S ha^{-1}, S_I – 15 kg S ha^{-1}, S_{II} – 30 kg S ha^{-1}, Se_0 – 0 g Se ha^{-1}, Se_I – 10 g Se ha^{-1}, Se_{II} – 20 g Se ha^{-1}, date of application: tillering stage (BBCH 22-24), stem elongation stage (BBCH 31-34).

Table 3

Zinc content (mg kg^{-1} dm) in the straw of spelt wheat (*Triticum spelta* L.) and common wheat (*Triticum aestivum* L.)

Selenium dose (B)	Sulfur dose (A)												Mean			
	S_0				S_I				S_{II}							
	Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear	
Spelt wheat (*Triticum spelta* L.)																
I selenium application date (C)																
Se_0	3.33	6.23	3.33	4.30^{ab}	8.10	6.40	8.10	7.53^{ab}	4.30	5.33	4.30	4.64^{ab}	5.24	5.99	5.24	5.49
Se_I	2.73	5.80	2.73	3.75^{ab}	5.13	4.65	5.13	4.97^{ab}	9.60	8.60	7.02	8.41^{ab}	5.82	6.35	4.96	5.71
Se_{II}	4.10	5.47	4.10	4.56^{ab}	4.54	5.40	5.89	5.28^{ab}	3.07	4.35	3.07	3.50^{a}	3.90	5.07	4.35	4.44
	3.39	5.83	3.39	4.20	5.92	5.48	6.37	5.93	5.66	6.09	4.80	5.52	4.99	5.80	4.85	5.21
II selenium application date (C))																
Se_0	3.33	6.23	3.33	4.30^{ab}	8.10	6.40	5.13	6.54^{ab}	4.30	5.33	7.80	5.81^{ab}	5.24	5.99	5.42	5.55
Se_I	9.37	5.83	9.37	8.19^{b}	4.73	5.03	6.70	5.49^{ab}	5.47	5.95	5.47	5.63^{ab}	6.52	5.60	7.18	6.44
Se_{II}	6.00	8.62	6.00	6.87^{ab}	4.27	6.40	5.93	5.53^{ab}	7.37	9.47	7.37	8.07^{ab}	5.88	8.16	6.43	6.83
	6.23	6.89	6.23	6.45	5.70	5.94	5.92	5.85	5.71	6.92	6.88	6.50	5.88	6.58	6.34	6.27
Mean																
Se_0	3.33	6.23	3.33	4.30	8.10	6.40	6.62	7.04	4.30	5.33	6.05	5.23	5.24	5.99	5.33	5.52
Se_I	6.05	5.82	6.05	5.97	4.93	4.84	5.92	5.23	7.54	7.28	6.25	7.02	6.17	5.98	6.07	6.08
Se_{II}	5.05	7.05	5.05	5.72	4.41	5.90	5.91	5.41	5.22	6.91	5.22	5.79	4.89	6.62	5.39	5.64
	4.81	6.36	4.81	5.33	5.81	5.71	6.15	5.89	5.69	6.51	5.84	6.01	5.44	6.19	5.60	5.74
$LSD_{0,05}$	A - n.s. B - n.s. C - n.s. AxB - 2.16 AxC - n.s. BxC - n.s. AxBxC - 3.44															

cont. Table 3

Selenium dose (B)	Sulfur dose (A)												Mean			
	S_0				S_I				S_{II}							
	Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear	
Common wheat (*Triticum aestivum* L.)																
I selenium application date (C)																
Se_0	2.73	3.97	5.33	4.01^{ab}	2.80	3.40	8.10	3.50^{a}	2.93	7.13	4.30	4.70^{ab}	2.82	4.83	4.55	4.07
Se_I	2.53	6.67	4.23	4.48^{ab}	6.73	5.40	4.73	5.93^{ab}	3.47	9.90	6.37	6.58^{ab}	4.24	7.32	5.42	5.66
Se_{II}	4.03	6.13	4.73	4.96^{ab}	3.03	6.10	4.27	3.93^{ab}	3.00	4.65	4.47	4.04^{ab}	3.35	5.63	3.96	4.31
	3.10	5.59	4.76	4.48	4.19	4.97	4.21	4.46	3.13	7.23	4.96	5.11	3.47	5.93	4.64	4.68
II selenium application date (C)																
Se_0	2.73	3.97	5.33	4.01^{ab}	2.80	3.40	4.30	3.50^{a}	2.93	7.13	4.03	4.70^{ab}	2.82	4.83	4.55	4.07
Se_I	3.50	13.13	5.33	7.32^{ab}	2.23	7.67	5.40	5.10^{ab}	2.83	14.15	5.37	7.45^{b}	2.85	11.65	5.37	6.62
Se_{II}	3.17	3.53	2.80	3.17^{ab}	4.20	5.80	4.00	4.67^{ab}	4.37	6.80	5.67	5.61^{ab}	3.91	5.38	4.16	4.48
	3.13	6.88	4.49	4.83	3.08	5.62	4.57	4.42	3.38	9.36	5.02	5.92	3.20	7.29	4.69	5.06
Mean																
Se_0	2.73	3.97	5.33	4.01	2.80	3.40	6.20	3.50	2.93	7.13	4.17	4.70	2.82	4.83	4.55	4.07
Se_I	3.02	9.90	4.78	5.90	4.48	6.54	5.07	5.52	3.15	12.03	5.87	7.02	3.55	9.49	5.40	6.14
Se_{II}	3.60	4.83	3.77	4.07	3.62	5.95	4.14	4.30	3.69	5.73	5.07	4.83	3.63	5.51	4.06	4.40
	3.12	6.24	4.63	4.66	3.64	5.30	4.39	4.44	3.26	8.30	4.99	5.52	3.34	6.61	4.67	4.87
$LSD_{0,05}$	A - n.s. B - 1,26 C - n.s. AxB - n.s. AxC - n.s. BxC - n.s. AxBxC - n.s.															

Values marked with the same letters do not differ significantly ($p \leq 0.05$). LSD for: A – sulphur dose, B – selenium dose, C – selenium application date, AxB, AxC, BxC, AxBxC – interactions; significant differences at $p \leq 0.05$,

S_0 – 0 kg S ha^{-1}, S_I – 15 kg S ha^{-1}, S_{II} – 30 kg S ha^{-1}, Se_0 – 0 g Se ha^{-1}, Se_I – 10 g Se ha^{-1}, Se_{II} – 20 g Se ha^{-1}, date of application: tillering stage (BBCH 22-24), stem elongation stage (BBCH 31-34).

(1997). The latter authors observed an increase in zinc content in oilseed rape and a decrease in hemp ketmia grown on soils with high selenium abundance. A stimulating effect of selenium on zinc uptake by plants was also reported by Arvy et al. (1995). An increase in zinc content in wheat grain under selenium fertilisation was reported by Ducsy et al. (2021).

The average copper content in spelt grain in the present study was 2.52 mg kg^{-1} d.m., and in common wheat grain 3.00 mg kg^{-1} d.m. (Table 4). In straw, the average copper content was 2.34 mg kg^{-1} d.m. and 2.90 mg kg^{-1} d.m., respectively (Table 5). These values are consistent with the results of Rachoń and Szumiło (2009), who reported copper contents of 2.85 and 2.99 mg kg^{-1} d.m. depending on wheat species, as well as with those of Kulczycki and Grocholski (2004). Other authors (Cegielska, Gromulska 2008, Knapowski et al. 2016) reported considerably higher copper contents. For feed production, a copper content of 4.0 mg kg^{-1} d.m. is considered optimal (Gorlach 1991). In spelt grain, sulphur fertilisation was associated with an increase in copper content compared with unfertilised plots. The lower dose increased copper content by 4%, and the higher dose by 13%. Application of the lower selenium dose increased copper content by 9%. Earlier selenium application resulted in a 7% increase in copper content in spelt grain. In common wheat grain, sulphur fertilisation increased copper content by 11% and 7%, respectively. In contrast, both lower and higher selenium doses reduced copper content, by an average of 8% and 17%. The timing of selenium application did not significantly affect copper content in common wheat grain. In spelt straw, the lower sulphur dose increased copper content by 10%, while the higher dose reduced it by 6% compared with the unfertilised control. Foliar selenium feeding increased copper content in most treatments, by 7% and 4%, respectively. Later selenium application increased copper content by 8% compared with the earlier date. In common wheat straw, sulphur fertilisation had no clear effect on copper content. However, selenium application increased copper content, with the stronger effect observed at the lower dose (7%). Later selenium application was associated with an average 7% decrease in copper content in common wheat straw.

Gondek and Gondek (2010) obtained the highest copper contents in plots not fertilised with sulphur and in those where sulphur was applied together with a compound fertiliser. Klikocka et al. (2018) and Barczak et al. (2019) also reported a positive effect of sulphur fertilisation on copper content in test plants. In the study by Gondek and Gondek (2010), the copper content of spring wheat straw was not affected by the applied mineral fertilisation. Schiavon et al. (2013) reported a reduction in copper content in tomato roots and leaves under selenium fertilisation. Similarly, He et al. (2004) demonstrated an inhibitory effect of selenium on copper uptake, noting that the addition of only 1 mg Se kg^{-1} soil significantly reduced copper uptake in oilseed rape.

A similar relationship was observed in wheat grain (Ducsay et al. 2021). Arvy et al. (1995) reported a stimulating effect of selenium on copper uptake

Table 4

Copper content (mg kg^{-1} dm) in the grain of spelt wheat (*Triticum spelta* L.) and common wheat (*Triticum aestivum* L.)

Selenium dose (B)	Sulfur dose (A))												Mean			
	S_0				S_I				S_{II}							
	Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear	
Spelt wheat (*Triticum spelta* L.)																
I selenium application date (C)																
Se_0	2.70	2.20	1.40	2.10[a]	2.40	3.50	1.77	2.56[a]	2.27	4.00	1.73	2.67[a]	2.46	3.23	1.63	2.44
Se_I	2.60	4.00	0.80	2.47[a]	3.73	3.30	2.23	3.09[a]	3.33	3.30	1.43	2.69[a]	3.22	3.53	1.49	2.75
Se_{II}	2.37	3.60	1.63	2.53[a]	3.10	3.00	1.41	2.50[a]	2.60	4.40	1.73	2.91[a]	2.69	3.67	1.59	2.65
	2.56	3.27	1.28	2.37	3.08	3.27	1.80	2.72	2.73	3.90	1.63	2.75	2.79	3.48	1.57	2.61
II selenium application date (C)																
Se_0	2.70	2.20	1.40	2.10[a]	2.40	3.50	1.77	2.56[a]	2.27	4.00	1.73	2.67[a]	2.46	3.23	1.63	2.44
Se_I	3.23	3.80	1.43	2.82[a]	1.97	2.60	1.53	2.03[a]	3.63	3.90	1.00	2.84[a]	2.94	3.43	1.32	2.57
Se_{II}	2.07	3.50	1.33	2.30[a]	1.60	3.50	1.40	2.17[a]	2.77	3.30	1.10	2.39[a]	2.15	3.43	1.28	2.29
	2.67	3.17	1.39	2.41	1.99	3.20	1.57	2.25	2.89	3.73	1.28	2.63	2.52	3.37	1.41	2.43
Mean																
Se_0	2.70	2.20	1.40	2.10	2.40	3.50	1.77	2.56	2.27	4.00	1.73	2.67	2.46	3.23	1.63	2.44
Se_I	2.92	3.90	1.12	2.65	2.85	2.95	1.88	2.56	3.48	3.60	1.22	2.77	3.08	3.48	1.41	2.66
Se_{II}	2.22	3.55	1.48	2.42	2.35	3.25	1.41	2.34	2.69	3.85	1.42	2.65	2.42	3.55	1.44	2.47
	2.62	3.22	1.34	2.39	2.54	3.24	1.69	2.49	2.81	3.82	1.46	2.69	2.66	3.43	1.49	2.52
$LSD_{0,05}$	A - n.s. B - n.s. C - n.s. AxB - n.s. AxC - n.s. BxC - n.s. AxBxC - n.s.															

cont. Table 4

Selenium dose (B)	Sulfur dose (A))												Mean			
	S_0				S_I				S_{II}							
	Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear	
Common wheat (*Triticum aestivum* L.)																
I selenium application date (C)																
Se_0	3.63	3.50	1.23	2.79[a]	4.47	3.80	2.23	3.50[a]	4.47	4.90	0.80	3.39[a]	4.19	4.07	1.42	3.23
Se_I	5.40	3.50	1.50	3.47[a]	3.63	3.60	1.77	3.00[a]	2.37	4.30	1.93	2.87[a]	3.80	3.80	1.73	3.11
Se_{II}	1.73	3.40	2.60	2.58[a]	2.37	2.90	3.00	2.76[a]	1.63	3.50	2.80	2.64[a]	1.91	3.27	2.80	2.66
	3.59	3.47	1.78	2.94	3.49	3.43	2.33	3.09	2.82	4.23	1.84	2.97	3.30	3.71	1.98	3.00
II selenium application date (C)																
Se_0	3.63	3.50	1.23	2.79[a]	4.47	3.80	2.23	3.50[a]	4.47	4.90	0.80	3.39[a]	4.19	4.07	1.42	3.23
Se_I	2.17	3.40	2.73	2.77[a]	2.30	4.30	3.00	3.20[a]	1.97	2.50	3.60	2.69[a]	2.15	3.40	3.11	2.89
Se_{II}	2.27	2.90	2.57	2.58[a]	1.77	4.10	2.77	2.88[a]	2.57	3.50	3.30	3.12[a]	2.20	3.50	2.88	2.86
	2.69	3.27	2.18	2.71	2.85	4.07	2.67	3.19	3.00	3.63	2.57	3.07	2.85	3.66	2.47	2.99
Mean																
Se_0	3.63	3.50	1.23	2.79	4.47	3.80	2.23	3.50	4.47	4.90	0.80	3.39	4.19	4.07	1.42	3.23
Se_I	3.79	3.45	2.12	3.12	2.97	3.95	2.39	3.10	2.17	3.40	2.77	2.78	2.98	3.60	2.42	3.00
Se_{II}	2.00	3.15	2.59	2.58	2.07	3.50	2.89	2.82	2.10	3.50	3.05	2.88	2.06	3.39	2.84	2.76
	3.14	3.37	1.98	2.83	3.17	3.75	2.50	3.14	2.91	3.93	2.21	3.02	3.08	3.69	2.23	3.00
$LSD_{0,05}$	A - n.s. B - n.s. C - n.s. AxB - n.s. AxC - n.s. BxC - n.s. AxBxC - n.s.															

Values marked with the same letters do not differ significantly ($p \leq 0.05$). LSD for: A – sulphur dose, B – selenium dose, C – selenium application date, AxB, AxC, BxC, AxBxC – interactions; significant differences at $p \leq 0.05$,
S_0 – 0 kg S ha^{-1}, S_I – 15 kg S ha^{-1}, S_{II} – 30 kg S ha^{-1}, Se_0 – 0 g Se ha^{-1}, Se_I – 10 g Se ha^{-1}, Se_{II} – 20 g Se ha^{-1}, date of application: tillering stage (BBCH 22-24), stem elongation stage (BBCH 31-34).

Table 5

Copper content (mg kg^{-1} dm) in the straw of spelt wheat (*Triticum spelta* L.) and common wheat (*Triticum aestivum* L.)

Selenium dose (B)	Sulfur dose (A)												Mean			
	S_0				S_I				S_{II}							
	Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear	
Spelt wheat (*Triticum spelta* L.)																
I selenium application date (C)																
Se_0	2.00	2.30	2.60	2.30[a]	1.80	4.07	1.80	2.56[a]	1.30	2.30	2.17	1.92[a]	1.70	2.89	2.19	2.26
Se_I	1.10	2.50	2.87	2.16[a]	2.47	2.10	2.60	2.39[a]	1.97	2.90	2.07	2.31[a]	1.85	2.50	2.51	2.29
Se_{II}	2.13	2.10	2.07	2.10[a]	1.80	2.30	2.18	2.09[a]	1.50	3.50	2.33	2.44[a]	1.81	2.63	2.19	2.21
	1.74	2.30	2.51	2.19	2.02	2.82	2.19	2.35	1.59	2.90	2.19	2.23	1.79	2.67	2.30	2.25
II selenium application date (C)																
Se_0	2.00	2.30	2.60	2.30[a]	1.80	4.07	1.80	2.56[a]	1.30	2.30	2.17	1.92[a]	1.70	2.89	2.19	2.26
Se_I	2.23	2.50	2.17	2.30[a]	2.43	2.60	5.37	3.47[a]	2.03	1.30	2.07	1.80[a]	2.23	2.13	3.20	2.52
Se_{II}	1.83	2.50	3.73	2.69[a]	2.33	2.10	2.17	2.20[a]	2.10	1.90	3.73	2.58[a]	2.09	2.17	3.21	2.49
	2.02	2.43	2.83	2.43	2.19	2.92	3.11	2.74	1.81	1.83	2.66	2.10	2.01	2.40	2.87	2.42
Mean																
Se_0	2.00	2.30	2.60	2.30	1.80	4.07	1.80	2.56	1.30	2.30	2.17	1.92	1.70	2.89	2.19	2.26
Se_I	1.67	2.50	2.52	2.23	2.45	2.35	3.99	2.93	2.00	2.10	2.07	2.06	2.04	2.32	2.86	2.41
Se_{II}	1.98	2.30	2.90	2.40	2.07	2.20	2.18	2.15	1.80	2.70	3.03	2.51	1.95	2.40	2.70	2.35
	1.88	2.37	2.67	2.31	2.11	2.87	2.65	2.55	1.70	2.37	2.43	2.17	1.90	2.54	2.59	2.34
$LSD_{0,05}$	A - n.s. B - n.s. C - n.s. AxB - n.s. AxC - n.s. BxC - n.s. AxBxC - n.s.															

cont. Table 5

Selenium dose (B)	Sulfur dose (A)												Mean			
	S_0				S_I				S_{II}							
	Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear		Iyear	IIyear	IIIyear	
Common wheat (*Triticum aestivum* L.)																
I selenium application date (C)																
Se_0	2.33	2.20	3.70	2.74[a]	2.87	2.70	3.30	2.96[a]	1.80	2.40	4.00	2.73[a]	2.33	2.43	3.67	2.81
Se_I	3.17	3.00	2.70	2.96[a]	3.13	2.90	2.44	2.82[a]	3.27	2.50	4.43	3.40[a]	3.19	2.80	3.19	3.06
Se_{II}	2.87	2.70	5.43	3.67[a]	3.80	2.80	1.47	2.69[a]	4.40	3.00	1.90	3.10[a]	3.69	2.83	2.93	3.15
	2.79	2.63	3.94	3.12	3.27	2.80	2.40	2.82	3.16	2.63	3.44	3.08	3.07	2.69	3.26	3.01
II selenium application date (C)																
Se_0	2.33	2.20	3.70	2.74[a]	2.87	2.70	3.30	2.96[a]	1.80	2.40	4.00	2.73[a]	2.33	2.43	3.67	2.81
Se_I	2.63	2.30	2.83	2.59[a]	3.90	2.70	3.03	3.21[a]	3.37	2.90	2.70	2.99[a]	3.30	2.63	2.85	2.93
Se_{II}	3.40	2.20	2.00	2.53[a]	3.03	2.20	3.10	2.78[a]	3.20	3.10	1.40	2.57[a]	3.21	2.50	2.17	2.63
	2.79	2.23	2.84	2.62	3.27	2.53	3.14	2.98	2.79	2.80	2.70	2.76	2.95	2.52	2.90	2.79
Mean																
Se_0	2.33	2.20	3.70	2.74	2.87	2.70	3.30	2.96	1.80	2.40	4.00	2.73	2.33	2.43	3.67	2.81
Se_I	2.90	2.65	2.77	2.78	3.52	2.80	2.74	3.02	3.32	2.70	3.57	3.20	3.25	2.72	3.02	3.00
Se_{II}	3.14	2.45	3.72	3.10	3.42	2.50	2.29	2.74	3.80	3.05	1.65	2.84	3.45	2.67	2.55	2.89
	2.79	2.43	3.39	2.87	3.27	2.67	2.77	2.90	2.98	2.72	3.07	2.92	3.01	2.61	3.08	2.90
$LSD_{0,05}$	A - n.s. B - n.s. C - n.s. AxB - n.s. AxC - n.s. BxC - n.s. AxBxC - n.s.															

Values marked with the same letters do not differ significantly ($p \leq 0.05$). LSD for: A – sulphur dose, B – selenium dose, C – selenium application date, AxB, AxC, BxC, AxBxC – interactions; significant differences at $p \leq 0.05$,

S_0 – 0 kg S ha^{-1}, S_I – 15 kg S ha^{-1}, S_{II} – 30 kg S ha^{-1}, Se_0 – 0 g Se ha^{-1}, Se_I – 10 g Se ha^{-1}, Se_{II} – 20 g Se ha^{-1}, date of application: tillering stage (BBCH 22-24), stem elongation stage (BBCH 31-34).

by the studied plants, which is consistent with the results of the present study. In contrast, Wu and Hung (1992) found no relationship between selenium fertilisation and copper content.

The accumulation of micronutrients in plants is influenced by ionic antagonism in the soil. Under conditions of high phosphate ion availability in the plant growth environment, Zn^{2+} uptake is reduced, thereby limiting its accumulation in wheat grain, as confirmed by field experiment results (Recena et al. 2021, Hui et al. 2025). The accumulation of Cu and Zn is also affected by their mutual antagonism in the soil, which may negatively influence their uptake and accumulation in plants (Rahman et al. 2022). Nitrogen fertilisation acts as an important modifying factor in Zn and Cu uptake. Application of ammonium ions (NH_4^+), due to their acidifying effect, increases the mobility and thus the availability of Cu^{2+} and Zn^{2+} ions to plants. In contrast, nitrate-based fertilisation (NO_3^-) alkalises the plant growth environment, leading to reduced micronutrient availability. The uptake of copper and zinc by plants also depends on the presence of sulphate ions, which is particularly evident when these micronutrients are supplied as copper and zinc sulphates. Balanced sulphur fertilisation enhances Zn uptake by wheat, although the synergistic effect strongly depends on soil properties and the applied fertiliser doses (Recena et al. 2021).

CONCLUSIONS

In a study on the effect of sulphur and selenium fertilisation on zinc and copper content in winter spelt wheat (*Triticum spelta* L.) cv. Rokosz and winter common wheat (*Triticum aestivum* L.) cv. Astoria, differentiated plant responses were observed depending on species, fertiliser dose, and timing of application. The average zinc content of spelt wheat grain was lower than values reported in the literature. Selenium, particularly at lower doses, increased zinc content in both grain and straw of the tested plants, with the effect being stronger when Se was applied later, especially in common wheat (*Triticum aestivum* L.). Sulphur application decreased zinc content in common wheat grain but increased its level in straw. The average copper content was below the optimum level for fodder. Sulphur generally increased copper content in the grain of both wheat species, although higher doses sometimes had the opposite effect. Selenium application had varying effects: in common wheat it decreased grain copper content, while in spelt wheat it increased copper content in straw. Selenium had a beneficial effect on zinc content, particularly in spelt grain, with lower doses and later application being most effective. In common wheat, the effect was also positive, although weaker. Sulphur fertilisation decreased zinc content in common wheat grain but increased it in the straw of both wheat species, suggesting a redistribution of this micronutrient within the plant under sulphur application. Copper content increased in the grain of both wheat species under sulphur fertilisation, particularly at lower doses, while in spelt straw higher doses appeared to reduce copper content. At lower doses, selenium may have increased copper content in spelt wheat straw; however, overall it reduced copper content in common wheat grain, consistent with other studies indicating an inhibitory effect of selenium on copper uptake. Comparative studies are planned on different fertilisation strategies, including soil application, foliar application, and innovative nanofertilisers containing sulphur and selenium, to evaluate their effectiveness in enhancing Zn and Cu accumulation in wheat grain and straw.

Author contributions

M.S.B.,M.K-S, – conceptualization, formal analysis, methodology, investigation, visualisation, writing, original draft preparation, M.S.B. supervision; R.C. – writing, review and editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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