

MINERAL PROFILE OF THE WINTER WHEAT GRAIN: EFFECTS OF SOIL TILLAGE SYSTEMS AND NITROGEN FERTILIZATION

DOLIJANOVIĆ, Ž.¹ – ROLJEVIĆ NIKOLIĆ, S.^{2*} – KOVAČEVIĆ, D.¹ – DJURDJIĆ, S.³ –
MIODRAGOVIĆ, R.³ – JOVANOVIĆ TODOROVIĆ, M.² – POPOVIĆ DJORDJEVIĆ, J.^{4*}

¹Department of Field and Vegetable Crops, Faculty of Agriculture, University of Belgrade,
St. Nemanjina 6, 11080, Belgrade, Serbia
(phone: +381-11-441-3321)

²Institute of Agricultural Economics, Belgrade, St. Volgina 15, 11060 Belgrade, Serbia
(phone: +381-11-697-2842, fax: +381-11-697-2848)

³Department for Agricultural Engineering, Faculty of Agriculture, University of Belgrade,
St. Nemanjina 6, 11080, Beograd, Serbia
(phone: +381-11-441-3449)

⁴Department of Food Technology and Biochemistry, Faculty of Agriculture, University of
Belgrade, St. Nemanjina 6, 11080, Belgrade, Serbia
(phone: +381-11-441-3142)

*Corresponding authors

e-mail: svetlana_r@iep.bg.ac.rs (S. Roljević Nikolić, ORCID ID: 0000-0002-3139-0289);
jelenadj@agrif.bg.ac.rs (J. Popović Djordjević, ORCID ID: 0000-0003-4057-3826)

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Abstract. The aim of this study was to analyze the impact of various systems of soil tillage and nitrogen doses on the mineral composition of the grain of the common winter wheat cultivar (*Triticum aestivum* ssp. *vulgare*), cv. Azra selected for the conventional intensive production. The field experiment was conducted on luvic chernozem in completely randomized blocks. Wheat was grown under three soil tillage systems: conventional tillage, mulch tillage and no-tillage, and the experiment included two doses of N fertilization (60 and 120 kg ha⁻¹). Concentrations of eighteen elements (As, Al, Ba, Ca, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Ni, S, Sr, P, V and Zn) in wheat grain samples were determined by means of inductively coupled plasma with optical emission spectrometry (ICP–OES). The results indicated that concentrations of the studied elements in the wheat grain were significantly affected by the tillage systems and fertilization rates ($p < 0.001$), as well as by the interaction of these two factors. A smaller dose of nitrogen fertilizer (60 kg ha⁻¹) had a significantly better impact on the concentration of macro- and microelements in the wheat grain than the dose of 120 kg N ha⁻¹. The reduced tillage systems and lower nitrogen rates in nutrition had a better effect on the increase of the content of the studied elements in the wheat grain than the conventional cultivation which applied higher nitrogen rates.

Keywords: wheat grain, production system, fertilization, macroelements, microelements, ICP-OES

Introduction

Wheat (*Triticum aestivum* L.) is one of the most significant cultivated species and the staple food for more than 50% of the global population (Rizwan et al., 2016). The average per capita consumption of wheat in developed countries and developing countries amounts to 95 kg and 61 kg, respectively (FAO, 2016). In Serbia, the average per capita consumption of wheat is 180 kg, which is significantly higher than the consumption rates in most European countries (USDA, 2017). Due to its high global

consumption in the form of different food products, wheat is considered to be an important source of minerals in the everyday human nutrition.

The bioavailability of microelements in cereals is generally low (Kan, 2015; Dapkekar et al., 2018) and the concentrations of trace elements that are valuable for human nutrition steadily decrease with an increase in yield, which has adverse consequences, including 'hidden hunger' (Cakmak et al., 2010). Approximately half of the global population has zinc deficiency, and anaemia induced by iron deficiency is the most common phenomenon in Europe (Kan, 2015; Winiarska-Mieczan et al., 2019). In this context, the increase of micronutrient concentrations such as Fe and Zn in cereal grain has become a high-priority research area (Cakmak et al., 2010; Kutlu, 2018). Certain heavy metals (Zn, Cu and Ni) are necessary for biological processes in the human organism and they represent integral and catalytic components of proteins and enzymes, but they can be toxic in an overdose (Tiwari and Lata, 2018). On the other hand, toxic or non-essential elements such as As, Cd, Cr and Pb have no biological function in the human body and can be harmful for human health even in extremely low concentrations in case of long-term exposure (Lei et al., 2015; Zhang et al., 2019). What is more, these metals are responsible for the depletion of certain essential elements (Abbas et al., 2017).

The optimal application of fertilizers is an important factor for obtaining high yield and high quality products. Among the elements of mineral nutrition, nitrogen has the most important role in forming the yield (Litke et al., 2018), quality and utilizable value of the wheat grain (Blandino et al., 2016). Numerous studies have shown that the application of nitrogen fertilizer can promote the accumulation of certain microelements in the wheat grain, such as Fe and Zn (Kutman et al., 2011; Singh et al., 2018). This effect is explained by the fact that the sufficient supply of nitrogen increases the content of proteins in the grain, and the proteins are where Fe and Zn are accumulated (Barunawati et al., 2013). Similarly, previous research has shown that the concentration of Fe and Zn has a significant positive correlation with the content of proteins and P in the wheat grain (Zhao et al., 2009).

There has recently been an increasing interest in reduced soil tillage, stimulated by ecological and economic reasons (Sharma et al., 2011). According to some authors, the methods of reduced soil tillage increase soil density and inadequate distribution of nitrogen, phosphorus and microelements (Klikocka and Marks, 2018), which can consequently affect the uptake of mineral matter and thus influence the yield and crop quality. However, Stanisławska-Glubiak and Korzeniowska (2012) did not determine significant differences between various tillage systems regarding the content of microelements in wheat plants, except in respect of P and Mg in the early growth phases when the concentration of these elements was higher in the NT and MT systems than in CT. A similar study indicated that the NT system did not lead to the impairment of the quality of wheat and maize grains in terms of the primary mineral content, which is extremely significant for the nutritional value of the wheat grain (Stanisławska-Glubiak and Korzeniowska, 2012).

Scientific literature is still deficient in information regarding the impact of tillage on the chemical content of the wheat grain. Thus, the aim of this research was to determine the impact that tillage systems and different fertilization rates, within the applied cultivation technology, had on the differences in the content of the studied elements in the wheat grain of the cultivar Azra.

Materials and methods

Field experiment

The examination of the effects of different tillage systems and fertilization rates on the content of macro- and microelements in the wheat grain was conducted at the research field “Radmilovac” that belongs to the Faculty of Agriculture (44°45’ N, 20°35’ E, Serbia) in the growing season of 2016-2017. The soil type was luvisol chernozem (WRB, 2014), with the following characteristics: $\text{pH}_{(\text{H}_2\text{O})}$ 8.04, total content of N 13%, available forms of phosphorus 22.18 mg P_2O_5 100 g^{-1} dry weight and potassium 19.10 mg K_2O 100 g^{-1} dw, content of humus in the topsoil layer 2.45%.

The study used the winter cultivar of common wheat (*Triticum aestivum* ssp. *vulgare*), cv. Azra, selected for the conventional intensive production. Cv. Azra is a winter hardy cultivar of medium late maturity. It is very well-resistant to *Erysiphe graminis* DC. and well-resistant to *Puccinia striiformis* f. sp. *tritici* (*Pst*). Its hectolitre weight amounts to 80 - 85 kg, protein content is 11.5 - 13.0%, and it belongs to the B1-B2 quality group. The previous crop was maize (*Zea mays* L.).

A two-factor field trial was arranged using split plot design in three replications, with the area of the elementary plot amounting to 6 m^2 . The examined factors were tillage and fertilization. Three different tillage systems were used and two levels of fertilization were applied (*Fig. 1*).

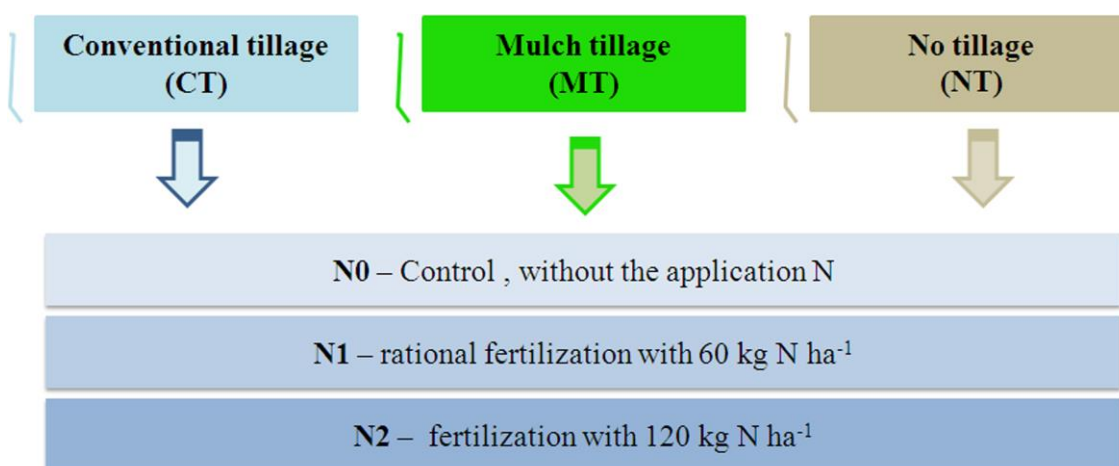


Figure 1. Experiment design

1. Conventional tillage (CT) - ploughing using a mouldboard plough at 25 cm and pre-sowing tillage using a disc harrow and a harrow,
2. Mulch tillage (MT) - tillage performed using a chisel plough at 25 cm with more than 30% of maize crop residues on the soil surface and the pre-sowing tillage using a disc harrow and a harrow,
3. No tillage (NT) - seeds were sown directly into unploughed soil, with the complete maize crop residues left on the surface.

Basic fertilization by NPK fertilizers was conducted during the autumn in the same quantity for all treatments. Spring fertilization was performed in the tillering phase using the KAN mineral fertilizer (25-27% N) and it differed in terms of pure nitrogen

quantities. Sowing was conducted on October 28th, 2016 with the crop density of 650 seeds m⁻². Standard cultivation measures in wheat production were applied and harvest was performed on June 29th, 2017.

Grain sample preparation for chemical analysis

In the grain of Azra cultivar, concentrations of macroelements: calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P) and sulphur (S), as well as microelements: aluminium (Al), arsenic (As), barium (Ba), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), nickel (Ni), strontium (Sr), vanadium (V) and zinc (Zn) were determined.

The representative samples were made using a random square method as described by Korunović and Stojanović (1989). The wheat grain samples were ground in the laboratory mill SJ-500 (producer “Metron”), and then prepared for the chemical analysis. Both soil and grain samples were subjected to the process of “wet digestion” as described in our previous work (Popović-Djordjević et al., 2019).

The determination of the content of the elements was performed using the inductively coupled plasma-optical emission spectroscopy (ICP–OES) analysis. The analysis was conducted using the instrument Thermo Scientific iCAP 6500 Duo ICP (Thermo Fisher Scientific, Cambridge, UK). The calibration of the instrument was performed by means of two certified multielement ICP–OES standards: Multi–Element Plasma Standard Solution 4, Specpure® (Alfa Aesar GmbH & Co KG, Germany) and SS–Low Level Elements ICV Stock (10 mg L⁻¹K) (VHG Labs, Inc–Part of LGC Standards, Manchester, NH 03103 USA). The analytic process was verified by the certified reference material EPA Method 200.7 LPC Solution (ULTRA Scientific, USA). The correlation of the measured concentrations with the certified values amounted to 96–104%.

Statistical analysis

Data processing was performed in the SPSS statistical package. The method for analyzing the variance (*F test*) for dual factor examination was used, and the significance of differences between the treatments was tested with the LSD test at the significance level of $p < 0.05$. Statistical data are given in *Table A1* in the *Appendix*.

Results

Concentration of macroelements in the wheat grain

The total content of the examined macroelements, as well as their average values are presented in *Table 1*. The total content of macroelements detected in the NT system (9968.11 mg kg⁻¹) was higher ($p < 0.05$) than in the MT and CT systems, by 1.7% and 5.3%, respectively. Differences in the treatment by means of nitrogen fertilization were also significant, where the content of macroelements in the control (9968.11 mg kg⁻¹) was higher by 2.3 and 3.2% than at the N1 and N2 levels, respectively.

The concentration of the examined macroelements, expressed in mg kg⁻¹, was in the following order: P (4058.89) > K (3350.33) > Mg (1169.55) > S (996.89) > Ca (171.33). The analysis of variance showed the statistically significant variability of macroelement concentrations among the studied tillage systems, as well as among the rates of nitrogen fertilization (*Table A1*). Considering the differences in the tillage

systems, the results indicate that the lowest concentration of all studied macroelements was detected in the CT system. The highest concentration of Ca ($178.67 \text{ mg kg}^{-1}$), K ($3410.33 \text{ mg kg}^{-1}$), Mg ($1236.33 \text{ mg kg}^{-1}$) and P ($4167.00 \text{ mg kg}^{-1}$) was detected in the NT system, while the highest concentration of S ($1016.33 \text{ mg kg}^{-1}$) was found in the MT system.

Table 1. Concentration of macroelements (mg kg^{-1}) in the wheat grain, cv. Azra

Tillage	N Rate	Ca	K	Mg	P	S	Total content of macroelements
CT	N0	164 ± 2	3652 ± 34	1300 ± 7	4470 ± 14	1031 ± 1	10617.00
	N1	173 ± 4	3244 ± 24	1094 ± 14	3917 ± 12	965 ± 6	9393.00
	N2	141 ± 2	2926 ± 22	942 ± 26	3388 ± 17	983 ± 6	8380.00
MT	N0	165 ± 3	3287 ± 32	1139 ± 27	3973 ± 22	981 ± 6	9545.00
	N1	177 ± 3	3462 ± 34	1180 ± 27	4180 ± 19	1040 ± 5	10039.00
	N2	186 ± 2	3351 ± 11	1162 ± 23	4101 ± 4	1028 ± 3	9828.00
NT	N0	189 ± 4	3271 ± 26	1192 ± 11	4007 ± 17	954 ± 3	9613.00
	N1	178 ± 3	3333 ± 33	1158 ± 6	4023 ± 10	979 ± 2	9671.00
	N2	169 ± 3	3627 ± 11	1359 ± 4	4471 ± 9	1011 ± 2	10637.00
Tillage	CT	159.33	3274.00	1112.00	3925.00	993.00	9463.33
	MT	176.00	3366.67	1160.33	4084.67	1016.33	9804.00
	NT	178.67	3410.33	1236.33	4167.00	981.33	9968.11
N Rate	N0	172.67	3403.33	1210.33	4150.00	988.67	9925.00
	N1	176.00	3346.33	1144.00	4040.00	994.67	9701.00
	N2	165.33	3301.33	1154.33	3986.67	1007.33	9615.00

Data are expressed as mean \pm standard deviation ($n = 3$)

The concentration of macroelements in the grain significantly varied depending on different levels of nitrogen fertilization (*Tables 1 and A1*). In the control group, there was a significantly higher concentration of P ($4150.00 \text{ mg kg}^{-1}$), K ($3403.33 \text{ mg kg}^{-1}$) and Mg ($1210.33 \text{ mg kg}^{-1}$) than in the N1 and N2 variant. The highest concentration of Ca ($176.00 \text{ mg kg}^{-1}$) was in the cases when the dose of 60 kg N ha^{-1} was applied, while the sulphur concentration in N2 ($1007.33 \text{ mg kg}^{-1}$) was higher by approximately 2% than in the N0 and N1 variants (*Fig. 2*).

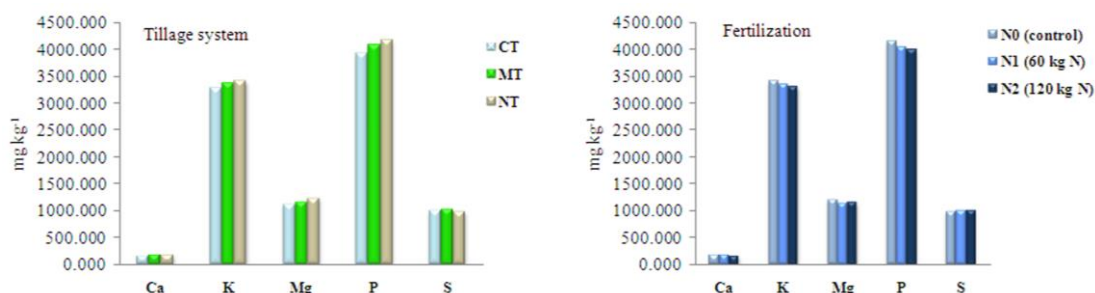


Figure 2. Changes of macroelement concentration (mg kg^{-1}) in the grain within different tillage systems and levels of fertilization

Observing the interaction between the tillage systems and the levels of nitrogen fertilization, it can be noticed that the highest average value of Ca ($189 \pm 4 \text{ mg kg}^{-1}$) was recorded in the NT system with the N0 variant, and it was significantly higher than in other tillage systems and levels of nitrogen fertilization. It is interesting that the differences in the K concentration were not significant between the CT system with the N0 variant ($3652 \pm 34 \text{ mg kg}^{-1}$) and the NT system with the N2 variant ($3627 \pm 11 \text{ mg kg}^{-1}$). On the other hand, the concentrations of Mg ($1300 \pm 7 \text{ mg kg}^{-1}$), P ($4470 \pm 14 \text{ mg kg}^{-1}$) and S ($1031.0 \pm 1.0 \text{ mg kg}^{-1}$) in the CT system with the variant N0 were significantly higher than in other tillage systems and fertilization levels. The obtained results indicate that the reduced tillage and/or application of lower nitrogen rates do not decrease the macroelement contents in the wheat grain (Fig. 2).

Concentration of microelements in the wheat grain

The total content of microelements in the wheat grain and their average concentrations are presented in Table 2. The research results showed that the microelement content in the grain was affected by the tillage system and fertilization rate, as well as by the interaction of these factors (Table A1). There is a small but significant difference regarding the microelement content between the CT system ($103.691 \text{ mg kg}^{-1}$) and NT system ($102.048 \text{ mg kg}^{-1}$). Considering the differences between the rates of nitrogen fertilization, it can be noticed that the total content of microelements in the N2 variant ($76.096 \text{ mg kg}^{-1}$) is significantly lower than in N1 ($108.229 \text{ mg kg}^{-1}$) and N0 ($104.880 \text{ mg kg}^{-1}$).

The concentrations of Al, Cr and Hg were below the limit of detection (LOD) in all studied grain samples of Azra cultivar. The concentrations of detected microelements, expressed in mg kg^{-1} , were in the following order: Fe (38.10) > Zn (27.90) > Mn (22.26) > Cu (3.87) > Ba (2.65) > Sr (0.83) > V (0.35) > Ni (0.29) > As (0.11) > Co (0.06).

The content of Fe detected in the NT system ($46.170 \text{ mg kg}^{-1}$) was significantly higher than in the MT ($23.390 \text{ mg kg}^{-1}$) and CT system ($44.740 \text{ mg kg}^{-1}$). The highest concentration of Fe in the amount of $47.330 \text{ mg kg}^{-1}$ was detected in the variant of fertilization with the smaller dose of nitrogen (60 kg ha^{-1}), while the lowest concentration ($20.200 \text{ mg kg}^{-1}$) was detected in the application of nitrogen at the rate of 120 kg ha^{-1} (Fig. 3). The interaction of the examined factors indicates that the highest Fe concentration ($101.3 \pm 1.0 \text{ mg kg}^{-1}$) was obtained in the NT system with the N0 variant, and it was significantly higher than in other tillage systems and fertilization levels (Table 2).

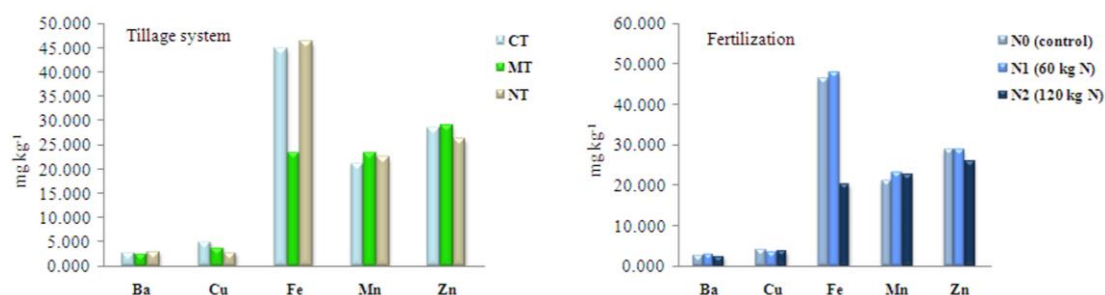


Figure 3. Changes of Ba, Cu, Fe, Mn and Zn concentration (mg kg^{-1}) in the grain within different tillage systems and levels of fertilization

Table 2. Concentration of microelements (mg kg⁻¹) in the wheat grain, cv. Azra

Tillage	N rate	As	Ba	Co	Cu	Fe	Mn	Ni	Sr	V	Zn	Total content of microelements
CT	N0	<LOD	2.32±0.02	0.06±0.02	6.61±0.06	21.12±0.07	21.4±0.3	1.30±0.09	0.688±0.005	0.284±0.002	34.21±0.06	87.992
	N1	0.013±0.003	2.68±0.02	0.25±0.02	2.61±0.05	96.7±0.8	22.6±0.4	0.36±0.06	0.697±0.003	0.53±0.05	29.0±0.2	155.440
	N2	<LOD	2.97±0.03	<LOD	5.98±0.07	16.4±0.3	19.3±0.3	<LOD	0.79±0.02	<LOD	22.2±0.2	67.640
MT	N0	0.177±0.004	2.94±0.05	0.012±0.003	2.06±0.06	16.57±0.09	21.0±0.4	<LOD	0.909±0.008	0.164±0.003	27.47±0.10	71.302
	N1	<LOD	3.66±0.02	0.018±0.003	6.52±0.07	28.3±0.3	24.9±0.4	0.5±0.2	0.878±0.003	0.78±0.02	31.95±0.02	97.506
	N2	<LOD	0.62±0.03	0.084±0.010	2.60±0.05	25.3±0.4	23.8±0.3	0.023±0.004	0.855±0.004	0.65±0.04	27.66±0.07	81.592
NT	N0	0.24±0.02	2.459±0.008	0.050±0.009	3.84±0.05	101.3±1.0	20.8±0.3	0.171±0.02	0.905±0.007	0.38±0.02	25.2±0.2	155.345
	N1	0.34±0.03	2.56±0.02	0.041±0.006	1.93±0.05	18.3±0.2	22.1±0.2	0.068±0.002	0.793±0.004	0.320±0.005	25.29±0.05	71.742
	N2	0.19±0.05	3.66±0.02	0.018±0.002	2.67±0.07	18.9±0.4	24.4±0.2	0.17±0.03	0.908±0.004	<LOD	28.14±0.10	79.056
Tillage	CT	0.004	2.657	0.103	5.066	44.740	21.100	0.553	0.725	0.271	28.470	103.691
	MT	0.059	2.407	0.038	3.727	23.390	23.233	0.174	0.881	0.531	29.027	83.467
	NT	0.257	2.893	0.036	2.813	46.170	22.433	0.136	0.869	0.233	26.210	102.048
N Rate	N0	0.139	2.573	0.041	4.170	46.330	21.067	0.490	0.834	0.276	28.960	104.880
	N1	0.118	2.967	0.103	3.687	47.770	23.200	0.309	0.789	0.543	28.747	108.229
	N2	0.063	2.417	0.034	3.750	20.200	22.500	0.064	0.851	0.217	26.000	76.096

Al, Cr and Hg were below LOD; Data are expressed as mean ± standard deviation (n = 3)

Significant differences between the tillage systems were also detected regarding the concentration of Zn. In the NT system ($26.210 \text{ mg kg}^{-1}$) it was lower by 9.7% and 7.9% than in the MT and CT systems, respectively. Considering the variants of nitrogen fertilization, Zn concentration was significantly lower than in the control, particularly in the N2 variant (10.2%). Observing the interaction of the examined factors, it can be noticed that the highest Zn concentration was detected in the CT system with the control variant ($34.21 \pm 0.06 \text{ mg kg}^{-1}$), in the MT system with the N1 variant ($31.95 \pm 0.02 \text{ mg kg}^{-1}$), and in the NT system with the N2 variant ($28.14 \pm 0.10 \text{ mg kg}^{-1}$) (Table 2).

When it comes to Mn, there were also significant differences regarding the tillage system and fertilization rate. The highest Mn concentration was detected in the MT system ($23.233 \text{ mg kg}^{-1}$), and it was higher than in the CT and NT systems by 10.1% and 3.6%, respectively. The Mn concentration in the N1 variant ($23.200 \text{ mg kg}^{-1}$) was higher than in the N0 and N2 variants by 10.1% and 3.2%, respectively.

The application of the conventional tillage system resulted in the significantly higher concentrations of Cu (5.066 mg kg^{-1}), Ni (0.553 mg kg^{-1}) and Co (0.103 mg kg^{-1}) than in the NT and MT systems, while there were no significant differences regarding nickel and cobalt between the NT and MT systems. It was also noticed that the concentrations of Cu and Ni in the grain decreased with the increased rate of nitrogen fertilization. Therefore, the highest concentrations of these microelements were detected in the variant N0 (4.170 mg kg^{-1} and 0.490 mg kg^{-1}). The concentration of Co in the wheat grain was the highest when the application of nitrogen was at the rate of 60 kg ha^{-1} and the differences between the variants N0 (0.041 mg kg^{-1}) and N2 (0.034 mg kg^{-1}) were not significant.

The highest Ba concentration was detected in the NT system (2.893 mg kg^{-1}), which was higher ($p < 0.05$) by 20.2% and 8.9% than in the MT and CT systems, respectively. Significant differences were also obtained at different fertilization rates. The Ba concentration in the N2 variant (2.417 mg kg^{-1}) was lower by 6.1% and 18.5% than in the control and N1 variant, respectively.

The content of Sr in plants is highly variable. The research results showed that the Sr concentration in the grain was the lowest in the CT system (0.725 mg kg^{-1}), and it was lower than in the MT and NT system by 17.7% and 16.5%, respectively. When it comes to the differences in fertilization rates, the lowest Sr concentration (0.789 mg kg^{-1}) in the grain was detected when applying the dose of 60 kg N ha^{-1} , while its concentration was the highest (0.851 mg kg^{-1}) at the rate of 120 kg N ha^{-1} .

The highest concentrations of V were detected in the MT system (0.531 mg kg^{-1}) and at the nitrogen application at a rate of 60 kg ha^{-1} (0.543 mg kg^{-1}). The intensity of soil tillage had an impact on the increase of As content, while the application of N fertilizers decreased the concentration of this trace element in the wheat grain (Fig. 4).

Discussion

A high yield of good quality is based on the proper use of agro-technical measures, primarily on the appropriate soil tillage and adapted crop fertilization. In modern agriculture, it is important to understand the effects of these measures since they can affect the product quality (Klikocka and Marks, 2018), and also contribute to the protection and preservation of soil fertility.

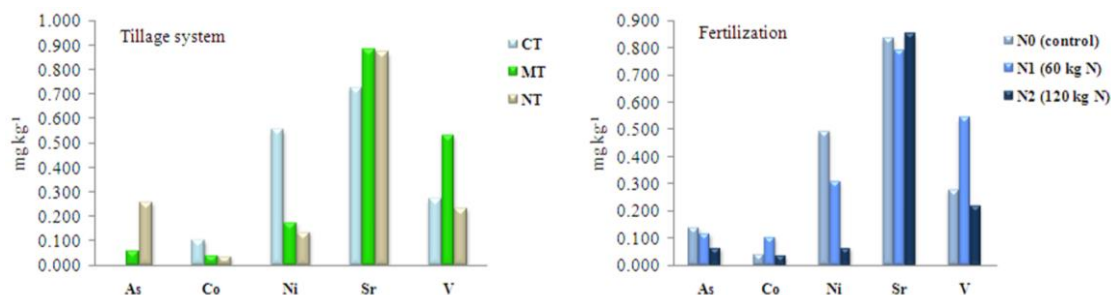


Figure 4. Changes of As, Co, Ni, Sr and V concentration (mg kg^{-1}) in the grain within different tillage systems and levels of fertilization

It was determined that the mineral content in the wheat grain, cv. Azra, differed significantly depending on the tillage system. The grain originating from the NT system had a significantly higher content of macroelements than in the MT system (1.7%) and CT system (5.3%). The highest concentrations of Ca ($178.67 \text{ mg kg}^{-1}$), K ($3410.33 \text{ mg kg}^{-1}$), Mg ($1236.33 \text{ mg kg}^{-1}$) and P ($4167.00 \text{ mg kg}^{-1}$) were detected in the NT system ($p < 0.001$), while the highest S concentration ($1016.33 \text{ mg kg}^{-1}$) was found in the wheat grain cultivated in the MT system ($p < 0.05$). The obtained results suggest that the reduced tillage systems can have a positive effect on the concentration of macroelements in the wheat grain. According to De Vita et al. (2007), the NT system is more efficient than the CT in arid environments since it decreases the evaporation of moisture from soil surface, which ensures its better availability to plants. Furthermore, Wozniak (2010) states that the reduced tillage and lowered rates of N fertilizers increase the content of minerals in the wheat grain.

Nitrogen has a crucial role in the vegetative growth and crop quality (Faizy et al., 2017; Litke et al., 2018). However, the intensive application of mineral fertilizers has different local and global effects on the environment and human health. The research results have shown that the lowest average concentrations of Ca ($165.33 \text{ mg kg}^{-1}$), K ($3301.33 \text{ mg kg}^{-1}$) and P ($3986.67 \text{ mg kg}^{-1}$) were detected in the variant with the highest rate of nitrogen fertilization (120 kg ha^{-1}). Studying the nitrogen application in protected areas, Bar-Tal et al. (2001) concluded that the application of larger doses of nitrogen decreased the assimilation of Ca, which is probably a consequence of the increased concentration of NH_4^+ . Other studies showed that higher rates of N fertilizer application increased the content of phosphorus and potassium in the wheat grain (Gaj and Gorski, 2014; Faizy et al., 2017), which can be affected by numerous factors such as assortment, crop maturity, soil type and content, fertilizer type and rate (Jākobsone et al., 2015; Kan, 2015). In this study, fertilizer application did not have a positive effect on the increase of Mg concentration in the wheat grain. Nutrition treatment at the N rates of 60 and 120 kg ha^{-1} had similar values of Mg content, and they were significantly lower than in the control treatment ($1210.33 \text{ mg kg}^{-1}$). Considering the studied macroelements, only S had a detected rise of concentration connected to the increase of N fertilizer dose. Similarly, Jamal et al. (2010) stated that there was a high positive correlation between the nitrogen and sulphur contents in wheat.

The analysis of variance of the obtained results showed that tillage had a statistically significant effect on concentration of microelements in the grain of cv. Azra. The highest ($p < 0.05$) concentrations of Fe ($46.170 \text{ mg kg}^{-1}$), Ba (2.893 mg kg^{-1}) and As (0.257 mg kg^{-1}) were detected in NT, while the concentrations of Zn ($29.027 \text{ mg kg}^{-1}$),

Mn (23.233 mg kg⁻¹), Sr (0.881 mg kg⁻¹) and V (0.531 mg kg⁻¹) in the MT system were significantly higher than in the CT and NT systems (Figs. 3 and 4). Arsenic is an environmental and food-chain contaminant, and inorganic As is a class-one carcinogen (Zhao et al., 2010). Wozniak and Makarski (2012) underline that the availability of microelements in well aerated soils is lower than in the less aerated ones, so it can be concluded that the multiple aeration of ground in the ploughing system depresses the availability of Zn and Cu, unlike in the ploughless tillage. The assimilation of Fe, Cu, Mn and Zn in the reduced tillage systems is easier not only for wheat but also for weeds in the calcareous soil (Taghizadeh et al. 2014). In addition, Santiago et al. (2008) found out that there was a higher concentration of Mn, Cu and Zn in the plants cultivated in the NT system than in the ones from CT and MT systems. Higher concentration of Mn in the conditions of reduced tillage (23.233 mg kg⁻¹ and 22.433 mg kg⁻¹) than in the case of conventional tillage (21.100 mg kg⁻¹) can be explained by the better moisture content in the soil in MT and NT systems, which was confirmed by other studies (Wozniak and Makarski, 2012). The decrease of mineral content in the food is considered to be related to the intensive agricultural practices which can result in their depletion from the soil (Kan, 2015).

Reduced tillage increases the content of organic matter and preserves soil fertility more than the conventional management practices (De Sanctis et al., 2012). Rizwan et al. (2016) say that organic amendments could be used to reduce the uptake of toxic trace elements by crop plants. The research results have shown that the lowest concentrations of microelements such as Co (0.036 mg kg⁻¹), Cu (2.813 mg kg⁻¹) and Ni (0.136 mg kg⁻¹) were detected in the NT system (Figs. 3 and 4). Other authors also underlined the tendency of heavy metals to make stable compounds with the organic matter and thus lower their bioavailability (Kabata Pendias, 2011).

Understanding the interactions between nitrogen and other elements is the key for the improvement of the efficiency of fertilizer application (Domagała-Świątkiewicz and Gastoł, 2013). The highest total content of microelements (108.229 mg kg⁻¹) was recorded with the nitrogen application at a dose of 60 kg ha⁻¹, while the lowest (76.096 mg kg⁻¹) was detected when the dose of 120 kg ha⁻¹ was applied. Also, the lowest concentrations of Ba (2.417 mg kg⁻¹), Co (0.034 mg kg⁻¹), Ni (0.064 mg kg⁻¹), V (0.217 mg kg⁻¹), Zn (26.000 mg kg⁻¹) and Fe (20.200 mg kg⁻¹) were detected when applying the highest nitrogen dose (Figs. 3 and 4). The average Ba concentration in plant tissues ranges between 2 and 13 mg kg⁻¹ and the higher concentrations of Ba are found in the plants from aridic zones (Kabata Pendias, 2011), whereas V is relatively easily taken up from acid soil, and some studies show that it is related to the process of biological nitrogen fixation (Bjekić, 2014). Fe availability to plants is dictated by the redox potential and pH soil reaction. In soils of higher pH, Fe is oxidized and is mostly in the form of insoluble ferric oxides which are less available to plants (Morrissey and Guerinot, 2009). In this case, the nitrate form of nitrogen (brought by a higher fertilization rate) probably increased pH and thus lowered the Fe uptake by plants. Zhao et al. (2009) underlined that Zn and Fe concentrations were significantly and positively correlated with P content, which was confirmed in this study since the lowest values of all three elements were detected in the N2 variant.

On the other hand, numerous authors state that larger doses of N fertilizer increase the microelement content in wheat, and some of the studies include the combined application of nitrogen and certain microelements (Klikocka and Marks, 2018; Kutman et al., 2011). Years-long research has shown that the application of higher nitrogen rates

leads to the increase in Fe, Zn and Cu concentrations, but it does not affect Mn concentration in the wheat grain (Shi et al., 2010). Later studies showed that the applied forms of N fertilizer had different effects on Mn content in plants (Klikocka and Marks, 2018). Svečnjak et al. (2013) reported that the content of trace elements depended primarily on the bioavailability of the minerals, but that different wheat cultivars could absorb different element levels from the soil, and that the same wheat cultivar could absorb different mineral levels from different soils.

The results have shown that Ni concentration (0.064 mg kg^{-1}) in the grain was the lowest in the variant with the highest rate of nitrogen application (*Fig. 4*), which can be the consequence of soil acidity, organic matter content and sorption capacity (Shaheen et al., 2017; Wlasniewski et al., 2019). The study of Wlasniewski et al. (2019) did not record a significant impact of mineral nutrition on the varying of nickel quantities in the wheat and barley grain, but it noticed a certain tendency of reducing the content of this metal in the wheat grain with the increase of NPK dose (Wlasniewski et al., 2019).

Conclusion

The results of the research have shown that the tillage system and level of nitrogen fertilization in top dressing, as well as the interaction between these two factors had an important effect on the content of macro- and microelements in the grain of winter wheat cultivar Azra. The lowest content of the examined macroelements, as well as the highest concentration of microelements Co, Cu and Ni, was observed in the grain produced in the CT system. On the other hand, the highest concentrations of microelements Fe, Ba and As were detected in the grain produced in the NT system. In the grain from the MT system, the concentrations of Mn, Sr, V and Zn were the highest in comparison with two other tillage systems. Significant differences in concentrations of macro- and microelements were detected in the grain produced with different levels of nitrogen fertilization. The highest total content of macroelements, as well as highest concentrations of Mg, K and P, was detected in the control. In the grain treated with a smaller dose of nitrogen (60 kg ha^{-1}), the highest total content of microelements, as well as the highest concentrations of Co, Ba, Fe, Mn and V, was detected. The obtained results indicated that reduced tillage and/or reduced nitrogen fertilization did not cause the reduction in macroelement content in the grain. Moreover, it had a positive effect on the content of nutritionally important minerals (Ca, Cu, Fe, K, Mg, Mn, P and Zn) in respect to the conventional tillage system with higher levels of nitrogen applied.

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APPENDIX

Table A1. Statistical analysis of element concentration in the grain of the winter wheat cv. Azra

Source	Element	df	Mean square	F	Sig.	LSD 0.05
Tillage	As	2	0.154	362.664	0.000	0.020
	Ba	2	0.532	752.796	0.000	0.026
	Co	2	0.013	117.656	0.000	0.011
	Cu	2	11.561	3261.705	0.000	0.059
	Mn	2	10.453	102.261	0.000	0.317
	Ni	2	0.522	88.627	0.000	0.076
	V	2	0.194	354.068	0.000	0.023
	Zn	2	19.942	1198.658	0.000	0.128
	Sr	2	0.068	1006.157	0.000	0.008
	Ca	2	988.000	111.150	0.000	2.951
	Fe	2	1464.952	6012.113	0.000	0.489
	K	2	121440.704	6.194	0.009	26.405
	Mg	2	35356.333	103.280	0.000	18.316
	P	2	136254.333	625.658	0.000	14.609
	S	2	2858.333	160.781	0.000	4.174
	Total content of microelements	2	1135.636	1022.625	0.000	1.043
	Total content of macroelements	2	607921.000	146.041	0.000	63.87
N_rate	As	2	0.013	30.723	0.000	0.020
	Ba	2	0.723	1022.287	0.000	0.026
	Co	2	0.013	116.592	0.000	0.011
	Cu	2	0.621	175.213	0.000	0.059
	Mn	2	10.643	104.120	0.000	0.317
	Ni	2	0.408	69.313	0.000	0.076
	V	2	0.219	399.735	0.000	0.023
	Zn	2	24.569	1476.780	0.000	0.128

	Sr	2	0.009	136.048	0.000	0.008	
	Ca	2	268.000	30.150	0.000	2.951	
	Fe	2	2167.143	8893.884	0.000	0.489	
	K	2	80891.370	4.126	0.033	26.405	
	Mg	2	11464.333	33.489	0.000	18.316	
	P	2	62433.333	286.684	0.000	14.609	
	S	2	817.333	45.975	0.000	4.174	
	Total content of microelements	2	2808.550	2529.061	0.000	1.043	
	Total content of macroelements	2	230508.000	55.375	0.000	63.87	
Tillage * N_rate	As	4	0.017	40.107	0.000	0.035	
	Ba	4	4.249	6008.766	0.000	0.045	
	Co	4	0.021	190.516	0.000	0.018	
	Cu	4	16.906	4769.854	0.000	0.102	
	Mn	4	9.913	96.978	0.000	0.548	
	Ni	4	0.506	85.937	0.000	0.132	
	V	4	0.162	296.055	0.000	0.04	
	Zn	4	55.920	3361.161	0.000	0.222	
	Sr	4	0.008	115.694	0.000	0.014	
	Ca	4	591.500	66.544	0.000	5.112	
	Fe	4	5437.586	22315.675	0.000	0.846	
	K	4	385470.370	19.661	0.000	45.735	
	Mg	4	60688.833	177.280	0.000	31.725	
	P	4	528309.333	2425.910	0.000	25.303	
	S	4	4020.333	226.144	0.000	7.23	
		Total content of microelements	4	5241.089	4719.530	0.000	1.807
		Total content of macroelements	4	2355310	565.818	0.000	110.627