THE EFFECT OF THE BACTERIUM *BACILLUS MEGATERIUM* VAR. *PHOSPHATICUM*, THE AMINO ACID L-ALPHA PROLINE AND MINERAL FERTILISATION WITH NITROGEN ON HEAVY METAL CONTENT IN SPRING WHEAT (*TRITICUM AESTIVUM* L.) GRAIN

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Abstract. The objective of the present work was to determine the effect of the bacterium *Bacillus megaterium var. phosphaticum*, the amino acid L-alpha proline and mineral fertilisation with nitrogen on heavy metal content in spring wheat (*Triticum aestivum* L.) grain destined for human consumption. Field research was conducted on a family-owned farm located in Krzymosze, Poland. Two factors were examined: factor I – biological products: control where no biological products were applied, *Bacillus megaterium var. phosphaticum*, L-alpha proline, *Bacillus megaterium var. phosphaticum* + L-alpha proline; factor II – mineral fertilisation with nitrogen: unfertilised control, 60 kg N$\cdot$ha^{-1}, 90 kg N$\cdot$ha^{-1}, and 120 kg N$\cdot$ha^{-1}. Grain samples were taken to determine Cu, Zn, Cd, Cr and Ni contents. The research demonstrated that weather conditions during the growing season had a significant influence on the heavy metal content of spring wheat grains. Increasing rates of mineral fertilisation with nitrogen contributed to an increase in the spring wheat grain content of heavy metals, an exception being zinc content in the grain of spring wheat fertilised with 120 kg N$\cdot$ha^{-1}. The lowest heavy metal content was recorded in the grain of spring wheat treated with the bacteria *Bacillus megaterium var. phosphaticum* + the amino acid L-alpha proline, regardless of whether the crop was unfertilised or fertilised with the rates of 60 and 90 kg N$\cdot$ha^{-1}.
Keywords: *phosphorus bacteria, mineral fertilisation, biostimulator, content in grain metal*

Introduction

Spring wheat grain is a raw material for the food industry so its content of heavy metals should be as low as possible. Some of these metals, e.g. Ni, Cu and Zn, are microelements necessary for biomolecules, and to maintain cell structure, function and proliferation (Khajeh et al., 2010). The metals are potentially toxic if present in excess in food. They may be the cause of genetic disorders (Zheng et al., 2008). Other metals, e.g. Cd, Pb and Cr are carcinogenic substances and they are responsible for the occurrence of conditions such as Alzheimer's disease, Parkinson's disease, multiple sclerosis and osteoporosis. Also, they negatively affect such organs as the heart, kidneys and lungs as well as the immune system (Jamova and Valko, 2011; Zakir et al., 2011; Pirsaheb et al., 2015). Numerous research have revealed excessive amounts of heavy metals in cereals and rice grown in various countries. This is due to the fact that currently harmful substances in the environment are pesticide residues and heavy metal compounds. Reducing the emission of heavy metal compounds is more difficult to achieve. Their main sources are the steel industry, energy, mining and transport. That is why it is of paramount importance to constantly monitor and check cereal grain for heavy metals. The value of guidance documents and safety standards as established by Food Safety Authorities (*Table 2*) around the world cannot be overstated in terms of protecting public health and providing

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guidance and assistance to stakeholders, not least farmers and processors (Thielecke and Nugent, 2018). Refining grains will reduce the presence of many contaminants but it also removes 50 to 80% of phytonutrients from whole grains. The studies demonstrated that it is these phytonutrients, (vitamins, minerals and fibres) that may exert a potentially protective effect against toxic metals in particular (Thielecke and Nugent, 2018). Further, the consumer also has a choice in mitigating any risk from contaminants, and to do so best by continuing to eat a healthy balanced diet, rich in nutrient dense foods, and including whole grain foods (Thielecke and Nugent, 2018).

According to Shicheng et al. (2018), mineral fertilisation with nitrogen increases the wheat grain content of heavy metals. Jastrzębska and Kostrzewska (2019) have demonstrated that an application of the phosphorus-dissolving bacteria *Bacillus megaterium*, compared to mineral fertilisation of spring wheat, contributed to a decline in the soil content of heavy metals. In the research, the phosphorus-dissolving bacteria were applied with fertiliser as ash produced from sewage sludge, and they reduced heavy metal content in the soil as opposed to mineral fertilisation. Also Asfa et al. (2020) observed lower phytotoxicity of heavy metals after inclusion of microbiology in wheat cultivation. Apart from microorganisms, modern agriculture also recommends an application of biostimulants, including amino acids (Calvo et al., 2014). Biostimulants serve to control and enhance plant life processes, and thus they increase plant resistance to stress and stimulate the development of rooting (Du Jardin, 2015). In this respect, it is a novel approach to apply the bacteria *Bacillus megaterium var. phosphaticum* and the amino acid L-alpha proline which willhopefully contribute to a decline in heavy metal content in spring wheat grain. As there is no research into this issue, it was attempted to determine the impact of the bacteria *Bacillus megaterium var. phosphaticum*, the amino acid L-alpha proline and mineral fertilisation with nitrogen on heavy metal content in the grain of spring wheat destined for human consumption.

Materials and Methods

A field experiment was conducted on a family-owned farm in Krzymosze near Siedlce, Poland, in 2017-2019. The trial was set up on soil classified as Stagnic Luvisol and whose contents of available macroelements was as follows: P 8.2, K 18.7, and Mg 4.8 mg⊙100 g^{-1} soil. Other contents were as follows: Mn 131, Cu 4.9, Zn 5.5, Pb less than 16.2, Cd 0.14, Cr less than 12.3 and Ni 12.4 mg⊙kg^{-1} soil. Soil reaction was neutral and humus content was 1.88%. The experiment was a split-block arrangement with three replicates. The experimental factors were as follows: factor I – biological products: control where no biological products were applied, *Bacillus megaterium var. phosphaticum*1 l⊙ha^{-1}, L-alpha proline 2 g⊙ha^{-1}, *Bacillus megaterium var. phosphaticum*1 l⊙ha^{-1}+ L-alpha proline 2 g⊙ha^{-1}; factor II – mineral fertilisation with nitrogen: unfertilised control, 60 kg N⊙ha^{-1} (pre-plant), 90 kg N⊙ha^{-1} (60 kg N⊙ha^{-1} pre-plant + 30 kg N⊙ha^{-1} at the stem elongation stage), and 120 kg N⊙ha^{-1} (60 kg N⊙ha^{-1} pre-plant + 30 kg N⊙ha^{-1} at the stem elongation stage + 30 kg N⊙ha^{-1} as foliar application of 8% solution of urea at the early ear formation stage).

Spring wheat cv. Mandaryna was grown after maize. Phosphorus and potassium fertiliser rates were adjusted to soil contents of available forms of these elements and were as follows: P 30.8 kg⊙ha^{-1} and K 99.6 kg⊙ha^{-1}. Mineral fertilisation with nitrogen was applied as described for factor II above. Spring wheat was sown in early April at the amount of 500 grains per 1 m^{2}. Biological products were applied once at the stage of

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spring wheat tillering. Spring wheat was harvested in early August. From each plot after the grain was harvested, a representative sample with a volume of 250 g was taken. Then it was ground and intended for chemical analysis. During harvest, grain samples were taken in each plot to determine microelements. Cu, Zn, Mn, Pb, Cd, Cr and Ni contents were determined by means of inductively coupled plasma optical emission spectrometry (ICP-OES) using an emission spectrometer Perkin Elmer Optima 8300.

The results for each trait tested were subjected to ANOVA suitable for the split-block design. When significant sources of variation were confirmed, their means were separated using Tukey test. Calculations were performed in MS Excel 12.0. The results for each characteristic were subjected to analysis of variance following the mathematical model: $y_{ijl} = n + a_i + g_i + e_{ij}^{(1)} + b_l + e_{jl}^{(2)} + ab_{il} + e_{ijl}^{(3)}$, where a = 1.. 4; b = 1, 2, … 4; n = 1, 2, 3 (number of replicates); y_{ijl} – value of the examined characteristic; a_i – effect of i-th level of factor A; g_j– effect of replicates (blocks); $e_{ij}^{(1)}$ – error 1 resulting from the interaction: factor A × replicates; b_l – effect of l-th level of factor B; $e_{jl}^{(2)}$ – error 2 from the interaction: factor B × replicates; ab_{il} – effect of the interaction: factor A × factor B; $e_{ijl}^{(3)}$ – random error. The course of weather conditions in the years of the research was varied (*Table 1*).

Table 1. *Weather conditions in the growing season of spring wheat according to the Zawady Meteorological Station*

Years	Month					Mean
	IV	V	VI	VII	VIII	
Mean air temperature ºC						
2017	6.9	13.9	17.8	16.9	18.4	14.8
2018	13.1	17.0	18.3	20.4	20.6	17.9
2019	9.8	13.3	17.9	18.5	19.9	15.9
Long-term (50yr) mean	8.2	14.2	17.6	19.7	19.1	15.8
Rainfall sum, mm						
2017	59.6	49.5	57.9	23.6	54.7	245.3
2018	34.5	27.3	31.5	67.1	24.5	184.9
2019	5.9	59.8	35.9	29.7	43.9	175.2
Long-term (50yr) mean	37.4	47.1	48.1	65.5	43.5	241.6

The most favorable year for the cultivation of spring wheat was 2017, when the highest amount of rainfall was recorded. Worse weather conditions were recorded in 2018, with a lower total of precipitation and an average air temperature higher than the long-term average. The strongest rainfall shortage was recorded in 2019. The average air temperature oscillated around the long-term average.

Table 2 shows the regulatory autorities’ permissible metal limits WHO/FAO.

Table 2. *Metal levels in food by WHO/FAO*

Metal	Concentrations (µg/g)
Cd	0.10
Cu	73.00
Cr	2.30
Pb	0.30
Zn	100.00

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Results and Discussion

Heavy metal contents in spring wheat grain were significantly affected by weather conditions and their interaction with biological products (*Table 3*).

Table 3. *Spring wheat grain content of heavy metals according to biological products applied in 2017-2019, mg kg^{-1} DM*

Biological products (A)	Years (Y)		
	2017	2018	2019
Manganese			
Control	82.14c	79.49d	77.68d
Bacillus megaterium var. phosphaticum	72.32a	72.69b	71.07b
L-alpha proline	78.98b	76.34c	74.72c
Bacillus megaterium var. phosphaticum + L-alpha proline	71.14a	68.51a	66.89d
Means	76.15C	74.26B	72.59A
Zinc			
Control	18.59a	20.00a	20.65a
Bacillus megaterium var. phosphaticum	23.16c	24.57c	25.22c
L-alpha proline	21.59b	23.05b	23.71b
Bacillus megaterium var. phosphaticum + L-alpha proline	25.68d	27.08d	27.72d
Means	22.26A	23.68B	24.33C
Copper			
Control	6.24c	5.37b	5.01b
Bacillus megaterium var. phosphaticum	5.30a	4.43a	4.07a
L-alpha proline	5.49ab	4.62a	4.26a
Bacillus megaterium var. phosphaticum + L-alpha proline	5.08a	4.23a	3.87a
Means	5.53C	4.66B	4.30A
Nickel			
Control	0.769c	0.669c	0.638a
Bacillus megaterium var. phosphaticum	0.638b	0.538b	0.508b
L-alpha proline	0.671b	0.573b	0.543b
Bacillus megaterium var. phosphaticum + L-alpha proline	0.556a	0.458a	0.427a
Means	0.659B	0.560A	0.529A
Chromium			
Control	0.463c	0.378d	0.355c
Bacillus megaterium var. phosphaticum	0.310a	0.225b	0.203a
L-alpha proline	0.355b	0.271c	0.248b
Bacillus megaterium var. phosphaticum + L-alpha proline	0.319a	0.189a	0.181a
Means	0.362B	0.266A	0.247A

In 2017, characterised by the highest precipitation sum, there was recorded an increase in manganese, copper, nickel and chromium contents as well as a decline in zinc content in spring wheat grain compared with the dry years 2018 and 2019 whose precipitation

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sums were much lower. Similarly, Radkowski et al. (2020a) reported a decline in microelements in the year with a higher precipitation sum compared with dry years. In the present study, an interaction was confirmed. The lowest manganese, copper, nickel and chromium contents and the highest zinc content were recorded in spring wheat grain in 2018-2019, when precipitation was lower, in the plot treated with the bacteria *Bacillus megaterium var. phosphaticum* + the amino acid L-alpha proline. By contrast, the highest heavy metal contents, excluding zinc, were found in the control plot, where no biological products had been applied, in 2017 characterised by the highest precipitation sum.

Statistical analysis demonstrated a significant influence of the experimental factors and their interaction on manganese content in spring wheat grain (*Table 4*).

Table 4. *Manganese content in spring wheat grain (means across 2017-2019), mg⋅kg^{-1} DM*

Biological products (A)	Mineral fertilisation with nitrogen, kg N⋅ha^{-1} (B)				Means
	Control	60	90	120	
Control	76.08d	78.50d	80.77d	83.24d	79.65D
Bacillus megaterium var. phosphaticum	71.32b	72.59b	73.86b	74.33b	73.03B
L-alpha proline	74.47c	75.74c	77.01c	79.48c	76.68C
Bacillus megaterium var. phosphaticum + L-alpha proline	66.14a	68.41a	69.68a	71.15a	68.85A
Means	72.00A	73.81B	75.33C	77.05D	-

Compared to control, biological products contributed to a decline in the spring wheat content of manganese. Also the study by Arhar and Ahmad (2018) showed that nitrogen-fixing bacteria reduced Mn uptake by wheat plants. In the present study, the lowest concentration of Mn was recorded in spring wheat grain following an application of the bacteria *Bacillus megaterium var. phosphaticum*and the amino acid L-alpha proline. Kandii et al. (2016) reported a decline in Mn content in wheat after amino acids had been applied in combination with mineral fertilisation with nitrogen. The same relationship was observed by Radkowski et al. (2020). In the present study, mineral fertilisation with nitrogen significantly influenced manganese content in spring wheat grain. Increasing rates of mineral nitrogen fertiliser contributed to a significant increase in Mn content in spring wheat grain. This finding corresponds with results reported by Hellal et al. (2012) and Radkowski et al. (2020a,b). In the experiment discussed here, an interaction was confirmed indicating that the lowest manganese content was found in the grain of spring wheat grown in plots treated with the bacteria *Bacillus megaterium var. phosphaticum*and the amino acid L-alpha proline and not fertilised with mineral nitrogen. By contrast, the highest values were found in units fertilised with 120 kg N⋅ha^{-1} and untreated with biological products. However, also in this case the spring wheat grain content of Mn was lower than the standards quoted by WHO/FAO, and was safe for humans (Adefarati et al., 2017).

Zinc content in spring wheat grain was significantly affected by the experimental factors and their interaction (*Table 5*).

Biological products significantly increased zinc content in spring wheat grain compared with control where no biological products were applied. Zinc plays an important role in plant metabolism. Both zinc excess and shortage considerably limit plant growth and development (Ociepa-Kubicka and Ociepa, 2012). It should be

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mentioned that plants growing in a polluted environment may accumulate high concentrations of this microelement, which poses a serious threat to human health (Srinivas et al., 2002; Sharma et al., 2004; Luo et al., 2013). Research by Fytianos et al. (2001), Demirezen and Ahmet (2006), Muchuweti et al. (2006) as well as Mahamed et al. (2012) demonstrated a low zinc content in cereal grain. In the present study, the highest concentration of zinc was recorded in spring wheat grain following an application of the bacteria *Bacillus megaterium var. phosphaticum*and the amino acid L-alpha proline. Also Athar and Ahmad (2018) reported increased zinc contents in cereal grain due to an application of bacteria. Radkowski et al. (2020a) demonstrated that an application of amino acids increases Zn concentration in spring wheat grain as well. In the experiment reported here, mineral fertilisation with nitrogen increased Zn content in spring wheat grain up to the rate 90 kg N$\cdot$ha^{-1}. The highest rate, that is 120 kg N$\cdot$ha^{-1}, was followed by a significant decline in the spring wheat grain content of zinc. The same relationship was demonstrated in research by Kandii et al. (2016) and Radkowski et al. (2020a,b). In the present work, an interaction was confirmed indicating that the highest zinc content was present in spring wheat grain following an application of the bacteria *Bacillus megaterium var.phosphaticum*, the amino acid L-alpha proline and mineral fertilisation with nitrogen at the rate of either 60 or 90 kg N$\cdot$ha^{-1}, it being the lowest in the control unit where no biological products had been applied, at all the rates of mineral fertilisation with nitrogen.

Table 5. *Zinc content in spring wheat grain (means across 2017-2019), mg kg^{-1} DM*

Biological products (A))	Mineral fertilisation with nitrogen, kg N$\cdot$ha^{-1} (B)				Means
	Control	60	90	120	
Control	19.17a	20.65a	19.79a	19.37a	19.75A
Bacillus megaterium var. phosphaticum	23.05b	24.25b	26.44b	23.53b	24.32C
L-alpha proline	20.16a	23.53b	24.89b	22.54b	22.78B
Bacillus megaterium var. phosphaticum + L-alpha proline	25.54c	26.96c	28.60c	26.18c	26.82D
Means	21.98A	23.85B	24.93C	22.91B	-

Statistical analysis demonstrated a significant impact of the experimental factors and their interaction on copper content in spring wheat grain. Biological products used in the study contributed to a significant drop in copper content in spring wheat grain compared with control. The lowest concentration of copper was recorded in spring wheat grain following an application of the bacteria *Bacillus megaterium var. phosphaticum*and the amino acid L-alpha proline. Research by Garcia-Fraile et al. (2015) and Vejan et al. (2016) revealed that plant growth stimulating bacteria occur around roots and are responsible for nutrient mobilisation, heavy metal sequencing and break-down of toxic elements present in soil. Also Athar and Ahmad (2018) reported that *Azotobacter* reduces Cu content in wheat grain. A similar relationship was observed by Radkowski et al. (2020a) who applied amino acids while growing wheat. In the present study, increasing rates of mineral fertilisation with nitrogen were followed by an increase in copper content in spring wheat grain. However, the content associated with the highest rate of mineral fertilisation with nitrogen, that is 120 kg N$\cdot$ha^{-1}, was lower than the permissible standards set by the WHO/FAO and is not harmful to humans (Adefarati et al., 2017). In studies by

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Hellal et al. (2012), Kandii et al. (2016) and Radkowski et al. (2020b), there was observed an increase in the spring wheat grain content of copper following increasing rates of mineral fertilisation with nitrogen. In the study reported here, there was confirmed an interaction which indicated that the lowest copper content was present in spring wheat grain after an application of the bacteria *Bacillus megaterium var. phosphaticum*, the amino acid L-alpha proline and mineral fertilisation with nitrogen at the rate of up to 90 kg N⊙ha^{-1}. The highest copper content was determined in control grain produced without using biological products at the highest rate of mineral fertilisation with nitrogen, that is 120 kg N⊙ha^{-1}.

Lead and cadmium contents in spring wheat grain were insignificantly affected by the experimental factors, and their concentration in spring wheat grain was too low to be detected by an emission spectrometer Perkin Elmer Optima 8300. This may be explained by the fact that the fields where the experiment was set up were located relatively far away from communication routes. Hence, spring wheat grain met standards the set by the WHO/FAO for lead and cadmium contents. Despite the low Cd and Pb contents in spring wheat grain, they must be constantly monitored and checked.

Statistical analysis demonstrated a significant influence of the experimental factors and their interaction on nickel content in spring wheat grain (*Table 6*).

Table 6. *Nickel content in spring wheat grain (means across 2017-2019), mg kg^{-1} DM*

Biological products (A)	**Mineral fertilisation with nitrogen, kg N⊙ha^{-1} (B)**				**Means**
	Control	**60**	**90**	**120**	
Control	0.591c	0.683c	0.721c	0.772c	0.692C
Bacillus megaterium var. phosphaticum	0.493a	0.534b	0.575b	0.644b	0.562B
L-alpha proline	0.512b	0.576b	0.613b	0.681b	0.596B
Bacillus megaterium var. phosphaticum + L-alpha proline	0.427a	0.453a	0.494a	0.547a	0.480A
Means	0.506A	0.562A	0.601B	0.661B	-

The applied biological products significantly reduced the concentration of nickel in spring wheat grain compared with untreated control. In their work, Jastrzębska and Kostrzewska (2019) pointed to the fact that phosphorus-dissolving *Bacillus* bacteria contributed to reduction in nickel content in spring wheat grain. Similarly, Sarfaz et al. (2019) and Figueiredo et al. (2011) reported that also rhizobacterine limits heavy metal content in plants. Plant growth promoting rhizobacteria (PGPR) are responsible for stimulating plant growth, heavy metal sequencing and break-down of toxic elements present in soil (Garcia-Fraile et al., 2015). A similar relationship was reported by Athar and Ahmad (2018) who examined *Azotobacter*. Naveed et al. (2020) and Turan et al. (2018) demonstrated that an application of bacteria contributed to a decline in the spring wheat grain content of nickel. In the present work, also an application of the amino acid L-alpha proline was followed by a decline in nickel content in spring wheat grain, particularly when combined with the bacteria *Bacillus megaterium var. phosphaticum*. It was confirmed by Popko et al. (2018) who demonstrated that an application of biostimulants contributes to a decline in the spring wheat content of nickel. In the study reported here, increasing rates of mineral fertilisation with nitrogen, in particular the rate 120 kg N⊙ha^{-1}, were followed by an increase in nickel content in spring wheat grain.

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However, even in the case of the highest N rate, Ni content in spring wheat grain was low and did not exceed standards set by the WHO/FAO and was not harmful to humans (Adefarati et al., 2017). This finding was confirmed by Shicheng et al. (2018) in their research on an application of mineral fertilisation with NPK. In the experiment reported here, an interaction was found and it indicated that the lowest nickel content was present in spring wheat grain following an application of the bacteria *Bacillus megaterium var. phosphaticum*and the amino acid L-alpha proline and no mineral fertilisation with nitrogen, or an application of either 60 or 90 kg N·ha^{-1}. By contrast, it was the highest for the untreated control where the rate of mineral fertilisation with nitrogen was either 90 or 120 kg N·ha^{-1}.

Chromium content in spring wheat grain was significantly affected by the experimental factors and their interplay (*Table 7*).

Table 7. *Chromium content in spring wheat grain (means across 2017-2019), mg kg^{-1} DM*

Biological products (A)	**Mineral fertilisation with nitrogen, kg N·ha^{-1} (B)**				**Means**
	Control	**60**	**90**	**120**	
Control	0.314b	0.362c	0.423c	0.495c	0.399C
Bacillus megaterium var. phosphaticum	0.186a	0.214a	0.261a	0.323a	0.246A
L-alpha proline	0.211a	0.261b	0.317b	0.376b	0.291B
Bacillus megaterium var. phosphaticum + L-alpha proline	0.158a	0.185a	0.223a	0.272a	0.210A
Means	0.217A	0.256A	0.306B	0.367C	-

Biological products significantly reduced chromium content in spring wheat grain compared with untreated control. Also Jastrzębska and Kostrzewska (2019) reported that the phosphorus-dissolving bacteria *Bacillus megaterium* reduced chromium concentration in spring wheat grain. Sarfaz et al. (2019), Figueiredo et al. (2011) and Rizvi et al. (2020) demonstrated that bacteria incorporated into soil reduce chromium content in wheat. Also in the present study, an application of the amino acid L-alpha proline contributed to a decline in chromium content in spring wheat grain compared with control. Similarly, Popko et al. (2018) found that biostimulants reduce heavy metals in wheat. In the experiment reported here, the lowest chromium content was recorded in spring wheat grain following an application of the bacteria *Bacillus megaterium var. phosphaticum*and the amino acid L-alpha proline. Mineral fertilisation with nitrogen affected chromium content in spring wheat grain, too. The highest chromium content was determined in spring wheat grain following the highest rate of mineral fertilisation with nitrogen, that is 120 kg N·ha^{-1}. Despite this, the chromium content was low and did not exceed standards set by the WHO/FAO, and was safe for humans (Adefarati et al., 2017). Also research by Shicheng et al. (2018) demonstrated that mineral fertilisation is associated with an increase in the spring wheat grain content of chromium. An interaction was confirmed in the study reported here which indicated that the highest chromium content was present in spring wheat grain following an application of the bacteria *Bacillus megaterium var. Phosphaticum* and the amino acid L-alpha proline and no mineral fertilisation with nitrogen or at the rates of 60 or 90 kg N·ha^{-1}.

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Conclusions

1. Weather conditions during the growing season significantly affected heavy metal contents in spring wheat grain.
2. The examined biological products significantly reduced heavy metal contents in spring wheat grain, excluding zinc content.
3. Increasing rates of mineral fertilisation with nitrogen contributed to an increase in the spring wheat grain content of heavy metals. The only exception was zinc content determined in the grain of spring wheat fertilised with 120 kg N⋅ha^{-1}.
4. The lowest heavy metal contents were recorded in the grain of spring wheat treated with the bacteria *Bacillus megaterium var. phosphaticum* + the amino acid L-alpha proline, whether the cereal was unfertilised or fertilised with 60 and 90 kg N⋅ha^{-1}.
5. Research on the use of bacillus bacteria and biostimulants in spring wheat cultivation should be continued and the content of heavy metals in the grain should be constantly monitored.

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