ASSESSMENT OF MICRO AND MACROELEMENTS UPTAKE IN MAIZE PLANTS IN RESPONSE TO DIFFERENT NITROGEN AND PHOSPHORUS FERTILIZATION RATES

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Abstract

This paper aims to evaluate the effects of nitrogen and phosphorus mineral fertilization, applied in varying doses, on the quality of maize crops grown in acidic soils. The study is based on a long-term bifactorial experimental design, involving progressively increasing doses of nitrogen and phosphorus (arranged in a $5P \times 5N$ scheme, with N_0 - N_{160} combined with P_0 , P_{80} , P_{120} , and P_{160}), alongside lime application. The findings offer valuable insights into the long-term impacts of these fertilization practices on crop quality. Results show that long-term fertilization combined with calcic amendments induces statistically significant changes in the macroelements content of maize leaves specifically nitrogen, phosphorus, potassium, calcium, and magnesium and the microelements content, including copper, zinc, manganese, and iron.

Key words: acidic soil, maize, mineral fertilization.

INTRODUCTION

Maize is one of the most important cereal crops in the world, alongside wheat and rice, serving as food for both humans and animals and as a primary feedstock for biogas production (Singh et al., 2024).

Currently, in the context of global warming and population growth, it becomes increasingly important to obtain high-quality crops (Bojtor et al., 2021). In this regard, soil quality must be considered, as soil acidity and nutrient availability are among the primary factors limiting crop growth (Gondek & Mierzwa-Hersztek, 2023).

To achieve better harvests, it is essential to determine the plant's chemical composition as a basis for fertilizer application (Kalra, 1998). Assessing the nutritional status of maize plants under different nitrogen and phosphorus application rates provides insight into fertilizer use efficiency (BąK et al., 2016; Pasley et al., 2019).

In Romania, acidification and excessive moisture are the most pronounced degradation processes of clay-illuvial soils in north-western Transylvania. Pedological and agrochemical studies conducted in the area indicate that approximately 60% of agricultural land exhibits an acidic reaction. Soil acidity is a natural phenomenon in regions with an excess water regime (Kurtinecz, 2010). As a result, since autumn 1961, it has been considered a major imperative to establish long-term experiences located on the acidic soils of Livada Agricultural Research Station (SCDA Livada).

These experiments offer the possibility to monitor changes in soil characteristics under the effects of limestone and fertilization amendments and how these changes are reflected in the quantity and quality of crops. The nutritional status of a plant can be evaluated by measuring its total nutrient content relative to its dry matter.

The objective of this study is to evaluate the uptake of essential macronutrients, including nitrogen, phosphorus, potassium, calcium, and magnesium, as well as the absorption of micronutrients such as copper, zinc, manganese, and iron, under the influence of nitrogen and phosphorus mineral fertilization. The concentration of an element is directly

influenced by its presence in the soil and its interactions with other micro or macroelements.

MATERIALS AND METHODS

The research presented in this paper was carried out on one of the representative soils of northwestern Transylvania, specifically on a luvic brown soil.

The experimental field is in the central part of the area occupied by clay-illuvial soils, at SCDA Livada, Satu Mare County, within a stationary experimental setup (Figure 1).

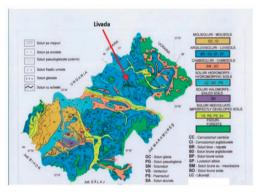


Figure 1. Soil map of Satu Mare County (Asvadurov&Boeriu, 1983)

Experiments on the amendment and fertilization of acidic soils at SCDA Livada were initiated in 1961, with a grain rotation system in place. In 2022, maize was sown (Figure 2).



Figure 2. Maize Field View - SCDA Livada

In the long-term experiment conducted at SCDA Livada, mineral fertilization was applied annually, with lime treatments performed every 4-6 years. The experiment was bifactorial, evaluating the effect of phosphorus and nitrogen fertilization (with five progressively increasing dose levels each, in a $5P \times 5N$ design, based on P_0 , P_{40} , P_{80} , P_{120} , P_{160}) on the quality of maize plants.

Seventy-five samples of maize leaves were harvested and analysed for their macro- and micronutrient composition.

After removing impurities and washing, the plant material is dried initially on filter paper at room temperature and subsequently in an oven with ventilation at 70°C. The leaves were ground to pass through a 2 mm sieve.

Total nitrogen content was determined by a wet digestion procedure using sulfuric acid, followed by sodium hydroxide distillation, and sulfuric acid titration (Kjeldahl method).

The plant samples were mineralized with a mixture of nitric, perchloric, and sulfuric acid followed by colorimetric determination of total phosphorus content at a spectrophotometer with ammonium vanadate-molybdate solution (Răuță & Chiriac, 1980).

In the same extract, potassium content was determined at the flame photometer, and those of calcium, magnesium, copper, zinc, manganese, and iron were determined by atomic absorption.

The results were analysed using variance analysis (ANOVA), followed by Tukey's interval test for multiple comparisons between all mean values (Ceapoiu, 1968). For all graphical representations, means followed by the same letter do not differ significantly at the 5% level (P<0.05) according to Tukey's HSD test.

RESULTS AND DISCUSSIONS

For harmonious development, maize plants require specific minerals at different growth stages. Therefore, determining the content of macroelements such as nitrogen, phosphorus, potassium, calcium, and magnesium in various plant organs can indicate the optimal doses of mineral fertilizers needed to achieve the best possible yields in corn crops (Gaj et al., 2020). As is well known, inorganic fertilizers rapidly release the nutrients that plants require (Makinde & Ayoola, 2010). This fact can be easily observed by analysing the nutrient content

of plants harvested from soils fertilized with these products and the absence of visible symptoms of nutrient deficiencies.

The nitrogen in ammonium nitrate, a commonly used nitrogen fertilizer, is present in both ammonium and nitrate forms. While the nitrate form is readily available for plant absorption, the ammonium form is quickly converted into nitrate by soil bacteria through the nitrification process and it becomes available for plant absorption. After absorption, nitrogen is transported into the leaves where it contributes

to photosynthesis, which helps the growth and development of maize plants (Wierzbowska et al., 2022)

Chemical analyses of maize plants harvested from the experimental field revealed that increasing doses of nitrogen fertilization led to statistically significant changes in the average nitrogen content of maize leaves. A statistically significant increase was observed starting from the N_{100} dose. The mean nitrogen content ranged from 1.78% in the N_{50} fertilized variant to 3.33% in the N_{200} fertilized variant (Figure 3).

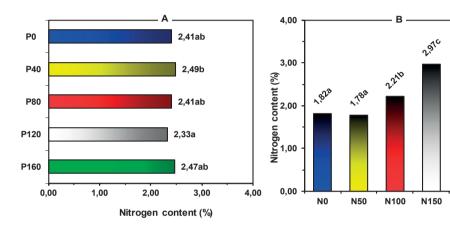


Figure 3. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Nitrogen Content in Maize Leaves

Phosphorus fertilization and nitrogen application enhance root system development and facilitate better nitrogen absorption (Graciano et al., 2006; Blandino et al. 2022). Conversely, an adequate phosphorus level formation of promotes the adenosine triphosphate (ATP), which plays a crucial role in nitrogen metabolism (Khan et al., 2023). Considering these aspects, the P₄₀ dose led to a statistically significant increase in nitrogen content in this study.

Phosphorus plays a crucial role in nutrient storage and transport within plants, but its absorption is influenced by soil pH and the presence of compounds that can immobilize phosphate ions.

Alley et al. (2019) state that due to the low mobility of phosphorus in the soil, applying

phosphorus fertilizers can increase the amount of phosphorus available to plants.

N200

The analysis of the chemical characteristics of maize leaves indicated that nitrogen fertilization caused statistically significant changes in the average phosphorus content of maize plants compared to the control (Figure 4).

Phosphorus levels decreased from 0.47% in the No variant to 0.26% in the variant with the highest nitrogen dose, likely due to soil acidification caused by increased nitrogen application, which reduced phosphorus absorption in the plants (Koper & Lemanowicz, 2008). In contrast, increasing phosphorus doses resulted in a higher average phosphorus content in leaves compared to the unfertilized variant for P_{80} , P_{120} , and P_{160} , with statistically significant differences.

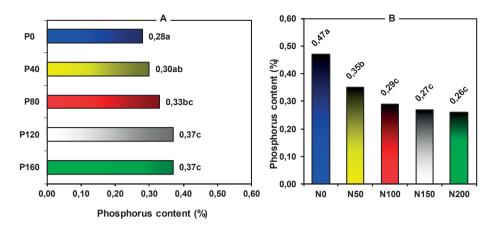


Figure 4. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Phosphorus Content in Maize Leaves

Although the other phosphorus fertilizer doses increased mean phosphorus levels compared to the control, these changes were not statistically significant.

Potassium, along with nitrogen and phosphorus, is one of the essential macronutrients for plants, playing a crucial role in plant metabolism and

enhancing their resistance to water stress, diseases, and pests (BąK et al., 2016).

The influence of phosphorus fertilization on the average potassium content in maize leaves decreased compared to the control as the fertilizer dose increased (Figure 5).

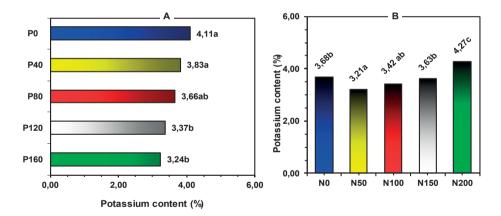


Figure 5. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Potassium Content in Maize Leaves

The low bioavailability is due to calcium and magnesium ions being present, and not potassium being included in the fertilization formula used in the experiment (Kalra, 1998; Malakshahi Kurdestani et al., 2024). The determined potassium (K) content falls within the range of 3.0-4.0% K, which is considered After applying the highest nitrogen dose, maize leaves had a significantly higher potassium content than unfertilized and other fertilized varieties. The application of high doses of

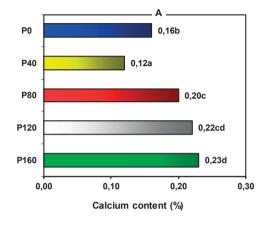
sufficient for the seedling stage according to Dalović et al. (2020). Fertilization with increasing nitrogen doses led to lower average potassium content values than the control variant, possibly due to the dilution effect stemming from the increase in dry biomass in the fertilized variants (Nenova et al., 2019) nitrogen may increase nutritional demand, stimulating potassium absorption. Nitrogen positively influences root system development and boosts metabolic processes, leading to a

higher rate of potassium absorption from the soil (Pasley et al., 2019).

Calcium is an element that supports the development of plants, having an important role in protecting plants from the strong sun rays; calcium regulates osmotic and ionic processes in cell membranes (Gaj et al., 2018).

In this study, maize was cultivated on moderately to strongly acidic soil (pH 5.2),

which resulted in a low Ca and Mg content in an antagonistic relationship between K (Figure 6). The application of phosphorus fertilization resulted in a slight increase in the average calcium content values of the analysed samples, despite all fertilization variants showing low calcium levels ranging between 0.11% and 0.23% (Răuță, & Chiriac; 1980, Kelling et al., 2000).



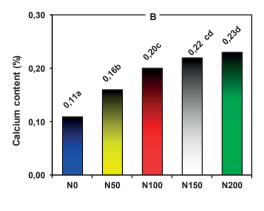


Figure 6. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Calcium Content in Maize Leaves

Nitrogen fertilization stimulates root development and increases transpiration, facilitating the absorption and transport of calcium into the leaves from the first dose of applied nitrogen. However, nitrogen fertilization did not increase the calcium content in the leaves beyond low levels.

Magnesium, an essential component of chlorophyll, is a vital nutrient for plant development. It plays a key role in photosynthesis, contributing to higher yield (Gaj et al., 2018). The magnesium content was in the same range of low values as the calcium content (Figure 7).

Long-term fertilization and calcium amendments induce statistically significant changes in micronutrient contents.

Copper, a micronutrient that supports plant defence mechanisms and photosynthesis, was

one of the chemical characteristics evaluated in maize plants harvested from the experimental Increasing doses of phosphorus fertilization led to copper immobilization, reducing its bioavailability for maize plants. As a result, the average copper content in maize leaves ranged between 5.9 mg/kg and 6.9 mg/kg, which falls within the normal concentration range (Figure 8). On the other hand, the reduction of Cu concentration could be partly explained by a decrease in the colonization of roots by arbuscular mycorrhizal fungi (AMF) due to increasing P application (Zhang et al., Nitrogen fertilization has caused increased copper mobility in the soil, making it more accessible to maize roots. Plants have a more intensive metabolic rate at high nitrogen levels and demand more micronutrients, including copper.

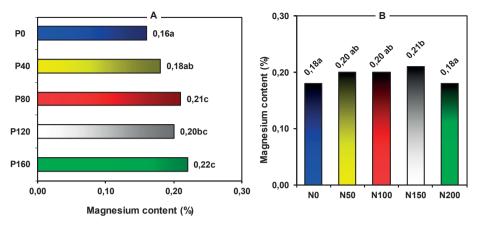


Figure 7. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Magnesium Content in Maize Leaves

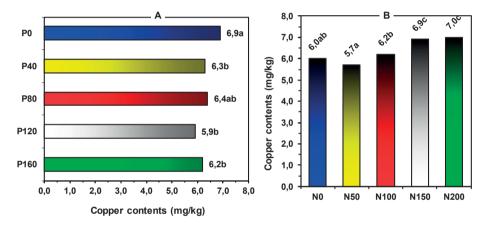


Figure 8. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Copper Content in Maize Leaves

This may explain the statistically assured increases in mean copper content values in maize plants.

The highest values of 6.9 mg/kg and 7.0 mg/kg were observed at high nitrogen fertilization rates, N₁₅₀ and N₂₀₀, respectively.

Iron, which is absorbed into plants by the root system, supports photosynthesis and contributes to increased nitrogen uptake by the plant. The iron content values in maize samples indicate the same blocking effect following fertilization with different doses of phosphorus as for copper. Nitrogen fertilization decreased the average iron content in maize leaves, with the lowest values obtained in N_{100} and N_{150} fertilized variants. These changes are statistically assured against

the control (Figure 9). The explanation could be due to the dilution effect induced by biomass expansion or the fact that nitrogen in large quantities can influence the balance of nutrient absorption. Fe competes with transition metals such as Cu, Zn, and Mn in its uptake, transport, and chemical reaction with plant cells (Rai et al., 2021). A higher copper supply may limit iron availability. Zinc plays a key role in calcium within transport plants, supporting development of leaves and grains. concentration in maize leaves follows a decreasing trend with increasing phosphorus application, with significant reductions observed at high P₁₂₀ and P₁₆₀ doses. These changes are statistically assured (Figure 10).

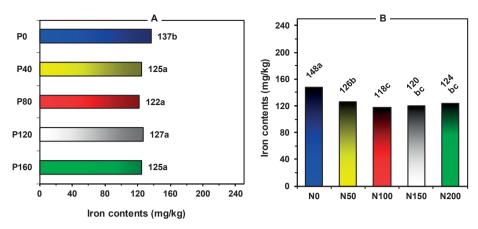


Figure 9. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Iron Content in Maize Leaves

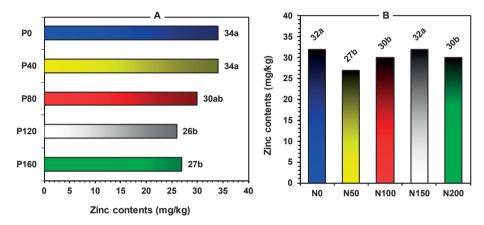


Figure 10. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Zinc Content in Maize Leaves

Moraghan (1985) reported that Zinc deficiencies induced by phosphorus in plants typically result from plant dilution effects, limited zinc translocation, or an imbalance between phosphorus and zinc.

Nitrogen fertilization has resulted in variations in mean zinc content ranging from 27 mg/kg to 32 mg/kg, which fall within the range of normal concentrations for maize plants (Răuţă & Chiriac, 1980). Manganese plays an important role in enzyme activity, facilitating nitrogen assimilation. Manganese contents found in maize plants indicated inhibited absorption of this micronutrient, with the effect becoming more pronounced as the applied phosphorus dose increased (Figure 11).

These changes are statistically assured compared to the variant without phosphorus fertilization.

Fertilization with increasing doses of nitrogen caused an increasing trend of manganese content in the analysed samples. Because maize can accumulate large quantities of heavy metals, nitrogen can influence the balance of other nutrients. reducing competition between manganese and other metals, such as iron or zinc, and allowing for more efficient manganese absorption (Wyszkowski & Brodowska, 2021). The acidification caused by nitrogen fertilization favours manganese absorption in the plant, leading to statistically significant increases compared to the control and between variants. starting from the N_{80} Micronutrients are required in smaller quantities than macronutrients for plant growth and development, but this does not diminish their importance in plant nutrition (Arnon & Stout, 1939; Johnson & Mirza, 2020).

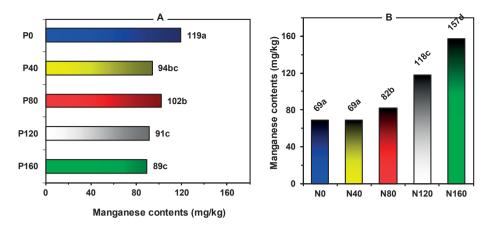


Figure 11. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Manganese Content in Maize Leaves

A deficiency in any essential element can negatively impact crop yields, making it crucial to determine the optimal fertilization formula to prevent any nutrient deficiencies.

The nutritional status of plants, reflected in their macro- and micronutrient content, can be influenced not only by nutrient deficiencies or excesses but also by a complex interplay of environmental factors such as water availability, climate, and other conditions (Houmani & Corpas, 2024).

CONCLUSIONS

The application of phosphorus had a favourable effect on nitrogen valorisation due to the stimulation of the development of the root system. Following fertilization with increasing doses of phosphorus, increases in nitrogen, phosphorus, calcium, and magnesium contents were observed from the analysed maize plants. Dose fractionation of nitrogen predicted the depressive effect of excessive nitrogen doses such as inhibiting the absorption of other nutrients (e.g., potassium, calcium, magnesium, manganese) copper. due to nutritional imbalances or the decrease in the efficiency of nitrogen use through leaching losses.

Upon applying progressively increasing nitrogen doses, statistically significant increases were recorded in the concentrations of nitrogen, potassium, calcium, and copper in maize leaves compared to the control variant. Calcium amendment and long-term fertilization induce changes in the chemical composition of maize

plants, reflecting the cumulative effect of previous treatments. Therefore, interpreting these results in the context of climate change is valuable for identifying the optimal fertilizer doses required for sustainable crop management.

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REFERENCES

Alley, M. M., Martz, M. E., Jr., Davis, P. H., Hammons, J. L., & Walsh, O. (2019). Nitrogen and phosphorus fertilization of corn (Publication 424-027). Virginia Cooperative Extension, Virginia Tech. https://www.pubs.ext.vt.edu.

Arnon, D. I., and P. R. Stout. "The essentiality of certain elements in minute quantity for plants with special reference to copper." *Plant Physiology*, vol. 14, no. 3, 1939, pp. 371–375.

https://doi.org/10.1104/pp.14.3.371

- Asvadurov, H., & Boeriu, I. (1983). Solurile judeţului Satu-Mare. I.C.P.A., O.J.S.P.A., Satu-Mare, Redacţia de propaganda Agricolă, Bucuresti.
- Bak, K., Gaj, R., & Budka, A. (2016). Accumulation of nitrogen, phosphorus and potassium in mature maize under variable rates of mineral fertilization. Fragmenta Agronomica, 33(1), 7–19.
- Blandino, M., Battisti, M., Vanara, F., & Reyneri, A. (2022). The synergistic effect of nitrogen and phosphorus starter fertilization sub-surface banded at sowing on the early vigor, grain yield and quality of maize. European Journal of Agronomy, 137, 126509. https://doi.org/10.1016/j.eja.2022.126509
- Bojtor, C., Illés, Á., Mousavi, S. M. N., Széles, A., Tóth, B., Nagy, J., & Marton, C. L. (2021). Evaluation of the nutrient composition of maize in different NPK fertilizer levels based on multivariate method analysis. *International Journal of Agronomy*, Article 5537549, 13 pages. https://doi.org/10.1155/2021/5537549
- Ceapoiu, N. (1968). Metode statistice aplicate în experiențele agricole și biologice. București: Editura Agro-Silvică.
- Đalović I, S Šeremešić, Y Chen, D Milošev, M Biberdžić, A Paunović (2020). Yield and nutritional status of different maize genotypes in response to rates and splits of mineral fertilization. *International Journal of Agriculture and Biology*, 23, 1141–1148. https://doi.org/10.17957/IJAB/15.1396
- Gaj R., Budka A., Górski D., Borowiak K., Wolna-Maruwka A., Bak K., (2018). Magnesium and calcium distribution in maize under differentiated doses of mineral fertilization with phosphorus and potassium. *Journal of Elementology*, 23(1), 137-150. https://doi.org/ 10.5601/jelem.2017.22.3.1491
- Gaj, R., Szulc, P., Siatkowski, I., & Waligóra, H. (2020). Assessment of the effect of the mineral fertilization system on the nutritional status of maize plants and grain yield prediction. *Agriculture*, 10(9), 404. https://doi.org/10.3390/agriculture10090404.
- Gondek, K., & Mierzwa-Hersztek, M. (2023). Effect of soil supplementation with mineral-organic mixtures on the amount of maize biomass and the mobility of trace elements in soil. Journal Name, Soil & Tillage Research, volume 226. https://doi.org/10.1016/j.still.2022.105558
- Graciano, C., Goya, J. F., Frangi, J. L., & Guiamet, J. J. (2006). Fertilization with phosphorus increases soil nitrogen absorption in young plants of *Forest Ecology* and Management 236, 202–210.
- Houmani, H., & Corpas, F. J. (2024). Can nutrients act as signals under abiotic stress? *Plant Physiology and Biochemistry*, 206, 108313. https://doi.org/10.1016/j.plaphy.2023.108313
- Kelling, K. A., Combs, S. M., & Peters, J. B. (2000). Plant analysis as a diagnostic tool. Retrieved from http://www.soils.wisc.edu/extension/publications/hori zons/2000/Plant%20Analysis%20as%20Tool.pdf
- Khan, F.; Siddique, A.B., Shabala, S.; Zhou, M.; Zhao, C. (2023). Phosphorus plays key roles in regulating plants' physiological responses to abiotic stresses. *Plants*, 12(15), 2861. https://doi.org/10.3390/plants12152861

- Kalra, Y. P. (Ed.). (1998). Handbook of methods for plant analysis. CRC Press.
- Koper, J., & Lemanowicz, J. (2008). Effect of varied mineral nitrogen fertilization on changes in the content of phosphorus in soil and plant and the activity of soil phosphatases. *Ecological Chemistry and Engineering* S. 15(4), 465–471.
- Kurtinecz, P. (2010). Die Dauerdüngungs- und Kalkungsversuche aus Nord-West Rumänien. Archives of Agronomy and Soil Science, 49(5), 503-510. https://doi.org/10.1080/03650340310001594772
- Makinde, E. A., & Ayoola, O. T. (2010). Growth, yield and NPK uptake by maize with complementary organic and inorganic fertilizers. *African Journal of Food, Agriculture, Nutrition and Development*, 10(3). https://doi.org/10.4314/ajfand.v10i3.54078
- Malakshahi Kurdestani, A., Francioli, D., Ruser, R., Piccolo, A., Maywald, N. J., Chen, X., & Müller, T. (2024). Optimizing nitrogen fertilization in maize: The impact of nitrification inhibitors, phosphorus application, and microbial interactions on enhancing nutrient efficiency and crop performance. Frontiers in Plant Science, 15, 1451573. https://doi.org/10.3389/fpls.2024.1451573
- Moraghan, J. (1985) 8. Plant tissue testing for micronutrient deficiencies and toxicities. Fertilizer Research, 7, 201–219. https://doi.org/10.1007/BF01049001
- Nenova, L., Benkova, M., Simeonova, T., & Atanassova, I. (2019). Nitrogen, phosphorus, and potassium content in maize dry biomass under the effect of different levels of mineral fertilization. *Agricultural Science and Technology*, 11(4), 311-316. https://doi.org/10.15547/ast.2019.04.052
- Pasley, H. R., Cairns, J. E., Camberato, J. J., & Vyn, T. J. (2019). Nitrogen fertilizer rate increases plant uptake and soil availability of essential nutrients in continuous maize production in Kenya and Zimbabwe. Nutrient Cycling in Agroecosystems, 115, 373–389. https://doi.org/10.1007/s10705-019-10016-1
- Rai, S., Singh, P. K., Mankotia, S., Swain, J., & Satbhai, S. B. (2021). Iron homeostasis in plants and its crosstalk with copper, zinc, and manganese. *Stress Biology*, 1(1), 100008. https://doi.org/10.1016/j.stress.2021.100008
- Răuță, C., & Chiriac, A. (1980). Metodologie de analiză a plantei pentru evaluarea stării de nutriție minerală. Academia de Științe Agricole și Silvice.
- Singh, R., Sawatzky, S., Thomas, M., Akin, S., Raun, W. R., Zhang, H., &Arnall, D. B. (2024). Micronutrients concentration and content in corn as affected by nitrogen,phosphorus, and potassium fertilization. Agrosystems, *Geosciences & Environment*, 7, e20568. https://doi.org/10.1002/agg2.20568
- Johnson, V.J & Mirza A. (2020). Role of macro and micronutrients in the growth and development of plants. *International Journal Current Microbiology* and Applied Sciences, 9(11):576-587. https://doi.org/10.20546/ijcmas.2020.911.071
- Wierzbowska, J., Sienkiewicz, S., & Światły, A. (2022).
 Yield and nitrogen status of maize (Zea mays L.)
 fertilized with solution of urea-ammonium nitrate

- enriched with P, Mg or S. *Agronomy*, 12(9), 2099. https://doi.org/10.3390/agronomy12092099
- Wyszkowski, M., Brodowska, M.S. (2021). Potassium and nitrogen fertilization vs. trace element content of maize (*Zea mays* L.). *Agriculture*, *11*, 96. https://doi.org/10.3390/agriculture11020096.
- Zhang, W., Zou, C., Chen, X., Liu, Y., Liu, D., Yang, H., Deng, Y., & Chen, X. (2020). Phosphorus application decreased copper concentration but not iron in maize grain. *Agronomy*, 10, 1716.

https://doi:10.3390/agronomy10111716