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ORIGINAL PAPER

Role of humic acids-based fertilisers and nitrogen fertilisers in the regulation of the macroelement content in maize biomass*

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Abstract

Mineral fertilisers are applied to soil to replenish or maintain at the necessary level its abundance of available nutrients, and to optimise the genetic production potential of plants. Humic substances are biologically active compounds of organic matter in the soil that effectively support mineral fertilisers in the cultivation of various crops. The aim of this study was to determine the effect of increasing doses of humic acids-based fertilisers (HAs) on the regulation of the macroelement content in maize grown on two soils (loamy sand and sand) fertilised with different nitrogen fertilisers (ammonium nitrate - AN, urea - U and urea and ammonium nitrate solution - UAN). Nitrogen fertilisation had a greater effect on the macroelement content of plants on sand. The highest macroelement content in maize grown on this soil was found on soils fertilised with UAN. Urea induced similar but smaller changes in the K, P and Na content of maize, but not in Mg and Ca. Urea also had the most favourable effect on plant biomass. HAs tended to increase the macroelement content of plants. On the sand, HAs had the greatest effect on the UAN fertilised objects, causing an increase in Mg, P, Ca and K compared to the unfertilised object. On loamy sand, the changes in their content in maize were less clear. HAs can be a good source of essential elements for plants by supporting the effectiveness of mineral fertilisation.

Keywords: humic acids, nitrogen fertilisers, soil material, maize biomass, macroelements

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INTRODUCTION

One of the most important agrotechnical measures determining the volume and profitability of crop production is fertilisation (Blecharczyk et al. 2023). Mineral fertilisers are applied to soil in order to replenish or maintain the required level of available nutrients, and to optimise the genetic production potential of plants (Masclaux-Daubresse et al. 2010). Nitrogen (N) is the basic element of life, forms an essential part of biomass and is involved in all physiological and metabolic processes in plants (Grzyb et al. 2021). It is an essential component of proteins, deoxyribonucleic acid (DNA), chlorophyll, vitamins and phytohormones, as well as energy compounds such as adenosine triphosphate (ATP) (Abrol et al. 2012). Nitrogen strongly activates growth processes, stimulates leaf growth, increases photosynthetic production, influences plant development and morphology, and affects plant uptake and use of other macro- and micronutrients (Ai et al. 2017).

In agriculture, N fertilisation determines crop yield (productivity) and soil fertility. Nitrogen is an effective yield factor (Anas et al. 2020), and there is a close relationship between the plant's response to N nutrition and biomass accumulation. Photosynthetic products account for more than 90% of plant dry matter, hence N plays a fundamental role in yield formation by influencing the photosynthetic process and its efficiency (Makino 2011). Therefore, proper, rational management of this component is essential for global food security (Martínez-Dalmau et al. 2021). On the other hand, an inordinate and excessive use of N fertilisers not only reduces technical and economic efficiency, affecting the profitability of agricultural production, but also causes environmental problems and threatens animal and human health (Luo et al. 2022). Unused N from agricultural production is dispersed into the environment and contributes to several regional and global environmental problems, such as soil acidification, ozone depletion and climate change, in particular groundwater and surface water pollution and eutrophication (Ward 2009, Martínez-Dalmau et al. 2021). The addition of reactive (reduced) N to the soil in the form of nitrate (NO_3^-), ammonium nitrogen (NH_4^+) or urea (U), which is rapidly hydrolysed to NH_4^+ ions, significantly increases yields (Khajuria 2016). However, excessive use of N fertilisers results in low N use efficiency (NUE) – Peng et al. (2010) and does not benefit yield. NUE does not exceed 50% for most crop species (Ahmed et al. 2017). Excess N results in overgrowth of aerial vegetative biomass and in poor root development, delayed maturity and seed setting, reduced resistance to disease, insects and lodging, and it ultimately leads to significant reductions in crop yield (Luo et al. 2022). On average, 65% of applied mineral N is lost from the plant-soil system through nitrate leaching to aquatic ecosystems and/or volatilisation of gases (ammonia and nitrous oxides) or is used by soil microorganisms (Canellas et al. 2013, Martínez-Dalmau et al. 2021). Unlike NH_4^+ ions, NO_3^- ions are not adsorbed by negatively charged soil colloids. Therefore, depending on the soil type and rainfall intensity, NO_3^- ions migrate down the soil profile and are easily washed out of the soil (Khajuria 2016). Such losses cause serious environmental problems and also reduce ecosystem productivity (Zhang et al. 2023).

Maize is a crop with high yield potential and high nutrient requirements (Shrestha et al. 2018). On average, maize requires 26-28 kg N, 11-13 kg phosphorus (P_2O_5) and 30-35 kg potassium (K_2O) to produce 1 tonne of grain (including straw) (Sugiono, Krismawati 2020). Its high nutrient requirements mean that fertilisation, especially with N, has a significant impact on its yield (Gehl et al. 2005). Increased N fertilisation can only stimulate yield increases up to a certain level up to 200 kg N ha^{-1} (Laskari et al. 2022), beyond which there may be a reduction in yield. Furthermore, as mentioned above, the application of excessively high N doses, especially on light soils, leads to a concentration of residual nitrates in the soil and contamination of groundwater, and poses a risk to human health (Huang et al. 2021). Therefore, measures should be taken to develop strategies to increase food production while reducing the excessive use of mineral fertilisers, especially N fertilisers (Xu et al. 2021). As reported by Brodowska et al. (2022), one such approach could be the application of humic substances (HS) to the soil. These substances prolong the action and increase the efficiency of these fertilisers (Cappelini et al. 2020) and stimulate nitrate (NO_3^-) uptake (Leite et al. 2020). In a study by Piccolo et al. (1992) it was shown that barley seedlings after application of HS had a significantly higher NO_3^- uptake than controls. According to these authors, this was due to the lowering of the pH at the root surface by the aforementioned substances, which facilitated the transport of nitrate ions by symport with hydrogen ions (H^+/NO_3^-).

HS are biologically active compounds of soil organic matter (Leite et al. 2020) that are effectively used in the cultivation of various crops (Symanowicz et al. 2022). Their positive effects on growth and yield have been reported in the cultivation of potato (Ekin 2019), pumpkin (Esho, Saeed 2017), tomato (Suman et al. 2017) and durum wheat (Delfine et al. 2005), among others. HS stimulate germination (Shahryari et al. 2011; Šerá and Novák 2022), as well as leaf development in crops and root growth, especially lateral roots and trichomes (Eyheraguibel et al. 2008). They exhibit auxin-like activity, influencing mechanisms that stimulate plant growth (Nardi et al. 2021), improve the nutritional status of plants and increase translocation of elements from root to shoot (Bondareva, Kudryasheva 2021). By influencing phytohormonal pathways or the action of carrier and structural genes and proteins responsible for physiological processes, the addition of HS regulates plant development (Canellas, Olivares 2014), increases chlorophyll synthesis (Shah et al. 2018) and tolerance to external stressors (Canellas et al. 2015, Giovanardi et al. 2016). HS affect the solubility and migration of elements, causing partial detoxification of toxic ions, e.g. Cr (Yang et al. 2021), while facilitating the uptake of macronutrients (e.g. K, Ca and P) and micronutrients (e.g. Zn, Mn and Fe) important for normal plant metabolism

(Çelik et al. 2010). In addition, HS have positive effect on soil properties (Symanowicz, Toczko 2023). HS have buffering properties in the pH range 5.5-8.0, thus helping to maintain a constant soil pH and counteracting soil acidification. This effect is most likely based on OH^- chemisorption and H^+ physisorption (Pertusatti, Prado 2007). They also act as carbon reservoirs and actively participate in the carbon cycle in nature (da Silva et al. 2021), and are a source of N, P and S due to their ability to bind biogenic elements (Bondareva, Kudryasheva 2021). There are also numerous literature reports on changes in the chemical composition of crops treated with HS (Katkat et al. 2009, Belal et al. 2019; Wyszkowski et al. 2023*a*). A 50% increase in nitrate content in leaves of maize seedlings treated with HS compared to controls was shown by Quaggiotti et al. (2004). They also showed that these substances can have a direct effect on the expression of genes encoding a putative maize nitrate transporter (ZmNrt2.1) and the maize H^+-ATPase isoform (Mha2). Khaled and Fawy (2011) showed that foliar application of 0.1% humic acids (HAs) significantly increased maize dry matter (by 23%) and uptake of N (by 24%), P (by 25%), K (by 39%), Ca (by 39%), Mg (by 58%) and Na (by 63%) compared to the control experiment.

The rational and sustainable management of N fertilisers in agricultural systems is challenging, but crucial given the impact on yield and quality and the potential for environmental pollution (Anas et al. 2020, Zhang et al. 2023). The use of biostimulants, including HS, can reduce the economic and environmental costs associated with N fertiliser application while maintaining soil ecological services and yield quality. Therefore, a study was conducted to determine the effect of applied organic matter in the form of HAs on the macronutrient content of maize biomass grown on two soils fertilised with different N fertilisers.

The aim of this study was to determine the effect of humic acids-based fertilisers on the macroelement content of the maize (*Zea mays* L.) biomass grown on two different soils fertilised with different N fertilisers.

MATERIALS AND METHODS

Methodology of the plant growing experiment

The research was based on a pot experiment. It investigated the effect of increasing doses of HAs and different forms of N fertiliser (ammonium nitrate – AN 34% N, urea – U 46% N and urea and ammonium nitrate solution – UAN 32% N) on the growth, development and macroelement content of maize (*Zea mays* L.) on two soil materials: loamy sand and loamy clay (IUSS Working Group WRB 2015). Detailed information on the characteristics of these soils and HAs is provided in previously published papers (Brodowska et al. 2022, Wyszkowski et al. 2023*b*). HAs (organic-mineral fertiliser Humik – CALFERT, Warsaw, Poland) in doses of 0, 0.05, 0.10 and

0.15 g kg^{-1} were applied to the soil three times: before sowing and during the vegetative growth of the test plant (maize): at the stage of 5 leaves (BBCH15) and at the stage of intensive shoot growth stage (BBCH30). The content of macroelements in the Humik was as follows: 40.00 g N, 0.436 g P, 41.50 g K and 3.150 g Mg kg^{-1}. The N dose applied was the same at all sites and was 160 mg N kg^{-1} soil. Phosphorus and potassium fertilisers were also added to each pot in equal doses of 60 mg P (Super FosDar 40) and 170 mg K kg^{-1} soil (KCl 50).

All mineral fertilisers (N, phosphorus and potassium) and the first dose of HAs were thoroughly mixed with 9 kg of soil and transferred to polyethylene pots. Kadryl variety maize (*Zea mays* L.) seeds were then sown at a rate of six per pot. Constant soil moisture (60% of maximum water capacity) was maintained throughout the plant growing experiment. The maize was harvested at the tassel emergence stage (BBCH59). At the time of maize harvest, samples of plant material were taken for laboratory analysis.

Methodology for laboratory and statistical analysis

Samples of the maize biomass were cut, dried at 60°C and ground. Wet mineralisation of plant samples was carried out in concentrated 96% sulphuric acid with 30% H_2O_2 in a Speed-Digester K-439 (Ostrowska et al. 1991). The mineralised samples were analysed for: P – by vanadium-molybdenum colorimetric method (Ostrowska et al. 1991), K, Ca, Mg and Na – by ASA atomic absorption/emission spectrometry (Ostrowska et al. 1991) on a SpectrAA 240FS spectrophotometer. The content of N and sulphur content has been published in another paper (Brodowska et al. 2022). Before setting up the experiment, the granulometric composition of the soil was analysed by laser using a Mastersizer 3000 (PN-R-04032).

The Statistica 13 package (TIBCO Software Inc. 2021) was used for statistical calculations. They were performed using a three-factor ANOVA analysis of variance, the Tukey's HSD test ($p \leq 0.01$), principal component analysis (PCA), percentage of observed variability and the Pearson's simple correlation coefficients.

RESULTS

Nitrogen fertiliser forms and humic acid doses, as well as soil quality, had a clear and mostly significant effect on plant growth and development (Figure 1) and on the macroelement content of the maize biomass (Tables 1-2).

Maize growth and development was dependent on soil type. Maize had a higher biomass on loamy sand than on sand (Figure 1). Urea had a more favourable effect on maize growth and development than AN on both soils, and UAN only on the second soil (loamy sand). The application of HAs had

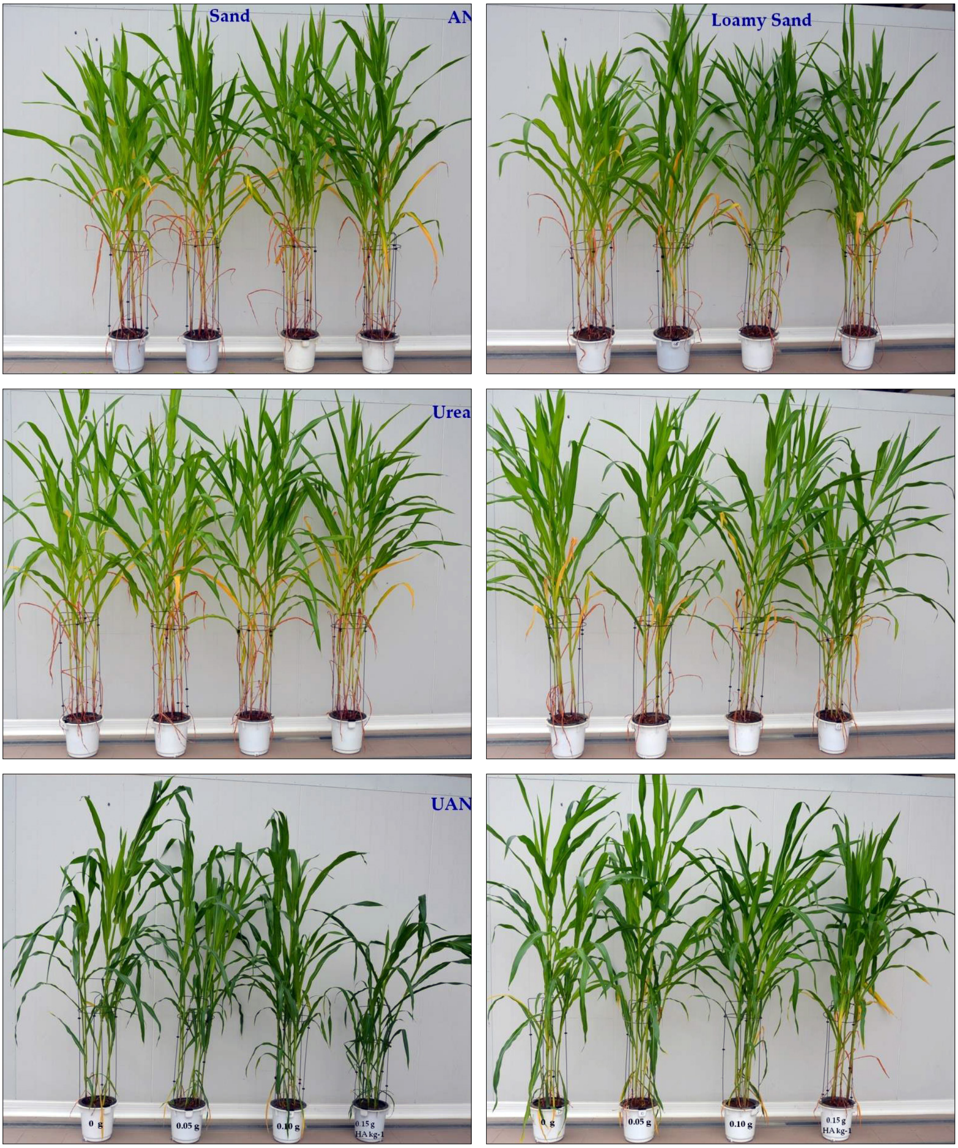

Fig. 1. Maize at the end of tassel emergence phase (BBCH 59)

a positive effect on maize biomass. In most of the test series, the average (0.10 g kg^{-1}) and, in the series with U on sand, the first (0.05 g kg^{-1} soil) dose of HAs proved to be optimal. The effect of HAs on plants was strongly negative in the series with UAN on sand and positive on loamy sand, but only for the first dose (0.05 g kg^{-1} soil).

The content of most of the elements tested in maize depended on the soil type, with the greatest differences recorded for P and Mg (Tables 1-2). Their

Table 1

Content of phosphorus, potassium and sodium in maize biomass (g kg^{-1} DM)

HAs dose (g kg^{-1} of soil)	Sand				Loamy sand			
	AN	U	UAN	average	AN	U	UAN	average
Phosphorus (P)								
0	2.251^{a-c}	2.521^{a-c}	2.551^{a-c}	2.441^{B}	2.276^{ab}	1.977^{ab}	2.158^{ab}	2.137^{A}
0.05	2.160^{ab}	2.465^{a-c}	2.496^{bc}	2.374^{B}	1.916^{a}	2.021^{a}	2.043^{a}	1.993^{A}
0.10	2.349^{a-c}	2.322^{a-c}	2.892^{c}	2.521^{B}	1.975^{ab}	2.159^{ab}	2.378^{ab}	2.171^{A}
0.15	2.055^{ab}	2.479^{a-c}	3.152^{c}	2.562^{B}	2.037^{a}	2.225^{ab}	1.893^{ab}	2.052^{A}
Average	2.204^{A}	2.447^{B}	2.773^{C}	2.474^{B}	2.051^{A}	2.096^{A}	2.118^{A}	2.088^{A}
r	-0.411	-0.400	0.923^{*}	0.785^{*}	-0.537	0.982^{*}	-0.291	-0.125
Potassium (K)								
0	9.31^{ab}	9.16^{ab}	10.76^{ab}	9.74^{A}	11.01^{ab}	10.57^{ab}	10.56^{ab}	10.71^{A}
0.05	9.31^{a}	9.70^{ab}	10.73^{ab}	9.91^{A}	11.51^{ab}	10.83^{ab}	11.23^{ab}	11.19^{A}
0.10	10.83^{ab}	10.36^{ab}	13.72^{b}	11.64^{A}	11.15^{ab}	12.07^{ab}	12.59^{ab}	11.93^{A}
0.15	8.92^{ab}	10.09^{ab}	16.17^{b}	11.73^{A}	12.23^{ab}	12.40^{ab}	9.20^{ab}	11.28^{A}
Average	9.59^{B}	9.83^{C}	12.84^{AB}	10.75^{A}	11.47^{AC}	11.47^{AC}	10.90^{AB}	11.28^{A}
r	0.053	0.854^{*}	0.946^{*}	0.924^{*}	0.778^{**}	0.963^{*}	-0.249	0.626^{*}
Sodium (Na)								
0	0.380^{a-c}	0.488^{a-f}	0.538^{d-f}	0.469^{A}	0.538^{d-f}	0.548^{d-f}	0.544^{a-e}	0.544^{A}
0.05	0.402^{ab}	0.490^{b-f}	0.529^{d-f}	0.474^{A}	0.531^{b-f}	0.544^{d-f}	0.395^{a-f}	0.490^{A}
0.10	0.421^{a-c}	0.527^{a-f}	0.545^{d-f}	0.498^{A}	0.549^{ef}	0.566^{f}	0.398^{a-f}	0.504^{A}
0.15	0.469^{a-d}	0.542^{b-f}	0.571^{c-f}	0.527^{A}	0.559^{d-f}	0.560^{f}	0.414^{a}	0.511^{A}
Average	0.418^{B}	0.512^{A}	0.546^{A}	0.492^{A}	0.544^{A}	0.555^{B}	0.438^{A}	0.512^{B}
r	0.975^{*}	0.954^{*}	0.821^{*}	0.963^{*}	0.852^{*}	0.730^{*}	-0.702^{*}	-0.474

HAs – humic acids, r – correlation coefficient, * significant at $p \leq 0.01$. Values with different letters ($^{A-C}$, $^{a-f}$) – significantly different at $p \leq 0.01$.

content in maize was 16 and 29% lower respectively on loamy sand than on sand. For K, Na and Ca, the relationships were reversed, with relatively small differences between these soils, not exceeding 5%.

The highest macroelement content in maize grown on sand was found in soils fertilised with UAN (Tables 1-2). UAN fertilisation increased Mg content by an average of 22%, P by 26%, Na by 31%, Ca by 33% and K by 34% compared with the AN series. Urea induced similar but smaller changes in the K, P and Na contents of maize, in contrast to Mg and Ca. The direction of the changes in K and Na content of maize after UAN application was reversed on loamy sand. Under its influence, the K content decreased by an average of 5% and the Na content by 20% compared to the series with AN. No major changes were observed in the contents of most of the elements tested (except Ca) in maize after U application and in the

Table 2

Content of magnesium and calcium in maize biomass (g kg^{-1} DM)

HAs dose (g kg^{-1} of soil)	Sand				Loamy sand			
	AN	U	UAN	average	AN	U	UAN	average
Magnesium (Mg)								
0	2.217^{d-h}	2.050^{a-g}	2.427^{f-g}	2.232^{B}	1.633^{a-d}	1.270^{a}	1.402^{a}	1.435^{A}
0.05	2.019^{a-g}	2.095^{a-f}	2.498^{gh}	2.204^{B}	1.619^{a-d}	1.701^{ab}	1.664^{a-d}	1.662^{A}
0.10	2.360^{e-h}	2.195^{b-g}	2.749^{h}	2.435^{B}	1.638^{a-d}	1.763^{a}	1.716^{a-e}	1.706^{A}
0.15	2.081^{c-h}	2.116^{c-h}	2.893^{h}	2.364^{B}	1.716^{a-c}	1.767^{a-e}	1.899^{a-e}	1.794^{A}
Average	2.169^{B}	2.114^{C}	2.642^{B}	2.308^{B}	1.652^{A}	1.625^{A}	1.670^{A}	1.649^{A}
r	-0.057	0.636^{*}	0.981^{*}	0.741^{*}	0.793^{*}	0.839^{*}	0.970^{*}	0.946^{*}
Calcium (Ca)								
0	2.245^{ab}	2.218^{a}	2.778^{ab}	2.414^{A}	2.485^{ab}	2.109^{ab}	2.758^{ab}	2.451^{A}
0.05	2.492^{ab}	2.230^{ab}	2.827^{ab}	2.516^{A}	2.666^{ab}	2.502^{ab}	2.869^{ab}	2.679^{A}
0.10	2.691^{ab}	2.357^{ab}	3.217^{ab}	2.755^{A}	3.089^{ab}	2.589^{ab}	3.188^{ab}	2.955^{A}
0.15	2.261^{ab}	2.373^{ab}	4.074^{b}	2.903^{A}	3.140^{ab}	2.822^{ab}	2.296^{ab}	2.752^{A}
Average	2.423^{AB}	2.294^{B}	3.224^{C}	2.647^{A}	2.845^{AC}	2.505^{AB}	2.778^{A}	2.709^{A}
r	0.151	0.935^{*}	0.921^{*}	0.990^{*}	0.962^{*}	0.968^{*}	-0.373	0.732^{*}

Explanations see Table 1.

contents of P, Mg and Ca after incorporation of UAN into the second soil (loamy sand) compared to the AN fertilised objects. UAN contributed to a 12% reduction in the Ca content of maize on loamy sand.

The effect of HAs on the macroelement content of the maize biomass was generally favourable (Tables 1-2). HAs generally increased their content in plants. However, their effect depended on the soil type and the form of N fertiliser.

On the sand, HAs had the greatest effect on the UAN-fertilised objects, increasing Mg by a maximum of 19%, P by 24%, Ca by 47% and K by 50% compared to the unfertilised objects (Tables 1-2). Similarly, the average dose of HAs (0.10 g kg^{-1}) had a weaker effect on the content of the above-mentioned elements in the series with AN and on the concentration of K and Mg in maize in the pots with U, while the highest dose of HAs (0.15 g kg^{-1}) affected the Na content in the pots with AN and U and Ca in the pots with U. It should be noted, however, that on the sand, the effect of HAs on the Na content of maize was greater in the plots with AN (+23%) and U (+11%) than in the plots with UAN (+6%) – Table 2.

Changes in the content of the tested macroelements in maize after application of HAs to a second soil (loamy sand) were less clear (Tables 1-2). HAs had the most favourable effect on the contents of P (+13%), K (+17%), Ca (+34%) and Mg (+39%) in the series with U and Ca (+26%) in the pots with AN, compared to the object without their application. They also reduced the P content in the AN pots and the Na content in the maize in the UAN pots. For the other elements and test series, the changes were smaller and their direction was often ambiguous.

The PCA analysis (Figure 2) showing the cumulative effect of the studied factors on the macroelement content of maize indicates that their contribution accounted for 58.02% of the correlation of the data set in group one and 21.23% in group two. The Na content vector was slightly shorter than the vectors of the other elements, indicating that it was less correlated with the factors studied. The highest positive correlation was found between K and Ca and between Mg and P content, and a negative correlation between plant biomass and P, Mg and K content in maize. There was also a small but negative correlation between plant biomass and plant Ca content. The scattering of the results in Figure 3 indicates a greater cumulative effect of HAs (especially the medium and highest dose) on the macroelement content of the maize biomass on objects fertilised with UAN than with U and AN, especially on sands.

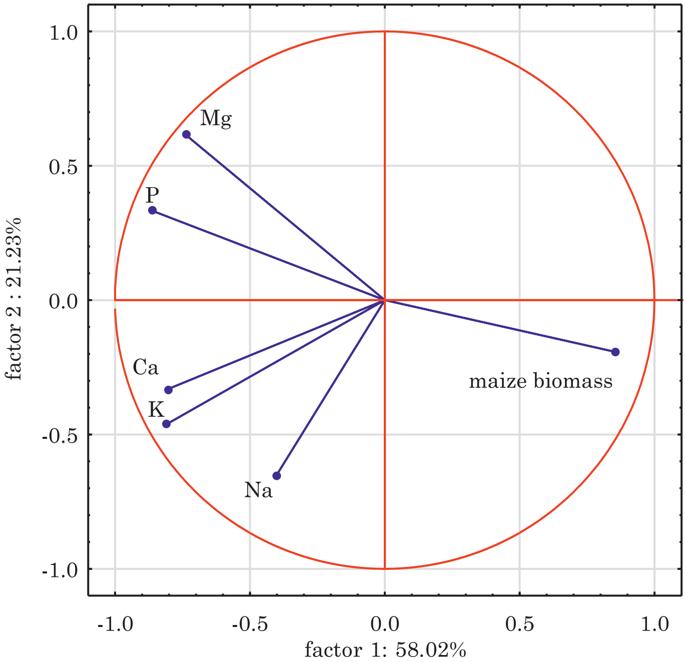

Fig. 2. Biomass and content of macroelements in maize illustrated with the PCA method. Key: vectors represent variables (biomass and content of P, K, Na, Mg and Ca in maize)

After calculating the percentage of observed variability using the $\eta 2$ coefficient, it was shown that the macroelement content of the maize biomass was primarily dependent on the form of N fertiliser (Figure 4). The type of N fertiliser had a stronger effect than the other factors on the Ca (42.2%),

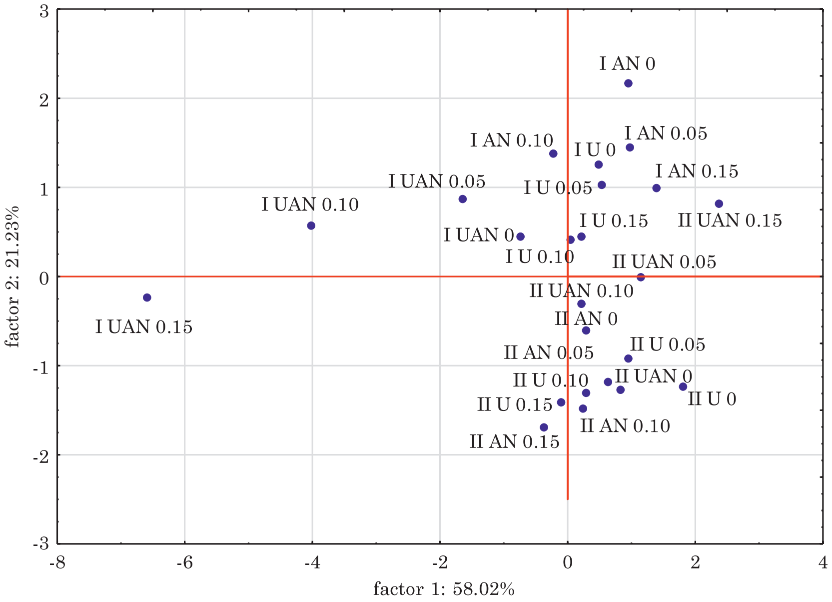

Fig. 3. Effect of humic acids on content of macroelements in maize biomass illustrated with the PCA method.
Points show the samples with elements: I – sand, II – loamy sand, AN – ammonium nitrate, U – urea, and UAN – solution of urea and ammonium nitrate using 0, 0.05, 0.10, and 0.15 g HAs kg^{-1} of soil

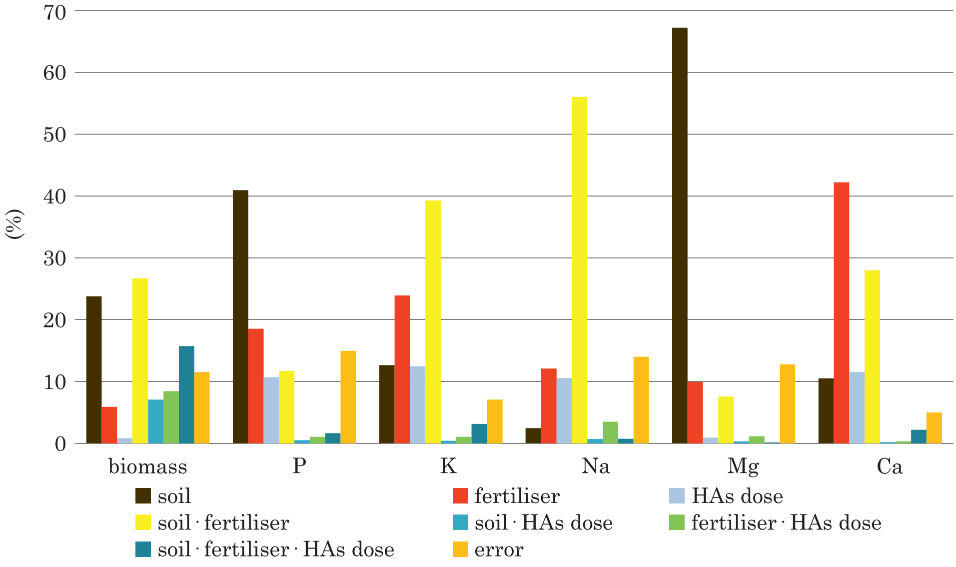

Fig. 4. Relative influence of factors on the biomass and the content of macroelements in maize (%).
Key: biomass and content of P, K, Na, Mg and Ca in maize, Has – humic acids

K (23.9%) and Na (12.1%) content, the soil type on the Mg (67.2%) and P (41.0%) content and the plant biomass (23.8%). It should also be noted that the interaction of fertiliser and soil type had a greater effect on Na (56.0%) and K (39.3%) content in maize than the same factors analysed separately.

DISCUSSION

In our study, N fertilisation significantly affected the growth and development of maize and the macronutrient content of its aerial biomass. A similar trend was observed by Szulc (2009), who showed a significant effect of N doses (0-150 kg N ha^{-1}) on the elements of maize yield structure, harvest index and macronutrient assimilation. He recorded the highest N uptake after the application of a dose of 90 kg N ha^{-1}. Further increases in the N dose resulted in a significant decrease in the magnitude of this characteristic. On the other hand, the highest uptake of P, K, Mg, Ca and Na was observed at a dose of 120 kg N ha^{-1}. The positive effect of N fertilisation on maize productivity has also been found by Abbasi et al. (2013) and Szulc et al. (2013). A high N supply stimulates root growth and development and increases the uptake of other nutrients (Amtmann et al. 2009), which may explain the accumulation of Mg, P, Na, Ca and K determined in the present experiment in the maize biomass recorded grown on sand after the application of UAN, as well as Ca, P and Mg after UAN application on loamy sand. In their experiment, Wierzbowska and Żuk-Gołaszewska (2014) showed that N fertilisation (U at doses: N_0, $N_{0.5}$, $N_{1.0}$ g pot^{-1}) significantly differentiated morphological parameters (plant height, number of branches, stem weight) of fenugreek and yield components (thousand seed weight, seed weight) and macronutrient content of aerial biomass. According to the authors, the highest applied N dose increased the content of N (by 68%), Na (by 8%), Mg (by 17%) and P (by 3%) in the leaves of the tested plant. Under the same conditions, fenugreek stems and pericarps also responded with an accumulation of selected macronutrients. The greatest changes were observed for P and Na content in pericarps (57% and 32% increase, respectively) and N and Na content in stems (19% and 11% increase, respectively). It seems that the positive effect of N fertiliser on crop yield and chemical composition should be attributed to its stimulating effect on chlorophyll biosynthesis, CO_2 assimilation and biomass accumulation (Wierzbowska et al. 2014), as well as its influence on rhizosphere processes and root growth and development (Shapiro et al. 2016), which consequently translates into a higher plant demand for other nutrients (Fageria 2001). These dependencies are in line with the results of our research, because the most macroelements in maize biomass were recorded under the effect of UAN. Urea caused analogous but lesser changes in the content of K, P and Na in maize. Nitrogen fertilisation affected the content of plant macroelements more on the sand than the loamy sand.

In our study, HS had a positive effect on maize yield and increased the macronutrient content of biomass. They had the strongest effect on sand in UAN-fertilised plots, causing an increase in Mg, P, Ca and K contents compared to control plots. The results obtained confirm the study conducted by Mohamed et al. (2017), who evaluated the effect of foliar application of HA (4 cm^3 dm^{-3}) and N fertiliser (0, 90 and 120 kg N fed^{-1}) on the growth and some biochemical parameters and macronutrient content (N, P, K) in leaves and grains of two maize hybrids. The authors considered the combination of humic acid with N fertiliser at a dose of 90 kg fed^{-1} to be the most favourable fertilisation method. Under these conditions, the N content in leaves (by 12%-23%) and grain (by 6%-16%), the P content in leaves (by 16-17%; only hybrid T.W.C.-310) and grain (by 28%; only hybrid S.C. Pioneer 30K09) and the K content in leaves (by 31%-79%) and grain (by 22%-44%) increased compared to the control (no fertilisation). In addition, this fertiliser treatment increased the chlorophyll, dry matter, total carbohydrate and protein content of the grain. According to the authors, these changes are a consequence of the increased efficiency of photosynthesis and protein synthesis in response to humic acid treatment, which activates physiological and biochemical processes in plants (Nardi et al. 2002). An increase in K, P, Ca, Mg and N in the biomass of wheat after the application of humic acid (foliar, 13 mg dm^{-3}) and half the recommended dose of N fertiliser was shown by El-Bassiouny et al. (2014). The beneficial effect of an organic combination of HS and N fertilisation has also been shown by Nazli et al. (2020) and Ehsan et al. (2014). The increase in the macronutrient content in plant biomass after the application of HS is related to their stimulating effect on root H^+-ATPase activity and the uptake of nutrients from the soil by roots and the translocation of elements to the growing parts of plants (Mora et al. 2010, Canellas, Olivares, 2014). In addition, HS facilitate the formation of soil macroaggregates by positively influencing the physical (Yang, Antonietti 2020), chemical and biological properties of the soil (Ren et al. 2022). In this way, they increase the soil's capacity to retain water and fertiliser, thereby prolonging their effect (Liu et al. 2020) and increasing the availability of macronutrients, especially N, P and K (Leite et al. 2020).

The effect of humic amendments is to maintain a high concentration of nutrients close to the root zone so that plants can take up and use them more efficiently, resulting in higher crop quality and yields. This is supported by a study by Azeem et al. (2015) in which the combined application of N (as U; 160 kg N ha^{-1}) and HAs (4.5 kg ha^{-1}) significantly increased the biological yield, leaf area, growth rate and grain yield of maize. The beneficial effect of HAs on maize yield was probably due to an increase in organic matter content, soil total N availability (Baldotto et al. 2019) and an increase in its assimilation. Improvements in maize vegetative growth traits such as plant height and leaf chlorophyll a and b content after HAs application at 20 mg dm^{-3} were also shown by Bakry et al. (2009). In their experiment, Abdo et al. (2022) showed a positive effect of combined fertilisation with AN (335 g kg^{-1};

285 kg N ha^{-1}) and HAs (3 g dm^{-3}) in maize cultivation. The authors demonstrated that it is possible to reduce conventional NPK fertilisation by 25% with the combined use of biostimulants. This type of fertilisation increased ear length, grain yield and grain protein content compared to the control (mineral fertilisation only). Macronutrient content and uptake were also positively correlated with the addition of HAs. It had a stimulating effect on the accumulation of N (by about 14%), P (by about 20%) and K (by about 7%) in the maize grain compared to the series without its application. Based on the literature data, it can be concluded that the effect of HAs on plant uptake of mineral nutrients is complex and results from their multidirectional action. HS are thought to regulate nutrient assimilation by (1) stimulating soil microbial activity (HS are a carbon source for microorganisms and stimulate the proliferation of PGPB – (plant growth promoting bacteria) Maji et al. (2017, Olivares et al. (2017), (2) regulating the expression of genes encoding the main nutrient transporters in roots (Tavares et al. 2016), and (3) increase the activity of enzymes involved in the use of these nutrients (e.g. nitrate reductase, aspartate aminotransferase, glutamine synthetase, glutamate synthase) – de Moura et al. (2023). This is confirmed by a study by Ertani et al. (2011), in which the application of HAs (1 mg C dm^{-3}) increased the activity of glutamine synthetase (GS) in maize roots (by 65%) and leaves (by 93%) and glutamate synthase (GOGAT) in roots (by 176%) and leaves (by 204%) compared to a parallel control series. Both enzymes play an important role in N assimilation.

In an experiment conducted by Çelik et al. (2010), soil application of HS (1 and 2 g kg^{-1}) had a positive effect on dry matter yield and macronutrient uptake of maize grown under stress conditions (calcareous and saline soil). The researchers showed the highest uptake of N (227.06 g pot^{-1}), as well as P (29.61 g pot^{-1}), Mg (46.19 g pot^{-1}) and Na (5.14 g pot^{-1}) in the series with the lower addition of HS. In contrast, a significant effect was observed for K (304.43 g pot^{-1}) and Ca (59.38 g pot^{-1}) at the sites with the highest applied HS dose. According to the authors, when humus was added to the soil, the chelating sites present in the soil became saturated with macronutrients, allowing plant roots to take up the cations thus adsorbed by cation exchange. Osman et al. (2013) evaluated the effects of two commercial N fertilisers (U, anhydrous ammonia; 200 kg ha^{-1}) and foliar application of humic and/or fulvic acid (5 g dm^{-3}) on the yield and macronutrient content of rice straw and grain. The authors showed that anhydrous ammonia together with foliar application of humic and fulvic acids significantly improved rice productivity and quality as well as post-harvest soil N content. This fertilisation treatment also resulted in increased N, P and K contents in rice straw and grain compared to the control series (without their addition). Othman et al. (2020) also showed a positive effect of HS on the macronutrient content of wheat grain and straw. After the application of humic acid potassium salt (6% solution for foliar application), the authors recorded an increase in the content of N (by 50%), P (by 19%) and K (by 100%) in grain. The content of these elements also increased in the straw of the tested plant. The greatest changes were observed for P, which increased by 24% compared to the control. HS, by increasing total and residual N in the soil (Yang et al. 2007), accelerate its uptake and use by plants. In turn, their high content in aerial biomass promotes P uptake from the soil by influencing tap-root growth and plant metabolism (Duncan et al. 2018, Grohskopf et al. 2019) and by increasing the solubility and availability of P as a result of lowering soil pH following NH_4^+ uptake (Fageria et al. 2001). In addition, HS increase the permeability of cell membranes, resulting in increased assimilation and accumulation of nutrients (Tahir et al. 2011). This may explain the concentration of P, Mg, Ca and K in the maize biomass found in the present experiment. In our study HAs had the strongest effect on UAN-fertilised sand soil increased in Mg, P, Ca and K of maize biomass.

Crop management is increasingly focused on reduction of production costs by reducing inputs (Bulgari et al. 2015). One way of doing this may be through the use of biostimulants, which aim to regulate and accelerate plant growth and development and increase yield levels, by changing the chemical composition of plants (Paradiković et al. 2011). The use of HS as biostimulants corresponds to the goals of sustainable agriculture, as their use not only increases the efficiency of nutrient use by stimulating plant growth, but also enables the reduction of the use of mineral fertilisers (Ziosi et al. 2013). As a result, it is possible to achieve yields similar to or higher than by using conventional fertilisation, without having a negative impact on the environment. Alternative crop feeding strategies are also gaining interest because of the depletion of natural resources, the fear of biodiversity loss and the need to ensure food security. For these reasons, research on this topic has a substantive and practical rationale. Further research (under field conditions) with other plant species and on heavier soils is necessary.

CONCLUSIONS

All the studied factors (soil type, form of N fertiliser and addition of HAs to the soil) influenced the the content of selected macroelements in maize and in plant biomass. The content of P and Mg in maize was lower on loamy sand than on sand, in contrast to biomass and other macroelements.

Nitrogen fertilisation had a greater effect on the macroelement content of plants on sand. The highest macroelement content in maize grown on this soil was found on soils fertilised with UAN. Urea induced similar but smaller changes in the K, P and Na content of maize, but not in Mg and Ca. Urea also had the most favourable effect on plant biomass. Changes in the macroelement content of maize on loamy sand were smaller and often inconclusive.

HAs tended to increase the macroelement content of plants. However, their effect depended on the soil type and the form of N fertiliser. On the sand, HAs had the greatest effect on UAN-fertilised objects, causing an increase in Mg, P, Ca and K compared to the unfertilised object. On loamy sand, the changes in their content in maize were less clear. HAs caused an increase in P, K, Ca and Mg content in the series with U and Ca content in the pots with AN, compared to the object without their application. They also caused a decrease in P in the AN plots and in Na in the maize in the UAN plots. HAs can be a good source of essential elements for plants by supporting the effectiveness of mineral fertilisation.

Author contributions

M.S.B., M.W. – conceptualization and methodology, N.K. – analysis, M.W., N.K., M.S.B. – writing-review and editing, M.W., M.S.B. – supervision, M.W., M.S.B. – funding acquisition. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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