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EFFECT OF pH AND IONIC Al^{3+} and Cu^{2+} ON THE CONCENTRATION OF MAGNESIUM IN *TRIFOLIUM PRATENSE* L.

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Abstract. The objective of the work was to determine the content of magnesium in plants of *Trifolium pratense* L. which grew in an environment contaminated with aluminium or copper. The growth and development of the plants was carried out in hydroponics, with strictly controlled composition and pH of the growth medium. Copper was added to the medium (as an additional application) in a solution of $CuSO_4{\cdot}5H_2O$, and aluminium in the form of a solution of $AlCl_3$, at concentrations of Al^{+3} and Cu^{+2} of 20 and 100 mg dm^{-3}. The concentration of magnesium, aluminium and cooper was determined by means of a ICP sequential emission spectrometer D-820 Hilger Analytical (UK) in extracts from the roots and aboveground parts. Decreasing pH of the medium did not cause any statistically significant changes in the concentration of the metals studied. There was a significant increase in the concentration of aluminium and copper in roots and aboveground parts of *Trifolium pratense* L. and lower magnesium concentration for plants that were grown in an environment contaminated with Al^{+3}/ Cu^{+2} at a concentration of 100 mg dm^{-3}. In the lower concentrations of both stressors (20 mg dm^{-3}) a decrease was noted in the level of magnesium in the whole plant, and a statistically significant increase in the concentration of aluminium and copper in the roots of *Trifolium pretense* L. In the aboveground parts of the plants that grew in medium supplemented with 20 mg dm^{-3} of Al^{+3}/Cu^{+2}, the increase of the concentration of these metals was not statistically significant.

Keywords: aluminium, copper, magnesium, *Trifolium pratense* L.

INTRODUCTION

Magnesium (a macroelement) is an essential alkali metal for plants, used by the plants in the synthesis of nucleic acids and proteins. This metal also plays a key role in a proper functioning of the various metabolic pathways. The role of magnesium in the plant is primarily the activation of over 300 enzymes, including: RNA polymerase, ATPases, protein kinases, phosphatases, glutathione synthesis and carboxylase (Shaul 2002, Swaminathan 2003). In addition, magnesium ion occupies a central place in the porphyrin system of chlorophyll and has the ability of aggregating its particles. Thus magnesium has a large influence on the course of the two phases of photosynthesis. Magnesium is a macro mineral that plays an active role in maintaining a healthy balance of the ion in the plant (Mercik *et al*. 1984, Shaul 2002). Therefore, an inadequate supply of magnesium in the roots impairs a number of physiological and metabolic processes of the plant. The consequence of this is a quantitative and qualitative deterioration of yields (McRitchie, 1992). According to Grześkowiak (2006), a soil that is poor in magnesium is particularly bad for crops. This especially relates to the species of *Trifolium pratense* L. with which the study reported in this publication is concerned. Plants absorb magnesium from the soil solution throughout the ontogeny, therefore the concentration of available magnesium in the soil is a very important factor for normal development of plants. Magnesium deficiency occurs most often in light soils, overly acidic. Most of the soils in Poland (about 60%) show the pH up to 5.5, i.e. belong to very acid (pH up to 4.5) or acid (pH 4.6-5.5) soil categories (Filipek *et al.* 2006). The problem of availability of magnesium for plants in such soils is closely related to the concentration of aluminium. At pH <5 aluminium present in the soil undergoes dissociation to a greater degree, which increases the absorption of the metal through the root system. This process also reduces the uptake of magnesium and lowers its content in plants (Ericsson et al. 1998). Acidic reaction of soil is also a factor influencing the solubility and phyto- and bioavailability of most heavy metals, including copper which, under optimal conditions, is taken up by plants as a micronutrient (Hlavay et al. 2004). Excessive amount of mobile (easily available for plants) ion of both copper and aluminium in the environment (soil solution or medium) of root growth results in an increased uptake of these ions by the roots of plants. This leads to a deficit of other elements necessary for the proper functioning of the plant, including magnesium.

The aim of this study was to determine whether and how low pH and high concentrations of aluminium (redundant element) and copper (microelement) influence the uptake and transport of magnesium ions in the plant *Trifolium pratense* L.

MATERIALS AND METHODS

The experiments were conducted on roots and aboveground parts of meadow clover (*Trifolium pratense L.*) cv. Jubilatka. Plant seeds were sown in aerated polyethylene containers fitted with Styrofoam inserts with pre-drilled holes in the underside of the pad to secure the mesh. Filter paper was provided between the insert and the mesh. There were 1 g *Trifolium pretense* L. seeded in each container (5 dm^3). After germination, 60 plants were left and the filter paper was removed.

The growth and development of the plants was carried out in hydroponics, with strictly controlled mineral composition and pH of the medium. The medium was prepared according to a modified Hoagland medium supplemented with a complete set of micronutrients (Starck 2012). The level of the medium was controlled every 24 hours and, if necessary, supplemented with distilled water; the medium was entirely replaced every 7 days. The growth and development of the plants was performed in a daily cycle of 16/8 hours (day/ night) and 296 K room temperature in the daytime and at 289 K overnight. Sodium lamps WLS 400 were used (light intensity = 200 Lx). In the initial phase of growth and development, plants grew in a medium at pH = 7. Differentiation of pH and the addition of aluminium and copper were applied for four weeks after germination. The incubation period under conditions of stress was 14 days. The pH of the medium was maintained at pH = 7 ± 0.2 for the control material and pH = 4.5 ± 0.2 for the stress caused by the low pH and the presence of copper ions and aluminium. The medium was adjusted with 0.1mol·dm^{-3} KOH solution and 0.1 mol·dm^{-3} HCl solution. The experiment was repeated three times. Copper was added to the medium in a solution of $CuSO_4 \cdot 5H_2O$ and aluminium in the form of a solution of $AlCl_3$ in a concentration of Cu^{+2} and Al^{+3} 20 and 100 mg·dm^{-3}. In order to wash the collected plant roots of interchangeably adsorbed ions, they were rinsed with 0.01 mol·dm^{-3} HCl and three times with distilled water and then separated from the aboveground parts of the plants. The plant material was dried at 378 K for 24 hours, and then dry-mineralised (Szatanik-Kloc *et al*. 2010). The concentrations of magnesium, aluminium and cooper in extracts from the roots and aboveground parts were determined using an ICP sequential emission spectrometer D-820 Hilger Analytical (UK). Statistica version 10.0 was used for statistical analysis.

RESULTS AND DISCUSSION

The concentration of aluminium and magnesium in the extracts of the roots and aboveground parts of *Trifolium pratense* L. is presented in Table 1.

An increase of aluminium concentration in the medium increased the metal content in the roots and aboveground parts of the test plants. Plants accumulate aluminium primarily in the roots, and the process of transportation of aluminium to the aboveground parts occurs in a small degree. Also in the case of the clover under study, the root was also the “first accumulator” of toxic forms of aluminium. In the aboveground parts of *Trifolium pratense* L. which grew in medium supplemented with 20 mg·dm^{-3} of aluminium, about 1000-fold lower content of the metal was recorded as compared with the roots. Increase in the content of Al^{3+} in the aboveground parts of plants which were stressed with aluminium at 20 mg dm^{-3} (in relation to that of the control plants) was not statistically significant. According to

Ma and Hiradate (2000), one of the factors that may determine the low degree of penetration of aluminium into the aboveground parts is the high affinity of the metal ion to O_2 donors (such as carboxyl groups of polygalacturonic acid and polysaccharides) that immobilise aluminium in the apoplast of roots. Very high concentrations of aluminium in the substrate (such as used in the experiment aluminium concentration of 100 mg dm^{-3}) may result in a statistically significant increase in the content of the metal in the aboveground parts, which was also observed in the tested plants of *Trifolium pratens*e L. As a result, aluminium stress can lead to an interference with the apoplast transport (short), which in turn (in some cases) can reduce the penetration of aluminium into the aboveground parts, but it also may lead to a deficiency of other micro- and macroelements, including magnesium. In the experiment, both the presence of aluminium ions and the reduction of the pH of the medium in which the plants grew resulted in a reduced content of magnesium in *Trifolium pratense* L. Statistically significant differences in the content of magnesium were observed in the roots and aboveground parts of the plants that grew in medium supplemented with all aluminium doses applied in the experiment. The lowering of pH of the medium did not affect significantly the content of aluminium and magnesium in the test *Trifolium pratense* L. In an environment with low pH, aluminium is present in the soil at a greater degree of dissociation, as it increases the absorption of the plant root system. Under the conditions used in the experiment, the medium was equivalent to a soil solution with Al^{3+} ions added in a form easily available for plants, because the content of the element in the analysed plant was determined by the concentration of aluminium in the solution.

Table 1. Magnesium and aluminium ($g{\cdot}kg^{-1}$ dry mass.) concentration in roots and aboveground parts of *Trifolium pretense* L stressed with Al (average value from 3 replicates, ± standard deviation). F – value of the statistic F for analysis of variance. Values indicated in bold differ significantly ($p< 0.05$) from control (pH7) values in the object. Tukey's RIR test.

Variant	Roots		Aboveground parts	
	Mg^{2+} (g kg^{-1})	Al^{3+} (g kg^{-1})	Mg^{2+} (g kg^{-1})	Al^{3+} (g kg^{-1})
pH7	9.3 ± 0.25	0.002 ± 0.001	15.1 ± 0.25	0.0003 ± 0.0002
pH4.5	8.9± 0.10	0.002 ± 0.001	14,7 ± 0.49	0.0003 ± 0.0003
pH4.5 + 20Al*	**6.2 ± 0.3**	**50.4 ±0.7**	**11.6 ± 0.3**	0.0024 ± 0.0003
pH4,5 + 100Al*	**2.4 ± 0.1**	**58.5 ± 0.9**	**6.9 ± 0.03**	**1.27 ± 0.01**
F	716.5 p<0.0001	9329.5 p<0.0001	423,6 p<0.0001	48220 p<0.0001

*Number in front of the element symbol means Al^{3+} ions concentration in the nutrient solution (mg dm^{-3})

Limitation of uptake of e.g. magnesium ions is associated with antagonistic interaction between the aluminium and magnesium ions, or a change in biochemical reactions occurring in the cytoplasm. According to Starck (2012), 5-10% of magnesium is in the cell walls, in conjunction with pectins. Aluminium limits cell wall saturation, therefore, the ability of plants to bind magnesium ions. In the second part of the experiment, the environment of *Trifolium pratense* L. growth was contaminated with copper. Copper, as already mentioned, is extracted by plants as a micronutrient (cation). In plants it activates many enzymes, and e.g. it is a component of catechol oxidase, ascorbate oxidase and plastocyanin (Buchanan *et al*. 2000, Szwejkowska 2000, Starck 2012). In addition, it plays an important role in the process of photosynthesis, respiration, metabolism of nitrogenous compounds and carbohydrates. Is also involved in the metabolism of cell membranes and regulates the synthesis of DNA and RNA (Drevensek *et al*. 2003, Misra and Draper 1998). In an environment contaminated with copper, the excess of the metal can reduce the uptake and transport of other micronutrients and macronutrients such as magnesium (Sheldon and Menzies 2005). Copper and magnesium concentrations in the extract from roots and aboveground parts of *Trifolium pratense* L. are shown in Table 2. It was noted that the copper concentration in the roots and aboveground parts of *Trifolium pratense* L. increased in direct proportion to the heavy metal content in growth medium. It was also observed that higher copper content in the roots and aboveground parts was accompanied by a correspondingly smaller magnesium content. Transport of magnesium ions between roots and the aboveground part of *Trifolium pratense* L. suggests that the uptake and upward transport of magnesium may be changed by excess copper levels.

Table 2. Magnesium and copper concentration in roots and aboveground parts of clover stressed with Cu (average value from 3 replicates,± standard deviation).
F – value of statistic F for analysis of variance. The values indicated in bold differ significantly ($p < 0.05$) from control (pH7) values in the object. Tukey's RIR test.

Variant	Roots		Aboveground parts	
	Mg^{2+}(g kg^{-1})	Cu^{2+}(g kg^{-1})	Mg^{2+}(g kg^{-1})	Cu^{2+}(g kg^{-1})
Controls – pH7	9.3 ± 0.25	0.002 ± 0.001	15.1 ± 0.25	0.0003 ± 0.0002
pH4.5	8.9± 0.10	0.002 ± 0.001	14,7 ± 0.49	0.0003 ± 0.0003
pH4.5 + 20Cu*	**6.2 ± 0.3**	**50.4 ±0.7**	**11.6 ± 0.3**	0.0024 ± 0.0003
pH4.5 + 100Cu*	**2.4 ± 0.1**	**58.5 ± 0.9**	**6.9 ± 0.03**	**1.27 ± 0.01**
F	716.5 p<0.0001	9329.5 p<0.0001	423,6 p<0.0001	48220 p<0.0001

*Number in front of the element symbol means Cu^{2+} ions concentration in the nutrient solution (mg dm^{-3}).

In plants that grew at pH 4.5 (without an additional application of Cu^{2+}) there were no significant changes in the concentration of copper and magnesium, in comparison with the plants in the control objects - pH7. Analysis of variance revealed significant changes in the concentration of magnesium and copper in the roots and aboveground parts of *Trifolium pratense* L. compared to plants originating from the control objects. The post-hoc test – Tukey's RIR test (analysing real significant differences in average) showed that both concentrations of copper (20 and 100 mg dm^{-3}) applied in the experiment significantly altered the magnesium and copper content in the roots and aboveground parts of *Trifolium pratense* L. The only exception was the aboveground parts of plants growing in the medium with copper at a concentration of 20 mg·dm^{-3}, in which the increase of the heavy metal content was not statistically significant. According to Alaoui-Sosse et al. (2004), plants accumulate copper mainly in the roots, but in a strongly polluted environment elevated levels of the metal occur also in the aboveground parts. Based on the study reported herein, we can draw the following conclusions.

CONCLUSION

1. Lowering pH of the medium in which *Trifolium pratense* L. grew did not affect significantly the concentration of aluminium, copper and magnesium in the studied plants.
2. The addition of aluminium and copper to the medium caused an increase in the concentration of these metals in the roots and aboveground parts of the test plants of *Trifolium pratense* L. Only in the aboveground parts of the plants that grew in medium supplemented with 20 mg dm^{-3} of Al^{+3}/Cu^{+2} the increase in the concentration of these metals was not statistically significant.
3. Both aluminium and copper ions resulted in lower magnesium concentration in the roots and aboveground parts of the plants studied.

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WPŁYW pH ORAZ JONÓW Al^{3+} i Cu^{2+} NA ZAWARTOŚĆ MAGNEZU W *TRIFOLIUM PRATENSE* L.

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Streszczenie. Celem pracy było określenie zawartości magnezu w roślinach *Trifolium pratense* L., które rosły w środowisku skażonym glinem lub miedzią. Wzrost i rozwój roślin prowadzono w hydroponice, przy ściśle kontrolowanym składzie i pH pożywki. Do pożywki dodano miedź, (jako dodatkową aplikację) w formie $CuSO_4 \cdot 5H_2O$ lub glin w formie $AlCl_3$ w stężeniach 20 i 100 mg·dm^{-3}. ·Zawartość glinu , magnezu i miedzi w ekstraktach z korzeni i części nadziemnych oznaczono Emisyjnym Sekwencyjnym Spektrometrem ICP D-820 Hilger Analytical. Obniżenie pH pożywki nie wpłynęło istotnie na zmiany zawartości badanych metali. W badanych roślinach *Trifolium pratense* L, które rosły w środowisku skażonym Al^{+3}/Cu^{+2} w stężeniu 100 mg·dm^{-3} stwierdzono istotny wzrost zawartości glinu i miedzi zarówno w korzeniach, jak i częściach nadziemnych oraz obniżenie zawartości magnezu. Przy niższym stężeniu obu stresorów (20 mg·dm^{-3}), odnotowano spadek zawartości magnezu w całej roślinie i istotne zwiększenie zawartości glinu i miedzi w korzeniach *Trifolium pratense* L. W częściach nadziemnych roślin, które rosły w pożywce dodatkiem 20 mg·dm^{-3} jonów Al^{+3}/Cu^{+2} , wzrost zawartości tych metali nie był statystycznie istotny.

S ł o w a k l u c z o w e : glin, miedź, magnez, *Trifolium pratense* L.