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MACROELEMENTS STATUS IN LETTUCE AFFECTED BY DIFFERENT FORMS OF PHOSPHORUS FERTILIZATION

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Abstract

Lettuce (Lactuca sativa L.) is annual vegetable,belonging to the family of Asteraceae. Soils in Croatia are generally poor in phosphorus, so phosphorus fertilization is of great significance. The aim of this research was to determine the effect of fertilization by different phosphorus fertilizers on the status of macroelements in lettuce. The field fertilization experiment was set up with lettuce according to the Latin square method with three fertilization treatments: T1 (control, without fertilization), T2 (phosphorus in polyphosphate form) and T3 (phosphorus in orthophosphate form). Nitrogen was determined by the Modified Kjeldahl method, phosphorus spectrophotometricaly, potassium flamephotometricaly, calcium, and magnesium atomic absorption spectrometricaly. The highest content in 100 g of fresh lettuce of phosphorus (28.24 mg P/100 g) and magnesium (48.54 mg Mg/100 g) were determined in the T1 treatment, while the highest content in 100 g of fresh lettuce of nitrogen (204.34 mg N/100 g), potassium (189.63 mg K/100 g) and calcium (122.85 mg Ca/100 g) were determined in the T3 treatment.

Key words: *Lactuca sativa L., magnesium, minerals, nitrogen, potassium.*

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is annual herbaceous vegetable belonging to the Asteraceae family. It originates from West Asia and East Africa; for instance in Egypt it has been used in the diet for 2500 years (Lešić et al., 2016).

Lettuce is a vegetable crop that has high nutritional and health value and is best consumed fresh. Fresh lettuce leaves contain about 91-96% water, 0.8-2.3% protein, 0.1-0.4% fat, 0.1% sugar, 0.54-1.5%fiber, 1.4% mineral substances. Leaves are also rich in vitamins C, B1, B2 and carotene, as well as in fiber. Energy rate (fat, protein and carbohydrates) is low, which is why nutritionists have recommended it as a staple meal for anyone who has to take care of being overweight (Matotan, 2004; Parađiković, 2009; Lešić et al., 2016).

During the growing season for quality growth and development of lettuce, all the biogenic macronutrients, that the plant uptake through the roots from the soil solution, are needed. That is why it is important that biogenic macronutrients are in an available or physiologically active form for the plant in optimal concentrations.

Nitrogen is an integral part of proteins, participates in the formation of amino groups in amino acids, participates in the structure of nucleotides where it is located in purine and pyrimidine bases, and plays an important role in the human body too. It is essential in the biochemistry of many non-protein components such as coenzymes, photosynthetic pigments, secondary metabolites and polyamines. Nitrogen is an integral part of the enzyme nitrate reductase and nitrite reductase, whose main function is the conversion of the nitrate form of nitrogen to ammonia (Maathius, 2009).

The plant uptakes nitrogen in its mineral forms as NO_3^- and NH_4^+ ions (Marschner, 1995). The ammonia ion can bind to soil colloids while the nitrate form has great mobility in the soil and can be leached, as it cannot be bind to soil colloids due to its negative charge. The plant will uptakesoil nitrogen that is not immobilized (Haynes, 1986). Plants growing on acidic soils and soils of poorer mechanical composition uptake better the NH_4^+ ion, compared to plants that grow on alkaline soils and soils of suitable water-air relationships that better uptake NO_3^- ion (Maathius, 2009).

Nitrogen content in lettuce leaves, according to literature data range from 1.13 to 5.02 % N in dry weight (Table 1).

Table 1. Nitrogen content in lettuce leaves (% N in dry weight)

Source	% N in dry weight
Tosic et al. (2019)	3.69-5.02
Wojciechowska et al. (2019)	2.56-3.32
Yarsi et al. (2019)	2.45-3.43
Solaimanet al. (2019)	3.80-4.10
Zandvakili et al. (2019)	1.13-4.08
Broadley et al. (2003)	4.2

According to Landis and Sreenis (2009) phosphorus is a macronutrient that is of great importance for plant growth and development, as well as it is part of every cell of all living organisms.

In acidic soils, which occupy about 40% of the total arable land, phosphorus is bound to aluminum and iron into insoluble compounds and as such is unavailable to plants (Vance, 2001). In alkaline soils, which occupy about 25% of the total arable land, phosphorus is bound to calcium into low soluble calcium phosphates. Therefore, phosphorus deficiency is one of the major limiting factors for successful agricultural production (Lynch and Brown, 2001).

Plants uptake phosphorus in anionic forms as $H_2PO_4^-$ and HPO_4^{2-}, and their concentration depends on soil pH (Kastori, 1983). Phosphorus in plant has a function in storing and releasing energy by storing all the solar energy transformed by the photosynthesis process into the chemical bonds between the phosphorus atoms in the ATP molecule. It is an integral part of the phospholipids that is the cell membrane consist of (Landis and Sreenis, 2009).

According to different authors lettuce phosphorus content ranged from 21 to 68 mg P/100 g of fresh weight (Table 2).

According to Landis (2009) potassium is uptaken by the plant root as the K^+ cation. Potassium is mobile in plants and its concentration decreases by the time. Potassium plays a key role in photosynthesis, phloem assimilate transport, nitrogen metabolism and storage processes of reserve compounds (Vukadinović & Vukadinović, 2011).

The potassium plant needs in large quantities. It plays an important role in the regulation of water in plants, effects the opening and closing of stomata, the synthesis of ATP and the activation of more than 60 enzymes, and therefore is also important for the synthesis of proteins, sugars and fats (Bergmann, 1992).

When potassium deficiency occurs, a decrease in stomata activity occurs too, which affects three important physiological processes: the inability to absorb CO_2 in photosynthesis, decrease of transpiration and turgor, and impedes the absorption of oxygen (Landis, 2009).

Potassium content in lettuce leaves ranged from 133 to 530 mg K/100 g fresh weight (Table 3).

Table 2. Phosphorus content in lettuce leaves (mg P/100 g fresh weight)

Source	mg P/100 g fresh weight
Mou (2009)	32
USDA (2018)	29
Botanical – online (2018)	23
Anilakumar et al. (2017)	30
Lešić et al. (2016)	21-68

Table 3. Potassium content in lettuce leaves (mg K/100 g fresh weight)

Source	mg K/100 g fresh weight
Mou (2009)	198
USDA (2018)	194
Botanical – online (2018)	257
Anilakumar et al. (2017)	247
Lešić et al. (2016)	133-530

Calcium is uptaken by the plant root in the Ca^{2+} ionic form (Vukadinović & Lončarić, 1998) and it has a significant importance in the process of respiration and photosynthesis (Maathius, 2009). Calcium plays a role in both increasing and decreasing the availability of other nutrients, for example increasing the availability of manganese in soil and reducing the availability of phosphorus (Maathius, 2009) due to soil pH or antagonism. It participates in the repair of soil structure by neutralizing soluble humic acids in insoluble calcium humates, which contributes to stable soil

structure (Znaor, 1996). It has a beneficial effect on the processes of ammonification, nitrifycation, biological nitrogen fixation and sulfur oxidation in soil (Vukadinović & Vukadinović, 2011).
Calcium has also an important role in plant growth. It participates in the composition of calcium pectinate, phytin salts, the composition of crystalline bodies, oxalates and calcite, and calcium phosphate buffer (Jug, 2016). It strengthens cell walls and stabilizes biomembranes, increases disease resistance and protects against toxins (Lešić et al., 2016).
Different authors quote calcium content in lettuce leaves from 13 to 60 mg Ca/100 g fresh weight (Table 4).

Table 4. Calcium content in lettuce leaves (mg Ca/100 g fresh weight)

Source	mg Ca/100 g fresh weight
Mou (2009)	40
USDA (2018)	36
Botanical - online (2018)	32
Anilakumar et al. (2017)	33
Lešić i sur. (2016)	13-60

Magnesium is an essential element that plants need for normal growth and development, and plays an important role in the plant's defense mechanism during abiotic stress. Plants uptake it in the Mg^{2+} ion form (Senbayram et al., 2015).
Magnesium affects the metabolism of carbohydrates, fats and proteins and activates a large number of enzymes (Vukadinović & Vukadinović, 2011). Magnesium is present in the chlorophyll molecule from 25 to 30%, while the remaining percentage is present in more mobile forms. Due to its good mobility in plants, magnesium can be easily translocated from the older parts of the plant and root to the younger parts, where it plays a role in the formation of the chlorophyll molecule (Senbayram et al., 2015). Therefore, the magnesium deficiency occurs in old leaves.
Magensium content in lettuce leaves ranged from 7 to 23 mg Mg/100 g fresh weight (Table 5).
Croatian soils are generally poor in phosphorus, so fertilization with phosphorus is of great importance. Fertilizers with phosphorus in the form of orthophosphates are most common used, while phosphorus in the form of polyphosphates are also available on the market.
Therefore, the aim of this research was to determine the effect of fertilization by different phosphorus fertilizers on the status of macroelements in lettuce.

Table 5. Magnesium content in lettuce leaves (mg Mg/100 g fresh weight)

Source	mg Mg/100 g fresh weight
Mou (2009)	7
USDA (2018)	13
Botanical - online (2018)	13
Anilakumar et al. (2017)	14
Lešić et al. (2016)	7-23

MATERIALS AND METHODS

Field fertilization experiment was set up in Velika Kosnica in Zagreb County (Croatia) with lettuce (*Lactuca sativa* L.), cultivar 'Aquarel' (Bejo). The experiment was set up using the Latin square method with three fertilization treatments: T1 - control, without fertilization; T2 - 500 kg/ha YaraMila Complex (55 kg/ha P_2O_5; Producer Yara, NPK 12-11-18 + 3MgO + 8S + B, S, Fe, Mn, Zn; phosphorus in form of polyphosphates); T3 - 370 kg/ha NPK 15-15-15 (55 kg/ha P_2O_5; Producer Petrokemija; phosphorus in form of orthophosphates).
Planting of lettuce seedlings was conducted on 18 June 2018. Rows spacing was 39 cm and 27 cm in rows. Standard agricultural technology was used, plowing was carried out in the spring. Application and incorporation of fertilizers was performed manually. Harvesting of lettuce was carried out at once on 6 August 2018 and average lettuce head samples were taken for chemical analysis.
Prior to setting up the fertilization experiment, a chemical soil analysis was performed (Table 6) which showed that the soil was alkaline, with poorly humus content, well supplied with nitrogen, poorly supplied with physiologically active phosphorus and rich in physiologically active potassium.
Average head lettuce samples were delivered to the Analytical Laboratory of the Department of Plant Nutrition, Faculty of Agriculture, University of Zagreb, where chemical analyses were performed. The lettuce leaves were cut into small pieces, dried at 105°C, after which

they were ground and homogenized. To determine the macronutrients content, the ground samples were digested with concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a microwave oven, after which phosphorus was determined spectrophotometrically, potassium by flame photometer, and calcium and magnesium by atomic absorption spectrometry AOAC (2015). Nitrogen was determined by the Adjusted Kjedahl Method (HRN ISO 11261: 2004). The dry matter was determined gravimetrically by drying to a constant mass.

Table 6. Soil chemical properties prior to field experiment set up

pH	H_2O	8.16
	nKCl	7.35
%	Humus	2.68
	N	0.17
AL-mg/100 g	P_2O_5	9.5
	K_2O	26
%	$CaCO_3$	3.8
	CaO	-

Statistical data analyses were performed using the SAS System for Win program ver 9.1 (SAS Institute Inc.)(SAS, 2002-2003). Analysis of variance (ANOVA) was performed following a Latin square experimental design. A Tukey's multiple comparison test (Tukey's HSD) was applied to identify differences among treatments.

RESULTS AND DISCUSSIONS

Dry matter content in lettuce leaves

Graph 1 shows the dry matter (DW) content in the analyzed lettuce leaves samples. No statistically significant differences were found in the dry matter content according to the fertilization treatments T1, T2 and T3, which ranged from 8.22 to 8.85%. The highest dry matter content was determined in the T3 treatment where phosphorus was applied in the form of orthophosphate.

According to Koudela and Petříková (2008) lettuce dry matter values range from 7 to 10%, and according to Anilakumaret al. (2017) this value is 5.4%. So, literature values are similar to the values obtained in this research.

Macroelemets content in dry weight of lettuce leaves

Nitrogen content in lettuce dry matter in the three fertilization treatments ranged from 2.29 to 2.38% N DW (Graph 2). The highest nitrogen content was determined in T2 treatment where phosphorus was applied in the form of polyphosphate (2.38% N DW), and the lowest nitrogen content was determined in T1 treatment without fertilization (2.29% N DW). Values obtained in this research are mostly lower compared to the literature data (Table 1), although Zandvakili et al. (2019) reported also even lower values that were obtained in this research.

Phosphorus content in lettuce dry matter in the three fertilization treatments ranged from 0.31 to 0.34% P DW (Graph 2). The highest phosphorus content was determined in T1 treatment where was no fertilization (0.34% P DW), while the lowest phosphorus content was determined in T2 and T3 treatment where phosphorus was applied in the form of polyphosphate and orthophosphate (0.31% P DW). Phosphorus data determined in this research is lower than the literature data, of 0.50% P DW (Broadley et al., 2003).

Potassium content in lettuce dry weight in the three fertilization treatments ranged from 2.15 to 2.24% K DW (Graph 2). The highest potassium content was determined in T1 and T2 treatment (2.24% K DW). The lowest potassium content was determined in T3 treatment where phosphorus was applied in the form of orthophosphate (2.15% K DW). The obtained values are almost twice lower than reported by Broadley et al. (2003) who reported 4.5% K DW in lettuce.

Calcium content in lettuce dry weight in fertilization treatments ranged from 1.35 to 1.42% Ca DW (Graph 2). The highest calcium content was found in T1 treatment where there was no fertilization and was 1.42% Ca DW, while the lowest was found in T2 treatment where phosphorus was applied in the form of polyphosphate (1.35% Ca DW). The average value of calcium in lettuce dry matter was 1.39% Ca DW, and is higher than reported in the literature, of 0.50% Ca DW (Vukadinović & Vukadinović, 2011).

Magnesium content in lettuce dry matter in all fertilization treatments ranged from 0.48 to 0.57% Mg DW (Graph 2).

The highest magnesium content was determined in the T1 treatment with no fertilization (0.57% Mg DW), and the lowest magnesium content was determined in the T2 treatment where phosphorus was applied in the form of polyphosphate (0.48% Mg DW).

The average magnesium content in lettuce dry matter was 0.52% Mg DW, and value obtained in this research is higher than reported,of 0.15 to 0.35% Mg DW (Vukadinović & Vukadinović, 2011).

Microelemets content in fresh weight of lettuce leaves

Nitrogen content in fresh weight (FW) of lettuce in the three fertilization treatments ranged from 192 to 204 mg N/100 g FW (Graph 3). The highest nitrogen content was determined in T3 treatment where phosphorus was applied in the form of orthophosphate (204 mg N/100 g FW).

The lowest nitrogen content was determined in T1 treatment where there no fertilization was applied (192 mg N/100 g FW).

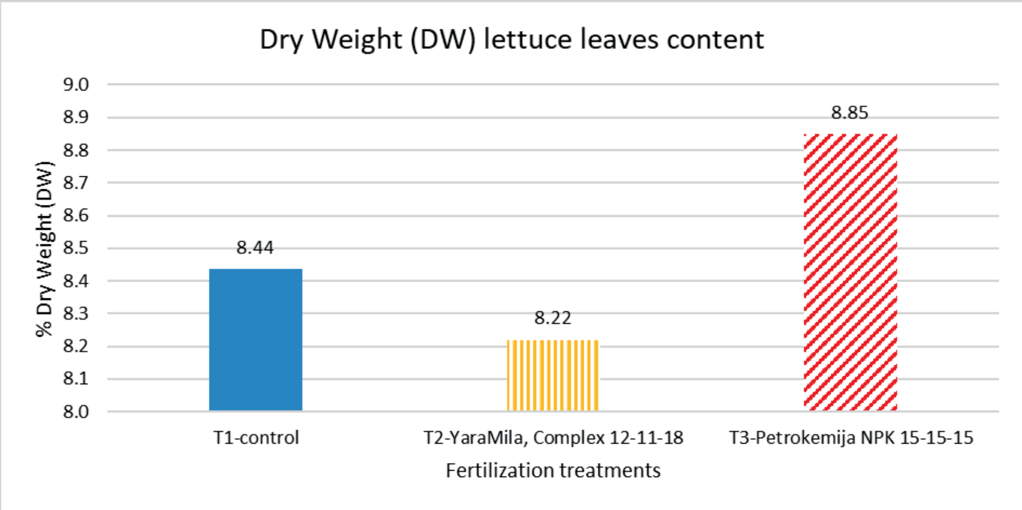
Phosphorus content in lettuce fresh weight regardless fertilization treatments ranged from 25 to 28 mg P/100 g FW (Graph 3).

The highest phosphorus content was determined in T1 treatment with no fertilization applied (28 mg P/100 g FW), while the lowest content was determined in T2 treatment where phosphorus was applied in the form of polyphosphate (25 mg P/100 g FW). The values obtained in this research are lower than the values from literature (29 and 30 mg P/100 g FW (USDA, 2018 and Anilakumar et al., 2017, respectively)).

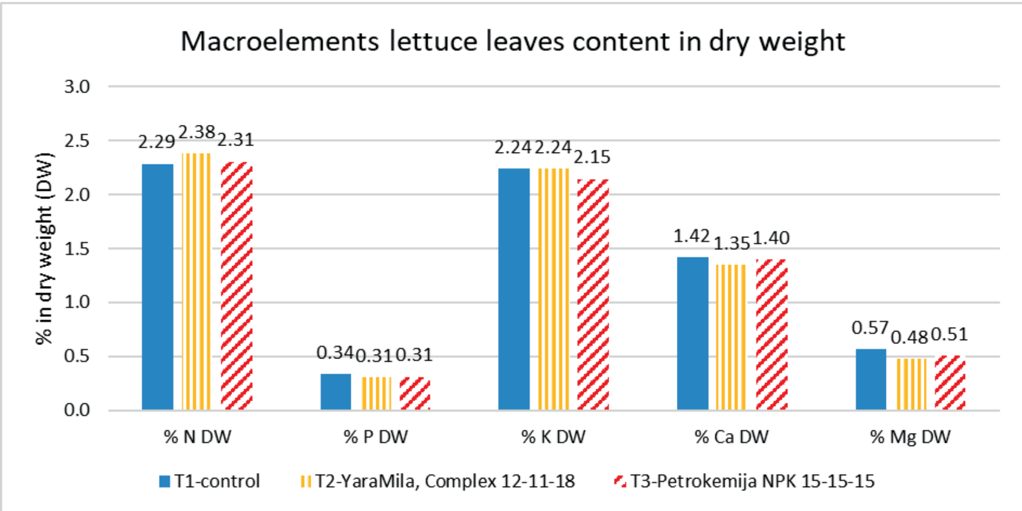
Potassium content in lettuce fresh weight in all fertilization treatments ranged from 184 to 190 mg K/100 g FW (Graph 3). The highest potassium content was determined in T3 treatment where phosphorus was applied in the form of orthophosphate (190 mg K/100 g FW), and the lowest content was found in T2 treatment where phosphorus was applied in the form of polyphosphate (184 mg K/100 g FW). The values obtained in this research are similar to those of Mou (2009) and USDA (2018) and lower than the values reported in Botanical – online (2018).

Calcium content in lettuce fresh weight regardless fertilization treatments ranged from 111 to 123 mg Ca/100 g FW (Graph 3). The highest calcium content was determined in T3 treatment where phosphorus was applied in the form of orthophosphate (123 mg Ca/100 g FW), and the lowest content was determined in T2 treatment where phosphorus was applied in the form of polyphosphate (111 mg Ca/100 g FW). The average value obtained in this research of 118 mg Ca/100 g FW is much higher than those reported in the literature (13 and 40 to mg Ca/100 g FW (Lešić et al., 2016 and Mou, 2009, respectively).

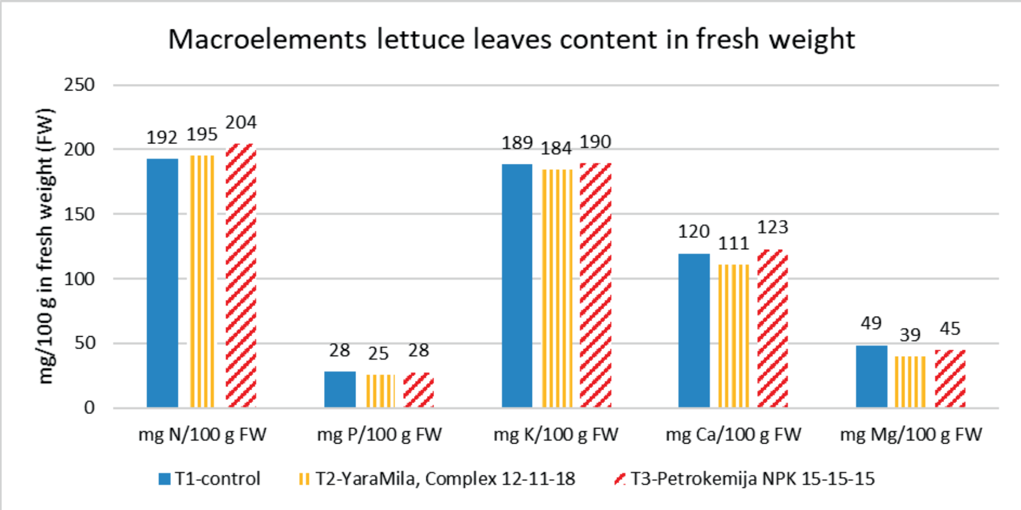
Magnesium content of fresh lettuce regardless fertilization treatments ranged from 39 to 49 mg Mg/100 g FW (Graph 3). The highest magnesium content was determined in the T1 treatment without fertilization (49 mg Mg/100 g FW), and the lowest content was determined in the T2 treatment where phosphorus was applied in the form of polyphosphate (39 mg Mg/100 g FW). The average value obtained in this research of 44 mg Mg/100 g FW are much higher than literature data (7-23 and 14 mg Mg/100 g FW (Lešić et al., 2016 and Anilakumar et al., 2017, respectively).



Graph 1. Dry Weight (DW) lettuce leaves content (% DW) according to different fertilization



Graph 2. Macroelements lettuce leaves content in dry weight(%in DW) according to different fertilization



Graph 3. Macroelements lettuce leaves content in fresh weight (mg/100 g in FW) according to different fertilization

CONCLUSIONS

This research has determined the status of macroelements in fertilized lettuce with different forms of phosphorus. The research was conducted in VelikaKosnica, Zagreb County (Croatia), using the Latin square method with three fertilization treatments.

The highest total dry matter (DM) content in lettuce leaves was determined in the T3 treatment (8.85% DM) where phosphorus was applied as orthophosphate.

Treatment T1 resulted with the highest content of minerals in dry weight (DW) of lettuce as follows: phosphorus 0.34% P DW, potassium 2.24% K DW, calcium 1.42% Ca DW and magnesium 0.57% Mg DW, while T2 treatment, where phosphorus in the form of polyphosphate was applied, resulted with the highest content of nitrogen (2.38% N DW).

In the fresh weight (FW) of lettuce in the T3 fertilization treatment, where phosphorus was applied in the form of orthophosphate, the highest content of nitrogen (204 mg N/100 g FW), potassium (190 mg K/100 g FW) and calcium (123 mg Ca/100 g FW) were determined, while in T1 treatment the highest content of phosphorus (28 mg/100 g FW) and magnesium (49 mg Mg/100 g FW) were determined.

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