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ORIGINAL PAPER

EFFECT OF DIFFERENTIATED IRON NUTRITION ON THE CONTENT OF MACRONUTRIENTS IN LEAVES OF LETTUCE (*LACTUCA SATIVA* L. VAR. *CAPITATA* L.) CULTIVATED IN PEAT SUBSTRATE*

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Abstract

Natural factors and human intervention, especially fertilization, change the ion dynamics in soil and substrates. Nutrients are often available in different chemical forms (mineral and organic), and as for iron, both forms, i.e. sulfates and chelates, are commonly used. The purpose of the study was to determine the influence of different sources and levels of iron, on the content of macronutrients in leaves of lettuce cultivated in peat substrate. The experiments were conducted in the spring and autumn season in a three-year cycle. They were set up under controlled, greenhouse conditions. Six sources of iron were applied (two sulfates and four chelated forms). The total content of N, P, K, Ca, Mg, S and Na was determined. The results were analyzed statistically with the Newman-Keuls test at a significance level of level $\alpha = 0.05$. The differentiated iron nutrition (both source and level) influenced the mineral composition of lettuce. In the spring cultivating season, mineral forms of iron in comparison to a chelate, caused significantly lower content of nitrogen and potassium. In the autumn cultivating season, after an application of mineral forms of iron compared to chelated ones, lettuce plants contained significantly more calcium and sulfur. To describe the relationship between the experimental factors, correlation and curvilinear regressions were determined. The iron nutrition significantly influenced the content of macronutrients in lettuce leaves. Negative correlation between the level of iron in the substrate and the content of phosphorus and calcium in the leaves was found. Positive correlation was found between the content of iron in the substrate and the content of sodium in the plants.

Keywords: fertilization, plant samples, iron compounds, chelates, sulfates, chemical composition.

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INTORDUCTION

The chemical composition of the plant depends primarily on the amount of available nutrients in the soil (Walker, Connolly 2008). Both macro- and micronutrients in the substrates and soil solution are affected by natural factors (pH, bicarbonate content, nutrient content and ballast content) and human activity (fertilization and pollution) (Titchenal, Dobbs 2007). Among these factors, one of the most important contributing element is fertilization (Bourn, Prescott 2002). Both the fertilization methods as well as growing season can influence macro- and micronutrient composition of lettuce (Kaplan et al. 2008, Masarirambi et al. 2010, Leogrande et al. 2013).

Iron is an essential micronutrient for the growth and development of plants. Despite of the presence of iron in the soil/substrate, symptoms of deficiency are often observed. Definition of iron bioavailability is described as the portion of total iron that can be easily assimilated by living organisms (Harmsen et al. 2005). This micronutrient remains in an unavailable forms for plants, especially in high pH environments, with excess of other ions (like K, Ca, Mg), bicarbonates or excessive humidity and low availability of oxygen. Mineral or organic fertilizers in the form of chelates are used to supplement iron deficiencies. So far, there is no clear evidence of the effect of individual sources of iron on the uptake of macronutrients from the substrate and their content in plants (Gunes et al. 1998, Hansen et al. 2004, Trujillo-Reyes et al. 2014, Satbhai et al. 2017).

The purpose of the study was to determine the influence of sulfate and chelated iron sources applied at different levels, on the content of macronutrients in lettuce leaves cultivated in peat substrate.

MATERIAL AND METHODS

The experiment with lettuce (*Lactuca sativa* L. var. *capitata* L.) cv. Sunny was carried out in a three-year cycle, in the spring and autumn season, under controlled conditions, in the greenhouse of the Department of Plant Nutrition, Poznan University of Life Sciences. The plants were grown in 6 dm^3 non-flow containers. The variant included 4 containers with 4 plants each (16 plants). Completely randomized, two-factorial experiments in 4 replications were set up with a separate control treatment (Table 1).

No fertilization with iron was applied in the control, and the content of the nutrient was 25 mg Fe dm^{-3} of substrate. High peat was used for cultivation, limed to pH_{H_2O} 6.5 and enriched with mineral nutrients to levels (mg dm^{-3}): N – 180, P – 140, K – 220, Zn – 20, Cu – 5, Mn – 20, B – 1, Mo – 1. The following chemically pure salts were used: $Ca(NO_3)_2$, $NH_4H_2PO_4$, KNO_3, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $MnSO_4 \cdot H_2O$, $Na_2B_4O_7 \cdot 10H_2O$, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$.

Table 1

Experimental factors

Source of Fe	mineral	$FeSO_4 \cdot 7H_2O$ – 20.07% Fe $Fe_2(SO_4)_3 \cdot nH_2O$ – 27.93% Fe
	chelate	FeDTPA – 9% Fe FeIDHA – 8.7% Fe FeEDDHMA – 6.5% FeEDTA + DTPA - 8% Fe
Level of Fe	substrate (mg dm^{-3})	50 75 100 150

After liming, the Ca and Mg content was sufficient, at Ca – 2045 mg dm^{-3}, Mg – 160 mg dm^{-3}, thus these nutrients were not supplemented. Iron was applied in the consecutive years according to the scheme of experiment (Table 1), in spring: on 16.04, 01.04, 06.04 and in autumn: on 10.09, 09.09, 10.09.

Lettuce seedlings in the two-leaf phase were planted in containers at around mid-April and mid-September. At the physiological maturity stage, 34, 35 and 29 days after planting seedlings in the spring seasons and 40, 38 and 41 days after planting seedlings in the autumn seasons, plants were harvested. Average samples were taken for chemical analysis, combining four halves of lettuce heads from each container. The plant material, after drying in an extractor dryer, was homogenized and mineralized.

The total content of N, P, K, Ca, Mg and Na was determined after mineralization in concentrated sulfuric acid (96%) and the content of S was assayed after mineralization in a mixture of nitric and perchloric acid in a volume ratio of 3 : 1. The analyses of N – Kjeldahl, P – colorimetric method, K, Ca, Mg, Na – by atomic absorption spectrometry (AAS), S – nephelometric method were performed.

The results were analyzed statistically by two-factorial analysis of variance. To determine the differences, the Newman-Keuls test was used at a significance level $\alpha = 0.05$.

The relationship between the level of iron in the substrate and the content of macronutrients in plants was determined. Correlation and curvilinear regression was determined.

RESULTS AND DISCUSSION

For spring experiments, the average nutrient content in lettuce leaves was shown in the range from 25 to 150 mg Fe dm^{-3}, and in the autumn it varied from 25 to 100 mg Fe dm^{-3} substrate. Especially in autumn, in the variant with the highest Fe levels in the form of chelates, plant decay was found.

The content of macronutrients in lettuce was dependent on the source and level of Fe in the substrate (Figures 1 - 4). Many authors report differences in the content of macronutrients in plants under different cultivation methods or fertilization (DZIDA, JAROSZ 2006, ALBORNOZ, LIETH 2015, KOWALSKA

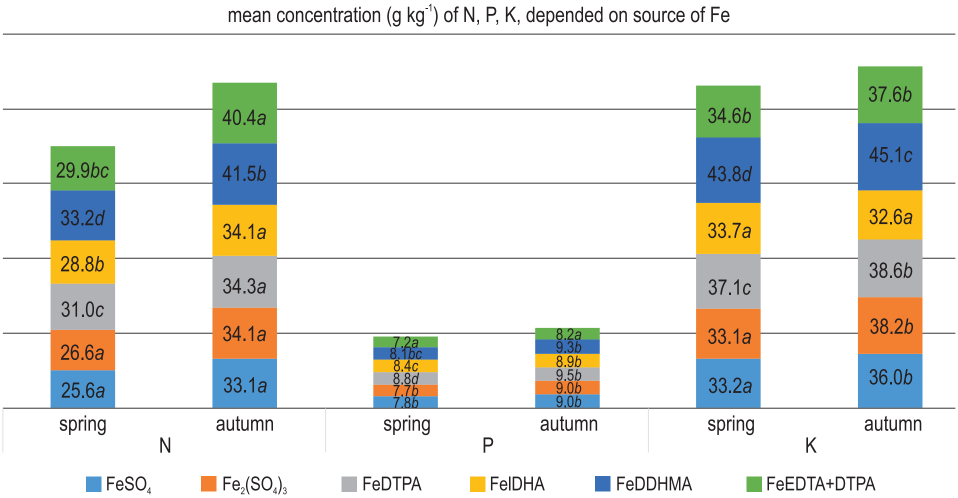

Fig. 1. The content of nitrogen, phosphorus and potassium in lettuce cv. Sunny depending on the source of iron in the substrate.
Means non-significantly different were marked with the same letters

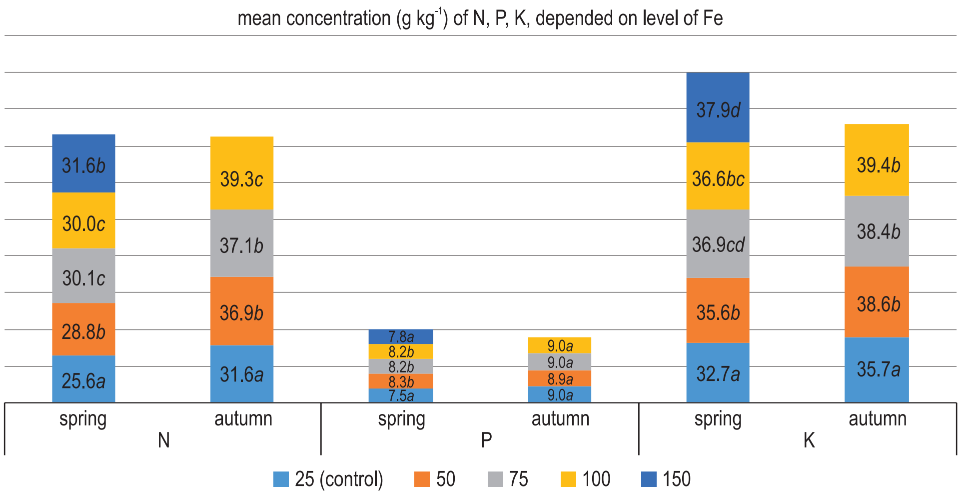

Fig. 2. The content of nitrogen, phosphorus and potassium in lettuce cv. Sunny depending on the level of iron in the substrate.
Means non-significantly different were marked with the same letters

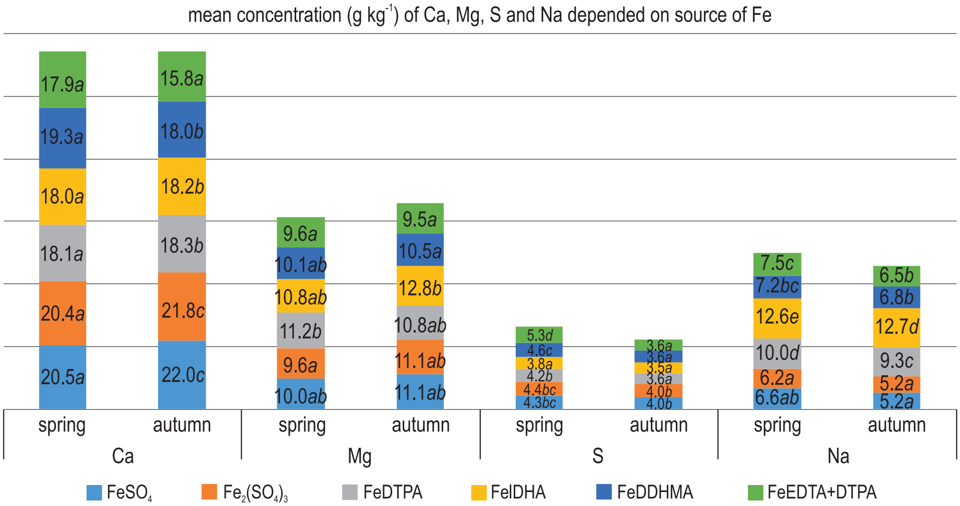

Fig. 3. The content of calcium, magnesium, sulfur and sodium in lettuce cv. Sunny depending on the source of iron in the substrate.
Means non-significantly different were marked with the same letters

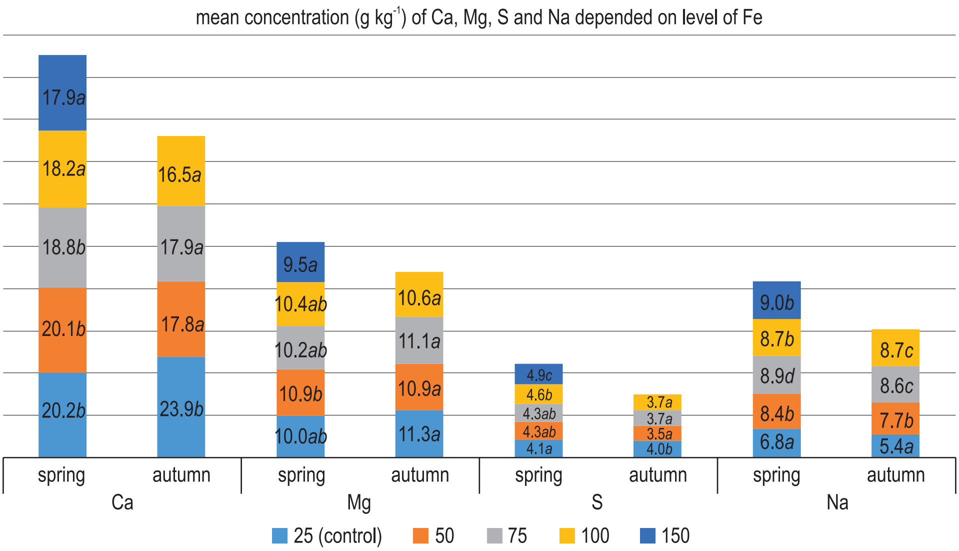

Fig. 4. The content of calcium, magnesium, sulfur and sodium in lettuce cv. Sunny depending on the level of iron in the substrate.
Means Average non-significantly different were marked with the same letters

et al. 2015, Sofo et al. 2016). In spring, the application of Fe in the mineral form decreased the total N content (Figure 1) in lettuce compared to the chelated form. There were no significant differences in autumn among the treatments, except Fe-EDDHMA with the highest content of nitrogen. Depending on the source of iron, the average N content in spring was from 25.6 g kg^{-1} to 33.2 g kg^{-1}, and in autumn it ranged from 33.1 g kg^{-1} to 41.5 g kg^{-1}. Increasing Fe level in the substrate caused an increase of the nitrogen content (Figure 2) in lettuce leaves – in spring from 25.6 g kg^{-1} to 31.6 g kg^{-1} and in autumn from 31.6 g kg^{-1} to 39.3 g kg^{-1}. Fallovo et al. (2009) found a similar relation in lettuce cultivated in soilless cultures, with the lower nitrogen content in the spring cultivation (46.2 g kg^{-1} N) and higher content in the consecutive growing season (48.0 g kg^{-1} N). Similarly, Kaplan et al. (2008) found higher nitrogen content in most treatments during autumn cultivation than in the spring. In this study, the nitrogen content in plants was generally lower than the levels considered as optimal by other authors. Adams et al. (1978), as cited in Windsor, Adams (1987), report the optimal values from 39.0 g kg^{-1} to 50.0 g kg^{-1} N. According to de Kreij et al. (1990), the optimum nitrogen content in lettuce cultivated in a greenhouse is 56.0 g kg^{-1}, and Mills, Jones (1996) maintain that it should be in the range of 42.0 g kg^{-1} to 56.0 g kg-1. Albornoz, Lieth (2015) in lettuce grown with different concentration of nutrients reported nitrogen content from 51.9 g kg^{-1} to 71.1 g kg^{-1}.

Many studies indicate a non-significant dependence of the phosphorus content on fertilization (except phosphorous fertilization) (Dzida, Jarosz 2006, Fallovo et al. 2009), although the significant proven range content is narrow (Hoque et al. 2010, Dzida at al. 2012, Kleiber 2014, Kowalska et al. 2015, Mzini, Winter 2015). White, Brown (2010) reported 2.0 g kg^{-1} - 5.0 g kg^{-1} d.m. of P as a sufficient concentration for non-tolerant crop plants and >10.0 g kg^{-1} d.m. P as a toxic amount. Similarly, Kim et al. (2016) found from 2.4 to 3.0 g kg^{-1} P in crisphead and romaine lettuces. The content of phosphorus (Figures 1, 2) in lettuce under the influence of mineral and chelated iron sources, both in spring and autumn, was similar. On average, in spring 7.8 g kg^{-1} of P was found with mineral sources of iron treatments and 8.1 g kg^{-1} was found with chelated sources of iron. In autumn, the average phosphorus content for mineral and chelated sources was identical: 9.0 g kg^{-1}. In spring, the increasing level of iron up to 50, 75 and 100 mg Fe mg^{-3} of the substrate, caused a significant increase in the phosphorus content in plants compared to the control treatment. The level of 150 mg Fe mg^{-3} of the substrate caused a slight decrease in the P content compared to the other levels. There was no significant difference between the control and the level of 150 mg Fe mg^{-3} of the substrate. In autumn, the level of iron did not affect the phosphorus content in lettuce. These values were similar to the ones obtained by other authors in lettuce experiments: 5.5 to 7.6 g kg^{-1} (Berry et al. 1981, as cited in Windsor, Adams 1987), 6.2 to 7.8 g kg^{-1} (de Kreij et al. 1990, Mills, Jones 1996). In lettuce cultivated in NFT cultures under the influence of FeHEDTA chelate or Fe^{2+} and ligninsulfonic acids or Fe^{3+} and ligninsulfonic acids, Demeyer et al. (2001*a*) found the phosphorus content in the range from 8.1 g kg^{-1} to 9.5 g kg^{-1}. Ylivainio et al. (2004) showed significant differences in the phosphorus content between control (4.3 g kg^{-1}) and FeEDDHA (5.6 g kg^{-1}) in lettuce cultivation in acidic sand. In lettuce cultivation in alkaline sand, the phosphorus content was not dependent on iron sources used in the experiment ($FeSO_4$, FeEDTA, FeEDDS, FeEDDHA). Demeyer et al. (2001*b*) found a similar phosphorus content in lettuce leaves grown in NFT with FeHEDTA chelate or with Fe^{2+} and polyphosphates mixture or with Fe^{3+} and polyphosphates mixture. Fallovo et al. (2009) reported a lower mean phosphorus content in lettuce cultivated in the soilless substrate, 5.5 g kg^{-1} in spring and 5.9 g kg^{-1} and summer cultivation, although the differences were not significant.

In tomato cultivation, Chohura et al. (2007, 2012) did not find any significant influence of the chelates (FeEDTA, FeEDTA + DTPA, FeEDTA + HEEDTA, FeDTPA) on the content of phosphorus in fresh fruits of tomato. This agrees with the results reported by Roosta et al. (2015) from an experiment with chelates and mineral sources of iron in lettuce. Avramaki et al. (2007) showed that increasing iron doses in the form of FeEDDHA chelate caused lower phosphorus uptake.

In spring, a higher potassium content was observed in plants grown with iron chelates than with iron sulfates (Figure 1). In both growing cycles, most potassium, similarly as nitrogen, was found in lettuce grown with FeEDDHMA chelate. The highest and lowest values of potassium in lettuce, depending on the source of iron, were found in spring: 33.1 g kg^{-1} and 43.8 g kg^{-1}, and in autumn: 32.6 g kg^{-1} and 45.1 g kg^{-1}. Regardless of the source of iron, the level of potassium in plants (Figure 2) was from 32.7 to 37.9 g kg^{-1} in spring and from 35.7 to 39.4 g kg^{-1} in autumn. Similar potassium content was observed by Ylivainio et al. (2004), who tested different sources of iron in lettuce grown in alkaline sand (iron sulfate II and different iron chelates), although the content of this element did not differ significantly. Hoque et al. (2010) reported similar values of K in lettuce under differentiated NPK fertilization. Chohura et al. (2012) did not show a significant effect of FeEDTA, FeEDTA + HEEDTA and FeDTPA chelates on the potassium content in fruits from tomato grown in peat substrate. Other authors indicated that the potassium content in leaves of lettuce was higher. According to de Kreij et al. (1990), an optimum potassium content in lettuce ranges from 78.2 g kg^{-1} to 136.8 g kg^{-1}. Mills, Jones (1996) claimed that the potassium content in lettuce grown in a field ranges from 66.0 g kg^{-1} to 90.0 g kg^{-1}. Demeyer et al. (2001*a*) reported that after the use of FeHEDTA chelate, the content of potassium was 90.0 - 95.0 g kg^{-1}. Fallovo et al. (2009) showed that lettuce grown in soilless substrate contained 58.6 g kg^{-1} K in the spring cultivation and 71.1 g kg^{-1} K in the summer cultivation. In this study, the effect was the same for a source of iron but not for its dose.

Mineral and chelated iron sources, in the spring and autumn cycles, had a similar effect on the calcium content in lettuce (Figure 3). More calcium was found in the variant with iron sulfates than in the variant with iron chelates, but these differences were statistically significant only for plants grown in autumn. Depending on the source of iron, the calcium content in the lettuce was: 17.9 - 20.5 g kg^{-1} in spring and 15.8 - 22.0 g kg^{-1} in autumn, while depending on the level of iron (Figure 4) it reached 17.9 - 20.2 g kg^{-1} in spring and 16.5 - 23.9 g kg^{-1} in autumn. These values are slightly larger than the ones considered optimal by DE KREIJ et al. (1990), i.e. from 8.0 to 12.0 g kg^{-1} Ca, or by HOQUE et al. (2010), i.e. 3.9 g kg^{-1} and 7.3 g kg^{-1} for romaine lettuce and 3.7 g kg^{-1} and 4.4 g kg^{-1} Ca for iceberg lettuce. WHITE, BROWN (2010) reported a range from 0.5 to 10.0 g kg^{-1} Ca as sufficient for plants. ASHKAR, RIES (1971), as cited in WINDSOR, ADAMS (1987), found that the calcium content in healthy lettuce was 16.0-30.0 g kg^{-1}. In lettuce cultivated in NFT after an application of FeHEDTA chelate, DEMEYER et al. (2001*b*) found 9.7 to 12.4 g kg^{-1} Ca. FALLOVO et al. (2009) reported an even lower content of Ca in lettuce 8.9 g kg^{-1} and 9.4 g kg^{-1} Ca, with no significant differences between cultivation seasons. CHOHURA et al. (2012) did not show a significant effect of chelates on the Ca content in tomato fruits.

Magnesium content in the leaves of lettuce, depending on the source of iron, ranged from 9.6 to 11.2 g kg^{-1} in spring and from 9.5 to 12.8 g kg^{-1} in autumn (Figure 3), and depending on the applied level it varied from 9.5 to 10.9 g kg^{-1} in spring and from 10.6 to 11.3 g kg^{-1} in autumn (Figure 4). However, DE KREIJ et al. (1990) and MILLS, JONES (1996) found that the optimum magnesium content in lettuce was lower and ranged from 2.4 to 7.3 g kg^{-1}. FALLOVO et al. (2009) indicated significant influence of the season of cultivation on the magnesium content of lettuce cultivated in spring and summer 4.8 g kg^{-1} and 5.5 g kg^{-1} Mg, respectively, but there was no significant interaction between the growing season and nutrient solution. CHOHURA et al. (2012) did not show a significant effect of chelates on the Mg content in tomato fruits. Similarly, MZINI, WINTER (2015) and KOWALSKA et al. 2015 did not report a significant effect of the tested treatments on the Mg content in lettuce and tomato.

An inconsistent impact of different sources and levels of iron on the sulfur content in lettuce has been demonstrated (Figures 3, 4). The sulfur content varied from 3.8 to 5.3 g kg^{-1} (depending on the iron source) and 4.1 - 4.9 g kg^{-1} S (depending on the iron level) in spring, and from 3.5 to 4.0 g kg^{-1} S and 3.5-4.0 g kg^{-1} S, respectively, in autumn. The content of sulfur in almost all leaf samples was greater than that determined as optimal for lettuce by DE KREIJ et al. (1990), i.e. less than 3.0 g kg^{-1} S, and by MILLS, JONES (1996), i.e. 2.6 to 3.2 g kg^{-1} S. According to WHITE, BROWN (2010), a sufficient concentration of sulfur is between 1.0 to 5.0 g kg^{-1}.

In spring and autumn, the highest amount of sodium was contained in lettuce grown in the substrate with FeIDHA chelate (Figure 3). This content was double the amount found in lettuce fed with mineral iron. A high content of sodium was also shown in lettuce grown in the substrate with FeDTPA chelate. Lettuce treated with iron sulfate II demonstrated the leaf content of sodium not significantly different from that obtained in the variant with FeEDDHMA chelate. CHOHURA et al. (2012) did show significant a effect of chelates FeEDTA, FeEDTA + HEEDTA and FeDTPA on the Na content in tomato fruits.

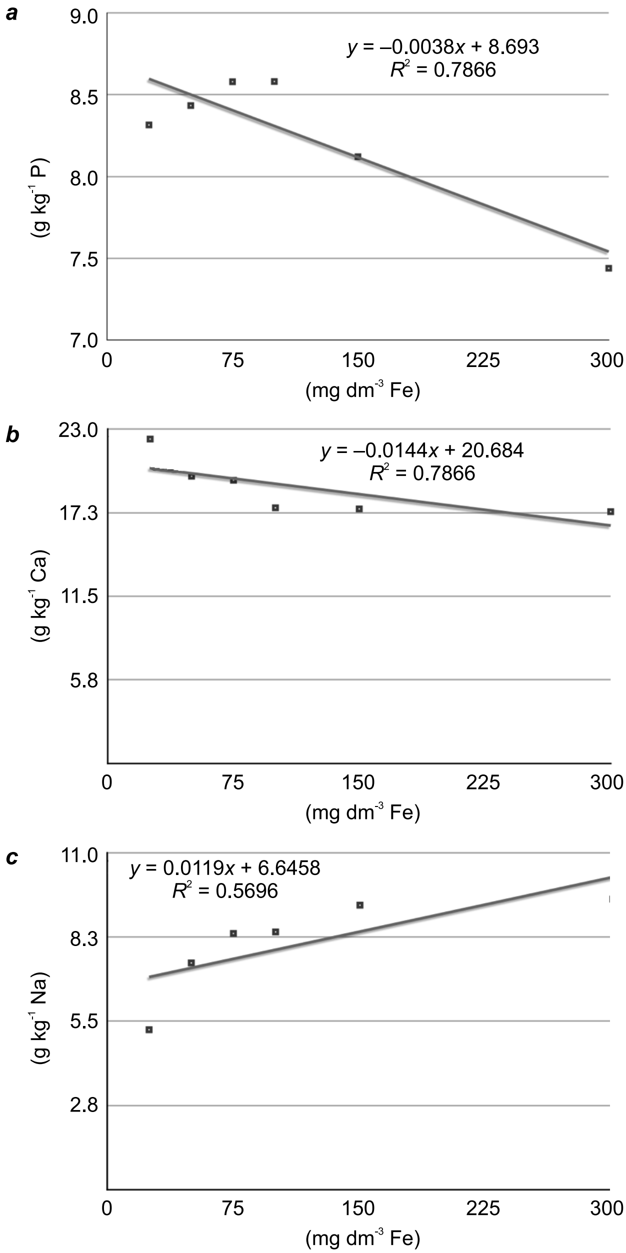

Fig. 5. Effect of the iron level on the phosphorus (*a*), calcium (*b*) and sodium (*c*) content in lettuce leaves

Regardless of the source of iron (Figure 4), at levels from 50 to 150 mg Fe dm^{-3}, the average sodium content in the plants was similar and significantly higher than that in the control plants. In autumn, the average content of sodium in lettuce depended on the form and level of applied iron. A higher sodium content was observed in the plants were iron was applied in the form of chelates compared to the mineral forms. With FeIDHA chelate, the content of sodium in lettuce was higher by 26% than that in lettuce supplied FeDTPA chelate and by about 46% than in lettuce nourished with FeEDTA + DTPA chelates. As the amount of iron in the substrate increased from 25 to 75 mg Fe dm^{-3}, the sodium content in the lettuce increased. In Kleiber's (2014) studies, the Na concentration in lettuce was unaffected by fertilization.

The analysis of dependence showed that the increase of iron levels in the substrate had no significant effect on the nitrogen, potassium, magnesium and sulfur content in plants. Statistically significant relationships were demonstrated between the iron level and the percentage content of phosphorus, calcium and sodium in the lettuce (Figure 5).

Significant negative correlation was found for phosphorus and calcium and a positive correlation for sodium. Increasing iron levels in the substrate resulted in a decrease in P and Ca content and an increase in Na content in the leaves of lettuce. The curvilinear regression equations describing these relationships explain respectively 78% variation for P, 53% for Ca, and 56% for Na. Tyksiński (1987), analyzed the interactions between iron doses and the content of soluble macronutrients in lettuce. The author stated antagonism between the growing iron content in the substrate and the phosphorus content in the lettuce and synergism between the growing iron doses in the substrate and the calcium and magnesium content in the plants. The differences in these relationships are probably due to other methods of macronutrient determination in the author's and own studies.

CONCLUSIONS

1. The differentiated iron nutrition significantly influenced the content of macronutrients in lettuce leaves.

2. In spring, when iron was applied in mineral forms, the plants contained significantly less nitrogen and potassium than after an application of iron chelates.

3. In the autumn crop cycle, plants fed with mineral iron compounds compared to chelated compounds contained significantly more calcium and sulfur.

4. Regardless of the source, increasing content of iron in the substrate caused an increase of the nitrogen and potassium content in the plants.

5. A significant negative correlation was found between the level of iron in the substrate and the content of phosphorus and calcium in the leaves of the lettuce.

6. A significant positive correlation was found between the content of iron in the substrate and the content of sodium in the plants.

7. The application of iron in the form of chelate FeEDDHMA caused a significant increase of the concentrations of nitrogen and potassium, whereas the chelated form of FeIDHA caused a significant increase of the concentration of sodium in lettuce leaves.

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