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The content of macroelements in white mulberry (*Morus alba* L.) leaves

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The aim of this study was to evaluate the agrochemical and biological quality indicators of soil and to determine the best harvest time to maximize the nutrient contents of white mulberry leaves (*Morus alba* L.) from two different cultivars. Mulberry leaves were analysed to determine the seasonal changes in the contents of Ca, K, P, Mg and S.

The study was carried out on 7-year-old mulberry trees belonging to *Morus alba* L. cultivated in an organic farm in the Kaunas District of Lithuania in 2016 and 2017. The soil agrochemical and biological indicators and mulberry leave samples were analysed two times during the tree vegetation period. The soil samples were collected in May and September (in the 2nd ten-day period) and leaves in June and September (in the 2nd ten-day period). The study revealed that a decreasing tendency for soil agrochemical indicators was observed throughout the entire period of growth of mulberry trees. At the end of the vegetation period the contents of nitrogen, available phosphorus and available potassium significantly decreased by 7.93, 24.85 and 25%, respectively, compared with that taken in May. At the end of the mulberry tree vegetation period the microbial biomass and dehydrogenase activity in the soil significantly decreased, by 20.39 and 17.95%, respectively, compared with that taken in the beginning of the vegetation period. The study revealed that harvest months had a statistically significant impact on the levels of the examined elements among the two *Morus* cultivars. It was found that the main macroelement in the leaves of both cultivars was Ca followed by K, P, Mg and S. However, the highest Ca, Mg, P and S contents in 'Turchanka' leaves increased with maturity. Importantly, the amount of macronutrients in the mulberry leaves varied in accordance with the cultivar and the harvest time.

These results can help farmers to select a cultivar and harvest time that would allow them to produce mulberry leaves with superior quality. Leaves collected from mulberry plants grown in Lithuania plantations may be used as a food supplement to help meet the recommended daily intakes of some macrominerals for adults.

Keywords: harvest time, macroelements, leaves, white mulberry

INTRODUCTION

In recent years, medicinal plants have been widely used in the treatment and prevention of diseases because they have a lower cost and fewer adverse effects in the body. Mulberry is a perennial, deep-rooted plant capable of thriving under diverse agro-climatic conditions. The white mulberry is the most cold-hardy of the three species *Morus alba* L., *M. rubra* L. and *M. nigra* L., although this varies from one clone to another (Ercisli, Orhan, 2007).

The mulberry plant is one of the conventional herbs that have been used in medicine since time immemorial due to its chemical composition and pharmacological functions. Almost all parts of the mulberry plant are used in Chinese and Indian medicine and in the food industry (Yang et al., 2010). However, the most important feature of the white mulberry is its high value in terms of healthfulness. White mulberry, whose leaves are rich in phenolic compounds and flavonoids (Thabti et al., 2012), contain a wide range of macronutrients and trace elements, amino acids (Butt, 2008). Mulberry-leaf tea has increased in popularity over the past decades because of its antidepressant, antibacterial, hypoglycaemic and antioxidant effects (Jeszka-Skowron et al., 2017). All of these properties of the white mulberry show that this plant may be used multi-directionally.

Furthermore, some authors (Kim et al., 2014; Levickiene et al., 2017) have studied the quality, nutritional potentials and chemical compositions of different *Morus* species. Mineral elements play important roles in biological reactions and have structural functions. The mulberry leaves contain a variety of nutrients, including macroelements (Radojković et al., 2014; Srivastava et al., 2006). As reported by many authors, the chemical contents of plant (mulberry) leaves are influenced by many factors, such as genetics, environment, climatic conditions, irrigation, fertilizing and soil conditions (Chetri et al., 1999). Moreover, mineral contents in leaves also change seasonally (Marschner, 1995).

The purpose of the present investigation was to evaluate the agrochemical and biological quality indicators of soil, as well, for the first time, the effect of the harvest time on mineral elements in the leaves of white mulberry plants grown in Lithuania. These results will be important in order to select cultivars with higher mineral contents and strengthen the value-added uses of the leaf in nutritional, industrial and pharmaceutical applications. Mulberry leaves can be used as natural colour and flavour enhancers to improve the composition, functionality and quality of food products.

RESEARCH METHODS AND CONDITIONS

The white mulberry cultivars 'Plodovaja 3' and 'Turchanka' originate from Ukraine. The field experiment was conducted on an organic farm in the Kaunas District of Lithuania between 2016 and 2017.

The experimental plots were arranged in a randomized design with four replicates, each with 6 trees. The distance between rows was 5 m and the distance between mulberry trees was 4 m. Trees were planted in 2010 and were not fertilized. Between the rows of mulberry trees white clover (*Trifolium repens* L.) was grown. The clover was cut and left on the soil as mulch. No diseases and pest control products were used in the experimental plots.

According to the data of the Kaunas Meteorological Station, the meteorological growing conditions in 2016 were favourable for mulberry vegetation because it was warm. In May compared to the Standard Climate Normal (SCN is the 30-year average from 1981 to 2010), the precipitation amount was 1.15 times more. September was dry and warm. The May of 2017 was favourable for the growth of mulberry trees. The average air temperature was very close to the SCN, the precipitation amount was by 7.2 mm more compared to SCN. September was warm and very wet, and the precipitation amount was 1.6 times more compared to SCN.

Of both cultivars, in each replication of the treatment, composite soil samples were taken two times (in May and in September in the 2nd ten-day period) from 5 places from the arable layer (0–20 cm depth) using an agrochemical auger. The samples were air-dried, crushed in a porcelain mortar and sieved with a 2 mm sieve. The soil samples were analysed for: the soil pH by a potentiometric method using a pH-meter in 1 N KCl extract (LST ISO 10390:2005), the total nitrogen % by the Kjeldal method, the available P_2O_5 concentration mg kg^{-1} by the CAL method using a spectrophotometer Beckman DU-40, the available K_2O concentration mg kg^{-1} by the CAL method using a flame photometer Corning PC-410, the soil activity of dehydrogenase after the M. A. Tabatabai (1982) and M. Järvan (2014) methods (ISO/FDIS 23753-2) and the microbial biomass by the chloroform fumigation-extraction method (ISO 14240-2:1997).

The leaves from each mulberry cultivar were manually collected representing the whole tree by collecting from four sides, under the same conditions once in June and September, in the 2nd ten-day period.

The laboratory sample was made up of 60–70 leaves from each tree. The leaves were frozen at −35°C, lyophilized using a freeze-drying plant sublimator 3 × 4 × 5 (ZIRBUS Technology GmbH, Bad Grund, Germany), ground into a fine powder for 1 min at 8000 rpm in a laboratory mill (Grindomix GM 200, Retsch GmbH, Haan, Germany) and stored in an airtight container until further use. The chemical analyses of the mulberry leaves were conducted in the Agrochemical Research Laboratory of the Lithuanian Research Centre for Agriculture and Forestry.

Powdered samples of the mulberry leaves were transformed into solutions and analysed. Potassium (K) and phosphorus (P) were measured photometrically after wet digestion in sulphuric acid. Calcium (Ca), magnesium (Mg) and sulphur (S) were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) after acid digestion in HNO_3:H_2O_2 (5:3, v/v) in a microwave reaching 200°C after 20 min. The solution was maintained at this temperature for 2 h. The procedures were performed according to the standard method (LST EN 15510:2017).

Chemical analyses were performed in triplicate. The experimental data were statistically analysed with ANOVA tests using the Statistica software version 7.0 (StatSoft, USA). Averages of the research data were calculated, and the Tukey's HSD test ($p < 0.05$) was applied to estimate the statistical significance of the differences between the data.

RESULTS AND DISCUSSION

Soil agrochemical and biological indicators

Soil fertility is determined by the optimal regime of the major nutrients such as nitrogen, phosphorus and potassium. When these elements are in short supply in the soil, mulberry performs and yields poorly.

The results averaged over the two experimental years of the white mulberry cultivars 'Plodovaja 3' and 'Turchanka' agrochemical and biological indicators of the soil are shown in Table 1. The data show that in the beginning of the mulberry trees vegetation period significantly higher contents of nitrogen, available phosphorus and available potassium in the soil were accumulated in May. At the end of the vegetation period the contents of nitrogen, available phosphorus and available potassium significantly decreased by 7.93, 24.85 and 25%, respectively, compared with that taken in May (Table 1). In our experiment, during all mulberry trees vegetation period pH_{KCl} was close to neutral acidity (6.41–6.81). From the mulberry trees vegetation beginning until the end of the season the contents of nutrients in the soil were decreasing because of the uptake by growing plants.

One of the most important and most accurate soil biological activity indicators are soil microbial biomass and soil enzyme activity. The increase in the biomass of microorganisms in the soil indicates that the capacity of microorganisms and the degradation of organic matter are higher. Also, this indicator is important for the assessment of organic matter deposits in the soil. Activity of dehydrogenase can show the characteristics of microbiological activity in the soil. The enzyme is active and reliably reflects the activity of active soil microorganisms**.** Our study showed that the amount of soil microbial biomass and dehydrogenase activity were significantly higher in the beginning of the mulberry tree vegetation (Table 1). A decreasing tendency for the soil microbial biomass and dehydrogenase activity has been observed throughout the entire period of growth of mulberry trees. The microbial biomass and dehydrogenase activity in the soil

Table 1. **The soil agrochemical and biological indicators, average 2016–2017**

Time of soil samples	Soil agrochemical and biological indicators					
	pH_{KCl}	Nitrogen, %	P_2O_5, mg kg^{-1}	K_2O_5, mg kg^{-1}	Soil microbial biomass C, µg C g^{-1}	Soil dehydrogenase, activity, µg g^{-1}
May	6.41^A	0.164^A	165^A	264^A	412^A	39^A
September	6.81^A	0.151^B	124^B	198^B	328^B	32^B

Different capital letters (A and B) in the same column represent significant differences between treatments, at $p < 0.05$.

significantly decreased, by 20.39 and 17.95%, respectively, compared with that taken in May.

Content of macroelements in white mulberry leaves

The imbalance of essential elements disturbs normal biochemical functions of the body, which may lead to several pathological conditions (Bordoloi et al., 2016). In this study, all of the macronutritive elements showed variation between the leaves of the two mulberry cultivars dependent upon the harvest time.

Mulberry is a good source of minerals and provides nutritionally useful amounts of most of them, including calcium (Ca), potassium (K), phosphorus (P), magnesium (Mg) and sulphur (S). Elements, such as Ca, K, P, Mg and S, in the leaves of the two different cultivars of white mulberry are depicted in Table 2.

Ca is an essential element responsible for bone strength and regulation of numerous functions in cells and tissues, such as muscle contraction, exocytosis and dental care prevention of colon cancer and reduction of obesity (Bronner, Pansu, 1998).

The results showed that the dominant macroelement in the leaves was Ca. According to the mean results of our experiment, the harvest time had a significant effect on the Ca content in mulberry leaves. The Ca content was found to be significantly higher in the leaves of both cultivars in September. In this context, it is worth mentioning that the Ca content in the young 'Turchanka' and 'Plodovaja 3' leaves decreased significantly by 59.4 and 47.5%, respectively, when harvested in June. The results are comparable to earlier reports. Noordulin et al. (2015) described that different mulberry genotypes and harvest time also influenced the calcium content. Other scientists have also confirmed the dependence of Ca levels on the characteristics of a cultivar (Radojković et al., 2014).

The increase of its content may be attributed to the deposition of Ca as calcium pectate in the middle lamella and the replacement of starch deposits by calcium oxalate crystals, which help sequester calcium in the leaves (Shear, Faust, 1980).

K is necessary for the formation of sugars, starches and carbohydrates and is important in protein synthesis and cell division in all parts of the plant. K is known as an activator of enzymes as well as a prominent factor in osmoregulation, which is important for the functions of enzymes and protein metabolism (Wayers, Paterson, 2001). It helps plants to adapt to unfavourable environmental conditions.

The results indicate that the K content in leaves from the two mulberry cultivars ranged from 1613 to 1860 mg 100 g^{-1} DM (Table 2). The significantly highest amount of K was found in the 'Plodovaja 3' leaves harvested in June. Imran et al. (2010) reported much lower amounts of this element in the *Morus alba*. In our investigation, the lowest content was determined in both cultivars harvested in September. A similar result was reported by Nooruldin et al. (2015), in which the K content in the leaves exhibited a decline with maturity. He found that the K content of mulberry leaves harvested from May to October was between 1306 and 1887 mg 100 g^{-1} DM. As reported by many authors, chemical contents of plant leaves were influenced by many factors such as genetics, environment, climatic conditions, irrigation, fertilizing and soil conditions (Asrey et al., 2007; Chetri et al., 1999). Moreover, mineral contents in leaves change seasonally (Marschner, 1995). If it is presumed that characteristics such as the soil,

Table 2. **The content of macroelements in white mulberry leaves, mg 100 g^{-1} DM, average 2016–2017**

Cultivar	Harvest time	Macroelements content				
		Ca	K	P	Mg	S
'Turchanka'	June	1121^{C}	1681^{B}	379^{C}	232^{C}	50^{C}
	September	2762^{A}	1675^{B}	693^{AB}	337^{A}	104^{A}
'Plodovaja 3'	June	1386^{B}	1860^{A}	637^{B}	216^{C}	73^{B}
	September	2639^{A}	1613^{C}	702^{A}	275^{B}	80^{B}

Different capital letters (A, B, and C) in the same column represent significant differences between treatments, at $p < 0.05$.

environment, climate factors and fertilizer application in the garden where all the trees were planted are homogenous, as reported earlier, these differences may be due to the genetic characteristics of the genotypes (Kazankaya et al., 2008).

P is necessary for photosynthesis, protein formation and almost all aspects of growth and metabolism in plants (Wayers, Paterson, 2001). Our findings show that the P contents in the leaves of the mulberry cultivars varied from 379 to 702 mg 100 g^{-1} DM (Table 2). A significantly lower P content (379 mg 100 g^{-1} DM) was found in the leaves of the 'Turchanka' harvested in June. The content of this mineral was significantly higher in 'Plodovaja 3' leaves harvested in September compared with those in June. However, the highest P content (693 mg 100 g^{-1} DM) in the 'Turchanka' leaves occurred in September. These differences between the cultivars could be associated with genetic differences. Nooruldin et al. (2015) reported that the P content decreased significantly with maturity (150 to 261 mg 100 g^{-1} DM), which is similar to the findings of Satpal et al. (2004), who reported a similar trend while working with the mulberry plant.

Mg is a critical structural component of the chlorophyll molecule and is necessary for the functioning of plant enzymes in the production of carbohydrates, sugars and fats (Weyers, Paterson, 2001).

Comparisons were also drawn between the results of the Mg content identified in the lyophilized mulberry leaf samples harvested over two months. The Mg content ranged from 232 to 337 mg 100 g^{-1} in 'Turchanka', while in 'Plodovaja 3' it ranged from 216 to 275 mg 100 g^{-1} (Table 2). The Mg content in 'Turchanka' and 'Plodovaja 3' significantly increased by 31.2% and 21.3%, respectively, with maturity, from June until September. Similar observations were reported by Nooruldin et al. (2015). They discovered that the Mg content significantly increased with maturity. According to Radojković et al. (2014), the first macroelement found in the extract of the *M. alba* and *M. nigra* leaves was Mg and the second was Ca. The increasing trend of Ca and Mg with the age of the leaf could be attributed to a limited mobility of these elements in the phloem (Leece, Gilmour, 1974).

S-containing amino acids, cystine, cysteine and methionine, are of great significance in the structure and function of proteins and enzymes. One of the most important S-containing peptides is glutathione, which has many important functions in plants and animals, including protection against oxidative stress (Malhotra, 1998).

Our findings show that the maximum S content was recorded in both cultivars during September. The amount of this element was significantly higher in 'Turchanka' leaves (104 mg 100 g^{-1} DM) compared with that of 'Plodovaja 3' leaves (80 mg 100 g^{-1} DM). Several authors found greater variations in levels of nutrients in different mulberry cultivars, indicating strong genetically controlled traits (Nooruldin et al., 2015; Satpal et al., 2004). We can declare that the S content in the leaves exhibited decline with maturity, whereas the significantly lowest (50 mg 100 g^{-1} DM) amount of S was detected in the young leaves from 'Turchanka'.

The research results of this study enable a purposeful selection of the desirable macroelements according to the mulberry leaf harvest time.

CONCLUSIONS

1. A decreasing tendency for soil agrochemical indicators has been observed throughout the entire period of growth of mulberry trees. At the end of the vegetation period the contents of nitrogen, available phosphorus and available potassium significantly decreased by 7.93, 24.85 and 25%, respectively, compared with that taken in May. At the end of the mulberry tree vegetation period the microbial biomass and dehydrogenase activity in the soil significantly decreased, by 20.39 and 17.95%, respectively, compared with that taken in the beginning of the vegetation period.

2. The contents of calcium and phosphorus in the leaves of both white mulberry cultivars 'Turchanka' and 'Plodovaja 3' increased in the course of the growing season. The highest contents of calcium and phosphorus in leaves of both cultivars were determined at the end of the experiment, i.e. in September.

3. The significantly highest amount of K (1860 mg 100 g^{-1} DM) was found in the 'Plodovaja 3' leaves harvested in June.

4. The significantly highest contents of magnesium (337 mg 100 g^{-1} DM) and sulphur (104 mg 100 g^{-1} DM) were determined in the leaves of

'Turchanka' at the end of the growing season, in September.

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MAKROELEMENTŲ KIEKIS BALTOJO ŠILKMEDŽIO (*MORUS ALBA* L.) LAPUOSE

Santrauka

Tyrimo tikslas – įvertinti dirvožemio agrocheminius ir biologinius kokybės rodiklius bei nustatyti baltojo šilkmedžio veislių 'Turchanka' ir 'Plodovaja 3' lapuose makroelementų sudėties įvairavimą vegetacijos metu.

Lauko eksperimentas vykdytas Kauno rajone esančiame ekologiniame šilkmedyne 2016–2017 metais. Dirvožemio agrocheminėms bei biologinėms savybėms nustatyti mėginiai buvo sudaryti gegužės ir rugsėjo mėn. (II-ąją dekadą), o baltojo šilkmedžio lapai makroelementų kiekybinei analizei buvo rinkti birželio ir rugsėjo mėn. (II-ąją dekadą). Eksperimento rezultatai parodė, kad dirvožemio agrocheminių rodiklių kiekio mažėjimo tendencija pastebėta per visą baltųjų šilkmedžių vegetacijos laikotarpį. Baigiantis vegetacijai, rugsėjo mėn., azoto, judriojo fosforo ir judriojo kalio kiekiai dirvožemyje, kuriame augo 'Turčianka' ir 'Plodovaja 3' veislės baltieji šilkmedžiai, sumažėjo atitinkamai 7,93; 24,85 ir 25 %, palyginti su vegetacijos pradžios (gegužės mėn.) dirvožemio tyrimų rezultatais. Dirvožemio, kuriame augo 'Turčianka' ir 'Plodovaja 3' veislės baltieji šilkmedžiai, mikroorganizmų biomasės sankaupos ir dehidrogenazės aktyvumas esmingai sumažėjo atitinkamai 20,39 ir 17,95 %, palyginti su vegetacijos laikotarpio pradžia. Tyrimų rezultatai parodė, kad, priklausomai nuo skynimo laiko, abiejų veislių lapuose visos vegetacijos metu dominavo makroelementas kalcis. Makroelementų (kalcio, fosforo, mangano, sieros) kiekiai šilkmedžių veislių 'Turčianka' ir 'Plodovaja 3' lapuose per visą vegetaciją didėjo. Didžiausi Ca, Mg, P ir S kiekiai nustatyti rugsėjo mėn. skintuose veislės 'Turčianka' lapuose. Patikimai didžiausias kalio kiekis (1860 mg 100 g^{-1} s. m.) nustatytas birželio mėn. skintuose 'Plodovaja 3' veislės lapuose.

Gauti rezultatai gali padėti ūkininkams pasirinkti vertingesnę šilkmedžių veislę ir tinkamą lapų skynimo laiką. Lapai, surinkti iš Lietuvoje auginamų šilkmedžio augalų plantacijų, gali būti naudojami kaip maisto papildas, taip pat kaip natūralūs spalvos, skonio stiprikliai maisto produktų sudėčiai, funkcionalumui ir kokybei pagerinti.

Raktažodžiai: baltasis šilkmedis, lapai, makroelementai, skynimo laikas