

THE CONTENT OF PHENOLIC GLYCOSIDES AND MACROELEMENTS (K, Ca, Mg) IN THE BARK OF HERBAL WILLOWS

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Abstract. The willows with high phenolic glycosides concentrations are used in herbal production. The knowledge about these metabolites are well documented, but less is known about macroelement concentration in the bark of herb willows. The aims of this study were to evaluate the content of phenolic glycosides and macroelements in the bark of *Salix alba*, *S. daphnoides* and *S. purpurea* originating from their natural habitats and cultivated on the two different soil types. The study was carried out in 2009 and 2010 on a 5–6 year-old plantation. All the species cultivated on the loess soil were characterized by the highest concentration of phenolic glycosides in the bark. The soil type under willow plantation determined both the content of phenolic glycosides and the content of macroelements in the bark of all the three willow species. Calcium primarily determined the difference in the chemical composition of the bark. Among the species studied, *Salix alba* was characterized by the highest content of K, Ca and Mg in the bark, compared to the other species studied, irrespective of the soil conditions.

Key words: *S. alba*, *S. daphnoides*, *S. purpurea*, willow bark, soil conditions

INTRODUCTION

Willows have an ability to synthesize salicylates, phenolic acids, flavonoids, and condensed tannins in their barks, leaves, and buds [Tegelberg et al. 2003, Julkunen-Tiitto et al. 2005, Poblócka-Olech et al. 2010]. Secondary metabolites play an important role in plant-herbivore and plant-fungus interactions [Heiska et al. 2007]. Many of these compounds act as toxins and deterrents against herbivores and pathogens and protect plants against abiotic hazards such as UV-B radiation [Tegelberg et al. 2003, Turtola et al. 2006].

Recently, there have been extensive efforts to evaluate the chemopreventive role of substances present in natural products [Hostanska et al. 2007]. Willow bark is included in the Polish Pharmacopeia and in the European monograph as a constituent of many herbal drugs, dietary supplements and weight loss enhancement remedies [Bisset and Wichtl 2001, Schmid et al. 2001]. Willow bark preparations can be used for the traditional symptomatic indications of fever, infections, mild rheumatic complaints, headache and chronic pain syndromes and extract of bark have antioxidant abilities [Schmid et al. 2001, Krauze-Baranowska and Szumowicz 2004, Gawlik-Dziki et al. 2012, Gawlik-Dziki et al. 2013]. Clinical studies have shown that herbal drugs obtained from willow bark are better tolerated by patients than synthetic derivatives [Schmid et al. 2001]. Unlike large doses of aspirin, willow bark does not seem to have any side effects [Zielonka et al. 2000], therefore many people have begun to turn back to willow bark as an alternative to aspirin.

Most willow species constitute a basic floristic element of vegetation and are frequent in river valleys and peatlands [Lorens et al. 2003, Sugier and Czarnecka 2010, Sugier et al. 2011]. Due to the high concentration of salicylates and rapid growth, many of them are recommended for herbal production and are a promising source of herbal drugs in the pharmaceutical industry [Sulima et al. 2006, Heiska et al. 2008, Förster et al. 2010, Sugier et al. 2011]. In Poland, such species as *S. alba*, *S. daphnoides* and *S. purpurea* originating from natural habitats and field-cultivated are mainly used to produce *Salicis cortex* [Krauze-Baranowska and Szumowicz 2004, Sulima et al. 2006, Sugier and Sugier 2007a].

Macroelements play an important role in many metabolic processes of living organisms. They have drawn the attention of researchers in studies on raw material from many herbs [Seidler-Łożykowska et al. 2008, Desideri et al. 2010, Nurzyńska-Wierdak et al. 2011]. In the case of willows, the data about macronutrient composition of *S. viminalis* and other willow species established to produce biomass for energy [Adler 2005] are well documented. However, much less is known about their concentration in the bark of herb willows e.g. *S. alba*, *S. daphnoides* and *S. purpurea*. The aims of this study were: (i) to evaluate the content of phenolic glycosides in the bark of *S. alba*, *S. daphnoides* and *S. purpurea* in field cultivation on two different soil types, (ii) to evaluate the content of the macroelements in willow bark of the species studied, and (iii) to indicate the macroelement which determined the chemical composition of raw material.

MATERIAL AND METHODS

The present studies involved selected *S. alba*, *S. daphnoides* and *S. purpurea* willow clones with the highest phenolic glycoside contents, originating from their natural habitats and cultivated on the two different soil types. The study was carried out in 2009 and 2010 on a 5–6 year-old plantation. The experiment was a completely randomized block design with three replicates conducted in the area of experimental fields at the University of Life Sciences in Lublin located on sandy soil (heavy loamy sand) (51°33'N; 22°44'E) and on loess soil (silt-loam) (51°15'N; 22°35'E). The plantation was estab-

lished at the spacing of 40 × 20 cm. Each plot was 16 m². The sandy soil was characterized by an average content of humus (1.41%), very low phosphorus (17.3 mg·kg⁻¹), very low potassium (33.2 mg·kg⁻¹), very low magnesium (15.0 mg·kg⁻¹) and strong acid reaction (pH KCl – 4.1). The loess soil contained an average content of humus (1.29%), very high phosphorus (98.3 mg·kg⁻¹), average potassium (134.5 mg·kg⁻¹), very low magnesium (15.8 mg mg·kg⁻¹) and acid reaction (pH KCl – 4.7). Mineral fertilization was applied in spring before the beginning of vegetation in both soil types: N – 20 kg, P – 13.1 kg, K – 49.8 kg calculated per 1 ha.

Willow shoots were harvested in November every year (2009–2010) in three replicates. The plant material was collected in the form of 60 annual shoots (20 entire shoots per plot) from every taxon. The shoots were washed with deionized water. Bark was separated from the wood by peeling and subsampled for chemical analysis.

The bark material sampled for salicylate analysis was dried at room temperature and intensively mixed and homogenized. After drying, the phenolic glycosides content calculated on salicin was determined by means of the HPLC technique according to Polish Pharmacopeia [2002] in the laboratory of Labofarm in Starogard Gdański.

The willow bark sampled for macroelement analysis was dried (80°C), weighed, and milled on a Retsch ball mill. Macroelements were extracted using a HNO₃/H₂O₂ mixture during microwave digestion. Cooled digests were diluted, filtered, and made up to 25 ml. Concentrations of K, Ca and Mg were measured in these digests by flame atomic absorption spectrophotometry (AAS, Z-8200, Hitachi, Japan).

The results presented are mean values from two study years. Significant differences between the mean values of phenolic glycosides and macroelement content of the bark were analysed using the ANOVA method and Tukey's post hoc tests. Statistical significance was set at $p \leq 0.05$. The samples were ordinated using the content of macroelements in willow bark by Principal Component Analysis (PCA). Before analyses, the data were centred and log-transformed. The Statistica and MVSP programs were used.

RESULTS AND DISCUSSION

The highest amounts of phenolic glycosides were found in the bark of *S. daphnoides* in relation to the other species studied both in the sand and loess soils, and the difference between the mean values were significant (fig. 1). The content of second metabolites was between 9.81% and 13.54%, and was to some extent similar to the data obtained in our previous field studies and higher in relation to violet willow clones taken from a natural habitat [Sugier and Sugier 2007a, 2007b]. In other studies, however, the concentration in the bark of this species was lower [DAB 1996, Szabo and Botz 1999]. The content of phenolic glycosides in the bark of violet willow cultivated on the sand soils was significantly lower in relation to the material taken from plants cultivated on the loess soil. Based on literature data [Bisset and Wichtl 2001, Poblócka-Olech et al. 2007, 2010, Förster et al. 2010], it was concluded that the clones of *S. daphnoides* studied may be classified as a rich sources of phenolic glycosides.

A somewhat lower content of the metabolites studied was characteristic for the bark of *Salix purpurea* (fig. 1). The contents of phenolic glycosides were in the range

6.48–7.52% on the sandy soil to 6.91–9.35% on the loess soil and the differences between the mean values were significant. The content of the second metabolites was to some extent similar to the data obtained in our previous field studies on this species [Sugier and Sugier 2007b] and higher in relation to data of other researches [DAB 1996, Szabo and Botz 1999].

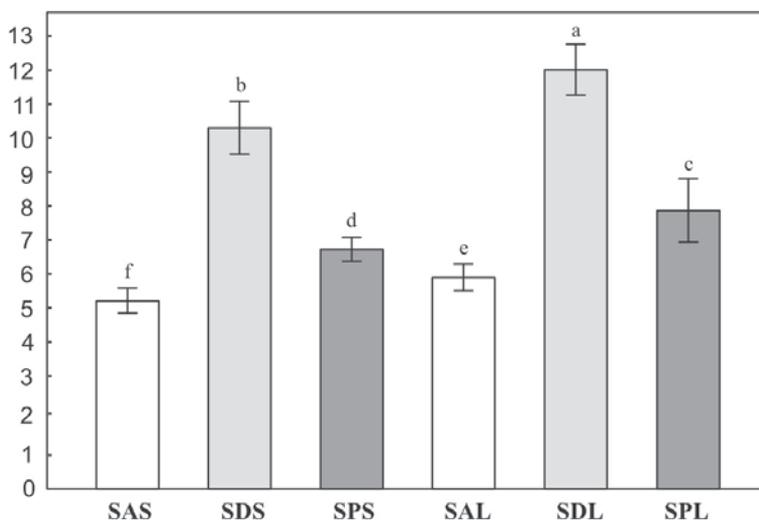


Fig. 1. The phenolic glycosides content in the bark of willows cultivated on the different soil types; SAS – *S. alba* on the sandy soil, SDS – *S. daphnoides* on the sandy soil, SPS – *S. purpurea* on the sandy soil, SAL – *S. alba* on the loess soil, SDL – *S. daphnoides* on the loess soil, SPL – *S. purpurea* on the loess soil, different letters indicates statistical differences ($p < 0.05$)

The bark of *Salix alba* was characterised by the lowest concentration of phenolic glycosides, although it was a few times higher than the minimum found in Polish Pharmacopeia [2002]. The mean content of phenolic glycosides in the bark of these species cultivated on the sandy soil (5.51%) was significantly lower in relation to the material taken from plants cultivated on the loamy soil (6.21%), (fig. 1).

The content of phenolic glycosides in the bark of all the studied willows was higher in relation to the concentration of the substances studied in the bark of violet willow originating from their natural habitats [Sugier and Sugier 2007a, 2007b]. However, the content was lower compared to the content of active substances in the bark of *S. myrsinifolia* taken from natural habitat [Sugier et al. 2011] and the cultivated plants [Heiska et al. 2005, 2008]. It was concluded that the selection and cultivation of herbal willow clones increased the phenolic compounds in the bark and leaves [Sugier and Sugier 2007a, 2007b, Förster et al. 2010].

The presence of phenolic glycosides may depend on environmental factors such as light and nutrient availability [Larsson et al. 1986, Heiska et al. 2005, Sugier and Sugier 2007b]. A fall in the content of phenolic glycosides under the influence of mineral fer-

tilization was also observed by other researchers [Heiska et al. 2005]. In the case of our results, there is an evident relationship between the kind of soil and content of phenolic glycosides in *Salix* bark. Statistically significant differences in the content of phenolic glycosides were noted in the case of all the studied species cultivated on the different soils (fig. 1). It was concluded that the type of soil influenced the phenolic compound content.

The results of the chemical analyses confirmed differences and some similarities in the macroelement composition of the willow barks analyzed (fig. 2). The mean concentration of potassium was the highest in the white willow bark, compared to purple willow and violet willow cultivated on the two soil types. It was $9.52 \text{ g}\cdot\text{kg}^{-1}$ in the plants cultivated on the sandy soil, and $8.63 \text{ g}\cdot\text{kg}^{-1}$ on the loess soil. The content of K in the bark of *S. purpurea* and *S. daphnoides* cultivated on the sand and loess soils were comparable.

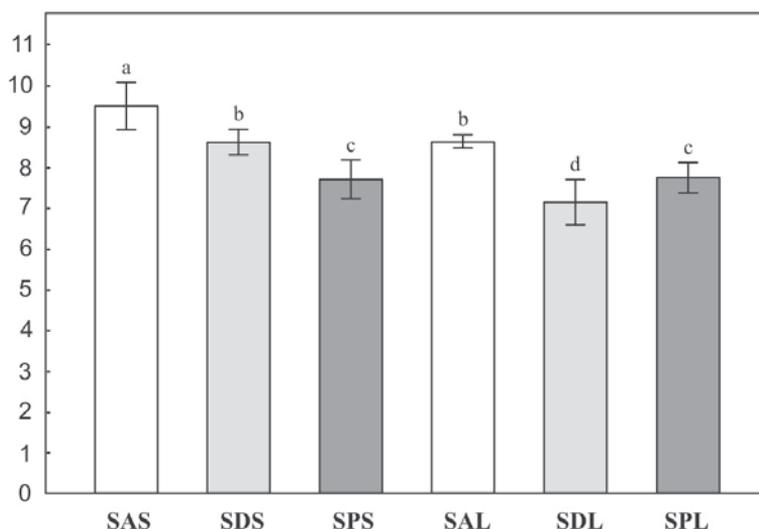


Fig. 2. The potassium content in the bark of willows cultivated on the different soil types; SAS – *S. alba* on the sandy soil, SDS – *S. daphnoides* on the sandy soil, SPS – *S. purpurea* on the sandy soil, SAL – *S. alba* on the loess soil, SDL – *S. daphnoides* on the loess soil, SPL – *S. purpurea* on the loess soil, different letters indicates statistical differences ($p < 0.05$)

In white willow bark, the K content was higher than in the bark of this species studied by Desideri et al. [2010], higher than in bark of *S. viminalis* [Adler 2005, Wróbel 2006] and in relation to K content from willows used to biomass production [Hytönen and Kaunisto 1999, Bungart et al. 2000]. In comparison to the bark material of the other shrub species [Desideri et al. 2010], the bark of the white willow is rich in K.

In the violet willow bark, mean value of K was between $8.63 \text{ g}\cdot\text{kg}^{-1}$ on the sandy soil and $7.16 \text{ g}\cdot\text{kg}^{-1}$ on the loess soil, and in the purple willow bark – between $7.72 \text{ g}\cdot\text{kg}^{-1}$ and

7.76 g·kg⁻¹ respectively (fig. 2). The K content was similar to the content of this macroelement in the bark of *S. viminalis* [Adler 2005, Wróbel 2006]. In the case of the two species, the difference between the content of K in plants cultivated on the two soils was statistically significant.

The highest content of calcium was found in the bark of the white willow – mean 17.19 g·kg⁻¹ on the loess soil and 10.05 g·kg⁻¹ on the sandy soil (fig. 2). In the case of the violet willow, it was between 15.16 g·kg⁻¹ on the loess soil and 10.15 g·kg⁻¹ on the sandy soil and in the bark of the purple willow – between 8.91 g·kg⁻¹ and 6.80 g·kg⁻¹, respectively. The bark of *S. purpurea* was characterized by the lowest content of Ca on both the sandy soil and loess soil in relation to the other species studied.

The content of Ca in the bark of all the species studied cultivated on the loess soil was ca. 50% higher in relation to content this macroelement in plants cultivated on the sandy soil, and the difference was statistically significant (fig. 3). In relation to the chemical composition of the bark material of *S. alba* and *S. viminalis* [Adler 2005, Wróbel 2006] and the other shrub species [Desideri et al. 2010], the Ca content in the bark of the violet willow and purple willow was much lower. However, the content of this macroelement in the bark of the white willow was comparable to that in literature data [Adler 2005, Wróbel 2006, Desideri et al. 2010]. *Salix alba* has a high capability of Ca accumulation in the bark. Based on literature data [Desideri et al. 2010], it was concluded that the bark of *S. alba* could contain even over 18 g·kg⁻¹ Ca and might be classified as a rich source of Ca, likewise as other herbs [Seidler-Łożykowska et al. 2008, Desideri et al. 2010, Nurzyńska-Wierdak et al. 2011].

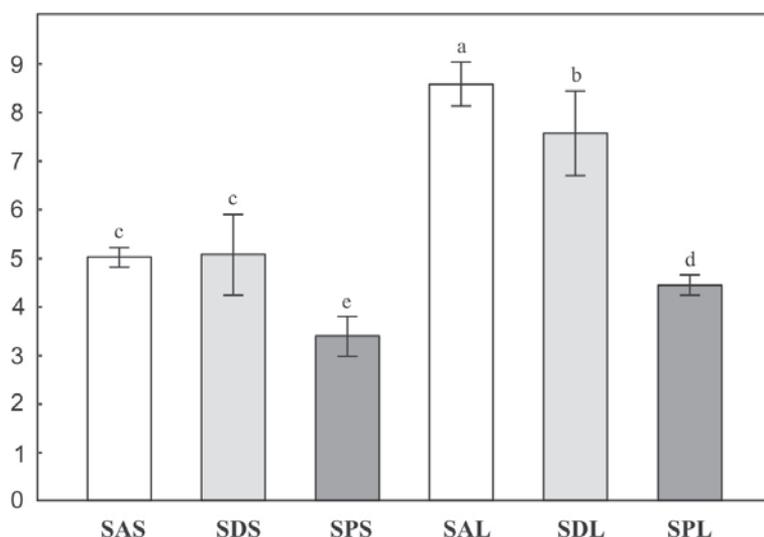


Fig. 3. The calcium content in the bark of willows cultivated on the different soil types; SAS – *S. alba* on the sandy soil, SDS – *S. daphnoides* on the sandy soil, SPS – *S. purpurea* on the sandy soil, SAL – *S. alba* on the loess soil, SDL – *S. daphnoides* on the loess soil, SPL – *S. purpurea* on the loess soil, different letters indicates statistical differences ($p < 0.05$)

Among the species studied, the content of Mg in the white willow bark was the highest both on the sandy soil and on the loess soil (fig. 4). The mean content was $1.56 \text{ g}\cdot\text{kg}^{-1}$ on the sandy soils, and almost two-fold higher in comparison to the other species. The concentration of Mg in the white willow plants taken from the loess soil was $1.41 \text{ g}\cdot\text{kg}^{-1}$, whereas in the violet willow and in the purple willow it was $1.14 \text{ g}\cdot\text{kg}^{-1}$ and $1.01 \text{ g}\cdot\text{kg}^{-1}$, respectively. The difference between the mean content of Mg in the bark of *S. alba* and *S. purpurea* cultivated on two soil types was statistically significant. However, the concentration of this macroelement was considerably lower in relation to the content in raw material from other bark of herb shrubs [Desideri et al. 2010]. The Mg concentration in the bark of *S. daphnoides* and *S. purpurea* was comparable to that in *S. viminalis* [Hytönen and Kaunisto 1999, Adler 2005, Wróbel 2006].

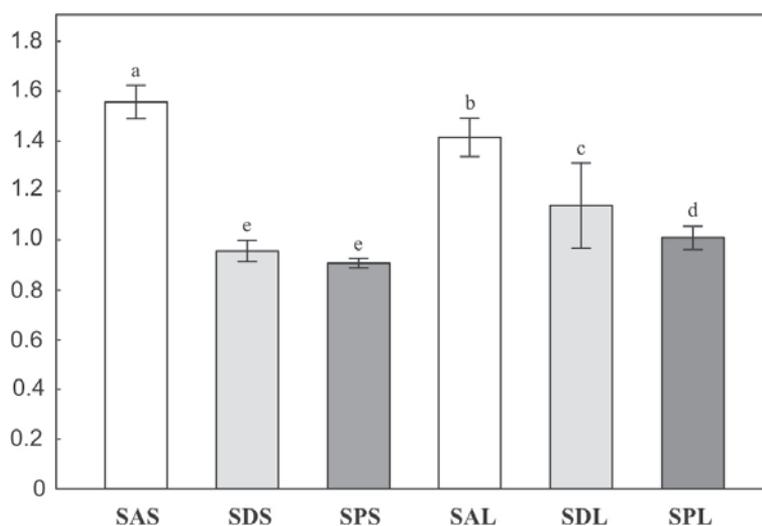


Fig. 4. The magnesium content in the bark of willows cultivated on the different soil types; SAS – *S. alba* on the sandy soil, SDS – *S. daphnoides* on the sandy soil, SPS – *S. purpurea* on the sandy soil, SAL – *S. alba* on the loess soil, SDL – *S. daphnoides* on the loess soil, SPL – *S. purpurea* on the loess soil, different letters indicates statistical differences ($p < 0.05$)

The PCA ordination of the samples based on the macroelement composition in the bark of the willows studied is presented in figure 5. The Principle Component Analysis extracted two axes with eigenvalues of 0.128 and 0.035. The PCA axis I accounted for 76% variation in the macroelement composition while PCA axis II accounted for 21% variation. PCA axis I was correlated with Ca and Mg and it indicated a gradient of these macroelements. In turn, axis II was correlated with Mg, K, and Ca and it indicated a gradient of these nutrients.

The Principal Component Analysis ordination indicated that the differentiation in the macroelement composition of the willow bark is explained by the variation in Ca

(fig. 5). The points representing the samples of the white willow form two groups. The first one (SAL) is localized on the right side of the diagram and is characterized by the highest Ca content; the second one (SAS) near the vector representing the K gradient is characterized by the highest potassium content. However, the SPS samples are located on the left site of the diagram and have the lowest concentration of Ca and Mg. Agglomeration of SAS, SAL and SPL samples provides evidence that the macroelement composition in the willow bark inside these groups is more similar. However, a different situation is found in the case of SPS, SPL, and SDL. They occupy a larger place of the ordination space and the content of the nutrients is more different than in SAS, SAL and SPL (fig. 5).

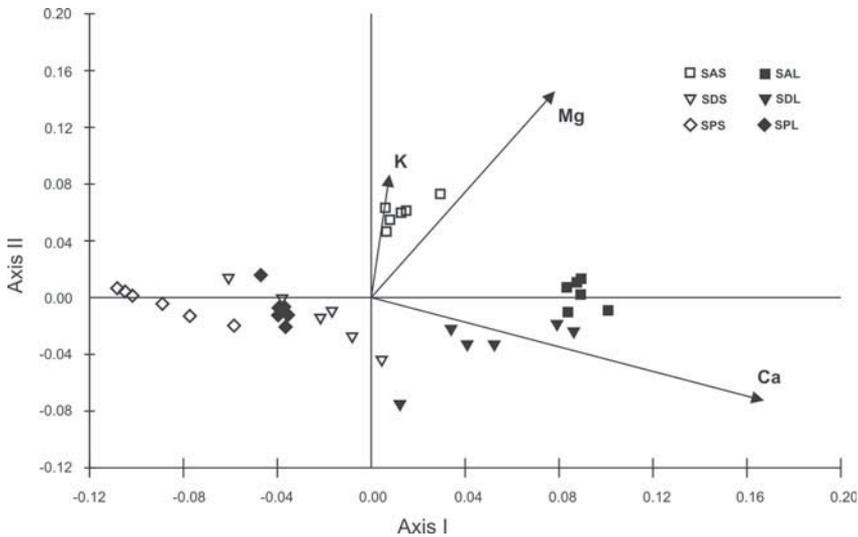


Fig. 5. Results of PCA analysis; SAS – *S. alba* on the sandy soil, SDS – *S. daphnoides* on the sandy soil, SPS – *S. purpurea* on the sandy soil, SAL – *S. alba* on the loess soil, SDL – *S. daphnoides* on the loess soil, SPL – *S. purpurea* on the loess soil, different letters indicates statistical differences ($p < 0.05$)

There are a many parameters influencing the phenolic compounds in willows e.g. climate, herbivore pressure, physiological age, time of the harvest or cultivation method [Heiska et al 2005, Sulima et al. 2006, Heiska et al. 2008, Förster et al. 2010]. Our studies have confirmed the fact that the soil type determines the content of phenolic glycosides. Moreover, the clones of herbal willows originating from their natural habitats exhibit a high content of active compounds in field conditions, which is consistent with the results of other researches [Förster et al. 2010]. Soil conditions influence the composition and variability of macroelements in the bark of herbal willows. Furthermore, the willow species studied have a different ability to accumulate the macroelements studied.

CONCLUSIONS

1. All the willow species cultivated on the loess soil were characterized by the highest concentration of phenolic glycosides in the bark. The soil type under willow plantation determined both the content of phenolic glycosides and the content of macroelements in the bark of all the three willow species studied.

2. The selected willow clones of *S. alba*, *S. daphnoides* and *S. purpurea* originating from their natural habitats were characterized by an even higher content of phenolic glycosides in the field conditions and can be a good source of quality raw material.

3. *Salix alba* was characterized by the highest content of potassium, calcium and magnesium in the bark, compared to the other species studied, irrespective of the soil conditions. The bark of this species is rich in calcium. Among the macroelements studied, calcium primarily determined the difference in the chemical composition of the bark.

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ZRÓŻNICOWANIE ZAWARTOŚCI GLIKOZYDÓW FENOLOWYCH I MAKROELEMENTÓW (K, Ca, Mg) W KORZE WIERZB ZIELARSKICH

Streszczenie. Wierzby charakteryzujące się dużą zawartością glikozydów fenolowych są wykorzystywane jako surowiec zielarski. Koncentracja tych metabolitów w korze jest stosunkowo dobrze udokumentowana, natomiast niewiele jest informacji odnośnie zawartości makroelementów. Celem badań było określenie zawartości glikozydów fenolowych oraz makroelementów (K, Ca, Mg) w korze *Salix alba*, *S. daphnoides* i *S. purpurea*, pozyskanych z naturalnych siedlisk występowania tych gatunków i uprawianych na różnych jednostkach glebowych. Badania były przeprowadzone w latach 2009 i 2010 na pięcio- i sześcioletniej plantacji. Wszystkie badane gatunki uprawiane na glebie lessowej charakteryzowały się większą zawartością glikozydów fenolowych w korze w porównaniu z uprawianymi na glebie piaszczystej. Właściwości gleby determinowały zarówno zawartość glikozydów fenolowych, jak i zawartość makroelementów w korze wszystkich ocenianych gatunków *Salix* sp. Z badanych pierwiastków zawartość wapnia w *Salicis cortex* była najbardziej zróżnicowana. *Salix alba* odznaczała się największą zawartością potasu, wapnia i magnezu w korze w porównaniu z pozostałymi gatunkami, niezależnie od warunków glebowych.

Słowa kluczowe: *S. alba*, *S. daphnoides*, *S. purpurea*, kora wierzby, glikozydy fenolowe, makroelementy

Accepted for print: 29.11.2012