

***Salix myrsinifolia* Salisb. AS A SOURCE OF PHENOLIC GLYCOSIDES: DISTRIBUTION AND CHARACTERISTIC OF HABITAT CONDITIONS IN THE MID-EASTERN POLAND**

Danuta Sugier¹, Piotr Sugier², Maciej Pawełek³,
Urszula Gawlik-Dziki¹

¹University of Life Sciences in Lublin

²Maria Curie-Skłodowska University in Lublin

³Labofarm in Starogard Gdański

Abstract. In Poland, *Salix myrsinifolia* Salisb. reaches the south-western border of the dense geographical range. Therefore, the number of natural stands of this species is limited. The aims of this study were: to evaluate the content of phenolic glycosides in dark-leaved willow bark taken from natural sites, and to investigate the habitat conditions and natural resources of this species in mid-eastern Poland. In the sites of *S. myrsinifolia* the annual shoots were taken, and the content of phenolic glycosides in willow bark was determined. The plant communities with the share of this species were characterised, and pH, organic matter, macro- and microelements in soils were determined. The bark of the dark-leaved willow is characterised by a very high content of phenolic glycosides and is considered to be potential natural source of salicylates. The plant communities with share of this species were formed spontaneously on highly transformed calcareous fens where peat was mined and on agriculturally developed peatlands. The number of natural stands is small, and gather from natural habitats is limited. Therefore, this species can be a good source of quality raw material during cultivating.

Key words: dark-leaved willow, phenolic glycosides, natural resources

INTRODUCTION

Dark-leaved willow (*Salix myrsinifolia* Salisb.) is a Euro-Siberian willow species, which represents the boreal type of distribution. In Europe, this species can be found in Scandinavia, in the north region of the European part of Russia and in the Baltic states

Corresponding author – Adres do korespondencji: Danuta Sugier, Department of Industrial and Medicinal Plants, University of Life Sciences in Lublin, 15 Akademicka Str., 20-950 Lublin, e-mail: danuta.sugier@up.lublin.pl

[Zieliński 1976, Hultén and Fries 1986]. This species is widespread in the north of Britain [Stewart et al. 1994] but rare in Ireland, where it is restricted to a few sites in the Northeast [Harron 1992], and in the Alps [Hultén and Fries 1986]. Outside of Europe, this species is present in the west of Siberia. In Poland, *S. myrsinifolia* reaches the south-western border of the dense geographical range from the Warmia Lowland and Olsztyn Lakeland to the region of Polesie Lubelskie and the valley of the Upper Bug river. It was reported from a number of localities in the north-eastern part of the country [Zieliński 1976, Faliński 1998, Zając and Zając 2001]. In mid-eastern Poland, *S. myrsinifolia* was recorded mainly in Polesie Lubelskie and Pagóry Chełmskie regions, rarely in the other areas of Lublin province [Fijałkowski 1994].

Dark-leaved willow is more winter tolerant than other *Salix* spp. [Lumme and Törmälä 1988]. Therefore, *S. myrsinifolia* clones were selected for biomass forestry in the north of Europe [Pohjonen 1991, Honkanen 1994]. Many of them are characterised by a high content of phenolic glycosides, which are typical secondary substances of plants of the family *Salicaceae* [Julkunen-Tiitto 1989, Kolehmainen et al. 1994], and play an important role in plant-herbivore and plant-fungi interactions [Sipura 1999, Hakulinen and Julkunen-Tiitto 2000, Veteli et al. 2003]. This willow species has also shown a great clonal variation in phenolic chemistry and biomass production [Julkunen-Tiitto and Meier 1992, Turtola et al. 2006], allowing selection of the promising clones for herbal willow cultivation. Dark-leaved willow is regarded as a suitable willow species for herbal drug production due to the rapid growth and high concentration of salicylates in leaves and twigs [Julkunen-Tiitto and Meier 1992, Heiska et al. 2008, Paunonen et al. 2009].

Salix purpurea L., *S. daphnoides* Vill. and *S. alba* L. are a very popular herbal species affirmed in the natural habitats and field-cultivated in Poland [Szcukowski et al. 2002, Krauze-Baranowska and Szumowicz 2004, Sulima et al. 2006, Sugier and Sugier 2007a, 2007b]. The phenolic glycosides contained in willow bark of this species are known for their anti-inflammatory, analgesic and fever-reducing effects, and have been shown to relieve rheumatic disturbances, infections, and headache [Chrubasik et al. 2000, Schmid et al. 2001, Biegert et al. 2004, Krauze-Baranowska and Szumowicz 2004, Khayyal et al. 2005]. The knowledge about content of phenolic glycosides in dark-leaved willow bark, current distribution of this species at the edge of its dense geographical range and environmental conditions is poor. Therefore, our main aims in the present study were as follow: (i) to evaluate the content of phenolic glycosides in dark-leaved willow bark taken from natural sites, (ii) to investigate and evaluate the habitat conditions of plant communities with the share of this species, and (iii) to evaluate the distribution and natural resources of *S. myrsinifolia* in mid-eastern Poland.

MATERIAL AND METHODS

The tree sites with shrubs of *S. myrsinifolia* were located in the Sawin peatland. In each site, three willows were randomly selected for determination of active components. The annual shoots from each of the three individuals were collected for chemical analysis at the end November every year (2007–2009). Bark was separated from wood with

a preparation knife and was chopped with scissors. After drying, the content of phenolic glycosides recalculated into salicylates was determined by means of the HPLC technique according to methods in Farmakopea Polska VI [2002] in the laboratory Labofarm in Starogard Gdański. Differences of content of phenolic glycosides in bark between the years were tested using Kruskal-Wallis nonparametric test. Data analyses were carried out in Statistica 5.1 program.

Additionally fifteen sites of *S. myrsinifolia* representing Polesie Lubelskie and Polesie Wołyńskie regions were selected (fig. 1). The sites of dark-leaved willow were located on organic soils in the Sawin peatland (PS), in the Krowie Bagno peatland near Lubowierz and Lubowierzek lakes (PL), and near Hańskie lake (PH). We have studied literature and examined the *S. myrsinifolia* specimens from the Herbarium at the Institute of Biology, Maria Curie-Skłodowska University, Lublin [LBL].

In the chosen plant communities with the share of *S. myrsinifolia*, phytosociological relevés were made using an eleven-degree scale, with + symbol for the species cover less than 5%, 1 – for cover of 5–10%, 2 – for 10–20%, ..., 10 – for 90–100%. Simultaneously, in chosen sites, the soil samples of topsoil (0 to 20 cm) were taken six times from each site by means of a manual core-drill. The soil material was intensively mixed, homogenized and dried at room temperature. The reaction of the dried samples was analyzed in distilled water and in 1 M KCl. Organic matter was determined by loss ignition at 550°C in a muffle furnace. The content of available P, K Ca, Mg, Fe, Mn, Ni, Cu, Zn, and Pb extracted in 0.5 M HCl in the organic soils was measured using Atomic Absorption Spectrophotometry. All data analyses were carried out in the Statistica 5.1 program. The Principal Component Analysis (PCA) and Detrended Correspondence Analysis (DCA) were performed using the MVSP program.

RESULTS AND DISCUSSION

A very high content of phenolic glycosides was determined in the bark of *S. myrsinifolia* on the basis of a preliminary chemical analysis. The bark of the dark-leaved willow was characterised by the phenolic glycoside content ranging from 14.34–30.08%, and mean value 22.38% in the year 2007, 23.67% in the year 2008 and 24.27% in 2009 (fig. 1). The mean content of phenolic glycosides in the willow bark was higher in 2009 than in 2008 and 2007, but the differences were not statistically significant. The standard variation calculated showed that the interspecific variability was higher in 2008 than in 2007 and 2009 (fig. 1).

From among the several willow species used as herbal raw material, the bark of *S. myrsinifolia* was characterised by the highest concentration of phenolic glycosides. The content of salicylates in the bark of *S. myrsinifolia* was higher in comparison to other herbal willows, such as: *S. purpurea*, *S. daphnoides*, *S. alba* and *S. pentandra* L. [Julkuunen-Tiitto 1989, Szabo and Botz 1999, Ikonen et al. 2002, Szczukowski et al. 2002, Sulima et al. 2006, Sugier and Sugier 2007a, 2007b, Föster et al. 2010].

The concentration of phenolic glycosides in the bark of *S. myrsinifolia* recorded in this study was higher in comparison to the data obtained from cultivated specimens of willow bark in Finland [Heiska et al. 2008]. Furthermore, the content of salicylates in

the bark of dark-leaved willow was higher in comparison to the data obtained from the leaves and shoot tips of this species [Ruuhola et al. 2000, Julkunen-Tiitto et al. 2005]. Our results correspond with the concentrations of phenolic compounds noted in the leaves of field-cultivated *S. myrsinifolia* clones under different rust-infection levels [Hakulinen and Julkunen-Tiitto 2000], and with the content of salicylates noted in the leaves of another boreal willow species – *Salix myrsinites* L. [Julkunen-Tiitto et al. 2005, Nyman and Julkunen-Tiitto 2000].

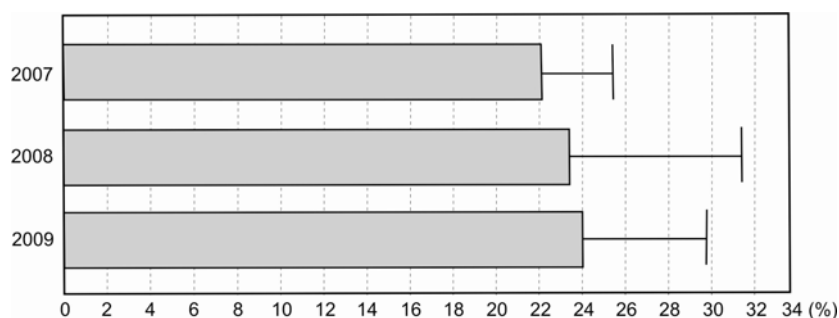


Fig. 1. Content of phenolic glycosides in dark-willow bark in the years 2007–2009

Ryc. 1. Zawartość glikozydów fenolowych w korze wierzby czarniawej w latach 2007–2009

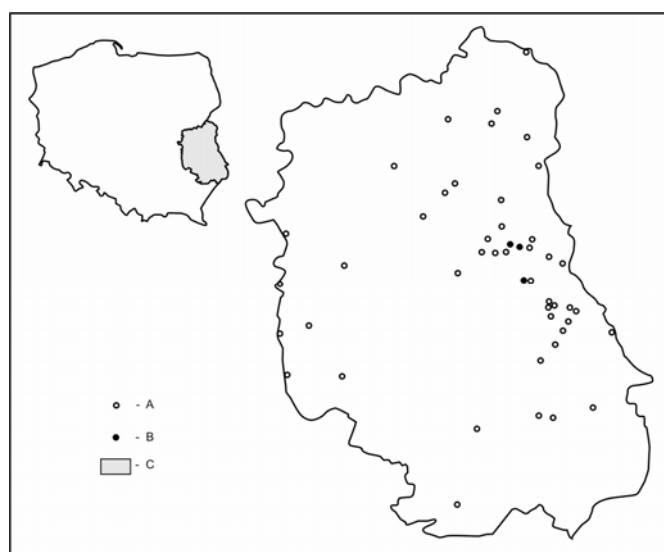


Fig. 2. Distribution of *Salix myrsinifolia* sites in the mid-eastern Poland. A – data on the basis of studied literature confirmed by phytosociological relevés in the last 50 years, B – studied sites, C – Lublin province.

Ryc. 2. Rozmieszczenie stanowisk *Salix myrsinifolia* w Polsce środkowowschodniej. A – na podstawie danych zawartych w literaturze potwierdzonych przez zdjęcia fitosocjologiczne w ciągu ostatnich 50 lat, B – badane stanowiska, C – województwo lubelskie

Among the *Salix* spp. only *S. purpurea* and *S. alba* are recognized as medicinal plants in Poland [Szafer et al. 1986, Rutkowski 2007]. The concentration of salicylates recorded in this study was a many times higher than the minimum reported in Farmakopea Polska VI [2002]. Therefore, this species should certainly be treated as yet another taxon which can be recognized as a medicinal plant and commonly used in the pharmaceutical industry.

In all the phytocoenoses studied (fig. 2), *S. myrsinifolia* was characterised by relatively small coverage. In the Sawin peatland, it constituted a maximally 10% of the shrub cover. In the other communities, only single individuals of this species were reported. The DCA results indicate floristic differentiation of the communities with the share of *S. myrsinifolia* (fig. 3). A very small group of phytosociological relevés representing PS is placed on the left part of the diagram. These communities (*Salicetum pentandro-cinereae*) are characterised by the high coverage of shrubs: mainly *S. cinerea* L., *S. pentandra*. The herbaceous layer contained a big share of *Urtica dioica* L., *Rubus idaeus* L. and *Carex acutiformis* Ehrh., whereas the coverage of *Molinia caerulea* (L.) Moench was negligible. The floristic composition of the communities is remarkably different from the communities on the right side of the diagram – there are phytosociological relevés of all the three fen sites studied. They are highly dense and cover a small part of the ordination area, which testifies to their marked floristic similarity. This group includes *Molinietum caeruleae* phytocoenoses which can be found on all the peatlands studied as well as *Salicetum pentandro-cinereae* located on the peatland near Hańskie Lake. *Molinia caerulea* clearly predominates in the herbaceous layer; its high coverage value ranging from 40 to 100% seems to determine the great similarity in this group of phytocoenoses. Other species with great frequency and coverage are *Potentilla erecta* (L.) Raeusch. and *Lysimachia vulgaris* L..

The top horizon of the organic soils examined differed significantly in a majority of the parameters studied. The considerable distance of PS, PL and PH sites in the PCA ordination space (fig. 4) confirms the difference in the content of the factors between the sites. The gradients of P, K, Fe, Ca, Mg and Pb are highly correlated with the first axis and have the biggest effect on soil difference, whereas OM, Cu, Zn and Ni displayed the least effect.

The reaction of the PS soil was usually neutral and lightly acidic, and that of PL and PH – slightly acid and acid (fig. 5). The average OM content of soils of all the sites was similar – 82.3 % in PS, 84.1% in PL and 83.2% in PH. The content of available phosphorus in PS was moderate, and the content of this element in PL and PH was very low [Zalecena 1990]. The K content was comparable and very low in the PL and PH soils, and considerably higher in PS [Zalecena 1990]. The Ca and Mg contents were comparable in the PL and PH soils, and clearly higher in PS. The Fe content was comparable in PL and PH soils and several fold higher in PS soils (fig. 6). The high content of Fe and Mn may result from the fact that peat was mined in the Sawin peatland. The enrichment of muck layers of the peat-muck soils in P, Fe and Mn resulted from organic matter becoming muck [Kalembasa et al. 2008, Kalembasa and Becher 2009]. Chemical analyses revealed that the mean contents of heavy metals can be arranged in the following decreasing series for PS: Fe > Mn > Pb > Ni > Zn > Cu; for PL: Fe > Pb > Ni > Mn > Cu > Zn; and for PH: Fe > Mn > Pb > Zn > Cu > Ni (Fig. 5). The concentration of Ni, Cu, Zn and Pb was relatively low in all the organic soils studied.

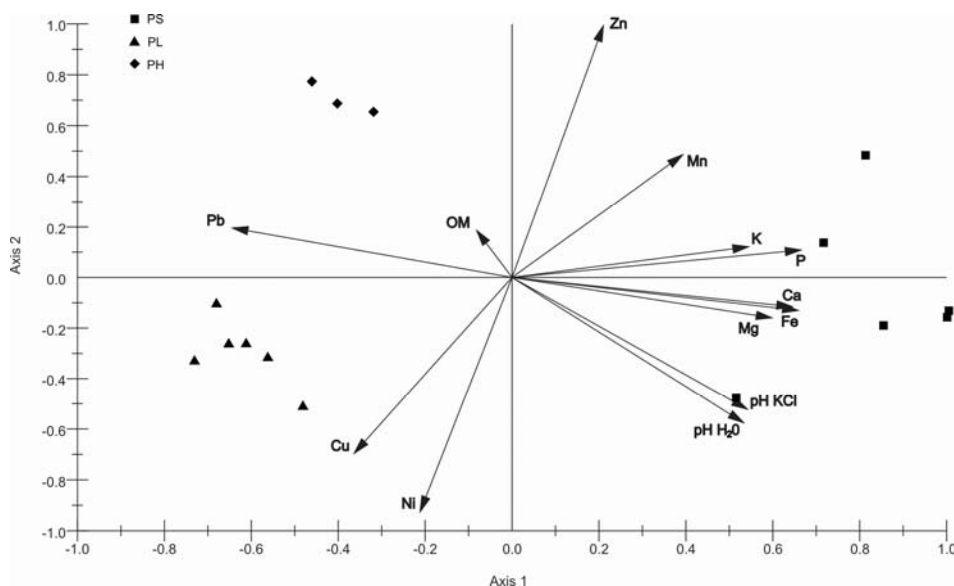


Fig. 4. Results of PCA analysis; PS – peatland near Sawin, PL – Krowie Bagno peatland near Lubowierz and Lubowierzek lakes, PH – Krowie Bagno peatland near Hańskie lake
 Ryc. 4. Rezultaty analizy PCA; PS – torfowisko koło Sawina, PL – torfowisko Krowie Bagno w pobliżu jezior Lubowierz i Lubowierzek, PH – torfowisko Krowie Bagno w pobliżu jeziora Hańskie

Salix myrsinifolia is characterised by a considerable number of localities (mainly in one region) and lack of dynamic tendency [Zarzycki et al. 2002]. In NE-Poland, this species occurs in exploited fens, where peat undergoes oxidative decomposition, and beside abandoned drainage ditches [Faliński 1998]. The species often occurs abundantly with *S. cinerea* and *S. pentandra*, but it avoids habitats that are permanently waterlogged. In the Kampinoski National Park, the dark-leaved willow was found in such plant communities as: *Molinietum caeruleae*, *Caricetum appropinquatae* and *Salicetum pentandro-cinereae* [Ferchmin 2009]. This species is relatively rarely found in the Środkowomazowiecka Lowland [Wierzba et al. 2008]. *S. myrsinifolia* is regarded as an endangered species out in Western Pomerania and Wielkopolska regions – outside the dense geographical species range [Żukowski and Jackowiak 1995, Jackowiak et al. 2007].

Distribution of *S. myrsinifolia* on the floristic maps of Lublin province [Fijałkowski 1994] indicates widespread occurrence of this species. Fijałkowski and Chojnacka-Fijałkowska [1990] reported this taxon in such associations as: *Caricetum appropinquatae*, *Caricetum davallianae* and *Cladietum marisci*. Most observations, however, originate from the years 1947–1967, which were followed by drainage and a period of peatland management; therefore, it is evident that many habitats of this species were strongly transformed. Fijałkowski [1994] estimates that the loss of localities resulting from the anthropogenic activity was in the range of 30% to 50%, compared to the 50. and 80. of the last century. In her studies of the *Cladium mariscus* (L.) Pohl habitats in

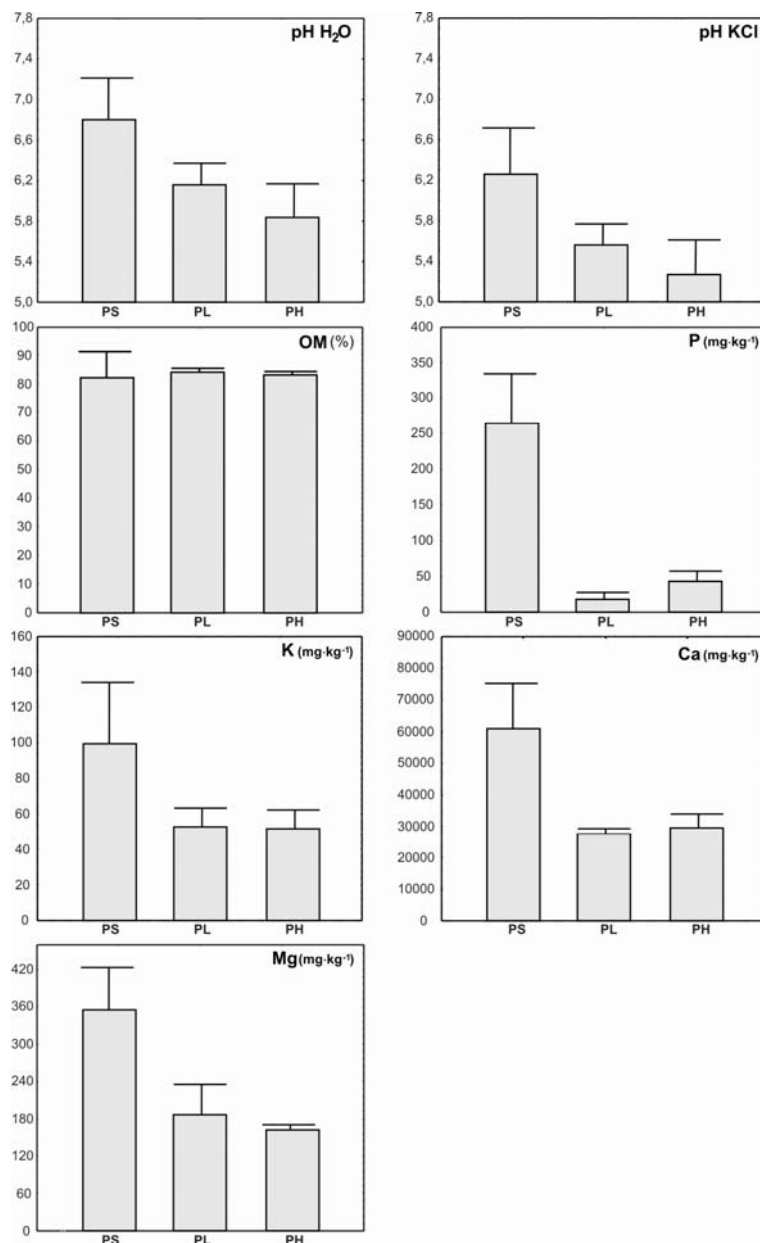


Fig. 5. Average value and standard deviation of studied soil properties; PS – peatland near Sawin, n = 6, PL – Krowie Bagno peatland near Lubowierz and Lubowierzek lakes, n = 6, PH – Krowie Bagno peatland near Hańskie lake, n = 3

Ryc. 5. Wartość średnia i odchylenie standardowe badanych właściwości gleb; PS – torfowisko koło Sawina, n = 6, PL – torfowisko Krowie Bagno w pobliżu jezior Lubowierz i Lubowierzek, n = 6, PH – torfowisko Krowie Bagno w pobliżu jeziora Hańskie, n = 3

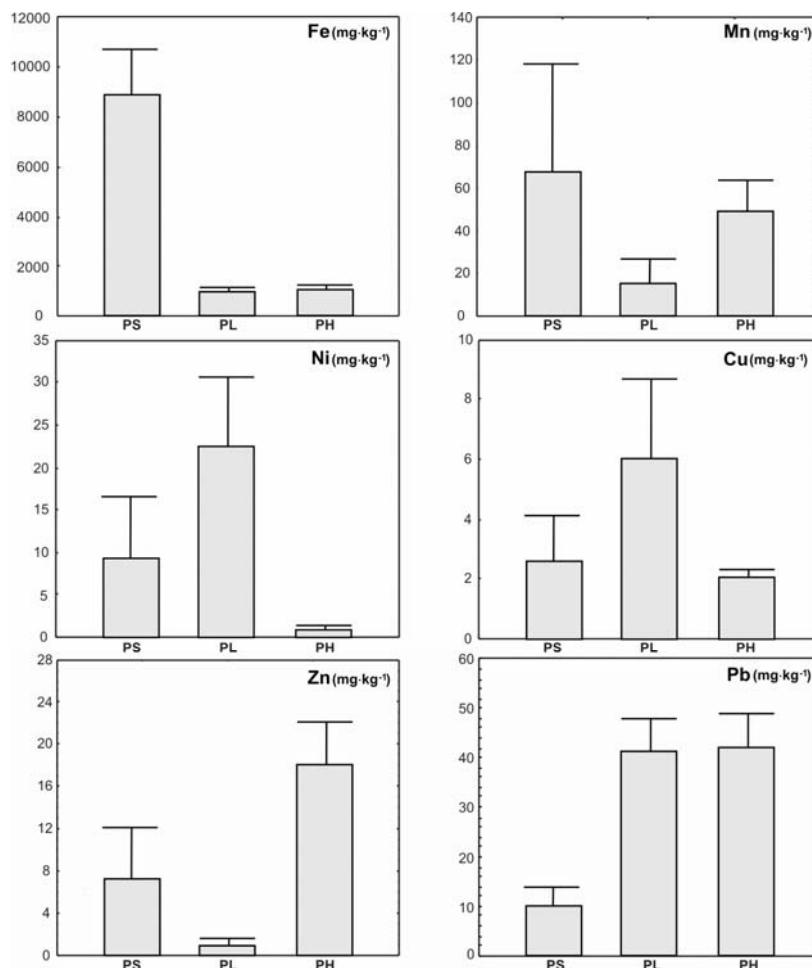


Fig. 6. Average value and standard deviation of heavy metal contents in the studied soils; PS – peatland near Sawin, n = 6, PL – Krowie Bagno peatland near Lubowierz and Lubowierzek lakes, n = 6, PH – Krowie Bagno peatland near Hańskie lake, n = 3

Ryc. 6. Wartość średnia i odchylenie standardowe metali ciężkich w badanych glebach; PS – torfowisko koło Sawina, n = 6, PL – torfowisko Krowie Bagno w pobliżu jezior Lubowierz i Lubowierzek n = 6, PH – torfowisko Krowie Bagno w pobliżu jeziora Hańskie, n = 3

Lublin province, Buczek [2005] did not confirm the presence of *S. myrsinifolia* in *Cladietum marisci* patches. In 2007 three localities of this species were found along the edges and on the railway embankment in Kotlina Hrubieszowska and Padół Zamojski regions [Wrzesień personal communications, LBL]. Our own verification of some localities mentioned by Fijałkowski [1994] did not confirm the presence of this species in numerous habitats similar to natural ones. *S. myrsinifolia* has not been reported from

lakeland peatlands in the Łęczna-Włodawa lakeland [Sugier and Popiołek 1998, 1999, Sugier 2002, Sugier and Czarnecka 2010]. According to Kucharczyk [2001] *S. myrsinifolia* is rare on alluvia (Vistula river) in riparian scrub and on terraces outside the embankments in wet willow scrub.

In N-E Poland a tendency for *S. myrsinifolia* was noted to occur in habitats which have been very highly modified by human activity [Faliński 1998]. This species occurred in wooded valleys, in clearings and along ditches, in settled clearings, along the causeways, and it was present in ditches between abandoned fields. *S. myrsinifolia* seems to show the strongest association with dried-out peatlands and ruderalized habitats, being almost entirely absent from pristine forests and well-preserved peatlands [Faliński 1998]. All the localities of this species presented in this work, situated at the edge of their geographical range, are characterised by a quite high degree of habitat transformation. In fact, they are secondary habitats and were formed as a result of peat exploitation (the Sawin peatland) and peatland management and transformation thereof into agriculturally utilised meadows (Krowie Bagno peatland). In the past, these habitats were dominated by species that are typical of calcareous fens and whose local populations have become almost completely extinct [Fijałkowski and Chojnacka-Fijałkowska 1990, Buczek 2005]. Due to the increasing anthropopressure, *S. myrsinifolia* occurs mainly on sites transformed by peat mining and agricultural development.

CONCLUSIONS

1. The bark of the dark-leaved willow was characterised by a very high content of phenolic glycosides and was considered to be potential natural source of salicylates for the pharmaceutical industry.

2. All the *S. myrsinifolia* sites under study were characterised by a relatively high degree of transformation. These communities were formed spontaneously on highly transformed calcareous fens where peat was mined and on agriculturally developed peatlands. The dark-leaved willow mainly occurs in *Molinietum caeruleae* and *Salicetum pentandro-cinereae* communities and its share in the shrub layer is low.

3. The dark-leaved willow habitats were characterised by neutral and slightly acidic reaction, a very low and also moderate content of P, and by low K and relatively high Ca and Fe content.

4. The relatively rare occurrence of this species in mid-eastern Poland does not offer an opportunity to use willow bark in the pharmaceutical industry. The number of natural stands is small, therefore this species can be a good source of quality raw material during cultivating.

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***Salix myrsinifolia* Salisb. ŹRÓDŁEM GLIKOZYDÓW FENOLOWYCH:
ROZMIESZCZENIE I CHARAKTERYSTYKA WARUNKÓW
SIEDLISKOWYCH W POLSCE ŚRODKOWOSCHODNIEJ**

Streszczenie. W Polsce *Salix myrsinifolia* Salisb. osiąga południowo-zachodnią granicę zwartego geograficznego zasięgu, toteż liczba naturalnych stanowisk tego gatunku jest ograniczona. Celem badań było określenie zawartości glikozydów fenolowych w korze wierzby czarniawej ze stanowisk naturalnych, ocena warunków siedliskowych oraz zasobów tego gatunku w Polsce środkowo-wschodniej. W korze pobranej z jednorocznych pędów *S. myrsinifolia* oznaczono zawartość glikozydów fenolowych. Scharakteryzowano zbiorowiska roślinne z udziałem tego gatunku, a w pobranych próbkach glebowych oznaczono pH, materię organiczną oraz makro- i mikroelementy. Kora wierzby czarniawej charakteryzowała się bardzo wysoką zawartością glikozydów fenolowych i może być

traktowana jako potencjalne źródło salicylanów. Zbiorowiska roślinne z udziałem tego gatunku wykształciły się spontanicznie na torfowiskach węglanowych przekształconych w związku z eksploatacją torfu oraz rolniczo zagospodarowanych. Liczba naturalnych stanowisk *Salix myrsinifolia* jest niewielka, a pozyskiwanie kory ze stanu naturalnego ograniczone. Celowe wydaje się wprowadzenie tego gatunku do uprawy.

Słowa kluczowe: wierzba czarniawa, glikozydy fenolowe, naturalne zasoby

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