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CHARACTERISTICS OF ABIOTIC CONDITIONS AND HEAVY METALS CONCENTRATION IN BARK OF THE PHARMACEUTICAL WILLOW *Salix purpurea* L. IN THE ŁĘCZNA-WŁODAWA LAKELAND

Danuta Sugier[*], Piotr Sugier[**]

[*] Department of Industrial and Medicinal Plants, University of Life Sciences
15 Akademicka str., 20-950 Lublin, danuta.sugier@up.lublin.pl
[**] Department of Ecology, Institute of Biology, Maria Curie-Skłodowska University
19 Akademicka str., 20-033 Lublin, piotr.sugier@poczta.umcs.lublin.pl

Summary. The aims of this study were to characterise the abiotic habitat conditions of *Salix purpurea* L., and to verify whether purple willow, which may be a potential raw material for *Salicis cortex,* has the ability to accumulate a high quantity of Zn, Pb and Cd in the bark. The study was carried out in 2008. Eighteen sites of *Salix purpurea* representing different habitats were selected. At each of the sites samples of the topsoil were taken, and pH, OM, macro- and microelements were determined. Simultaneously, in chosen sites the annual willow shoots were cut, and samples of the bark were prepared chemical analysis. The content of Zn, Pb and Cd in milled bark samples was determined.

The *S. purpurea* habitats studied are highly diverse in terms of physicochemical properties. This is determined by such parameters as Fe, Ca, Mn, P, Zn and Pb levels. The studied soils are characterised by a relatively low content of heavy metals. Cadmium, a high concentration of which was observed in mineral soils and in the bark of willows growing thereon, is an exception. The high heavy metal content in *S. purpurea* bark disqualifies it as herbal raw material and indicates that this crop should be cultivated under controlled conditions.

Key words: *Salix purpurea,* heavy metals, pharmaceutical willow, raw material

INTRODUCTION

Willows have an ability to synthesize a diverse complement of such phenolic compounds as salicylates, cinnamic acid derivatives, flavonoids, and condensed tannins, which are important secondary compounds [Heiska *et al*. 2008]. *Salix* species with high salicylate concentrations are recommended for herbal

production and are a promising source of herbal drugs in the pharmaceutical industry [Sulima *et al.* 2006, Förster *et al*. 2009]. *Salix purpurea* is one of the willow species characterised by a substantially high content of phenolic glycosides in its bark [Schmid 1998, Szczukowski *et al*. 2002, Sugier 2005, Sulima *et al*. 2006, Sugier and Sugier 2007]. At present, such species as *S. alba* and S. *purpurea* originating from natural habitats are mainly used to produce *Salicis cortex*.

Several studies have shown that *Salix* spp. exhibit the capacity to accumulate high levels of Cd, Pb and Zn [Meers *et al*. 2007], especially in leaves and bark [Landberg and Greger 1996, Rosselli *et al*. 2003], and exhibit high tolerance for these metals [Landberg and Greger 1996, Pulford *et al*. 2002, Pulford and Watson 2003, Rosselli *et al*. 2003]. Also, other studies show a similar trend of higher heavy metal concentrations in the upper horizons of soils [Urban 2004, Czarnecka and Sugier 2006, Kalembasa *et al*. 2006, Wójcikowska-Kapusta and Urban 2008], where main root biomass is situated, compared to deeper layers. The upper layers of organic soils accumulate heavy metals coming mostly from atmospheric deposition. Bark which can be used as raw material in the pharmaceutical industry is an important sink for biologically available metals [Pulford *et al.* 2002, Pulford and Watson 2003]. Therefore, it is vital to assess the habitat conditions of *S. purpurea* – a very valuable willow species due to its phenolic compounds. It is also essential to pay special attention to the content of heavy metals which may disqualify willow bark as herbal raw material. The aims of this study were:

– to characterise the abiotic habitat conditions of the pharmaceutical willow *Salix purpurea* growing spontaneously on organic and mineral soils, with special emphasis on the heavy metal content;

– to verify whether purple willow, which may be a potential raw material for *Salicis cortex,* has the ability to accumulate a high quantity of Zn, Pb and Cd in the bark.

MATERIAL AND METHODS

Salix purpurea is a common species in Poland; it constitutes a basic floristic element of brushwood association *Salicetum triandro-viminalis*, frequent in the valleys of lowland rivers, especially in the Vistula river valley and its tributaries [Matuszkiewicz 2006]. In the Łęczna-Włodawa Lakeland, purple willow is a common element of fens, including calcareous fens. It is found in the association *Salicetum pentandro-cinereae*, and also forms the shrub layer in meadow communities. It occurs less frequently on mineral soils in that region.

The study was carried out in 2008. Eighteen sites of *Salix purpurea* representing different habitats were selected. Six sites of purple willow were located on organic soils in the peatland near Sawin (OSS). Six sites were located on organic soils in the Krowie Bagno peatland (OSK), and six sites on mineral soils – on the edge of the Krowie Bagno peatland (MSK).

In each of the sites samples of the 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 and homogenised, and dried at room temperature. The pH of the dried samples was analysed in distilled water and in 1 M KCl. Organic matter was determined by loss ignition at 550^{o}C in a muffle furnace. Available P and K forms were assessed by means of the Egner-Rhiem method. The content of Ca, Mg, Fe, Mn, Ni, Cu, Pb, Zn and Cd, extracted in 0.5 M HCl in the organic soils and 1 M HCl in the mineral soils, were measured using Atomic Absorption Spectrophotometry.

Simultaneously, in chosen sites, the annual willow shoots were cut in November 2008. The shoots were washed thoroughly with deionised water. Bark for chemical analysis was separated from the wood by peeling and dried at 40^{o}C to constant weight and subsequently milled. Milled bark samples were dissolved in a microwave in concentrated nitric acid. The content of Zn, Pb and Cd were measured using Atomic Absorption Spectrophotometry.

The normality of the data distribution was analysed using the Shapiro-Wilk test. The non-parametric Kruskall-Wallis test was used when the hypotheses for the parametric test were not confirmed. All data analyses were carried out in Statistica 5.1 program. The Principal Component Analysis (PCA) was performed using the program MVSP.

RESULTS AND DISCUSSION

The soils of the study sites differed significantly in a majority of the parameters studied. The considerable distance of the OSS, OSK and MSK sites in the PCA diagram (Fig. 1) confirms the diversity of the content of the parameters

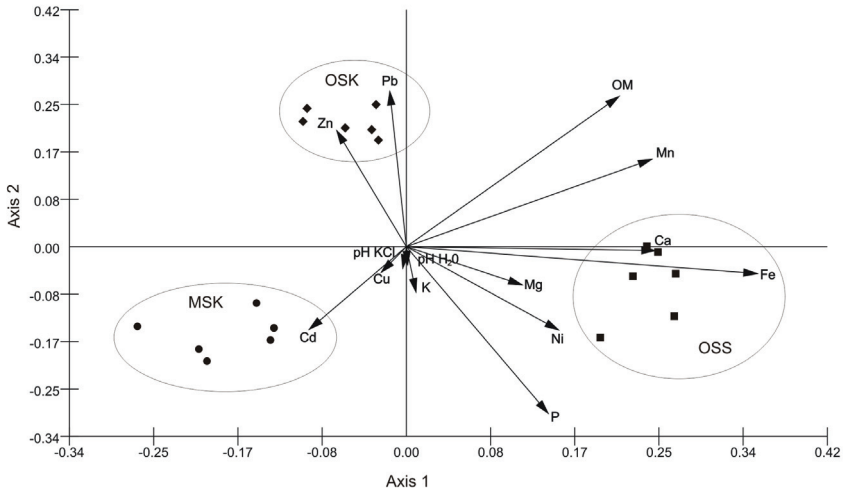

Fig. 1. Results of PCA analysis: OSS – organic soils in the peatland near Sawin, OSK – organic soils in the Krowie Bagno peatland, MSK – mineral soils – edge of Krowie Bagno peatland

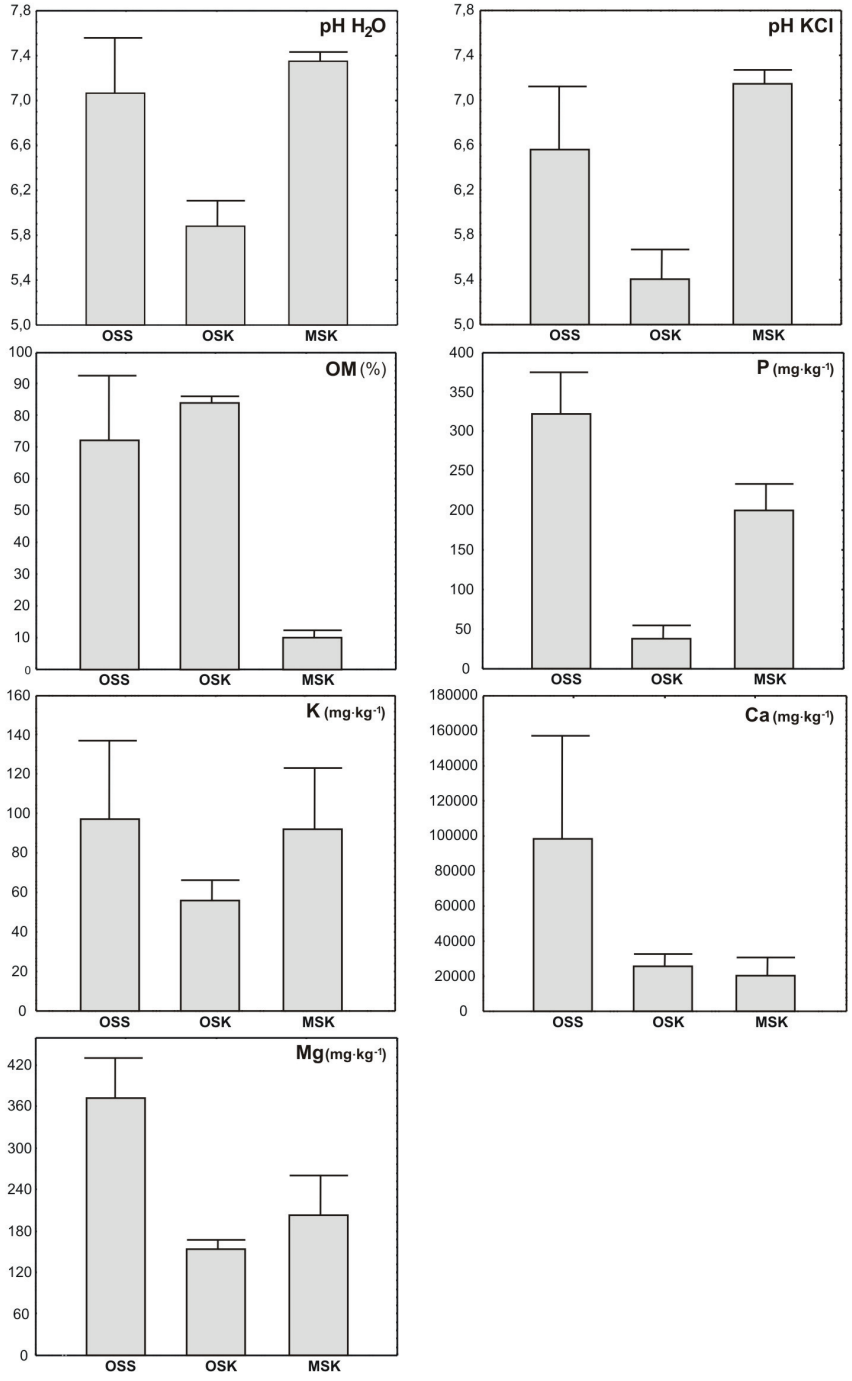

Fig. 2. Average value and standard deviation of studied soil properties; OSS – organic soils in the peatland near Sawin (n = 6), OSK – organic soils in the Krowie Bagno peatland (n = 6), MSK – mineral soils – edge of Krowie Bagno peatland (n = 6)

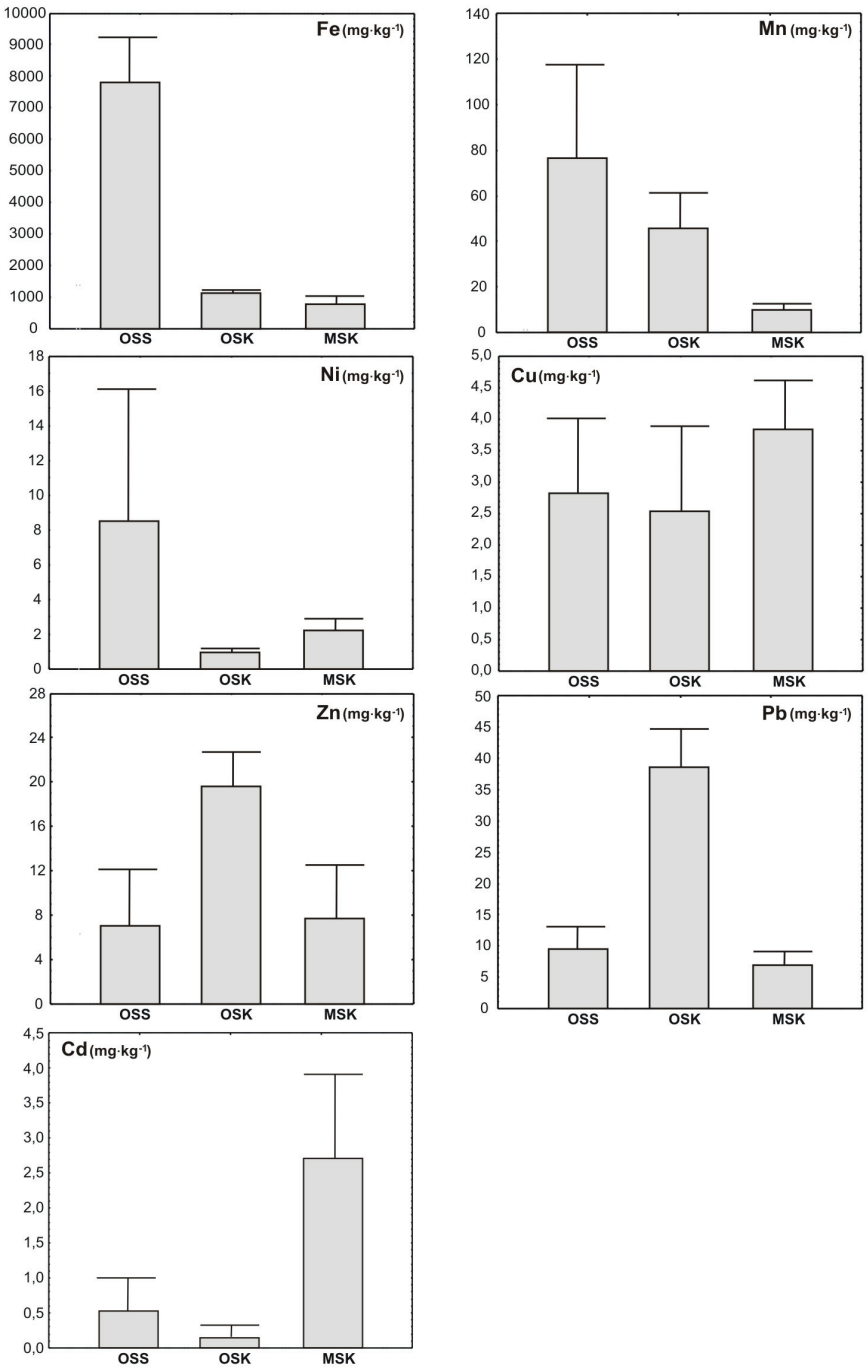

Fig. 3. Average value and standard deviation of heavy metal contents in the studied soils; OSS – organic soils in the peatland near Sawin (n = 6), OSK – organic soils in the Krowie Bagno peatland (n = 6), MSK – mineral soils – edge of Krowie Bagno peatland (n = 6)

of interest not only between the mineral and organic soils, but also between the organic soils (OSS and OSK). Fe, Ca, Pb, Zn, P, OM, Mn and Cd had the biggest effect on soil diversity, whereas pH H_2O, pH KCl, K, Cu, Mg and Ni displayed the least effect.

The reaction of the OSK soil was usually acidic, OSS – neutral, and that of MSK – alkaline (Fig. 2). The average OM content was 72.5% in OSS, 84.2% in OSK and 10.2% in MSK. The content of available phosphorus in MSK was very high [Fertilisation recommendations 1990]. The content of this element in OSS was moderate, and in OSK it was very low. The largest quantity of available phosphorus was accumulated in muck layers [Pawluczuk 2006]. Hence, there was a relatively high content of this element in the dehydrated surface layer in OSS. The K content was considerably low in the organic soils and low in the mineral soils [Fertilisation recommendations 1990]. The Ca and Mg contents were comparable in the OSK and MSK soils, and several-fold higher in OSS. Moreover, the OSS soils were characterised by a high Fe content (Fig. 3). The high content of these elements may result from the fact that peat was mined in the Sawin peatland. Consequently, the top layer of the soil is dehydrated and undergoes the muck formation process. Several studies have also demonstrated that the enrichment of muck layers of the peat-muck soils in P, Fe and Mn resulted from organic matter becoming muck [Urbaniak and Sapek 2004, 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 OSS: Fe > Mn >Pb > Ni > Zn > Cu > Cd; for OSK: Fe > Mn > Pb > Zn > Ni > Cu > Cd; and for mineral soils Fe > Mn > Zn > Pb > Cu > Cd > Ni (Fig. 3). The concentration of Pb, Ni, Zn and Cu was relatively low, both in organic and mineral soils. Cd, the content of which in some sites on mineral soils was higher than the maximum permissible concentration, was an exception [Regulation of the Ministry of the Environment 2002]. The high Cd concentration, in turn, might result from fertilisation of the neighbouring fields with phosphorous fertilisers containing relatively high amounts of this element [Jarosz and Nowińska 1992].

The soils of the study localities differed in most of the parameters investigated in a statistically significant manner (Tab. 1). Only for Cu were the differences between the contents of this element statistically insignificant. However, no

Table 1. Differences in heavy metal contents and studied soil properties between the three habitats of *Salix purpurea*

pHH_2O	pHKCl	OM	P	K	Ca	Mg
**	***	**	**	*	**	**
Fe	Mn	Ni	Cu	Zn	Pb	Cd
***	**	***	n.s.	**	**	**

*$0.01 < P \leq 0.05$, **$0.001 < P \leq 0.01$, ***$P \leq 0.001$, n.s. – no significant; OSS – organic soils in the peatland near Sawin (n = 6), OSK – organic soils in the Krowie Bagno peatland (n = 6), MSK – mineral soils – edge of Krowie Bagno peatland (n = 6)

differences in the heavy metal contents were observed in *S. purpurea* bark (Fig. 4). The statistical analysis did not demonstrate differences in the concentration of Zn, Pb and Cd. The maximum permissible content of the metals in the bark were: 0.3 $mg \cdot kg^{-1}$ for Cd, 2 $mg \cdot kg^{-1}$ for Pb, and 50 $mg \cdot kg^{-1}$ for Zn [Kabata-Pendias *et al*. 1993, Regulation of the Minister of Health 2003]. The concentration of Zn and Cd in the bark sampled from all the sites considerably exceeded the abovementioned values. The Pb content was the only value that was within the norm [Regulation of the Minister of Health 2003]. The Zn content in the bark on OSK soils was over 12-fold higher in comparison to Zn concentration in the soil. It was similar for Cd: its content in the bark sampled from OSS was over 2-fold higher, and in the bark sampled from OSK over 4-fold higher compared to the content of this element in the soils. The highest Cd content was reported from plants growing on mineral soils; it was on average 4-fold higher than the maximum permissible concentration [Regulation of the Minister of Health 2003]. The permissible concentrations were also exceeded in the case of Zn; in OSK, the concentration of this metal was on average almost 5-fold higher, while in MSK it was 3-fold higher.

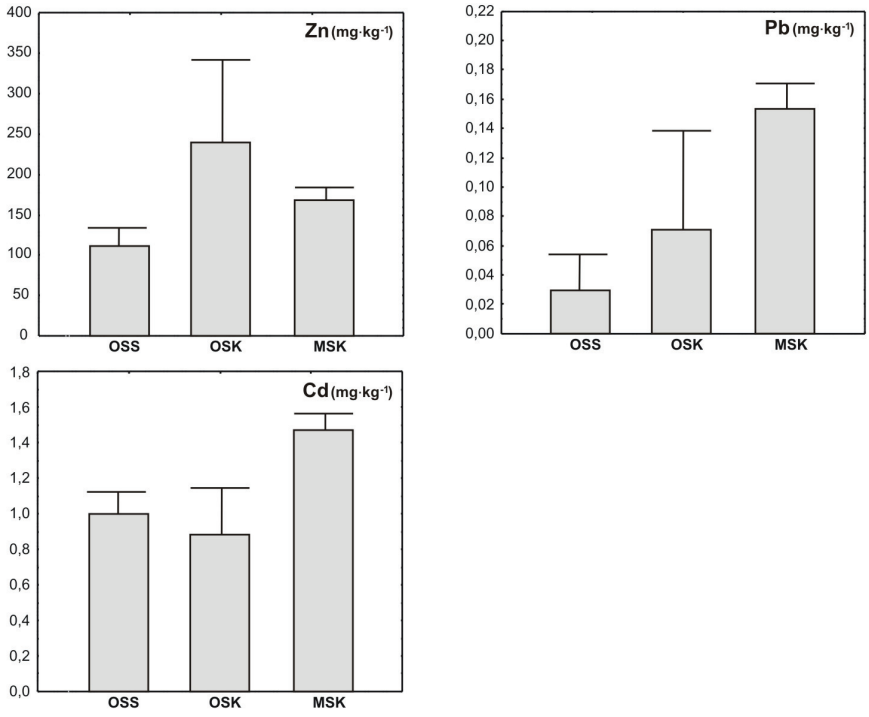

Fig. 4. Average value and standard deviation of chosen heavy metal contents in bark; OSS – organic soils in the peatland near Sawin (n = 6), OSK – organic soils in the Krowie Bagno peatland (n = 6), MSK – mineral soils – edge of Krowie Bagno peatland (n = 6)

Salix purpurea with a high salicylic glycoside content of the bark is recommended for herbal production and is a promising source of herbal drugs [Schmid 1998, Szczukowski *et al.* 2002, Sugier 2005, Sulima *et al*. 2006, Sugier and Sugier 2007, Förster *et al*. 2009]. As it is clear from the present study, this species displays an ability to accumulate Zn and Cd in the bark in amounts that are several-fold higher than the maximum permissible content [Regulation of the Minister of Health 2003]. Landberg and Greger [1996] reported high concentration of heavy metals in the shoots of this species as well. *S. purpurea* from the study localities has the ability to accumulate large amounts of Zn and Cd in the bark, which limits its potential as herbal raw material.

CONCLUSIONS

1. The *Salix purpurea* habitats studied are highly diverse in terms of physicochemical parameters, even within the same peatlands. This is determined by such parameters as Fe, Ca, Mn, P, Zn and Pb levels.
2. The studied soils are characterised by a relatively low content of heavy metals which may be transported and deposited in *S. purpurea* shoots. Cadmium, high concentration of which was observed in the mineral soils and in the bark of willows growing thereon, is an exception.
3. The high heavy metal content in *S. purpurea* bark disqualifies it as herbal raw material and indicates that this crop should be cultivated under controlled conditions.
4. In the case of obtaining raw material from its natural state, attention should be paid to the content of heavy metals in the bark, since even with a relatively low content thereof in the soil the concentration of these metals in the bark of the studied species may be considerably high.

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CHARAKTERYSTYKA WARUNKÓW ABIOTYCZNYCH ORAZ ZAWARTOŚĆ METALI CIĘŻKICH W KORZE WIERZBY FARMACEUTYCZNEJ *Salix purpurea* L. NA POJEZIERZU ŁĘCZYŃSKO-WŁODAWSKIM

Streszczenie. Celem badań była charakterystyka warunków siedliskowych *Salix purpurea* L. oraz sprawdzenie, czy wierzba purpurowa, która może być potencjalnym źródłem surowca *Salicis cortex*, ma zdolność akumulacji dużych ilości Zn, Pb i Cd w korze. Badania przeprowadzono w roku 2008 na osiemnastu stanowiskach wierzby purpurowej reprezentujących różne typy siedlisk. W każdym z nich z wierzchniego poziomu pobrano próbki glebowe i oznaczono w nich pH, materię organiczną, makro- i mikroelementy. Jednocześnie na badanych stanowiskach ścięto jednoroczne pędy, a pobraną korę przygotowano do analiz chemicznych. W zmielonych próbkach oznaczono Zn, Pb i Cd. Gleby badanych stanowisk są bardzo zróżnicowane pod względem większości badanych czynników. Decydują o tym głównie takie parametry jak Fe, Ca, Mn, P, Zn i Pb. Gleby charakteryzują się stosunkowo małą zawartością metali ciężkich. Wyjątek stanowi kadm, którego wysoką koncentrację stwierdzono w glebach mineralnych oraz korze rosnących na nich wierzb. Duża zawartość metali ciężkich w korze *S. purpurea* ogranicza możliwości pozyskiwania surowca zielarskiego ze stanu naturalnego i skłania do uprawy tego gatunku w kontrolowanych warunkach.

Słowa kluczowe: *Salix purpurea,* metale ciężkie, wierzba farmaceutyczna, surowiec zielarski