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ORIGINAL RESEARCH PAPER

Impact of road transport on soil physicochemical characteristics and heavy metal concentrations in the bark of purple willow (*Salix purpurea* L.)

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Abstract
The aim of this study was to determine the impact of road transport on soil physicochemical characteristics and concentration of heavy metals in the bark of purple willow. The study was carried out at two groups of sites situated along a national road at a distance of 5–10 m and ca. 100 m from the road. At each of the sites, annual willow shoots were cut and surface soil samples were taken. The concentrations of Zn, Pb, and Cd in bark samples were measured and of K, Ca, Mg, Zn, Cr, Cu, Ni, Mn, Pb, and Cd in the soil. The concentrations of Mn and Pb were predictably higher in soils located near the road, which may indicate an impact of road transport on the soil content of these metals. The concentrations of Zn, Pb, and Cd in *S. purpurea* bark from the sites located at distances of 5–10 m and ca. 100 m from the road were similar. However, the Cd concentration in the bark exceeded the maximum permissible concentration in the dried material, despite the low Cd concentrations in the soils at of all the sampling sites. It is important to pay due attention to the concentrations of this heavy metal in this plant material when it is intended for pharmaceutical use, even in that obtained from plants growing on soils qualifying as "uncontaminated". *Salix purpurea* used for *Salicis cortex* should therefore be cultivated under controlled conditions.

Keywords
purple willow; bark; road transport; contaminants; heavy metals; Zn; Pb; Cd

Introduction

Willows (*Salix* species) have an ability to synthesize diverse phenolic compounds such as salicylates, cinnamic acid derivatives, flavonoids, and condensed tannins, which are important secondary metabolites [1–4]. Willows with high salicylate concentrations, e.g., *S. purpurea* L., *S. daphnoides* Vill., *S. fragilis* L., and *S. myrsinifolia* Salisb., are recommended for herbal production and are a promising source of herbal drugs [5–11]. Phenolic glycosides are very important for human health, and willows as herbal drug plants have a long-lasting history in remedy traditions [3]. *Salix* extracts and bark drugs are in current use for the treatment of pain and inflammation [12–15]. Several studies have however shown that *Salix* species exhibit a capacity to accumulate high concentrations of heavy metals, especially in the leaves and in and on the bark [16–20]. Wood and bark are important sinks for biologically-available metals, whose concentrations in wood are frequently lower than in roots and bark [21,22]. Particularly noteworthy are Cd and Zn, which are more easily transported to the shoots [23] and accumulated in the wood and bark, i.e., the pharmaceutical raw material from willow.

The development of road transport contributes to accumulation of various types of pollutants in soils, including heavy metals particularly when located in the immediate vicinity of busy communication routes [24–27]. A significant number of such areas close to road systems are used agriculturally, which may therefore pose a toxicological threat to crop plants and soil organisms due to the increasing concentrations of heavy metals, especially in their bioavailable forms [28]. In general, the heavy metal concentrations in surface soils near roads decreases with increasing distance from the road [29–33]. Among these are Cd, Pb, and Zn which are classified as metals with a very high degree of potential hazard [28,29] and can thus pose a threat to living organisms occupying these habitats. Furthermore, particular attention should be paid to plant species which are a source of herbal raw materials for man. Although they are introduced into cultivation, many species are also obtained from both natural or anthropogenic habitats. Very often, herbal plants occur along roadsides and are collected due to easy access and transport. Unfortunately, medicinal plants frequently exhibit a capacity to accumulate toxic elements selectively, and the heavy metal concentrations in many herb plant species can exceed permissible safe concentrations [34–37]. Some herbal plants such as the common dandelion are actually regarded as good indicators of anthropogenic toxic metal pollution of the environment [24,25,38]. Food chain transfers may also ultimately result in accumulation of elements in the human organism and, in the extreme, eventual disturbance in physiological processes [39,40].

Willow bark is obtained for the production of natural aspirin from various willow species in both natural and anthropogenic localities [41–43], and the purple willow is a common component of plant communities occurring on the roadsides and accompanying ditches in Poland. In the current literature, much attention is paid to heavy metal contamination of herbs [24,25,34–37]. However, less information is available on the concentration of these elements in pharmaceutically utilized *Salix* tissues [44]. It therefore seems important to examine the ability of pharmaceutical willow to accumulate heavy metals in the bark and the dangers related to their presence in the raw material obtained for the pharmaceutical industry and, hence, in drugs produced from the milled bark. It is also important to understand the relationship between heavy metals in soils and bark of pharmaceutical willow species. The aim of this study was to determine the impact of road transport on selected soil physicochemical characteristics and the concentrations of heavy metals in purple willow bark.

Material and methods

Description of the study site

The study was carried out at sites situated along the national road No. 82 in mideastern Poland (51°25′22″ N, 23°14′10″ E). Six sites of *S. purpurea* located at a distance of 5–10 m from the road (SPR) and six sites distant >100 m from the road (SPN) were selected.

Plant and soil materials

At each of the sites, annual willow shoots were cut manually in November 2014. Simultaneously, soil samples were taken from topsoil (0–20 cm) at six loci from each site by means of a core-drill. The soil material was thoroughly homogenized and air-dried at room temperature and then sieved through a 2-mm stainless steel sieve. Willow shoots were washed thoroughly with deionized water. Bark for chemical analysis was separated from the wood by peeling, dried at 40°C to constant weight, and milled afterwards. Milled bark samples were digested in a microwave oven in closed Teflon vessels using a mixture of concentrated Suprapur nitric acid and hydrogen peroxide. The pH of the soil samples was measured in distilled water (v/v 1:5) and in 1 M KCl (v/v 1:5) with a pH glass electrode using the supernatant after mixing. Organic matter (OM) content was determined by loss-on-ignition at 550°C in a muffle furnace. Phosphorous was determined colorimetrically using the Egner-Riehm method. The concentrations of Zn, Pb, and Cd in the digested bark samples and those of K, Ca, Mg, Zn, Cr, Cu, Ni,

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Pb, and Cd from soil extracted in 1 M HCl were measured by flame atomic absorption spectrophotometry (Perkin Elmer 1100B).

Statistical analysis

The Levene's test and Shapiro–Wilk test was used to ascertain the homogeneity of variances in the data sets and to determine the normality of the distributions of the data. Mann–Whitney test was used to detect significant differences between specific sample mean pairs. Variation in the soil characteristics and heavy metal concentrations in the bark were explored using principal components analysis (PCA) [45]. Before analysis, the soil data were centered and log transformed, and the bark data were centered and standardized. The PCA analyses were conducted using the multivariate statistical package (MVSP) [46]. All other statistical analyses were carried out using the Statistica 6.0 software package [47].

Results

Metals in the soil

The physical and chemical characteristics of the topsoil horizon from the sites sampled are summarized in Tab. 1. The results of the PCA showed considerable variation in these soil characteristics (Fig. 1, Tab. 2) and the data revealed clear differentiation between the sites. The first two principal components account for most of the variation in the data set (79.5% of the total variance). The first axis explains 49.4% of the total variance and represents Ca, Mg, and P. The second axis accounts for a further 30.1% and mostly represents Zn and Ca (Fig. 1). The SPN sites placed on the left-hand side of the ordination diagram show higher Ca, P, and Mg than the SPR sites located on the right-hand side. Furthermore, the larger dispersion of points representing the SPN sites in the ordination space indicates that they are more variable in terms of the parameters measured, especially Zn.

The reaction of the soil at SPR and SPN was usually neutral (Tab. 1) and the mean organic matter content in these surface soils was 10.23% for SPR and 10.15% for SPN. The concentrations of all the macroelements studied in the SPR soils were slightly higher than in the SPN soils, and the differences are statistically significant in the case of P, Ca, and Mg. The analyses reveal that the mean concentrations of heavy metals can be arranged in the following decreasing series: Fe > Mn > Zn > Pb > Cu > Cd > Ni for SPR, and Fe > Zn > Mn > Pb > Cu > Cd > Ni for SPN. The concentrations of Fe, Ni, Mn, and Pb were higher for SPR than SPN soils. However, statistically significant differences are confirmed only for the two latter metals. The concentrations of Cu, Zn and Cd in the soil samples were all similar.

Metals in plant tissues

The Zn, Pb, and Cd concentrations in the willow bark are presented in Tab. 3. The results of the PCA show the variation in the heavy metal concentrations in the bark of *S. purpurea* (Fig. 2, Tab. 4). The first two principal components account for most of the variance in the data set (40.6% of total variance). The first axis explains 26.2% and represents Zn, Pb, and Cd. The second axis accounts for 14.4% and mostly represents Pb and Cd. The points representing the heavy metal concentrations in the bark on the ordination diagram are characterized by large variabilities, and it is not possible to show a clear boundary between the two groups analyzed. The willow bark has relatively high heavy metal concentrations; the mean for Zn, Pb, and Cd in the bark ranges from 168 mg kg^{-1} in SPR to 151 mg kg^{-1} in SPN, from 0.23 mg kg^{-1} in SPR to 0.30 mg kg^{-1} in SPN, and from 1.08 mg kg^{-1} in SPR to 1.51 mg kg^{-1} in SPN, respectively. The concentrations of Zn, Pb, and Cd in the *S. purpurea* bark at the SPR and SPN sites are similar and no statistically significant differences were found.

Tab. 1 Characteristics of the topsoil horizon for the *S. purpurea* sites located at a distance of 5–10 m from the road (SPR; *n* = 6) and sites of distant >100 m from the road (SPN; *n* = 6).

Soil Characteristic	SPR				SPN			
	Mean	Min	Max	*SD*	Mean	Min	Max	*SD*
pH (H_2O)	7.34	7.23	7.43	0.07	7.06	6.27	7.36	0.38
pH (KCl)	7.14	6.97	7.30	0.10	6.80	5.74	7.19	0.52
OM (%)	10.23	7.08	11.95	1.76	10.15	4.60	16.28	4.78
P (mg kg^{-1})	186.92 [b]	130.92	248.75	40.42	71.51 [a]	43.64	98.35	23.10
K (mg kg^{-1})	88.60	61.60	137.20	25.34	66.93	30.80	118.80	27.33
Ca (mg kg^{-1})	23,646 [b]	16,720	34,520	5,878	6,313 [a]	4,280	10,136	2,334
Mg (mg kg^{-1})	222.13 [b]	145.60	259.20	40.71	115.67 [a]	86.40	168.80	30.01
Fe (mg kg^{-1})	826	536	1168	216	730	304	1,464	359
Mn (mg kg^{-1})	10.83 [b]	8.12	13.08	1.77	6.39 [a]	2.48	9.84	2.82
Ni (mg kg^{-1})	2.50	1.44	3.44	0.69	1.91	0.88	2.72	0.58
Cu (mg kg^{-1})	4.05	2.84	4.92	0.69	5.73	2.56	9.56	2.86
Zn (mg kg^{-1})	8.24	3.28	16.96	4.28	8.91	6.08	12.60	2.00
Pb (mg kg^{-1})	7.85 [b]	6.08	9.88	1.47	5.49 [a]	4.32	8.08	1.35
Cd (mg kg^{-1})	2.93	1.24	5.04	1.36	5.02	2.00	8.36	2.61

Different letters indicate significant differences according to the Mann–Whitney test. All elemental data relate to the 1 M HCl-extractable concentrations measured.

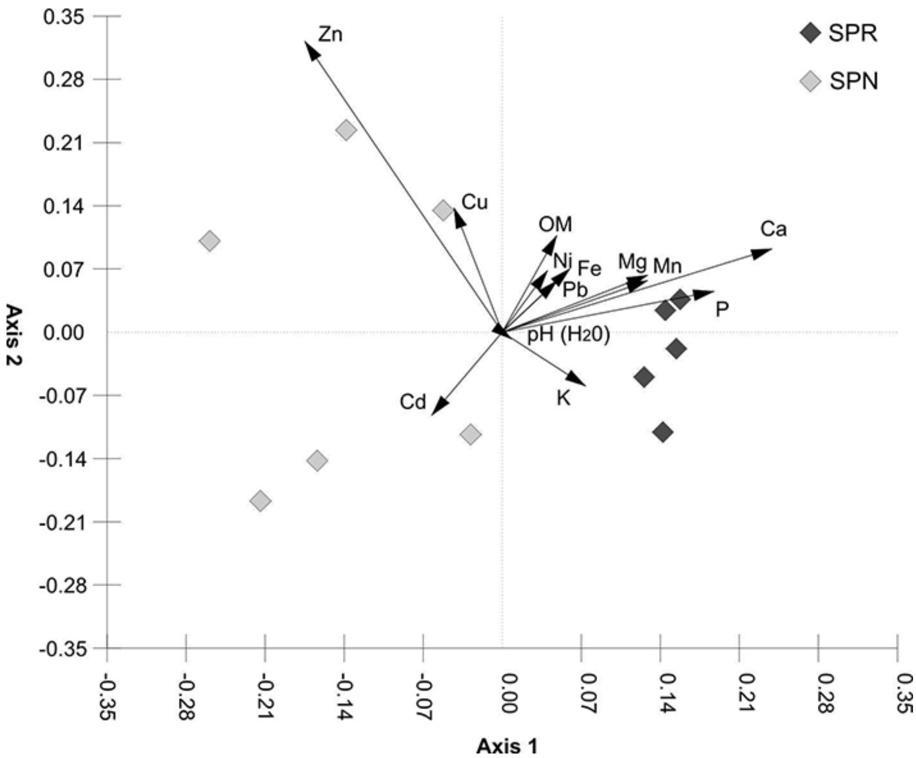

Fig. 1 Results of a PCA based on the soil physicochemical characteristics of *S. purpurea* sites. SPR – sites of *S. purpurea* located at a distance of 5–10 m from the road; SPN – sites of *S. purpurea* distant >100 m from the road.

Tab. 2 Results of a PCA based on the soil physicochemical characteristics of *S. purpurea* sites. (**A**) Eigenvalues and variance (%) explained by the first two PCA axes; (**B**) Loading components for each variable associated with the two axes.

Heavy metals	Axis 1	Axis 2
(A)		
Eigenvalues	0.285	0.174
Percentage	49.39	30.08
Cumulative percentage	49.39	79.47
(B)		
pH (H_2O)	0.02	−0.018
OM	0.115	0.255
P	0.446	0.107
K	0.175	−0.142
Ca	0.568	0.219
Mg	0.306	0.15
Fe	0.142	0.167
Mn	0.306	0.136
Ni	0.095	0.162
Cu	−0.102	0.328
Zn	−0.415	0.766
Pb	0.116	0.136
Cd	−0.148	−0.219

Tab. 3 Concentrations of selected heavy metals (Zn, Pb, Cd) in the bark of *S. purpurea* at the studied sites located at a distance of 5–10 m from the road (SPR; n = 6) and sites of distant >100 m from the road (SPN; n = 6).

	SPR				SPN			
Heavy metal	Mean	Min	Max	*SD*	Mean	Min	Max	*SD*
Zn (mg kg^{-1})	167.92	141.00	187.90	15.89	150.73	120.40	173.20	18.60
Pb (mg kg^{-1})	0.23	0.12	0.63	0.18	0.30	0.12	0.51	0.16
Cd (mg kg^{-1})	1.08	0.58	1.53	0.33	0.99	0.57	1.51	0.30

Discussion

In the present study, the values of all the macroelements analyzed were slightly higher in the SPR soils than in the SPN soils, and the differences were statistically significant in the case of P, Ca, and Mg. The greatest attention should, however, be paid to the heavy metals. The concentrations of 1 M HCl-extractable Fe, Ni, Mn, and Pb were also higher in the SPR than in the SPN soils but statistically significant differences were confirmed only for Pb and Mn. The general trend presented above is in good agreement with the results from other research confirming the fact that the concentrations of heavy metals in soils located near roads decrease with increasing distance from roads, and so their highest concentrations is close to the transport route [30,33]. As demonstrated by Modrzewska and Wyszkowski [32], the concentrations of Pb, Cd, Cr, and Ni in soils located directly adjacent to the road (25 m) were higher than that of these elements in the top layer of soil taken from distances of 50 m and 100 m. Cadmium, Pb, and Zn are classified as metals with a very high degree of potential hazard to human health [28,29,48–51]. The permissible concentrations of Zn, Ni, and Pb in soils used for agriculture are <300 mg kg^{-1}, <100 mg kg^{-1}, and 100 mg kg^{-1}, respectively [52]. Therefore, the concentrations determined in SPR and SPN soils are below these limits, taking

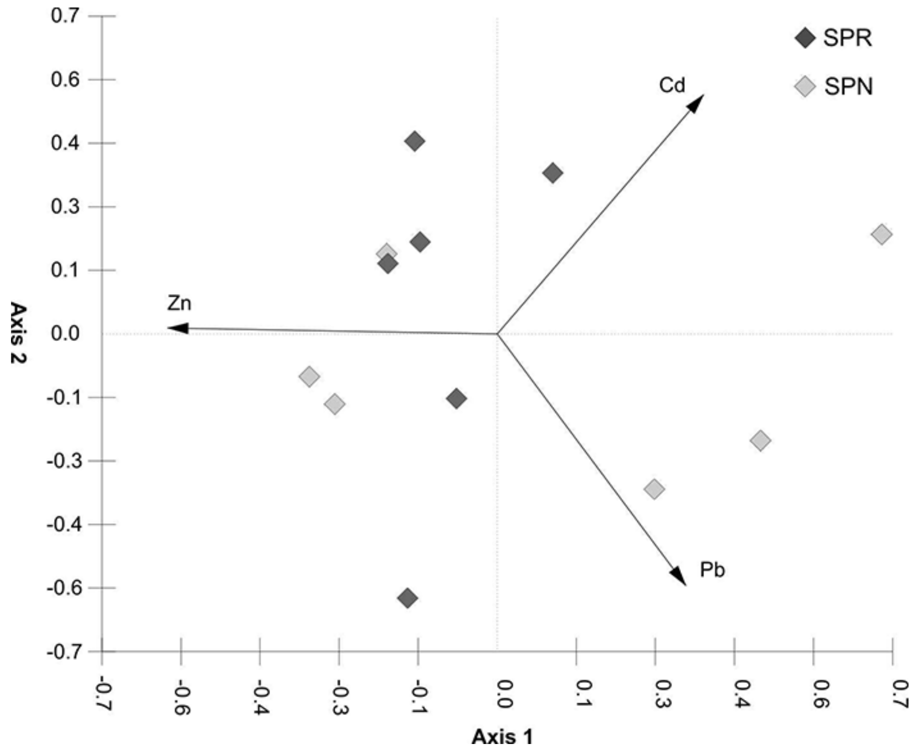

Fig. 2 Results of a PCA based on the Zn, Pb, and Cd concentration in bark of *S. purpurea*. SPR – sites of *S. purpurea* located at a distance of 5–10 m from the road; SPN – sites of *S. purpurea* distant >100 m from the road.

Tab. 4 Results of a PCA based on the Zn, Pb, and Cd concentration in bark of *S. purpurea*. (**A**) Eigenvalues and variance (%) explained by the first two PCA axes. (**B**) Loading components for each variable associated with the two axes.

Heavy metals	Axis 1	Axis 2
(A)		
Eigenvalues	3.662	2.019
Percentage	26.16	14.42
Cumulative percentage	26.16	40.58
(B)		
Zn	−0.763	0.017
Pb	0.436	−0.725
Cd	0.477	0.689

into account the fact that forms extracted in 1 M HCl constitute about half of the total content. The Pb concentration is obviously related to the overall pollution gradient in space and time. The highest increases in environmental Pb concentrations in the last decade of the twentieth century reflected emissions originating from the combustion of leaded gasoline by traffic [48,49] or local sources of contamination from industrial areas [50]. Similarly, the Mn concentration in the surface soil layers may be connected with the increased level of motorization [51]. Manganese also originates from fuel combustion which probably explains the higher concentrations of Mn and Pb in the surface layers of the SPR soils in comparison with those at the SPN sites (Tab. 1). The Cd concentration was highly variable between sites; in a majority of cases, it was >4 mg kg^{-1}, and it exceeded 6 mg kg^{-1} at four sites. Both the SPR and SPN soils may therefore be considered as contaminated with Cd [52]. It should be added that the extractable Cd fraction here is only a part of the total concentration in the soil. Fertilizers often contain elevated amounts of heavy metals (notably Cd in phosphates), which can increase the concentrations of the available forms of these elements in the soil when there has been long-term fertilizer application [53,54]. The high Cd background concentration in the region where there are no point sources of pollution might therefore result from fertilization of the neighboring fields with phosphatic fertilizers containing relatively high amounts of this element in addition to the parent geology [28,55–57].

In studies on the concentrations of heavy metals in herbal raw materials, soils located along roads are often characterized by higher heavy metal concentrations [58]; similarly, the plants growing in these soils. Prior to the present research, it was assumed that the concentrations of heavy metals in soils located along roads would be higher than in the soils sampled at a distance of >100 m from the road. The results of our analysis showed a completely different picture. In the present study (Tab. 3), the Pb concentrations in the bark from the SPR sites (0.23 mg kg^{-1}) and SPN sites (0.30 mg kg^{-1}) were normal [59] and did not exceed the maximum permissible concentration in the dried raw material [60]. Lead is mainly accumulated in the roots of plants as it is only weakly translocated [23]. This may account for its relatively low concentration in the bark of *S. purpurea* from both the SPR and SPN sites. Therefore, from the point of view of the concentration of this metal, willow bark can be regarded as a safe raw material that can be used in the pharmaceutical industry. However, the Cd concentrations are somewhat different (Tab. 4); its concentration in the bark from SPR (1.08 mg kg^{-1}) and SPN (0.99 mg kg^{-1}) exceeds the maximum permissible concentration in the dried material [60]. Despite the low Cd concentration in the soil at both SPR and SPN, that of this metal in the bark was high. The problem of accumulation of excessive Cd amounts has been described in willows by other workers (e.g., [44]), as well as in other herbal plants. Increased concentrations of this element have been found in rosemary, tarragon, lovage, ribwort plantain, and common nettle raw materials [61,62]. Unfortunately, this fact disqualifies the raw materials from their use in the pharmaceutical industry. Cultivation of purple willow taken from natural habitats can provide a glycoside-rich raw material [42] and can reduce the risk of *Salicis cortex* contamination with heavy metals, especially Cd. Several other studies have shown that *Salix* species exhibit a capacity to accumulate high concentrations of Cd, Pb, and Zn, especially in leaves and bark [16–20], all of which are used as pharmaceutical materials. Wood and bark are important sinks for biologically available metals [22,63], and metal concentrations in wood are frequently lower than in roots and bark [60]. Particularly noteworthy are Cd and Zn, which are more easily transported to shoots [23] and accumulated in wood and bark. These tissues are slow to enter the decomposition cycle and therefore accumulated metals can be immobilized in a metabolically inactive compartment for a considerable time [22,63].

Salix purpurea is a common species in Poland found mostly beside rivers and streams in, for example, *Salicetum triandro-viminalis* associations. It frequently grows on arable land, peatlands, and along roads. Some authors [42] emphasize the selection and introduction of willows with a high salicylic glycosides concentration for cultivation under controlled conditions. We strongly support this proposal, taking into account the capacity of *S. purpurea* for heavy metal accumulation in and on its bark, as demonstrated in this study (Tab. 4), and the risk of their inclusion in the food chain.

Conclusions

Given the widespread occurrence of the purple willow, its bark can be obtained as a raw material from both natural and anthropogenically-transformed stands, such as those located near roads. This poses a threat of contamination of the raw material with heavy metals and, as shown in this study, such a danger in the case of Cd also occurs at a considerable distance from transport routes. A low concentration of the extractable forms of Zn or Cd in the soils does not ensure an equally low level of this element in the bark. It seems important to pay attention to the concentration of these heavy metals in plant materials intended for pharmaceutical use, even in those obtained from plants growing on soils qualifying as uncontaminated. *Salix purpurea* plants can accumulate concentrations exceeding the acceptable limits in their bark. This has implications for the cultivation of this willow for pharmaceutical purposes in controlled conditions, i.e., on soils low heavy metal status.

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Wpływ transportu drogowego na właściwości gleb i zawartość metali ciężkich w korze wierzby purpurowej (*Salix purpurea* L.)

Streszczenie

Celem badań było określenie wpływu transportu drogowego na właściwości gleb i zawartość metali ciężkich w surowcu (korze) wierzby purpurowej. Badania przeprowadzono na stanowiskach położonych wzdłuż drogi krajowej w odległości około 5–10 m i około 100 m od drogi. W każdym z nich wycięto roczne pędy wierzby i pobrano próbki gleby. Określono koncentrację Zn, Pb i Cd w próbkach kory oraz zawartość K, Ca, Mg, Zn, Cr, Cu, Ni, Pb i Cd w glebie. Zawartość Cu, Zn i Cd w badanych glebach była podobna. Z kolei zawartość Mn i Pb była wyższa w glebach położonych przy drodze, co może wskazywać na wpływ transportu drogowego na zawartość tych metali. Koncentracja Zn, Pb i Cd w korze *S. purpurea* pobranej z miejsc położonych w odległości ok. 5–10 m i ok. 100 m od drogi była podobna. Jednak zawartość Cd w pozyskanym surowcu przekraczała maksymalne dopuszczalne jego stężenie. Istotne jest zatem zwrócenie szczególnej uwagi na zawartość kadmu w materiale roślinnym przeznaczonym do wykorzystania w przemyśle farmaceutycznym, nawet pozyskiwanym z roślin rosnących na glebach zakwalifikowanych jako niezanieczyszczone. Dlatego też *S. purpurea* przeznaczona do wykorzystania jako *Salicis cortex* powinna być uprawiana w kontrolowanych warunkach.