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Original Research

Contamination by Pb of *Hacquetia epipactis* Scop. DC and *Urtica dioica* L. Growing in Nature Reserves Affected by Local and Transboundary Emissions

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Abstract

The object of our study was to estimate Pb content in the legally-protected plant *Hacquetia epipactis,* as well as stinging nettle (*Urtica dioica*), in the nature reserves laid in the border zone between Poland and the Czech Republic. The following forms of Pb reserves in soil were determined for biotopes of these plants: pseudo-total content and speciation forms, as well as soluble and insoluble forms in dust settled on leaves. Migration of Pb was analyzed from forms directly bioavailable (exchangeable and adsorbed) and indirectly bioavailable (organically bound and carbonates) in soil in the soil-rhizome system. We also assessed the migration of Pb in settled dust system – leaves. Research shows that *H. epipactis* has the ability to accumulate Pb in the form of exchangeable and adsorbed Pb from the soil and soluble forms Pb in settled dust on the leaves.

Keywords: plants, metals, speciation analysis of soil, settled dust, nature reserves

Introduction

Most of the chemoecological studies have covered issues referring to the range of succession and prevalence of selected plants species, often determined by an excess or deficiency of selected elements in the natural environment. A number of plant species occur as relict forms, and, along with their biotopes, they exhibit various pathways of colonizing untypical land ecosystems. In consequence, phytosocioligists may encounter specific and, simultaneously, interesting phytogeographic examples. These dynamic and phytogeographic phenomena refer also to *Hacquetia epipactis* (Scop.) DC, (in Polish: cieszynianka wiosenna), a plant that in Poland is under strict species protection [1]. *H. epipactis* occurs in the natural environment of the Cieszyńskie foothills (Pogórze Cieszyńskie) and Silesian Beskids (Beskid Śląski) [2], and in selected biotopes of the Lubelszczyzna region [3], in the area of Mogilany [4]. The Northern border of range of occurrence of this species runs through Poland. Literature addressing *H. epipactis* is relatively rich [5-11]. It discusses the classification of communities, pharmacognostic properties, and synecological effects. In contrast, data is lacking on chemoecological studies referring to the accumulation of selected elements and their migration in the soil – plant (*H. epipactis*) – settled dust system.

In the fertile broadleaved forests (Pogórze Cieszyńskie) where this plant species occurs, permanent changes have been undergoing change as a consequence of persistent and uncomfortable transboundary emissions of dust originating

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from smelter plants and power plants, as well as local emission from the combustion of coal, often with high incineration [12, 13]. In consequence, *H. epipactis* is exposed to strong effects of specified environmental conditions, which is speculated to be the main reason of diminishing communities of *H. epipactis* occurrence [8, 14].

The objective of the study was to obtain reliable results on the presence of Pb in the biotope of *H. epipactis* and its potential effect on the intoxication of particular morphological parts of the plant. The aim of this study was:

- determine the cumulative features *H. epipactis* for Pb, in a trans-border impact of the emissions of particulate matter
- determine the resources of Pb ions in soil and dust settled on the leaves, which potentially can be accumulated by rhizomes and leaves
- determine the statistical characteristics and co-occurrence of Pb in rhizomes and leaves
- carrying out similar research on *U. dioica*

Material and Methods

Research Location

Nature reserves near Cieszyn (southern Poland), including the Municipal Woodland on the Puńcówka (Lasek Miejski nad Puńcówką), the Municipal Woodland on the Olza (Lasek Miejski nad Olzą), and Kopce are within the influence of increased metal immissions (Fig. 1). Those reserves were created to maintain and protect the population of *H. epipactis*. In that area, elements occur in the soil as a result of a smelter plant-power plant transboundary emission from the Ostrawsko-Karwiński Mining Region (Zagłębie Ostrawsko-Karwińskie) [15], and emission from local household hearths on the administrative area of the city of Cieszyn.

Fig. 1. Location of the tested reserves.

- Lasek Miejski nad Puńcówką (LMP), latitude 49º44′10.6″N, longitude 18º37′51.7″E – reserve floristic, area 6.96 ha, located on the right bank of the valley Olza, at the mouth of the river Puńcówka to Olza. LMP adjacent to the center of Cieszyn and factory buildings, public buildings and sports and recreation centers. Used as a municipal park
- Lasek Miejski nad Olzą (LMP), latitude 49º43′48″N, longitude 18º37′44″E, reserve floristic, area 3.23ha, situated on a steep right bank of the valley Olza, in the southern part of Cieszyn
- Kopce (K), latitude 49º46′41.38″N, longitude 18º36′37.5″E, forest reserve with an area of 14.76ha, located on the right bank of the valley Olza, in the northern city of Cieszyn, this reserve is the least altered floristically from among the respondents

Studied Plant Species

Botanists acknowledge *H. epipactis* as a migrating relic, growing in fertile, broadleaved forests and requiring a specific microclimate, soil, floristic composition, and the coexistence of other plant species. It should be emphasized, however, that *H. epipactis* is a plant characterized by high habitat requirements. *H. epipactis* grows well on clay soils with a neutral or barely acidic reaction and with a high moisture content. A high volume of atmospheric precipitation facilitates its vegetation and strong leaching of calcium carbonate from the soil. It is one of the earliest blooming species of plants in spring. The blooming and fruit bearing of *H. epipactis* span a long period and its vegetative season ends in October, hence this plant is not a typical geophyte. Inflorescences are shedding blossoms at the end of the blooming season. *H. epipactis* bears fruits in May and June, and its leaves take a dark-green color that hinders finding the plant in known biotopes. The major factors that negatively affect the succession of *H. epipactis* include: disturbances in the circuit of ground water, excessive thinning of tree sand (which facilitates deposition of fine-dispersive dusts in the profile of forest height), synanthropization of habitats, and the picking of single plants of this strictly protected species.

Sample Collection and Analytical Procedures

In each of the analyzed reserves, five sites of sample collection were selected: for *H. epipactis* – in the area of natural assemblages of occurrence in the reserves and for *U. dioica* – synanthropic stations on the edges of the reserves. The environmental material was collected in the spring of 2007, in the blooming season of *H. epipactis*.

Soil

Soil samples were taken from the rhizosphere level of the stands of the tested species occurrence (level Ol). On every stand five subsamples of soil were taken. A total of 75 samples of soil were collected. Soil samples in laboratory conditions, after being air-dried, ground in porcelain mortar were homogenization in sieves with a 1 mm cut-off. Samples were stored in polyethylene containers.

Pseudo-Total Contents of Metals in Soil

Samples of air-dried soil (1.000 g) were acidified with 15 cm^3 of spectrally pure HNO_3 (V) by heating in a water bath to 90-95ºC for one hour. In addition, the mineralizate was hot-flushed with 1% HNO_3. Mineralizate was supplemented with distilled water to 50 cm^3 [16].

Speciation Analysis of Soil

The occurrence of selected chemical forms of metals (speciation) in the soil was analyzed by means of extraction from soil samples (1.000 g) with the following solutions:

1. Exchangeable form – 1M KNO_3
2. Adsorbed form – 0.5 M KF, pH=6.5
3. Organically bound – 0.1 M $Na_4P_2O_7$
4. Carbonates – 0.1 M EDTA
5. Sulphides – 6 M HNO_3
6. Residues – 65% HNO_3

The samples were treated sequentially with subsequent reagents for 24 h at a ratio of 1:40, and then centrifuged (4000 rpm). After decanting, the remaining soil was rinsed with 10 cm^3 of redistilled water [17]. The resources of elements in the soil are divided into directly-bioavailable (exangeable and adsorbed forms) and indirectly (potentially)-bioavailable (organically bound and carbonates) [18-20].

Soil pH

Concentration of hydrogen ions in the soil was determined in a suspension of 1 M KCl. Determined potentiometrically with an Elmetron pH-meter CP-55.1.

Plant Material

The various morphological parts of plants (rhizomes and leaves) of *H. epipactis* and nettle were washed with distilled water and air-dried. Weighed portions (1.000 g) were acidified with 10 cm^3 of spectrally pure HNO_3 (V), by heating the solution to a temperature of 60ºC±1ºC. Clear mineralizate was transferred quantitatively to 25 cm^3 measuring flasks and filled with redistilled water [16].

Settled Dust

Dust settled on the leaves was obtained by washing the leaf lamina with distilled water (1000 cm^3). The resultant washings were filtrated through a hard filter. Soluble and insoluble forms of metal compounds in water were obtained. The suspension and the filtrate were treated separately with 3 cm^3 of spectrally pure HNO_3 (V). Clear solutions were transferred into measuring flasks with a volume of 25 cm^3 and filled with redistilled water [21].

Determination of Metals

The content of the following metals: Pb, Cd, Cu, Cr, Co, Ni, Mn, Fe, and Zn was determined by the atomic absorption spectrometric method (AAS) using a Philips Pye Unicam SP-9 spectrometer. The limited detection of the assays reached 0.01 $mg\cdot kg^{-1}$ and was controlled by means of comparative determinations conducted in the reference material: for plants CTA-VTL-2 (ICH and TJ), and soil CRM 277 (BCR). Validation of the methodology applied was carried out in cooperation with the Central Mining Institute in Katowice and the Department of Inorganic Chemistry at Silesia Technical University based on parallel determinations in 3 samples of soil and plants and in 3 samples of the reference material.

Statistical Analysis

Statistical analysis was conducted using Statistica 8 software and involved determinations of: geometrical mean, excess, kurtosis, variability coefficient, and Pb content corresponding to the 10th and 95th percentiles. And also determined correlation coefficients.

Results

The main reserves of Pb in the natural environment of *H. epipactis* include total contents of this element in soil and dust settled on leaves (Tables 1 and 2). The frequency of Pb occurrence in the settled dust as well as in the soil usually resembled normal distribution, but with a prevalence of left-sided distribution in the case of the settled dust and right-sided distribution in the case of soil. The pseudo-total content of Pb in soil from biotopes of *H. epipactis* ranged from 84 to 95 μg/g, with the variability of its occurrence reaching 18-44% (Table 1).

The average contribution of soluble forms of Pb in the dust settled on leaves is the highest in the LMO reserve 102.8 $mg\cdot kg^{-1}$ and the LMP reserve 69.6 $mg\cdot kg^{-1}$, and the lowest in the K reserve 33.5 $mg\cdot kg^{-1}$. The contribution of the insoluble forms of Pb fluctuates between 101 and 137 $mg\cdot kg^{-1}$ (Table 2). A typical characteristic of Pb occurrence in soluble and insoluble forms in the dust settled on leaves is the higher variability of Pb occurrence in soluble form (38-52%) as compared to the insoluble form (18%), except for the LMO biotope (61%). The environmental content of the soluble forms of Pb in the examined biotopes of *H. epipactis* is lower in the insoluble form and reaches 8-56 $mg\cdot kg^{-1}$ and 77.5-104.7 $mg\cdot kg^{-1}$ for the soluble and insoluble forms, respectively (Table 2).

Reserves of Pb being the most significant to *H. epipactis* and *U. dioica* occur in forms both directly and indirectly bioavailable from the soil (Table 3). On the stations of *H. epipactis* (on example of Kopce reserve), the contents of Pb occurring in particular chemical forms in the soil were as follows ($mg\cdot kg^{-1}$): exchangeable form 74.24, adsorbed form 8.98, organically bound 7.89, carbonates 14.52 (in the

Table 1. Pseudo-total Pb content in soil in the site of *H .epipactis* and *U. dioica* of the tested reserves (mg·kg^{-1}).

Reserve	Position	Geometric mean	Standard deviation	Percentile		Coefficient		
				10	95	Excess	Kurtosis	Variability %
LMP	*H. epipactis*	84.35	15.31	58.94	94.99	-1.98	3.94	18
	U. dioica	46.18	22.19	24.97	84.69	0.96	1.59	44
LMO	*H. epipactis*	96.10	23.57	79.50	137.58	1.58	2.69	24
	U. dioica	51.58	12.53	36.69	65.83	-0.40	-2.18	24
K	*H. epipactis*	95.76	46.48	41.73	163.07	-0.34	-0.43	44
	U. dioica	37.98	24.77	25.74	86.03	2.13	4.66	59

LMP – reserve Lasek Miejski nad Puńcówką
LMO – reserve Lasek Miejski nad Olzą
K – reserve Kopce

Table 2. Pb occurrence in soluble (S) and insoluble (I) fraction of the dust settled on the leaf surface of *U. dioica* of the tested reserves (mg·kg^{-1}).

Reserve	Fraction of settled dust	Geometric mean	Standard deviation	Percentile		Coefficient		
				10	95	Excess	Kurtosis	Variability %
LMP	S	69.63	38.94	49.53	144.15	2.06	4.39	52
	I	101.47	19.98	77.52	133.39	0.60	2.01	19
LMO	S	102.76	41.49	56.31	152.42	-0.35	-2.14	38
	I	137.04	94.39	87.83	319.51	2.04	4.28	61
Kopce	S	33.46	22.66	7.99	63.51	-0.64	-0.24	55
	I	137.94	25.45	104.76	168.32	-0.41	-0.41	18

LMP – reserve Lasek Miejski nad Puńcówką
LMO – reserve Lasek Miejski nad Olzą
K – reserve Kopce

biotope of *U. dioica*, the respective value accounted for (mg·kg^{-1}): exchangeable form 12.67, adsorbed form 6.79, organically bound 0.78, carbonates 16.51 (Table 3). In the case of Pb occurrence in the bioavailable forms in the rhizosphere of *U. dioica*, the content of Pb is comparable to those of Mn, Fe, and Zn. Among the potentially-bioavailable forms, noteworthy is the content of Pb on stations of *H. epipactis*, i.e. the content of Pb occurring in the form of carbonates accounts for 14.5 mg·kg^{-1}, whereas on stations of *U. dioica* the respective value reaches 16.5 mg·kg^{-1}.

Pb co-occurrence with chosen elements in the bioavailability and potential bioavailability forms in the soil of the 'Kopce' reserve is presented in Table 4.

Under conditions of the observed emission of Pb, the environmental content of Pb in rhizomes of *H. epipactis* was the highest in the Kopce reserve (8.12 mg·kg^{-1}) and the lowest in the LMP reserve (2.76 mg·kg^{-1}). It is worth emphasizing the relatively high variability of Pb occurrence in rhizomes of *H. epipactis* compared to the examined nettle originating from the same biotope (Table 5). The coefficient of variability of Pb occurrence in the rhizomes of *H. epipactis* accounted for 40-60%, whereas in the roots of nettle it was 20-69%.

The content of Pb in leaves of *H. epipactis* ranged from 2.8 to 4.7 mg·kg^{-1}, while in leaves of nettle – from 3.36 to 5.88 mg·kg^{-1}. The highest content of Pb in nettle leaves was observed in the LMP reserve. In turn, in the Kopce reserve, concentrations of Pb in the leaves of plants were comparable for both examined species (Table 6).

Pb co-occurence with chosen elements in leaf and rhizome of *H. epipactis* and *U. dioica* of the reserve 'Kopce' is presented in Table 7.

Discussion

At the beginning, it should be emphasized that the discussion directly related to the accumulation of Pb by *H. epipactis* is restricted owing to a lack of data on the subject. The presented results, depicting changes in the content of Pb in the main morphological parts of this plant are pioneering. Also, the scope of research on the occurrence of Pb in the major environment reserves for *H. epipactis* is innovatory, and is the first ever to consider the speciation analyses of soil.

Due to the high physiogeographical differences, the environmental content of Pb corresponding to the 10th per-

Table 3. Metal content in select chemical forms in the soil in the site of *H. epipactis* and *U. dioica* of Kopce Reserve (mg·kg^{-1}).

Chemical form of the element	Site																	
	H. epipactis									*U. dioica*								
	Metals																	
	Pb	Cd	Cu	Cr	Co	Ni	Mn	Fe	Zn	Pb	Cd	Cu	Cr	Co	Ni	Mn	Fe	Zn
Exchangeable	74.24	1.79	9.98	1.39	2.15	4.27	150.58	230.15	38.17	12.67	0.54	3.40	1.27	1.05	1.04	10.79	15.33	11.43
Adsorbed	8.98	0.42	1.99	1.51	0.77	1.65	24.29	48.04	5.04	6.79	0.03	1.78	1.87	1.11	0.94	3.05	19.12	1.44
Organically bound	7.89	0.01	1.63	1.08	1.10	0.1	36.82	410.12	5.49	0.78	0.09	2.06	0.84	0.05	0.10	39.55	255.19	3.35
Carbonates	14.52	0.17	2.67	1.15	1.43	0.33	82.28	663.12	3.12	16.51	0.16	4.52	1.27	1.06	2.38	77.66	982.94	7.27

Table 4. Pb co-occurrence with chosen elements in the bioavailability and potentially bioavailability forms in the soil of Kopce Reserve.

Type	Chemical form of element	Site															
		H. epipactis								*U. dioica*							
		Metals															
		Cd	Cu	Cr	Co	Ni	Mn	Fe	Zn	Cd	Cu	Cr	Co	Ni	Mn	Fe	Zn
		Correlation coefficient															
Bioavailability	Exchangeable	0.95	0.95	-0.15	-0.76	0.46	-0.42	0.68	0.92	0.93	0.94	-0.34	0.36	0.76	0.97	-0.19	0.75
	Adsorbed	-0.20	-0.42	0.18	0.09	-0.15	0.45	0.93	0.41	0.96	-0.05	0.73	0.30	-0.14	0.87	0.84	0.70
Potential bioavailability	Organically bound	1.0	0.66	0.31	-0.51	-21	0.22	0.18	0.86	-0.38	-0.26	0.32	1.0	0.97	0.70	0.96	0.15
	Carbonates	0.65	0.29	0.38	-0.03	0.02	0.15	0.38	0.14	-0.31	-0.24	-0.31	0.44	-0.37	0.55	0.87	0.21

Table 5. Pb content in the rhizome *H. epipactis* and *U. dioica* of the tested reserves (mg·kg^{-1}).

Reserve	Plant species	Geometric mean	Standard deviation	Percentile		Coefficient		
				10	95	Excess	Kurtosis	Variability %
LMP	*H. epipactis*	6.99	3.21	2.76	11.18	-0.96	0.95	42
	U. dioica	2.71	1.48	1.59	5.40	1.45	2.24	50
LMO	*H. epipactis*	7.21	2.17	4.96	9.96	-0.15	-2.46	29
	U. dioica	1.55	1.25	1.07	3.98	2.02	4.12	69
K	*H. epipactis*	14.17	9.77	8.12	30.23	0.87	-1.64	60
	U. dioica	1.25	0.79	0.56	2.66	0.97	1.07	56

LMP – reserve Lasek Miejski nad Puńcówką
LMO – reserve Lasek Miejski nad Olzą
K – reserve Kopce

Table 6. Pb content in the leaf *H. epipactis* and *U. dioica* of the tested reserves (mg·kg^{-1}).

Reserve	Position	Geometric mean	Standard deviation	Percentile		Coefficient		
				10	95	Excess	Kurtosis	Variability %
LMP	*H. epipactis*	2.82	0.74	2.17	3.93	0.57	-1.18	25
	U. dioica	5.88	1.42	3.62	7.30	-1.73	3.40	24
LMO	*H. epipactis*	3.98	0.50	3.38	4.61	0.05	-1.65	13
	U. dioica	3.36	2.10	1.65	5.97	-0.34	-3.05	54
K	*H. epipactis*	4.71	1.38	3.36	6.80	0.14	-1.58	28
	U. dioica	4.65	1.08	3.35	6.04	-0.05	-1.38	23

LMP – reserve Lasek Miejski nad Puńcówką
LMO – reserve Lasek Miejski nad Olzą
K – reserve Kopce

centile on such a study area was highly diversified, namely: in the biotope of *H. epipactis* in the K reserve it accounted for: 41.7 mg·kg^{-1}, in the LMP reserve 58.9 mg·kg^{-1}, and 79.5 mg·kg^{-1} in the LMO reserve. Similar differences were observed for incidental concentrations of Pb (95 percentil), the highest values of which (163 mg·kg^{-1}) were recorded in the K biotope and slightly lower ones in the LMO reserve 137 mg·kg^{-1}, and LMP reserve 95 mg·kg^{-1}. The content of Pb in the soils of the nature reserves examined exceeded (by about two times) the permissible value for soils of the protected areas. According to the Regulation of the Minister of Environment on quality standards of soils and quality standards of ground, this value was stipulated at a level of 50 mg·kg^{-1} [22].

Apart from the forms directly- and indirectly-bioavailable from soil, considerable reserves of Pb are accumulated in dust settled on plants. In this case, the close proximity of the biotopes of *H* to the Ostrawsko-Karwiński Mining Region and Steelworks in Trzyniec (Czech Republic) (ca. 10 km away) coupled with diversified topographic and physiographical conditions of the area under study, make that the imission of Pb in particular biotopes differed substantially, i.e. as much as ca. 70 mg·kg^{-1} in the soluble form and ca. 36 mg·kg^{-1} in the insoluble form of settled dust. For comparison, the content of soluble and insoluble forms was over the range of 33.5-102.8 mg·kg^{-1} and 101-137 mg·kg^{-1}, respectively. These values are comparable to the contents of Pb noted in dust settled in the vicinity of a power station and processing plants of ores of non-ferrous metals [23].

Nevertheless, apart from the route of foliar fertilization, the final accumulation proceeds through Pb absorption by rhizomes of *H. epipactis* and nettle from ion-exchangeable and absorbable forms, and in the case of a lowered reaction of soil – also from organically bound and carbonates. It is interesting, however, that despite large reserves in the soil and their bioavailability, the absorption of this element by rhizomes of *H. epipactis* is rather moderate. This may result both from the fact of bioaccumulation of the element in the organic layers of soil, as well as from the existence of mechanisms of Pb discrimination by that species or the competition for the receptor by a number of elements [24].

In comparing the pseudo-total content of Pb in the soil with contents of Pb forms directly bioavailable to *H. epipactis*, it may be observed that the ion-exchange forms constitute 75% of total Pb content; in the case of *U. dioica* the corresponding value accounts for ca. 33%. The adsorbable forms constitute another 10% of total Pb content, and the

Table 7. Pb co-occurence with chosen elements in leaf and rhizome of *H. epipactis* and *U. dioica* of the reserve 'Kopce'.

Morph. part	Position															
	H. epipactis								*U. dioica*							
	Metals															
	Cd	Cu	Cr	Co	Ni	Mn	Fe	Zn	Cd	Cu	Cr	Co	Ni	Mn	Fe	Zn
	Correlation coefficient															
Leaf	-0.12	0.22	0.84	0.41	0.67	0.96	0.89	0.77	0.33	0.75	0.18	1.0	0.86	0.21	0.51	-0.83
Rhizome	0.98	0.07	-0.05	0.67	0.75	0.73	0.88	0.20	0.94	0.30	-0.61	1.0	0.34	0.39	0.94	0.21

full percentage contribution is completed by organic links and carbonates. In the case of *H. epipactis*, this is an alarming phenomenon, for the rhizomes permanently accumulate the directly-bioavailable forms. Those reserves are supplied regularly from the potentially-bioavailable forms, since acid rains are observed to occur in this area. The reaction of the precipitation ranges from 2.3 to 5.5. Chemical analysis showed that pH_{KCl} of the soil was within the range 5.36 to 7.25 in LMP reserve and from 4.84 to 6.99 Kopce Reserve. In LMO reserve pH_{KCl} of the soil was the least differentiated and was 7.22 on average.

An important issue of significance at the so-called "stage of toxicological bioavailability" is the co-occurrence of Pb with the other elements. The high coefficients of correlation describing a change in the content of Pb along with a change in the concentrations of particular elements for the four chemical forms of occurrence examined point to the common origin of those elements, e.g. from emission or phenomena observed at the stage of toxicological availability. The coefficients of correlation having the same direction of changes indicate a similar character of chemical stress in respect to the plant species analyzed.

In the case of *H. epipactis*, being at the stage of toxicological availability, of high significance in competing for a receptor, i.e. rhizome for ion-exchange forms of Pb, are Cd, Cu, Fe, and Zn (Table 4). This is indicated by significant coefficients of Pb correlation with those elements ($p \leq 0.05$) over the range of 0.68-0.95. Similar correlations are observed for organically bound Pb (0.66-1.0), except for Fe ($r=0.18$). In turn, the role of Pb carbonates is emphasized by coefficients of correlation referring to Cd (0.65), Cr, and Fe (0.38). Strong antagonists of Pb in the exchangeable form are Co (-0.76) and Mn (-0.42), as well as Organically bound Co (-0.51). It is typical that, apart from antagonism of Cu (-0.42) and synergism of Mn and Zn (0.45), and Fe (0.93), the adsorbed forms are of no significance to the other elements. Attention should also be paid to the negligible role of Ni ions in competing for the receptor, as compared to Pb and Mn in the potentially-bioavailable forms.

When it comes to *U. dioica*, the occurrence of elements in the soil in the exchangeable form and adsorbable form is of greater current significance (Table 4). For instance, the synergistic role in relation to Pb, in both bioavailable forms, is exhibited by Cd, Mn, and Zn, as well as by Cu in the rhizosphere of *U. dioica*. Likewise in the case of *H. epipactis*.

The co-occurrence of Pb in leaves of *H. epipactis* (Table 7) displays a character of directly-proportional changes with Cd, Co, Ni, Fe, and Zn ($r=0.41$-0.96, $p \leq 0.004$). In the case of *H. epipactis* rhizomes, corresponding changes referred to Co, Ni, Mn, and Fe. In the rhizomes, a distinct directly proportional correlation could be observed between Pb and Cd, which accounted for 0.98 in *H. epipactis* and for 0.94 in nettle. In addition, Cu was found not to affect significantly changes in the content of Pb. In the leaves of *U. dioica*, directly proportional relations were observed between Pb and, Cu, Ni, and Fe, whereas indirectly proportional relations were noted in respect to Zn. In contrast, in the rhizosphere of *U. dioica* attention should be paid to the directly proportional relations of Pb with Cd and Fe and indirectly proportional relations with Cr.

As a result of the above-described phenomena and the co-occurrence of Pb with other elements in the soil and in dust settled on leaves, the contents of Pb accumulated in rhizomes of *H. epipactis* from biotopes of the investigated reserves ranged from 7 mg·kg^{-1} (LMP, LMO) to 14 mg·kg^{-1} (K). In comparison, the content of Pb in roots of plants collected in a recreational area reached 2.35 mg·kg^{-1} for *Tanacetum vulgare* and 4.57 mg·kg^{-1} for *Solidago virgaurea* and was 3-6-fold higher, on average, for *H. epipactis* [25].

Also noteworthy is the significantly lower content of Pb in the roots of nettle as compared to *H. epipactis*. In terms of area analysis, greater exposure of *H. epipactis* to Pb was observed in Kopce Reserve, followed by the LMP and LMO reserves. The content of Pb in leaves of *H. epipactis* and *U. dioica* from the examined reserves is similar, i.e. 2.8-4.7 mg·kg^{-1} and 3.36-5.88 mg·kg^{-1}. This mode of the occurrence of average contents of Pb was also confirmed by values corresponding to the 95th and the 10th percentiles, at the concomitant low variability of Pb content in leaves (Table 6).

Conclusions

1. The content of Pb in the soils of the nature reserves examined exceeded (by about two times) the permissible value for soils of the protected areas. It may be observed that the ion-exchange forms constitute 75% of total Pb content. The adsorbable forms constitute another 10% of total Pb content.

2. *Hacquetia epipactis* possesses a capacity to accumulate Pb from the form of Pb directly bioavailable from soil.
3. A change in the content of Pb in particular morphological parts of *Hacquetia epipactis* is determined by physiogeographical conditions in respect to the major emitter of contaminants (Steelworks in Trzyniec) and a common proportion of selected speciation forms of Pb in the soil.

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