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Cent. Eur. J. Biol. • 9(3) • 2014 • 320-330
DOI: 10.2478/s11535-013-0267-9

Central European Journal of **Biology**

Chemical composition of the leaves of *Reynoutria japonica* Houtt. and soil features in polluted areas

Research Article

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Received 06 June 2013; Accepted 14 October 2013

Abstract: The study was conducted on six sites that are dominated by Japanese knotweed (*Reynoutria japonica*) and that vary in the level of industrialization and habitat transformation by humans. The aim of the research was to investigate the chemical-physical features of soil under a closed and dense canopy of *R. japonica*, the chemical composition of the *R. japonica* leaves, and to compare the content of certain elements in the soil-plant-soil system. The soil organic carbon (C_{org}) content varied from 1.38±0.004% to 8.2±0.047% and the maximum in leaves was 49.11±0.090%. The lowest levels of total nitrogen (N_{tot}) in soil were recorded on the heavily disturbed sites (till 0.227±0.021%). Soil pH varied greatly, ranging from acidic (pH=4.0) to neutral (pH=7.7). Heavy metal content differed significantly among the study sites. At all of the sites, both in the case of soil and plant leaves, Zn was a dominant element and its concentration ranged from 41.5 to 501.2 mg·kg^{-1} in soils and from 38.6 to 541.7 mg·kg^{-1} in leaves. Maximum accumulations of P (2103.3±15.3 mg·kg^{-1}) and S (2571.7±17.6 mg·kg^{-1}) were observed on the site that had been influenced by agricultural practices. The results obtained showed that *R. japonica* is able to accumulate high levels of heavy metals.

Keywords: *Reynoutria japonica* • *Invasive species* • *Leaf chemistry* • *Soil chemistry* • *Nutrient compounds* • *Heavy metals* • *Disturbed habitat*

1. Introduction

Geographically, alien plant species are able to adapt to a wide range of ecological conditions. This ability allows them to quickly colonize areas beyond their natural distribution range [1,2]. Invasive alien species show a wide range of responses to environmental conditions, and in different spatial scales they often colonize highly disturbed and fragmented habitats, especially within towns, cities, and their surrounding areas. Invasive plants are able to modify the soil environment and thereby facilitate the further encroachment of new individuals of the same or other invasive species [3].

There is a great deal of evidence that invasive species may modify the physical and chemical properties of soils [4]. This includes having an impact on the biogeochemical cycles of N and other elements [5-8], on the quantity and quality of soil organic matter [9], and on soil pH [10]. Changes to the chemical properties of soil due to the influence of vegetation have been observed in many different ecosystems [5,11-15], and recently special attention has been focused on the role of alien species in soil nutrient enrichment [16,17]. Different plant species, including alien ones, have been shown to directly impact the modification of biotic soil environments [7,10,18]. Invasion of geographically alien plant species may not only have an impact on the chemical properties of soils but also on the different processes that take place in an ecosystem. One alien plant species that may cause significant changes in bioceonoses and the pedosphere is *Reynoutria japonica*.

The natural distribution range of *Reynoutria japonica* includes Japan, Korea, Taiwan, and the northern part of China [19,20], where it predominantly occurs as a pioneer species on volcanic cones [21,22] and plays the role of a nurse plant. *R. japonica* was introduced to Europe from its natural range in Asia as a decorative

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plant in 1825, and within the same year it was named the most interesting decorative plant of the year by The Society of Agriculture and Horticulture in Utrecht [23]. Therefore, Japanese knotweed was used in Europe to stabilize mobile sands and in the reclamation of derelict lands [24]. The first notes on the presence of *R. japonica* in Poland date to the second part of the 19th century [25].

In Poland, Japanese knotweed predominantly grows in secondary, anthropogenic habitats, including areas along roads, railway tracks, dumping sites of urban and industrial waste, around cemeteries, and in town parks. It less frequently occurs in natural ecosystems, mainly along river valleys, forest edges, and in places which were partially disturbed by human activities, such as corridors of rivers that flow through damp forests, whose banks have been straightened or laid with concrete. *R. japonica* tolerates a wide spectrum of soil conditions and may occur in both very poor, acidic soils and in more neutral, rich ones [26].

Outside its natural distribution range, *Reynoutria japonica* does not possess any specific habitat requirements, allowing it to grow in many different environments. In the USA, its presence has been noted in a wide range of habitats, including dry sandy soils, waterlogged soils, dumping sites, rocky shores, and floodplains with very damp soils [27]. Because fragmented parts of *R. japonica* rhizomes and stems germinate very quickly and easily, the species can be easily dispersed by water and animals [28]. However, its distribution is mainly facilitated by human activity, namely by the massive movement of topsoil that contains parts of Japanese knotweed propagules [29].

In most cases information regarding the distribution of *Reynoutria japonica* is limited to information about the places where the species grows, whereas information about the characteristics of its habitats is not provided. Furthermore, apart from some very general and limited information, *e.g.*, stating that *R. japonica* occurs in sands, loams, or gravels, along river corridors, etc., no data on the type of soil where it grows is available [27].

Reynoutria japonica has been the subject of many detailed investigations with varying objectives. These have included research on the historic and current distribution ranges of the species [*e.g.*, 30,31], predictions of the impact of potential global climate changes on its future distribution ranges [19], its genetic diversity [32], and also on its influence on native species of plants and animals [25,26,33,34]. At present, among the available, published data, there is a lack of sufficient information about the influence of *R. japonica* on the physical and chemical properties of soil that forms under its canopy [4,35] and on the impact of the chemical composition of *R. japonica* leaves on the soil chemical properties. In its natural distributional range in Japan, this species is the first to colonize the fresh, volcanic lava. In this way, similar to many other early-succession species, it hastens the soil-formation processes by decreasing the soil bulk density, increasing water capacity and also the content of soil organic matter and nutrients compounds [36]. Furthermore, during primary succession in the soils forming under the canopy of *R. japonica* on Mt. Fuji, higher concentrations of different forms of nitrogen were recorded compared with neighbouring sites that were unoccupied by Japanese knotweed [37]. However, to the best of the authors' knowledge, no data on the impact of *R. japonica* on soil formation is available. Thus, the aim of our study was to estimate the physical and chemical properties of soils under a dense cover of *R. japonica* and determine the chemical composition of leaves of that species within habitats that differ distinctively in level of anthropogenic transformation.

2. Experimental Procedures

2.1 Study sites

The experiments were conducted on sites that differed in the level of transformation as a result of human pressure within an area located in northern and southern Poland (Table 1). Six sites representing different levels of environmental disturbance from very low to extremely high were chosen (Table 1). Site selection was based on the following criteria:

I. the massive presence of a single-species cover of *Reynoutria japonica* and

II. a transformed sequence of genetic soil horizons that reflected different degrees of former human pressure. Prior to site selection, preliminary soil profile samples were taken using an Edelman-corer (ø=7.5 cm).

A site representing the lowest level of disturbance (1 – very low) was located at the edge of a pine wood that had been planted on the habitat of a broadleaved forest. Apart from the small input of mineral material of an external origin, the soil of Site 1 is characterized by an undisturbed pattern of soil horizons. In the case of the next two sites (Site 2 – low, and Site 3 – medium), which are situated within direct proximity of the riverbeds of regulated rivers: a mountain river (site 2) and a river flowing through agricultural land (site 3). The soil structure at these sites has been transformed by the supply of sediments originating from both floodwater and runoff from arable fields. Another important feature of the soils of sites 2 and 3 was the mechanical destruction of the topsoil horizons by agricultural practices and

Site name	Disturbance level	Site attributes				Site coordinates
		Habitat	Soil	Vegetation		
Ryn	1 – very low	edge of a pine forest	haplic cambisol (eutric)	Single-species patches of *R. japonica* with a high share of ruderal and nitrophilous species		53° 57' 12,40" N 21° 33' 47,57" E
Szczyrk	2 – low	zone adjoining the river bed of a regulated, mountainous river	fluvic endogleic cambisol			49° 41' 42,11" N 18° 59' 08,74" E
Ligota	3 – medium	zone adjoining the river bed of a regulated river flowing through arable fields	fluvic cambisol			49° 52' 25,68" N 18° 57' 19,76" E
Sosnowiec-Zagórze	4 – high	cemetery border with the dumping site of organic topsoil material	Hortic anthrosol			50° 16' 48,79" N 19° 10' 22,69" E
Sosnowiec-Dębowa Góra	5 – very high	dumping site with building materials and used electronic equipment	technic regosol, technic anthrosol			50° 15' 37,84" N 19° 08' 48,48" E
Chorzów	6 – extreme	spoil heap of coal-mining excavation material, with a high share of fine-grained material	technic regosol, technic anthrosol			50° 17' 58,42" N 18° 58' 34,10" E

Table 1. Characteristics of the study sites.

construction work performed to regulate the river banks. Soils of the sites representing disturbance levels 4–6 are located on the borders of a graveyard (Site 4), on a waste dumping site (Site 5), and on the edge of a post-coal mining spoil heap which had been transformed as the result of the deposition of industrial and urban waste (Site 6). The most important features of the sites are presented in Table 1.

2.2 Soil sampling

Soil samples for chemical analyses were taken from the organic accumulation layer (A) that represents the main rooting area of most herb layer species, including *Reynoutria japonica*. At the laboratory, air-dried samples were sieved (<2 mm) and analyzed, following the standard procedures given by Bednarek *et al.* [38], for pH measured potentiometrically in H_2O and in 1 mol L^{-1} KCl using a glass electrode, total organic C (%) according to the Tiurin method, total N content (%) using the Kjeldahl method, $CaCO_3$ using the Scheibler method, and exchangeable Al^{3+}, Ca^{2+}, Mg^{2+}, Na^+, K^+ after sample extraction in 1 mol L^{-1} CH_3COONH_4 at 7.0 pH using Atomic Absorption Spectrometry analysis and hydrolithic acidity (H_h) according the Kappen method. In addition, the total element content in the soil was analyzed after wet mineralization in nitrohydrochloric acid ($3HCl+HNO_3$) and assayed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) [39].

2.3 Plant leaf sampling

Leaves of *Reynoutria japonica* for chemical analyses were sampled at the end of the vegetation season in late September and early October. At least ten undamaged leaves from different individuals were collected at each site. Latex gloves were used during manual sampling to ensure isolation of the sampled plant material from human skin. Preliminary preparation of samples for analyses included washing leaves with distilled water, drying at room temperature, and at 105°C followed by homogenization. Sampling procedures of plant material and its preparation for the laboratory analyses were performed according to the instructions given by MacNaeidhe [40] and Markert [41].

The total content of the following elements was measured in the plant material: Ca, Mg, K, Na, Fe, Al, Zn, Cd, Pb, P, S, Cr, Cu and Ni. Similarly to the procedures for the soil samples, the total element content in plant tissues was analyzed after wet mineralization in nitrohydrochloric acid and assayed using ICP-OES [39]. Each sample was analyzed in triplicate for all properties being investigated, both in the case of soil and leaf samples.

2.4 Statistical analyses

Significant differences in the content of measured soil chemical parameters among the study sites and differences in the leaf concentrations of the analyzed elements among the sites were estimated using the Kruskal-Wallis test. In addition, the Spearman correlation rank was applied in order to determine whether there is a relationship between the concentration of heavy metals in the plant tissues of *Reynoutria japonica* leaves and in the soil samples. Furthermore, linear regression analysis was carried out to assess the influence of the heavy metal content in the soil on their concentration in plant tissues. All statistical analyses were done using the Statistica 10.0 package.

3. Results

3.1 Chemical properties of soils in sites along the disturbance gradient

The C_{org} content in the soils of the study sites studied varied from 1.38±0.004% (Site 3 – medium) to 8.2±0.047% (Site 6 – extreme) and significant differences according to the Kruskal-Wallis test were observed only between those two sites (P=0.0154). The highest N_{tot} content was recorded in the soils of the sites that represented a high level of disturbance (mean till 0.227±0.021%); however, the differences among the sites were not significant (Table 2). The $CaCO_3$ content showed a distinct difference between two highly disturbed sites, namely between Site 4, located at the edge of a graveyard, and Site 6, located on the edge of a coal-mine spoil heap (P =0.0073).

Soils of the study sites are characterized by a wide range of pH, and can be classified as varying from very acidic, through acidic, weakly acidic, neutral, and alkaline. The pH values were between 4.0 and 7.5 in KCl and from 5.0 to 7.7 in H_2O (Table 2). According to the result of the Kruskal-Wallis test, statistically significant differences (P=0.0017 and P=0.0019, for pH in H_2O and in KCl, respectively) were recorded between Site 2 and Sites 3 and 6. The highest levels of hydrolithic acidity (H_h) were noted for the soil from Site 2 (mean 8.32±3.07 cmol(+)·kg^{-1} (Table 2), and this value was significantly higher (P=0.0063) than those recorded for Sites 3 and 6 (1.283±0.04 and 1.207±0.253 cmol(+)·kg^{-1}, respectively).

All the exchangeable cations (Al^{3+}, Ca^{2+}, K^+, Mg^{2+} and Na^+) varied greatly among the sites investigated (according to the Kruskal-Wallis test, p values ranged from 0.0017 to 0.0153). The highest value for Ca^{2+} was noted in the soil of Site 3. Al^{3+} was highest in the case of Site 2, K^+ and Mg^{2+} were highest at Site 6, and Na^+ was highest at Sites 4 and 5 (Table 2).

The highest Al, Ca, Fe, Mg, P, S, and Na contents were recorded in soils from Site 6 (spoil heap), which represented the highest level of disturbance. These differences were confirmed by the results of the Kruskal-Wallis test (Table 2). Significant differences in heavy metal content in soils were detected among the study sites. The highest concentrations of Cr (27.4±0.458 mg·kg^{-1}), Cu (79.7±0.400 mg·kg^{-1}), and Ni (36.7±0.500 mg·kg^{-1}) were recorded at Site 6 (spoil heap), whereas the highest concentrations of Pb (158.07±0.25 mg·kg^{-1}), Cd (6.7±0.04 mg·kg^{-1}), and Zn (501.2±0.252 mg·kg^{-1}) were recorded at Site 4, which is located around the cemetery. The rank of the decreasing content of heavy metals in the soils from the study sites is as follows (Table 2):

Site 1 – very low: Zn>Pb>Cr>Cu>Ni>Cd,
Site 2 – low: Zn>Cr>Pb>Ni>Cu>Cd,
Site 3 – medium: Zn>Cr>Pb>Ni>Cu>Cd,
Site 4 – high: Zn>Pb>Cu>Cr>Cd>Ni,
Site 5 – very high: Zn>Pb>Cu>Cr>Ni>Cd,
Site 6 – extreme: Zn>Cu>Pb>Ni>Cr>Cd.

3.2 Element concentration in *R. japonica* leaves

The highest C_{org} content, reaching 49.1±0.090%, was recorded in leaves from Site 2, and this result was significantly higher than that of the leaves from Sites 6 (spoil heap) and 4 (waste dump near the cemetery). The highest concentrations of Al (361±3.6 mg·kg^{-1}), Ca (19,623.3±25.2 mg·kg^{-1}), and Fe (312.3±2.5 mg·kg^{-1}) were observed in the leaves collected on sites with high, very high, and extreme disturbance levels, whereas the highest Mg concentration (6,351.7±7.6 mg·kg^{-1}) was recorded on the least disturbed sites. Leaves from Site 3, which had a medium disturbance level (agricultural pressure), had the highest concentrations of P (2,103.3±15.3 mg·kg^{-1}) and S (2,571.7±17.6 mg·kg^{-1}).

The heavy metal concentrations in *Reynoutria japonica* leaves from the study sites are given below:

Site 1 – very low: Zn>Cu>Cr>Pb>Ni>Cd,
Site 2 – low: Zn>Cu>Cd>Pb>Ni>Cr,
Site 3 – medium: Zn>Cu>Pb>Cr>Ni>Cd,
Site 4 – high: Zn>Pb>Cu>Cd>Cr>Ni,
Site 5 – very high: Zn>Pb>Cu>Cr>Ni>Cd,
Site 6 – extreme: Zn>Cu>Pb>Cr>Ni>Cd.

3.3 Comparison of selected elements' content in the 'soil-plant-soil' system

The elemental concentrations found in soil and leaves are very diverse. The Fe concentration was higher in soil (from 6,608.3±38.2 to 45,920±53 mg·kg^{-1}, respectively) than in leaves (from 137±1.0 to 266.3±1.5 mg·kg^{-1}) for all of the sites studied. The same situation was also observed in the case of Al. An opposite situation was observed for Ca and Mg, for which the concentrations in leaves were higher than in soils (Table 2). Reverse relations in concentration of Ca and Mg were observed in the case of very high and extreme sites (Sites 5 and 6). Moreover, the P (2,103.3±15.3 mg·kg^{-1}) and S (2,571±17.6 mg·kg^{-1}) concentrations were higher in the leaves than in the soils in mentioned sites (700.3±0.6 and 199.7±1.5 mg·kg^{-1}, respectively) . The content of the elements in the soil and plants that were investigated are shown in Table 2.

The Cu, Cr, Ni, and Pb concentrations were higher in the soils than in *Reynoutria japonica* leaves for all of the study sites, regardless of their disturbance level. Furthermore, in the case of Cu, a clear tendency of

	Disturbance level/Site name	1 – Ryn	2 – Szczyrk	3 – Ligota	4 – Sosnowiec-Zagórze	5 – Sosnowiec-Dębowa Góra	6 – Chorzów	*p*-level
Soil	C_{org} (%)	2.63 (±0.003)	2.16 (±1.81)	1.38 (±0.004)^{a}	2.88 (±0.003)	4.22 (±0.06)	8.20 (±0.05)^{b}	0.0154
	N_{tot} (%)	0.193 (±0.002)	0.196 (±0.15)	0.137 (±0.006)	0.210 (±0.01)	0.227 (±0.02)	0.223 (±0.01)^{b}	n. s.
	$CaCO_3$ (%)	0.027 (±0.01)	0.013 (±0.02)	0.347 (±0.012)	0.000^{a}	0.067 (±0.06)	1.44 (±0.03)^{b}	0.0073
	pH ($H_2$0)	6.0 (±0.02)	5.0 (±0.38)^{a}	7.4 (±0.02)^{b}	6.3 (±0.15)	6.7 (±0.10)	7.7 (±0.09)^{b}	0.0017
	pH (KCl)	5.6 (±0.02)	4.0 (±0.08)^{a}	7.1 (±0.01)^{b}	6.0 (±0.01)	6.1 (±0.11)	7.5 (±0.08)^{b}	0.0019
	Hh (cmol(+)kg $^{-1}$)	5.027 (±0.04)	8.32 (±3.07)^{a}	1.283 (±0.04)^{b}	5.713 (±0.03)	6.107 (±0.14)	1.207 (±0.25)^{b}	0.0063
	Al^{3+} (cmol(+)kg $^{-1}$)	0.011 (±0.001)	1.081 (±0.18)^{a}	0.004 (±0.001)^{b}	0.014 (±0.002)	0.018 (±0.003)	0.006 (±0.001)^{b}	0.0018
	Ca^{2+} (cmol(+)kg $^{-1}$)	9.1 (±0.01)^{b}	2.363 (±0.54)^{a}	12.943 (±0.031)	6.567 (±0.03)	7.1 (±0.05)	8.543 (±0.03)^{b}	0.0017
	K^{+} (cmol(+)kg $^{-1}$)	0.105 (±0.002)^{a}	0.177 (±0.07)	0.143 (±0.002)	0.201 (±0.001)	0.209 (±0.002)	0.693 (±0.002)^{b}	0.0153
	Mg^{2+} (cmol(+)kg $^{-1}$)	0.907 (±0.01)	0.491 (±0.20)^{a}	0.45 (±0.002)^{a}	0.836 (±0.002)	0.877 (±0.01)	1.817 (±0.021)^{b}	0.0027
	Na^{+} (cmol(+)kg $^{-1}$)	0.033 (±0.002)	0.025 (±0.01)^{a}	0.035 (±0.003)	0.083 (±0.003)^{b}	0.085 (±0.003)^{b}	0.032 (±0.002)	0.0052
	Al (mg kg^{-1})	6410 (±36.10)^{a}	17184 (±2767.0)	17000 (±10.00)	4296.7 (±45.10)^{a}	10971 (±62.10)	22110 (±36.10)^{b}	0.0027
	Ca (mg kg^{-1})	2403.3 (±15.30)	803.2 (±113.7)^{a}	4996.7 (±15.3)^{b}	1798.3 (±12.60)	3500 (±20.00)	20613 (±32.10)	0.0017
	Fe (mg kg^{-1})	7696.7 (±15.30)^{a}	18700 (±3617)	20900 (±20)	6608.3 (±38.20)^{a}	9210 (±36.10)	45920 (±52.90)^{b}	0.0027
	K (mg kg^{-1})	898.3 (±7.60)	2650 (±278)^{a}	2803.3 (±5.80)^{a}	501 (±3.60)^{b}	1026.7 (±20.80)	2096.7 (±15.30)	0.0027
	Mg (mg kg^{-1})	1201.7 (±17.60)^{b,c}	2555 (±283.9)	2396.7 (±15.30)	499.3 (±3.10)^{b}	3200 (±20.00)^{c}	7076.7 (±87.40)^{a,c}	0.0027
	P (mg kg^{-1})	401.3 (±3.20)	297.3 (±111.00)^{a}	498.30 (±7.60)	700.3 (±0.60)^{b}	697.3 (±2.10)	698.3 (±7.60)	0.0034
	S (mg kg^{-1})	199.7 (±1.50)^{b}	200.7 (±111.30)	201 (±3.60)^{b}	300.7 (±2.10)	511.7 (±7.60)	698.3 (±17.60)^{a}	0.0119
	Na (mg kg^{-1})	49.5 (±0.20)^{b}	58.6 (±1.10)^{b,c}	88 (±1.00)	63.4 (±0.10)	150 (±0.20)^{c}	445.4 (±0.60)^{a,c}	0.0017
	Cd (mg kg^{-1})	0.137 (±0.01^{b}	0.41 (±0.22)^{b,c}	0.73 (±0.02)	6.743 (±0.04)^{a,c}	4.233 (±0.06)^{c}	1.433 (±0.02)	0.0017
	Cr (mg kg^{-1})	11.63 (±0.06)^{a}	22.68 (±2.88)	24.6 (±0.10)	9.7 (±0.20)^{a}	15.16 (±0.06)	27.4 (±0.46)^{b}	0.0026
	Cu (mg kg^{-1})	5.867 (±0.15)^{b}	10.567 (±4.09)^{b,c}	17.16 (±0.06	25 (±1.00)	41 (±1.00)^{c}	79.7 (±0.40)^{a,c}	0.0017
	Ni (mg kg^{-1})	4.567 (±0.06)^{b}	13.167 (±1.46)	20.3 (±0.2.00)^{c}	5.433 (±0.06)^{b,c}	11.033 (±0.15)	36.7 (±0.50)^{a,c}	0.0017
	Pb (mg kg^{-1})	16.367 (±0.15)	15.317 (±3.32)^{b}	20.66 (±0.06)^{b}	158.07 (±0.25)^{a}	120.3 (±0.60)	43.067 (±0.15)	0.0026
	Zn (mg kg^{-1})	41.53 (±0.31)^{b}	62.95 (±12.69)^{b,c}	92.167 (±0.12)	501.2 (±0.25)^{a,c}	401.17 (±0.76)^{c}	274.8 (±4.34)	0.0017

Table 2. Soil chemical properties and chemical composition of the soils and leaves.

The same letter in superscript means no significant difference according to the Kruskal-Wallis test; n.s. – not significant.

	Disturbance level/Site name	1 – Ryn	2 – Szczyrk	3 – Ligota	4 – Sosnowiec-Zagórze	5 – Sosnowiec-Dębowa Góra	6 – Chorzów	*p*-level
Leaves	C_{org} (%)	47.72 (±0.03)	49.11 (±0.09)^a	48.85 (±0.04)	47.60 (±0.05)^b	47.92 (±0.08)	47.51 (±0.28)^b	0.0091
	N_{tot} (%)	1.356 (±0.002)^a	1.547 (±0.01)	2.787 (±0.002)^b	2.167 (±0.003)	1.367 (±0.01)	1.954 (±0.003)	0.0054
	Al (mg kg^{-1})	219 (±3.60)	279.7 (±5.50)	141 (±3.60)^a	266.7 (±15.30)	361 (±3.60)^b	241 (±3.60)	0.0060
	Ca (mg kg^{-1})	18516 (±15.30)	14210 (±36.10)	14883 (±76.40)	18016 (±76.40)	2406 (±3.60)^a	19623 (±25.20)^b	0.0054
	Fe (mg kg^{-1})	137 (±1.00)^a	204.7 (±0.60)	190.3 (±2.10)	312.3 (±2.50)^b	297 (±2.00)	266.3 (±1.50)	0.0053
	K (mg kg^{-1})	6266 (±15.30)^a	11793 (±15.30)	15463 (±15.30)^b	11826 (±25.20)	6645 (±42.70)	9190 (±36.10)	0.0058
	Mg (mg kg^{-1})	6351.7 (±7.60)^a	2951.7 (±7.60)	2670 (±10)^b	3880 (±21.80)	3003 (±2.10)	5958.3 (±7.60)	0.0054
	P (mg kg^{-1})	1251 (±3.60)	1238.3 (±12.60)	2103.3 (±15.30)^a	1721.7 (±12.60)	1123.3 (±25.20)^b	1433.3 (±15.30)	0.0060
	S (mg kg^{-1})	1341.7 (±7.60)^a	1563.3 (±20.80)	2571.7 (±17.60)^b	2378.3 (±7.60)	1766.7 (±15.30)	2310 (±36.10)	0.0054
	Na (mg kg^{-1})	85.7 (±0.60)	74.3 (±1.20)	2718 (±2.60)^a	106.7 (±1.50)	66.7 (±1.50)^b	75.3 (±1.50)	0.0062
	Cd (mg kg^{-1})	0.267 (±0.02)	2.17 (±0.01)	0.167 (±0.06)^a	5.277 (±0.02)^b	1.073 (±0.03)	0.333 (±0.02)	0.0053
	Cr (mg kg^{-1})	1.033 (±0.06)	1.553 (±0.03)	1.01 (±0.01)^a	1.287 (±0.01)	1.837 (±0.02)^b	1.057 (±0.01)	0.0087
	Cu (mg kg^{-1})	5.107 (±0.02)	5.153 (±0.02)	7.887 (±0.01)^a	7.227 (±0.02)	3.747 (±0.04)^b	7.407 (±0.02)	0.0053
	Ni (mg kg^{-1})	0.47 (±0.02)^a	1.637 (±0.02)^b	0.657 (±0.01)	0.723 (±0.03)	1.263 (±0.02)	0.79 (±0.01)	0.0053
	Pb (mg kg^{-1})	0.867 (±0.06)^a	2.1 (±0.10)	1.067 (±0.06)	9.767 (±0.15)^b	4.467 (±0.06)	4.867 (±0.06)	0.0052
	Zn (mg kg^{-1})	38.633 (±0.40)^a	75.767 (±0.15)	40.5 (±0.10)	541.7 (±7.64)^b	64.67 (±0.15)	55.27 (±0.218)	0.0054

continued **Table 2.** Soil chemical properties and chemical composition of the soils and leaves.

The same letter in superscript means no significant difference according to the Kruskal-Wallis test; n.s. – not significant.

increasing concentration in soil content at sites with a higher disturbance level was observed. The Cd concentration at Sites 1 and 2, which had a very low and low disturbance level, respectively, were higher in leaves as compared to the soil (Site 1 = 0.267±0.015, Site 2 = 2.17±0.010 $mg\cdot kg^{-1}$). The Zn concentration was higher in leaves than in the soil at Site 2 (75.8±0.15) and Site 4 (541.7±7.638 $mg\cdot kg^{-1}$), whereas at the other study sites the concentration was higher in the soil (Table 2).

3.4 Heavy metal concentrations in the soil and leaves

The Spearman correlation rank showed a significant, positive relationship between the soil and leaf concentrations for Pb (r=0.88, *p*=0.000001), Zn (r=0.63, P=0.005) and Cd (r=0.53, *p*=0.02). According to the results of the regression analysis, the content of those metals in the soil had a positive impact on their concentrations in *Reynoutria japonica* leaves (r^2 for Pb, Cd and Zn reached 0.78; 0.61 and 0.47, with *p*=0.00000007; 0.00008; and 0.001, respectively).

4. Discussion

Reynoutria japonica maintains a relatively high growth rate even in conditions with a N deficiency [37] or in soils that have been contaminated with heavy metals [42]. For these reasons, there is a very wide range of results available in the published data on the influence of *R. japonica* on the chemical properties of soils under its canopy. Many authors emphasize the role of alien, invasive species, including Japanese knotweed, on the increase in the availability of nutrients compared to sites that are not invaded by such species [4,43-45].

Our studies showed distinctive differences in the physical and chemical properties of soils among the sites. Such results are mainly due to the types of the initial and current substrates the soil is composed of, especially due to their distinctively anthropogenic character. The chemical properties of soils developing under *Reynoutria japonica* canopies are very diverse; for example, their pH level is very wide. In the case of the sites that we investigated, it ranged from 4.0 to 7.7. Barney *et al*. [27] recorded similar pH values in Canada (4.5–7.4), whereas in Wales, Palmer [46] mentioned the 3–8 range, and in the case of Belgium, the soil pH under *R. japonica* varied from 4.4 to 7.3 [45]. Such a wide range in pH is due to the character of the soil substrate and the concentration of soluble alkaline mineral elements.

The C_{org} and N_{tot} content of soils under a dense canopy of *Reynoutria japonica* is very uneven, mainly as a result of the deposition of external material of a mineral character in the case of sites located within town borders, or organic sediments from floodwater or arable fields. The latter situation occurs on sites located on less disturbed land, outside urban areas. A similar situation with comparable values of organic matter content under a *R. japonica* canopy was described by Vanderhoeven *et al.* [45] and Ehrenfeld [5]. Both the above- and below-ground parts of Japanese knotweed play a role in the increase in soil total organic matter content. In this way, the species influences the content of soil organic matter and indirectly the soil chemistry. As a geophyte, *R. japonica* leaves the ground bare in the winter, which in turn influences the functioning of the soil, especially its physical and chemical properties. Japanese knotweed possesses annual, above-ground stems that can reach 3 metres in height and which allow it to produce big patches of very dense canopy composed of a single species. In addition, the rhizomes of *R. japonica* grow very quickly and may reach a depth of 2 metres [29], which allows the species to play an important role in the cycling of many elements in the 'soil-plant' system. Many studies also have been performed concerning pollution in contaminated areas using tree leaves and soils as biological indicators [47].

The maximum concentration of P in the soils under the *Reynoutria japonica* that we studied reached a value of 700 $mg\cdot kg^{-1}$, exceeding the levels recorded by Vanderhoeven *et al*. [45]. The maximum soil P concentration measured by these authors was 29.85 $mg\cdot kg^{-1}$ for the sites occupied by *R. japonica*, whereas at sites free of that species, the P concentration only reached 23.55 $mg\cdot kg^{-1}$. At our study sites, the P concentration was related to the character of the deposits introduced by humans. Barney *et al*. [27] provided a similar explanation for the observed P concentration. According to Dassonville *et al.* [4], in Europe, the wide range of nutrient content under the cover of alien species is strictly related to the initial habitat conditions, *i.e*., the soil conditions present under the anthropogenic layer of sediments and deposits. The rhizomes of *R. japonica* may grow deep into the natural mineral horizons where they reach the alkaline elements. Previous observations by Dassonville *et al.* [4] in this respect are confirmed in the results of our studies.

The Ca concentration in the soils investigated ranged from 803.2 to >20,000 $mg\cdot kg^{-1}$ and the Mg concentration ranged from 499.3 to >7000 $mg\cdot kg^{-1}$. These values are significantly higher than those given by Vanderhoeven *et al.* [45], which reached 576–4560 $mg\cdot kg^{-1}$ for Ca and 76.1–275.1 $mg\cdot kg^{-1}$ for Mg. The differences observed are the result of the disturbance level of a habitat, and in the case of our study sites, it is also due to the presence of sediments of different grain sizes, which originated from coal combustion and building materials (Sites 5 and 6). Both Ca and Mg are very mobile elements in soil-vegetation systems [15] and alien species have an impact on the biogeochemical cycles of many elements. Thus, as suggested by Ehrenfeld [5], alien species may directly modify the concentration of many elements in the soils in this way.

The concentrations of heavy metals in soils under the *Reynoutria japonica* canopy were high and in most cases they corresponded with the results obtained by other authors [4,48]. The maximum Fe content at our study sites exceeded the level of 45000 mg·kg^{-1} (the minimum reached 6,608.3 mg·kg^{-1}), which is comparable with the data from sites located in Wrocław, in south-western Poland (>47,000 mg·kg^{-1}), and in Prague, Czech Republic (>43,800 mg·kg^{-1}) that were given by Sołtysiak *et al.* [48]. These authors conducted their studies along roadsides, within and outside the cities mentioned above. It is worth emphasizing that the minimum concentrations of Fe in the soils were distinctively higher at our study sites than in localities from Wrocław (740 mg·kg^{-1}), although they were significantly lower than the minimum values recorded for the Prague sites (>2,7000 mg·kg^{-1}) that were given by Sołtysiak *et al.* [48].

The Zn content in soils varied, with the lowest level recorded on the least disturbed site (Site 1 = 41.5 mg·kg^{-1}). The maximum Zn concentration that we recorded exceeded 500 mg·kg^{-1} (Site 4), although it is lower than the maximum level of Zn recorded in the soils in Wrocław (>2,600 mg·kg^{-1}) [48]. The higher Zn concentration in soils at Site 4 compared to the other sites may be related to the presence of the metals contained in the wastes from the neighbouring cemetery that were dumped in the topsoil. On the other hand, the minimum value for Zn content, 41.5 mg·kg^{-1}, was higher than that given by Sołtysiak *et al.* [48] for Wrocław (10.5 mg·kg^{-1}), but lower than the one for the Prague site (54.7 mg·kg^{-1}). The results given by Vanderhoeven *et al.* [45] indicate much lower concentrations of Zn in soils under a canopy of *Reynoutria japonica* in Belgium (4.8–24.2 mg·kg^{-1}).

The Pb, Cu, and Cr concentrations in the soils of our study sites reached the following ranges: Pb from 15.3 (Site 2) to 158.1 mg·kg^{-1} (Site 4), Cr from 9.7 (Site 4) to 27.4 mg·kg^{-1} (Site 6), and Cu from 5.9 (Site 1) to 79.7 mg·kg^{-1} (Site 6). In most cases, the concentrations did not differ from the natural geochemical background and did not exceed the concentration norms for Poland [49]. The Cd concentration was the lowest among all of the heavy metals investigated and varied from 0.14 (Site 1) to 6.7 mg·kg^{-1} (Site 4). Such a range of values is influenced by the presence and types of anthropogenic deposits located at those sites. In the case of Pb, its maximum concentration (158.1 mg·kg^{-1}) was higher than those recorded for Prague (68.4 mg·kg^{-1}), but lower than the values for Wrocław (203.0 mg·kg^{-1}). The higher values for Wrocław were probably due to the location of the sites near roads with high traffic, in contrast with most of our sites, which were situated away from busy roads. The Cr concentrations at all of our study sites were lower than in Prague and Wrocław [48]. In Belgium, Vanderhoeven *et al.* [45] observed a much lower level of soil contamination with Cu (0.11–0.34 mg·kg^{-1}), so their results do not correspond with ours or those obtained by Sołtysiak *et al.* [48].

4.1 Chemical composition of Reynoutria japonica leaves

The maximum concentration of P in the leaves of Japanese knotweed that we studied exceeded the level of 2,100 mg·kg^{-1}, which is probably due to the input of natural and artificial fertilizers (organic and mineral), which contain high amounts of P (Site 3, which was influenced by agricultural activities) and the transport of sediments during high-water periods. An additional important source of P in this area was the uncontrolled inflow of communal sewage containing high amounts of detergents into the river catchment. Higher concentrations of P than those we observed were reported by Dassonville *et al.* [35] (>2400 mg·kg^{-1}) and Vanderhoeven *et al.* [45] for Belgium, which was explained as the consequence of the decomposing biomass from the highly productive ecosystems present in that area [16]. Kourtev *et al.* [10] showed that in experimental conditions, soil microbial communities that form under a dominant alien plant species may influence the biochemical processes that take place in the soil and the content and cycling of many nutrients in the soil environment.

The maximum Ca concentrations in *Reynoutria japonica* leaves from our study sites varied from 2,406 to >19,500 mg·kg^{-1}and Mg from 2,670 to >6,300 mg·kg^{-1}. Such high values may reflect the soil conditions on those sites. According to the results obtained by Dassonville *et al.* [35], it is clear that Ca concentrations in plant material may exceed the level of 22,000 mg·kg^{-1}, which is a much higher figure than those that we observed. The minimum Ca levels that we obtained were also lower than those reported by other, already mentioned authors who gave the values of 5,700 and 7,490 mg·kg^{-1}, respectively. An opposite situation is observed in the case of Mg concentration in *R. japonica* leaves when compared with the results obtained by other researchers [5,13,48]. Both the minimum and the maximum values of Mg concentration that we recorded are higher than those given by Dassonville *et al.* [4], Dassonville *et al.* [35], and

Vanderhoeven *et al.* [45], who recorded a maximum level of 4,136 mg·kg^{-1} and a minimum of 618–1,150 mg·kg^{-1}.

The Fe concentration in *Reynoutria japonica* leaves did not depend on the level of the disturbance of a site and reached comparable levels at all the sites studied, varying from 137.0 to 312.3 mg·kg^{-1}, whereas the values recorded by Sołtysiak *et al.* [48] for Wrocław and Prague were 224.5 and 227.0 mg·kg^{-1}, respectively.

The Zn concentration in *Reynoutria japonica* leaves varied between 38.6 and 541.7 mg·kg^{-1}, and these values were similar to those recorded for the soils of those sites. Other authors also reported low Zn concentrations in leaves, below the level of 62.9 mg·kg^{-1}, from other parts of Europe [4,45,48].

Maximum concentrations of Pb, Cu, Cd, and Cr in the leaves of *Reynoutria japonica* reached the following ranges: Pb = 0.87–9.77 mg·kg^{-1}, Cu = 3.75–7.89 mg·kg^{-1}, Cd = 0.17–5.28 mg·kg^{-1}, and Cr = 1.01–1.84 mg·kg^{-1} (Table 2). These results are comparable with those of Sołtysiak *et al.* [48]; our maximum values correspond with the results for Wrocław, and our minimum ones correspond with those for Prague. Only in the case of Pb was the maximum concentration of that element in the leaves of *R. japonica* noticeably higher than the one given by Sołtysiak *et al.* [48] for Wrocław. It should also be stressed that the maximum Cu concentration that we observed was similar to the results reported by Dassonville *et al.* [35] (7.8 mg·kg^{-1}) and Vanderhoeven *et al.* [45] (7.84 mg·kg^{-1}) for Belgium.

The potential application of *Reynoutria japonica* as a hyperaccumulator of heavy metals has already been investigated in Europe [50] and in Japan [51]. In those cases, Japanese knotweed populations from areas with soils contaminated with Cu (3,000 mg·kg^{-1} of dry mass), Zn (10,000 mg·kg^{-1}), and Cd (100 mg·kg^{-1}) were analyzed. The results of those investigations showed that *R. japonica* accumulates these metals in both its rhizomes and its leaves, especially in the cell walls [51]. A typical, average level of the above-mentioned elements in soils not contaminated with heavy metals reached 20, 110, and 2 mg·kg^{-1} of dry mass, for Cu, Zn, and Cd, respectively. The concentrations of those metals recorded in plant tissues were 2,300, 6,700, and 62 mg·kg^{-1} of a dry mass, respectively. It appears that in comparison with other plant species growing on soils rich in heavy metals, *R. japonica* accumulates much higher amounts of these elements. Similar studies conducted by Hulina and Dumija [50] pointed to a high concentration of Cu, Zn, Pb, and Cd in *R. japonica* leaves. In addition, Tateno and Hirose [52] observed that amounts of organic nitrogen, ammonium, and nitrates in the soil were a few times higher under a canopy of *R. japonica* than in places unoccupied by that species. Thus, according to those authors [52], Japanese knotweed plays an important role in the process of enrichment of soil with nutrients during primary succession.

5. Conclusions

Reynoutria japonica is a species with wide ecological amplitude, and thus is able to adapt and grow in very diverse ecological conditions. Outside its natural geographical distribution range, these features allow the species to rapidly colonize areas with soil that was mechanically disturbed due to human pressure. Although Japanese knotweed may also occur in relatively weakly disturbed sites, it encroaches and establishes much more dynamically in highly disturbed and deregulated ecosystems.

Soils under a dense canopy of *Reynoutria japonica* are characterized by a wide variety of their physical and chemical parameters, which in turn depend on the type of the anthropogenic materials (inflow/sediments) that dominate the soil substrates. Thus, the type of human pressure is a key factor that shapes the physical-chemical soil conditions independent of the level of habitat disturbance. Although *R. japonica* is a species that possesses the ability to modify soil conditions, in the case of ecosystems that are highly transformed due to human pressure, the soil-forming role of Japanese knotweed is of secondary importance. Due to its phalanx growth form, *R. japonica* may easily invade any available habitats that are free of competition and consequently inhibit or eliminate the native herb layer species from such sites. This, in turn can lead to the development of extensive, single-species patches of Japanese knotweed in highly transformed urban and industrial areas.

Reynoutria japonica is able to grow on sediments or soils with a very wide pH range. Furthermore, it possesses a high level of tolerance for soils/substrates that have been severely contaminated with heavy metals. Our studies showed that Japanese knotweed is not only able to accumulate P in its leaves, but also some other heavy metals. *R. japonica* is regarded as a dangerous, invasive species in Europe (for example, in the UK active control of *R. japonica* populations is a statutory obligation that is regulated by a legislative act).

Acknowledgements

The authors would like to thank for two anonymous reviewers, whose comments were valuable to the final version of this paper.

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