

Short Communication

Effect of Soil Artificially Polluted with Lead on an Invasive *Fallopia x bohemica*: A Case Study from Central Europe

Justyna Sołtysiak, Teresa Brej*

Department of Botany and Plant Ecology, Wrocław University of Environmental and Life Sciences, Wrocław, Poland

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Abstract

The presented study was carried out to estimate the effects of lead on an invasive *Fallopia x bohemica*. The plant material was hexaploid hybrid, which was collected from riparian habitat of Wrocław (SW Poland) and cultivated under greenhouse conditions. Plants were grown in control pots and in pots with soil artificially polluted with various doses of Pb (50, 100, 200 mg·kg⁻¹) for 98 days. To determine the ability of tested hybrids to grow under stress conditions we tested their morphological characteristics (fresh and dry weight of aboveground parts, fresh and dry weight of underground parts, length of shoots, length and width of leaves, number of leaves). The lead content in the rhizomes was also determined for roots and leaves separately. In the greenhouse experiment, the rhizomes of *Fallopia x bohemica* regenerated well in the polluted soil. The increase in the concentration of lead in soil did not negatively affect its growth and morphological characteristics. The tested hybrid accumulated lead, mainly in underground parts. The mean Pb content in plants from the soil where 200 mg·kg⁻¹ of Pb was added was more than 20 times higher than in control plants. The results suggest that the invasive *Fallopia x bohemica* is tolerant to lead pollution, what can facilitate its intensive expansion on anthropogenic soils, where the level of this metal is usually high.

Keywords: invasive plant species, Japanese Knotweed *s.l.*, hybrid, lead

Introduction

Pollution with heavy metals is one of the most noticeable environmental problems due to their adverse ecological effects reported by many publications [1-3]. Toxic metals contaminating the soils create difficult

conditions for most plants growing in them. They may cause various morphological, physiological, and biochemical dysfunctions in plants, either directly or indirectly, and cause damaging effects [2]. Heavy metal toxicity in plants varies with plant species, specific metal, content of metal, its chemical form, soil composition and pH. Some metals, including Cu, Mg, Co, Zn and Cr, are essential to plants in trace amounts, but only when metals are present in bioavailable forms, and at excessive levels they have the potential to become toxic to plants [4]. Others, such as Mg,

*e-mail: justyna.soltysiak@upwr.edu.pl

Co, and Zn, have unknown biological functions and can cause disorders even in relatively lower contents [5].

Fallopia x bohemica (Chrtk et Chrtková) J.P. Bailey is an interspecific hybrid between two Asian herbaceous perennial species *Fallopia japonica* var. *japonica* (Houtt.) Ronse Decraene and *Fallopia sachalinensis* (F. Schmidt ex Maxim.). All belong to the *Fallopia* genus, (Polygonaceae), but as it creates a polyploid complex of species it is often referred to as Japanese Knotweed *s.l.* [6]. Members of the *Fallopia* genus differ in their genetic variation. Among them, *Fallopia x bohemica* shows the highest genetic variability and three ploidy levels ($2n = 44, 66$ and 88) [6, 7], and show various invading abilities. *Fallopia x bohemica* is considered the most troublesome plant of the *Fallopia* genus [8]. The hybrid spreads faster than the parental species and is ecologically better adapted to human-influenced environments due to its higher regenerative ability [7]. *Fallopia x bohemica* strongly competes with other plant species, even with its parents at the areas where taxa co-occur [9]. Moreover, among invasive taxa of the *Fallopia* genus, the hybrid has the greatest resistance to chemical eradication [10]. In Central Europe, the *Fallopia* genus willingly invades habitats changed by human influence. They occur frequently in places with a high level of anthropogenic impact, including areas along roads and railway tracks [7,11]. The *Fallopia* genus can inhabit soils polluted by heavy metals. In some areas of Central Europe, Japanese Knotweed *s.l.* can grow on soils where the maximum values of heavy metals reach up to: $79.7 \text{ mg}\cdot\text{kg}^{-1}$ (Cu), $158.1 \text{ mg}\cdot\text{kg}^{-1}$ (Pb) and $500 \text{ mg}\cdot\text{kg}^{-1}$ (Zn) in the case of *Fallopia japonica* [12], and $51.6 \text{ mg}\cdot\text{kg}^{-1}$ (Cu), $216.3 \text{ mg}\cdot\text{kg}^{-1}$ (Pb) and $448.8 \text{ mg}\cdot\text{kg}^{-1}$ (Zn) for *Fallopia x bohemica* [13]. It was also observed that the hybrid range in such places increases quickly [13].

In this study, we examined the effect of lead on *Fallopia x bohemica* growing under greenhouse conditions. This metal was tested because it belongs to the major pollutants connected with anthropogenic activities and it is toxic to most plants species. We hypothesized that the *Fallopia x bohemica* hybrid is tolerant to lead pollution, which can facilitate its intensive expansion on anthropogenic soils, where the content of this metal is usually high. The presented experiment is the first step to verify this hypothesis. Its main goal was to answer the questions: 1) Does the presence of high lead doses in soils affect the regeneration ability of *Fallopia x bohemica* from rhizome fragments?, 2) Does the tested hybrid tend to accumulate

Pb?, and 3) Does Pb influence *Fallopia x bohemica*'s growth and its chosen morphological characteristics?

Materials and Methods

Greenhouse Experiment

To evaluate the response of *Fallopia x bohemica* to lead pollution in soil, a greenhouse investigation was carried out. In this study, we used *Fallopia x bohemica* hybrid with chromosome number (66) and ploidy level ($2n = 66$). The experiment was designed with three doses of lead (50, 100 and $200 \text{ mg}\cdot\text{kg}^{-1}$), which were applied into plastic pots, each containing 5 kg of soil with known chemical properties (Table 1). The pots with soil without the addition of Pb were treated as the control. All lead treatments and the control were tested in four replications. Plants were grown from the last week of May to the end of August under natural light and temperature conditions. During the experiment, the air temperature varied from 20 to 30°C . The research material was fresh rhizome fragments of *Fallopia x bohemica*. *Fallopia* taxa as invasive plants, spread mainly in a vegetative way on newly settled areas, usually by rhizome fragments [4]. For this reason, the rhizomes were used to establish the plants in the experiment. The plant material for the experiment was collected from riparian habitat of the Odra River at $51^\circ06.035'\text{N}$, $17^\circ04.643'\text{E}$ in the city of Wrocław (SW Poland). The locality was selected on the basis of published data [13] and represents a place where the content of lead does not exceed its average level, which for uncontaminated Polish soils is reported as $18 \text{ mg}\cdot\text{kg}^{-1}$ [14].

The dug-out rhizomes were cleaned from the soil with a brush and rinsed with distilled water. Next, they were divided into 10-centimeter fragments of the same weight (14,5 g). Single rhizome fragments were placed at a depth of 5 cm into the Pb treatment and control pots. Plants in the greenhouse were grown for 98 days. To determine the ability of the tested hybrid to grow under stress conditions, its morphological characteristics (fresh and dry weight of aboveground parts, fresh and dry weight of underground parts, length of shoots, length and width of leaves, number of leaves) were measured. Next, the lead concentration in plant tissues was determined. Lead belongs to metals that accumulate in underground parts of plants [5, 15]. Generally, the content of Pb in plant organs tends to decrease in the following order: roots>leaves> stems> flowers [15].

Table 1. Chemical properties of soil used in the greenhouse experiment.

pH		N _{og}	C _{og}	K ₂ O	P ₂ O ₅	MgO	Agronomical Category
K _{Cl}	H ₂ O	[%]		[mg100·g ⁻¹]			
5.9	6.3	0.092	1.4	32.6	13.5	4.7	Medium soil

Taking this into account, underground (rhizomes with roots) and aboveground organs (leaves) of *Fallopia x bohemica* were treated separately, as different parts of the plants accumulate Pb. The stems and flowers as organs that contain a smaller amount of this metal were not analyzed. Plant material was washed with distilled water. Soil particles were removed carefully from the underground parts of plants. Plant samples were air-dried at 85°C and milled with a stainless-steel grinder. Next, the samples were digested in a mixture of HCl and HNO₃ (in a ratio 3:1) [16]. Concentrations of heavy metals in plant tissues were analyzed by the ICP-OES technique using a Varian 220 analytical machine. Each sample was analyzed in four replicates.

Statistical Analysis

The Shapiro-Wilk W test was used to check the normal distribution of data. The homogeneity of variance was checked by Levene's test. The data of normal distribution were analyzed using one-way ANOVA with post-hoc testing of significance of differences using Tukey's test, in which the test statistic (F) and probability value (p) were taken into account. The data for which a normal distribution was not found, or the homogeneity of variance was not obtained, were analyzed using non-parametric methods, the Kruskal-Wallis test, where attention was paid to the test statistic (H) and probability value (p). To determine the relationship between the studied biometric features

of *Fallopia x bohemica*, principal correspondence component analysis (PCA) was used. Data used in the analyses were transformed logarithmically. Statistical analyses were conducted using the STATISTICA v. 12 (StatSoft Inc., 2014) package.

Results and Discussion

The *Fallopia* genus has massive vegetative regeneration abilities, especially from rhizome segments. Brock et al. [17] have shown that in natural conditions, a new plant can be created from a small rhizome segment (even up to approximately 0.7 g in weight). The results of the presented study showed that high doses of lead (50, 100, 200 mg·kg⁻¹) did not induce a delay of the regeneration of rhizomes of *Fallopia x bohemica*. New plants regenerated after 4 days in control pots as well as in all pots with contaminated soils.

It is known that excessive lead accumulation in plant tissue impairs various morphological, physiological, and biochemical functions [18]. Common changes in morphological characteristics of plants induced by excess lead can include the reduction in size and thickness of leaf blade or shrinking and distortion of the leaves, and lead can also decrease plant height [19]. The variability of all studied morphological features of *Fallopia x bohemica* are shown in Fig. 1. Based on the analysis of the PCA diagram, it was concluded that the first canonical axis cumulated 29.5% of the total

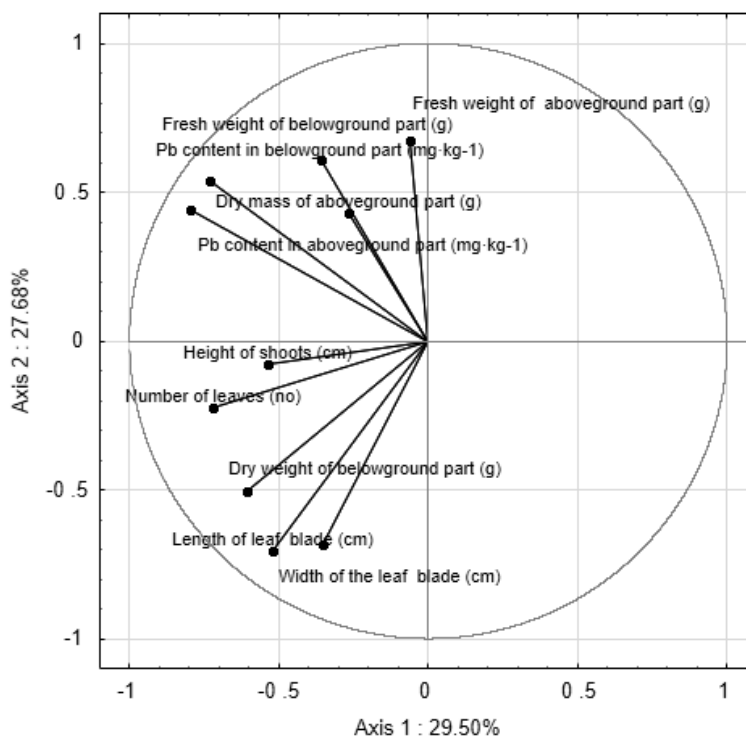


Fig. 1. Results of PCA in the first and second canonical axes; analysis was prepared for all biometric features and the content of Pb in underground and aboveground parts of plant bodies.

Table 2. Comparison of morphological characteristics of *Fallopia x bohemica* and the content of lead in aboveground and underground part of plants from pots polluted with various levels of Pb; the table shows mean values with standard errors; different letters present significant differences obtained after Tukey's test* and Kruskal-Wallis test($P \leq 0.05$).

Characteristics		Control	50 mg·kg ⁻¹	100 mg·kg ⁻¹	200 mg·kg ⁻¹
Dry weight of under-ground	g	87.86a (±12.68)	68.98a (±11.57)	74.76a (±5.52)	102.16a (±14.78)
Fresh weight of under-ground*	g	479.66a (±85.18)	445.33a (±38.83)	469.66a (±71.18)	556.33a (±195.91)
Length of the leaves*	cm	12.03a (±0.28)	10.73a (±0.44)	10.91a (±0.07)	12.13a (±0.83)
Width of the leaves	g	8.20a (±0.19)	7.22a (±0.38)	6.75a (±0.38)	7.82a (±0.37)
Height of shoots	cm	69.43a (±9.20)	64.93a (±6.06)	70.06a (±9.27)	75.86a (±3.19)
Dry mass of above-ground shoots	g	16.84a (±1.48)	22.98b (±1.45)	22.11ab (±0.55)	22.24 ab (±1.26)
Fresh mass of above-ground shoots *	g	137.33a (±0.88)	226.66a (±74.78)	153.66a (±22.51)	206.0a (±97.62)
The number of leaves	nb	102.0ab (±5.70)	71.0a (±8.54)	100.0ab (±5.84)	116.0b (±12.98)
Pb content in above-ground parts*	mg·kg ⁻¹	1.23a (±0.31)	1.36a (±0.37)	5.12a (±1.12)	9.09a (±1.41)
Pb content in under-ground parts*	mg·kg ⁻¹	1.45a (±0.05)	9.57ab (±0.91)	13.43ab (±1.58)	29.50b (±4.18)

variance, while the second axis cumulated 27.68%. The results have shown a correlation between features of the two study groups, fresh weight of aboveground and underground parts, dry mass of aboveground, Pb content in aboveground and underground and the second represented height of shoots, number of leaves, dry weight of underground parts, length of leaf blade, and width of the leaf blade. Analysis of variance indicated that *Fallopia x bohemica* from the soils artificially contaminated with lead were not very different from control plants. Statistically significant differences for the characteristics were found in the case of dry mass of aboveground shoots ($F = 5.144$; $p = 0.028$) and number of leaves ($F = 4.604$; $p = 0.0373$). The lack of a negative effect (even in the presence of a high Pb dose) on the morphological characteristics of the hybrid can be an evidence of this plant's tolerance to lead. The low impact of heavy metals (Cr, Cu, Cd, Zn and Pb) on the growth of Japanese Knotweed *s.l.* was shown by Michalet et al. [20]. After a three-month experiment, the final height of plants was not reduced by metal pollution. Moreover, in some cases, plant height was even significantly greater in comparison to control. Authors have concluded that the presence of toxic metals in soil had a rather stimulating effect on the *Fallopia* genus growth, depending on pollution level.

Our investigation has proven that the studied *Fallopia x bohemica* hybrid is able to accumulate lead (in underground organs mainly). The statistically significant differences were found between underground parts of control plants and plants from pots polluted

with different doses of Pb ($H = 10.00$; $p = 0.185$). Plants from pots where particular doses of Pb were added accumulated a high amount of this metal compared to the control (Table 2). In the underground part of plants grown in soil with the addition of 200 mg·kg⁻¹, the mean Pb concentration was more than 20 times higher than in control plants. These results of the greenhouse experiment are corresponding with field research showing the potential of heavy metal uptake (in underground parts) by the *Fallopia* genus [13, 21]. A high lead concentration ability was also found in other invasive plants, e.g., in *Solidago canadensis* from soils polluted with Pb in China [22] and in *Conium maculatum*, which is spreading on contaminated roadside soil in Cook County in the USA [23]. The investigation has shown that both species have developed some tolerance to heavy metals. Zhang et al. [24] have proven that higher tolerance to Pb stress can enable an invasive *Solidago canadensis* to outcompete the native species and may promote its rapid invasion on Pb-contaminated soils. The tolerant *Solidago canadensis* grows better in comparison with native species, e.g., *Kummerowia striata* [24], and is able to reduce native plants under monoculture successfully [22].

The plants are characterized by various abilities to distribute and accumulate heavy metals. Some differences can be found even between genotypes of the same species [25]. Berchová-Bímová et al. [26] have investigated the role of different cytotypes of three *Fallopia* taxa in Cd, Pb and Zn absorption. In this study, the concentrations of heavy metals were

significantly higher in underground parts of plants (roots and rhizomes) than in aboveground parts [26]. In the case of Cd and Zn, the taxa did not respond differently to metal exposure. A significant difference in Pb content was found between the ploidy levels. The amount of Pb in tetraploid plants was significantly lower than in hexaploid and octoploid plants. In the greenhouse experiment, the hexaploid hybrid of *Fallopia x bohémica* was tested, which can partly explain its observed ability to accumulate lead. However, this requires further, detailed study.

Conclusions

The results of our greenhouse experiment have shown that high content of Pb in soils does not affect the invasive *Fallopia x bohémica*. In soils contaminated with lead, the plant has regenerated well from small rhizome fragments. The lead has not influenced *Fallopia x bohémica*'s growth and its morphological characteristics. Moreover, the tested hybrid has accumulated large amounts of Pb, mainly in underground parts. Based on the results of the greenhouse experiment, we conclude that the tested hybrid of *Fallopia x bohémica* has tolerance to lead. This important characteristic can facilitate its invasion success, especially in anthropogenic habitats, which are often polluted by lead. The study of these issues will be continued. It is also worth checking the response of the hybrid to other toxic metals.

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