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,
Fallopia x bohemica (Chrtek et Chrtková) J.P. Bailey is an interspecific hybrid between two Asian herbaceous perennial species Fallopia japonica var. japonica (Hoult.) Ronse Decraene and Fallopia sachalinensis (F. Schmidt ex Maxim.). All belong to the Fallopia genus, (Polygonaceae), but as it creates a polycomplex 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 mg kg\(^{-1}\) (Cu), 158.1 mg kg\(^{-1}\) (Pb) and 500 mg kg\(^{-1}\) (Zn) in the case of Fallopia japonica [12], and 51.6 mg kg\(^{-1}\) (Cu), Pb 216.3 mg kg\(^{-1}\) (Pb) and 448.8 mg 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 bohemia 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 bohemia 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 mg 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°06.035’N, 17°04.643’E in the city of Wroclaw (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 mg 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   | K\(_{\text{Cl}}\) | H\(_2\text{O}\) | N\(_{\text{eq}}\) | C\(_{\text{eq}}\) | K\(_{\text{O}}\) | P\(_{\text{O}}\) | MgO | Agronomical Category |
|------|----------------|--------------|--------------|------------|----------|----------|-----|---------------------|
| 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 variance. This diagram shows the relationships between the studied biometric features and the content of Pb in underground and aboveground parts of plant bodies.

![Graph showing PCA analysis](image-url)
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 = 5.145; p = 0.038), length of the 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 plants under 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

| Characteristics                  | Control | 50 mg kg⁻¹ | 100 mg kg⁻¹ | 200 mg kg⁻¹ |
|---------------------------------|---------|------------|-------------|-------------|
|                                 | g       | (±12.68)   | (±11.57)    | (±5.52)     | (±14.78)     |
| Fresh weight of underground*    | g       | (±85.18)   | (±38.83)    | (±71.18)    | (±195.91)    |
| Length of the leaves*           | cm      | 12.03a     | 10.73a      | 10.91a      | 12.13a       |
|                                 |         | (±0.28)    | (±0.44)     | (±0.07)     | (±0.83)      |
| Width of the leaves             | g       | 8.20a      | 7.22a       | 6.75a       | 7.82a        |
|                                 |         | (±0.19)    | (±0.38)     | (±0.38)     | (±0.37)      |
| Height of shoots                | cm      | 69.43a     | 64.93a      | 70.06a      | 75.86a       |
|                                 |         | (±9.20)    | (±6.06)     | (±9.27)     | (±3.19)      |
| Dry mass of above-ground shoots | g       | 16.84a     | 22.98b      | 22.11ab     | 22.24 ab     |
|                                 |         | (±1.48)    | (±1.45)     | (±0.55)     | (±1.26)      |
| Fresh mass of above-ground shoots* | g   | 137.33a    | 226.66a     | 153.66a     | 206.0a       |
|                                 |         | (±80.88)   | (±74.78)    | (±22.51)    | (±97.62)     |
| The number of leaves            | nb      | 102.0ab    | 71.0a       | 100.0ab     | 116.0b       |
|                                 |         | (±5.70)    | (±8.54)     | (±5.84)     | (±12.98)     |
| Pb content in above-ground parts* | mg kg⁻¹ | 1.23a      | 1.36a       | 5.12a       | 9.09a        |
|                                 |         | (±0.31)    | (±0.37)     | (±1.12)     | (±1.41)      |
| Pb content in under-ground parts* | mg kg⁻¹ | 1.45a      | 9.57ab      | 13.43ab     | 29.50b       |
|                                 |         | (±0.05)    | (±0.91)     | (±1.58)     | (±4.18)      |
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 bohemica* 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 bohemica*. In soils contaminated with lead, the plant has regenerated well from small rhizome fragments. The lead has not influenced *Fallopia x bohemica’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 bohemica* 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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