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JAPANESE KNOTWEED *(REYNOUTRIA JAPONICA)* AS A PHYTOREMEDIATOR OF HEAVY METALS

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ABSTRACT
Recently, plant remediation techniques - phytoremediation - have been developed. Japanese Knotweed (*Reynoutria japonica*), is an invasive species with negative impacts on the environment and the economy. In order to assess the tolerance of highly invasive weeds to heavy metals, an experiment was conducted in which this plant was grown in control soil and in soils contaminated with different concentrations of Pb, Cd. The content of heavy metals in the soil did not eliminate the ability of *Reynoutria japonica* roots to regenerate. In soil contaminated with any concentration of Pb, the shoots of this plant grew at a similar rate as the control plants, and had the same morphological characteristics. Higher concentrations of cadmium and lead Cd (100, 200 mg kg^{-1}), Pb (2000 mg kg^{-1}) have extended rhizome regeneration compared to control plants. In soils contaminated with lower concentrations of Cd or Pb shoots grew at a similar rate as control plants. Chemical analysis of heavy metal content showed that this weed accumulated large amounts of metals when grown in soil contaminated with heavy metals A relatively high intake of Cd, aboveground plants. At a cadmium concentration of 100 mg kg^{-1}, more than 537 times the amount of cadmium accumulated in the aboveground part of the plant, as opposed to that in control. The ability of Japanese Knotweed (*Reynoutria japonica*), to regenerate from parts of the rhizome, to grow and develop under stressful conditions in the presence of heavy metals indicates a high tolerance to heavy metals.

Key words: Japanese Knotweed (*Reynoutria japonica*), phytoremediation, heavy metal accumulation

INTRODUCTION
Phytoremediation is a method that uses plants to remove or stabilize pollutants in the environment. The goal of remediation of contaminated areas due to human activity is to restore such ecosystems to their original state. Due to the application to a large number of pollutants, as well as the possibility of implementation on various surfaces, and due to lower environmental impact and lower costs, phytoremediation is considered a green technology. The toxicity of heavy metals in plants varies depending on plant species, metal specificity, metal content, its chemical form, soil composition and pH. Some metals, including Cu, Mg, Co, Zn, and Cr, are essential for plants in trace amounts, but only when metals are present in bioavailable forms, and in increased amounts can become toxic to plants (Nagajyoti, 2010). Mg, Co and Zn, have unknown biological functions and can cause disturbances even at relatively lower concentrations.

Phytoremediation consists of four different technologies used by plants and each has a different mechanism for remediation of soils, sediments and waters contaminated with heavy metals. These include:

1. Phytoaccumulation (Phytoextraction) - the use of plants, with a large biomass and the ability to accumulate metals and appropriate soil additives to transport and concentrate metals from the soil to the aboveground parts of plants, which will then be removed through conventional agronomic measures.
2. Phytostabilization - the use of plants in order to reduce the bioavailability of pollutants in the environment; in this case the plants stabilize the contaminated soil rather than clean it.
3. Rhizofiltration - the use of the root system of plants for the absorption and adsorption of pollutants, mainly metals, from water.
4. Phytovolatilization - the use of plants for the extraction of certain, volatile, metals from the soil, and then their release through the leaves into the atmosphere (Muthusaravanan et al. 2018)

Invasive species are one of the main problems of modern ecology and are considered a significant component of global change associated with human activity (Sołtysiak, 2020).

Earlier research has found that these plant species have the following characteristics: strong and rapid growth, high fertility, strong competitiveness (Sharma et al., 2005). Furthermore, it was observed that in order to become a good phytoremediator they must have a wide tolerance in the environment and be adapted to different conditions (Bradley et al. 2010). Cadmium and lead were chosen as pollutants because they are widespread in the environment, especially as soil contaminants. The experiment was carried out in natural environmental conditions where the reaction to stressful conditions of the Japanese Knotweed (*Reynoutria japonica*) caused by the presence of heavy metals was monitored (Sołtysiak et al. 2011a, 2014b, Rahmonov et al. 2014a, 2019b).

Japanese knotweed, *Reynoutria japonica* (*Polygonaceae*), synonym *Fallopia japonica*

It is native to East Asia, naturally distributed in China, Japan, North and South Korea and Taiwan (Bailey 2003, CABI 2020). Japanese Knotweed, (*Reynoutria japonica*) was widespread as an ornamental plant in the 19th century, while today it is considered the worst European invasive alien plant species (Nentwig et al. 2018), as it spreads in almost all European countries.

Japanese knotweed, (*Reynoutria japonica*) is a tough herbaceous, rhizomatous perennial, in the native (natural, native) area, tolerates different soil acidity, and tolerates pH values in a wide range from 3 to 8 , prefers moist soil, is thermophilic, tolerates higher concentrations of nitrogen and heavy metals, adapts the soil to itself, and by secreting toxic substances prevents the growth of other plants, thus rapid growth. Habitats are areas near rivers and streams, neglected terrains and shrubs, along roads, by settlements (Trinajstić et al. 1994).

Some authors (Alcorta et al., 2004, Newman and Reynolds, 2004) cite the issue of introducing potentially invasive species used in phytoremediation that do not belong to native species.

The term invasive alien species refers to plants that have been intentionally or unintentionally introduced into areas outside their natural habitat. These species show a very rapid adaptation to conditions in the new and foreign environment, but also a great ability to reproduce and a large number. Invasive species, Japanese knotweed, (*Reynoutria japonica*), is a honey and ornamental species that stabilizes mobile soils along river banks, but changes the soil physically and chemically, inhibits the growth of other plants, takes away light, adapts to almost all possible habitat conditions, destroys native flora and fauna and is resistant to eradication attempts (Novak et al., 2010).

Japanese knotweed, *(Reynoutria japonica)* is also invasive and appears along the river, and its presence can serve two purposes: suppression of invasive attack and remediation of contaminated soil (Nguyen, L.2002).

Some authors have investigated different species (taxa), *Reynoutria japonica* ie the ability of their growth in localities, which during growth were exposed to high content of Cd, Pb and Fe in the soil. High concentrations of heavy metals are found in the roots and rhizomes of all species. These results showed a certain ability of *Reynoutria japonica* to absorb heavy metals, indicating a high phytoremediation potential (Berchová-Bímová, K. et al. 2014). Increased concentrations of Cd, Cr, Cu, Pb and Zn are acute toxins for most plant species. Plant growth at increased concentrations of heavy metals (so-called "metal stress") includes morphological and structural changes of aboveground shoots, such as deformation and chlorosis of leaves, necrosis of leaves, changes in leaf color, red and purple color of leaves, brown edges of leaves, decreases number and size of leaves, biomass decreases, growth inhibition and final extinction (Benyó et al. 2016).

Preliminary field studies in the city of Wrocław, Poland, have shown that the increased content of heavy metals in the soil does not reduce the invasion of *Reynoutria japonica*, but on the contrary, it is competitive with other plant species (Sołtysiak et al. 2014).

The aim of this study was to determine:

1) Ability of highly invasive Japanese Knotweed, (*Reynoutria japonica*) for regeneration from rhizome parts in soils contaminated with different concentrations of Pb and Cd.

2) Possibility of accumulation of heavy metals (Pb and Cd) in its underground and aboveground parts.

MATERIAL AND METHODS

Plant material of *Reynoutria japonica* studied in this experiment was collected from the city of Bihać (Bosnia and Herzegovina). The rhizomes of *Reynoutria japonica* were then cleaned in the laboratory, purified with distilled water and cut into pieces of equal length and weight. Prepared plant materials (fragments of rhizome *Reynoutria japonica* were planted in plastic pots, one rhizome in each pot with a content of 5 kg of soil (from the greenhouse). Chemical analysis of the soil used in the study, shown (Table 1.)

Table 1. Chemical analysis of the soil used in the research

Parameters	**Unit of Measurement**	**Results**
Depth	cm	0-30
Hygroscopic moisture (Hy)	%	4,70
Organic matter	%	21,42
Mineral matter	%	78,58
Humus	%	0,54
Active acidity		7,07
pH KCl-u		6,95
NH_3-N	mg kg^{-1}	3,68
NO_3-N	mg kg^{-1}	7,05
NO_3^-	mg kg^{-1}	30,38
P	mg kg^{-1}	1,62
PO_4^{3-}	mg kg^{-1}	4,88
P_2O_5	mg kg^{-1}	3,79
SO_4^{2-}	mg kg^{-1}	27,13
K_2O	mg kg^{-1}	9,22
K	mg kg^{-1}	7,59
Ca^{2+}	mg kg^{-1}	553,48
Mg^{2+}	mg kg^{-1}	81,39

The experiment was set up with certain concentrations of heavy metals, lead and cadmium, applied directly to plastic pots, each of which contained 5 kg of soil with known chemical properties (Table 1). Pots without the addition of heavy metal were treated as controls.

Solutions of the corresponding salts were prepared with a solution of lead nitrate Pb $(NO)_3$, 1 mol dm^{-3} cadmium sulphate $CdSO_4$ 0.1 mol dm-3, and then diluted and adjusted to the mass of the soil to define the exact mass concentration of the ions present to be removed. remediation. The concentration of pollutants is determined in accordance with the maximum permissible concentrations of pollutants prescribed by the Rulebook on determining the permitted amounts of harmful and dangerous substances in the soil and the methods of their testing (official Gazette of the Federation of Bosnia and Herzegovina, 72/09).
The low concentration (LC) of lead ions (Pb^{2+}) was 1000 mg, cadmium (Cd^{2+}) 20 mg per kilogram of soil. The high concentration (HC) of lead ions (Pb^{2+}) was 2000 mg, cadmium (Cd^{2+}) 100 mg per kilogram of soil.

All treatments with lead and cadmium were tested, as well as control in three replications (LC, HC, Control). The plants were grown for 45 days, from mid-July to the end of August under natural light temperature conditions.

Plant growth monitoring, measurements and analysis of heavy metal concentrations

During the experiment, the growth and development of *Reynoutria japonica* was monitored. After the experiment, the concentration of heavy metals in the aboveground organs (stem and leaves) and underground organs (roots) was determined.

The plant material was washed with distilled water, then dried at room temperature. Samples of accurately weighed mass (about 1g), previously homogenized, dry plant material, annealed and prepared for heavy metal analysis. In the sample thus prepared, the values on the Atomic Absorption Spectrophotometer, Perkin Elmer AAS 800, were read by flame technique. The analyzes were performed in the Laboratory of the Biotechnical Faculty of the University of Bihać.

RESULTS AND DISCUSSION

The obtained results of the analyzed plant material with standard deviation and the results of statistical data processing (One Way Anova) depending on the applied concentration of a part of the plant are presented in Tables 2,3. For experimental research, a plant culture Japanese knotweed (*Reynoutria japonica*) was selected, which shows very fast adaptation to conditions in new and foreign environments, but also a great ability to reproduce, it is adaptable to almost all possible habitat conditions, (Novak et al., 2010, Bradley et al., 2010).

During the control treatment, the plants looked healthy in the first stages of growth and development. By monitoring the parameters of plant growth and reproduction, no significant changes were observed on the young leaves of the plant, also plants grown in soil contaminated with low metal concentrations, developed normally and achieved the expected development characteristic of this species.

Results of the analyzed plant material on the content of heavy metals

Based on a one-way analysis of variance (One-Way ANOVA and Tukey test), a significant effect of metal concentration (High Concentration-HC and Low Concentration-LC) on phytoremediation potential depending on the part of the plant (root, stem and leaf) was determined ($p \leq 0,05$).

Table 2. Values of tested heavy metal, lead, Pb in plant material (mg kg^{-1})

Lead, Pb	Root	Stem	Leaf
Control	2,56±0,29^{b}	1,39 ±0,83^{a}	1,05±0,81^{a}
Low Concentration-LC	79,22±0,50^{c}	23,91±0,59^{b}	30,97±0,61^{a}
High Concentration-HC	293,66±0,87^{c}	86,03±1,37^{a}	162,22±0,0,96^{b}
ANOVA	$p \leq 0,05$	$p \leq 0,05$	$p \leq 0,05$

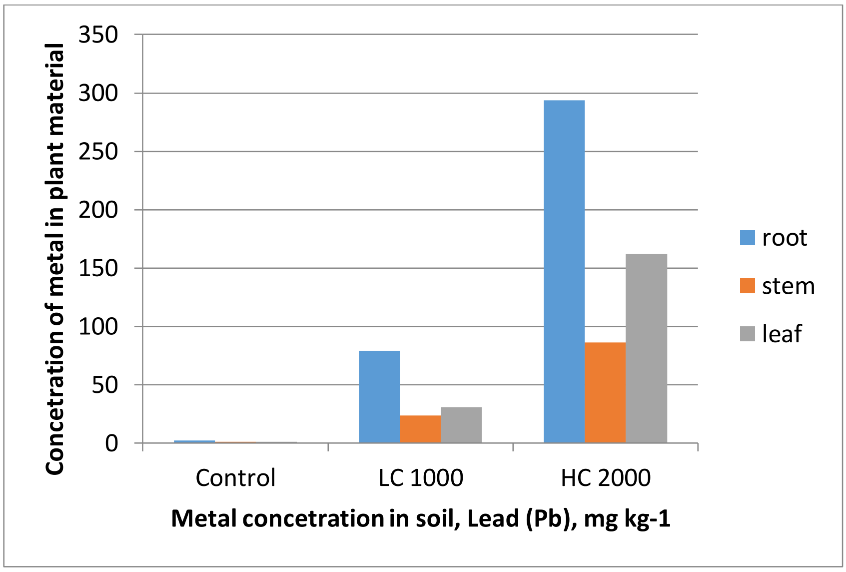

Figure 1. Concentration of Pb in plant material

The highest concentration of Pb was determined during the treatment of HC in the root system of Japanese knotweed, (*Reynoutria japonica*) in the amount of 293.66 mg kg^{-1}, a lower value was recorded in the leaves (162.22, mg kg^{-1}), while the lowest concentration was recorded in the stem in the amount of 86,03 mg kg^{-1}. Based on the results of the Tukey test, it was found that there are significant differences in the amount of heavy metal adsorbed in parts of the plant ($p \leq 0.05$), and the largest amount of metal in HC and LC was determined in the root system, significantly lower in leaves and lowest in stem. In the control treatment, there is no significant difference in heavy metal adsorption between the stem and the leaves.

Table 3. Values of tested heavy metal cadmium, Cd in plant material

Cadmium, Cd	Root	Stem	Leaf
Control	1,86±0,64^{a}	1,1±0,50^{a}	1,52±1,69^{a}
Low Concentration-LC	9,89±1,13^{c}	191,80±2,71^{b}	228,66±3,95^{a}
High Concentration-HC	101,80±1,13^{c}	546,60±2.72^{b}	811,90±3,80^{a}
ANOVA	$p \leq 0,05$	$p \leq 0,05$	$p \leq 0,05$

A statistically significant difference in the adsorption of cadmium, Cd from the soil to different parts of the plant, as well as the dependence of the applied treatment (HC and LC) was found. The highest concentration of Cd was adsorbed during the treatments, by the leaves of Japanese knotweed, *(Reynoutria japonica*) in concentrations of 811.90 mg kg^{-1} and 228.66

mg kg^{-1}, in relation to lead, Pb, the lowest concentration was determined in the root system in amounts of 9.89 mg kg^{-1} and 101.80 mg kg^{-1}

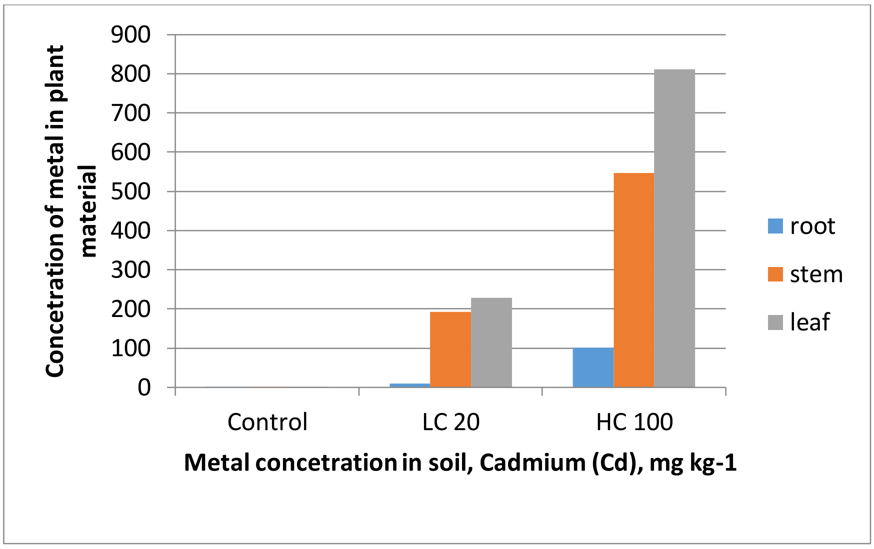

Figure 2. Concentration of Cd in plant material

Plants with phytoremediation ability are characterized by the accumulation of lead in the root system, and according to Mitrić et al., (2013), this in a way represents a form of protection of the aboveground part. Natural concentrations of lead in plants range from 5 to 10 mgkg-1 (Radojević and Bashin, 1999). The concentrations of lead in the tested plants during the treatment with low and high concentration are significantly higher. Lead belongs to the metals that accumulate in the underground parts of plants. In general, the lead content in plant organs tends to decrease in the following order: roots> leaves> stems> flowers (Scharma P., Dubey S., 2005). The results of the research confirm the ability of *Reynoutria japonica,* while to absorb heavy metals (lead and cadmium), and great phytoremediation potential.

Lead mainly accumulated in the underground parts of *Reynoutria japonica*, while the aboveground parts contained smaller amounts of this metal. Plants grown in the sample contaminated with lead (HC, 2000 mgkg-1) contained 115 times more of this element than the control. Among the studied metals, the concentration of Cd was found in the largest quantities. For example, in the case where the cadmium concentration was 100 mg kg-1 the plants (aboveground part) accumulated more than 537 times the amount of cadmium than that found in the control.

Furthermore, the results of the research showed that the *Reynoutria japonica* shows tolerance to heavy metals. Even high concentrations of heavy metals (Cd and Pb) in the soil did not completely eliminate the ability to regenerate the root, the rhizome of the Japanese knotweed, (*Reynoutria japonica*). Similar results were obtained by Michalet et al. (2017), who investigated the influence of heavy metals on rhizome regeneration and growth of Japanese knotweed, (*Reynoutria japonica*). Vukovic et al. (2019) observed that *Reynoutria japonica* has the ability to root from plant parts, rhizomes as well as the ability to rapidly regenerate rhizomes under different levels of soil contamination, making it an invasive species, especially at sites of anthropogenic contamination at higher amounts of heavy metals. Some metals, such as Pb, did not have a negative effect on the growth of Japanese knotweed, so grew at a similar rate as control plants. Only at a higher concentration of Cd (100 mg kg^{-1}) was growth retarded observed, indicating symptoms of "metal stress" in plants involving morphological and structural changes of aboveground shoots, such as leaf deformity and chlorosis, leaf necrosis, red and purple leaf color, brown leaf edges, reduced leaf number and size, reduced biomass, inhibited growth and eventual death (Benyó et al. 2016). According to Radojevic and Bashkin, 2006, 3-8 mg kg^{-1} Cd in soil is a critical concentration for plant growth. In the case of the Japanese knotweed, even a higher concentration of this metal in the soil 20 mg kg^{-1}) did not have a toxic effect on plant growth.

Analysis of metal concentrations in plant tissues showed a high potential of *Reynoutria japonica* for the uptake of heavy metals and their intensive accumulation in aboveground parts (in case of Cd) and underground parts (in case of Pb), which was confirmed by Böhmová & Šoltes, 2017, and Rahmonov at al., 2019.)

The Translocation factor is defined as the ratio of the metal concentration in the aboveground part of a plant to the metal concentration in the root, and is used to determine the efficiency of the plant in translocating heavy metal from the root to the aboveground part. The translocation factor indicates an enhanced ability of plants to bioaccumulate heavy metals compared to the control sample. The obtained values for TF are the most important test that can be used to assess the phytoremediation potential of the plant (Angelova et al., 2016).

Table 4. Translocation factor (TF)

Element	Control	Low Concentration	High Concentration
Pb	0,41	0,39	0,55
Cd	3,50	23,12	7,96

Plants with a translocation factor less than 1 can be used as phytostabilizers (metal concentration in the underground part of the plant), and as phytoextractors if they have a translocation factor greater than 1, transport and concentrate metals from the soil to the aboveground parts of plants (Takarina et al. 2017).

The obtained results confirmed the previous research that the Japanese knotweed, as an invasive species, spreads very quickly, and shows a great tolerance for the presence of heavy metals. By knowing the biological properties of such plant species, it is possible to successfully manage invasive species, as confirmed, (Yasin et al. 2019). Therefore, it is very important to continuously conduct field research in the future.

CONCLUSIONS

On the basis of the results of the presented research, the following conclusions can be drawn:

1. The presence of heavy metals in contaminated soil does not eliminate the ability to regenerate the roots of Japanese knotweed, *Reynoutria japonica*.
2. Metals such as Pb do not have a toxic effect on plant development, which was observed in this experiment. In soil contaminated with any concentration of Pb, the shoots of this plant grew at a similar rate as the control plants, and had the same morphological characteristics as control.
3. High concentrations of Cd (200 mg kg^{-1}), can delay the rapid growth of Japanese knotweed, *Reynoutria japonica*
4. The increased concentration of all metals in the soil did not have a negative impact on plant growth. This fact proves that the Japanese knotweed, *Reynoutria japonica*, accumulates heavy metals efficiently when grown in soils contaminated with heavy metals. This was especially observed in the case of Cd. In the aboveground parts of the plant from the samples with the addition of HC Cd 100 mg kg^{-1}, 537 times more Cd was found, compared to the control.

5. The ability of this invasive weed to regenerate from parts of the rhizome, grow and develop under stressful conditions, in the presence of elevated concentrations of some metals (Pb, Cd) proved that the Japanese knotweed, *Reynoutria japonica,* has a high tolerance to heavy metals.

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