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The improving influence of laser stimulation on phytoremediation capabilities of selected *Silene vulgaris* ecotypes

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Abstract: The aim of the paper is to improve the phytoremediation features of the metallophyte *Silene vulgaris* through photo-stimulation of seeds using a semi-conductive laser. Seeds of two *Silene vulgaris* ecotypes were used in the experiment. One type of seeds – "Wiry" ecotype – originated from a site contaminated with heavy metals (a serpentinite waste heap), and the other ecotype – "Gajków" – was collected on a site with naturally low heavy metal content. The seeds of both types were preconditioned with laser light with previously fixed doses: C(D0), D1, D3, D5, D7, D9. The basic radiation dose was $2.5 \cdot 10^{-1}$ J·cm^{-2}. The soil for the experiment was serpentinite weathering waste. The seeds and plants were cultivated in the controlled conditions of a climatic chamber. Laser light indeed stimulated seed germinative capacity but better effects were obtained in "Wiry" ecotype, originating from a location contaminated with heavy metals. In the case of morphological features, a significant differentiation of stem length was found for different ecotypes, dosages and the interactions of these factors. The study showed a strong influence of laser radiation on selected element concentrations in above-ground parts of *Silene vulgaris*, though "Wiry" ecotype clearly accumulated more heavy metals and magnesium than the "Gajków" ecotype.

Introduction

Conventional methods of post-mining or post-industrial site re-cultivation are usually expensive and often ineffective. In many cases they contribute to further degradation effects, for instance through destroying natural habitats (Mierek-Adamska et al. 2009, Rostański et al. 2015). As a result, alternative methods such as phytoremediation are developed, where plants are used as organisms cleansing the environment from toxic substances, e. g. heavy metals (Mierek-Adamska et al. 2009, Moreira et al. 2015). According to Ernst (1996), particular vegetation spontaneously growing in these locations, e. g. metallophytes, should constitute the basis for rational, fast and efficient re-cultivation of post-exploitation sites (Chaney et al. 2005, Ciarkowska and Hanus-Fajerska 2008). Vascular plant index lists 145 species linked to soils rich in metal in Poland (Jędrzejczyk-Korycińska 2006, Kasowska and Koszelnik-Leszek 2014, Nowak et al. 2011, Rostański et al. 2015, Wierzbicka and Rostański 2002, Żołnierz 2007). One of them is *Silene vulgaris* whose ecotypes spontaneously grow on degraded post-mining and post-industrial sites (Kandziora et al. 2007, Koszelnik-Leszek and Bielecki 2013, Nadgórska-Socha et al. 2011, Wierzbicka and Panufnik 1998). However, small size and low biomass yield (Murakami and Ishikawa 2007) limit the use of metallophytes in attempts. Pre-sowing laser radiation conditioning is one of the pro-ecological methods used to stimulate plants growth (Dobrowolski et al. 1987, Dobrowolski 1996, Dobrowolski and Różanowski 1998). It was proved that laser light stimulates the germination, growth and size of harvest (Danaila-Guidea et al. 2011, Dobrowolski 1986, Dobrowolski et al. 1987, Hernandez et al. 2010, Prośba-Białczyk et al. 2012, Szajsner 2009).

For that reason, during the pot growth test conducted in controlled conditions of a climatic chamber, attempts were made to establish the influence of laser radiation on the seed quality (germination capacity and rate) of selected *Silene vulgaris* ecotypes seeds. Additionally, selected morphological features of the control group and the experimental group were studied. Moreover, the capacity to absorb heavy metals in various radiation dosages was studied. The experiment was conducted on serpentinite soil from a serpentinite heap, naturally rich in heavy metals.

Materials and methods

The seeds of two *Silene vulgaris* ecotypes and plants cultivated from them constituted the study material.

General characteristics of selected Silene vulgaris ecotypes and their habitats

The “Gajków” (non metallicolous) ecotype originates from a natural habitat in the village Gajków located to the south-east of Wrocław (51°3′25.021″N, 17°11′9.415″E). The “Wiry” (serpentine) ecotype grows on a small post-mining serpentinite heap near a village called Wiry located close to the western slopes of Mount Ślęża (50°50′12.773″N, 16°37′3.724″E).

Serpentinites are peculiar rocks whose occurrence in Poland is limited to Lower Silesia (Żołnierz 2007). The particular chemistry of serpentinites, formed through transformed ultra-alkali intrusive rocks, results in unique soil rich in magnesium and low in calcium (low Ca:Mg ratio), and additionally rich in Ni, Cr and Co in values exceeding noted values from other Lower Silesian soils (Żołnierz 2007).

Silene vulgaris (Moench) Garcke is a perennial plant from the Caryophyllaceae family. The species is commonly found in Europe, northern Africa, Asia and both Americas (Koszelnik-Leszek and Bielecki 2013). In Poland the species occurs on grasslands, fields and in forests, but it can also be found in synanthropic plant communities as an element of ruderal habitats such as serpentinite or calamine heaps or other post-mining areas (Koszelnik-Leszek and Bielecki 2013).

Silene vulgaris is a bioindicator of heavy metal contamination and its presence has been recorded in areas both naturally rich in heavy metals and contaminated by human activity (Bratteler et al. 2002, Kazakou et al. 2010, Koszelnik-Leszek and Bielecki 2013, Nadgórska-Socha et al. 2011, Rostański et al. 2015, Wierzbicka and Panufnik 1998). The literature provides examples of the species’ unique adaptation capabilities leading to the formation of separate ecotypes adapted to extreme habitat conditions. Apart from *Silene vulgaris* ecotypes immune to lead and zinc, there are also ones able to tolerate an abundance of copper and nickel (Koszelnik-Leszek and Bielecki 2013).

Pot growth test

The laboratory experiment using a semi-conductive laser (model CTL-1106MX with a power of 200 mW and a wavelength of 670 nm) consisted in pre-sowing irradiation of seed conditioning of selected *Silene vulgaris* ecotype seeds with previously determined radiation dosages. The dosages were as follows: single – (D1), three – (D3), five – (D5), seven – (D7) and nine-fold (D9) fold basic dosage of $2.5 \cdot 10^{-1}$ $J \cdot cm^{-2}$. The duration of individual exposure was 4.1 min. The control group C (D0) consisted of seeds without any dosages. The irradiated seeds of both *Silene vulgaris* ecotypes were then planted in pots with 1 kg of weathering waste originating from the serpentinite heap in Wiry (20 pieces per pot). Each combination of ecotype and dosage was performed in three repetitions. Plants were cultivated in the control conditions of a climatic chamber (Sanyo model MLR-351).

Germination capacity and rate (ISTA 2008) were determined for every dosage and ecotype. After 6 weeks of the experiment the height and width of leaf blades were calculated, and after 8 weeks leaf blades were collected and secured for further analyses, which determined the influence of radiation dosages on metal accumulation and growth stimulation. The collected samples (above-ground plant parts) were subjected to dry mineralization. The contents of Ni, Cr, Co, Zn and Mg were determined using the AAS method on SpectrAA 220 Fast Sequential equipment.

The soil used in the experiment was weathering waste from a serpentinite heap. Before the experiment the material was analyzed for selected parameters. Soil pH was noted potentiometrically at 1 mol $KCl \cdot dm^{-3}$. Phosphorus and potassium were recorded using Egner-Riehm method, magnesium by Schachtschabel method. Total and available heavy metal content was determined using the method of Atomic Absorption Spectrophotometry with the use of SpectrAA 220 Fast Sequential equipment.

Statistical analysis. Seed quality and morphological features

The obtained results were analyzed statistically (Statistical Tool for Agricultural Research 2012) using two way analysis of variance with factor I as the two selected ecotypes of *Silene vulgaris* and factor II as the radiation dosages. Fisher-Snedecor distribution was used to determine the relevance of the studied variance sources. Duncan’s new multiple test range was used to determine homogeneous groups.

The chemical composition of plant material

Statistical analyses were conducted using Statistica ver. 12 software (StatSoft Inc. 2014). Normal distribution was checked using Kruskal-Wallis test. Variance analysis and Fisher’s exact test were used to determine the differences in element concentrations in the selected *Silene vulgaris* ecotypes. Variance homogeneity was determined using Levene’s test. The data for which normal distribution or variance homogeneity were not obtained were analyzed using Kruskal-Wallis test.

Results and discussion

Serpentinite soil used in the pot growth experiment was characterized by high heavy metal content (Tab. 1). The concentrations of Ni, Cr, Co and Zn (both total and available) exceeded mean values of these elements for Poland and Central Europe (Kabata-Pendias 2011). The values were, however, typical of Polish serpentinite occurrences (Żołnierz 2007). The analysis showed that material from the excavation heap was characterized by a particularly low concentrations of phosphorus and potassium (for Poland) and, at the same time, particularly high or even toxic concentration of magnesium (again, for Poland). The recorded pH (KCl) of 7.8 is typical of serpentinite habitats in Lower Silesia (Żołnierz 2007).

Laser radiation as a physical factor modifies biochemical and physiological processes and influences enzyme systems improving the germination, growth and crop performance of plants (Dobrowolski et al. 1987, Hernandez et al. 2010, Szajsner 2009).

In the pot growth experiment the germination capacity and rate were significantly higher in the “Wiry” ecotype (Fig. 1a). Seed germinative capacity underwent a significant increase after five- and nine-fold semi-conductive laser irradiation, by, respectively, 13.3 and 14.2% as compared with the control seeds (Fig. 1b). The interaction of ecotypes with provided radiation dosages led to the conclusion that for the “Gajków” ecotype the D9 dosage provided a 16% capacity increase. The “Wiry” ecotype showed stimulation under the influence of D5 dosage – 16% increase as compared to the control group (Fig. 2). The germination rate underwent no significant change under laser irradiation (Fig. 1b), but there was an interaction between ecotypes and dosages. The "Gajków" ecotype showed stimulation after D3, D7 and D9 seed irradiation (10% to over 13%), whereas the "Wiry" ecotype responded to D3 and D5 by 13% to 15% (Fig. 3).

In the case of morphological features there were significant differences between stem lengths among ecotypes, dosages and factor interactions. The above-ground parts of the "Gajków" ecotype (1.91 cm) were shorter than in the 'Wiry' ecotype (2.43 cm) – Fig. 4a. From among the radiation doses, D5 showed significant lengthening of the stem (Fig. 4b). The obtained interaction showed a strong stimulating effect of seedbed irradiation with dosages D5 and D9 as compared to the control stems of the "Wiry" ecotype. For the "Gajków" ecotype, no significant influence of laser irradiation was noted (Fig. 5). Leaf blade width showed no changes in either ecotype after pre-sowing irradiation of seed (Fig. 6).

Table 1. Characteristics of the soil material (degraded) in conducted experiment

Element	Mobile form (Total form) [mg·kg^{-1}]	Element	Mobile form [mg·100g^{-1}]
Ni	273.4 (1765.9)	**Mg**	80.3
Cr	6.4 (373.1)	**P**	0.8
Co	24.9 (153.1)	**K**	2.9
Zn	97.0 (350.6)	Element	Volue (1M KCl)
		pH	7.8

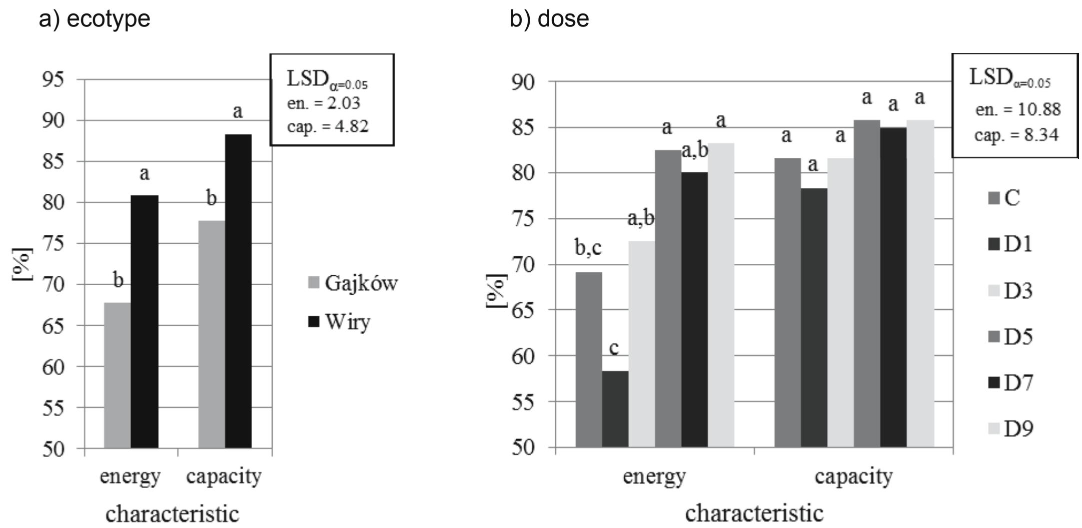

Fig. 1. Effect of stimulation of *Silene vulgaris* seeds – homogeneous groups

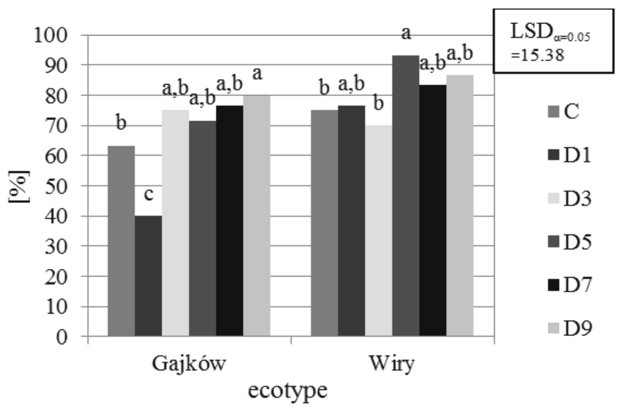

Fig. 2. Effect of stimulation of *Silene vulgaris* seeds on energy germination – interaction ecotype × dose, homogeneous groups

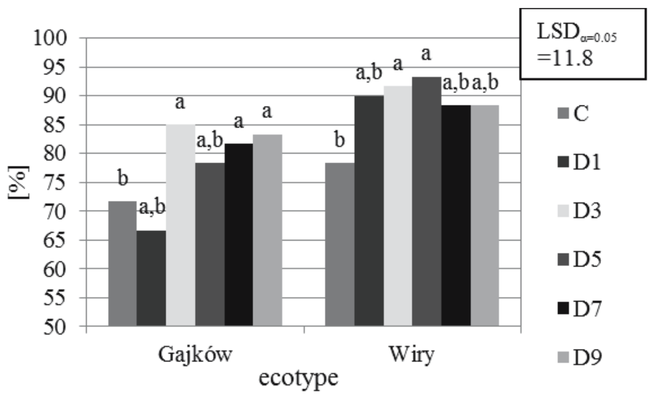

Fig. 3. Effect of stimulation of *Silene vulgaris* seeds on capacity germination – interaction ecotype × dose, homogeneous groups

In the studies of spring barley (Rybiński et al. 2003) and of cultivated plants with pre-sowing irradiation of (Klimont 2006) it was observed that germination capacity and germination rate increased, the caryopses number increased and anomalous seedlings number decreased (onion).

Spring barley research showed an increase of assimilation surface in inflorescence and flag leaves after helium-neon laser irradiation (Rybiński and Garczyński 2004).

Chemical analyses were conducted to compare the studied populations of *Silene vulgaris* in terms of heavy metal content after seedbed stimulation of seeds planted in experimental serpentinite soil. Data in Table 2 suggest that selected ecotypes reacted differently to seedbed conditioning. The differences in accumulated metals were visible in both ecotypes originating from control areas (C). The differences may result from habitat conditions and the resulting ecotype adaptation. Lower metal concentrations in above-ground parts of the "Wiry" ecotype (serpentinite heap) could be a consequence of defense mechanisms inhibiting the absorption and flow of metals from under-ground to above-ground parts. These mechanisms may not be present in the "Gajków" ecotype originating from a habitat with naturally low heavy metal concentrations (Zenk 1996).

The conducted calculations showed a significant influence of laser irradiation on the concentrations of selected elements in the above-ground parts of the studied *Silene vulgaris* ecotypes. In the case of the "Wiry" ecotype the highest concentrations of magnesium was recorded after D3 and D5 dosages ($F=68.59$; $p<0.0001$); for nickel after D3 ($F=776.794$; $p<0.0001$); for Co between dosages D3-D9 ($F=21.93$; $p=0.000012$). Zinc ($F=1.08$; $p=0.42$) and chromium ($H=12.69$; $p=0.003$) concentrations did not change significantly. In the case of the "Gajków" ecotype the highest magnesium concentrations were recorded after D3 ($F=34.83$; $p=0.0000$); zinc – C(D0)-D3 ($F=37.71$; $p=0.0000$); nickel – D9 ($H=15.83$; $p=0.0073$); and cobalt – D3 ($F= 25.24$; $p=0.0000$). No significant influence of irradiation was recorded in the case of chromium concentrations in *S. vulgaris* of that ecotype ($F=1.53$; $p=0.25$).

In the case of plants usually used in phytoremediation (Horn 2000, Moreira et al. 2015) pro-ecological usage of laser biotechnology caused an increase in pollution resistance and changed the level of toxic compound accumulation in plant tissues (Borowiak et al. 2016, Grygierzec 2013, Grygierzec and Gowin 2010, Jakubiak and Śliwka 2008, 2009, Śliwka and Jakubiak 2010). In the studies on increasing the phytoremediation

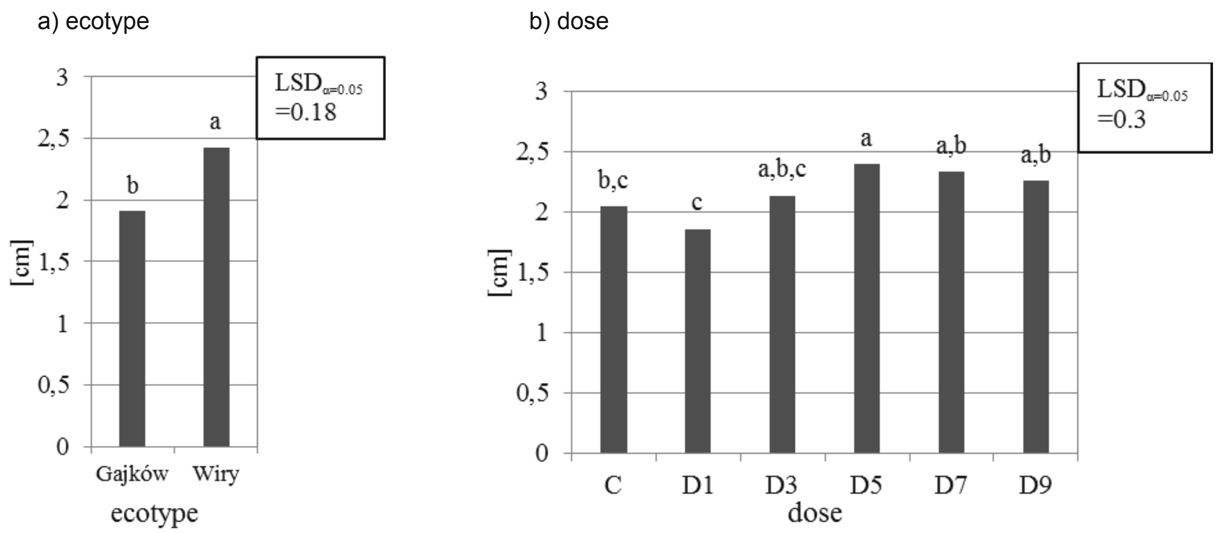

Fig. 4. Effect of stimulation of *Silene vulgaris* seeds on seedling length plant height-homogeneous groups

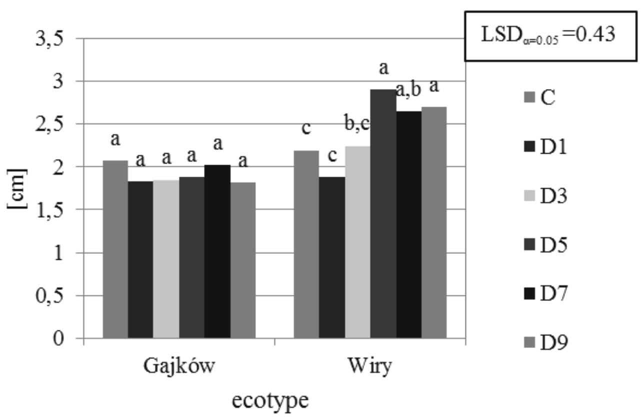

Fig. 5. Effect of stimulation of *Silene vulgaris* seeds on seedling length plant height-interaction ecotype × dose, homogeneous groups

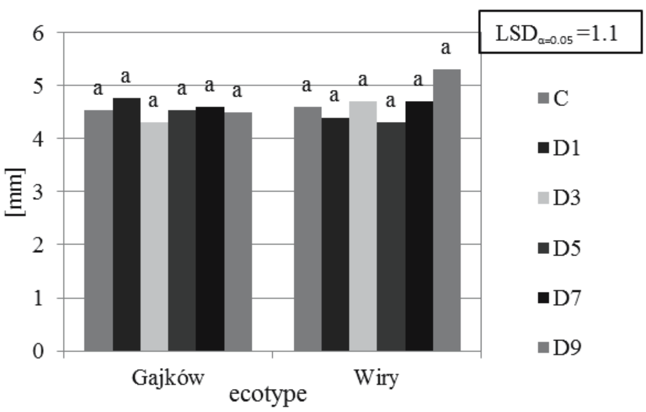

Fig. 6. Effect of stimulation of *Silene vulgaris* seeds on width leaf blade-homogeneous groups

Table 2. Content of analyzed elements in the above-ground parts of selected ecotypes *Silene vulgaris*. Means values (±SE). Different letters indicate significant differences obtained by the LSD Fisfera or Kruskal-Wallis test (p≤0.05)

Ecotype	Dose	Mg	Zn	Ni	Cr	Co
		[g·kg^{-1}]	[mg·100g^{-1}]			
Wiry	C (D0)	23.8±1.51a	12.7±1.00a	77.8±2.58a	5.2±0.91a	6.5±0.68a
	D1	21.6±0.86a	12.9±1.40a	77.7±3.04a	6.3±0.46a	6.0±0.66a
	D3	54.6±0.79c	15.7±0.81a	611.2±10.76d	23.6±3.65a	23.1±1.40b
	D5	58.6±1.50c	14.8±1.18a	531.9±10.98c	24.4±1.97a	21.4± 1.44b
	D7	40.7±3.34b	14.2±1.18a	390.1±6.47b	19.5±2.71a	19.6 ±2.15b
	D9	43.8±1.86b	14.0±0.87a	407.2±9.83b	24.4±1.90a	18.4±2.49b
Gajków	C (D0)	43.8±1.86b	12.8±0.51c	95.8± 2.81ab	7.5±1.47a	12.9±2.10b
	D1	45.2±0.92b	13.1±0.81c	79.6±3.51ab	9.8±1.58a	10.3± 1.06ab
	D3	60.2±2.63c	14.3±0.53c	71.5±6.37a	12.7±1.59a	26.9± 1.77c
	D5	26.1±3.31a	8.5±0.30a	106.1±8.45ab	9.2±0.95a	8.1 ±0.94a
	D7	28.3±1.71a	6.7±0.47b	138.1±9.65ab	8.5±0.65a	10.1 ±1.08ab
	D9	32.6±1.91a	6.4±0.66b	257.5±14.2b	8.9±1.94a	9.2 ±0.96ab

capability of *Festuca rubra* (Grygierzec and Gowin 2010) and *Poa pratensis* (Grygierzec 2013) it was determined that seedbed stimulation of both species increased their bioaccumulation coefficients (BC) for selected heavy metals.

In his studies, Różanowski (2000) conducted laser bio--stimulation of *Salix viminalis* cuttings and determined that the irradiation caused a greater accumulation of heavy metals in roots and shoots.

The results obtained by Śliwka and Jakubiak (2010) confirmed the significant influence of laser light on the increased speed of cell division, which consequently led to an increase in biomass of the hydrophytes *Lemna minor* and *Iris pseudoacorus* and indirectly caused the faster uptake of biogenic elements, which are the main cause of water eutrophication.

In the course of the conducted experiment it was recorded that, irrespectively of the irradiation dosage, the leaf blades and stems in the "Gajków" ecotype (from areas with naturally low concentrations of heavy metals) have anthocyanin color. This effect was not recorded in the serpentinite ecotype "Wiry". Increased anthocyanin synthesis may be connected with their role in removing the toxic effect of trace elements on plants. According to Hale et al. (2002) these compounds may directly protect cells from the harmful effect of metal ions by forming lasting compounds. Increased anthocyanin accumulation could be the effect of water management distortions and water deficit in plants (Grzesiuk et al. 2008) and it could also be connected with immunity deficiency to heavy metals in the soil, which, in turn, might be the first indicator of insufficient heavy metal detoxification caused by plant physiology dysfunction (Kandziora et al. 2007).

Conclusions

The conducted pot growth experiment led to the formulation of the following conclusions:

1. Pre-sowing irradiation of seed significantly increased the germination rate and capacity of seeds and caused the lengthening of *Silene vulgaris* stems. For the "Gajków" ecotype the germination rate and capacity stimulation were observed after D9 dosage, whereas for the "Wiry" ecotype the D5 dosage also stimulated the lengthening of above-ground parts.
2. Leaf blade width showed no significant changes after irradiation.
3. The irradiation caused significant changes in the concentrations of heavy metals in above-ground parts of *Silene vulgaris*. However, the "Wiry" ecotype originating from a serpentinite heap showed better metal accumulation capabilities than the "Gajków" ecotype originating from areas with naturally low heavy metal content.

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Wpływ stymulacji laserowej nasion na polepszenie właściwości fitoremediacyjnych wybranych ekotypów *Silene vulgaris*

Streszczenie: Celem pracy doświadczalnej była próba polepszenia właściwości fitoremediacyjnych metalofitu *Silene vulgaris* poprzez fotostymulację nasion przy użyciu lasera półprzewodnikowego. Badaniom eksperymentalnym poddano nasiona dwóch ekotypów *Silene vulgaris*. Nasiona pierwszego, ekotyp – „Wiry”, pochodziły z obszaru zanieczyszczonego metalami ciężkimi (hałda odpadów serpentynitowych) a nasiona drugiego, ekotyp – „Gajków”, zebrano z obszaru o naturalnie niskiej zawartości metali ciężkich. Nasiona obu ekotypów, przedsiewnie kondycjonowano promieniami światła laserowego odpowiednio wcześniej ustalonymi dawkami: D0, D3, D5, D7, D9. Dawka podstawowa promieniowania wynosiła $2{,}5 \cdot 10^{-1}$ $J \cdot cm^{-2}$. Podłożem eksperymentalnym, w które wysiano napromieniowane i kontrolne nasiona była zwietrzelina pochodząca z hałdy odpadów serpentynitowych. Nasiona i wyrosłe z nich rośliny uprawiano w warunkach kontrolowanych fitotronu. Światło laserowe istotnie poprawiało energię i zdolność kiełkowania nasion, z tym jednak że lepsze efekty laserowej biostymulacji obserwowano u ekotypu Wiry, pochodzącego z obszaru zanieczyszczonego metalami ciężkimi. W przypadku badanych cech morfologicznych wykazano istotne zróżnicowanie długości łodyg dla ekotypów, dawek jak i interakcję badanych czynników. Na podstawie przeprowadzonych obliczeń wykazano istotny wpływ promieniowana laserowego na zmianę koncentracji wybranych pierwiastków w częściach nadziemnych *Silene vulgaris,* z tym jednak że rośliny ekotypu Wiry kumulowały wyraźnie więcej metali ciężkich i magnezu, niż ekotyp Gajków.