https://doi.org/10.17221/50/2020-PSE

Garden pansy (*Viola* × *wittrockiana* Gams.) – a good candidate for the revitalisation of polluted areas

Klaudia Sychta, Aneta Słomka, Elżbieta Kuta*

Department of Plant Cytology and Embryology, Institute of Botany, Faculty of Biology, Jagiellonian University, Cracow, Poland

**Corresponding author: klaudia.michno@doctoral.uj.edu.pl*

Citation: Sychta K., Słomka A., Kuta E. (2020): Garden pansy (*Viola* × *wittrockiana* Gams.) – a good candidate for the revitalisation of polluted areas. Plant Soil Environ., 65: 272–280.

Abstract: In the current studies, heavy metal tolerance level, accumulation efficiency and sexual reproduction were determined in *Viola* × *wittrockiana*, a non-metallophytic ornamental cultivar in comparison to *V. tricolor*, a metallophyte, after zinc (Zn) or lead (Pb) treatment (0, 10, 100 and 1 000 ppm) in pot experiments. The seed germination frequency that was not reduced in comparison to the control, the effective Zn absorption from the soil and exclusion strategy for Pb, as well as the regular sexual reproduction of *V.* × *wittrockiana* treated with heavy metals all indicate the tolerance of this plant to heavy metals. The lack of a seed set under experimental conditions of *V.* × *wittrockiana* was due to the absence of pollinators, rather than the negative impact of heavy metals, as pollen viability and ovule development were normal under the treatments. The results indicate that *V.* × *wittrockiana* represents similar tolerance to *Viola* metallophytes and could be considered as a good material for the reclamation of polluted areas. The exceptional tolerance to heavy metals, the ability to initiate new generations in heavy-metal-burdened soil, which are additionally coupled with the unique beauty, make the garden pansy a good candidate to be potentially used in the future for phytoremediation purposes.

Keywords: contamination; metalliferous site; hyperaccumulator; phytotoxic metal; urban area

Although heavy metals are naturally present in the soil, anthropogenic contamination is a global problem in the modern world, mainly due to industrial activity. In the soils contaminated with heavy metals, the concentrations of trace elements, such as Cd, Cr, Cu, Ni, Pb and Zn, are several thousand times higher than in non-contaminated areas. Permanent plant cover is crucial to prevent further spreading of the contaminants. Tolerant plant species are used to remove contaminants (e.g. heavy metals) from the soil in the process of phytoremediation and the reclamation of polluted areas (Tangahu et al. 2011). Tolerant plants, colonising metalliferous sites, are classified as excluders, indicators or (hyper)accumulators. Hyperaccumulating plants can accumulate 10–1 000 times higher concentrations of metals than the concentration in the soil, whereas indicators accumulate heavy metals nearly in the same concentrations as occuring in the ground (Tangahu et al. 2011, van der Ent et al. 2013). Excluders can grow over a wide range of phytotoxic metals until physiological mechanisms lose control and allow unregulated uptake, resulting in the death of the plant. Plants used for reclamation do not necessarily need to be (hyper) accumulators, especially as their number is limited (ca. 700 species), even excluders could be used as they stabilise the soil and prevent harmful particles from spreading (van der Ent et al. 2013, Reeves et al. 2017).

Metallophytes are very frequent in the Violaceae family, with the *Hybanthus* species of Violaceae known to be Ni hyperaccumulators and numerous species in the genus *Viola* L. described as being able to tolerate high concentrations of heavy metals present in the soil (Bothe and Słomka 2017, Reeves et al. 2017). In calamine soils enriched with Zn, Pb and Cd, *V. lutea* ssp. *calaminaria* (Ging.) Nauenb. and ssp. *westfalica* Nauenb., *V. tricolor* L., *V. baoshanensis* W.S.Shu, W.Liu &

Supported by the National Science Centre, Project No. 2019/32/T/NZ8/00451, and by the Dean of the Faculty of Biology of the Jagiellonian University, Project No. DS/D/WB/IB/10/2019 (for Klaudia Sychta).

https://doi.org/10.17221/50/2020-PSE

C.Y.Lan and *V. yedoensis* Makino thrive, while in serpentine soils with Ni, Fe, Mn and Mg, *V. vourinensis* Erben. occurs. In turn, *V. macedonica* Boiss. & Heldr., *V. arsenica* Beck and *V. allchariensis* Beck are tolerant to metalloid As (Banásová et al. 2006, Ernst 2006, Psaras and Constantinidis 2009, Gao et al. 2015). Violets colonising calamine and serpentine soils were the scientific objects and models of long-term *in vivo* studies, focusing on the accumulation of heavy metals, physiological defense mechanisms, morphological and genetic variability, genetic differentiation, species origin, the impact of heavy metals on the genome, and reproductive processes (Słomka et al. 2008, 2011, 2017, Kuta et al. 2014, Migdałek et al. 2013). To explain the cellular mechanisms of heavy metal accumulation and their tolerance, as well as the ability of the metal-loaded surviving cell to divide and regenerate into plants, *in vitro* studies of suspended cells have recently been developed (Sychta et al. 2018, 2020).

Viola × *wittrockiana* Gams. (garden pansy) is a common bedding plant worldwide, exhibiting a wide range of flower colours, cold tolerance, long flowering and compatibility to broad climatic conditions as well as other charming characteristics, such as odour and taste (Fernandes et al. 2017). Initially, the species was selected as an inter-specific hybrid resulting from the crossing of at least three *Viola* sect. Melanium Ging. species (pansies), *V. tricolor* L., *V. lutea* Hudson and *V. altaica* Ker-Gawler (Kroon 1972). The total height and flower size is significantly larger in *V.* × *wittrockiana* than in its ancestors (Wittrock 1895). Increasing ploidy levels of garden pansy could be useful for the creation of new genotypes characterised by new desirable ornamental traits e.g. larger flowers, flower colours or tolerance to biotic or abiotic stresses (Dalbato et al. 2005, 2013). Little is known about their physiological and developmental responses to environmental factors. It is known that pansies are resistant to low temperatures. Flowering, flower size and shoot weight increase simultaneously with decreasing temperatures (Adams et al. 1997). Currently, *V.* × *wittrockiana* is one of the dominant plant species among ornamental bedding plants in the U.S. market and, during the past 60 years, much of the innovative pansy breeding has been performed in Germany, the United States and Japan (Du et al. 2018).

The studies aimed to evaluate the tolerance level to Zn and Pb and accumulation efficiency of these metals in the roots and shoots of the non-metallophytic cultivar *V.* × *wittrockiana* (a species not known for its tolerance to heavy metals) compared to the pseudo-metallophyte *V. tricolor*, in relation to the practical use of this relatively large ornamental plant with beautiful flowers for revitalisation of contaminated sites.

MATERIAL AND METHODS

Plant material. *Viola tricolor* seeds were collected from the Bukowno heap next to an operating smelter (Poland, 50°16'37''N, 19°28'05''E). Calamine ($ZnCO_3$, $ZnSiO_3$), sphalerite (ZnS) and galena (PbS) were exploited in this region. The mean Zn concentration in the soil of Bukowno is 6 725 ppm, Pb 1 769 ppm and soil pH 6.9 (Słomka et al. 2011).

Viola × *wittrockiana* seeds were purchased from a commercial supplier (Polan, Poland).

Determination of seed germination frequency in pot experiments. Altogether, 420 seeds (30 seeds/taxon/treatment) of *V. tricolor* and *V.* × *wittrockiana* were sown into pots containing peaty soil (pH 5.5–6.5; NaCl < 1.9 g/dm^3; C_{org} ~90%; Zn ~150 mg/dm^3; Pb ~0.25 mg/dm^3) supplemented with Zn – $Zn(NO_3)_2$ (10, 100, 1 000 ppm) or Pb – $Pb(NO_3)_2$ (10, 100, 1 000 ppm) in stable laboratory conditions (25 ± 3 °C with a 16-h photo-period under cool-white fluorescent lamps (flux 70–100 μmol/m/s)). Soil without heavy metals was used as a control. The frequency of seed germination was determined successively up to 30 days after sowing (the germination rate of the seeds was uneven). The plants of *V. tricolor* grew indoors in pots until flowering and seed set (ovules not analysed embryologically). *V.* × *wittrockiana* bloomed indoors but did not produce seeds (ovules were embryologically studied).

Determination of pollen viability. Seventy flowers (five flowers/taxon/treatment) of *V. tricolor* and *V.* × *wittrockiana* after the treatment with Zn (10, 100 and 1 000 ppm) or Pb (10, 100 and 1 000 ppm) or control growth in pots in laboratory conditions were collected after 90 days of heavy metal treatment. One large bud (nearly open flower) per plant from five plants was fixed in 96% ethanol and glacial acetic acid (3:1, *v*/*v*) and stored at 4 °C for further analyses. Isolated pollen grains were stained on microscopic slides using the Alexander's dye (Singh 2003). The frequency of viable pollen grains was counted under a light microscope. The number of analysed pollen grains varied from ~600 to ~1200.

Ovule development in *V.* × *wittrockiana*. Twenty-eight flowers at different developmental stages (from bud to fully open) of the *V.* × *wittrockiana* cultivar growing outdoors and 10 open flowers of plants culti-

https://doi.org/10.17221/50/2020-PSE

vated in laboratory conditions, non-treated (control) and treated with 1 000 ppm of Zn or 1 000 ppm of Pb, were prepared for embryological analysis according to Kwiatkowska et al. (2019). Embryological analysis was performed using a microscope (Nikon E80i, Tokyo, Japan) equipped with a digital camera under bright-field illumination. The pistil and stigma from five flowers of plants cultivated outdoors were observed using a scanning electron microscope (SEM) and a stereoscopic microscope. SEM observation of floral elements followed the procedure described by Kuta et al. (2012). To analyse ovules in the ovaries of plants treated and non-treated with heavy metals, carpels from five flowers per treatment were dissected and the ovule size and shape were evaluated with a stereoscopic microscope (Opta-Tech SK, Warsaw, Poland).

Estimation of Pb and Zn content in roots and shoots of heavy-metal-treated plants of *V.* × *wittrockiana* and *V. tricolor.* Forty-two plants (three plants/taxon/treatment) were harvested after 60 days of heavy-metal treatment (the day of seed sowing is considered as the first day), pre-washed and rinsed with 1 mol/L ethylenediaminetetraacetic acid (EDTA) (Sigma-Aldrich, St. Louis, USA) solution followed by deionised water, separated into roots and shoots, dried at 100 °C and weighed.

Samples of the roots or shoots of *V. tricolor* and *V.* × *wittrockiana* for atomic absorption spectrometry (AAS) analysis were prepared according to the procedure described by Sychta et al. (2018). The samples were prepared in three technical replications.

Determination of bioaccumulation factor and translocation factor. Zinc or lead content were used to determine the bioaccumulation factor (BF) – the ratio of concentration of heavy metals in plant tissues (shoots and roots) to the concentration of heavy metals in the soil (Reboredo et al. 2018), and the translocation factor (TF) – the ratio of metal concentration in the shoots to the concentration in the roots. The values > 1 of BF and TF indicate the ability to hyperaccumulate (Shumaker and Begonia 2005).

Statistical analysis. One-way ANOVA followed by the Tukey's test was performed to determine the significance of seed germination frequency between treatments within particular species and the number of ovules in the ovary between treatments in *V.* × *wittrockiana*. Two-way ANOVA followed by the Tukey's test was performed to determine the significance of differences in the frequency of viable pollen grains between treatments and species. Three-way ANOVA was applied to assess the influence of concentration of particular heavy metals, parts of the plant (root/shoot) and taxon. All analyses were performed using Statistica 13 (Statsoft, Cracow, Poland) and Microsoft Excel 2016 (Redmond, USA).

RESULTS

Seed germination frequency in non-metallophytic *V.* × *wittrockiana* was not affected by heavy metals. The germination frequency (percentage) of *V.* × *wittrockiana* and *V. tricolor* in soil without heavy metals was high (66.7% and 86.7%). Seed germination of *V. tricolor* decreased simultaneously with increasing Zn concentration and in the highest Zn concentration (1 000 ppm) it was decreased by 30% compared to the control. The seed germination frequency of *V.* × *witrockiana* in Zn treatment did not differ from the control. Pb treatment did not influence seed germination in either *V. tricolor* and *V.* × *wittrockiana* (Table 1). Overall, non-metallophytic *V.* × *wittrockiana* showed higher tolerance to Zn than the metallophyte *V. tricolor* as the germination percentage was not diminished in any heavy metal treatment.

Regular male and female germline development in *V. tricolor* and in *V.* × *wittrockiana* under heavy metal stress indicates tolerance to heavy metals. The pollen viability of both taxa after treatment with Zn or Pb was very high (~90%) and did not differ significantly from the control plants (Figure 1), which indicates a lack of disturbance in the male reproductive system,

Table 1. Seed germination frequency (%) of *Viola* × *wittrockiana* and *V. tricolor* after treatment with zinc (Zn) or lead (Pb) or with deionised water (control) for 30 days

Taxon	Control	Pb			Zn		
		10 ppm	100 ppm	1 000 ppm	10 ppm	100 ppm	1 000 ppm
V. × *wittrockiana*	66.7^{a}	63.3^{a}	70^{a}	76.6^{a}	60^{a}	63.3^{a}	60^{a}
V. tricolor	86.7^{a}	90^{a}	76.6^{ab}	83.3^{a}	90^{a}	70^{ab}	56.7^{b}

Means bearing the same letters indicate statistical indifferences ($P \leq 0.05$; Tukey's test) according to one-way ANOVA

https://doi.org/10.17221/50/2020-PSE

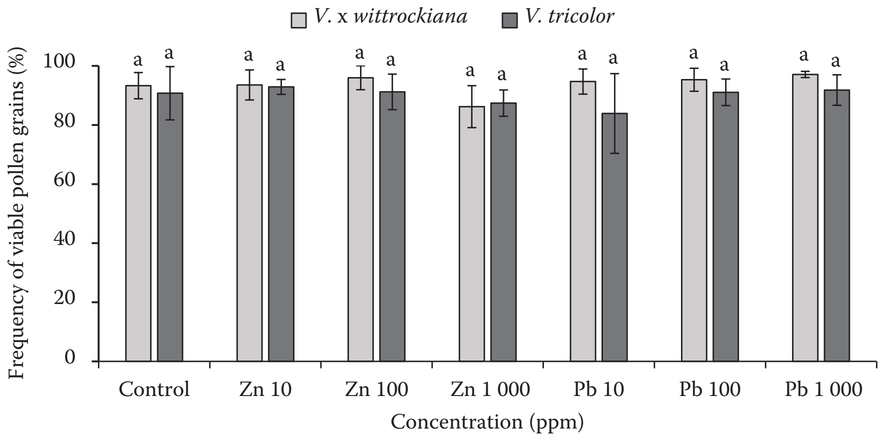

Figure 1. Pollen viability of *Viola* × *wittrockiana* and *V. tricolor* after the treatment of plants with zinc (Zn) or lead (Pb) or with deionised water (control) in pot experiments. Means bearing the same letters indicate statistical indifferences ($P \leq 0.05$; Tukey's test) according to two-way ANOVA

a known phenomenon resulting from the genotoxicity of Zn and Pb especially during microsporogenesis. The *V. tricolor* plants formed seed capsules fully filled with seeds after each treatment (data not shown).

To explain the lack of seed set by *V.* × *wittrockiana* growing indoors (experimental conditions, lack of pollinators), the female gametophyte (FG), embryo and endosperm development of plants cultivated

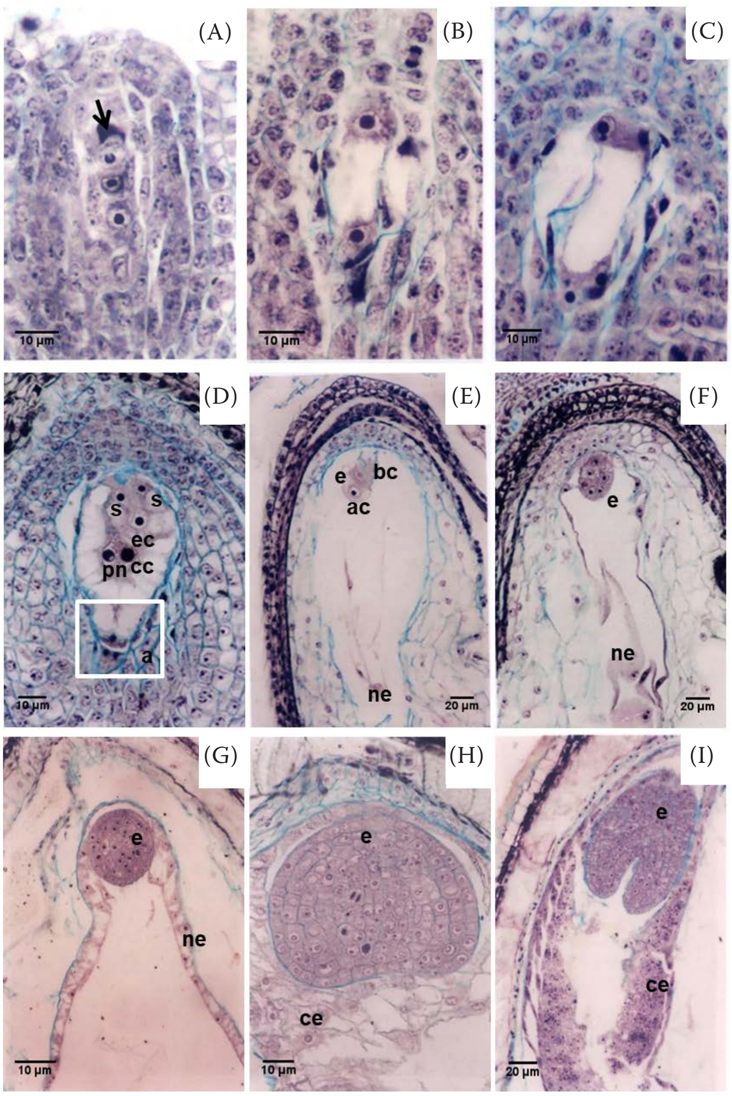

Figure 2. Female gametophyte (FG), embryo and endosperm development of *V.* × *wittrockiana*. A – tetrad of megaspores, micropylar megaspore degenerated (arrow); B – 2-nucleate FG; C – 4-nucleate FG; D – 7-celled FG, two visible synergids (s), an egg cell (ec), central cell (cc) with two polar nuclei (pn), three antipodal cells (a, insert); E – 2-celled embryo (e) with apical (ac) and basal cell (bc), visible nuclear endosperm (ne); F, G – globular stage of embryo (e), surrounded by nuclear endosperm (ne); H, I – heart (H) and torpedo (I) stage of embryo (e) and cellular endosperm (ce). A–I – longitudinal sections of ovules stained with Heidenhain's hematoxylin combined with alcian blue

https://doi.org/10.17221/50/2020-PSE

outdoors (the presence of pollinators) were analysed in detail. The FG have developed according to the Polygonum type from chalazal megaspore of a linear tetrad (Figure 2A) passing the 2-, 4-, 8-nuclear stages to 7-celled with the egg apparatus at the micropolar pole, the central cell with two polar nuclei and three short-lived antipodes (Figure 2B–D). An Asterad type embryo, without a suspensor, was surrounded by nuclear endosperm at the 2-celled and globular stage (Figure 2E–G), and by cellular endosperm in the heart and torpedo stage (Figure 2H, I). In plants cultivated outdoors under the SEM microscope, a special arrangement of five filamentless stamens with appendices tightly surrounding the style ended with head-like stigma with stigmatic cavity and stigmatic lip, the latter covered with papillae were visible. At the edges of the stigma there were two lateral outgrowths covered with hairs preventing self-pollination (Figure 3A, B).

The enlarged ovaries were fully filled with viable ovules in *V.* × *wittrockiana* plants treated and non-treated with Zn or Pb growing indoors (laboratory conditions) (Figure 3C–E). At this stage, anatropous, bitegmic and crassinucellate ovules were enlarged, regular in size and shape, and attached to the placenta. However, in such ovules, unfertilised FG degenerated (Figure 3F, G) and only shrunk FG instead of the predicted 7-celled FG or embryo and endosperm was observed in all ovules. The average number of ovules per ovary of plants growing outdoors was 90, cultivated indoors after treatment with Pb – 110, with Zn – 117, in the control – 123. There were no statistical differences in the number of ovules per ovary between all treatments.

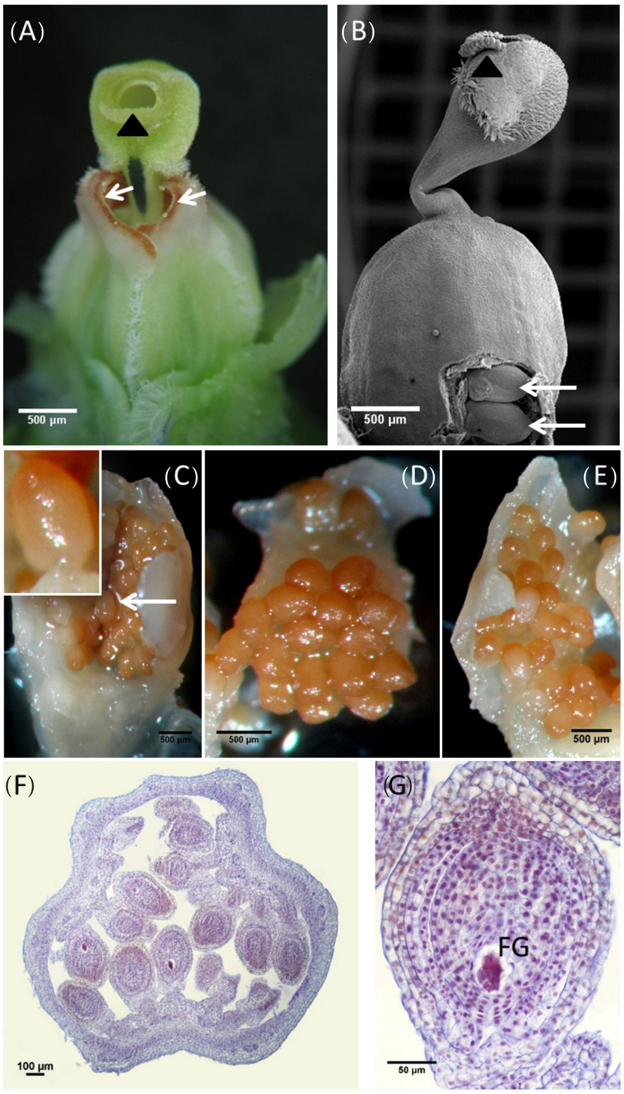

Figure 3. Pistil, ovary, ovules of *Viola* × *wittrockiana* – a non-treated with heavy metals (A, B) and after treatment with lead (Pb) (C–G). A – pistil ended with head-like stigma with cavity and lip (arrowhead), ovary tightly surrounded by five stamens, style by anther appendices (arrows); B – pistil, enlarged ovary, visible ovules (arrows) inside the ovary and lateral outgrowths with hairs (arrowhead on stigma); C – open ovary filled with normally developed ovules (arrow), single ovule inserted; D, E – separated carpels of the ovary with ovules attached to the placenta; F – transversal section of the tripartite ovary with ovules; G – normally developed ovule, degenerating female gametophyte (FG) inside the ovule due to the lack of fertilisation; B – a scanning electron microscope microphotograph

https://doi.org/10.17221/50/2020-PSE

High Zn and Pb accumulation efficiency in *V.* × *wittrockiana* in comparison with *V. tricolor*. Three-way ANOVA for Pb accumulation revealed no statistical differences between the taxa. There was a statistically significant impact for parts of the plant, concentration of Pb, the interaction between the taxa and metal concentration and the interaction between parts of the plant and metal concentration. Three-way ANOVA for Zn accumulation revealed statistically significant differences between the taxa, parts of the plant, concentration and the interaction between the taxa, parts of the plant and Zn concentration (Table 2).

Lead was accumulated at higher concentrations in the roots than in the shoots. However, the concentration in plants was lower than in the soil (Table 3). The BF and TF values for Pb were much lower than 1, except for BF at lower concentrations (10 and 100 ppm for *V. tricolor* and 10 ppm for *V.* × *wittrockiana*) of this element applied to the soil (Figure 4).

A significant increase of Zn accumulation in roots and shoots was found in *V. tricolor* after 1 000 ppm and in *V.* × *wittrockiana* after treatment with 100 and 1 000 ppm. The accumulation of Zn in the whole plant was about four times higher than the concentration applied to the soil. *Viola* × *wittrockiana* accumulated Zn in roots and shoots in nearly the same concentration (1 924.74 *vs.* 1 823.87 ppm at 1 000 ppm). The differences in Zn accumulation between roots and shoots was higher in *V. tricolor* (1 222.66 *vs.* 2 718.71 ppm at 1 000 ppm) (Table 3). The BF and TF values for Zn of both taxa reached ~1 or higher, depending on the concentration (Figure 4).

DISCUSSION

The results of this study showed high tolerance to Zn and Pb of non-metallophytic *V.* × *wittrockiana.* This common garden plant seems to be another metal-tolerant violet, which makes us willing to conclude that the tolerance to heavy metals in *Viola* is innate (constitutive) character, was present in the ancestor and was inherited by almost all descendants. This is speculated on the comparison of suspended cell tolerance between three metallophytes from other families (*Silene vulgaris* subsp. *humilis*, *Arabidopsis hallerii* and *Armeria maritima*) clearly showing that violets, even non-metallophytes, tolerate higher Zn and

Table 2. Three-way ANOVA results for the effect of the taxon, part of the plant and concentration on heavy metal accumulation by plants treated with zinc (Zn) or lead (Pb)

Treatment	SS	*df*	MS	*F*-value	*P*-value
Pb experiment					
Taxon	295.48	1	295.48	0.0557	0.815
Part of the plant (root *vs.* shoot)	136 618.09	1	136 618.09	25.7537	0.000***
Concentration	669 539.76	3	223 179.92	42.0715	0.000***
Taxon × part of the plant	23 997.51	1	23 997.51	4.5237	0.041*
Taxon × concentration	152 005.27	3	50 668.42	9.5514	0.000***
Part of the plant × concentration	122 090.60	3	40 696.86	7.6717	0.000***
Taxon × part of the plant × concentration	68 186.88	3	22 728.96	4.2846	0.011*
Error	169 752.80	32	5 304.77		
Zn experiment					
Taxon	40 912.64	1	40 912.64	0.4156	0.000***
Part of the plant (root *vs.* shoot)	743 685.73	1	743 685.73	7.5552	0.009**
Concentration	28 565 653.53	3	9 521 884.51	96.7351	0.000***
Taxon × part of the plant	407 802.82	1	407 802.82	4.1429	0.050
Taxon × concentration	126 415.47	3	42 138.49	0.4281	0.734
Part of the plant × concentration	826 668.93	3	275 556.31	2.7994	0.055
Taxon × part of the plant × concentration	1 524 851.83	3	508 283.94	5.1637	0.005**
Error	3 149 840.99	32	98 432.53		

SS – sum of squares; *df* – degrees of freedom; MS – mean square; *F*-value – value of *F* distribution; *P*-value – probability value; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

https://doi.org/10.17221/50/2020-PSE

Table 3. Zinc (Zn) and lead (Pb) accumulation (ppm) in dry weight of roots and shoots of *Viola* × *wittrockiana* and *V. tricolor* after 60 days of growing in pots with Zn or Pb or without heavy metal (control)

Concentration		*V.* × *wittrockiana*		*V. tricolor*	
		root	shoot	root	shoot
Pb	0	5.18 ± 4.88[a]	1.28 ± 2.15[a]	0.00 ± 1.25[a]	1.33 ± 1.23[a]
	10	36.47 ± 28.32[a]	2.98 ± 6.33[a]	29.71 ± 19.16[a]	13.88 ± 1.13[a]
	100	58.88 ± 15.48[ab]	26.82 ± 7.34[a]	405.02 ± 121.02[c]	29.84 ± 10.45[a]
	1 000	455.37 ± 97.87[c]	276.90 ± 44.47[bc]	345.15 ± 124.44[c]	103.90 ± 29.63[b]
Zn	0	64.43 ± 25.16[a]	188.83 ± 39.60[a]	68.61 ± 9.32[a]	33.26 ± 15.10[a]
	10	82.46 ± 14.14[a]	183.97 ± 61.32[a]	33.45 ± 11.36[a]	84.35 ± 11.76[a]
	100	278.12 ± 122.00[b]	411.48 ± 58.77[b]	53.08 ± 26.38[a]	254.66 ± 53.66[a]
	1 000	1 923.74 ± 397.80[cd]	1 822.87 ± 452.62[cd]	1 222.66 ± 365.30[bc]	2 718.71 ± 584.35[d]

Means bearing the same letters indicate statistical indifferences ($P \leq 0.05$; Tukey's test) according to three-way ANOVA

Pb concentration than the metallophytes from other families (Sychta et al. 2018, Miszczak et al. unpubl.). The ornamental character of *V.* × *wittrockiana* could also be treated as deeply desirable especially for the reclamation of urban areas.

Enormous amounts of heavy metals and extreme conditions negatively influence seed germination, seedling survival and reduce the diversity of plants on waste heaps (Wierzbicka and Rostański 2002). The evident features of plant tolerance to heavy metals is the high frequency of seed germination, survival and further development of the seedlings, adult plant flowering and seed set after treatment with heavy metals. Both of the studied taxa, *V.* × *wittrockiana* and *V. tricolor*, exhibit these characteristics. Neither garden pansy nor *V. tricolor* develop an accumulation strategy, although they accumulate high concentrations of Zn both in the roots and shoots and Pb mainly in the roots. Neither of violets can be classified as Zn-hyperaccumulator, because the plant accumulation is much lower than 10 000 ppm according to the definition of Baker et al. (2000). Assuming lower concentration, as suggested by van der Ent et al. (2013), the advisable concentration of 3 000 ppm is not reached, though *V. tricolor* is close to achieving it. It transports Zn from roots to shoots more effectively than *V.* × *wittrockiana*, which was also confirmed under *in situ* conditions (Słomka et al. 2011). These definitions are, however, quite risky, because they do not take into consideration the accumulation efficiency which depends on the metal concentration in the soil, as presented in the current study, and the other factors in the natural environment. A better definition seems to be that (*i*) a hyperaccumulator needs to accumulate in leaves 2–3 orders of magnitude higher than in plant leaves on normal soils, and (*ii*) at least one order of magnitude greater than the usual range in plant leaves on metalliferous soils (van der Ent et al. 2013). It was noticed

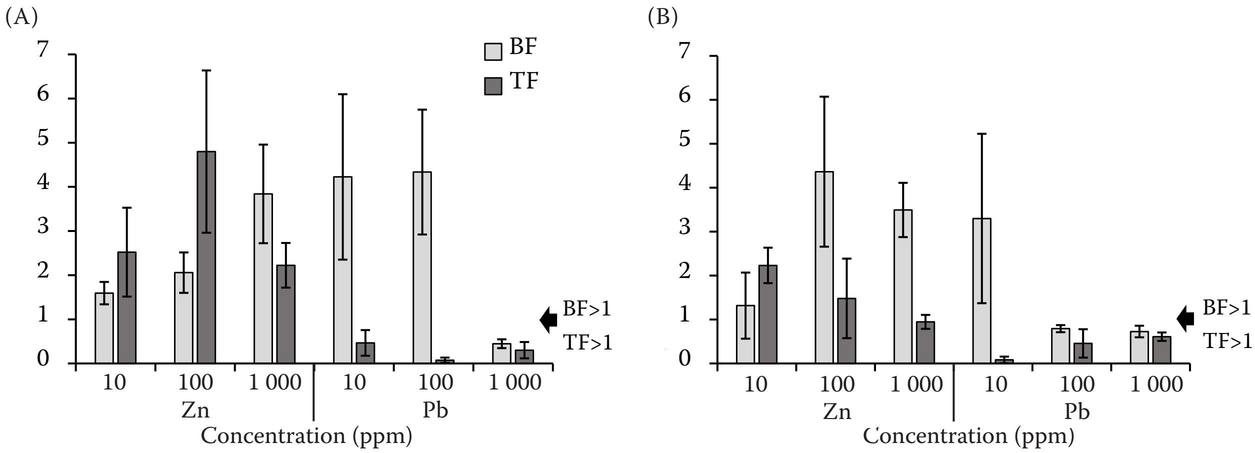

Figure 4. Bioaccumulation factor (BF) and translocation factor (TF) for plants of (A) *Viola tricolor* and (B) *V.* × *wittrockiana* in pot experiments after 60 days of treatment with zinc (Zn) or lead (Pb)

https://doi.org/10.17221/50/2020-PSE

that, with increasing heavy metal content in the soil, the concentration of particular heavy metals in plants increased simultaneously. The hyperaccumulation abilities should be supported by the bioaccumulation factor (Reboredo et al. 2018), and the translocation factor, showing the effectiveness of plants in the transport of heavy metals to the shoots (Shumaker and Begonia 2005). Plants with high BF and TF values could be useful for the phytoremediation of polluted areas. The BF and TF values for Zn were higher or nearly 1 in *V.* × *wittrockiana* and *V. tricolor*, which indicates that these species could be useful for Zn phytoextraction, whereas for Pb, both values were mostly much lower than 1, suggesting an exclusion strategy for Pb (Li et al. 2007).

The female and male germlines development of *V.* × *wittrockiana* were not affected by the heavy metal stress. The plants produced flowers, pollen viability reached over 90%, and large viable ovules filled the ovaries. Seeds in controlled conditions were not produced due to the lack of pollinators, as agent-mediation is a requirement for successful fertilisation and self-pollination is almost impossible in this species (Kroon 1972, Dalbato et al. 2013). The costs of tolerance to heavy metals measured as the disturbances in sexual reproduction do not apply in the case of *V.* × *wittrockiana*. Usually, the higher the frequency of degeneration processes and disturbances in ovules, stamens, embryo and endosperm development, the lower the tolerance and the sexual reproduction. These processes in *Viola metallophytes* and pseudometallophytes of calamine and serpentine soils is slightly impaired which, however, does not deplete the population (Bothe and Słomka 2017, Słomka et al. 2017). It should be kept in mind that *V.* × *wittrockiana*, an ornamental plant, does not occur naturally in polluted soil whereas all these studies were performed on plants growing in their natural metalliferous sites.

From our studies, it is clear that *V.* × *wittrockiana* could be a new garden plant useful for phytoremediation and reclamation of polluted areas, extending the list of tolerant ornamental plants with economic and aesthetic values, such as *Calendula officinalis*, *Althaea rosea*, *Osmanthus fragrans*, *Ligustrum vicaryi*, *Cinnamomum camphora*, *Loropetalum chinense* and *Euonymus japonicus* (Liu et al. 2008, Wu et al. 2011, Ho et al. 2014, Peng et al. 2018).

The knowledge about the tolerance and adaptation of plants to heavy metals is the basis for further actions aimed at phytoremediation, reclamation and revalorisation of polluted areas. This study shows that *V.* × *wittrockiana*, an ornamental plant, tolerates and also accumulates Pb and Zn in its tissues. The tolerance of *V.* × *wittrockiana* established based on seed germination, pollen viability and the ability to reproduce under heavy metal stress, is comparable to *V. tricolor*, a metallophyte abundantly inhabiting areas polluted with Zn and Pb. As a perennial, highly tolerant plant, with a relatively high biomass and deep root system, as well as large beautiful flowers, *V.* × *wittrockiana* is a potentially useful plant for the reclamation of polluted sites, and providing aesthetic values to urbanised areas.

Acknowledgment. The authors thank MSc Joanna Opoka for sharing some of the results and photographic documentation from her master's thesis completed in the Department of Plant Cytology and Embryology, Institute of Botany, Jagiellonian University and professor Jerzy Bohdanowicz from the Gdańsk University for scanning electron microscope documentation.

REFERENCES

Adams S.R., Pearson S., Hadley P. (1997): An analysis of the effects of temperature and light integral on the vegetative growth of pansy cv. universal violet (*Viola* × *wittrockiana* Gams.). Annals of Botany, 79: 219–225.

Baker A.J.M., McGrath S.P., Reeves R.D., Smith J.A.C. (2000): Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal polluted soils. In: Terry N., Bañuelos G. (eds.): Phytoremediation of Contaminated Soil and Water. Boca Raton, CRC Press, 85–107. ISBN 9780367399436

Banásová V., Horak O., Čiamporová M., Nadubinská M., Lichtscheidl I. (2006): The vegetation of metalliferous and non-metalliferous grasslands in two former mine regions in Central Slovakia. Biologia, 61: 433–439.

Bothe H., Słomka A. (2017): Divergent biology of facultative heavy metal plants. Journal of Plant Physiology, 219: 45–61.

Dalbato A.L., Kobza F., Suchánková P. (2005): Polyploidy effects on frost tolerance and winter survival of garden pansy genotypes. Horticultural Science, 32: 138–146.

Dalbato A.L., Kobza F., Karlsson L.M. (2013): Effect of polyploidy and pollination methods on capsule and seed set of pansies (*Viola* × *wittrockiana* Gams). Horticultural Science, 40: 22–30.

Du X.H., Wang M.G., Słomka A., Liu H.C. (2018): Karyologic and heterosis studies of the artificial inter- and intraspecific hybrids of *Viola* × *wittrockiana* and *Viola cornuta*. HortScience: a publication of the American Society for Horticultural Science, 53: 1300–1305.

Ernst W.H.O. (2006): Evolution of metal tolerance in higher plants. Forest Snow and Landscape Research, 80: 251–274.

https://doi.org/10.17221/50/2020-PSE

Fernandes L., Casal S., Pereira J.A., Saraiva J.A., Ramalhosa E. (2017): Edible flowers: a review of the nutritional, antioxidant, antimicrobial properties and effects on human health. Journal of Food Composition and Analysis, 60: 38–50.

Gao J., Luo M., Zhu Y., He Y., Wang Q., Zhang C. (2015): Transcriptome sequencing and differential gene expression analysis in *Viola yedoensis* Makino (Fam. Violaceae) responsive to cadmium (Cd) pollution. Biochemical and Biophysical Research Communications, 459: 60–65.

Ho J.R., Ma H.W., Wang Y.C., Ko C.H., Chang F.C., Feng F.L., Wang Y.N. (2014): Extraction of heavy metals from contaminated soil by *Cinnamomum camphora*. Ecotoxicology, 23: 1987–1995.

Kuta E., Bohdanowicz J., Słomka A., Pilarska M., Bothe H. (2012): Floral structure and pollen morphology of two zinc violets (*Viola lutea* ssp. *calaminaria* and *V. lutea* ssp. *westfalica*) indicate their taxonomic affinity to *Viola lutea*. Plant Systematics and Evolution, 298: 445–455.

Kuta E., Jędrzejczyk-Korycińska M., Cieślak E., Rostański A., Szczepaniak M., Migdałek G., Wąsowicz P., Suda J., Combik M., Słomka A. (2014): Morphological *versus* genetic diversity of *Viola reichenbachiana* and *V. riviniana* (sect. *Viola*, Violaceae) from soils differing in heavy metal content. Plant Biology, 16: 924–934.

Kroon G.H. (1972): Some aspects of the pollination mechanism of *Viola tricolor* L. and *Viola* × *wittrockiana* Gams. Acta Botanica Neerlandica, 21: 630–632.

Kwiatkowska M., Żabicka J., Migdałek G., Żabicki P., Cubała M., Bohdanowicz J., Słomka A., Jędrzejczyk-Korycińska M., Sliwinska E., Sychta K., Marcussen T., Thiele K., Kuta E. (2019): Comprehensive characteristics and genetic diversity of the endemic Australian *Viola banksii* (section *Erpetion*, Violaceae). Australian Journal of Botany, 67: 81–98.

Li M.S., Luo Y.P., Su Z.Y. (2007): Heavy metal concentrations in soils and plant accumulation in a restored manganese mineland in Guangxi, South China. Environmental Pollution, 147: 168–175.

Liu J.N., Zhou Q.X., Sun T., Ma L.Q., Wang S. (2008): Identification and chemical enhancement of two ornamental plants for phytoremediation. Bulletin of Environmental Contamination and Toxicology, 80: 260–265.

Migdałek G., Woźniak M., Słomka A., Godzik B., Jędrzejczyk-Korycińska M., Rostański A., Bothe H., Kuta E. (2013): Morphological differences between violets growing at heavy metal polluted and non-polluted sites. Flora – Morphology, Distribution, Functional Ecology of Plants, 208: 87–96.

Peng Z., Guo Z.H., Cao X., Xiao X.Y., Liu Y., Shi L. (2018): Phytostabilization potential of ornamental plants grown in soil contaminated with cadmium. International Journal of Phytoremediation, 20: 311–320.

Psaras G.K., Constantinidis T. (2009): Two new nickel hyperaccumulators from the Greek serpentine flora. Fresenius Environmental Bulletin, 18: 798–803.

Reboredo F.H., Pelica J., Lidon F.C., Ramalho J.C., Pessoa M.F., Calvão T., Simões M., Guerra M. (2018): Heavy metal content of edible plants collected close to an area of intense mining activity (southern Portugal). Environmental Monitoring and Assessment, 190: 484.

Reeves R.D., Baker A.J.M., Jaffré T., Erskine P.D., Echevarria G., van der Ent A. (2017): A global database for plants that hyperaccumulate metal and metalloid trace elements. New Phytologist, 218: 407–411.

Shumaker K., Begonia G. (2005): Heavy metal uptake, translocation, and bioaccumulation studies of *Triticum aestivum* cultivated in contaminated dredged materials. International Journal of Environmental Research and Public Health, 2: 293–298.

Singh R.J. (2003): Plant Cytogenetics. Boca Raton, CRC Press. ISBN: 0-8493-2388-6

Słomka A., Libik-Konieczny M., Kuta E., Miszalski Z. (2008): Metalliferous and non-metalliferous populations of *Viola tricolor* represent similar mode of antioxidative response. Journal of Plant Physiology, 165: 1610–1619.

Słomka A., Kuta E., Szarek-Łukaszewska G., Godzik B., Kapusta P., Tylko G., Bothe H. (2011): Violets of the section Melanium, their colonization by arbuscular mycorrhizal fungi and their occurrence on heavy metal heaps. Journal of Plant Physiology, 168: 1191–1199.

Słomka A., Kwiatkowska M., Jędrzejczyk-Korycińska M., Bohdanowicz J., Poznańska P., Shuka L., Borucki W., Kuta E. (2017): Insight into "serpentine syndrome" of Albanian, endemic violets (*Viola* L., Melanium Ging. section) – Looking for unique, adaptive microstructural floral, and embryological characters. Plant Biosystems – An International Journal Dealing with all Aspects of Plant Biology, 151: 1022–1034.

Sychta K., Słomka A., Suski S., Fiedor E., Gregoraszczuk E., Kuta E. (2018): Suspended cells of metallicolous and nonmetallicolous *Viola* species tolerate, accumulate and detoxify zinc and lead. Plant Physiology and Biochemistry, 132: 666–674.

Sychta K., Słomka A., Sliwinska E., Migdałek G., Kuta E. (2020): From cells highly tolerant to Zn and Pb to fully fertile plants – Selection of tolerant lines with *in vitro* culture. Plant Physiology and Biochemistry, 146: 231–237.

Tangahu B.V., Abdullah S.R.S., Basri H., Idris M., Anuar N., Mukhlisin M. (2011): A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. International Journal of Chemical Engineering, 939161.

Van der Ent A., Baker A.J.M., Reeves R.D., Pollard A.J., Schat H. (2013): Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant and Soil, 362: 319–334.

Wierzbicka M., Rostański A. (2002): Microevolutionary changes in ecotypes of calamine waste heap vegetation near Olkusz, Poland: a review. Acta Biologica Cracoviensia. Series Botanica, 44: 7–19.

Wittrock V.B. (1895): Viola-Studier – A contribution to the history of the pansies having special reference to their origin. Acta Horti Bergiani, 2: 441–522.

Wu F.Z., Yang W.Q., Zhang J., Zhou L.Q. (2011): Growth responses and metal accumulation in an ornamental plant (*Osmanthus fragrans* var. *thunbergii*) submitted to different Cd levels. International Scholarly Research Notices, 738138.

Received: January 29, 2020
Accepted: April 20, 2020
Published online: June 1, 2020