Ann. Bot. Fennici 50: 142–148 ISSN 0003-3847 (print) ISSN 1797-2442 (online)
Helsinki 12 April 2013

Preliminary studies on the morphological variability among *Arabidopsis halleri* populations from contrasting habitats

Izabella Franiel & Karolina Steindor*

*University of Silesia, Department of Ecology, ul. Bankowa 9, PL-40-007 Katowice, Poland (*corresponding author's e-mail: karolina.steindor@us.edu.pl)*

Received 16 Jan. 2012, final version received 1 Feb. 2013, accepted 7 Feb. 2013

Franiel, I. & Steindor, K. 2013: Preliminary studies on the morphological variability among *Arabidopsis halleri* populations from contrasting habitats. — *Ann. Bot. Fennici* 50: 142–148.

Different environmental conditions can cause specific modifications during the development of plants. In this study, we investigated the effect of contrasting habitats (a Zn-Pb dump and a forest site) on morphological and reproductive traits of 154 plants of the perennial herb *Arabidopsis halleri*. Base on the results of our preliminary investigation we distinguished morphological and functional traits, which differentiate populations of *A. halleri* from forest and dump habitats. Discriminant analysis distinguished the seed number and the length of the longest rosette leaf as the most distinctly variable traits.

Introduction

Phenotypic plasticity is the capability of a single genotype to express different phenotypes in different environments, and it is assumed to be a mechanism for individual adaptation to environment heterogeneity. Adaptive plasticity is commonly expressed in traits directly related to fitness and enables plants to persist under particular environmental conditions (Sultan 2000). Twenty years ago, developmental and physiological plasticity was reported in terrestrial plants, insects, fish and small mammals. In more recent studies, plasticity was scrutinized most intensively in plants, because the effects of environment are clearly manifested in their growth. In addition, the latest papers focused on such aspects of plasticity that are directly related to the functional and reproductive success of plants (Sultan 1995, 2000, 2003, Alpert 1999).

Plants also demonstrate great morphological plasticity in their response to the environment, such as the number of neighboring plants, light, nutrient and water availability, or pollution (Sekimura *et al.* 2000, Gianoli 2002, Navas & Garnier 2002, Zvereva & Kozlov 2005). It can be displayed in vegetative and reproductive traits such as leaf size and shape, leaf area, trichome densities, or the number and weight of seeds (Chester & Shaver 1982, Lynn & Waldren 2001, Kozlov & Zvereva 2007, Przedpełska & Wierzbicka 2007).

Zinc (Zn) and lead (Pb) ores have been extracted in Poland since the 12th century, and their exploitation and a primitive metal smelting technology resulted in large quantities of waste particularly in the Upper Silesia region (Skubała 2011). Wastes from Zn and Pb ore mining and processing are very hostile environments for organisms because of the high concentrations of toxic elements (Cd, Zn, Pb, As). However, many plant species are able to grow in such habitats (Cabała *et al*. 2004).

Our research focuses on plants originating from very different habitats, that is from a beech forest and a Zn-Pb smelter dump. The complex composition of the deposited industrial wastes (high amounts of heavy metals) and the structure of the dump (the way of heaping up, the lack of soil) produced a contaminated, dry and unproductive environment. However, plants that manage to establish themselves in such harsh conditions can profit from the high light availability and low inter- and intrapopulation competition.

The aim of our study was to answer the questions (i) whether populations of *Arabidopsis halleri* from two contrasting habitats differ in selected morphological and functional traits, and, if so, (ii) which of the morphological traits of *A. halleri* are the most variable.

Material and methods

Study species

Arabidopsis halleri is a self-incompatible, insect-pollinated perennial herb, which forms rosettes and propagates by stolons. Stems, growing from the base of a rosette, are decumbent, and simple or branched at their upper parts. Blades of the basal leaves are orbicular to broadly ovate, slightly pinnatifid or lyrate-pinnatifid. Stem leaves are shortly petiolate, ovate to oblong or lanceolate, toothed or rarely lobed, with the size decreasing towards the stem tip. The plant produces a large number of oblong, brown seeds placed in linear, flattened, torulose siliquas (Al-Shehbaz & O'Kane 2002, Van Rossum *et al*. 2004). The chromosome number (n) is 8. *Arabidopsis halleri* prefers mesic environments and occurs in meadows under intensive competition, as well as at heavy-metal contaminated sites, being regarded as a zinc hyperaccumulator (Sarret *et al*. 2002). The species ranges from France to Taiwan and has various subspecies; in Europe it is distributed in mountainous areas with continental climate (Mitchell-Olds 2001, Van Rossum *et al*. 2004).

Site characteristics and sampling

The Zn-Pb dump (DS) is situated in the northern section of Wełnowiec Ditch (*Rów Wełnowiecki*), east of the ”Silesia Non-ferrous Metal Plant”. The site includes areas in two towns: Katowice and Siemianowice (49°04´N, 18°55´E). The studied plants grew on the top of a post-flotation sludge waste-heap (33 m high), composed of waste originating from complex Zn-Pb ore enrichment processes, dolomites, clays and extremely toxic silts (Zn 11 078 mg kg^{-1}, Pb 1331 mg kg^{-1}, Cd 9 mg kg^{-1} d.m. soil; Franiel & Więski 2005).

“Uroczysko Buczyna” is a patch of semi-natural beech (*Fagus sylvatica*) forest (FS) located in the vicinity of the city of Chorzów (50°16´N, 18°57´E). The soil is mainly brown podzol formed of loamy sand with a relatively low high metal content (Zn 100 mg kg^{-1}, Pb 196 mg kg^{-1}, Cd 1 mg kg^{-1} d.m. soil). The plant samples were collected from marginal forest patches constituting fragments of the *Tilio-Carpinetum* association. The herb layer (at the level of 80%) was well developed with the cover of grasses (*Festuca gigantea* and *Deschampsia caespitosa*) and *Arabidopsis halleri* (Franiel & Fiałkiewicz 2007).

Both sites (the dump area and the Uroczysko-Buczyna forest) are in the climatic zone of Central Uplands and have the maximum precipitation in June (158.7 mm in Uroczysko Buczyna, and 133.8 mm in the dump area) and the minimum precipitation in March (28.9 mm, and 22.3 mm, respectively). July is the warmest month (20.1 °C in the dump area, and 19.3 °C in Uroczysko Buczyna) and December is the coldest one (–5.1 °C and –5.6 °C, respectively). The maximum radiation intensity at the dump area was PAR-1265 E m^{-2} s^{-1}, whereas in the forest understory it was 141 E m^{-2} s^{-1} (Phytophotometer FR-20, Optel Opole, Poland; with clear sky conditions at ground level in the first week of July).

Plant material was sampled randomly from 154 (77 from either site) flowering individuals within a distance of one meter from each other. That distance should be sufficient to make sure that the collected individuals represent different genotypes (Van Rossum *et al*. 2004). According to our concept, an individual is a rosette (= ramet), which can be easily distinguished and counted (Falińska 2002). The total number of flowers per individual was recorded at the beginning of the study (17 July 2009). In either habitat, the examined plants were labeled and harvested at the end of the fructification period. The following traits were measured in the laboratory: (1) the number of flowering axes, (2) the average flowering axe length, (3) the number of flowering axe leaves, (4) the rosette diameter, (5) the longest rosette leaf length, (6) the longest rosette leaf breadth, (7) the flowering axe weight, (8) the rosette weight, (9) the number of flowers per individual, (10) the number of fruits per individual, (11) the number of seeds, (12) the maximum siliqua length. For dry weight measurements, the material was dried at 50 °C to constant weight.

Statistical methods

The differences in traits of *A. halleri* between groups were evaluated using a *t*-test. Prior to testing, all the data were log(x + 1)-transformed to stabilize variances, and standardized to allow comparisons of different variables.

We carried out a cluster analysis (CA) to arrange the collected data according to some organized structures or groups by analyzing the similarities in the studied areas. The clusters are sets of related data, and the data belonging in two separate clusters should differ significantly. The assumption of a higher degree of similarity within the examined sites was made in view of CA, based on the average values of particular traits for each of the 154 selected individuals. The dendrogram was created using the Ward method, which allows the fusion of clusters on the basis of minimizing within-cluster variance.

The likeness between the objects is established on the basis of an appropriate index or Euclidean distance. By applying such analyses it is possible to form groups of objects whose members resemble one another as closely as possible in respect to specified features, while at the same time they differ substantially from the members of neighboring sets.

A reduction in the number of variables was obtained with the principal component analysis (PCA), in which a smaller number of uncorrelated components were obtained, each of which was equivalent to a certain percentage of the total variability. In order to determine which variables discriminated between the two *A. halleri* populations, the discriminant function analysis with the stepwise method was used. Variables with the tolerance lower than 0.01 were rejected (Castillo-Rivera *et al*. 2000). The conclusion on the discriminatory power was reached based on Wilks' λ statistics, which describes overall separation of the groups. The discrimination analysis was also used as a criterion for classification, allowing us to assess the percentage of individual objects correctly assigned to specified morphotypes. The analyses were performed with the software Statistica 7.0 PL.

Results

Mean values of all investigated *A. halleri* traits differed significantly ($p < 0.001$) between the dump site and the forest site (Table 1). Plants from the polluted environment had higher biomass of vegetative parts, a higher number of flowering axes and the flowering axes had more leaves. However larger rosette leaves were characteristic of plants from the forest site.

The reproductive traits, such as the number of flowers, fruits and seeds were about three to four times higher in plants growing on the dump.

Our cluster analysis (CA) results showed a clear distinction between the two habitats (Fig. 1).

Contrary to CA, PCA allows to confirm the hypothesis in terms of describing the range of variability in the groups regarding the principal components, while at the same time identifying the variables responsible for the discrimination of groups. The results of the scree test allowed us to distinguish the two components that accounted for 64% of the resulting variability (Fig. 2). The closer the vectors representing the input variable are plotted, the stronger the positive correlation between the corresponding variables is (DS 4, 5, 6, 7, 8, 10 and FS 4, 5, 7, 8). If these vectors are perpendicular, then the variables are not correlated (group DS *versus* FS).

The results of the discriminant function analysis confirmed the differences in the morphomet-

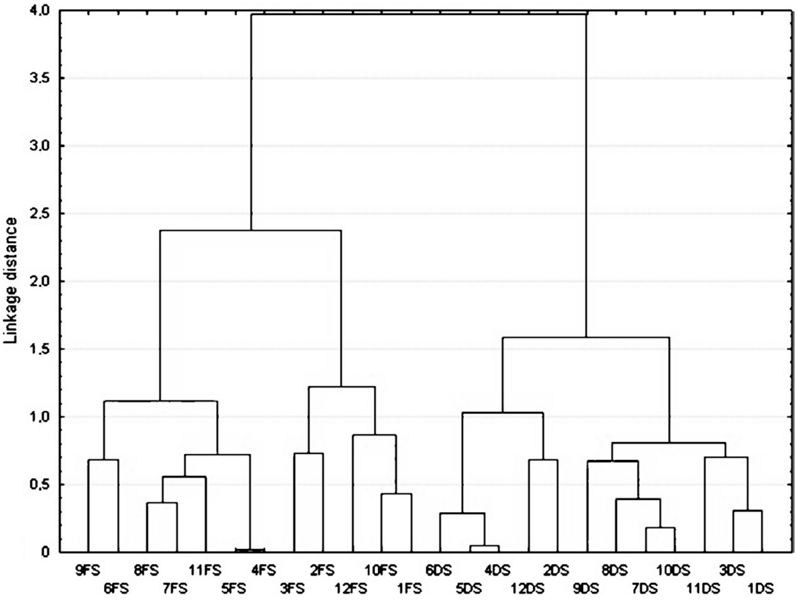

Fig. 1. A hierarchical clustering based on the 24 measured traits of plants from two habitats (DS, FS). Dendrogram based on 1 – *r* distance using the Ward method. For abbreviations *see* Table 1).

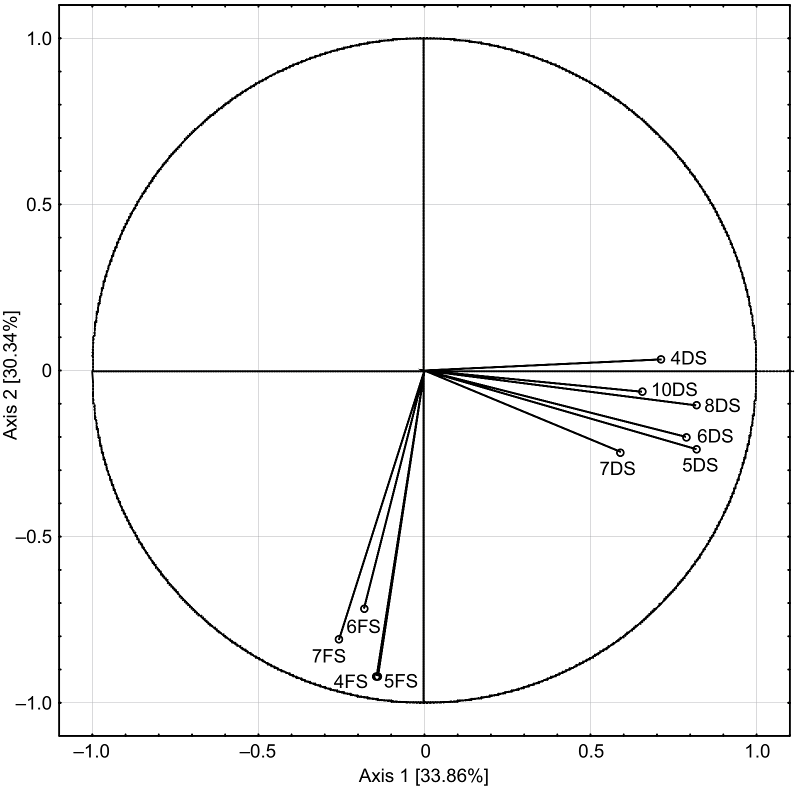

Fig. 2. Configuration of points representing the variables (factor loadings > 0.7) in the system of the first two principal component axes.

rics of the two populations (Wilks' $\lambda = 0.213$; $F_{7,146} = 76.669$, $p < 0.001$). The partial values of Wilks' λ (Table 2) revealed that the studied populations are primarily discriminated by the number of seeds and the length of the longest rosette leaf. The parameter values of the classification function (not shown) were used for *a posteriori* classification in order to determine whether the empirical data corresponded with the model. The classification matrix correctly distinguished 97% of the individuals characterized as the "dump site morphotype" and 100% of the individuals of the "forest site morphotype".

Discussion

Arabidopsis halleri is a widely spread species with a wide ecological amplitude (Mitchell-Olds 2001, Van Rossum *et al*. 2004). It has a broad tolerance to different environmental conditions, forming specialized populations in mountain habitats, shaded forests and heavy-metal contaminated areas (Al-Shehbaz & O'Kane 2002, Pauwels *et al*. 2006).

Plants can respond to their environments through their developmental plasticity in many aspects of their phenotypes. Our investigation showed clear differences in the examined traits of the dump and forest populations, which suggests there are two distinct morphotypes (Table 1 and Fig. 1). The DS population had significantly higher biomass of vegetative parts, the larger number of flowering axes and flowering axes had more leaves. One of the reasons could be the greater light availability at the open dump site (maximum PAR = 1265 E m^{-2} s^{-1}). Similarly, in the experiment of Nilsson and D'Hertefeldt (2008) *Aegopodium podagraria* produced higher biomass in unshaded pots.

In the forest populations of *A. halleri*, the rosette leaves were longer and wider. Shading can contribute to an increase in the leaf area, rosette size and stem elongation (Gianoli 2002, Jimenez-Ambriz *et al*. 2007). The other distinct trait that differentiated the populations between the studied habitats was the input into sexual reproduction. Plants from the dump site produced about four times more flowers and about three times more fruits and seeds than those from the forest (Table 1). This phenomenon was also observed in *Vaccinium myrtillus* and *V. vitis-idea* under heavy metal stress: the proportion of sexually reproducing clones was higher in polluted habitats, and the reproductive clones had more generative flowering axes and higher fruit yield under the impact of pollution (Zvereva & Kozlov 2005). *Chamerion angustifolium* and *Cornus suecica* had a larger size and higher flower production at polluted sites (Kozlov & Zvereva 2007).

PCA and discriminant analysis pointed out the generative traits (the number of fruits and seeds) as the most important for the distinction of the two morphotypes (Table 2 and Fig. 2). The importance of the sexual effort at the unfavorable dump site might result from several causes. Firstly, in dump areas resources are very scarce (especially water), which can induce plants to produce large numbers of small flowers. This is a compromise based on the diversion of water essential for photosynthesis to the development of flowers (Tucic & Miljkovic 2010). The resource-cost hypothesis proposed by Galen

Table 1. *Arabidopsis halleri* population characteristics and *t*-test results.

Traits	Dump site (DS)		Forest site (FS)		df	*t*	*p*
	Mean	SD	Mean	SD			
1. Number of shoots	4.14	2.20	1.36	0.56	152	10.72	< 0.001
2. Mean shoot lenght (cm)	10.03	4.11	15.18	7.53	152	–5.26	< 0.001
3. Number of shoot leaves	12.83	7.70	6.95	5.48	152	5.46	< 0.001
4. Rosette diameter (cm)	4.12	1.15	5.28	2.40	152	–3.82	< 0.001
5. Longest rosette leaf length (cm)	2.30	0.59	3.10	1.34	152	–4.89	< 0.001
6. Longest rosette leaf breadth (cm)	0.37	0.11	0.59	3.11	152	–5.01	< 0.001
7. Shoot weight (g dry weight)	0.07	0.05	0.02	0.02	152	7.36	< 0.001
8. Rosette weight (g dry weight)	0.13	0.08	0.01	0.02	152	11.68	< 0.001
9. Number of flowers per individual	13.52	9.07	3.29	2.99	152	9.40	< 0.001
10. Number of fruits per individual	31.08	0.47	11.40	9.13	152	6.86	< 0.001
11. Number of seeds	24.92	8.18	7.16	3.11	152	17.81	< 0.001
12. Maximal siliqua length (cm)	1.92	0.47	1.34	0.63	152	6.36	< 0.001

Table 2. Parameters of the discriminant analysis function (12th step).

Traits	Wilks' λ	Partial Wilks' λ	*F* remove	*p*	Tolerance	1 – tolerance
Number of seeds (11)	0.29	0.73	52.93	< 0.0001	0.64	0.36
Longest rosette leaf lenght (5)	0.23	0.92	12.00	0.0007	0.68	0.32
Number of flowers per individual (9)	0.23	0.95	8.16	0.0049	0.81	0.19
Maximal siligua lenght (12)	0.22	0.96	6.15	0.0143	0.89	0.11
Average shoot lenght (2)	0.23	0.95	8.18	0.0048	0.84	0.16
Longest rosette leaf breadth (6)	0.22	0.97	4.04	0.0463	0.76	0.24
Rosette weight (8)	0.22	0.99	1.84	0.1765	0.53	0.47

(1999) implies that the water availability in particular acts as a selection factor underlying the reproductive allocation cost in flowering plants.

Although *A. halleri* is a potentially clonal plant, Van Rossum *et al*. (2004) suggested that the clonality remains a marginal mode of this species' reproduction. Sexual reproduction seems to be the most important strategy of propagation. One of the elements significant to generative reproduction is successful pollination (in this case by insects). The large number of small flowers at the dump sites might also be an evolutionary outcome of pollinator selection pressure, as pollinator-mediated selection promotes plants with an attractive floral display and the xeric environment favors plants producing smaller and numerous flowers (Galen 1999). Secondly, the seed establishment ratio in highly polluted areas is frequently low (Levit 1980, Mishra & Choudhuri 1999, Kuriakose & Prasad 2008). The cost-benefit model described by Loehle (1987) predicts the increased allocation to sexual reproduction, because seeds may disperse to more favorable sites or persist in the soil, and the successful colonization depends on variables such as seed dispersal, seed longevity, germination or fast growth rate after the establishment (Chester & Shaver 1982).

In this study we distinguished morphological and functional traits, which differentiate populations of *A. halleri* from forest and dump habitats. Whether those traits reflect local adaptation or phenotypic plasticity needs testing in further investigations in controlled conditions. Similar research was conducted on *Arabidopsis arenosa* populations from dump and forest habitats, which confirmed the species adaptation to the unfavorable environment of the Zn-Pb dump (Przedpełska & Wierzbicka 2007).

Acknowledgments

We would like to thank Ms. E. Kaźmierczak for her valuable comments and improvements on previous versions of this manuscript. The work was supported by grant of Polish Ministry of Science and Higher Education in 2009–2011.

References

Al-Shehbaz, I. A. & O'Kane, S. L. Jr. 2002: Taxonomy and phylogeny of *Arabidopsis*, Brassicaceae. — *The Arabidopsis Book* 11: 1–22.

Alpert, P. 1999: Effects of clonal integration on plant plasticity in *Fragaria chiloensis*. — *Plant Ecology* 141: 99–106.

Cabała, J., Teper, E., Teper, L., Małkowski, E. & Rostański, A. 2004: Mineral composition in rhizosphere of plants grown in the vicinity of a Zn-Pb ore flotation tailings pond. Preliminary study. — *Acta Biologica Cracoviensia Series Botanica* 46: 65–74.

Castillo-Rivera, M., Kobelkowsky, A. & Chavez, A. M. 2000: Feeding biology of the *Citharichthys spilopterus* Bothidae in a tropical estuary of Mexico. — *Journal of Applied Ichthyology* 16: 73–78.

Chester, A. L. & Shaver, G. R. 1982: Reproductive effort in cotton grass tussock tundra. — *Holartic Ecology* 5: 200–206.

Falińska, K. 2002: *Przewodnik do badań biologii populacji roślin*. — PWN, Warszawa.

Franiel, I. & Fiałkiewicz, B. 2007: Morphological variability of *Cardaminopsis halleri* L. HAYEK populations from areas differing in anthropopressure level. — *Polish Journal of Environmental Studies* 165: 677–683.

Franiel, I. & Więski, K. 2005: Leaf features of silver birch *Betula pendula* Roth. Variability within and between two populations uncontaminated *vs*. Pb-contaminated and Zn-contaminated site. — *Trees* 191: 81–88.

Galen, C. 1999: Why do flowers vary? The functional ecology of variation in flower size and form within natural plant populations. — *Bioscience* 49: 631–640.

Gianoli, E. 2002: Phenotypic responses of the twining vine *Ipomoea purpurea* Convolvulaceae to physical support availability in sun and shade. — *Plant Ecology* 16:

21–26.

Jimenez-Ambriz, G., Petit, C., Bourrie, I., Dubois, S., Olivieri, I. & Ronce, O. 2007: Life history variation in the heavy metal tolerant plant *Thlaspi caerulescens* growing in a network of contaminated and noncontaminated sites in southern France. Role of gene flow, selection and phenotypic plasticity. — *New Phytologist* 173: 199–215.

Kozlov, M. V. & Zvereva, E. L. 2007: Does impact of point polluters affect growth and reproduction of herbaceous plants? — *Water Air and Soil Pollution* 186: 183–194.

Kuriakose, S. V. & Prasad, M. N. V. 2008: Cadmium stress affects seed germination and seedling growth in *Sorghum bicolor* L. Moench by changing the activities of hydrolyzing enzymes. — *Plant Growth Regulation* 54: 143–156.

Levitt, J. 1980: *Response of plants to environment*. — Academic Press, New York.

Loehle, C. 1987: Partitioning of reproductive effort for clonal plants, a benefit-cost model. — *Oikos* 49: 199–208.

Lynn, D. E. & Waldren, S. 2001: Morphological variation in populations of *Ranunculus repens* from the temporary limestone lakes Turloughs in the west of Ireland. — *Annals of Botany* 87: 9–17.

Mishra, A. & Choudhuri, M. A. 1999: Monitoring of phytotoxicity of lead and mercury from germination and early seedling growth indices in two rice cultivars. — *Water Air and Soil Pollution* 114: 339–346.

Mitchell-Olds, T. 2001: *Arabidopsis thaliana* and its wild relatives, a model system for ecology and evolution. — *Trends in Ecology and Evolution* 1612: 693–700.

Navas, M.-L. & Garnier, E. 2002: Plasticity of whole plant and leaf traits in *Rubia peregrina* in response to light, nutrient and water availability. — *Acta Oecologica* 23: 375–383.

Nilsson, J. & D'Hertefeldt, T. 2008: Origin matters for level of resource sharing in the clonal herb *Aegopodium podagraria*. — *Evolutionary Ecology* 22: 437–448.

Pauwels, M., Frerot, H., Boninin, I. & Saumitou-Laprade, P. 2006: A broad-scale analysis of population differentiation for Zn tolerance in an emerging model species for tolerance study, *Arabidopsis halleri* (Brassicaceae). — *Journal Compilation 2006 Europaean Society for Evolutionary Biology* 19: 1838–1850.

Przedpełska, E. & Wierzbicka, M. 2007: *Arabidopsis arenosa* (Brassicaceae) from a lead–zinc waste heap in southern Poland — a plant with high tolerance to heavy metals. — *Plant and Soil* 299: 43–53.

Sarret, G., Saumitou-Laprade, P., Bert, V., Proux, O., Hazemann, J.-L., Traverse, A., Marcus, M. A. & Manceau, A. 2002: Forms of zinc accumulated in the hyperaccumulator *Arabidopsis halleri*. — *Plant Physiology* 130: 1815–1826.

Sekimura, T., Roose, T., Li, B., Maini, P. K., Suzuki, J. & Hara, T. 2000: The effect of population density on flowering axe morphology of herbs in relation to light capture by leaves. — *Ecological Modeling* 128: 51–62.

Skubała, K. 2011: Vascular flora of sites contaminated with heavy metals on the example of two post industrial spoil heaps connected with manufacturing of zinc and lead products in Upper Silesia. — *Archives of Environment Protection* 37(1): 57–74.

Sultan, S. 1995: Phenotypic plasticity and plant adaptation. — *Acta Botanica Neerlandica* 44: 363–383.

Sultan, S. 2000: Phenotypic plasticity for plant development, function and life history. — *Trends in Plant Science* 512: 537–542.

Sultan, S. 2003: Phenotypic plasticity in plants: a case study in ecological development. — *Evolution & Development* 5: 25–33.

Tucic, B. & Miljkovic, D. 2010: Fluctuating asymmetry of floral organ traits in natural populations of *Iris pumila* from contrasting light habitats. — *Plant Species Biology* 25: 173–184.

Van Rossum, F., Bonnin, I., Fenart, S., Pauwels, M., Petit, D. & Saumitou-Laprade, P. 2004: Spatial genetic structure within a metallicolous population of *Arabidopsis halleri*, a clonal, self-incompatible and heavy-metal-tolerant species. — *Molecular Ecology* 13: 2959–2967.

Zvereva, E. L. & Kozlov, M. V. 2005: Growth and reproduction of dwarf shrubs *Vaccinium myrtillus* and *V. vitis-ideae*, in a severely polluted area. — *Basic and Applied Ecology* 6: 261–274.