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Morphological variability of *Cardaminopsis halleri* (L.) Hayek from selected habitats in the Silesian Upland (Southern Poland)

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Abstract: *Cardaminopsis halleri*, a known hyperaccumulator, shows considerable morphological variability in the territories with a high level of heavy metal (Zn, Cd, Pb) pollution. Therefore, the presented studies were designed as an attempt to explain the scope and causes of observed variability. Plants from *Cardaminopsis halleri* populations was collected in the vegetation seasons of 2003 and 2004 in the vicinity of two sources of zinc and lead pollution and from two control localities. For each individual, 15 quantitative variables (traits) and 12 qualitative ones were analysed. Statistically significant differences were shown for qualitative and quantitative traits, attesting to high morphological variability between the investigated populations of *Cardaminopsis halleri*. Populations exposed to strong heavy metal pollution were characterised by significantly smaller size of rosette and stem leaves in comparison to control populations. Chromosome numbers of all individuals in the studied populations is the same (2n=16), which proves that the observed differences are not a result of polyploidy. The populations from Bieszczady (control localities) differ significantly from the populations from Silesia with regard to a number of qualitative and quantitative characteristics. High heavy metal pollution is one of important factors which influence the morphological variability of investigated *Cardaminopsis halleri* populations.

Key words: variability, morphology, heavy metal pollution, *Cardaminopsis halleri*

1. Introduction

In the course of evolution some plants have developed a network of defence mechanisms which enable them to grow and reproduce in polluted ecosystems (Baranowska-Morek 2003). An interesting evolutionary phenomenon within this group are so-called hyper-accumulators (Macnair 2002) which store over 1% of metal in the dry weight of aerial organs, what corresponds to a 10 to 1000-fold increase in accumulation in comparison to other plant species (Wójcik 2000).

One of the best known hyperaccumulators is *Cardaminopsis halleri* (L.) Hayek (Wierzbicka & Rostański 2002; Małkowski & Kurtyka 2003). It is a mountain plant which occurs in Poland in the mountain ranges of the Carpathians and Sudetes, also found in the adjacent territories of the Upper and Lower Silesia as well as the Sandomierz Lowland (Sychowa 1985; Zając & Zając 2001). This species is characterised by an increased uptake of heavy metals, especially zinc and cadmium, from the substrate, faster translocation of these elements in the shoots and resistance to higher metal concentrations in tissues in comparison with species which are not hyperaccumulators (Małkowski & Kurtyka 2003). Its occurrence within the Silesian Upland is probably linked to the presence of heavy metal-rich soils (Wierzbicka & Rostański 2002). The metallurgy of zinc and lead ores in the area has developed predominantly within the Tarnowskie Góry Ridge, Katowice Upland and Jaworzno Hills, where the highest number of *Cardaminopsis halleri* localities is also found (Fiałkiewicz 2005).

It has, however, been noticed that Haller's rock-cress populations which grow on the polluted ground differ significantly in their morphology from populations originating from territories less exposed to the sources of pollution (Fig. 1).

A similar phenomenon has been observed for *Silene vulgaris* and *Dianthus carthusianorum* which grow on abandoned zinc mine spoil heaps in the vicinity of

Olkusz. Detailed studies allowed to discern some microevolutionary changes which occur in the case of investigated species (Wierzbicka 2002; Wierzbicka & Rostański 2002).

In the present study, an attempt was made to explain the scope and causes of morphological variability in *Cardaminopsis halleri* populations occurring in locations with varying degree of environmental pollution.

2. Material and methods

The plant material used for investigations was collected in the vegetation seasons of 2003 and 2004 in the following four sites of occurrence of abundant *Cardaminopsis halleri* populations in the following vicinities: **MS** – Miasteczko Śląskie metal works near Tarnowske Góry (51 plants), **KW** – Silesia metal works in Katowice-Wełnowiec (51 plants), **BB** – Forests in Bibiela near Tarnowskie Góry (51 plants) and **UG** – Forests and meadows near Ustrzyki Górne, Połonina Caryńska and the Tarnica tourist trail (21 plants).

The collected specimens were subjected to morphological biometric analysis with regard to 15 quantitative traits as well as 12 qualitative traits used to differentiate the investigated populations (Table 1). These traits were selected on the basis of taxonomic keys, original studies on the same topic as well as own observations made in the field and in the KTU Herbarium.

Cytogenetic studies were also performed in order to determine the chromosome number in somatic cells of plants from investigated populations.

For determination of statistical significance of results obtained from the measurements, the following methods were applied: arithmetic mean, standard error, min-max range, Sheffe's statistical significance test, cluster analysis.

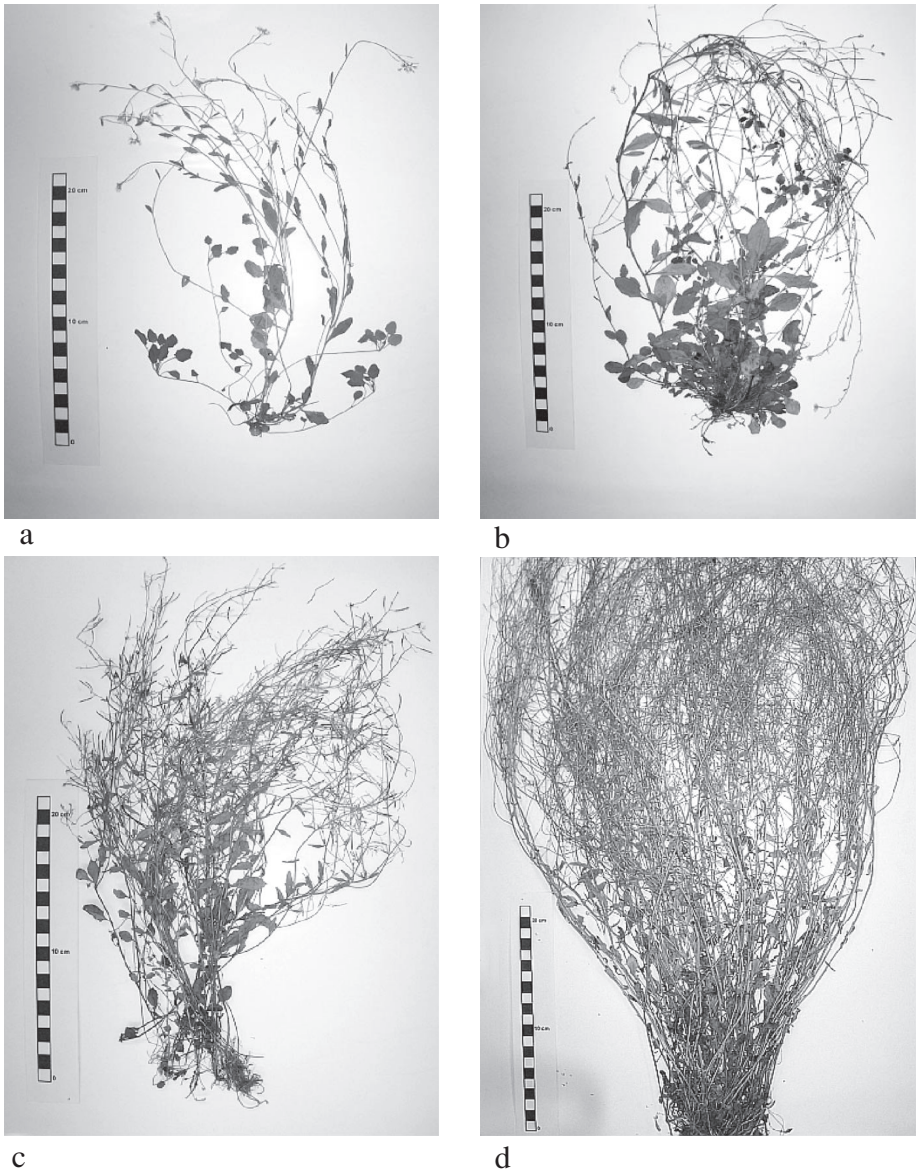

Fig. 1. Aspects of individuals of *Cardaminopsis hallerii* from investigated populations
Explanations: a – Ustrzyki Górne, b – Bibiela, c – Katowice-Wełnowiec, d – Miasteczko Śląskie

Table 1. Quantitative and qualitative traits which were used in morphological biometrical analysis (according to Jones & Akeroyd 1993; Al. Shehbaz & O'Kane 2002)

Quantitative traits	Number of trait	Qualitative traits
Height	1	Presence of stolons
Aerial stems number	2	Stem hair shape
Aerial stem branches number	3	Stem hairiness
Rosettes number per individual	4	Rosette leaf hair shape
Maximal rosette leaf length	5	Rosette leaf hairiness
Maximal rosette leaf width	6	Rosette leaf shape
Maximal lower stem leaf length	7	Bud (and flower pedicel) hairiness
Maximal lower stem leaf width	8	Siliqua shape
Maximal upper stem leaf length	9	Seed shape
Maximal upper stem leaf width	10	Presence of membrane rim on seeds
The shortest siliqua length	11	Seed colour
The longest siliqua length	12	Seed mucilaginous coating
Total fruits and flowers per individual	13	
Seed length	14	
Seeds number per siliqua	15	

All statistical analyses and their graphic representations were performed using „Statistica 6.0" software.

3. Results

3.1. Variability of quantitative traits

The investigated populations show highly diversified height of individuals. It ranges from 110 mm to 850 mm. Individuals from the Miasteczko Śląskie (MS) population are typified by the highest number of aerial stems with a maximal value of 56, and the highest number of side branches (with a maximum of 340). Plants from Katowice-Wełnowiec (KW) and Bibiela (BB) have a similar number of side branches, while the lowest values for this trait have been observed for individuals from Ustrzyki Górne (Figs. 2a, b). All of the studied populations have similar number of rosettes per individual (Fig. 2c). Individuals from the MS population are characterised by the highest number of flowers and siliquas per individual (with a maximal value of 1032), while the maximal number of generative organs in plants from the Bieszczady population was only 100 (Fig. 2d). Individuals from MS and KW populations have similar dimensions of rosette and stem leaves which are considerably smaller than for the Silesian control population from Bibiela. In the case of UG, leaf dimensions are smallest (Figs. 2 e-h).

3.2. Variability of qualitative traits

Investigated populations MS, KW, BB, UG show high variability within and between them with regard to qualitative characteristics (Fig. 3). The most variable traits include: stem hairness, shape of rosette leaves, hairness of rosette leaves, shape of rosette leaf hairs, hairness of pedicels, seed shape, presence of membrane rim on seeds, mucilaginous coating of seeds. The highest intra-population variability with regard to these traits is observed among individuals from MS, KW, BB. Large differences were observed in the hairness of the stem (Fig. 3a). Hairs of rosette leaves were straight, bifurcate or stellate and their density varied (Figs. 3b, d). Flower buds were naked or pubescent. Flower pedicels were naked or hairy in individuals from Miasteczko Śląskie, Bibiela and Bieszczady. Rosette leaves were typified by variable shape. They were lyrate, undivided, oval or cordate, in the case of individuals from Miasteczko Śląskie sometimes cuspidate at the tip (Fig. 3c). Variable seed shape was observed – they were round or oblong-ovate, with or without a membranaceous margin, light brown or maroon, with mucilaginous or viscous coating.

3.3. Cluster analysis of investigated populations

Cluster analysis carried out for all investigated populations with regard to quantitative and qualitative traits allowed to establish that the most divergent population is the one from MS region (Fig. 4). The largest differences were observed between MS and UG; also KW and BB individuals differed considerably from MS individuals with regard to morphological characteristics. The highest similarity was shown for individuals from populations KW and BB.

3.4. Chromosome number

Results obtained from the cytogenetic studies of representative individuals from the populations from Miasteczko Śląskie, Katowice-Wełnowiec, Bibiela and Ustrzyki Górne do not differ from the values found in literature. In all populations chromosome number in somatic cells is 2n=16.

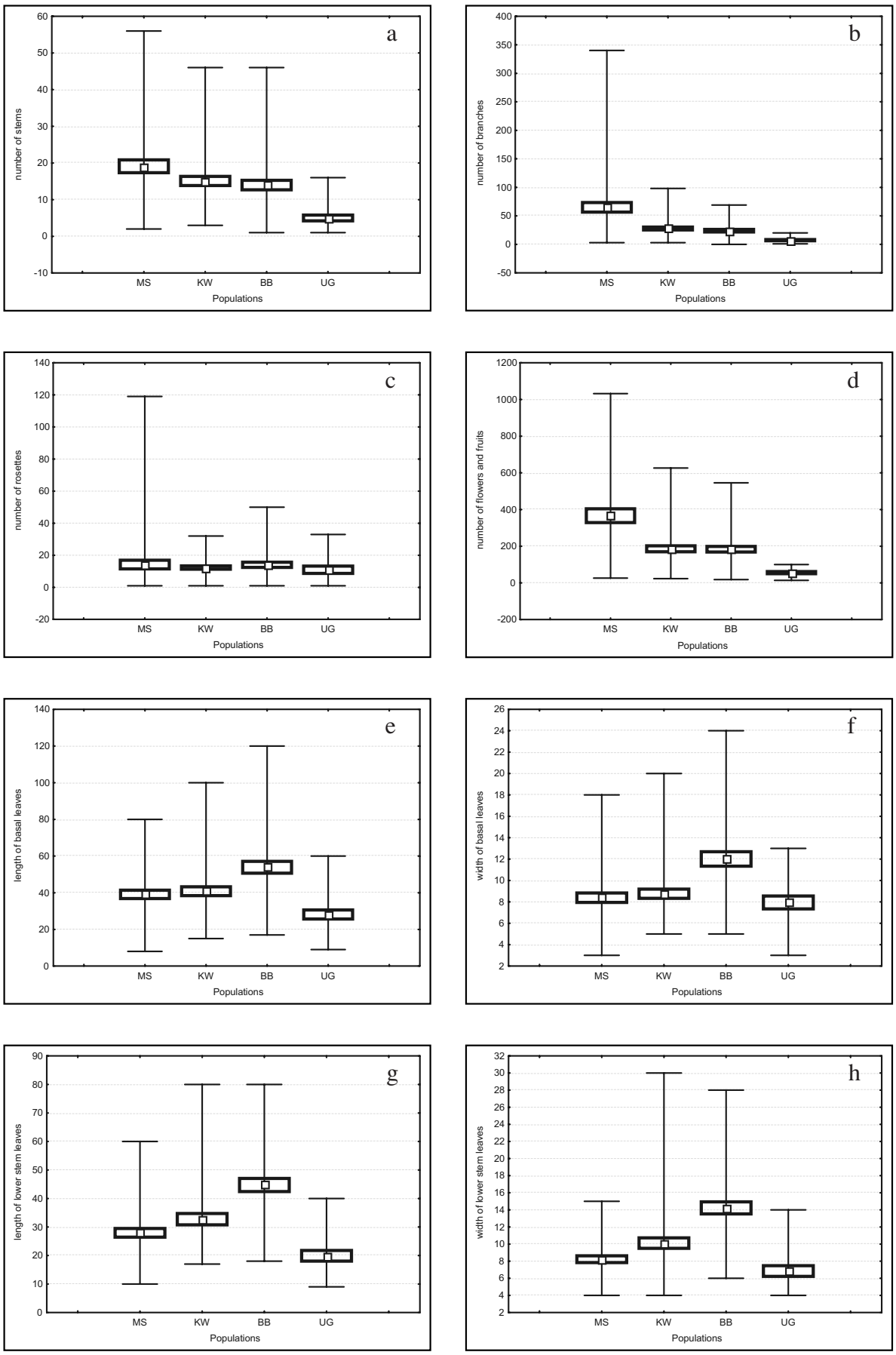

Fig. 2. Variability of quantitative traits among the studied populations
Explanations: MS – Miasteczko Śląskie, KW – Katowice-Wełnowiec, BB – Bibiela; UG – Ustrzyki Górne; a - number of stems, b - number of aerial stem branches, c - number of rosettes per individual, d - number of flowers and siliquas per individual, e - length of longest rosette leaf, f - width of broadest rosette leaf, g - length of longest lower stem leaf, h - width of broadest lower stem leaf

4. Discussion

Cardaminopsis halleri – species commonly considered as a mountain one (Zając 1996) – is characterised by a relatively broad ecological amplitude. It also occurs in post-industrial areas (Rostański 1997, 2000; Pasierbiński & Rostański 2001; Mańczyk & Rostański 2003). It forms a mosaic of populations which are highly diversified with regard to individual traits. This is a response to diverse factors which influence its variability.

In Silesia, studies on variability affected by environmental pollution with heavy metals have been performed on *Arabidopsis thaliana* (Kilian *et al*. 1985) as well as of *Cardaminopsis arenosa* (Rostański *et al*. 1989, 2005; Rostański 1993; Myśliwiec 2003). Rostański *et al*. (2005) have shown a significant impact of the pollution level on the morphological traits of *Cardaminopsis arenosa*.

Harlender (2003) and Micyk (2004) in their ecological studies on *Cardaminopsis halleri* populations growing on an abandoned zinc metal works spoil heap in Katowice-Wełnowiec have confirmed statistically significant differences in shoot length and number, rosette leaf size and number, rosette diameter, fruit length, number of flowers and fruits, as well as in shoot and rosette weight.

The presented results confirm the impact of environmental pollution on plant characteristics such as number of ground-level shoots, rosette leaf dimensions, number of flowers and fruits as well as length of siliquas. The recorded statistical significance of differences between studied populations seems conspicuous with regard to the size of stem leaves and the number of side branches. Poorly differentiated traits include plant height and the number of rosettes with the mean values is similar for all investigated populations despite high variability of both features.

Biometrical comparisons of quantitative traits have shown greatest differences with regard to the parameters stated for the population from Miasteczko Śląskie. This

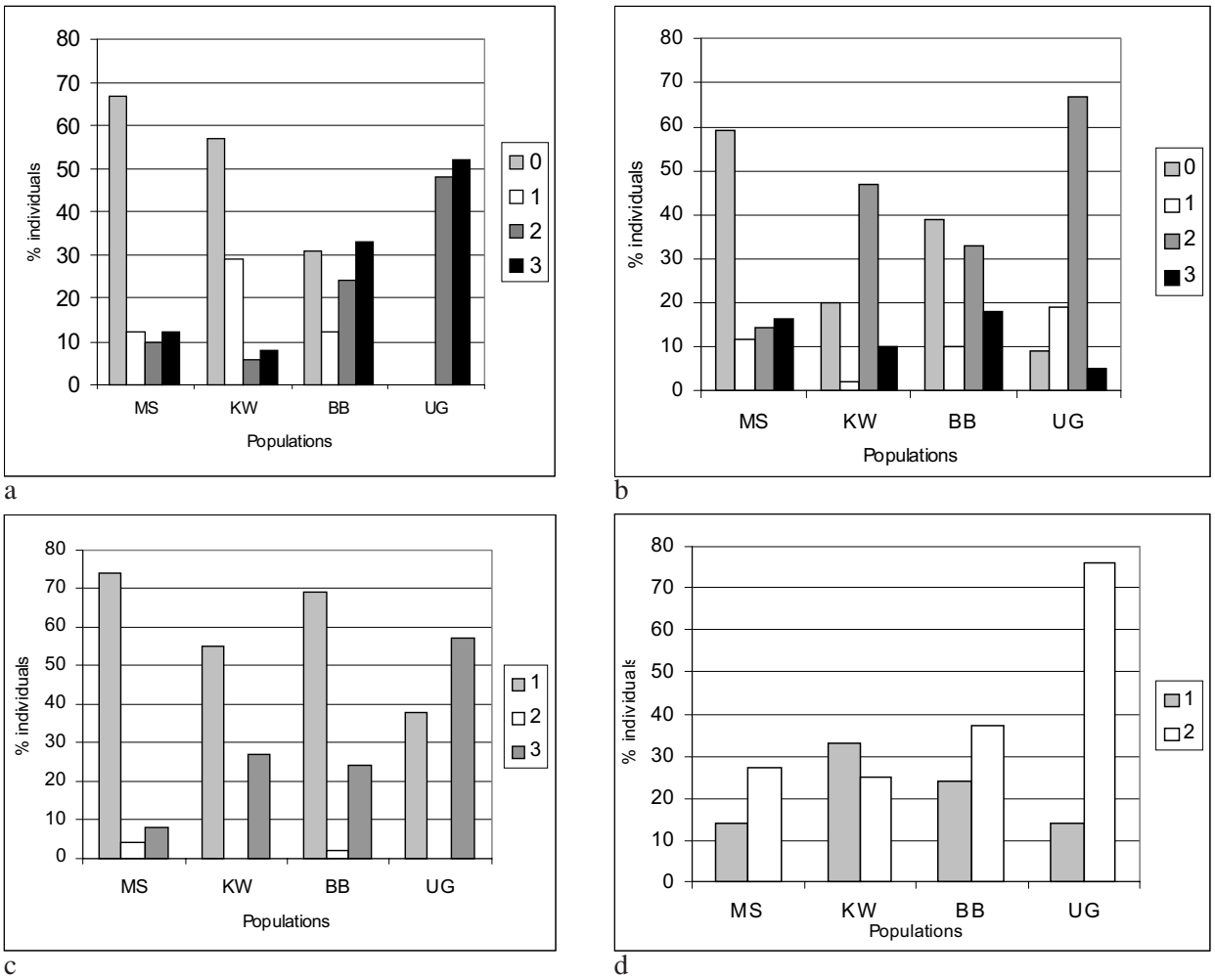

Fig. 3. Variability within and between investigated populations with regard to qualitative traits
Explanations: a – hairness of stem (0-naked, 1-weakly hairy, 2-hairy up to the half, 3-all hairy); b – hairness of rosette leaves (0-naked, 1-several hairs on top, 2-few hairs on whole leaf surface, 3-intensely hairy); c – shape of basal leaves (1-lyrate, 2-lyrate, cuspidate at the tip, 3-whole, not divided); d – shape of hairs on rosette leaves (1-stellate and straight, 2-bifurcate and straight). Population names as in Fig. 2

has been also confirmed by the performed cluster analysis (Fig. 4).

In the case of Bieszczady populations, the different climate and habitat conditions make them considerably distinct from the Silesian populations. This is reflected in the diversification of the number of side branches, siliqua length and seed size as well as the number of seeds per siliqua and the number of flowers and fruits (Fig. 2). Similar observations were made in the case of qualitative traits, e.g.: hairiness, leaf and seed shape.

Control cultivation of *Cardaminopsis halleri* in identical conditions has given different results. Size, shape and colour of rosette leaves in population from Bieszczady differed considerably from the population originating from a polluted area. Explanation of the causes of observed variability, however, requires further studies.

Conducted analysis has enabled us to make a revision of available literature data. *Cardaminopsis halleri* shows a much broader range of morphological features variability in comparison with descriptions provided by Mądalski (1967) and Sychowa (1985). Sychowa quotes the plant height as being within the range from 6 to 60 cm, while individuals from Miasteczko Śląskie reached a height of up to 85 cm. Variability range for siliqua size (5-33 mm) and seed size (1-1.6 mm) also differs from the values given in the literature (Fiałkiewicz 2005).

of seeds, which according to Mądalski (1967) are the features typical only for *Cardaminopsis arenosa*.

The observed intra-population variability may be linked to the phenomenon of hybrid formation. Jones & Akeroyd (1993) reported the occurrence of intermediate forms between *Cardaminopsis halleri* subsp. *halleri* and subsp. *ovirensis* in the populations from the Tatra mountains.

Investigated populations of *Cardaminopsis halleri* from Miasteczko Śląskie and Katowice-Wełnowiec are characterised by high phenotypic variability. These differences have also been observed during control cultivation (Fiałkiewicz 2005). It is important to determine whether these changes will persist in further generations. It has been suggested that *Cardaminopsis halleri* shows phenotypic plasticity in response to the variable environmental conditions (Harlender 2003). This is a genetically conditioned range of responses to the specific changes which usually do not occur randomly. Often different species show similar reactions related to environmental transformations (Jones & Wilkins 1977). This phenomenon demands further studies with a higher number of replicated investigations.

Similar studies have been performed with the Carthusian pink (*Dianthus carthusianorum*). Populations from spoil heaps were typified by numerous shoots, smaller leaf dimensions and they demonstrated a number of adaptations

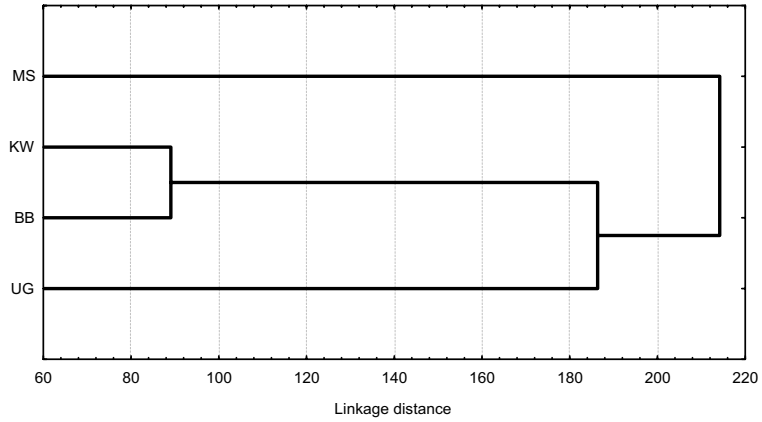

Fig. 4. Cluster analysis of investigated populations with regard to qualitative and quantitative traits. Population names as in Fig. 2

The described species is characterised by large intra-population and inter-population variability with regard to hairness of the stem, leaves, buds and flower pedicels, shape of hairs, leaves and seeds and mucilaginous coating of seeds. In majority of cases the variability of these traits are considerably different from the literature data. Characteristics which have not been hitherto described include hairy flower pedicels and mucilaginous coating

to the difficult habitat conditions occurring on the spoil heap. A sufficient number of replicated studies made possible distinguishing a heavy metal-rich soil ecotype for this species (Wierzbicka 2002; Wierzbicka & Rostański 2002).

According to the literature (Sychowa 1985; Al. Shehbaz & O'Kane 2002; Hall 2002), a proper chromosome number for *Cardaminopsis halleri* is 2n=16. Results

obtained from the cytogenetic studies of representatives of the populations from Miasteczko Śląskie, Katowice Wełnowiec, Bibiela and Ustrzyki Górne do not diverge from the values found in the literature.

5. Conclusions

Establishment of statistically significant differences with regard to qualitative and quantitative traits attests to considerable morphological variability among investigated populations of *Cardaminopsis halleri*.

The Silesian populations from Miasteczko Śląskie and Katowice-Wełnowiec are characterised by smaller dimensions of rosette and stem leaves, which sets them apart from the population from Bibiela (control). It may be concluded that these are bioindicative traits showing a specific variability in the areas with high heavy metal pollution.

Populations from Bieszczady are significantly different from populations from Silesia with regard to qualitative and quantitative traits, but their intra-population variability is restricted. It reflects the influence of geographical distance and divergent habitat conditions on the distinct character of mountain individuals.

The studied populations from Silesia are characterised by considerable intra-population variability with regard to hairiness, shape of leaves and seeds, mucilaginous coating of seeds.

Individuals from Miasteczko Śląskie are the most divergent population of *Cardaminopsis halleri* (number of generative organs and side branches).

Considerable variability observed between the studied populations with regard to quantitative and qualitative traits may be caused by phenotypic plasticity of the species as a response to the differentiation of environmental conditions.

Chromosome number for all individuals from the investigated populations is the same and equals 2n=16. This is a proof that the reported morphological variability is not a result of polyploidy (differences in chromosome number).

Pollution of the environment with heavy metals (Zn, Pb, Cd) has a significant influence on the level of morphological variability in the investigated populations of *Cardaminopsis halleri*. The higher is the load of pollutants in the environment, the grater is the observed variability.

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