

# OVULES, FEMALE GAMETOPHYTES AND EMBRYOS ARE MORE SENSITIVE TO HEAVY METAL POLLUTION THAN ANTHERS AND POLLEN OF *CARDAMINOPSIS ARENOSA* (L.) HAYEK (BRASSICACEAE), A MEMBER OF CALAMINE FLORA

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Reproductive processes including male and female lines, embryo and endosperm development were studied in *Cardaminopsis arenosa* (syn. *Arabidopsis arenosa*) growing on two metalliferous sites (Bukowno and Bolesław, S. Poland), rich in Zn, Pb, Cd and other metals. Disturbances of developmental processes and necroses observed in anthers and ovules influenced plant fertility and seed set of plants from both metal-polluted sites. In anthers, disturbances and necrosis during male meiosis and pollen development occurred at low frequency (4–5%). Pollen grain viability was very high, reaching over 90%. In ovules the frequency of abnormal meiosis, female gametophyte developmental disturbances and necrosis was high, 23.5–28% depending on site. The polluted environment also affected embryo and endosperm. Necrosis of whole generative structures decreased plant fertility. This study indicates that the range of disturbances and necroses in embryological structures and processes (at gametophyte level) gives a set of useful characters to determine plant tolerance to stress, complementary to many tolerance characters at the sporophyte level of plant ontogenesis.

**Key words:** *Cardaminopsis arenosa*, colonization, female gametophyte, meiosis, metallophytes, plant reproduction, pollen grains, post-industrial areas.

## INTRODUCTION

Plants colonizing areas polluted with heavy metals have to develop adaptations to harsh conditions that influence not only their physiological processes but also reproduction (e.g., de Knecht et al., 1995; Sharma et al., 1995; Czapik, 2002; Rao, 2006; Słomka et al., 2008; Mohsenzadeh et al., 2011; Yousefi et al., 2011). The longer colonization lasts, the less the cost of adaptation; this will be reflected in a lack of evidence of dysfunction of the plant organism under pollution (e.g., Antonovics et al., 1971; Ernst, 1999). Old waste heaps are good areas for investigating plants' adaptation to heavy metal pollution (e.g., Wierzbicka and Panufnik, 1998; Wierzbicka and Pielichowska, 2004; Wierzbicka and Rostański, 2002). The Olkusz region is Poland's oldest mining settlement, chartered in 1299. Lead, iron, zinc, and also silver, limestone, sand, coal and marble have been extracted and processed at sites in this area (Bolesław,

Bukowno, Olkusz) since the Middle Ages (Drobnik, 2004). These industrial activities dramatically altered the local environment, leaving waste heaps, excavations, hydrological disruption, secondary enrichment of soil with heavy metals, and air pollution.

The old waste heap in Bolesław (S. Poland), the study area, has more than a century of history. The habitat conditions are extreme, with strong insolation and winds, physiological drought, low soil nutrient content and high concentrations of heavy metals in soil. Nevertheless, plants spontaneously colonized these sites and a calamine flora adapted to this harsh environment was formed, including species such as *Armeria maritima*, *Biscutella laevigata*, *Dianthus carthusianorum*, *Silene vulgaris*, *Viola tricolor* and *Cardaminopsis arenosa*, the latter considered a bioindicator of soil contaminated with lead and zinc. Many of these plants are metallophytes (Wierzbicka and Rostański, 2002 and references cited therein).

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There is a lot of information relating to the sporophytic phase of ontogenesis of these calamine taxa. Among the research areas studied are the flora of the waste heap, levels of toxic metals in soil and in plant organs, morphological traits of specimens, mycorrhiza, mechanisms of adaptation, tolerance, and the strategies plants evolved to live on the Bolesław waste heaps (e.g., Wóycicki, 1913; Dobrzańska, 1955; Godzik, 1993; Antosiewicz, 1995; Grodzińska et al., 2000; Wierzbicka and Rostański, 2002; Szarek-Łukaszewska et al., 2004; Wierzbicka and Słysz, 2005; Przedpeńska and Wierzbicka, 2007; Olko et al., 2008; Słomka et al., 2008, 2011). However, little is known about the gametophytic phase of plant ontogenesis and the initial development of the new sporophyte in the very specific environment of contaminated sites (Kranter and Colville, 2011).

Properly formed gametes, regular double fertilization and embryo development are indispensable to successful sexual reproduction. Environmental pollution with heavy metals in soil, water and air negatively affects plant embryological processes. Previous embryological studies have investigated plants from postindustrial (metalliferous) sites in the Legnica-Głogów Copper Basin, the zinc waste heap in Katowice-Wełnowiec and calamine heaps in the Olkusz Industrial Region (Poland), calamine heaps in Germany and Belgium and the Hame-Kasi iron and copper mine (Hamedan, Iran), covering species from different families and genera, for example *Capsella bursa-pastoris*, *Chenopodium botrys*, *Chondrilla juncea*, *Cirsium vulgare*, *Echium vulgare*, *Lotus corniculatus*, *Ranunculus repens*, *Reseda lutea*, *Vicia cracca* (Izmailow, 2000, 2002a,b; Kościńska-Pająk, 2000, 2002; Czapik and Kaźmierska, 2002; Czapik et al., 2002; Izmailow and Biskup, 2003; Biskup and Izmailow, 2004; Łuczynska and Izmailow, 2008; Mohsenzadeh et al., 2011; Yousefi et al., 2011), and violets from the *Viola* and *Melanium* sections (Siuta et al., 2005; Hildebrandt et al., 2006; Słomka et al., 2010, 2012; Migdalek et al., 2013).

We examined the effects of growing in an environment polluted with heavy metals on generative reproduction in the metallophyte *Cardaminopsis arenosa* (L.) Hayek (synonym *Arabidopsis arenosa* (L.) Lawalrée), analyzing processes in ovules and anthers.

## MATERIAL AND METHODS

### SITE DESCRIPTION

The plants originated from three localities in southern Poland: (1) an old calamine waste heap in Bolesław (B); (2) the old Michalska outcrop located

TABLE 1. Developmental processes in ovules of *Cardaminopsis arenosa* L. (Hayek) from control site. MMC – megaspore mother cell; FM – functional megaspore; ES – embryo sac

Stage of development	Number of ovules
1 archesporial cell	33
2 archesporial cells	2
MMC	73
Dyad	2
Tetrad of megaspores	1
Tetrad with FM	50
Tetrad with FM + archesporial cell	1
Two FM in tetrad	1
1-nucleate ES	19
1-nucleate ES + 1-nucleate ES	1
2-nucleate ES	19
2-nucleate ES + 8-nucleate ES	1
4-nucleate ES	20
8-nucleate ES	7
Mature ES	169
Zygote + nuclear endosperm	55
2–3 celled proembryo + nuclear endosperm	13
Quadrant stage of proembryo + nuclear endosperm	36
Octant stage of proembryo + nuclear endosperm	24
Proembryo at early globular stage + nuclear endosperm	80
Proembryo at globular stage + nuclear endosperm	116
Embryo at heart-shaped stage + cellular endosperm	33
Embryo at torpedo stage + cellular endosperm	11
Mature embryo	13
Disturbances and necrosis in ovules	7 (1%)
$\Sigma$	787

on the north side of the Bolesław zinc smelter in Bukowno (Bk); and (3) Kostrze near Cracow (reference material).

Both calamine heaps are highly contaminated with heavy metals. In Bolesław the levels in soil (mg/kg) were as follows: Zn 37,799–48,483, Pb 1647–1701, Cd 171–233, Ni 35.2–36.3, Fe 50,900–57,900, Mg 46,300–53,300, Ca 106,000–131,450 (Godzik, 1991, 1993) and Tl 43–78 (Wierzbicka et al., 2004). The rhizosphere of examined specimens contained (mg/kg) Ca 16,850, Mg 13,460, Fe 22,460, Zn 37,060 and Cd 210 (Kwiatkowska, unpubl. data).

In Bukowno the levels ( $\mu\text{g/g}$ ) in soil were Pb 42–3570, Zn 234–12,400, Cd 2–73.2, Ni 0.2–234 and Cu 1.6–376 (Verner et al., 1996). The rhizosphere of examined specimens contained (mg/kg) Ca 5440, Mg 5020, Fe 14,950, Zn 25,400 and Cd 330 (Kwiatkowska, unpubl. data).

The rhizosphere of examined specimens from the uncontaminated Kostrze locality contained (mg/kg) Ca 5250, Mg 1480, Fe 8990, Zn 80 and Cd 2 (Kwiatkowska, unpubl. data).

#### PLANT MATERIAL

We collected 40–100 flowers from 10–20 plants per population. Embryological processes were analyzed in ~3800 ovules in total from all sites (polluted and reference). Flowers in various development stages were fixed in situ in a mixture of 96% ethanol and glacial acetic acid (3:1 v/v) for 24–48 h and stored in 70% ethanol at 4°C pending analyses. For ovules and anthers the paraffin method was used. Briefly, fixed material dehydrated in a graded ethanol series (30% to 100%) was embedded in paraffin and sectioned 10 µm thick on a rotary microtome (Reichert). Slides were stained with Heidenhain's hematoxylin combined with alcian blue and mounted in Entellane (Aldrich). For pollen viability (stainability) studies, pollen grains were stained with 1% acetocarmine (Singh, 2003). The frequency of viable pollen was estimated from 1000 pollen grains for each locality.

#### STATISTICS

The significance of differences in embryological data between populations was checked with chi-square tests in Microsoft Office Excel 2007.

### RESULTS

#### PROCESSES PROCEEDING REGULARLY IN ANTHERS AND OVULES OF PLANTS FROM NON-METALLICOLOUS AND METALLICOLOUS POPULATIONS

In anthers the tetrads of microspores were formed after regular, simultaneous meiosis (Figs. 1, 2). The pollen grains were shed at the three-celled stage (Fig. 3). The frequency of viable pollen grains as indicated by acetocarmine staining was very high, reaching 98% in plants from the unpolluted site and over 90% in those from both polluted areas (Bolesław 95%, Bukowno 92.5%).

The archesporial cell functioning as the megaspore mother cell (MMC) developed in campylotropous, tenuinucellate, bitegmic ovules (Fig. 4). A linear megaspore tetrad was formed after regular meiotic division (Fig. 5). An 8-nucleate (7-celled) female gametophyte was formed from the chalazal megaspore after three mitotic divisions, and consisted of an egg apparatus (egg cell accompanied by 2 synergids) at the micropylar pole, 3 antipodal cells at the chalazal pole, and the central cell with 2 polar nuclei (Figs. 6–8). In

~2% of the analyzed ovules, 2 or 3 archesporial cells underwent development or 2 megaspores of the same tetrad started to develop. After double fertilization, the embryo developed according to Onograd type; that is, the zygote divided transversely, forming a 2-celled proembryo with basal and apical cells. Further transverse division of the basal cell gave rise to a filamentous suspensor (Fig. 9). The embryo proper developed from the apical cell via quadrant, octant, globular, heart, torpedo and cotyledonous stages (Figs. 10–12). A hypophysis was formed and produced the initials of the root cortex and cap primordium. Endosperm developed according to nuclear type (Tab. 1). Typical development was observed in 99% of the ovules and 100% of the analyzed anthers of flowers from the unpolluted site.

In material from the polluted localities the frequency of regularly proceeding processes leading to production of the female gametophyte in ovules was reduced to 72% (Bolesław) and 76.5% (Bukowno); regularity of processes during embryogenesis was reduced to 73.5% (Bolesław) and 80% (Bukowno). Anther development, microsporogenesis and microgametogenesis were regular in 94.7% (Bolesław) and 95.6% (Bukowno) of the investigated anthers (Tabs. 1–3).

#### DEVELOPMENTAL DISTURBANCES AND NECROSES IN SPECIMENS FROM POLLUTED SITES

The disturbances and necroses observed in anthers and ovules at different stages of their development affected plant fertility and seed set.

In anthers from polluted sites, premature degeneration of the tapetum (Fig. 13), microsporocytes, tetrads (Fig. 14), microspores, pollen grains (Fig. 15) and whole anthers (Fig. 16), and disturbances of male meiosis and pollen grain formation occurred at low frequency, reaching ~4% (9 of 205 analyzed flower buds) in material from Bukowno and ~5% (14 of 264 analyzed flower buds) in material from Bolesław. The differences between these two populations were not significant ( $N=469$ ;  $\chi^2=0.21$ ;  $0.5 < P < 0.9$ ).

Ovules from polluted areas were smaller, measuring 1/2–2/3 the size of same-stage ovules from the unpolluted site. Female gametophytes were distinctly shorter and sometimes only slightly flexed, and they ripened prematurely before reaching the proper size and campylotropous shape.

Various kinds of disturbances and degeneration were observed at different development stages in ovules of plants from both polluted sites. Degenerative changes not part of the normal differentiation and aging process affected megasporocytes, whole dyads, tetrads, whole female gametophytes at differentiation or maturation stages, sin-

gle elements of the mature female gametophyte (synergids, egg cell, polar nuclei), and early-stage antipodals (Figs. 17, 18). Abnormalities were found in 28% of analyzed ovules from Bolesław and in 23.5% of those from Bukowno but the differences between those populations were not significant ( $N=913$ ;  $\chi^2=2.51$ ;  $0.1 < P < 0.2$ ) (Tab. 2).

The results from observations of male and female gametophyte development make it evident that anthers and pollen are less sensitive to heavy metal pollution than female gametophytes. The differences are statistically significant for both the Bolesław specimens ( $N=943$ ;  $\chi^2=60.971$ ;  $P < 0.001$ ) and those from Bukowno ( $N=439$ ;  $\chi^2=32.057$ ;  $P < 0.001$ ).

Similar processes accompanied embryogenesis, endosperm development and silique formation, leading to reduced fertility. Necrotic zygotes (Fig. 19), degeneration of whole proembryos, embryos and endosperm at various development stages, and abnormally formed suspensors or embryos proper were observed in 26.5% of the ovules from Bolesław and 20% of those from Bukowno (Tab. 3, Figs. 20–23); the differences between the polluted populations were statistically significant ( $N=1573$ ;  $\chi^2=5.515$ ;  $P < 0.01$ ). In maturing and mature siliques, 25.7% of the seeds from Bolesław (314 of 1174 analyzed ovules) and 3.3% of those from Bukowno (25 of 731 analyzed ovules) were aborted (Fig. 24).

The frequency of degeneration of whole flower buds, opened flowers or ovules from the two metallophilous populations was similar: 12% (94 of 773 analyzed flower buds) for Bolesław and 11% for Bukowno (28 of 262 analyzed flower buds); the difference was not significant ( $N=1035$ ;  $\chi^2=0.409$ ;  $0.5 < P < 0.9$ ).

## DISCUSSION

According to Xiong and Peng (2001), in plant ontogenesis the gametophytic phase is much more sensitive to stress conditions than the sporophytic phase; the sensitivity of gametophytes to heavy metal pollution is several times higher than for sporophytes, and depends on the species and its sensitivity threshold. In *Viola tricolor*, heavy metals in soils affected reproductive processes more than morphological traits (Słomka et al., 2012). *Cardaminopsis arenosa* is very well adapted to heavy metals and shows heritable xeromorphic adaptation to a harsh waste-heap environment (Przedpeńska and Wierzbicka, 2007). The present embryological study makes it clear that embryological processes in that taxon are vulnerable to abiotic stress, especially in the female line.

The response to stress conditions differs depending on the species and the stage of embryogenesis; some processes are more sensitive than

others. Within the same taxon, populations might respond differently depending on the type of pollution (e.g., *Cirsium arvense*, *Capsella bursa-pastoris*, *Echium vulgare*). More sensitive to environmental pollution are species in early stages of colonization (newcomers) or less plastic species in which sufficient tolerance mechanisms have not yet developed and generative reproduction is disturbed, as in *Ranunculus repens* and *Vicia cracca* (Izmailow, 2000, 2002a,b; Czapik et al., 2002; Czapik and Kaźmierska, 2002; Izmailow and Biskup, 2003; Biskup and Izmailow, 2004). The metallophytes that have formed permanent calamine populations in the Bolesław industrial area through the last hundred years, such as *Arabidopsis arenosa*, *Armeria maritima*, *Dianthus carthusianorum*, *Biscutella laevigata*, *Silene vulgaris* and *Viola tricolor*, are already adapted to adverse environmental conditions and show some degree of stability and resistance to metals (Wierzbicka and Panufnik, 1998; Załęcka and Wierzbicka, 2002; Wierzbicka and Słysz, 2005; Przedpeńska and Wierzbicka, 2007; Olko et al., 2008; Słomka et al., 2012). In *C. arenosa*, a member of the old calamine flora of Bolesław, we found that reproductive processes were disturbed, leading to reduced seed set. Surprisingly, ovules, female gametophytes, embryos and endosperm were more affected than anthers and pollen. In embryos at different development stages we observed numerous groups of necrotic cells. Such necrotic cells excluded from metabolism were observed in root of *Zea mays* under the influence of Cu; copper accumulated in significant quantities in the individual cell, causing disintegration and finally cell death, while other cells in the tissue functioned properly; this is a recognized part of the resistance strategy of *Zea mays* to deal with copper toxicity (Quouninou et al., 1995). The presence of metal ions in embryos might trigger a mechanism directing them into single cells or groups of cells, causing their death, so that the remaining cells can grow properly. Under this interpretation, the presence of necrotic cells in embryos would be an element of resistance/tolerance to toxic ions present in the environment, which are transported, albeit in smaller quantities, to flowers and developing fruits. The coefficients of zinc transport from roots to flowers and seeds show that this metal was transported to aboveground parts in plants from Bolesław (B) and Bukowno (Bk) at lower levels (flowers/roots: Bolesław 0.3 B, Bukowno 0.4 Bk; seeds/roots: Bolesław 0.2, Bukowno 0.3) than in control material (flowers/roots 1.0; seeds/roots 0.8), indicating that mechanisms of restricted transport and tolerance developed in this taxon (Kwiatkowska, unpubl. data). The content of mineral compounds in the seed depends on their accumulation in the rhizosphere, absorption by roots, transport to shoots via xylem,

TABLE 2. Megasporogenesis and female gametophyte development in ovules of *Cardaminopsis arenosa* L. (Hayek) from polluted sites. MMC – megaspore mother cell; FM – functional megaspore; ES – embryo sac

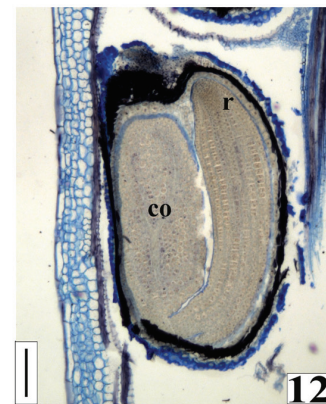
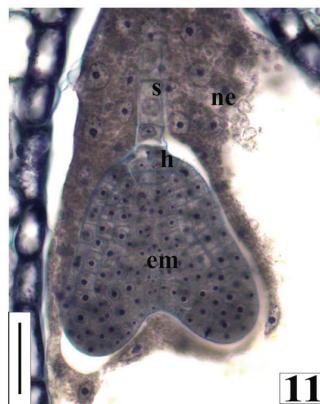
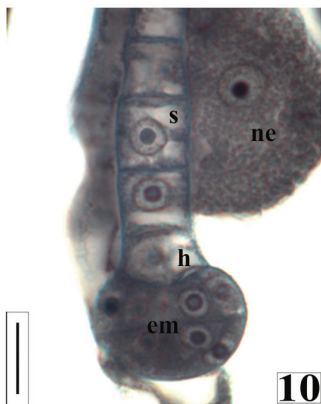
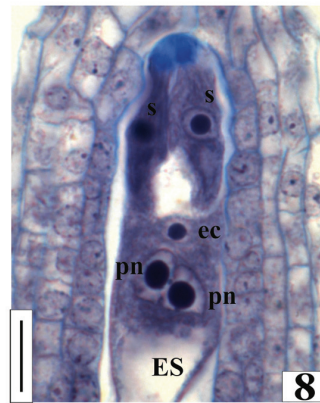
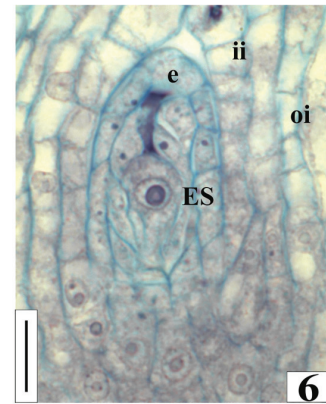
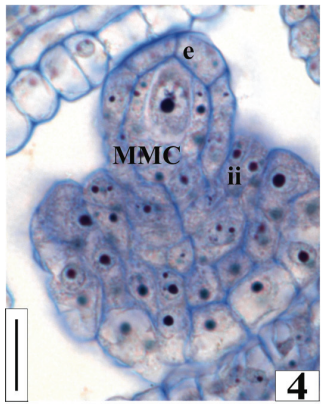
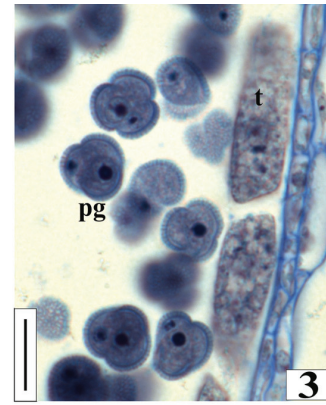
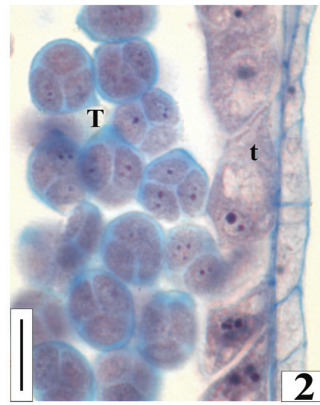
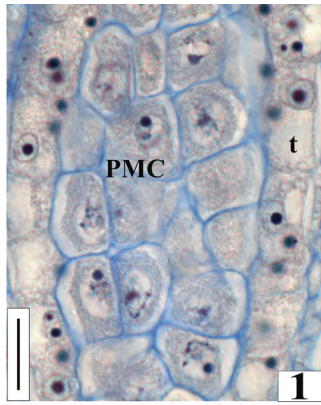
Stage of development	Bolesław		Bukowno	
	Ovules with disturbances and necrosis	All analyzed ovules	Ovules with disturbances and necrosis	All analyzed ovules
1 archesporial cell				12
2 archesporial cells	1	2		
MMC	1	3	1	16
MMC + archesporial cell		1		
Dyad		2		1
Dyad + tetrad of megaspores	1	1		
Tetrad of megaspores		1	1	2
Tetrad of megaspores + MMC + archesporial cell	1	1		
Two tetrads of megaspores + archesporial cell	1	1		
Tetrad of megaspores with MF	2	37		11
Tetrad of megaspores with MF + archesporial cell			1	1
Tetrad of megaspores with MF + dyad	1	1		
Tetrad of megaspores with MF + tetrad of megaspores	1	1		
Tetrad of megaspores with MF + tetrad of megaspores + archesporial cell			1	1
1-nucleate ES		12	1	6
2-nucleate ES	5	18		2
4-nucleate ES	11	29	2	8
5-nucleate ES	3	3	1	1
6-nucleate ES	1	1		
7-nucleate ES	2	2		
4-nucleate ES + 4-nucleate ES	1	1		
4-nucleate ES + 4-nucleate ES + 2-nucleate ES	1	1	1	1
8-nucleate ES	10	19	2	8
Mature ES	153	542	43	163
Mature ES + Mature ES			1	1
$\Sigma$	196 (28%)	697	55 (23.5%)	234

and finally through phloem to developing seeds (Kranner and Colville, 2011; Waters and Sankaran, 2011). High doses of metals in the soil may even inhibit seed production (Brun et al., 2003).

Disturbances observed in embryological processes may be due to direct effects of metal ions

in cells, such as induction of oxidative stress, genotoxicity, and direct damage of organelles (Briat and Lebrun, 1999; Zenk, 1996), or may be caused by the plant's expenditure of energy on a number of detoxification processes, leading to a shortage of energy for proper development of gametophytes

**Figs. 1–12.** Regular embryological processes in flowers from control site. Longitudinal sections of anthers and ovules. **Fig. 1.** Microspore mother cells (PMC) at prophase I, tapetum (t). **Fig. 2.** Tetrads of microspore (T), tapetum (t). **Fig. 3.** Two- and three-celled pollen grains (pg), tapetum (t). **Fig. 4.** Megaspore mother cell (MMC) in tenuinucellate ovule, nucellar epidermis (e), inner integument (ii). **Fig. 5.** Dyad (D) in developing ovule. Micropylar cell at prophase II, chalazal cell at metaphase II. Nucellar epidermis (e), inner integument (ii), outer integument (oi). **Fig. 6.** Mononuclear embryo sac (ES) derived from chalazal megaspore. Above, degenerating sister megaspores. Nucellar epidermis (e), inner integument (ii), outer integument (oi). **Fig. 7.** Binuclear embryo sac (ES) surrounded by nucellar epidermis (e). **Fig. 8.** Micropylar pole of mature female gametophyte (embryo sac, ES), two polar nuclei at fusion (pn). Egg apparatus consists of an egg cell (ec) and two synergids (s). **Fig. 9.** Few-celled proembryo with undivided apical cell (a) and suspensor (s), nuclear endosperm (ne). **Fig. 10.** Proembryo at early globular stage (em) with protodermis and developing hypophysis (h). Suspensor (s), nuclear endosperm (ne). **Fig. 11.** Embryo at heart stage (em) with hypophysis (h) surrounded by nuclear endosperm (ne). **Fig. 12.** Mature embryo at U-shaped stage. Embryo root (r), cotyledons (co). Bars in Figs. 1–10 = 20  $\mu$ m; in Fig. 11 = 50  $\mu$ m; in Fig. 12 = 200  $\mu$ m.



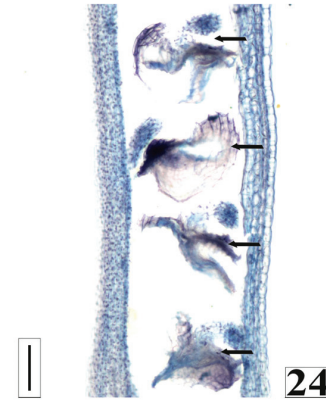
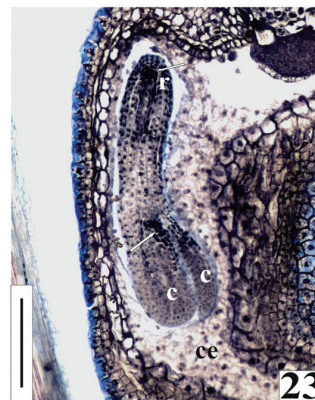
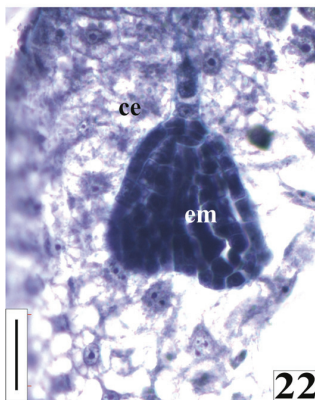
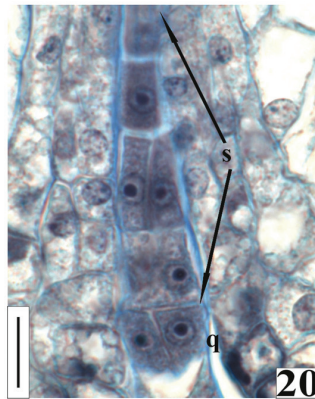
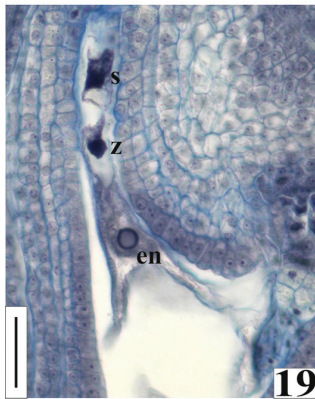
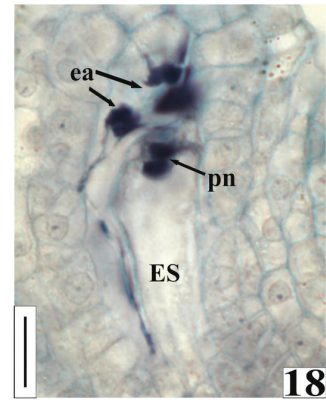
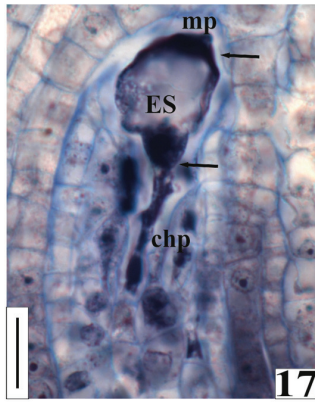
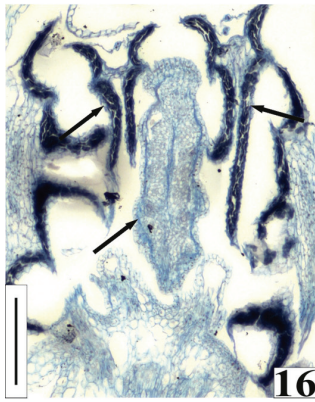
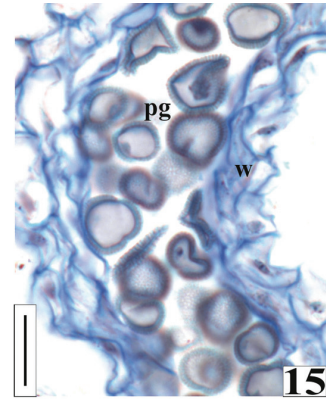
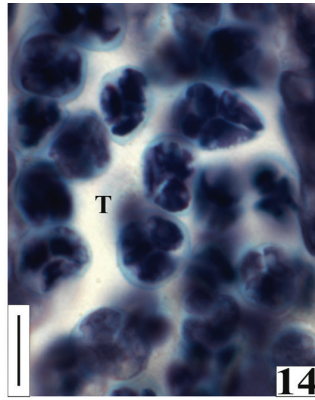
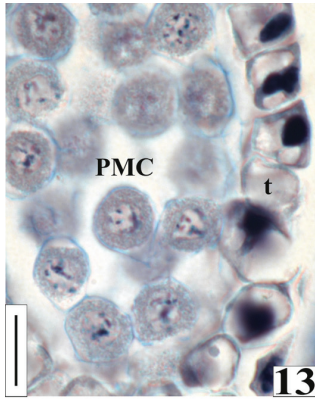


TABLE 3. Embryogenesis in ovules of *Cardaminopsis arenosa* L. (Hayek) from polluted sites

Stage of development	Bolesław		Bukowno	
	Ovules with disturbances and necrosis	All analyzed ovules	Ovules with disturbances and necrosis	All analyzed ovules
Zygote + nuclear endosperm	25	135	8	44
2-celled proembryo + nuclear endosperm	23	62	4	19
3–8 celled proembryo + nuclear endosperm	2	11	7	23
Quadrant stage of proembryo + nuclear endosperm	3	15	5	21
Octant stage of proembryo + nuclear endosperm	2	13	4	17
Proembryo at early globular stage + nuclear endosperm	8	54	10	56
Proembryo at globular stage + nuclear endosperm	58	146	38	160
Embryo at heart-shaped stage + cellular endosperm	55	106	18	56
Embryo at torpedo-shaped stage + cellular endosperm	34	86	11	36
Embryo with growing cotyledons/ mature embryo	18	232	41	299
$\Sigma$	228 (26.5%)	860	146 (20%)	731

and seed production. The latter was documented in our study. Besides the heavy metals stress, harsh environmental conditions (strong insolation, wind erosion, unsuitable soil profiles, low share of water-retaining soil aggregates) contributed to making the ovules smaller and the female gametophytes shorter and only slightly flexed. They ripened prematurely before reaching proper size and campylotropous shape. The specimens entered the generative phase earlier to accelerate seed production, indicating an "r" life history strategy. Rostański and Wierzbicka (2002) found that some plants from the calamine flora of the Bolesław heap represent an "r" strategy and xeromorphic adaptations: lower biomass of aerial parts, fewer shoots, fewer leaves, more flowers, and rapid entry to the generative phase (versus reference populations). These sporophytic traits along with disturbances of embryological processes can be considered costs of tolerance.

## CONCLUSIONS

This study showed that the range of disturbances and necroses in embryological structures and processes furnishes a good set of characters to determine stress tolerance in plants. The male and female lines differ in their sensitivity to metal pollution. In *Cardaminopsis arenosa* the male line is more resistant, manifested in production of viable pollen at very high frequency.

## AUTHORS' CONTRIBUTION

Both authors contributed to the conception and design, acquisition of data, analysis and interpretation of data, and drafting or critical revision of the paper.

MK conception and design, material collection, slide preparation, data acquisition, contribution to

**Figs. 13–24.** Disturbances in male and female generative lines of plants from two contaminated sites: Bolesław (B) and Bukowno (Bk). **Fig. 13.** Precocious degeneration of tapetum layer (t), microsporocytes (PMC) at prophase I, (B). **Fig. 14.** Necrotic (degenerating) tetrads of microspores (T), (B). **Fig. 15.** Deformed and nonviable pollen grains (pg), degeneration of anther wall (w), (B). **Fig. 16.** Necrosis in generative structures of flower bud; anthers and ovules in ovary (arrows), (B). **Fig. 17.** Embryo sac (ES) at early stage of gametogenesis. Clamped space of embryo sac at chalazal pole (chp) and degeneration of nuclei and cytoplasm at both poles – micropylar (mp) and chalazal (arrows), (B). **Fig. 18.** Mature embryo sac (ES) with degenerated egg apparatus (ea) and two polar nuclei (pn), (Bk). **Fig. 19.** Embryo sac after fertilization with proper primary endosperm cell (en) and degenerated zygote (z) and synergid (s), (B). **Fig. 20.** Proembryo at quadrant stage (q) with abnormally massive suspensor (s), (Bk). **Fig. 21.** Proembryo at globular stage (em) with necrotic cells in hypophysis (h) and suspensor (s) (arrows), (B). **Fig. 22.** Necrosis of whole embryo at heart stage, cellular endosperm (ce), (B). **Fig. 23.** Embryo with growing cotyledons. Groups of necrotic cells in root (r) and cotyledons (c) (arrows), cellular endosperm (ce), (B). **Fig. 24.** Inhibition of seed development. Part of silique with completely degenerated developing seeds (arrows), (Bk). Bars in Figs. 13–15, 17, 18, 20, 21 = 20 µm; in Fig. 16 = 200 µm; in Figs. 19, 22 = 50 µm; in Fig. 23 = 200 µm; in (Fig. 24) = 100 µm.



interpretation of data, drafting of the manuscript; RI participation in data analysis, interpretation of the results, critical revision of the manuscript.

The authors declare that there are no conflicts of interest.

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