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REPRODUCTION OF *ECHIUM VULGARE* L. (BORAGINACEAE) AT CONTAMINATED SITES

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Reproduction of *Echium vulgare* L. was examined in situ at two contaminated sites (Silesia, Poland): a zinc spoil heap in Katowice-Wełnowiec, and the surroundings of the Żelazny Most copper post-flotation waste reservoir. Plants from uncontaminated sites (near Rymanów) were studied as the control material. We compared the reproductive capacity of plants in the stress conditions to that of plants from uncontaminated sites. Degenerative processes and abnormalities found in the reproductive organs of plants from polluted sites, more intensified in the population from Żelazny Most, resulted in lowered fertility of plants. In germination tests on standard soil and from polluted sites, seeds from the control plants had the lowest germination on soil from Żelazny Most, and seeds from Żelazny Most had significantly delayed germination and had higher germination on standard soil than on polluted soil from their site; on the other hand, seeds from Katowice-Wełnowiec had higher germination on waste heap soil than on standard soil. The results suggest that among the populations from polluted sites, the one from Katowice-Wełnowiec is more advanced in selection for tolerance.

Key words: *Echium vulgare* L., heavy metals, pollution, embryological disturbances, germination.

INTRODUCTION

Knowledge of the embryology of plants growing in contaminated habitats lags behind that of other areas such as the ecology, physiology or genetics of these plants. Very few papers present the data needed to estimate the reproductive strategy from an embryological point of view. Only some of them refer to processes in the reproductive organs of plants from contaminated habitats compared with those from natural uncontaminated sites (Izmaiłow, 2000; Kościńska-Pająk, 2000; Czapik et al., 2002; Czapik and Kaźmierska, 2002; Izmaiłow, 2002a,b; Kościńska-Pająk, 2002a,b).

This study on *Echium vulgare* examines whether the generative structures of a taxon typically occurring in ecologically disturbed sites are sensitive to the extremely difficult conditions of a polluted environment.

MATERIALS AND METHODS

We examined embryological processes in *Echium vulgare* at two contaminated sites in the Lower and Upper Silesian Industrial Region (Poland). The specimens of one population grew in the close vicinity of the Żelazny Most copper post-flotation reservoir located in the Legnica-Głogów Copper District. The soils near the reservoir (particularly at its eastern side) are highly contaminated with heavy metals (Pb, Cu, Cd, Co, Hg) and As (Krajewski, 1993; Piątkowski and Skibicki, 1997; Kijewski, 1998). The site of the second studied population, the zinc spoil heap in Katowice-Wełnowiec, has high concentrations of zinc, lead and cadmium (Tokarska-Guzik et al., 1991). Plants from two uncontaminated pastures near Rymanów (southeast Poland) were studied as the control material. The studied plants were tetraploids (2n = 32).

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Flower buds, flowers at anthesis and ovaries after anthesis were fixed in ethanol/acetic acid (3:1); paraffin sections 8 mm thick were stained in Heidenhain's hematoxylin and alcian blue. Micro- and megasporogenesis, embryo sac development and embryo and endosperm formation were analyzed in materials fixed in situ. In view of gynodioecism and gynomonoecism occurring in *E. vulgare*, microsporogenesis was studied in hermaphrodite flowers. Processes in ovules and seed development were studied in 170 ovules from the control plants, 350 ovules from Katowice-Wełnowiec, and 270 ovules from Żelazny Most.

Germination tests were done on seeds harvested from plants growing at the contaminated sites and from the control plants. The true fruit – nutlet – will be referred to as the seed. Each test sample comprised 250 seeds, on account of the low number of specimens in the populations at the polluted sites. The seeds were sown on soil from their polluted sites and on standard soil; the control seeds were sown on unpolluted soil and soil from both contaminated sites. Germination was scored in days of emergence of the radicles. The final germination count was made after 12 days.

After four weeks the seedlings were transplanted to a 1:1 mixture of polluted and standard soil.

RESULTS

CONTROL PLANTS

Microsporogenesis and pollen development were studied in hermaphrodite flowers. The anthers are tetrasporangiate with secretory-type tapetum. Disturbances in meiotic divisions were not detected. Cytokinesis was simultaneous; tetrahedral tetrads were formed. Mature pollen grains were uniform in size and three-celled at shedding stage.

Ovules of *Echium* are hemianatropous, unitegmic and crassinucellate. The vascular strand branches in the integument. The archesporial cell (1–3) functions as the megaspore mother cell; after meiotic divisions a linear tetrad is formed, and the chalazal megaspore develops into a Polygonum-type embryo sac (ES). As a rule a single functional ES was observed. The mature ES consists of the egg cell and two synergids (one is of persisting type), the central cell with two polar nuclei which fuse before fertilization, and three antipodals. At this stage of development it is common for some ESs to degenerate without visible signs of fertilization, and fruits can consist of 3(2) nutlets.

Embryo development conforms to the Chenopodiad type; the uniseriate suspensor at the globular stage of the embryo consists of 6–8 cells. The mature embryo fills the seed and is differentiated into two cotyledons, the shoot apex, hypocotyl and radicle.

A specific feature of endosperm development is the formation of a lateral haustorium composed of two coenocytic 8-nucleate cells with polyploid nuclei (Małecka, 1975). Its higher lateral cell is in contact with the young proembryo and degenerates at its globular stage, whereas an enlarged lower lateral cell is visible still at the late torpedo-shaped stage. The invariable localization of the haustorial cells close to the funiculus suggests their participation in nutrient transport to the embryo and endosperm proper.

PLANTS FROM CONTAMINATED SITES

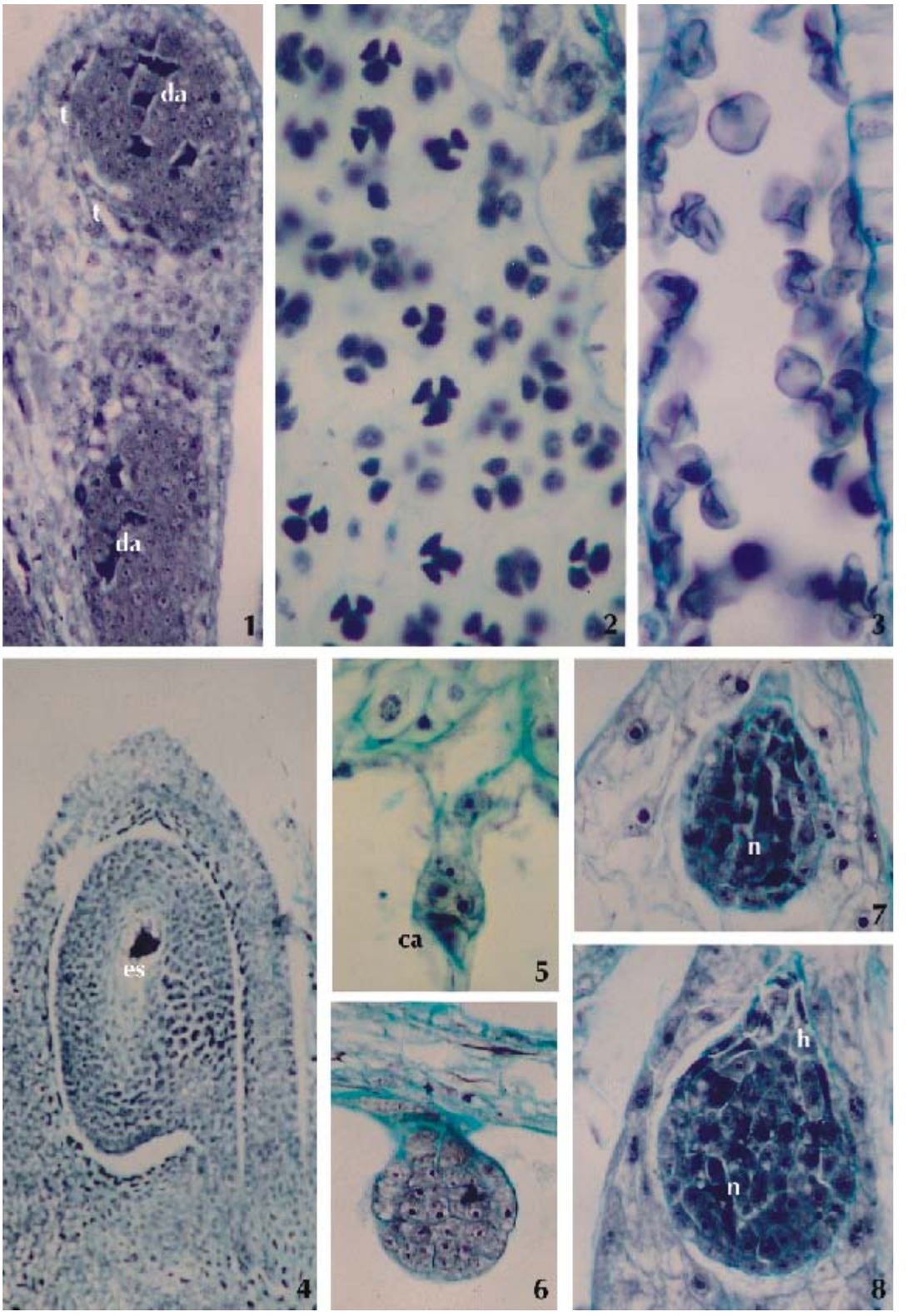
In plants from the waste heap in Katowice-Wełnowiec and from the vicinity of the Żelazny Most reservoir, disturbances and degenerative processes were found in part of the anthers (Figs. 1–3) and pistils or maturing seeds (Figs. 4–12), whereas regular development occurred in the other ones (Figs. 13–15).

Plants from the waste heap in Katowice-Wełnowiec

Degenerative processes or irregularities in the reproductive organs were observed in a small number of the studied flowers.

Necrotic regions in the archesporium and tapetum were observed sporadically in very young anthers (Fig. 1). At the tetrad stage, some anther loculi were filled with wholly degenerated tetrads, and only a few tetrads were viable or else 1–2 micro-

Figs. 1–8. Degenerative processes in anthers and ovules of *Echium vulgare* from polluted site in Katowice-Wełnowiec. **Fig. 1.** Degeneration of cell groups in archesporium (da) and tapetum (t). × 380. **Fig. 2.** Tetrads composed of all degenerated, some degenerated and all viable microspores. × 760. **Fig. 3.** Section of anther with high number of degenerated pollen grains and microcytes. × 760. **Fig. 4.** Longitudinal section of ovule with degenerated content of ES (es), surrounded by typically developed vegetative tissue of ovule. × 190. **Fig. 5.** Five-celled proembryo with aborted apical cell (ca). × 880. **Fig. 6.** Globular proembryo without suspensor. × 440. **Figs. 7, 8.** Globular proembryos with necrotic cells (n) and atypical intercellular spaces; proembryo with irregular cell arrangement in hypophysis (h) in Fig. 8. × 190.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spores in a tetrad appeared alive (Fig. 2). In samples of mature pollen the frequency of degenerated pollen grains reached ~35% (rarely ~90%) (Fig. 3).

In ovules, degeneration of ES at various developmental stages was observed. Rarely it was the stage of 2–4-nucleate ES; more often, degeneration of the egg apparatus or total degeneration of mature ES without fertilization occurred (Fig. 4). The frequency of the latter stage was similar to that of mature ES degeneration in the control material.

Disturbances in early embryogenesis were found in a few flowers and included (a) degeneration of the apical cell of the young proembryo (Fig. 5), whereas in other typical proembryos that cell was viable, destined to form the prominent part of the embryo proper (Fig. 13); (b) deviated embryos in two ovules were formed without a suspensor (Fig. 6), and typically at the same globular proembryo stage a several-celled suspensor was visible in other ovules of the same plants (Fig. 14) as well as in the control material; and (c) in several embryos, small necrotic regions, intercellular spaces in the embryo proper and/or an irregular cell arrangement in the hypophysis (Figs. 7, 8).

In a few ovules the endosperm haustorium aborted at the initial stage of its formation, before zygote division (Fig. 9), whereas in the typical course of endosperm development it could be observed still at the late torpedo-shaped stage of embryo (Fig. 15).

Plants from the vicinity of the Żelazny Most reservoir

In the studied material, developmental disturbances and degeneration were of low variability but occurred with higher frequency than in plants from Katowice-Wełnowiec.

Examination of microsporogenesis showed a low level of meiotic disturbances (laggards in anaphase II). Precocious degeneration of tapetal cells was not observed; on the other hand, several flowers showed enormous growth of cells in the tapetum. Degenerative processes started at the tetrad stage when some microspores were aborted; degeneration was intensified after microspore separation. The frequency of degenerated pollen grains and microcytes reached ~90% in some flowers.

Ten percent of the flowers showed destructive processes in the tissues of the receptacle and very advanced degeneration of all ovules (Fig. 10); in another 10% of the flowers, the chalazal zone of the nucellus was degenerated in the vicinity of the funicular vascular strand (Fig. 11). Degenerated ESs starting from one-nucleate stage to mature ES before anthesis were observed in other ovules. These processes reduced fruiting; the frequency of ovules with degenerative processes reached ~80% of the studied material.

The embryos in maturing seeds were formed typically, except for a few in which the apical region of the radicle degenerated (Fig. 12).

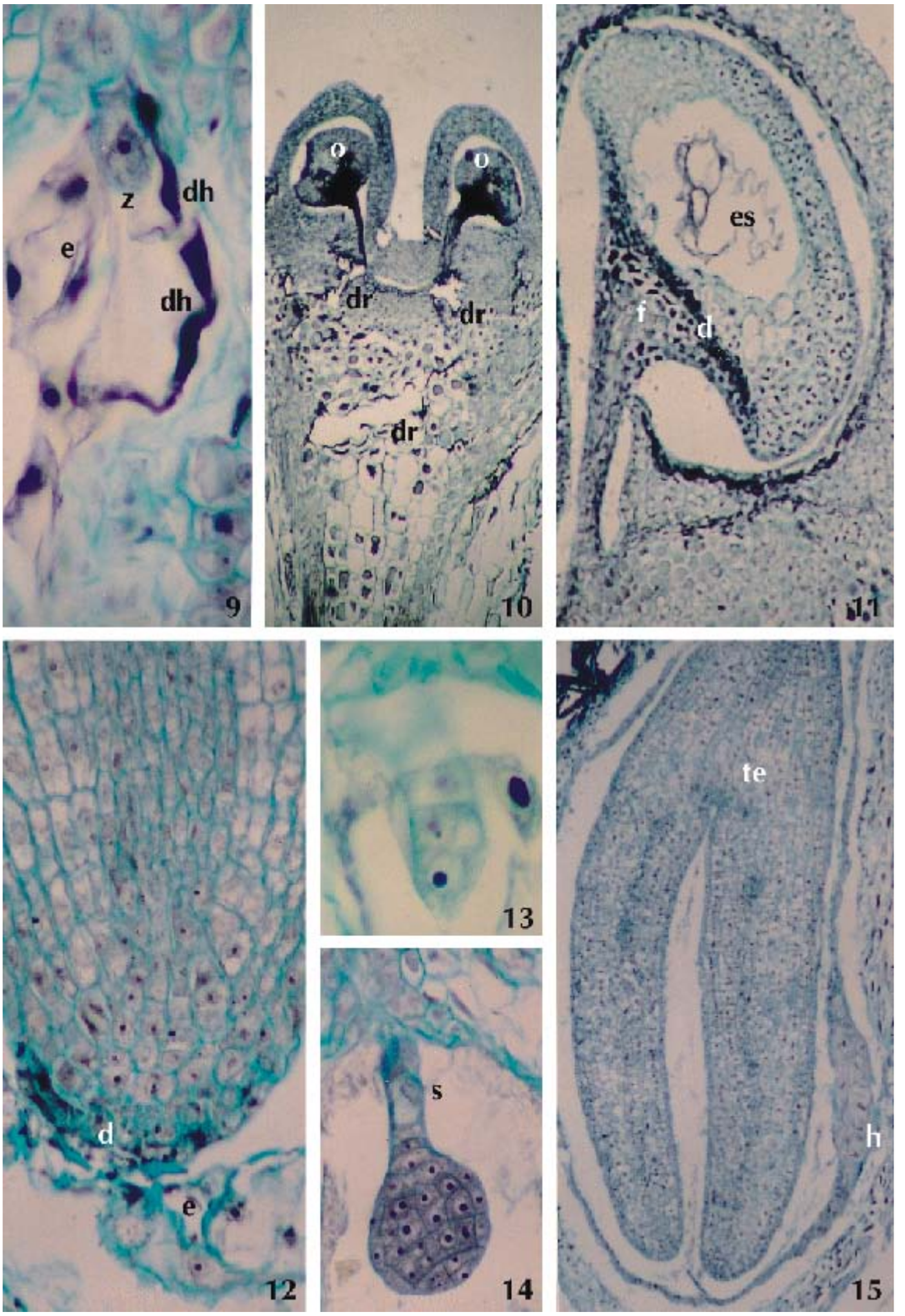
GERMINATION TESTS

Figure 16 shows the percentage of seedlings emerging in each test. The seeds from control plants sown on standard unpolluted soil, on soil from the waste heap in Katowice-Wełnowiec and on soil from Żelazny Most had the lowest germination (71.2%) in the latter test; on unpolluted soil it was 94%. There was also a distinct difference in the germination rate: on soil from Żelazny Most ~19% of the seeds germinated as late as the 7th day, whereas on standard soil germination was fast and almost synchronous (76% germinating on the 2nd day and 18% on the 3rd day).

The seeds harvested from the Katowice-Wełnowiec waste heap population had higher germination on heap soil (77.6%) than on unpolluted soil (67.2%).

On the other hand, seeds collected from the plants at Żelazny Most had lower germination on their polluted soil (64.4%) than on standard soil (78.8%). In both tests, polluted and unpolluted soil, a good proportion of the seed samples from Żelazny Most had significantly delayed germination (9th and 8th days, respectively).

Figs. 9–12. Degenerative processes in material from polluted sites. **Fig. 9.** Material from Katowice-Wełnowiec. **Figs. 10–12.** Material from Żelazny Most. **Fig. 9.** Median section of ES with zygote (z) and early stage of endosperm development; degenerated endosperm haustorium at the stage of two lateral coenocytes (dh); viable endosperm proper (e). × 760. **Fig. 10.** Longitudinal section of ovary and receptacle; degeneration of ovules (o), destruction of large cell groups in receptacle (dr). × 80. **Fig. 11.** Longitudinal section of ovule with degenerated chalazal zone of nucellus (d) near funicular vascular strand (f); embryo sac (es). × 190. **Fig. 12.** Longitudinal section of radicle tip with degenerated apical region (d); endosperm proper (e). × 380. **Figs. 13–15.** Regular development of embryo and endosperm in material from polluted site in Katowice-Wełnowiec. **Fig. 13.** Typical five-celled proembryo. × 880. **Fig. 14.** Typical globular proembryo with five-celled suspensor (s). × 440. **Fig. 15.** Section of typically formed young seed still containing well-developed endosperm haustorium (h) at the stage of torpedo-shaped embryo (te). × 80.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During the next four weeks of cultivation on polluted soil the seedlings showed poor development and soon were arrested at early juvenile stage. Cotyledon chlorosis was observed in some seedlings (~2%). Transplantation to unpolluted-polluted soil (1:1) stimulated better development and growth of seedlings for a few weeks.

DISCUSSION

The results of this study essentially confirm our previous research on *Vicia cracca*, *Ranunculus repens*, *Capsella bursa-pastoris* (Izmaiłow, 2000, 2002a,b), *Cirsium arvense* (Czapik and Kaźmierska, 2002), and *Chondrilla juncea* (Kościńska-Pająk, 2000, 2002a,b), which showed various kinds of disturbances and degeneration processes occurring in different frequencies in male and female organs of plants from contaminated habitats.

In all the mentioned taxa, embryological processes were compared with those in material from unpolluted sites, where developmental irregularities and degeneration generally were found as rare phenomena. Therefore their occurrence in plants from polluted areas was treated as effects of external stress and as reactions to various unfavorable conditions: excessive levels of heavy metals in soil and groundwater, nutrient deficits, drought conditions and wind erosion of contaminated soil. Embryological observations revealed some disturbances and degenerative processes, reflected in lower fertility of plants and lower seed quality.

Among the specimens of *E. vulgare* from the two contaminated sites, those from the waste heap in Katowice-Wełnowiec seemed better adapted to their polluted site than those from the vicinity of the Żelazny Most reservoir. At the latter site the *E. vulgare* population is at an early stage of succession; its specimens occurred with low frequency at a site of very early vegetation development and probably grew under very unfavorable conditions. The germination test results for seed samples of those plants suggest that they were more sensitive to pollutants than were the maternal plants of the seeds harvested from the waste heap in Katowice-Wełnowiec.

The delayed germination of both the control seeds and those from Żelazny Most, particularly evident in the samples on polluted soil from Żelazny Most, might suggest that the fruit covering and seed coat in *E. vulgare* is permeable to heavy metals ions; the most probable explanation of the delay, however, is contact of the radicle with ions when it starts to pierce

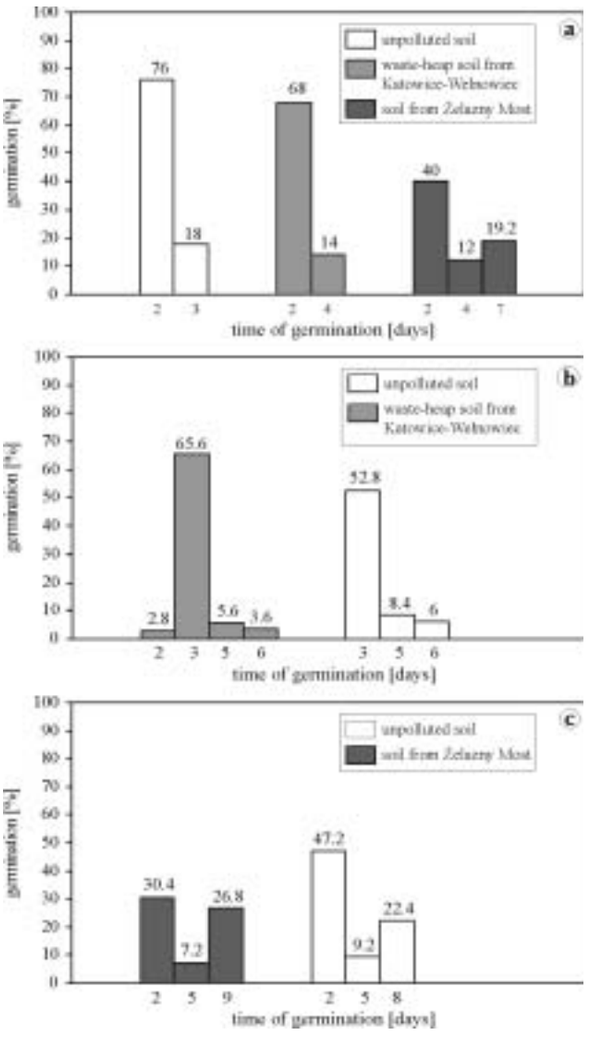

Fig. 16. Germination dynamics of *Echium vulgare* seeds on unpolluted and polluted soil. (**a**) Seeds from the control plants, (**b**) Seeds from the waste heap plants from Katowice-Wełnowiec, (**c**) Seeds from plants grown at Żelazny Most.

the fruit-seed coat. Mukherji and Maitra (1977), Woźny et al. (1982) and Fargasova (1994) gave examples of taxa – *Oryza sativa*, *Lupinus luteus* and *Sinapis alba*, respectively – whose seeds lost some degree of germination ability as a result of the inhibitory effect of lead. Wierzbicka and Obidzińska (1998) in their comprehensive studies on the effect of lead and barium ions on seed imbibition and germination in different plant species showed that delayed germination in some tested taxa was the effect of toxic ions.

The studied plants of *Echium* complete the life cycle in their contaminated sites, but the reactions

of the generative organs in situ, their lower fertility and their susceptibility to the effect of pollutants on seed germination in tests indicate that these populations have not yet achieved successful selection for tolerance to their unfavorable environments.

Studies dealing with *Silene vulgaris* (Wierzbicka and Panufnik, 1998), *S. vulgaris* and *Dianthus carthusianorum*, the dominant species on calamine waste heaps in the Olkusz Ore District (Poland) (Wierzbicka and Rostański, 2002), showed genetically established adaptations to the harsh conditions of waste heaps. In the authors' opinion, the studied waste-heap populations of these taxa demonstrate an "r" type life strategy (Falińska, 1996): they show reduction of biomass accompanied by earlier entry into the generative phase, and the plants are distinguished by large reproductive effort and increased fertility.

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