Original Research

Evaluation of Seed Germination Ability of Native Calamine Plant Species on Different Substrata

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Abstract

The purpose of our work was to evaluate the seed germination ability of native species representing calamine populations of *Alyssum montanum*, *Biscutella laevigata* (Brassicaceae), and *Dianthus carthusianorum* (Caryophyllaceae). As substrata for germination, calamine substrate on which populations of examined plant species were grown in natural conditions, and horticultural substrate constituting a mixture of sand and garden soil were used. *A. montanum* and *B. laevigata* seeds demonstrated a high ability to germinate on calamine substrate, which was characterized by large contents of soluble forms of zinc (115.1 mg·kg^{-1}), lead (0.91 mg·kg^{-1}), and cadmium (3.12 mg·kg^{-1}), and low water capacity (18.95% g/g). The seed germination ability of calamine *Dianthus carthusianorum* ecotype was comparable on both studied substrata types.

Keywords: seed germination ability, metallophytes, contaminated environment

Introduction

Plant communities growing in areas with high content of heavy metals in the substrate are known to be composed of numerous species capable of accumulating metallic elements in aboveground or underground organs. Research made on populations representing metallophytes, which occur both on areas with the increased geochemical background as well as on secondary dump habitats, showed that the concentration of certain metal ions in plant tissues can be much higher than in specimens growing in uncontaminated areas [1-4]. Until the late 1980s flowers were ranked among the most protected organs from the influence of harmful metal ions. For example it was then proved that in ovule, and mainly in developing embryo sack, ions of lead were not present, in spite of strong contamination of vegetative organs with this element, and constantly seeds do not contain this element. Moreover, in the majority of species the seed coat constitutes the effective barrier against penetrating of toxic ions. On that account the content of harmful elements in the seed coat is usually two- to four-times higher than in the embryo [5, 6].

In the case of lead the tightness of this barrier is a species characteristic, for instance among representatives of Fabaceae, Brassicaceae, and Poaceae there are known particular species for which the seed coat does not constitute sufficient protection against penetrating of lead ions [7]. However, studies initiated at the end the 20th century [8, 9] showed that generative organs can also contain harmful elements. In numerous species growing on contaminated habitats, like *Vicia cracca* [10], *Cirsium arvense* [11], *Ranunculus repens* [12], *Capsella bursa-pastoris* [13], *Chondrilla juncea* [14], *Echium vulgare* [15], and *Viola guestphalica* [16] were documented disruptions during

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developmental processes of the embryo sac, and in both embryo and endosperm as well. Simultaneously, it was observed that earlier stages of the embryo development were usually much more sensitive to pollution by heavy metals than later ones. Disorders in the processes of microsporogenesis and pollen grain development were also registered [12, 14, 17]. Intoxicants occurring in soils of industrial lands also can cause delaying, or even hampering seed germination [6]. The negative influence of heavy metals on this process was observed among others at *Echium vulgare*, *Phaseolus vulgaris*, *Pisum sativum*, *Raphanus sativus*, *Linum usitatissimum*, and *Sinapis alba*, whereas at *Lactuca sativa*, *Soya hispida*, *Cucumis sativus*, *Triticum vulgare*, *Allium cepa*, and *Zea mays* a destructive influence of harmful ions on the process of seed germination was not observed [7, 18]. Thus, in spite of the existence of barriers limiting the penetration of certain ions that were created by individual taxonomical groups in the course of their evolution, heavy metals can penetrate into generative organs, and hence contribute to reduction in the reproductive success. Selection of species that would be able to realize the full lifecycle in such harsh habitat conditions that undoubtedly dominate in chemically contaminated areas can thus prove to be unusually helpful in the reconstruction of habitats degraded by industrial activity. Plants species representing local metallophytes are significant material in the course of reclamation, and at the same time they can be used in certain phytoremediation technologies [19-22]. *Biscutella laevigata* (Brassicaceae) accumulates heavy metals both in underground organs, and in shoots, while *Dianthus carthusianorum* (Caryophyllaceae) only in the root system [1-4]. Numerous *Alyssum* (Brassicaceae) species also are capable of accumulating high concentrations of heavy metals in their tissues, and more than 50 species belonging to that genus accumulate nickel in amounts above 2.5% of dry matter of shoots [23]. *Alyssum montanum* was chosen as considered plant material.

The aim of our work was to evaluate seed germination ability using the above-mentioned native local species representing calamine flora growing in the Olkusz Ore – bearing area for the reason of the planned production of such plant material. Afterward, in the course of cultivation it is additionally planned to apply some techniques of vegetative propagation commonly used in horticultural practice. We focused on increases in the effectiveness of these valuable genotypes propagation, as the application of such species can significantly contribute not only to lower the toxicity of heavy metals, but also to accelerate the process of creating the vegetation cover on contaminated industrial wastes. On this account, we suggest that the production of native local metal-tolerant species, which can be applied during restoration of degraded areas, is an issue worth undertaking.

Material and Methods

Seed Sample Collection

The area of the fruits collecting from which seed samples were obtained is located in Małopolska province, (district Olkusz, commune Bolesław), on the border of the Silesian Upland and the Kraków-Częstochowa Jura. Plant material was taken in the vegetation period of 2010, from specimens covering the following sites:

(A) above a 100-year-old slag heap, from which seed samples of two plant species from the Brassicaceae Burnett (Cruciferae Juss.) were acquired: *Alyssum montanum* L. and *Biscutella laevigata* L.

(B) uncultivated meadow with the dominance of species growing in full light, where seed samples of calamine *Dianthus carthusianorum* L. ecotype (classified within the family *Caryophyllaceae* Juss.) were collected.

Harvest of ripened fruits from single *A. montanum* and *B. laevigata* populations was begun on 23 May, and from the *D. carthusianorum* population on 27 June. In every case the collection was conducted three times, in two-week intervals, and seed samples were prepared separately from every time of the fruit collection. Seeds were sown on:

(1) calamine substrate, on which respective population of examined plant species were grown in natural conditions,

(2) horticultural substrate prepared by mixing sand and Vitahum garden soil at a 1:3 ratio.

Chemical Analyses of Substrata

Properties of applied substrata were determined with the following methods: soil organic carbon and total nitrogen content with the use of automatic TOC-TN 1200 analyzer, pH_{H_2O} potentiometrically according to Lityński et al. [24] field capacity FC in Richards chambers on porous plates according to Klute [25], total content of Zn, Pb, and Cd in substrata samples digested in nitric (V) and chloric (VII) acids (after Ostrowska et al. [26]), and soluble forms extracted by 0.01 M $CaCl_2$ at pH 7 Houba et al. [27] with AAS method, using apparatus PU 9100x.

Experimental Procedure

In greenhouse conditions 720 seeds of every plant species were sown in two replications with 30 seeds in every variant of the experiment: from three dates of fruit collection, two seed sowing dates (30.08 and 7.09), and on two types of substrata. In the greenhouse the first seedlings were observed after fourteen days since the moment of sowing. Just then observation was begun, and the number of seedlings was summed up every two days. The evaluation was finished when a number of germinating seeds did not change in the significant way, thus the seedlings were counted after three weeks from the date of sowing. An evaluation of seed germinating ability also was conducted in laboratory conditions. In each of two replications 30 seeds were sown to Petrie dishes inlaid with blotting paper (control). Conditions of seed germination were established on the basis of International Regulations of the Evaluation of Seeds [28]. The recommendations for germinating conditions of *B. laevigata* and *A. montanum* were established as for *Brassica oleracea*, whereas in the case of *D. carthusianorum* – for *Dianthus caryophyllus*. Seeds were germinated on wet blotting paper at 20ºC. The experiment was repeated three times.

Table 1. Selected physical and chemical properties of substrates used in the experiment. Mean values together with standard deviation (SD) are provided.

Substrate	pH in H_2O	SOC* $g \cdot kg^{-1}$	TN** $g \cdot kg^{-1}$	FC*** % g/g	Total content of heavy metals [$mg \cdot kg^{-1}$]			Contents of soluble forms of heavy metals [$mg \cdot kg^{-1}$]		
					Zn	Pb	Cd	Zn	Pb	Cd
Calamine	7.5	45.1±0.3	6.5±0.1	18.95±0.25	9,021±10	2,500±9	102.8±1.8	115.1±1.2	0.91±0.05	3.12±0.03
Horticultural	5.8	215.2±0.6	12.1±0.3	46.06±0.32	195.2±5.5	95.2±2.5	0.9±0.1	2.34±0.4	0.01±0.0	0.02±0.0

*soil organic carbon content, ** total nitrogen content, *** field capacity

Results

Characterization of Substrata Used in the Experiment

In greenhouse conditions seeds were sown on two types of substrata with diversified properties. Calamine substrate was characterized by a small content of organic carbon and total nitrogen, i.e. a small amount of organic substance. However, it contained large amounts of zinc, lead, and cadmium, exceeding admissible values defined by the Ministry of the Environment in the Regulation on standards of the quality of the soil and the quality of the earth [29] for industrial lands (group C). Admissible values defined in the Regulation for Zn, Pb, and Cd in the described substrate were exceeded about 9-fold for Zn, 6-fold for Pb, and 7-fold for Cd. Horticultural substrate used in the experiment was characterized by a large content of organic carbon and total nitrogen, while the levels of Zn, Pb, and Cd ranged within admissible values defined in above-mentioned Regulation for 0-30 cm depth layer of agricultural lands (group B). The field water capacity, which is a good indicator of the water retention ability, was much lower in the calamine than in the horticultural substrate (Table 1).

An alkaline reaction of calamine substrate was probably the main cause of relatively low amounts of Zn, Pb, and Cd extracted by $CaCl_2$ solution at pH=7, which are considered soluble forms of heavy metals directly available for plants. The solution of calcium chloride extracted about 1.3%, 0.04%, and 3.0% of total forms of Zn, Pb, and Cd, respectively (Table 1). Content of soluble forms of Zn, Pb, and Cd determined in the horticultural substrate was much lower than in the calamine one, all the same share of these metals in their total content was similar in both types of substrata.

Seed Germination Ability

Distinctive differences in the seed germinating ability of plant species representing the Brassicaeae family have been observed, depending on the kind of applied substrata. Both *A. montanum* and *B. laevigata* showed greater ability of seed germination on the substrate taken from the stand on which populations of examined species of plants appeared in natural conditions, in comparison with the horticultural one. In the case of *A. montanum*, grown from seed samples collected on 13 and 27 of June, almost 7-fold and 5-fold higher numbers (respectively) of seedlings were obtained on the calamine comparing to the number of seedlings obtained on the other substrate type. However, the ability of *B. laevigata* seed germination on calamine substrate amounted on average to 26 percent, and was only about twice as high as on the horticultural substrate (Table 2).

The differences in the ability of *D. carthusianorum* (Caryophyllaceae) for seed germination depended on the type of used ground, but did not prove to be so distinct, as in case of two evaluated species from the *Brassicaceae* family. The ability of seed germination of this species was only slightly higher on the substrate with the increased content of heavy metals than on the horticultural soil (Table 2). Simultaneously, the ability of seed germination on the substrate with the increased content of heavy metals was relatively similar to the control, conducted in laboratory conditions according to guidelines of the International Regulations of the Evaluation of Seeds [28]. Moreover, on the ability of *A. montanum* and *B. laevigata* seed germination the date of the fruits collection exerted distinctive influence. Seeds of *A. montanum* obtained on 23 May and on 13 June germinated more poorly, comparing to seeds collected on 27 June, or did not germinate at all as in the case of *B. laevigata* (Table 2), while in the case of calamine *D. carthusianorum* ecotype, a distinct influence of the date of fruits collection in the field on the ability of seed germination was not noticed, which indicates the possibility of obtaining viable seedlings independently of the date of material acquisition.

Discussion of Results

Nowadays we should seek constant improvement of available techniques that allow us to purify ecosystems contaminated by heavy metals. Physical and chemical methods applied either in order to remove or deactivate these elements are known to be relatively expensive and, what is more important, they are extremely harmful for organisms living in the substrate. Moreover, the use of such techniques can occasionally result in secondary pollution of the environment. Bioremediation counterweighs such difficulties, being a favourable alternative that benefits from microorganisms and plants in the course of pollutant elimination [21, 30-32].

Reconstruction of habitats destroyed by human activity is a legal obligation in many countries. On strongly degraded or devastated areas habitat conditions must be created

Table 2. The mean percentages of *A. montanum*, *B. laevigata*, and *D. carthusianorum* seed germination ability on two substrata types. Mean values together with standard deviation (SD) are provided.

Plant species	Sowing date	a*			b			c		
		Control	Horticultural substrate	Calamine substrate	Control	Horticultural substrate	Calamine substrate	Control	Horticultural substrate	Calamine substrate
Alyssum montanum	30.08	0	0	0	40.1±3.2	10.1±3.2	26.4±2.1	56.9±3.2	0	60.4±3.1
	7.09	10.1±3.2	0	0	43.2±2.5	0	52.3±2.3	80.1±1.1	30.1±2.1	83.2±1.4
Biscutella laevigata	30.08	0	0	0	0	0	0	50.1±4.1	13.9±5.3	37.9±4.2
	7.09	0	0	0	10.9±5.1	0	0	77.2±2.2	10.1±3.2	15.3±5.1
Dianthus carthusianorum	30.08	93.0±2.1	43.2±5.3	80.1±1.1	97.1±2.1	23.1±4.2	80.1±1.1	83.2±1.4	50.9±3.1	93.0±2.1
	7.09	80.1±1.1	47.9±3.2	87.2±1.1	90.1±2.4	70.1±2.3	83.2±1.4	93.0±2.1	77.2±2.2	77.2±2.2

*a, b, c – terms of seed collection: *A. montanum* and *B. laevigata*: a – 23.05.2010; b – 13.06.2010; c – 27.06.2010; *D. carthusianorum*: a – 27.06.2010; b – 11.07.2010; c – 25.07.2010.

from the base. The flora are being implemented for the purpose of restoring biological life, protecting soil against erosion, and limiting further contamination of the environment [20, 21, 30, 31, 33, 34]. Developing efficient and cost-effective growing methods of native species that subsequently could be renewed by self-sowing or by vegetative propagation will be beneficial for the process of the natural succession. With the aim of obtaining plant material of *A. montanum*, *B. laevigata*, and *D. carthusianorum,* it is possible to apply seed sowing under covers as the economic, simple and universally used method of garden plants propagation.

During seed imbibition only tissues hydrating at about 85-95% leads to the seedling formation, which constitutes the transitional stage between the period of the embryonic and the postembryonic development of mature plants. Calamine substrate used in the experiment was characterized by low water retention (18.95% g/g). Thus a higher ability of seed germination ability on this substrate type in comparison with the horticultural one could be a result of the greater availability of water accumulating on the surface of irrigated calamine substrate, which is what brought about the more effective seed imbibition. In the case of *A. montanum* and *B. laevigata* seeds, it clearly facilitated activation of storage materials by switching over to the catabolic phase, which in turn enabled embryos to grow and develop efficiently [35, 36]. On the other hand the results of research are known, proving that treating seeds of some particular species with solutions of zinc can facilitate germination [37]. Consequently, high ability of seed germination of tested plant species on calamine substrate also could be caused by a large content of the soluble form of zinc, amounting to 115.1 $mg \cdot kg^{-1}$.

Remarkable influence of the term of the collection of *A. montanum* and *B. laevigata* seeds on their germination presumably is pointing at the physiological immaturity of seeds during the harvest conducted on 23 May and 13 June 2010. Climatic conditions such as temperature, solar exposure, and the amount and distribution of rainfall influence seed ripening, and hence if they are unfavorable, cause lowering of the sowing material quality [35]. Therefore, on account of unfavorable weather conditions that were present in mid-2010 (intense and long-term rainfall), most probably *A. montanum* and *B. laevigata* seeds were collected in these terms before their maturity. They could also be dormant then, which is one of the adaptive strategies of wild plants [2, 19, 36, 38, 39].

Thus, a limiting factor in the greenhouse production of discussed species may be the low ability of seed germination, and hence a small number of obtained seedlings, which is particularly essential in the case of *A. montanum* and *B. laevigata*. In order to confirm that it is an effect of disruptions in processes of the generative propagation resulting from the negative influence of extreme environmental conditions occurring on slag heaps, embryological research is indispensable. Independent of discussed problems, the relatively high size of the population and the specimens' expansion on areas of younger slag heaps, prove the existence of genotypes advanced in the adaptation to difficult conditions occurring on zinc-lead slag heaps covered by some of these species [40, 41].

Conclusions

From obtained results subsequent conclusions can be drawn:

(1) Calamine substrate used in the experiment was very strongly polluted with zinc, lead, and cadmium, but its alkaline reaction lowered the solubility of these metals, and their amount directly available to plants.

(2) The use of calamine substrate had a positive effect on the germination ability of *Alyssum montanum*, *Biscutella laevigata*, and *Dianthus carthusianorum* seeds.

(3) In order to get large amounts of plant material as a result of generative propagation the seed collection of species representing the Brassicaceae family and belonging to calamine flora should be conducted in the second-half of June.

(4) Large amount of the *Dianthus carthusianorum* seedling was obtained regardless of the term of acquiring sowing material.

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