**Brief communication**

**РАСТИТЕЛНИ АДАПТАЦИИ ЗА РАСТЕЖ ВЪРХУ Zn-Pb ОТПАДЪЧНИ НАСИПИ – ПРИМЕР С *DIANTHUS CARTHUSIANORUM***

**PLANT ADAPTATIONS TO GROWTH ON ZN-PB WASTE DEPOSITS – A CASE OF *DIANTHUS CARTHUSIANORUM***

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**Резюме**

Целта на проведеното изследване е да се проучат механизмите на толерантност към стрес от Cd, Pb и Zn в растението *Dianthus carthusianorum*, което е основен растителен вид, колонизиращ отпадъчен насип край Bolesiaw около Olkusz, Южна Полша. Два екотипа от *D. carthusianorum* – единият, растящ върху отпадъчен насип (calamine екотип), а другият – върху незамърсена почва около Люблин (контрола), са анализирани, за да се идентифицират морфологични и физиологични черти, както и генетични маркери, свързани с толерантността. Установено е, че съществува съществена вариабилност между двата екотипа *Dianthus carthusianorum*. Типът calamine показва по-висока толерантност, но механизмите на тази толерантност предстои да бъдат проучени. Нашите резултати не подкрепят важната роля на глутатиона и на органичните киселини за повишената толерантност към метали в calamine екотипа.

**Abstract**

The aim of the study was to investigate the mechanisms of tolerance to Cd, Pb and Zn excess stress in *Dianthus carthusianorum*, one of the dominant species colonizing an old Zn-Pb waste heap in Bolesław near Olkusz, Southern Poland. Two ecotypes of *D. carthusianorum*, one growing on the waste heap (calamine ecotype) and the other on unpolluted soil near Lublin (control ecotype) were analysed to identify their morphological and physiological traits as well as genetic markers. Significant phenotypic and genetic variability exists between *Dianthus carthusianorum* ecotypes originating from waste deposits and non-polluted areas. The calamine ecotype showed higher metal tolerance; however, the mechanisms of this tolerance remain to be elucidated. Our results do not support the important role of glutathione, phytochelatins or organic acids in the enhanced tolerance to metals of the waste heap ecotype.

**Ключови думи:** *Dianthus carthusianorum*, кадмий, олово, цинк.
**Key words:** *Dianthus carthusianorum*, cadmium, lead, zinc.

Waste deposits left over from ore mining and smelting activities are especially unfavourable environments for plant growth and development. Not only extremely high metal (Zn, Pb, Cd) contents in the substratum but also water and nutrient deficit as well as strong insolation and winds pose strong selective pressure on plants occurring in such habitats. Therefore, the vegetation spontaneously colonising such waste deposits represent an interesting object to investigate the mechanisms of adaptation to growth in environments highly contaminated by metals.

It was noted that some plants occurring on an old, about 130 years old Zn-Pb waste heap in Bolesław near Olkusz, Southern Poland, are distinguished by specific morphological features such as dwarfism, stronger growth of underground parts over aboveground parts, small and narrow leaves and extended time of flowering. In some plant species, e.g. *Silene vulgaris*, *Biscutella laevigata* and *Dianthus carthusianorum,* these traits were proved to be persistent in three successive generations despite cultivation under optimal conditions on a substrate devoid of heavy metals and with adequate water supply (Wierzbicka and Rostański, 2002). Hence, it was concluded that in these plant species microevolutionary processes and strong selection of specimens that are best adapted to local conditions led to the development of a separate ecotypes, the so-called calamine ecotypes. The adaptations of these ecotypes to their growth habitat include particular anatomical alterations that allow them to tolerate water deficit; they were also shown to exhibit elevated tolerance to heavy metals. However, the mechanisms of this tolerance are not fully understood.

The aim of this study was to investigate the mechanisms of tolerance to Cd, Pb and Zn excess stress in *Dianthus carthusianorum*, one of the dominant species colonizing this waste deposit. Two ecotypes of *D. carthusianorum*, one growing on the Zn-Pb waste heap in Bolesław (calamine ecotype) and the other on unpolluted soil near Lublin (control ecotype) were analysed to identify their morphological and physiological traits as well as genetic markers.

The plants of the calamine ecotype differed significantly from the plants of the control ecotype in terms of their morphology both when grown in their natural habitats and when cultivated under uniform environmental conditions in a controlled growth room. The calamine plants were smaller, possessed fewer leaves per plant, their leaves were shorter and narrower, their stems were also shorter and with a smaller number of flowers per inflorescence.

To confirm that some genotype adaptations had occurred in these plants, we isolated DNA from the leaves of the first generation of plants grown under uniform controlled conditions and analysed it using molecular markers. Two polymerase chain reaction (PCR)-based markers, random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) systems were used in this study.

Taking into consideration 21 primers giving polymorphic bands (17 RAPD and 4 ISSR primers), we were able to prove a very distinct genetic polymorphism between plants originating from the waste heap and the non polluted area.

Based on the growth parameters (EC100 – effect of concentration, root elongation, root viability, root and shoot biomass) of plants cultivated hydroponically and exposed to Zn (50-1000 $\mu$M), Cd (5-50 $\mu$M) or Pb (15-30 $\mu$M), we demonstrated higher metal tolerance of the calamine ecotype in spite of the fact that both ecotypes accumulated similar concentrations of Zn and Cd and the waste heap ecotype accumulated even more Pb than the control ecotype.

The question arose then about the mechanisms of such enhanced metal tolerance. At the cellular level, metal tolerance may be achieved in different ways (Hall, 2002; Yadav, 2010). First of all, substantial amounts of metal ions may be bound to cell wall components thus preventing their uptake into the protoplast. To avoid metal toxicity in the cytoplasm, chelation of metal ions by various ligands takes place, followed by metal sequestration into the vacuole. Two groups of metal ligands are of major importance in metal detoxification in the cytoplasm. The first one consists of thiol peptides, glutathione (GSH) and its derivatives – phytochelatins (PC). Both ligands bind metal ions, and subsequently, such complexes are translocated into the vacuole. Another important group of ligands involved in metal chelation in the cytoplasm as well as metal storage in the vacuole are organic acids. The accumulation of these two groups of ligands was the subject of further investigation in this study.

Glutathione accumulation in the metal untreated plants was higher in the waste heap ecotype; however, after exposition to the metals in the nutrient solutions, it did not exhibit a uniformed pattern under various metal treatments and, in general, it was not correlated in any specific way with the various metal concentrations. Zinc did not induce PC synthesis; accumulation of these peptides was found in the plants treated with Cd as well as in the roots of plants treated with Pb. Phytochelatin concentration in the roots was not correlated with the Cd concentration in the solution; in the shoots, however, the accumulation of these thiol peptides increased with increasing Cd concentration in the growth medium, similarly as it increased in the roots of plants treated with increasing concentrations of Pb. Most remarkably, PC accumulation was higher in the plants from the unpolluted site. There was no correlation between any metal concentration in the growth medium and either malate or citrate concentration in the plants of both ecotypes. The citrate concentrations were in general higher in the waste heap ecotype, whereas no such correlation could be found for malate.

Plants growing in their natural habitats did not accumulate PC; the GSH level was higher in the calamine ecotype, similarly as the level of citrate; however, the level of malate was higher in the control ecotype.

Histochemical staining of roots and leaves with dithizone revealed slight differences in localization of the metals between these two ecotypes. In the leaves of the control ecotype, much more Cd was found in the epidermal cells, and especially in the stomatal cell walls. On the contrary, in the leaves of the calamine ecotype more Cd-ditizone precipitates were found in the leaf bundles. The highest amounts of all the metals studied were accumulated in the root tips; in more distinct root parts they were mainly found in the vascular bundles. Baranowska-Morek and Wierzbicka (2004) detected numerous Pb deposits in the cytoplasm and vacuoles of the root tip cells, whereas no Pb was found in the cell cytoplasm of the waste heap ecotype and it was only detected in the cell walls and the vacuoles. This may at least partially explain the higher Pb toxicity in the control ecotype.

In summary, a significant phenotypic and genetic variability exists between *Dianthus carthusianorum* ecotypes originating from waste deposits and non-polluted areas. The calamine ecotype showed higher metal tolerance; however, the mechanisms of this tolerance remain to be elucidated. Our results do not support the important role of glutathione, phytochelatins or organic acids in the enhanced tolerance to metals of the waste heap ecotype. Further studies are necessary in order to find the exact localization of Cd, Zn and Pb at the tissue and ultracellular levels and to explain its contribution to metal tolerance of these plants.

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