

## **An advanced field experimental design to assess plant tolerance to heavy metal pollution**

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Only a limited number of vascular plant species can survive and reproduce in toxic metalliferous environments. Among these species, pseudometallophytes are particularly interesting, as their metallicolous (M) populations on metalliferous soils and non-metallicolous (NM) populations on non-metalliferous soils show very pronounced ecological differences. Pseudometallophytes thus provide excellent opportunities for multidisciplinary research to improve phytoremediation and phytomining.

Numerous methods have been developed to investigate plant adaptation to metal pollution, the majority of which has been conducted under controlled laboratory conditions. Although these efforts have significantly advanced our understanding of mechanisms underlying metal tolerance in plants, populations must be reciprocally transplanted to clearly identify natural selection. Only then is it possible to test, whether the fitness of native plants is higher than that of nonnative populations and thereby prove local adaptation.

Here, we present an enhanced field experimental design aimed at verification of local adaptation to habitats with different levels of heavy metal soil contamination. At two M and two NM sites, we established a total of 12 plots (4 sites x 3 plots each), removed the existing local vegetation, and collected soil samples for chemical analyses (5 samples per plot). Plant collection (N= 480) from all four selected populations was established under laboratory conditions prior to the transplant experiment. Genotypes were randomly distributed within each plot (240 x 270 cm) and planted along a regular spaced grid (30x30cm cell size) in spring 2015. Measurements will start in spring 2016, by which time plants are expected to have acclimatized to the local conditions.

For the two subsequent years, growth, survival, fitness, life cycle and herbivory consumption will be monitored for each transplant. On a weekly basis, we will record: 1) pictures of each transplant to determine the plant development, 2) the percentage of leaves showing visible symptoms of Zn toxicity (i.e. leaf chlorosis, mottling, necrosis, anthocyanescence), and measurement of 3) number of stems, 4) length of the longest stem, 5) effective photosystem II yield, and 6) chlorophyll content. Upon termination of the experiment, we are going to harvest all plant material for genetic, physiological and chemical analyses.

This ongoing project is conducted in southern Poland and as a study object we chose the model pseudometallophyte *Arabidopsis halleri* (Brassicaceae). However, our enhanced experimental design can easily be adapted to other locations and species, thereby facilitating the intercomparisons between results obtained by different researchers and from a variety of geographic locations. The obtained plant material can be used in interdisciplinary approaches in support of efforts to improve environmental health and landscape quality at polluted sites.