SUMMARY

Environmental pollution with trace metal elements (TMEs) has been high due to natural and anthropogenic sources. The presence of toxic metals such as Cd or very high content of essential micronutrients such as Zn poses a serious threat to the environment. Plants take up TMEs from the soil and accumulate them in their tissues, which can pose a threat for health of humans consuming plants containing higher concentrations of these metals. Therefore, there is a need to develop new and effective techniques of removal of heavy metals form the environment or limiting their negative influence on living organisms.

The aim of the project was to acquire deeper knowledge on the mechanisms involved in the uptake and accumulation of trace metal elements (TME) by plants and in the resistance to their toxic effects. The so-called metal hyperaccumulators, as *Arabidopsis halleri*, are plants that can live on soils heavily polluted by TME and have the ability to accumulate extraordinarily high concentrations of these metals in above-ground tissues. *A. halleri* can be used as a model-species to study the biological and molecular mechanisms involved in the tolerance and accumulation of high concentration of metals in the soil. This species is characterized by high tolerance to high concentrations of Cd and Zn. It is also capable of accumulating very large quantities of Zn in the shoots, named Zn hyperaccumulators, and also of Cd in some populations, named Cd hyperaccumulators. *Arabidopsis arenosa*, a closely related species to *A. halleri*, also shows high tolerance to both metals but is reported as a non-hyperaccumulator. Previous studies showed that *A. arenosa* accumulates metals mainly in the roots, translocating only a small portion of Cd and Zn from roots to shoots. In Southern Poland both species can be observed on the same sites. The experiments were designed to study inter and intra –specific variability between metallicolous and non- metallicolous populations of *A. halleri* and *A. arenosa* in order to identify physiological parameters and genes responsible for contrasting behaviour in their adaptation to metal contamination. In order to achieve the goal, the investigations were divided into stages that constitute separate chapters in this work.

First chapter includes field investigations as well as laboratory experiments. Both one metallicolous and one non-metallicolous population of *A. halleri* and *A. arenosa* (four populations in total) from Southern Poland were thoroughly investigated to determine physiological differences in their native site and upon different Cd treatments in the hydroponic experiments. The total concentration of TMEs and bioavailable fractions of Cd, Pb and Zn in the original soil were analysed. The concentration of these elements was also analysed in roots and leaves of all investigated populations. Moreover, the photosynthetic apparatus activity and

pigment content were compared in all populations growing in their natural habitat. The ploidy of each population was analysed to confirm that investigated populations of *A. halleri* are diploid and *A. arenosa* are tetraploid to rule out genetic material exchange between two species. In hydroponic culture plants were exposed to 50, 100 and 200 μM of Cd for 24 days. Afterwards the same analyses as *in situ* were performed. For the first time mineral composition of *A. arenosa* combined with analysis of photosynthetic apparatus activity was performed both in field and in controlled conditions. Our results show that metallicolous *A. arenosa* is as tolerant to heavy metals as the metallicolous population of hyperaccumulator *A. halleri*. Moreover, the metallicolous population of *A. arenosa* was shown to hyperaccumulate Cd and Zn.

The second chapter contains the analysis of the response of metallicolous populations of *A. halleri* and *A. arenosa* exposed to extremely high Cd (1.0 mM) or Zn (5.0 mM) concentration. Differences in Cd and Zn accumulation, tolerance, photosynthetic apparatus performance and pigment content changes were assessed after 7 days of treatment. *A. arenosa* accumulated more Cd than *A. halleri* while the latter accumulated more Zn. Treatment with extreme Cd and Zn concentrations confirmed extreme level of tolerance of both metallicolous *A. arenosa* and *A. halleri* populations. Both species differed in the response of photosynthetic apparatus when exposed to Cd and Zn at high concentration, suggesting different mechanisms involved in metal homeostasis.

The third chapter focused on evaluation of the correlation between physiological responses and gene expression in *A. halleri* and *A. arenosa* metallicolous and non metallicolous populations. In this chapter expression of genes underlying physiological differences between the metallicolous and non-metallicolous populations of *A. halleri* and *A. arenosa* was analysed. Plants were treated in hydroponic experiment with 5 μM Cd or 150 μM Zn for 10 days. Lower concentration of metals than in 1st experiment was chosen to analyse the gene expression under mild stress in both hypertolerant metallicolous and sensitive non-metallicolous populations. During the treatment growth of plants, level of Cd and Zn tolerance, mineral composition and pigment content was analysed. The expression level of genes involved in the uptake, translocation or detoxification of Cd and Zn, as well as the biosynthesis of flavonoids (*HMA2*, *HMA3*, *HMA4*, *MTP1*, *IRT1*, *IRT3*, *FRD3*, *FRO2*, *ZIF1*, *NRAMP3*, *NRAMP4*, *OPT3*, *NAS2*, *LDOX* and *F3H*) was analysed. We observed contrasting accumulation of Cd and Zn in *A. halleri* and *A. arenosa* metallicolous populations. Expression level of genes showed differences not only between species but also between populations. The type of treatment (Cd or Zn) also had different effect on all populations in regards to some genes.

The results acquired in this project fill the gap in the physiological characteristics of *A. halleri* species both in the field and in controlled hydroponic conditions. The in depth physiological characterisation of *A. arenosa* from metalliferous site identified this population as a new hyperaccumulator of Cd and Zn. These results may serve as a good basis for future studies on the phenomenon of hyperaccumulation. Moreover, observed the contrasting expression of genes involved in metal uptake, translocation and detoxification between *A. halleri* and *A. arenosa* suggests that *A. arenosa* might be a good new model to study metal homeostasis and tolerance in plants.