## Reg. No: 2021/41/B/NZ9/04123; Principal Investigator: dr in . Joanna Cerazy-Waliszewska

Arsenic (As) is a highly phytotoxic element that occurs naturally in the earth's crust. Contamination with this metalloid is a serious global problem. Although arsenic enters the environment through natural geogenic processes, its exposure rapidly heightened through industrialization and formerly used As-based herbicides.

In aerobic environments, including soil, moderately toxic forms of arsenate - As(V) are the most common. Less toxic methylated forms of As(V) such as monomethylarsenic acid (MMA) or dimethylarsenic acid (DMA) also exist in most of contaminated soils. Yet, the most harmful inorganic form of arsenic for plants - reduced arsenite - As(III), constitutes substantial part of total As contamination.

The problem of soil contamination with As affects mainly the vicinities of the areas of e.g. copper, silver and gold extraction, e.g. in Poland in Zloty Stok. Phytoextraction and phytoremediation are the methods of recultivation of contaminated sites and flotation wastes, and are founded on the use of plant species capable of growing on contaminated soils, with simultaneous immobilization of the pollutants in the plant organs. So far, trees as poplars, maples or willows, were predominantly exploited for soil decontamination. Nonetheless, last years more attention is paid on fast-growing plants, including non-food grasses of C4 photosynthesis, especially *Miscanthus* × *giganteus*. *Miscanthus* is an example of multiple uses. The *Miscanthus* genus includes about 20 species of perennial grasses characterized by rapid biomass growth. For many years, intensive research has been carried out on the use of *Miscanthus* for bioenergetic purposes, not only for the production of pellets or briquettes, but also for second generation fuels e.g bioethanol. However, perennial growth, including that on marginal lands, and large amount of biomass produced could be considered as additional attributes enhancing phytoremediation potential of *Miscanthus*.

The pilot project (Miniature 3) showed, regardless of differences between tested giant miscanthus (M. × giganteus) cultivars (genotypes), several interesting phenomena. For the first, negative effects on investigated traits were more intensive in case of lower concentrations of arsenic forms (As(III), As(V), and DMA) tested. Moreover, the most abundant As form in plants was DMA and other organic compounds, regardless of an As form applied. Nevertheless, the most harmful aftermaths for plant growth and physiology were observed As(III) application. Changes in functioning of photosynthetic apparatus the most likely resulted from decreased activity of RuBisCo and probably other enzymes of C4 CO<sub>2</sub> assimilation, and deterioration of photosystems through damages in thylakoids and other cell membranes, as indicated by increased electrolyte leakage.

Summarising, the results of the pilot project indicated the need of continuation of studying mechanisms of *Miscanthus* response to As stress, as well as made possible to formulate relevant research hypotheses.

The first hypothesis is that during As phytoextraction, plants of *Miscanthus* species, which are exposed to less toxic organic As forms (e.g. DMA) may accumulate this metalloid in higher concentrations. Simultaneously, plants developed mechanisms of rapid As transformation from highly toxic inorganic As, mostly trivalent, to more tolerable and deposited organic As compounds as DMA, and/or other complexes. The question why and how taken up As is detoxificated via transformation to DMA/other organic forms and how this process affects plant metabolism needs to be resolved. The second hypothesis is that due to metabolic antagonism between arsenic and phosphorus, As may disrupt many metabolic pathways, and arsenolipids might replace phospholipids in membranes. High level of AsLp in cell membranes may decrease their integrity and make them more susceptible to damage. Membrane dysfunction, especially in case of thylakoids, subsequently results in deterioration of photosynthetic apparatus, both light phase associated with photosystems functions, and dark phase of  $CO_2$  assimilation.

The aim of the project is to establish basic model of arsenic phytoextraction and tolerance of C4 perennial grasses the example of *Miscanthus*  $\times$  *giganteus*. This model will be developed as the result of comprehensive and interdisciplinary studies on arsenic phytoextraction and its effects for two genotypes of *M*.  $\times$  *giganteus* ('Illinois' and 'Nagara') characterized by distinct reaction to As.

Described studies raised several questions about details of the reaction to As contamination in case of *Miscanthus* – being not only energy or utility crop, but also considered as a model for other C4 perennial grasses. The reaction to As needs to be investigated on subsequent levels of plant functioning: molecular, metabolic, physiological and finally growth and development. We expect that transcriptome analyses will allow to not only determine scale of changes in expression of genes responsible for cell homeostasis, but also to identify genes or gene families specifically induced/repressed by As stress.

We believe that knowledge gained in the outlined topics will contribute to determine key response elements of M. × *giganteus*, and in prospect – other C4 plants, to As contamination. The project results will be meaningful both for basic sciences, like **functional genomics**, **plant metabolomics**, **physiology**, **and ecology**, as well as disciplines of utilitarian importance, such as **phytoremediation**, **agronomy**, **bioenergetics and plant biotechnology**.