ORAL PRESENTATIONS

Ecotoxicological evaluation of soils in the Olkusz Ore-bearing Region

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The Olkusz region is one of the most polluted areas across Poland and Central Europe. Our aim was to estimate environmental threats affecting living organisms in the vicinity of Olkusz due to contamination with heavy metals. Furthermore, we wanted to investigate factors that might influence development of calamine grassland and metal-tolerant plant populations in the region.

We examined toxicity of soils with combination of bioassays (plants, crustaceans and bacteria; short- and long-term tests; detection of mortality and growth inhibition) and chemical analyses (mineralization with nitrogen acid and extraction with: water, 0.01 M $CaCl_2$, 0.02 M EDTA in acetic buffer pH 4.65, acetic acid according to BCR protocol; detection with AAS and ICPMS). Moreover, we performed plant and bacterial tests of mutagenicity to assess genotoxic effects of soils. We examined samples from: "the oldest" and "young" zinc-lead waste heap in Bolesław; forest in the vicinity of Olkusz; centre of Olkusz; post-flotation tailings landfilled in Bolesław and reference (non-contaminated) site in Central Poland.

Toxicity of investigated soils was confirmed by a set of various biotests, based on reactions of different organisms. The most serious toxic effects were caused by post-flotation tailings and soil from zinc-lead waste heap in Bolesław. The biological effects of soils were related to amount of bioavailable forms of heavy metals. Water extracts that were used in tests, contained only very low amount of elements (0.1–1.0% of total concentration). However, despite relatively low metal concentration, they were lethal for tested organisms. We also demonstrated that up to 50% of total metals was extracted from soils under low pH. This finding clearly point out potential risk of increase of the toxicity of soils in the vicinity of Olkusz due to change of soil acidity or redox conditions.

Both *Allium* and *umu* mutagenicity test yielded negative results, indicating the lack of hazard of genotoxic effects caused by soils from the Olkusz region. This result is significant for explanation of potential factors influencing the development of tolerant plant species across the Olkusz region, showing that the main force of micro-evolutionary processes on zinc-lead soils is selection rather than mutations.

Rhizosphere microbial communities of *Arabidopsis arenosa* (L.) and *Arabidopsis halleri* (L.) from heavy-metal polluted sites

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Heavy metals are known to pose a serious threat to soil organism and whole ecosystem. Although heavy metals exert a negative influence on living organisms, some plants are able to grow in metal-contaminated environment. Among them, *Arabidopsis halleri* (L.) and *Arabidopsis arenosa* (L.) received a lot of attention. The first one is a model for metal accumulation studies as a facultative zinc and cadmium hyperaccumulator, while the second occurs on moderately Zn-polluted soils and coincide with *Arabidopsis halleri* (L.) species (Przedpełska and Wierzbicka, 2007; Huguet et al., 2012).

The aim of the study was to compare the structural and functional biodiversisty of rhizosphere microbial communities of *Arabidopsis halleri* (L.) and *Arabidopsis arenosa* (L.) sampled at various heavy metal-polluted sites in Upper Silesia, Poland. In order to estimate structural and functional diversity of microbial communities, phospholipid fatty acid analysis (PLFA) and BIOLOG EcoPlates™ were used, respectively. The total, Zn- and Cd- resistant microbial fractions were determined using standard plate cultures. Additionally, physico-chemical parameters of tested soils were measured. Obtained data was subjected to multiple statistical analyses.

Analyzed soils were found to be significantly contaminated with bioavailable fraction of zinc and cadmium ions. For majority of tested parameters, a distinct rhizosphere effect was evident. Statistical analysis revealed, that *Arabidopsis arenosa* (L.) and *Arabidopsis halleri* (L.) show high degree of similarities, in terms of microbial structural and functional biodiversity. However, a various pattern of microbial diversity occurred at different sampling sites. This suggests, that although rhizospheres of analyzed plant species tend to be similar with regard to individual sites, a soil characteristics plays a major role in the development of specific microbial communities.

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Zinc tolerance and distribution in *Plantago lanceolata* L. – an interdisciplinary approach

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Plantago lanceolata L. (narrowleaf plantain) is a cosmopolitan, perennial and medicinal plant. It colonizes zinc-lead waste heaps in the Olkusz Region. We studied the morphological character and the level of tolerance to zinc of two populations of *P. lanceolata*, originated from: (i) zinc-lead waste-heap in Bolesław near Olkusz and (ii) non-contaminated site in Central Poland (Tuszyn Las). Plants originated from seeds collected in the field and grown under controlled conditions. The tolerance to zinc was examined both the short- and long-term tests.

Populations from zinc-lead waste heap in Bolesław and from the reference site differed in terms of plants' morphological character. Plants originated from Bolesław had fewer narrower leaves. However, generative shoots were more numerous and they appeared earlier, in comparison with the reference population. These differences indicate the morphological and ecological adaptations to grow under extremely unfavourable environment. Moreover, population from Bolesław showed an increased tolerance to zinc. The difference was proved for various plant development stages: (i) in the seedling stage (5 mg/L Zn^{2+}), (ii) in fully developed plant stage (75 mg/L Zn^{2+}). We conclude that population of *P. lanceolata* from the zinc-lead waste heap in Bolesław near Olkusz developed an inducible tolerance to zinc.

To examine the reasons of the tolerance phenomenon within *P. lanceolata* species, we used a combination of chemical and biological methods of detection of zinc in plants' organs, tissues and cells. Results of the measurement of zinc concentration (ICPMS) and zinc imaging in light, confocal and electron microscope, as well as semi-quantitative co-localization of various elements in tissues (LA ICPMS) will be presented.

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Contamination of food crops grown on soils with elevated content of heavy metals

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The Upper Silesia region is characterized by high contamination of heavy metals in soils. It is a result of past and present intensive mining and processing activity of non-ferrous metal ores in this area. The soils with the highest concentration of heavy metals are located in the Piekary Śląskie area, where agricultural fields cover as much as 50% of area (Nieć et al., 2013, Central Statistical Office). The essential health-risk factor may be consumer's consumption of the edible plants cultivated in studied area. Cadmium (Cd) and lead (Pb) were analysed in collected soil and vegetable samples to assess the risk. 21 soil samples and seven species of vegetables cultivated in the interested area (red cabbage, white cabbage, savoy cabbage, broccoli, carrot, potato, parsley) were collected. The concentration of heavy metals (Cd and Pb) was determined by optical emission spectroscopy with inductively coupled plasma (ICP OES). The soils pH was identified. The significant exceedance of the maximum allowable content in the majority of the soil and vegetable samples were indicated (range of concentrations: Cd – 3,52–68,47 mg/kg soil dry weight; Pb – 72,65–1516,51 mg/kg soil dry weight; range of concentrations for vegetables: Cd – 0,14–1,85 mg/kg wet weight.; Pb – 0,16–5,82 mg/kg wet weight). The allowable contents of heavy metals concentrations in vegetables in the case of cadmium were exceeded over 18 times, and in the case of lead – over 50 times (Comission Regulation EU, 2011).

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CENTRAL STATISTICAL OFFICE, LOCAL DATA BANK. http://www.stat.gov.pl

COMISSION REGULATION (EU) No 420/2011 of 29 April 2011 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs.

Phytoextraction and ecological catalysis: symbiosis for future

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Phytotechnologies such as metals phytoextraction are very attractive solutions for the reclamation of contaminated brownfields. However, the economic potential of these technologies remains poor, due to a lack of credible outlets for metal-contaminated biomass. Strong efforts are still necessary to recycle metals from contaminated-biomass, so as to develop the full economic potential of phytoextraction. This is particularly true in a context of resource depletion, with increasing metals prices and market volatility.

In this communication, we aim to present a new outlet for metal contaminated-biomass. Phytoextraction generates plant wastes, which are valued through an innovative concept of ecological recycling. Because certain plants are able to hyperaccumulate primary or strategic metals as Ni^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+} and Pd^{2+} can be directly used as catalysts in organic fine chemical reactions. Ecocatalysts derived from hyperaccumulators are useful for the synthesis of molecules with high added value in fine and industrial chemistry (aroma and cosmetics with the "natural" label, drugs and oligomers with biological interests, highly functionnalized aromatic heterocycles, chrial structures, key intermediate in various industrial chemical process and biopecticides). They allow the development of substitutive reagents to oxidants forbidden by REACH too. The new catalysts represent very efficient alternative to catalysts derived from metallurgy, with a three dimension domain of chemistry/ecology/environment.

This original approach brings the first perspective of enhancing the unique biomass and establishes a new field of Green Chemistry: Ecocatalysis. It is already a Green Revolution in Chemistry. Phytoextraction are source of innovation in organic synthesis and organic synthesis is the driving force of developed phytotechnologies. These results are the first use of metal-rich biomass in organic synthesis and constitute a strong encouragement for the economic development of phytoextraction programs for metal-bearing soils.

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Changes in species composition of calamine grassland after the removal of woody plants – effect of a conservation management

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In Olkusz region (the Silesian-Cracow Upland) due to very long time, lasting several hundred years, zinc and lead mine activity post-industrialized landscape was formed. The characteristic features of this area are waste heaps rich in heavy metals (Zn, Pb, Cd). These objects are grown by specific calamine vegetation which developed as a result of the spontaneous succession. One of such examples is more than 100 years old grassland in Bolesław, which unique nature and presence of many rare and protected plants make this object very precious from nature conservation viewpoint. This area was subjected to protection as an site of ecological interest also because of the occurrence of *Biscutella laevigata*. Until 80s of 20th century the area was only grown by single trees (*Pinus sylvestris*). The dry and toxic substratum limited succession of vegetation. In recent years Scots Pine rapidly spread what now is the main threat to calamine grassland. It was advisable to start active protection including such treatments as trees and shrubs removal (Kowolik et al., 2010).

In vegetation season 2008 in the area of 0.8 ha in systematic grid every 8 m permanent study plots were established. In total 120 plots (each of 1m^2) floristic inventory was done and cover of ground shaded by Scots Pine was assessed. In floristic composition species of alkaline, warm biotopes and heavy metal tolerant plants prevailed. During winter 2011 trees and shrubs of 75% study plots were eradicated. In 2013 new floristic inventory and analysis were performed. The species composition was changed; total number of species increased and number of species in particular plots both uncovered ones and those ones under Scots Pine canopy. New herbaceous plants appeared, among others, *Anthoxanthum odoratum, Reseda lutea* or *Trifolium repens* and shrub and tree seedlings: *Acer pseudoplatanus, Betula pendula, Populus tremula* and *Frangula alnus*. The contribution of grassland species increased in study plots especially in uncovered plots. The number of plots with participation of *Agrostis capillaris, Campanula rotundifolia, Dianthus carthusianorum, Linum catharticum, Scabiosa ochroleuca* or *Silene vulgaris* increased. The further monitoring of the object is needed to estimate the effects of trees and shrub removal.

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The use of chlorophyll fluorescence fingerprints as reliable bioindicator for heavy metals stress detection in plants

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Various chlorophyll fluorescence parameters have been used to detect different types of stress such as: low and high light, low and high temperature, water scarcity, salinity, nutrient deficiency, heavy metals and others. Typically, under stress conditions the value of some measured or calculated chlorophyll fluorescence parameters decrease, while others increase. However the level of these changes can indicate not only to alterations in photosynthetic efficiency of the sample, but also to a damage (non-reversible changes) of the photosynthetic apparatus. Heavy Metals are widely known as environmental pollutants resulting from manmade activities (Kalaji and Łoboda, 2007; Saxena et al., 2011). Photosynthesis is usually suppressed by high concentrations of heavy metals (Romanowska-Duda et al., 2005; Shaw et al., 2014), but the effect of an individual heavy metal can be specific for a given plant species and even cultivar. In this work, the application of chlorophyll a fluorescence kinetics is discussed as an informative tool for studying the effects of different environmental stresses, especially heavy metals, on photosynthesis process. A number of examples is given to illustrate the effects of short and long-term exposure to heavy metals on the quantum efficiency of photosynthetic appartus (Kalaji and Łoboda, 2007; Tuba et al., 2010). This work suggests that some of chlorophyll fluorescence parameters could be a good indicators to monitor the negative influence of heavy metals at early stages of their action.

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Impact of polluted environments on reproductive processes of plants representing different metallophyte status

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Summary of studies on the influence of polluted with heavy metals environments on sexual reproduction of plants from different genera and families and representing the status of obligatory and facultative metallophyte as well as so called new comers is present. The investigations focused on gametophytic phase of plant ontogenesis, double fertilization and seed development in specimens growing at post-industrial areas were initiated in the late 1990s by deceased Professor Romana Czapik and are continued till today in the Deartment of Plant Cytology and Embryology (e.g., Czapik, 2002; Izmaiłow, 2000; Kościńska-Pająk, 2000; Izmaiłow and Biskup, 2004; Słomka et al., 2012).

External environmental factors occurring on waste heaps (e.g., heavy metals in the substrate, strong insolation and winds, water deficit) reduced plant fertility resulting from developmental disturbances and degenerations in male and female lines. The frequency of abnormal male and female meiosis, degenerations of whole flower buds, ovules, anthers, archesporial cells, microsporocytes, microspores, pollen grains, precocious degeneration of anther tapetum, female gametophyte, embryo and endosperm cells, whole pro/embryos was highest in so called new comers, the taxa just started to colonize metalifferous sites. Therefore, the extend/scale/percentage of disturbances and necroses in embryological structures and processes could be a good feature to determine the plant tolerance to stress. Ecological plant embryology may be useful, complementary to characters at sporophytic level to examine the plant response to environmental stress.

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The conditioning of the industrial tourism within selected areas of Metropolitan Association of Upper Silesia

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The article focuses on issues related to preservation of the natural environment in areas of resource mining within the Upper Silesian Metropolitan Union (GZM) for tourism and recreation. Underground and surface mining has largely transformed the surface features and hydrological conditions, and caused changes in the orogen. The proceedings related to recostructurization of the mining industry include actions aimed and preservation of some of such areas as areas of environmental value. The article presents in more detail natural (geotechnical and hydrogeological) conditions which favor preservation and further development of post-mining areas. It also presents places which are of environmental value in terms of geodiversity which can be attractive for industrial tourism and geotourism. The paper also includes assessment of such areas in terms of their usefulness for tourism and recreation.

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Environmental protection and management of two metalliferous sites in Nord – Pas-de-Calais (France)

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During one century and a half, agricultural soils and natural areas located in Nord – Pas-de-Calais (France) have been greatly polluted by three major lead and zinc metallurgical industrial plants. These soils have been impacted by the storage of by-products (slag heaps exposed to weathering) and by the fallen of factory smokes. Industrial activities then indirectly helped the formation of calamine substrates (van Halluwyn et al., 1987), composed of metallophyte species such as *Armeria maritima* subsp *halleri*, *Arabidopsis halleri, Silene vulgaris* var. *humilis* and *Viola calaminaria*. These calamine substrates have been observed in Auby and Mortagne-du-Nord (both french towns). The concerned areas, approximately 400 000 squared metres, are mainly controled by local entities (Lemoine, 2012). They are also protected by Natura 2000 network. The environmental management is assumed by governmental entities specialized in Nature preservation (e.g. Nord departement Council, Scarpe-Escaut Regional Nature Park). Ecological restorations and research programs are their two main concerns.

The preservation of Peru park (Auby, France) is a textbook case. This polluted area was threatened by a project which consisted in the application of decontamination techniques. In order to preserve its calamine soil and its rich vegetation, important information and consultation procedures were conducted towards neighborhoods inhabitants (Delhaye et al., 2011).

An unexpected butterfly has recently been discovered on this area, the Mazarine Blue (*Cyaniris semiargus*). This insect, unknown in rest of the territory, may be using a flower, *Armeria maritima* subsp *halleri*, as a host-plant (Lemoine, 2013).

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Relationships between soil depth and arbuscular mycorrhizal colonization of grasses roots in soils contaminated with heavy metals

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It was demonstrated that most of seed plants species forms the symbiosis with the arbuscular mycorrhizal fungi (AMF). It was also documented that in non-contaminated soil the colonization of plant roots by AMF decreases with soil depth. However there is a dearth of data on AMF colonization at different depths in soils contaminated by heavy metals. The aim of the current study was to compare root colonization by AMF in different plant species and at various soil depths.

Investigations were conducted at two sites contaminated with Cd and Zn and at a control site. The roots and soil samples were collected at three depths (0 – 10, 10 – 20, 20 – 35 cm). Mycorrhizal research was carried out on roots of *Deschampsia caespitosa, Festuca ovina* and *Molinia caerulea*. Mycorrhizal colonization assessment was carried out according to Trouvelot method. The content of bioavailable metals in soil was assessed by extraction with 0,01 M $CaCl_2$.

It was found that at non-contaminated site AMF colonization of *D. caespitosa* roots decreased with soil depth. Conversely, for the same plant species AMF colonization increased with soil depth at the first contaminated site. The same tendency was observed for *F. ovina* and *M. caerulea* at the second contaminated site. The relationship of AMF colonization observed in both contaminated soils was negatively correlated with concentration of bioavailable forms of Cd and Zn.

The data presented above indicate that in soils contaminated with heavy metals AMF colonization increases with increasing soil depth, in contrast to non-contaminated soils. This opposite trend in root colonization by AMF is caused by high concentration of bioavailable forms of Cd and Zn in upper soil layers.

Genetic architecture of cadmium tolerance in the hyperaccumulator *Arabidopsis halleri*: fine-mapping and variability within the species

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The hyperaccumulators constitute powerful models to study the mechanisms of metal homeostasis, adaptation to extreme environment and evolution of complex selected traits. In the last few years, important progress has been achieved in our understanding of the mechanisms underlying metal tolerance and accumulation in the hyperaccumulator *Noccaea caerulescens* and *Arabidopsis halleri*. In *A. halleri*, three QTL for Cd tolerance were found using an interspecific cross between this species and a non-tolerant relative (Courbot et al., 2007). The major QTL was shown to co-localize with the HMA4 gene which was functionally validated (Hanikenne et al., 2008). Nevertheless, it was demonstrated that Cd detoxification in *A. halleri* requires expression of additional genes (Hanikenne et al., 2008). In order to pinpoint novel candidate genes, we have conducted the fine-mapping of the QTL CdTol3. We localized CdTol3 within a region of 2cM representing 70 genes in *A. thaliana*. Among these genes, two showed annotation linked to Cd and four are more expressed in *A. halleri* than in *A. thaliana* (Talke et al., 2006). In order to investigate the role of these genes in Cd tolerance, their expression profile in *A. halleri* was estimated and *A. thaliana* KO mutants were phenotyped. In parallel, genetic architecture of Cd tolerance and accumulation was investigated in an *A. halleri* population from a non-contaminated site. This population showed a smaller Cd tolerance than the one of the metallicolous population used in the study of Courbot et al. (2007). The objective is to compare the genetic architecture in populations from different edaphic type and displaying different levels of tolerance and accumulation.

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Population genetics of *Arabidopsis halleri* in Europe

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Arabidopsis halleri is a facultative montane and pseudometallophyte species involved in numerous studies of the adaptation to anthropogenic metal stress. In order to test the genetic representativeness of European accessions used in theses studies, we investigated the population genetic structure of *A. halleri* in Europe, using the phylogeographic framework. Microsatellite and nucleotide polymorphisms from the nuclear and chloroplast genomes, respectively, were used for genotyping. Large-scale population structure was characterized by a significant phylogeographic signal among two major genetic units. Localization of the phylogeographic break suggested long-standing isolation among populations on either side of the Alps. This was assumed to reveal allopatric glacial survival in Southern and Central Europe. Genetic isolation was shown to be maintained in Western Europe by the high summits of the Alps, whereas admixture was detected in the Carpathians. In this context, *A. halleri* accessions from either unit could reflect distinct evolutionary histories, and recent adaptation to metal stress within either unit should be considered as independent events.

Glutathione-mediated detoxification system in plants

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In last years we observe a gradual increase of heavy metal contamination in environment , metals presence, depending on their oxidation state, can be highly reactive, resulting in fitotoxicity. It manifests in the growth inhibition, nutrient composition heavy metal accumulation and functioning of the detoxicative system in these plants (Yadav, 2010).

Copper and zinc are essential components for normal growth and development of all organisms, as they serve as a cofactor for many physiological processes. However, it can be highly toxic at excessive level. Lead and cadmium are phytotoxic and nonessential for plants. Among the studied ions only Zn didn't inhibit growth of pea plants and in the first hours of cultivation caused an increase in root length. The growth and development of plants in heavy-metal polluted environment depends on their ability to activate efficient antioxidant and detoxicative mechanisms.

In the plants exposed to metal ions was observed increase of oxidative stress markers such as MDA, level of oxidative proteins, GSH/GSSG and ascorbate ratios, phytochelatins. The generation and localization of ROS in pea roots was determined by the confocal microscopy. In the early hours after metals application a increase of glutathione metabolism and antioxidative enzymes activity was measured. During a prolonged treatment the LA ICP MS was applied in order to map the elements in plant tissues in vivo.

Cu and Cd were the most toxic for plants, because they caused large generation of ROS in the pea cells. In the *Pisum sativum* roots exposed to four heavy metals, we observed the highest accumulation of Zn and Cu.

The response of pea plants to Pb, Cu, Cd, and Zn ions seems to depend on many factors: presence of other heavy metals in the environment, mineral content, plant species, condition and etc.

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Characterization of endophytic *Brevibacterium casei* MH8a strain and its use for the enhancement of Cd, Zn, Cu uptake by white mustard

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The excessive release of heavy metals into the environment poses a very serious problem worldwide. Due to the high toxicity of these metals, there is a need to remove them from contaminated soils. One of the most promising strategies is phytoextraction, which is defined as the use of plants to take up pollutants from contaminated soil. In recent years it has been revealed that plant-microbe interactions also play an important role in enhancing metal phytoextraction. Therefore, application of microbes has opened up a promising research area for the improvement of phytoextraction efficiency.

The aim of this study was to test whether the multiple-tolerant bacterium *Brevibacterium casei* MH8a isolated from metal-contaminated soil is able to promote plant growth and enhance Cd, Zn and Cu uptake by *Sinapis alba* L. under laboratory conditions. Moreover, the ability of inoculated, rifampicin-resistant strain ($MH8a^{rf}$) to colonize plant tissues was tested. Additionally, the mechanisms of the plant growth promotion and the ecological consequences of bioaugmentation on autochthonous microorganisms were examined.

MH8a strain exhibited the ability to produce 1-amino-cyclopropane-1-carboxylic acid deaminase, indole 3-acetic acid and HCN but was not able to solubilize inorganic phosphate and siderophore production. Soil inoculation with MH8a significantly increased plant biomass as well as the accumulation of Cd (208%) and Zn (86%) and Cu (39%) in plant shoots in comparison with those grown in non-inoculated soil. MH8arf was also isolated from roots as well as leaves of inoculated plants. This result indicated the endophytic properties of tested strain. DGGE and PLFA analysis revealed that the introduced MH8a had only a short-term influence on the structure of indigenous microbial communities. The plant growth-promoting properties of MH8a and its ability to increase the metal uptake connected with its long-term survival and short-term impact on autochthonous microorganisms make the strain a good candidate for the promotion of plant growth and phytoremediation efficiency.

Dysfunction of carbonic anhydrase Cah3 protein affects *Chlamydomonas* tolerance to Cd-induced oxidative stress

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Cadmium is known to be reactive oxygen species (ROS) inducer and photosynthesis inhibitor, in addition its toxicity depends on CO_2 concentration. Many microalgae respond to CO_2 limitation by the induction of a carbon concentrating mechanism (CCM). There are evidences that a CCM is the result of an interaction between DIC transport system and a compartmentation of Rubisco and carbonic anhydrase (CA, EC. 4.2.1.1). Cah3 is one of CAs located on the luminal side of thylakoid, functionally associated with the electron donor side of photosystem II. In this study we try to elucidate a role of Cah3 in tolerance of algal cells to cadmium induced oxidative stress.

Chlamydomonas reinhardtii wild type (WT) and its Cah3-deficient mutant (CC-2699) grown at 2.5% CO_2 concentration were exposed to $CdCl_2$ (95 M). In both strains the expression of ROS scavenging enzymes: catalase (CAT), ascorbate peroxidase (APX), chloroplastic and mitochondrial isoforms of superoxide dismutase (SOD) was analyzes. The intracellular level of hydrogen peroxide and photosynthetic oxygen evolution was also monitored.

Untreated cells of CAH3-def. mutant exhibited 3-fold higher expression of chloroplastic (Fsd1) and mitochondrial (Msd3) isoforms of SOD, CAT (Cat1) and APX (Apx1) as compared to WT strain. It was accompanied with 2-fold higher intracellular H_2O_2 concentration in the mutant cells than in WT ones. In WT cells, Cd treatment resulted in 7-fold increase in H_2O_2 concentration along with significant increase in transcript level of Fsd1 (4-fold), Msd3 (3-fold) and Apx1 (2.5-fold) genes. In CAH3-def. mutant cells only 1.2-fold increase in H_2O_2 concentration and 1.5-fold change of antioxidative enzymes transcript was noticed. Photosynthetic activity and CA total activity was more affected by Cd in WT than mutant cells, but in the both photosynthesis was transiently inhibited.

In conclusion, due to markedly higher level of transcripts of ROS scavenging enzymes, cells of CAH3-def. mutant possess a better ability to overcome oxidative stress induced by cadmium than WT cells.

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Zinc content in the substrate and flowering time in *Arabidopsis arenosa* (Brassicaceae)

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Flowering time is an important character that enables plants to adapt to their local environments and the onset of flowering is thought to be one of the key events in plant life cycle influencing most important plant characteristics as: seed set, interactions with pollinators and thus genetic variability. Taking into account an important role that zinc plays in major life processes in plants we were interested to see if it has also an impact on the onset of flowering. This question has never been raised before and the metal has never been considered as a factor influencing flowering time in plants. It seemed to us, that *Arabidopsis arenosa* may be a convenient plant model to investigate this question. Previous research (Przedpełska and Wierzbicka, 2007) showed that two different population types exist within *A. arenosa*: (1) metallicolous populations – growing on heavy-metal-polluted calamine soils rich in Zn, (2) non- metallicolous populations – growing on soils with normal Zn content.

The present study was aimed to test the following hypotheses:

1. Zn content in the growth medium promotes the onset of flowering in *A. arenosa*.
2. Changes in the onset of flowering induced by Zn depend on Zn concentration employed.
3. Zn-induced early onset of flowering is a universal plant response present within the species and is not an effect of stress or physiological adaptation to high Zn content in the environment.

Our results indicate that Zn ions present in the growth medium promote early flowering in *A. arenosa* and that this effect may depend on Zn concentration used. Zn-induced early flowering in *A. arenosa* seems to be a universal plant response present within the species and is not an effect of stress or physiological adaptation to high Zn content in the environment.

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The collection of metallophytes in the Scientific Herbarium of the University of Silesia (KTU-HMP)

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Collection of metallophytes in the University of Silesia Scientific Herbarium (KTU) (Rostański and Gerold-Śmietańska, 2008) currently comprises about 2,000 specimens of vascular plants, collected in Southern Poland (Silesian-Cracow Monocline) and other metalliferous (heavy-metal-enriched) sites in Europe: the Harz Mountains, North Rhine-Westphalia (Germany), La Calamine, Moresnet and Plombières (Belgium).

The collection includes:

1. Specimens of known metallophytes and hyperaccumulators from historical localities (e.g. V*iola calaminaria, Thlaspi caerulescens, Minuartia verna, Armeria maritima* ssp. *halleri*).
2. Specimens collected from anthropogenically polluted areas with known content of metals in the soil (published data).
3. Specimens collected from the typical heavy-metal-polluted areas (e.g. dumps, sedimentation pools, flotation dumping grounds etc.) with unknown level of metal contamination (unpublished data).
4. Specimens from experiments, grown on substrates enriched with heavy metals (published or in press studies, exact metal concentration in the substrate is usually known).

The collection is complemented by a database of morphotypes and genotypes of metallophytes, containing data about their occurrence, the date and authorship of field observation or experiment. Each specimen will be eventually scanned and high quality image will be made available through the Internet.

The presentation of this collection is also intended to be an invitation for researchers to deposit their plant material from the field excursions or laboratory experiments in order to preserve it and make it available for other researchers.

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Biomineralization on conifers growing on ultramafics under Mediterranean bioclimate (California, Spain)

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Ultramafic rocks are common geological materials which weathering originates soils of basic or neutral pH, high levels of magnesium, nickel, iron, and other metallic elements such as chromium or cobalt, and low contents of nutrients, specifically Ca. The characteristics of these habitat entails a specific vegetation known as serpentine or ultramafic vegetation that usually shows marked differences with vegetation of adjacent substrates as a high level of endemic taxa.

Some good examples of ultramafic territories with a Mediterranean macrobioclimate are found in SW Europe (Andalusia, Spain) and SW North America (California, USA). The western Spanish Betic Ranges include the largest area of ultramafic rocks (> 400 km^2) in the Iberian Peninsula. The vast distribution area of the ultramafics throughout Californian landscape covers from the Coastal Mountain Ranges to the foothills of Sierra Nevada in a W-E transect and from the Mountains of Klamath-Siskiyou to the southern Coastal Mountain Ranges. All these territories are characterized by high endemicity due to their numerous specialized taxa known as serpentinophytes, both obligate (serpentine endemics) and facultative (able to grow also on other substrates).

Conifer species support the inhospitable conditions of ultramafic soils ranging on a wide spectrum of habitat features. We are working on facultative and obligate serpentinophyte tree conifers using a comparative study by means of inductively coupled plasma mass spectrometry (ICP-MS), scanning electron microscopy coupled with energy dispersive X-ray analysis (SEM-EDX) and X-Ray diffraction analysis (RX).

Results indicate the absence of Ni-hyperaccumulators, but the presence of high concentrations of other metals (Fe, Al, Co, Cr). In general, all the conifers studied had Ca/Mg ratios over 1, and processes of biomineralization of Ca, Mg and other elements, being whewellite the most common biomineral in these taxa.

This kind of studies can help to understand the species adaptation to extreme habitats (such as ultramafics) and support the strategies for plant conservation in these peculiar territories.

Relationships between root hairs and toxic effect of Cd on photosynthesis in barley (*Hordeum vulgare* L.)

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The aim of the present study was to find relationships between root hair development and the toxic effect of Cd on photosynthesis in barley.

Experiments were carried out with barley cv. Dema and its root hair mutants (*rhp1.a* – root hair primordia only; *rhs2.a* – root hairs significantly shorter; *rhi1.a* – root hair pattern irregular) (Janiak and Szarejko, 2007). Three-days-old seedlings of barley were cultivated in the hydroponic system with the basal medium for 3 weeks and then 10 or 25 μM Cd for the next 5 days were applied. On the 5th day of the cadmium treatment, the chlorophyll *a* fluorescence with Pocket-PEA Fluorimeter (Hansatech Instruments Ltd., England) and the length of root systems were measured.

It was found, that all selected parameters (energy absorbed, maximum trapping flux, electron transport, dissipation, and active reaction centers per excited cross-section leaf area) quantifying the behavior of PSII of barley leaves were higher for cv. Dema in comparison with the mutants in the control medium. By contrast, in the presence of Cd all parameters studied were lower in cv. Dema when compared to mutants.

In the control medium the roots were longer in mutants than in cv. Dema. Cadmium had no influence on the roots length in Dema and *rhp1.a*, whereas in mutant *rhi1.a* an increase in cadmium concentration enhanced root length. In *rhs2.a* Cd caused a decrease in roots length.

On the basis of the above data it is proposed that reduction of root hairs, and in consequence – of the root system absorption area, diminish the toxic effect of cadmium on photosynthesis in barley. The toxic effect of cadmium significantly decreased the length of roots in *rhs2.a*, which could explain the lack of negative influence of Cd on all selected parameters of photosynthesis in this mutant.

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Do lichens function as enclaves for mite fauna on bare toxic post-industrial dumps?

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In this project we examined oribatid fauna of strongly contaminated post-smelting dumps (southern Poland) that exists in the substrate and is associated with the most frequent lichen *Cladonia rei*. In spite of the possible deterrent or even toxic effects of several lichen products, some of the oribatid mite species feed on lichens (Seyd and Seaward, 1984). Although studies of oribatid mites exposed to metal contamination were carried out in the past (e.g. Caruso et al., 2009; Skubała and Zaleski, 2012), their response to such strong pollution as observed at the studied sites and the association of mites with lichens in contaminated habitats have not been elucidated so far. In total 2936 specimens of Oribatida, representing 50 oribatid species, were sampled on ten dumps and the reference site.

Both abundance and species richness of oribatid fauna collected from *Cladonia rei* thalli were significantly higher than recorded in the dump's substrate. Thalli of *C. rei* act as an island for soil oribatid mites on extremely contaminated post-smelting dumps. Oribatid mites may resist heavy metal contamination by selecting less-contaminated microsites and they possibly use them for feeding on fungal mycelia and organic matter debris that is trapped in the lichen thalli. The pool of oribatid species able to persist in extremely high doses of heavy metals was comparatively broad. However, only one species *Tectocepheus velatus* was able to achieve high abundances on all dumps. Three different responses of species (tolerant, sensitive and indifferent) to heavy metal contamination were recognised. Redundancy analysis indicates that highly elevated levels of heavy metals, as well as K content, C/N ratio and pH value, were the main factors that influence the composition and distribution of species.

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Adaptation, microevolution and speciation of violets (*Viola* L., Violaceae) from metalliferous sites

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The review of our long-term studies on adaptation, morphological variability, genetic differentiation and speciation under the influence of heavy metal polluted environment is based on selected representatives of *Viola* L. genus. Nine taxa from two sections (Viola, Melanium) occuring on calamine (Zn/Pb) and serpentine (Ni/Cr) soils, representing status of facultative metallophyte (growing at metalliferous and non-metaliferrous sites) and obligatory metallophyte (growing at metalliferous sites exclusively) were studied. Biosystematic investigations including morphological, microstructural, embryological traits, different nuclear molecular markers (AFLP, ISSR, ITS) combined with estimation of arbuscular mycorrhizal colonization and soil characteristic allowed us to establish the impact of polluted environment on: (1) intra- and interpopulation morphological and genetic differentiation in facultative metallophytes, (2) the origin (ancestor taxon findings) of several obligatory metallophytes, (3) the role of mycorrhiza (AMF) in metal tolerance, (4) tolerance's costs revealed in sexual reproduction (Hildebrandt et al., 2006; Słomka et al., 2008, 2011, 2012; Kuta et al., 2012, 2014).

In conclusion: polluted with heavy metals environments are an excellent "laboratory" to investigate microevolutionary and speciation processes. Interpopulation genetic differentiations of *Viola tricolor* and *V. riviniana* (including interspecific hybridization) are the examples of ecotypes formation (metallicolous vs. non-metalicolous populations). Obligatory metallophytes occurring exclusively on calamine soils (*V. lutea* ssp. *calaminaria* and *V. lutea* ssp. *westfalica*) evolved from non-metallophyte taxon *V. lutea*. The origin of several species from serpentine soils (*V. albanica, V. dukadjinica, V. raunsiensis, V. macedonica, V. aetolica*), which are genetically (ITS markers) indistinguishable but well morphologically defined, is still uncertain. All violets, with the exception of *V. albanica*, were AMF positive, all showed slight to moderate disturbances in reproductive processes but seeds production allow them to form permanent, rich in individuals populations.

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Soil heavy metal pollution at sites left by historical Zn-Pb ore mining: implications for the environment

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This study surveyed sites left by former Zn-Pb mining in western Małopolska (S Poland), focusing on old heaps of mining waste rock ("warpie") located in agricultural land and suburban wasteland. Topsoil samples were taken from 65 heaps (macro-scale study) and characterized using many parameters, including heavy metal contamination, macronutrient content, pH and texture. Moreover, plant species richness and composition as well as soil microbial parameters (biomass, urease and arylsulphatase activities, the colonization potential of arbuscular mycorrhizal fungi) were estimated. At five sites, a short transect was delineated from heaps towards their surroundings (micro-scale study) to estimate the impact of the heaps on metal concentration in the adjacent agricultural soil. The total heavy metal concentration in the heap soil varied greatly across sites, ranging from 5 to 522 mg Cd kg^{-1}, 94 to 23006 mg Pb kg^{-1}, 6 to 51 mg Tl kg^{-1} and 394 to 70435 mg Zn kg^{-1}. The values were very high compared to these measured in the soil of the control areas: 2–5 mg Cd kg^{-1}, 13–67 mg Pb kg^{-1}, 1–17 mg Tl kg^{-1} and 63–476 mg Zn kg^{-1}. In the micro-scale study, the amount of heavy metals in soil generally decreased when increasing the distance from the heaps. Despite this trend, the metal concentration in agricultural soil sampled near the heaps remained well above the values recorded from the control sites.

Soil pollution, represented by exchangeable Cd, was strongly correlated with available P content (negatively) as well as species composition of plant communities. The lowest values of all microbial parameters were recorded for the most polluted sites. This might be not only the effect of high metal concentrations and low P availability in soil, but also the effect of the plant community identity: the *Carlino acaulis-Brometum erecti* association dominating on most heaps differed between sites with high (the *festucetum ovinae* variant) and low (the typical and *rubietum caesi* variants) heavy metal concentrations.

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Vegetation of thermal soils in Iceland – an interesting model for studies on plant adaptation

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Geothermal activity is very common in Iceland. This natural phenomenon attracted attention of many scientists, especially geologist as well as experts in the utilisation of geothermal energy. Although geothermal systems create unique environments for plant growth, research focused on geothermal vegetation is still in its initial phase. Very few studies have addressed the problem of geothermal ecosystems and vast majority of published works is purely descriptive.

In Iceland geothermal systems can be divided into two main categories: low and high temperature type (Arnórsson et al., 2008). Low temperature areas (with temperature below 150°C in the uppermost 1000 m of the system) are located throughout Iceland and can be classified as a tectonic phenomenon. High temperature areas (with temperature above 200°C in the uppermost 1000 m of the system) extends from south-western to north-eastern part of the country and follow the zone of active volcanism (Arnórsson et al., 2008).

Vegetation of geothermal areas in Iceland was examined during the last decade. The main aim of this project was to characterise vegetation in relation to physical and chemical parameters of the substrate (Elmarsdóttir et al., 2003; Elmarsdóttir and Vilmundardóttir, 2009). Results showed that geothermal environments in Iceland are characterised by the presence of gradients in substrate temperature, humidity, pH and element content that have an influence on species diversity.

The presence of steep environmental gradients as well as clear differences in plant distribution along these gradients suggest that local adaptation might be one of the forces responsible for the emergence of the flora of geothermal sites. We believe that geothermal environments in Iceland may serve as an excellent "field laboratory" for studies on plant response to such abiotic stress factors as heat, pH and element content.

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The problem of pollution on the railroad tracks in north-eastern Poland

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There are very few articles focused on research concerning environment pollution caused by railway transport. Among them we can mention some studies carried out in the north-east of Poland by the research group from Faculty of Biology, Warsaw University. The results of those proved the railway transport to be a source of soil and organisms pollution with heavy metals, PAH, PCB, oil-derivative compounds and pesticides (Wiłkomirski et al., 2011).

The species composition of plant communities occupying railway areas has been recently described for north-eastern Poland (Galera et al., 2011, 2012). Among the found species, *Geranium robertianum* L. was distinguished from the rest by its abundance on railroad tracks in north-eastern Poland, although usually it prefers shade and fertile forest habitats. The railway embankment is an extreme environment for plants, with lack of water and nutrients, as well as high insolation and regular herbicides treatment. It was shown that separate form of *G. robertianum* had arised on the railroad tracks, known as 'railway-wandering plant' (Wierzbicka et al., 2013).

We have carried out assessment of toxicity and pollution levels of soils collected from railroad tracks of the chosen train stations in north-eastern Poland. The contaminants detected in the soils were oil-derivative compounds, PAH, PCB and heavy metals, which caused growth inhibition or death of test organisms from all of the trophic levels. However, concentration values of these substances in the soils did not exceed polish standards for communication areas. This indicates a synergistic effect of contaminants on organisms.

Based on the obtained results we found that railway transport can be a potential threat for environment.

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