POSTERS

Plant tests as a tool to assess toxicity of soils from the Olkusz Region

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Plant tests are sensitive and cost-effective tools for investigation of biological effects of toxic substances. They are commonly used in detection of chemicals and pharmaceuticals. We aimed to verify applicability and sensitivity of two plant tests to assess toxicity of environmental samples – soils from the Olkusz Ore-bearing Region.

Soil samples originated 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. We used *Allium* test, in which inhibition of *Allium cepa* L. adventitious roots growth and cell divisions (mitotic index) during cultivation in soil were examined. Onions was cultivated for 4 days in soils. Afterwards, concentrations heavy metals in *A. cepa* roots were measured. We also applied *Lemna* test, in which inhibition of vegetative reproduction and surface of plants was measured. Individuals of *Lemna minor* were incubated for 7 days in water extracts of soils. Ultrastructural changes in cells were observed in transmission electron microscope.

The *Allium* test demonstrated that the most serious toxic effects were caused by post-flotation substrate, followed by the substrate from "young" zinc-lead waste heap in Bolesław. Root growth was inhibited. The intensity of cell divisions in meristematic region of root tip decreased within 24–96 hours; however, there were no mutagenic effects, e.g. disturbed chromosomes, micro-nuclei, etc. Onion roots that were cultivated in the most polluted soils, accumulated high concentrations of zinc, lead, cadmium and thallium. High content of lead was also detected in roots of *A. cepa* grown in soil from Olkusz. The *Lemna* test confirmed the toxicity of abovementioned samples. Plants incubated in water extracts showed strong inhibition or lack of vegetative reproduction despite relatively low concentration of heavy metals in solutions. We confirmed high sensitivity of *Lemna* test in environmental pollution monitoring. Plants exhibited clear differences on the cell level, manifested in increasing degradation of chloroplast, nucleus and cytoplasm structure.

Photosynthetic activity of *Chlamydomonas reinhardtii* wild type and CC-2699 mutant under Cd-stress

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Carbonic anhydrase (CA, EC 4.2.1.1) catalyze the reversible reaction $CO_2+H_2O \leftrightarrow HCO_3^-+H^+$ providing a substrate (CO_2) for Rubisco. To date, three gens families encoding distinct classes of CAs have been identified (α-, β- and γ-CA) in *Chlamydomonas reinhardtii*. Among them, α-type lumenal CAH3 is the key enzyme required for the optimal function of the water oxidation complex at the donor side of PSII. Cadmium inhibits photosynthesis, but the exact mechanism of this inhibition remains unclear. The aim of this work was to assess whether CAH3-deficiency affects photosynthetic activity of *Chlamydomonas reinhardtii* cells grown in Cd-induced stress conditions.

C. reinhardtii wild type (WT) and CC-2699 mutant (CAH3-deficient) grown at 2.5% CO_2 concentration, were exposed to $CdCl_2$ (95 μM). In the 3rd, 6th and 12th h of exposure, photosynthetic O_2 evolution, total CA (CAtot.) activity, CAH3 protein amount, chlorophyll *a* fluorescence (OJIP), Rubisco large subunit (*Rbs L*) expression and total activity of superoxide dismutase ($SOD_{tot.}$) were examined.

The western blot analysis allowed us to confirm the presence of CAH3 protein in WT strain but not in CAH3-def. mutant. In mutant cells, up to 3-fold lower $CA_{tot.}$ activity as compared to WT was detected which suggests the significant contribution of CAH3 in total CAs activity. Over 4-fold higher level of *Rbs L* transcript in CAH3-def. cells could be interpreted as partial complementation for low CAs activity, thus low CO_2 supply for carboxylation reactions. The control cells of both strains showed similar photosynthetic O_2 evolution but WT appeared to be more sensitive to Cd than mutant. Although the OJIP measurements showed that photochemical energy conversion was transiently inhibited in Cd-treated cells of both strains, O_2 evolution and $CA_{tot.}$ activity were inhibited only in WT. Because in CAH3-def. cells $SOD_{tot.}$ activity was up to 2.5-fold higher as compared to WT, we suppose that one the reasons of higher mutant's resistance to Cd may be more efficient defense against oxidative stress induced by this metal.

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The influence an incineration ash for phytoavailable of heavy metals

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Incineration ash applied to the soil, due to its physicochemical specific properties to the immobilization of heavy metals in the soil, thereby limiting their transport to the plants (Antonkiewicz, 2011; Derkowski, 2001). The aim of the study was to determine the effect of incineration ash and increasing of cadmium in the soil on yield and uptake of heavy metals by plants.

Studies on the effects of incineration ash introduced into the soil contaminated with cadmium, zinc and lead on yield and uptake of heavy metals by maize (*Zea mays* L.) was carried out under the three-year pot experiment. In a pot experiment incineration ash applied to the soil in an amount of 23,33 g · pot^{-1}, corresponding to 20 t · ha^{-1} and the increasing doses of cadmium in an amount of 3–15 mg Cd · kg^{-1} d.m. soil. The experiment scheme included also a control containing a mineral soil and ash only. The use of ash and cadmium in amounts of 3–5 mg · kg^{-1} d.m. to the soil significantly influenced the increase on the yield of the aerial parts and roots of maize. However applying successive doses of cadmium (7,5–15 mg · kg^{-1}) to influenced in a significant decrease on the yield of the tested plant. Research shows that applying incineration ash depression decreased the yield of maize.

Increasing doses of cadmium applied to the soil mixed with ash significantly influenced the content of the metal in maize, whereas reduced content of Zn and Pb in the tested plant. In measuring the Cd content in maize by the number of border drawn by IUNG-PIB was found exceeding the permissible content of this metal in maize. However the content of Zn and Pb in maize corresponded to meet the same requirements to the fodder quality. Increasing doses of cadmium applied to the soil mixed with the ash uptake influenced the increase of this element by maize, and to reduced uptake of Zn and Pb.

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DNA damage in neuroblasts of grasshoppers from variously polluted sites, treated with metals in laboratory conditions

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The main aim of this study was to assess the amount of DNA damage in grasshoppers larvae originating from variously polluted meadows, and treated with metals in the laboratory. This way we tried to estimate the role of possible preadaptation of the insects to heavy metals.

Adult grasshoppers (*Chorthippus brunneus*) were collected at two heavy metal polluted sites (Olkusz, PI* = 12.1, and Szopienice, PI = 22.7), and at reference one (Pilica, PI = 0.53). The insects initiated the maternal culture and egg pods laid by the females from the sites were successively collected. After the diapause (12 weeks at 4°C) the larvae, immediately after hatching, were exposed to Zn, Cu Cd, or Ni for 24 h in laboratory conditions. The level of DNA damage was detected by comet assay method.

The level of DNA damage in *C. brunneus* larvae cells caused by biogenic metals (Zn, Cu) was site- and dose-dependent, that suggests some form of physiological adaptation to metals in their habitats. The significant increase of damage in larvae originating from Pilica at Cu concentration as high as 100 μg/ml was observed. Offspring from Olkusz site showed the highest DNA damage at Cu concentration of 1000 μg/ml. Moreover, the level of DNA damage in cells of larvae originated from Olkusz was not affected by Zn. Nickel provoked the highest DNA damage at concentration of 10 μg/ml, whereas at concentration of 100 μg Zn/ml the level of damage was low, and comparable to the control groups in grasshoppers from all examined populations. Application of Cd to larvae at concentrations covering the range of 0–10 μg Cd/ml of drinking water during 24 h did not cause unequivocal elevation of DNA damage. Apparently cadmium (or any byproducts of its action) does not reach the larvae brain. It is also probable that either the concentration of Cd or the duration of exposure were not high enough or long enough to affect DNA in neuroblasts of larvae.

*Pollution Index – $PI = C_1/C_{p1} + C_2/C_{p2} + \cdots + C_n/C_{pn}$

where C_1, C_2, … C_n the concentrations of particular contaminants in a given area and C_{p1}, C_{p2}…C_{pn} the maximum permissible levels of contaminants.

The possibility of beech forest regeneration on habitats transformed by exploitation of zinc and lead ores

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In the Silesian Upland zinc and lead ores are associated with Middle Triassic dolomites and limestones. Traces of their exploitation are visible until today in many places of the Upland as "warpie"-called excavations, i.e. funnel-shaped pits surrounded by a ring of overburden. Some of them are currently covered with forest or scrub.

Beech forest growing on the site of the former zinc and lead ore mining in the area of four Triassic hills in the Trzebiesławice vicinity, north east to Dąbrowa Górnicza was investigated. Beech woods are natural potential vegetation of these hills. Forest existing here currently developed as a result of forest regeneration in places where it was significantly destroyed. On the map of 1982, parts of the hills mentioned above disturbed by excavations are designated as areas covered by sparse forest. Currently the beech stand is 50–90 years old. Sixty phytosociological relevés made in the parts of hills disturbed by exploitation were the basis for the assessment of forest regeneration. Floristical analysis of the undergrowth composition in these phytocenoses revealed that they were rich and characterised by large share (qualitative and quantitative) of ancient woodland plants and small share of non-forest plants. Some thermophilous and calciphilous species were also observed, even in places with northern exposure and situated at the foot of hills. This is undoubtedly the effect of ore mining, which resulted in the extraction of limestone to the surface.

Statistical analysis identified 5 separated groups among investigated phytocoenoses: patches without the dominants but with significant share of different woodland species, 2 types of patches dominated by *Mercurialis perennis* (one with significant share of ancient woodland species and other with smaller their participation) and 2 types of patches with *Convallaria majalis* as a dominant (with the same group of species as differentiating factor). Floristic differences reflect the different values of habitat factors (soil moisture, fertility and pH as well as light conditions) occurring here in various combinations. The obtained results allow to conclude that the investigated beech forests reached the optimal stage of renewal despite the fact that they were formed in areas very disturbed by former exploitation of heavy metal ores.

Assessment of lead and cadmium concentrations in *Viscum album* L. herb in Silesian Upland

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European mistletoe *Viscum album* L. is a semi-parasitic shrub native to Europe and western and southern Asia. In Poland it parasitizes mainly on poplars *Populus* sp., lime trees *Tilia* sp., birches *Betula* sp., maples *Acer* sp., rowans *Sorbus* sp., and fruit trees (Strzelecka and Kowalski, 2000). Mistletoe herb *Visci herba* is was collected from wild-growing specimens (van Wyk and Wink, 2004; Senderski, 2009). Active principles of this raw material are toxic proteins: viscumin and lectin (Olsenet al., 1982; Urech et al.,2006). Other compounds are: flavonoids, liganes and other phenylopropanes, biogenic amines, cyclophenols, phytosterols (Kohlmünzer, 2000; Frohne, 2007). Herb infusions and extracts administered internally have cardiotonic, antihypertensic, antisclerotic, and anticancic action (Strzelecka and Kowalski, 2000; Lamer-Zarawska et al., 2007). Lectin in larger doses induces cancer cell apoptosis. Cytotoxic action is also reported for viscumin (Stirpe et al., 1982; Frohne, 2010). Standarised preparations containing lectins ML I are immunostimulants (Frohne, 2010).

In 2013 we started a study on capability of harvesting *Visci herba* from Silesian Upland. A preliminary assessment of usefulness of this stock entails Cd and Pb concentrations in this stock. Relationships between Pb and Cd level in stock and situation of localities from which samples were collected can be pictured, especially in relation to pollution emitters.

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Analysis of cadmium tolerance trait in *Arabidopsis halleri* (Brassicaceae)

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Arabidopsis halleri is considered as one of the best model to study adaptation to extreme metallic conditions. While Cd tolerance seems to be constitutive, the mechanisms underlying the trait is still poorly understood. Courbot et al. (2007) have investigated the genetic architecture of Cd tolerance in *A. halleri* using interspecific crosses (BC1) between this species and *A. lyrata* ssp *petraea* (non-tolerant and non-accumulator relative). A major QTL region was identified, co-localizing with *HMA4*, whose role as genetic determinant in Cd tolerance has already been confirmed in *A. halleri* by means of RNAi (RNA interference) (Hanikenne et al., 2008). Nevertheless, although responsible for 43% of the phenotypic variability for Cd tolerance, *HMA4* has been proved to be necessary but not sufficient for determining this trait (Hanikenne et al., 2008). After fine-mapping, it has been possible to identify a candidate gene for the second QTL (23.7% of phenotypic variance).The candidate gene *CdTol2* codes for a cation transporter localized in the vacuolar membrane, especially in the mesophyll tissue. Preliminary analyses showed a correlation between *CdTol2* expression and Cd tolerance in BC1 individuals. For a better understanding of Cd tolerance in *A. halleri*, we are currently making the functional analysis of the candidate gene *CdTol2* (expression profile, *A. thaliana* KO mutants, expression of *AhCdTol2* in *A. thaliana* mutant).

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Zn-Cd-cross-homeostasis – molecular and physiological approach

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Cadmium (Cd) is one of the most toxic non-essential element for living organism even at low concentration. Cd is taken up and transported across plant membranes mainly by transporters for metals that are essential for plants such as zinc (Zn), iron (Fe), manganese (Mn), calcium (Ca).

In comparison to other annual crop plants, tobacco (*Nicotiana tabacum*) accumulates Cd in significant quantitis in shoots. When it grows on low-Cd contaminated soil Cd concentration in shoots is higher than in roots. For this reason tobacco is an interesting object to study mechanism contributing to the uptake and distribution of Cd. It has also potential for fitoremediation purpose.

HMA4 is a transmembrane protein exporting Zn and Cd from the cytoplasm to the apoplast. It is expressed in cells surrounding xylem vessels and involved in the control of root-to-shoot translocation of Zn and Cd. Thefore, we transformed tobacco with *Arabidopsis thaliana HMA4* gene under control of CaMV35S promoter in order to further increase Cd concentration to shoots for biotechnological purpose. Transgenic and wild type (WT) plant were grown in the medium with a range of Zn and Cd concentrations (low/high Cd, low/high Zn). The observed differences between transgenic and WT in Cd and Zn accumulation depend on Zn and Cd concentration in the medium and their mutual proportions. For example, at low Cd/low Zn regimens, Cd concentration in roots and shoots of transgenic plants was reduced in comparison to WT. Upon high Cd/low Zn, Cd concentration in shoots of transgenic plants was higher than in WT. At both these conditions Zn concentration in shoots was higher in transgenics than in WT.

Further it was examined how the expression of *AtHMA4* affects the expression of Zn homeostasis genes in roots upon a range of Zn and Cd concentrations.

Based on performed analysis the major pathways that were altered in transgenics, and possibly contribute to the generation of phenotype of transgenic tobacco plants could be proposed.

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The plant cell wall in response to trace metals

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Tip growing protonemata of *Funaria hygrometrica* were used as model for studying the effects of Pb exposure (1000 μM Pb, 4h) on plant cell walls (CWs). The experiments showed that plant CWs were markedly remodeled. The most striking difference was the appearance of CW thickenings (CWTs) containing high amount of low-methylesterified pectins (up 40%) which were absent from the tip CW of control protonemata and are known as the compound able to bind Pb^{2+}.

Recently, CWTs formation has also been detected in root tips of both *Populus tremula* × *P. tremuloides* and *Arabidopsis thaliana* after Pb exposure (1000 μM Pb 4h and 32 μM Pb 24h respectively). Microscopic analysis (transmission electron microscopy – TEM) of CWTs showed that they varied in size and morphology but they usually indicated high amount of Pb. Immunogold method, with use JIM5 antibody, showed moreover that CWTs were especially abundant in low-methylesterified pectins (JIM5 pectin epitope) – able to bind metal ions. Furthermore, gold particles indicating the occurrence sites of JIM5 pectin epitope were commonly placed adjacent to Pb deposits. It indicated a close colocalization of the two compounds. This result was additionally supported in poplar roots by X-ray microanalysis connected with TEM.

Obtained results showed that plant CWs may be intensively modified in response to Pb. The most striking difference is the appearance of CWTs containing high level of low-methylesterified pectins and able to accumulate large amount of Pb deposits. Therefore, we can conclude that CWTs appear to be a very important repository for Pb^{2+}. Moreover, CWTs formation in different species and types of plant cells suggests that it may be a common plant tolerance strategy to Pb.

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Cabbage can overwhelm stress triggered by chemical enhancers of phytoextraction

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Cabbage can be used in phytoremediation due to its fast growth and large biomass allowing to take up heavy metals (Kusznierewicz et al., 2012), although its capacity to remove e.g. cadmium from soil is 8-fold lower than that of *Thlaspi caerulescens* (Bączek-Kwinta et al., 2011). As the use of synthetic chelators can increase heavy metal accumulation in plants used for phytoextracion (Luo et al., 2005), the aim of the study was to establish the benefits of using EDDS (S,S-ethylenediaminedisuccinic acid) and citric acid in a field study. The chemicals (50 mM) were added to the soil once, 6 weeks before cabbage harvesting. Control plants were treated with distilled water. All treatments comprised 5 replicates (plants).

EDDS was more efficient as the enhancer of metal accumulation by plants than the citric acid, although it rapidly triggered the toxicity symptoms on leaves (wilting, photosynthesis alterations and necroses). Plants were gradually overwhelming stress, however, and EDDS treatment increased cadmium, iron, manganese and zinc contents in their heads, but the effect was slight. Based on the results, the advantage of the use of synthetic chelators in case of cabbage-mediated phytoextraction is not obvious. Interestingly, both enhancers altered distribution of some metals within the individual plant, hence their mode of action can be discussed in terms of physiological effect.

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Late cultivars of cabbage can better cope with cadmium-involved stress than the early one

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The usefulness of white cabbage (*Brassica oleracea* ssp. *capitata* L. *f. alba*) for phytoremediation of heavy metals (cadmium and zinc) due to the rapid growth and a large biomass of its heads has been already described (Bączek-Kwinta et al., 2011, Kusznierewicz et al., 2012). As the photosynthetic apparatus supplies the whole plant with sugars necessary for various physiological processes and, in consequence, results in the yield, the aim of the study was to estimate the influence of cadmium on the condition of photosynthetic apparatus of cabbage. Total chlorophyll content as well as the fluorescence parameters of chlorophyll *a* were established during plants' vegetation in greenhouse, in the soil spiked with two cadmium concentrations, whereas Cd content in plants was assayed by inductively coupled plasma atomic emission spectroscopy.

At the beginning of vegetation, cadmium triggered a decline in total chlorophyll content. The effect was sustained in plants of early cultivar, whereas it ceased in the specimens of the late ones. The fluorescence parameters of chlorophyll *a*: F_v/F_m, NPQ and ETR revealed marked changes, differentiated among the cultivars similarly to the pattern of chlorophyll content alterations. The yield of the heads was diminished, but to the lower extent in plants of late cultivars, which accumulated up to 2-fold more Cd than the early one. Hence, late cultivars of cabbage can be useful for phytoremediation of soils contaminated with this element.

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Differences in hsp70 level in *Xerolycosa nemoralis* from polluted areas

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Heat shock proteins (hsps), also known as stress proteins (sps) or chaperons play a crucial role in the development of stress resistance and adaptation to the environment (Fink, 1999). They are highly phylogenetically conserved proteins and classified according to their molecular weight (Sørensen et al., 2003).

Recently, heat shock proteins (mainly hsp70) are main object in ecotoxicology research (Sørensen et al., 2003). Hsp70 is the largest and relatively well known family of stress proteins. The principal reason of popularity of this family is a fast synthesis induction under stress condition (high temperature, UV radiation, changes in pH, viral infections, toxic metals, etc.). Scientist claim that hsp70 in good biomarker of proteotoxicity in various organism (Feder and Hofmann, 1999).

Spiders live in industrial changed areas are exposed to the negative effects of contaminants, like heavy metals, petroleum etc.. In addition, their place in trophic networks increases this risk of exposure to harmful substances (Hopkin, 1989). Literature date indicate a vague role of hsp70 in preadaptation to contaminations in spiders from variously polluted areas (Wilczek, 2005; Wilczek, 2008).

This work presents the preliminary results of research on the temperature induction of hsp70 in both sexes *Xerolycosa nemoralis* (Lycosidae) from variously polluted slagheap areas. Our results indicate that hsp70 synthesis is not a main defence mechanism to thermal stress in *X. nemoralis*.

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Selected enzymes activity in soils contaminated with heavy metals in *Betula pendula* Roth and *Pinus sylvestris* L. stands

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Soil enzymes are group of enzymes playing an important role in maintaining soil ecology, physical and chemical properties, fertility and soil health. Soil enzyme activities are considered to be good bioindicators, reflecting natural and anthropogenic disturbances. Studies on the impact of different trees on soil microorganisms have shown that the activity and number of microorganisms in the soil may differ depending on the tree species (Zak et al., 2003; Wardle et al., 2004; Cesarz et al., 2013). To study a host tree effect on soil enzymes activity we selected silver birch (*Betula pendula* Roth.) and Scots pine (*Pinus sylvestris* L.) plots naturally regenerated at heavy metals contaminated areas in southern Poland. The control sites (non-contaminated with heavy metals) are located in Kórnik study forest.

The number of living microorganisms at the contaminated sites, estimated by the dehydrogenase activity was higher at the pine plots. This result suggests that at the polluted plots pines are creating more favorable conditions for microorganisms development in the contrary to natural stands where the birch has positive role. Activity of urease and both phosphatases is higher in the birch plots which is related to nutrients inflow from decomposition of annually lost leaves. However the annual leaves loss in birch may increase the total heavy metal concentration in the soil and negatively affect the microogranisms activity. Moreover acid phosphatase was negatively affected by total Ni, Ag and Zn content in soil. Also urease was negatively correlated with total Ag, Zn and Cd.

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A comparison of the effects of $PbCl_2$ and Met_3PbCl on slowly activating (SV) channels in red beet (*Beta vulgaris* L.) taproots

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Lead (Pb) is one of the hazardous heavy metal pollutants of the environment. It exerts a wide range of adverse effects on growth and development (Małkowski et al., 2002; Sharma and Dubey, 2005; Kurtyka et al., 2012) wherein organolead, in general, is considerably more toxic than inorganic compounds of this metal (Przestalski et al., 2000). Pb ions influence, among others, membrane transport systems i.a. in tonoplast (Trela et al., 2012). In the vacuolar membrane of higher plants there are two major ion currents mediated by non-selective cation channels: SV channel (slowly activating) and FV channel (fast activating). Under conditions usually applied to patch-clamp experiments on vacuoles (symmetrical 100 mM K^+, 1 mM luminal Ca^{2+}) SV channels activate at positive potentials.

The aim of the present study was to compare the effect of inorganic lead ($PbCl_2$) and its organic compound – trimethyllead chloride (Met3PbCl) on SV channels properties in the red beet vacuoles. The vacuoles were isolated by using of nonenzymatic method from red beet taproots. The SV channels were studied by the patch-clamp technique in the whole-vacuole or the (cytoplasmic side-out) excised-patch configuration using EPC-7 amplifier (List-Medical-Electronic, Darmstadt, Germany).

It was found that the organolead has more inhibitory effect on SV channel activity as compared to the inorganic compound of this metal.

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Ecological factors and arbuscular mycorrhiza of *Ambrosia artemisiifolia*, an invasive plant species colonizing anthropogenic habitats – example from Upper Silesia, Poland

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Ambrosia artemisiifolia (Common ragweed), an invasive alien plant which spreads on a large scale on farmland, and along roads and railway tracks in many European countries, causes economic losses in agriculture and brings increased sensitization to ragweed allergens in human populations (Tokarska-Guzik et al., 2011).

The aim of the presented study was to monitor the actual state of the spread of *A. artemisiifolia* in Upper Silesia, using the example of selected ragweed populations occupying different anthropogenic habitats, namely: a roadside (O – Orzesze and Ż – Żory) and a railway track (TG – Tarnowskie Góry). The analysis of ragweed biometrical indicators, ecological factors and arbuscular mycorrhiza allow the prediction of its future trends of spread on a regional scale, and has implications for control strategies.

The results of the plant's biometric characteristics showed that the average values of morphometric attributes (length of stem, number of lateral branches, length of male and female inflorescences, length of the main root, dry weight) were higher for the roadside population (Ż) compared to the railway population (TG) and the second roadside population (O). Soil analysis implied lower levels of organic carbon, phosphorus and nitrate nitrogen in the roadside population (Ż), in comparison to the other populations. These results contradict the higher growth of ragweed in the Żory site, and indicate that future studies are still required for its explanation. *Ambrosia artemisiifolia* populations in Upper Silesia were found to have arbuscular mycorrhizal colonization, confirming previous reports from Europe. Mycorrhizal plants of this species were found in all the investigated populations. Arbuscular mycorrhizal structures like arbuscules, as well as vesicles, coils and hyphae, were found in the roots of *A. artemisiifolia*. The intensity of mycorrhizal colonization was very high in all stands but slightly higher in the railway population.

The obtained results suggest that ecological factors like soil properties and mycorrhiza can have a positive impact on ragweed plants' vigor. However, its invasive success can also be influenced by other factors such as the age of the population and type of habitat.

ACKNOWLEDGMENTS: The studied populations were registered in 2013 as a part of the SMARTER *Ambrosia* survey, focused on Ambrosia population dynamics, by monitoring selected established, unmanaged populations yearly in 2014–2016 (SMARTER – Sustainable management of *Ambrosia artemisiifolia* in Europe).

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Cadmium and zinc phytoextraction with willow (*Salix viminalis* L.)

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Phytoextraction is promising and the main technology to remove heavy metals from the soil, using hyperaccumulators which uptake and translocate heavy metals to aboveground tissues. On the other hand, that contaminations may interfere with the physiological processes of plants, alter their morphology or result in biomass production. Species used for the phytoextraction should tolerate the toxic effects of the target element and have a high growth rate (Ali et al., 2013). Although many willow species are not recognized as hyperaccumulators, their properties combine heavy metal accumulation with high biomass yields (Lewandowski et al., 2006).

In order to understanding the mechanisms of *Salix viminalis* L. tolerance, the studies were conducted in a model system – woody cuttings grown in hydroponic culture, under Cd and Zn influence (0.5 mM and 1.5 mM), at controlled light, thermal and humidity conditions. Accumulation of the tested heavy metals, the content of potentially complexing amino acids, and low molecular weight organic acids (LMWOA) in roots and shoots were determined. Moreover, the morphometric measurements of the willow cuttings were performed.

Cd accumulation was more intense in the roots, whereas Zn ions in the shoots. Under the tested concentrations both metals inhibited the growth and changed their metabolism. The level of majority LMWOA was lower at Cd and Zn addition, but the content of amino acids increased, particularly in the roots. The complexity of willow response to Cd and Zn is discussed.

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Metal management in *Erica andevalensis* an endemic plant from the mining district of SW Iberian Peninsula

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Erica andevalensis (Ericaceae) is an endemic heath that grows in the mining district located in the southwest of the Iberian Peninsula, associated to the Iberian Pyritic Belt, a great mass of metallic sulphides that extends from Portugal to Seville.

In this territory *E. andevalensis* grows on fluvial terraces and gullies caused by erosion in tailings and mining areas. This species and the vegetation communities that form are bound to extremely acid waters and soils with low content of nutrients (Ca, Mg, K, P) and high concentrations of Fe, Pb, Cu, As, S among other elements. Therefore, it is consider a species with a high level of adaptation.

A number of researches on this species have ascertained its capability of accumulate Mn, bioindicate Cu and exclude from its aerial parts other elements highly concentrated in its habitat as Pb and As. It is known that this species present metal concentrations in its leaves and stems among 73–514 ppm for Fe, 124–778 ppm for Mn, 76–738 ppm for Al, 9–16 ppm for Cu, 0.77–9.80 ppm for Pb, and 0.41–6.85 ppm for As; and despite the low availability of nutrients it can concentrate Ca in a range of 2012–5942 ppm, 223–3771 ppm for K and 883–2566 ppm for Mg.

The recent studies about these subjects indicate the existences of multiple strategies involving roots that plants could use to cope with the excess of metals, but most of them are centered in helophytic grasses. Little is known about the entrance of metals in *E. andevalensis* roots and the possible existence of bio-mineralization processes that this plant could use to avoid a free flow of metals to its aerial parts.

By using different analytical techniques (inductively coupled plasma mass spectrometry (ICP-MS), scanning and transmission electron microscopy coupled with energy dispersive X-ray analysis (SEM and TEM-EDX) metal concentration and distribution in *E. andevalensis* roots from Rio Tinto mining area (Huelva, Spain) has been studied.

Results show that the roots are the part of the plant with the highest metal concentration. Metals could be found mainly in its surface and inside the most external layers of the root tissues (epidermis, cortex), as a part of the cell walls or even crystallized in mineral forms.

Genetic differentiation of metallicolous and non-metallicolous populations of *Echium vulgare* in Poland

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Echium vulgare is an autotetraploid (2n = 4× = 32), biennial or perennial, monocarpic species. It is a typical ruderal plant, which colonises lands disturbed by human activity including areas polluted with heavy metals (HMs). Occurrence of *E. vulgare* on Zn-Pb waste heaps as well as on uncontaminated areas allows investigation of microevolutionary processes resulting in adaptation to HMs (Dresler et al., 2014) as well as genetic differentiation in response to HM stress. In the present study, intrapopulation genetic diversity and differentiation between metallicolous (MP, MB) and non-metallicolous (NM) populations were elucidated using random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP), and simple sequence repeat (SSR) molecular markers. The coefficients of intrapopulation genetic diversity were slightly lower in the NM than in the MP and MB populations as assayed using all marker systems studied. The higher genetic diversity within the metallicolous populations corresponded to a higher percentage of polymorphic fragments and number of private bands. Analysis of molecular variance (AMOVA), depending on the molecular marker system used, showed that 80–90% of total variation resided within populations, while 9–14% of the total genetic diversity was due to differentiation among all populations and 0–6% resulted from differentiation among non-metallicolous and metallicolous populations. In conclusion, this study has demonstrated that all four types of marker assays have different properties: AFLP was the most efficient in revealing the high level of polymorphism; however, ISSR and SSR markers were more efficient in showing the high differentiation between the populations studied.

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Accumulation of trace elements in the lichens with different growth forms of thalli

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Accumulation and processing of trace elements by lichens are one of the most interesting and widely studied aspects of lichenology. Lichen thalli lack root systems and protective waxy cuticles and absorb water via the whole surface, so that elements that are present in the atmosphere and those in the substrate can penetrate into the lichens. Therefore, these cryptogams can accumulate elements at levels exceeding their metabolic requirements.

Lichens occurring on soils or rocks are in intimate contact with lithic sources of nutrients. We examined accumulation capacity of epigeic lichens having ability to grow on extremely contaminated post-industrial dumps. Lichens with different growth forms were considered: crustose – *Baeomyces rufus, Diploschistes muscorum*; foliose – *Cladonia symphycarpa, Peltigera didactyla*; fruticose – *Cladonia cariosa, C. furcata, C. pyxidata, C. rei* and *Stereocaulon nanodes*. Various elements were determined in the lichen and corresponding substrate samples using analytical PIXE method: Al, Si, P, S, Cl, Sc, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Ge, Pb, Se, Br, Rb, Sr, Y, Zr, Mo, Cd, Sn.

Several noteworthy results can be emphasized. Crustose lichens accumulate relatively higher amounts of elements than others. However, *Stereocaulon nanodes* proved to be a hyperaccumulator, especially in relation to Mn, Fe, Cu, Pb, Sr, and Br. Lead concentrations in all lichens varied independently of the metal levels in the substrate. This suggest that local air pollution could be a strong interfering factor that increases the Pb content in lichens. Trace elements, such as: Al, Si, Sc, V, Cr, Co, Ni, Ga, Ge, Rb, Y, Zr, Mo, Sn, present in the substrate sometimes in large amounts, usually were below detectable limit in lichen samples. These elements do not penetrate into the thalli or are accumulated at very low level. Symptomatic and alarming are very high contents of Cl in all measured lichen samples. This suggests a considerable local salinity of the habitat.

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Soil contamination by heavy metals in Silesia – past or still current problem?

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Upper Silesian Industrial Region is historically associated with mining and processing industry of non-ferrous metal ores. Intensive industrial activity conducted over the years has contributed to soils contamination with heavy metals. Cadmium, lead and zinc once introduced into the soil stay there for a long time. Accumulation of heavy metals in agricultural and allotment garden soils is a serious public-health-related risk factor. Another important source of exposure to heavy metals, beyond ingestion route, is oral exploration especially in case of children (during outdoor games – dirty toys and hands). The aim of this study was to evaluate the content of heavy metals such as cadmium, lead and zinc in soils from 14 cities of the Upper Silesian Industrial Region. Particular attention was paid to allotment gardens, cultivated fields, playgrounds and playing fields. The study was performed in order to answer the question whether the heavy metals in the soils of Upper Silesia are still the problem or belong to the past. The studied soils (568 samples) were collected from the agricultural fields (155 samples), allotment gardens (138 samples), playgrounds and playing fields (together 275 samples). The content of heavy metals (Cd, Pb and Zn) was determined by ICP OES. In over 70% of the soil samples the maximum allowable content of heavy metals has been exceeded. The highest number of exceedance of the limit value was recorded in soils collected from allotments (80%). In 5 out of 14 cities the exceedance of the maximum allowable content were the highest. Those cities are: Piekary Śląskie (100%), Siemianowice Śląskie (90%), Katowice (86%), Sosnowiec (81%) and Dąbrowa Górnicza (76%). In the studied soils the content of heavy metals was differentiated. Expanding monitoring of heavy metals in soil is recommended. The education campaign directed to residents of the Upper Silesia also seems appropriate.

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Gene action and heterosis in maize (*Zea mays* L.) under normal and drought conditions

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Genetic systems controlling quantitative traits of maize using North Carolina Design II mating design among nine parental lines and their 20 F1's were studied under normal and drought stress conditions. Highly significant differences existed among studied genotypes, revealing a large amount of variability among them under both conditions. The significant values of mean square for parents vs. crosses were observed, indicating the importance of heterotic values and no-additive genetic variance in the inheritance of these traits under the two conditions. Some lines and their F1 crosses showed drought susceptibility index "s" values less than one revealing relative drought resistance. The results showed that the magnitudes of non-additive genetic variance σ2D were larger than those of additive ones σ2A for most studied traits, indicating that the non-additive gene action was pronounced in the inheritance of these traits. Therefore, these promising crosses could be utilized in maize breeding program to improve these traits under favorable and drought stress. This finding could be emphasized by the estimate values of narrow sense heritability.

Ecological catalysts derived from Zn hyperaccumulating plants: Preparation and catalytic activity in Diels-Alder and Garcia Gonzalez reactions

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Metal-hyperaccumulating plants, or metallophytes, extract trace metals (TM) from soil and concentrate them in their shoots. Exploring the utility of these plants led to the development of phytoextraction techniques that represent an opportunity to remove TM from contaminated soils, *e.g.*, contaminated mine sites, which in turn represent a major environmental problem worldwide.

Herein, we demonstrated that metallophytes could be the basis of a novel, plant-inspired, metallo-catalytic platform with synthetic potential in important processes in organic chemistry.

Zn hyperaccumulating plants *Noccaea caerulescens* and *Anthyllis vulneraria* were used as the starting material for preparation of novel Lewis acid ecological catalysts that efficiently mediate the Diels-Alder reaction. High yields, very good regio- and diastereoselectivity, low catalyst loading and the possibly of its recovery and reuse, along with mild reaction conditions, eco-friendly treatment and short reaction time, are the key advantages of the presented approach (Escande, 2014).

Additionally, these novel ecological catalysts were found to catalyse the Garcia Gonzalez reaction, a highly valuable route for preparation of polyhydroxy furans starting from carbohydrates (hexoses and pentoses) and -dicarbonylated compounds (Escande, 2014).

Further experimentation is required in order to fully determine the potential of the new plant based catalysts, but there is no doubt that they will continue to display very interesting properties for cutting-edge "Green Chemistry". Additionally, the use of metal hyperaccumulator plants as a source of metals for applications as catalysts in chemical processes is of interest for the phytoremediation of mine sites contaminated with heavy metals.

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Specificity of epiphytic habitats in areas with elevated content of heavy metals; a case study from Katowice town (S Poland)

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In urban areas air pollution is mentioned as one of the major factors limiting the occurrence of epiphytic bryophytes (Zechmeister and Hohenwallner, 2006). Heavy metals and sulphur compounds influence negatively the essential physiological processes, e.g. may disturb the development of spores and the growth of protonema (Basile et al., 1995). The purposes of the investigation conducted in Katowice were analysis of tree bark pollution by heavy metals and sulphur – regarding localization, seasonal variability and tree species. The content of contamination in bark and mosses (exposed in "moss bags") were analyzed.

The average content of heavy metals in bark of poplar (*Populus xcanadensis*) varied among the examined sites. Higher levels were found close to the roads and vicinity of metalworks. The highest sulphur content was registered in Muchowiec (6100 mg kg^{-1} in June) – it is possibly a result of the sports airport located nearby, as well as the close presence of metalworks. Seasonal variability of contaminations was also observed. In comparison with exposed *Sphagnum fallax*, higher levels of examined metals were detected in poplar bark.

The content of heavy metals in bark varied among the analyzed 10 tree species (from "Janów" locality). For instance, the level of lead in most cases remained less than 80 mg kg^{-1}; the higher – 135,7 mg kg^{-1} – was detected in bark of *Robinia pseudoacacia*. The content of heavy metals in *Pleurozium schreberi* exposed on the bark of different tree species also varied. For instance, the highest cadmium content was registered in moss exposed on the bark of *Populus tremula* (89,47 mg kg^{-1}), the lowest was from *Picea pungens* (55,02 mg kg^{-1}). Inflow of contamination with stemflow seems to be one of the crucial factors determining a quality of epiphytic habitats in urban areas.

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Heavy metal accumulation in *Betula pendula* Roth trees growing on polluted sites in Upper Silesia

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This study was undertaken to examine to what degree heavy metal pollution affect the plant material of *Betula pendula* Roth growing on contaminated sites (dumps) in Upper Silesia. The study focused on the investigation of the seeds biometrical characteristics and germination capacity as well as heavy metal (Zn, Pb, Cd) concentrations of birch trees grown on industrial dumps. A sampling procedure was established for seed collection from different zinc-lead dumps in comparison to the non-polluted site (ca 60 km from Katowice). Analysis of the results showed that even though there were no significant differences in seed heavy metal accumulations and only a few in seed biometrical characteristics. There were significant differences of mean germination time as well as peak germination value in comparison zinc-lead polluted vs non-polluted sites.

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Natural and historical values of the Olkusz Ore-bearing Region, southern Poland

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The Olkusz Ore-bearing Region (OOR) has been exploited, with varying intensity, since 12th century. The centuries of mining and processing of Ag-Pb-Zn ores and sand resulted in degradation of the landscape and destruction of the natural vegetation. The area is dominated by excavations and waste heaps created by old and modern mining and metallurgy. The substrate in these areas usually contains amounts of heavy metals that are above average (especially Zn, Cd, and Pb), at the same time it is low in nutrients and dry, which makes it difficult for plants to colonize. Exploited mining areas (open pits, sand pits, waste heaps) are recalimed involving mainly afforestation with pine tree, larch and birch. Currently the OOR is characterized by a great diversity of habitats and hence richness of species. Within its small area (48 km^2) with a mosaic of habitats: from dry to the wet, from open areas to forest, on a limestone or sand substrate, 810 taxa of vascular plants were found (species, subspecies, hybrids, varieties). Among them there are 39 species that are protected (including 32 strictly protected, and 7 partially protected ones) such as: *Ophioglossum vulgatum, Goodyera repens, Malaxis monophyllos, Epipactis helleborine, E. atrorubens, Hepatica nobilis, Botrychium lunaria* and others. The communities of valuable calamine grasslands have developed in the OOR on substrates contaminated with Zn and Pb as a result of natural succession. The grasslands on waste heaps are rich in species, among which there is *Biscutella laevigata*, for which the OOR is the second, next to the Tatra Mountains, position in Poland. On the other hand, sand grasslands are poor in species. They are dominated by *Festuca ovina* and *Armeria maritima*. In the OOR many others, rare in Poland species have been found. In the forest, positions of rare fungi were found: *Suillus cavipes, Geastrum minimum* and *Scleroderma septentrionalne*. For the latter species the OOR is the second position next to Kampinoska Forest. Valuable components of lichen biota of this area are very rare species such as *Vezdaea leprosa, Agonimia vouauxii* and *Thelocarpon imperceptum*. These are their only positions in Poland. So far in the OOR, two NATURA 2000 areas were created in order to protect the valuable calamine grasslands.

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Upper Silesia heavy-metal sites – the characteristic of nature

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Poland's largest resources of zinc and lead ores are located in the southern part of the country (Silesia and Lesser Poland), where they were mined and processed since the Middle Ages. In connection with the development of numerous mining and metallurgical centres in the area, numerous new landforms evolved including open pit mines, shafts, adits and waste heaps. They have a substantial impact on surface water and groundwater, soils and consequently on plants and animals. High concentration of heavy metals (zinc, lead and cadmium) in the substrate has led to the emergence of new habitats with no counterparts in the natural environment.

There were about 90 industrial sites connected with heavy metal mining and processing in Silesia including 40 mines and about 50 smelters and rolling mills. Currently there is no ongoing mining activity and zinc smelting is done only in one smelter in Miasteczko Śląskie. A large part of the industrial areas has been transformed and developed. Heavy metal habitats in Upper Silesia can be classified (according to Baker et al., 2010) as:

- secondary sites: mining areas, spoil and slag heaps, ore processing and concentration (beneficiation) areas.
- tertiary sites: sites polluted as a result of atmospheric deposition in the vicinity of metal smelters or alluvial deposition of metal-enriched substrates by sedimentation in river floodplains and on raised riverbanks.

Different plant communities developed in heavy-metal-polluted sites in the Upper Silesia, and there are several species that can be treated as dominant in these communities: *Arabidopsis halleri, A. arenosa, Festuca ovina, Silene vulgaris, Deschampsia caespitosa, Anthyllis vulneraria, Lotus corniculatus, Daucus carota, Viola tricolor, Plantago lanceolata* and *Echium vulgare*.

Investigations focused on interactions between plants and environment is an interesting topic, and results of such studies certainly have a potential to be applied in practice.

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Plant-aphid-hoverfly, tritrophic transfer of metals in urban area

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The aim of our study was to examine the effect of metal transfer in tritrophic model: plant -phytophagous pest – predator in urban area. Our model was consisted of: host plant: *Philadelphus coronarius*, herbivorous insect: aphid *Aphis fabae* and aphidophagous predators: larvae of three species of hoverflies: *Syrphus ribesii, Syrphus vitripenis, Epistrophe bifasciata*. The plants and insects were collected in reference place, Zagaje Stradowskie and in overcrowded streets of Kraków. We measured the concentration of: Cd, Cu, Ni, Pb, Zn in animals and calculated bioaccumulation factor (BF) for aphids (using the data collected earlier for plant, Kafel et al., 2010) and hoverfly larvae.

P. coronarius bushes were mainly characterized by elevated levels of metals: lead and zinc, when compared data for samples collected in Krakow and Zagaje Stradowskie (Kafel et al., 2010). In the case of *A. fabae*, elevated amounts of: cadmium, copper and zinc were detected, when compared animals from both research areas. Syrphides family representatives differentiated in the accumulation of metals, and it was species specific. However, it was found that two representatives of *Syrphus* sp. differentiated higher accumulation of copper and nickel. For the other hoverfly, *E. bifasciata* higher bioaccumulation of cadmium was found.

Generally, we did not observe the biomagnification process along examined tritrophic chain. It must be depicted that in the case of aphids high BF (>2) was calculated for copper and zinc. In the case of hoverflies inhabiting bushes in Kraków, we do not calculated BF higher than two, and the highest oscillated around 1,7 in the case cadmium (the case of *E. bifasciata*). It seems that hoverflies from urban area had more effective mechanisms of metal removing.

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Scale insects (Hemiptera, Coccoidea) of heavy metals contaminated habitats in Southern Poland

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The faunistic research was conducted in heavy-metal-polluted habitats between 2009 and 2013. Study plots were established in seven post-industrial wastelands situated within the boundaries of Upper Silesia and Małopolska Upland. We recorded the presence of twenty four species of scale insects. They belong to six families, of which the largest is Pseudococcidae. Most of species occurring in contaminated habitats are common in Poland e.g. *Trionymus perrisii* (Signoret, 1875) (Pseudococcidae) and *Parthenolecanium corni* (Bouché,1844) (Coccidae), but some of them are rare. The most interesting species is Polish cochineal *Porphyrophora polonica* (Linnaeus, 1758) (Margarodidae), which was found in two localities. The species is considered to be endangered. We also recorded the presence of another rare species: *Coccura comari* (Künow, 1880) (Pseudococcidae). A large population of that species was recorded in one study plot. Species characteristic for xerothermic habitats such as *Greenisca brachypodii* (Borchsenius and Danzig, 1966) (Eriococcidae) and *Lecanopis formicarum* Newstead, 1893 (Coccidae) were also found to be present in post-industrial wastelands.

Scale insect species found in metal-contaminated habitats were collected from trees, bushes (e.g. all species that belong to the family Diaspididae) and grasses (e.g. most species of Pseudococcidae).

Heavy metal bioaccumulation and physiological responses in selected populations of *Vaccinium myrtillus* L. and *Vaccinium vitis-idaea* L.

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The purpose of this study was to examine possible relationships between the availability of metals in soil (Cd, Pb, Zn) and their concentrations in the leaves of *Vaccinium myrtillus* L. and *Vaccinium vitis-idaea* L. Analysis also concerned the physiological responses of plants from three heavily polluted sites (immediate vicinity of Miasteczko Śląskie zinc smelter, ArcelorMittal Poland S.A. iron smelter in Dąbrowa Górnicza-Łosień and Jaworzno III power plant in Jaworzno) and one potentially clean site (Pazurek nature reserve in Jaroszowiec Olkuski). All of the sites are situated in the southern parts of Poland in the Śląskie or Małopolskie provinces. The contents of non-protein thiols, proline and activity of guaiacol peroxidase and superoxide dismutase in the leaves of *Vaccinium myrtillus* L. and *Vaccinium vitis-idaea* L. were measured.

In soil, the highest content of Cd, Pb and Zn (HNO_3 extracted and $CaCl_2$ extracted) were detected at the Miasteczko Śląskie site. Additionally, there was a clear difference in the concentrations of metals between the fraction of soil extracted with HNO_3 and the fraction extracted with $CaCl_2$. At all sites a several times lower concentration of the examined metals was determined in the fraction of soil extracted with $CaCl_2$.

Significant positive relationships were found in Cd, Pb and Zn concentrations between soil and plants. The highest concentrations of Cd, Pb and Zn were noted in the leaves of *V. myrtillus* and *V. vitis-idaea* from the site in Miasteczko Śląskie (*V. myrtillus*: 5.54 mg kg^{-1} d.w., 182.15 mg kg^{-1} d.w., 256.35 mg kg^{-1} d.w.; V. *vitis-idaea*: 10.6 mg kg^{-1} d.w., 405.5 mg kg^{-1} d.w., 385.6 mg kg^{-1} d.w., respectively).

In *V. myrtillus* we found a positive correlation between the level of non-protein thiols and Cd, Pb, Zn concentrations, and also between proline and the same metals. In *V. vitis-idaea* leaves an increasing trend in superoxide dismutase activity accompanied an increase in Cd, Pb and Zn concentrations. At the same time the increased levels of all metals in the leaves of *V. vitis-idaea* were accompanied by decreased activity of guaiacol peroxidase.

Effects of *Festuca ovina* L. on the properties of soil contaminated with heavy metals

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Sheep fescue *Festuca ovina* L. is a grass species typical of dry, poor habitats. Due to the considerable resistance to high concentrations of heavy metals in soil, it is a regular component of plant communities developing in post-industrial sites, such as those in the Olkusz Zn-Pb mining area (S Poland). On sandy soils contaminated with heavy metals, sheep fescue often forms dense, almost monospecific grasslands. This species can enter also more fertile habitats (e.g. with brown or limestone soil) and seems to displace there other plants. The aim of this study was to determine the effect of sheep fescue on physicochemical and biological properties of brown soil. This was done by comparing the vegetation patches colonized recently and dominated by sheep fescue with those representing multispecies assemblages (comprising of *Leontodon hispidus, Avenula pubesces, Achillea millefolium, Crepis biennis* and other mesophilous species) in terms of several soil parameters: texture, pH, the total and exchangeable concentrations of elements (C, N, P, K, S, Ca, Mg, Zn, Pb, Cd), the density of enchytraeids, and the respiration and biomass of soil microorganisms. The soil under sheep fescue grassland contained less silt particles and essential elements (N, P, K, Ca) compared to the soil under multispecies grassland; in addition, it was characterized by a lower activity of soil mesofauna and soil microorganisms. The two types of grasslands did not differ in pH and the concentration of C and heavy metals in soil. The results suggest, that sheep fescue can reduce soil fertility and thereby hinder the growth and reproduction of other, more demanding species. In the longer term, such changes in soil may promote the mobilization of heavy metals and thus further exacerbate habitat conditions.

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Effect of copper mine post-flotation sediment on chemical composition of herbaceous plants colonizing the Wartowice tailings pond (Lower Silesia, Poland)

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The response of herbaceous plants poorly colonizing post flotation tailings from copper mining industry were analyzed in comparison to soil environment. Five species *Agrostis stolonifera, Calamagrostis epigejos, Cerastium arvense, Polygonum aviculare* and *Tussilago farfara*, were examined for chemical composition with particular regard to heavy metals contents, especially copper as the effect of the growing substrate properties.

The plant samples collected from the tailings did not exhibit the excess of copper permissible as feeding purposes, probably due to the protective action of carbonates and pH >8.0 in the sediment (Spiak et al., 2009).

Multivariate analyses comprising the plant as well soil and sediment chemical composition revealed that among 13 studied elements the most significant in the plants variability were Cu, Mg, Na, Ca and the samples from the soil tend to have lower levels of these elements than from the sediment. The samples from the tailings were differentiated, for example samples of *C. arvense* and *P. aviculare* were distinguished by the highest levels of Cu, Mg, Na and Ca while the samples of *A. stolonifera* and *C. epigejos* were setting apart by the lowest contents of Fe. The analyses also showed that the levels of Cu, Na, Mg and than Ca and Fe in plants were positively relative to the substrate concentration of Mn, Ca, Mg, Cu as well as negatively to the contents of Zn and P. These results reflected ion antagonism occurrence, because the ratios of Fe:Mn, Fe:(Cu+Zn) and Ca:P in plants tissues collected from the sediment were significantly wider than from the natural soil as a result of high manganese, copper and calcium contents in the sediment.

The study provided evidence that the adverse chemical composition of the growth substrate changed chemical composition of tested plants compare to those from the soil environment. The extremely sparse plant colonization of the post flotation sediment results from the physical and chemical properties of the copper ore flotation tailings.

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Habitat adaptation of vascular plants spontaneously colonizing spoils from serpentine mining in Lower Silesia (Poland)

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Spoils from serpentine mining, as habitats for plants development, feature a combination of unfavorable properties resulting from specific traits of serpentine weathered rocks, such as xerism, low richness in macronutrients along with high levels of magnesium and large content of heavy metals, mainly nickel, chromium and cobalt (Kazakou et al., 2010). These stressful conditions can govern the process of succession and reduce the pool of plant species that are able to colonize the serpentine post-mining sites (Koszelnik-Leszek et al., 2013).

In the study, species of vascular plants spontaneously occurring on serpentine waste dumps and quarries in Lower Silesia were analyzed with regard to: geographical-historical status, life forms and selected ecological factors, i.e. light and trophic preferences, soil moisture and reaction, resistance to increased heavy metals content in the soil, as well seed dispersal modes and occurrence of mycorrhiza. The flora was relatively poor, and consisted of 113 species of vascular plants. The most abundant families were: Asteraceae, Poaceae, Fabaceae and Caryophyllaceae. The most numerous were species related to dry grassland communities, particularly of the Festuco-Brometea class, which included taxa endangered in the region of Lower Silesia, like *Avenula pratensis, Salvia pratensis* and *Festuca valesiaca*. The flora was dominated by apophytes and hemicryptophytes. The spoils hosted heliophilous species which prefer mesic and dry habitats, moderately poor in nutrients and featuring neutral soil reaction. On two study sites distinguished by the highest nickel levels, about 30% of the flora were the species that tolerate an increased content of heavy metals in the soil.

The studied flora was dominated by the species well adapted to the distinctive properties of serpentine habitat, particularly the low nutrient availability and the water deficiency. The huge nickel contents in serpentine substrata promoted the occurrence of species that tolerate increased heavy metals content in the soil.

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Expression of *AtHMA4* alters tissue specific transcription profile of tomato leaves exposed to Zn

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Engineering of enhanced Zn uptake and accumulation in the shoots is a major goal in biofortification. Heterologous expression of *HMA4* in plants have been considered a useful strategy to obtain altered metal distribution in tissues for purpose of biofortification. *Arabidopsis HMA4* transformation facilitates Zn root-to-shoot translocation, but in Zn-supply dependent manner (Siemianowski et al., 2011; Barabasz et al., 2012). It implies that distinct effects of *HMA4* expression on Zn root:shoot distribution in transgenics grown under different Zn supply regimes results from the interaction between the transgene activity and the molecular background of the host plant at varying metal concentrations in the medium.

The focus of this study was an impact of *AtHMA4* transformation on genes transcription profiles in tomato. The aim was to identify genes differentially regulated in 35S::*AtHMA4*-expressing and wild-type tomato under a range of Zn supply. As a first step, the transcription profiles were compared between transgenics with enhanced Zn root-to-shoot translocation and WT-plants (grown at 5 μM Zn). Selected indicative genes will be used in further study to analyse the molecular mechanism underlying the difference in Zn root:shoot distribution between tested plant lines exposed to low-to-high Zn conditions.

RNA was isolated from the Laser Microdisected spongy parenchyma and palisade parenchyma of leaves of transgenic and WT plants.

The *AtHMA4* expression modified four important metabolic categories of genes specifically for the examined tissues:

(1) Transcription factors involved in Fe/Zn homeostasis: (a) bZIP known to be involved in the Zn metabolism; (b) WRKY (response to abiotic stress).
(2) Key metal transporters and metal uptake facilitators (NRAMPs and FRO1), downregulation of NtYSL1 homologue.
(3) Genes encoding proteins involved in modification of the structure of the cell wall (more strongly downregulated in palisade parenchyma);
(4) Genes from the ethylene pathway (aminocyclopropane-1-carboxylate oxidases, ethylene receptors)

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Diversity of the functional analysis of vegetation of areas connected with processing of lead and zinc ores

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The aim of research was functional analysis of vegetation, which developed on the area connected with processing of lead and zinc ores in the area of the Silesian Upland. In order to recognise diversity of vegetation 99 samples were collected from wastelands such as: settling and washing tanks, wastes produced in smelters, vicinity of artificial water reservoirs. Abundance of species was estimated on the modified Braun-Blanquet cover-abundance scale. We used DCA analysis in order to determine the direction and range of vegetation variability in the data set. Average Ellenberg's indicator values for each sample were calculated and correlated with species scores on I and II axes of DCA. Beta-Flexible clustering method and Soerensen coefficient were used in order to classify samples. Diagnostic, dominant and constant species were determined for each group of samples. Vegetation units were compared taking into account chosen diversity measures such as Shannon-Wiener diversity index, Evenness, share of species representing different ecological groups and life history traits (life span, canopy height, SLA, reproduction type, flowering onset, life strategy, seed mass, seed number).

As a result of classification 8 vegetation units were recognised, mainly dominated by grass species such as: *Phragmites australis, Calamagrostis epigejos, Deschampsia caespitosa, Agrostis capillaris, Festuca ovina*, other perennial species such as *Solidago gigantea, Solidago canadensis, Reynoutria japonica*, and biennials such as *Echium vulgare, Melilotus officinalis, Picris hieracioides.* Groups of plots were clearly separated by moisture, fertility (axis I) and soil reaction (axis II). First DCA axis separates poorer in species samples dominated by *Phragmites australis*, which occurred in wet, more fertile places (washing tanks, places around artificial ponds), with higher share of rush (the *Phragmitetea australis* class) and nitrophilous species (the *Artemisietea vulgaris* class) from more richer in species patches with *Echium vulgare* and *Festuca ovina*, which occurred in open, drier, poor sites, with higher share of ruderal species (the *Onopordion acanthii* alliance) and grassland species (*Festuco-Brometea* class) in their floristic composition. This gradient of fertility and moisture separates perennial, tall herbs, flowering later during growing season, which reproduce vegetatively and by seeds from biennials, smaller species, flowering early during growing season, reproducing mainly by seeds.

The influence of water sorbing geocomposites on grass biomass cultivated on soil substitute – field experiment

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Soil substitutes are a still underestimated material, which could replace the top layer of the soil in the degraded areas, providing an environment for plant growth and development. They are produced mostly by mixing the organic fraction (sewage sludges, compost) with mineral fraction (sand, fly ashes) (Reynolds et al., 1999; Zhang et al., 2008; Gupta et al., 2010). The use of artificial soil for remediation purposes is also suggested. Such materials should have organizing capacity for creation of plant cover and will: diminish rain water migration in soil profile, increase water retention, provide better management of micro and macro-elements and create appropriate environment for good development of microorganisms.

Adequate water condition is a key factor in the healthy growth of plants in the soil substitutes. It could be achieved by using geocomposites – containing superabsorbents, which properties help in water retention and support plant growth in drought periods. Experiments conducted by the IETU confirmed the positive impact of geocomposites on the growth and quality of grass biomass when cultivated on soil substitute. The results could be implemented in creation of plant cover using soil substitutes on degraded areas, where isolation of contaminants can diminish existed environmental and health risk.

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Interactive effects of Cd and Pb on the elongation growth and membrane potential of maize (*Zea mays* L.) coleoptile cells

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Among heavy metals, cadmium (Cd) and lead (Pb) are one of the most serious environmental pollutants which are well known as a highly phytotoxic agents (Seregin and Ivanov, 2001; Kurtyka et al., 2011a). Many of the toxic effects of these metals are related, in part, to their destructive influence on structure and function of plant membranes which in consequence causes growth inhibition (Karcz and Kurtyka, 2007; Kurtyka et al., 2011b; 2012). In this study, we evaluated the effects of 0.1 mM Cd and Pb on the IAA-induced elongation growth of 1 cm-long coleoptile segments obtained from 4-d-old etiolated maize seedlings. The cadmium and lead contents in maize segments and effects of both metals on membrane potential (E_m) on parenchymal cells were also examined.

It was found that cadmium and lead administered into the incubation medium at 0.1 mM decreased the elongation growth of maize coleoptile segments. When Cd and Pb were added together their toxic effect was comparable with that observed in the presence of Cd alone. Furthermore Cd caused depolarization of E_m of coleoptile cell whereas Pb brought about membrane hyperpolarization. The mechanism of toxic effect of Cd and Pb on the elongation growth of maize coleoptile segments is discussed taking into account effect of both metals on plasma membrane permeability and accumulation of Cd and Pb in plant tissue.

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Under pressure: observations of Indian mustard's daily dealings with trace metal stress

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The presence of trace metals in excess cause the generation of the Reactive Oxygen Species, followed by the activation of the elements of the defense system (Gill and Tuteja, 2010). In an attempt to reduce oxidative stress plants employ a variety of enzymes, of which a very important role is played by superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) (Gill and Tuteja, 2010; de Pinto et al., 2012). Different species and varieties differ in their ability to sustain toxic effects of trace elements. Hyperaccumulators, like Indian mustard (*Brassica juncea*), are highly resistant to these stressors (Seth, 2012).

In our experiment we aimed to test hyperaccumulators response to oxidative stress caused by lead, zinc, copper and cadmium ions, in individual and binary combinations of metals on plants grown on peat pellets. Indian mustard seedlings response was measured after treatment with 50 μM of metal ions in individual treatments and 25 μM of each metal ions in binary combinations (ZnCd, CuZn, CdPb, ZnPb, CuPb, CuCd).

SOD activity was significantly increased after 24 hours of incubation with metals in all treated roots (except for Pb^{2+}). The highest response was elicited by the ZnCd combination. At the next time-points (48, 72, 96h) we observed that binary combinations stimulated SOD to a higher extent than the individual metal treatments. Highest activation of SOD was observed in treatments with essential metals (Cu, Zn), while CAT and APX activity in roots was high especially in incubations with non-essential metals (24, 48, 72 hours) and with copper (72, 96 hours). Activity of CAT and APX in aboveground parts was higher in treatments with copper and its binary combinations (24, 48 hours).

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Competitive mechanisms of metals (Cu, Zn, Cd, Pb) during their intake by pea plants

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Presence of single heavy metals (Cd, Pb, Cu, Zn) induces ROS (reactive oxygen species) production and causes oxidative stress in plants. While applied in two-element combinations, trace metals impact organisms in a more complex way. To assess the resultant effect we treated pea grown hydroponically with trace metals in variants: CuPb, CuCd, CuZn, PbCd, ZnPb, ZnCd at concentrations of 25 M for each metal ion. Abiotic stress inhibited root elongation growth, decreased biomass production, induced changes in root colour and morphology. It changed rate of ROS production, protein oxidation, malondialdehyde content, increased activity and altered gene expression of defence enzymes (superoxide dysmutase, catalase, ascorbate peroxidase, glutathione reductase, γ-glutamylcysteine synthetase).

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Concentration of bioavailable forms of heavy metals in soils. Extraction with 0.01 M $CaCl_2$ – effect of different time of extraction

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There is a large number of methods to obtaine bioavailable fraction of heavy metals from soils. Among them extraction with 0.01 M $CaCl_2$ is increasingly used. However different authors use different time of extraction. Thus the aim of the present study was to investigate the effect of time of extraction on the content of bioavailable metals in soils.

Experiment was carried out with two soils which vary with heavy metals content (CF1 – medium contaminated; SW – highly contaminated). Extraction was conducted with 3 g of air-dry soil and 30 ml 0.01 M $CaCl_2$ for 1, 2, 3, 4 and 5 hours, and content of Cd, Cu, Pb and Zn were measured.

The content of bioavailable forms of cadmium and copper did not vary significantly irrespecitve of the soil and time of extraction. Conversly, concentration of lead and zinc for soil CF1 diminish from 3rd hour because of reabsorption, whereas for soil SW reabsorption was not observed.

On the basis of the presented data and data from literature (Pueyo et al., 2004) it is concluded that optimal time of soil extraction with 0.01 M $CaCl_2$ is 2 hours.

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In vitro cultures of *Biscutella laevigata*

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The metallurgical industry contributes to the formation of wastes which are serious threat to the environment. New effective ways to prevent spread of such contamination in the ecosystems are still looking for. In nowadays restoration schemes special attention is paid on plant species representing local, well adapted populations (Muszyńska et al., 2013). Thus, as donor material to start *in vitro* cultures we have used aseptic *Biscutella laevigata* calamine ecotype seedlings, obtained in aseptic conditions from seed samples taken from population growing in the Olkusz Ore-bearing Region.

In experimental work we focused on obtaining the lines with increased resistance to stress conditions. Therefore we needed to: (1) optimize the micropropagation protocol (Hanus-Fajerska et al., 2012); (2) manipulate the culture media by supplementation with lead nitrate and cadmium chloride (0.1; 0.5 or 1.0 μM Pb $(NO_3)_2$; 0.5 μM, 2.5 μM and 5.0 μM $CdCl_2$). In such a case micropropagation efficiency was evaluated and culture physiological status was determined by measuring the contents of photosynthetic pigments, chlorophyll fluorescence and antioxidant activity in shoots; (3) initiate callus culture on modified MS medium supplemented with different levels and kinds of PGR (KIN, BAP, NAA, 2,4-D) or chosen additives, and optimize the protocol; (4) optimize isolation (primary explants – leaf mesophyll, secondary explant – callus tissue; composition of enzyme mixture: concentration and type of enzymes, and osmoticum, the time of incubation) and subsequent stages of protoplast culture. Isolated protoplasts were suspended in liquid media to obtain microcolonies, and changes during subsequent days of culture were observed microscopically.

The supplementation of Pb^{2+} and Cd^{2+} ions to the culture medium had a positive impact on studied *Biscutella laevigata* ecotype, and on regenerated microplants. The detailed results obtained through these research will be presented and discussed during poster session.

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Element accumulation patterns in selected metallophytes colonizing contaminated soil

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In this study, we used metallophytes to determine the concentrations of contaminants in ecosystems affected by metalliferous mining and smelting, the main sources of heavy metal pollution. We investigated the distribution of elements in three pseudometallophytes species: *Cardaminopsis arenosa, Plantago lanceolata* and *Plantago major*, naturally occurring at metalliferous and non-metalliferous sites in southern Poland. The accumulation of Al, Cd, Cu, Fe, Mn, Pb, Zn, as well as Ca, P, Na and K in shoots and roots was measured. The accumulation of nutrient elements (ANE) in the studied plants was also analyzed. Quantification of soil and plant pollution was studied using TF (translocation factor), MR (mobility ratio), EF (enrichment factor) for Zn, Pb and Cd. The level of the accumulated nutrient elements (ANE) was visibly higher in plants from melalliferous sites than non-contaminated ones. Also higher potassium share in ANE was found in the shoots of *C. arenosa* and *P. lanceolata* from metalliferous sites.

Comparing the levels of trace elements in shoots of studied plants, the highest content of Cd, Zn, Pb, Al, Fe and Mn was found in *C. arenosa*, which better reflected metal concentrations in the metalliferous and non-metalliferous soil than other plants. Levels of Zn and Cd were above toxic thereholds in *C. arenosa* shoots. Cd, Zn in *P. lanceolata* shoots and Pb in all investigated plants were in the ranges known as toxic for plant tissues.

Among the studied plant species the translocation factor (TF) ranged between 0.24–6.2, 0.14–4.5, 0.14–2.5, mobility ratio (MR) 0.01–11, 0.001–1.49, 0.01–0.39 and enrichment factor (EF) 3.65–53.6, 1–15.8, 2.6–75.1 for Cd, Zn and Pb respectively. Almost in all cases the plants had enrichment coefficient >2, which reflected their high metal accumulation potential. Among the studied plants, *C. arenosa* and *P. lanceolata* showed better accumulation and phytostabilisation potential than *P. major*.

Floristic and phytocoenotic biodiversity of the oldest Zn-Pb post-mining areas in selected sites of the Silesian Uplands (S Poland)

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The largest centres of mining and processing of zinc and lead ores in Poland are located in the north-eastern part of the Silesian Uplands, mainly near the towns of Tarnowskie Góry and Olkusz. The occurrence of these ores is related to the Middle Triassic dolomites (Malon et al., 2013).

Extraction of minerals has been carried out on this territory since the Middle Ages, but there are currently active mines only in the Olkusz Ore-bearing Region.

The subject of the study comprised the 8 oldest (over 100 years) selected post-mining sites located in the town of Dąbrowa Górnicza and its surroundings. Extraction of ores by means of opencast mining was carried out there. The vegetation has developed on these sites mainly spontaneously. Trees have been planted on the three sampled sites, but the herb layer plants appeared there as a result of natural succession. The main aim of the study was to identify and compare the floristic and phytocoenotic diversity of the described sites.

Xerothermic grasslands and warm thickets of the *Festuco-Brometea* and *Trifolio-Geranietea sanguinei* classes, as well as forests of the *Querco-Fagetea* class were the main types of plant communities which developed there. There were 98 vascular plant species recorded on the sites analysed. Among them about 60% of the species were ranked to the list of species occurring in the Polish metalliferous areas (Rostański et al., 2014). The studied areas are distinctive due to the occurrence of many rare and endangered elements of native flora. The localities of 21 plant species protected by law in Poland were confirmed there. Identifying the spontaneously developed vegetation of the post-mining areas is very important for local biodiversity and valuable for nature conservation. Moreover, some important conclusions concerning the prospects for degraded area reclamation can be drawn.

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Aphids (Insecta, Hemiptera, Aphidomorpha) related to plants in heavy metals habitats

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Ecological community (biocoenosis) formed on soils with a high concentration of heavy metals are characterized with particular flora. Extremely inhospitable physical and chemical conditions (abiotic components) of such habitats (high toxicity of the soil, high temperature, shortage of nutrients and water) exert strong selection pressure on their biotic components. The research on plants, i.e. the producers in a food chain, which occur in metalliferous areas, has been conducted for several decades. It is interesting whether this pressure affects a higher level of a trophic pyramid, i.e. primary consumers, particularly insects. Sucking phytophages, like aphids, are model animals for this kind of research. Most Aphidomorpha are monophages with a high level of correlations in the relation insect – host plant. Thus it was decided to study the diversity of aphids in extreme habitats and check whether a strong pressure exerted by abiotic factors can affect the quality (species composition) and abundance of aphids.

The study was conducted on 2 study sites near Olkusz (S Poland). The selected sites represent rare plant communities in Europe, calamine grasslands (*Violetea calaminariae*), which constitute part of European Natura 2000 conservation programme, namely 'Armeria' and 'Pleszczotka'. Also comparative material was collected from xerotermic grasslands (*Festuco-Brometea*) and sand ones (*Koelerio glaucae-Corynephoretea canescentis*) formed on the soil with similar physical and chemical characteristics, but without excess heavy metal content.

The research helped to establish that calamine grasslands are characterised by a high diversity of aphid species, on the other hand, the comparative analysis proved that species composition of such aphidocoenoses is similar to aphid communities related to xerothermic grasslands, with a significant element of specialized species in both aphidocoenoses (K-strategy type), often rare on a higher than local scale. It was also observed that a strong habitat pressure in the form of heavy metal concentration in the soil does not limit aphid infestation (abundance of local populations).

The model of restrained heavy metals accumulation in the lichen *Cladonia rei*

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There are known to be some lichens which are associated with metal-enriched substrates and have the unique ability to grow in strongly contaminated areas. Tolerance to the presence of heavy metals inside thalli or exclusion of elements are the primary adaptations described for growth in polluted sites. *Cladonia rei* is a typical fruticose and epigeic lichen. It is heavy metal tolerant species that grows both in contaminated and uncontaminated sites and is known as a rapid colonizer of bare ground (Osyczka and Rola, 2013a).

The phenomenon of mass occurrence of *Cladonia rei* in post-smelting slag dumps was studied in relation to its metal accumulation capacity. The study was conducted in terms of a wide spectrum of Zn, Cd, Pb, As contamination. Various regression models were considered to find the best fitted one that greatly reflects the dependencies between concentrations of these elements in the lichen and corresponding substrate. Specific non-linear regression models described by a power function reflected relationships between Zn and Cd contents in thalli and in the host substrate in the most reliable way. This indicates that with increasing content of these elements in the substrate, the accumulation capacity of *C. rei* decreases (Osyczka and Rola, 2013b). The relationship for As was also noted, but none significant model was found. Contrarily, Pb concentrations in the thalli varied independently of the metal levels in the substrate. There are other important sources which affect the concentration of this element in the thalli.

The restrained accumulation pattern may be an important attribute of *C. rei* which facilitates its colonization of extremely contaminated substrates and highlights its ecological importance as sturdy pioneer in affected sites.

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The influence of water sorbing geocomposites on *Miscanthus* × *giganteus* biomass cultivated on heavy metals contaminated arable soil – field experiment

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Cultivation of energy crops (i.e. *Miscanthus* × *giganteus*) creates the possibility of using agricultural land excluded from the food production, set aside and polluted by industry, however such approach might lead to excessive concentrations of metals in plant tissues (Pogrzeba et al., 2012, 2013). The important issue in setting up of energy crop plantations is to provide water and nutrients – the crucial parameters for determination of successful plant growth (Tonn et al., 2012). This is mainly due to the fact, that contaminated areas usually are poor in nutrients and have improper water relations. Therefore, there is a need to find the satisfied solutions for adequate water regime. The key parameter in creation of the energy crop plantation (especially in case of *Miscanthus* × *giganteus*) is adequate water relations in the first year of plantation. The use of geocomposites (water superabsorbents), could help in proper growth and plant development towards good biomass as a stored energy. After two years of field experiment no clear relationship between presence of geocomposites in the soil and *M. giganteus* growth and uptake of macroelements were observed. However, introducing of geocomposites to the soil may result in lower heavy metals content in plant tissues.

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Distribution of Pb in *Populus tremula* × *P. tremuloides* and *Arabidopsis thaliana* root tip tissues

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Pb rather immobile trace metal is mainly accumulated within the plant roots. Therefore, the aim of the study was to estimate the regions of Pb accumulation and its relationship to the level and distribution of low-methylesterified pectins (a cell wall compound able to bind metal ions).

The objects of the study were root tips of *Populus tremula* × *P. tremuloides* and *Arabidopsis thaliana* treated with $PbCl_2$ (1000 μMPb 4h, control 4h H_2O). Low-methylesterified pectins were identified by immunogold method (JIM5 antibody); Pb – by rodizonic acid, X-ray microanalysis connected with transmission electron microscopy (TEM) and as electron-dense deposits in TEM.

Detection of Pb by rodizonic acid showed that Pb deposits were present in most cells building root tip tissues in both plant species. However, it was possible to indicate a special zone which showed especially high Pb accumulation. We called it the "accumulation zone". It included the very tip tissues as cap cells (especially columella) and apical meristematic cells. Moreover, it extended above the very tip and included, e.g. epidermal cells and adjacent to them cortex cells.

Generally, the highest Pb accumulation was detected in the same regions where high amount of JIM5 pectin epitope occurred. There were (1) CWs contact sites of the neighboring cells, (2) CWs adjacent to intercellular spaces (IS – in particular the inner site of the CW) and moreover (3) IS spaces. Generally JIM5 pectin epitope and Pb deposits showed close colocalization what was detected by microscopic methods and X-ray microanalysis.

To sum up, the root tips were the regions of especially high Pb accumulation in both plant species. It was possible to distinguish a special "accumulation zone" in the root tip which indicate particularly high Pb accumulation. Furthermore, accumulation of Pb in studied plant species was mainly dependent on the low-methylesterified pectins level.

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Responses of cryptogamic species to heavy metal contamination – a case study in psammophilous grasslands

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Due to their pioneer nature lichens and bryophytes constitute a large biomass and play a fundamental role in poor grassland areas. These organisms are also known as effective and rapid colonisers of bare ground in strongly contaminated habitats (Osyczka and Rola, 2013a). Heavy metal contamination generally leads to a decrease in biodiversity and impoverishment of cryptogamic communities. On the other hand, cryptogams are regarded as stress tolerators and some of them are strongly linked to metal-rich substrates (Osyczka and Rola, 2013b).

This study determines changes in the structure of cryptogamic vegetation of poor psammophilous grassland along a pollution gradient near a zinc smelter and defines the most important habitat factors affecting species composition and richness. Lichens and bryophytes were examined in study plots along six transects in 4 distance zones and the physicochemical properties of corresponding soil samples were analysed.

Four different responses of species to substrate contamination were identified, with a distinct group of species resistant to and favoured by metal contamination. In the zone most affected by pollution, cryptogamic vegetation is quite abundant and consists of specific and constant assemblage of the most tolerant pioneer species. Although species richness decreases as one approaches the smelter, the replacement of certain sensitive species by resistant ones was observed along a pollution gradient. Species richness was negatively affected by the total Zn as well as exchangeable Cd and Zn to a remarkable extent. Cryptogamic communities clearly dominated by the lichen *Cladonia rei* demonstrate high bioindicative value and indicate potential contamination of the substrate. The changes in community structure could be a useful tool in metal pollution biomonitoring.

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The potential pathways of plants pollution in the emission region of Copper Smelter GŁOGÓW – current condition of agricultural environment in this region

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Current condition of agricultural environment in the vicinity of Copper Smelter GŁOGÓW was investigated. Studies were carried out in the years 2008–2013. Copper, lead, zinc, cadmium and arsenic levels were determined by means of AAS in the soils and cultivated plants threatened by air and dust emissions from the Smelter.

The aim of the studies was: 1) to find out whether the soils around the Smelter are suitable to agricultural farming; 2) to assess the consumption value of the plants cultivated in those soils, taking as a criterion the content of heavy metals detected in their edible parts.

The current concentration of heavy metals and arsenic in the agricultural environment around the Copper Smelter GŁOGÓW does not seem to exert a phytotoxic impact on agrocenoses because the condition of investigated crops do not differ from those in the areas outside the emission. The studies revealed that heavy metals, either accumulated in the soils or coming from the current emission, did not contaminate the cultivated plants. The results obtained prove the effectiveness of eco-friendly activities taken in recent years by the Smelter for the sake of the neighboring agricultural environment.

Previous studies proved that chemical stabilization of pH in investigated soils within the range 6,8–7,2 inactivates mobility of metals and arsenic and decreases availability of mentioned elements to the roots of crops cultivated in these soils. Using sequential chemical extraction method of Zeien and Brümmer fractions of Cu, Pb, Zn, Cd and As in soils influenced by emissions from the Smelter were investigated. The fractional distribution of metals and arsenic in soil samples in large degree depended on the type of studied element. Relatively the highest contents of heavy metals and arsenic were determined in fractions hard accessible for plants.

The studies revealed that heavy metals and arsenic accumulated in majority of soils in the vicinity of Copper Smelter GŁOGÓW are strongly bound in the soil sorptive complex and their accessibility for plants cultivated in these soils is significantly reduced.

Bioindication of an environment using a bark of *Pinus sylvestris* as a biotest to determine the pollution emitted by steelworks

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Investigation of environmental contaminations by heavy metals and their spreading are very important in the context of sustainable development. Main phytotoxic substances emitted by iron works are sulphur oxides and nitrogen oxides that cause acidification of rain and dust which may cause alkalinisation of rain. Dusts contain mostly iron, calcium and magnesium, and smaller quantities of heavy metals such as cadmium, zinc and lead. Therefore bark of *Pinus sylvestris* has been studied. It was collected from trees growing in different distances from the emitter Mittal Steel Poland SA Katowice branch Dąbrowa Górnicza – the largest ironworks in Poland. Variation of pH and calcium content was not related to the distance from the emitter and the place of sample collection. The results obtained for bark of *P. silvestris* showed significantly increased content of cadmium, lead, copper and iron in samples from the immediate vicinity of steelworks in comparison to samples from places situated away from the smelter and in relation to control. This trend was also evident in the case of magnesium but was slightly weaker. The content of manganese was 2–3 times higher in controls.

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Heavy metals (Zn, Pb, Cd, Tl) rich phases on roots of early-succession plants growing on the wastes from historical Zn-Pb mining and processing (Southern Poland)

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The research was done in the south-east part of Silesia-Kraków Upland (Olkusz region, Poland). Three studied areas of former and contemporary Zn-Pb mining, processing and flotation had been selected. All of them had been devoid of soil and plant cover, because of industrial human activity and they had been contaminated by the high concentrations of heavy metals (Zn, Pb, Cd, Tl and others) (Cabala et al., 2011). They are very interesting research plots where the processes of spontaneous succession and initial soil cover development could be observed. Simultaneously, the diversity of primary and secondary phases containing heavy metals is also worth investigating (Rożek, Cabała, 2013). Roots of *Festuca ovina* and *Corynephorus canescens* were investigated using the Scanning Electron Microscope (SEM, EDS) for recognition of particular groups of metal-bearing (Zn, Pb, Cd, Fe, Mn) minerals, shreds of fungi and algae. Soils and sediments were investigated using AAS and XRD methods.

Significant volume of metals occuring in polymineral aggregates characterized by complex structure and chemical composition. Forms and mineralogical type of metals-bearing phases occurring on rhizodermis have an essential influence on plant toxicity connected with the uncommon heavy metals and metalloids (Babula et. al., 2008).

The investigation was focused on forms and elemental composition of primary and secondary minerals present in rhizodermis of selected plants species. In rhizospheres, there were identified the secondary metalliferous phases and also mineral grains in forms and chemical compositions different from the minerals occurring in primary and oxidized Zn-Pb ores. The formation of such forms on roots may be connected with the defensive reaction of plants to high concentrations of heavy metals. The presence of secondary, uncommon mineral phases prove the occurrence of metals in dissolved ionic forms during the plant vegetation, which had increasing the pool of bioavailable metals.

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Thallium and arsenic in the needles of Scots pine on the post-mining areas

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Thallium (Tl) and arsenic (As) accompany sulphide metal ores (e.g. Zn, Pb, Fe), and they are spreading in environment mainly by mining and smelting ores. These elements are toxic for organisms even in low concentrations. They are ballast elements for plants, but they can be easily taken up and can disturb nutrients uptake (e.g. K, P) (Meharg and Hartley-Whitaker, 2002; Vaník et al., 2013). The study aim was to assess Tl and As concentrations in needles of Scots pine (*Pinus sylvestris* L.) from reclaimed post-mining areas and their impact on pine mineral nutrition. Six study sites were situated in the area related to mining and processing of Zn-Pb ores (the Olkusz Ore-bearing Region – the OOR) and two outside it. The content of Tl and As, as well as N, P, K, Ca, Mg, Fe, Zn, Pb, Cd from each study site was measured in surface soil and one-year needles. The concentrations of Tl and As in soil and in needles from the OOR were low, compared to published data from this region (Wierzbicka et al., 2004; Cabała, 2009; Dmuchowski et al., 2011). They were very high only in soil on old mining wastes. In pine needles any significant correlation between Tl and K or As and P indicating on the disturbances in the nutrients uptake wasn't detected. However Tl can negatively impact on N content – negative correlation between them was found.

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Transfer of heavy metals in forest habitats: soil – clonal plants

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The aim of this study was to evaluate the extent of soil contamination with selected heavy metals and the transferability of these contaminants into rhizome of the dominant clonal plant *Carex brizoides* L. in forest habitats. Soil samples were collected from 20 sites of the Silesian Upland (Upper Silesia, southern Poland):10 with the presence of *Carex brizoides* in the undergrowth – rhizome of *Carex briozides* was sampled in these sites, and 10 patches where this species was absent. The transfer of Zn, Pb, Cd, Cu, Fe from soil into the rhizome of *Carex brizoides* L. was studied in the oak-hornbeam habitats presenting the different degree of degradation. *Carex brizoides* rhizomes were digested in 8 ml of HNO_3 and 2 ml of H_2O_2, using Milestone Ethos One microwave oven. Concentration of heavy metals in plant material and soil (HNO_3 and $CaCl_2$-extractable) was analysed using flame AAS (Thermo Scientific™ iCE 3500). The transfer of heavy metals from soil to rhizome of *Carex brizoides* was determined by transfer factor (TF) value.

Generally, the concentrations of heavy metals were similar in the soil of both forest habitat groups. The difference in total concentration of Zn, Fe and bioavailable Pb was statistically significant ($p<0.05$). Correlation between the concentration of heavy metals in soil and rhizome of *Carex brizoides* was positive for bioavailable concentrations of Pb, Cd and Cu ($p<0.05$). The TF for Cu was higher than other metals i.e. Pb and Cd. The transfer factor value was greather than 1 for Zn, Pb, Cd and Cu. The value of TF showed that clonal plants – *Carex brizoides*, play a significant role in the transfer of certain heavy metals in forest habitats.

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New data on the mechanism of hormesis in plants

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Term hormesis describes the stimulation phase in a growth response curve that is induced by low concentrations of toxic substances, e.g. heavy metals.

Experiments were carried out with etiolated 4-day-old maize seedlings cv. 'Kosmo 230', which grew for the last 24 hours in hydroponic systems filled with control medium or the control medium with $PbCl_2$ or $CdCl_2$ at the concentration of 10^{-5}, 10^{-4} or 10^{-3} M. After 24 hours of incubation in the presence of the metals the growth of seedlings organs (roots, mesocotyls, coleoptiles) was measured. Subsequently coleoptile sections were excised. In one batch of segments the content of IAA and H_2O_2 was determined spectrophotometrically, whereas the second batch was used to elongation growth test.

Both metals diminish significantly the growth of roots, mesocotyls and coleoptiles of seedlings. On the contrary, coleoptiles segments excised from seedlings treated with Pb or Cd at the concentration of 10^{-3} M grew better than segments excised from control seedlings when incubated in the control medium. To explain the mechanism of this hormetic effect the content of IAA and H_2O_2 were measured in the sections. It was found that the higher growth of segments excised from treated seedlings was correlated with higher content of both IAA and H_2O_2 when compared to the control. It should be emphasized that the highest content of both IAA and H_2O_2 was observed in segments excised from seedlings treated with 10^{-3} M Cd or Pb.

The data would seem to suggest that the mechanism of hormesis induced by heavy metals is related to higher content of IAA and H_2O_2 in the plant tissues. Although the presence of H_2O_2 is necessary, the auxin level must be seen as a major factor contributing to hormetic effect.

Heavy metals in edible *Agaricus* species, sources of contamination

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Basidiocarps of the saprotrophic species of genus *Agaricus* are an important components of the diet for some animals and humans as well. Trophic pathways (nutrition trails) with such mushrooms make important part of ecosystem.

Parra (2008) discussing selected, specialist researches of other authors cites a number of issues associated directly with accumulation of heavy metals in basidiocarps of *Agaricus* species – indicating mainly as contamination of natural environment. *Agaricus* species in the culture may be subject of contamination as well. Source of pollutions may be different e.g.: chemicals used during preparing of the substrate or contamination of biological material. For this reason, taking care about cultivations safety is necessary. Some publications pay attention on this type of contaminations, for example associated with arsenic (As) and mercury (Hg) accumulation (Vetter and Berta, 2005; Soeres et al., 2005).

Radionuclid accumulation by wild edible basidiocarps fungi is presently important issue in environmental toxicology. Radioactive cesium (137Cs) is absorbed in small amount by *Agaricus* species over which the research is conducted (*Agaricus arvensis, A. campestris, A. macrosprus, A. sylvaticus, A. sylvicola, A. subperonatus*) (Barnett et al., 1999).

General conclusion: So, in the industrial region as Silesia, even so delicious fungus as *Agaricus* have to be collected and eaten very carefully.

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Accumulation of heavy metals by the parasitic species on the example of genus *Phellinus*

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Due to dependencies that occur between fungus and plants we distinguish 3 groups of fungi: saprotrophic, symbiotic and parasitic. Parasitic fungi are considered as week indicator of metals accumulation (including heavy metals). This is due to the fact that tree is a specific filter, for this reason there is no relationship between concentration of this substances in soli and basidiocarp. There have been found large quantities of these substances in the fruiting bodies.

Living organisms have different abilities to accumulate heavy metals e.g. *Phellinus badius* basidiocarps strongly accumulate metals such as lead (Pb), copper (Cu), cadmium (Cd) or silver (Ag) compared with wood bark (*Pinus radiata*), but less than marine algae *Durvillea potatorum* (Matheickal, 1997). There are only a few studies about heavy metals accumulation. It is said that the parasitic fungi accumulate metals equally or heavily than mycorrhizal or saprotrophic fungi. During the researches it has been shown, that *Phelinus* genus fungi cause white rot of wood. At the time of biochemical transformations fungi absorb heavy metals which penetrate into the wood and are accumulated in fruiting bodies.

Heavy metals which are accumulated in *Phellinus igniarius* basidiocarps have been used for ages by Native Alaskans. Basidiocarps are burning (this process additionally increases the concentration of metals) then their ashes are mixing with tobacco and water and finally they are chewed (Blanchette, 2001, 2002). Such high concentrations of metals and heavy metals causes alkalization of the oral cavity environment and better absorption of tobacco. When the ashes of *Phellinus igniarius* are mixed with tobacco it added "a powerful kick" to a human who is chewing it.

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Genotoxic effects of cadmium in female and male *Steatoda grossa* (Theridiidae) spiders

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Spiders as predators and polyphages are generally recognized as macroconcentrators of metals, including cadmium. Considerable accumulation of these xenobiotics in their bodies is favored by high absorption from their diet and also by low metabolism rate in midgut glands, where the metal loads are generally higher than in other organs.

The aim of this study was to assess the genotoxic effects of Cd on hemocytes and midgut glands cells of web building spiders *Steatoda grossa* (Theriididae), exposed to metal under laboratory conditions. The analyses were conducted on adult females and males, fed for four weeks with cadmium-contaminated flies *Drosophila hydei* (grown on a medium suplemented with 0.25 mM $CdCl_2$). The comet assay, that provides quantitative measure of the DNA-strand breaks (Collins, 2009), was used to evaluate the DNA damage caused by the metal. Cadmium contents was measured in whole spider bodies by the AAS method.

Cadmium body burdens in females in the group receiving the metal in the diet was six times lower than in males (females: 0.116 μg . g^{-1} dry weight; males: 0.696 μg . g^{-1} dry weight) suggesting, that females may have more effective mechanisms that control the metal uptake via digestive tract or its elimination from the body. Irrespectively of sex, spiders fed preys contaminated with cadmium, had significantly higher median values of the comet parameters: tail DNA (TDNA), tail length (TL) and olive tail moment (OTM), in comparison with the control. The level of DNA damage was higher in midgut glands cells of males than in females (TDNA – 6 times, TL – 6 times), while genotoxic effect of cadmium in hemocytes was higher in females (TDNA – 3 times, TL – 2 times). The obtained results indicate that cadmium in spiders has a strong genotoxic effects and may cause DNA damage by acting even in small concentrations, however severity of the damage seems to be sex -and internal organ- dependent. The comet assay can be considered as sensitive tool for measuring the deleterious effect of cadmium on DNA integrity in spiders.

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Contents of selected heavy metals in medicinal plant *Arctostaphylos uva-ursi* (L.) Spreng from various localities in southern Poland

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The bearberry *Arctostaphylos uva-ursi* (L.) Spreng is a valuable medicinal plant. The harvested herbal material are entire or fragmented leaves *Uvae ursi folia*. Leaf extracts in human body have mainly antibacterial effect in the urinary system (Frohne, 2010). In Poland *A. uva-ursi* is the species strictly protected by law, therefore the acquiring its raw material is not allowed by law. However, in few localities in southern Poland occurrence of this species on habitats influenced by human activity were observed, i.e. along railway lines running across coniferous forest complexes (Bacler, 2009; Stebel et al., 2013). *A. uva-ursi* grows here on protective and technical belts of railway lines. Samples of *A. uva-ursi* leafs and soil were collected from four localities – two natural places and two from neighbourhood of railway lines. The main purpose of the investigation was to compare contents of selected heavy metals (cadmium, iron, lead, manganese and zinc) in herbal materials from plants growing in natural and influenced by human habitats.

Dry samples were cleaned of foreign components and dried in air ambient temperature. After drying, the samples were mineralized using 69.0–70.0% nitric acid 'BAKER INSTRA-ANALYZED for Trace Metals Analysis'. Content of heavy metals were determined by atomic absorption spectrometry with electro-thermal atomization (ETAAS) using ZL 4100 Perkin Elmer with the Zeeman's method of background correction.

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Quercus robur L. and *Quercus rubra* L. as biomonitors of heavy metal pollution

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Plants are considered to be good bioindicators of metal pollution and trees are often used to assess the level of heavy metal contamination especially in cities, where their occurrence is widespread. In this study concentrations of Pb, Zn and Cd were measured in leaves, twigs and bark of *Quercus robur* L. and *Q. rubra* L. and in soils in 8 towns in southern Poland (Tychy, Lędziny, Jaworzno, Olkusz, Pszczyna, Bielsko-Biała, Bieruń, Oświecim) in order to evaluate the level of pollution in this area and the suitability of chosen organs as pollution monitors. Additionally the accumulation factor was calculated to evaluate the element accumulation at the urbanized sites compared to relatively unpolluted site of Roztocze National Park (Maisto et al., 2004).

The highest levels of investigated elements were found in Olkusz and Jaworzno in all examined organs (beside Pb in leaves) as well as in soils, in other cities the concentration levels were similar. In case of Pb and Cd the concentrations were exceeding the physiological level almost in every investigated city in all organs, in case of Zn only in bark of *Q. robur* in Olkusz (Kabata-Pendias and Pendias, 1999). The accumulation factor suggest that all investigated cities are threatened with Pb and Zn pollution, Jaworzno and Olkusz additionally with Cd pollution. The concentrations of investigated elements in leaves, twigs and bark were positively correlated with soil concentrations suggesting their atmospheric origin, however the concentration of Pb in leaves of both tree species was negatively correlated with the content in the soil. Statistical differences between concentrations of heavy metals in both tree species were observed only in case of Zn and Pb in bark samples. Bark of *Q. robur* showed greater capacity of metal accumulation. The concentration of metals in selected organs can be arranged as follows: leaves < twigs < bark.

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Sex dependent cadmium and copper concentration in the wolf spiders *Xerolycosa nemoralis* (Lycosidae) during their development on variously contaminated sites

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Heavy metals, including Cd and Cu, belong to the group of the most persistent pollutants that can accumulate in the organisms. Spiders as predators play an important role in substance turnover in the trophic webs. As secondary consumers they are significantly exposed to heavy metals that are deposed mainly in midgut gland cells. Metal level in the spiders depends on sex, developmental stage, hunting strategy and kind of metal (Wilczek, 2008).

The aim of the study was to assess whether, and how, food quality (polluted with cadmium/copper vs unpolluted) determines the metal levels in spider body in relation to developmental stage, sex, body part (prosoma, opisthosoma) and original habitat. In the study eggs, embryos as well as juveniles and female and male adults of the wolf spider *X. nemoralis* (Lycosidae) were used. The spiders were collected from two sites: Katowice-Wełnowiec (50°17' N, 19°00'E), heavily polluted with metals and Pilica (50°28' N, 19°39' E) – reference.

In the laboratory the spiders were fed Cd- and Cu-intoxicated fruitflies (*Drosophila melanogaster*) for 21 days. Control spiders obtained unpolluted food. Cd and Cu concentrations were measured by AAS method (atomic absorption spectrometry).

Food supplementation with metals caused the increase in their concentrations in prosoma and opisthosoma, irrespectively of the site of spider origin. Metal concentration in females was higher than in males. The age of spiders did not change the Cu and Cd concentration in prosoma. In opisthosoma metal concentrations in adults were higher than in remaining stages.

Among cocoons, eggs and embryos Cd concentration increased as follows: embryos < eggs < cocoons, irrespectively of the habitat. This may be the result of genetic material protection against metals. For Cu concentration the order was different: cocoons < eggs < embryos since this metal plays an important structural and regulatory role. Lower mass and higher number of the eggs was found in cocoons from Wełnowiec, in comparison to the reference ones. This confirms the ability of energy allocation changes under increased metal concentration in food.

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The effect of cadmium on secondary metabolites, structure and ultrastructure of St. John's wort (*Hypericum perforatum* L.) leaves

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Increasing environmental pollution and civilization diseases caused that many researches highlight the threat of water and soil contamination with heavy metals, like lead and cadmium (Cd). The exposure of plants to the cadmium leads to phytotoxicity. However, some plants (including medicinal plants such as *Hypericum perforatum* L.) are hyperaccumulators, which means that they can accumulate relatively high concentrations of the metals in their cells (Pesko, 2010). There is no data so far concerning the effect of cadmium on ultrastructure of St. John's wort leaves. Therefore, the aim of our study was to describe the cadmium related changes in leaves cell organisation and ultrastructure as well as in active compounds (anthranoids, polyphenols) levels in the shoots of *Hypericum perforatum* L.

The experiments were carried out on *in vitro* cultures of St. John's wort using Murashige and Skoog medium (MS) with Cd (25 and 400 μM). Shoots were collected at 3, 7, 11, and 17 days of plant growth and used for phytochemical analyses. Microscopic examination was performed using leaf fragments collected on 7th and 17th day of the culture. The hypericin, polyphenols and cadmium content were determined using HPLC-FL, spectrophotometric (with Folin's reagent) and graphite furnace atomic absorption spectrometry (GFAAS) method, respectively.

The exposure to cadmium did not affect the hypericins ($p>0.05$), but increased the polyphenols content in the shoots ($p=0.015$ and $p=0.00001$ on the 17th day of culture on medium with Cd 25 μM and 400 μM, respectively). The microscopic examination revealed polyphenols as black spherical deposits and small granules in the plant cells. Degradation changes in the structure of chloroplast, increased cell vacuolation, swollen mitochondrial cristae as well as lower leaves blades thickness were observed for the plant cultured on media with both cadmium concentrations.

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Energy budget of snail *Cepaea nemoralis* under cadmium exposure

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The aim of this study was to estimate energetic budget of the land snail under cadmium exposure. During one week exposure to this metal (two concentrations of Cd in the artificial food were used: 1 and 13 μg Cd · g^{-1} dry weight) parameters of food utilization were determined. In addition, the relations between analyzed components of energetic budget and extend of metal exposure were assessed.

For the investigations land sail *Cepaea nemoralis* (Pulmonata: Helicoidea) was chosen. This species can live in strongly contaminated habitats and due to ability to accumulate great amount of metals in its tissues can be used as bioindicators of environment pollution (Dallinger and Rainbow, 1993).

Parameters and indices of energetic budget in adult individuals of *C. nemoralis* showed low production in spite of large consumption and big assimilation efficiency. It may give the evidence for altered energy allocation due to cadmium in the diet and in tissues.

Analysis of components of energetic budget confirms their dependence on cadmium exposure. Net production, index of assimilation efficiency, index of gross and net production decreased with the metal concentration in the food. The greatest amount of consumption, faeces production, food assimilation and respiration in the animals from Cd1 group were stated, whereas in the snails from control and Cd2 group these indices were similar, however lower than in Cd1 group. This may reflect hormetic effect at lover exposure level.

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The application potential of metallophyte plant species collected in the BIOGEO-SILESIA ORSIP database

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In the 21st century studies of natural resources have become a vital tool in evaluating the impact of man on the ecosystem. Today's observations of nature are based on modern methods of collecting, storing, handling, processing, sharing and disseminating data. Accessible through open-access databases and geoportals, the results of such research can become an effective tool used in the management of natural resources.

Silesia province is currently establishing an Open-access Regional Spatial Information System (ORSIP) whose purpose is to set up a regional open-access digital platform integrating referential and thematic spatial data resources. This database, created by researchers from the University of Silesia in collaboration with Silesia province, covers information cross-referenced to 18 sets of biodiversity data and 1 set of data on geosites. Data from scientific research are the basic source of information (Tokarska-Guzik et al., in press).

One of the 18 sets of biodiversity data is dedicated to the collection of information about the flora and vegetation of post-industrial sites, including sites contaminated by heavy metals such as lead and zinc. These database provide a tool for gathering information about vascular plant species occurrence, species composition, and the abundance of vegetation patches recorded on sites contaminated by heavy metals, as well as detailed information about the condition of biotic and abiotic sites.

Data collected in this way provide the possibility to perform a range of analysis which can help in answering the following questions and many others: on what kinds of habitat types do metallophyte species occur?; are the species accompanying metallophyte species specific for the site conditions or the species?

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Floristic values of heavy metal contaminated areas in the Silesia-Cracow Uplands

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Areas with a high concentration of heavy metals in the soil can be divided into three types of habitats: primary, secondary and tertiary sites (Baker et al., 2010). The Silesia-Cracow Uplands is the region with particularly frequent occurrence of these habitats. One can find here old ore mines or storage places of mining waste material as well as areas secondarily contaminated by the atmospheric deposition of metal-enriched industrial dusts. Plants that are tolerant to heavy metal toxicity and are able to survive and reproduce on metalliferous soils are called metallophytes. Interestingly, many protected and endangered species can be found among Polish metallophytes.

The aim of the study was to assess the floristic value of metalliferous areas of the Silesia-Cracow Uplands. The study was based on published literature and herbarium collections. The list of metallophytes followed Rostański et al. (2014). Species protected by law and endangered were defined according to Parusel and Urbisz (2012).

We recorded the presence of 10 species protected by law and 25 threatened taxa. Particularly valuable in the aspect of nature protection are: *Antennaria dioica, Biscutella laevigata, Epipactis palustris, Erysimum odoratum, Gentiana cruciata, Gentianella germanica, Malaxis monophyllos, Prunella grandiflora, Pulsatilla patens, Thesium alpinum*.

Our results suggest that some of the metalliferous habitats located in Silesia-Cracow Uplands should be legally protected due to their high floristic value.

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The effect of lignite and green compost on bioavailability of Pb, Cd, Zn and microbial population in heavy-metal contaminated soil

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The effect of lignite and green compost on the bioavailability of Pb, Cd and Zn and number of cultivated soil microorganisms in heavy metal-contaminated soil during an aided phytostabilization was studied. During the first season of *Festuca arundinacea* growing in soil amended with lignite (PL), the amount of bioavailable fractions of Pb, Zn and Cd declined about 2.5-fold, whereas in soil enriched with green compost (PC) it was 4- to 6-fold reduced. During the experiment the number of psychrophiles, actinomycetes and fungi in PL soil increased from $1.35 \cdot 10^7$ to $2.05 \cdot 10^8$, $1.44 \cdot 10^3$ to $1.27 \cdot 10^4$ and $1.13 \cdot 10^4$ to $2.39 \cdot 10^4$ CFU g^{-1} dw^{-1} soil, respectively. In turn, in PC soil the increase in the number of actinomycetes and fungi were only observed. The number of actinomycetes increased from $3.26 \cdot 10^3$ at the beginning of the experiment to $2.88 \cdot 10^4$ CFU g^{-1} dw^{-1} soil at the end, while the number of fungi from $8.6 \cdot 10^4$ to $1.81 \cdot 10^5$ CFU g^{-1} dw^{-1} soil. At this time, the number of psychrophiles in PC soil did not change significantly. It was confirmed that the aided phytostabilization supported by lignite and green compost is an effective method to decrease the content of bioavailable fraction of Pb, Cd and Zn in heavy-metal contaminated soil. Additionally, it was demonstrated that both stabilizers caused the increase in the number of selected groups of microorganisms in tested soil.

Is *Biscutella laevigata* subsp. *woycickii* a thallium hyperaccumulator?

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The *Biscutella laevigata* L. plants are known as being able to hyperaccumulate thallium. There are two locations of this species in Poland: in the Tatra Mountains and on the calamine waste heap in Bolesław near Olkusz. The Tatra population belongs to subsp. *gracilis* Mach.-Laur., whereas the zinc-lead waste heap population has been lately accepted as a distinct subspecies, *B. laevigata* subsp. *woycickii* (Wąsowicz, 2014; Wąsowicz et al., 2014; Wierzbicka et al., 2014).

The soil from the Bolesław location contains a big amount of thallium (15.2–43 $mg \cdot kg^{-1}$ d.m.), however, in the form available to plants – only 1,1 $mg \cdot kg^{-1}$ d.m. Plants of the two *Biscutella laevigata* subspecies, in different growth phases, have been cultivated in this soil for one vegetation season. The Tl concentration in the plants after this period amounted to 98.5 $mg \cdot kg^{-1}$ d.m. on average. The biggest Tl amount was transported to leaves – 164.9 $mg \cdot kg^{-1}$ d.m. on average, max. 588.2 $mg \cdot kg^{-1}$ d.m. Next, EDTA was added to the soil to enhance the Tl bioavailability. The Tl concentration in plants increased to 109 $mg \cdot kg^{-1}$ d.m., and its biggest quantity was detected in leaves – 138.4 $mg \cdot kg^{-1}$ d.m. (max. 1100 $mg \cdot kg^{-1}$ d.m.). The translocation index was 6.1.

Although the plants of both subspecies do not contain thallium in the amount indicating hyperaccumulation (over 500 mg kg^{-1} d.m.), the high root-shoot translocation index suggests that they are potentially able do hyperaccumulate Tl. This ability common to the plants from both locations seems to be a characteristic species feature.

We conclude that *Biscutella laevigata* subsp. *woycickii* is not a thallium hyperaccumulator, however, these plants show a high potential for accumulating it.

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Application of ecological indicators values for analysis of heavy metal presence in the habitats of selected plant communities

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Indicator values are universal tool used to define the condition of natural habitats based on the species composition of phytocoenoses. There are two lists of indicator values, Ellenberg's of Middle European's plants (Ellenberg and Leuschner, 2010) and Zarzycki's of Polish flora (Zarzycki et. al., 2002), which are most popular in Poland. In both scales values related to resistance to increased heavy metal content in the soil (M) occur.

In this study the suitability of both M values were determined to identify the content of heavy metals in soils, as well as habitats were also characterized using remaining indicator values: light (L), temperature (T), continentality (K), soil moisture (W or F), trophy (Tr or N), soil acidity (R), soil granulometric (D) and organic matter content (H). The soil properties, such as concentration of Cd and Zn, pH and the content of organic matter were measured in 5 phytocoenoses.

Results show discrepancy between M values of two indicator values lists and no relationship between abundance of species considered to be metalophytes and content of heavy metals measured in the soils were found. PCA analysis was also performed on all mentioned above indicator values, species composition data and the soils properties. Results of preformed PCA analysis indicate more significant role of soil properties measured in phytocenoses than all of the indicator values.

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Grasslands of historical Zn-Pb mining sites in western Małopolska (S Poland)

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This study characterizes the *Carlino acaulis-Brometum erecti* association occurring on the relics of the former Zn-Pb mining (small mine waste heaps termed locally 'warpie') in western Małopolska (S Poland). The association – investigated at 65 sites – had a typical (of calcareous grassland) plant species composition (the *CB* variant, N=13), when developing at low and moderate concentrations of heavy metals in soil (on average 17 mg Cd kg^{-1}) and high distances from the edge of the forest (on average 175 m). The *CB* variant changed into the *festucetum ovinae* variant (subassociation) (*CBF*, N=38) at high levels of metal contamination (127 mg Cd kg^{-1}) or the *rubietum caesi* variant (CBR, N=14) near the forest (28 m). The *CB, CBF* and *CBR* variants were described in terms of the total coverage, the species richness and the number of species belonging to different phytosociological classes, functional groups, Grime's CSR strategies and seed dispersal types; the data were collected from 4 m^2 plots. The three variants differed significantly in the total coverage (it averaged 96.5, 90.5 and 98.2 % for *CB*, *CBF* and *CBR*) but were similar as far as the species richness is concerned (20, 20 and 17 species per plot for *CB*, *CBF* and *CBR*). The *CB* variant, in many respects, was intermediate between the *CBF* and *CBR* variants. Although dominated by *Brachypodium pinnatum*, the *CB* variant was composed of many species, mostly from the *Festuco-Brometea* (60–70%) and *Molinio-Arrhenatheretea* (20–30 %) classes. The *CBF* variant with *Festuca ovina* had less stable species composition than the previous one as indicated by the admixture of species from other classes, e.g. *Koelerio-Corynephoretea, Calluno-Ulicetea* or *Asplenietea trichomanis*. This variant distinguished by the highest number of forbs, some of which were stress-tolerant plants (including facultative metalophytes), and the lowest number of plants dispersed zoochorically. The *CBR* variant with *Rubus caesius*, developed in the proximity of the forest, had more competitors, mainly woody plants typical of forest habitats, and less plants dispersed anemochorically compared to the *CB* and *CBF* variants.

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Molinia caerulea root colonization by arbuscular mycorrhizal fungi in various metal contaminated sites

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In Poland *Molinia caerulea* is a diagnostic species of moist grasslands (the *Molinion* alliance) and wet coniferous forests (the *Molinio-Pinetum*). It is a facultative halophyte but not indicator of resistance of increased heavy metal content in the soil (Zarzycki et al., 2002).

It has arbuscular type of endomycorrhiza. In the Silesian Upland this grass was frequently recorded not only in semi-natural and natural habitats but also on metal-contaminated sites (Rostański, 2006). The aim of this study was to examine what is the level of the mycorrhizal colonization in *Molinia caerulea* roots obtained from vegetation patches occurring in two different types of habitats. We expected that level of mycorrhizal colonization depends on harshness of the environment and will be higher in individuals obtained from contaminated habitats.

For detailed research 9 localities were chosen in the Silesian Upland: 4 in the areas connected with zinc and lead industry and 5 in moist grasslands. In each locality we selected 1 study plot further divided into 5 subplots. We made floristic records and collected soil samples and root samples of 5 individuals of *Molinia caerulea*. Soils samples were analysed in reference to: content of heavy metals (Pb, Cd, Zn, Fe) and chosen physical and chemical parameters (granulometric composition, electrical conductivity, pH, P_2O_5, K_2O, Mg, Ca, Na, Cl, $N\text{-}NH_4$). The extent of mycorrhization was evaluated according to Trouvelot method by determination following parameters (F% – mycorrhizal frequency, M% – intensity of the mycorrhizal colonisation, A% – arbuscule abundance).

The preliminary results confirmed differences in level of mycorrhizal colonization in different types of habitats. However, in contrast to our expectations, it was lower in areas contaminated with heavy metals.

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Physiological mechanisms of plant adaptation to growth in metalliferous areas

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Waste deposits left over mining and processing of Zn-Pb ores poses extremely harsh environments for establishment of vegetation. Nevertheless, some plant species spontaneously colonise such areas. These plants (so called 'pseudometallophytes') usually represent common plant species but exhibit a greater ability to resist, tolerate, or thrive in toxic metalliferous habitats compared with members of the same species from unpolluted soils. This is related to development of a variety of mechanisms enabling them to avoid, resist, or detoxify the metals within their tissues.

The aim of the present study was to identify the physiological mechanisms of adaptation of several plant species to growth on Zn-Pb deposits in southern Poland. In particular, we assessed the metal accumulation strategy by determining the ratio of the metal content in plant shoots to its content in the substrate (bioconcentration coefficient, BC) as well as metal localisation in shoots using histochemical staining with dithizone. Moreover, accumulation of organic acids, glutathione (GSH), and phytochelatins (PCs) was determined in order to assess intracellular mechanisms of metals detoxification in plants growing on the waste deposits in comparison with the populations of these species from unpolluted areas.

All the plant species studied [*Dianthus carthusianorum* L., *Echium vulgare* L., *Daucus carota* L., *Silene vulgaris* (Moench) Garcke] exhibited a metal excluding strategy as shown by the very low BC values (below 0.01 for Zn and Pb, and below 0.05 for Cd for all species). Additionally, the metals were excluded from the shoot tissues by deposition thereof in epidermal, stomatal, and trichome cells as revealed by the visualisation of the metal complexes with dithizone. The concentrations of malate, citrate, succinate, and acetate varied considerably between the species studied, however the differences in accumulation of these acids between metallicolous and nonmetallicolous populations were in most cases insignificant. The waste heap plants did not produce PCs, although the GSH concentrations were higher in metallicolous than in nonmetallicolous populations. In conclusion, intracellular metal chelating by organic acids or PCs is not crucial for adaptation of the studied plants to growth in metalliferous areas; instead, metal exclusion from the tissues seems to be of major importance.

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Zinc and cadmium effect in ontogenesis of *Spodoptera exigua*

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Metals accumulation in plants is known as one of their defense mechanisms against herbivorous insects. Beet armyworm *Spodoptera exigua* is an agricultural insect, highly polyphagous pest, especially dangerous in tropical and subtropical areas of the world. There are examples of their strains differing in resistance to environmental xenobiotics.

The aim of our study was to assess the consequences of cadmium and zinc exposure on the growth parameters of *S. exigua* larvae as well as metal accumulation in developmental stages (larvae, pupae and adults) and in pupal cases. We compared these parameters between individuals originating from two breeding strains (control and cadmium – metal stressed over 100 generations). The larvae from the control and cadmium strains were fed the diet contaminated with cadmium (44 mg Cd/kg dry weight of larval diet) and with zinc (50 mg Zn/kg dry weight), as single stressor or in mixture.

In the present study, we found the differences in survival of the larvae and the duration of the larval stage. The larval survival was lower in the group of larvae originating from the cadmium strain fed both cadmium- and zinc-contaminated food and from the control strain fed the diet contaminated with zinc in comparison to the control group. The duration of the larval stage was longer for animals fed zinc-contaminated and cadmium- and zinc-contaminated food than for insects exposed to cadmium alone or to control animals.

In the groups of animals exposed to cadmium, the elevated concentration of this metal was observed in the bodies of 5th stage larvae, pupae, adults and pupae cases when compared with control data. In the cases of group of animals exposed to zinc, such elevation was only detected in the bodies of 5th stage larvae and pupae.

We found some differences between animals from both examined strains, related to survival rate, cadmium accumulation in larvae and in pupal cases. We did not found such differences in metal accumulation in adult insects.

Metal accumulation and defence response of ladybird *Subcoccinella vigintiquatuorpunctata* fed on *Silene vulgaris* inhabiting metal contaminated areas

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The possibility of existence of the animal in antropogenically changed ecosystems depends e.g. on efficiently acting systems of biochemical defence. *Subcoccinella vigintiquatuorpunctata* is the only one ladybird regarded as a pest of many crops, feeding on plants such as: alfalfa (*Medicago* L.), clover (*Trifolium* L.), potato (*Solanum tuberosum* L.), white beet (*Beta vulgaris* L. subsp. *vulgaris*) and many plants from Fabaceae family.

The aim of our research was to assess the level of metals (Zn, Cd, Cu, Pb) in insect and its food – metallophyte *Silene vulgaris* L., as well as to determine the influence of food source on adult ladybird *S. vigintiquatuorpunctata* using selected biomarkers of cellular stress: glutathione transferase (GST) activity, carboxylesterase (CarE) activity, H_2O_2 and glutathione levels and total antioxidant capacity (TAC) as well as heat shock proteins and metallothionein level.

S. vulgaris and the insects were collected from three localities: 250 (S250 site) and 450 (S450 site) meter distance from non-ferrous mining-metallurgic smelter "Szopienice S.A". in Katowice-Szopienice, Poland, which was one of the biggest emitters of particles containing zinc and cadmium and from a calamine soil area in Dąbrowa-Górnicza-Strzemieszyce (DG site), where Zn and Pb ores were exploited in the 19th century.

The highest Zn and Cd concentrations in plants from the S250 site were detected. High Pb level in insects' body from the DG site was connected to the elevated level of this metal in plants growing in this area. The Pb level measured in plants and in animals from the DG site was statistically higher than detected for *S. vulgaris* and insects from the S450 site. The opposite tendency was assessed for copper. The lowest level of detoxifying enzymes was detected in animals from the S250 site, simultaneously they were characterised by the highest TAC level. It points out the important contribution of other biomarkers, tested separately. We detected statistically important differences in GST and CarE activity between ladybirds from both sites in Szopienice. Among stress proteins, the lowest metallothionein level was evaluated in animals from the S450 site. Therefore, it seems, that TAC level could be suitable biomarker for detecting the effects of metal exposure in *Subcoccinella vigintiquatuorpunctata*.