Attachment 3

# SUMMARY OF POST-DOCTORAL DISSERTATION

**presenting scientific achievements and publications**


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## 1. PERSONAL DATA

**First name and surname**

Kinga Drzewiecka

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## 2. DIPLOMAS AND SCIENTIFIC DEGREES

– **Master of Science in Commodity Science - 2001 r.**

Faculty of Commodity Science, Poznań Academy of Economics

(currently: Poznań University of Economics and Business)

**Thesis entitled:**

„The accumulation of polycyclic aromatic hydrocarbons in apples" carried out in the Department of Instrumental Analysis

Supervisor: prof. dr hab. Jacek Kozioł
Reviewer: prof. dr hab. Alicja Maleszka

– **Degree of doctor of the agricultural sciences in the field of horticulture - 2007 r.**

Faculty of Horticulture, August Cieszkowski Agricultural University of Poznań

(currently: Faculty of Horticulture and Landscape Architecture, Poznań University of Life Sciences)

**Doctoral dissertation entitled:**

„Salicylic acid in plant reactions to selected environmental stressors" carried out in Department of Chemistry

Supervisor: prof. dr hab. Piotr Goliński
Reviewers:
prof. dr hab. Monika Kozłowska
(Department of Plant Physiology, Poznań University of Life Sciences)
prof. dr hab. Barbara Tomaszewska
(Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University of Poznań)

## 3. INFORMATION ON EMPLOYMENT HISTORY

- II-VI.2001 – **internship**, Department of Instrumental Analysis, Faculty of Commodity Science, Poznań Academy of Economics (currently: Poznań University of Economics and Business),
- 2001-2005 – **Ph.D. student**, Faculty of Horticulture, August Cieszkowski Agricultural University of Poznań Wydziale Ogrodniczym (currently: Faculty of Horticulture and Landscape Architecture, Poznań University of Life Sciences),
- 2002-2007 – **research assistant (half time)**, Chemistry Department, August Cieszkowski Agricultural University of Poznań (currently: Poznań University of Life Sciences),
- 2007-2008 – **research assistant**, Chemistry Department, Poznań University of Life Sciences,
- 2008-obecnie – **assistant professor**, Chemistry Department, Poznań University of Life Sciences.

## 4. DESCRIPTION OF THE SCIENTIFIC ACHIEVEMENT CONSTITUTING THE BASIS FOR THE APPLICATION FOR THE INITIATION OF THE POSTDOCTORAL DEGREE CONFERRAL PROCEDURE

a) in accordance with art. 16 item 2 of the Act of 14 March 2003 on scientific degrees and scientific titles and on degrees and title in the field of art (the Journal of Law Dziennik Ustaw no. 65, item 595 with later amendments) I hereby submit the scientific accomplishment entitled:

**Interactions of heavy metals, metalloids and their forms – the effect on selected physiological parameters in phyto- and mycoremediation**

b) The presented scientific accomplishment is documented by a series of **6** scientific publications (including 5 papers, of which I am the first author) with the total score of points according to the classification of the Ministry of Science and Higher Education **175** and the total IF score according to the Journal Citation Reports (JCR) database **13.424** (according to the year of publication):

I. **Drzewiecka K.**, M. Mleczek, M. Gąsecka, Z. Magdziak, P. Goliński (2012) Changes in *Salix viminalis* L. cv. 'Cannabina' morphology and physiology in response to nickel ions - hydroponic investigations. Journal of Hazardous Materials, 217-218: 429-438.

IF$_{2012}$=3.925; 45 pkt. MNiSW

II. Gąsecka M., M. Mleczek, **K. Drzewiecka**, Z. Magdziak, T. Chadzinikolau, I. Rissmann, P. Goliński (2012) Physiological and morphological changes in *Salix viminalis* as a result of plant exposure to copper. Journal of Environmental Science and Health, Part A Toxic/Hazardous Substances and Environmental Engineering, 47: 48-557.

$IF_{2012}$=1.252; 20 pkt. MNiSW

III. **Drzewiecka K.**, M. Mleczek, M. Gąsecka, Z. Magdziak, A. Budka, T. Chadzinikolau, Z. Kaczmarek, P. Goliński (2017) Copper and nickel co-treatment alters metal uptake and stress parameters of *Salix purpurea* × *viminalis*. Journal of Plant Physiology, 216:125-134.

$IF_{2016}$=3.121; 35 pkt. MNiSW

IV. **Drzewiecka K.**, M. Gąsecka, P. Rutkowski, Z. Magdziak, P. Goliński, M. Mleczek (2017) Arsenic forms and their combinations induce differences in phenolic accumulation in *Ulmus laevis* Pall. Journal of Plant Physiology, 220: 34–42.

$IF_{2016}$=3.121; 35 pkt. MNiSW

V. **Drzewiecka K.**, M. Mleczek (2017) Salicylic acid accumulation as a result of Cu, Zn, Cd and Pb interactions in common reed (*Phragmites australis*) growing in natural ecosystems. Acta Physiologiae Plantarum, 39:182.

$IF_{2016}$=1.364; 25 pkt. MNiSW

VI. **Drzewiecka K.**, M. Siwulski, M. Mleczek., P. Goliński, K. Sobieralski (2012) Bioaccumulation of heavy metals from artificially enriched **substrates** and their impact on physiology of King Oyster mushroom (*Pleurotus eryngii*). Fresenius Environmental Bulletin, 21: 1666-1674.

$IF_{2012}$=0.641; 15 pkt. MNiSW

The individual contribution of the post-doctoral degree applicant in the preparation of the above-mentioned scientific publications is shown in Attachment no. 4. Declarations of the co-authors of the publications presented above together with the declaration of their individual contributions are given in Attachment no. 6. None of the above-mentioned publications was a part of a monographic series in another post-doctoral degree conferral (habilitation) procedure.

c) Discussion of the scientific objective of the above-mentioned publications and obtained results

**Introduction:**

In nature the simultaneous occurrence of metals is a common phenomenon, leading to synergistic and antagonistic interactions, both between toxic elements and metals essential for plant growth and development. Observed interactions affect the uptake of minerals from the substrate as well as the degree of accumulation and translocation of heavy metals to aboveground organs, while they also considerably modify their toxicity (KOVÁČIK ET AL. 2012, GUALA ET AL. 2010, LI ET AL. 2009). It is crucial to consider interactions between metals, metalloids and their forms of varying toxicity in view of the application of biological methods using plants for bioindication and phytoremediation of metal and metalloid pollutants (KOMANICKA ET AL. 2013, FRIESL ET AL. 2006, PULFORD & WATSON 2003). Currently, mixed pollution of soil and water possess a serious ecological threat, mainly in heavily degraded industrial and post-industrial areas. The problem requires the use of environment-friendly clean-up methods for gradual regeneration of soil and restoration of biodiversity. However, the use of phyto- or mycoremediation requires species/taxa showing high tolerance and well recognized mechanisms of their response to the pollutants under conditions of their co-existence in the environment.

The current state of knowledge does not provide us with extensive information on the overall effect of metals on the metabolism of plants under stress conditions, which may result from the complexity of the analysis and problems with the interpretation of interdependencies between metals, leading to a decrease or an increase in their toxicity. The general aim of the research documented in the presented accomplishment was to verify mechanisms of plant response to the action of a single stress factor (metal) in relation to more complex experimental systems investigated under controlled conditions and in relation to natural environmental conditions. The above-mentioned publications describe the effect of interactions between metals/metalloids on the levels of selected metabolites - indicators of oxidative stress in plants either already used or exhibiting considerable potential applicability in phytoremediation (common osier, European white elm and common reed) and significantly contribute to current knowledge on plant response to the action of toxic elements.

Elevated contents of heavy metals and metalloids in the soil, including also micronutrients (e.g. Cu, Zn, Ni), cause symptoms of phytotoxicity in plants and have a negative effect on the condition of entire ecosystems. The toxic effect of these elements is manifested as disturbed water relations and nutrition in plants, reduced photosynthetic activity as well as oxidation of cellular components, which all results from the reaction of metal/metalloid ions with thiol groups of proteins leading to conformation changes, competitive inhibition of uptake of other ions (macro- and micronutrients) as well as substitution of metals from the group of prosthetic (particularly antioxidative) metalloenzymes (CHEN ET AL. 2009, ERNST 2006). Depending on the redox activity/potential, metal/metalloid ions may directly – in Haber-Weiss and Fenton reactions (e.g. $Cu^{2+}$), and indirectly – by inducing cell membrane NADPH oxidase (e.g. $Ni^{2+}$, $Cd^{2+}$) generate reactive oxygen species (ROS) leading to oxidative stress (SHAHID ET AL. 2014, GAJEWSKA & SKŁODOWSKA 2007, VAN ASSCHE & CLIJSTERS 1990). Plant resistance to the action of heavy metals is a complex phenomenon and it is based on avoidance mechanisms (symbiosis with soil microorganisms, excretion of protons and organic acids to the substrate in order to bind them in the rhizosphere and reduce metal bioavailability/mobility), tolerance (metal binding by functional groups found in the cell wall, reduced transport through the cell membrane, efficient chelation by organic acids and formation of complexes with peptides – metallothioneids and phytochelates and further storage in the vacuole), as well as non-specific plant response to oxidative stress covering biosynthesis of several secondary metabolites (HALL 2002).

Stress metabolomics is an extremely complex problem investigated by specialists in many fields of science, e.g. biologists, physiologists and geneticists. As it was shown in the case of hyperaccumulator plants (ASSUN ET AL. 2003), enhanced accumulation of metals is genetically independent of tolerance mechanisms; however, both phenomena require further studies to indicate metabolites involved both in the non-specific plant response to oxidative stress and mechanisms of heavy metal detoxification. Such a complex function is ascribed to phenolic compounds, which enhanced biosynthesis accompanies various stress conditions (GĄSECKA ET AL. 2017, KOVÁČIK ET AL. 2008, BIAŁOŃSKA ET AL. 2007, MICHALAK 2006). Phenolic compounds are a broad spectrum of metabolites including flavonoids, phenolic acids, stilbenes, tannins, etc., which (together with the polymeric form - lignin) act as antioxidants, chelators and structural components. Changes in the composition of the phenolic fraction and intensified biosynthesis of these metabolites are observed in plants growing naturally in degraded areas and they serve as biochemical indicators of environmental pollution.

Among phenolic compounds we need to focus on salicylic acid acting as a regulator of plant growth and development, as well as a regulatory and signalling substance in complex mechanisms of defence response to several stress factors (POPOVA ET AL. 1997, RASKIN 1992). Participation of salicylic acid in the hypersensitive response (HR) and systemic acquired resistance (SAR) in plants infested by pathogens has been clarified; however, functioning of this metabolite under oxidative stress caused by abiotic factors, particularly of anthropogenic origin, remains ambiguous and requires further research. Obviously, studies comprising the presented accomplishment broaden our knowledge on the role of salicylic acid in plant response to the toxic effect of metal and metalloid ions and confirms its complexity. The function of a stress signal transductor – also served by the volatile methyl ether – concerns communication between underground and aboveground organs both within a given plant and between plants growing in the immediate vicinity. In the phenomenon of oxidative stress, this compound shares signalling mechanisms with reactive oxygen species influencing expression of the NPR1 gene – a key regulator in SAR, and inducing a cascade of protein kinases (mitogen-activated protein kinases - MAPK). In this way the activity of antioxidant enzymes, the level of glutathione and expression of mitochondrial genes are regulated and as a consequence the oxidative status of the cell is modulated (BERKOWITZ ET AL. 2016, SINGH ET AL. 2016, SAMUEL ET AL. 2005). As a representative of phenolic acids it also exhibits chelating and antioxidant properties of considerable importance in the detoxification of metal and metalloid ions. The first report on the induction of biosynthesis of salicylic acid under the influence of heavy metals, published in 2002 by PÁL ET AL., documented an increase in the contents of this metabolite in leaves of maize seedlings treated with cadmium. Next, FREEMAN ET AL. (2004, 2005) showed its enhanced accumulation among Thlaspi species exhibiting hyperaccumulator properties in relation to nickel and zinc. According to those authors, this metabolite blocks the activity of phytochelatin synthase, thus maintaining the high level of glutathione exhibiting antioxidant action and limiting the accumulation of ROS and the level of oxidative stress. Additionally, exogenous salicylic acid considerably reduces cadmium accumulation in leaves and thus the toxicity of this metal, which was confirmed for seedlings of flax and rice produced from seeds treated with this metabolite. However, in the case of nickel an increased translocation of the metal to the photosynthetic organ and a decrease in biomass parameters were observed, which indicates a significant effect of the metal characteristics on its interaction with the metabolite (BELKADHI ET AL. 2012, KOVÁČIK ET AL. 2009, POPOVA ET AL. 2008, CHOUDHURY & PANDA 2004).

In view of the complex function of salicylic acid in plant response to stress factors, its biosynthesis and action may play a key role in plant resistance to the toxic effects of heavy metals and metalloids, particularly in the case of their coexistence and interactions in the environment influencing the resulting plant response and effectiveness of phytoremediation. The individual contribution of the post-doctoral degree applicant in the presented series of publications comprises the analysis of contents of salicylic acid in the free form - biologically active (SA) and its glucoside being a storage form of the metabolite (SAG) - using liquid chromatography coupled with the highly selective and sensitive fluorimetric detection. Moreover, a proper selection of statistical methods to analyse recorded results made it possible to diversify the effect of heavy metals on the induction of salicylic acid biosynthesis in the case of their interactions and to determine differences in the levels of other metabolites – both primary and secondary – connected with phenolic metabolism and immune response in plants (simple sugars, total content and profile of phenolic compounds, glutathione).

Apart from phytoremediation, an effective method of biological remediation of soils to remove xenobiotics and metallic pollutants is provided by mycoremediation, utilising properties of white-rot fungi (WRF), including edible mushrooms from the genera *Pleurotus*, *Lentinula*, *Pholiota* etc. (POINTING 2001). These fungi are characterised by the capacity to completely degrade lignin, making it possible for them to overgrow wood and colonise tree trunks. This characterisitc is determined by the capacity of the mycelium to produce and excrete extracellularly to the substrate a complex of lignin-modifying enzymes (LME), the so-called ligninases, including lignin peroxidase, a manganese-dependent peroxidase and laccase. These enzymes, thanks to their low specificity in relation to the oxidised substrate, also degrade compounds similar in their structure to lignin, including polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), dioxins, pesticides (e.g. DDT, lindane, pentachlorophenol), explosives (e.g. TNT) and selected organic solvents (e.g. carbon tetrachloride). Enzymatic degradation of organic pollutants by ligninase is based on the non-specific free radical mechanism initiated by peroxidases and involving secondary substances released by the mycelium to the substrate, i.e. oxalic acid – a donor of electrons and 3,4-dimethoxybenzyl (veratryl) alcohol and hydrogen peroxide, i.e. mediators enhancing the oxidative potential of the ligninolytic enzyme complex (MESTER & TIEN 2000). White-rot fungi also exhibit a unique capacity to absorb and accumulate heavy metals (KALAC & SVOBODA 2000). Studies conducted on fungal cultures of the popular oyster fungus (*Pleurotes ostreaus)* showed an accumulation of 10 μg $g^{-1}$ d.m. for copper and 5 μg $g^{-1}$ d.m. for zinc (at the 5 mM addition of the metal to the substrate), while the addition of 3 mM lead caused accumulation of 20 μg $g^{-1}$ d.m. (SANGLIMSUWAN ET AL. 1993). Over many years many studies documented the accumulation of toxic elements in the mycelium and fruiting bodies of edible fungi, while only scarce publications concerned mechanisms of the exceptional tolerance and efficient detoxification of heavy metals by these organisms. Probably, apart from the efficient biosorption of metals by polysaccharides of the cell walls, fungi are equipped with a cellular mechanism consisting in the formation of stable complexes of metal ions with proteins and amino acids, thus reducing their toxicity (COLLIN-HANSEN ET AL. 2007). Similarly as it is the case with phytoremediation, the problem of the effect of metal interactions in the substrate on the efficiency of the remediation process concerns also this method and the diverse effect of ions on the enzymatic activity of ligninase may play a key role in limiting mycelium growth and overgrowth of the polluted substrate, and as a consequence it may reduce metal availability. This problem was one of the research tasks in the project, of which I was the head in the years 2009-2011 and concerning applicability of white-rot fungi in mycoremediation of soils polluted with polycyclic aromatic hydrocarbons and biosorption of heavy metals.

**Discussion of the scientific aims and results presented in the above-mentioned publications:**

## SYNERGISTIC AND ANTAGONISTIC INTERACTIONS BETWEEN METALS AND THEIR IMPACT ON THE METABOLISM OF COPPER AND NICKEL TREATED ENERGY WILLOW

*Drzewiecka K., M. Mleczek, M. Gąsecka, Z. Magdziak, P. Goliński (2012) Changes in Salix viminalis L. cv. 'Cannabina' morphology and physiology in response to nickel ions - hydroponic investigations. Journal of Hazardous Materials, 217-218: 429-438.* (45 pkt. MNiSW; $IF_{2012}$=3.925)(Attachment. 4, publication: IB1)

*Gąsecka M., M. Mleczek, K. Drzewiecka, Z. Magdziak, T. Chadzinikolau, I. Rissmann, P. Goliński (2012) Physiological and morphological changes in Salix viminalis as a result of plant exposure to copper. Journal of Environmental Science and Health, Part A Toxic/Hazardous Substances and Environmental Engineering, 47: 48-557.* ( 20 pkt. MNiSW; $IF_{2012}$=1.252) (Attachment 4, publication: IB2)

*Drzewiecka K., M. Mleczek, M. Gąsecka, Z. Magdziak, A. Budka, T. Chadzinikolau, Z. Kaczmarek, P. Goliński (2017) Copper and nickel co-treatment alters metal uptake and stress parameters of Salix purpurea × viminalis. Journal of Plant Physiology, 216:125-134.* (35 pkt. MNiSW; $IF_{2016}$=3.121) (Attachment 4, publication: IB3)

A significant ecological problem observed in industrialised regions, particularly in the vicinity of non-ferrous metal mines and smelting works, is connected with mixed metallic contamination. This concerns e.g. copper and nickel, and results from the minerals containing these elements present simultaneously in the extracted and processed ores (most often sulfide). The electronic and electro-plating industries are additional sources of the coincident emissions of both these metals, compounding this problem.

Phytoremediation, including phytoextraction, is a promising and rapidly developing method to remediate polluted areas at relatively low financial outlays. However, mixed pollutants require the application of plant species exhibiting high tolerance and accumulation capacity in relation to a broad spectrum of metals, determining the efficiency of this process. Apart from phytoextraction of heavy metals, application of tolerant species (including those from the genera *Salix* an *Populus*) and their hybrids has an advantageous effect on soil composition and texture and reduces metal bioavailability, thus promoting the expansion of less tolerant species and restoration of biocenotic balance in the modified ecosystem.

The studies presented in the above-mentioned publications concerned a hybrid of purple willow and basket willow - *Salix purpurea* × *viminalis* (previously termed *Salix viminails* L. cv. "Cannabina"), exhibiting both a considerable growth rate and biomass production, as well as relatively high tolerance to heavy metals in comparison to other woody species. Moreover, a significant trait of this hybrid is also connected with its high accumulation capacity in relation to copper, zinc, chromium and cadmium confirmed both in model studies and *in situ*. In view of its high utility value, this hybrid shows a significant application potential in phytoremediation of toxic elements and reclamation of strongly contaminated areas.

The aim of this study was to indicate differences in the metabolic response of seedlings of the *Salix purpurea* × *viminalis* hybrid to elevated concentrations of copper and nickel in the substrate and to determine the effect of the coincident presence of these metals on their accumulation and metabolic toxicity indexes. Both elements belong to the group of transition metals and serve the role of micronutrients essential for normal physiological function of plants. Despite their chemical similarity, copper is an element of much greater physiological importance than nickel, serving the function of a co-factor for several enzymatic proteins, including antioxidants. As a consequence, mean copper content in organs of higher plants depending on the species and organ is ~1-5 μg $g^{-1}$ d.m., while that of nickel is only 2-4 ng $g^{-1}$ d.m. Despite the considerable difference in the basal contents, toxicity thresholds of both metals for most plants are comparable, i.e. ~20-30 and 10-50 μg $g^{-1}$ d.m., respectively, for copper and nickel. Although the mechanisms of toxicity for the two metals are relatively well known, no systematic analysis has been conducted to date on the joint effect of these elements on the activation of defence mechanisms and the response of plants used in phytoremediation.

In the presented studies 1-year old stem cuttings from a 3-year old *Salix purpurea* × *viminals* rootstock were grown under controlled conditions in the Knop medium supplemented with copper ($Cu^{2+}$) (Attachment 4, point IB1) or nickel ions ($Ni^{2+}$) (Attachment 4, point IB2) and their equimolar mixtures (Attachment 4, point IB3). Metals were added to the medium in the form of nitrate hydrates readily soluble in water to obtain concentrations of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mM of each of the metals. After 14-day culture analyses were conducted on biometric parameters of cuttings (total root mass, mean length of roots, shoots and leaves, and leaf area), accumulation of copper and/or nickel as well as levels of selected metabolites (total phenolic contents, contents of soluble carbohydrates, the level of glutathione as well as contents of salicylic acid both in the free form and bound in glucoside). In order to determine differences in the response of the hybrid to the presence of copper and nickel in the medium, as well as the effect of their interactions to the investigated stress parameters the recorded results were verified in relation to unifactorial systems using respective statistical methods, including multiple regression analysis, contrast analysis and Principal Component Analysis (PCA).

After 14-day culture the presence of both copper and nickel in the medium had a highly negative effect on growth and development of willow cuttings. In the case of the applied mixture, the reduction in the values of biometric parameters of plants was significantly correlated with the levels of both metals in the medium, while growth inhibition affected mainly the root system, for which a decrease in biomass in relation to the control by ~90% was recorded at the highest applied concentration (3 mM). At the same time, reduction in the mean leaf length amounted to ~54%, while that of the photosynthetically active area was by only ~41%. An antagonistic effect of nickel in relation to copper was also shown for the mean shoot length resulting from the limitation of copper accumulation in these organs, caused by the competitive action of nickel.

The level of copper and nickel accumulation in individual organs of the hybrid was strongly correlated with the increasing level of metals in the medium ($R^2$=0.9264-0.9871), with the highest contents detected in the roots (~20 and 14 μg $g^{-1}$ d.m. at 3 mM, for copper and nickel, respectively) and in lignified shoots (~14 μg $g^{-1}$ d.m. at 3 mM for both metals). At the same time, translocation of metals to the aboveground organs was low, amounting to ~26 and 23% for nickel and copper, respectively, in relation to their contents in roots. At the simultaneous occurrence of both metals in the substrate, a slight, although statistically significant, stimulation of their uptake to leaves was observed.

The presence of metals in the medium led to disturbances in the metabolism of carbohydrates and a considerable increase in contents of soluble sugars (glucose, fructose and sucrose) in leaves of plants exposed to copper and nickel in relation to the control specimens. This increase was significantly correlated with the level of metals in the solution, with the strongest dependence observed for sucrose ($R^2$=0.7439) and slightly weaker for simple sugars ($R^2$≈0.66). At the simultaneous presence of copper and nickel in the medium a synergistic increase was observed in the accumulation of glucose and fructose in leaves of the hybrid plants, while an addition of nickel had a competitive effect in relation to copper and reduced the accumulation of sucrose.

Both metals strongly induced the accumulation of phenolic compounds in leaves of hybrid plants, including salicylic acid both in the free form and as glucoside. As a result of activation of defence response, the total content of the metabolite increased on average from ~2.6 to 60 μg $g^{-1}$ f.m., reaching the highest level at the concentration of metals in the mixture at 1.5 mM. In contrast, at higher concentrations (2-3 mM) a gradual decrease was observed in the accumulation of this metabolite. The contrast analysis showed a synergistic action of nickel in relation to copper in the induction of biosynthesis of phenolic metabolites and the accumulation of free – biologically active – form of salicylic acid at the simultaneous occurrence of both metals in the medium.

Enhanced accumulation of salicylic acid was accompanied by an increase in the production of glutathione in leaves of hybrid plants, significantly correlated with the level of metals in the medium ($R^2$=0.6487). At the same time, no accumulation of phytochelatins was recorded in this organ. The highest content of tripeptide - at 218 μg $g^{-1}$ f.m. and amounting to ~340% contents in leaves of the control plants - was recorded at a concentration of 2.5 mM of both metals in the medium. Similarly as in the case of phenolic metabolites, the synergistic effect of the coincident content of copper and nickel in the substrate and intensification of glutathione accumulation was observed in relation to unifactorial systems.

Conclusions:

- The joint effect of heavy metals on their bioaccumulation, biomass parameters and levels of primary and secondary metabolites essential for plant response to stress results from their interactions - additive, synergistic or antagonistic. The identification of the type of these interactions is crucial for the application of plants in phytoremediation and land reclamation, in which we observe mixed metal contamination in soil.
- The occurrence of copper and nickel in the substrate at comparable concentrations enhances translocation of both metals to leaves of the hybrid *Salix purpurea* × *viminalis.*
- Accumulation of metals in the photosynthetically active tissue leads to changes in secondary metabolism, including enhanced accumulation of phenolic compounds and glutathione exhibiting chelating and antioxidant properties. The level of copper in leaves is linearly correlated with the content of glutathione, while detoxification of nickel involves both phenolic metabolites and glutathione.
- Enhanced biosynthesis of phenolic metabolites in plants exposed to the action of copper and nickel results from disturbances in primary metabolism and as a consequence, the accumulation of water soluble sugars (glucose, fructose and sucrose) serving as signalling substances and donors of structural carbon.
- The simultaneous occurrence of copper and nickel in the substrate has a synergistic effect on the level of primary (glucose, fructose) and secondary metabolites (phenolic compounds, glutathione), which indicates an increase in their effect on willow plants and intensification of mechanisms of intracellular defence response in relation to individual metals.

- Copper induces the accumulation of sucrose in willow leaves to a greater extent than nickel, under the influence of which a stronger accumulation of salicylic acid is recorded. This indicates significant differences in the response of the hybrid to elevated contents of these metals in the substrate.
- Nickel induces enhanced biosynthesis of glutathione, which level is correlated with the content of salicylic acid in willow leaves (mainly in the free form), which confirms the participation of this phenolic acid in the induction of glutathione biosynthesis and probable inhibition of phytochelatin synthase.

## THE INFLUENCE OF INORGANIC AND ORGANIC ARSENIC FORMS AND THEIR INTERACTIONS ON PHENOLIC METABOLISM IN *ULMUS LAEVIS*

*Drzewiecka K., M. Gąsecka, P. Rutkowski, Z. Magdziak, P. Goliński, M. Mleczek (2017) Arsenic forms and their combinations induce differences in phenolic accumulation in Ulmus laevis Pall. Journal of Plant Physiology, 220: 34–42. (35 pkt. MNiSW; IF2016=3.121)* (Attachment 4, publication: IB4)

Soil contamination with arsenic (As) is an increasingly serious problem in many regions worldwide, resulting mainly from the extraction of iron and copper sulfide minerals rich in arsenic. Even in south-western Poland, as a result of the operations of the mining industry, the content of this metalloid in the soil reaches 18 g $kg^{-1}$ d.m. and exceeds 450-fold the upper limit of background values. Apart from the mining and smelting industries, arsenic contamination of soils is also caused by the application of pesticides based on arsenic compounds and irrigation of fields. In the case of agricultural areas the problem of arsenic contamination of soils and as a consequence contamination of crops is presently found in many leading food producing countries, such as China, India, Pakistan or the United States.

Both in the soil environment and in plant cells arsenic undergoes reversible enzymatic and non-enzymatic changes between its inorganic ($As^{III}$ and $As^{V}$) and organic forms - mainly monomethyl- and dimethyl arsenic (MMA and DMA, respectively) varying in bioavailability and toxicity. The mechanism of $As^{V}$ toxicity is based on the competitive elimination of inorganic phosphorus from important biochemical reactions such as ADP phosphorylation in mitochondria, while in the case of $As^{III}$ enzyme inhibition is observed as a result of metalloid binding with the sulfhydryl group. Moreover, both inorganic forms of arsenic cause overproduction of reactive oxygen species, resulting in oxidative stress. Organic forms of arsenic are considered to be less toxic to plants, although at present we have only limited data on the mechanisms of their phytotoxicity.

The aim of the study was to determine the effect of inorganic and organic arsenic forms and their combination on the metabolism of phenolic compounds, particularly the accumulation of salicylic acid. The object of that study was European white elm (*Ulmus laevis*) - a species with the confirmed high potential, in comparison to other woody plant species, for application in phytoremediation of arsenic contaminated soils. Two-year elm cuttings were grown in a greenhouse on the Knop medium containing 0.06 mM arsenic in the form of sodium metaarsenate ($NaAsO_2$), disodium arsenate ($Na_2AsO_4$) or dimethylarsinic acid ($(CH_3)_2As(O)OH$) as well as equimolar mixtures of two or three components. After 3-month culture contents of free and bound salicylic acid were determined in the harvested plant material (roots and leaves). At the same time total content of phenolic compounds was recorded and their profile was identified using ultraperformance liquid chromatography (UPLC). In order to determine the relationship between the accumulation of arsenic, contents of phenolic metabolites and the level of oxidative stress, the degree of cell membrane damage was also determined in the reaction of malondialdehyde with thiobarbituric acid.

The presence of arsenic in the substrate (irrespective of its form) each time caused an enhanced accumulation of salicylic acid in elm leaves, reaching as much as ~4.2 µg $g^{-1}$ f.m. (the total content of the free form and form bound with glucose) and equivalent to ~300% contents of the metabolite recorded in the control plants. At the same time, after 3-month culture no accumulation of salicylic acid was detected in the root system, which confirms an intensive transport of free salicylic acid (as a product of the shikimate pathway) from the root zone to the aboveground parts of plants in order to protect the photosynthetic organs, as well as the effect of light on the induction of the phenylpropanoid pathway. A considerable increase in the contents of this metabolite in the photosynthetically active tissues at the simultaneous low and stable level in roots was observed previously in various species of plants exposed to long-term action of both biotic and abiotic stressors. An increase in the accumulation of free salicylic acid (SA) in leaves in relation to the control was comparable for all the applied arsenic forms ($As^{III}$, $As^{V}$, DMA). In the case of a mixture of inorganic forms ($As^{III}$ and $As^{V}$), this effect was additive, while the addition organic arsenic (DMA) considerably reduced the effect of inorganic forms on the level of the metabolite in leaves. At the same time, a significant increase was observed in the contents of malondialdehyde, indicating the intensification of lipid oxidation in cell membranes and the protective action of salicylic acid in plant response to the toxic action of arsenic. The antagonistic action of DMA in relation to $As^{III}$ and $As^{V}$ was also observed for the reaction of SA glycosylation (the degree of its conversion to glucoside), which indicates the negative effect of organic arsenic on the storage mechanism of the active form of this metabolite in leaves. This assumption is also confirmed by the strong correlation between the contents of SA and gentisic acid – the first product of catabolic SA reactions, reported in leaves (*r*=0.90) and the accumulation of gentisic acid in roots under the influence of DMA. Moreover, the accumulation of salicylic acid in leaves is accompanied by an increase in the contents of C6C3 phenylpropanoids and their derivatives, i.e. caffeic, chlorogenic, *p*-coumaric and synapic acids as well as flavonoids catechin and vitexin exhibiting strong antioxidant properties (*r*=0.78-0.55). In turn, a negative correlation was observed between the contents of salicylic and gallic acids (*r*=-0.42), which indicates the competitive biosynthesis of these two metabolites. In view of the accumulation of individual phenolic compounds and speciation of arsenic in organs of elm trees it was found that an increase in the level of free salicylic acid in the photosynthetically active tissue resulted mainly from the intensive accumulation of $As^{V}$ in roots (*r*=0.80) and it was the effect of activation of both the phenylpropanoid pathway in leaves and the shikimate pathway in roots, as well as the transport of the metabolite from roots to the aboveground organs of plants.

Conclusions:

- The presence of arsenic in the substrate causes changes in the phenolic metabolism in underground and aboveground parts of European white elm, which character and intensity depend on the form of the metalloid
- Intensification of the phenolic metabolism under the influence of arsenic is found mainly in leaves, while to a lesser extent it affects roots of elm trees, which results from the effect of light on the activity of enzymes of the phenylpropanoid pathway
- Accumulation of organic forms of arsenic in the roots (including DMA and MMA) induces the shikimate pathway and enhanced accumulation of protocatechuic acid – a dihydroxy derivative of benzoic acid with strong chelating and antioxidant properties
- In contrast to roots, the presence of arsenic in the substrate (except for $As^{III}$) causes inhibition of the shikimate pathway and induction of the competitive phenylpropanoid pathway with the accompanying accumulation of C6C3 acids (caffeic, *p*-coumaric and chlorogenic) in leaves
- The presence of arsenic in the substrate causes an increase in the level of free salicylic acid in the photosynthetically active tissue primarily with the intensive accumulation of $As^{V}$ in the roots and resulting from the activation of both the shikimate pathway and the phenylpropanoid pathway, and the transport of the metabolite from the roots to the aboveground organs

- Organic arsenic has a negative effect on the level of free salicylic acid in elm leaves and glucosylation of this metabolite, thus reducing the level of glucoside with the storage function
- The joint effect of inorganic arsenic forms on the accumulation of salicylic acid is additive in character, while the presence of DMA reduces the accumulation of this metabolite in elm leaves caused by $As^{III}$ and $As^{V}$
- Limited accumulation of salicylic acid in elm leaves caused by DMA is accompanied by the intensification of oxidative stress and as a consequence intensification of cell membrane damage. This indicates a key role of this metabolite in the response of this species to the toxic action of arsenic

**THE INFLUENCE OF MULTI-METAL POLLUTION OF NATURAL WATER ECOSYSTEMS ON SALICYLIC ACID ACCUMULATION IN COMMON REED**

*Drzewiecka K., M. Mleczek (2017) Salicylic acid accumulation as a result of Cu, Zn, Cd and Pb interactions in common reed (Phragmites australis) growing in natural ecosystems. Acta Physiologiae Plantarum, 39:182. (25 pkt. MNiSW; IF2016=1.364)* (Attachment 4, publication: IB5)

Application of macrophytes, including sensitive species (e.g. lesser bulrush) and tolerant species with accumulator properties (e.g. common reed) in bioindication of water quality is considered to be of increasing importance by ecologists and ecotoxicologists. Moreover, aquatic plant species with high tolerance and accumulation capacity are used in phytoextraction of heavy metals from waters and reclamation of degraded areas. An example of such a macrophyte is common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) - a species inhabiting the littoral zone even in strongly polluted water bodies and rivers and spread over almost all climatic zones. The aim of the presented study was to determine the applicability of salicylic acid as a biochemical indicator for the joint action of mixed metal contamination on this species. Moreover, another objective of the study was to verify the unifactorial experimental systems under controlled conditions in relation to natural environmental conditions, under which we most frequently observe the simultaneous occurrence of pollutants considerably modifying their toxicity.

Common reed plants were harvested four times in the vegetative season of 2014 from four water bodies located in the catchment of the Bogdanka River. The sampling stations were situated both in suburban areas and in the direct vicinity of the city centre of the Poznań agglomeration in order to diversify the sources and levels of metal pollutants. In view of the strong anthropopressure found in the analysed area and the considerable surface run-off of precipitation water, the catchment of the Bogdanka - together with the above-mentioned water bodies - has been investigated for years in terms of the level, distribution and seasonal fluctuations in pollutants in the abiotic and biotic elements of their ecosystems. In the collected plant material (leaves and rhizomes) analyses were also conducted on the contents of selected metals, which levels in the previous years exceeded the mean phytotoxicity threshold (Cu, Pb, Cd) or mean natural contents in plant tissues (Zn).

Each time the mean annual contents of the investigated metals in organs of the common reed showed higher values in the rhizomes than in the photosynthetically active organs, with the highest accumulation recorded for zinc, medium for copper and lead, while the lowest in the case of cadmium (irrespective of the tested organ).

The greatest value of the leaf/rhizome translocation index was recorded for zinc (0.93), it was lower and comparable for lead and cadmium (0.70 and 0.65), while it was lowest for copper (0.40). Accumulation of all the investigated metals in both organs of common reed showed a slow increase in the course of the vegetative period, despite the intensive increment in the biomass of the macrophyte in the summer months and the typically observed effect of "natural dilution" of metals.

Along with the assays for metal contents analyses were conducted for free and bound salicylic acid in the harvested plant material. At each time points the contents of the metabolite recorded in leaves were many times greater than in the rhizomes of common reed, with the highest contents reaching ~25 and 0.6 μg $g^{-1}$ f.m. (for leaves and rhizomes, respectively). These results considerably exceeded values documented in literature for the control plants (not exposed to the action of stressors) and ranging from several ng to approx. 1 μg $g^{-1}$ f.m. (depending on the species). Enhanced biosynthesis of salicylic acid was found in the periods of the greatest plant growth, which is accompanied by intensive uptake of nutrients from the substrate. In the rhizomes the highest level of salicylic acid was recorded already in May. Next, along with the increase in leaf biomass a considerable decrease was recorded in the contents of the metabolite in therhizomes, probably caused by the transport of its active form to photosynthetically active organs of the macrophyte. Enhanced accumulation of salicylic acid in leaves was shown in July, followed by the gradual decrease in September and November, probably resulting from catabolic reactions, emission of volatile methyl ester or its polymerisation to lignin. It should be stressed here that over the entire vegetative period, with an increase in the accumulation of salicylic acid in leaves a logarithmic decrease was observed in its contents in the rhizomes. This indicates a key role of the investigated metabolite mainly in the protection of the photosynthetic system of the plants against unfavourable environmental factors. Moreover, the percentage shares of the free form in the total contents of salicylic acid (the total of the free and glucoside forms) was significantly higher in the rhizomes than in the leaves (mean ~80 and 58%, respectively), indicating an active storage mechanism for the metabolite mainly in the aboveground organs.

Multiple regression analysis showed a better goodness of fit for the linear dependence ($R^2$=0.9969-0.9876, $p \leq 0.001$) when considering the effect of all analysed metals on the level of salicylic acid than it was in the case of analyses of the effect of a single variable (metal) ($-0.48 \leq r \leq 0.44$). This confirmed the assumption on the existence of an interaction between metals influencing the resulting level of oxidative stress and activation of intracellular response mechanisms. Salicylic acid content in leaves of common reed was strongly correlated with the accumulation of zinc in the rhizomes, while the presence of this metal in the aboveground organs stimulated the conversion of this metabolite to the glucoside form. At the same time, with an increase in the accumulation of copper a decrease was observed in the effect of zinc on the induction of biosynthesis of salicylic acid and its accumulation in both organs. These results confirm the competitive character of both metals observed in the environment and definitely indicate the antagonistic action of copper in relation to zinc also in the induction of intracellular mechanisms of plant response to stress. For cadmium a positive effect was shown on the accumulation of salicylic acid only in the rhizomes, while increased accumulation of this metal in leaves had no significant effect on the level of the metabolite. In the case of lead an increase in the contents of the metal in the leaves led to a reduction in the level of free salicylic acid and simultaneously induced its storage in the form of glucoside.

Conclusions:

- Heavy metal pollution in natural ecosystems induces a complex plant response to stress, among other things it leads to an enhanced biosynthesis of salicylic acid – a key phenolic metabolite, serving regulatory and signalling functions
- The level of salicylic acid, the degree of its translocation from the roots to the aboveground parts of plants as well as binding with glucose (conversion to the storage form) are the result of antagonistic and synergistic interactions between metal pollutants accumulated by plants
- Among the analysed metals, the greatest capacity to induce the accumulation of salicylic acid is found for zinc and concerns both the rhizomes and leaves of common reed
- In the case of the coincident occurrence in the environment, copper shows a strong antagonistic action in relation to zinc not only at the stage of uptake and accumulation, but

also in the intracellular induction of plant response to stress and the biosynthesis of salicylic acid

- An increase in the level of cadmium causes an enhanced accumulation of salicylic acid in the rhizomes
- Enhanced accumulation of lead, when found together with other metals, limits the accumulation of salicylic acid in the photosynthetically active organs and stimulates the formation of the storage form (glucoside)
- Salicylic acid content may be a biochemical indicator of the joint effect of environmental pollutants on plants and the level of oxidative stress

## THE INFLUENCE OF METAL IONS IN SUBSTRATE AND THEIR INTERACTIONS ON LACCASE ACTIVITY, METAL BIOACCUMULATION AND YIELD OF KING OYSTER MUSHROOM

*Drzewiecka K., M. Siwulski, M. Mleczek., P. Goliński, K. Sobieralski (2012) Bioaccumulation of heavy metals from artificially enriched substrates and their impact on physiology of King Oyster mushroom (Pleurotus eryngii) Fresenius Environmental Bulletin, 21: 1666-1674. (15 pkt. MNiSW; $IF_{2012}$=0.641)* (Attachment 4, publication: IB6)

King oyster mushroom (*Pleurotus eryngiii*) belongs to the group of white rot fungi gaining in popularity among mushroom growers thanks to the cultivation requirements comparable to those of the commonly grown oyster mushroom (*P. ostreatus*) in culture on lignocellulose substrates as well as attractive taste and valuable health-promoting properties. In nature it is a saprophytic fungus common in central Asia and in the Mediterranean, where it successfully colonises the soil environment. In recent years model studies showed the considerable potential of this species to degrade stable organic pollutants in the soil as well as applicability in mycoextraction of heavy metals and radionuclides. The study presented in this publication concern the capacity of the king trumpet mushroom to accumulate selected heavy metals and the effect of these pollutants on mycelium growth and yielding. Analyses were also conducted on the effect of metals on the activity of laccase – a ligninolytic enzyme determining the degradation of the substrate and mycelium growth as well affecting the efficiency of xenobiotic degradation. Earlier studies confirmed the domination of laccase in the enzymatic complex secreted by the mycelium of *P. eryngii* to the substrate and were the basis for the assumption on the activity of this enzyme as a physiological indicator of the effect of soil/substrate pollutants in this species. Both the interactions of heavy metals and coincident occurrence of metal and organic pollutants are common phenomena in the degraded environment and - through the effect of metals on laccase activity - they may considerably reduce the effectiveness of mycoremediation. The presented publication provides the first information on the effect of the coincident occurrence and potential interactions between metals on their accumulation, physiological parameters, mycelium growth and yielding of *P. eryngii*.

The king trumpet mushroom was grown under controlled conditions adding bivalent ions of heavy metals, i.e. cadmium, cobalt, copper, nickel and zinc, to standard growing substrate. Metal salts were added at the stage of substrate preparation to the culture obtaining their contents at 1, 5 and 10 mmol $kg^{-1}$ f.m. (mM). Additionally, an equimolar combination of the above-mentioned metals was applied, i.e. 1 mM of each metal.

Among the used metals copper, cobalt and nickel most strongly limited mycelium growth (~50% in relation to the control at the 10 mM addition), while this effect showed a linear dependence on the contents of the metal in the substrate. The addition of zinc and cadmium inhibited mycelium growth at ~10% irrespective of the applied concentrations. At the addition of metals in the form of a mixture, the observed decrease in mycelium growth was comparable to that at the 1 mM addition of individual metals singly (Cu, Co and Ni).

Addition of micronutrients (Cu, Zn) in the adopted concentration range had no negative effect on the yield of king trumpet mushroom. The other metals (Co, Ni, Cd) at lower concentrations (1 and 5 mM) induced mycelium yielding, while at the 10 mM addition of these metals a decrease was observed in the yield on average by 50% in relation to the control. The addition of metals in the form of a mixture caused a slight reduction in yielding in relation to the 1 mM metal addition in the unifactorial system.

Accumulation of metals in fruiting bodies of *P. eryngii* increased in proportion to the contents of metals in the substrate, reaching the highest values for Zn>Co>Ni>Cu>Cd, respectively, amounting to ~68, 57, 24, 15 and 4 mg $kg^{-1}$ DW at the highest applied concentration (10 mM). The value of the bioaccumulation factor (BAF) was on a medium and low levels, amounting to 0.101, 0.097, 0.039, 0.024 and 0.003, respectively. The addition of the metal mixture resulted in an almost two-fold increase in the accumulation of nickel with the accompanying considerable decrease in the uptake of copper and cobalt (by ~40 and 30% in relation to the addition of a single metal). In the case of the other metals (Zn, Cd) no significant effect was observed for the coincident occurrence of metals on their accumulation.

Addition of copper, cobalt and nickel strongly induced laccase activity at the complete overgrowing of the substrate at the mycelium incubation stage. The observed increase in enzyme activity was from ~200 to ~1300, 1100 and 650 nkat $kg^{-1}$ FW, respectively, at the 10 mM addition of metals to substrate. The other metals (Zn, Cd) and the addition of the metal mixture only slightly induced the activity of laccase in comparison to the control. At a concentration of 1 mM the simultaneous application of metals significantly reduced the effect of copper and nickel on the activity of this enzyme.

Laccase activity was also determined in the substrate following the harvest of the fruiting bodies. In contrast to the substrate after the incubation stage, the addition of metals led to the inhibition of activity of this enzyme already at the 1 mM addition, i.e. from ~18000 for the control to ~16000, 7500, 6000, 3900 and 3700 nkat $kg^{-1}$ FW, respectively, for Zn, Co, Cd, Ni and Cu. At the highest concentration (10 mM) this activity was ~80-600 nkat $kg^{-1}$ FW, which amounted to only ~0.45-3.33% laccase activity in the control. The addition of metals in the form of a mixture drastically reduced the activity of this enzyme in comparison to the 1 mM addition of a single metal and it was almost 100-fold in relation to the control.

Conclusions:

- The presence of Cu, Co and Ni in the substrate inhibits mycelium growth in *P. eryngii* directly proportionally to the metal concentrations (within the range of 1-10 mM) despite the considerable induction of laccase activity in the substrate. The other metals (Zn and Cd) limit mycelium growth only by ~10% (irrespective of concentrations) and only slightly induce laccase activity
- In the case of a mixture of metals, their negative effect on substrate overgrowing is not additive in character, while it lowers the induction of laccase to the level in the control
- In terms of the production of biomass of fruiting bodies the king trumpet mushroom is characterised by a higher resistance to the action of heavy metals than most plants used in phytoremediation of soils loaded with metal pollutants and it yields even at concentrations of 10 mM
- The presence of heavy metals at concentrations of 1-5 mM has no negative effect on the yield volume and even slightly induces fruiting body development. Only at a concentration of 10 mM for Co, Ni and Cd a reduced yielding is observed in *P. eryngii*
- Metals found in the form of a mixture have no significant effect on the yield volume in relation to the control culture
- Among the analysed metals *P. eryngii* accumulates Zn the strongest, while for Cd the accumulation is weakest, with the levels of metals in the fruiting bodies being directly

proportional to the concentrations of the metals in the substrate (within the adopted range of concentrations, i.e. 1 – 10 mM)

- Values of the bioaccumulation factor indicate medium and weak sorption properties (depending on the metal) of this species in relation to the applied metals
- At the mixed metal contamination of the substrate Ni exhibits strong competitive properties, which results in an almost two-fold increase in its accumulation (at the concentration of 1 mM) and a reduction of Cu and Co uptake
- In the substrate after yield harvest the presence of metals (particularly Ni and Cu) drastically reduces laccase activity, while the effect is additive in the case of their mixture, which may exclude the possibility of repeated yielding and mycoextraction of metals, as well as the degradation of xenobiotics by mycelium after the fruiting stage

**Concluding remarks:**

The most important accomplishments of the presented studies include the determination of:

- The varied effect of copper and nickel on the induction of oxidative stress and the production of salicylic acid in hybrid willow with significant applicability in the phytoremediation of soils contaminated with heavy metals in response to their elevated contents in the substrate.
- The interactions between copper ąnd nickel observed as a result of their coincident occurrence in the substrate and influencing phytotoxicity and intensity of oxidative stress.
- The effect of various arsenic forms and their mutual transformations and interactions on the level of oxidative stress and the accumulation of salicylic acid in leaves of European white elm.
- The complex function of salicylic acid in the response of woody plants to stress caused by heavy metals and metalloids in comparison to other products of primary and secondary metabolism, including water soluble sugars, other phenolic compounds as products of two pathways (the shikimate pathway and the phenylpropanoid pathway) and glutathione.
- Applicability of salicylic acid as a biomarker of stress caused by mixed metal pollution in plants coming from natural aquatic ecosystems.
- The effect of heavy metals and their interactions on the activity of laccase – a key lignocellulose enzyme determining the suitability of white rot mycelium for applications in mycoremediation of metal and xenobiotic pollutants.

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## 5. PRESENTATION OF THE PROFESSIONAL CAREER, INCLUDING OTHER FORMS OF SCIENTIFIC, RESEARCH AND TEACHING ACTIVITY

My graduation with distinction from the studies in Commodity Science in 2001 at the Poznan University of Economics and Business (then the Poznan Academy of Economics) and the preparation of my Master's thesis under the supervision of the late Prof. dr hab. Jacek Kozioł – head of the Department of Instrumental Analysis, have provided me with the skills and knowledge to undertake scientific and research work and investigate problems spanning from chemistry, biology and environmental protection, based on the objective chemical analytics of organic compounds (including those with bioactive and toxic properties). Already during my studies I participated in a 2-week internship at the Department of Chemistry, the Poznan University of Life Sciences (then the August Cieszkowski Agricultural University of Poznan) under the supervision of Dr Marian Kostecki in the area of instrumental methods of chemical analysis, particularly practical application of liquid chromatography in biological studies and quantification of metabolites of pathogenic fungi in cereal kernels. Moreover, during my last year of studies I took the semester internship at the Department of Instrumental Analysis, the Poznan Academy of Economics, where I was an assistant teacher at laboratory classes in Instrumental Analysis for 2nd year students of Commodity Science.

Directly after graduation I started 3rd cycle studies at the PhD Studies at the Faculty of Horticulture of the former August Cieszkowski Agricultural University of Poznan, conducting research at the Department of Chemistry of that university under the supervision of its Head - Prof. dr hab. Piotr Goliński. From June 2002 to the defence of my PhD dissertation in 2007 I was employed at the Department on the position of assistant lecturer at ½ required amount of working hours and participated actively in trainings in chemical analysis and operation of state-of-the-art research apparatus for the dynamically developing equipment base of the Department of Chemistry, including liquid chromatographs in two configurations with detectors facilitating analyses of a broad spectrum of organic compounds in natural samples and a gas chromatograph coupled with the new-generation mass spectrometer. At the same time, I initiated cooperation with the Department of Plant Physiology and the Department of Ecology and Environmental Protection of the former August Cieszkowski Agricultural University of Poznan concerning oxidative stress caused by environmental factors of anthropogenic origin (heavy metals, tropospheric ozone) in indicator plants. Thanks to the support of Prof. dr hab. Monika Kozłowska and incorporation into the studies on biomonitoring of ozone conducted by Dr hab. Klaudia Borowiak, I specified the objectives and research hypotheses of my PhD dissertation.

The selection of the research problems was definitely influenced by international environmental courses which I took in 2001 and 2002, including a course in estimation of the effect of tropospheric ozone on tree stands organised within the ICP-Forest Programme at the OTC experimental station (Open Top Chamber) in Lottecaldo, Switzerland and the Moggio Level II plot in Italy as well as an intensive course in bioremediation of soils contaminated with heavy metals and xenobiotics organised within the framework of the Socrates Programme in Tartu, Estonia, entitled: "Management and treatment of polluted soils in Europe". My participation in the above-mentioned courses, apart from broadening my knowledge in the impact of pollutants on the natural environment made me aware of the scale of this problem and convinced me of the need to conduct this line of research. I considered it to be particularly justified to investigate the application of plants with high tolerance to heavy metals in the restoration of species biodiversity and reclamation of extremely contaminated and degraded areas as a result of unlimited expansion of the mining industry over the last decades. In turn, species/varieties of sensitive plants may be used in pollution biomonitoring - including the air, exposed to transboundary transfer over large distances and affecting to a considerable degree areas of low anthropopressure and seemingly ecologically unpolluted. In both cases, the primary aspect of my research interests focused on the metabolomics of oxidative stress and the function of plant secondary metabolites in the modification of the varied resistance and accumulation properties of plants used in phytoremediation and biomonitoring of pollutants.

In 2003 I was awarded the scholarship of the Dekaban Foundation, within the framework of which I took a five-month internship at the Michael Smith Biotechnology Laboratory at the University of British Columbia in Vancouver under the supervision of Prof. Brian Ellis and the direct supervision of Dr Markus Samuel (at present Professor at the University of Calgary). Within the framework of this scholarship I participated in many monographic seminars and I conducted some of the research for the project financed by the Natural Sciences and Engineering Research Council of Canada, covering determinations of salicylic acid and its glucoside in leaves of cultivated tobacco (*Nicotiana tabacum* L.), including plants of the wild type and NahG transgenic plants exposed to biotic and abiotic stressors (tobacco mosaic virus, bacterial elicitor proteins and ozone). Obtained results definitely showed a key role of salicylic acid in non-specific response of tobacco plants to stress, both of biotic and abiotic origin and confirmed the hypothesis on the applicability of this metabolite as a biomarker of the negative effect of stress factors on plants and the intensity of oxidative stress. This convinced me to verify these conclusions in the w experimental systems investigated by me. What is more, these studies for the first time proved the mechanism of intracellular transduction of stress signals and induction of genes encoding proteins connected with pathogenesis via protein kinase activated specifically by the investigated metabolite. This accomplishment was published in a journal of high international renown in 2005 (Attachment 4, publication: IIA21).

In the years 2003-2005 I participated in a research project financed by the Ministry of Science and Higher Education, entitled: Assessment of the effect of selected trace metals on the level of plant indicators of environmental stress in aquatic ecosystems (Attachment 4, point 2F7) headed by Prof. dr hab. Piotr Goliński, and realised in cooperation with the Department of Plant Physiology and the Department of Green Areas of my University. Research initiated in this project, covering biomonitoring of heavy metals in aquatic ecosystems of the Poznań Agglomeration, is continued to the present and has been the subject of numerous diploma theses, including M.Sc. thesis, of which I was the scientific supervisor (Attachment 4, point IIID3). Many original papers published in international journals presented research within this project, including a paper documenting the varied effect of heavy metal accumulation on the level of salicylic acid in organs of a common macrophyte – common reed (*Phragmites australis*) analysed under natural conditions at a mixed metal pollution (Attachment 4, prace: IB6, IIA2, IIA16, IIA20).

In 2007 I defended my PhD dissertation entitled "Salicylic acid in plant response to selected environmental stresses" and by the decision of the Council of the Faculty of Horticulture of the August Cieszkowski Agricultural University of 25 September 2007 I received the PhD degree in agricultural sciences in the field of horticulture. Since 1 March I have been employed at the Department of Chemistry, the Poznan University of Life Sciences at the position of adiunkt [associate professor]. In the first years after the defence of my PhD dissertation I participated as a contractor in two research projects concerning fungal diseases of important crop plants (mainly fusariosis of asparagus and winter wheat) and the accompanying oxidative stress (Attachment 4, point IIF4, IIF6). In cooperation with the Department of Phytopathology, the Poznan University of Life Sciences and the Laboratory of Medicinal Physics of the Adam Mickiewicz University in Poznań we showed varied virulence of the common *Fusarium* species and the capacity to induce oxidative stress in organs of investigated crop plants. Moreover, conducted experiments also confirmed the effect of accumulation of fusarium toxins on the overproduction of free oxygen radicals and the level of oxidative stress in asparagus shoots along with the accompanying activation of defence mechanisms and enhanced biosynthesis of salicylic acid (Attachment 4, publications: IIA9, IIA14).

In the years 2009-2012 I participated in the project entitled: "Studies on the response of selected plant species to tropospheric ozone", headed by Dr hab. Klaudia Borowiak, in which – as a contractor - I conducted research being a continuation of the subject of my PhD dissertation and extended to include crop plant species (white beans) and ornamental plant species (petunia) characterised by considerable sensitivity to the presence of ground surface ozone (Attachment4, point IIF3). The long-term cooperation with the Department of Ecology and Environmental Protection PULS has resulted in a series of diploma theses (including a M.Sc. thesis, of which I was the scientific supervisor, and a Bachelor's thesis in English prepared under by supervision and the scientific supervision of Prof. dr hab. Piotr Goliński) (Attachment 4, point IIID3, IIID4), as well as numerous publications from the joint areas of environmental protection and plant physiology discussing the negative effect of ozone on crop plants and the mechanism of intracellular plant response to ozone stress (Attachment 4, publications: IIA11, IIA13, IIA15, IIA19, IIB6).

In the years 2012-2017 with a cooperation with Prof. dr hab. Iwona Morkunas from Department of Plant Physiology of Poznań Univeristy of Life Sciences, I participated in research focused on the impact of biotic stressor, pea aphid (*Acyrthosiphon pisum* Harris) of different size of population, on the generation of signalling molecules in leaves of pea seedlings (*Pisum sativum* L. cv. Cysterski) within a project granted by the National Science Center of Poland (NCN) (Attachment 4, point: IIF2). The scope of my activity in this project was the evaluation of free and glucoside bound salicylic acid in plant material. The results of the experiment were presented in original research publication entitled: „Differential induction of *Pisum sativum* defense signaling molecules in response to pea aphid infestation" published in the Plant Science journal in 2014 (Attachment 4, publication: IIA4). Further, considering pea aphid as an important indicator of the environment condition I participated in research concerning the influence of abiotic stress factor (lead) and lead-induced signal molecules on the reaction of pea seedlings to *A. pisum* infestation. The obtained results were presented on international conferences and published as an original research work entitled: „The influence of lead on generation of signalling molecules and accumulation of flavonoids in pea seedlings in response to pea aphid infestation" in the Molecules journal (Attachment 4, publication: IIA1). Additionally, with a cooperation with Prof. dr hab. Iwona Morkunas, I was involved in research on the relation between the level of carbohydrates and secondary metabolites in *Lupinus luteus* L.cv. Juno infected with *Fusarium oxysporum* f.sp. *lupini* (Attachment 4, point: IIF4). Within this scope I carried out the HPLC analyses of salicylic acid content in inoculated seedlings.

A simultaneous direction of my research concerned the mechanism of toxicity of heavy metals and the complexity of plant response to their elevated concentrations in the substrate, particularly in species and varieties of plants used or showing high potential for applications in phytoremediation. Since 2008 I have been participating in research conducted in cooperation with the Department of Plant Physiology and the Department of Silviculture, the Poznan University of Life Sciences, and - in recent years - with the Department of Biochemistry and the Department of General Botany of the Faculty of Biology, the Adam Mickiewicz University. Within the framework of this research area I participated in two research projects (one completed – Attachment 4, point IIF5, and one currently in progress – Attachment 4, point IIF1), covering studies on the phytoremediation potential of native tree species in terms of their survival rates and accumulation capacity in culture on substrate extremely contaminated with heavy metals (Cu, Zn and Pb) and metalloids (As). The primary objective of my research was to determine the role of salicylic acid in the response of these plants to stress caused by toxic elements, particularly the interactions between heavy metals and considerably modifying their toxicity at their simultaneous occurrence in the substrate. Moreover, I attempted to determine the mechanism of function of this metabolite in comparison to other products of primary and secondary metabolism participating in plant response to stress caused by heavy metals and metalloids. I am presenting the original papers documenting my activity in this research subject as the scientific accomplishment constituting the basis for the application for the degree of doktor habilitowany [post-doctoral degree] (Attachment 4, publications: IB1, IB2, IB3, IB4). Moreover, within the framework of a project completed in 2010, I participated in the adaptation of methodology of profiling low-molecular organic acids applying high performance liquid chromatography in the reverse phase system. This method found a specific application in studies on the rhizosphere of plants used in phytoremediation, aiming at the determination of the profile of exudates secreted by plants to the substrate in order to reduce mobility of toxic elements and a simultaneous increased availability of minerals. I participated also in studies concerning the effect of the molar ration of calcium and magnesium ions in the medium and the addition of compost spent after garden mushroom growing on bioavailability and the accumulation of heavy metals and as a result on their phytotoxicity. In these studies as a measure of the level of induction of the physiological response of common willow to stress e.g. the content of salicylic acid and photosynthetic activity (Attachment 4, publications: IIA3, IIA6, IIA7, IIA10, IIA17, IIB5).

In the years 2009-2012 as the concept proponent and head of my research project studies I realised on the suitability of white rot fungi in remediation of soils and sewage contaminated with heavy metals and organic compounds (particularly polycyclic aromatic hydrocarbons - PAHs) (Attachment 4, point IIIF2). I conducted this research with the scientific help of Prof. dr hab. Marek Siwulski from the Department of Vegetable Crops and in cooperation with the Department of Physics of my University and the Department of Medicinal Physics, the Adam Mickiewicz University in Poznań. Obtained results definitely confirmed high applicability of representatives of these organisms in accumulation of heavy metals from contaminated substrate and indicated the unique capacity of their mycelium for extracellular degradation of xenobiotics based on the reactions catalysed by enzymes secreted to the substrate. Moreover, as a result of conducted experiments it was found that similarly as in the case of phytoremediation, a significant problem affecting the efficiency of mycoremediation is connected with the occurrence of mixed pollution, particularly coincident occurrence of heavy metal ions significantly influencing the activity of lignocellulose enzymes. Obtained results were presented partly in an M.Sc, thesis prepared under my supervision (Attachment 4, point IIID1) and published in the form of original papers (Attachment 4, publications: IB6, IIA8, IIA12, IIA18, IIB4).

I am a co-author of three chapters in English language monographs published in the years 2012-2015 by Springer, which subjects reflect my research works. Particularly, these publications constitute a review of the latest literature reports concerning the role of salicylic acid in oxidative stress caused by abiotic factors (Attachment 4, publication: IIB2, IIB3), as well as methods of biomonitoring and remediation of PAH pollutants (Attachment 4, publication: IIB1). I am a co-author of numerous conference papers presented as oral presentations (Attachment 4, publications: IIH1-IIH7) and posters (Attachment 4, publications: IIIB1-IIIB30) at national and international scientific conferences. Cooperating with editorial boards of international journals I prepared several reviews of manuscripts, including adjudication reviews, on subjects consistent with my research from the overlapping fields of plant physiology, biochemistry and environmental protection (Attachment 4, point IIII). On commission of NSC I prepared 4 reviews of applications for financing of research projects in the Opus and Preludium competitions (Attachment 4, point IIIH).

Within the framework of my teaching activity since the beginning of my professional life I have been giving classes in chemistry courses and in instrumental analysis for students of most faculties at my University (Attachment 4, point IIIC1). In the years 2009 and 2011 I was the supervisor of internships for students of Environmental Protection realised at the Department of Chemistry. For many years I have been actively participating in programmes and initiatives aiming at the popularisation of science and promotion of the Poznan University of Life Sciences among students of elementary and secondary schools such as the Researchers' Night, the Poznań Festival of Science and Art, the University of Young Discoverers, academic classes for junior and senior high school students and laboratory workshops for young children (Attachment 4, point IIIA1-IIIA3, IIIC2, IIIC3). Starting from this year I am the scientific supervisor for the Students' Scientific Club for Applied Chemistry Fans at the Faculty of Wood Technology, the Poznan University of Life Sciences (Attachment 4, point IIIJ4).

In the years 2009-2010 I was a member of the Council of the Department of Chemistry, while since 2015 I have been involved in planning of class schedules and reporting on working hours for employees. I also serve the function of a member of the University Election Committee at the Poznan University of Life Sciences in the years 2015-2019. Moreover, in 2015 I was a member of the team appointed to prepare changes in the curricula and programmes for 2$^{nd}$ cycle studies at the Faculty of Wood Technology (Attachment 4, point IIIG2, IIIJ4).

A detailed specification of scientific, teaching and organisational accomplishments is presented in Attachment 4.

## 6. SUMMARY OF SCIENTIFIC PUBLICATION ACTIVITY

| Achievement | No. | ∑ IF * | ∑ MNiSW score* |
|---|---|---|---|
| 1. Original research publications | | | |
| a) indexed in Journal Citation Report (list A) | | | |
| *Acta Biologica Cracoviensia, Botanica* | 1 | 0.586 | 13 |
| *Acta Biologica Hungarica* | 1 | 0.504 | 20 |
| *Acta Physiologae Plantarum* | 3 | 4.472 | 75 |
| *Acta Scientiarum Polonorum, Hortorum Cultus* | 2 | 1.239 | 40 |
| *Applied Magnetic Resonance* | 1 | 0.820 | 13 |
| *Archives of Environmental Protection* | 2 | 1.152 | 28 |
| *Central European Journal of Biology* | 1 | 0.633 | 20 |
| *Ecotoxicology and Environmental Safety* | 1 | 2.294 | 32 |
| *Fresenius Environmental Bulletin* | 5 | 3.185 | 71 |
| *International Journal of Phytoremediation* | 1 | 1.770 | 25 |
| *Journal of Hazardous Materials* | 1 | 3.925 | 45 |
| *Journal of Environmental Science, Part A* | 1 | 1.252 | 20 |
| *Journal of Plant Physiology* | 2 | 6.242 | 70 |
| *Molecules* | 1 | 2.861 | 30 |
| *Photosyntetica* | 1 | 0.862 | 25 |
| *Plant Journal* | 1** | 6.969 | 24 |
| *Plant Science* | 1 | 3.607 | 35 |
| *Planta* | 1 | 3.263 | 40 |
| **Total:** | **27** | **45.636** | **626** |
| **- including series for application** | **6** | **13.424** | **175** |
| b) peer-reviewed journals (list B) | | | |
| *Ars Separatoria Acta* | 1 | - | 4 |
| *Electronic Journal of Polish Agricultural Universities* | 1 | - | 4 |
| *Folia Horticulturae* | 1 | - | 14 |
| **Total:** | **3** | - | **22** |
| **Total number of original publications {1}:** | **30** | **45.636** | **648** |
| 2. Conference abstracts and post-conference papers | | | |
| a) oral presentations | 7 | - | - |
| b) posters | 30 | - | - |
| **Total {2}:** | **37** | - | - |
| 3. Monographs (book chapters) {3} | 3 | - | 14 |
| **Total {1+2+3}:** | **70** | **45.636** | **662** |
| **- including series for application** | **6** | **13.424** | **175** |

* according to the year of publication
** Publisher prior to PhD conferral

## 7. BIBLIOMETRIC SUMMARY OF PUBLICATION ACTIVITY

| **Biometric parameter** | **Score** |
|---|---|
| *No. of original publications according to Web of Science (WoS) – list A* | 27 |
| *Total impact factor (IF) according to the Journal Citation Reports (JCR), for the year of publication* | 45.636 |
| *Total MNiSW score according to the year of publication* | 662 |
| *Total number of citations (without auto-citations) according to Web of Science (WoS)* | 158 |
| *Hirsch Index (IH) according to Web of Science (WoS)* | 8 |