Attachment No 2

Wojciech Pokora, Ph.D.

A summary presentation of scientific achievements

University of Gdańsk
Faculty of Biology
Department of Plant Physiology and Biotechnology
Gdańsk, 2018

1. Name nad Surname: Wojciech Pokora

2. Diplomas and scientific degrees:

Ph.D. diploma in biology, 2004, University of Gdańsk, Faculty of Biology, Geography and Oceanology.
The title of the doctoral dissertation, conducted at the Department of Plant Physiology:
"The role of superoxide dismutases in the adaptation of green algae *Scenedesmus* to oxidative stress induced by abiotic contaminants of anthropogenic origin".
Promoter: Prof. dr hab. Zbigniew Tukaj

Master's degree in biotechnology in the field of phytopathology, 2000, Intercollegiate Faculty of Biotechnology of the University of Gdańsk and the Medical Academy in Gdańsk.
The title of the master's thesis, conducted at the Department of Biotechnology and Plant Protection of the Intercollegiate Faculty of Biotechnology of UG and AMG:
'Characterization of germplasm of wild species of the *Solanum* genus, cultivars of potato S.*tuberosum* and somatic hybrids S.*brevidens x S.tuberosum'.*
Promoter: Prof. dr hab. Ewa Łojkowska

3. Information on employment in scientific units.

From April 1, 2004 to the present: assistant professor at the Department of Plant Physiology and Biotechnology, Faculty of Biology, University of Gdańsk.

4. Indication of achievement * resulting from art. 16 sec. 2 of the Act of 14 March 2003 on academic degrees and academic titles, and on degrees and titles in the field of art (Journal of Laws No. 65, item 595, as amended):

a) title of scientific achievement: "The participation of hydrogen peroxide in the adaptation of microalgae to the stress associated with photosynthesis disturbances and in the course and regulation of their cell cycle"

b) Publications included in the scientific achievement:

1. **Pokora W.**, Tukaj Z., 2010. The combined effect of anthracene and cadmium on photosynthetic activity of three *Desmodesmus* (Chlorophyta) species. Ecotoxicol. Environ. Saf. 73(6): 1207-1213. **IF: 2,34; MNiSW: 30.** I estimate my percentage share at 75%.

2. **Pokora W.**, Tukaj Z., 2013. Induction time of Fe-SOD synthesis and activity determine deferent tolerance of two *Desmodesmus* (green algae) strains to chloridazon: a study with the synchronous cultures. Pest. Biochem. Physiol. 107: 68-77. **IF: 1,9; MNiSW: 25.** I estimate my percentage share at 80%.

3. **Pokora W.**, Baścik-Remisiewicz A., Tukaj S., Kalinowska R., Pawlik-Skowrońska B., Tukaj Z., 2014. Adaptation strategies during cell cycle of two *Desmodesmus armatus* strains (green microalgae) revealing different tolerance to cadmium: A study with light-

induced synchronized cultures of algae. J. Phycol. 171: 69-77. **IF: 2,2; MNiSW: 30.** I estimate my percentage share at 40%.

4. **Pokora W.**, Aksmann A., Baścik-Remisiewicz A., Dettlaff-Pokora A., Rykaczewski M., Gappa M., Tukaj Z., 2017. Changes in nitric oxide/hydrogen peroxide content and cell cycle progression: Study with synchronized cultures of green alga *Chlamydomonas reinhardtii*. J. Plant Physiol. 208: 84-93. **IF: 3,121; MNiSW: 35.** I estimate my percentage share at 60%.

5. **Pokora W.**, Aksmann A., Baścik-Remisiewicz A., Dettlaff-Pokora A., Tukaj Z., 2018. Externally applied hydrogen peroxide modifies cell cycle of *Chalmydomonas reinhardtii*. J. Plant Physiol. 230: 61-72. **IF: 3,121; MNiSW: 35.** I estimate my percentage share at 75%.

c) Discussion of the scientific purpose of the above work and results achieved, and their possible applicability.

The scientific achievement, constituting the basis for the applying for the postdoctoral degree has been presented in five publications, concerning the role of enzymes responsible for hydrogen peroxide generation and scavenging during the adaptation of microalga cells to stress resulting from disturbances in the photosynthesis process as well as participation of hydrogen peroxide and nitric oxide in the course and regulation of the cell cycle of green alga *Chlamydomonas reinhardtii*. In all of the papers, I am the first author and in three of them I am also the corresponding author. The total IF of these publications is 13,42, the sum of the MNiSW points is 155.

The participation of hydrogen peroxide in the adaptation of microalgae to the stress associated with photosynthesis disturbances and in the course and regulation of their cell cycle

Modulation of "oxide-reductive homeostasis" is one of the reasons of the stressful impact of abiotic factors towards plants. It can lead to the occurrence of imbalance between the formation and neutralization of reactive oxygen species (ROS), known as the oxidative stress phenomenon (Mittler, 2017). It is believed, that the slight disruption of this homeostasis plays a role in the regulation of a number of physiological and biochemical processes (Foyer, 2018), and some of ROS, especially hydrogen peroxide, but also reactive nitrogen species (RNS), such as nitric oxide, may act as signaling molecules (Neil et al., 2002). Exposition of plant cells to sub-lethal doses of xenobiotics usually causes a significant increase in the amount of generated ROS, known as the oxidative stress phenomenon, and is revealed by the

increased activity of antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT) or peroxidase (PX) (Torres at al., 2008). The activity of superoxide dismutases, catalase and peroxidases stays in the close interdependence, mainly due to the fact, that the product of one enzyme is a direct substrate for the others. Their catalytic activity is a factor that primarily determines the amount of hydrogen peroxide present in the plant cell. In addition, intermediate products of superoxide anion radical reduction to the molecule of oxygen and water accomplish a number of regulatory and signaling functions in cell (Mittler, 2017). Hydrogen peroxide is believed to play a key role in regulation of gene expression of a number of enzymes, both involved in the neutralization of ROS (SOD, GPX, APX), but H_2O_2 is also involved in the induction of expression of PR proteins (pathogenesis related), proteins from the HSP family (heat shock proteins) or proteins associated with the course and regulation of the cell cycle (e.g., histone kinases).

The production and neutralization of reactive oxygen species seems to be crucial for the proper functioning of the cell. Therefore, the efficiency of enzymatic and non-enzymatic mechanisms responsible for ROS level control has a substantial impact on the proper development of the cell, the course of photosynthesis and respiration processes, but also determines the adaptation ability of the plant cell to adverse environmental conditions. In my Ph.D. dissertation " The role of superoxide dismutases in the adaptation of green algae *Scenedesmus* to oxidative stress induced by abiotic contaminants of anthropogenic origin " carried out in the Department of Plant Physiology, University of Gdańsk, under the supervision of Prof. dr hab. Zbigniew Tukaj, I examined the individual and combined impact of xenobiotics to a number of strains of unicellular green algae. Analysis of the toxicants effects revealing different mechanisms of action (heavy metal - cadmium, polycyclic aromatic hydrocarbon - anthracene and herbicide inhibiting the process of photosynthesis - chloridazone) indicated the phenomenon of oxidative stress induction as a universal mechanism of cell response to these substances, and the increased activity of antioxidative enzymes such as superoxide dismutase, catalase or ascorbate peroxidase, was interpret as one, that determine microalgal cells ability for adaptation of to the conditions of chemically induced stress. I also demonstrated a large variation in the sensitivity (reduction of population growth, photosynthetic activity) of closely related *Desmodesmus* (formerly *Scenedesmus*) strains to the toxicants studied (PhD thesis, Pokora, 2003 and also: Pokora et al., 2003, Tukaj and Pokora, 2006). After completing my P.hD. I continued the studies on the effects of xenobiotics towards green algal cells, focusing on the mechanism of their action on the photosynthesis process, studied at the population level (**Pokora et al., 2010**, work No. 1), as

well as on the indication of physiological and biochemical features of algal cells, that determine their sensitivity or tolerance to excess of ROS generated under stress conditions. In these research I applied an experimental model based on synchronous cultures. In this type of culture the results of studies could be interpreted at the level of events taking place in a single cell, taking into account the course of its cell cycle (**Pokora et al., 2013, 2014**, works 2 and 3). In turn, the use of model organism *Chlamydomonas reinhardtii* enabled me to characterize the cell cycle and related to it changes in free radical cell homeostasis, analyzed at the physiological, biochemical and molecular level (**Pokora et al., 2017**, work No. 4). I have shown that the modification of the reciprocal NO and H_2O_2 ratio, induced in the selected phase of the *Chlamydomonas* cell cycle, by modulating redox homeostasis, may accelerate or delay the duration of the cell cycle, increase the number of replication rounds occurring in one cell cycle, modify biomass and volume, and accelerate the release of daughter cells (**Pokora et al., 2018**; work No. 5). These works constitute my scientific achievement set as a cycle of studies presenting the role of free radical homeostasis in adaptation of algal cells to stress conditions, analyzed both at the population and cellular level and the role of hydrogen peroxide and nitric oxide in the course and regulation of the green algal cell cycle.

Hydrogen peroxide in adaptation of microalgal cells to stress associated with photosynthesis disturbances - population level

The increase in the activity of antioxidant enzymes detected in the cells of *Desmodesmus* (green alga) treated with cadmium, anthracene and their combination, was interpret as a key mechanism responsible for adaptation of these organisms to chemically induced stress (Tukaj and Pokora, 2006). Since the cellular response to cadmium and anthracene, applied individually and together, manifested mainly due to induction of chloroplast-associated protective mechanisms, the effect of these two substances on the process of photosynthesis in *Desmodesmus* strains was analyzed in details. The rate of photosynthetic oxygen evolution and chlorophyll *a* fluorescence induction and quenching measured by pulse amplitude modulation method (PAM), (**Pokora and Tukaj, 2010**, work No. 1) was applied. The results showed a characteristic relationship between superoxide dismutase activity and the value of the qN coefficient, corresponding to the non-photochemical dissipation of energy absorbed by the photosynthetic apparatus (Thiele et al., 1997). In green algae, the value of qN is mainly related to the change of ΔpH and the conversion of anteraxanthin and zeaxanthin in the xanthophyll cycle (Garcia-Mendoza et al., 2002). In cells treated solely with anthracene or cadmium, I observed an increased SOD activity or increased non-chemical energy dissipation. On the other hand, when cells were

treated simultaneously with two toxicants, both the protective mechanisms mentioned, undergoes activation. The increase in SOD activity occurred just after 2 h of exposure, but the change in qN was visible about 6 hours after exposure to toxicants. Analysis of the rate of photosynthetic oxygen evolution and PS II quantum efficiency (φ PS II, Genty et al., 1989) showed any significant decrease in the efficiency of photosynthesis. Thus, the elevated superoxide dismutase activity and /or other processes associated to the non-photochemical dissipation of energy absorbed by photosystem II could be considered as sufficient mechanisms, required to protect the photosynthetic apparatus of algae against the effects of elevated levels of RFT induced by cadmium and anthracene. Experiments using batch cultures, conducted in optimal and suboptimal conditions have, however, some limitations. Under such culture conditions, the algal cells grow and divide in an randomized way - asynchronously. At the same time, the sensitivity of a detailed organism may depend on the phase of its ontogenetic development and the rate of photosynthetic apparatus maturity, thus results obtained with asynchronous culture are a sum of the reaction of all developmental stages of organisms in the population. To analyze the adaptive mechanisms of microalgae to stress induced by abiotic factors at the level of a single cell and simultaneously take into account the stage of its ontogenetic development and the maturity of the photosynthetic apparatus, further research were conduct using an experimental model based on synchronous cultures.

Hydrogen peroxide in adaptation of microalgal cells to stress associated with photosynthesis disturbances – single cell level

In the synchronous culture, microalgae are kept under optimal growth conditions (rich mineral medium, high CO_2 supply, light intensity close to the photosynthesis saturation point E_k). Under alternating periods of light and dark, the cells grow and divide in the same time - synchronously. All organisms are at the same stage of their ontogenetic development - at the same moment of their cell cycle (Andersen, 2005, Harris, 2009). In the case of organisms I studied, it lasts for no longer than 24 hours. Such synchronous culture allows to analyze biological samples of large numbers of "biologically" unified organisms, while the interpretation of the obtained results can be done at the level of a single cell. The changes in physiological, biochemical and molecular processes resulting from cell development and their correlation with phases of the cell cycle can also be taken into account. In the case of *Desmodesmus* and *Chlamydomonas* genus, the interpretation of results is also possible at the level of a single chloroplast, because these organisms possess only one such organelle (Andersen, 2005). My previous studies indicated significant difference in the sensitivity of

closely related *Desmodesmus* strains towards heavy metals and herbicides (Tukaj and Pokora, 2006). Selected representatives of these toxicant - cadmium and chloridazone, were then supplied to the culture of synchronously growing algae cells. That set of experiments aimed to identify specific features of tested algae, determining their different tolerance to oxidative stress and correlation of stress related biochemical and physiological changes with the course of algal cell cycle (**Pokora and Tukaj, 2013**; work No. 2, **Pokora et al., 2014**, work no.3). Comprehensive analysis of the mechanisms responsible for adaptation of cells of two *Desmodesmus* strains to cadmium-induced stress (**Pokora et al. 2014**, paper No. 3) included the measurements of the activity and amount of protein of detailed SOD isoforms, phytochelatin synthesis and total glutathione pool assay, as well as, the induction of chaperone from HSP family proteins biosynthesis. The cells' growth of resistant strain was not inhibited, while it's photosynthetic activity decreased in the first hours after exposure, then recovered to the level recorded in control cultures. This was correlated with cells' ability to detoxify reactive oxygen species, that was significantly higher and induced in a much shorter time than in the sensitive strain. The increased activity of chloroplast SOD isoform (Fe-SOD), as well as the significantly higher level of glutathione, were pointed out as the factors differentiating the strain more resistant to cadmium from the sensitive one. On the other hand, analysis of two closely related strains of *Desmodesmus*, that in asynchronous cultures characterized with a distinctly different sensitivity to the photosynthesis inhibiting herbicide - chlorydazone, was performed. Here, the synchronous culture of algae was also applied. (**Pokora and Tukaj, 2013**, work No. 2). In the cells of the sensitive strain, the chloridazone caused a reduction in the number of cell divisions, and thus led to a decrease in the number of daughter cells released at the end of the cell cycle. In cells of the resistant strain, the herbicide, not only, does not inhibit cell' growth and division, but it accelerated the cell cycle, leading to an increase of the number of daughter cells released. It was a result of occurrence of an additional round of replication in the one cell cycle sequence. At the same time, the energy trapped in the reaction center (RC) was similar in both strains, but the amount of energy absorbed by single RC was twice as high in the sensitive strain, so that the non-chemical energy dissipation in the cells of the sensitive strain significantly exceeded the value obtained for the resistant one. The control cells of both strains differed significantly in the amount of Fe-SOD isoforms present in the cell: FSD 1 and FSD 2. At the same time, differences in the activity of these isoforms were slender. In the cells of sensitive strain, although the enzyme activity was significantly higher, I did not notice an increase of this activity, as well as, increase in the amount of newly synthesized SOD protein, in response to

herbicide treatment. In cells of the resistant strain, despite lower initial content of Fe-SOD isoforms protein, the induction of their biosynthesis occurred within 2 hours after exposure to the herbicide, leading to enzyme activity increase and the efficient disproportionation of the superoxide anion radical to hydrogen peroxide. At the same time, in the cells of this strain, the increase in the protein amount and activity of chloroplast SOD isoforms correlated with the increase in the number of active PS II reaction centers and the restoration of the photosynthesis process efficiency to the level recorded in control cells. The obtained results indicated that in the case of microalgae, the "velocity" of the induction of adaptation mechanisms may be the overriding factor over their "constitutive" efficiency. Occurrence of the link between kinetics of changes in the biosynthesis and activity of proteins determining hydrogen peroxide production, photosynthesis efficiency and changes in the cell development profile - shortening / increasing the number of replication rounds or inhibiting the cell cycle of green algae, was the premise for me to undertake a more detailed study on the relationship between kinetics of changes in the intracellular hydrogen peroxide concentration and the course of the cell cycle of green algae.

Hydrogen peroxide in the course and regulation of microalgae cell cycle

H_2O_2 acts as a signal molecule in plants (Alscher et al., 1997) and algae (Petrov and Van Breuseg, 2012), and its formation both, under physiological conditions as well as in response to stress factors suggests the participation of H_2O_2 in signal transduction leading to regulation expression of a number of proteins. In addition to hydrogen peroxide, also nitric oxide is considered as a signal molecule (Sakihama, 2002). In higher plant cells, NO is formed mainly from arginine with the participation of nitric oxide synthase (NOS). In the cells of the green algae, including *Chlamydomonas*, the conversion of NO_2 in the nitrate reductase (NR) catalyzed reaction is considered to be the evolving pathway for NO formation (Sakihama, 2002). The simultaneous formation of NO and H_2O_2 in response to stress, suggests the occurrence of the interaction between H_2O_2 and NO during cells' response to environmental stimuli. Available literature data indicate, that the manifestation of the signaling role of H_2O_2 and NO in plants may occurs via the modulation of expression of protein directly related to the regulation of the cell cycle (cyclins and cyclin-dependent kinase) (Mittler, 2017). My further experiments were carried out using a unicellular green alga *Chlamydomonas reinhardtii*. The known genome sequence (www.chlamy.org) makes

this organism widely used model in molecular and physiological studies (Grossman et al., 2007), also using synchronous cultures, enabling the study of the cell cycle. During *Chlamydomonas* cell cells multiple and overlapping divisions occur, leading to the formation of 2^n daughter cells, where one cycle includes "n" replication rounds, during which the nucleus and chloroplast divide. Each of the stages of division processes, including DNA replication, mitosis and cytokinesis, is preceded by a growth phase in which the formation of cell organelles takes place and energy reserves are collected (Cross and Umen, 2015). The mechanism regulating the transition of the G1 phase into S (G1 / S) is associated with the passage of the cell cycle through the G1 / S control point. The cell size and, probably, the duration of the G1 phase is crucial (Matsumara et al., 2003). This point is called the commitment point, and after its passage, further cell division, progeny cells formation and their release can occur without the participation of light energy (Hirt, 1996). In this part of my research, I aimed to characterize the *Chlamydomonas* cell cycle in terms of the changes in the amount of hydrogen peroxide and nitric oxide generated in cells (**Pokora et al., 2017**, paper No. 4). In my research model I applied the conditions cultures in such a way, that the cells passes 3 replication rounds in one cell cycle. The cell cycle characteristics was performed in three areas: a) the progression of the cell cycle, analyzed at the level of selected cyclins and cyclin-dependent kinases expression, what allowed to determine the typical phases (G1, S, M), cell cycle characteristic points (G1/S, S/M), the beginning of cytokinesis as well as determine the kinetic of daughter cells release; b) the functioning of photosynthetic apparatus, parameterized by measuring efficiency of energy trapping and utilization in photochemical processes and non-chemical decomposition of absorbed energy excess (analysis of kinetics of induction and quenching of chlorophyll a fluorescence in vivo - OJIP test) and efficiency of photosynthetic oxygen evolution and quantitative changes in the composition of photosynthetic pigments; c) maintaining redox homeostasis of reactive oxygen and nitrogen species followed by expression analysis (the amount of transcript present in the cell and activity) of enzymes involved in their formation and neutralization in the cytosol, chloroplast and mitochondrion. In my research I had indicated the occurrence of circadian oscillations in the production of NO and H_2O_2 in *Chlamydomonas* cells. These oscillations were in a line with changes in activity and relative transcript abundances of superoxide dismutase enzymes, catalase and peroxidase - in the case of hydrogen peroxide and changes in photosynthetic activity as well as activities and transcripts abundances of nitrate and nitrite reductase - in the case of nitric oxide. These results are consistent with reports of the circadian rhythm related oscillations of NR expression and enzymes involved in H_2O_2 metabolism (SOD, CAT,

PX) in other microalgal species (Velasco, 1989). I have also demonstrated that the changes in the NO / H_2O_2 ratio overlap specific cell cycle moments such as start of G1 phase, G1 / S and S / M checkpoint transition, cytokinesis induction and induction of daughter cell release (**Pokora et al., 2017**; work No. 4). On the basis of the obtained results, I established and verified the hypothesis assuming that the change of the intracellular ratio of $H2O_2$ / NO, via their exogenous application and/or quenching, may modifies the course of the *Chlamydomonas* cell cycle (**Pokora et al. 2018**, paper No. 5). For this purpose, the modification of H_2O_2 / NO ratio was induced by adding a stabilized solution of hydrogen peroxide to the synchronous culture, so that its concentration per cell, reached 150% of the value that was detected in the control culture, in the same moment of cell cycle. I chose four moments of cell development:

- young progeny cells, entering the cell cycle, before exposure to light (variant a)
- cells with the lowest NO / H_2O_2 ratio, before reaching the first commitment point, exhibiting high photosynthetic activity, (variant b)
- cells with a maximum NO / H_2O_2 ratio, before cytokinesis begins (variant c),
- mature mother cells, in which the highest during the entire cycle NO / H_2O_2 ratio was recorded and the cytokinesis process began (variant d).

The obtained results indicated that, depending on the phase of the *Chlamydomonas* cell cycle, H_2O_2, through a mild modification of redox homeostasis, may accelerate or delay the duration of cell cycle, increase the number of replication rounds occurring in one cell cycle, modify progeny cell' biomass and volume, and accelerate the release of daughter cells. These reported changes were indicated as the result of better adaptation of cells to light exposure after the dark period (variant a). The change in the NO / H_2O_2 ratio at the moment when its value is the lowest (variant b) led to the increase in the efficiency of electron transport between PS II and PS I, while the amount of energy absorbed by RC remained unchanged. Such an increase in photosynthetic process efficiency along with the increase of cell potential to neutralize generated ROS, an increased transcript level of cyclin (CYC) and cyclin-dependent kinases (CDK) as well as higher amount of DNA resulted in cells ability to pass an additional - the fourth round of replication, and thus increasing the number of daughter cells released at the end of the cycle. On the other hand, the increase in the amount of H_2O_2 present in the cell, at the time when the NO / H_2O_2 ratio was the highest (variant c) led to a delay in the beginning of the cytokinesis process. In case of *Chlamydomonas*, the chloroplast division correlates with the cytokinesis process, during which the photosynthetic activity of the cell is significantly reduced (Cross and Umen, 2015). By delaying these events,

the prolonged time of photosynthetic activity was not sufficient to induce another round of cell division, but led to an increase in the biomass of daughter cells released at the end of the cycle. Finally, the application of hydrogen peroxide after the cytokinesis process (variant d) indicated, that increase in the H_2O_2 level can be an important element of the sequence of events leading to the acceleration of the moment of daughter cells release from mother cells. The above results seem to be very promising in a light of the upcoming development of microalgae mass-cultures based technologies, that aim to increase the efficiency of biomass production in bioreactors, biofuel production, as well as obtaining algae cells with desired features, e.g. in bioremediation. The connection between my results and the physiological basis of the hormesis phenomenon (Calabrese et al., 2007) and the regulation of the cell cycle is particularly interesting. Presented in my achievement state of knowledge provides a potential opportunity to control the development of a population of microalgae cells and may contribute to the better explanation of some unexpected results (stimulation of population growth) recorded in toxicological experiments where the effects of low doses of toxicants are analyzed (EC10 / 24). In addition, according to my best knowledge, research on the interaction / interaction of ROS and RNS in the term of the course of the cell cycle of algal cells is innovative and has not been conducted in any other research team.

The summary of the most important elements of the presented scientific achievement, being the basis for my applying for the postdoctoral degree are:

1. Identification of chloroplast isoforms of superoxide dismutase as key enzymes determining the ability of *Desmodesmus* and *Chlamydomonas* cells adaptation to stress induced by toxicants belonging to the pollutants most frequently recorded in the aquatic environment: heavy metals - presented on the example of cadmium and pesticides - presented on the example of herbicide chloridazone.

2. Indication of the "velocity" of the induction of adaptation mechanisms as a superior factor over the "degree" of induction of these mechanisms, as the factor that determine different sensitivity to a given toxicant in-between closely related strains of green algae.

3. Demonstration of time coincidence between cell cycle events, such as the transition of the G1 / S and S / M check-points or induction of daughter cell release with the oscillations in the level of signaling molecules - hydrogen peroxide and nitric oxide, demonstrated using green alga *Chlamydomonas reinhardtii*.

4. Evidenced, that an externally induced change in the H_2O_2 / NO ratio, induced at selected moments in the cell cycle may lead to its modification, what is manifested by an increased number of replication rounds, and thus a higher number of daughter cells released from one mother cell, increased biomass of progeny cells and increased adaptation abilities of young progeny cells to intense light exposure.

References (includes items not listed in point 4b):

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5. Discussion of other scientific and research (artistic) achievements.

Research papers

Beyond the pares concerning the scientific achievement presented above, my output consists of 12 manuscripts (total IF 15,62, MNiSW: 270), which I am a co-author. Below there is a brief overview of these works.

5.1.1. Scientific scope: The role of superoxide dismutases in adaptation of green algae *Desmodesmus* to stress induced by abiotic factors

1. **Pokora W.**, Reszke J., Tukaj Z., 2003. Changes in superoxide dismutases (SODs) activities and isoforms profiles during growth of some *Scenedesmus* species, strains and mutants grown in batch-cultures. Acta Physiol. Plant. 25: 375 - 384. **IF: 1,34; MNiSW:20.**
2. Tukaj Z., **Pokora W.**, 2006. Individual and combined effect of anthracene, cadmium and chloridazon on growth and activity of SOD izoformes in three *Scenedesmus* species. Ecotoxicol. Environ. Saf., 65: 323-331. **IF: 2,34; MNiSW:30**.
3. **Pokora W.**, Aksmann A., Tukaj Z., 2011 a. Functional characteristics of green alga *Scenedesmus obliquus* (Chlorophyceae): 276-6 wild type and its two photosystems deficient mutants cultured under photoautotrophic, mixotrophic and heterotrophic conditions. Phycol. Res. 59: 259-268. **IF: 0,95; MNiSW:20.**
4. **Pokora W.**, Detlaff-Pokora A., Tukaj Z., 2011 b. Expression of superoxide dismutase isoforms in *Desmodesmus subspicatus* cells exposed to anthropogenic contaminants. Polish J. Environ. Stud. (20: 605 - 610). **IF:0,54; MNiSW:15.**
5. Aksmann A., **Pokora** W., Tukaj Z., 2001. SOD activity in *Scenedesmus armatus* cells treated with cadmium and two aromatic hydrocarbons.,. Acta Physiol. Plant., 23: 31. **IF: 1,34; MNiSW:20.**
6. Aksmann A., **Pokora W.**, Baścik-Remisiewicz A., Tukaj Z., 2004. Combined effect of anthracene and cadmium induces oxidative stress in algal cells**.** Acta Physiol. Plant. 26: 216. **IF: 1,34; MNiSW:20.**
7. **Pokora W.**, Tukaj Z., 2007. The activity of SOD isoforms in cells of three *Desmodesmus obliquus* green alga strains grown under photoautothropic, mixotrophic and heterotrophic conditions. Acta Biochim. Pol. 54: 191. **IF: 1,23; MNiSW:20.**

The set of manuscripts mentioned above consist a presentation of the results concerning the enzyme: superoxide dismutase (SOD) and its isoforms present in the cells of green alga *Desmodesmus* (formerly *Scenedesmus*). SODs are responsible for converting of superoxide anion radical to hydrogen peroxide. This enzyme constitutes the so-called the first line of defense against oxidative stress conditions, and the efficiency of scavenging of superoxide anion radical into hydrogen peroxide is crucial in protecting the cell from the harmful effects of more toxic ROS, such as the hydroxyl radical (Mittler, 2017). In these papers, I presented the physiological and biochemical characteristics of a series of strains of green algae *Desmodesmus,* that included kinetics of their populations growth and changes in photosynthetic activity as well as changes in the activity and profile of superoxide dismutase

isoforms detected during the growth of these algae in control conditions, on mineral substrates (Pokora et al., 2003) as well as under different trophic conditions (Pokora et al., 2007, 2011 a). Analysis of the protein profile of SODs, indicates the presence of one Cu /Zn-SOD isoform, 1-2 Mn-SOD isoforms and 1-3 Fe-SOD isoforms in all tested species. The SOD isoenzymatic profile is characteristic for each species and does not change during the development of the population, whereas there are differences in the SOD profile of the same species / strains in different types of culture (intensive, aerated and stationary). Irrespective of the rate of population growth, the composition of the culture medium and the studied organism, the Fe - and Mn - SOD activity is dominant, and the intensification of culture and the mixototrophic growth of algae stimulates the production of superoxide anions in their chloroplasts, what may be the origin of oxidative stress. This phenomenon was demonstrated with the wildtype strain of green algae *Desmodesmus obliquus* and its' two mutants with misfunctional PS II and PS I respectively (Pratt and Bishop, 1968). All these strains were characterized in details due to the functionality of the photosynthetic apparatus (efficiency of photosynthetic oxygen production, curve analysis) induction and quenching of chlorophyll a fluorescence and *in vivo* - OJIP test) during their growth in various trophic conditions. The conditions were set, so that the photosynthesis process (photoautotrophy) and mitochondrial respiration (heterotrophy) were the dominant, or balanced (mixotrophy) sources of metabolic energy in the cell (Pokora at al., 2011 a). The verification of photosystem activity in mutants' cells was performed by their comparison with a wild-type strain treated with specific PS II inhibitor (DCMU: (3- (3,4-dichlorophenyl) -1,1-dimethylurea)) and PS I inhibitor (MV: 1,1'-dichloride) dimethyl-4,4'-bipyridinium). The role of mitochondrial metabolism in cells, where the photosynthesis is inhibited, was analyzed using cultures carried out under heterotrophic conditions, with no supply of light energy. I confirmed the total dysfunction of PS II in one of the mutants, while the analysis of kinetics of chlorophyll fluorescence induction and quenching, together with the application of a specific PS I inhibitor (MV) indicated, that in the cells of mutant described as an organism with non-functional PS I, a certain fraction of photosystems I is active. The fact, that PS I mutant, when cultured in the heterotrophic conditions grew much weaker than the wild strain and PS II mutant, raising some doubts whether the mutation in this strain concerns photosynthetic activity only. The experiments confirmed also the important role of superoxide dismutases in the protection of the photosynetic apparatus of green algae against ROS excess mediated damages, especially in cells with malfunction of PS I, where the probability of superoxide anion generation is significantly higher (Allen et al., 2003).

My research was also focused on the influence of cadmium and aromatic hydrocarbons and herbicides blocking the photosynthesis process on the activity of SOD in *Desmodesmus* cells revealing different sensitivity to these substances (Pokora et al., 2006, 2011 b). These studies allowed to determine the nature of interactions between the studied xenobiotics (Tukaj and Pokora, 2006). The $EC_{50/24}$ values (concentration, that inhibit population growth by 50% after 24 hours of culture) for cadmium, anthracene and chloridazone indicated anthracene as the most toxic, followed by chloridazone, while cadmium caused relatively the lowest inhibition of the population growth of *Desmodesmus*. In the natural environment, these substances occur commonly, not as a sole ones, but in the mixtures (Babu et al., 2002), therefore I analyzed the types of interactions between these compounds, when applicated to algal cultures in two- and three-component mixtures. To parametrize these interactions, I used the mathematical formulas described by Wang et al. (1995) and Colby et al. (1967), allowing to determine both the type and direction of interaction between the investigated factors. The most common form of interaction for the substances I studied was antagonism (the actual effect was less than expected one) and addition (effects caused by the use of compounds alone were summarized). These two types of interactions were characteristic of mixtures containing the herbicide affecting photosynthesis - chloridazone. The combination of anthracene and cadmium led to joint enhancement of the toxic effects of these substances, giving a synergistic effect. At the same time, in cells derived from cultures treated with both cadmium and anthracene, I noticed a significant increase in the activity of chloroplast isoforms of superoxide dismutase. That indicated an increased production of superoxide anion radical subsequently in these cells, converted then into hydrogen peroxide. One of the manuscript (Pokora et al., 2011 b) represents a methodical manner, where, using the example of control and treated with toxicants cultures of green algae, I demonstrated the usefulness of the method applied to analysis of activity of isolated from algal cells, individual SOD isoforms, separated using native polyacrylamide electrophoresis combined with staining specific for the activity of SOD. The technique based on the densitometric analysis of the intensity and area of the bands originated from the native enzyme and comparison of the results with the reference values obtained for the enzymatic standard enabled me to determine the separate activity of each individual SOD isoenzymes.

1. Allen, J. F. (2003). State transitions--a question of balance. *Science*, *299*(5612), 1530-1532.
2. Babu, T. S., Tripuranthakam, S., & Greenberg, B. M. (2005). Biochemical responses of the aquatic higher plant *Lemna gibba* to a mixture of copper and 1, 2-dihydroxyanthraquinone: Synergistic toxicity via reactive oxygen species. *Environmental Toxicology and Chemistry*, *24*(12), 3030-3036.

3. Colby, S. R. (1967). Calculating synergistic and antagonistic responses of herbicide combinations. *Weeds*, *15*(1), 20-22.
4. Mittler, R. (2017). ROS are good. *Trends in Plant Science*, *22*(1), 11-19.
5. Pratt, L. H., & Bishop, N. I. (1968). Chloroplast reactions of photosynthetic mutants of *Scenedesmus obliquus*. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *153*(3), 664-674.
6. Wang, J., Zhang, M., Xu, J., & Wang, Y. (1995). Reciprocal effect of Cu, Cd, Zn on a kind of marine alga. *Water Research*, *29*(1), 209-214.

5.1.2. Scientific scope: Analysis of *Chlamydomonas reinhardtii* mutants with functional defects of the photosynthetic apparatus in terms of their ability to tolerance of stress induced by cadmium and anthracene

1. Aksmann A., **Pokora** W., Baścik-Remisiewicz A., Dettlaff-Pokora A., Wielgomas B., Dziadziuszko M., Tukaj Z., 2014. Time-dependent changes in antioxidative enzyme expression and photosynthetic activity of *Chlamydomonas reinhardtii* cells under acute exposure to cadmium and anthracene. Ecotox. Environ. Saf. 110: 31-40 **IF: 2,482; MNiSW: 30.**
2. Aksmann, A., **Pokora W.**, Baścik-Remisiewicz A., Dettlaff-Pokora A., Tukaj Z., 2016. High hydrogen peroxide production and antioxidative enzymes expression in the *Chlamydomonas reinhardtii cia3* mutant with an increased tolerance to cadmium and anthracene. Phycol. Res. 64: 300-311. **IF: 1,338; MNiSW: 25.**

3. **Pokora W.**, Aksmann A., Baścik-Remisiewicz A., Dettlaff-Pokora A., Tukaj Z., 2014. "Dysfunction of carbonic anhydrase Cah3 protein affects *Chlamydomonas* tolerance to Cd-induced oxidative stress". Acta Biol. Cracov.56: 36. **IF 0,73; MNiSW: 20.**
4. Aksmann A., Baścik-Remisiewicz A., **Pokora W.**, Dettlaff-Pokora A., Tukaj Z., 2014. „Photosynthetic activity of *Chalmydomonas reinhardtii* wild type and CC-2699 mutant under Cd-stress". Acta Biol. Cracov.56: 49. **IF: 0,73; MNiSW: 20.**

The mentioned above manuscripts present a series of experiments carried out using intensive cultures of *Chlamydomonas reinhardtii*, where the influence of both anthracene (ANT) and cadmium (Cd) on increased H_2O_2 production in algal cells was shown (Aksmann et al. 2014). A role of disturbances in the functioning of the photosynthetic apparatus on the cells' ability to chemically induced stress tolerance was investigated (Aksmann et al., 2016). In order to explain, how do algae neutralize the harmful effects of oxidative stress, a series of experiments was carried out to and the sequence of molecular and physiological changes that take place in cells exposed to ANT and Cd was proposed (Aksmann et al. 2014). The analysis of chlorophyll *a* fluorescence *in vivo* parameters showed that photosynthetic disturbances in cells treated with ANT and Cd are transient: photosynthetic activity, diminished the most at the sixth hour of cell exposure to toxicants, than, within several hours recovered to the level comparable with one in control cells (Aksmann et al., 2014). The observed decrease in the

efficiency of the photosynthetic apparatus and the increase in the amount of non-photochemically dissipated energy was mainly caused by a 50% reduction of the fraction of active PS II reaction centers. At the same time, the relative abundance of transcripts of genes encoding SOD and CAT increased, followed by a progressive rise in the activity of these enzymes. That enabled the restoration of redox homeostasis and the subsequent, gradual increase in photosynthetic activity of cells. As a result of the analysis of changes in the number of transcripts and the activity of individual antioxidant enzyme isoforms, CAT and mitochondrial Mn-SOD isoform were found as ones that protect the mitochondrion against RFT, whereas the chloroplast originated Mn-SOD isoform is the main enzyme involved in chloroplast protection. Any dysfunction of the photosynthetic apparatus seems to lead to increased sensitivity of algal cells to oxidative stress. Surprisingly, studies performed using *C. reinhardtii* cia3 mutant indicated, that it was less sensitive to ANT and Cd than wild type strain (WT) (Aksmann et.al., 2015). The cia3 mutant does not possess one of the carbonic anhydrase isoforms (CAH3), one of the enzymes responsible for the efficient assimilation of CO_2 (Hanson et al., 2003, Shutova et al., 2008). This organism is characterized by low acclimatization possibilities to changes in CO_2 concentration in the environment. As a result, oxidative stress caused by inhibition of photosynthetic processes is likely to occur in its' cells. To determine the causes of different sensitivity of the mutant and the wild strain to the toxicants, we focused on the determination of the amount of H_2O_2 produced by the cells as well as on the analysis of the antioxidant enzymes expression and activity. Under control growth conditions, the production of H_2O_2 by cia3 mutant cells exceeded the values recorded for WT. Similarly, the levels of transcripts of SOD encoding genes and activity of its isoforms were markedly, and in case of APX and CAT, slightly higher than in wild-type cells. In the latter, as a result of ANT and Cd treatment, symptoms of oxidative stress appeared in the rapid increase in H_2O_2 production, while in the case of a mutant these changes were less significant. The obtained results suggest that a high, constitutive level of expression and activity of antioxidant enzymes is associated with a large amount of H_2O_2 produced "physiologically" by mutant, resulting in the occurrence a "permanent" state of mild oxidative stress in these cells. In a light of this, we assume, that this organism has better potential to neutralize ROS generated as a result of exposure to toxic substances.

1. Hanson, D. T., Franklin, L. A., Samuelsson, G., & Badger, M. R. (2003). The *Chlamydomonas reinhardtii* cia3 mutant lacking a thylakoid lumen-localized carbonic anhydrase is limited by CO2 supply to rubisco and not photosystem II function in vivo. *Plant physiology*, *132*(4), 2267-2275.

2. Shutova, T., Kenneweg, H., Buchta, J., Nikitina, J., Terentyev, V., Chernyshov, S., ... & Junge, W. (2008). The photosystem II-associated Cah3 in *Chlamydomonas* enhances the O2 evolution rate by proton removal. *The EMBO journal*, *27*(5), 782-791.

5.1.3. Scientific scope: oxidative stress in corn coleoptiles exposed to naphthoquinones.

1. Kurtyka R., **Pokora W.**, Tukaj Z., Karcz W., 2016. Effects of juglone and lawsone on oxidative stress in maize coleoptile cells treated with IAA. AoB Plants 8: plw073. **IF: 2,24; MNiSW: 30.**

Naphthoquinones are substances produced by bacteria and fungi, as well as products of secondary metabolism of higher plants, widespread in nature. Their biological activity is based on two main mechanisms; one of them is the induction of oxidative stress caused by interaction with chloroplast and mitochondrial electron transport chains (Cheniany et al., 2013), while the other one depends on direct interaction with macromolecules, where quinones act as electrophiles. Redox homeostasis is considered to be one of the factors involved in the regulation of plant growth via auxin activity (Schopfer et al., 2002), but little is known about the interaction between ROS produced by quinones and auxin action (IAA). We compared the effect of two naturally occurring naphthoquinones, juglone (JG, 5-hydroxy-1,4-naphthoquinone) and lawsone (LW, 2-hydroxy-1,4-naphthoquinone) on the induction of oxidative stress in isolated segments of maize coleoptiles. Maize coleoptiles are used as a typical experimental model for research on the auxin-dependent growth mechanism and potential interactions between auxin and other factors affecting cell growth (Schopfer et al., 2002). It was found, that the increased production of hydrogen peroxide and the increased activity of antioxidant enzymes (SOD, PX and CAT) in maize coleptiles were higher under of lawsone than juglone treatment, regardless of the presence of auxin (IAA) in the incubation medium. Moreover, both naphtoquinones caused an increase in the activity of Cu / Zn-SOD isoenzymes, what suggests, that after juglone and lawsone exposure, hydrogen peroxide was produced primarily in the cytosol and cell wall, where the enzyme is located. Interestingly, the cell's potential to neutralize hydrogen peroxide was determined by the activity of PX and CAT. The activity of catalase was pointed as the main enzymatic mechanism responsible for removing hydrogen peroxide, generated as a result of oxidative stress induced by JG or LW. Therefore, we hypothesized that the formation of hydrogen peroxide, which was more effectively induced by LW, was the main factor responsible for the differences between the toxicity of LW and JG in maize coleoptiles. The oxidative stress induced by JG and LW is therefore one of the mechanisms of allelopathic action of quinones towards plants, while the

oxidation of auxins (IAA), which is possible under such conditions, probably does not play a role in this process.

1. Cheniany, M., Ebrahimzadeh, H., Vahdati, K., Preece, J. E., Masoudinejad, A., & Mirmasoumi, M. (2013). Content of different groups of phenolic compounds in microshoots of *Juglans regia* cultivars and studies on antioxidant activity. *Acta physiologiae plantarum*, *35*(2), 443-450.
2. Schopfer, P., Liszkay, A., Bechtold, M., Frahry, G., & Wagner, A. (2002). Evidence that hydroxyl radicals mediate auxin-induced extension growth. *Planta*, *214*(6), 821-828.

5.2. Other indicators of scientific activity

I presented the results of my research as the posters and oral speeches while participating in both, national (8) and international (12) scientific conferences.

I participated in the realization of 11 research projects, financed by the Committee for Scientific Research (2 grants), Ministry of Science and Higher Education (1 grant), University of Gdańsk (7 grants) and National Science Center (1 grant). In 7 of these projects, including the MNiSW and KBN grants, I was the grant manager. I prepared and submitted to the Ministry of Environment Protection an application for the permission the for the in-door use of GMO organisms in the Department of Plant Physiology and Biotechnology (decision No. 13/2014, No. in the register: 01-102 / 2013). According to the permission, I am the person responsible for supervising the proper handling of GMO organisms in the Department of Plant Physiology and Biotechnology.

Twice I received a team award of the Rector of the University of Gdańsk for a multi-author scientific publication cycle.

In 2013, I was on a research internship at the Umea Plant Science Center (Umea, Sweden),

In the years 2013-2018, I made a total of 17 reviews of scientific publications manuscripts in international (JCR list) journals. I participated in the preparation of two chapters in the "Guide to exercises in plant physiology", edited by Z. Tukaj. Moreover, in addition to conducting laboratory exercises in the subject of "Plant physiology", I am the sole author of the lectures and laboratory experiments protocols, to a series of lectures and laboratory exercises for students of Faculty of Biology, University of Gdańsk. These include: "Plant biotechnology" (30 h of lectures, 30 h of laboratory exercises, 30 h of auditorium exercises), "Methods of *in vitro* cultures" (lecture 30 or 15 h), "Secondary metabolites of plants" (lecture 15 h), "*In vitro* cultures in plant breeding" (lecture 15 h and 30 h laboratory exercises). In addition, I prepare and conduct lectures and exercises within the subjects run by a wider group of people, such as "Plant Physiology II", "Plant originated substances in medical diagnostics" and "Actual research at the Faculty of Biology, University of Gdańsk".

So far I have been the scientific supervisor of 14 masters' degree students, including the to be a promoter of four of them and the promoter of four bachelor's thesis. I also actively participated in the provision of the course program of new Faculties of study at UG - " Experimental Genetics and Biology" and the English-language, inter-faculty course "Bio-Innovation and Entrepreneurship". I also involved in the actions for the popularization of science by participating, as an organizer and exhibitor, at the Baltic Science Festival, Scientist's Night, I conduct the scientific workshops "Discover the work of a biologist" and perform some popular-science lectures as part of the program "Invite a scientist to school" and "Olivian Art Academy. " I was also a member of the team developing and supervising the substantive project and execution of the functional and spatial concept of the new building of the Faculty of Biology UG (current headquarter of the Faculty), ensuring optimal conditions for work and scientific development of the Chair.

A detailed description of my scientific, teaching and organizational achievements can be found in a separate attachment (Annex No. 3).

Gdańsk, 27.09.2018

Wojciech Pokora