Anna Aksmann, PhD

Summary of professional accomplishments

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

Gdańsk, 2016

APPENDIX 2

1.   Name and surname: ANNA AKSMANN

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2.   Diplomas and academic degrees:

PhD diploma in biological sciences in the field of biology, 2005, University of Gdańsk, Faculty of Biology, Geography and Oceanology.
The title of PhD thesis:
„The importance of photosynthetically active radiation and UV-B in the effects of tricyclic aromatic hydrocarbons on planktonic green algae of the genus *Scenedesmus*"
Thesis supervisor: Professor Zbigniew Tukaj, Department of Plant Physiology, Faculty of Biology, Geography and Oceanology, University of Gdańsk.

MSc diploma in biology in the field of general biology, 1995, University of Gdańsk, Faculty of Biology, Geography and Oceanology.
The title of MSc thesis:
„The role of light in growth and perforation processes of coleoptiles of *Triticum aestivum* L. var. Halisa".
Thesis supervisor: Krystyna Burkiewicz, PhD, Department of Plant Physiology, Faculty of Biology, Geography and Oceanology, University of Gdańsk.

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3. Information on employment in scientific institutions.

From 01.04.2005 until now: assistant professor in Department of Plant Physiology and Biotechnology, Faculty of Biology, University of Gdańsk.
From 01.10.1997 to 31.03.2005: assistant in Department of Plant Physiology, Faculty of Biology, Geography and Oceanology, University of Gdańsk. This includes 1-year of maternity leave (October 1999 – September 2000).

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4. Scientific achievement according to Act of 14 march 2003 on Academic Degrees and Titles and on Degrees and Title in Art, art. 16, par. 2 (Journal of Laws No. 65, item 595, with changes):

a) the title of scientific achievement:

Mechanism of toxicity of polycyclic aromatic hydrocarbons towards unicellular green algae.

b) publications belonging to the scientific achievement:

1. **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: 1.42. MSHE[1]:25. I estimate my contribution for 40%.

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[1] "MSHE" describes paper's score according to Ministry of Science and Higher Education, Poland

2. **Aksmann A**., Pokora W., Baścik-Remisiewicz A., Dettlaff-Pokora A., Wielgomas B., Dziadziuszko M., Tukaj Z. 2014. Time-dependent changes in antioxidative enzymes expression and photosynthetic activity of *Chlamydomonas reinhardtii* cells under acute exposure to cadmium and anthracene. Ecotoxicol. Environ. Saf. 110: 31-40.
IF: 2.762. MSHE: 30. I estimate my contribution for 60%.

3. Baścik-Remisiewicz A., **Aksmann A**., Żak A., Kowalska M., Tukaj Z., 2011. Toxicity of cadmium, anthracene and their mixture to *Desmodesmus subspicatus* estimated by algal growth-inhibition ISO standard test. Arch. Environ. Contam. Toxicol. 60 (4): 610-617.
IF: 1.927. MSHE: 25. I estimate my contribution for 45%.

4. **Aksmann A.,** Shutova T., Samuelsson G., Tukaj Z., 2011. The mechanism of anthracene interaction with photosynthetic apparatus: a study using intact cells, thylakoid membranes and PS II complexes isolated from *Chlamydomonas reinhardtii*. Aquat. Toxicol. 104 (3-4): 205-210.
IF: 3.761. MSHE: 45. I estimate my contribution for 70%.

5. **Aksmann A**., Tukaj Z., 2008. Intact anthracene inhibits photosynthesis in algal cells: A fluorescence induction study on *Chlamydomonas reinhardtii* cw92 strain. Chemosphere, 74: 26-32.
IF: 3.054. MSHE: 24. I estimate my contribution for 85%.

c) <u>discussion of the scientific goals of the work and the results achieved, together with a discussion of their possible use:</u>

Scientific achievement being the basis for applying for post-doctoral degree is presented in five monothematic publications concerning the mechanism of the toxic effect of polycyclic aromatic hydrocarbons, with anthracene as an example, on the unicellular green algae. In four of these works I am both the first author and the corresponding author, and **total IF** for these publications is **12.924**.

Polycyclic aromatic hydrocarbons (PAHs) and their derivatives for many years are the subject of scientific research, since these substances belong to the main anthropogenic environmental pollutants. Their toxicity, mutagenicity and potential for bioaccumulation pose a threat to human health and life. The problem of PAHs toxicity towards plant organisms was often considered in research in the field of ecotoxicology and plant physiology, but the results obtained, focused mainly on the growth effects of PAHs action, have not given a clear answer about the primary causes of their toxicity.

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I began my studies on PAHs toxicity towards unicellular algae, as an assistant employed in the Department of Plant Physiology (Faculty of Biology, University of Gdańsk), under the guidance of Professor Zbigniew Tukaj. My research, funded mainly by a State Committee for Scientific Research (one grant) and by University of Gdańsk (four grants), has focused on two aromatic hydrocarbons: anthracene (ANT) and phenanthrene (PHE). These studies were summarized in the doctoral dissertation entitled „The importance of photosynthetically active radiation and UV-B in the effects of tricyclic aromatic hydrocarbons on planktonic green algae of the genus *Scenedesmus*". My results allowed to demonstrate, among other things, that the toxicity of PAHs and their derivatives ($PAH_{deriv.}$) strongly depends on the intensity of photosynthetically active radiation (PAR) and on the availability dissolved inorganic carbon (DIC) in the environment (Aksmann and Tukaj 2004). The study also indicated that closely related strains of algae can vary drastically in their susceptibility to PAHs and $PAH_{deriv.}$ and that the isomeric forms of these substances, although similar in structure, have a different level of toxicity to the test organisms (Aksmann and Tukaj 2004, Tukaj and Aksmann 2007). Since confrontation of the above results with literature data has not given a clear answer as to the possible reasons for the observed differences in toxicity of PAHs and to the different response of algae strains, in further experiments I decided to investigate the primary causes of the PAHs impact on the unicellular green algae.

Seeking mechanisms underlying the toxicity of PAHs, I focused on ANT – hydrocarbon with high toxicity and photosensitizing properties (Huang et al. 1997, Aksmann and Tukaj 2004). It is worth noting that the subject of my interest was the effect of anthracene in its original form, while most of the available literature data as a cause of ANT toxicity pointed to its vulnerability to sunlight-dependent photomodification, which results in ANT conversion into quinones (Huang et al. 1997, Mallakin et al., 1999). The toxicity of quinones follows mainly from the possibility of replacing natural cellular components of the redox system (e.g. plastoquinone, ubiquinone), which causes an abnormality of basic metabolic processes. In opposite, the mechanism of toxicity of unchanged PAHs is not so obvious.

Due to the hydrophobic nature of PAHs, they accumulate in plant cells, primarily in chloroplast, which results in photosynthesis disruption (Duxbury et al., 1997). In

studies of photosynthetic apparatus function under stress conditions, valuable tools are non-invasive methods based on the measurement of chlorophyll fluorescence *in vivo*: a method of pulse amplitude modulation (PAM), or a method of Kautsky curves analysis (test OJIP). Therefore, in the first stage of my research I decided to use these methods to analyze changes in the course of photosynthesis in ANT-treated algal cells. The use of a model unicellular alga *Chlamydomonas reinhardtii* and OJIP method allowed to show that ANT changed the curve of chlorophyll fluorescence induction in a way corresponding to inhibition of electrons flow on the donor side of photosystem II (PS II), probably between quinones Qa and Qb, which was accompanied by reduction of the quantum efficiency of PS II (**Aksmann and Tukaj 2008**). Moreover, the results suggested that the site of ANT action is not only PS II, but also the electron transport chain from PS II to photosystem I (PS I) and probably PS I itself. The above was supported by the fact that the efficiency of the photosynthetic oxygen evolution, depended on the harmonious operation of both photosystems, was in ANT-treated cells inhibited more than would indicate fluorescence parameters based on the operation of the PS II only. To examine the mentioned problem, I decided to make a more detailed analysis of the impact of ANT on photosynthetic apparatus, using the thylakoids and PS II complexes isolated from *Chlamydomonas reinhardtii*. These experiments, performed during one of my stays in Umea Plant Science Center (Umea, Sweden), enabled to demonstrate clearly that ANT does not act directly on photosystem II (**Aksmann et al. 2011**). Interestingly, it was shown that ANT interaction with isolated thylakoids resulted in stimulation of the electrons flow through a photosynthetic electron transport chain (ETC) (**Aksmann et al. 2011**). Since similar results are obtained for electrophilic substances (Lotina-Hennsen et al. 1998) and for photophosphorylation-uncoupling agents (McCarty 1980), this effect of ANT could have theoretically two reasons. First, ANT could act as an artificial electron acceptor, taking them away from one of the ETC carriers. However, in the light of the literature data it seemed unlikely due to the low reduction potential of ANT (Vanýsek 2004). The second possible mechanism of the stimulatory effect of ANT on electrons transport in ETC was related to hydrophobic properties of the hydrocarbon. It is known that PAHs accumulate in protein-lipid membranes (Duxbury et al., 1997) causing a conformational changes in them. Thus, it was hypothesized that ANT, by increasing the thylakoid membrane

permeability to protons and reduction of proton gradient, acts as a photophosphorylation uncoupler, which *in vitro* accelerated the electrons transport and *in vivo* resulted in inhibition of photosynthesis. This hypothesis was verified by analyzing the chlorophyll *a* fluorescence *in vivo* using PAM technique. This method allows to estimate the non-photosynthetic fluorescence quenching parameters (NPQ, qEmax) whose values depend on the proton gradient across the thylakoid membrane (Demmig-Adams and Adams, 2006; Müller et al., 2001). Indeed, my research has shown that ANT causes a decrease in the values of NPQ and qEmax in *C. reinhardtii* cells (**Aksmann et al. 2011**), which confirms the uncoupling effect of this substance and enables to explain both the stimulation of electrons flow in an *in vitro* system (isolated thylakoids) and the inhibition of photosynthesis *in vivo* (intact cells).

It is worth mentioning that the above-described study focused exclusively on ANT as a substance acting individually, while in contemporary research in the field of toxicology a question of particular importance is coexistence in the aquatic environment various groups of chemical contaminants (De Zwart and Posthuma 2005). In an aquatic environment polycyclic aromatic hydrocarbons (including ANT) often coexist with heavy metals (Gust, 2006). Biological effects of such mixtures of toxic substances are often difficult to predict due to their interactions (Faust et al. 2003). Therefore, the subject of my further consideration was a joint effect of ANT and cadmium (Cd), as the representative of heavy metals, on green algae cells. To investigate the complex interactions that occur during the treatment of plant organism with mixtures of toxic substances, toxicological test standard ISO (ISO 2004 protocol 8692) was chosen, with green algae *Desmodesmus subspicatus* as an indicator organism. To better characterize the changes occurring in plant cells subjected to combined action of ANT and Cd, the recommended ISO end-point, namely the population growth, has been supplemented with parameters describing photosynthetic processes. The results showed that a mixture of ANT and Cd applied at low concentrations (corresponding to toxicological values EC_{10}) influences *D. subspicatus* stronger than one could expect from the sum of the effects of substances acting independently. Thus, the interaction between toxicants was synergistic, both in relation to population growth and in relation to the general state of the photosynthetic apparatus (**Baścik-Remisiewicz et al. 2011**). Since the conditions of the algal ISO test (poor medium, low initial density of the population) are expected to reflect

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natural environmental conditions, it may be assumed that the toxicity of PAHs present in water bodies can be significantly enhanced by heavy metals coexisting with them. Since both these groups of substances are inducers of ROS (reactive oxygen species) formation in a cell, a natural choice of further research was to analyze the parameters of oxidative stress in algae treated with ANT and Cd in the context of the acclimation potential of these organisms to chemically-induced stress.

Series of experiments based on intensive *Chlamydomonas reinhardtii* cultures demonstrated that both ANT and Cd caused increased production of H_2O_2 by treated cells (**Aksmann et al. 2014**). Despite a clear symptoms of oxidative stress, the growth of *C. reinhardtii* population was only partially inhibited, which means that a large fraction of the cells was able to continue growth and division. It is known that oxidative stress initiate within the cell various preventive/corrective processes at molecular, biochemical and physiological levels. To explain how algae can neutralize the effects of oxidative stress caused by the action of ANT and Cd, series of experiments was planned to follow time-dependent molecular and physiological changes occurring in the treated cells (Aksmann et al. 2014). Analysis of acclimation processes at the molecular level included changes in the expression of genes encoding the major antioxidant enzymes: superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and changes in these enzymes activity, with particular reference to chloroplast and mitochondrial SOD isoforms. To characterize cell response at physiological level, photosynthetic activity of the organism was investigated, through the analysis of chlorophyll *a* fluorescence parameters *in vivo*.

The results showed that the photosynthesis disruption in cells treated with ANT and Cd was transient: photosynthetic activity, strongly reduced in the sixth hour of cells exposure to toxicants, after several hours of exposure returned to a state close to the control cells (**Aksmann et al. 2014**). The analysis of chlorophyll *a* fluorescence parameters shows that decrease in the overall efficiency of photosynthetic apparatus and a threefold increase in the amount of energy dissipated in non-photochemical way resulted mainly from 50% reduction of the fraction of active PS II reaction centers. At the same time in the treated cells significantly increased the level of transcripts of SOD- and CAT- encoding genes and, subsequently, one could observe the progressive increase in the activity of these enzymes. Analysis of changes in the transcripts level and activity of individual isoforms of antioxidant enzymes helped

me to draw interesting conclusion that CAT and the mitochondrial Mn-SOD isoform protect mitochondria against ROS, while the main enzyme that protects chloroplast is chloroplastic Mn-SOD isoform. Since stress conditions caused a decrease in activity of chloroplastic Fe-SOD and APX, strong expression of the gene encoding the chloroplastic Mn-SOD was recognized as the kind of "complementation" designed to support effective neutralization of ROS in the chloroplast. On the basis of comprehensive interpretation of these results it is assumed that cell response to the stress caused by ANT and Cd begins with stimulation of the SOD- APX- and CAT-encoding genes expression, followed by an increase in these enzymes activity which, in turn, allows gradual restoration of the photosynthetic activity of the cells. In conclusion, in cells defense against stress caused by toxic substances, a special role seems to play the maintenance of redox homeostasis of the cell.

An important relationship between ROS metabolism and the degree of cells resistance to toxicants was confirmed in the next series of studies, the purpose of which was to investigate the possible causes of different reaction of wild-type (WT) *C. reinhardtii* and its cia3 mutant to ANT and Cd (**Aksmann et al. 2016**). Dysfunctional mutant cia3 lacks of CAH3 − one of the isoformes of carbonic anhydrase (CA), belonging to the enzymes responsible for the efficient uptake of CO_2 (Hanson et al. 2003, Shutova et al. 2008). This dysfunction causes low acclimation capacity of cia3 mutants to changes in CO_2 concentration in the environment, whereby are exposed to oxidative stress resulted from inhibition of photosynthetic processes. Thus, it could be expected that this type of dysfunction sensitizes cia3 cells to toxicants. Surprisingly, studies have shown that cia3 mutant is less sensitive to ANT and Cd than WT, which was manifested, among others, by stronger inhibition of photosynthesis and population growth of treated WT (**Aksmann et al. 2016**). Looking for the reason(s) of the different tolerance of both strains to Cd and ANT, we paid special attention to differences in ROS production and scavenging in the strains, because both Cd and ANT are known to be oxidative stress inducers (Huang et al. 1997; Szivák et al. 2009; Aksmann et al. 2014). Our data demonstrated a significantly higher H_2O_2 production in the control mutant cells compared to the WT ones. At this point the question arises why cia3 produces more H_2O_2 than the wild-type cells. To answer to this question it is necessary to consider that the CAH3 protein supports the effective function of water oxidation complex in

PS II (Shutova et al., 2008). Cia3 mutant cells, deprived of CAH3 support, have less efficient PSII than the WT cells (Villarejo et al., 2002; **Aksmann et al. 2016**) and an optimal electron transport in these cells is maintained by overproduction of PS II reaction centres. Thus, the increased number of PS II reaction centres with their reduced efficiency in mutant cells can possibly explain high H_2O_2 production in the control cia3. Lowering of electron transport efficiency is usually accompanied by enhanced ROS production (Apel and Hirt 2004), among other superoxide (O_2^{-}), that is further dismutated by SODs to hydrogen peroxide. As regards ROS-scavenging enzymes, we have found that the transcript levels for SOD isoforms, as well as the isoenzymes activity were also higher in the cia3 cells than in the WT ones (**Aksmann et al. 2016**). However, there were no significant differences in the transcript level of APX and CAT in both strains, and slightly elevated activity of the enzymes was observed in the cia3 cells. On the other hand, in WT cells treated with ANT and Cd, clear symptoms of oxidative stress could be seen in a sharp increase in H_2O_2 production, whereas in the case of cia3 cells these changes were less pronounced. In toxicants-treated WT cells also SODs expression and activity was higher than in cia3 ones, but APX and CAT activities were similar in both strains. Since high induction of ROS-scavenging enzymes expression and activity enhanced the resistance of cells to chemically induced stress (Foyer and Noctor 2005, Pokora and Tukaj 2010; Pokora and Tukaj 2013), we assume that the relatively high initial expression and activity of SODs in cia3 mutant result in its higher tolerance to Cd- and ANT-induced stress.

Summarizing the overall results described in the above series of publications, **probable sequence of events occurring in the algal cells exposed to anthracene** can be presented. The toxic effects of this substance begins presumably as non-specific effects on chloroplast protein-lipid membrane, leading to changes in the structure of the thylakoids and to photophosphorylation uncoupling. This, in turn, inhibits CO_2 assimilation which provokes an overproduction of ROS leading to damage to the photosystem. In such conditions, the cell protective mechanisms are activated, among which an important role is played by antioxidant enzymes, maintaining redox homeostasis of cells and influence the organism's sensitivity to hydrocarbon-provoked stress.

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References *(includes items not listed in 4b)*

1.   Aksmann A., Tukaj Z. 2004. The effect of anthracene and phenanthrene on the growth, photosynthesis and SOD activity of green alga *Scenedesmus armatus* depend on the PAR irradiance and CO_2 level. Arch. Environ. Contam. Toxicol. 47: 177-184.

2.   Apel K., Hirt H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55: 373-399.

3.   De Zwart D., Posthuma L. 2005. Complex mixture toxicity for single and multiple species: proposed methodologies. Environ. Toxicol. Chem. 24(10): 2665-2676.

4.   Demmig-Adams B., Adams W.W. 2006. Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. New Phytol. 172(1): 11-21.

5.   Duxbury C. L., Dixon D. G., Greenberg B.M. 1997. Effects of simulated solar radiation on the bioaccumulation of polycyclic aromatic hydrocarbons by the duckweed Lemna gibba. Environ. Toxicol. Chem. 16(8): 1739-1748.

6.   Faust M., Altenburger R., Backhaus T., Blanck H., Boedeker W., Gramatica P. et al. 2003. Joint algal toxicity of 16 dissimilarly acting chemicals is predictable by the concept of independent action. Aquat. Toxicol. 63:43–63.

7.   Foyer C.H., Noctor G. 2005. Redox homeostasis and antioxidant signaling: A metabolic interface between stress perception and physiological responses. Plant Cell 17:1866–1875.

8.   Gust K.A. 2006. Joint toxicity of cadmium and phenanthrene in the freshwater amphipod *Hyalella azteca*. Arch. Environ. Contam. Toxicol. 50:7–13.

9.   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 CO_2 supply to rubisco and not photosystem II function *in vivo*. Plant Physiol. 132: 2267–2275.

10.   Huang X.D., McConkey B.J., Babu T.S., Greenberg B.M. 1997. Mechanisms of photoinduced toxicity of photomodified anthracene to plants: inhibition of photosynthesis in the aquatic higher plant *Lemna gibba* (duckweed). Environ. Toxicol. Chem. 16(8): 1707-1715.

11.   ISO 2004. International Organization for Standardization. Water quality - Fresh algal growth inhibition test with unicellular green algae. ISO 8692:2004 (E).

12.   Lotina-Hennsen B., Achnine L., Ruvalcaba N.M., Ortiz A., Hernández J., Farfán N., Aguilar-Martínez M. 1998. 2,5-Diamino-p-benzoquinone derivatives as photosystem I electron acceptors: synthesis and electrochemical and physicochemical properties. J. Agr. Food Chem. 46(2): 724-730.

13.   Mallakin A., McConkey B.J., Miao G., McKibben B., Snieckus V., Dixon D.G. et al. 1999. Impacts of structural photomodification on the toxicity of environmental contaminants: anthracene photooxidation products. Ecotoxicol. Environ. Saf. 43:204–212.

14.   McCarty R.E. 1980. Delineation of the mechanism of ATP synthesis in chloroplast. Use of uncouplers energy transfer inhibitors and modifiers of CF1. A. San Pietro (Ed.), Methods in Enzymology, vol. 69Academic Press, New York/London, 1980

15.   Müller P., Li X.P., Niyogi K.K. 2001. Non-photochemical quenching. A response to excess light energy. Plant Physiol. 125(4): 1558-1566.

16.   Pokora W., Tukaj Z. 2010. The combined effect of anthracene and cadmium on photosynthetic activity of three *Desmodesmus* (Chlorophyta) species. Ecotoxicol. Environ. Saf. 73: 1207–1213.

17.   Pokora W., Tukaj Z. 2013. Induction time of Fe-SOD synthesis and activity determine different tolerance of two *Desmodesmus* (green algae) strains to chloridazon: A study with synchronized cultures. Pestic. Biochem. Physiol. 107: 68–77.

18.   Shutova T., Kenneweg H., Buchta J., Nikitina J., Terentyev V., Chernyshov S., Andersson B., Allakhverdiev S.I., Klimov V.V., Dau H., Junge W., Samuelsson G. 2008.

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Photosystem II-associated Cah3 in *Chlamydomonas* enhance the O_2 evolution rate by proton removal. EMBO J. 27:782–791.
19.   Szivák R., Behra R., Sigg L. 2009. Metal-induced reactive oxygen species production in *Chlamydomonas reinhardtii* (Chlorophyceae). J. Phycol. 45: 427–435.
20.   Tukaj Z., Aksmann A., 2007. Toxic effects of anthraquinone and phenanthrenequinone upon *Scenedesmus* strains (green algae) at low and elevated concentration of CO_2. Chemosphere 66:480–487.
21.   Vanýsek P. 2004. Reduction and oxidation potentials for certain ion radicals. D.R. Lide (Ed.), Handbook of Chemistry and Physics (85th ed.), CRC Press, USA, 2004.
22.   Villarejo A., Shutova T., Moskvin O., Forssén M., Klimov V.V., Samuelsson G. 2002. A photosystem II-associated carbonic anhydrase regulates the efficiency of photosynthetic oxygen evolution. EMBO J. 21(8): 1930-1938.

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5. Discussion of the other scientific achievements.

5.1. Publications

Besides the publications that comprises the main scientific achievement outlined above, my achievement consists of 9 publications (**total IF: 18.058**), of which I am a co-author. Below is a brief discussion of these works.

5.1.1. Filed: toxicity of polycyclic aromatic hydrocarbons and their derivatives towards green algae.

1) Tukaj Z., Bohdanowicz J., **Aksmann A**. 1998. A morphometric and stereological analysis of ultrastructural changes in two *Scenedesmus* (Chlorococcales, Chlorophyta) strains subjected to diesel fuel oil pollution. Phycologia 37 (5): 388-393.
2) **Aksmann A**., Tukaj Z. 1999. Wpływ antracenu i fenantrenu na glony *Scenedesmus armatus (Chlorophyta)* w zależności od natężenia napromieniowania PAR. Zeszyty Problemowe Postępów Nauk Rolniczych 469: 279-284.
3) **Aksmann A**., Boryń K., Tukaj Z. 2004. Rola procesu fotomodyfikacji w toksycznym oddziaływaniu policyklicznych węglowodorów aromatycznych na mikroglony *Scenedesmus.* Zeszyty Problemowe Postępów Nauk Rolniczych 496: 439-448.
4) **Aksmann A**., Tukaj Z. 2004. The effect of anthracene and phenanthrene on the growth, photosynthesis and SOD activity of green alga *Scenedesmus armatus* depend on the PAR irradiance and CO_2 level. Arch. Environ. Contam. Toxicol. 47: 177-184.
5) Tukaj Z., **Aksmann A.**, 2007. Toxic effects of anthraquinone and phenanthrenequinone upon *Scenedesmus strains* (green algae) at low and elevated concentration of CO_2. Chemosphere 66: 480–487.

A series of five publication listed above presents the results of research on physiological and structural effects of polycyclic aromatic substances on single-celled

green algae. In the first set of experiments a mixture of polycyclic aromatic hydrocarbons (PAHs) was used in the form of an aqueous extract of fuel oil (**paper 1**). In strain sensitive to PAH, namely *Desmodesmus* (former *Scenedesmus*) *microspina,* extract oil caused ultrastructural changes like an increasing cell surface, a strong vacuolization, an increase of the total volume of the mitochondria and a reduction in the volume of the chloroplast and nucleus. In insensitive strain *Scenedesmus quadricauda* these changes were much less pronounced, and in the case of the nucleus and the microsomal fraction, even increase in the volume was observed compared to the control. These changes suggested that in strains sensitive to aromatic hydrocarbons major changes occur in the course of key plant cell processes: respiration and photosynthesis.

Because diesel fuel toxicity is the result of an effect of many different substances, in further research, focused on the physiology of the cell (**papers 2 - 4**), I have selected two petroleum aromatic hydrocarbons, anthracene (ANT) and phenanthrene (PHE), known for their toxicity to plant organisms (Huang et al. 1993). Due to the fact that the two hydrocarbons are able to absorb solar radiation, experiments were focused on the role of photosynthetically active radiation (PAR) and UV-B in ANT and PHE toxicity. The obtained results enable to estimate the importance of the photosensitization (impact of PAR) and photomodification (impact of UV-B) processes in PAH toxicity. Because of this latter phenomenon, the study included also the main photoproducts of examined hydrocarbons: anthraquinone (ANTQ) and phenanthrenequinone (PHEQ), respectively (**paper 5**).

The results led to the conclusion that, in regard to green algae of the genus *Desmodesmus* (formerly *Scenedesmus*) ANT is forty times more toxic than PHE, which resulted from PAR-dependent photosensitizing action of ANT. Interestingly, ANT photomodification by UV-B significantly decreased its toxicity which was related to almost twice lower toxicity of its main photoproduct, ANTQ. The opposite results were obtained for PHE, whose photoproduct (PHEQ) turned out to be a hundred times more toxic than its parent hydrocarbon. The cause of such a high toxicity of PHEQ was its photosensitizing property. Analysis of photosynthetic parameters of algae cells treated with ANTQ and PHEQ showed that one of the reasons of their toxicity was inhibition of electron flow in photosynthetic chain. Very interesting result was obtained by comparing the impact of ANTQ and PHEQ on two

closely related *Desmodesmus* strains. It was shown that different sensitivity of the two strains to the tested quinones resulted, inter alia, from unequal superoxide dismutase (SOD) activity in the cells, which is strongly associated with the organisms tolerance to oxidative stress.

References

1. Huang X-D., Dixon D.G., Greenberg B.M. 1993. Impacts of UV radiation and photomodification on the toxicity of PAHs to the higher plant *Lemna gibba* (Duckweed). Environ. Toxicol. Chem. 12: 1067-1077.

5.1.2. Filed: the use of metabolomics in research of Chlamydomonas reinhardtii acclimation to low CO_2 concentration.

1) Renberg L., Johansson A. I., Shutova T., Stenlund H., **Aksmann A**., Raven J. A., Gardeström P., Moritz T., Samuelsson G., 2010. A metabolomic approach to study major metabolite changes during acclimation to limiting CO_2 in *Chlamydomonas reinhardtii*. Plant Physiol. 154(1): 187-196.

Chlamydomonas reinhardtii is one of green algae possessing CCM − carbon concentrating mechanism − which is activated under CO_2 shortage in the environment (Giordano et al. 2005). CCM induction include changes in expression of dozens of genes, as was demonstrated in numerous studies (see, e.g. Miura et al. 2004; Yamano et al., 2008; Yamano, and Fukuzawa 2009), but knowledge about metabolic changes occurring during CCM induction was still very limited. Study described in the work by Renberg et al. (2010), in which I participated during my two stays at Umea Plant Science Center (Sweden), were designed to examine changes in the level of key cell metabolites during CCM induction.

The use of GC-TOF MS (gas chromatography-time of flight mass spectrometry) enabled to identify 128 metabolites (amino acids, lipids, carbohydrates), whose level significantly changed after the transfer of cells to the low CO_2 conditions. The obtained metabolomic results fitted well to transcriptomic data presented by Yamano et al. (2008), describing the relationship between CCM induction and transient stimulation of photorespiration, which allowed us to propose a model for regulation of metabolic changes in the cells *C. reinhardtii* during acclimation to low concentrations of CO_2 in environment.

References

1. Giordano M., Beardall J., Raven J.A. 2005. CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. Annu. Rev. Plant Biol. 56: 99–131.

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2. Miura K., Yamano T., Yoshioka S., Kohinata T., Inoue Y., Taniguchi F., Asamizu E., Nakamura Y., Tabata S., Yamato K.T., et al. 2004. Expression profiling-based identification of CO_2-responsive genes regulated by CCM1 controlling a carbon-concentrating mechanism in *Chlamydomonas reinhardtii*. Plant Physiol. 135: 1595–1607.
3. Yamano T., Fukuzawa H. 2009. Carbon-concentrating mechanism in a green alga, *Chlamydomonas reinhardtii*, revealed by transcriptome analyses. J. Basic Microbiol. 49:42–51.
4. Yamano T., Miura K., Fukuzawa H. 2008. Expression analysis of genes associated with the induction of the carbon-concentrating mechanism in *Chlamydomonas reinhardtii*. Plant Physiol. 147: 340–354.

5.1.3. Filed: factors influencing the cell cycle of green algae

1) Grabski K., **Aksmann A**., Mucha P., Tukaj Z., 2010. Conditioned medium factor produced and released by *Desmosdemus subspicatus* and its effect on the cell cycle of the producer. J. Appl. Phycol. 22 (4): 517 - 524.
2) Pokora W., **Aksmann A.**, Baścik-Remisiewicz A., Dettlaff-Pokora A., Rykaczewski M., Gappa M., Tukaj Z., 2016. "Changes in nitric oxide/hydrogen peroxide content and cell cycle progression: study with synchronized cultures of green alga *Chlamydomonas reinhardtii*". J. Plant Physiol. doi: 10.1016/j.jplph.2016.10.008

The cell cycle is a sequence of processes that occur in the cell between two consecutive mitotic divisions. These processes consist of the growth phase and the reproductive phase, and are regulated by both the intracellular circadian rhythm, as well as by environmental factors (Cross and Umen 2015). Modification of green algae cell cycle progression (shortening or lengthening) can occur in response to such environmental stimuli as: changes in the light intensity and duration of light period, temperature fluctuations, the presence of chemicals (toxins or growth promoters). Some of these substances are produced by algal cells.

Planktonic algae growing in both natural conditions and in a laboratory, produce and secrete into the culture medium various organic substances. Medium obtained after cells removing from the culture is called conditioned medium (CM). CM added in suitable proportions to the fresh medium can change its properties, e.g. affecting the speciation of metals and reducing the toxicity of chemical substances (Lombard et al. 2005; Torelli et al. 2008). There are also reports that CM may contain substances of the growth-promoter character (Sakamoto et al., 1996), although these substances have not yet been isolated and described. In studies described in work by Grabski et al. (2010) (**paper 1**) we used both asynchronous and synchronous *Desmodesmus subspicatus* cultures, which enabled us to examine stimulating influence of CM on the cell cycle of the organism. The results revealed that the CM,

twice diluted with fresh culture medium (CM/2), strongly stimulates the growth of *D. subspicatus* population. Analysis of chlorophyll *a* fluorescence parameters together with the rate of photosynthetic oxygen evolution measurements made it possible to show that the probable reason of growth stimulation was the increase in efficiency of photosynthetic processes in cells grown in CM/2. As a result, in cultures grown in CM/2 increased the percentage of cells which pass four, instead of three, commitment points during their cell cycle. This, in turn, resulted in releasing the sixteen, instead of eight, daughter cells. The final result was higher density of CM/2 populations in relation to the control population, grown in a fresh culture medium.

In the progression of cell cycle of green algae and other plant cells, the key elements are the signal cascades leading to activation of the appropriate transcription factors. Signaling molecules in these cascades are e.g. hydrogen peroxide and nitric oxide, which modulate the signal cascades regulating the synthesis of cyclins (CYCs) and the cyclin-dependent kinases (CDKs), allow the passage of cells through the consecutive steps of the cycle.

Because activity of the enzymes responsible for the formation of H_2O_2 (superoxide dismutase, SOD) and NO (nitrate reductase, NR and nitrite reductase, NIR) in algal cells is subjected to circadian oscillations (Carvalho et al. 2004, Lehner et al. 2009), it may be assumed that changes in the H_2O_2/NO concentration ratio is one of the elements regulating cell cycle progression. To verify this hypothesis, we investigated changes in the activity of SOD, NR and NIR and changes in the concentration of NO and H_2O_2 during the course of cell cycle of *Chlamydomonas reinhardtii* (**paper 2**). It was found that the increase in H_2O_2 concentration observed at the beginning of a cycle, originated from the activity of chloroplast isoforms Fe-SOD and Mn-SOD. Their activity increased because of oxidative stress associated with starting the photosynthesis in young daughter cells transferred from darkness to light. On the other hand, sharp increase in the NO concentration immediately before the transition of cells from light to dark phase of the cycle, was caused by increased activity of NR and NIR. It has been observed that this increase in the concentration of NO is independent of photosynthesis and that it is accompanied by increased activity of chloroplast isoform Mn-SOD, which resulted in the increased production of H_2O_2. At the same time the cell division begins, which was confirmed by analyzing the expression profile of genes encoding CYCs and CDKs. These results

clearly indicate a close relationship between the *Chlamydomonas* cell cycle and changes in the intracellular concentrations of H_2O_2 and NO, which in turn is associated with fluctuations in the expression and activity of enzymes responsible for the formation and neutralization of these signaling molecules.

References
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2. Cross F.R., Umen J.G. 2015. The Chlamydomonas cell cycle. The Plant J. 82: 370-392.
3. Lehner C., Kerschbaum H. H., Lütz-Meindl U. (2009). Nitric oxide suppresses growth and development in the unicellular green alga *Micrasterias denticulata*. J. Plant Physiol. 166(2): 117-127.
4. Lombardii A.T., Hidalgo T.M.R., Viera A.A.H. 2005 Copper complexing properties of dissolved organic materials exuded by the freshwater microalgae *Scenedesmus acuminatus* (Chlorophyceae). Chemosphere 60:453–459.
5. Sakamoto B., Nagai H., Hokama Y. 1996 Stimulators of *Gambierdiscus toxicus* (Dinophyceae) growth: the possible role of gambieric acid-A as an endogenous growth enhancer. Phycologia 35:350–353.
6. Torelli A., Marieschi M., Castagnoli B., Zanni C., Gorbi G., Corradi G.M. 2008. Identification of *S2-T A63*: a cDNA fragment corresponding to a gene differentially expressed in a Cr-tolerant strain of the unicellular green alga *Scenedesmus acutus*. Aquat Toxicol 86:495–507.

5.1.4. Filed: the impact of trophic conditions on the functioning of photosynthetic mutants of green alga Scenedesmus obliquus.

1) Pokora W., **Aksmann A**., Tukaj Z. 2011. Functional characteristics of green alga *Scenedesmus obliquus* (Chlorophyceae): 276-6 wild type and its two photosystems deficient mutants cultured under photoautothropic, mixotrophic and heterotrophic conditions. Phycol. Res. 59:259-268.

Mutants of green algae are now commonly used in the physiological, biochemical and molecular studies of plant cells (Ochiai et al. 2007, Harris 2009). These organisms may also be useful in toxicology studies for precise determination of the mechanisms of chemicals' toxicity at physiological and/or molecular level. Such a potential tool seems to be *Scenedesmus obliquus* mutants with dysfunctional photosystems PS I or PS II (Pratt and Bishop 1968). The use of such mutants eliminates the need to use artificial inhibitors of photosynthesis thus prevents the possible interactions between the inhibitors and tested substances. However, before such dysfunctional organisms could be used in experiments, their physiology and biochemistry must be thoroughly understood. Thus, the objective of our study was to present a thorough functional characterization of wild-type strain and two photosynthetic mutants of *S. obliquus*.

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An analysis of photosynthetic and respiratory activity of the above organisms as well as measurements of activity of a key antioxidant enzyme – superoxide dismutase have demonstrated that for all three strains optimal growth conditions was mixotrophy. Surprisingly, both photosynthetic mutants were capable to grow photoautotrophically despite the low chlorophyll content in the cells and low intensity of photosynthetic oxygen evolution. Analysis of chlorophyll fluorescence induction curves confirmed the high degree of dysfunctionality of PS II in PSII-def. mutant, however, in PSI-def. mutant a fraction of PS I turned out to be active. What's more, heterotrophic growth of PSI-def. mutant was much worse than growth of wild-type strain and PSII-def. mutant, causing reasonable doubts whether a mutation in this strain affects only the photosynthetic activity. The conclusion of the work was, therefore, that the PSII-def. mutant can be considered as fully blocked in its photosystem II activity but PSI-def. mutant should be considered only as a strain on the limited capacity of photosystem I.

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2. Harris E. H. 2009. The Chlamydomonas Sourcebook. Vol. I: Introduction to Chlamydomonas and Its Laboratory Use. Elsevier Academic Press, San Diego, CA.
3. Pratt L.H., Bishop N.I. 1968. Chloroplast reactions of photosynthetic mutants of *Scenedesmus obliquus*. Biochim. Biophys. Acta 153: 664–74.

5.2. Other scientific activities

The results of my research was presented in the form of posters and lectures during **20 scientific conferences**, both national and international.

I participated in **11 research projects** funded by the Ministry of Science and Higher Education (2 projects), University of Gdańsk (8 projects) and National Science Centre (1 project). In seven of these projects I was a **project leader**, including NCN project entitled "The role of ELIPs (early light induced proteins) in acclimatization of *Chlamydomonas* cells to anthropogenic pollution of the aquatic environment". I also participated in the preparation of a project request for the Minister of the Environment on the use of GMOs in the Department of Plant Physiology and Biotechnology (Decision No 13/2014, No. registry: 01-102 / 2013).

APPENDIX 2

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Three times I received **the team award of the Rector of the University of Gdansk** for multi-authors series of scientific publications.

In the years 2007 – 2013 I was on six short-term **scientific interships** in Umea Plant Science Center (Umea, Sweden) and in year 2010 I participated in **scientific workshops** about using chlorophyll fluorescence in physiological studies, organized by the Department of Molecular Plant Physiology, University of Warsaw.

In the years 2007 – 2016 I prepared in total **30 reviews** of manuscripts of scientific publications for international journals.