**MSc ARIEL KAMIŃSKI, ABSTRACT OF DOCTORAL DISSERTATION**

Increasing eutrophication of water has led to higher frequency and intensity of cyanobacterial blooms. One of the most important consequences of this is the release of
a wide range of bioactive secondary metabolites into water that can be toxic to humans and animals. One of the most common freshwater neurotoxins is anatoxin-a (ANTX-a), the first cyanotoxin to be isolated and structurally analyzed. It has been found in many countries all over the world including Canada, Finland, France, Italy, Iran, Japan, Kenya, Poland, Sweden and the USA. It is synthesized by cyanobacterial genus such as: *Anabaena*, *Aphanizomenon*, *Arthrospira*, *Cylindrospermum*, *Microcystis*, *Nostoc*, *Oscillatoria*, *Phormidium*, *Planktothrix* and *Raphidiopsis.* ANTX-a is a potent postsynaptic nicotinic agonist and mimics the neurotransmitter acetylcholine. The difference between them is that ANTX-a is not degraded by acetylcholinesterase. Its action overstimulates muscles, causing convulsions, abdominal breathing, cyanosis and paralysis, possibly leading to death by asphyxiation when respiratory muscles are affected.

 The data on the effects of physicochemical factors on ANTX-a stability are limited. Earlier studies documented its high lability; the half-life of ANTX-a at pH 8 or 10 was approximately 14 days under natural photoperiod conditions. In this doctoral dissertation in controlled laboratory conditions has been demonstrated high resistance of ANTX-a breakdown under some physicochemical factors similar to those periodically occurring in environment. Stability of ANTX-a molecule was pH dependent. Acidification of the sample stabilized, while alkalization accelerated its degradation. When aqueous solution of ANTX-a was submitted to impact of high photosynthetically active radiation, temperature ≤ 20 ˚C, acidic pH, presence of cyanobacterial photosynthetic pigment – phycocyanin C, it was relatively stable for a long period of time (9 weeks). Nevertheless, some physicochemical factors such as: UV-B irradiation, high temperature (100 °C), alkaline pH accelerated its decomposition. The fastest decomposition of ANTX-a molecule was observed for its aqueous solution at pH 9,5: after 1 h of UV-B irradiation (ANTX-a concentration lowered by 84 %) and after 1 h of treatment at 100 ˚C (decrease of 83 % relative to its initial concentration). Analysis of the MS/MS spectra proved the presence of degradation products with *m/z* of 180 Da, 184 Da and 198 Da. None of them have been previously described. The factors accelerated degradation of ANTX-a molecule can be used in water treatment technology.

 There are numerous reports of harmful impact of ANTX-a on animals, but data on its effects on plants are limited. In this doctoral dissertation was examined the influence of ANTX-a on *Lemna trisulca* and degree of its biodegradation and bioaccumulation by this macrophyte.
*L. trisulca* inhabits similar habitats as cyanobacteria able to synthesize ANTX-a. Moreover, it is small in size, fast growing, widespread and resistant to harmful effects of naturally occurring concentrations of toxin (1,75 µg·mL-1). In the concentrations ≤ 5 µg ANTX-a·mL-1 the induction of oxidative stress in plants, manifested by changes in *i.a.* activity of antioxidant enzymes, content of total protein and photosynthesis pigments, respiratory and photosynthetic efficiency was not detected. Significant decreases in biomass content by 30 % was demonstrated in samples cultivated in media containing 12,5 µg ANTX-a·mL-1 after 24 day of experiment. It was correlated with a significant decrease in the photosynthetic efficiency. Only after 14 days of *L. trisulca* cultivation in media containing 25,0 µg ANTX-a·mL-1 the decrease in chlorophyll *b* content was observed*.*

One gram of fresh weight (f.w.) of *L. trisulca* cultured for 14 days in the media containing 250 µg ANTX-a removed 86 % of the initial toxin concentration. The analyzed macrophyte strongly binds adsorbed ANTX-a. The quantity of toxin released to media was minimal: from 3,5 % to 5,1 % of the initial bioaccumulated value. Within 14 days the content of accumulated ANTX-a in the macrophyte decreased by 76 % (from initial 19,3 µg ANTX-a·g-1 f.w.), 71 % (from 37,3 µg ANTX-a·g-1 f.w.) and 47 % (from 63,7 µg ANTX-a·g-1 f.w.). The data shows that part of the ANTX-a was degraded. Mass spectra analyses did not indicate transformation of ANTX-a to already known forms. These findings suggest that *L. trisulca* has much potential as a phytoremediation agent for stabilization of aquatic environments.

This study also evaluated the interaction between the aquatic macrophyte *Lemna trisulca* and the cyanobacterium *Anabaena flos-aquae* during co-cultivation. The macrophyte drastically inhibited the biomass accumulation of the cyanobacterium (after 32 days of co-cultivation of both organisms, by 86,9 % compared with the control). By contrast, the biomass of the macrophyte was similar in both groups – experimental and control.. *L. trisulca* had
a stabilizing effect on the media pH. Over 32 days of cultivation, the macrophyte readily adsorbed phosphate ions from the media, resulting in a systematic increase in the N/P ratio, limiting this nutrient for cyanobacterium. Furthermore, under this condition, reductions in ANTX-a concentrations by 78,6 % in the media and by 99,9 % in the cyanobacterial cells were detected. Presumably, *L. trisulca* simultaneously accumulated and degraded the toxin inside of its tissues. During 32 days of *A. flos-aquae* and *L. trisulca* co-cultivation, no increases were observed in the total phenolic concentrations in the media or in the extractable fractions for both organisms. Presented results suggest that after environmental studies *L. trisulca* may be a natural factor used for the biological inactivation of this cyanobacterium and its toxin. Furthermore, these results have an ecological significance for the prevention of cyanobacterial blooms in water ponds.