

Little is known on the toxicity of cyanobacterial oligopeptides beyond microcystins. Therefore, the aim of this project is to study the effects of individual cyanobacterial oligopeptides such as aeruginosin, aeruginosamides, anabaenopeptins, cyanopeptolins and in combination with cyanotoxins (microcystin-LR and cylindrospermopsin) on organisms representing different trophic levels of aquatic ecosystems: plants-duckweed (*Lemna minor*, *Spirodela polyrhiza*) and invertebrates such as rotifers (*Brachionus calyciflorus*) crustaceans (*Daphnia* sp., *Thamnocephalus platyurus*), insects (*Chironomus* sp.), and fish (cell lines: rainbow trout gill cell line (RTgill-W1) and carp leucocyte cell (CLC) line). We propose the following hypotheses which will be verified during the studies: **a) individual cyanobacterial oligopeptides other than cyanotoxins (microcystin-LR, cylindrospermopsin) induce alterations of survival, growth, behavioural, physiological and biochemical parameters in aquatic organisms, b) cyanobacterial oligopeptides enhance adverse effects induced by common cyanobacterial toxins (microcystin-LR, cylindrospermopsin).** The project includes several novelites:

1. Regarding the current state of knowledge, no data exist on the sub-lethal effects of cyanobacterial oligopeptides (beyond microcystins) at organismal level measured by sensitive and early, physiological and biochemical (and also behavioural in case of animals) responses of aquatic organisms, also there exist some single studies concerning lethal effects on crustaceans. Use of the fluorescent microscopy will enable for the first time a creation of maps of cytotoxic effects in crustaceans and rotifers exposed to cyanobacterial oligopeptides.
2. Majority of the available reports regarding effects of cyanobacterial oligopeptides is limited to the responses of single enzymes. The proposed project will give a broader insight into the functioning of selected animals under the pressure of individual cyanobacterial oligopeptides and their mixtures which occur in nature. The study will be focused on their effects at the organismal (*in vivo*) level.
3. The novelty of this project will also include determination of the influence of these cyanobacterial metabolites on selected cellular parameters of fish. Rainbow trout gill (RTgill-W1) and carp leucocyte cell lines (CLC) will be used as sensitive indicators.

The experimental organisms will be exposed to:

- individual pure oligopeptides,
 - mixture of individual oligopeptides with microcystin-LR (cyanotoxin)
 - mixture of individual oligopeptides with cylindrospermopsin (cyanotoxin)
 - mixture of all studied oligopeptides.
- at their increasing concentrations compared to controls.

Since cyanobacterial oligopeptides are highly bioactive and effects on different biological processes may be expected, a range of various endpoints (survival, physiological, biochemical) and in case of animals also behavioural will be determined at standardized time points: 4h, 24h, 48h and 72h for animals, 48h and 96h for plants. In experimental plants leaf biomass, root length chlorophyll content and oxygen production as physiological endpoints will be determined. In invertebrates, in addition to survival, physiological and biochemical endpoints, behavioural indicators will be evaluated. The project will also include determination of effects induced by oligopeptides and their combinations with microcystin-LR and cylindrospermopsin on fish gill and leucocyte cell lines. Since microcystin-LR was reported to alter liver architecture that may be related to disturbances of cytoskeleton, other cyanobacterial oligopeptides may be expected to induce similar effects. The project will also provide data for explanation of mechanisms of action of cyanobacterial oligopeptides and cyanotoxins in the tested organisms. Determination of kinases activity will help in evaluation whether oligopeptides and their mixtures with cyanotoxins induce disturbances on kinases/phosphatases balance. The project will provide valuable exotoxicological data. The results will enrich the current knowledge on the effects of cyanobacterial congeners of individual oligopeptides and their mixtures of oligopeptides with cyanotoxins which occur in nature. The results will give basis for elaboration of guidelines for environmental risk assessment regarding cyanobacterial oligopeptides. The results project will also enable to consider some most sensitive endpoints of the model organisms as parts of ecotoxicological early warning systems (EWS) that would be used for monitoring of aquatic reservoirs and fish farms.