

# **On Toxicant-Induced Succession in Periphyton Communities: Effects of Single Chemicals and Chemical Mixtures**

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Akademisk avhandling för filosofie doktorexamen i miljövetenskap med inriktning mot fysiologisk botanik, som enligt beslut i lärarförslagsnämnden i biologi kommer att offentligas försvaras fredagen den 20e mars 2009, kl. 10.00 i föreläsningssalen, Institutionen för Växt- och Miljövetenskaper, Carl Skottbergs gata 22B, Göteborg.

Examinator: Prof. Anna-Stina Sandelius

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**Abstract:** This thesis deals with the effects of single chemicals and chemical mixtures on biological communities. Ecosystems are exposed to complex mixtures of chemicals, and because combination effects can occur, it is necessary to assess the joint environmental risk from co-occurring contaminants. Two concepts are available to predict joint toxicity if the concentrations and toxicities of the single chemicals are known. Concentration Addition (CA) is believed to apply for mixtures of similarly acting chemicals, whereas the opposite concept of Independent Action (IA) was developed for dissimilarly acting chemicals. Most validation efforts of CA and IA have been performed under relatively simple biological conditions using single species tests. However, because the aim is to predict the risks of mixtures to complex and variable systems in nature, it is necessary to test the applicability of CA and IA on at least a community-level of biological complexity. Natural communities contain a tremendous biological diversity and thus a multitude of potential targets susceptible to toxicant exposure. In addition, species interact and a reduced ecological fitness of one species can thereby indirectly affect other species. This has the consequence that similarly acting chemicals on a biochemical/physiological level might be dissimilarly acting in a multispecies community, and ecological interactions alone are out of scope of both CA and IA. Natural marine periphyton communities were sampled from the environment on artificial substrata and incubated over a period of ecological succession together with single chemicals and chemical mixtures. Patterns and magnitude of toxicant-induced succession in the microalgal and cyanobacterial component of the biofilm were assessed by species counts using microscopy and/or chemotaxonomic analysis of pigment profiles. The resulting toxicant-induced changes were then used to assess similarity or dissimilarity between the chemicals in their ecological mode of action, and the precision with which CA and IA could predict effects from reference mixtures of similarly and dissimilarly acting chemicals. On a biochemical level similarly acting photosystem II-inhibiting herbicides acted similarly also in the periphyton communities, and CA provided an accurate prediction for their joint toxicity. CA was equally powerful for a mixture of non-congeneric PSII inhibitors assumed beforehand to have a less similar ecological mode of action. IA was slightly better than CA for predicting the joint effect from a mixture of dissimilarly acting pharmaceuticals and personal-care products. At a mixture concentration that caused a 50% reduction in biomass, IA deviated from the observed effect only by a factor of 1.1. However, lower concentrations of this mixture caused a stimulation of biomass, a phenomenon which is outside the scope of both CA and IA. The mixture studies further strengthens the evidence that joint toxicity needs to be considered in risk assessments even if the chemicals do not cause a discernible effect on their own. Furthermore, the findings show that both CA and IA are suitable to predict risks from co-occurring pollutants on community structure. The human pharmaceutical clotrimazole is regarded as a priority pollutant to the marine environment and its ecotoxicity was specifically evaluated. Clotrimazole is a fungicide that inhibits sterol synthesis at 14 $\alpha$ -demethylase (14DM). Periphyton communities exposed to 50 pmol/L clotrimazole had a reduced 14DM functioning, and 500 pmol/L clotrimazole had profound effects on sterol biosynthesis which coincided with a decrease in biomass. Because up to 100 pmol/L clotrimazole has been detected in the marine environment, this indicates that the current use of clotrimazole is associated with a high environmental risk.