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of Periphyton Communities Using
HPLC Pigment Analysis**

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Ecotoxicological Investigations of Periphyton Communities Using HPLC Pigment Analysis

The main objective of this thesis has been to prove that HPLC pigment analysis (high performance liquid chromatography) can be a useful method for monitoring community shifts in periphytic algae in pesticide studies. The HPLC pigment analysis uses the different chemotaxonomic pigments in the periphyton to identify the corresponding algal class while their three-dimensional growth form as community attached to substrate often resists separation for microscopic analysis.

The effect of two different photosystem II inhibiting herbicides, terbuthylazine and isoproturon, on periphyton was investigated in two outdoor mesocosms studies. A regression design with six control enclosures and seven treatments was used. Periphyton was sampled on glass slides and the results of the HPLC pigment analysis from the samples were verified with microscopic algal counting. The HPLC method according to Wright *et al.* (1991) was adjusted for the use with periphyton and multiple linear regression was chosen for calculation of class composition from marker pigment amounts. With HPLC pigment analysis and microscopy, the same pattern in community shifts could be observed.

Bacillariophyceae and Chlorophyceae, especially the filamentous green algae, were the most sensitive algal classes in both herbicide studies. One Chlorophyceae, *Chlamydomonas globosa*, was tolerant to both herbicides and even increased in numbers after treatment. Cyanobacteria were less sensitive, as well as Chrysophyceae, which also developed tolerance to TBA in middle concentrations (100 and 200 µg/l TBA). Cryptophyceae had increased percentages especially in the highest treatment in both studies (400 µg/l).

Additionally, the ecotoxicological values like NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration) for the effects of the whole periphyton as well as for different algal classes were comparable, and even the Principal Response Curves showed a comparable reaction of the community with both methods.

The influence of sublethal herbicide doses on cell pigment content was observed in the mesocosm studies and confirmed by single species tests.

Physical and chemical parameters as well as phytoplankton, and additionally in the TBA study macroinvertebrates and bacteria, were investigated to complete the information of changes in functional and structural parameters in the systems. The two physical parameters, oxygen and pH-value, reacted more sensitive to the herbicides in both studies than the periphyton. Investigations of the periphyton in comparison to phytoplankton showed that class compositions were different in the two communities as well as classes were differently affected by the herbicides suggesting that phytoplankton investigations alone are not sufficient to characterize all effects on primary producers.

All these results prove that the HPLC pigment analysis can provide a powerful tool to investigate periphyton as well as phytoplankton in ecotoxicological studies. Microscopic analysis is recommended to be used additionally but can be reduced to representative samples.