ECOTOXIC EFFECTS OF HYDROXAMIC ACID ALLELOCHEMICALS AND SELECTED DEGRADATION PRODUCTS ON AQUATIC ORGANISMS

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INTRODUCTION

Allelochemicals are exuded by many plant roots under natural conditions. Those substances will cause an effect to other organisms living in the soil nearby and will be degraded afterwards. The type of effect could be manifold, but often it will suppress pathogens or competitors of the producer. Nevertheless, neither accumulation nor a drift over far distances is to await (Korte, 1992). If such allelochemicals (or any similar substances) are identified, isolated or produced and applied in bigger amounts as a natural pest control, a drift or transport into so called 'non target' environments may occur (Crossland et.al., 1982). By application on top of the soil, in difference to the natural appearance in the rhizosphere, other organisms may be affected. However, if allelochemicals are intended to be used as pesticide replacement, ecotoxicity data have to be generated for quality assurance and consumer protection reasons. It was part of the EU research project FATEALLCHEM (details see below) to characterise allelochemicals for their effects against target as well as against non target organisms. Results of the project part dealing with ecotoxicity of allelochemicals against aquatic organisms are presented and discussed in the lecture.

MATERIAL AND METHODS

The investigated 10 substances were three former identified parent hydroxamic acid allelochemicals, namely Benzoxazolin-2(3H)-one (BOA), 6-Methoxy-benzoxazolin-2(3H)-one (MBOA), 2,4-Dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one (DIMBOA). Two identified biodegradation products deriving from MBOA and five biodegradation products deriving from BOA were chosen later. BOA was bought from Fluka (Art.No.: 12825) and the rest of the substances were obtained from F. Macias, Universidad de Cadiz (UCA). The degradation studies as base for choosing metabolites had been performed by Fomsgaard et.al. 2003.

Prior to the use in the biotests, the substances had been dissolved in DMSO. The whole dilution series necessary for generating a dose-response relation had been made in the solvent as well. The concentration in the DMSO was 1000 times higher than the final start concentration in the biotest, so that exactly 1 µl DMSO-solution per ml biotest was pipetted and therefore the solvent addition to each biotest vessel had been exactly the same 0,1%.

The marine bacteria *Vibrio fischeri* was obtained together with the ready to use testkit Lumis Tox from Dr. Lange, Germany, Art.No.: LCK 486, which is in accordance to EN-ISO 11348-3.

The freshwater green algae *Selenastrum capricornutum* was obtained from ATCC, USA, Art. No.: 22662. The soil green algae *Chlorella pyrenoidosa* was isolated from a standard soil of the University of Applied Sciences, Vienna, (Fritz, 1999) and characterised at the IFA-Tulln according to Bellinger 1992. Both algae were used in an aquatic growth inhibition biotest according to the OECD guideline No. 201.

The freshwater micro-crustaceae *Daphnia magna* STRAUSS was obtained in 1997 from the Higher School for Chemistry, Vienna, and kept in continuous culture at the IFA-Tulln. The organisms were used in the aquatic immobilisation biotest according to the OECD guideline No. 202.

NOEC-values had been read directly out of the inhibition results and are given as the highest test concentration not showing any effect significantly different from the blind. EC50-values were obtained by non-linear curve fit to the Weibull function and a calculation using the function indices (OECD, 2003).

RESULTS

For the purpose of this abstract the results are summarised as EC_{50}-values. The values have to be seen as preliminary, since not all statistical analysis (especially fugitives) had not been applied and some necessary repetitions have not been done so far.

Table 1. Preliminary EC_{50} values as calculated from the results of the aquatic biotests for allelochemicals and their degradation products, using the 3-parameter Weibull equation. Symbol key: Da = *Daphnia magna*, Chl = *Chlorella pyrenoidosa*, Sel = *Selenastrum capricornutum*, Vib = *Vibrio fischeri* (LumisTox)

	EC_{50} [µmol/l]			
Substance	**Da (48h)**	**Chl (72h)**	**Sel (72h)**	**Vib (0,5h)**
DIMBOA	>9,5	>47	>47	44
MBOA	>12	>61	>61	1,2
AMPO	>41	8,8	0,22	68
AAMPO	>7	>7	>7	>7
BOA	>15	>74	>74	>15
AP	3,5	>92	3,0	>92
HPAA	>13	>13	>13	>13
HPMA	>10	>51	>51	>51
APO	1,5	1,3	0,73	9,4
AAPO	8,6	27	1,7	11

DISCUSSION

The ecotoxicity of the three parent substances BOA, MBOA and DIMBOA against four aquatic test organisms was generally very low (the EC_{50} was not reached, even with the highest tested concentration). BOA and DIMBOA did show uncommon dose-response-relations against green algae which may be based on their comparably rapid biodegradation and a much higher ecotoxicity of the metabolites.

Based on that chronologically first results a set of possible degradation metabolites were selected and tested subsequently. Some of them did show dramatically higher ecotoxicity than the parent allelochemicals. The metabolites with the highest toxicity did have structural similarities, those are first of all the phenoxazinones: APO (3-Aminophenoxazin-2-one), AAPO (3-Acetamidophenoxazin-2-one) and AMPO (2-Amino-8-methoxyphenoxazin-3-one). The toxicity was reduced in the acetylated metabolites (even in the case of the highest toxic APO when transformed into AAPO) which could be denoted as detoxifying process for the primary metabolites.

Currently there is no clear evidence about degradation during the runtime of a ecotoxicity test. Probably there was a partial degradation or other, not yet tested metabolites did occur or the

metabolites did undergo further degradation. In that sense the current discussion has to be seen as preliminary, in the upcoming month detailed answers will be searched for.

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