

Forschungsdepartment Ökologie
Fachgebiet Systematik und Ökophysiologie

Ecotoxicological Investigations of Periphyton Communities Using HPLC Pigment Analysis

Sonja Eser

Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften (Dr. rer. nat.)

genehmigten Dissertation.

Vorsitzender: Univ.-Prof. Dr. W. Höll

Prüfer der Dissertation:

1. Univ.-Prof. Dr. W. Huber
2. Univ.-Prof. Dr. A. Melzer

Die Dissertation wurde am 12.03.2001 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am 24.07.2001 angenommen.

Berichte aus der Biologie

Sonja Eser

**Ecotoxicological Investigations
of Periphyton Communities Using
HPLC Pigment Analysis**

Shaker Verlag
Aachen 2001

Die Deutsche Bibliothek - CIP-Einheitsaufnahme

Eser, Sonja:

Ecotoxicological Investigations of Periphyton Communities
Using HPLC Pigment Analysis / Sonja Eser.

Aachen : Shaker, 2001

(Berichte aus der Biologie)

Zugl.: München, Techn. Univ., Diss., 2001

ISBN 3-8265-9444-4

Copyright Shaker Verlag 2001

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior permission of the publishers.

Printed in Germany.

ISBN 3-8265-9444-4

ISSN 0945-0688

Shaker Verlag GmbH • P.O. BOX 1290 • D-52013 Aachen

Phone: 0049/2407/9596-0 • Telefax: 0049/2407/9596-9

Internet: www.shaker.de • eMail: info@shaker.de

ACKNOWLEDGEMENT

First of all I want to thank Prof. Dr. W. Huber for offering me that fascinating and worthwhile subject and his advice and support in preparing my doctoral thesis.

I also want to thank Prof. Dr. A. Melzer who kindly accepted to be the second examiner.

My thanks also go to the American Cyanamid Company, Princeton, USA, for their financial support of this work and in particular Dr. Gary Mitchell, who offered much advise and discussion in planning and reporting of the TBA study.

My special thanks go to Prof. Dr. E. Elstner, Technische Universität München, and his scientific staff for offering me the opportunity to use their HPLC system for the pigment analysis and giving me plenty of time for my extensive studies. Everybody was extremely helpful and offered assistance in every respect.

Sincere thanks to Dr. Harald Schempp, who introduced me to the work with the HPLC analysis, helped with every problem of the system (and there were many) and was always prepared to discuss or to cheer me up.

I am very grateful for the support of my colleagues, especially of Mrs. Roswitha Rümelin for helping with the analyses, Dr. Karin Neugebaur-Büchler for introducing me in algal identification, and Dipl. Biol. Herbert Grünwald for lots of discussions and support with computer problems.

Thanks to Mr. Achim Lüdecke for the entertaining help with the outdoor samplings.

Many thanks also to Dr. Uta Raeder and the scientific staff at the Limnological Research Station Iffeldorf for helping me to get started with the periphyton, helping with diatom identification, and especially for many helpful discussions.

I am very grateful for the valuable help with the HPLC method and pigment calculation programs, which I received from Dr. S. W. Wright and Dr. H. W. Higgins, CSIRO, Hobart, Australia and from Mr. H. Schmid, WWA Kempten.

I also want to thank Prof. Dr. Dr. h. c. mult. H. K. Lichtenhaller, Universität Karlsruhe, for much information about shade-type adaptation and Dr. P.J. Van den Brink, Alterra, Wageningen, Netherlands, for advice with the PRC calculations of pigments. Thanks also to Mrs. Buhr and Dr. M. Stähler, BBA, Kleinmachnow, for advise with algal cultures and for the helpful HPLC column.

Thanks also to Dr. Schierle, Hoffman-La Roche, Basel, who kindly provided carotenoid samples and to Mr. J. Alkofer, Novartis, who turned up with a bottle of Gardoprim just in time.

Many thanks to Dr. Daniela Nicastro, Dipl. Biol. Peter Ludwig, Mrs. Evelyn Rädler, Mrs. Ingrid Assmann, Mrs. Ines Baumer-Huber and again Dipl. Biol. Herbert Grünwald for proofreading the manuscript.

A very special thank you to my mother who supported my study and this thesis in every respect and a big hug to my husband who accompanied me cheerfully through all ups and downs of the work.

CONTENTS

1 INTRODUCTION	1
1.1 Ecology of periphyton.....	2
1.2 Monitoring with periphyton	5
1.3 HPLC pigment analysis	6
1.4 Objective of the presented thesis.....	8
2 MATERIAL AND METHODS	10
2.1 Location and construction of mesocosms.....	10
2.2 Introduction of enclosures and artificial substrata	11
2.3 Herbicides	12
2.3.1 TERBUTHYLAZINE	12
2.3.1.1 Characteristics	12
2.3.1.2 Treatment procedures of mesocosm with TBA	14
2.3.1.3 Method of TBA analytic	15
2.3.2 ISOPROTURON	16
2.3.2.1 Characteristics	16
2.3.2.2 Treatment procedures of mesocosm with IPU	18
2.3.2.3 Method of IPU analytic	19
2.4 Analysing of physical and chemical water parameters	21
2.4.1 WATER CHEMISTRY.....	21
2.4.2 WATER QUALITY PARAMETERS.....	22
2.5 Macroinvertebrate sampling.....	22
2.6. Periphyton sampling and preparation for HPLC.....	23
2.6.1 USE OF ARTIFICIAL SUBSTRATA FOR PERIPHYTON SAMPLING	23
2.6.2 ARTIFICIAL SUBSTRATE FOR HERBICIDE STUDIES.....	24
2.6.3 PERIPHYTON SAMPLING IN TBA STUDY	24
2.6.4 PERIPHYTON SAMPING IN IPU STUDY	25
2.6.5 MICROSCOPIC ANALYSIS OF ALGAE	25
2.7 Phytoplankton	25
2.8 Macrophyte mapping	25
2.9 Bacteria	26

2.10 Single species tests.....	26
2.10.1 SINGLE SPECIES TEST WITH TBA – GROWTH INHIBITION TEST	28
2.10.2 SINGLE SPECIES TESTS WITH IPU	28
2.11 HPLC pigment analysis	29
2.11.1 HPLC METHOD FOR SEPARATION OF PIGMENTS	30
2.11.2 STANDARD PREPARATION AND CALIBRATION OF HPLC SYSTEM	32
2.11.3 PREPARATION OF PERiphyton SAMPLES FOR HPLC ANALYSIS.....	34
2.11.4 CALCULATION OF CLASS COMPOSITION FROM PIGMENT DATA	35
2.12 Computer programs and statistic methods	36
2.12.1 T-TEST (ONE POPULATION)	37
2.12.2 LINEAR REGRESSION ANALYSIS.....	37
2.12.3 DIVERSITY INDEX	38
2.12.4 EVENNESS.....	39
2.12.5 DISTANCE COEFFICIENT.....	39
2.12.6 CLUSTER ANALYSIS.....	40
2.12.7 PRC ANALYSIS	40
3 RESULTS.....	43
3.1 Preliminary investigations	43
3.1.1 USE OF ARTIFICIAL SUBSTRATA	43
3.1.2 SELECTION OF HPLC METHOD AND PIGMENT PREPARATION TECHNIQUE	44
3.2 Terbutylazine (TBA) study	47
3.2.1 TBA CONCENTRATION AND HALF-LIFE	47
3.2.2 PHYSICAL PARAMETERS.....	48
3.2.2.1 Oxygen content.....	48
3.2.2.4 pH-value.....	51
3.2.1.2 Conductivity	53
3.2.1.3 Temperature	53
3.2.1.4 Alkalinity (HCO_3^-)	54
3.2.3 WATER CHEMISTRY PARAMETERS	56
3.2.3.1 Potassium, calcium, sodium, silicate and hardness	56
3.2.3.2 Nitrate, ammonium, phosphate and total phosphorus.....	56
3.2.4 MACROINVERTEBRATES	57
3.2.4.1 <i>Cloeon</i> sp. (Baetidae)	58

3.2.4.2 Red Chironomidae.....	59
3.2.4.3 <i>Chaoborus crystallinus</i> (Chaoboridae).....	59
3.2.4.5 Diversity, evenness and distance coefficient of the macroinvertebrate community	60
3.2.5 MACROPHYTES	62
3.2.6 FUNGI AND BACTERIA	63
3.2.7 PHYTOPLANKTON	64
3.2.8 PERIPHYTON.....	67
3.2.8.1 Microscopic analyses of periphyton	67
3.2.8.1.1 Important species	67
3.2.8.1.2 Development of different algal classes in the periphyton	74
3.2.8.1.3 Species richness.....	80
3.2.8.1.4 Total abundance	82
3.2.8.1.5 Diversity, evenness and distance coefficient (RAD) of periphyton community	82
3.2.8.2 Results from HPLC pigment analyses	85
3.2.8.2.1 Chlorophyll a amount.....	85
3.2.8.2.2 Lutein	87
3.2.8.2.3 Fucoxanthin	88
3.2.8.2.4 Alloxanthin	90
3.2.8.2.5 Zeaxanthin	91
3.2.8.3 Comparison of HPLC with microscopic results for the periphyton	93
3.2.8.3.1 Regression of marker pigments to biovolume and cell numbers of algal classes.....	93
3.2.8.3.2 Comparison of class development over time	96
3.2.8.3.2.1 Class development calculated from microscopy	96
3.2.8.3.2.2 Class development calculated from HPLC analysis.....	99
3.2.9 PRINCIPAL RESPONSE CURVES (PRC)	103
3.2.9.1 PRC of physical parameters.....	103
3.2.9.2 PRC analysis of periphyton abundance data	104
3.2.9.3 PRC of HPLC pigment data	107

3.3 Isoproturon (IPU) study.....	110
3.3.1 IPU CONCENTRATION AND HALF-LIFE	110
3.3.2 PHYSICAL PARAMETERS	111
3.3.2.1 Oxygen content.....	111
3.3.2.2 pH-value.....	114
3.3.2.3 Conductivity	116
3.3.2.4 Alkalinity.....	117
3.3.2.5 Temperature	118
3.3.3 WATER CHEMISTRY PARAMETERS	119
3.3.3.1 Potassium, calcium, sodium, silicate and hardness	119
3.3.3.2 Nitrate, ammonium, phosphate and total phosphorus.....	119
3.3.4 MACROPHYTE MAPPING.....	120
3.3.5 PHYTOPLANKTON.....	120
3.3.6 PERiphyton.....	124
3.3.6.1 Microscopic analysis of periphyton	124
3.3.6.1.1 Important species	124
3.3.6.1.2 Development of different algal classes in the periphyton	131
3.3.6.1.4 Species richness.....	136
3.3.6.1.5 Total abundance	138
3.3.6.1.6 Diversity, evenness and distance coefficient (RAD) of periphyton community	139
3.3.6.2 Result from HPLC pigment analysis	141
3.3.6.2.1 Chlorophyll a amount.....	141
3.3.6.2.2 Lutein	143
3.3.6.2.3 Chlorophyll b	145
3.3.6.2.4 Fucoxanthin	145
3.3.6.2.5 Zeaxanthin	147
3.3.6.2.6 Alloxanthin	149
3.3.6.3 Comparison of HPLC with microscopic results for the periphyton....	150
3.3.6.3.1 Regression of marker pigments to biovolume and cell numbers of algal classes.....	150
3.3.6.3.2 Comparison of class development over time.....	152
3.3.6.3.2.1 Class development calculated from microscopy	152
3.3.6.3.2.2 Class development calculated from HPLC analysis.....	154

3.3.7 PRINCIPAL RESPONSE CURVES (PRC)	158
3.3.7.1 PRC of physical parameters.....	158
3.3.7.2 PRC analysis of periphyton abundance data	159
3.3.7.3 PRC of HPLC pigment data	163
3.4 Single species tests.....	166
3.4.1 SINGLE SPECIES TESTS WITH TBA	166
3.4.2 SINGLE SPECIES TESTS WITH IPU	168
3.4.3 EFFECTS OF HERBICIDES ON PIGMENT CONTENT PER CELL.....	169
4 DISCUSSION	176
4.1 Artificial substrate and architecture of periphyton	176
4.2 HPLC method, pigment extraction and calculations.....	178
4.2.1 COMPARISON OF HPLC AND EXTRACTION METHODS	178
4.2.2 CALCULATION OF CLASS COMPOSITION FROM PIGMENT AMOUNTS	179
4.3 Terbuthylazine study	181
4.3.1 PESTICIDE ANALYTIC	181
4.3.2 PHYSICAL AND CHEMICAL PARAMETERS	181
4.3.3 MACROINVERTEBRATES	183
4.3.4 MACROPHYTES	184
4.3.5 PHYTOPLANKTON	185
4.3.6 PERIPHYTE INVESTIGATED WITH MICROSCOPY	185
4.3.7 COMPARING PERIPHYTE TO PHYTOPLANKTON COMMUNITY.....	190
4.3.8 PERIPHYTE INVESTIGATED WITH HPLC PIGMENT ANALYSIS	190
4.3.9 COMPARISON OF MICROSCOPIC AND PIGMENT DATA – TBA	192
4.3.9.1 Regression of marker pigment to biovolume and cell number.....	192
4.3.9.2 Comparison of class development.....	192
4.3.9.3 Comparison of Principal Response Curves	193
4.3.9.4 Comparison of ecotoxicological results	194
4.4 Isoproturon study	197
4.4.1 PHYSICAL AND CHEMICAL PARAMETERS AND IPU HALF-LIFE.....	197
4.4.2 MACROPHYTES	198
4.4.3 PHYTOPLANKTON.....	198
4.4.4 PERIPHYTE INVESTIGATED WITH MICROSCOPY	199

4.4.5 COMPARISON OF PERIPHYTON TO PHYTOPLANKTON COMMUNITY	201
4.4.6 PERIPHYTON INVESTIGATED WITH HPLC PIGMENT ANALYSIS	201
4.4.7 COMPARISON OF MICROSCOPIC AND PIGMENT DATA - IPU	202
4.4.7.1 Regression of marker pigments to biovolume and cell numbers	202
4.4.7.2 Comparison of class development.....	202
4.4.7.3 Comparison of Principal Response Curves.....	203
4.4.7.4 Comparison of ecotoxicological results	204
4.5 Influences of herbicides on pigment composition of cells	206
4.6 Comparison of effects from TBA and IPU treatment.....	211
4.7 Conclusions.....	212
 5 SUMMARY	 215
5.1 English.....	215
5.2 German.....	217
 6 APPENDIX: Pigment composition of algae	 219
 7 REFERENCES	 223

Figures

Fig. 1: Mesocosm with introduced enclosures at research station Grünschwaige	11
Fig. 2: Scheme of metabolic pathway for terbutylazine in plants (modified after Ciba-Geigy 1991) ...	13
Fig. 3: Pathways for the biodegradation of isoproturon (after Lehr <i>et al.</i> 1996)	17
Fig. 4: Mix of algal cultures measured with the HPLC (method after Wright <i>et al.</i> 1991).....	33
Fig. 5: Preparation of pigment extracts	34
Fig. 6: Comparison of two HPLC analysis methods for the same periphyton sample.....	46
Fig. 7: Concentration and half-life of terbutylazine in single enclosures.....	47
Fig. 8: Relative oxygen content (in %) at bottom of enclosures during TBA study.....	48
Fig. 9: Linear regression of oxygen content bottom (% of controls) for day 42	50
Fig. 10: pH-values during TBA study.....	51
Fig. 11: Conductivity ($\mu\text{S}/\text{cm}$) during TBA study	53
Fig. 12: Temperature at the top of enclosures during TBA study	54
Fig. 13: Alkalinity as HCO_3^- (mg/l) during TBA study	55
Fig. 14: Numbers of <i>Cloeon</i> sp. in different treatments during TBA study	58
Fig. 15: Numbers of red Chironomidae in the different enclosures	59
Fig. 16: <i>Chaoborus crystallinus</i> numbers in enclosures in the TBA study.....	60
Fig. 17: Shannon index (diversity) for the macroinvertebrate community in the TBA study.....	61
Fig. 18: RAD index for macroinvertebrate community in comparison to the controls (mean)	62
Fig. 19: Bacteria numbers shown as % deviation from controls (according to Lozano 1992).....	63
Fig. 20: Phytoplankton development in the controls (mean) and the highest treatment of TBA (400 $\mu\text{g}/\text{l}$)	65
Fig. 21: Chlorophyll <i>a</i> amount in the phytoplankton - TBA study.....	66
Fig. 22: <i>Achnanthes minutissima</i> (Bacillariophyceae) – cell numbers/ cm^2 in the treated enclosures (TBA study)	68
Fig. 23: <i>Oscillatoria</i> sp. (Cyanobacteria) – cell numbers/ cm^2 in the treated enclosures (TBA study) ...	70
Fig. 24: <i>Chlamydomonas globosa</i> (Chlorophyceae) – cell numbers/ cm^2 in the treated enclosures (TBA study)	71
Fig. 25: <i>Chromulina cf. nitens</i> (Chrysophyceae) – cell numbers/ cm^2 in the treated enclosures (TBA study)	73
Fig. 26: Cell number development of Bacillariophyceae over time with TBA	74
Fig. 27: Cell number development of Chlorophyceae over time with TBA	76
Fig. 28: Cell number development of Cryptophyceae over time with TBA	77
Fig. 29: Cell number development of Chrysophyceae over time with TBA	78
Fig. 30: Cell number development of cyanobacteria over time with TBA	79
Fig. 31: Species numbers during study with TBA	80
Fig. 32: Total abundance of algae cells in the periphyton – TBA study.....	82
Fig. 33: Shannon indices of periphyton community – TBA study	83
Fig. 34: Evenness of periphyton community of TBA study	84

Fig. 35: RAD indices of periphyton community in treated enclosures against controls (mean) with TBA	84
Fig. 36: Chlorophyll a amount (ng/cm ²) in the periphyton during the TBA study	85
Fig. 37: Lutein amount (ng/cm ²) in periphyton during TBA study.....	87
Fig. 38: Fucoxanthin amount (ng/cm ²) in periphyton TBA study.....	89
Fig. 39: Alloxanthin amount (ng/cm ²) in enclosures TBA study	91
Fig. 40: Zeaxanthin amount (ng/cm ²) in enclosures TBA study	92
Fig. 41: Linear regression of fucoxanthin amount to Bacillariophyceae biovolume – TBA study.....	94
Fig. 42: Linear regression for chlorophyll a to total biovolume – TBA study.....	95
Fig. 43: Class development of periphyton over time in the controls (mean) and treated enclosures – microscopy (TBA study).....	98
Fig. 44: Class development of periphyton over time in controls (mean) and treated enclosures – pigment analysis (TBA study)	100
Fig. 45: Linear regression of chlorophyll a calculated and chlorophyll a measured (TBA study).....	102
Fig. 46: Principal Response Curve of physical parameters (DO-pH-alkalinity-conductivity syndrome) – TBA study	103
Fig. 47: Principal Response Curve for abundance data of the periphyton (TBA study)	106
Fig. 48: PRC of periphyton abundance data combined to classes (TBA study).....	107
Fig. 49: Principal Response Curve of periphyton pigment data (TBA study)	108
Fig. 50: Half-life of isoproturon in single enclosures	110
Fig. 51: Relative oxygen content (%) at the bottom of enclosures during IPU study	112
Fig. 52: Linear regression of relative oxygen content on day 14 of IPU study	113
Fig. 53: pH-values during IPU study.....	114
Fig. 54: Conductivity (μ S/cm) during IPU study	116
Fig. 55: Alkalinity (HCO_3^-) development during IPU study	117
Fig. 56: Temperature at the top of enclosures during IPU study	118
Fig. 57: Phytoplankton development in the controls (mean) and the highest treatment (400 $\mu\text{g/l}$) of IPU study	121
Fig. 58: Chlorophyll a amount in phytoplankton treated with IPU	123
Fig. 59: <i>Chlamydomonas globosa</i> (Chlorophyceae) - cell numbers/cm ² in the treated enclosures (IPU study)	125
Fig. 60: <i>Geminella interrupta</i> (Chlorophyceae) - cell numbers/cm ² in the treated enclosures (IPU study)	126
Fig. 61: <i>Mougeotia</i> sp. (Chlorophyceae) - cell numbers/cm ² in the treated enclosures (IPU study) ...	127
Fig. 62: <i>Achnanthes minutissima</i> (Bacillariophyceae) - cell numbers/cm ² in the treated enclosures (IPU study)	128
Fig. 63: <i>Oscillatoria limnetica</i> (cyanobacteria) - cell numbers/cm ² in the treated enclosures (IPU study)	129
Fig. 64: <i>Chromulina</i> cf. <i>spongifera</i> (Chrysophyceae) - cell numbers/cm ² in the treated enclosures (IPU study)	130
Fig. 65: Cell number development of Chlorophyceae over time with IPU	131

Fig. 66: Cell number development of Bacillariophyceae over time with IPU	133
Fig. 67: Cell number development in cyanobacteria over time with IPU	134
Fig. 68: Cell number development of Chrysophyceae over time with IPU	135
Fig. 69: Development of species number during the IPU study.....	136
Fig. 70: Development of total abundance of periphytic algae during IPU study.....	138
Fig. 71: Shannon indices for periphyton community with IPU	140
Fig. 72: RAD indices for the periphyton community in treated enclosures against the controls (mean) with IPU	140
Fig. 73: Chlorophyll a amount (ng/cm ²) in the periphyton in IPU study	141
Fig. 74: Lutein amount (ng/cm ²) in periphyton during IPU study	144
Fig. 75: Chlorophyll b amount (ng/cm ²) in periphyton during IPU study	145
Fig. 76: Fucoxanthin amount (ng/cm ²) in periphyton during IPU study	146
Fig. 77: Zeaxanthin amount (ng/cm ²) in periphyton during IPU study	148
Fig. 78: Alloxanthin amount (ng/cm ²) in periphyton during IPU study	149
Fig. 79: Class development of periphyton over time in the controls (mean) and the treated enclosures – microscopy (IPU study)	153
Fig. 80: Class development of periphyton over time in controls (mean) and treated enclosures – HPLC analysis (IPU study)	155
Fig. 81 Comparison of calculated and measured chlorophyll a (IPU study).....	157
Fig. 82: Principal Response Curve of physical parameters (DO-pH-alkalinity-conductivity syndrome) of IPU study.....	158
Fig. 83: Inverse regression analysis according to Liber et al (1992) for periphyton abundance data on day 70 (IPU study)	161
Fig. 84: Principal Response curve with abundance data of periphyton (IPU study).....	162
Fig. 85: Principal response Curve of periphyton pigment data (IPU study).....	163
Fig. 86: Linear regressions of C _{dt} values from pigment PRC on day 70 (IPU study).....	165
Fig. 87 Single species test with <i>Nitzschia palea</i> and TBA - inhibition of growth.....	167
Fig. 88: Single species test with <i>Ankistrodesmus spiralis</i> and TBA - inhibition of growth.....	167
Fig. 89: Single species test with <i>Achnanthes minutissima</i> and IPU - inhibition of growth.....	168
Fig. 90: Inhibition of growth: single species test with <i>Ankistrodesmus spiralis</i> and IPU.....	169
Fig. 91: Pigment content (pg/cell) in <i>Achnanthes minutissima</i> treated with IPU	170
Fig. 92: Pigment content (pg/cell) in <i>Ankistrodesmus spiralis</i> treated with IPU.....	171
Fig. 93: Pigment content (pg/cell) in <i>Nitzschia palea</i> after treatment with TBA.....	172
Fig. 94: Pigment ratio Chl a/fucoxanthin in <i>Achnanthes minutissima</i> with IPU	173
Fig. 95: Changes in pigment ratio (Chl a/fucoxanthin) in <i>Nitzschia palea</i> with increasing TBA concentration	173
Fig. 96: Pigment ratio Chl a/lutein in <i>Ankistrodesmus spiralis</i> with increasing IPU concentration.....	174
Fig. 97: Chl a and cell numbers in comparison measured in <i>Achnanthes minutissima</i> with increasing IPU concentration	175
Fig. 98: Observed (LOEC) and calculated (NOEC) effect concentrations in the TBA study in comparison. In addition, the EC ₁₀ and EC ₅₀ values are shown from single species tests.	195

Fig. 99: Observed (LOEC) and calculated (NOEC) effect concentrations in the IPU study in comparison. In addition, the EC ₁₀ and EC ₅₀ values are shown from single species tests.....	205
Fig. 100: Factors influencing the chlorophyll a content in algae	207

Tables

Table 1: Physical and chemical characteristics of TBA (Perkow 1983/1988).....	12
Table 2: Chromatographic conditions for analyzing terbutylazine	15
Table 3: Physical and chemical properties of IPU (Industrieverband Agrar 1990).....	16
Table 4: Chromatographic conditions for analyzing isoproturon.....	19
Table 5: Methods of water analysis.....	21
Table 6: Settings for single species tests.....	27
Table 7: Chromatographic conditions for pigment analysis (modified after Wright <i>et al.</i> 1991)	31
Table 8: Parameters used in linear regression analysis	38
Table 9: Significant deviation of relative oxygen content from controls of TBA treatments and sampling days	49
Table 10: Linear regression of oxygen content (%) at the bottom of enclosures and calculated NOEC	50
Table 11: Significant deviation of pH-value from controls of TBA treatments and sampling days	52
Table 12: Linear regression of pH of enclosures and calculated NOEC	52
Table 13: Significant deviation of <i>Achnanthes minutissima</i> cell numbers from controls of TBA treatments and sampling days	69
Table 14: Significant deviation of <i>Chromulina cf. nitens</i> cell numbers from controls of TBA treatments and sampling days	73
Table 15: Significant deviation of Bacillariophyceae cell numbers from controls of TBA treatments and sampling days	75
Table 16: Significant deviation of Chlorophyceae cell numbers from controls of TBA treatments and sampling days	76
Table 17: Significant deviation of Chrysophyceae cell numbers from controls of TBA treatments and sampling days	79
Table 18: Significant deviation of species numbers from controls of TBA treatments and sampling days	81
Table 19: Linear regression of species richness and calculated NOEC	81
Table 20: Linear regression of Chl a and calculated NOEC	86
Table 21: Significant deviation of lutein from controls of TBA treatments and sampling days	88
Table 22: Significant deviation of fucoxanthin from controls of TBA treatments and sampling days ...	90
Table 23: Regression coefficients of pigment amount (ng/cm^2) to algal biovolume ($\mu\text{m}^3/\text{cm}^2$).....	93
Table 24: Regression coefficients of pigment amount (ng/cm^2) to cell numbers of algal classes (numbers / cm^2).....	93
Table 25: Linear regression for C_{dt} values of PRC abundance data with classes combined and TBA	105
Table 26: Linear regression of pigment data was calculated.....	109
Table 27: Significant deviation of relative oxygen content from controls of IPU treatments and sampling days	111

Table 28: Linear regression analysis for oxygen content bottom (as % of control) and calculated NOEC	113
Table 29: Significant deviation of pH from controls of IPU treatments and sampling days	115
Table 30: Linear regression of pH (as % of controls) and calculated NOEC	115
Table 31: Significant deviation of alkalinity from controls of IPU treatments and sampling days	118
Table 32: Significant deviation of <i>Chlamydomonas globosa</i> from controls of IPU treatments and sampling days	124
Table 33: Significant deviation of <i>Achnanthes minutissima</i> from controls of IPU treatments and sampling days	127
Table 34: Significant deviation of Chlorophyceae from controls of IPU treatments and sampling days	132
Table 35: Significant deviation of cyanobacteria from controls of IPU treatments and sampling days	134
Table 36: Significant deviation of species richness from controls of IPU treatments and sampling days	137
Table 37: Linear regression analysis for species numbers (as % of control) and calculated NOEC ..	137
Table 38: Linear regression of total abundance (as % of control) and calculated NOEC	139
Table 39: Significant deviation of chlorophyll a from controls of IPU treatments and sampling days ..	142
Table 40: Linear regression analysis of chlorophyll a (% of controls) and calculated NOEC	143
Table 41: Significant deviation of fucoxanthin from controls of IPU treatments and sampling days ..	146
Table 42: Linear regression analysis for fucoxanthin (% deviation from controls) and calculated NOEC	147
Table 43: Linear regression analysis of zeaxanthin and calculated NOEC	148
Table 44: Regression coefficients of pigment amount (ng/cm^2) to algal biovolume ($\mu\text{m}^3/\text{cm}^2$)	151
Table 45: Regression coefficients of pigment amount (ng/cm^2) to cell numbers of algal classes (numbers / cm^2)	151
Table 46: Conversion factors used for calculation of class composition	154
Table 47: Linear regression of C_{dt} values for physical data from PRC	159
Table 48: Linear regression of C_{dt} values for abundance data from PRC	161
Table 49: Linear regression of C_{dt} values for pigment data from PRC	164
Table 50: Measured EC_{50} values of single species tests with TBA	166
Table 51: Measured EC_{50} and EC_{10} values of single species tests with IPU	168
Table 52: Pigment contents (pg/cell) in <i>Ankistrodesmus spiralis</i> with IPU	171
Table 53: Changes in pigment ratios (mean) of species tested.....	174
Table 54: Literatur data of pigment ratios used.....	180
Table 55: Pigment composition of Bacillariophyceae	219
Table 56: Pigment composition of Chrysophyceae.....	220
Table 57: Pigment composition of Cyanobacteria.....	221
Table 58: Pigment composition of Chlorophyceae.....	221
Table 59: Pigment composition of Cryptophyceae.....	222
Table 60: Pigment composition of Dinophyceae.....	222

ABBREVIATIONS

a.i.	active ingredient
Chl a	chlorophyll a
DO	dissolved oxygen
EC ₅₀	effective concentration (50%)
EC ₁₀	effective concentration (10%)
Fig.	Figure
HPLC	High performance liquid chromatography
IPR	ion-pairing reagent
IPU	Isoproturon
LOEC	lowest observed effect concentration
NOEC	no observed effect concentration
PSII	photosystem II
PRC	Principal Response Curve
p-value	probability
r	correlation coefficient
RAD	Relative Absolute Distance
RPC	Reversed phase chromatography
rpm	round per minute
sign.	Significant
TBA	Terbutylazine
X ₀ , X _L , X _U	NOEC calculated as crossing point with control mean, lower and upper standard deviation