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Pulse Effects of Herbicides on Periphyton in Streams and Recovery

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Content

Sammenfatning og konklusioner

Inden for de sidste 10 år er det blevet bevist, at pesticider ofte forekommer i målelige koncentrationer i danske åer og vandløb. Der har især været fokus på insekticidernes mulige effekter, da mange insekter og krebsdyr er meget følsomme over for denne stofgruppe. Insekticidernes effekter i åer og vandløb er synlig, da resultatet ofte er en markant nedgang i populationen af insekter og krebsdyr. De fleste overvågningsprogrammer til åer og vandløb medtager insekter og krebsdyr, hvilket gør det muligt at spore pesticidernes effekter på disse organismer. Der er observeret både flere krebsdyr og flere insekter, som driver med strømmen eller er døde, i åer og vandløb, som har været udsat for lave koncentrationer af insekticider. Herbicidernes effekter på mikroalger i åer og vandløb er ofte mindre synlig og således endnu sværere at detektere. Dertil kommer, at mikroalger kun yderst sjældent er medtaget i overvågningsprogrammerne. Det er desuden svært at forudsige herbicidernes effekter på perifyton i åer og vandløb ud fra de traditionelle toksicitetstest med enkeltarter, da disse test ikke medtager påvirkninger, som forekommer på samfundsniveau, f.eks. forskydning i artssammensætning, heterotrof nedbrydning af pesticider, pesticiders fastklæben til overflader, osv. Perifyton er fæstnet til overflader i en polysaccharid matrix sammen med heterotrofe mikrober, svampe og dødt organisk materiale, hvilket kan virke som en effektiv barriere, der forhindrer en eksponering. Der mangler generelt publicerede data for herbicidernes effekter på mikroalger knytter til overflader (perifyton).

I nærværende projekt har vi undersøgt fire forskellige herbiciders effekter på naturlige samfund af perifyton fra åer og vandløb. De udvalgte herbicider var metribuzin, hexazinon, isoproturon og pendimetalin. Der blev undersøgt effekter på fotosyntetiske aktivitet og artssammensætning.

I nærværende projekt påvirkede isoproturon, hexazinon og metribuzin perifyton ved lave koncentrationer, og ved markant lavere koncentrationer end de effektkoncentrationer, som er offentliggjort for standard vækstest med enkeltarter. Lave koncentrationer af hexazinon stimulerede fotosyntesen, mens pendimetalin ikke påvirkede perifytonets fotosyntetiske aktivitet i nærværende undersøgelse. Et signifikant resultat af dette projekt er, at selv en korttidseksponering for metribuzin påvirkede artssammensætningen i perifyton samfund.

Summary and conclusions

During the last decade, it has been documented that pesticides frequently occur in measurable concentrations in Danish streams. Especially the possibly effects of insecticides have been in focus, since many invertebrates are very sensitive to insecticides. The effects of the insecticides are often visible, since they result in a reduction of the invertebrate population during runoff events. In monitoring programs of streams invertebrates are mostly included, which leaves it possible to detect effects by pesticides on crustaceans and insects. Increased drift and mortality of crustaceans and insects have been found in stream exposed to low concentrations of insecticides. Effects of herbicides on the microalgae in streams are less obvious and therefore even more difficult to document. In addition microalgae are only on rare occasions included in monitoring programs. Furthermore, effects of herbicides on periphyton in streams are difficult to predict from the traditional single-species toxicological tests, since these tests do not include influences, which occur on community level, for instance displacements in species composition, heterotrophic degradation of the pesticides, adhering of pesticides to surfaces, etc. The periphyton is attached to surfaces in a polysaccharide matrix along with heterotrophic microbes, fungi and detritus, which may serve as an effective barrier preventing the transfer of a contaminant. Generally, published data on effects of herbicides on periphyton communities are lacking.

In the present study, effects of four different herbicides on natural communities of periphyton from streams were investigated. The selected herbicides were metribuzin, hexazinone, isoproturon and pendimethalin. Effects on photosynthetic activity and composition of the periphyton communities were investigated.

In the present study, isoproturon, hexazinone and metribuzin affected the periphyton at low concentration, and at distinctly lower concentration than the effect concentrations published for standard single-species growth-test. Low concentrations of hexazinone stimulated the photosynthesis, while pendimethalin did not affect the photosynthetic activity of the periphyton in the present investigation. A significant result of this study was that even a short-term exposure to metribuzin affected the composition of the periphyton community.

1 Introduction

During the last decade it has been documented that pesticides frequently occur in measurable concentrations in Danish streams (County of Fyn, 1999; County of Aarhus, 1999; NERI, in prep). A significant transport of pesticides from agricultural areas to streams has also been documented throughout Europe and North America (Kreuger, 1999; Baker & Richard, 1989; Lundbergh et al. 1995). Pesticides are transported to the streams by drift, drainage and runoff, and the highest concentrations of pesticides have been found during the spraying season and during periods of high precipitation (Kreuger, 1998; County of Fyn, 1988, Liess & Schulz; 1999). Frequency and concentration of pesticides in stream water can be very variable. In Danish streams pesticides have occurred in 7-60% of the samples analysed and the concentrations have ranged between 0.001-3 μgl^{-1} (NERI, in prep). When sampling for pesticides in stream water in an agricultural area in southern Sweden 1990-1996, selected herbicides were found in 73% of the water samples with concentrations up to 45 μgl^{-1} (Krueger, 1998).

Especially the possibly effects of insecticides have been in focus, since many invertebrates are very sensitive to insecticides. While the effects of insecticides on macroinvertebrates in the streams have been documented repeatedly (e.g. Pusey et al. 1994, Liess and Schultz 1999, Sibley et al. 1991, Aanes and Bække 1994), the effects of herbicides on the autothrophic organisms in streams are less obvious and therefore even more difficult to establish. Lotic environments are dynamic systems, highly influenced by seasonal changes which affects the influence of the herbicide on periphyton (Guasch et al. 1997). Furthermore, effects of herbicides on periphyton in streams are difficult to predict from the traditional single-species toxicological tests, since these tests do not include influences which occur on community level, for instance displacements in species composition, heterotrophic degradation of the pesticides, adhering of pesticides to surfaces, etc. The periphyton is attached to surfaces in a polysaccharide matrix along with heterotrophic microbes, fungi and detritus, which may serve as an effective barrier preventing the transfer of a contaminant (Wang et al. 1999). Hence it is important to include this complex matrix in the experiments, as well as approaching natural physical condition in experimental test designs, when investigating effects of contaminants on the periphyton. Furthermore, in the single-species tests the possibility of recovery is not investigated, although this is highly relevant, since algal populations are opportunistic with short generation times and might be able to recover fast.

In this project effects of selected herbicides on natural communities of periphyton from streams were investigated. The selected pesticides were metribuzin, hexazinon, isoproturon and pendimethalin. Effects on photosynthesis and species composition of the epiphytic communities were investigated. Furthermore the recovery was studied by transferring the periphyton to clean water (only for metribuzin experiment).

2 Materials and Methods

2.1 Achieving the natural populations of epiphytes

Three frames each containing 170 round glass discs with a diameter of 1 cm were positioned in the mesothrophic and uncontaminated (due to almost no agricultural activity in the catchment area) stream Esrum Mølleå, located in the northern part of Sjælland, Denmark, in September 1999 and May 2000. After 2-3 weeks the glass discs were visibly coloured by colonising epiphytes growing on the glass surfaces and they were transported to the laboratory nearby. The glass discs were gently transferred to a white tray containing stream water and the discs were sorted with regard to density of attached epiphytes. Only glass discs, which were uniformly covered with epiphytes, were used in the experiments. Each disc selected was transferred to a glass vial containing 10 ml filtered stream water added 100 µM $NaNO_3$, 16 µM Na_2HPO_4, and 50 µM $Na_2SiO_3 \cdot 5H_2O$, to ensure that nutrients were not limiting for the growth of the periphyton in the experiments.

2.2 Exposure to herbicides

In September 1999, the glass discs with epiphytes were exposed to pendimethalin, isoproturon, metribuzin, or hexazinone for 24 hours at concentrations 0, 0.4, 2, 10, and 50 μgl^{-1} in triplicates. For isoproturon and metribuzin, additional concentrations of 250 and 1250 μgl^{-1} were included in the experiments. Furthermore, for studying the effects of short-term exposure to pesticides, periphyton was also exposed to hexazinone, isoproturon and pendimethalin in the concentrations 0, 0.4, 2, 10, and 50 μgl^{-1} in triplicates for 1 hour. The glass vials were incubated at ***in situ*** temperature (16 °C) and light intensity (250 µE $m^{-2}s^{-1}$) in an incubator in the laboratory. The effect of the pesticides on the periphyton was determined by measuring the photosynthetic activity by adding 1µCi ^{14}C (The International Agency for ^{14}C Determination, Denmark) to the vials by the end of the exposure period and incubating the vials for one hour as described above. Adding acetic acid until pH 2 stopped the incubations. The water was evaporated by drying the samples at 60 °C. The release of incorporated ^{14}C-carbon was enhanced by addition of 1 ml concentrated dimetylsulfoxide. After half an hour 10 ml scintillation cocktail (UltimaGold, Packard) was added. The activity, as disintegration per minute (dpm), was calculated from the counts per minutes (cpm) data using an external standard technique and appropriate correction factors. The abiotic carbon fixation in all experiment <0.5% was estimated as the fixation of ^{14}C in samples killed by addition of formalin to a final concentration to 1%.

2.2.1 Effect of varying exposure time

In May 2000 the effect of a varying exposure time (1- 48 h) was studied at different concentrations (0, 0.4, 2, 10, and 50 μgl^{-1}) of metribuzin. 270 vials each containing one glass disc with natural community of periphyton were prepared for the experiment. The periphyton were incubated with metribuzin in the different concentrations, and after 1, 2, 6, 12, 24, and 48 hours, the photosynthetic activity was determined in triplicates as described above. In

order to determine the effect of metribuzin on the group composition of periphyton, 3 vials from each concentration were sampled from the experiment after 2, 24 and 48 hours. The 3 glass discs with periphyton from the same concentration were wrapped in a GF/F filter, immediately frozen in liquid nitrogen, and analysed within two months by HPLC as described below.

2.2.2 Recovery experiment

Vials were sampled 1, 2, 6, 12, 24, and 48 hours after the addition of Metribuzin for the recovery experiment. The Metribuzin containing stream water was decanted from each vial and immediately replaced by fresh filtered stream water with added nutrients. After 48 hours in fresh water the primary production was determined in triplicates as described above. Corresponding recovery of the group composition of the epiphytic communities exposed 2, 24, or 48 hours, was investigated after 48 hours in herbicide-free water.

2.3 Calculation of effect concentrations

No effect concentration (NEC) and effect concentrations (EC_{50}) were calculated for the photosynthetic activity measurements using log-linear interpolation as described in Petersen & Gustavson (1998). In some instances NEC could not be determined. Instead the lowest observed effect concentration (LOEC) was determined.

2.4 Pigment analyses

For HPLC analyses of the pigment composition the filter package with the 3 glass discs were thawed and placed in 6 ml 90 % acetone, sonicated on ice for 10 minutes and extracted for 24 hours at 4 °C. The filter and cell debris were filtered from the extract using disposable syringes and 0.2 µm Teflon syringe filters. 1ml extract and 0.3 ml water was transferred to HPLC vials and the vials were placed in the cooling rack of the HPLC. The samples were injected into a Shimadzu LC-10A HPLC system according to the method described by Wright et al. (1991), although the linear gradient was modified slightly: 0 min: 100 % A, 2 min: 100 % B, 2.6 min: 90 % B/10 % C, 13.6 min: 65 % B/35 % C, 20 min: 31 % B/69 % C, 28 min: 100 % B, 31 min: 100 % A. The HPLC system was calibrated with pigment standards from The International Agency for ^{14}C Determination, DHI – Water and Environment, Denmark. Peak identities were routinely confirmed by diode array.

3 Results

3.1 Effects of herbicides on the photosynthesis

Isoproturon, metribuzin and hexazinone had all distinct effects on the photosynthetic activity, which was reduced at increasing concentrations, while pendimethalin did not show any effects (Fig. 1). Short-term and long-term exposure affected the algae community differently: exposure to isoproturon for 1 hour had no effect at the lowest concentration, but after 24 h of exposure the photosynthesis was reduced even at the lowest concentration used, i.e. 0.4 µg l^{-1}.

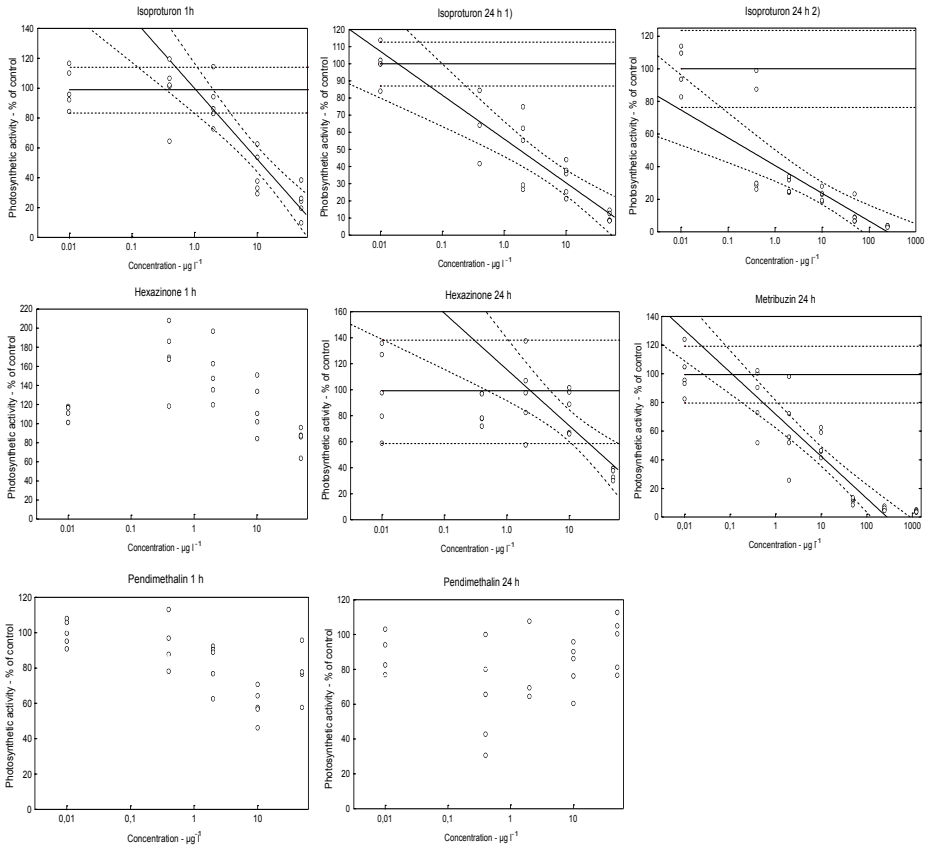

Figure 1. Effect on the photosynthetic activity of epiphytic algae when exposed to 4 different herbicides at different concentrations for 1 and 24 hours.

NEC was determined to 1.00 µgl^{-1} for the periphyton in short-term exposure to isoproturon, while NEC was 0.019 and <0.01µgl^{-1}, after 24 hours exposure to isoproturon (Table 1). The calculated effect concentration EC_{50} of isoproturon was higher in short-term exposure (11.3 µgl^{-1}) than in the two long-term exposure experiments (1.74 and 0.53 µgl^{-1}, respectively) (Table 1).

The response to hexazinone during short-term exposure stimulated the photosynthesis at the three lowest concentrations (0.4, 2, and 10 μgl^{-1}, Fig .1), and NEC and EC_{50} could not be determined. This effect was not found in long-term exposure (Fig. 1), where NEC of hexazinone was 2.29 μgl^{-1}, and EC_{50} was relatively high, i.e. almost 33 μgl^{-1} (Table 1). Metribuzin was highly toxic to the periphyton at low concentrations; NEC was 0.11 μgl^{-1} and EC_{50} was 5.57 μgl^{-1} after long-term exposure (Table 1, Fig. 1).

Table 1. No Effect Concentrations (NEC) and Effect Concentrations (EC_{50}) for the herbicides investigated during 1 and 24 h of exposure.* Stimulating effect.

	NEC	EC_{50}
	$\mu g\ l^{-1}$	
Isoproturon 1 h	1.00	11.28
Isoproturon 24 h 1)	0.019	1.74
Isoproturon 24 h 2)	<0.01	0.53
Metribuzin 24 h	0.11	5.57
Hexazinon 1 h	-*	-*
Hexazinon 24 h	2.29	32.88
Pendimethalin 1 h	No effects	No effects
Pendimethalin 24 h	No effects	No effects

The epiphytic algal community sampled in May 2000 was less sensitive to metribuzin than the community sampled in September 1999 with regard to their photosynthetic response. NEC increased from 0.11 μgl^{-1} in September to 2.35 μgl^{-1} in May and EC_{50} was 5.57 μgl^{-1} in September and 15.23 μgl^{-1} in May after 24 and 23 hours, respectively (Table 1 & 2). The metribuzin treatment had even in some instances a stimulating effect on the photosynthesis in May at the lowest concentrations (Table 1 & Fig 2). EC_{50} varied during the exposure period between 9.57 μgl^{-1} and 34.00 μgl^{-1} (Table 2) and was in average 24.54 ± 10.24 (S.D.), and there was no trend of increasing or decreasing toxicity as function of duration of exposure (Fig. 2 & Table 2).

Table 2. No Effect Concentrations (NEC) and Effect Concentrations (EC_{50}) for metribuzin during different exposure periods. * Stimulating effect.

Hours of exposure	NEC	EC_{50}
	$\mu g\ l^{-1}$	
1	-*	34.00
2	0.6	9.57
6	-*	33.16
18	-*	23.73
23	2.35	15.23
48	1.37	31.56

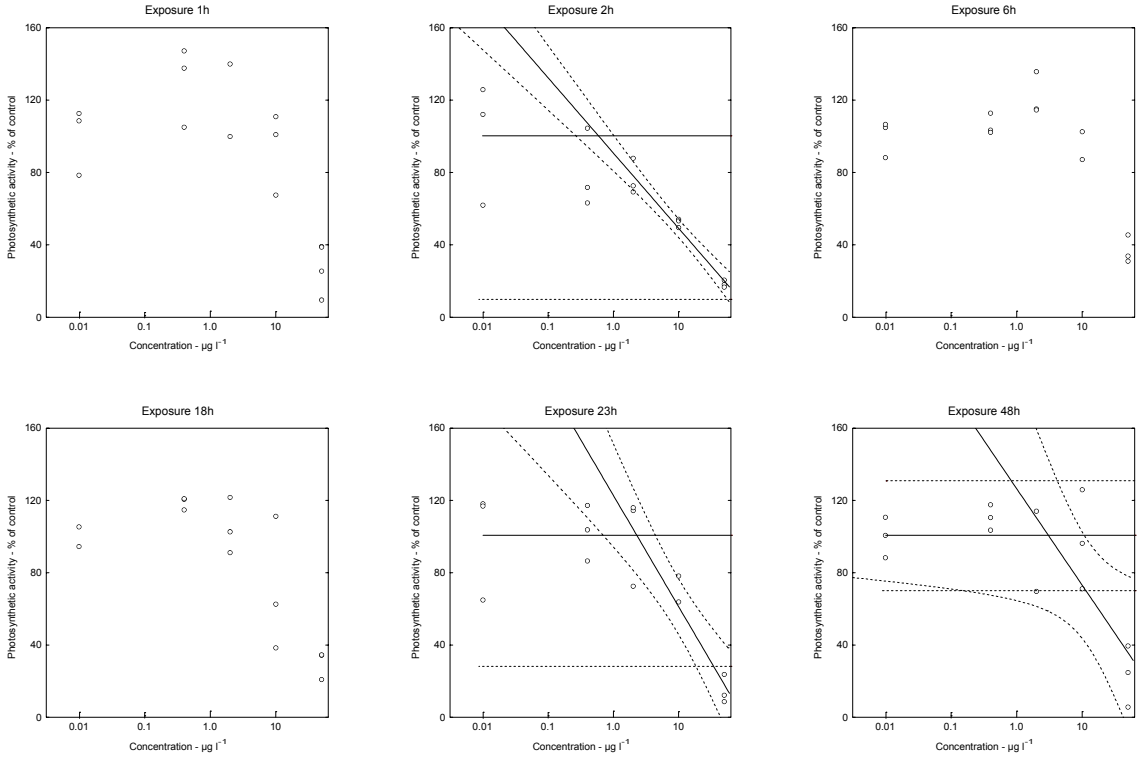

Figure 2. Exposure to metribuzin in 1, 2, 6, 18, 23, and 48 hours.

3.2 Recovery

The photosynthesis activity of the periphyton recovered almost completely after 48 hours in fresh water. The pulse effects of metribuzin on the photosynthetic activity was only short-term and apparently reversible (Fig. 3).

3.3 Effect on the algae group composition

The phytoplankton pigments detected indicated that especially diatoms (detected by fucoxanthin, diadinoxanthin and sporadically presence of diatoxanthin) and chlorophytes (detected by chlorophyll ***b***, lutein, violaxanthin, and neoxanthin) were abundant on the glass discs. Also cyanobacteria (detected by zeaxanthin) were present, although in lower abundances (comparing the concentrations of the algae pigments on the plates) (Fig. 4). Metribuzin had a stimulating effect at 0.4 and 2 µg l^{-1} on the production of chlorophyll ***a*** (Chl ***a***) after exposure for two hours (Fig. 4). However after 23 h, the Chl ***a*** concentration was slightly increased at 0.4 µg l^{-1} but reduced at 2 µg l^{-1}, and at 48 h the Chl ***a*** concentration was reduced even at 0.4 µg l^{-1} (Fig. 4). During recovery the Chl ***a*** concentration increased in all experiments, and although Chl ***a*** was visibly affected by metribuzin during the exposure period, the periphyton resumed growth at all concentration after transfer to herbicide free medium (Fig. 4). Only at the highest metribuzin concentrations after 48 h, the Chl ***a*** concentration was still reduced in the recovery experiments compared to the lower concentrations, but had increased from 6 µg Chl ***a*** l^{-1} to 15 µg l^{-1} during the 48 hours of recovery.

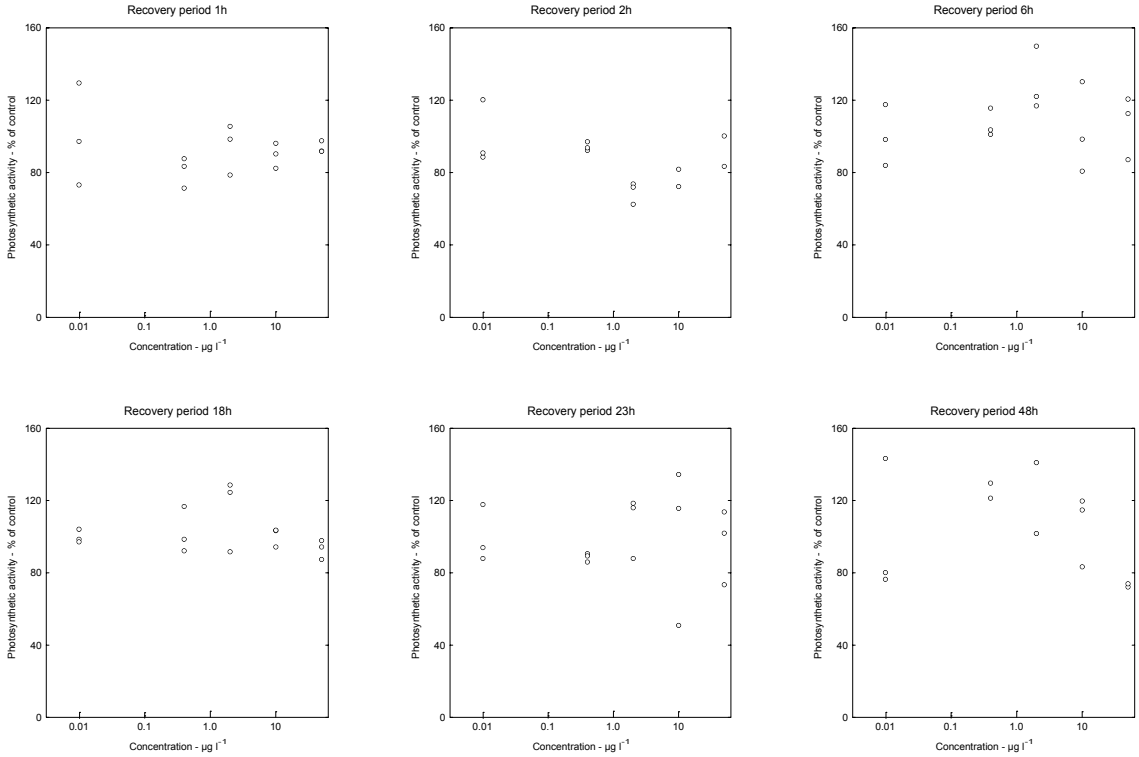

Figure 3. Recovery after 1, 2, 6, 18, 23, and 48 hours of exposure to metribuzin.

Especially chlorophytes were negatively influenced by exposure to metribuzin. While chlorophytes generally decreased due to metribuzin exposure, diatoms in particular, but also cyanobacteria apparently were the groups responsible for the general increase in Chl ***a*** at lower metribuzin concentrations, since they increased at all concentrations except at the highest concentration, 50 µg l^{-1} (Fig. 4). During the recovery experiment both diatoms and especially cyanobacteria recovered well, i.e. no difference in the concentration in the controls compared to the metribuzin treatments, and only diatoms were affected at 50 µg l^{-1} after long-term exposure. The chlorophytes, however, were affected at all metribuzin concentrations even after 48 hours in fresh water, also after only short-term exposure to metribuzin (Fig. 4).

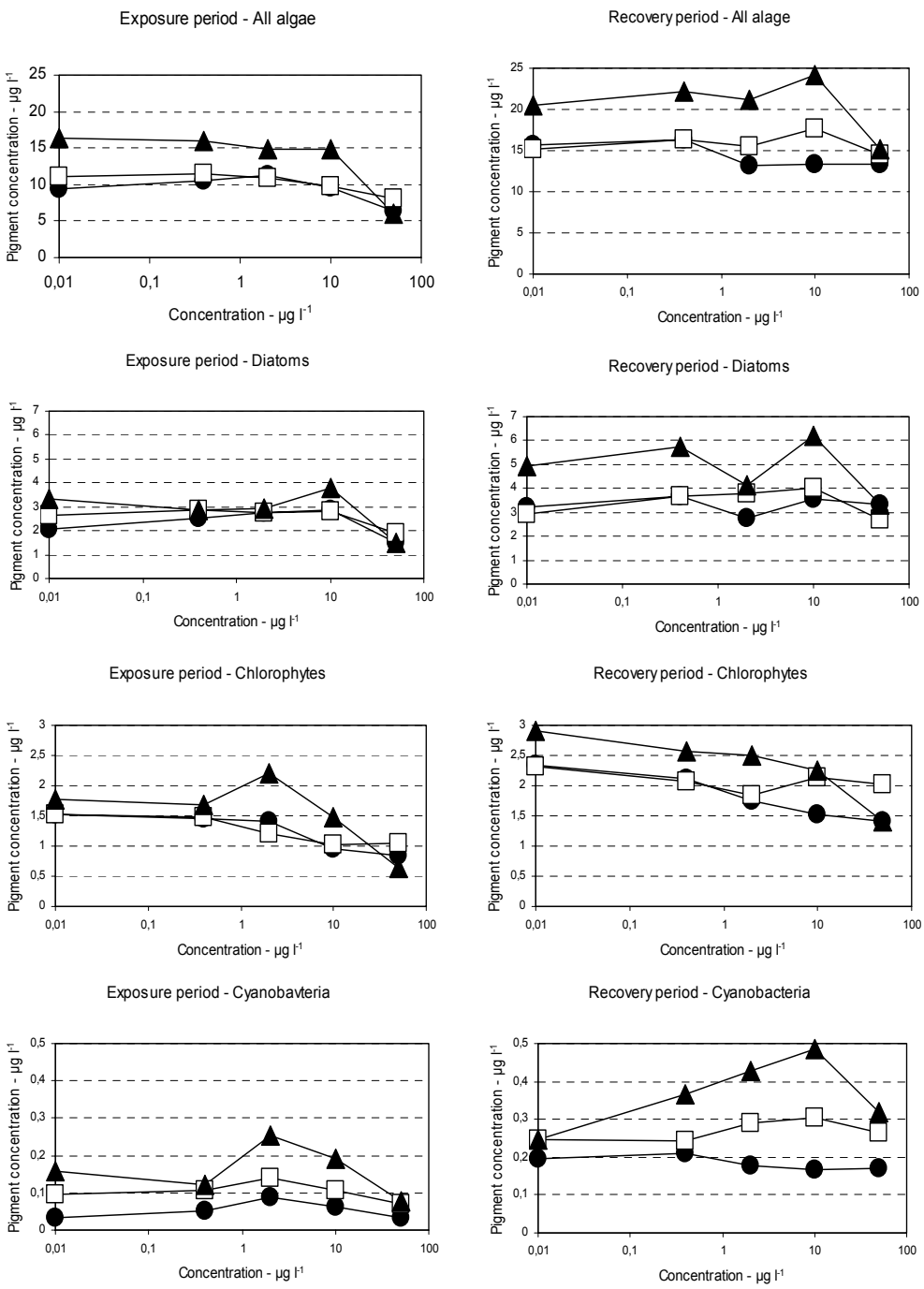

Figure 4. Effect of metribuzin on the different phytoplankton groups during exposure and recovery. Circles: 2 hours of exposure. Squares: 23 hours of exposure, triangles: 48 hours of exposures.

4 Discussion

4.1 Effects of herbicides on the photosynthesis of epiphyton

Periphyton are highly relevant as test organisms in toxicity tests since they constitute the most important food item for benthic fauna in streams, and effects on the productivity and biomass of the algae caused by toxic substances may have impact on the entire food web. Bonilla et al.(1998) found in experiments with episammon (mikroalgae on sandgrains), periphyton and phytoplankton that the different algal communities showed similar sensitivity to the herbicide simazine, but different sensitivity to the herbicide paraquat, where periphyton was the most sensitive.

Generally only few data for pulse effect of herbicides on periphyton communities is published. In the present study isoproturon affected the photosynthesis activity severely at quite low concentrations. NEC in 24-hour test was below 0.02 µg l^{-1}, lowest observed effect concentration (LOEC) was 0.4 µg l^{-1} (Fig. 1), and EC_{50} were the lowest detected in these experiments, i.e., 0.53-1.74 µg l^{-1} (Fig. 1 & Table 1). All values are distinctly lower than EC_{50} value published for standard single-species growth-test over 72 or 96 hour: EC_{50} between 12-40 µg l^{-1} has been published for the planktonic microalgae ***Chlorella pyrenoidosa***, ***Scenedesmus subspicatus*** and ***Chlamydomonas reinhardtii*** (Traunspurger et al. 1996; Anton et al. 1993). These results illustrate the difficulty to predict what the effects under natural conditions will be from the standard single-species toxicological tests, since these tests do not include influences which occur on community level, for instance displacements in species composition, competition between species, etc.

Compared to the frequent occurrence and concentration of isoproturon detected in Danish and Swedish streams, the effect concentration for periphyton is low. In Danish streams (Lillebæk and Odder Bæk) isoproturon is found in about 25% of the water samples and in concentration between 0.011-0.068 µg l^{-1} (NERI, Denmark 2001). In Swedish streams isoproturon has been detected in concentration up to 10 µg l^{-1} (Krueger 1998).

Metribuzin was highly toxic to the periphyton community at low concentrations. In the experiment September 1999 NEC was 0.11 µgl^{-1} and EC_{50} was 5.57 µgl^{-1} in 24 hour-test (Table 1, Fig. 1). Only very few data on the toxicity of Metribuzin have been published. In a single-species test with ***Selenastrum capricornutum*** an EC_{50} (96 hours) of 43 µgl^{-1} was found (Fairchild et al. 1997). This value is distinctly higher than the effect concentration found in the present study in September 1999 for periphyton communities.

The effect concentration EC_{50} of isoproturon and metribuzin decreased by 1-2 orders of magnitude when exposure time increased from 1-2 hours to 24 hours (Table 1 & 2). This result implies that the effects of pesticides on periphyton communities largely depend on the duration of the exposure to the herbicides. This result is important to take into account in the risk evaluation of pulses of pesticides. This was also the case for hexazinone, although the effect concentration could not be estimated for the short-term exposure

experiment because of inconsistency in the dose-response curves and lack of points in the calculation of NEC and EC_{50}. The inconsistency was caused by stimulation at the three lowest concentrations (0.4, 2, and 10 μgl^{-1}, Fig. 1) of hexazinone compared to controls and inhibition at the highest concentration. After 24 hours exposure the stimulation of the photosynthesis disappeared (Fig. 1), the dose-response curve was consistent, and NEC and EC_{50} for hexazinone was 2.29 and 32.88 μgl^{-1} (Table 1). Such stimulation in a short-term experiment is commonly found as a short-term response to toxic stress. However, the stimulation is obviously a response to the toxicant, and it is difficult to evaluate its effect.

Pendimethalin did not show any effects on the natural community of periphyton at concentrations up to 50 μgl^{-1}. The solubility of pendimethalin in water is relative low, i.e., about 275 μgl^{-1} and pendimethalin has a relatively high affinity for particles (Pesticide Manual), both properties that indicate the bio-availability for algae may be low. Swedish investigations indicated that the transport of pendimethalin from agriculture to streams probably is small (Kreuger 1998). In contradiction to the Swedish results pendimethalin has been found in Danish stream in concentrations up to 0.077 μgl^{-1} (NERI, Denmark 2001).

Metribuzin affected the periphyton sampled in September 1999 and in May 2000 very differently. The community sampled in May was less sensitive to metribuzin than the community sampled in September, and the photosynthesis in May was even stimulated at the lowest concentrations (Table 1 & 2, Figure 1 & 2). These results confirm the hypothesis that natural communities are highly variable due to influence by physical, chemical, and biological parameters, and toxic substances have different impact on and result in different effect concentrations of the algal communities depending on season, species composition, nutrient status etc. Unfortunately the group composition was not analysed in September. Communities typically consist of many different species that differ largely in sensitivity to toxicants. The difference in sensitivity found may be explained by the dominance of metribuzin tolerant/sensitive species in the communities sampled in May. In the case that tolerant species dominated the community the risk of the toxicant will be underestimated. Because of the variations in sensitivity of phytoplankton communities at different locations and at different times of the year, it has been advocated that ^{14}C-assimilation tests with phytoplankton communities are unsuitable in hazard evaluations of toxicants (Kusk & Nyholm 1991). However, single-species tests are not a good alternative since variability in sensitivity for toxicants may differ up to three orders of magnitude between different species and no general sensitive algae species have been identified (Blanck et al. 1984, Wängberg and Blanck 1988, Källqvist and Romstad 1994).

In the recovery experiment following metribuzin exposure, EC_{50} did not reveal any increasing or decreasing trend in toxicity as function of the duration of the exposure, but varied in a range between 9.57 and 34.00 $\mu g\ l^{-1}$ and NEC and LOEC were not differing much throughout the 48 h exposure experiment (Figs. 2 and Table 2). The inhibition of the photosynthesis due to metribuzin exposure was therefore not depending on the duration of the exposure to this toxicant. This was also found in the study by Bonilla et al (1998) in experiments with natural communities of microalgae and the herbicide simazine, while the inhibition due to exposure to the herbicide paraquat was

dependent on the exposure time, which increased from 18 to 76 % between 30 min and 24 h of exposure.

The recovery of the primary production was almost complete after 48 hours in clean water even at the highest concentrations where the metribuzin inhibited the primary production by 80%. The result indicates that the pulse effect of metribuzin on periphyton is reversible even at very high concentrations. However this was not the case since the composition of the periphyton was affected even by short-term exposure (2 hours) at the lowest concentration tested (0.4-µg l^{-1}).

4.2 Effects of metribuzin on the individual algal groups

Since the concentration of accessory pigments are related the Chl ***a*** concentration at equal light intensity (Schlüter et al. 2000), the change in the concentrations of the detected pigments can be used for assessing the development of the biomass of the respective groups as effects of the treatment with the herbicide metribuzin. For chlorophytes and diatoms, several specific pigment were detected (neoxanthin, violaxanthin, lutein (chlorophytes) and diadinoxanthin and diatoxanthin (diatoms), data not shown), and the development of these pigments followed the diagnostic pigments, chlorophyll ***b*** and fucoxanthin, respectively, closely, indicating that this method is very robust. The chlorophytes were the most affected group by exposure to metribuzin and were almost always reduced due to the metribuzin treatment, while especially cyanobacteria, but also diatoms at the lowest concentrations, i.e., 0.4, 2, and 10 µg l^{-1} (Fig. 4), were stimulated due to the metribuzin exposure. This gives evidence of a different response to metribuzin within the periphyton community, where the negative impact on chlorophytes and positive impact on diatoms and cyanobacteria causes displacements in the composition of the algae community within 48 h. Displacements in the biomass of such functional different algae groups, result in changed food availability and quality for the grazers which ultimately will have impact on the entire food web. At the highest applied metribuzin concentration, 50-µg l^{-1}, the biomass of all phytoplankton groups was reduced compared to the control. Consequently, potential indirect impact on higher trophic levels of metribuzin and other pesticides that may affect the composition and biomass of periphyton warrant further investigations.

The induced changes in composition of the communities and the fast recovery of the photosyntethic activity are in good agreement to the responses found in other studies including effects of toxicants on algae communities e.g. tributyl-tin (Blanck and Dahl, 1996, Petersen and Gustavson 1998), atrazine (Gustavson & Wängberg 1995), arsenate Blanck and Wängberg 1988). The most likely direct effects of herbicides on periphyton communities in streams may be exclusion and inhibition of sensitive species.

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