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Short-term effects of herbicides on primary productivity of periphyton in lotic environments

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Freshwater algae are quite sensitive to herbicides that enter running water ecosystems through direct application, aerial drift, and/or watershed run-off. However, due to a lack of suitable methodologies, few studies examine the effects of such contamination on naturally occurring attached algal communities under field conditions (i.e., exposure regimes using pulsed doses or brief episodes of peak concentrations to simulate surface run-off during storm events). This paper describes a method for determining the acute short-term effects of four herbicides (hexazinone, atrazine, tebuthiuron and metolachlor) on the net primary productivity (NPP) of periphytic algae in the field using a portable bankside incubator; NPP was measured by monitoring changes in oxygen production (mg O_2 per m^2 upper surface of rock substrate per h and mg O_2 h per mg chlorophyll using the light–dark technique. All herbicides with photosynthetic inhibition as a mode of action significantly reduced NPP. The lowest observed effect concentrations (LOECs) for the herbicides were 43 μg hexazinone l^{-1}, 109 μg atrazine l^{-1} and 137 μg tebuthiuron l^{-1}. The no observed effect concentrations (NOECs) for these chemicals were <43 μg hexazinone l^{-1}, 93 μg atrazine l^{-1} and 52 μg tebuthiuron l^{-1}. Metolachlor did not significantly reduce NPP at the concentrations that were tested (range 19.6–274 μg l^{-1}). However, community respiration (which included respiration by invertebrates) was significantly reduced at the highest metolachlor concentration (274 μg l^{-1}). Community respiration was not significantly affected by any concentration of the other three herbicides used.

Keywords: periphyton; herbicides; net primary productivity; lotic; methodology.

Introduction

Algae and cyanobacteria are aquatic biota which form a major component of the submerged and attached freshwater community (i.e., the epilithon or periphyton) in lotic environments. These microorganisms may influence the fate and biological effects of contaminants in aquatic ecosystems and provide food and habitat for a variety of aquatic animals (Weitzel, 1979; Paul and Duthie, 1989; Boston *et al.*, 1991). Herbicides can enter lotic ecosystems either by accidental overspray of the stream surface during aerial or ground application (Thompson *et al.*, 1991) or through mobilization of chemicals by rain or snowmelt, etc. (Wauchope, 1978; Norris *et al.*, 1983; Muir and Grift, 1987). Such contamination may expose periphytic algae to high concentrations of herbicides for short periods of time before dilution, sorption and/or transformation can ameliorate effects.

Episodic exposures to contaminants may be brief but still detrimentally affect sensitive species with possible indirect effects on other trophic levels (Boston *et al.*, 1991).

Freshwater algae are structurally simple compared with terrestrial plants, but these organisms can be sensitive to herbicides because they share many common metabolic characteristics with the target organisms of most pesticides; for example, inhibition of photosynthesis has been reported in single-celled species as well as with more complex plant species (Stratton, 1987; Boston *et al.*, 1991). Several studies have examined the effects of continuous exposure of periphyton to low concentrations of herbicides, particularly atrazine, under controlled laboratory conditions in artificial stream channels (Kosinski, 1984; Hamala and Kollig, 1985; Lynch *et al.*, 1985; Krieger *et al.*, 1988). However, few studies have evaluated responses of naturally occurring periphytic communities to herbicides under field conditions. There is a strong need for short- and long-term toxicity test methods suitable for analyzing responses of attached algae to contaminants under more realistic conditions, especially using field exposure regimes and pulsed-doses or brief exposures of peak concentrations to simulate surface run-off during storm events (Jones *et al.*, 1986; Jurgenson and Hoagland, 1990; Veersteeg, 1990; Boston *et al.*, 1991; Thompson *et al.*, 1991). This paper describes a method for determining the acute responses of algae to herbicides under field conditions by monitoring changes in oxygen production using the light–dark technique (Vollenweider, 1974).

Materials and methods

Herbicides used in experiments

Two *S*-triazine compounds, atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine as a wettable powder [WP], 85.5%) and hexazinone (3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione; liquid emulsifiable concentrate [EC], 240 g active ingredient l^{-1}, a urea-based herbicide (tebuthiuron; *N*-[5-(1,1-dimethylethyl)-1,3,4-triadiazol-2-yl]-*N,N'*-dimethylurea; WP, 80%) and a chloroacetamide (metolachlor; 2-chloro-*6'*-ethyl-*N*-(2-methoxy-1-methylethyl)-acet-*o*-toluidide; EC 960 g active ingredient l^{-1}), were used in these experiments. The herbicides were chosen to represent chemicals extensively used in agriculture, e.g., atrazine and metolachlor (Moxley, 1989), or with potential widespread use in forestry, e.g., hexazinone and tebuthiuron (Feng *et al.*, 1989; Campbell, 1990, 1991). The mode of action of atrazine, hexazinone and tebuthiuron is inhibition of photosynthesis through blockage of electron transport between photosystems II and I (Sassaman and Jacobs, 1986; Solomon *et al.*, 1988; Eisler, 1989). Metolachlor is thought to affect protein, terpenoid and gibberellic acid syntheses (Kent *et al.*, 1991). A stock solution of each herbicide was prepared on the day of each experiment by diluting the formulated product with distilled water. The concentrations of herbicides that were evaluated were chosen to bracket those likely to occur in stream water from run-off events or during overspray (Bovey *et al.*, 1978; Forney and Davis, 1981; Neary *et al.*, 1983, Sassaman and Jacobs, 1986; Chesters *et al.*, 1989).

Determination of effects on primary productivity

The effects of each herbicide on the primary productivity of naturally occurring periphytic communities on submerged rocks in a stream were measured using a specially constructed portable bankside incubator (Fig. 1) modified from a design developed at the

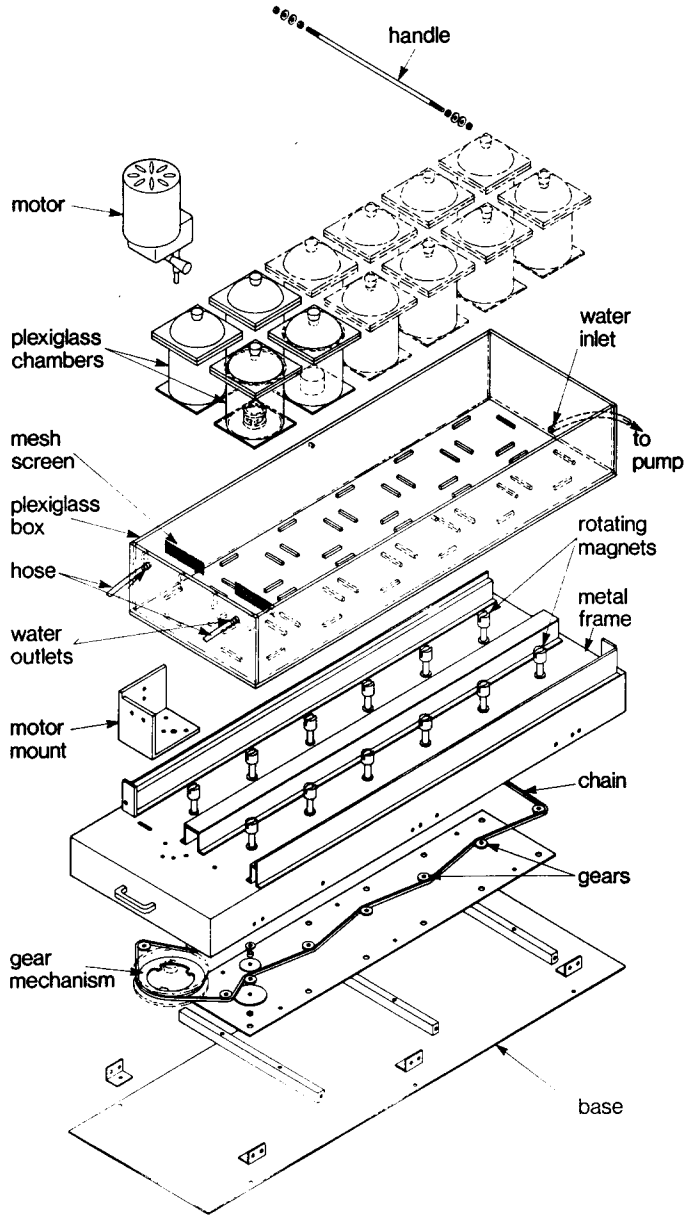

Fig. 1. Streambank incubator.

University of Guelph, Guelph, Canada (Rosenfeld, 1989; Rosenfeld and Roff, 1991). All experiments were performed between June and August 1990, on a level area of land in full sunlight adjacent to Limestone Creek, a low-order stream in a deciduous-forest watershed in southern Ontario (43°26′0.5 N latitude, 79°53′0.1 W longitude). The algal component of the periphytic community in this stream was composed mainly of filamentous cyanophytes, green algae and diatoms.

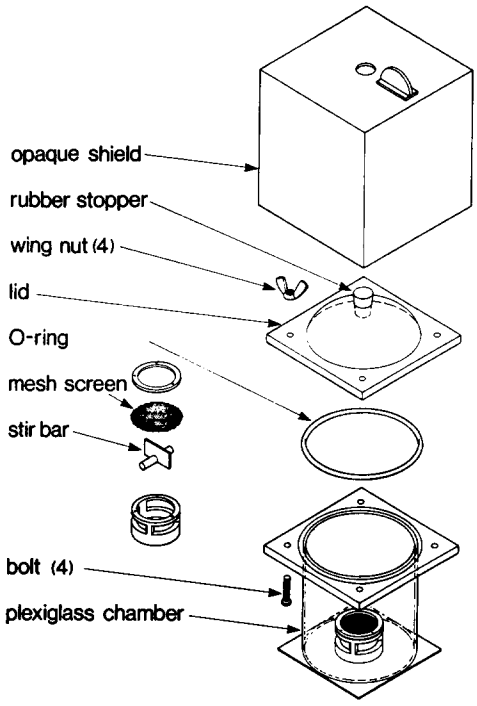

Fig. 2. Incubation chamber with baffles.

To conduct an experiment, twelve rocks (5–10 cm diameter) were selected randomly from a riffle area of the streambed which was not shaded by canopy, and placed on a mesh stand in individual incubation chambers (Fig. 2). Each incubation chamber consisted of a 1.5 l cylindrical plexiglass container, 12 cm diameter and 10 cm long, and was filled with stream water which had been sparged with purified nitrogen gas to lower the oxygen concentration to 3–4 mg l^{-1} below saturation (Rosenfeld and Roff, 1991). Care was taken to exclude air bubbles in a chamber during filling; chambers observed to have bubbles were refilled before an experiment was started.

The chambers were incubated within the larger plexiglass tank (Fig. 1) which rested on a metal base housing twelve vertically rotating magnets driven by a chain and gear mechanism and a motor powered by a portable gas generator. Stream flow within each chamber was simulated by rotating the magnets which, in turn, circulated water within each chamber by spinning a magnetic stir bar fitted with vertical baffles. This procedure generated a flow rate of about 0.03–0.08 m s^{-1} at half the radius from the center of the chamber. Stream water was circulated through the plexiglass tank with a 12 V bilge pump, to maintain the temperature of the chambers as close as possible to that in the stream. In each experiment, the temperature of the water in the chambers never exceeded the temperature in the stream by more than 2 °C. Incubations were generally conducted between 11:00 and 15:00 h.

Six of the twelve incubation chambers were made opaque with shields covered with brown paint to exclude light and six were exposed to ambient light conditions. In each

experiment, three light and three dark chambers were dosed with a single concentration of a given herbicide; three light and three dark chambers were not dosed and served as controls.

Primary production was estimated using the light–dark method (Vollenweider, 1974); oxygen concentrations were monitored with a digital YSI model 58 oxygen meter and a YSI oxygen temperature probe. The oxygen meter was calibrated daily against air-saturated distilled water. Because algal respiration cannot be separated from respiration by micro-heterotrophs in periphytic communities, experiments actually measured community respiration and net primary production.

Incubations ranged from 20 min to approximately 4 h, depending on the intensity of the photosynthetically available radiation (PAR, measured in μE m^{-2} s^{-1}), the temperature of the stream water and the metabolic activity of the epilithon layer. Dark incubations usually were longer than light incubations to get a measurable oxygen change because respiratory metabolism typically was less than photosynthetic metabolism. A Li-Cor model LI550 integrating quantum meter was used to measure PAR above the water surface during each incubation. Incubations were conducted only on sunny days when light intensities were >500 μE m^{-2} s^{-1}, in order to reduce variability in results due to cloud cover.

At the end of each experiment, intact incubation chambers were taken to the laboratory in coolers containing ice. The volume of water in each incubation chamber was measured by decanting it into a graduated cylinder. Organic layers on all upper rock surfaces were quantitatively sampled for chlorophyll *a*. All periphyton was removed by rinsing and scraping the surface of each rock using a small wire brush, and a spray bottle containing distilled water. Subsamples of periphyton (10 ml) were filtered onto 0.45 μm GF/C filters, homogenized in acetone, and the concentrations measured spectrophotometrically using standard procedures (APHA, 1975). The upper surface area of each rock was estimated by weighing an outline of the rock drawn on paper of known areal weight. NPP and respiration was expressed as changes in mg O_2 per m^2 per h and mg O_2 per h per mg chl *a* for periphyton communities in treated and control chambers, for each herbicide concentration. The statistical significance of the herbicidal effects was determined using comparison of means (Mendenhall, 1971) at $p \leq 0.05$ for each experimental concentration versus a control.

Determination of pesticide residues

1000 ml water samples from the treated and control chambers were extracted in 4 l glass amber containers with 200 ml dichloromethane (DCM) followed by 2 × 100 ml DCM (pesticide grade; Caledon Laboratories). Combined extracts were dried over Na_2SO_4, transferred to a 500 ml round bottom flask with 4 ml isooctane and concentrated to a known volume by rotary evaporation at 45 °C. The flask was rinsed with isooctane, and the residue transferred quantitatively to a test tube and concentrated to 1 ml in a stream of dry nitrogen. Further concentration or dilution was made as appropriate for gas chromatographic analysis.

Analsyes of the four herbicides were performed using a Hewlett-Packard 5890 Series II gas chromatograph with a splitless injector, DB-5 column, N/P detector and an autosampler. Dimensions of the column were 0.25 ID × 30 m length with 0.25 ID μm film thickness; injector and detector temperatures of 200 °C and 300 °C, respectively, were used. The initial column temperature was 80 °C and the program rate was 4 °C min^{-1} to 280 °C with zero time final hold. Specific gas flow rates for the helium carrier gas

(ultrahigh purity), nitrogen make-up, air and hydrogen (prepurified) were 1–1.5, 25, 117 and 3.1 ml min^{-1}, respectively. A standard mixture of the herbicides was used to determine the retention times and calibrate the detector responses. Percent recovery of each herbicide was determined by spiking MilliQ™ water and Limestone Creek water with the lowest and highest concentrations of each herbicide, and extracting these samples as described above. Concentrations of herbicides measured in each chamber under experimental conditions were then corrected for recovery efficiencies.

Results and discussion

A variety of streambank incubators that enclose periphytic communities under field conditions has been used in ecological studies to assess gross and net primary productivity of periphyton in lotic environments (e.g., Paul *et al.*, 1989; Fuller and Bucher, 1991; Rosenfeld and Roff, 1991). The use of one such incubator for determining short-term effects of herbicides on net photosynthetic activity of naturally occurring algal periphytic communities is an adaptation of a design that shows promise in ecotoxicological studies.

Percentage recoveries of atrazine, hexazinone, tebuthiuron and metolachlor in spiked Milli-Q™ and Limestone Creek stream water are shown in Table 1. Efficiencies of extraction ranged from 74.4% to 95.4% for atrazine, 80.5% to 103.4% for hexazinone and 82.9% to 98.9% for metolachlor. The variability in recovery of tebuthiuron from water was greater (range 62.9–129.3%), but still within the limits of acceptability.

NPP of the epilithic stream communities placed in control chambers during *in situ* incubation of natural substrates varied throughout experiments but usually ranged between 500 mg O_2 and 1500 mg O_2 per m^2 per h (Figs 3–6). In general, NPP was greater at higher levels of PAR, but there was no statistically significant correlation of NPP with light levels. These results were similar to values obtained by Rosenfeld (1989) and Rosenfeld and Roff (1991) in their determinations of the availability of autochthonous carbon on rock substrates in uncanopied streams in southern Ontario, including Limestone Creek, used in this study; NPP in these studies averaged 1580 ± 920 mg O_2 per m^2 per h at unshaded upstream sites and 1220 ± 220 mg O_2 per m^2 per h at downstream unshaded sites. Rosenfeld and Roff (1991) found that PAR had the highest correlation with NPP, but temperature of incubation was also a factor which affected primary production. Light saturation of NPP is thought to occur at intensities >400 μE per m^2 per s, thereby allowing other environmental variables to influence NPP (Jasper and Bothwell, 1986; Steinman and McIntire, 1987). Respiration rates within control chambers in this study (Fig. 7a–d) were also similar to values of 353 ± 99 and 404 ± 296 obtained at upstream and downstream unshaded sites in southern Ontario, respectively (Rosenfeld and Roff, 1991).

The three photosynthetic inhibitors (atrazine, hexazinone and tebuthiuron) each had significant effects on NPP but not respiration of algal communities on rock substrates. The concentrations needed to lower significantly the NPP were similar to those that could occur during overspray or run-off conditions in agricultural or forestry application (Figs 3–6, 7a–c). For example, hexazinone significantly depressed NPP at concentrations $\geq$ 43 $\mu g\ l^{-1}$ (Fig. 3). Hexazinone is a triazine herbicide with temporary registration in Canada for broad-spectrum control of woody plants in forestry management such as in conifer release programs. Before 1991, registration of hexazinone was restricted to

Table 1. Mean percent recoveries of water samples spiked with four herbicides

Type of water	Nominal concentration ($\mu g\ l^{-1}$)							
	25	200	50	500	125	500	50	100
	Atrazine		Hexazinone		Tebuthiuron		Metolachlor	
MilliQ								
% Recovery (±SE)	94.8 ± 5.7	95.4 ± 6.2	89.1 ± 4.6	80.5 ± 18.5	62.9 ± 19.7	109.6 ± 25.8	84.8 ± 11.3	98.9 ± 3.8
CV	5.9	6.5	5.2	22.9	31.3	23.5	13.3	3.8
Stream								
%Recovery (±SE)	89.1 ± 5.6	74.4 ± 11.9	90.1 ± 18.5	103.4 ± 17.0	N.A.[a]	129.3 ± 9.9	83.5 ± 14.1	82.9 ± 10.8
CV	6.5	16.2	20.5	16.4		7.7	17.2	13.0

[a]N.A. = not available.

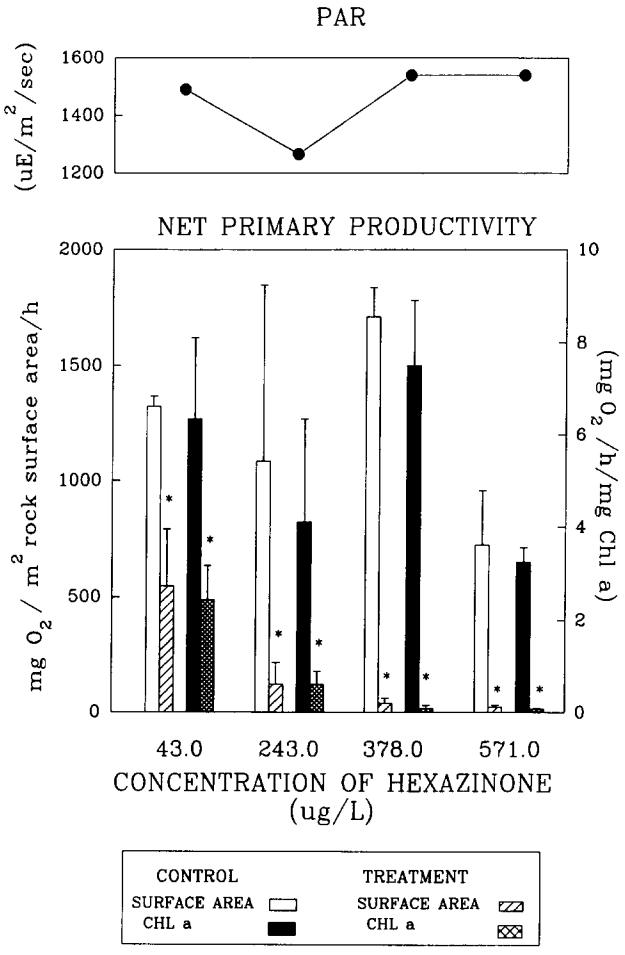

Fig. 3. NPP of periphyton in control and hexazinone-dosed incubator chambers; oxygen production is expressed both on an area basis (upper surface of rock substrate) and algal biomass (mg chlorophyll *a*). Daily PAR is indicated on the upper figure. Asterisks indicate statistical significance at $p \leq 0.05$.

ground application and only small quantities of this herbicide were used (McLeay, 1988). However, hexazinone recently received registration for aerial forest vegetation management at maximum rates of 4.0 kg a.i. per ha (Campbell, 1991). An increase in the use of this herbicide in forestry applications is anticipated. Hexazinone is highly soluble in water (about 3000 mg l^{-1}), persistent, and has a high potential for moving off the targeted site (Feng *et al.*, 1989; Allender, 1991). Several studies have reported concentrations of 15–442 μg hexazinone per l in surface run-off from forested watersheds for up to two years after it was applied (Neary *et al.*, 1983; Legris and Couture, 1987; Lavy *et al.*, 1989). Solomon *et al.* (1988) found a significant dose-dependent change in oxygen concentrations in hexazinone-treated enclosures in a bog lake in northeastern Ontario for two weeks following surface application simulating an overspray scenario

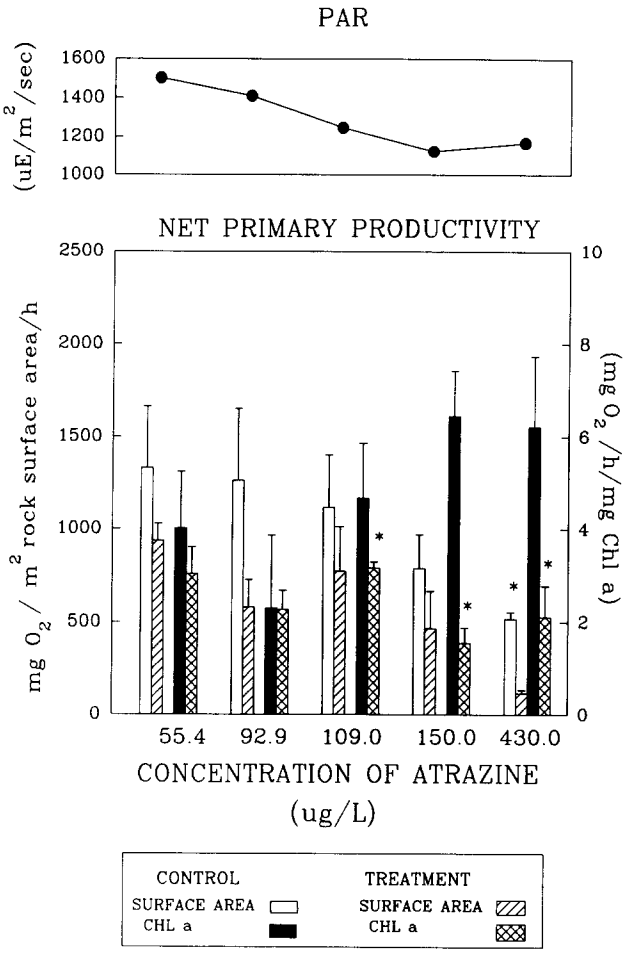

Fig. 4. NPP of periphyton in control and atrazine-dosed incubator chambers.

with an initial aquatic concentration of 200 $\mu g\ l^{-1}$. In a similar study, Thompson *et al.* (1993a) found that chronic exposure of the phytoplankton community in enclosures to levels of hexazinone > 10 $\mu g\ l^{-1}$ lowered oxygen concentrations in the water column and reduced the biomass of most algal taxa. Secondary impacts of hexazinone on the zooplankton communities within the enclosures were also evident, and were attributed to reductions in phytoplankton biomass and concentrations of dissolved oxygen (Thompson *et al.*, 1993b). These results and those from the present study suggest that primary productivity in aquatic ecosystems could be reduced through inhibition of photosynthesis at concentrations of hexazinone which may occur in either run-off or overspray situations; however, the duration of such effects in lotic ecosystems is not yet known. Additional effects on fish and invertebrates in river and stream watersheds have not been reported (Rhodes, 1980; Mayack *et al.*, 1982).

In contrast to the paucity of information about the impacts of hexazinone in aquatic ecosystems, there is much published information about the effects of atrazine on

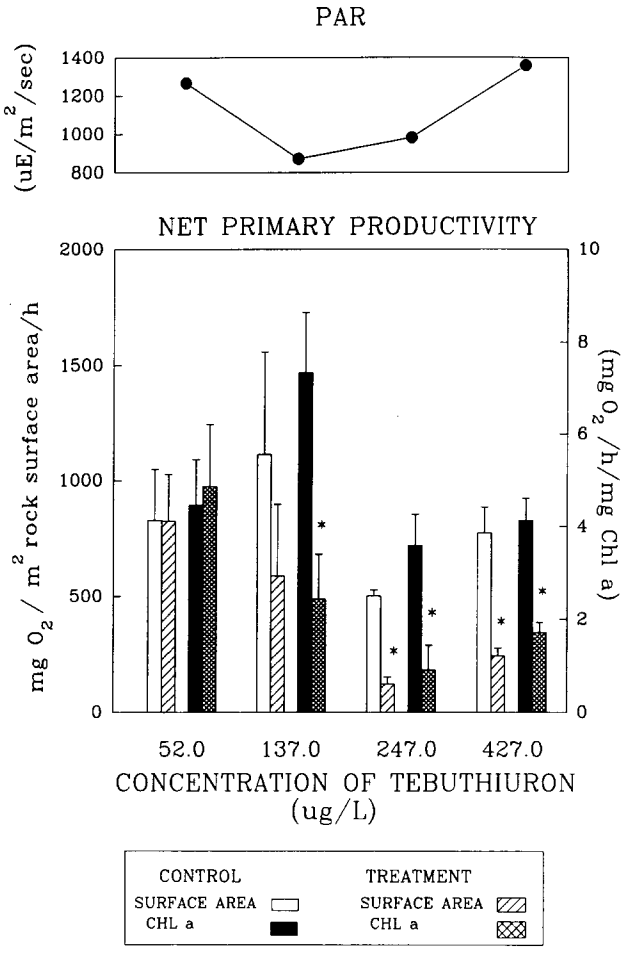

Fig. 5. NPP of periphyton in control and tebuthiuron-dosed incubator chambers.

periphytic communities. Atrazine is one of the most extensively used herbicides in North America and this chemical has been recorded in many surface waters at concentrations ranging from 0.1 μg l^{-1} to 30.3 μg l^{-1}. Concentrations of 27–69 μg l^{-1} have been shown to occur in run-off adjacent to treated cornfields after spring application (Krieger *et al.*, 1988; Eisler, 1989) and peak residues in bulk run-off (sediment + water) at concentrations as high as 1000 μg l^{-1} have been reported in catastrophic events (Wauchope, 1978; Forney and Davis, 1981). In the present study, concentrations of atrazine $\geqslant$109 μg l^{-1} reduced NPP of periphyton communities within 4 h, although these results were significant only when NPP was standardized to algal chl *a* (see Fig. 4). Higher concentrations of 430 μg atrazine per l were required to reduce significantly the oxygen production of the entire periphyton community. Other studies have found that NPP of periphyton in artificial stream channels decreases within days following addition of similar or lower concentrations of atrazine (about 100 μg l^{-1}), but exposure in these experiments was continuous (Hamala and Kollig, 1985; Moorhead and Kosinski, 1986; Pratt *et al.*, 1988). In contrast, Jurgensen and Hoagland (1990) found that short term exposure (24 h) of

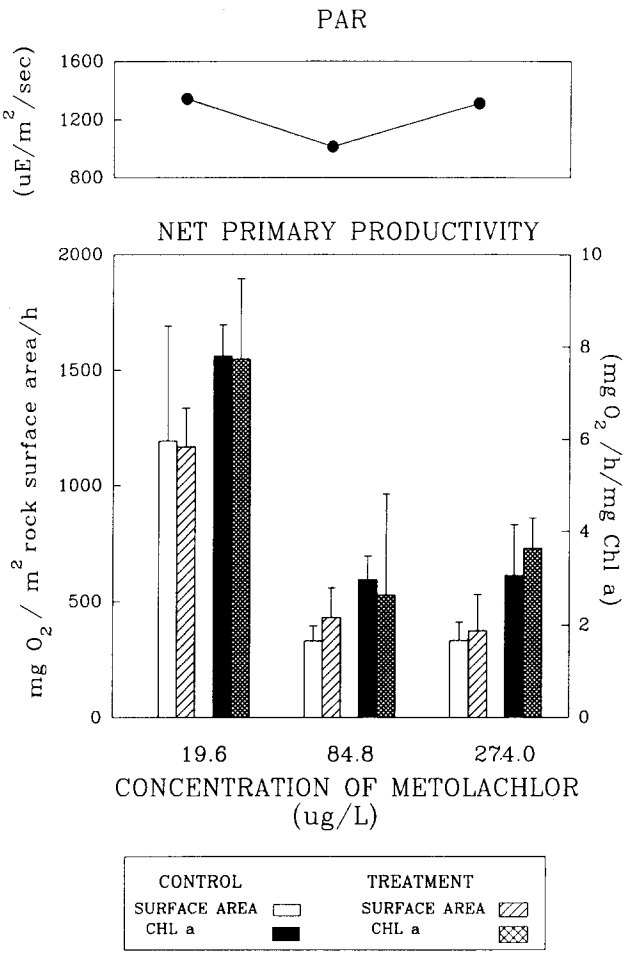

Fig. 6. NPP of periphyton in control and metolachlor-dosed incubator chambers.

attached algal communities in a small, spring fed stream in western Nebraska to concentrations of atrazine as high as 100 μg l^{-1} did not have a significant long term effect on cell densities of the dominant algae or the ash-free dry weight biomass of the periphyton community. Rocchio and Malanchuk (1986) applied single daily doses of atrazine (50–150 μg l^{-1}) in aquatic systems and found that dissolved oxygen production declined to zero in all treatments. However, after dosing stopped, all systems recovered within 48 h, and no permanent ecosystem damage was detected. These results suggest that effects of higher concentrations or pulses of atrazine on periphyton in lotic environments may be transitory if exposure duration is short; once exposure has ceased, nonlethal photosynthetic inhibition may be reversed. However, the severity of the effects may depend on the species composition of the community. For example, in a study of the impact of atrazine on periphyton in large volume enclosures, Herman *et al.* (1986) found that, although the cyanobacteria were drastically affected by concentrations in the range 80–140 μg l^{-1}, these concentrations had no obvious impact on green algae or diatoms.

Tebuthiuron is a broad-spectrum, soil-applied herbicide used in rights-of-way manage-

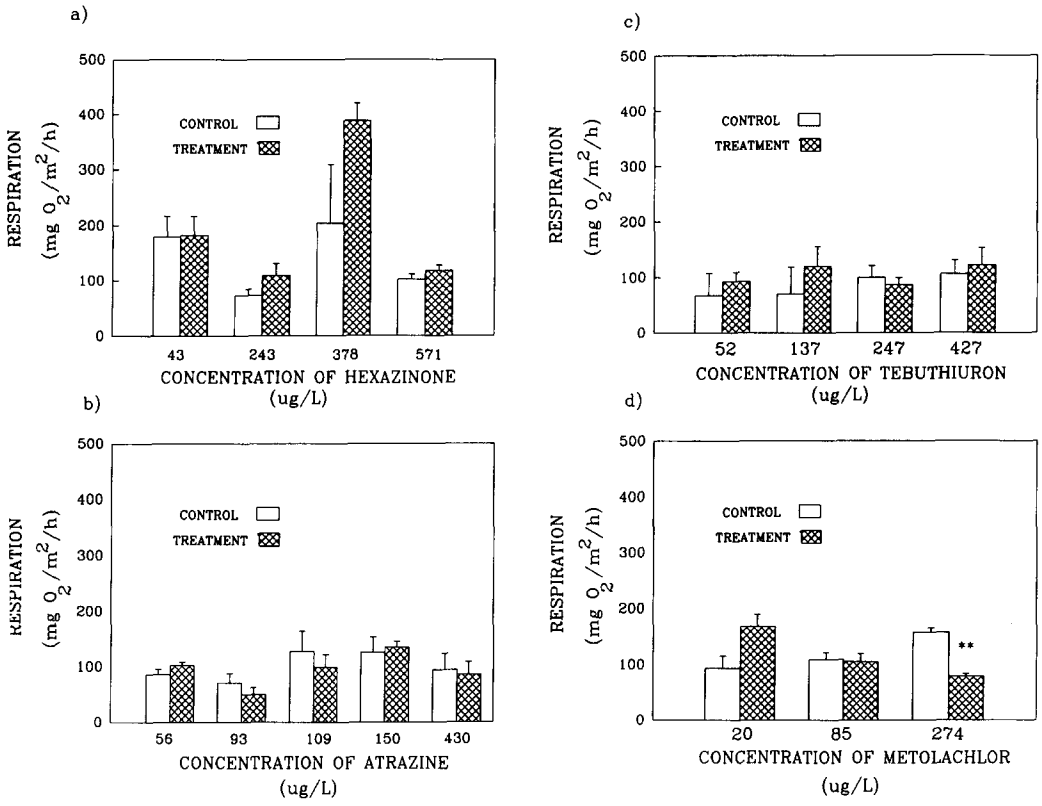

Fig. 7. Community respiration (mg O_2 per m^2 per h) of periphyton exposed to (a) hexazinone, (b) atrazine, (c) tebuthiuron, (d) metolachlor.

ment where total control of woody vegetation, annual weeds and some perennial grasses is required (Emmerich *et al.*, 1984). It is moderately mobile in soil and leaches slowly; it is also very persistent, with a half-life of 2–7 years in soils (Johnsen and Morton, 1989). In water, tebuthiuron does not undergo hydrolysis under normal environmental ranges of pH and temperature. Fairly high concentrations of tebuthiuron have been measured in flowing water and catchment ponds up to three months following treatment of watersheds; 70–2230 $\mu g\ l^{-1}$ were reported for surface run-off (Bovey *et al.*, 1978; Sassaman and Jacobs, 1986). In the present study, concentrations of $\geq$137 μg tebuthiuron per l significantly reduced the NPP of algae; concentrations of $\geq$247 μg tebuthiuron per l detrimentally affected community productivity (see Fig. 5). Concentrations great enough to inhibit NPP are possible in treated watersheds based on the results of the above studies. Adams *et al.* (1985) and Blaise and Harwood (1991) found that concentrations $\geq$50 $\mu g\ l^{-1}$ over six or seven days were required to reduce the growth of the green alga *Selenastrum capricornutum* in single-species laboratory tests. Temple *et al.* (1991) suggest that 200–500 μg tebuthiuron l^{-1} is the nominal dose required in mesocosms before phytoplankton primary production effects could be detected but, once effects were noted, indirect effects such as a depression in chironomid density were noted, and attributed to changes in algal species composition. Price *et al.* (1989) found that 180 μg

tebuthiuron l^{-1} did not lower algal production in 400 ml microcosms. These results, plus the reported persistence of tebuthiuron, suggest that the productivity of attached algae may be decreased in watersheds where tebuthiuron is used extensively and concentrations $\geqslant 100\ \mu g\ l^{-1}$ occur.

Metolachlor is one of the most extensively used herbicides in Ontario, with 1.7 million metric tons of active ingredient used to control annual grasses and broadleaf weeds in crops such as corn and sorghum in 1988 (Moxley, 1989). Metolachlor is very water soluble (530 mg l^{-1}), and leaches readily, leading to speculation that concentrations of metolachlor in surface waters may be high, particularly, early in the growing season after first application. Ambient concentrations of 0.27–4.4 μg metolachlor per l have been reported for agricultural watersheds (Chesters *et al.*, 1989), with maximum concentrations of 3–293 $\mu g\ l^{-1}$ in storm run-off events (Buttle, 1990). In the present study, metolachlor had no significant effects on NPP of periphyton communities at the concentrations tested; however, a significant decrease in periphytic respiration was evident at a metolachlor concentration of 274 $\mu g\ l^{-1}$. St. Laurent *et al.* (1992) found that concentrations of $\geqslant 50\ \mu g$ metolachlor per l reduced growth of the green alga *S. capricornutum* in 96 h microplate algal assay experiments, but Walsh *et al.* (1991) reported that the LOEC for metolachlor for two freshwater wetland plant species was 250–500 $\mu g\ l^{-1}$. The specific mode of action of metolachlor is unknown, but it is thought to interfere with protein synthesis, terpenoid synthesis and mitotic activity; therefore, it is not surprising that immediate effects of metolachlor on oxygen production were not noted in the present study. However, the use of the chemical provided a good means of checking on the validity of effects on photosynthesis observed with the other compounds (i.e., it served as a 'negative control'). Techniques for measuring metabolic activities other than photosynthesis or longer-term experiments with metolachlor might be needed to detect possible detrimental effects of this herbicide on periphyton. With regard to the effects on respiration (Fig. 7d), it is worth noting that monitoring oxygen concentrations of a periphytic community does not exclude respiration by invertebrates and bacteria. Thus, it is possible that metolachlor inhibits one or more metabolic enzyme(s) in aquatic animals. There is little information about the toxicity of metolachlor to other aquatic organisms, but acute effects have been reported in the range 0.78–26.0 mg l^{-1} for fish and invertebrates (Kent *et al.*, 1991). These concentrations are much higher than those used in the present study.

The methods described in this paper provide a rapid *in situ* technique for determining the short term effects of photosynthetic inhibitors on naturally occurring periphytic communities in rivers and streams, using concentrations similar to those that can be found in storm run-off events or overspray situations. A criticism of this and other short term toxicity tests is that short term tests may not be as sensitive as longer term tests, and effects may be reversible. For example, Versteeg (1990) found that the EC50's for four day growth, CO_2 fixation and O_2 generation of the green alga *S. capricornutum* exposed to atrazine were 0.05 mg l^{-1}, 0.10 mg l^{-1} and 0.38 mg l^{-1} respectively. However, as suggested by Pratt *et al.* (1988), a decrease in functional activity such as photosynthesis may indicate more serious effects on community structure, although the risk of permanent damage depends on the duration of exposure. The key advantage of short term algal tests is speed and flexibility for assessing the interactive effects of environmental factors, such as light, water temperature, etc., on toxicity (Versteeg, 1990). The technique described in this paper could be modified to determine recovery of photosynthetic

activity by returning the rocks to the stream environment (with suitable identifying markers) and then periodically monitoring oxygen production of these communities through time. In addition, the use of enclosed chambers is amenable to ^{14}C uptake experiments which provide alternative assessments of toxicity.

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