Chapter 10

Phytoremediation of Herbicide-Contaminated Surface Water with Aquatic Plants

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There is current interest in the use of artificial wetlands and macrophyte-cultured ponds for the treatment of agricultural drainage water, sewage, and industrial effluents. Aquatic plant-based water treatment systems have proved effective and economical in improving the quality of wastewaters containing excess nutrients, organic pollutants, and heavy metals. This investigation was conducted to test the hypothesis that herbicide-tolerant aquatic plants can remediate herbicide-contaminated waters. The addition of *Ceratophyllum demersum* (coontail, hornwort), *Elodea canadensis* (American elodea, Canadian pondweed), or *Lemna minor* (common duckweed) significantly ($p \leq 0.01$) reduced the concentration of [^{14}C]metolachlor (MET) remaining in the treated water. After a 16-day incubation period, only 1.44%, 4.06%, and 22.7% of the applied [^{14}C]MET remained in the water of the surface water systems containing *C. demersum*, *E. canadensis*, or *L. minor* whereas 61% of the applied [^{14}C]MET persisted in the surface water systems without plants. *C. demersum* and *E. canadensis* significantly ($p \leq 0.01$) reduced the concentration of [^{14}C]atrazine (ATR) in the surface water. Only 41.3% and 63.2% of the applied [^{14}C]ATR remained in the water of the vegetated systems containing *C. demersum* and *E. canadensis*, respectively. Eighty-five percent of the applied [^{14}C]ATR was detected in the water of the *L. minor* and nonvegetated systems. Our results support the hypothesis and provide evidence that the presence of herbicide-tolerant aquatic vegetation can accelerate the removal and biotransformation of metolachlor and atrazine from herbicide-contaminated waters.

Herbicides in Surface and Subsurface Waters. Runoff/erosion of pesticides from agricultural fields is believed to be the largest contributor to water quality degradation in the midwestern United States. Atrazine, alachlor, cyanazine, and metolachlor are the major herbicides used in Iowa and the Midwest (*1, 2*). The intense use of these relatively water soluble and mobile compounds threatens the integrity of surface and subsurface waters (*3, 4*). Approximately 1 to 6% of the applied herbicides may be lost to the aquatic environment by runoff and drainage

depending on the slope of the field, tillage practices, presence or absence of subsurface drains, and the quantity and timing of rainfall after application (*5-7*). Monitoring studies have detected herbicides in surface waters (*3, 8, 9*), tile-drain water and groundwater (*5,10,11*). Goolsby et al. (*3*) and Thurman et al. (*8*) reported frequent detection of metolachlor, alachlor, cyanazine, atrazine, and the atrazine degradation products deethylatrazine and deisopropylatrazine in rivers and streams of the midwestern United States. Atrazine and metolachlor were the two most frequently detected herbicides. Measurable amounts of atrazine were reported in 91%, 98%, and 76% of the preplanting, postplanting, and harvest surface waters sampled. Metolachlor was detected in 34%, 83%, and 44% of the preplanting, postplanting and harvest-season waters sampled, respectively.

Problems Associated with Pesticide-Contaminated Water. The presence of pesticides in surface water is a concern for human health and the health of aquatic ecosystems (*12*). Contamination of surface waters with pesticides exposes nontarget microorganisms, plants, and animals to compounds that may have an adverse effect on individual organisms or biotic communities. Aquatic insects and other aquatic arthropods are particularly susceptible to insecticides, whereas herbicides may suppress the growth of aquatic vegetation (*13-16*). The primary concern involving human exposure to pesticide-contaminated waters involves long-term exposure to low concentrations through drinking water (*13*). Conventional water treatment processes (filtration, clarification, chlorination, softening, and recarbonation) do little to reduce the levels of pesticides in drinking water (*13, 17, 18*). Pesticide concentrations are significantly reduced only when advanced processes such as ozonation, reverse osmosis, or granular activated carbon are used. In areas where water treatment facilities lack advanced treatment processes, the concentration of pesticides in the finished drinking water will be similar to the concentrations found in the surface water or groundwater source (*17*).

Phytoremediation of Contaminated Water. There is current interest in the use of artificial wetlands and macrophyte-cultured ponds for treating wastewater (agricultural drainage water, sewage, and industrial effluents) (*19-23*). Aquatic plant-based water treatment systems have proved to be effective and economical in improving the quality of wastewater effluents (*24-27*). Floating and emergent aquatic plants including water hyacinth (*Eichhornia crassipes* Mart.), elodea (*Egeria densa* P.), duckweed (*Lemna* and *Spirodela* spp.), pennywort (*Hydrocotyle umbellata* L.), common arrowhead (*Sagittaria latifolia* L.), common reed (*Phragmites australis*), and pickerelweed (*Pontederia cordata* L.) reduce the levels of total suspended solids and nutrients (N and P) in wastewater by solid filtration, nutrient assimilation, and microbial transformation (*19-28*). In addition, aquatic plants and their associated microbiota have contributed to the removal and biotransformation of xenobiotic compounds from contaminated waters and sediments. Microbiota of cattail roots (*Typha latifolia* L.) and duckweed plants (*L. minor*) accelerate the biodegradation of surfactants (*29*). Curly leaf pondweed (*Potamageton crispus* L.), common duckweed (*L. minor*), and their epiphytic microbes contributed to the removal and degradation of pentachlorophenol from a stream, and various duckweed plants (*Lemna* and *Spirodela* spp.) have been shown to accumulate metals (aluminum, cadmium, copper, lead, and mercury) from aqueous solutions (*30-32*).

Previous research provides evidence that aquatic plants can remediate wastewaters containing excess nutrients, organic pollutants, and heavy metals. This investigation was

conducted to test the hypothesis that herbicide-tolerant aquatic plants can remediate herbicide-contaminated waters. Experiments were setup to evaluate the ability of two submerged aquatic plants (*Ceratophyllum demersum* L. and *Elodea canadensis* Rich.) and one floating aquatic plant (*Lemna minor* L.) to remediate metolachlor or atrazine contaminated waters. Metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide] controls annual grass weeds and broadleaf weeds in corn, soybeans, peanuts, and potatoes. Atrazine [6-chloro-*N*-ethyl-*N*'-(1-methylethyl)-1,3,5-triazine-2,4-diamine] inhibits photosynthesis of susceptible grassy and broadleaf weeds in corn, sorghum and turf grass (*33*). Our results support the hypothesis and demonstrate the presence of herbicide-tolerant aquatic vegetation can accelerate the removal and biotransformation of metolachlor and atrazine from herbicide-contaminated waters.

Materials and Methods

Chemicals. Metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide] (CGA 24705, 97.3 % pure); [U-ring-^{14}C]metolachlor ([^{14}C]MET) (98.9% pure); the metolachlor degradates *N*-(2-ethyl-6-methylphenyl)-2-hydroxy-*N*-(2-methylethyl)-acetamide (CGA 40172, 98.4% pure) and 4-(2-ethyl-6-methylphenyl)-5-methyl-3-morpholinone (CGA 40919, 99.8% pure); [U-ring-^{14}C]atrazine ([^{14}C]ATR) (98.2% pure); [U-ring-^{14}C]deethylatrazine (94.8% pure); [U-ring-^{14}C]deisopropylatrazine (92.9% pure); [U-ring-^{14}C]didealkylatrazine (98.8% pure); and [U-ring-^{14}C]hydroxyatrazine (97.5% pure) were gifts from the Ciba-Geigy Corporation, Greensboro, NC.

Surface Water and Aquatic Plant Sample Collection. Surface water and aquatic plants *Lemna minor* L. (common duckweed), *Elodea canadensis* Rich. (American elodea, Canadian pondweed), and *Ceratophyllum demersum* L. (coontail, hornwort) were collected from the Iowa State University Horticulture Station Pond, Ames, Iowa. The aquatic plants were selected as a result of their abundance and availability. Pond water samples were collected in sterile 4-L bottles and stored at 4 ± 2°C. Aquatic plants were collected and maintained, at 25 ± 2°C, in aquaria containing distilled water and Hoagland's nutrient solution with a 14:10 (L:D) photoperiod.

Experimental Design. Experiments were conducted to evaluate the degradation of metolachlor or atrazine in vegetated- and nonvegetated-surface-water incubation systems. Each experimental variation [herbicide (metolachlor, atrazine) x aquatic plant (*L. minor*, *E. canadensis*, *C. demersum*) x the duration of the incubation period (0-16 days)] was replicated a minimum of three times. Analysis of variance and least square means determined significance between treatments.

Surface Water/Plant Incubation Systems. French square bottles were filled with 150 ml of a water solution containing pond water/Hoagland's nutrient solution/ultra-pure water (1:1:4 v/v/v). A pond water/Hoagland's nutrient solution/ultra-pure water mixture was used rather than 150 ml of pond water in order to make the study more reproducible for other researchers. [^{14}C]MET or [^{14}C]ATR was added to the water at a rate of 200 μg/L. This rate was chosen to represent a runoff concentration and to ensure there was enough radioactivity for the detection of metabolites. Aquatic plants (3 g) were added to 150 ml of the treated water solutions and

placed in a temperature-controlled room set at 25 ± 2°C with a 14:10 (L:D) photoperiod. Three replicate vegetated- and nonvegetated-incubation systems were dismantled on each of the designated incubation days. The herbicides and their degradates were extracted from the water solutions and the plant tissues, and a mass balance was determined.

Water Extraction and Analysis. At the completion of each test, the aquatic plants were removed from the water solutions by using vacuum filtration and were rinsed with ultra pure water. The plant rinsate was added to the filtrate. A portion of the treated water was counted with a liquid scintillation spectrometer to determine the quantity of radioactivity remaining in the water. The herbicides and herbicide degradates were removed from the remaining water with a solid phase extraction (SPE) process. Supelclean Envi-18 6-cc solid phase extraction cartridges (Supelco, Inc., Bellefonte, PA) were positioned on a 12-port Visiprep Solid Phase Extraction Vacuum Manifold (Supelco, Inc., Bellefonte, PA) and activated with 18 ml (3 column volumes) of certified ethyl acetate followed by 18 ml of certified methanol and finally 18 ml of ultra-pure water. The water samples were drawn through the activated cartridges by using an applied vacuum (50 kPa). Once the entire sample had been drawn through the extraction cartridge, the packing was dried by drawing air through the cartridge for approximately 15 minutes. The cartridges were eluted with 10 ml certified methanol followed by 5 ml of certified ethyl acetate. The radioactivity of the effluent (post-SPE water sample) and the methanol and ethyl acetate eluates was determined with liquid scintillation techniques. The quantity of metolachlor, atrazine, and their degradates in the methanol eluates were characterized by thin layer chromatography (TLC).

Plant Extraction and Analysis. Plant tissues were extracted three times with certified methanol. The volume of the extract was reduced with a rotary evaporator and the plant extracts were characterized by TLC. Dry-extracted plant tissues were mixed with hydrolyzed starch and combusted in a Packard sample oxidizer (Packard Instrument Co.) to determine the activity of the nonextractable residues. The $^{14}CO_2$ produced from the combusted plant material was trapped in Carbo-Sorb E and Permafluor V (Packard Instruments Co.). Liquid scintillation spectroscopy was used to quantify the radioactivity in the plant extracts and the combusted plant tissues.

Thin-Layer Chromatography. A portion of the methanol eluates from the water samples or plant extracts, representing 70,000 dpm (0.03 μCi), was concentrated under nitrogen in a warm-water bath. [^{14}C]MET, *N*-(2-ethyl-6-methylphenyl)-2-hydroxy-*N*-(2-methylethyl)-acetamide, 4-(2-ethyl-6-methylphenyl)-5-methyl-3-morpholinone and the water and plant extracts from the metolachlor-treated systems were spotted on 20-cm by 20-cm glass plates containing a 250-μm layer of normal-phase silica gel 60 F-254. The TLC plates were developed in a hexane/methylene chloride/ethyl acetate (6:1:3 v/v/v) solvent system (*34*). [^{14}C]ATR, [U-ring-^{14}C]deethylatrazine, [U-ring-^{14}C]deisopropylatrazine, [U-ring-^{14}C]didealkylatrazine, [U-ring-^{14}C]hydroxyatrazine, and the reduced water and plant extracts from the atrazine-treated systems were spotted on normal phase silica gel plates and developed in a chloroform/methanol/formic acid/water (100:20:4:2 v/v/v) solvent system (Ciba-Geigy). An ultraviolet lamp (254 nm) was used to locate the nonradiolabeled standards and the location of the radiolabeled standards and extracted compounds was determined by autoradiography using Kodak X-Omat diagnostic film (Eastman Kodak Co., Rochester, NY). The silica gel of

each spot was scrapped into vials containing 5 ml of Ultima Gold scintillation cocktail (Packard Instrument Co., Downers Grove, IL) and the radioactivity in each sample was quantified on a liquid scintillation spectrometer. The ^{14}C mass balance was determined for each system. Percentage of applied ^{14}C in the degradation products was summed and reported as the percentage of applied ^{14}C associated with total degradation products in the water or plant extracts. A report of the individual degradation products and the percentage of applied ^{14}C associated with the individual degradation products will not be discussed in this chapter. Information regarding the degradation products will be written in a paper to be submitted to the journal of *Environmental Toxicology and Chemistry*.

Results

Reduction of Metolachlor and Atrazine in the Water of the Vegetated Incubation Systems. The concentrations of [^{14}C]MET and [^{14}C]ATR were significantly reduced ($p \leq 0.01$) in the water of the vegetated surface water incubations systems. After 16 days, 22.7%, 4.06%, and 1.44% of the applied [^{14}C]MET remained in the water of the vegetated incubation systems that contained *L. minor* (common duckweed), *E. canadensis* (American elodea), and *C. demersum* (coontail), respectively (Figure 1). Sixty-one percent of the applied [^{14}C]MET was detected in the water of the nonvegetated incubation systems. The quantity of the [^{14}C]ATR that remained in the water of the atrazine-treated *E. canadensis* (63.2%) and *C. demersum* (41.4%) vegetated incubation systems were significantly ($p \leq 0.01$) reduced compared with the nonvegetated incubation systems (85.0%) (Figure 2). The water of the *L. minor* incubation systems (84.9%) contained levels of [^{14}C]ATR comparable to the concentrations found in the water of the nonvegetated incubation systems (85.0%). Half-lives of [^{14}C]MET and [^{14}C]ATR in the water of the vegetated and nonvegetated incubation systems were calculated assuming first-order reaction kinetics (Table I). The significant reduction in the concentration of [^{14}C]MET and [^{14}C]ATR in the water of the vegetated incubation systems may be the result of 1) the herbicide attaching to the surface of the plant, 2) the accumulation, sequestering, and degradation of the herbicide in the plant, or 3) the degradation of the herbicides in the water.

Plant Uptake of ^{14}C. Replicates of the metolachlor- or atrazine-treated vegetated incubation system containing either *L. minor* or *C. demersum* were extracted and analyzed immediately following the herbicide treatment (day 0) and 4, 8, 12, and 16 days after the addition of the herbicide. Vegetated incubation systems containing *E. canadensis* were extracted and analyzed on day 0, 4, and 16. After 16 days, less than 25% of the applied ^{14}C was detected in the *L. minor*, *E. canadensis*, or *C. demersum* plants of the metolachlor- or atrazine-treated vegetated incubation systems (Tables II & III). Significantly greater quantities of ^{14}C were associated with the plant tissues of the metolachlor-treated systems compared with the atrazine-treated systems ($p \leq 0.01$), which may be the result of the greater water solubility of metolachlor (metolachlor = 530 mg/L at 20°C, atrazine = 33 mg/L at 27°C). Metolachlor may be more bioavailable and more readily absorbed and translocated in plants than atrazine as a result of its increased water solubility. Plants of the metolachlor-treated *L. minor*, *E. canadensis*, and *C. demersum* systems contained 7.57 ± 0.09%, 20.3 ± 3.07%, and 23.2 ± 0.02% of the applied ^{14}C after 16 days. Aquatic plants from the atrazine-treated systems contained 1.21 ± 0.05%, 11.7 ± 1.06%, and 9.23 ± 1.17% of the applied ^{14}C in the *L. minor*, *E. canadensis*,

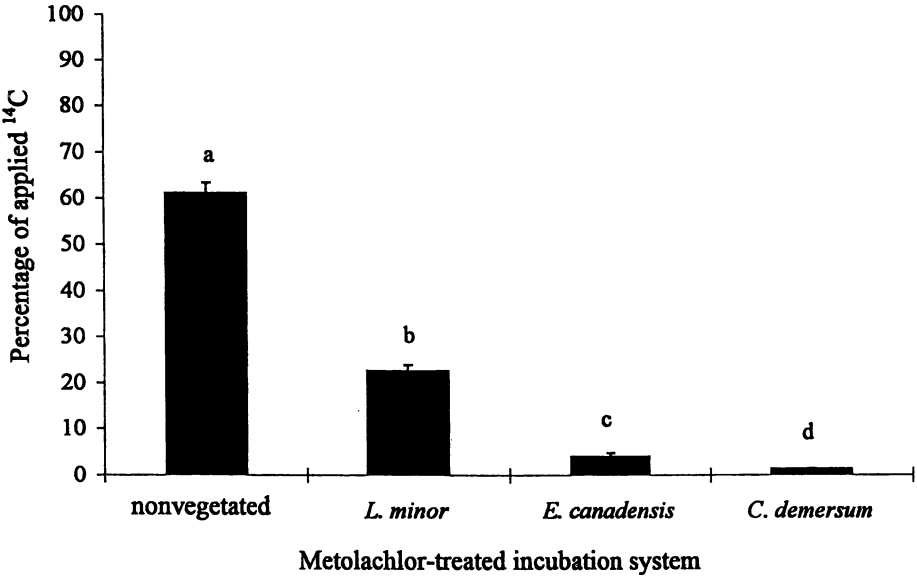

Figure 1. Percentage of applied [^{14}C]metolachlor remaining in the water of the nonvegetated- and vegetated-surface water incubation systems after 16 days.

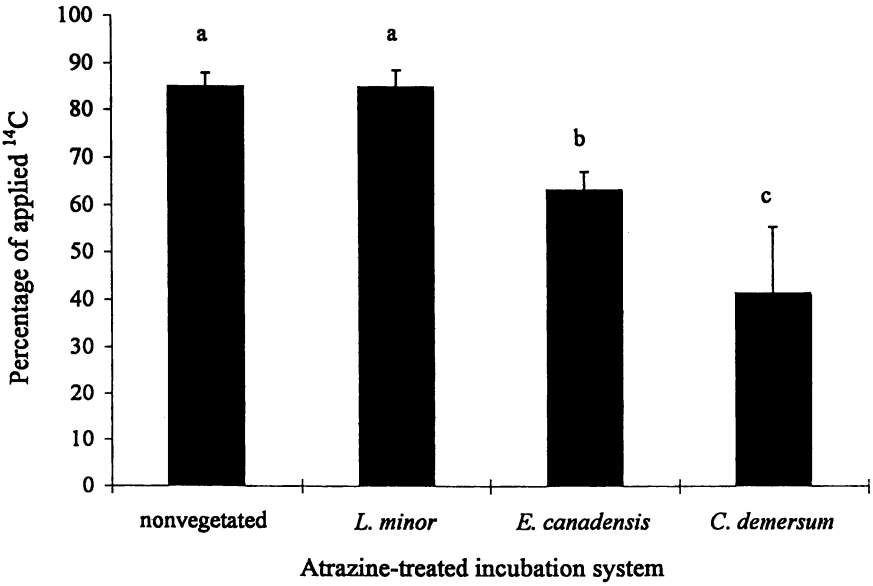

Figure 2. Percentage of applied [^{14}C]atrazine remaining in the water of the nonvegetated- and vegetated-surface water incubation systems after 16 days.

Table I. First-order half-lives of metolachlor and atrazine in the water of the nonvegetated- and vegetated-surface-water incubations systems

Incubation system	Half-life (days)[a]	
	Metolachlor	Atrazine
Nonvegetated	32 ($r^2 = 0.83$)	144 ($r^2 = 0.76$)
Vegetated - *Lemna minor*	8 ($r^2 = 0.95$)	78 ($r^2 = 0.45$)
Vegetated - *Elodea canadensis*	3 ($r^2 = 0.99$)	25 ($r^2 = 0.93$)
Vegetated - *Ceratophyllum demersum*	3 ($r^2 = 0.61$)	12 ($r^2 = 0.73$)

[a]The half-lives were calculated assuming first-order reaction kinetics. The natural log of the percentage of applied [^{14}C]metolachlor or [^{14}C]atrazine remaining in the water was plotted with time (days). Half-lives were calculated based on the percentage of applied [^{14}C]metolachlor or [^{14}C]atrazine remaining in the water. The rates of metolachlor or atrazine metabolism, plant uptake, and the release of the parent compound or its metabolites from the plant are not known. The calculated half-lives do not reflect this rate data.

Kruger et al.; Phytoremediation of Soil and Water Contaminants
ACS Symposium Series; American Chemical Society: Washington, DC, 1997.

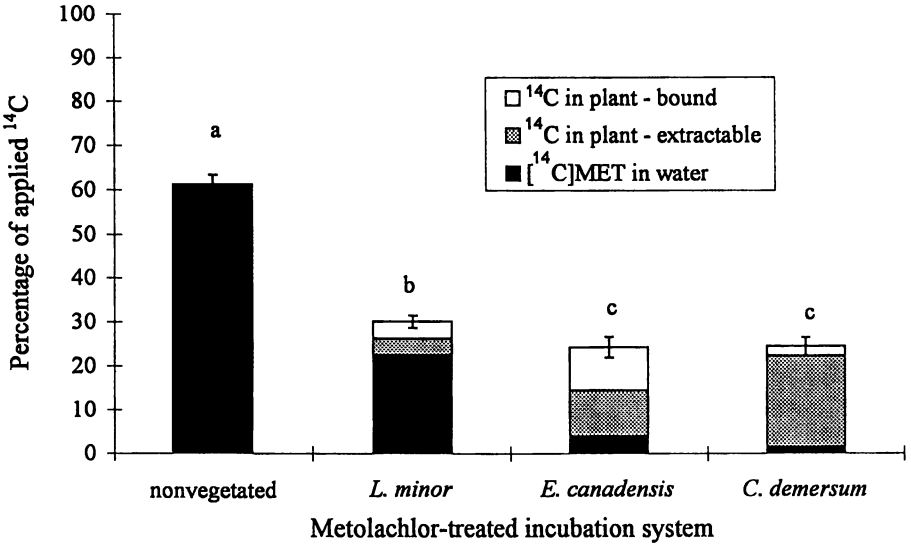

Figure 3. Significance of plant uptake in the reduction of [^{14}C]metolachlor from the water of the vegetated incubation systems. A comparison of the percentage [^{14}C]metolachlor remaining in the water of the nonvegetated incubation system with the summation of the percentage [^{14}C]metolachlor in the water of the vegetated incubation system and the percentage ^{14}C in the plant.

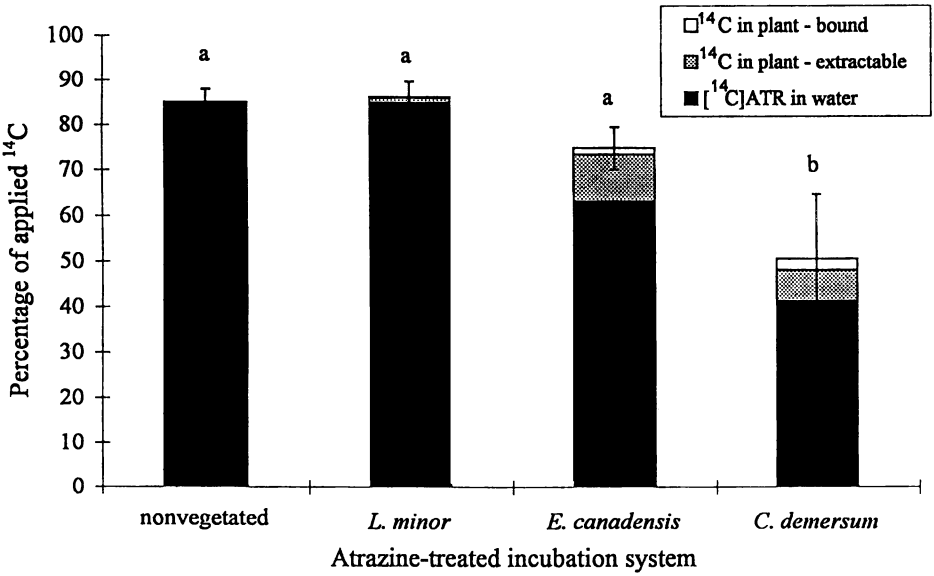

Figure 4. Significance of plant uptake in the reduction of [^{14}C]atrazine from the water of the vegetated incubation systems. A comparison of the percentage [^{14}C]atrazine remaining in the water of the nonvegetated incubation system with the summation of the percentage [^{14}C]atrazine in the water of the vegetated incubation system and the percentage ^{14}C in the plant.

and *C. demersum* systems, respectively. Based on the results of our investigation and the assumption that there was no rapid and significant plant uptake, metabolism, and release of the herbicide degradation products from the plant into the water between the extraction intervals (days 0, 4, 8, 12, and 16), plant uptake of ^{14}C by the aquatic vegetation did not, by itself, account for the significant reduction in the concentrations of [^{14}C]MET detected in the water of the vegetated incubation systems. Examination of the data presented in Figure 3 shows the summation of the percentage of applied [^{14}C]MET remaining in the water of the vegetated incubation systems plus the percentage of the applied ^{14}C associated with the plant tissues (extractable and nonextractable) represents a significantly smaller ($p \leq 0.01$) portion of the applied herbicide than the percentage of applied [^{14}C]MET remaining in the water of the nonvegetated-incubation systems. Similar results were seen in the atrazine-treated *C. demersum* system (Figure 4). These results suggest the significant ($p \leq 0.01$) reductions of [^{14}C]MET in the water of the *L. minor*, *E. canadensis*, and *C. demersum* systems and [^{14}C]ATR in the water of the *C. demersum* system did not occur predominantly as the result of plant uptake and the sequestering of the herbicide in the plant. Additional factors such as the degradation of the herbicide in the water or the degradation of the herbicide in the plant and the subsequent release of the herbicide and degradates into the water seem to be more important. Addition of the ^{14}C percentage in the *E. canadensis* to the percentage of [^{14}C]ATR in the water of the *E. canadensis* system was not significantly different from the percentage of [^{14}C]ATR remaining in the water of the nonvegetated system. This suggests that the accumulation of ^{14}C in the *E. canadensis* and the degradation of [^{14}C]ATR in the water were equally important to the significant reduction of [^{14}C]ATR.

Degradation of Metolachlor and Atrazine in the Water and Plant Tissues. Metolachlor, atrazine, and a number of the degradation products of metolachlor and atrazine were detected in the water extracts and plant extracts of the metolachlor- or atrazine-treated vegetated incubation systems. In the metolachlor-treated *L. minor*, *E. canadensis*, and *C. demersum* systems, the plant extracts and the water extracts contained significantly ($p \leq 0.01$) greater quantities of total ^{14}C (metolachlor and degradates) (*lines*) than [^{14}C]MET (*bars*) (Figure 5). The significantly reduced quantities of [^{14}C]MET relative to the total ^{14}C measured in the water and plant extracts and the detection of metolachlor degradates in these extracts indicate that the significant reduction ($p \leq 0.01$) of the [^{14}C]MET in the water of the vegetated systems occurs, in large part, as a result of degradation. The presence of herbicide degradates in the water and plant extracts may result from 1) the degradation of the herbicide in the water, 2) the degradation of the herbicide in the plant, 3) the degradation of the herbicide in the water and the accumulation of the herbicide degradates in the plant, or 4) the degradation of the herbicide in the plant and the release of the herbicide degradates into the water. Results from these vegetated incubation studies cannot definitively determine the location of the herbicide degradation. Our data (Table II) show significantly greater quantities of metolachlor degradates were found in the water fraction of the vegetated-incubations systems compared with the quantity of total ^{14}C detected in the plants ($p \leq 0.01$). The percentage of applied ^{14}C associated with the metolachlor degradates in the water of the vegetated incubation systems were at least 2.5 times greater than the percentage of applied ^{14}C detected in the plants (extractable and nonextractable) throughout the duration of the incubation. Less than twelve percent of the ^{14}C associated with the plant extracts was identified as [^{14}C]MET. This represents less than one percent of the total applied [^{14}C]MET. These results suggest that either 1) the majority of the

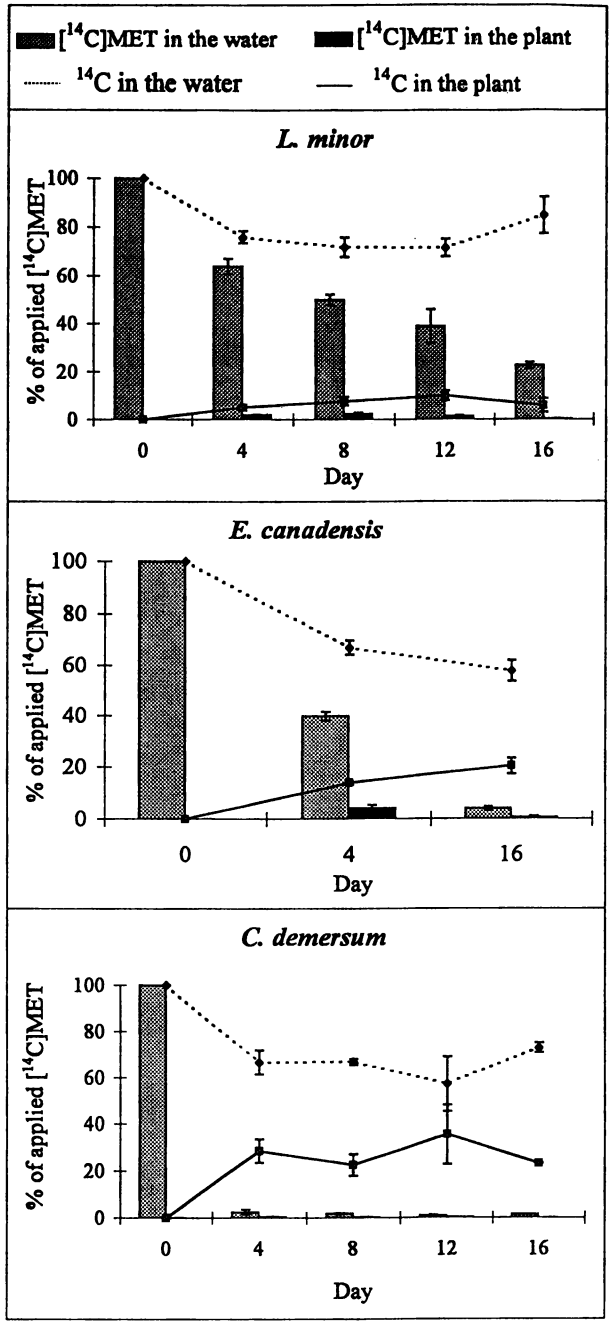

Figure 5. Percentage of applied ^{14}C and [^{14}C]metolachlor detected in the water and plant extracts of the vegetated and nonvegetated metolachlor-treated surface water incubations systems. ([^{14}C]MET = [^{14}C]metolachlor; ^{14}C = total radioactivity (parent + degradates).

Table II. Mass balance of the metolachlor-treated nonvegetated- and vegetated-surface-water incubation systems after sixteen days

	Water			Plant				Mass balance
				Extractable		Unextractable		
Incubation system	[^{14}C]MET[a] (%)[b]	[^{14}C]Degradates (%)[b]	Total [^{14}C] (%)[b]	[^{14}C]MET[a] (%)[b]	[^{14}C]Degradates (%)[b]	[^{14}C]Bound (%)[b]	Total [^{14}C] (%)[b]	Total [^{14}C] (%)[b]
Nonvegetated	61.2 ± 2.29	19.9 ± 2.20	81.1 ± 0.10	----	----	----	----	81.1 ± 0.10
Vegetated - *L. minor*	22.7 ± 1.30	56.2 ± 0.37	78.8 ± 1.64	0.43 ± 0.47	3.19 ± 2.18	3.95 ± 1.80	7.57 ± 0.09	86.4 ± 1.73
Vegetated - *E. canadensis*	4.06 ± 0.79	53.7 ± 3.51	57.7 ± 4.15	0.60 ± 0.52	9.83 ± 1.41	9.91 ± 1.71	20.3 ± 3.07	78.1 ± 7.22
Vegetated - *C. demersum*	1.44 ± 0.07	71.4 ± 2.09	72.9 ± 2.16	0.02 ± 0.02	20.9 ± 2.34	2.20 ± 0.25	23.2 ± 2.18	96.0 ± 4.34

[a][^{14}C]MET = [^{14}C]metolachlor.
[b](%) = percentage of applied ^{14}C.

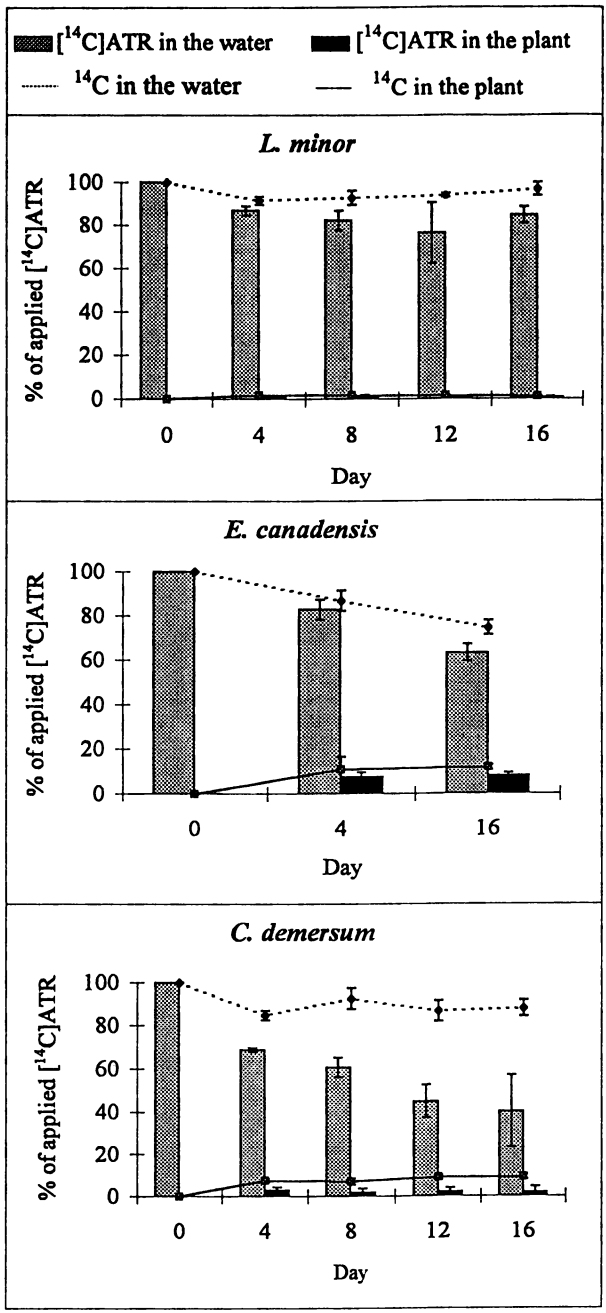

Figure 6. Percentage of applied ^{14}C and [^{14}C]atrazine detected in the water and plant extracts of the vegetated and nonvegetated atrazine-treated surface water incubation systems. ([^{14}C]ATR = [^{14}C]atrazine; ^{14}C = total radioactivity (parent + degradates).

[^{14}C]MET degradation occurred in the water of the metolachlor-treated vegetated incubation system or 2) the herbicides were rapidly taken up into the plants, metabolized, and released into the water solution within the 4-day intervals between the extraction and analysis of the incubation systems. Additional experiments need to be conducted in order to determine if the herbicides are degraded by microorganisms in the water or transformed in the plant and released into the water. In the vegetated and nonvegetated incubation systems we did not account for the mineralization of metolachlor or atrazine to CO_2. Between 78% and 98% of the applied radioactivity was recovered in the metolachlor- and atrazine-treated systems (Tables II & III).

The degradation of [^{14}C]ATR in the vegetated incubation systems primarily occurred in the water phase. With one exception (the day-four water extract in the atrazine-treated *E. canadensis* systems), the percentage of applied [^{14}C]ATR (*bars*) remaining in the water of the vegetated incubation systems was significantly less than the percentage of the total ^{14}C (atrazine and degradates combined) (*lines*) remaining in the water ($p \geq 0.02$) (Figure 6). Less than 12% of the applied ^{14}C was found in the *L. minor*, *E. canadensis*, and *C. demersum* plants throughout the duration of the incubations. The levels of [^{14}C]ATR detected in the plant extracts were not significantly different from the total ^{14}C (extractable and nonextractable) measured in the plants. This indicates that the degradation of [^{14}C]ATR in the plants was minimal, assuming the plant uptake, metabolism, and release of atrazine transformation products was minimal during the 4-d time intervals between the extraction and analysis of the 0, 4, 8, 12, and 16-d incubation systems. With the exception of the *E. canadensis* system, the water of the atrazine-treated vegetated incubation systems contained a significantly ($p < 0.01$) greater quantity of atrazine degradates than the total quantity of ^{14}C that was detected in the plants (extractable and nonextractable) (Table III). The quantity of atrazine degradates in the water of the *L. minor* and *C. demersum* systems was ten times and five times greater, respectively, than the quantity of ^{14}C detected in the *L. minor* and *C. demersum* plants. These data suggest [^{14}C]ATR was predominately degraded in the water rather than in the aquatic plants. The absence of a large accumulation of ^{14}C into the plants preceding a significant decrease in the quantity of radioactivity detected in the plant (extractable and nonextractable) suggests that the degradation of atrazine and metolachlor occurred mostly in the water phase of the incubation system rather than in the plant.

Atrazine Versus Metolachlor. When we compare the atrazine-treated vegetated and nonvegetated systems with the metolachlor-treated vegetated and nonvegetated systems, a greater percentage of the applied herbicide ([^{14}C]ATR or [^{14}C]MET) persisted in the atrazine systems compared with the metolachlor systems (Figures 1 & 2, Tables II & III). A greater percentage of the applied herbicide was characterized as degradates in the water and the plant extracts of all three metolachlor-treated vegetated systems relative to the corresponding atrazine-treated systems (Tables II & III). In addition, metolachlor and/or metolachlor degradates were more readily taken up into the plant or attached to the surface of the plant (total ^{14}C in the plant) than atrazine and its degradates. Based on this investigation, metolachlor was more readily degraded than atrazine. These results agree with the monitoring studies of Goolsby et al. (*3*) and Thurman et al. (*8*); they reported that atrazine was more persistent than metolachlor, alachlor, or cyanazine in the surface waters of the midwestern United States.

Table III. Mass balance of the atrazine-treated nonvegetated- and vegetated-surface-water incubation systems after sixteen days

	Water			Plant				Mass balance
				Extractable		Unextractable		
Incubation system	[^{14}C]ATR[a] (%)[b]	[^{14}C]Degradates (%)[b]	Total [^{14}C] (%)[b]	[^{14}C]ATR[a] (%)[b]	[^{14}C]Degradates (%)[b]	[^{14}C]Bound (%)[b]	Total [^{14}C] (%)[b]	Total [^{14}C] (%)[b]
Nonvegetated	85.0 ± 2.98	8.83 ± 3.71	93.9 ± 5.19	----	----	----	----	93.9 ± 5.19
Vegetated - *L. minor*	84.9 ± 3.73	12.0 ± 1.87	97.0 ± 3.00	0.64 ± 0.48	0.35 ± 0.42	0.22 ± 0.11	1.21 ± 0.05	98.2 ± 3.05
Vegetated - *E. canadensis*	63.2 ± 3.84	11.4 ± 2.78	74.6 ± 3.31	7.90 ± 1.24	2.33 ± 1.03	1.47 ± 0.42	11.7 ± 1.06	86.3 ± 4.37
Vegetated - *C. demersum*	41.3 ± 14.0	46.1 ± 11.3	87.5 ± 3.19	1.82 ± 2.75	4.97 ± 2.38	2.44 ± 0.86	9.23 ± 1.17	96.7 ± 4.36

[a][^{14}C]ATR = [^{14}C]atrazine.
[b](%) = percentage of applied ^{14}C.

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***C. demersum* Versus *E. canadensis* Versus *L. minor*.** The presence of plants and the type of plant can make a significant difference in the quantity of metolachlor or atrazine that remains in the water. Our investigations demonstrated, with the exception of the atrazine-treated *L. minor* system, that the presence of aquatic plants significantly ($p \leq 0.01$) reduced the concentration of [^{14}C]MET and [^{14}C]ATR in the herbicide-contaminated waters (Figures 1 & 2). Lack of a significant difference in the concentration of [^{14}C]ATR in the *L. minor* incubation systems compared with the nonvegetated system may be attributed to the phytotoxicity of atrazine to the *L. minor* (*35, 36*). *C. demersum* was superior in the remediation of the metolachlor- and atrazine-contaminated waters. The herbicide-reduction efficiencies of the aquatic plants were, from most efficient to least efficient, *C. demersum* > *E. canadensis* > *L. minor* for both the metolachlor- and atrazine-treated systems. Degradation seems to be the predominant factor involved in the high herbicide-reduction efficiency of the *C. demersum* system. The quantities of atrazine and metolachlor degradates detected in the water of the vegetated incubation systems were, in descending order, *C. demersum* > *L. minor* = *E. canadensis*. The accumulation of the herbicides in *C. demersum* seemed to play a secondary role to degradation. Herbicide accumulation in the plants followed the order of *C. demersum* = *E. canadensis* > *L. minor* for the metolachlor- and atrazine-treated systems. This may be related to the surface area of the plant exposed to the herbicide-contaminated water. Both the *C. demersum* and *E. canadensis* are submerged aquatic plants whereas *L. minor* is a free-floating aquatic plant. The submerged aquatic plants would have a greater surface area exposed to the herbicide in relation to the floating *L. minor*.

Discussion

The purpose of our investigation was to evaluate the ability of aquatic plants to remediate herbicide-contaminated waters. Our results demonstrated the presence of herbicide-tolerant aquatic plants contributed to the accelerated dissipation of metolachlor and atrazine in the surface water incubation systems.

Aquatic plants can contribute directly or indirectly to the removal of pollutants from water and sediment. Direct interaction of the plant and contaminant would include the uptake and accumulation or metabolism of the xenobiotic compound within the plant. Research has shown that plants contain enzymes that transform and conjugate organic contaminants (*37-39*). Herbicides that are absorbed by herbicide-resistant plants can be transformed and conjugated by these enzymes to degradation products that may be stored in the vacuoles or cell walls of the plant cells (*37, 40*) or released from the plant back into the water. The tolerance of plants to metolachlor is often dependent on the plants' ability to rapidly conjugate metolachlor. In most cases, atrazine-resistant plants contain a different amino acid in the photosynthetic protein that will interfere with atrazine's ability to disrupt electron flow (*33*).

The dissipation of contaminants from water or sediment can be indirectly affected by plants as a result of the accelerated biodegradation of the compound in the phyllosphere or rhizosphere. Plants provide a favorable surface for the attachment of microorganisms (*41-43*), and they supply organic nutrients to epiphytic microorganisms, in the form of photosynthates and exudates, which stimulate microbial growth in the phyllosphere and rhizosphere (*43, 44*). In addition, certain plants can transport oxygen to anaerobic sediments and anoxic waters, which create oxidized microenvironments that stimulate the microbial degradation of organic substances (*45, 46*).

The presented data provide evidence that enhanced degradation is the predominant factor involved in the significant reduction of metolachlor and atrazine from the waters of the vegetated incubation systems. The sequestering of the atrazine or metolachlor or their degradation products in the plant was minimal. Additional experiments need to be conducted to determine if the accelerated degradation occurs as the result of degradation in the plant or as a result of enhanced biodegradation associated with epiphytic microorganisms in the phyllosphere or rhizosphere. Results of this investigation are similar to other phytoremediation studies that report the major mechanism of pollutant removal to be enhanced degradation (*29*, *47*).

Metolachlor was more readily degraded than atrazine in the nonvegetated and vegetated systems. Atrazine may be more recalcitrant to degradation as a result of its chemical structure or bioavailability to microorganisms or plants. Metolachlor has been shown to be primarily degraded by microorganisms in sediments (*10*) and a number of metolachlor degradation products were detected in microbial cultures (*48*, *49*). Laboratory studies have shown that atrazine, in surface water samples or aquatic solutions, was recalcitrant to microbial degradation (*50*). This may be the result of the resistance of the *s*-triazine ring to microbial attack (*51 as cited by 12*). Metolachlor is more water soluble than atrazine and therefore more bioavailable to plants and microorganisms. The greater solubility of metolachlor may account for the increased percentage of applied ^{14}C detected in the plants of the [^{14}C]metolachlor treated systems compared with the [^{14}C]atrazine treated systems. Greater plant uptake and bioavailability of metolachlor to the plants and epiphytic microorganisms contributes to the more rapid degradation of metolachlor compared with atrazine.

Conclusions

Our research has demonstrated that aquatic vegetation may be used to remediate herbicide-contaminated waters. With the exception of the atrazine treated *L. minor* system, concentrations of [^{14}C]MET or [^{14}C]ATR were significantly ($p \leq 0.01$) reduced in the water of the vegetated incubation systems after 16 days. In both the metolachlor- and atrazine-treated systems, the herbicide-reduction efficiencies of the aquatic plants were, from most efficient to least efficient, *C. demersum* > *E. canadensis* > *L. minor*. The results of our investigation suggest the significant ($p \leq 0.01$) reductions of [^{14}C]ATR in the water of the *C. demersum* system and [^{14}C]MET in the water of the *L. minor*, *E. canadensis*, and *C. demersum* systems did not occur predominantly as the result of the absorption and sequestering of the herbicides and their transformation products in the plants. Accelerated biodegradation seems to be more important than plant accumulation and storage to the enhanced dissipation of metolachlor and atrazine from the water of the vegetated systems. Additional experiments need to be conducted with surface-sterilized and non-sterilized plants to confirm whether the accelerated degradation of the herbicides was the result of xenobiotic metabolism in the plant or of enhanced biodegradation of the herbicides in the water do to increased microbial populations in the phyllosphere or rhizosphere of the aquatic plants.

Practical application of this research would be the construction of wetlands and macrophyte-cultured ponds for the phytoremediation of agricultural-drainage effluents from field runoff and tile drains. These aquatic macrophyte systems would provide a relatively maintenance-free and cost-effective means of remediating contaminated effluents before their release into streams, rivers, and lakes. Phytoremediation of wastewater effluents can reduce the levels of contaminants that enter natural waters, which would lessen the adverse impact of

pollutants on aquatic ecosystems, remove unwanted nitrates and pesticides from surface drinking water sources, and help meet public demands for higher water quality.

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