Arch Environ Contam Toxicol (2011) 60:261–271
DOI 10.1007/s00244-010-9596-0

Role of Vegetation in a Constructed Wetland on Nutrient–Pesticide Mixture Toxicity to *Hyalella azteca*

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Received: 29 March 2010 / Accepted: 16 August 2010 / Published online: 3 September 2010

Abstract The toxicity of a nutrient–pesticide mixture in nonvegetated and vegetated sections of a constructed wetland (882 m^2 each) was assessed using *Hyalella azteca* 48-h aqueous whole-effluent toxicity bioassays. Both sections were amended with a mixture of sodium nitrate, triple superphosphate, diazinon, and permethrin simulating storm-event agricultural runoff. Aqueous samples were collected at inflow, middle, and outflow points within each section 5 h, 24 h, 72 h, 7 days, 14 days, and 21 days postamendment. Nutrients and pesticides were detected throughout both wetland sections with concentrations longitudinally decreasing more in vegetated than nonvegetated section within 24 h. Survival effluent dilution point estimates—NOECs, LOECs, and LC_{50}s—indicated greatest differences in toxicity between nonvegetated and vegetated sections at 5 h. Associations of nutrient and pesticide concentrations with NOECs indicated that earlier toxicity (5–72 h) was from permethrin and diazinon, whereas later toxicity (7–21 days) was primarily from diazinon. Nutrient–pesticide mixture concentration–response assessment using toxic unit models indicated that *H. azteca* toxicity was due primarily to the pesticides diazinon and permethrin. Results show that the effects of vegetation versus no vegetation on nutrient–pesticide mixture toxicity are not evident after 5 h and a 21-day retention time is necessary to improve *H. azteca* survival to ≥90% in constructed wetlands of this size.

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Estimated world population in 2009 was ~6.7 billion (US Census Bureau 2009) with population growth projected to exceed 8 billion by 2025 (Yu 2008) and, as a result, the need for food and fiber will also increase. Food and fiber sources can be obtained through agriculture, which provides most of the world's needs for these commodities. However, efficient cultivation of crops requires significant use of both fertilizers and pesticides to maximize crop growth and productivity. Globally, ~109.6 and 41.4 million tons of nitrogen and phosphate fertilizers, respectively, were used in 2007 (FAO 2009). Although necessary, the use of these chemicals can lead to degradation of water quality of rivers, streams, and lakes in proximity to cultivated land. Conservation measures recommended by the US Department of Agriculture include cultural and physical best management practices (BMPs) such as reduced tillage, grass filter strips, vegetated drainage ditches, and constructed wetlands to mitigate soil loss, sedimentation, and degradation of water bodies receiving agricultural runoff (Locke et al. 2008).

Historically, natural wetlands provided multiple functions for physical, chemical, and biological processes affecting compounds such as nutrients and pesticides (Reddy and DeLaune 2008). These processes result in decreased impacts to rivers, streams, and lakes. Natural aquatic vegetation occurring in these wetlands has been shown to improve water quality by trapping and processing contaminants such as pesticides, nutrients, and sediments

(Vymazal 2007). However, there has been a continuing global decrease in the number of natural wetlands, as these marginal lands are converted to more productive cropland (Reddy and DeLaune 2008) and, as a result, these functions cannot be utilized. Because of this, constructed wetlands are increasingly being used as one of several BMPs in the United States to assist in mitigating nutrients and pesticides in agricultural runoff from reaching rivers, streams, and lakes via government programs such as the US Department of Agriculture, Natural Resources Conservation Service, Wetland Reserve Program. Although several studies have examined how constructed wetlands with or without vegetation mitigate toxicity of insecticides singly or in binary mixtures (Bouldin et al. 2007; Schulz et al. 2003; Sherrard et al. 2004), there is little information regarding the effects of complex nutrient–pesticide mixtures on aquatic biota in these systems and the influence aquatic vegetation might have under these conditions (Schulz and Peall 2001).

The purpose of this study was to assess the toxicity of a mixture of two nutrients—nitrogen and phosphorus—and two insecticides—the organophosphate diazinon [*O*,*O*-diethyl 0-2-isopropyl-6-methyl(pyrimidine-4-yl) phosphorothioate] and the pyrethroid permethrin [3-phenoxybenzyl (1*RS*)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate]—in vegetated and nonvegetated sections of a constructed wetland using *Hyalella azteca* 48-h whole-effluent bioassays.

Materials and Methods

Constructed Wetland Design

An experimental wetland was constructed at the University of Mississippi Field Station near Oxford, Mississippi, USA. The constructed wetland was evenly divided and hydraulically isolated with an earthen levee into two sections (882 m^2 each). One section contained no emergent vegetation and the other was naturally vegetated with emergent blunt spikerush (*Eleocharis obtuse*) and sallow sedge (*Carex lurida*) (95%) with small clusters of Vasey's grass (*Paspalum urvillei*) (5%). Both sections had an earthen embankment at the back of the wetland ~0.33 m high allowing for an average 0.16–0.17-m depth and a hydraulic retention time of 5.77 h and 5.59 h at maximum water volume capacity for nonvegetated and vegetated sections, respectively. The wetland slope was 8.6 mm/m (0.86% grade) and 9.3 mm/m (0.93% grade) for nonvegetated and vegetated sections, respectively. Each section had an estimated 12,000–15,000 L at the back of the wetland prior to amendment. Both sections were simultaneously amended over a 5-h period with a mixture of sodium nitrate (886.5 g N), triple superphosphate (270 g P), diazinon [37.62 g active ingredient (a.i.)], and permethrin (0.4104 g a.i.) dissolved in runoff (223,339 L) from a simulated runoff event. Runoff volume was based on that which would be expected from a 32-ha agricultural field generating inflow of ~6.2 L/s for a total volume of 111,670 L of runoff in each section. Contaminant mixture concentrations were determined from an estimated percent runoff from 32 ha for each constituent as follows: nitrogen, 4.5%; phosphorus, 0.97%; diazinon, 0.05%; and permethrin, 0.09% (McDowell et al. 1989; Moore et al. 2008). Water movement was via sheet flow and covered the surface of the inflow and middle sections with no preferential flow areas during the first 2–3 h of amendment and all water was fully impounded within respective sections with no outflow during amendment. Aqueous samples were collected at inflow, middle, and outflow points within each section 5 h, 24 h, 72 h, 7 days, 14 days, and 21 days after amendment began.

Nutrient and Pesticide Analyses

Approximately 250 mL of water were collected at each location during each sampling event, preserved on wet ice, and transported to the USDA-ARS National Sedimentation Laboratory (NSL), Oxford, Mississippi, USA for nutrient analysis. Water samples were analyzed for nitrogen as dissolved inorganic nitrogen (NH_4^+, NO_3^-, and NO_2^-), soluble reactive phosphorus (SRP), and total phosphorus (TP). The cadmium reduction method was used to analyze NO_3^- and NO_2^-, whereas NH_4^+ and SRP were analyzed using the phenate and ascorbic acid methods, respectively, according to standard methods (APHA 2005; Murphy and Riley 1962). TP was determined by persulfate oxidation digestion procedure followed by the ascorbic acid colorimetric method (APHA 2005). All analyses were performed using a ThermoSpectronic Genesys™ 10 ultraviolet (UV) spectrophotometer (Spectronic Instruments, Inc., Rochester, New York, USA) with detection limits of: 0.02 mg/L, NH_4^+; 0.01 mg/L, NO_3^-, NO_2^-, SRP, and TP. Nutrient recovery (%) and precision (RSD%), based on fortified samples, were evaluated. NH_4^+, NO_3^-, and NO_2^- showed acceptable mean recoveries of 95–105% with RSDs from 9.7 to 17.9%; SRP and TP also had good mean recoveries of 99–100% with RSDs from 4.2 to 5.0% based on standard quality assurance/quality control (QA/QC) practices (APHA 2005).

Approximately 500 mL of water were collected at each location during each sampling event, preserved on wet ice, and transported to the USDA-ARS NSL for pesticide analysis. Pesticides were extracted using pesticide-grade ethyl acetate, dried over anhydrous Na_2SO_4, and concentrated to near-dryness by rotary evaporation. The extract was then subjected to silica gel column chromatography cleanup and concentration to 1 mL volume under high-purity dry

nitrogen for gas chromatography (GC) analysis. Pesticide analysis was conducted using an Agilent Model 7890A gas chromatograph (Agilent Technologies, Inc., Waldbronn, Germany) equipped with dual Agilent 7683B series autoinjectors, dual split-splitless inlets, dual-capillary columns, an Agilent ChemStation, and the autoinjector set at 1.0 µL injection volume were used for all targeted pesticide analyses according to Smith and Cooper (2004) and Smith and Lizotte (2007). The Agilent 7890A GC was equipped with two micro electron-capture detectors (µECDs). For diazinon analysis, the analytical column was an Agilent HP 1MS capillary column, 30 m × 0.25 mm inner diameter × 0.25 µm film thickness. Column oven temperatures were: inlet at 85°C for 1 min; ramp at 25–185°C and hold for 20 min. The retention time was 11.20 min. For permethrin, the analytical column was an Agilent HP 1MS capillary column, 30 m × 0.25 mm inner diameter × 0.25 µm film thickness. Column oven temperatures were as follows: inlet at 75°C for 1 min; ramp at 35–230°C and hold for 15 min. Retention times were 15.43 min for *cis*-permethrin and 15.89 min for *trans*-permethrin. The carrier gas used was ultrahigh-purity (UHP) helium at 28 mL/min and the inlet temperature at 250°C. The µECD temperature was 325°C, with a constant makeup gas flow of 60 mL/min UHP nitrogen. Analytical detection limits for diazinon and permethrin were 0.1 µg/L. Pesticide recovery (%) and precision (RSD %), based on fortified samples, were evaluated. Diazinon showed acceptable recoveries of 100–128% with RSDs from 5.4 to 14.2%; permethrin also had good recoveries of 98–117% with RSDs from 1.8 to 10.3% based on standard QA/QC practices (APHA 2005).

Bioassays

Approximately 900 mL of water were collected at each location during each time period, hardness adjusted to about 100 mg $CaCO_3$/L using $CaCl_2$ and $NaHCO_3$, and transported to the USDA–ARS NSL for whole-effluent toxicity analysis. Bioassays using wetland samples were 48-h static nonrenewed aqueous exposures assessing *H. azteca* survival within seven serial dilutions (0.02, 0.10, 0.39, 1.56, 6.25, 25, and 100%) of four replicates each, according to modified US Environmental Protection Agency EPA) (USEPA 2000) protocol for *H. azteca* reference toxicity tests. All bioassays were conducted in a Powers Scientific incubator (Powers Scientific, Inc., Pipersville, Pennsylvania, USA) at 23 ± 1°C with a photoperiod of 16:8 h light:dark. Animals passing a 600-µm stainless-steel mesh sieve but retained by a 425-µm stainless-steel mesh sieve (~1–2 weeks old) were collected for the bioassays. Five *H. azteca* were placed in each replicate 88-mL polypropylene plastic test chamber with one 2-cm × 2-cm square sterile cotton gauze as the substrate. Aqueous exposures consisted of 100 mL hardness adjusted (~100 mg/L as $CaCO_3$) sample and/or control water with seven serial dilutions at 0.25 dilution factor. Control and dilution water, free from priority pollutants, were from a naturally spring-fed pond located at the University of Mississippi Field Station (UMFS) and having the following ranges of measured water quality parameters: dissolved oxygen (mg/L), 4.5–12.6; pH, 5.9–7.3; alkalinity (mg/L as $CaCO_3$), 8–16; hardness (mg/L as $CaCO_3$), 10–30; conductivity (µS/cm), 20.3–25.7; turbidity (NTU), 8.3–25.1; dissolved solids (mg/L), 9–86; suspended solids (mg/L), 0–28; TP (µg/L), 0–81; NH_4 (µg/L), 0–136; NO_3 (µg/L), 42–170; NO_2 (µg/L), 1–15; chlorophyll-*a* (µg/L), 0–23. Because of the very soft nature of the control and dilution water, hardness and alkalinity were adjusted using $CaCl_2$ and $NaCO_3$ at 100 mg/L.

Data Analysis

Statistical analyses were performed on 48-h *H. azteca* survival. Point estimates of no-observed effects effluent dilution fractions (NOECs) and lowest observed effects effluent dilution fractions (LOECs) were determined by analysis of variance (ANOVA) or Kruskal–Wallis (ANOVA on ranks) with Dunnett's multiple range test when appropriate. NOECs were based on the lack of statistically significant differences ($p > 0.05$) relative to controls and LOECs were the lowest effluent dilution fractions that yielded statistically significant differences ($p \leq 0.05$) relative to controls. Estimated 50% lethal effluent dilution fraction effects, LC_{50}s (%) were determined using probit analysis or trimmed Spearman–Karber methods, when appropriate (USEPA 2000). Pearson product moment correlation coefficients were generated to determine associations between log-normal-transformed measured nutrient and pesticide concentrations and log-normal-transformed NOECs at 5–72 h and at 7–21 days to examine temporal changes in concentration–responses. Acute (48–96 h) toxic units (TUs)—the measured concentration divided by the median lethal concentration—described by Pape-Lindstrom and Lydy (1997) were calculated for all components of the nutrient–pesticide mixture in this study. Calculated *H. azteca* TUs were 15.07 µg/L for diazinon (Burkepile et al. 2000), 0.037 µg/L for permethrin (Wheelock et al. 2005), and 39.8 mg/L for NH_4^+ (Ankley et al. 1995). TUs for NO_3^- and NO_2^- were 62.5 mg/L (Camargo et al. 2005) and 2.09 mg/L (Camargo and Alonso 2006) based on the amphipods *Echinogammarus echinosetosus* and *Eulimnogammarus toletanus*, respectively. These were used in lieu of *H. azteca* due to the lack of published effects concentrations. In addition, because there was no available published data on the acute toxicity of SRP or TP on crustaceans, phosphorus was not included in the model.

The sum of all TUs in a mixture provides a toxicity summation (Pape-Lindstrom and Lydy 1997) and a sum TU ($\sum$TU) was calculated for the nutrient–pesticide mixture in both nonvegetated and vegetated wetland sections. Linear regression was performed on log-normal-transformed TUs versus log-normal-transformed LC_{50}s to elucidate the likeliest sources of observed toxicity.

Results

Nutrient and Pesticide Concentrations

Concentrations of ammonium (NH_4^+) ranged from below detection limits to 0.03 mg/L and below detection limits to 0.02 mg/L in nonvegetated and vegetated sections, respectively (Table 1). Concentrations moderately increased after 24 and 72 h in nonvegetated and vegetated sections, respectively. However, no spatial trends in NH_4^+ were evident. Nitrates (NO_3^-) ranged from 0.16 to 2.42 mg/L and from 0.15 to 3.07 mg/L in nonvegetated and vegetated sections, respectively. As with NH_4^+, concentrations similarly decreased after 24 h in both treatment types. Nitrites (NO_2^-) ranged from 0.01 to 0.02 mg/L and from 0.01 to 0.03 mg/L in nonvegetated and vegetated sections, respectively. Again, as with NH_4^+, no spatial trends in NO_3^- were evident.

Concentrations of SRP ranged from below detection limits to 1.48 mg/L and from 0.05 to 2.59 mg/L in nonvegetated and vegetated sections, respectively (Table 1).

Table 1 Aqueous nutrient concentrations (mg/L) in a constructed wetland with nonvegetated and vegetated sections

			Time after amendment commenced					
Nutrient	Type	Location	5 h	24 h	72 h	7 days	14 days	21 days
NH_4^+	Nonvegetated	Inflow	ND	ND	0.02	0.02	0.03	0.03
		Middle	ND	ND	0.02	ND	0.03	ND
		Outflow	ND	ND	0.02	ND	ND	ND
	Vegetated	Inflow	ND	ND	ND	0.02	ND	ND
		Middle	ND	ND	ND	ND	ND	ND
		Outflow	ND	ND	ND	ND	ND	ND
NO_3^-	Nonvegetated	Inflow	1.29	1.56	0.16	0.16	0.17	0.17
		Middle	2.42	1.97	0.17	0.37	0.17	0.18
		Outflow	2.19	2.31	0.20	0.16	0.17	0.16
	Vegetated	Inflow	0.88	3.07	0.18	0.16	0.16	0.15
		Middle	2.02	1.24	0.17	0.16	0.15	0.15
		Outflow	2.69	1.68	0.16	0.16	0.16	0.16
NO_2^-	Nonvegetated	Inflow	0.01	0.02	0.01	0.02	0.02	0.01
		Middle	0.01	0.02	0.01	0.01	0.01	0.01
		Outflow	0.02	0.02	0.02	0.01	0.02	0.01
	Vegetated	Inflow	0.01	0.01	0.01	0.01	0.01	0.01
		Middle	0.02	0.02	0.01	0.01	0.01	0.01
		Outflow	0.02	0.03	0.01	0.01	0.01	0.02
SRP	Nonvegetated	Inflow	0.73	0.71	0.24	0.02	0.05	0.03
		Middle	1.48	0.34	0.14	0.08	0.04	0.03
		Outflow	1.13	0.25	0.11	0.02	0.01	ND
	Vegetated	Inflow	0.71	2.59	0.95	0.06	0.12	0.15
		Middle	1.79	2.20	0.95	0.12	0.13	0.06
		Outflow	1.10	0.41	0.12	0.07	0.14	0.05
TP	Nonvegetated	Inflow	1.02	1.46	1.46	1.02	1.42	1.87
		Middle	2.45	1.39	0.74	0.73	1.17	0.97
		Outflow	1.83	1.39	1.34	1.06	0.55	1.02
	Vegetated	Inflow	0.79	3.12	1.59	0.91	3.02	3.09
		Middle	2.38	2.26	1.53	0.94	2.93	2.19
		Outflow	2.06	1.59	1.48	0.27	2.41	1.81

ND below detection limit: 0.02 mg/L, NH_4^+; 0.01 mg/L, NO_3^-, NO_2^-, SRP, and TP

SRP concentrations decreased more in nonvegetated than vegetated sections and both treatment types similarly decreased from inflow to outflow and over time. TP ranged from 0.55 to 2.45 mg/L and from 0.27–3 to 12 mg/L in nonvegetated and vegetated sections, respectively. Moderate decreases in TP concentrations from inflow to outflow occurred during four sampling periods.

Diazinon concentrations ranged from 3.1 to 121.9 μg/L and from 2.2 to 128.2 μg/L in nonvegetated and vegetated sections, respectively (Table 2). Diazinon concentrations similarly decreased continuously throughout the 21-day observation period in both treatment types. Diazinon concentrations decreased from inflow to outflow only within the vegetated section during the 5-h time period. Otherwise, no spatial patterns in diazinon were evident. *Cis*-permethrin ranged from below detection limits to 4.2 μg/L and from below detection limits to 3.6 μg/L in nonvegetated and vegetated sections, respectively (Table 2). Similarly, *trans*-permethrin ranged from below detection limits to 3.6 μg/L and from below detection limits to 3.0 μg/L in nonvegetated and vegetated sections, respectively. Permethrin concentrations decreased within 72 h of amendment in both treatment types. As with diazinon, permethrin decreased from inflow to outflow within the vegetated section during the 5-h time period. Thereafter, spatial patterns in permethrin were not as evident.

Bioassay Responses

Water quality data were within parameters for acute aqueous bioassays according to the USEPA (2000) protocol for *H. azteca* reference toxicity tests. Water quality for the toxicity tests with field site water were as follows: temperature (°C), 22.7–23.2; dissolved oxygen (mg/L), 6.2–7.5; pH, 7.6–8.0; alkalinity (mg/L as $CaCO_3$), 54.2–74.1; hardness (mg/L as $CaCO_3$), 68.4–130.9; conductivity (μmhos/cm), 310.0–455.0; turbidity (NTU), and 7.4–37.6. *Hyalella azteca* survival in all controls (0% effluent) was ≥90% for all 48-h bioassays (Fig. 1). Within the first 5 h of amendment, ≤5% survival occurred in 1.56% effluent samples at all sites in the nonvegetated section (Fig. 1a–c), whereas 0, 15, and 70% survival occurred in 1.56% effluent samples at inflow, middle, and outflow, respectively, in the vegetated section (Fig. 1d–F). Resulting NOECs, LOECs, and LC_{50}s showed similar toxicities throughout the nonvegetated section, whereas the vegetated section had a decrease in toxicity with a 10-fold increase in LC_{50} values from inflow to outflow. Comparisons of nonvegetated versus vegetated at the outflow showed a greater than 10-fold difference in LC_{50} values (Table 3). By 24 h, differences in toxicity within and among vegetation types were less evident. Although NOECs ranged from 0.10 to 1.56%, LC_{50}s only ranged from 1.05 to 2.41% (Table 3) and dose–response curves

Table 2 Aqueous diazinon and permethrin concentrations (μg/L) in a constructed wetland with non-vegetated and vegetated sections

			Time after amendment commenced					
Pesticide	Type	Location	5 h	24 h	72 h	7 days	14 days	21 days
Diazinon	Nonvegetated	Inflow	114.2	86.6	36.5	17.5	3.7	3.1
		Middle	121.9	71.4	51.9	16.1	4.2	3.2
		Outflow	104.3	84.2	41.5	14.1	7.6	3.3
	Vegetated	Inflow	107.3	83.7	34.0	16.4	5.3	4.1
		Middle	128.2	106.2	60.5	19.4	8.3	2.6
		Outflow	80.9	76.7	44.3	28.5	6.2	2.2
Cis-permethrin	Nonvegetated	Inflow	2.9	0.6	ND	ND	ND	ND
		Middle	2.2	0.2	0.1	ND	0.1	ND
		Outflow	4.2	0.3	0.1	ND	ND	ND
	Vegetated	Inflow	3.6	0.4	0.2	ND	ND	0.1
		Middle	2.6	0.3	0.1	ND	ND	ND
		Outflow	0.5	0.1	ND	ND	ND	ND
Trans-permethrin	Nonvegetated	Inflow	2.5	0.4	ND	ND	ND	0.1
		Middle	1.8	0.1	ND	ND	0.1	ND
		Outflow	3.6	0.2	0.1	ND	ND	ND
	Vegetated	Inflow	3.0	0.3	0.1	ND	ND	ND
		Middle	2.2	0.2	0.1	0.1	ND	ND
		Outflow	0.3	0.1	0.2	ND	ND	ND

ND below detection limit of 0.1 μg/L

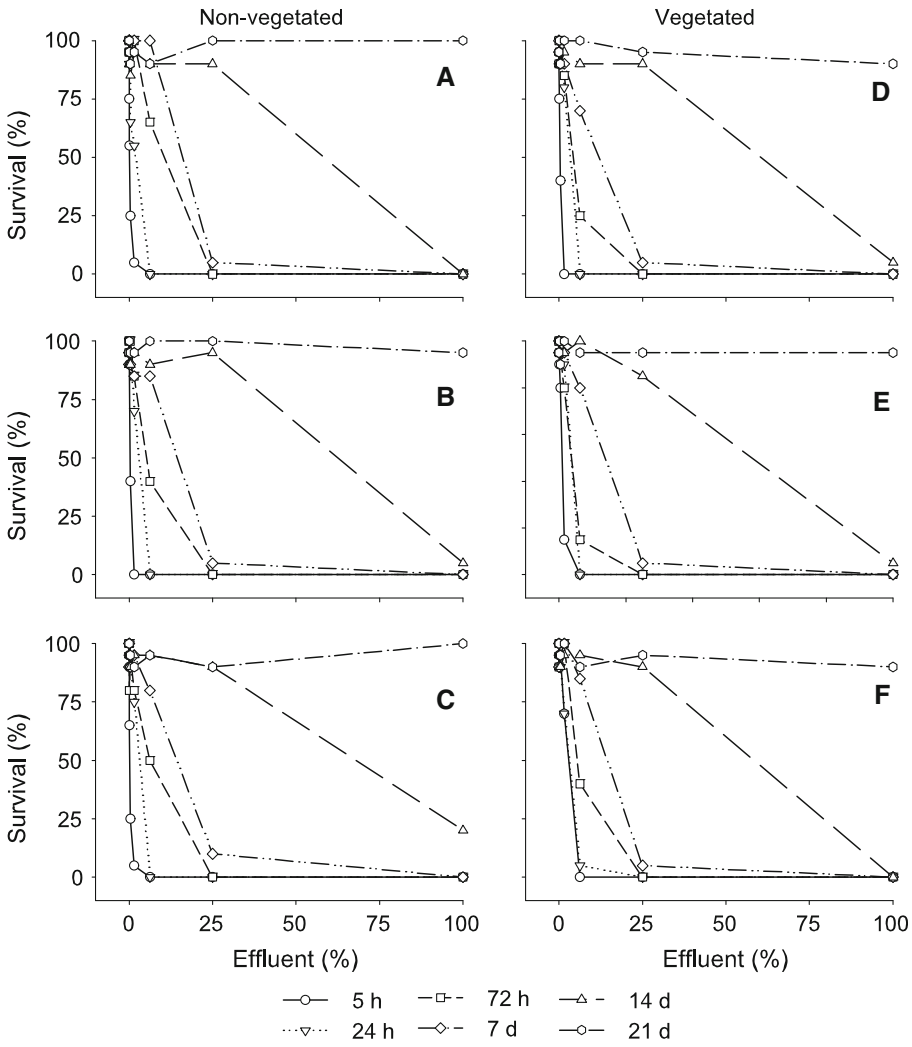

Fig. 1 *Hyalella azteca* 48-h exposure–response curves for nutrient–pesticide mixture effluent from nonvegetated: (**a** inflow, **b** middle, **c** outflow) and vegetated (**d** inflow, **e** middle, **f** outflow) sections of a constructed wetland

were similar (Fig. 1). At 72 h, NOECs and LOECs were very similar across vegetation types and locations, whereas LC_{50}s ranged from 8.85% at nonvegetated inflow to 2.54% at the vegetated midpoint (threefold difference). *Hyalella azteca* survival end points were increasingly similar spatially from 7 to 21 days after amendment, with no apparent differences in toxicity across vegetation types (Table 3). Toxicity of both nonvegetated and vegetated sections at all sites decreased over time, as shown in Table 3 and Fig. 1, and within 21 days, survival was ≥90% at 100% effluent for all samples.

Nutrient, Pesticide, and Bioassay Associations

Correlation analysis revealed significant associations with the amended nutrient–pesticide mixture in both nonvegetated and vegetated sections of the constructed wetland. For nutrients amended to the nonvegetated section, overall significant associations of *H. azteca* 48-h NOECs and concentrations of dissolved inorganic nitrogen, NH_4^+ and NO_3^-, and phosphorus as SRP occurred (Table 4). Within the first 72 h of amendment, NOECs were significantly associated with NH_4^+, NO_3^-, and SRP, but within the next 3 weeks, no significant association with any nutrients was evident. Nutrients amended to the vegetated section had significant overall associations between NOECs and NO_3^- and SRP only. Within the first 72 h postamendment, no significant associations between NOECs and nutrients was evident, and within the next 3 weeks, only NH_4^+ was significantly associated with NOECs (Table 4). For pesticides amended to both nonvegetated and vegetated sections, overall significant negative associations of NOECs and concentrations of diazinon and permethrin (*cis*, *trans*, and sum) were evident. A similar pattern occurred within 5–72 h of amendment in the nonvegetated section. However, no significant association of NOECs with diazinon occurred in the vegetated section during this time period. For the 7–21-day time period in both nonvegetated and vegetated sections, only diazinon concentrations were significantly associated with NOECs (Table 4).

Table 3 *Hyalella azteca* 48-h survival NOEC, LOEC, and LC_{50} values (%) exposed to nutrient–pesticide mixed effluent from nonvegetated and vegetated sections of a constructed wetland

		Nonvegetated			Vegetated		
Time	End point	Inflow	Middle	Outflow	Inflow	Middle	Outflow
5 h	NOEC	0.02	0.10	0.02	0.10	0.39	0.39
	LOEC	0.10	0.39	0.10	0.39	1.56	1.56
	LC_{50}	0.10	0.34	0.16	0.20	0.61	2.05
24 h	NOEC	0.10	0.39	0.39	0.39	1.56	1.56
	LOEC	0.39	1.56	1.56	1.56	6.25	6.25
	LC_{50}	1.03	1.79	1.98	1.92	2.37	2.41
72 h	NOEC	1.56	1.56	0.39	1.56	0.39	1.56
	LOEC	6.25	6.25	1.56	6.25	1.56	6.25
	LC_{50}	8.85	4.42	3.35	3.38	2.54	5.31
7 days	NOEC	6.25	6.25	6.25	1.56	6.25	6.25
	LOEC	25	25	25	6.25	25	25
	LC_{50}	12.5	9.83	10.46	6.75	9.92	10.72
14 days	NOEC	25	25	25	25	25	25
	LOEC	100	100	100	100	100	100
	LC_{50}	41.27	50.67	55.20	50.00	46.50	40.17
21 days	NOEC	100	100	100	100	100	100
	LOEC	>100	>100	>100	>100	>100	>100
	LC_{50}	>100	>100	>100	>100	>100	>100

Table 4 Pearson product moment correlation coefficients (r) of *H. azteca* 48-h survival versus nutrients and pesticides in nonvegetated and vegetated sections of a constructed wetland for the first three sampling periods (5–72 h), the last three sampling periods (7–21 days), and all six sampling periods (All)

	Nonvegetated NOEC			Vegetated NOEC		
Pesticide or nutrient	5–72 h ($n = 9$)	7–21 days ($n = 9$)	All ($n = 18$)	5–72 h ($n = 9$)	7–21 days ($n = 9$)	All ($n = 18$)
NH_4^+	0.817**	0.200	0.701**	−0.250	−0.802**	0.431
NO_3^-	−0.695*	−0.346	−0.805***	−0.293	−0.490	−0.654**
NO_2^-	0.197	−0.127	−0.147	0.506	0.201	−0.213
SRP	−0.792*	−0.428	−0.839***	−0.294	0.093	−0.741***
TP	−0.382	0.348	−0.386	0.312	0.663	0.241
Diazinon	−0.833**	−0.931***	−0.962***	−0.521	−0.895**	−0.942***
Cis-permethrin	−0.910***	−0.233	−0.781***	−0.762*	0.296	−0.630**
Trans-permethrin	−0.903***	0.387	−0.739***	−0.748*	−0.088	−0.616**
$\sum$Permethrin	−0.908***	0.149	−0.762***	−0.757*	0.090	−0.625**

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Although correlation does not imply causation, an attempt to ascertain likely causes of observed toxicity was done using a TU model approach as previously described to assess mixture toxicity. Linear regression models constructed for the nonvegetated section showed that diazinon TUs explained >83% of the LC_{50} variation followed by permethrin TUs, NO_3^- TUs, and NH_4^+ TUs. The $\sum$TU model explained >86% of the LC_{50} variation (Fig. 2). Based on calculated TUs used in these models, primary toxicity originated from the two insecticides diazinon and permethrin, with nitrogen well below toxic concentrations. Linear regression models constructed for the vegetated

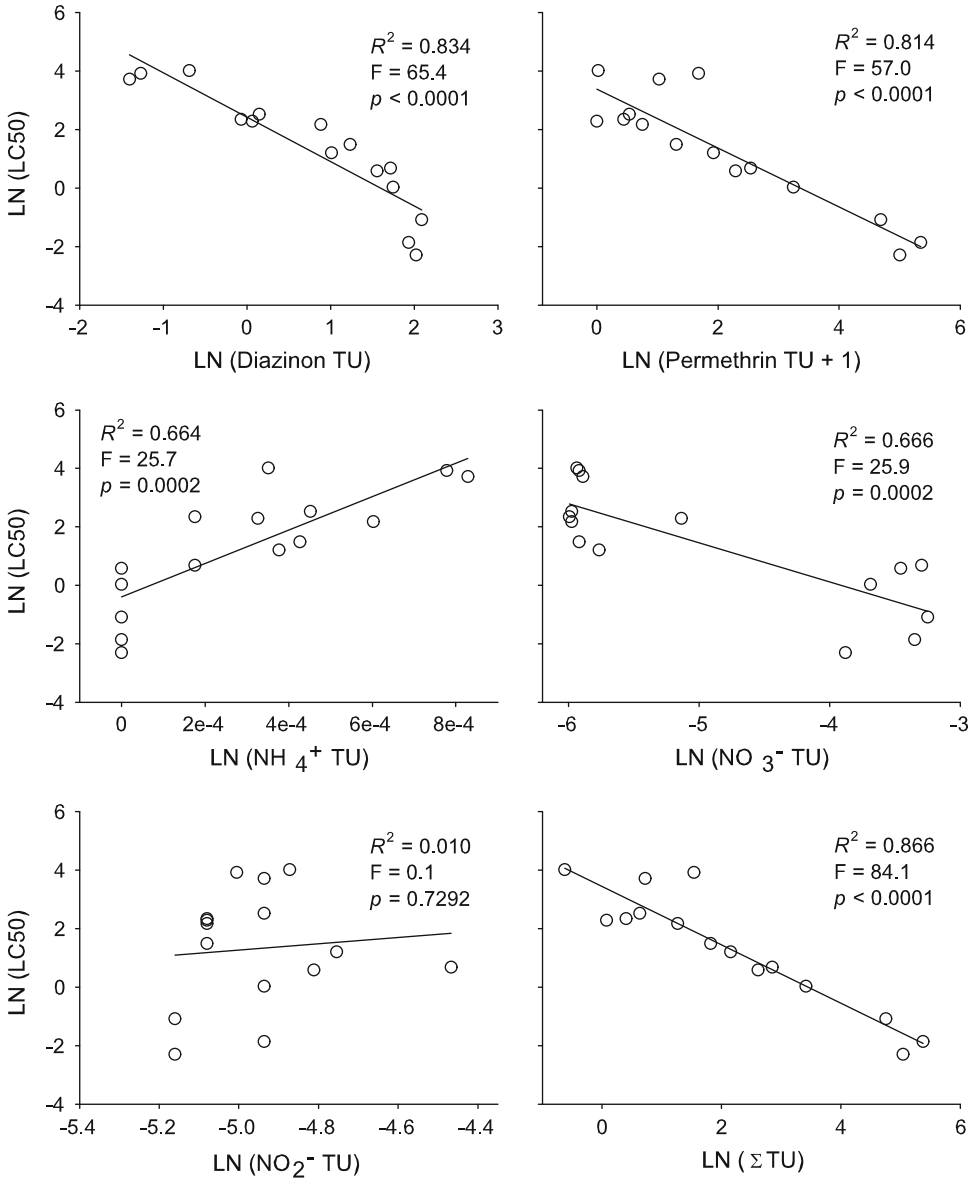

Fig. 2 Linear regression relationships ($N = 15$) between 48-h *H. azteca* LC_{50} and pesticide TUs, nutrient TUs, and $\sum$TU in the nonvegetated section of a constructed wetland

section showed similar patterns with diazinon TUs, explaining >85% of the LC_{50} variation followed by permethrin TUs and NO_3^- TUs. The $\sum$TU model explained 93% of the LC_{50} variation (Fig. 3). Again, primary toxicity originated from both insecticides, with NO_3^- well below toxic concentrations.

Discussion

Few studies have examined how efficiently constructed wetlands mitigate aqueous-phase nutrient–pesticide effluent mixture toxicity (Hunt et al. 2008; Schulz and Peall 2001). The present study observed significant decreases in aqueous-phase nitrogen and phosphorus concentrations within 7 days of amendment and is comparable with a number of studies examining the dissipation of nutrients from the wetland aqueous phase (Hunt et al. 2008; López-Flores et al. 2003; Vymazal 2007). A comprehensive review by Vymazal (2007) focused on key components necessary in constructed wetlands to maximize nutrient removal, primarily hydrology and vegetation type. The current study examined the influence of vegetation type while hydrology was constant.

Aqueous-phase diazinon concentrations dissipated within 7–14 days of amendment in both vegetated and nonvegetated sections and aqueous-phase permethrin concentrations dissipated within 72 h. These results are comparable with results previously reported by Moore et al. (2007, 2008, 2009a, b) and Bouldin et al. (2007) using emergent wetland vegetation. However, Hunt et al. (2008) did not observe this pattern for diazinon within constructed

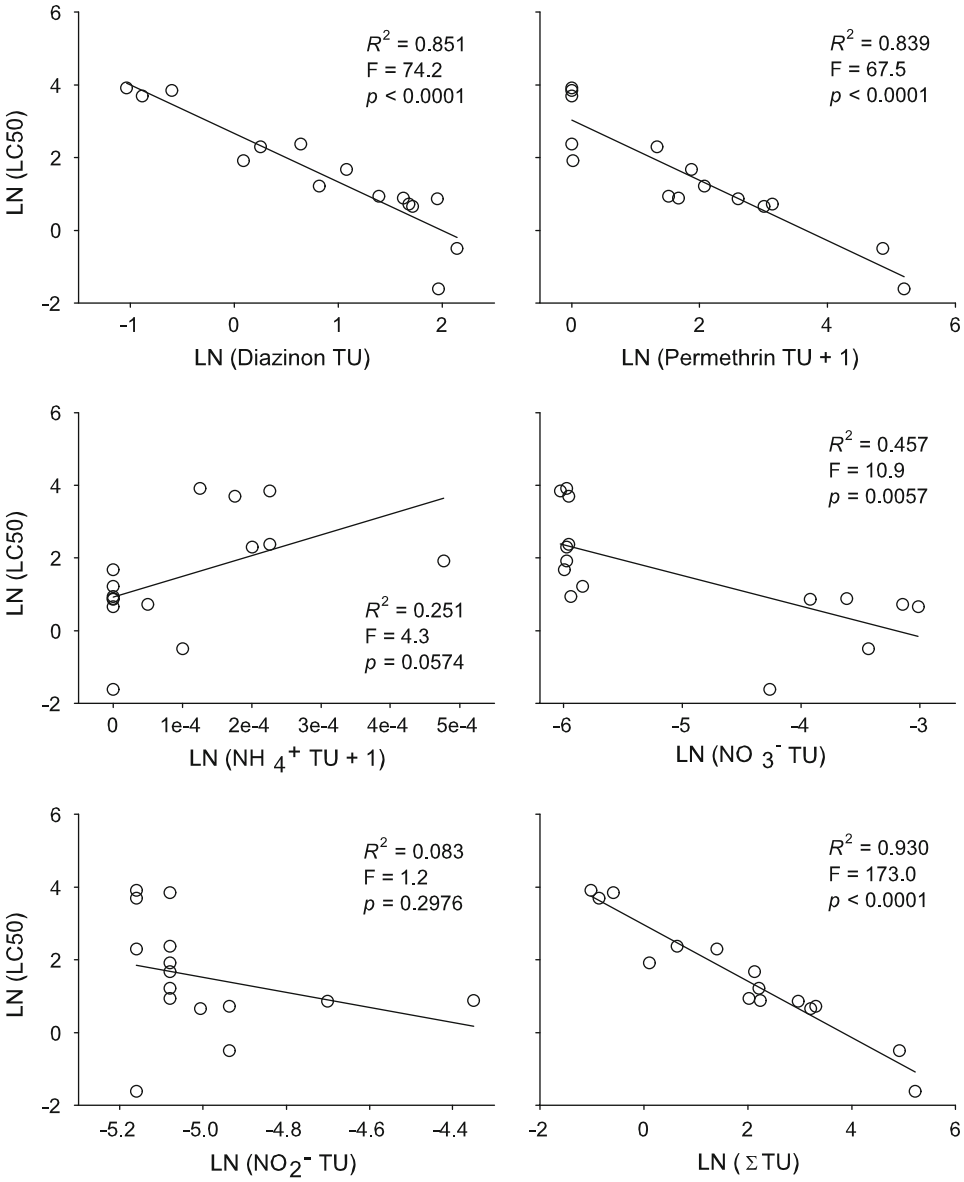

Fig. 3 Linear regression relationships ($N = 15$) between 48-h *Hyalella azteca* LC_{50} and pesticide TUs, nutrient TUs, and $\sum$TU in the vegetated section of a constructed wetland

sediment retention ponds vegetated with floating pennywort (*Hydrocotyle ranunculoides*). Because permethrin, a pyrethroid, is highly hydrophobic, it will rapidly (within hours) dissipate from the water column and sorb to organic and inorganic materials in contact with the water column (Allan et al. 2005; Sharom and Solomon 1981).

Nutrient–pesticide effluent toxicity to *H. azteca* after 48-h exposures varied spatially between vegetated and nonvegetated sections only within the 5-h sample. However, this difference in effluent toxicity between vegetated and nonvegetated section disappeared by 24 h. This is in contrast with other studies in which vegetation was observed to significantly and continuously decrease effluent toxicity compared with nonvegetation (Moore et al. 2009b; Schulz et al. 2003). Both Schulz et al. (2003) and Moore et al. (2009b), however, only assessed a single insecticide and did not include any nutrient amendment with either study. Nutrients amended to shallow aquatic systems with little or no vegetation often quickly lead to eutrophication of these systems (Scheffer 2004). Rapid eutrophication was indirectly observed in this study (via visual inspection) with the presence of algal blooms within the nonvegetated section by 72 h after amendment. Under eutrophic conditions, strongly hydrophobic insecticides such as pyrethroids can become significantly less toxic within 24 h (Smith and Lizotte 2007). As a result, mixtures of nutrients and insecticides can indirectly interact with one another and mitigate insecticide toxicity in shallow wetlands comparable with heavily vegetated wetlands. The direct effects of nutrients on aquatic crustaceans (e.g., *Ceriodaphnia dubia*, *Daphnia magna*, *Hyalella azteca*, etc.) potentially inhabiting constructed wetlands have not been well assessed (Camargo et al. 2005;

Camargo and Alonso 2006; Huddleston et al. 2000; Spieles and Mitsch 2000). Although phosphorus is not considered directly toxic to animals (Carpenter et al. 1998), this nutrient is a key component causing eutrophication and hypereutrophication, increasing algal blooms and other indirect negative effects. Various nitrogen species can cause direct and/or indirect effects on aquatic crustaceans exposed to constructed wetland effluents. Ammonia toxicity to *C. dubia* has been implicated in constructed wetland water samples during tertiary treatment of petroleum refinery effluent (Huddleston et al. 2000). Spieles and Mitsch (2000) observed significant decreases in invertebrate community indexes within constructed wetlands receiving nutrient-loaded municipal wastewater, creating hypereutrophic conditions and hypoxia.

Observed toxicity in the present study was associated with both nutrients and pesticides. However, correlation does not imply causation. Correlation in conjunction with known contaminant concentrations and known published effects concentrations provide a weight-of-evidence approach (Burkhardt-Holm and Schuerer 2007) in determining which compounds elicited the observed *H. azteca* mortality. In the present study, concentrations of both diazinon and permethrin were highly inversely correlated ($p < 0.01$) with *H. azteca* survival responses (LC_{50}s) regardless of vegetation type. Pesticide correlations were consistent with known published exposure response curves (Burkepile et al. 2000; Wheelock et al. 2005). In comparison, correlations with concentrations of varying nutrients were not as consistent with both positive and negative correlations with *H. azteca* LC*50*s (e.g., NH_4^+). Additionally, TUs derived from known published effects concentrations were much greater for both insecticides diazinon and permethrin where these TU values were nearly always >0.5 when significant mortality was observed. In contrast, NO_3^-, NO_2^-, and NH_4^+ TUs were always ≤0.05 regardless of observed mortality. Based on this approach, the pesticides diazinon and permethrin were the likely cause of observed mortality. Nutrients (specifically dissolved inorganic nitrogen) provided minimal if any additional toxicity, as ≥80% of the measured nitrogen was in the least toxic form, NO_3^-, with the most toxic form, NH_3, below detection limits.

Our results show that a 882-m^2 wetland with or without rooted emergent vascular vegetation and dosed with nitrogen, phosphorus, diazinon, and permethrin can induce toxicity in *H. azteca* for up to 3 days for permethrin and 14 days for diazinon. Additionally, results suggest that the effects of vegetation versus no vegetation on diazinon and permethrin toxicity to *H. azteca* showed that vegetation was more effective than no vegetation in mitigating toxicity at 5 h, but these differences were not evident after 5 h with the addition of nitrogen and phosphorus. Finally, wetlands of this size should impound influent agricultural runoff containing these contaminants for up to 21 days to fully mitigate potential ecological impacts on receiving streams, rivers or lakes.

Acknowledgments The authors wish to thank Lisa Brooks, James Hill, and Renee Russell for analytical assistance. Mention of equipment, computer programs, or a pesticide neither constitutes an endorsement for use by the US Department of Agriculture nor does it imply pesticide registration under FIFRA as amended. All programs and services of the USDA are offered on a nondiscriminatory basis without regard to race, color, national origin, sex, marital status, or handicap.

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