Water Air Soil Pollut (2011) 220:69–79
DOI 10.1007/s11270-010-0735-z

Effects of Vegetation in Mitigating the Toxicity of Pesticide Mixtures in Sediments of a Wetland Mesocosm

Richard E. Lizotte Jr. · Matthew T. Moore · Martin A. Locke · Robert Kröger

Received: 27 August 2010 / Accepted: 21 December 2010 / Published online: 13 January 2011

Abstract This study assessed effects of a mixture of two pesticides, diazinon and permethrin, on 48-h sediment toxicity to *Hyalella azteca* in a constructed wetland mesocosm containing non-vegetated and vegetated sections. Sediment samples were collected at inflow, middle, and back points within each section 5, 24, 72 h, 7, 14, and 21 days post-amendment. Pesticides were detected in sediments throughout non-vegetated and vegetated wetland sections. *H. azteca* 48-h survival varied across sampling period, wetland location, and vegetation type with lowest survival occurring within the first 72 h of the inflow and middle locations of the non-vegetated section. Sediment toxicity was ameliorated by 14 and 7 days within the non-vegetated and vegetated sections, respectively. Relationships between pesticide concentrations and animal survival indicated toxicity was from both diazinon and *cis*-permethrin in the non-vegetated section and primarily *cis*-permethrin in the vegetated section. Results show that vegetation ameliorated pesticide mixture 48-h sediment toxicity to *H. azteca* earlier and to a greater extent than non-vegetated constructed wetlands. A 21-day retention time is necessary to improve 48-h *H. azteca* sediment survival to ≥90% in wetlands of this size.

Keywords Diazinon · Permethrin · Constructed wetland · *Hyalella azteca*

R. E. Lizotte Jr. (✉) · M. T. Moore · M. A. Locke
USDA-ARS National Sedimentation Laboratory,
P.O. Box 1157, Oxford, MS 38655, USA
e-mail: Richard.lizotte@ars.usda.gov

R. Kröger
Department of Wildlife, Fisheries and Aquaculture,
Mississippi State University,
P.O. Box 9690, Starkville, MS 39762, USA

1 Introduction

Agriculture is essential in providing the necessary food and fiber for the global human population. Chemicals such as pesticides allow increased efficiency and productivity of crops by controlling plant and animal pests (Cooper and Dobson 2007). In conjunction with these benefits, agriculture is also a considerable non-point source of organic contaminants such as pesticides entering into aquatic systems (Reddy and DeLaune 2008). Constructed wetland mesocosms acting as processing buffers within a broader suite of agricultural best management practices can be used to help mitigate pesticide runoff from agricultural fields into receiving systems (e.g., lakes, rivers, streams) (Locke et al. 2008). These wetland systems have reduced concentrations of non-point source agricultural insecticides such as organophosphates and pyrethroids (Bouldin et al. 2005; Hunt et al. 2008; Moore et al. 2009). Such wetlands also have important functions in enhancing the water quality and ecological values of downstream receiving waters (Reddy and DeLaune 2008). Different compartments

(i.e., aqueous and sediment) have separate roles, as either sinks or sources, in determining effectiveness of wetlands in mitigating pesticide toxicity (Moore et al. 2007). For these reasons, the importance of elucidating potential effectiveness of wetlands in reducing pesticide toxicity to aquatic biota exposed to these different phases needs to be addressed.

Diazinon (*O*,*O*-diethyl *O*-[2-isopropyl-6-methyl-4pyrimidinyl] phosphorothioate) and permethrin [(3-phenoxyphenyl) methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane carboxylate] were used as model contaminants in a constructed wetland mesocosm designed to assess mitigation of runoff from an agricultural field. Diazinon is an organophosphate insecticide used on a variety of agricultural crops such as fruits, vegetables, and field crops for control of soil insects and other pests (USEPA 2004). Approximately 163,000 kg of diazinon as active ingredient (a.i.) was applied to agricultural crops in five states within the USA during 2006 with greatest applications occurring in California on lettuce crops (USDA NASS 2010). Diazinon can persist in aquatic environments for up to 6 months with a half-life of 39 days in treated river water under summer-time conditions (USEPA 2005). Due to the relatively high water solubility of diazinon, this pesticide does not partition to sediment in the aquatic environment as readily as more hydrophobic organophosphates (e.g., chlorpyrifos) and diazinon can desorb back into the aqueous phase more rapidly (Sharom et al. 1980; Zambonin et al. 2002). Permethrin is a type I (i.e., ester of chrysanthemic acid dihalovinyl analogue absent a cyano group at the α carbon position of the alcohol moiety) synthetic pyrethroid insecticide occurring as a racemic mixture of the *cis* and *trans* isomers and is a restricted use pesticide for vector (mosquito) control and agricultural fruit and vegetable crops due to its high toxicity to aquatic organisms (USEPA 2009). According to USDA NASS (2010), in 2006, more than 33,600 kg permethrin (a.i.) was applied to agricultural crops in 13 states within the USA, with greatest applications occurring in California on lettuce crops, similar to diazinon. Permethrin is an environmentally persistent pyrethroid with a half-life of 113–175 days in aquatic environments under anaerobic conditions. Due to its relatively low water solubility and hydrophobic properties, permethrin can rapidly partition to the sediment phase in aquatic environments (USEPA 2009). Hunt et al. (2006) and Zhang et al. (2008) observed both diazinon and permethrin to co-occur in stream water and sediment samples, respectively, in agricultural watersheds of California. As a result, both pesticides have the potential to contribute to non-point source contamination of aquatic environments (Anderson et al. 2003, 2006). For these reasons, there is a potential risk to benthic and epibenthic aquatic invertebrates from exposure to sediment-associated diazinon and permethrin mixtures.

Previous research has demonstrated how constructed wetlands with or without vegetation mitigate surface water (aqueous) toxicity of insecticides singly or in binary mixtures (Schulz et al. 2003a; Sherrard et al. 2004; Bouldin et al. 2007). However, there is less information regarding the effects of pesticide mixtures on sediment toxicity within these systems (Moore et al. 2007). The purpose of this study was to assess the use of a wetland mesocosm to mitigate ecological impacts of a simulated pesticide mixture of diazinon and permethrin in runoff from agricultural fields into receiving aquatic systems by using standard 48-h sediment bioassays with the freshwater test organism, *Hyalella azteca*. *H. azteca* (order: Amphipoda) is a freshwater crustacean and an epibenthic detritivore, closely associated with surficial sediments. *H. azteca* occurs in wetlands and lakes throughout much of North America and is an important food source for birds, fish, amphibians, and larger invertebrates (de March 1981).

2 Materials and Methods

2.1 Site Description and Sample Collection

A wetland mesocosm (1,890 m^2) located at the University of Mississippi Field Station was evenly divided and hydraulically isolated into non-vegetated and vegetated sections longitudinally with an earthen levee. Both sections were 882 m^2 with an earthen levee laterally at the back of the wetland approximately 0.33 m high allowing for an average 0.16–0.17 m depth and a hydraulic retention time of 5.59 and 5.77 h at maximum water volume capacity for vegetated and non-vegetated sections, respectively. Wetland slope was 8.6 mm/m (0.86% grade) and 9.3 mm/m (0.93% grade) for non-vegetated and vegetated sections, respectively. The non-vegetated

section contained no emergent vegetation and the vegetated section was naturally colonized with emergent *Eleocharis obtusa* and *Carex lurida* (95%) with patches of *Paspalum urvillei* (5%). Both sections had an estimated 12,000–15,000 L of standing water at the back of the wetland prior to amendment. A single simultaneous amendment occurred for 5 h with a mixture of diazinon (37.62 g a.i.), and permethrin (0.4104 g a.i.) homogenized in a 5,678-L dosing tank, with passive gravity-generated outflow through a 10.2 cm diameter gate valve. This simulated runoff from a 32-ha agricultural field during a rainfall event generating inflow of approximately 6.2 L/s for a total volume of 111,670 L of runoff in each section. 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 to 3 h of amendment and all water was fully impounded within respective sections with no outflow during amendment. Surficial (top 2.5 cm) sediment samples were collected using an acetone-washed steel diamond-shaped trowel 12 cm long × 6.5 cm wide at inflow, middle, and back points within non-vegetated and vegetated sections 5, 24, 72 h, 7, 14, and 21 days after amendment commenced. Approximately 500 g of surface sediment was collected at each location during each time period, preserved on wet ice, and transported to the USDA-ARS National Sedimentation Laboratory (NSL), Oxford, Mississippi, USA for sediment characterization, carbon, nutrient, and pesticide analysis as well as bulk sediment bioassays.

2.2 Physical and Chemical Sediment Analyses

Approximately 1 g (±0.1 g) of sub-sampled homogenized sediment was analyzed for total organic carbon using a Vario Max CNS (carbon-nitrogen-sulfur) instrument from Elementar Analysensysteme GmbH (Hanau, Germany) in CN mode with precision of less than or equal to 0.5% relative measurement, employing a thermal conductivity detector for measurements following tungsten catalytic tube combustion of samples and separation of gases. Post-combustion tubes were held at a temperature of 900°C, and the quartz tungsten reduction tube was at a temperature of 830°C to acquire desired component. Sediment particle size distribution assessing sand, silt and clay fractions from homogenized samples was determined using a Horiba model LA-90 laser scattering particle size distribution analyzer (Horiba Scientific, Kyoto-shi Kyoto, Japan) according to methods reported in Schaff et al. (2003).

Sediment samples prepared for pesticide analysis were air dried for 24–48 h, ground, homogenized, and 10 g sub-sampled. For pesticide extraction, subsamples were placed in 50-mL centrifuge tubes with 20 mL of ethyl acetate, sonicated for 1 min, and centrifuged at 2,000–2,500×*g* for 5 min. The solvent layer was transferred to a second 50-mL centrifuge tube, an additional 10 mL of ethyl acetate was added, and the extraction process was repeated. The extract was concentrated to near dryness using a nitrogen evaporator and solvent exchanged into 10 mL hexane/acetone 90:10 (azeotrope). Extracted samples were subjected to silica gel clean-up prior to 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 diazinon, *cis*-, and *trans*-permethrin analyses modified from Bennett et al. (2000). The Agilent 7890A GC was equipped with two micro electron capture detectors (μECDs). For diazinon, the analytical column was an Agilent HP 1MS capillary column, 30 m × 0.25 mm i.d. × 0.25 μm film thickness. Column oven temperatures were: initial at 85°C for 1 min; ramp at 25°C to 185°C and hold for 20 min. Retention time was 11.20 min. For *cis*- and *trans*-permethrin, the analytical column was an Agilent HP 1MS capillary column, 30 m × 0.25 mm i.d. × 0.25 μm film thickness. Column oven temperatures were: initial at 75°C for 1 min; ramp at 35°C to 230°C and hold for 15 min. Retention times were 15.43 min for *cis*-permethrin and 15.89 min for *trans*-permethrin. Carrier gas used was ultra-high purity (UHP) helium at 28 mL/min and inlet temperature at 250°C. The μECD temperature was 325°C with a constant make-up gas flow of 60 mL/min UHP nitrogen. Standard calibration curves were based upon ranges of concentrations from 0.1 to 1.0 mg/kg for diazinon and from 0.171 to 2.09 mg/kg for *cis*- and *trans*-permethrin, respectively, following standard quality assurance/quality control protocols (Eaton et al. 2005). Extraction efficiencies of diazinon and *cis*- and *trans*-permethrin fortified sediment samples were ≥90% with minimum detection limits of 0.001 mg/kg for all pesticides.

2.3 Sediment Bioassays

Bulk sediment bioassays were 48-h static non-renewed exposures assessing *H. azteca* survival according to modified protocols from Smith et al. (2007) and USEPA (2000) for *H. azteca* reference toxicity tests and acute sediment toxicity tests. *H. azteca* approximately 1 to 2 weeks old were collected by passing mixed-age animals through a 600-μm stainless steel mesh sieve and using only those retained by a 425-μm stainless steel mesh sieve for the bioassays. Exposure chambers were 88 mL polypropylene plastic cups containing 16 g wet weight of homogenized whole bulk sediment and 60 ml of overlying water. Five *H. azteca* were placed in each of four replicate exposure chambers with one 2×2-cm^2 sterile cotton gauze as substrate. All bioassays were conducted in a Powers Scientific Inc. incubator (Powers Scientific, Inc., Pipersville, Pennsylvania, USA) at 23±1°C with a photoperiod of 16:8 light/dark at the USDA-ARS NSL. Control sediment and overlying water, free from priority pollutants, were from a naturally spring-fed pond located at the University of Mississippi Field Station. Measured physicochemical characteristics of overlying water are reported in Table 1 and included temperature, dissolved oxygen, pH, alkalinity, hardness, conductivity, nitrite, nitrate, and ammonia using standard methods (Eaton et al. 2005).

2.4 Statistical Analyses

H. azteca 48-h survival data were ln(x+1) transformed (Schulz et al. 2003a) and analyzed using a one-way analysis of variance (ANOVA) with Dunnett's multiple range tests versus controls. Effects of time after amendment (time), vegetation type (type), and location within the wetland mesocosm section (location) on *H. azteca* survival were analyzed using a three-way ANOVA. Pearson Product Moment correlations of *H. azteca* survival versus pesticide concentrations in non-vegetated and vegetated sections of a wetland mesocosm were conducted. Because total organic carbon (TOC) can influence sediment pesticide toxicity (Nebeker et al. 1989), reported pesticide concentrations are often adjusted to the fraction of organic carbon content of the sediment (μg pesticide/g OC). For these analyses, pesticide concentrations were either unadjusted, adjusted to sediment TOC, adjusted to sediment sand fraction (sand), silt fraction (silt), clay fraction (clay), or adjusted to a combination of OC*silt to determine the strongest association between observed survival and pesticides.

3 Results

3.1 Sediment Characteristics and Pesticide Concentrations

Wetland mesocosm sediments from both vegetated and non-vegetated sections were predominantly sandy to silty loam with low TOC and clay fractions (<2%) (Table 2). Eight of 36 samples were almost exclusively silt (>97%). All control sediment samples were sandy loam with <1% TOC and had no detectable amounts of diazinon or permethrin. Both pesticides were detected in >90% of all sediment samples collected during the 21 day observation period (Table 3). Sediment diazinon concentrations in the non-vegetated section were detected in all 18 samples and ranged from 1 to 45.5 ng/g (Table 3). Diazinon

Table 1 Mean (SD) overlying water quality characteristics for *Hyalella azteca* 48-h sediment bioassay exposures

Characteristic	Control	Non-vegetated	Vegetated
Temperature °C	23.0 (0.3)	23.0 (0.3)	23.0 (0.3)
Dissolved oxygen mg/L	7.0 (0.7)	6.6 (0.5)	6.7 (0.6)
pH	7.4 (0.4)	7.2 (0.2)	7.1 (0.2)
Alkalinity mg/L as $CaCO_3$	65.6 (12.9)	65.6 (5.6)	63.7 (7.9)
Hardness mg/L as $CaCO_3$	102.5 (28.6)	108.2 (21.2)	108.2 (21.2)
Conductivity μS/cm	416.3 (78.7)	411.6 (52.9)	406.5 (59.1)
Nitrite mg/L	0.017 (0.003)	0.020 (0.004)	0.021 (0.007)
Nitrate mg/L	0.202 (0.077)	0.214 (0.082)	0.213 (0.089)
Ammonia mg/L	0.010 (0.008)	0.015 (0.011)	0.007 (0.005)

Table 2 Sediment characteristics (%) from non-vegetated and vegetated sections of a constructed wetland

Time	Characteristic	Non-vegetated			Vegetated		
		Inflow	Middle	Back	Inflow	Middle	Back
5 h	Sand	54.7	81.1	65.7	72.3	85.2	34.8
	Silt	44.7	18.7	33.5	24.7	14.7	63.6
	TOC	1.8	1.0	0.9	1.6	1.8	1.0
24 h	Sand	71.6	61.9	71.6	0.3	62.6	58.1
	Silt	28.1	37.8	27.6	98.0	36.7	40.2
	TOC	0.9	1.0	0.6	1.3	1.2	0.8
72 h	Sand	51.6	81.1	25.8	32.0	0.0	0.0
	Silt	48.0	18.9	72.6	66.8	98.0	97.2
	TOC	1.0	1.2	1.1	1.4	1.4	1.1
7 days	Sand	28.3	0.0	48.9	0.0	44.6	39.8
	Silt	70.8	98.7	50.1	98.1	54.4	58.6
	TOC	1.2	1.8	1.0	1.2	1.0	1.0
14 days	Sand	29.9	31.1	0.0	23.7	31.2	17.2
	Silt	68.9	67.6	97.5	74.9	67.1	80.0
	TOC	1.3	1.3	1.1	0.9	1.2	0.9
21 days	Sand	41.4	43.1	0.0	0.0	51.3	0.2
	Silt	58.0	55.8	98.4	98.4	47.8	97.5
	TOC	1.9	1.4	1.0	1.3	0.9	1.2

concentrations within non-vegetated section sediments varied spatially and temporally with peak concentrations in inflow samples at 5 h (45.5 ng/g), middle samples at 72 h (27.4 ng/g), and back samples at 7 days (18.8 ng/g) with typically decreasing concentrations towards 21 days. In contrast, diazinon concentrations in vegetated section sediments ranged from below detection limits to 47.2 ng/g and only 14 samples had detectable levels. Within the vegetated section, peak diazinon values occurred at 72 h at inflow (47.2 ng/g), middle (15.3 ng/g), and back (12.0 ng/g) sampling sites. Typically, inflow sediments had the greatest diazinon values and back sediments the lowest with decreasing concentrations from 72 h to 21 days.

Permethrin, a mixture of *cis*- and *trans*- isomers, was assessed in wetland mesocosm sediment samples for both isomers (Table 3). Sediment *cis*-permethrin in the non-vegetated section was detected in all 18 samples and ranged from 0.1 to 5.0 ng/g. Greatest sediment *cis*-permethrin concentrations occurred in inflow samples at 5, 24, and 72 h, and in back samples at 7, 14, and 21 days. *Trans*-permethrin in sediment was detected in only three samples and ranged from below detection limits to 0.3 ng/g. Within vegetated section sediments, *cis*-permethrin was detected in 17 of 18 samples and ranged from below detection limits to 5.6 ng/g. Greatest concentrations for each sampling time period occurred in inflow samples at 24 h, 14 days, and 21 days, and in back samples at 5 and 72 h. Sediment *trans*-permethrin occurred in only five samples and ranged from below detection limits to 2.1 ng/g.

3.2 Bioassays

H. azteca 48-h survival in diazinon and permethrin contaminated sediment varied spatially and temporally in both non-vegetated and vegetated sections of the constructed wetland (Fig. 1). Animal survival in non-vegetated sediments ranged from 5% to 100% with lowest survival occurring in inflow sediments within 7 days. Comparisons versus control survival showed significantly ($p<0.05$) lower survival in inflow and back at 5 h, inflow and middle at 24 h, all three sites at 72 h and 7 days, and similar survival at 14 and

Table 3 Diazinon and permethrin concentrations (ng/g) in sediment from non-vegetated and vegetated sections of a constructed wetland

Time	Pesticide	Non-vegetated			Vegetated		
		Inflow	Middle	Back	Inflow	Middle	Back
5 h	Diazinon	45.5	1.0	2.4	N	N	N
	Cis-Permethrin	5.0	0.9	1.3	3.4	2.7	3.5
	Trans-Permethrin	0.3	N	N	N	N	1.4
24 h	Diazinon	8.8	12.2	3.5	43.7	10.0	6.8
	Cis-Permethrin	1.9	N	TR	5.6	2.0	1.5
	Trans-Permethrin	N	N	N	1.6	N	N
72 h	Diazinon	17.0	27.4	17.2	47.2	15.3	12.0
	Cis-Permethrin	1.6	TR	0.8	2.4	0.8	5.2
	Trans-Permethrin	N	N	N	TR	N	TR
7 day	Diazinon	18.7	23.2	18.8	24.5	15.0	6.7
	Cis-Permethrin	1.2	TR	2.3	N	0.8	0.8
	Trans-Permethrin	N	N	TR	N	N	N
14 day	Diazinon	13.3	20.0	18.6	40.3	5.5	N
	Cis-Permethrin	0.8	0.9	3.0	3.4	0.9	0.5
	Trans-Permethrin	N	N	TR	2.1	N	N
21 day	Diazinon	5.8	11.8	11.7	13.4	4.1	3.8
	Cis-Permethrin	1.2	TR	1.7	0.8	TR	0.6
	Trans-Permethrin	N	N	N	N	N	N

N below detection limit of 1 ng/g, *TR* trace

21 days. By 14 days, average survival was ≥90% at all sites. In contrast, *H. azteca* survival in vegetated sediments ranged from only 50% to 100% with lowest survival in inflow and middle sediments within 72 h. Comparisons versus control survival showed significantly ($p<0.05$) lower survival at all three sites at 5 h, and inflow and middle sites at 24 h. By 21 days, average animal survival was ≥90% at all sites.

Results of the three-way ANOVA with time ($p<0.0001$), vegetation type ($p=0.0002$), and wetland location ($p=0.0035$) showed significant spatial and temporal variations in survival (Table 4). Survival increased with distance through the wetland from inflow to back and over time within the non-vegetated section; however, spatial patterns were not as clear in the vegetated sections even though temporal variation was similar. Significant interactions occurred between time x vegetation type ($p=0.0084$) and vegetation type x location ($p<0.0001$) elucidating the greater toxicity in the inflow of the non-vegetated section within 72 h. Significant interaction of time x location ($p=0.0041$) highlighted the greater toxicity at inflow and middle versus back of both vegetated and non-vegetated sections and a significant three-way interaction of time × vegetation type × location ($p=0.0486$) supported the much greater toxicity observed at the inflow of the non-vegetated wetland at 5, 24, and 72 h.

3.3 Sediment Pesticide Associations

Although both pesticides examined in the constructed wetland are insecticides, there were different patterns of associations between observed survival responses and measured concentrations (Table 5). Within the non-vegetated section, *H. azteca* survival was significantly associated with unadjusted diazinon concentrations ($r=-0.522$, $p<0.01$) and total pesticide concentrations (∑pesticides; $r=-0.526$, $p<0.01$). Pesticide concentrations adjusted to OC content minimally improved correlation coefficients for both diazinon and ∑pesticides (Table 5). Pesticide concentrations adjusted to sediment silt fraction, however,

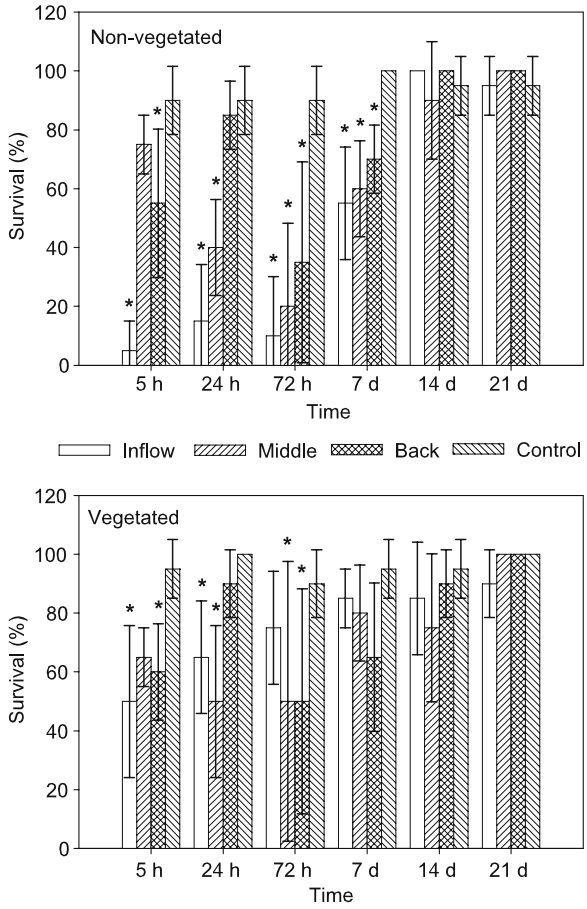

Fig. 1 *Hyalella azteca* 48-h mean survival (n=4) exposed to diazinon and permethrin contaminated sediment from non-vegetated and vegetated sections of a wetland mesocosm (*statistically significantly different from controls, p<0.05)

further elucidated associations between survival and diazinon (r=−0.679, p<0.001), *cis*-permethrin (r=−0.567, p<0.01), and ∑pesticides (r=−0.699, p<0.001). Pesticide concentrations adjusted to both OC and silt showed intermediate correlation coefficients with animal survival but sand and clay adjusted concentrations were not (p>0.05; Table 5). In contrast, within the vegetated section, *H. azteca* survival was associated with unadjusted *cis*-permethrin concentrations (r=−0.731, p<0.001) and ∑pesticides (r=−0.496, p<0.05) but not diazinon (r=−0.287, p>0.05). Adjusting pesticide concentrations from the vegetated section via OC, silt, or OC × silt did not improve correlation coefficients and sand and clay adjusted concentrations were, again, unassociated with animal survival (p>0.05; Table 5).

4 Discussion

Several studies have assessed effects of a single pesticide on sediment-associated aquatic animals in wetlands designed to mitigate agricultural runoff (Milam et al. 2005; Bouldin et al. 2007; Kröger et al. 2009). Few studies, however, included assessments of pesticide mixtures in wetlands designed to mitigate agricultural runoff (Bouldin et al. 2005; Moore et al. 2007). Assessments of pesticide mixtures in sediment toxicity instead of single, individual pesticides are more appropriate since agricultural runoff potentially entering a wetland mitigation system will consist of some type of pesticide mixture (Belden et al. 2006). As these pesticide mixtures dissipate from the water column, some fraction can enter different compartments of the system including biological compartments (sorption to plants, algae, and sediment organic carbon) and physical compartments (physical sorption to sediment particles: sand, silt, clay) at varying rates and concentrations depending upon the physicochemical properties of the pesticides. In the current study, *cis*-permethrin was detected in nearly every sample (97%) while diazinon was detected in 89% of samples and *trans*-permethrin was detected in only 22% of samples, thus affecting exposures of these insecti-

Table 4 *Hyalella azteca* 48-h sediment survival [ln (x+1) transformed] three-way analysis of variance with sampling period (time), vegetation type (type), and wetland location (location)

Source of variation	*df*	Sum of squares	Mean squares	*F* statistic	*p* Value
Time	5	68.79	13.76	17.72	<0.0001
Type	1	11.66	11.66	15.02	0.0002
Location	2	9.27	4.64	5.97	0.0035
Time × Type	5	12.75	2.55	3.29	0.0084
Time × Location	10	21.68	2.17	2.79	0.0041
Type × Location	2	16.35	8.18	10.53	<0.0001
Time × Type × Location	10	14.98	1.50	1.93	0.0486

Table 5 Pearson Product Moment correlation coefficients (r) of *Hyalella azteca* 48-h survival versus pesticides (n=24) in sediment from non-vegetated and vegetated sections of a constructed wetland

Pesticide	ng/g	μg/g OC	μg/g sand	μg/g silt	μg/g clay	μg/g OC × silt
Non-vegetated						
Diazinon	−0.522**	−0.576**	0.194	−0.679***	−0.333	−0.676***
Cis-Permethrin	−0.385	−0.364	0.237	−0.567**	−0.328	−0.524**
Trans-Permethrin	−0.173	−0.068	0.200	−0.278	−0.336	−0.175
∑Permethrin	−0.379	−0.351	0.238	−0.559**	−0.328	−0.520**
∑Pesticides	−0.526**	−0.578**	0.196	−0.699***	−0.333	−0.702***
Vegetated						
Diazinon	−0.287	−0.233	−0.258	−0.213	−0.205	−0.162
Cis-Permethrin	−0.731***	−0.623**	−0.288	−0.594**	−0.366	−0.643***
Trans-Permethrin	−0.223	−0.161	−0.346	−0.182	−0.161	−0.147
∑Permethrin	−0.692***	−0.543**	−0.289	−0.597**	−0.376	−0.607**
∑Pesticides	−0.496*	−0.335	−0.224	−0.342	−0.348	−0.261

*p<0.05; **p<0.01; ***p<0.001

cides and enantiomers to sediment-associated animals. Previous studies have observed *cis*-permethrin to occur in greater concentrations within sediments than *trans*-permethrin (Allan et al. 2005) which showed the *trans*-permethrin isomer dissipated more rapidly from sediment than the *cis*-permethrin isomer.

In the current study, the differences in *H. azteca* survival clearly showed the importance of vegetation in mitigating the toxicity of pesticide-contaminated wetland sediments. Animal survival was consistently greater throughout the vegetated section within the first 7 days of exposure to pesticide-contaminated wetland sediments compared to non-vegetated wetland sediments. Schulz et al. (2003b), who examined the effects of wetland vegetation on methyl-parathion toxicity to aquatic benthic macroinvertebrates where toxicity was reduced spatially and temporally in vegetated wetlands compared to non-vegetated ones. The presence of vegetation would provide an additional biological dilution effect of pesticide-laden influent via more surface area for sorption, uptake, and degradation thus reducing the amount of available pesticide mass to sorb to sediments. Reduction in pesticide amounts in vegetated wetland sediments would allow for greater likelihood of sediment biota survival, more rapid recovery of affected sediment biota and more rapid sediment recolonization of regions within the wetland affected by pesticide contamination. As a result, vegetation can play a significant role in mitigating sediment toxicity during the earliest phases of sorption–desorption of hydrophobic organic compounds such as pyrethroid insecticides. Such a role should not be underestimated since this mitigates impacts to non-target benthic organisms that may utilize constructed wetlands as habitat in addition to the primary role of mitigating impacts to lakes, rivers, and streams that would otherwise directly receive agricultural runoff.

Associations of wetland sediment *H. azteca* survival and the insecticide binary mixture were also influenced by vegetation. In non-vegetated wetland sediments, unadjusted pesticide concentrations of diazinon and ∑pesticides were negatively correlated with animal survival. The greatest number and strongest negative correlations, however, occurred between diazinon, *cis*-permethrin, and ∑pesticides and animal survival when pesticide concentrations were adjusted for sediment silt fraction. Intermediate negative correlations occurred with adjustments due to organic carbon content. These results suggest the influence of finer grained material in sediments is more important in determining acute sediment toxicity within the first 7 days than organic carbon content where vegetation is minimal. In contrast, vegetated wetland sediments showed strongest negative correlation between unadjusted *cis*-permethrin concentrations and animal survival and no association with observed diazinon concentrations (either unadjusted or adjusted). Neither sediment particle size nor

organic carbon content appeared to improve these correlations under vegetated conditions. These components could have less of a role in sediment toxicity as diazinon and permethrin are compartmentalized among macrophytes, sediment particles, and sediment organic carbon within the first week when toxicity was observed.

Few studies have directly assessed organophosphate sediment toxicity and, specifically, diazinon sediment toxicity (Bouldin et al. 2007; Smith et al. 2007). This is likely due to physicochemical properties of diazinon as the compound sorbs less readily to and desorbs more rapidly from sediments than pyrethroids (Bondarenko and Gan 2006; Gan et al. 2005). Effects of diazinon in sediment were observed at concentrations ranging from >20 ng/g for *H. azteca* 48-h survival (Smith et al. 2007) to >200 ng/g for *Chironomus dilutus* 10-day growth (Bouldin et al. 2007). In contrast, sediment toxicity of pyrethroids have been the focus of numerous studies (Maund et al. 2002; Amweg et al. 2005; Weston et al. 2004, 2008; Ng et al. 2008). Specifically, permethrin sediment toxicity was assessed by Amweg et al. (2005) using *H. azteca* 10-day sediment bioassays with LC50s ranging from 57 to 112 ng/g or 1.58–8.05 μg/g OC. In comparison, the present study showed 48-h *H. azteca* survival significantly decreased at sediment pesticide concentrations below those observed for either diazinon or permethrin individually. These differences can be explained by two factors. First, sediments examined in the current study that were collected within the first 72 h of dosing with diazinon and permethrin (both hydrophobic organic insecticides) quickly sorbed to sediments within this time frame (Maund et al. 2002). Within that same time frame, these insecticides, especially the pyrethroid permethrin, would also then begin rapidly desorbing (labile/reversible desorption phase; Xu et al. 2008) followed by progressively slower desorbing phases that coincide with decreasing pesticide bioavailability and resultant toxicity. Sediment permethrin and diazinon desorption would decrease as contact time between these pesticides and sediment increases, in a process known as sediment aging (Xu et al. 2008). Second, within organophosphate-pyrethroid mixtures, where the two insecticides have different modes of action, there is a possibility of greater than additive toxicity or synergism (Denton et al. 2003; Belden and Lydy 2006). Specifically, Denton et al. (2003) observed the combination of the organophosphate insecticide diazinon and the pyrethroid insecticide esfenvalerate interacted synergistically in causing acute toxicity to fathead minnow (*Pimephales promelas*). Since both diazinon and the pyrethroid permethrin were used in the current study, joint synergistic toxicity of both insecticides would elicit responses of *H. azteca* at concentrations below previously reported effects concentrations.

5 Conclusions

Results show that an 882-m^2 wetland with or without rooted emergent vascular vegetation and dosed with diazinon and permethrin can induce toxicity in *H. azteca* for up to 3 days. Additionally, results suggest that effects of vegetation versus no vegetation on diazinon and permethrin toxicity to *H. azteca* showed vegetation was more effective than no vegetation in mitigating toxicity within the first week but these differences were not evident by 14 days. 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 sediment-associated aquatic organisms.

Acknowledgments Authors thank Lisa Brooks, Sam Testa III, John Massey, James Hill, and Dan McChesney for analytical assistance. Mention of equipment, computer programs or a pesticide does not constitute 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 non-discriminatory basis without regard to race, color, national origin, sex, marital status, or handicap.

References

Allan, I. J., House, W. A., Parker, A., & Carter, J. E. (2005). Diffusion of the synthetic pyrethroid permethrin into bed sediments. *Environmental Science & Technology, 39*, 523–530.

Amweg, E. L., Weston, D. P., & Ureda, N. M. (2005). Use and toxicity of pyrethroid pesticides in the Central Valley, California, USA. *Environmental Toxicology and Chemistry, 24*, 966–972.

Anderson, B. S., Hunt, J. W., Phillips, B. M., Nicely, P. A., de Vlaming, V., Connor, V., et al. (2003). Integrated assessment of the impacts of agricultural drainwater in

the Salinas River (California, USA). *Environmental Pollution, 124*, 523–532.

Anderson, B. S., Phillips, B. M., Hunt, J. W., Connor, V., Richard, N., & Tjeerdema, R. S. (2006). Identifying primary stressors impacting macroinvertebrates in the Salinas River (California, USA): relative effects of pesticides and suspended particles. *Environmental Pollution, 141*, 402–408.

Belden, J. B., & Lydy, M. J. (2006). Joint toxicity of chlorpyrifos and esfenvalerate to fathead minnows and midge larvae. *Environmental Toxicology and Chemistry, 25*, 623–629.

Belden, J. B., Gillom, R. J., Martin, J. D., & Lydy, M. J. (2006). Relative toxicity and occurrence patterns of pesticide mixtures in streams draining agricultural watersheds dominated by corn and soybean production. *Integrated Environmental Assessment and Management, 3*, 90–100.

Bennett, E. R., Moore, M. T., Cooper, C. M., & Smith, S. (2000). Method for the simultaneous extraction and analysis of two current use pesticides, atrazine and lamda-cyhalothrin, in sediment and aquatic plants. *Bulletin of Environmental Contamination and Toxicology, 64*, 825–833.

Bondarenko, S., & Gan, J. (2006). Degradation and sorption of selected organophosphate and carbamate insecticides in urban stream sediments. *Environmental Toxicology and Chemistry, 23*, 1809–1814.

Bouldin, J. L., Farris, J. L., Moore, M. T., Smith, S., Stephens, W. W., & Cooper, C. M. (2005). Evaluated fate and effects of atrazine and lambda-cyhalothrin in vegetated and unvegetated microcosms. *Environmental Toxicology, 20*, 487–498.

Bouldin, J. L., Farris, J. L., Moore, M. T., Smith, S., & Cooper, C. M. (2007). Assessment of diazinon toxicity in sediment and water of constructed wetlands using deployed *Corbicula fluminea* and laboratory testing. *Archives of Environmental Contamination and Toxicology, 53*, 174–182.

Cooper, J., & Dobson, H. (2007). The benefits of pesticides to mankind and the environment. *Crop Protection, 26*, 1337–1348.

de March, B. G. E. (1981). *Hyalella azteca* (Saussure). In: Lawrence SG (ed) *Manual for the culture of selected freshwater invertebrates*. Canadian Special Publications: Fisheries and Aquatic Sciences, 54:61–77.

Denton, D. L., Wheelock, G. E., Murray, S. A., Deanovic, L. A., Hammock, B. D., & Hinton, D. E. (2003). Joint acute toxicity of esfenvalerate and diazinon to larval fathead minnows (*Pimephales promelas*). *Environmental Toxicology and Chemistry, 22*, 336–341.

Eaton, A. D., Clesceri, L. S., Rice, E. W., & Greenberg, A. E. (2005). *Standard methods for the examination of water and wastewater* (21st ed.). Washington: American Public Health Association, American Water Works Association, Water Environment Federation.

Gan, J., Lee, S. J., Liu, W. P., Haver, D. L., & Kabashima, J. N. (2005). Distribution and persistence of pyrethroids in runoff sediments. *Journal of Environmental Quality, 34*, 836–841.

Hunt, J. W., Anderson, B. S., Phillips, B. M., Tjeerdema, R. S., Richard, N., Connor, V., et al. (2006). Spatial relationships between water quality and pesticide application rates in agricultural watersheds. *Environmental Monitoring and Assessment, 121*, 245–262.

Hunt, J., Anderson, B., Phillips, B., Tjeerdema, R., Largay, B., Beretti, M., et al. (2008). Use of toxicity identification evaluations to determine the pesticide mitigation effectiveness of on-farm vegetated treatment systems. *Environmental Pollution, 156*, 348–358.

Kröger, R., Lizotte, R. E., & Moore, M. T. (2009). Survival, growth, and body residues of *Hyalella azteca* (Saussure) exposed to fipronil contaminated sediments from non-vegetated and vegetated microcosms. *Bulletin of Environmental Contamination and Toxicology, 83*, 369–373.

Locke, M. A., Knight, S. S., Smith, S., Cullum, R. F., Zablotowicz, R. M., Yuan, Y., et al. (2008). Environmental quality research in the Beasley Lake watershed, 1995-2007: succession from conventional to conservation practices. *Journal of Soil and Water Conservation, 63*, 430–442.

Maund, S. J., Hamer, M. J., Lane, M. C. G., Farrelly, E., Rapley, J. H., Goggin, U. M., et al. (2002). Partitioning, bioavailability and toxicity of the pyrethroid insecticide cypermethrin in sediments. *Environmental Toxicology and Chemistry, 21*, 9–15.

Milam, C. D., Bouldin, J. L., Farris, J. L., Schulz, R., Moore, M. T., Bennett, E. R., et al. (2005). Evaluating acute toxicity of methyl parathion application in constructed wetland mesocosms. *Environmental Toxicology, 19*, 471–479.

Moore, M. T., Lizotte, R. E., & Smith, S. (2007). Responses of *Hyalella azteca* to a pyrethroid mixture in a constructed wetland. *Bulletin of Environmental Contamination and Toxicology, 78*, 245–248.

Moore, M. T., Cooper, C. M., Smith, S., Cullum, R. F., Knight, S. S., Locke, M. A., et al. (2009). Mitigation of two pyrethroid insecticides in a Mississippi Delta constructed wetland. *Environmental Pollution, 157*, 250–256.

Nebeker, A. V., Schuytema, G. S., Griffis, W. L., Barbitta, J. A., & Carey, L. A. (1989). Effect of sediment organic carbon on survival of *Hyalella azteca* exposed to DDT and endrin. *Environmental Toxicology and Chemistry, 8*, 705–718.

Ng, C. M., Weston, D. P., You, J., & Lydy, M. J. (2008). Patterns of pyrethroid contamination and toxicity in agricultural and urban stream segments. In J. Gan, F. Spurlock, P. Hendley, & D. P. Weston (Eds.), *Synthetic pyrethroids: Occurrence and behavior in aquatic environments* (pp. 355–369). Washington: American Chemical Society.

Reddy, K. R., & DeLaune, R. D. (2008). *Biogeochemistry of wetlands: Science and applications*. New York: CRC.

Schaff, S. D., Pezeshki, S. R., & Shields, F. D. (2003). Effects of soil conditions on survival and growth of black willow cuttings. *Environmental Management, 31*, 748–763.

Schulz, R., Moore, M. T., Bennett, E. R., Milam, C. D., Bouldin, J. L., Farris, J. L., et al. (2003a). Acute toxicity of methyl-parathion in wetland mesocosms: assessing the influence of aquatic plants using laboratory testing with

Hyalella azteca. *Archives of Environmental Contamination and Toxicology, 45*, 331–336.

Schulz, R., Moore, M. T., Bennett, E. R., Farris, J. L., Smith, S., & Cooper, C. M. (2003b). Methyl parathion toxicity in vegetated and non-vegetated wetland mesocosms. *Environmental Toxicology and Chemistry, 22*, 1262–1268.

Sharom, M. S., Miles, J. R. W., Harris, C. R., & McEwen, F. L. (1980). Behavior of 12 insecticides in soil and aqueous suspensions of soil and sediment. *Water Research, 14*, 1095–1100.

Sherrard, R. M., Bearr, J. S., Murray-Gulde, C. L., Rodgers, J. H., & Shah, Y. T. (2004). Feasibility of constructed wetlands for removing chlorothalonil and chlorpyrifos from aqueous mixtures. *Environmental Pollution, 127*, 385–394.

Smith, S., Lizotte, R. E., & Moore, M. T. (2007). Toxicity assessment of diazinon in a constructed wetland using *Hyalella azteca*. *Bulletin of Environmental Contamination and Toxicology, 79*, 58–61.

US Department of Agriculture (USDA) National Agricultural Statistical Service (NASS). (2010). Agricultural chemical use database. http://www.pestmanagment.info/nass/. Accessed 30 March 2010.

US Environmental Protection Agency (USEPA). (2000). Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA 600/R-99/064 Washington, DC.

US Environmental Protection Agency (USEPA). (2004). Interim reregistration eligibility decision for diazinon. EPA 738-R-04-006 Washington, DC.

US Environmental Protection Agency (USEPA). (2005). Aquatic life ambient water quality criteria: Diazinon. EPA 822-R-05-006 Washington, DC.

US Environmental Protection Agency (USEPA). (2009). Reregistration eligibility decision for permethrin. EPA 738-R-09-306 Washington, DC.

Weston, D. P., You, J., & Lydy, M. J. (2004). Distribution and toxicity of sediment associated pesticides in agriculture-dominated water bodies of California's Central Valley. *Environmental Science & Technology, 38*, 2752–2759.

Weston, D. P., You, J., Amweg, E. L., & Lydy, M. J. (2008). Sediment toxicity in agricultural areas of California and the role of hydrophobic pesticides. In J. Gan, F. Spurlock, P. Hendley, & D. P. Weston (Eds.), *Synthetic pyrethroids: Occurrence and behavior in aquatic environments* (pp. 26–54). Washington: American Chemical Society.

Xu, Y., Gan, J., Wang, Z., & Spurlock, F. (2008). Effect of ageing on desorption kinetics of sediment-associated pyrethroids. *Environmental Toxicology and Chemistry, 27*, 1293–1301.

Zambonin, C. G., Losito, I., Cilenti, A., & Palmisano, F. (2002). Solid-phase microextraction coupled to gas chromatography-mass spectrometry for the study of soil adsorption coefficients of organophosphorus pesticides. *Journal of Environmental Monitoring, 2002*, 477–481.

Zhang, X., Liu, X., Luo, Y., & Zhang, M. (2008). Evaluation of water quality in an agricultural watershed as affected by almond pest management practices. *Water Research, 42*, 3685–3696.