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Toxicity Evaluation of Diazinon Contaminated Leaf Litter

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Diazinon (O,O-diethyl O-[2-isopropyl-6-methyl-4pyrimidinyl] phosphothiate) is an organophosphate pesticide with widespread use on a variety of agricultural crops, such as fruit trees, corn, and tobacco (Burkepile et al., 2000). Approximately 6 million kg of diazinon are applied annually in the United States and, as a result, are a potential contributer to nonpoint source contamination of aquatic environments (Banks et al., 2003). Within these aquatic environments, decaying plant materials and leaf litter can be a prominent physical feature, especially during autumn (Webster and Benfield, 1986; Federle and Ventullo, 1990). These materials have extensive surface areas conducive to sorption of soluble organic contaminants (Pierce et al., 1977). This allows for the potential entry of these compounds into detrital food webs (Odum and Drifmeyer, 1978) resulting in potential effects on aquatic ecosystems (Forrow and Maltby, 2000).

Despite the extensive presence of detrital material within some aquatic systems, such as wetlands, marshes, small streams, and heavily canopied rivers, ponds, and lakes, limited research or information exists on the influence of pesticide-contaminated detritus on aquatic ecosystems (Odum et al., 1969; Swift et al., 1988; Harrahy et al., 1994). With increasing use of constructed wetlands to mitigate nonpoint source contamination, there is a growing need to assess the role of detritus in understanding the fate and effects of various contaminants on nontarget aquatic organisms.

This study presents an assessment of leaf litter contamination in a constucted wetland and a toxicity evaluation using a standard laboratory bioassay with the freshwater test organism, *Hyalella azteca* Saussure.

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Materials and Methods

Leaf litter was simulated using postabcission Norway maple leaves (*Acer platanoides*) contained within polyester bags having 0.5 cm diameter mesh openings. Approximately 20 g (dry weight) of leaf litter was added to each bag, and approximately 25 g of stainless steel weights were added to prevent floating and to ensure all leaf litter remained submerged. Five leaf-litter bags were placed together in the center of each of three cells within a constructed wetland designed for the mitigation of agricultural contaminant runoff (e.g., sediment, pesticides, nutrients, etc.). Wetland cells included a sediment retention pond (SRP), primary wetland cell (1° cell), and a secondary (2°, finishing) wetland cell located adjacent to Beasley Lake in Sunflower County, Mississippi, USA. The constructed wetland was dosed with 0.16 mg/L diazinon (active ingredient) as Diazinon 4E®, and 403 mg/L suspended sediment (as a carrier), simulating a 1.3-cm rainfall event and runoff from a 14 ha agricultural field. Rainfall event was based on climatological data from the Beasley Lake weather station, whereas the contributing area (14 ha) is the actual drainage area feeding the constructed wetland. One leaf-litter bag was collected from each wetland cell at 8 h, 48 h, 7 d, 15 d, and 27 d after initial dosing.

Upon collection, leaf-litter samples were dried and analyzed for diazinon within 48 h after drying. Analytical chemistry was conducted according to Bennett et al. (2000), using a Hewlett-Packard 6890 gas chromatograph (Santa Clara, California,USA) equipped with dual HP 7683 ALS

Table 1. Mean ± SD physical and chemical water characteristics for diazinon-contaminated Norway maple leaf (*Acer platanoides*) litter aqueous exposure

Parameter	Control	SRP	1° Cell	2° Cell
Temperature (°C)	20.6 ± 0.3	20.6 ± 0.3	20.6 ± 0.3	20.6 ± 0.3
pH (s.u.)	7.9 ± 0.2	7.8 ± 0.2	7.8 ± 0.2	7.9 ± 0.2
Dissolved oxygen (mg/L)	7.6 ± 0.4	7.2 ± 0.4	7.1 ± 0.4	7.2 ± 0.5
Conductivity (μm hos/cm)	293 ± 96	293 ± 95	292 ± 95	292 ± 95
Hardness (mg/L as $CaCO_3$)	92 ± 9	91 ± 12	97 ± 8	94 ± 9
Alkalinity (mg/L as Ca CO_3)	70 ± 5	68 ± 0	68 ± 0	68 ± 0

SRP, sediment retention pond; 1° Cell, primary wetland cell; 2° Cell, secondary (finishing) wetland cell

autoinjectors. Briefly, samples were dried and ground, and diazinon was extracted by the addition of ethyl acetate. The mixture was sonicated and centrifuged (2000–2500 rpm). The extract was concentrated to near dryness (1 mL) using a nitrogen evaporator and solvent exchanged into hexane. Level of quantification for this analysis was 0.1 μg/kg. Recoveries based on fortified samples (5 replicates each at fortification levels of 0.01, 0.1, and 1.0 μg/kg) were >89%.

Forty-eight hour static, nonrenewal, aqueous toxicity tests using *Hyalella azteca* were conducted according to USEPA (1994) protocol. Animals, 4–5 d old, were collected for the experiment. Aqueous leaf-litter exposures consisted of 200 mL of overlying water, free from priority pollutants, obtained from the University of Mississippi Field Station (UMFS) (Moore et al., 1998) and three, 2 cm diameter Norway maple leaf discs as a substrate and food. Control leaf-litter was from preconditioned leaves used in *H. azteca* laboratory cultures. Contaminated leaf discs from leaf-litter bags were placed in three replicate exposure chambers per site (250 mL borosilicate glass beakers). Ten *H. azteca* were placed in each exposure chamber. Toxicity tests were conducted in a Powers Scientific, Inc. Animal Growth Chamber (Pipersville, Pennsylvania,USA) with a 16:8 h photoperiod. Bioassay endpoints measured were 48 h survival. Standard physical and chemical water characteristics were measured according to APHA (1998). *H. azteca* 48 h survival data were analyzed using descriptive statistics and one-way analysis of variance (ANOVA) with Dunnett's multiple range test versus controls. If data failed parametric assumptions, a Kruskal-Wallis one-way ANOVA on ranks was utilized with Dunn's multiple-range test vs controls. Linear regression was performed on survival vs pesticide concentration when significant effects were observed. Data analysis was conducted using SigmaStat® v.2.03 (Chicago, Illinois, USA) statistical software (SPSS, 1997).

Results and Discussion

Measured physical and chemical water characteristics of temperature, pH, dissolved oxygen, conductivity, hardness, and alkalinity are presented in Table 1. Chemical analysis revealed spatial and temporal variation in diazinon concentrations within leaf litter samples among three wetland cells (Table 2). Leaf litter samples from the first two wetland cells (SRP, 1° cell) had measurable amounts of diazinon within 8 h of dosing. Diazinon was not detected in the finishing cell, representative of hydraulic retention times >8 h of SRP and 1° cell within the constructed wetland. Leaf litter diazinon concentrations typically decreased with increasing time periods possibly caused, in part, by material degradation and desorption. After 15 d, SRP and 1° cell showed no measurable amounts of diazinon, however, the finishing cell remained contaminated 27 d post dosing. Similar spatial and temporal patterns of transfer/transformation were observed for two other organophosphate insecticides, chlorpyrifos and methyl parathion, within living aquatic macrophytes growing in constructed wetlands (Moore et al., 2002; Schulz et al., 2003).

Table 2. Measured diazinon concentrations (μg/kg dw) in Norway maple leaf (*Acer platanoides*) litter[a]

Time	SRP	1° Cell	2° Cell
8 h	56.9	576.5	BQ
48 h	62.1	176.7	2211.1
7 d	34.6	55.4	602.5
15 d	BQ	BQ	243.3
27 d	BQ	BQ	191.2

[a] Level of quantification limit is 0.1 μg/kg dw

BQ, below quantification limit; SRP, sediment retention pond; 1° Cell, primary wetland cell; 2° Cell, secondary (finishing) wetland cell

However, Odum and Drifmeyer (1978) noted that concentrations of organochlorine pesticides, such as DDT found in detritus, were much greater than those in live aquatic macrophytes. Although previous studies examined the fate of pesticides within constructed wetlands (Moore et al., 2000; Moore et al., 2001; Moore et al., 2002), these studies focused exclusively on three specific media: water, sediment ,and living plant material. Results of this study show that detrital (litter) material plays a role in this process. *Hyalella azteca* 48 h survival also varied spatially and

Table 3. 48 h mean ± SD percent survival of *Hyalella azteca* exposed to diazinon contaminated Norway maple leaf (*Acer platanoides*) litter

Time	Location				
	Control	SRP	1° Cell	2° Cell	r^2 [a]
8 h	83 ± 21 A	30 ± 10* B	3 ± 6* B	80 ± 0 A	0.648[†]
48 h	70 ± 17 A	13 ± 15* B	3 ± 6* B	10 ± 17* B	0.108
7 d	87 ± 6 A	90 ± 10 A	93 ± 6 A	0 ± 0* B	0.963[†]
15 d	90 ± 10 A	100 ± 0 A	97 ± 6 A	17 ± 15* B	0.940[†]
27 d	97 ± 6 A	97 ± 6 A	100 ± 0 A	90 ± 17 A	0.153
r^2 [b]		0.789[†]	0.652[†]	0.286[†]	

[a] Linear regression of survival vs diazinon concentration by location within time

[b] Linear regression of survival vs diazinon concentration by time within location

[†] Statistically significant linear relationship ($p < 0.05$)

SRP, sediment retention pond; 1° Cell, primary wetland cell; 2° Cell, secondary (finishing) wetland cell. Means with a different letter are significantly different temporally within each location ($p < 0.05$). Means with an asterisk (*) are significantly different spatially within each time frame ($p < 0.05$)

temporally in conjunction with measured diazinon concentrations (Table 3). Despite some variation in survival data (during 48 h and within 2° cell), spatial and temporal patterns were elucidated. Survival decreased significantly (as compared to controls) in the first two wetland cells (SRP, 1° cell) 8 h after diazinon dosing. After 48 h post dosing, all wetland cells showed decreased survival, ranging from 3% (in 1° cell) to 13% (in SRP) as compared to controls. Decreases in toxicity were apparent within the first two wetland cells 7 d after dosing, however, a decrease in toxicity within the finishing cell was not apparent until the last sampling period (27 d after dosing).

Linear regression analysis showed significant spatial relationships between survival and diazinon concentrations throughout the constructed wetland 8 h after dosing (r^2 = 0.648; F = 18.4; p = 0.002; Power = 0.916),168 h after dosing (r^2 = 0.963; F = 260.2; p <0.001; Power = 1.00), and 360 h after dosing (r^2 = 0.940; F = 157.5; p <0.001; Power = 1.00). Similar regression analysis across sampling periods showed significant temporal relationships between survival and diazinon concentrations within SRP (r^2 = 0.789; F = 48.6; p = <0.001; Power = 0.998), 1° cell (r^2 = 0.652; F = 24.3; p <0.001; Power = 0.972), and, to a lesser degree, finishing cell (r^2 = 0.286; F = 5.2; p = 0.040; Power = 0.542). Temporally, *H. azteca* 48 h survival was significantly associated with leaf litter diazinon concentrations during 8 h, 7 d, and 15 d time periods. However, greater survival variation at 48 h contributed to the lack of any significant relationship. Also, after 27 d, no relationship was observed, due, in part to the greater survival variation in the 2° cell. Spatially, animal survival was significantly associated with leaf litter diazinon concentrations within SRP and 1° cell. Although statistically significant, the association of leaf litter diazinon concentrations and survival within the 2° cell had a Power value below 0.8. This suggests a compromised sensitivity in the appropriateness of the linear model due, primarily, to the greater variation in survival data within this wetland cell. In general, there was a close relationship between measured diazinon concentrations and *H. azteca* survival responses throughout the study.

Few studies have attempted to assess pesticide toxicity specifically within detritus (Odum et al., 1969; Swift et al., 1988; Harrahy et al., 1994). More commonly, studies have shown the effects of contaminants on detrital processing rates (e.g., leaf processing) and associated biodiversity in streams (Bird and Kaushik, 1992; Scheiring, 1993; Forrow and Maltby, 2000; Pascoal et al., 2003). In the present study, detrital leaf litter remained acutely toxic to *H. azteca* for up to 15 d after dosing, showing a significant contribution to overall toxicity in the system. Aqueous acute (48–96 h) effects concentrations of diazinon to *H. azteca* ranged from approximately 4–15 μg/L (Collyard et al., 1994; Burkepile et al., 2000; Stuijfzand et al., 2000). In the current study, *H. azteca* survival was affected by leaf-litter diazinon concentrations of approximately ≥ 60 μg/kg (Table 3). This is 4–15-fold greater than published aqueous effects concentrations and suggests diazinon bound to detrital material is not as readily bioavailable to aquatic organisms as in the aqueous phase.

Based on responses of *Hyalella azteca* to diazinon contaminated leaf-litter, detritus-bound diazinon, initially serving as a contaminant sink during initial pesticide influx, may change to a source of diazinon contamination affecting nontarget aquatic organisms for days to weeks after entering a constructed wetland. Further studies are needed to elucidate the role of detritus in pesticide contamination within aquatic systems and associated effects on nontarget aquatic organisms.

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