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Diazinon Accumulation and Dissipation in *Oryza sativa* L. Following Simulated Agricultural Runoff Amendment in Flooded Rice Paddies

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Abstract Flooded post-harvest rice paddies were examined as systems for reducing diazinon (organophosphate insecticide) concentrations in stormwater runoff. Two paddies were cultivated in *Oryza sativa* L. and amended with a 3-h simulated stormwater diazinon runoff event. Initial diazinon adsorption peaked at 347 and 571 μg kg^{-1} (3% mass load reduction) for mean above-ground plant tissue concentrations in each pond, respectively. Subsequent senescence of above-ground tissue showed significant decreases in tissue mass (r^2=0.985) and adsorbed diazinon mass (90±4% and 82±1%) within 1 month of amendment. There were no corollary increases in water column diazinon concentrations. Furthermore, control *O. sativa* tissue placed within the treatment ponds had below-detectable levels of diazinon throughout the decomposition phase, suggesting a lack of within pond transference of dissipated diazinon. This study shows the relative effectiveness of diazinon adsorption by post-harvest rice plants and a potential mitigation strategy of senescence and pesticide degradation for contaminated tailwater.

Keywords Pesticide · Diazinon · Rice · Stormwater · Mitigation

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1 Introduction

Dormant season applications of pesticides, specifically organophosphates such as diazinon, in conjunction with frequent rainfall, result in contaminated runoff and off-site environmental risks. Organophosphates are of concern, as low concentrations (micrograms per liter) can be harmful to aquatic fauna (Giddings et al. 1996). Diazinon (*O*, *O*-diethyl *O*-(2-ispropyl-6-methyl-4-pyrimidinyl) phosphorothioate) is an organophosphate insecticide that inhibits neuronal acetylcholinesterase activity, resulting in neural excitation. Within the United States, 13 million tons of diazinon are applied annually, of which 20% treats agricultural produce and 80% treats various indoor and outdoor pests in urban environments (Banks et al. 2005). Post-2003, chemical manufacturing companies (e.g., Syngenta and Drexel Chemical Co.) have requested EPA to voluntarily cancel all product registrations containing diazinon (USEPA 2007). Diazinon has a moderate soil sorption (K_{oc}) of

1,750 and a water solubility between 40 mg L^{-1} (Evans et al. 1998) and 68 mg L^{-1} (Kratzer 1999) at 20°C.

California's concerns with diazinon nonpoint source contamination in agricultural surface waters is well documented (Domagalski 1996; Kratzer 1999; Epstein et al. 2001; Holmes and de Vlaming 2003; de Vlaming et al. 2004; Joyce et al. 2004; Schiff and Sutula 2004; Brady et al. 2006). Particular examples known in the literature are the Central Valley, including Sacramento and San Joaquin rivers, and Imperial Valley, including Alamo and New Rivers. In the Central Valley, orchard (almond and other stone-fruit) growers use diazinon in winter to control wood-boring insects. Winter rainfall results in high diazinon loads in both San Joaquin (Kratzer 1999) and Sacramento Rivers (Domagalski 1996; Brady et al. 2006). de Vlaming et al. (2004) reported high levels of mortality in *Cerodaphnia dubia* in the Imperial Valley watershed as a result of diazinon, with the extent of mortality being highly correlated with quantity of organophosphate applied within the watershed.

Along with almond and stone fruit production, a multitude of other agricultural products are also produced in California, including rice. At the time of winter and diazinon application, associated rice paddies are fallow post-rice harvest. Most often, paddies are flooded and rice stubble is left to senesce within rice paddies until the following year when the soil is once again prepared for cultivation. Flooding post-harvest rice paddies with contaminated drainage waters could be environmentally advantageous in tailwater recovery. Tailwater recovery is the reduction of contamination risk from tailwater to improve off-site water quality. Tailwater mitigation is important in systems where drainage waters are directed rapidly into receiving waters, and the potential for contamination is high. In these systems, water for reuse is pumped through fallow, post-harvest fields where biodegradation and microbial processes reduce pesticide loads and concentrations. Furthermore, post-harvest tailwater recovery could be beneficial in times of droughts and water shortages where runoff from irrigated fields can be collected, conveyed over fallow paddies for reuse or simply used for off-site water quality remediation. However, tailwater recovery would not be useful for systems were contaminants such as heavy metals and persistent agrochemicals tend to accumulate in the soil substrate.

Little information on diazinon absorption and adsorption to vegetation occurs in the literature. Kansouh and Hopkins (1968) examined the absorptive characteristics of diazinon in bean plants. Diazinon is initially absorbed and accumulated in high quantities in bean roots than anywhere else in the plant. Further studies have shown diazinon to be absorbed from leaf applications and translocated into the roots and root exudates (Gunner et al. 1966). Both studies noted a diffusion of diazinon from the plant into the aqueous medium along a concentration gradient. Sixty-eight percent of absorbed diazinon is transferred from the roots to aqueous nutrient solution in 2 days, indicating that diazinon will move freely from roots to water (Kansouh and Hopkins 1968). Laanio et al. (1972) described the metabolic fate of diazinon in rice plants and showed that up to 50% of labeled ^{14}C diazinon was rapidly absorbed and translocated in rice plants. Primary degradation mechanisms of hydrolysis of the ester bond and oxidation of the isopropyl side chain resulted in 50% of accumulated diazinon dissipating in 9 days.

If rice plants do indeed act as biodegradation agents for tailwater and stormwater runoff, what occurs post-tailwater recovery within flooded rice paddies, i.e., during above-ground rice tissue senescence? Literature on decomposition of above-ground vegetation has shown nutrients such as nitrogen and phosphorus leach from plant tissues during senescence (Kröger et al. 2007), but to date, few studies (Schueler 1995; Horst et al. 1996) have examined pesticide dissipation rates post-amendment.

This study took place in simulated rice paddies in Mississippi, where conditions surrounding California's diazinon concern and the potential management treatment plan were mimicked. This study used post-harvest rice paddies, amended with simulated enriched diazinon drainage effluent and examined (1) the uptake (adsorption and absorption) of diazinon by rice plants growing in amended rice paddies, (2) the dissipation of diazinon from decomposing rice plants, and (3) the potential transfer of the insecticide from the treated paddies to receiving waters.

2 Materials and Methods

2.1 Experimental Design and Setup

Common rice (*Oryza sativa* L.) was cultivated, amended with diazinon, and subsequently allowed to senesce in two adjacent treatment wetland cells (W1 and W2) at the University of Mississippi Field Station (UMFS), Abbeville, MS, USA (Fig. 1). A third cell (W3) at the UMFS provided a local non-contaminated system where rice was grown in similar substrate and climate conditions without the applied diazinon. Cells were prepared according to standard agricultural practices for rice cultivation. Cells were drained, dried, tilled, sown, and fertilized (90 kg ha^{-1} Triple 13 N/P/K). Water levels were increased and decreased as required for maximum germination. Prior to amendment, treatment cells outflow pipes were adjusted to increase water volumes to a theoretical 3 h hydraulic residence time, thus making water residence time roughly equal between the two cells despite the difference in cell configuration and dimensions. Each treatment cell was flooded with UMFS spring water to this theoretical residence time prior to amendment. Control cell water levels prior to and during the experiment were spring fed and were similar to the treatment cells. In November 2006, at 1 month post-harvest (rice harvested with remaining rice plants intact), treatment cells were amended with a storm runoff containing 0.1% of simulated applied diazinon (*Martins Diazinon 4E*; *a.i.* 47.5%) over an 80 ha field. The timing of the amendment would coincide with the dormant season application of diazinon to orchards in CA and thus diazinon would be at its peak concentration and load in drainage waters. Amendment occurred over 3 h through a 30 m PVC diffuser with regular spaced (±20 cm) diffuser holes (Fig. 1). A mixing chamber of 200 L amended a mean targeted diazinon concentration of 0.68 mg L^{-1} through 0.5-in. Tygon® plastic tubing plumbed into the PVC diffuser. Mean discharge rate for diazinon amended inflow was 0.0003 m^3 s^{-1} with a total discharge effluent water volume of 32,400 L for the duration of the amendment. Equal volume and flow rates occurred over the length of the diffuser and between treatment cells. Outflow discharge rate peaked 3–3.5 h post-amendment.

2.2 Sampling Design

Bulk water and plant samples were taken at five sampling transects within W1 and W2 (Fig. 1). The five transects in W1 and W2 were inflow (0 m), 12 (8) m, 24 (16) m, 36 (24) m, and outflow (50, 28 m), respectively. A bulk sample constituted a grab sample every 6 m across the width of the respective cell. Water samples were taken in acetone/hexane washed 1-L amber glass bottles every 30 min for 6 h and thereafter at 8, 24, 48, and 72 h. Additional outflow water samples were taken weekly till the cells were drained in the following spring (March 2007). Whole plant samples were taken at each transect every hour till 6 h and thereafter at 8, 24, 48, and 72 h and were wrapped in heavy-duty aluminum foil and placed in

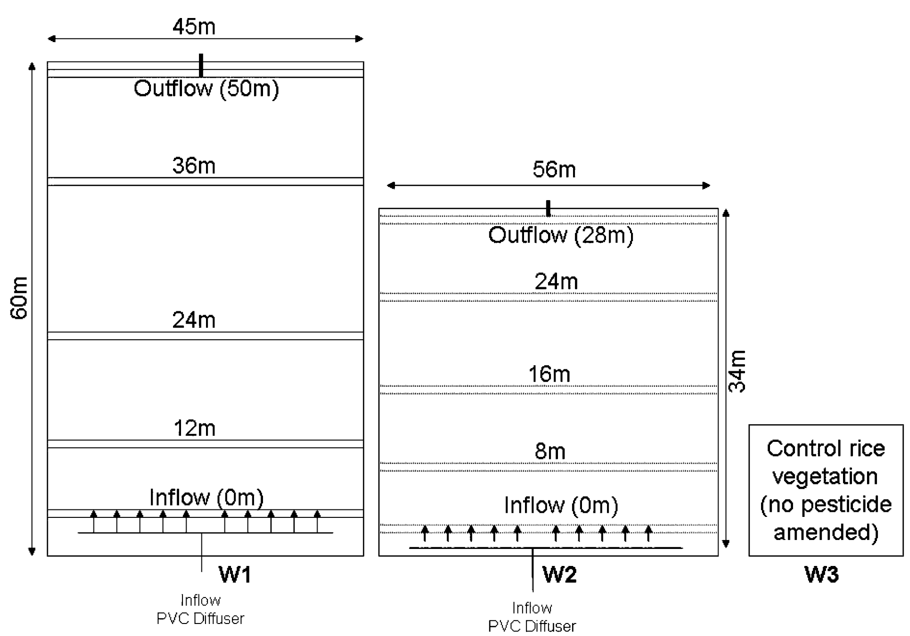

Fig. 1 Two wetland ponds were cultivated in *O. sativa* and amended with a 3-h diazinon storm runoff exposure. Plant decomposition samples (1–5) were stratified within each pond and sampled over a typical 4-month fallow period. Control plant samples (c) were placed at sample location three

labeled double-lock Ziploc™ bags. Bulk inflow and outflow samples were sampled in front of the discharge pipes, respectively, in both treatment cells. Water and plant loads/mass was determined by multiplying the known compartment concentration by the known water volume at all transects.

2.3 Plant Tissues and Decomposition

Variable N-concentrations and lignin content have been shown to influence decomposition rates (Cronk and Fennessy 2001). Decomposition rates were compared between diazinon amended rice (W1 and W2), control pond rice (W3), and rice grown at a neutral site (control neutral rice). The neutral site rice was grown off-site under similar climate and substrate conditions as in the cell experiments, but on a smaller scale. This comparison allowed an evaluation of the effects of pesticide sorption (amended vs. non-amended/control) on decomposition rates of rice.

Plant tissues were collected before and after pesticide amendment in all treatments. Pre-amendment rice samples were taken at all transects in both treatment cells (W1 and W2), and bulk was sampled within the control pond (W3) and control neutral site to establish baseline diazinon levels. Six hours post-amendment, a large bulk plant sample was taken from each transect in W1 and W2 and randomly within W3 and control neutral site. The first half of the sample represented the initial diazinon plant concentration prior to deployment and decomposition. The second half of each sample was used as the initial plant sample placed in the decomposition bags. Approximately 50 g WW (±30 g DW basis) of plant material from the respective transects and treatment cells were placed in accordingly labeled decomposition bags with 5 mm mesh diameter. Decomposition bags were redeployed at the respective transects on the soil surface and attached with stakes to avoid being moved. Control pond rice and control neutral rice was similarly placed in labeled decomposition bags and deployed at transect three in W1 (24 m) and W2 (16 m), respectively. These transects represented the halfway point for each respective treatment cell in terms of surface area and thus would retain water over a similar time period.

In cell W1 and W2, each sampling transect consisted of five replicate mesh bags. At transect three, W1 (24 m) and W2 (16 m), there were a further five control pond rice bags and five control neutral rice bags. A single decomposition bag was collected monthly, for 4 months, from each sampling location in W1 and W2. Each bag removed from the field was placed in a double-lock Ziploc™ bag to avoid cross-contamination within the ice chest. At the laboratory, individual bags were gently washed with DI water to remove accumulated sediment on the bag and sample. Samples were removed from the bags for preparation and analysis. To simulate dormant season field circumstances in CA, water levels were increased post-amendment in both treatment cells. Increasing water residence time is a commonly used management option to enhance pollutant removal processes and to attract wildlife.

2.4 Sample Preparation and Analysis

All samples were transported in ice chests back to the United States Department of Agriculture—Agricultural Research Service facility in Oxford, MS, USA. All plant samples post-washing were frozen for 48 h prior to drying. Samples were air-dried to a constant weight under similar environmental conditions. The effect of temperature and drying time on diazinon concentration was assumed to be negligible for all samples. Plant material was randomly ground using a Wiley Plant Mill (<2 mm) and subsequently mixed. After each sample, the plant mill was cleaned using compressed air to remove remaining residues. A 2 g sub-sample derived from the larger bulk sample was used for analysis. A 500 mL water sample was immediately fixed with 500 mg KCl and 25 mL distilled ethyl acetate and prepared for gas chromatography analysis within 48 h. Diazinon concentration in the water column was extracted following Bennett et al. (2000), Smith and Cooper (2004), and Smith et al. (2006). Diazinon in plant material was extracted using the following procedure. Two grams of finely ground plant material and 20 mL ethyl acetate were placed in 50 mL centrifuge tubes. Tubes were sonicated (Sonics GE600 sonicator) for 1 min in pulse mode using an 80% duty cycle. Following sonication, samples were centrifuged at 3,500 rpm using an IES HN-S centrifuge with a four-place rotor. The solvent layer was removed, a further 10 mL of ethyl acetate was added, and the above extraction process was repeated. Each extract was concentrated to near dryness under a UHP nitrogen using a nitrogen evaporator (N-EVAP, Organomation). Samples were subjected to silica gel columns pre-wet with hexane for clean up. Glass micro-columns were fitted with wool plugs, and 200°C activated silica gel (5 cm) was poured into the column. Concentrated sample extracts were added to each column and rinsed twice with approximately 1 mL of hexane. A 10 mL hexane/acetone eluent was added to sample, which was transferred into 16 mm×100 mm culture tube. The extract was further concentrated using the nitrogen evaporator, and ethyl acetate was added to bring the sample to a 1 mL volume (Bennett et al. 2000).

Both water and plant analytes were analyzed by gas chromatography-electron capture detection using HP model 6890 gas chromotagraph equipped with dual HP 7683 ALS autoinjectors, dual split-splitless inlets, dual capillary columns a HP Kayak XA chemstation (Smith and Cooper 2004), and a main 30 m×0.25 mm i.d. (0.25 μm film thickness) HP 5MS capillary column. A multi-level calibration procedure was used with standards and was updated every tenth sample. Repeated standards were 0.1, 1, and 10 ppb with 100% recovery of each. The limits of detection for diazinon aqueous and plant tissue analyses were 0.01 μg L^{-1} and 0.1 μg kg^{-1}, respectively. All samples were analyzed in triplicate from the original sub-sample.

Plant samples, both pre- and post-exposure, were calculated in parts per billion (micrograms per kilogram). Pearson linear correlations and two-sample, two-tailed, equal variance Student t tests were used for statistical comparisons (α=0.05). The diazinon tissue concentrations that are reported henceforth are a combination of two processes: adsorption and absorption. Without being able to distinguish between the two, the diazinon results reflect the total amount of pesticide sorbed to the plant.

3 Results and Discussion

3.1 Initial Diazinon Sorption

Initial aboveground rice tissue analysis determined that diazinon concentrations were below detection limits in rice plants sampled spatially within W1 and W2 prior to diazinon amendment. Furthermore, diazinon was not detected in either control pond rice or control neutral rice prior to deployment into W1 and W2. Post-diazinon amendment, overall (0–24 h) above-ground diazinon concentrations were a function of distance from the injection point (Fig. 2). Pearson's linear correlations of diazinon tissue concentrations and distance from injection point weakly correlated for treatment cell W1 (r^2=0.6231) and had no distinct trend in treatment cell W2 (r^2=0.0313) when including the outflow transect samples. However, by excluding the outflow samples from the correlation analysis,

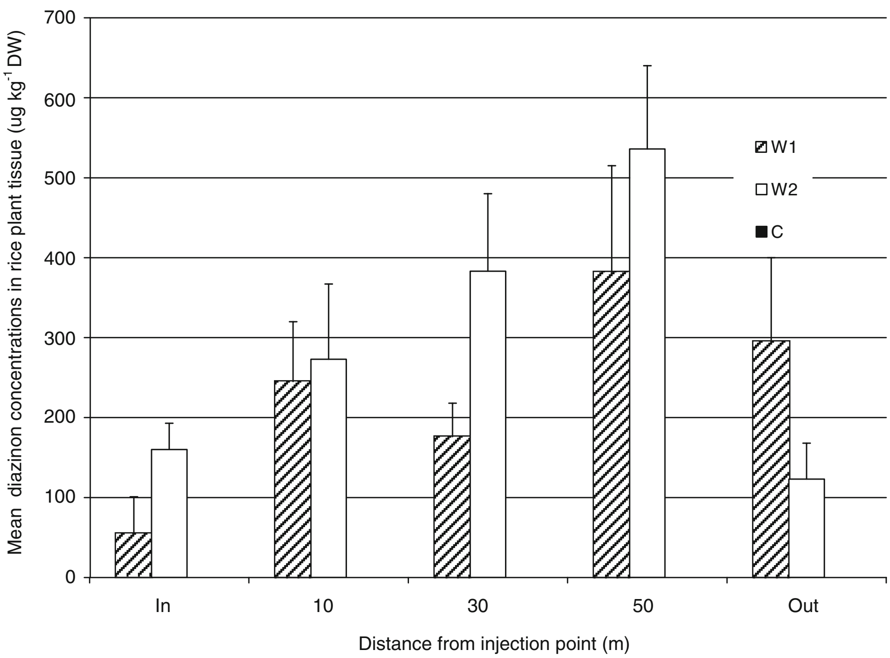

Fig. 2 Overall (0–24 h) mean diazinon concentrations in above-ground rice plant tissue (microgram per kilogram DW) as a function of distance from the injection point (meter; n=8). Concentrations represent total absorbed and adsorbed diazinon on the plant

statistically significant increases in diazinon tissue concentrations were associated with distance from the injection point for both W1 (r^2=0.7438) and W2 (r^2=0.9934). Furthermore, diazinon plant tissue concentrations increased with exposure time (Fig. 3). Pearson's linear correlations (r^2) of 0.541 (p=0.07) and 0.8033 (p<0.01) for W1 and W2, respectively, highlight weakly and strongly significant relationships. Thus, these data suggest that increased exposure time results in significant increases in overall diazinon tissue concentrations in the above-ground rice tissue. Syversen and Haarstad (2005) described pesticide residues in riparian buffer zone vegetation. Of the 11 different pesticides reported, diazinon showed significant accumulation and retention by above-ground riparian vegetation, where two concentrations (1.5 and 15 μg L^{-1}) resulted in 94–98% retention of diazinon. Under laboratory conditions, Laanio et al. (1972) demonstrated the ability of diazinon (^{14}C-labelled) uptake in rice. Uptake within the root mass remained constant throughout the experiment, while an increase in radioactivity over time in the shoots suggests rapid translocation of diazinon. In contrast, Kansouh and Hopkins (1968) reported that diazinon accumulated in higher quantities in bean roots than in any part of the bean plant. Eighty percent of the diazinon accumulated in the roots, while the remainder translocated to the stem and shoots in decreasing amounts (Kansouh and Hopkins 1968). Gunner et al. (1966) discovered that diazinon-swabbed bean plants rapidly translocated the diazinon from shoots to roots. Diazinon was detectable in the roots, and root exudates within 18 h of the swab application.

Interestingly, at the time of decomposition bag deployment (6 h) the mean water column diazinon concentration was 80.28±22 and 129±15 μg L^{-1} for W1 and W2, respectively. The mean initial amended above-ground tissue concentration deployed into each decomposition bag was 347±108 and 571±135 μg kg^{-1} for W1 and W2, respectively. This plant concentration range was similar to those reported elsewhere in the literature (Watanabe and Grismer 2001). Overall, this was a 434% and 442% increase in plant tissue concentration as compared to the water column. It is noted in the literature that plants have the capacity for luxury uptake, i.e., the ability to obtain higher than necessary concentrations of nutrients (Cronk and Fennessy 2001; Kröger et al. 2007), heavy metals (Outridge and Noller 1991; Jayaweera et al. 2008; Meyers et al. 2008), polychlorinated biphenyls (Macek et al. 2000; Alkorta and Garbisu 2001), and 2,4,6-trinitrotoluene (Hughes et al. 1997). However, very little is known concerning plant capacity in pesticide uptake. In this system, when converting the concentration sorbed to a mass balance of diazinon load mitigated within the entire wetland cell, the overall contribution of above-ground rice is low. Averaging the results over 6 h, the sorption to above-ground tissue accounted for 2.8±0.5% (W1) and 3.6±0.5% (W2) of total diazinon load mitigation, respectively.

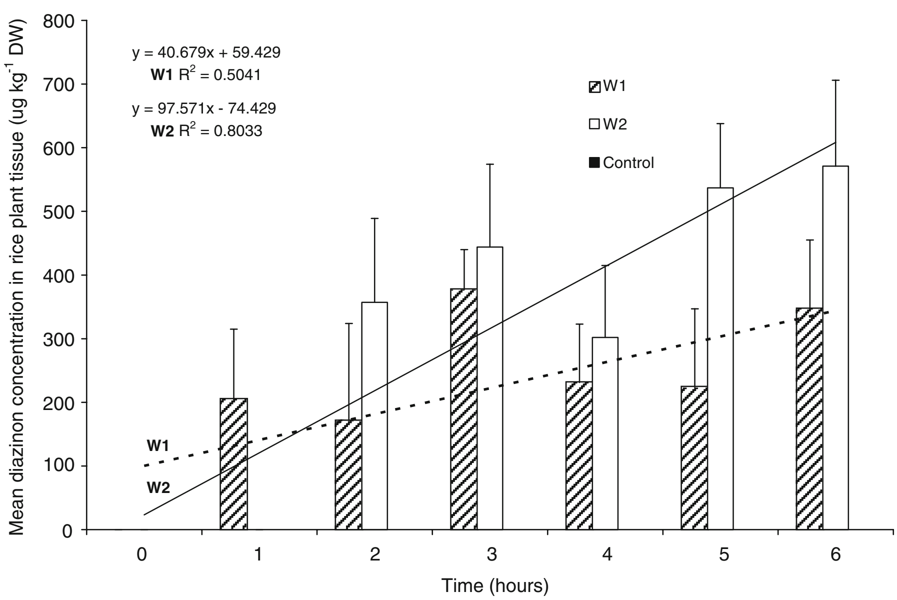

Fig. 3 Average diazinon concentrations per pond of above-ground rice plant tissue over time. Transect samples (n=5) were grouped per time sampling interval. Control samples include both control pond and control neutral rice

Water contributed over 52.4±2.5% and 60.3±4.4% for W1 and W2, respectively, with the remainder sorbing to sediment.

3.2 Spatial and Temporal Trends in Diazinon Dissipation

At deployment of the decomposition bags, there were no significant differences (p=0.23) between initial above-ground diazinon concentrations in W1 and W2. Likewise, the control pond and control neutral above-ground rice had below-detectable concentrations of diazinon when deployed into W1 and W2, respectively. A binomial regression (Fig. 4) showed a decrease (r^2= 0.9855) in rice plant weight with time for all treatments. There were no effects of site type (control pond/ control neutral/treatment) on decomposition rates and biomass loss.

The greatest percentage loss of diazinon, 90±4% and 82±1%, occurred 1 month post-amendment for W1 and W2, respectively (Fig. 5). There were no significant differences (p>0.05) in diazinon concentrations in decomposition samples spatially within W1 and W2 for any month sampled. Branham and Wehner (1985) identified fates of diazinon on turf grass and reported 90.3%, 64.5%, and 49.6% of degradation respectively for turf treatments after 3 weeks. However, research by Graebing and Chib (2004) demonstrated that diazinon was more susceptible to hydrolysis than photolysis because studies indicated that irradiated solution half-lives were similar to those of dark controls. The fact that within the flooded rice fields rice decomposition samples were saturated by a constant water cover increases the possibility of hydrolytic degradation and metabolism of diazinon. Horst et al. (1996) reported that irrigation in addition to rainfall, clipping, and degradation reduced pesticide concentrations in turf up to 82% by the second sampling (10 days).

All treatment and control replicates were below diazinon detection limit of 0.01 μg kg^{-1} 3 months post-amendment (Fig. 5). When drained (March, 2007), decomposed rice vegetation previously exposed to diazinon concentrations had no detectable diazinon concentrations in the above-ground tissues. Water column concentrations for diazinon 3 months post-amendment were less than 5 μg L^{-1} spatially throughout both ponds as compared with a maximum 6 h amendment concentration (time 0; Fig. 5). No diazinon was detected in any control pond or control neutral rice samples throughout the 4-month duration of the experiment. The low concentrations in the water column, as well the lack of diazinon in control samples, suggests that dissipated diazinon lacks transference within either treatment cells. Diazinon entering the ecological system can either volatize to the atmosphere, sorb to soils and plants, be taken up by aquatic organisms, or remain in solution (Larkin and Tjeerdama 2000). Diazinon remaining in solution is conducive to undesired transport to receiving

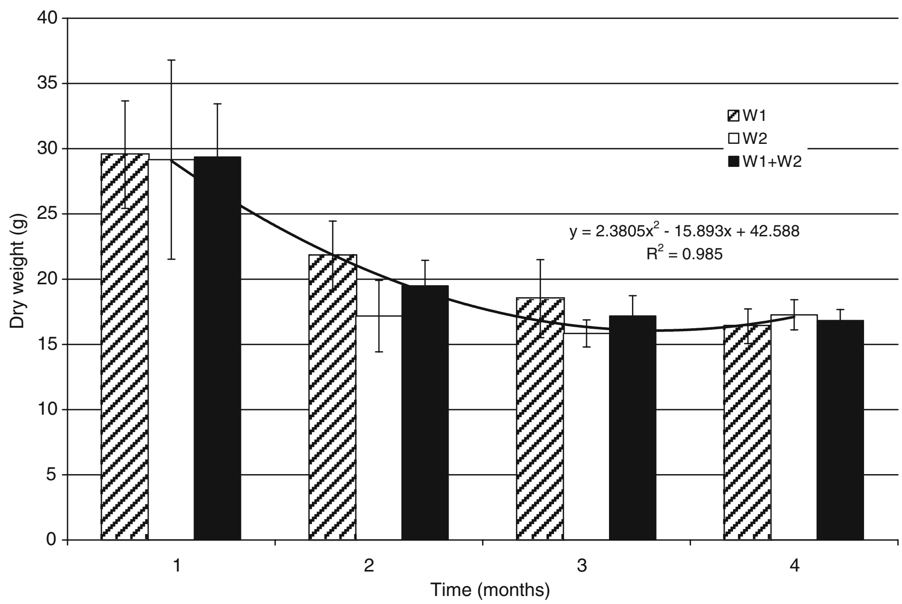

Fig. 4 Change in rice biomass weight with time (n=5). There were no significant differences between initial plant weights (month 1) and through time between W1, W2, and control (ANOVA; p>0.05)

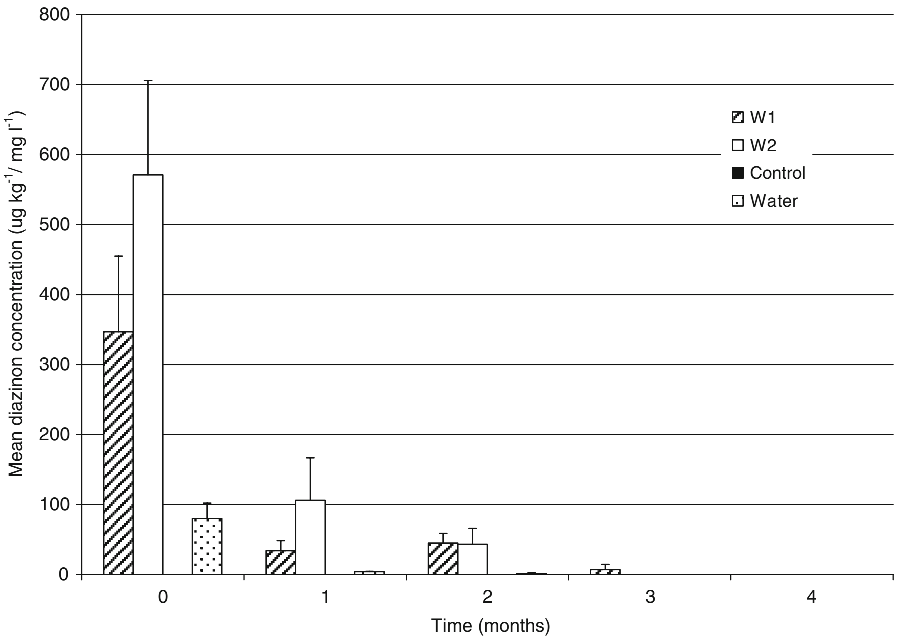

Fig. 5 Mean diazinon concentrations in senescent vegetation and water column over time (4 months post-amendment; n=5). Time 0 is the amended rice tissue concentrations, as well as water column concentrations at 6 h (initial decomposition)

systems, while adsorbed diazinon undergoes hydrolysis and photolysis. Organophosphate pesticides will undergo hydrolysis after application, converting to less toxic compounds or oxon analogues (Domagalski 1996). Oxon analogues, though more toxic than the parent, undergo faster hydrolysis relative to the parent organophosphate. The increased retention time simulated from this study clearly demonstrates the sorbance of diazinon, in a luxury capacity, to the vegetative compartment from the water column. Furthermore, the non-transference of sorbed diazinon between sediment, vegetative compartments within the system and the lack of diazinon in drained outflows, highlights the effective use of post-harvest rice paddies in aiding tailwater recovery. The lack of insecticide recycling suggests that diazinon dissipation occurs within the flooded field and poses no temporal threat to downstream aquatic environments when fields are drained.

This research, though aimed at rice paddies in California and potentially crayfish/rice farmers in Louisiana, can be modified for urban environments. Studies have shown the relationship between urban stormflows and pesticide application and fate within surface waters (Schueler 1995; Horst et al. 1996). Schueler (1995) detected low diazinon concentrations every month in urban streams in Atlanta, GA, with the highest measured concentration occurring in May, when spring pre-emergence applications are highest. Schueler (1995) furthermore suggested that diazinon exhibited the greatest risk of toxicity in urban stormwater situations.

4 Conclusion

Senescence of vegetation with adsorbed diazinon (e.g., post-harvest rice) in flooded tailwater recovery systems poses little discernible environmental concern for contamination of downstream ecosystems following draining of flooded rice fields. A lack of diazinon recycling and below-detectable diazinon concentrations in drain outflows suggest that diazinon dissipation occurs within the flooded agricultural system. Wetland biogeochemical conditions, variable water residence times, and vegetation can be introduced within detention basins and constructed wetlands to produce similar characteristics to the flooded rice paddies and aid in the hydrolysis and photodegradation of associated pesticides.

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