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Survival and Growth of *Hyalella azteca* Exposed to Three Mississippi Oxbow Lake Sediments

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The lower Mississippi River Delta (LMRD) has historically been an area of intensively cultivated agriculture. Consisting of portions of Missouri, Arkansas, Mississippi, and Louisiana, the LMRD is home to over 1.2 million hectares of cotton (*Gossypium hirsutum* L.), rice (*Oryza sativa*), corn (*Zea mays* L.), soybeans [*Glycine max* (L.) Merr.], and various other crops. In order to maintain the area's significant food and fiber production, exhaustive pesticide applications have been historically used. With increased land drainage, there are more opportunities for these pesticides to be transported to aquatic receiving systems, such as rivers, lakes, and streams.

Scattered throughout the LMRD are remnants of former river meanders known as oxbow lakes. These lakes have since been isolated from their original rivers, and they serve as historical benchmarks of agricultural contamination over the last century. By capturing runoff from a smaller contributing area (as opposed to an entire river's watershed), these oxbow lakes provide crucial insights not only into historical pesticide contamination, but also into potential ecosystem recovery and rehabilitation.

The objective for this research was to determine potential impairment of *Hyalella azteca* exposed to sediment collected from three Mississippi Delta oxbow lakes, using both analytical chemistry and bioassays. *H. azteca* are epibenthic macroinvertebrates, commonly found in permanent lakes, ponds, rivers, streams, and ditches across North America (de March 1981). The organism's ubiquity, ease of laboratory culture, and epibenthic nature make it a viable candidate for sediment bioassays.

MATERIALS AND METHODS

Sediment cores were collected from three Mississippi Delta oxbow lakes: Mossy Lake, Three Mile Lake, and Macon Lake. All three lakes are located in the intensively cultivated Bear Creek watershed in western Mississippi, USA. Mossy Lake is approximately three miles southwest of Morgan City in Leflore County. Three Mile and Macon Lakes are located in Sunflower County, approximately six and nine miles, respectively, east of Inverness, Mississippi. Cores were collected

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at various sediment depths (0-20 cm; 20-40 cm; 40-60 cm; 60-80 cm) from each lake for larger, in-depth analytical chemistry and sediment accumulation studies; however, only the shallow core (0-20 cm) was used for toxicity assessments, since it represented the fraction of the sediment-water interface where biological impairment may occur.

Individual core samples for toxicity assessments were collected using a 7.6 cm diameter PVC coring tube by manually plunging into sediments. Individual samples were collected and composited prior to being stored in acetone-hexane rinsed, 1 L glass containers. Samples were placed on ice and transported to the USDA-ARS NSL for immediate chemical extraction and use in bioassays.

H. azteca were cultured at the USDA-ARS National Sedimentation Laboratory, according to standard operating procedures (de March 1981). Ten-day static, non-renewal, whole sediment toxicity experimental exposures were conducted according to US EPA protocol (2000). Organisms that passed through a 425 μm mesh sieve, but were retained by a 250 μm mesh sieve, were collected and used in the experiment (approximately 4 d old). Sediment (40 g) from each lake sample was placed in three replicate exposure chambers per site (250 mL borosilicate glass beakers). Ten *H. azteca* were placed in each replicate exposure chamber along with three 2 cm maple leaf discs for food and substrate. Exposures were conducted in a Powers Scientific Animal Growth Chamber with a 16:8 h photoperiod. Overlying water, free from priority pollutants, was obtained from springs at the University of Mississippi Field Station (UMFS) (Gillespie et al. 1996; Moore et al. 1998). Control sediment was also collected from UMFS (Deaver and Rodgers 1996). Measured water chemistry parameters included dissolved oxygen, temperature, pH, conductivity, alkalinity, and hardness (APHA 1998) (Table 1). Toxicity endpoints measured included survival and growth (measured in both body length and weight). Body length was measured using a Videometric 150 Image analyzer with Videometric software.

Sediment samples were analyzed for a suite of 17 current and historic-use pesticides and metabolites. Analytical chemistry was performed according to Bennett et al. (2000) using a Hewlett-Packard 6890 gas chromatograph equipped with dual HP 7683 ALS autoinjectors and microelectron capture detectors. Fortified samples were prepared for each pesticide and recovery measured.

Descriptive statistics and one way analysis of variance (ANOVA) tests were performed on the survival and growth (length and weight) data. If normality of the data set failed, a Kruskal-Wallis one way ANOVA was employed (Sigma Stat v. 2.0).

RESULTS AND DISCUSSION

Ingersoll et al. (1998) described the practicality of using sub-lethal endpoints (growth and reproduction) in longer duration experiments ($\geq$ 10d) for sediments that may be marginally contaminated, as opposed to shorter duration toxicity tests

Table 1. Mean (± s.d.) overlying water quality characteristics of each lake sediment exposure.

Parameter	Control	Mossy	Three Mile	Macon
Temperature (°C)	24.3±0.2	23.9±0.1	24.1±0.4	23.9±0.2
pH (s.u.)	7.4±0.1	7.8±0.1	7.7±0	6.9±0.4
Dissolved oxygen (mg/L)	5.6±0.1	6.0±0.1	6.2±0	5.9±0.3
Alkalinity (mg/L $CaCO_3$)	64.2±30	73±6.0	60±0	41±3.0
Hardness (mg/L $CaCO_3$)	64.2±30	103±0	86±12	54±9.1
Conductivity (μmhos/cm)	307±29	291±15	327±12	273±11

measuring only survival in highly contaminated sediments. Survival was not observed to be significantly impaired in any of the three oxbow lake sediments (Table 2). Likewise, no observable significant differences were noted in *H. azteca* length when compared to controls. There was a significant difference in weight of *H. azteca* exposed to Three Mile Lake sediment; however, it was a significant increase in weight.

Of the 17 pesticides and metabolites screened in sample sediments, 11 were detected in Mossy Lake, 6 in Three Mile Lake, and 10 in Macon Lake. Based on fortified samples, extraction efficiencies for each pesticide were $\geq$ 90%. Organochlorine insecticides dieldrin (1,2,3,4,10,10-hexachloro-exo-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene), DDT (dichloro diphenyl trichloroethane), and DDT metabolites DDE (dichloro diphenyl dichloroethylene) and DDD (dichloro diphenyl dichloroethane) were measured in all three oxbow lake sediments (Table 3). Moon Lake, another extensively studied oxbow lake located within the Mississippi Delta, had a reported mean lake sediment sum DDT concentration of 235.45 μg/kg (Cooper, 1991). Given the proximity of these lakes to historical cotton-producing agricultural land, these measurements are not surprising.

Chlorfenapyr [4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluromethyl) pyrrole-3-carbonitrile), an insecticide applied under a 1995 Section 18 permit, was briefly used on Mississippi cotton. In 1999, the US EPA determined chlorfenapyr posed too significant a risk to continue application. Concentrations of chlorfenapyr were detected in each of the three lakes, with the highest concentration (9.649 μg/kg) in Macon Lake. This lake also had the highest concentrations of DDT, fipronil (5-amino-1-[2,6-dichloro-4-(trifluoromethyl) phenyl]-4-[(trifluoromethyl)sulfinyl]-1*H*-pyrazole-3-carbonitrile), lambda-cyhalothrin [lambda-cyano-3-phenoxybenzyl-3-(2-chloro-3,3,3-trifluroprop-1-enyl)-2,2-dimethyl cyclopropanecarboxylate], and cyanazine [2-(4-chloro-6-ethyl-amino-1,3,5-triazin-2-ylamino)-2-methylpropionitrile]. Even though the concentration of DDT measured in Macon Lake was five times that measured in Mossy Lake, there were no observed significant difference in *H. azteca* length and

Table 2. Mean survival and growth of *Hyalella azteca* exposed to three oxbow lake sediments (alpha = 0.05).

Site	Survival	Length (mm)	Weight (mg)
Control	99±2%	2.01±0.43	0.04±0.02
Mossy Lake	96±2%	2.16±0.28	0.05±0.01
Three Mile Lake	92±12%	2.64±0.49	0.1±0.03*
Macon Lake	81±4%	2.15±0.37	0.04±0.02

* denotes significant difference

Table 3. Pesticide concentrations (μg/kg) in three Mississippi oxbow lake sediments (0-20 cm depth).

Pesticide	Detection limit (μg/kg)	Mossy Lake	Three Mile Lake	Macon Lake
Alachlor	0.0005	bd	bd	bd
Metalochlor	0.001	bd	bd	bd
Trifluralin	0.0001	bd	bd	bd
Pendimethalin	0.0005	bd	bd	bd
Dieldrin	0.0001	1.008	0.520	0.985
DDE	0.0001	2.717	1.490	4.129
DDD	0.0001	2.988	2.013	2.760
DDT	0.001	12.065	6.210	63.240
Chlorpyrifos	0.0001	bd	bd	bd
Methyl parathion	0.001	2.638	bd	3.120
Chlorfenapyr	0.0005	2.377	0.373	9.649
Fipronil	0.0001	1.664	bd	3.175
Fipronil sulfone	0.0001	2.093	1.147	1.597
Bifenthrin	0.0001	0.270	bd	bd
Lambda-cyhalothrin	0.0001	1.629	bd	2.129
Atrazine	0.001	29.536	bd	bd
Cyanazine	0.0005	bd	bd	6.488

bd = below detection limit

weight between the two lakes. Although not statistically different, Macon Lake did have the lowest mean *H. azteca* survival of the three lakes.

Few studies have focused on pesticide toxicity associated with site-specific sediment concentrations (Douglas et al. 1993; Hoke et al. 1994; Swartz et al. 1994). Hoke et al. (1995) detailed development of sediment quality criteria for the organochlorine dieldrin, while Fairey et al. (2001) examined sediment quality guideline quotients for chemical mixtures. Most available pesticide field and laboratory data are for water exposures only (Johnson and Finley 1980; Siegfried

1993; Hoke et al. 1994, 1995). Maund et al. (1998) reported a *Daphnia magna* 72 h LC_{50} for unstirred sediment contaminated with lambda-cyhalothrin to be 47 μg/L. This concentration is 20 times greater than the highest lambda-cyhalothrin measured in the three evaluated oxbow lakes.

It has been reported that organism sensitivity to certain pesticides may be altered in the presence of additional pesticides. Belden and Lydy (2000) reported that while aqueous concentrations of atrazine posed little risk alone to *Chironomus tentans*, in concentrations ranging from 40-200 μg/L, it increased toxicity of chlorpyrifos, methyl parathion, and diazinon. Jin-Clark et al. (2002) reported on synergistic effects of chlorpyrifos toxicity to *C. tentans* when exposed in combination with atrazine and cyanazine. Synergistic effects on larval amphibians have also been reported for atrazine exposed with alachlor (Howe et al. 1998). Anderson and Lydy (2002) determined that atrazine concentrations $\geq$ 40 μg/L caused a significant increase in *H. azteca* toxicity when exposed in combination with the organophosphate insecticides chlorpyrifos, methyl parathion, and diazinon. While there is considerable literature available on synergistic effects of pesticides in water exposures, little to no information is available for these potential effects in sediment exposures.

The sediment quality triad approach (SQT) advocates the use of analytical chemistry, toxicity tests, and benthic assessments to determine potential sediment impairment (Chapman et al. 1997). Even though *H. azteca* are not always the most sensitive test species for toxicological assessments dealing with pesticides, they do serve as reliable indicators of potential impairment of sediments with bound pesticides. With the presence and potential chemical interactions of organochlorine, organophosphate, pyrazole, pyrethroid, and triazine pesticides, it is impossible to predict potential sediment toxicity by analytical chemistry alone. By utilizing two of the three SQT (analytical chemistry and *H. azteca* bioassays), it was determined that there was no observable impairment of Macon Lake, Mossy Lake, or Three Mile Lake sediments.

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