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Responses of *Hyalella Azteca* to Chronic Exposure of Mississippi Delta Sediments

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Abstract: *Hyalella azteca* was used to assess biological impairment in sediments from nine water bodies in the Mississippi Delta (i.e., lower Mississippi alluvial plain). Water bodies were categorized according to land use and implementation of agricultural best management practices (BMPs). Sediment samples were collected at three sites within each water body from June to July 2004 and analyzed for 17 current and historic-use pesticides and metabolites. Twenty-eight day *H. azteca* survival and growth were measured to assess the degree of biological impairment. No significant ($P > 0.05$) mortality occurred in animals exposed to sediments. Significant growth impairment was observed in sediments from all three 303(d) listed water bodies and two of three BMP oxbow lakes. Historic-use pesticides and metabolites were implicated in two of five biologically impaired water bodies. Complex contaminant mixtures often limit attempts to provide clear, definitive sources of biological impairment. In this study, even accounting for sediment characteristics such as sand-silt-clay fractions and organic carbon content did not further clarify sources of toxicity in some water bodies. Finally, results show that implementation of BMPs can mitigate biological impairment within lake sediments.

Key words: *Hyalella azteca*, chronic toxicity, sediment, pesticides, Mississippi Delta.

1. Introduction

The lower Mississippi River Alluvial Plain, commonly referred to as "the Delta", is one of the most intensive agricultural areas in the US [1] and produces a variety of crops including cotton (*Gossypium hirsutum*), soybeans (*Glycine max*), corn (*Zea mays*), and rice (*Oryza sativa*). The region comprises the southern portion of the Mississippi River basin and extends over 1100 km from southeastern Missouri to Louisiana at the Gulf of Mexico [2]. The Mississippi Delta is 18,130 km^2, and has a long growing season with conditions conducive to frequent pesticide use. Associated with this use is a concomitant potential for transport into nearby water bodies such as lakes, rivers and streams. The Mississippi Delta has numerous bayous, sloughs, and oxbow lakes, water bodies that have been physically isolated from their respective main river channels [3]. Because of extensive crop cultivation in the Delta, pesticide use is an integral part of the region [4]. Mississippi Delta water bodies often receive pesticide laden effluent from agricultural fields, primarily during storm events [5].

To meet the goals of the Federal Clean Water Act (CWA), the state of Mississippi in 1996 first compiled a list of impaired water bodies within the Yazoo Drainage Basin, including the Delta. The TMDL(Total Maximum Daily Loads) program is primarily focused on identifying the loading limits of contaminants, such as pesticides, in water bodies to ensure that appropriate standards are met for that water bodies' designated use (e.g., drinking, swimming, and fishing). Within this program, sediments have been recognized as both potential sinks and sources of contaminants. For 303(d) listed water bodies, an assessment of sediment

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contamination and toxicity can determine the extent that sediment adds to the overall degraded condition of the water body while contributing in the development of load allocations and attainment goals and strategies for the eventual water body TMDL [6].

Assisting the state of Mississippi in meeting the goals of the CWA, the US Department of Agriculture in 1994 implemented the Mississippi Delta Management Systems Evaluation Area, designated as a regional effort to evaluate best management practices (BMPs) that could mitigate non-point source pollution in the Delta [7]. Determining the ability of BMPs in mitigating pesticide contamination of sediment is an important part of understanding the extent of overall potential contamination in Delta water bodies and assists in assessing several concerns.

However, in order to better ascertain the extent that BMPs mitigate pesticide contamination, there is a need for a comparable reference condition. A likely candidate was found within the White River National Wildlife Refuge (NWR). NWR is located in the Delta portion of the state of Arkansas and contains water bodies with nearly identical geologic and hydrologic features as those in the Delta portion of the state of Mississippi. The refuge is comprised of 64,000 ha of which 61,600 ha are bottomland hardwood forest and includes 356 lakes interconnected with streams, sloughs and bayous. Anthropogenic impacts within the refuge are minimal with limited access during annual natural winter and spring flooding and less than 0.6% of the area (360 ha) designated as cropland [7].

Overall objectives of this study are summarized in four parts: 1) assess sediment quality impairment due to current agricultural practices; 2) determine impacts and degradation of aquatic systems (i.e., habitat) has occurred; 3) address limited availability of data on sediment pesticide contamination and/or impairment in Mississippi Delta water bodies with limited anthropogenic impact (control sites); and 4) ascertain the efficacy of BMPs in mitigating pesticide contamination of sediment. This study assesses biological impairment in Mississippi Delta water body sediments using *Hyalella azteca* during summer, 2004, in three category water bodies; control, BMP, and impaired (according to USEPA section 303(d) Clean Water Act).

2. Materials and Methods

Nine sampled watersheds were located in the Mississippi Delta and divided into three categories: NWR, control; BMP; 303(d), impaired. NWR watersheds were adjacent to the White River in Arkansas and included Columbus Lake and Lower White Lake located in Arkansas County, AR, and Upper Swan Lake located in Monroe County, AR (Fig. 1).

While NWR is a national wildlife refuge and relatively free of row crop agriculture (< 0.6% of total area), the White River drains much of the Arkansas portion of the Mississippi Delta which is significantly

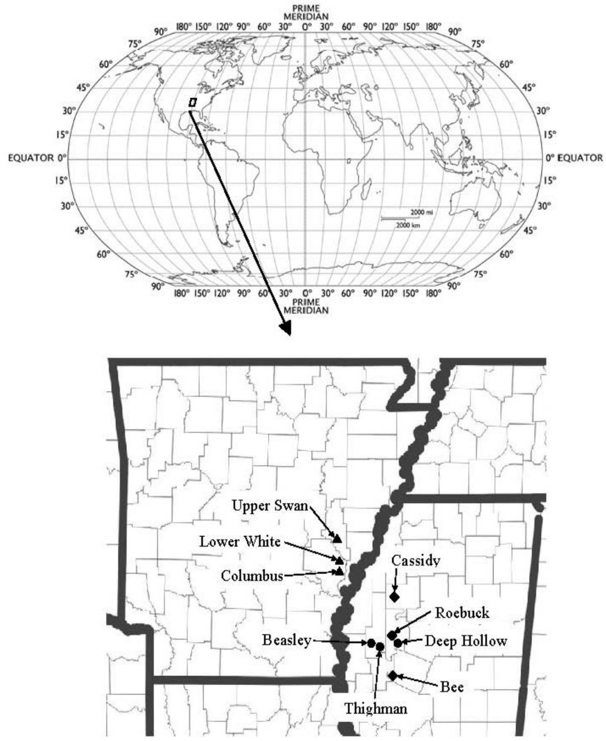

Fig. 1 Locations of water bodies studied within the Mississippi Delta, USA. White River National Wildlife Refuge water bodies (▲); Best Management Practices water bodies (●); USEPA Clean Water Act section 303d impaired water bodies (♦).

cultivated and a potential source of pesticide contamination during winter and spring flooding. BMP watersheds included Beasley Lake and Thighman Lake located in Sunflower County, MS, and Deep Hollow Lake located in Leflore County, MS. The 303(d) watersheds included Bee Lake located in Holmes County, MS, Cassidy Bayou located in Tallahatchie County, MS, and Roebuck Lake located in Leflore County, MS. Land use of BMP and 303(d) watersheds was primarily rowcrop agriculture with sediment and pesticide runoff accumulating in the study lakes.

Two surface sediment samples (top 5 cm) from each of three sites within each watershed (total of six samples per watershed) were collected during summer (June to July) 2004, placed in amber colored glass jars, preserved on ice and transported to the USDA-ARS National Sedimentation Laboratory, Oxford, MS for sediment characterization (Table 1) and pesticide analysis. Pesticide analysis was conducted with a Hewlett-Packard (HP) model 6890 gas chromatograph with a HP1MS capillary column [8, 9] to determine concentrations of 12 current-use pesticides (Tables 2-3), two historic-use pesticides, including pp' DDT and Dieldrin, and three metabolites, Fipronil sulfone, pp' DDD and pp' DDE (Table 4). Sediment samples were dried, ground, and pre-wetted with ultra-pure water followed by the addition of ethyl acetate. The mixture was sonicated and centrifuged (2000 – 2500 g). The extract was concentrated to near dryness using a nitrogen evaporator and solvent exchanged into hexane. Level of quantification for sediment analyses was 0.1 ng/g.

Twenty-eight day static non-renewal whole sediment toxicity tests using laboratory reared *H. azteca* were conducted according Nebeker et al. [10], ASTM [11] and USEPA [12] protocols, with modifications. Organisms, 4-5 d old, were collected for the experiment. Each sediment exposure consisted of 40 g wet weight sediment from a lake sample with 160 mL overlying hardness adjusted water (free from priority pollutants) from the University of Mississippi Field Station [13, 14] placed in four replicate exposure chambers per site along with two, 6 mm diameter maple leaf discs as substrate and food. Additional feeding of 0.1 ml of a 1:1 suspension rabbit chow:Tetramin® flake food (2 g/L) occurred at test initiation and every two days during week 1. Feeding increased throughout the test as follows: week 2, 0.5 ml of 2 g/L suspension; week 3, 0.5 ml of 4 g/L suspension; week 4, 0.5 mL of 10 g/L suspension. Toxicity tests were conducted in a Powers Scientific, Inc. Animal Growth Chamber with a 16:8 (light:dark) h photoperiod and a set temperature of 20±1℃. Measured physical and chemical water characteristics for sediment tests were temperature, pH, dissolved oxygen, conductivity, hardness, alkalinity, ammonium-N, nitrate-N, and nitrite-N [15]. Bioassay endpoints measured were survival and growth (as wet weight in mg).

Data were analyzed using descriptive statistics and one-way analysis of variance (ANOVA) with Tukey's multiple range test on survival and growth. If data failed parametric assumptions, a Kruskal-Wallace one-way ANOVA on ranks with SNK multiple range test versus controls was utilized. Pearsons Product Moment correlation coefficients were calculated on growth versus pesticide concentration and organic carbon (OC) normalized insecticide and metabolite concentrations when significant impairment was observed. Statistical significance level was set at 5 percent ($P \leq 0.05$) for all analyses [16]. Data analysis was conducted using SigmaStat® v.2.03 statistical software [17].

3. Results

Mississippi Delta sediments were characterized as predominantly silt loam (Table 1). NWR sediments were exclusively silt loam whereas two BMP and one 303(d) sediments were a sand to silt loam mix. Total organic carbon (TOC) fractions ranged from 1-6% with NWR sediments having the greatest TOC and BMP sediments the lowest. All lake sediments examined had detectable concentrations of at least 4 of the 17 current and historic-use pesticides and metabolites examined, however only the metabolite pp'-DDE was detected in every sample. Percent detections ranged from 37% in Columbus Lake sediments to 80% in Cassidy Bayou sediments (Tables 2-4). Current-use herbicides atrazine and metolachlor occurred most frequently and in the greatest concentrations in Mississippi Delta sediments, whereas pendimethalin was detected almost exclusively in Cassidy Bayou sediments (Table 2). Current-use insecticides methyl parathion and chlorfenapyr occurred most frequently and in greater concentrations within Delta sediments (Table 3). Chlorpyrifos occurred more often in BMP sediments whereas; fipronil and bifenthrin were observed more frequently in 303(d) listed sediments (Table 3). The current-use insecticide metabolite fipronil sulfone was detected in 93% of the samples; however, greatest concentrations occurred in Upper Swan Lake (NWR) and Lower White Lake (NWR) sediments (Table 4). Historic-use pesticides and metabolites were nearly ubiquitous within Mississippi Delta sediments [except Columbus Lake (NWR)], with pp' DDT concentrations being the greatest (Table 4).

Survival of *H. azteca* exposed to Mississippi Delta sediments for 28 d was not statistically significantly different among sites due to large variations in means from sites with greater than 20% overall mortality. However, clear trends were apparent. NWR sites had >90% survival at all sites examined, ranging from 92-100%. Animals exposed to Columbus Lake sediments had the highest overall survival and Lower White Lake the lowest (Table 5). BMP sites showed intermediate survival ranging from 79-100% with *H. azteca* exposed to Deep Hollow Lake sediments having the highest survival and Thighman Lake the lowest. Sediments from 303(d) listed water bodies elicited the poorest mean survival ranging from 58-96% with Cassidy Bayou having the highest survival and Roebuck Lake the lowest. Statistically significant ($P < 0.05$) 28d growth impairment of *H. azteca* was observed in sediments from all three 303(d) listed water bodies and two of three BMP oxbow lakes, Deep Hollow Lake and Beasley Lake (Table 5).

Cassidy Bayou [303(d)] had the greatest growth impairment with all sites having growth less than all NWR control sediment sites. Beasley (BMP) and Roebuck [303(d)] lakes were equally impaired with all sites having growth less than 8 of 9 NWR control sites. Intermediate growth impairment occurred in Deep Hollow Lake (BMP) sediments with growth less than 5 of 9 NWR control sites. Thighman Lake (BMP) sediments showed the least growth impairment with growth at only sites 2 and 3 less than 2 and 5 of 9 NWR control sites, respectively. Growth effects observed in Bee Lake [303(d)] were more complex. This was the only water body with significant within-lake growth effects where site 1 animals were much smaller than site two. Comparisons with NWR control sediments showed growth impairment was most evident at site one with growth less than all NWR sites, site three growth was less than 5 of 9 NWR sites, and site two was less than 4 of 9 NWR sites. Overall patterns of growth show 303(d) sediments <BMP sediments <NWR sediments.

Attempts to associate sediment pesticide concentrations with observed growth impairment in the Mississippi Delta were limited, even after normalizing for OC sediment content. There were no associations of overall growth and pesticide concentrations across all water body sediments examined. Within-lake associations between growth and sediment current-use pesticide concentrations were observed in only one water body, Thighman Lake (BMP) with Alachlor (Table 4). Within-lake associations between growth and historic-use pesticide, dieldrin, and the metabolite pp'-DDD were observed in the BMP water body,

Beasley Lake (dieldrin), and the 303(d) water body Cassidy Bayou (dieldrin and DDD) (Table 6). No associations among impaired animal growth and any measured sediment pesticides were observed in Deep Hollow Lake (BMP), Roebuck Lake [303(d)] or Bee Lake [303(d)] (Table 6).

Table 1 Sediment characteristics of samples collected from Lower Mississippi Alluvial Plain water bodies during summer 2004.

Water body	Class	Site	Sand (%)	Silt (%)	Clay (%)	TOC (%)
Upper Swan	NWR	1	31.6	68.1	0.3	5.8
		2	29.5	70.1	0.4	4.8
		3	20.3	79.2	0.5	4.4
Lower White	NWR	1	39.1	60.6	0.3	6.6
		2	36.1	63.7	0.2	5.6
		3	37.6	62.2	0.2	5.3
Columbus	NWR	1	15.4	83.7	0.9	4.8
		2	19.8	78.8	1.4	3.3
		3	29.6	68.6	1.8	1.5
Deep Hollow	BMP	1	46.3	51.7	2	1.6
		2	1.1	95.7	3.2	2.0
		3	16.0	81.3	2.7	2.1
Beasley	BMP	1	12.0	83.9	4.1	2.3
		2	0.8	91.2	8.0	2.3
		3	4.1	92.0	3.9	1.8
Thighman	BMP	1	1.8	92.9	5.3	2.2
		2	9.1	84.3	6.6	2.1
		3	45.9	51.6	2.5	1.1
Roebuck	303(d)	1	1.1	93.1	5.8	2.7
		2	6.4	89.2	4.4	2.1
		3	18.2	76.8	5.0	2.7
Cassidy	303(d)	1	28.0	68.5	3.5	1.7
		2	32.4	63.8	3.8	1.7
		3	60.2	37.5	2.3	1.4
Bee	303(d)	1	21.7	76.0	2.3	3.4
		2	28.6	70.1	1.3	3.5
		3	24.1	73.2	2.7	2.8

Table 2 Current-use herbicide concentrations (ng/g dw) in sediment samples collected from Lower Mississippi Alluvial Plain water bodies during summer 2004. ND = below detection limit (0.01 ng/g dw) and TR = below quantification limit (0.1 ng/g dw).

Water body	Site	Trifluralin	Pendimethalin	Atrazine	Cyanazine	Alachlor	Metolachlor
Upper	1	ND	ND	246.5	10.1	5.2	58.8
Swan	2	ND	0.2	809.3	27.3	0.6	394.6
	3	ND	ND	5510.4	44.0	ND	766.6
Lower	1	11.4	ND	1561.6	26.9	ND	413.4
White	2	ND	ND	950.5	15.9	0.1	107.1
	3	ND	ND	261.1	5.1	1.2	41.5
Columbus	1	ND	ND	62.9	ND	ND	9.1
	2	0.2	ND	22.9	ND	1.7	42.0
	3	ND	ND	ND	ND	ND	ND
Deep	1	TR	ND	ND	0.3	ND	ND
Hollow	2	ND	ND	8.9	ND	ND	9.3
	3	TR	ND	79.1	ND	0.1	5.1
Beasley	1	ND	ND	ND	ND	0.1	1.8
	2	ND	ND	5.2	ND	TR	2.4

(to be continued)

	3	ND	TR	52.9	1.0	0.2	10.2
Thighman	1	0.1	ND	102.3	4.8	3.0	43.8
	2	1.2	ND	1209.1	20.3	ND	19.8
	3	ND	ND	30.1	0.9	ND	3.9
Roebuck	1	ND	ND	201.4	2.4	ND	16.4
	2	ND	ND	67.7	ND	ND	6.2
	3	ND	ND	306.1	5.4	ND	46.9
Cassidy	1	0.4	67.0	14.2	0.3	ND	ND
	2	0.3	16.3	20.5	0.1	ND	ND
	3	1.5	1.1	72.8	0.6	0.9	ND
Bee	1	ND	ND	308.2	3.7	1.2	49.6
	2	ND	ND	465.6	4.0	20.5	51.2
	3	ND	ND	81.2	ND	1.1	16.3

Table 3 Current-use insecticide concentrations (ng/g dw) in sediment samples collected from Lower Mississippi Alluvial Plain water bodies during summer 2004. ND = below detection limit (0.01 ng/g dw) and TR = below quantification limit (0.1 ng/g dw).

Water body	Site	Chlorpyrifos	Methyl Parathion	Fipronil	Chlorfenapyr	Bifenthrin	-cyhalothrin
Upper	1	ND	3.4	2.1	20.1	3.0	11.8
Swan	2	14.2	189.3	1.3	6.0	2.0	ND
	3	ND	ND	0.3	2.0	0.2	ND
Lower	1	ND	92.3	1.1	6.4	0.8	0.1
White	2	ND	3.3	1.4	7.9	2.1	ND
	3	ND	3.3	0.8	1.5	2.3	ND
Columbus	1	12.3	ND	ND	ND	ND	ND
	2	23.2	ND	ND	0.7	ND	ND
	3	ND	4.4	ND	ND	ND	ND
Deep	1	3.4	12.5	ND	0.7	ND	ND
Hollow	2	8.8	6.9	0.4	0.5	ND	ND
	3	ND	12.4	0.4	0.8	ND	3.3
Beasley	1	4.1	6.4	ND	0.9	ND	ND
	2	ND	6.4	ND	0.7	ND	1.5
	3	6.2	13.1	0.4	1.1	ND	0.7
Thighman	1	35.5	ND	ND	1.5	ND	ND
	2	17.1	ND	0.4	0.9	0.3	ND
	3	ND	6.1	ND	0.8	ND	ND
Roebuck	1	ND	3.5	0.5	0.9	0.8	ND
	2	11.4	5.6	ND	0.3	ND	ND
	3	ND	10.9	0.7	0.6	1.3	ND
Cassidy	1	ND	5.8	0.5	0.1	0.1	ND
	2	ND	5.6	0.7	0.2	0.4	ND
	3	11.3	14.9	0.3	0.2	4.1	ND
Bee	1	12.1	5.5	0.7	2.8	1.3	ND
	2	ND	4.3	1.0	12.5	1.4	ND
	3	15.6	5.8	0.6	0.7	ND	ND

Table 4 Historic-use insecticide and metabolite concentrations (ng/g dw) in sediment samples collected from Lower Mississippi Alluvial Plain water bodies during summer 2004. ND = below detection limit (0.01 ng/g dw) and TR = below quantification limit (0.1 ng/g dw).

Water body	Site	Fipronil Sulfone	Dieldrin	pp'-DDT	pp'-DDD	pp'-DDE
Upper Swan	1	8.9	0.5	49.2	2.6	2.0
	2	4.4	1.5	7.6	2.5	0.6
	3	1.6	0.2	11.7	2.2	0.5
Lower White	1	4.3	0.1	117.4	1.5	2.0
	2	5.3	0.1	28.0	2.4	2.4
	3	0.4	0.2	27.5	1.1	0.3
Columbus	1	ND	ND	ND	ND	0.3
	2	0.2	0.1	3.3	1.0	0.2
	3	ND	0.1	2.9	ND	0.1
Deep Hollow	1	0.9	0.2	9.6	3.1	3.1
	2	0.8	0.2	7.4	3.3	4.2
	3	0.8	0.3	4.4	2.0	1.7
Beasley	1	0.6	0.1	6.8	1.7	2.3
	2	0.7	0.2	2.9	1.2	0.6
	3	0.9	0.3	9.8	3.7	7.3
Thighman	1	1.5	0.2	7.0	2.8	3.0
	2	1.1	0.6	8.7	3.4	7.8
	3	0.7	0.2	3.3	1.1	0.5
Roebuck	1	0.7	0.2	33.5	4.3	5.2
	2	TR	0.2	13.3	3.1	3.4
	3	0.3	0.1	15.9	2.9	3.1
Cassidy	1	0.1	0.3	19.0	4.5	4.0
	2	0.2	0.2	21.8	4.4	4.3
	3	0.2	0.2	123.7	3.9	7.9
Bee	1	2.6	1.0	25.1	3.4	5.3
	2	4.3	1.3	20.3	3.6	7.5
	3	0.1	0.2	12.7	2.0	3.3

Table 5 Mean 28 d Survival and growth (as wet weight) of Hyalella azteca exposed to watershed sediments collected from the Lower Mississippi Alluvial Plain during summer 2004. Mean values (n=4) with different letters are statistically significantly different (P < 0.05).

Watershed	Class	Site	Survival (%)	Growth (mg wet weight)
Upper Swan	NWR	1	96±8 A	3.0±0.2 ABC
		2	96±8 A	2.3±0.5 ABCDEF
		3	100±0 A	3.1±0.4 AB
Lower White	NWR	1	96±8 A	2.6±0.6 ABCDE
		2	92±10 A	3.2±0.5 A
		3	96±8 A	2.6±0.6 ABCDE
Columbus	NWR	1	100±0 A	3.1±0.5 AB
		2	96±8 A	3.3±0.6 A
		3	100±0 A	2.5±0.4 ABCDE
Deep Hollow	BMP	1	96±8 A	1.5±0.2 EFGHI
		2	96±8 A	1.3±0.5 FGHI
		3	100±0 A	1.7±0.5 EFGHI
Beasley	BMP	1	100±0 A	1.2±0.1 FGHI
		2	88±16 A	0.7±0.3 HI

(to be continued)

		3	88±25 A	0.6±0.3 I
Thighman	BMP	1	79±16 A	2.9±0.3 ABCD
		2	88±16 A	1.9±0.3 BCDEFG
		3	88±25 A	1.8±0.5 DEFGH
Roebuck	303(d)	1	71±28 A	0.8±0.4 GHI
		2	63±32 A	0.7±0.3 HI
		3	63±44 A	1.2±0.7 FGHI
Cassidy	303(d)	1	96±8 A	0.6±0.1 I
		2	88±8 A	1.2±0.5 GHI
		3	83±0 A	0.7±0.4 HI
Bee	303(d)	1	67±14 A	0.6±0.3 I
		2	58±22 A	1.9±0.3 CDEFG
		3	88±16 A	1.6±0.2 EFGHI

Table 6 Associations of Hyalella azteca growth with pesticide concentrations in sediments collected from watersheds in the Lower Mississippi Alluvial Plain during summer 2004. Bold values of Pearson Product Moment correlation coefficients (n=7) are statistically significant (P < 0.05).

Pesticide	Deep Hollow	Beasley	Thighman	Roebuck	Cassidy	Bee
pp’-DDT	0.026	-0.327	0.440	-0.087	-0.430	-0.464
pp’-DDD	-0.130	-0.647	0.455	-0.411	**-0.813**	-0.414
pp’-DDE	0.029	-0.489	0.223	-0.143	0.745	-0.238
Dieldrin	-0.100	**-0.830**	0.041	-0.167	-0.751	-0.234
-cyhalothrin	0.369	-0.573	N / A	N / A	N / A	N / A
Bifenthrin	N / A	N / A	0.012	0.006	-0.428	-0.344
Chlorfenapyr	-0.167	-0.460	0.556	-0.126	-0.376	0.037
Fipronil Sulfone	-0.295	-0.609	0.429	-0.242	-0.456	-0.131
Fipronil	0.390	-0.390	0.012	-0.007	-0.407	-0.218
Methyl Parathion	0.083	-0.613	-0.406	0.098	0.098	-0.343
Chlorpyrifos	0.184	-0.120	0.579	-0.390	-0.153	-0.583
Metolachlor	0.192	-0.449	0.585	-0.009	N / A	-0.431
Alachlor	0.331	-0.302	**0.827**	N / A	-0.380	0.116
Cyanazine	0.134	-0.390	0.104	0.042	-0.498	-0.198
Atrazine	0.228	-0.498	-0.237	-0.083	-0.471	-0.238
Pendimethalin	N / A	-0.390	N / A	N / A	-0.426	N / A
Trifluralin	0.418	N / A	-0.389	-0.342	-0.473	N / A
pp’-DDT-OC	0.141	-0.185	0.243	0.023	-0.412	-0.255
pp’-DDD-OC	-0.129	-0.626	0.295	-0.478	**-0.854**	-0.439
pp’-DDE-OC	0.029	-0.488	0.194	-0.140	-0.676	-0.207
Dieldrin-OC	-0.115	**-0.778**	-0.093	-0.227	**-0.807**	-0.247
-cyhalothrin-OC	0.369	-0.566	N / A	N / A	N / A	N / A
Bifenthrin-OC	N / A	N / A	0.012	0.007	-0.425	-0.363
Chlorfenapyr-OC	-0.193	-0.523	0.122	-0.169	-0.419	0.034
Fipronil-OC	0.381	-0.390	0.012	-0.006	-0.433	-0.236
Methyl Parathion-OC	0.035	-0.642	-0.406	0.060	-0.653	-0.322
Chlorpyrifos-OC	0.194	-0.150	0.562	-0.393	-0.153	-0.534

4. Discussion

Most previous studies examining pesticide contamination in Mississippi Delta water body surface sediments focused primarily on persistent organochlorine insecticides such as DDT and metabolites, dieldrin, and toxaphene [5, 15, 16, 20]. These legacy pesticides have been examined for several decades due to their persistence and continued potential risk to aquatic biota. Comparisons of Mississippi Delta sediment contamination from these historic-use pesticides and metabolites with the current study showed slowly decreasing peak concentrations of ΣDDT with 1275 ng/g in 1977 [18], 600 ng/g in 1982 [20], 129 ng/g in 1997 [5], and 70 ng/g in 2000[4]. In the current study, more than 90% of all sediments had <60 ng ΣDDT/g and only four of 27 sites had >34 ng ΣDDT/g (Table 1). Current use pesticide sediment contamination in the Mississippi Delta has been less intensively studied due to the transient nature of many compounds. Studies have focused primarily on insecticides, such as pyrethroids and organophosphates, because of their low water solubility and high affinity for sediment [8, 9]. This study showed sediment contamination by pyrethroids and organophosphates to be comparable with previous studies within the same region [4, 19, 21]. Sediment current-use herbicide concentrations in this study were also comparable to studies by Shea et al. [21] and Moore et al. [4] with atrazine frequently occurring and in the greatest concentrations, while pendimethalin and trifluralin were found infrequently.

To date, few studies have attempted to assess toxicity and pesticide contamination within Delta water body surface sediments for such a broad group of current-use chemicals [4, 21, 22]. Such studies typically assessed acute (96 h to 10 d) sediment toxicity using *H. azteca* [4, 21, 22]. The studies by Shea et al. [21] and Moore et al. [4] showed 10 d *H. azteca* survival and growth to be unaffected by acute exposure to pesticide contaminated Mississippi Delta oxbow lake sediments whereas Shea et al [21] observed significant survival impairment only in animals exposed to sediment pore water containing the maximum concentration of the suspected pesticide but not composite sediment pore water. In contrast, present research showed significant chronic (28d) sediment toxicity to *H. azteca* and pesticide contamination among watersheds studied.

Associating observed bulk sediment contaminant concentrations with aquatic invertebrate responses have been attempted by few researchers in the study region [21, 22] with limited results. More commonly, numerical sediment quality guidelines (SQGs) have been used as part of monitoring programs, sediment quality assessments and water quality assessments using threshold effects concentrations (TECs) and probable effects concentrations (PECs) in North America for a number of organic and inorganic contaminants [23]. However, SQGs are currently lacking for many current-use pesticides commonly used throughout the United States. As a result, attempts to close the data gap in SQGs include comparing published measured effects concentrations for pesticides of concern with measured bulk sediment pesticide loads. Current-use herbicide concentrations observed in this study showed trifluralin, pendimethalin, cyanazine and alachlor Delta sediment concentrations to be well below reported effects concentrations (Table 7). However, atrazine and, to a lesser extent, metolachlor Delta sediment concentrations were at levels that could potentially cause impairment [24]. Yet greatest *H. azteca* survival and growth coincided with highest concentrations of these two herbicides, suggesting that, despite concentrations as great as 5,000 ng atrazine/g and 700 ng metolachlor/g, they may not be bioavailable to aquatic invertebrates in Delta sediments. Current-use insecticide Delta sediment concentrations showed fipronil, fipronil sufone (metabolite) and chlorfenapyr were often below reported effects concentrations (Table 7). Organophosphate pesticides, chlorpyrifos and methyl parathion, occurred in concentrations that might be of concern based upon reported aqueous effects concentrations for these two insecticides (Table 7). But as with atrazine, no *H. azteca* impairment coincided with these elevated concentrations. Pyrethroids, bifenthrin and lambda-cyhalothrin, also occurred above reported effects concentrations in several Delta sediments (Table 7) and again, no animal impairment was observed.

Table 7 Published acute and chronic effects concentrations of 17 pesticides and metabolites examined to aquatic organisms.

Pesticide	Organism	Phase	Endpoint	Concentration	
Trifluralin	*Daphnia magna*	Aqueous	48 h LC50	500 ng/ml	EXTOXNET, 1996
Pendimethalin	*Daphnia magna*	Aqueous	48 h LC50	280 ng/ml	EXTOXNET, 1996
Atrazine	*Hyalella azteca*	Sediment	28 d NOEC	>2.5 ng/g	Wan et al., 2006
Cyanazine	*Hyalella azteca*	Aqueous	96 h NOEC	>200 ng/ml	Trimble and Lydy, 2006
Alachlor	*Echinogammarus tabaldii*	Aqueous	96 h LC50	13,000 ng/ml	Pantani et al., 1997
Metolachlor	*Hyalella azteca*	Sediment	28 d NOEC	>3 ng/g	Wan et al., 2006
Chlorpyrifos	*Hyalella azteca*	Aqueous	96 h LC50	0.07 ng/ml	Trimble and Lydy, 2006
Methyl Parathion	*Hyalella azteca*	Aqueous	96 h LC50	2.1 ng/ml	Anderson and Lydy, 2002
Fipronil	*Procambarus clarkia*	Aqueous	96 h LC50	14.3 ng/ml	Schlenk et al., 2001
Chlorfenapyr	*Hyalella azteca*	Sediment	10 d LC50	20.6 ng/g	Rand, 2004
Bifenthrin	*Hyalella azteca*	Sediment	10 d LC50	2 ng/g	Amweg et al., 2005
-cyhalothrin	*Hyalella azteca*	Sediment	10 d LC50	5 ng/g	Amweg et al., 2005
Fipronil Sulfone	*Procambarus clarkii*	Aqueous	96 h LC50	11.2 ng/ml	Schlenk et al., 2001
Dieldrin	*Hyalella azteca*	Sediment	10 d LC50	22,800 ng/g	Hoke et al., 1995
pp'-DDT	*Hyalella azteca*	Sediment	10 d LC50	4,200 ng/g	Schuytema et al., 1989
pp'-DDD	*Hyalella azteca*	Sediment	28 d EC50	240,000 ng/g	Ingersoll et al., 2005
pp'-DDE	*Hyalella azteca*	Sediment	10 d NOEC	2,100 ng/g	Hoke et al., 1994

This strongly supports the suggestion that observed impaired growth in animals exposed to Delta sediments is not due primarily to any individual current-use pesticide. Because all Delta sediments showed significant but varying degrees of mixture current-use pesticide contamination, there was a concern of mixture toxicity effects. While the most common assumption with mixtures is additive toxicity, several studies have observed synergistic effects on aquatic invertebrates with certain classes of current-use pesticides. Anderson and Lydy [25] and Trimble and Lydy [26] noted synergistic toxicity of the triazine herbicides atrazine and cyanazine mixed with organophosphate insecticides chlorpyrifos and methyl parathion in *H. azteca*. Other possible synergistic mixtures have been suggested by Bouldin et al. [27] with atrazine and the pyrethroid lambda-cyhalothrin in the midge (Chironomus tentans). Although all of these current-use pesticides occurred in Delta sediments, we were, again, unable to link these with observed growth impairment, further supporting the conclusion that current-use pesticides, even in potentially synergistic mixtures, did not occur in concentrations great enough to be bioavailable.

Historic-use organochlorine pesticides (OCs) and their metabolites observed in this study occurred below published reported effects concentrations (Table 7). However, MacDonald et al. [23] reported TECs for OCs, pp'-DDT and pp'-DDE, that were below our measured Delta sediment concentrations as well as TECs for dieldrin and pp'-DDD, that were just above our measured Delta sediment concentrations. Contamination by OCs has been implicated in observed toxicity of Mississippi Delta sediments by Shea et al. [21] as the most likely source in 11 refuges within the region. In addition, we observed associations of impaired *H. azteca* growth with OCs in Beasley Lake (dieldrin) and Cassidy Bayou (pp'-DDD and dieldrin). Finally, our results concur with MDEQ [28] listing of the studied 303(d) watersheds which had postings of warnings for fish consumption due to OC contamination. This strongly implicates OCs as the most likely source of observed impairment in animals exposed to Delta sediments and coincides with current observations that, in general, NWR watersheds had the lowest OC contamination and 303(d) listed watersheds the highest.

Overall, observed results of the current study were due to several contributing factors. First, patterns of chronic toxicity and pesticide contamination were indicative of agricultural land-use practices in and around these watersheds with influxes of materials occurring during storm events and seasonal flooding [21]. Second, these patterns were further elucidated by the degree of static or flow-through conditions within each watershed, with some watersheds (Beasley Lake, BMP; Deep Hollow Lake, BMP) receiving little or no additional flow from neighboring riverine systems [5] due to anthropogenic alteration of the landscape (e.g., drainage ditches, levees) compared with other more open watersheds (Upper Swan Lake, NWR; Lower White Lake, NWR; Columbus Lake, NWR) with extensive flood plains allowing greater transport of contaminants. Control (WRNWR) watersheds sediments were not chronically toxic to *H. azteca*, although they still received a significant influx of current-use pesticides. In this study, the complexity of contaminant mixtures limited our attempts to provide clear, definitive sources of toxicity. Accounting for sediment characteristics such as sand-silt-clay fractions and organic carbon content provided only limited additional associations with observed toxicity. Despite these limits, results show that application of BMPs surrounding similar watersheds can lessen the degree of biological impairment within delta watershed sediments.

4. Conclusions

Chronic (28d) toxicity was greater in 303(d) listed delta watershed surface sediments (all sites in all three watersheds) and lesser in BMP treated delta watershed surface sediments (four sites in two of three watersheds). No chronic toxicity was observed in any WRNWR control delta watershed surface sediment for any biological endpoint examined. Associations between observed chronic delta surface sediment toxicity and measured delta surface sediment current and historic-use pesticides and metabolites were limited. However; historic-use pesticides in delta surface sediments were implicated in 2 of 5 biologically impaired delta watershed surface sediments and current-use pesticides were implicated in one delta watershed surface sediment. Measured delta surface sediment characteristics, sand-silt-clay fractions and organic carbon content did not further clarify sources of chronic toxicity in some watersheds. Use of best management practice (BMP) treatments and technologies mitigated chronic toxicity of delta surface sediments compared to untreated watersheds (i.e., impaired, USEPA section 303(d) Clean Water Act).

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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.

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