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Hyalella azteca (Saussure) Responses to Coldwater River Backwater Sediments in Mississippi, USA

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Abstract Sediment from three Coldwater River, Mississippi backwaters was examined using 28 day *Hyalella azteca* bioassays and chemical analyses for 33 pesticides, seven metals and seven PCB mixtures. Hydrologic connectivity between the main river channel and backwater varied widely among the three sites. Mortality occurred in the most highly connected backwater while growth impairment occurred in the other two. Precopulatory guarding behavior was not as sensitive as growth. Fourteen contaminants (seven metals, seven pesticides) were detected in sediments. Survival was associated with the organochlorine insecticide heptachlor.

Keywords Floodplain sediments · Organochlorine pesticides

Riverine backwaters along lowland, floodplain rivers are ecologically important systems providing habitat for diverse flora and fauna. These systems can be impacted by adjacent agricultural land use that produces contaminated runoff. As part of an environmental quality assessment, the objective of the study was to assess sediment quality from four sites in three Coldwater River, Mississippi backwaters using 28 day *Hyalella azteca* bioassays and chemical analyses for 33 pesticides, seven metals and seven PCB mixtures. The test organism, *Hyalella azteca*, was chosen because it is a freshwater crustacean (Order: Amphipoda) that is an epibenthic detritivore found in close association with surface sediments. This animal occurs widely across North America in lakes, wetlands and backwater environments and is an ecologically important part of aquatic food webs (de March 1981).

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Materials and Methods

Three backwaters (shallow < 1.5 m river bends severed from the main river channel) along the Coldwater River in northern MS, USA were examined as part of an environmental quality assessment. These backwaters have intermittent hydroperiods: extremely shallow (<0.5 m) to dry during summer and fall, and wet with periodic flooding during winter and spring coinciding with rainfall and river stage (Table 1). The mean annual duration of connection to the river was determined for each backwater by surveying controlling elevations at each site, and comparing these elevations with once-daily river stages transferred to each site from nearby gages. The percentages of days with river connection at one and at both ends of the old channels were computed based on 45 years of record (1960–2005) (Shields et al. 2007). All three sites were bordered by a fringe ($\sim$5–20 m wide) of mature forest surrounded by fields intensively cultivated for soybeans or cotton. Three surface (top 5 cm) whole wet sediment samples ($\sim$2 kg) were collected at each site during June, 2006 using an acetone rinsed Ekman dredge sampler. Samples were placed in 1 L acetone/hexane triple washed amber colored glass jars fitted with a Teflon® – lined screw cap, preserved on wet ice and transported to the USDA-ARS National Sedimentation Laboratory (NSL), Oxford, MS for bioassays and contaminant analyses within 24 h of sampling.

Sediment samples were homogenized prior to bioassay and contaminant analysis. Briefly, samples were air-dried and analytical chemistry was conducted for 47

Table 1 Description of backwater sites that were sampled for this study; all sites are severed meander bends along the Coldwater River in northern Mississippi, USA

Location	Latitude–longitude	Length (m)	Width (m)	Flooding, % of time[a]
Site 1	34°39′43.10″ N, 90°13′18.15″ W	2,000	40	18/3
Sites 2 and 3	34°40′19.40″ N, 90°13′41.34″ W	2,500	40	18/2
Site 4	34°51′31.56″ N, 89°48′24.10″ W	350	20	24/10

[a] Percent of time backwater is connected to river/percent of time connection is strong enough to allow the river to flow through the backwater

contaminants: 33 pesticides, seven metals and seven polychlorinated biphenyl (PCB) mixtures. Pesticide analyses were conducted following Bennett et al. (2000) and USEPA (1984) using gas chromatography with a micro electron capture detector. Metal and PCB mixture analyses were performed by the Soil-Plant Analysis Laboratory, University of Louisiana–Monroe, Monroe, Louisiana using atomic absorption and inductively coupled plasma-atomic emission spectrometry (metals) or gas chromatography with a micro electron capture detector (PCB mixtures) using methods described by USEPA (1984). Extraction efficiencies of all fortified samples analyzed using quality assurance/quality control protocols were $\geq$90%.

Bioassays were 28 day static whole sediment toxicity tests following methods described by USEPA (1994), with modifications, using 4–5 days old *Hyalella azteca* reared at the NSL. Bioassays were initiated within 24 h of sample collection. Exposures consisted of 40 g w/w sediment sample with 160 mL overlying water (hardness adjusted and free from priority pollutants) from the University of Mississippi Field Station (UMFS) (Deaver and Rodgers 1996). Control sediment was also obtained from UMFS. Six *H. azteca* were placed in each of seven replicate exposure chambers (250 mL borosilicate glass beakers) per site. Animals were fed a 1:1 suspension mixture of rabbit chow:Tetramin® flake food at 1, 5, 10, 25 mg L^{-1} every 2 days during week 1, 2, 3, and 4, respectively, along with 2, 6 mm diameter maple (*Acer* sp.) leaf discs. Toxicity tests were conducted in a Powers Scientific, Inc. Animal Growth Chamber with a 16:8 (light:dark) h photoperiod and a temperature of 23 ± 1°C. Standard physical and chemical water characteristics for sediment tests (temperature, pH, dissolved oxygen, conductivity, hardness, alkalinity, ammonium-N, nitrate-N, and nitrite-N) were measured according to APHA (1998). Bioassay endpoints measured were survival, growth (mg w/w) and precopulatory guarding behavior as described by Blockwell et al. (1998).

Bioassay data were statistically analyzed with SigmaStat® v.2.03 statistical software (SPSS 1997) using descriptive statistics and one-way analysis of variance (ANOVA) on survival, growth (mg w/w) and precopulatory guarding behavior with Tukey's multiple-range test, when appropriate. When data failed parametric assumptions, a Kruskal–Wallace one-way ANOVA on ranks with Dunn's multiple range test was utilized. If endpoint effects were observed, Spearman Rank Order correlation analysis was conducted between response and measured contaminant concentration to determine associations. Statistical significance level was set at $\alpha = 5\%$ ($p \leq 0.05$) for all analyses.

Results and Discussion

Overlying water quality characteristics assessed during 28 days bioassays were as follows: temperature, 23.2–23.8°C; dissolved oxygen, 4.68–7.74 mg L^{-1}; pH, 5.6–7.2; alkalinity, 17.1–34.2 mg L^{-1} as $CaCO_3$; hardness, 51.3–102.1 mg L^{-1} as $CaCO_3$; conductivity, 262–385 µmhos cm^{-1}; nitrate-N, 0.12–4.28 µg L^{-1}; nitrite-N, 0–0.30 µg L^{-1}; ammonium-N 0–0.26 µg L^{-1}. Values were within acceptable limits for hardness adjusted water according to USEPA (1994) standards for chronic sediment toxicity testing using *Hyalella aztecea*. Several studies have examined sediment toxicity in Mississippi using *H. azteca* (Winger and Lasier 1998; Moore et al. 2004, 2007), however these were primarily acute 10 day exposures eliciting no adverse effects. Ingersoll et al. (2001) observed longer-term 28 day chronic sediment exposures to be more sensitive than acute exposures. In the current study *H. azteca* 28 day survival (11.9%) for sediments from the more frequently connected site 4 was significantly lower than for the control (92.9%). Although the least connected backwater (site 1) had only 66.7% survival, variability limited

Table 2 *Hyalella azteca* 28 days responses to sediment from three Coldwater River backwaters in Mississippi

Location	Survival (%)	Growth (mg w/w)	Precopulatory guarding behavior
Site 1	66.7 ± 21.5	1.1 ± 0.4[a]	0 ± 0[a]
Site 2	90.5 ± 13.1	2.5 ± 0.4	1.3 ± 1.3
Site 3	92.9 ± 13.1	1.8 ± 0.3[a]	1.3 ± 1.1
Site 4	11.9 ± 8.1[a]	N/A	N/A
Control	92.9 ± 13.1	3.0 ± 0.8	1.9 ± 0.9

N/A not applicable

[a] Statistically significantly different from controls

any conclusive evidence of decreased survival (Table 2). Growth was significantly impaired at sites 1 and 3 compared with controls. Precopulatory guarding behavior was not observed in animals exposed to the more lethal sediments from site 1; however, sediments from sites 2 and 3 were associated with behavior similar to controls.

Table 3 Characteristics and pesticide concentrations (ng g^{-1} d/w) of Coldwater River backwater sediments

Characteristic or pesticide	Site 1	Site 2	Site 3	Site 4	Control
Silt (%)	76.5	64.0	81.4	17.7	11.5
Clay (%)	5.1	2.5	6.0	0.1	0.1
TOC (%)	2.3	2.0	0.8	1.2	0.3
Trifluralin[a]	ND	ND	ND	ND	ND
Pendimethalin[a]	ND	ND	ND	ND	ND
Atrazine[a]	ND	ND	ND	ND	ND
Cyanazine[a]	ND	ND	ND	ND	ND
Alachlor[a]	ND	ND	ND	ND	ND
Metolachlor[a]	ND	ND	ND	ND	ND
Chlorpyrifos[a]	ND	ND	ND	ND	ND
Methyl Parathion[a]	ND	ND	ND	ND	ND
Chlorfenapyr[a]	ND	ND	ND	ND	ND
Bifenthrin[a]	ND	ND	ND	ND	ND
λ-cyhalothrin[a]	ND	ND	ND	ND	ND
Fipronil[a]	ND	ND	ND	ND	ND
Fipronil Sulfone[a]	ND	ND	ND	ND	ND
Aldrin[a]	22.5	8.75	2.91	TR	1.84
Dieldrin[a]	ND	ND	ND	ND	ND
p,p′-DDT[a]	ND	ND	ND	ND	ND
p,p′-DDD[a]	ND	ND	ND	ND	ND
p,p′-DDE[a]	ND	ND	ND	ND	ND
αBHC[b]	17.15	3.63	ND	1.26	ND
βBHC[b]	ND	ND	ND	ND	ND
δBHC[b]	ND	ND	ND	ND	ND
γBHC[b]	393.29	104.15	1.02	48.21	ND
Chlordane[b]	ND	ND	ND	ND	ND
Toxaphene[b]	ND	ND	ND	ND	ND
Endrin[b]	ND	ND	ND	ND	ND
Endrin aldehyde[b]	ND	ND	ND	ND	ND
Endosulfan I[b]	TR	ND	ND	TR	ND
Endosulfan II[b]	TR	TR	ND	TR	ND
Endosulfan sulfate[b]	ND	ND	ND	ND	ND
Heptachlor[b]	8.97	1.71	TR	56.15	TR
Heptachlor epoxide[b]	TR	TR	TR	TR	ND
Mirex[b]	ND	ND	ND	ND	ND
Methoxyclor[b]	ND	ND	ND	ND	ND

ND = below detection limit, TR = trace

[a] Detection limit 0.1 ng g^{-1}

[b] Detection limit 1.0 ng g^{-1}

Sediments from sites 1, 2 and 3 were soft mud, with about 80% finer than sand by weight. Site 4 sediments were about 80% sand, reflecting more frequent river flow (Table 3). Detection of pesticides was limited to only seven of 33 pesticides examined (Table 3) including organochlorine insecticides aldrin, αBHC, γBHC (lindane), heptachlor and heptachlor epoxide. Only aldrin and heptachlor were detected in all samples. Seven metals examined were observed in various concentrations in nearly all samples with lowest concentrations in the control sediment (Table 4) and were comparable with concentrations reported within Mississippi sediments (Cooper and Gillespie 2001; Knight and Cooper 1996; Winger and Lasier 1998). None of the seven PCB mixtures examined were at concentrations above detection limits (Table 4) and, again, were similar to results reported by Winger and Lasier (1998) for this region. Most frequent detections occurred from metals and legacy pesticides.

Using consensus-based numerical sediment quality guidelines developed by MacDonald et al. (2000) for freshwater ecosystems in North America, concentrations of detected contaminants in this study (pesticides and metals) were compared with reported probable effects concentrations (PECs) to elucidate likely sources of toxicity. Based upon reported metals PECs from MacDonald et al. (2000) (in mg kg^{-1} d/w: arsenic = 33.0; copper = 149; lead = 128; mercury = 1.06; chromium = 111; cadmium = 4.98; zinc = 459), observed metals concentrations in this study

Table 4 Metal (mg kg^{-1} d/w) and PCB (ng g^{-1} d/w) detection limits and concentrations in Coldwater River backwater sediments

Contaminant	Detection limit	Site 1	Site 2	Site 3	Site 4	Control
Metal						
Arsenic	0.007	1.48	2.13	5.21	2.74	0.38
Copper	0.003	19.25	16.75	12.50	7.00	0.50
Lead	0.015	20.50	20.25	18.25	8.50	2.25
Mercury	0.0004	0.105	0.121	0.076	0.083	0.030
Chromium	0.002	11.50	10.50	7.50	5.50	1.50
Cadmium	0.001	4.00	4.00	3.00	2.50	ND
Zinc	0.003	64.00	55.25	48.75	8.50	1.25
PCB mixture						
Aroclor 1016	1.0	ND	ND	ND	ND	ND
Aroclor 1221	1.0	ND	ND	ND	ND	ND
Aroclor 1232	1.0	ND	ND	ND	ND	ND
Aroclor 1242	1.0	ND	ND	ND	ND	ND
Aroclor 1248	1.0	ND	ND	ND	ND	ND
Aroclor 1254	1.0	ND	ND	ND	ND	ND
Aroclor 1260	1.0	ND	ND	ND	ND	ND

ND = Below detection limit

were not a likely source of toxicity. Most metals assessed in this study were from naturally occurring lithic sources in Mississippi Delta sediments. However several metals have been historically used and/or are currently used as agricultural pesticides and could be a source of potential contamination. Historically used lead-arsenical insecticides such as lead arsneate were used on cotton to control cotton boll weevil (*Anthonomus grandis*) (Cooper and Gillespie 2001) and currently used arsenical herbicides such as monosodium methane arsenate (MSMA) are used for weed control. Additionally, mercury was historically used as a fungicide and/or seed treatment in agriculture (Cooper and Gillespie 2001). Copper as copper sulfate has also been used as an herbicide, primarily as an algicide and/or aquatic herbicide and has been used by catfish farmers in the Mississippi Delta (Schrader and Harries 2001). Concentrations of organochlorine pesticide lindane observed at sites 1 and 4 were $>9\times$ the reported PECs (4.99 ng g^{-1} d/w), and heptachlor at site 4 was $3.5\times$ the reported PEC (16.0 ng g^{-1} d/w) for the slightly less toxic metabolite heptachlor epoxide (USEPA 2008), providing a conservative estimate of an unreported heptachlor PEC. Although MacDonald et al. (2000) reports a PEC for dieldrin (61.8 ng g^{-1} d/w) but not aldrin, both have similar toxicities (USEPA 2008) and the dieldrin PEC was used. Aldrin concentrations at sites 1–3 were above dieldrin PECs, but site 1 was $>10\times$ the PEC. There were no reported or comparable PECs for αBHC. Persistent organochlorine insecticide contamination continues to be reported in aquatic ecosystems throughout Mississippi wherever past or current agricultural practices occur (Knight and Cooper 1996; Moore et al. 2004, 2007). These historically used pesticide residues occur in agriculturally cultivated soils that can move via runoff during storm events either directly into backwaters or are deposited during high flow and flood events where they accumulate in sediments and potentially affect aquatic biota (Cooper 1987).

Effects on survival were associated with the organochlorine insecticide heptachlor ($r = -1.00$, $p = 0.017$, $n = 5$). Spearman Rank correlations did not show any significant associations with *H. azteca* growth or behavior. Based upon weight of evidence from reported PECs and associations with observed *H. azteca* responses, the most likely source of observed sediment toxicity was due to the organochlorine insecticide heptachlor.

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