Bull. Environ. Contam. Toxicol. (1997) 58:550-557

Bulletin of Environmental Contamination and Toxicology

Toxicity of Stormwater Treatment Pond Sediments to *Hyalella azteca* (Amphipoda)

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Received: 12 August 1996/Accepted: 6 January 1997

Stormwater runoff from highways and commercial, industrial, and residential areas contains a wide spectrum of pollutants including heavy metals, petroleum hydrocarbons, pesticides, herbicides, sediment, and nutrients (U.S. EPA 1991). Recent efforts to reduce the impacts of urbanization on natural wetlands and other receiving waters have included the construction of stormwatcr treatment ponds and wetlands. In addition to providing flood control, these systems improve water quality through settling, adsorption, and precipitation of pollutants. These processes remove up to 95% of metals, nutrients and sediment before they are discharged from the site (U.S. EPA 1991). The design of stormwater ponds to provide habitat for aquatic wildlife has prompted concern over the potential exposure of aquatic organisms to these contaminants (Campbell 1994).

Aquatic sediments concentrate a wide array of organic and inorganic pollutants. Although in many cases water quality criteria are not exceedcd, organisms living in or near the sediments may be adversely affected (Canfield et al. 1994). The availability of chemicals in sediments depends strongly on the prevailing chemistry. For example, toxicity of contaminants is controlled by the presence of iron oxides, organic materials, and insoluble sulfides (Tessier and Campbell 1987). Physical conditions of the sediment and water quality characteristics including pH, redox potential and hardness, also influence contaminant availability.

Studies have shown that heavy metals and nutrients carried by runoff concentrate in the sediment of stormwater ponds (Yousef et al. 1984; Campbell 1994). Although several investigations have assessed the toxicity of sediments in streams receiving urban runoff, there have been few studies of the toxicity of stormwater treatment pond sediments to aquatic organisms (Katznelson et al. 1995). This study was part of a large-scale assessment of the contaminant hazards of stormwater treatment ponds (Karouna-Renier 1995). The objective of this study was to evaluate the toxicity of sediments and water from stormwater ponds over a 10-d period to juvenile *Hyalella azteca.* Bioassay results were related to concentrations of acid volatile sulfides and metals of the tested sediments.

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Table 1. Drainage area characteristics of monitored ponds.

Pond	Land Use	%I	Drainage Area	Year	Surface Area
Patuxent	Reference	0%	NA	1990	0.02 ha
Baltimore-Wash. Parkway	Highway	20%	2.6 ha	1991	0.20 ha
Chapel Estates	Residential	30%	12.2 ha	1990	0.16 ha
Country Meadows	Residential	45%	29.8 ha	1989	0.37 ha
Executive Terrace	Commercial	42%	9.0 ha	1989	0.08 ha
Golden Triangle	Commercial	60%	8.7 ha	1991	0.20 ha
Lions Gate	Residential	60%	NA	1991	0.20 ha
Muirkirk Road	Residential	50%	46.9 ha	1990	0.21 ha
New Cut Road	Highway	20%	NA	1990	0.20 ha
Winchester Road	Highway	24%	17.3 ha	1990	0.24 ha

NA=not available, %I=(impervious cover/drainage area)* 100, Year = year of construction.

MATERIALS AND METHODS

Nine stormwater ponds located in Prince George's, Anne Arundel, and Howard Counties, Maryland were selected for toxicity testing based on a contemporaneous study of sediment metal concentrations and macroinvertebrate community data (Karouna-Renier 1995). Thc drainage areas of the study sites represented three land uses: commercial, residential, and highway (Table 1). Commercial drainage areas consisted primarily of office parks and small retail stores associated with each complex. Residential areas were composed of single family homes, townhouses and garden apartment buildings. Both commercial and residential ponds also received runoff from streets associated with the developments. Ponds serving highway drainages received runoff from interstate and limited access state roads of four to six lanes in width.

Stormwater ponds selected for the study met the following criteria: (1) presence of standing water throughout the year with a maximum depth of 2 m; (2) presence of emergent vegetation and open water areas; (3) constructed specifically to collect stormwater runoff; and, (4) located in the Atlantic Coastal Plain geologic province. All of the ponds selected for toxicity testing were 3-5 years old. Reference sediment, chosen to match experimental sediments in physico-chemical properties, was collected from a constructed wetland at the Patuxent Wildlife Research Center (Patuxent), Laurel, MD that did not receive any urban or road runoff (see Sparling et al. 1995 for construction details).

Sediment was collected from the littoral zone at the outflow. inflow, and mid-pond. In those ponds with more than one inflow area, the inflow farthest from the outflow was selected. Sediment samples were collected using a hand-held corer with a plastic sleeve on 22-23 June, 1994, 24-36 hours prior to initiation of the test. The top 2.5 cm of sediment from each bore were composited in acid-cleaned 1000-ml glass jars and immediately placed on ice. This fraction of stormwater pond sediments has been shown to contain the highest levels of contaminants (Yousef et al. 1984). Concurrently with the sediment collection, surface water for use in the bioassay was collected from each pond in 20 L, acid-washed,

Table 2. Initial water quality conditions of bioassay chambers.

Pond	pH	DO[a]	Hardness[a]	Alkalinilty[a]	Conductivity
Patuxent	8.05-8.26	8.0- 8,1	4.1 -4.3	152	260
Baltimore-Wash. Pkwy.	7.70-7.93	7.8- 8.0	3.5 -4.0	76	215
Country Meadows	7.98-8.07	7.5 -7.9	4.3 -4.5	72	510
Chapel Estates	7.38-7.53	6.8- 7.6	2.2 -2.6	64	345
Executive Terrace	762.773	6.5 -7.3	3.0- 3.3	80	500
Golden Triangle	7.73-7.77	7.0- 7.5	3.1 -3.3	64	415
Lions Gate	8.08-8.21	7.6- 8.1	4.3 -4.4	92	312
Muirkirk Road	7.86-8.05	7.1 -7.8	3.8 -4.1	72	240
New Cut Road	7.83-7.91	6.9- 7.8	3.6- 3.6	80	310
Winchester Road	7.58-7.85	7.4- 8.0	3.3 -3.4	68	720

[a]DO=mg/l, Hardness=mg/l Ca, Alkalinity=mg/l as $CaCO_3$, Conductivity=µmhos.

polyethylene jugs. The water samples were stored in the laboratory at the test temperature and were continuously aerated.

An intermittent-renewal sediment testing system was constructed as described by Zumwalt et al. (1994). The bioassays were conducted for 10 days at 22±2°C with a 16L:8D photoperiod in an environmental chamber at Patuxent. Three replicate exposure vessels were used per pond. Sediment (100 ml) and water (175 ml) were added to each vessel and allowed to settle and equilibrate for a period of 24 hr (Ankley et al. 1993a; Zumwalt et al. 1994). At the initiation of the assay, overlying water was renewed and 10 juvenile *H. azteca* (2-4 mm) obtained from a commercial distributor, were randomly added to each beaker.

The amphipods were fed 0.8 mg (dry weight) of a yeast-cerophyll-trout chow (YCT) mixture daily (Ankley et al. 1993a). Water (50 ml) collected from the ponds at the initiation of the study was delivered twice daily to each vessel. Aeration of the overlying water (2-3 bubbles/s) was provided by suspending a 14.6-cm long Pasteur pipet approximately 4 cm above the sediment surface to avoid any disturbance of the sediment.

Dissolved oxygen and pH were monitored every 48 hr in all beakers after the morning water renewal with electronic probes. Temperature was monitored daily in a randomly selected replicate per pond and in all replicates every 48 hr. Alkalinity, conductivity and hardness were determined at test initiation and termination using a chemical test kit and electronic probes, respectively. Initial overlying water quality characteristics are presented in Table 2.

At the end of the 10-d period, *H. azteca* were retrieved by gently passing the water and sediments through as 500-µm sieve. Missing organisms were presumed dead. Endpoints measured were survival rates, length, and dry weight. An ocular micrometer was used to measure body length from the base of the first antenna to the end of the third uropod along thc dorsal surface. After length was measured, the samples were dried in a desiccating oven at 50°C for 24 hours and mean dry weights of amphipods were obtained by dividing total weight per replicate by the number of recovered organisms.

Sediments used in the bioassay were analyzed for Acid Volatile Sulfides (AVS), simultaneously extracted metals (Cd, Cu, Ni), and Total Organic Carbon. AVS was measured by acidifying approximately 10 g of wet sediment with 1 N HCl. Reactive sulfides that were converted to gasified H_2S were trapped as Ag_2S and measured gravimetrically. Simultaneously extracted metals were analyzed by digesting sediments with lN HCl in glass beakers on a hot plate and diluted to volume with distilled water. The digestate was analyzed for Cd by graphite furnace AAS and for Ni and Cu by atomic emission using an argon plasma.

Total organic carbon was determined by burning acidified freeze-dried sediment using a LECO Model 523-300 induction furnace under an oxygen environment. The resultant CO_2 gas was detected and quantified with a Horiba PIR-2000 infrared detector. The output signal from the detector was sent to an Hewlett Packard 3396A integrator which reported the quantity of CO_2 as a peak area. All analyses were performed by the Geochemical and Environmental Research Group at Texas A&M. QA/QC included the analyses of procedural blanks, duplicates, reference materials, and spike recoveries. Recoveries of sulfide spikes averaged 98% from spiked sediments. Recoveries of Cu, Cd and Ni from spiked sediments were 123%, 105%, and 112%, respectively.

Statistical analyses were performed using a commercial software package (SAS 1986). The data were tested for normality using the Wilkes-Shapiro Test and homogeneity of variances was tested by plotting sample variances against sample means, followed by a test of correlation between the two. The proportions of surviving *H. azteca* were transformed to the arcsine of the square root. Length and weight of *H. azteca* were not transformed. Survival and weight were analyzed by one-way ANOVA. Length of *H. azteca* was analyzed using a nested ANOVA with mean square error terms derived from replicates

Table 3. Mean (± SD) survival, weight and length *of Hyalella azteca* exposed to water and sediments from stormwater treatment ponds in Maryland.

Pond	Survival (%)	Weight (µg)	Length (mm)
Patuxent	86.67 ± 0.06	97.0 ± 41.6	5.57±0.55
Baltimore-Wash Pkwy	90.00 ± 0.10	47.1 ± 14.8	5.61 ± 0.49
Country Meadows	96.67 ± 0.06	96.7 ± 60.3	6.44 ± 0.79
Chapel Estates	96.67 ± 0.06	159.3 ± 18.9	6.15 ± 0.67
Executive Terrace	90.00±0.10	117.9 ± 10.5	6.74 ± 0,80
Golden Triangle	86.67±0.12	183.3 ± 28.9	6.21 ± 0.82
Lions Gate	93.33 ± 0.06	97.0 ± 41.6	5.63 ± 0.68
Muirkirk Road	96.67 ± 0.06	155.6 ± 70.7	6.31 ± 0.74
New Cut Road	93.33 ± 0.06	136.7 ± 23.8	6.81 ± 0.75
Winchester Road	90.00 ± 0.10	85.1 ± 6.4	5.66 ± 0.52

nested within treatments. Specific contrasts used Tukey's HSD tests at $\alpha=0.05$. Thc Spearman Rank Correlation procedure was used to identify significant relationships among measures of toxicity and chemical variables.

RESULTS AND DISCUSSION

Overlying water quality parameters were within acceptable ranges (Ankley et al. 1993). Dissolved oxygen remained above 60%. saturation in all replicates throughout the duration of the study. Mean survival rates (±SD) for *H. azteca* exposed to reference sediments was 86.7±5.7%, exceeding the recommended control survival rate of 80% (Ankley et al. 1993). None of the sediments sampled from stormwater ponds were found to be toxic to *H. azteca* in the 10-d exposure. Mean survival rates for all test sediments ranged from 86.7% to 96.7% (Table 3). No significant differences in survival rates were observed among any of the ponds. Mean weights for the amphipods ranged from 47.1 µg to 183.3 µg (Table 3). However, no significant differences were observed from the reference.

Mean lengths of *H. azteca* ranged from 5.61 to 6.81 mm. *H. azteca* from New Cut Road (highway), Country Meadows (residential), and Executive Terrace (commercial) ponds were significantly longer ($p<.0001$) than the reference (Patuxent) (Table 3). Enhanced growth in bioassays has been observed in other investigations (Ingersoll and Nelson 1990; Schlekat et al. 1994). A potential causative factor is the presence of a supplemental food source such as organic particulate in those test sediments with enhanced growth. None of the sediments were sieved prior to testing to minimize the alteration of chemical composition and environment of the sediments. Thus, certain sediments may have had a food supply beyond that which was provided by the YCT.

It has been demonstrated that divalent metals tend to bind to the acid volatile sulfide (AVS) fraction of aquatic sediments, rendering them biologically unavailable. In the presence of excess AVS, free metal activity and toxicity are reduced. No acute toxicity is predicted

Table 4. Levels of total organic carbon (TOC), simultaneously extracted Cu and acid volatile sulfides (AVS) of sediments used in 10-day *Hyalella azteca* bioassays.

Pond	TOC (%)	AVS	Cu	Cu/AVS
Patuxent	1.13	3.0	2.2	0.73
Baltimore-Wash Pkwy	0.25	3.4	1.0	0.29
Chapel Estates	0.59	52.0	<1.0	<0.02
Country Meadows	0.66	76.0	<1.0	<0.0l
Executive Terrace	0.92	29.0	1.1	0.04
Golden Triangle	0.68	9.6	1.1	0.11
Lions Gate	1.35	30.0	<1.0	<0.03
Muirkirk Road	0.63	23.0	7.2	0.31
New Cut Road	0.83	56.0	<1.0	<0.02
Winchester Road	0.27	<2.0	<1.0	<0.5

Note: Concentrations of simultaneously extracted Cd and Ni were below detection limits (0.2 µg/g and 5.0 µg/g) and are not reported. Results expressed as µg/g dry weight.

when the ratio of simultaneously extracted metal (SEM) to AVS is less than one (DiToro et al. 1992). When the SEM to AVS ratio exceeds one, toxicity may or may not be observed, depending upon the presence of other sediment fractions such as organic carbon, which also play a role in controlling bioavailability. The interplay of these factors determines the actual availability of sediment metals to aquatic organisms.

Levels of AVS in the sediments from this study ranged from <2.0 µg/g to 76.0 µg/g (Table 4). Concentrations of simultaneously extracted metals (SEM) were consistently low. Copper ranged from <1.0 to 7.2 µg/g and levels of Cd and Ni in all of the samples were below the detection limits of 0.2 µg/g and 5.0 µg/g, respectively. Kemble et al. (1994) reported No-effect Concentrations (NEC) for amphipod length of 325 µg/g, 3.87 µg/g, and 5.23 µg/g of simultaneously extracted Cu, Cd and Ni. The NEC is the maximum concentration of a metal in sediment at which the toxicity indicator is not significantly affected. The levels of these metals in our samples were below the NEC. Ratios of CuSEM to AVS were <1 for all samples in this study, indicating that Cu is not bioavailable from the sediments during the sampling period.

AVS concentration was the only chemical parameter that significantly correlated with any of the toxicity endpoints. Both survival rate ($r=0.7543$, $p=0.0117$) and amphipod length ($r=0.6370$, $p=0.0476$) were correlated with AVS. The positive relationship we found may be indicative of the role AVS plays as an inhibitor of metal toxicity. Besser et al. (1996) also noted that the growth of *Chironomus tentans* (Diptera) generally correlated with differences in AVS concentrations. However, they found both positive and negative relationships. They attributed the differences to other sediment characteristics such as particle size, ammonia levels, and TOC. Sediment TOC in this study ranged from 0.25% to 1.35%.

Toxicity of metals in stormwater ponds may show seasonal variations. Howard and Evans (1993) found that the concentrations of sediment sulfides in lakes vary not only spatially, but seasonally as well. As oxygenated water is introduced to the hypolimnion during periodic turnover, sulfide concentrations in the sediment decrease. As a result, during times of AVS-minima, metals could become available, potentially exerting a toxic effect on macroinvcrtebrates. Thus, the toxicity of stormwater pond sediments may change as a result of this cycle.

It is evident that the lack of toxicity in this study is indicative only of the conditions at the time of sediment and water collection. All of the ponds in this study were quite young, fewer than 5 yr old. Only limited data are available on the changes in sediment metal concentrations as stormwater treatment ponds age. Further accumulation of contaminants in the sediments of older ponds may result in changes in metal availability, and therefore, toxicity.

Although dry-weather conditions in these ponds caused no apparent toxicity to invertebrates, wet-weather transport of contaminants into the ponds may change the invertebrate response. The variability in contaminant concentrations and bioavailability is highly site-specific, making it difficult to generalize about wet-weather toxicity. For example, Katznelson et al. (1995) observed toxicity in *Ceriodaphnia dubia* (Cladocera) in an urban stormwater treatment marsh during wet-weather but not in dry weather. Further research is needed to evaluate site-specific differences in wet-weather toxicity and seasonal changes in toxicity of stormwater pond sediments and water.

Acknowledgements. The authors thank V. Dang who helped collect water chemistry data. P. Lowe, N. Beyer and R. Eisler provided helpful comments on earlier drafts of this manuscript.

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