EVALUATION OF TOXICITY OF SEDIMENT SAMPLES COLLECTED FROM THE SANTA ANA NATIONAL WILDLIFE REFUGE, TEXAS

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Abstract

The objective of this study was to evaluate the toxicity of sediments collected from the Santa Ana National Wildlife Refuge, Hidalgo Co in southern Texas, USA. A 42-d toxicity test was conducted with the amphipod *Hyalella azteca* (28-d sediment exposure followed by 14-d water-only exposure) to evaluate sediments from the refuge. Endpoints measured were survival, growth, and reproduction. A total of 6 sampling stations on the refuge were tested along with a control sediment. None of the sediment samples from the Santa Ana Refuge were toxic to the amphipods. Survival, growth and reproduction (number of young/female) of amphipods were not significantly reduced in any of the 6 sediment samples compared to the control sediment. There was no correlation between biological endpoints and grain size of the sediments. A significant correlation was observed between pore-water unionized ammonia and amphipod growth at Day 28. Sediment samples were not analyzed for whole sediment chemistry, therefore no comparison between endpoints in the toxicity test to sediment chemistry was possible.

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Introduction

Agricultural, industrial and urban development along both banks of the Rio Grande and Rio Bravo rivers have increased in recent years resulting in an increase in effluent discharge and contaminated runoff into the river. Industrial effluent as well as domestic return flows from the Mexican side are often discharged untreated into the Rio Grande or a feeder stream (Eaton and Andersen 1987). Metals and pesticides were the chemicals exceeding screening levels (USEPA Human Health criteria for Consumption of Fish and Water) in water samples collected from the mainstream of the river (TNRCC 1994). Unionized ammonia and chloride were chemicals attributed to the toxicity of fathead minnows and cladocerans in samples from Anhuelo drain from Reynosa, Mexico (12.9 km miles upstream of the Santa Ana National Wildlife Refuge, NWR). Arsenic concentrations exceeded the screening levels in the Anhuelo drainage by 25 fold and by 19 fold at a location next to the refuge (TNRCC1998).

Sediments can act as a repository for a whole array of organic and inorganic contaminants, and can accumulate these contaminants to extremely high concentrations, even when water concentrations of contaminants are at or below acceptable water quality criteria (Shimp et al. 1971; Oschwald 1972; Medine and McCutcheon 1989). With the exception of rainfall (about 59.4 cm/year), the main source of surface water entering the NWR has been pumped in directly from the Rio Grande River or into the Santa Ana NWR from the river via an irrigation canal (Figure 1). However, recently, some of the ponds have been supplemented with well water.

The objective of this preliminary study was to conduct a chronic whole-sediment toxicity test with 6 sediment samples from the Santa Ana National Wildlife Refuge using the amphipod *Hyalella azteca* (USEPA 2000, ASTM 2001). The refuge receives water that may contain a vast array of possible contaminants. Therefore, the results of this study will provide the information for making decisions regarding future studies on the refuge including Toxic Identification Evaluations (TIE) followed by sediment chemistry to narrow down the class of compounds responsible for any mortality.

Methods

Sample Collection, Handling, and Storage

Personnel from the United States Fish and Wildlife Service collected sediment samples on October 4, 2001 from the Santa Ana National Wildlife Refuge (Figure 1). All sediment samples were collected using a 500 ml high-density polyethylene (HDPE) dipper (ladle) from about the upper 5 cm of the sediment surface. Each sediment sample was a composite of 10 to 12 scoops to obtain a minimum of 2 L of sediment/station (for amphipod testing and physical analyses of sediments). Samples were held in the dark on ice at 4^{o}C in HDPE containers before shipment to the Columbia Environmental Research Center (CERC) in Columbia, MO. Samples arriving at CERC were given an in-house CERC designation for use in testing (Table 1). The control sediment was sediment from West Bearskin Lake (Ankley et al. 1994, Ingersoll et al. 1998). Sediment toxicity tests were started within two weeks of sample collection from the field. Sediments were not sieved to remove indigenous organisms; however, large indigenous organisms and large debris were physically removed (using forceps) during homogenization of samples in the laboratory.

Culturing of Test Organisms

Mixed-age amphipods were mass cultured at 23° C with a luminance of about 800 lux using 80-L glass aquaria containing 50 L of CERC well water (hardness 283 mg/L as $CaCO_3$, alkalinity 255 mg/L as $CaCO_3$, pH 7.8; Ingersoll et al. 1998). Artificial substrates were placed in the amphipod culture aquaria (six 20 cm sections/aquarium of "coiled-web material"; 3M Corp., Saint Paul, MN). Amphipods used to start the tests were obtained by collecting amphipods that passed through a #35 U.S. Standard size (500-um opening) and were stopped by a #40 (425-um opening) sieve placed under water (ASTM 2001, Ingersoll et al. 2001). Amphipods were held in 3 L of water with gentle aeration and with a small amount of Tetramin® (flake fish food) and a maple leaf for 24 hours before the start of the test. Use of this sieving method resulted in an average amphipod length of 1.77 mm (0.06 SE) at the start of the exposure.

Sediment Exposures

Test sediments were homogenized in a stainless steel bowl using a plastic spoon and added to exposure beakers 1 d before test organisms were added (Day -1).

Subsamples of sediment were then collected for pore-water isolation and physical characterizations.

Toxicity tests with *Hyalella azteca* were conducted for a total of 42 d (28 d of sediment exposure followed by 14 d of water only exposure; Ingersoll et al. 1998, USEPA 2000, ASTM 2001). Endpoints measured in the amphipod exposures included survival and growth (length) on Day 28, survival on Day 35, and survival and growth on Day 42, and reproduction (number of young/female produced from Day 28 to Day 42). The purpose for transferring surviving amphipods from sediment to water at Day 28 is to monitor reproduction. At about Day 28, amphipods used to start the exposures begin to go into amplexus followed by release of their first brood (Ingersoll et al. 1998).

Amphipods were exposed to 100 ml of sediment with 175 ml of overlying water (CERC well water) in 300-ml beakers (eight replicates/treatment; 4 replicates for Day 28 survival and growth and 4 replicates for Day 28 to 42 survival, growth and reproduction) at 23^{o} C. The photoperiod was 16:8 h light:dark at an intensity of about 200 lux at the surface of the exposure beakers. Each beaker received 2 volume additions/d of overlying water starting on Day -1 (Zumwalt et al. 1994). One diluter cycle delivered 50 ml of water to each beaker (diluters cycled every 4 h ± 15 min). Tests were started on Day 0 by placing 10 amphipods (7-d old) into each beaker using an eyedropper. Amphipods in each beaker were fed 1.0 ml YCT (1.7 to 1.9 g/L stock solution) in a water suspension daily (USEPA 2000; ASTM 2001). Beakers were observed daily for the presence of animals, signs of animal activity (i.e., burrowing), and to monitor test conditions (mainly water clarity).

On Day 28, amphipods were isolated from each beaker by pouring off most of the overlying water, gently swirling the remaining overlying water and upper layer of sediment and washing the sediment through a No. 50 (300-:m opening) US Standard stainless steel sieve. The materials that were retained on the sieve were washed into a glass pan and the surviving amphipods were removed. Amphipods from 4 of the replicates were counted and preserved in 8% sugar formalin in a scintillation vial for subsequent length measurements (Kemble et al. 1994; Ingersoll et al. 1998).

Amphipods from the remaining 4 replicates/treatment were placed in a 300-ml beaker containing 175 ml of overlying water and a 3 cm x 3 cm piece of "coiled-web material" (3M Corp., Saint Paul, MN). Each beaker received two volume additions of water and 1.0 ml of the YCT suspension daily. Reproduction of amphipods was then measured on Days 35 and 42 by counting the number of young in each of these water-only beakers. Production of young amphipods in these beakers was monitored by removing and counting the adults and young in each beaker. On Day 35, the adults were returned to the same water-only beakers. On Day 42, adult amphipods were preserved with sugar formalin for growth and sex determination (mature male amphipods were distinguished by the presence of an enlarged second gnathopod). Amphipod length measurements were made using an EPIX imaging system (PIXCI® SV4 imaging board and XCAP software; EPIX Inc., Buffalo Grove, IL) connected to a computer and a microscope.

About 170 ml of pore water was isolated from sediment samples by centrifugation at 5,200 rpm (7000 g) for 15 min at 4^{o}C (Kemble et al 1994). Immediately after pore water was isolated, dissolved oxygen, pH, alkalinity, temperature, conductivity, total ammonia, and hardness were measured using methods outlined in Kemble et al. (1994; Table 2). About 20 to 50 ml of pore water was used to measure ammonia and a similar volume of pore water was used to measure the other water quality characteristics. A wide range in the water quality characteristics of the pore water was observed for pH (6.0 to 7.8), alkalinity (62 to 620 mg/L as $CaCO_3$), dissolved oxygen (4.0 to 6.7 mg/L), conductivity (829 to 2830 :mho/cm), total ammonia (1.27 to 8.71 mg/L), unionized ammonia (<0.002 to 0.0095 mg/L) and hardness (175 to 398 mg/L as $CaCO_3$; Table 2). Due to a lack of pore water generated, ammonia concentration was not determined in the Site SA-02 pore-water sample.

Conductivity, pH, alkalinity, hardness, dissolved oxygen, and total ammonia were measured in overlying test water on Day 0 (the day amphipods were stocked), Day 28, and Day 42 of the exposure. Conductivity and dissolved oxygen, in overlying water were also measured weekly. Methods used to characterize overlying water quality in the whole-sediment tests are described in Kemble et al. (1994). Temperature in the water baths holding the exposure beakers were monitored daily. Overlying water pH, alkalinity, total hardness, conductivity and total ammonia measurements were similar among treatments pH (8.3 for all treatments), alkalinity (239 to 252 mg/L as $CaCO_3$), dissolved oxygen (6.2 to 7.4 mg/L), conductivity (629 to 676 :mho/cm), total ammonia (0.061 to 0.157 mg/L), unionized ammonia (<0.001 to 0.0014 mg/L) and hardness (270 to 290 mg/L as $CaCO_3$); Table 3). Dissolved oxygen in the overlying water was at or above the acceptable level of 2.5 mg/L in all treatments throughout the study (ASTM 2001, USEPA 2000).

Physical characterization of sediment samples

Physical characterization of sediments included: (1) percentage water (Kemble et al. 1994), (2) particle size analysis using a hydrometer (Foth et al. 1982; Gee and Bauder 1986; Kemble et al. 1994), and (3) total organic carbon using a coulometric titration method (Cahill et al. 1987; Kemble et al. 1994). Precision and accuracy of the coulometric technique used were tested against National Bureau of Standards and Standard Reference Materials (NBS-SRM) with an error of less than 0.03% of the excepted values (Cahill et al. 1987).

Data Analysis and Statistics

Statistical analyses for the amphipod exposures were conducted using one-way analysis of variance (ANOVA) at $p = 0.05$ for all endpoints except length which was analyzed using a one-way nested ANOVA at $p = 0.05$ (amphipods nested within a beaker). Before statistical analyses were performed, data were tested for normality and transformed if needed. Amphipod growth data had a normal distribution and were not transformed before statistical analysis. Amphipod reproduction data (number of young/female) were square root transformed before statistical analysis. Variance treatment means for all survival data was heterogenous, therefore a rank analysis of variance was performed and mean differences determined using a T-test on ranked means (at $\forall = 0.05$). Spearman rank correlation procedures were also used to evaluate relationships between the responses of amphipods exposed to the field-collected sediments and the physical sediment characteristics or the water quality (pore water or overlying water) characteristics. Statistical significance for the rank correlations was established at 0.008 for all comparisons to minimize experiment-wise error (Bonferroni method; Snedecor and Cochran 1982). All statistical analyses were performed with Statistical Analysis System programs (SAS 2001).

Results and Discussion

Physical Characteristics of sediment samples

A broad range in grain size and TOC was observed in the sediment samples collected from the assessment areas (Table 4). Water content of sediments ranged from 32% for sediment from Site SA-05 to 64% for sediment from Sites SA-03 and SA-04. Sediment organic carbon content ranged from 1.3% in the control sediment to 6.8% in sediment from Site SA-06 (Table 4). Classification of the sediment samples for grain size varied from site to site (i.e., clay (SA-01 and SA-02), silt clay (SA-03), clay loam (SA-04) sandy loam (SA-06) while the control sediment and sediment from Site SA-05 were a loam (Table 4).

Sediment Exposures

Sediment samples from the Santa Ana Refuge were not toxic to the amphipods. Survival of amphipods was not significantly reduced in any of the 6 sediment samples relative to the control sediment (Table 5). Growth of amphipods was not significantly reduced compared to the control in any of the 6 samples (Table 5). Similarly, none of the sediment samples significantly reduced amphipod reproduction (number of young/female) compared to the control sediment (Table 5).

Indigenous organisms recovered at end of the 28-d sediment exposure included oligochaetes, and a mussel. Amphipods were observed in amplexus in all of the sediment treatments except for Site SA-05.

Comparison of Sediment Characteristics to Toxicity Responses

Relationships of physical characteristics of sediments to toxicity were evaluated using Spearman Rank correlation. The results of this evaluation showed that there were no significant correlations between survival, growth or reproduction (Table 5) and grain size, TOC, or percent water (Table 4). This finding is consistent with the results of earlier studies (USEPA 2000; ASTM 2001; Kemble et al. 2002) which showed that sediment particle size or TOC did not affect the response of *Hyalella azteca* in 28-d sediment exposures. We also evaluated relationships of pore-water and overlying water quality to the biological endpoints. There was significant positive correlation between 28-d amphipod growth and unionized porewater ammonia (r=0.8928). While this was a positive correlation, ammonia levels were well below threshold levels (levels expected to cause impairment; USEPA 2000). The lack of correlation may have resulted from the small number of sediment samples and the lack of toxic effects.

Summary

Sediment samples from the Santa Ana Refuge were not lethal to amphipods. Amphipod survival identified all 6 of the sediment samples as non-toxic (a significant reduction compared to the control sediment). Sublethal endpoints evaluated also identified none of the sediment samples as toxic (a significant reduction compared to the control).

The lack of chemistry data in this project made evaluation of biological endpoints to chemistry data impossible. Future studies at the refuge should include chemical analysis of sediment samples.

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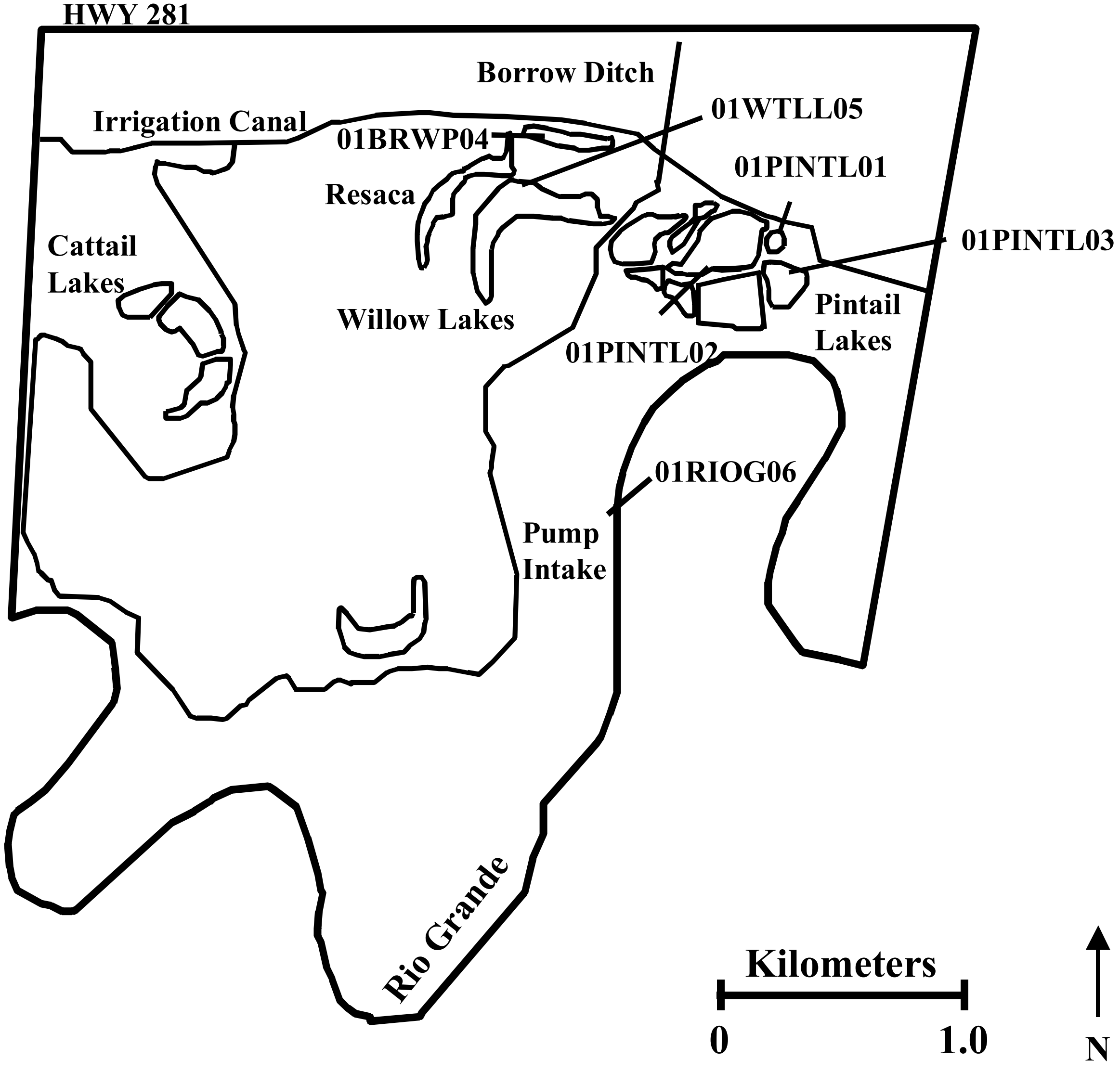

Figure 1. Location of sampling sites in the Santa Ana National Wildlife Refuge.

Table 1. Identification numbers for sediment samples used to conduct toxicity tests (NA= not applicable).

Site Location	Station Code	CERC #
NA	NA	Control
Pintail Lakes	01PNTL01	SA-01
Pintail Lakes	01PNTL02	SA-02
Pintail Lakes	01PNTL03	SA-03
Borrow Ditch	01BWP04	SA-04
Pump Intake	01R10G06	SA-05
Willow Lakes	01WILL05	SA-06

Table 2. Measured pore water water quality characteristics for exposures with Santa Ana sediment samples.

Sample Number	Temperature (°C)	D.O. (mg/L)	Conductivity (umho@25°C)	pH	Alkalinity (mg/L as $CaCO_3$)	Total Ammoia (mg/L)	Unionized ammonia (mg/L)	Hardness (mg/L as $CaCO_3$)
Control	17	4.0	829	6.0	62	5.22	0.0002	175
SA-01	14	4.5	1369	7.5	450	2.55	0.0023	370
SA-02	14	4.0	1460	7.8	212	NM	NM	390
SA-03	15	4.4	1660	7.8	286	2.52	0.0038	390
SA-04	15	4.1	2830	7.8	408	5.48	0.0095	398
SA-05	16	6.7	1773	7.5	620	8.71	0.0082	390
SA-06	15	5.1	2150	7.6	380	1.27	0.0013	390

NM = Not Measured

Table 3. Mean measured overlying water quality characteristics for exposures with Santa Ana sediment samples.

Sample Number	Temperature (^oC)	D.O. (mg/L)	Conductivity (umho@25^{o}C)	pH	Alkalinity (mg/L as $CaCO_3$)	Total Ammoia (mg/L)	Unionized ammonia (mg/L)	Hardness (mg/L as $CaCO_3$)
Control	23	7.4	629	8.3	239	0.157	0.0014	270
SA-01	23	7.0	660	8.3	250	0.076	0.0006	288
SA-02	23	7.0	654	8.3	250	0.063	0.0005	290
SA-03	23	6.3	661	8.3	252	0.095	0.0009	288
SA-04	23	6.5	663	8.3	247	0.111	0.0011	283
SA-05	23	6.2	676	8.3	250	0.122	0.0010	288
SA-06	23	6.8	654	8.3	244	0.061	0.0006	281

Table 4. Physical and chemical characteristics of sediment samples from Santa Ana Refuge.

Sample Number	TOC (%)	Water (%)	Particle Size (%)			Sediment Class
			Sand	Clay	Silt	
Control	1.3	40	53	17	30	Loam
SA-01	3.9	48	6	74	20	Clay
SA-02	4.8	60	39	34	28	Clay Loam
SA-03	5.6	64	4	67	29	Clay
SA-04	6.1	64	8	34	59	Silty Clay Loam
SA-05	2.2	32	53	22	25	Sandy Clay Loam
SA-06	6.8	49	28	26	46	Clay Loam

Table 5. Response of *Hyalella azteca* in 28-d exposures to sediment samples from Santa Ana Refuge and a West Bearskin control (WB) sediment (standard error in parenthesis). No significant differences were observed in the responses of amphipods between test sediments from the refuge and the control sediment ($p < 0.05$).

Sample Number	28-d Survival	28-d Length	35-d Survival	42-d Survival	42-d Length	# of young Per Female
Control	96 (2.63)	4.29 (0.06)	95 (2.89)	95 (2.89)	5.27 (0.09)	5.03 (0.92)
SA-01	93 (3.13)	4.67 (0.08)	98 (2.50)	98 (2.50)	5.26 (0.08)	5.00 (1.16)
SA-02	96 (3.75)	4.13 (0.07)	100 (0.00)	100 (0.00)	5.09 (0.08)	3.15 (0.81)
SA-03	95 (3.27)	5.09 (0.08)	100 (0.00)	100 (0.00)	5.36 (0.11)	7.73 (2.08)
SA-04	98 (2.50)	4.46 (0.07)	98 (2.50)	93 (4.15)	5.34 (0.09)	4.10 (0.86)
SA-05	95 (2.67)	5.10 (0.07)	95 (2.89)	93 (4.79)	5.79 (0.08)	11.08 (2.71)
SA-06	93 (2.50)	4.38 (0.07)	95 (2.89)	95 (2.89)	5.14 (0.09)	5.83 (1.09)