Pimephales promelas and Laboratory Bioassay Responses to Cadmium in Effluent Dominated Systems.

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ABSTRACT

Whole Effluent Toxicity tests predict individual responses, but do not measure natural population or community responses. Because southwestern U.S. rivers are often dominated by municipal effluents, more direct evaluations of ecosystem health are needed to properly evaluate fate and effects of metals in such aquatic systems. We designed lotic mesocosms to assess effluent and cadmium effects on stream biota and to determine relevance to regulatory criteria of standard laboratory tests. A municipal effluent served as source water for experimental streams, colonized by invertebrates from a nearby stream. Because of inherent temporal effluent variability, we characterized source water chemically (standard parameters hourly; alkalinity, hardness, TOC, total and dissolved metals at 0600, 1400, 2200) and biologically (*C. dubia* bioassays for 0600, 1400, and 2200 samples). Cadmium, a non-point contaminant in north Texas streams, was added to replicate units at 0, 0.22, and 2.22 mM during a 10-day study period. Cd concentrations were verified by GF-AAS. Adult fathead minnows were caged in stream pool sections on day –2 to acclimate. Stream riffles and pools were sampled on days 0 and 10 for macroinvertebrates, periphyton, system metabolism and fish biomarkers. Concurrent laboratory *C. dubia* and *P. promelas* bioassays were performed with stream water throughout this study. Compared to untreated streams, *P. promelas* vitellogenin, condition, hematocrit, GSI, and HSI were unaffected by 0.22 and 2.22 mM Cd treatments. Laboratory bioassays, benthic macroinvertebrates, periphyton and system metabolism were not affected by 0.22 mM Cd treatments. Our laboratory and field results indicate that municipal effluent constituents alter Cd bioavailability and effects. Current water quality criteria for metals based on hardness alone do not account for such constituents.

RESEARCH RATIONALE

• Rivers in the southwestern and south-central U.S. are often greater than 90% return flow from wastewater treatment plants. Questions remain concerning the degree to which assessment of single chemical effects reflects protection in "real-world" situations.

• Although WET tests are useful in predicting aquatic individual effects (Dickson et al. 1992), they are not meant to directly measure natural population, community or system responses.

• Interactions among municipal effluents and upstream contaminants can reduce agreement between WET test and field assessment results for a given discharge (La Point & Waller 2000). Effluent and upstream contaminant mixture effects may be cumulative (La Point & Waller 2000) or effluent constituents may ameliorate upstream toxicity (Eagleson et al. 1990).

• Standard WET test species, *Ceriodaphnia dubia* and *Pimephales promelas*, do not always protect receiving stream biota (Cook et al. 1999). Consequently, more direct evaluations of ecosystem health, using lotic mesocosm bioassessments, are needed to properly evaluate aquatic systems affected by wastewater discharges (La Point & Waller 2000).

• To examine regional effluent questions, we recently constructed the University of North Texas Stream Research Facility (UNTSRF) at the City of Denton, TX Pecan Creek Water Reclamation facility. UNT streams are conceptually similar to those described by Rodgers et al. (1996). Each stream consists of a mixing box, two riffle sections with different slopes, and a downstream pool. City of Denton, TX final treated effluent serves as source water for experimental streams. Effluent is pumped into reservoirs from which streams are supplied water by gravity flow. Streams are colonized by invertebrates from nearby Pecan Creek.

• Cadmium, a non-point source contaminant in north Texas, has been suggested as an endocrine modulating compound (Olsson et al. 1995; Guevel et al. 2000; Stoica et al. 2000; Thompson 2000). However, effects of cadmium on endocrine biomarkers of aquatic biota have not been evaluated under field conditions. This is of particular interest in systems affected by cadmium, and dominated by municipal effluent discharge.

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EXPERIMENTAL OBJECTIVES

• Evaluate effects of Cd and effluents on adult male *Pimephales promelas* endocrine endpoints.

• Evaluate bioavailability and effects of Cd on aquatic biota in model systems dominated by a municipal effluent.

EXPERIMENTAL APPROACH

• Streams were monitored monthly for periphyton and benthic invertebrates, and biweekly for water quality from May 2000 to August 2000.

• Replicate streams were nominally dosed with 0, 0.222, or 2.22 mM Cd using high precision peristaltic pumps from 25 August 2000 (Day 0) to 6 September 2000 (Day 12).

• City of Denton effluent (source water for UNTSRF) was characterized chemically and biologically during this study.

Chemical: 1. Hourly measures of pH, temperature, conductivity, dissolved oxygen, TDS, turbidity, and ammonia (Table 1).
2. Alkalinity, hardness, total organic carbon, and total and dissolved metals (Ag, Cd, Cu, Cr, Ni, Pb, Zn) determined on study days -2, 2, 5, 9 and 12 at 0600, 1400, and 2200 (Table 1).

Biological: *C. dubia* chronic bioassays with daily renewals at 0600, 1400, and 2200 (Table 1).

• Laboratory *C. dubia* and *P. promelas* 7-day WET bioassays conducted with water from each stream (Figure 4).

• Streams were sampled on study days 0 and 10 for benthic macroinvertebrates, periphytic biomass, and ecosystem productivity (Figures 1-3, 5) and sampled on day 12 for fish endpoints (Figure 4). Analysis for VTG performed on whole liver homogenates by SDS-PAGE, Western blotting and a monoclonal anti-VTG antibody from Cayman Chemical Co. that crossreacts with fathead minnows (Hemming et al. 2001). Hepatic protein content was determined using a bovine serum albumin standard and a Bio-Rad Protein Assay protein dye.

• Analysis of treatment effects performed by Repeated Measures ANOVA using SPSS following arc sine (square root (y)) transformation of proportional data and log(x+1) transformation of other benthic invertebrate data. Data are presented as untransformed means (±SE) unless otherwise noted.

Table 1. Mean water quality characteristics (±SD) of UNTSRF (City of Denton, TX effluent) at 0600, 1400, and 2200.

Parameter	0600	1400	2200
pH	6.90 (±0.10)	6.90 (±0.09)	6.88 (±0.09)
Temperature (°C)	29.3 (±0.18)	31.3 (±0.12)	30.8 (±0.10)
Dissolved Oxygen (mg/L)	4.5 (±0.29)	7.3 (±0.43)	4.2 (±0.23)
Alkalinity (mg/L, $CaCO_3$)	64 (±8.22)	69 (±8.94)	69 (±12.45)
Hardness (mg/L, $CaCO_3$)	145.6 (±11.52)	140 (±5.66)	142.4 (±9.21)
Total Organic Carbon (mg/L)	7.62 (±1.05)	7.80 (±2.05)	7.31 (±2.20)
Total Dissolved Solids (mg/L)	0.5751 (±0.0061)	0.5779 (±0.0018)	0.5765 (±0.0024)
Specific Conductance (us/cm)	898.4 (±9.7)	903.0 (±2.8)	900.6 (±3.9)
Turbidity (NTU)	9.17 (±3.20)	12.2 (±5.45)	9.43 (±3.86)
Cu (mM)			
Total, Dissolved	0.275 (±0.02), 0.23 (±0.016)	0.303 (±0.049), 0.271 (±0.052)	0.278 (±0.042), 0.262 (±0.009)
Ag, Cd, Cr, Ni, Pb, Zn (mM)			
Total, Dissolved	n.d., n.d.	n.d., n.d.	n.d., n.d.
Ceriodaphnia dubia 7-day Reproduction (Lab RHW = 22.3 (±1.34))	31.7 (±4.03)	31.6 (±4.97)	29.9 (±2.99)

Figure 1. Effects of 0, 0.222, and 2.22 mM Cd on *Physa* sp. Abundance (Cd, p=0.04; Cd x Time, p=0.03).

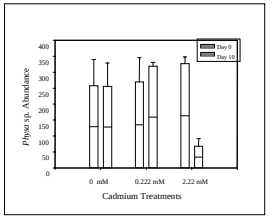


Figure 2. Effects of 0, 0.222, and 2.22 mM Cd on percentage of Chironomidae to total benthic macroinvertebrate abundance (Cd, p=0.07; Cd x Time, p=0.007).

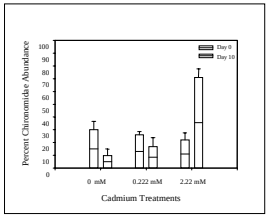


Figure 3. *Ceriodaphnia dubia* neonates produced during a 7-day bioassay for laboratory RHW, and 0, 0.222, and 2.22 mM Cd treated streams.

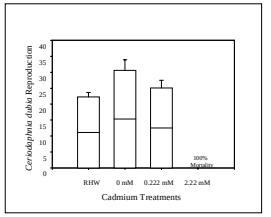


Figure 4. Effects of 0.222, and 2.22 mM Cd on adult male *Pimephales promelas* Hepatic- and Gonadal-Somatic Indices.

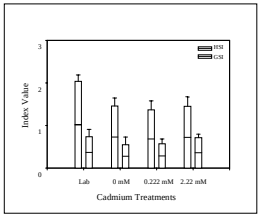
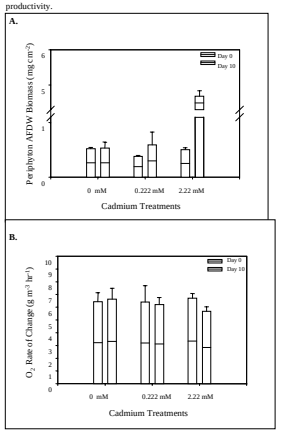


Figure 5. Effects 0, 0.222, and 2.22 mM Cd on A. periphytic biomass (AFDW) (Cd, p=0.004; Cd x Time, p=0.004) and B. gross system primary productivity.



CONCLUSIONS

Fish Responses

• No vitellogenin (VTG) detected in adult male *P. promelas* following a 12 day exposure. Condition, hematocrit, HSI, GSI and secondary sexual characteristics were unaffected by Cd x effluent treatments.

• City of Denton municipal effluent is seasonally estrogenic (see Allen et al. PH061, Hemming et al. PH064) but was not estrogenic during this study. Texas Woman's University and the University of North Texas contribute approximately 40% of the City of Denton's population. Data from subsequent studies in December 2000 (Allen et al. PH061) and May 2001 (Figure 6) support our observed lack of VTG induction and suggest that such seasonal estrogenicity is linked to population demographic changes when school is in session.

Laboratory and Stream Responses

• No adverse effects of Cd observed in 0.22 mM nominal treatments (Figures 1, 2, 3 & 5).

• Cd bioavailability and toxicity is altered by constituents of effluent dominated streams.

	Hardness of 140 mg/L
Current USEPA acute water quality criterion for Cd	= 0.051 mM
Current USEPA chronic water quality criterion for Cd	= 0.013 mM
No observed effect level of total Cd in this study	= 0.1245 mM
No observed effect level of dissolved Cd in this study	= 0.1201 mM

• Water quality criteria for metals based on hardness alone is conservative and does not account for other parameters influencing metal bioavailability and toxicity in southwestern U.S. receiving systems.

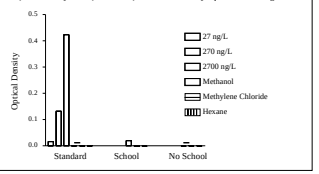
• Lotic mesocosms are valuable in determining multiple factors influencing chemical bioavailability in aquatic ecosystems.

CURRENT RESEARCH

• Evaluate *P. promelas* and system responses to long-term (90-day) cadmium exposure.

Do Seasonal Population Demographic Changes Influence Municipal Effluent Estrogenicity?

Figure 6. Yeast Estrogen Screening assay activity for estradiol standards (27, 270, 2700 ng/L) and solvent fractions (methanol, methylene chloride, hexane) extracted from SDB-XC extraction disks. Samples were collected on 2 May (School) and 16 May 2001 (No School). Estradiol toxicity equilivent = 14 ng/L.



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