TOXICITY OF BIOSOLID ELUTRIATES FROM DIFFERENT WASTEWATER TREATMENT PROCESSES TO *CERIODAPHNIA DUBIA*

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Abstract. Biosolids from wastewater treatment plants (WWTPs) have a high content of nutrients and organic matter and are used as a land fertilizer constituent. However, biosolids can contain many toxic chemicals, including heavy metals and synthetic organic chemicals. Thus, land application of biosolids may introduce these toxicants to water resources through runoff or soil leachate. The objective of this study was to assess the toxicity of biosolid elutriates from different WWTP processes to *Ceriodaphnia dubia*. Class B biosolids and composted biosolids (Class A) were collected from six southeastern WWTPs. Elutriate from each biosolid sample was used as the test solution in 48 hour acute toxicity tests. Results indicated that elutriates were highly toxic (LC50 range 11.2%-35.5%) to *C. dubia*. A decrease in toxicity (completely alleviated) was observed for one elutriate from a composted biosolids sample, but an increase in toxicity was observed in the other. Reductions in dissolved oxygen concentrations in some tests confounded toxicity results. Elevated concentrations of 4-nonylphenol (4-NP) were found in several biosolid samples that had high elutriate toxicity. Additional chemical analyses are being conducted to identify other toxic compounds in elutriates and biosolids. Anticipated benefits include selection of different treatment and disposal methods of biosolids to reduce potential for toxicants to enter surface waters.

INTRODUCTION

Georgia produces approximately 175,000 dry tons of biosolids per year from publicly owned wastewater treatment plants (WWTPs). Present options of disposal in Georgia fall into four major categories: landfilling, land application, incineration, and composting. Application to land, such as agricultural fields, is not only a better economic option, but is widely viewed as a way to increase soil quality through recycling nutrients and organic matter. However, biosolids have been shown to contain many toxic chemicals, including heavy metals and synthetic organic chemicals at high concentrations (Beck et al. 1996, Bhandari and Xia 2003, Fjallborg and Dave 2002, Wilson et al. 1996). After land-application, contaminants can enter surface waters by run-off during rainfall events or percolation through the soil. The amount and diversity of contaminants resulting from runoff has not been evaluated previously; however LaGuardia et al. (2001) found that nonylphenol could leach out of biosolids suggesting the possibility for contamination of surface waters. Current regulations limit concentrations of heavy metals in biosolids, but no standards exist for synthetic organic chemicals in biosolids.

Few studies have been conducted to determine the toxicity of biosolids on freshwater aquatic organisms. Biosolid elutriate has been shown to reduce survival and reproduction in cladocerans (Veerina et al. 2002, Hall and Hall 1989); however, toxicity has not been related to any specific toxicant within biosolids, and no information exists on toxicity of biosolid elutriates from different WWTP processes. The objective of the present study was to determine the toxicity of elutriates from biosolids collected from WWTPs that employ different post-treatment processes. These data will be incorporated into a preliminary assessment of the potential impact of biosolids on water resources within Georgia, and will provide toxicity data needed to establish criteria for their safer use as soil amendments.

EXPERIMENTAL METHOD

Ceriodaphnia dubia (water flea) is a freshwater zooplankton that is commonly used in effluent toxicity testing (US EPA, 1993). Organisms were cultured according to EPA protocol (US EPA, 1993) in very soft synthetic water (VSSW; 2.5% Perrier®) at 24-

26EC, under a 16 h light: 8 h dark photoperiod. Broodstock *C .dubia* were fed daily 100 :l of YCT (yeast, cerophyll and digested tetramin) and 100 :l *Selanastrum capricornitum* (US EPA, 1993). New broodstock organisms were cultured from healthy neonates every week, providing a steady supply of neonates from the third or fourth brood for toxicity testing.

Sample Preparation

Biosolids treated with both anaerobic and aerobic digestion and post-treatment composting were obtained from WWTPs in Georgia and South Carolina. Elutriates used in the toxicity tests were prepared according to EPA guidelines (US EPA/US ACE, 1998). Elutriate tests were performed to mimic the biosolid/water mixture that would occur during surface runoff or subsequent leaching from soils. Biosolids were added to VSSW water in a 1:4 ratio (250 g biosolids: 1000 ml VSSW water). VSSW was used because its water quality (hardness, 13 mg/L as $CaCO_3$; alkalinity, 14 mg/L as $CaCO_3$) was similar to surface waters near the locations of the selected WWTPs. The elutriate mixture was stirred on a magnetic stirring plate for one h at room temperature. After settling for one h, the elutriate was removed, centrifuged, and filtered (120 :m mesh) to remove particulates. Toxicity tests were initiated immediately following filtration.

Toxicity Tests

Acute toxicity tests (48 h) were conducted with each elutriate according to EPA protocol (US EPA, 1993). Each elutriate was diluted with VSSW to the following elutriate percentages: 75, 50, 25, 12.5, and 6.25%. *C. dubia* less than 24 hours old were used for tests, with five neonates exposed to 15 ml of test solution in 30 ml polypropylene cups, without food. Each of three replicate tests consisted of 3 cups of each elutriate sample plus a control (VSSW). Dissolved oxygen (DO); (YSI Model 85, Yellow Springs, OH), pH (Orion Model 720A, Beverly, MA), and temperature (YSI Model 85, Yellow Springs, OH) were measured at the beginning and end (48 h) of each test.

The concentration resulting in 50% mortality (LC50) of the exposed organisms was computed by the Trimmed Spearman Karber method (US EPA, 1993). Mortality was defined as no movement by the organism after gentle probing. Control mortality and reference toxicity tests served as QA/QC to evaluate test validity. For all tests, control mortality of #10% was required for test acceptance. Results from parallel tests with the reference toxicant, copper sulfate, were required to fall within our lab's control chart LC50 ranges (LC50 " 2 SD) for acceptance (US EPA, 1993). If results did not meet these criteria, tests were rejected and repeated.

RESULTS AND DISCUSSION

All reference toxicity data were within the acceptable range and no control mortality occurred during tests. Average LC50 values for *C. dubia* exposed to biosolid elutriates ranged from 11.2% to 35.5% for the six WWTPs (Table 1). Veerina et al. (2002) conducted a similar study and showed reduced survival and reproduction in *C. dubia*, but did not evaluate acute toxicity. In their study, biosolids were mixed with soil, which makes comparison with the present study difficult. Also, Veerina et al. (2002) did not attempt to determine the toxic components in the filtrate, which is a specific goal of our research.

The concentration of 4-Nonylphenol (4-NP) in biosolids was evaluated in a separate study (Xia and Pillar 2003) and results indicate elevated concentrations of 4-NP in some samples. The relation between the concentration of 4-NP in biosolids and their elutriates is unknown. In general, elutriates with high toxicity came from biosolids with elevated concentrations of 4-NP. For example location #10, which had the lowest toxicity (LC50 = 35.5%) had no detectable 4-NP, and the biosolid with the highest 4-NP concentration (1380 ppm) generated the elutriate yielding the highest toxicity (location #2). NP is toxic and bioaccumulative in aquatic organisms at low concentrations, and is reported as being an endocrine disrupting chemical (Lech et al. 1996). Tanaka and Nakanishi (2002) found NP to be highly toxic and suppress reproduction in an another daphnid species, *Daphnia galeata*. Many of these organic compounds and their metabolites are detected in WWTP effluents and sludges because they are not completely degraded by the treatment process (LaGuardia 2001, Bhandari and Xia 2003, Keller et al. 2003). Additional chemical characterization of biosolids and their elutriates will be necessary to identify the suite of potentially toxic components that are extracted into the aqueous elutriate phase, and thus present a threat to aquatic organisms.

Water quality data for all tests were acceptable (US EPA, 1993), except for DO. Measurements of DO were below the EPA recommendation of 4.0 mg/L in all samples, except for location #2 and the composted sample from location #7 (Table 1). Low DO typically

Table 1. Acute toxicity of diluted biosolid elutriates to *C. dubia.* Biosolids were obtained from WWTPs having different digestion processes for sludge treatment.

Location #	Treatment[a]	Mean Treatment Volume (mgd)[b]	DO (mg/L)[c]	4-Nonylphenol[d] in biosolids (ppm)	48 hr LC50[e] (% Elutriate)
2	Anaerobic	35	7.5 (7.02-8.39)	1380	11.2 " 3.4
7	Anaerobic	8	4.0 (0.87-7.16)	359	16.5 " 0.9
7	Composted	--	4.85, 5.30, 5.80[f]	45	NM[g]
9	Anaerobic	0.275	3.3 (1.50-4.42)	239	22.3 " 4.6
10	Aerobic	6.5	2.4 (0.44-4.94)	ND[h]	35.5 " 2.8
10	Composted	--	3.3 (1.60-5.12)	ND[h]	16.1 " 3.3
11	Anaerobic	80	3.1 (0.64-5.48)	805	27.3 " 3.3
12	Anaerobic	25	5.2 (3.29-6.28)	702	17.7 " 0.0

[a] Digestion process for biosolids
[b] Million gallons per day
[c] Mean (range), for dissolved oxygen in the lowest dilution with 100% mortality
[d] Data from Xia and Pillar, 2003
[e] Mean " SD, n = 3 tests
[f] Minimum DO recorded for each test (no mortality observed)
[g] No mortality was observed
[h] Not Detected

results from bacterial decomposition of organic material, a major component of biosolids. Aeration of elutriate samples before test initiation and renewal of solutions after 24 h were not successful in maintaining sufficient DO concentrations throughout the test duration. For some samples low DO may have contributed to elutriate toxicity (e.g., #7 and composted #10). However, for some samples, there is evidence that DO may not contribute to toxicity. For example, the elutriate sample with the highest toxicity (#2, LC50 = 11.2%) had DO values consistently above 7 mg/L. Conversely, the lowest DO concentrations were measured in elutriates having the lowest toxicity (# 10, LC50 = 35.5%). Reductions in DO below 3.0 mg/L have been shown to reduce feeding and respiration rates in other daphnids (Kring and O'Brien 1976, Heisey and Porter 1977). The combined effects of lowered DO with copper exposure resulted in increased toxicity to the mayfly, *Ephoron virgo;* however, the toxicity of diazinon was unaffected by low DO conditions (Van Der Geest et al. 2002). However, in the daphnids, *Daphnia magna* and *Daphnia pulex*, a 48 h LC50 value of 0.6 mg/L and 0.5 mg/L respectively has been reported for DO (Nebeker et al. 1992). There is no data on the lethal level of DO for *C. dubia*, and further investigation is needed on the relation of DO in biosolid elutriate toxicity.

Biosolids are often composted to promote additional removal of pathogens and further digest odor-causing organic matter. This process has been shown to be a cost-effective practice for both the WWTP and consumer, with a rapid increase in the number of biosolid composting projects. There is also the possibility of composting as a bioremediation approach before biosolids are land-applied. For two WWTP locations (#7 and 10), the effect of composting on biosolid toxicity was evaluated to see if a reduction in toxicity occurred. For location #7, toxicity was completely alleviated in the composted sample, while significant toxicity was measured in the noncomposted sample. In contrast, elutriate from location #10 had substantially increased toxicity following composting, with the mean LC50 reduced nearly 55% (Table 1). However, in this sample low DO concentrations again confounded the toxicity results. The composted biosolid from location #7 had lower concentrations of 4-NP compared to the uncomposted sample; however samples were collected at different times and may not be comparable. Further testing under laboratory-controlled composting conditions will determine the extent of biosolid composting as an effective bioremediation process.

In conclusion, this study provides evidence that land application of biosolids may be a source of toxicity to surface water environments. Biosolid elutriates can be toxic to *C. dubia* and potentially other aquatic organisms. Toxicity is most likely due to anthropogenic chemicals still present in biosolids after treatment. Reductions in DO caused by the microbial degradation of the high organic content of biosolids may also play a role in toxicity, especially in combination with certain types of toxicants. Future studies need to correlate toxicity studies with chemical analyses of biosolids and their aqueous elutriates. Tests also need to be designed to eliminate DO as a confounding factor. Finally, research on the potential for different compost methods to alleviate or reduce biosolid toxicity could lead to a reduction in contamination of water resources.

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