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Toxicity of Alum Sludge to *Ceriodaphnia dubia* and *Pimephales promelas*

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Alum ($Al_2(SO_4)_3$) is widely used in the flocculation-sedimentation step of the drinking water treatment process and in industrial settings to clarify waste streams and influents. Although widely used and commonly discharged, alum effluents contain extremely high levels of aluminum. Some studies examining the toxicity of alum have been published (Robinson and Perkins 1977; Boyd 1979; Lamb and Bailey 1981). However, studies evaluating the toxicity of alum effluents are rare. Aluminum toxicity has been widely studied, but most of these studies were conducted in dilute, low pH waters that are not typical of most receiving systems.

This study evaluated acute and chronic toxicity of a large drinking water treatment plant's "alum sludge" effluent to a freshwater fish and invertebrate. Objectives of this study were to: (1) evaluate alum sludge toxicity to *Ceriodaphnia dubia* and to *Pimephales promelas* larvae and juveniles, (2) monitor water quality parameters which may influence effluent toxicity, and (3) compare results of tests with each species.

MATERIALS AND METHODS

Two static renewal *C. dubia* and *P. promelas* chronic toxicity tests were conducted according to U.S. EPA (1985). All effluent was filtered through 26-µm mesh net to remove parasitic or predatory organisms. The dilution water in all tests was moderately hard reconstituted water (U.S. EPA 1985). Water chemistry parameters monitored during toxicity tests were evaluated according to APHA et al. (1985). Total aluminum was determined according to procedures for acid-extractable metals. Aqueous aluminum was determined from samples filtered through 0.22-µm pore size filters and preserved with metals-grade nitric acid. Water chemistry determinations were conducted at 24 hr intervals on fresh "renewal" (R) solutions and 24 hr old "before renewal" (BR) solutions. Minimum analyses conducted

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were: temperature, pH, dissolved oxygen, conductivity, alkalinity, and hardness in the control and 100 percent effluent exposures at renewal; and pH, dissolved oxygen, conductivity, and temperature before renewal of test solutions in the control, low, middle, and 100 percent effluent exposures.

A 96 hr "worst case" toxicity test was conducted with juvenile P. promelas exposed to unfiltered, continuously aerated effluent. This was done to simulate highly-turbid, "worst case" conditions of unfiltered effluent identical to that being discharged. These tests removed the artificial effects of filtering effluent prior to testing. Five P. promelas were exposed in triplicate in 4 l glass jars. Experimental conditions were identical to those in P. promelas seven-day larval tests except that solutions were not renewed.

Mortality and sub-lethal effects in seven-day tests were analyzed with Fisher's Exact Test, Dunnett's Procedure or Steel's Many One Rank Test (U.S. EPA 1985). Results of P. promelas "worst case" test were analyzed using Dunnett's Procedure. Statistical analyses were considered significant at alpha 0.05. No observed effects concentrations (NOEC), lowest observed effects concentrations (LOEC), and effluent chronic values (ChV) were calculated according to U.S. EPA (1985). The effluent ChV is the geometric mean of the NOEC and LOEC, and estimates the threshold level for toxicity.

RESULTS AND DISCUSSION

Ranges of water chemistry parameters determined from raw, unfiltered effluent are as follows: pH 6.02 - 6.40 (n=7), dissolved oxygen 0.6 - 1.0 mg/l (n=7), conductivity 425 - 455 µmhos/cm (n=6), turbidity 64,470 NTU (n=1), total suspended solids (TSS) 40,000 mg/l, total hardness 480 - 800 mg/l $CaCO_3$ (n=2), total aluminum 930 mg/l (n=1), aqueous aluminum 0.121 mg/l (n=1). Unfiltered effluent is characterized by very low dissolved oxygen concentrations, and very high TSS and total aluminum concentrations. The vast majority of aluminum in raw effluent is undissolved. Filtering effluent prior to use in chronic tests increased dissolved oxygen concentrations and pH, and decreased TSS, alkalinity, hardness, and conductivity of test effluents.

Water quality values and reproduction observed during C. dubia tests are presented in Table 1. The addition of effluent to control water reduced dissolved oxygen concentrations and pH, but pH of effluent exposures generally increased following renewal. The effluent continued to exert an oxygen demand through time. In 50 and 100 percent effluent, dissolved oxygen concentrations were reduced to as low as 4.8 and 2.8 mg/l, respectively. Changes in pH, dissolved oxygen concentration, and conductivity prior to test solution renewals indicates equillibration of test solutions was occurring. Mean aqueous aluminum concentrations of control, 50 and 100 percent effluent

Table 1. Range of water quality parameters and reproduction in *C. dubia* seven day tests. Numbers in parentheses are number of observations.

Exposure (% effluent)	pH	Dissolved Oxygen (mg/L)	Conductivity (µmhos/cm)	Aqueous Aluminum (mg/L)	Live Off-spring (X ± Range)
0, R	7.25-7.96(14)	7.6-8.6 (14)	260-355 (14)	0.024	34.2 ± 13.9
0, BR	7.30-8.09(14)	6.9-8.6 (14)	290-390 (14)	0.012	
6.3, R	7.01-7.73(10)	6.8-8.4 (10)	275-360 (10)	--	
6.3, BR	7.73-8.13 (7)	6.7-7.6 (7)	35-355 (7)	0.339	33.2 ± 8.4
12.5, R	7.09-7.33 (4)	6.5-8.2 (3)	310-365 (3)	--	
12.5, BR	7.47-8.11 (4)	6.3-7.3 (3)	320-345 (4)	--	35.3 ± 9.5
25, R	6.58-7.34 (7)	6.6-8.2 (6)	310-380 (6)	0.220	30.4 ± 5.6
25, BR	7.49-7.96 (6)	5.6-7.1 (6)	330-440 (6)	--	
50, R	6.68-7.29(13)	6.9-8.2 (13)	290-390 (13)	0.128, 0.149, 0.194	25.8 ± 5.9
50, BR	7.28-7.97(13)	4.8-7.4 (12)	330-450 (12)	0.271, 0.210, 0.208	
100, R	6.15-6.91(14)	6.0-8.4 (14)	385-460 (14)	0.285, 0.035, 0.045,	8.2 ± 5.2
100, BR	7.30-8.92(12)	2.8-7.1 (13)	360-470 (12)	0.052, 0.20, 0.147, 0.139, 0.134, 0.346	

Test temperatures 24 to 26°C (n=15), R = Renewal solutions, BR = Before Renewal.

were 0.018, 0.194, and 0.154 mg/l, respectively. Aqueous aluminum concentrations in renewal solutions of 50 and 100 percent effluent were generally lower than those in the 24 hr old before renewal solutions, indicating possible dissolution/desportion of aluminum over these 24 hr periods.

Control survival in *C. dubia* experiments was 100 percent and all effluent exposures had 80 to 100 percent survival. Mortality of effluent-exposed animals was not statistically different from controls. Delayed release of first broods was consistently observed for animals exposed to 100 percent effluent. In one experiment, first brood release occurred by day three for all females exposed to <100 percent effluent while those exposed to 100 percent effluent delayed first brood release until day four or five. In the second test, with the exception of one animal in 50 percent effluent, all animals exposed to <100 percent effluent released first broods by day four. Two animals exposed to 100 percent effluent delayed first brood release until day seven, while another did not release any young in seven days (microscopic examination of this animal on day seven revealed the presence of eyed young). Steel's Many One Rank Test indicated that reproduction of animals exposed to 100 percent effluent was significantly lower than controls. The NOEC and LOEC predicted by these experiments is 50 percent and 100 percent effluent, respectively. The geometric mean of these values is 71 percent, and represents the *C. dubia* effluent chronic value.

Delayed brood release and significant reductions in *C. dubia* reproduction observed in 100 percent effluent were likely due to the combined effects of reductions in pH and dissolved oxygen concentrations, physical stress due to high levels of suspended solids, and, possibly, the presence of aqueous aluminum. Although substantial mortality was not observed in these experiments, reductions in pH and dissolved oxygen concentrations likely contributed to delays and reductions in *C. dubia* reproduction. High suspended solids concentrations have been shown to reduce feeding efficiency and reproduction of another cladoceran, *Daphnia pulex*, (McCabe and O'Brien 1983) and may have been a factor reducing feeding efficiency and subsequent reproduction of *C. dubia*. High levels of total aluminum in the effluent (930 mg/l in raw effluent) would certainly have been lethal to this species if the aluminum were dissolved. Sublethal effects of aqueous aluminum in the range of concentrations observed in these experiments have not been assessed. Aqueous aluminum alone was probably not the factor exerting sub-lethal toxicity in 100 percent effluent since similar aqueous aluminum concentrations were observed in 50 percent effluent where delays and significant reductions in reproduction were not observed. Thus, low pH, low dissolved oxygen concentrations and high levels of suspended solids in 100 percent effluent likely induced the sub-lethal effects.

Water chemistry, and mortality and growth data from *P. promelas* seven-day tests are presented in Table 2. Addition of effluent

Table 2. Range of water quality parameters and mortality and growth data in *P. promelas* seven day tests.

Exposure (% eff-luent)	pH	Dissolved Oxygen (mg/L)	Conduc-tivity (µmhos/cm)	Aqueous Alluminum (mg/L)	Turbidity (NTU)	Total Suspended Solids (mg/L)	% Mortality (X ± Range)	Dry Weight (mg) (X + Range)
0, R	7.55-8.30	8.2-8.8	270-400	0.02	--	--	7.5 ± 12.5	0.49 ± 0.09
0, BR	7.57-7.91	5.1-7.2	235-375	--	0.5	--		
6.3, R	7.28-7.87	8.2-8.8	270-325	0.43, 0.07, 0.08	110-1450	10-1210	30 ± 10	0.39 ± 0.09
6.3, BR	7.59-7.90	5.7-7.2	290-355	0.07, 0.09	--	--		
12.5, R	7.33-7.89	8.2-8.8	275-350	--	180-850	35-7900(2)	12.3 ± 17.7	0.45 ± 0.06
12.5, BR	7.48-7.61	5.7-6.9	290-355	--	--	--		
25, R	7.15-7.65	7.9-8.6	290-350	--	240-4100	110-4300	12.5 ± 12.5	0.54 ± 0.05
25, BR	7.37-7.48	5.6-6.6	300-355	--	--	--		
50, R	7.08-7.43	6.7-8.4	300-380	0.25, 0.06	390-9610	240-6420	7 ± 11	0.54 ± 0.10
50, BR	7.20-7.66	5.3-6.6	315-370	0.07	--	14*		
100, R	6.99-7.33	6.5-8.3	330-400	0.31, 0.08, 0.07	531-830	630-4960	12.5 ± 17.5	0.43 ± 0.05
100, BR	7.05-7.49	5.3-6.0	340-380	0.19, 0.08, 0.05	--	4*		

Test temperatures 24 to 26°C (n=13), R = Renewal, BR = Before Renewal,
* = Samples siphoned from water column of settled exposure, BR.
-- Not measured.

to control water again reduced pH and dissolved oxygen concentration, with pH generally increasing during the 24 hr periods prior to renewal. Dissolved oxygen concentrations declined during these periods but not to the degree observed during C. dubia tests. This may relate to differences in test volumes and surface area of exposures. Changes in water chemistry parameters after renewal again indicate some equlibration of test solutions was occurring. Clear increases in alkalinity, hardness, and turbidity were again observed with the addition of effluent. Total suspended solids conentrations of > 1,000 mg/l were observed in all effluent exposures. TSS concentrations of 50 percent and 100 percent effluent declined by orders of magnitude during the 24 hr periods prior to test solution renewals due to rapid settling of solids. Mean total aluminum concentrations in 100 percent effluent were 111 mg/l. Mean aqueous aluminum concentrations in 6.3, 50 and 100 percent effluent were 0.13 to 0.15 mg/l. The highest aqueous aluminum concentration (0.432 mg/l) was measured in 6.3 percent effluent, again indicating some dissolution and/or desorption of aluminum may have occurred as a result of equillibration with control water.

Mean dry weights of P. promelas larvae did not reveal a clear dose-response relationship. Dunnett's Procedure found significant differences in mortality of controls and larvae exposed to 6.3 percent effluent. Drawing a conclusion as to the cause of significant mortality at 6.3 percent effluent would be tenuous due to the low number of tests performed. However, it is interesting that these mortalities occurred in the exposures where the highest concentration of aqueous aluminum was measured. Mean dry weights were not statistically different between any exposures. High levels of total aluminum in 100 percent effluent did not exert substantial mortality to P. promelas larvae because this aluminum was not dissolved. Precipitation of aluminum due to high effluent alkalinity, sorption and complexation with solids, and cation competition due to high effluent hardness were all factors which likely reduced aluminum toxicity.

In the P. promelas "worst case" experiment, mortality occurred only in 100 percent effluent (mean = 40 $\pm$ 20 percent mortality). A TSS concentration of 40,000 mg/l was measured in this effluent. Dissolved oxygen concentrations were 7 to 8 mg/l due to continuous aeration. Test solution pH ranged from 7.85 to 8.23 in controls, and from 7.06 to 7.38 and 6.47 to 6.82 in 50 percent and 100 percent effluent, respectively. Aqueous aluminum concentrations were always < 0.3 mg/l, indicating that greater than 99 percent of aluminum was undissolved since 930 mg/l total aluminum was previously measured in raw effluent. Highest dissolved aluminum concentrations (0.244 mg/l) in 6.3 percent effluent again indicate dissolution/desorption of aluminum as a result of equillibration with control waters. Aqueous aluminum concentrations in 50 and 100 percent effluent were 0.037 to 0.121 mg/l (n=5). Since raw effluent induced mortality to P. promelas juveniles only in 100 percent effluent a reliable LC50 could not be calculated. Chi square analysis indicated that mortality data were not normally distributed. Logit transformation normalized these data and subsequent analysis with Dunnett's Procedure found mortality of organisms exposed to 100 percent effluent was significantly different from all other exposures. The NOEC, LOEC, and effluent chronic value predicted by this experiment is 50 percent, 100 percent, and 71 percent effluent, respectively. These results agree with those of the C. dubia seven-day test. The cause of juvenile P. promelas mortality in 100 percent effluent exposures was likely physical stress due to high levels of suspended solids.

Reductions in pH and dissolved oxygen concentrations, high levels of suspended solids, and possibly aqueous aluminum were implicated as likely causes of toxicity in C. dubia tests while high suspended solids likely caused toxicity in the P. promelas "worst case" experiment. Total aluminum concentrations in seven-day tests were usually > 100 mg/l, while aqueous aluminum concentrations in all tests were < 0.5 mg/l. The vast majority of aluminum in the effluent was undissolved and apparently not available to exert substantial toxicity. The P. promelas "worst case" experiment indicated that unfiltered effluent was acutely toxic at high concentrations, and agreed well with results of C. dubia experiments. The utility of the C. dubia seven-day test is clear from the results of this research. The effluent did not exert substantial mortality to C. dubia, but sub-lethal effects at high effluent concentrations were quite evident. Sub-lethal effects to fathead minnows were not observed. Although dissolved aluminum may have played a role in exerting sub-lethal toxicity, results of water quality analyses indicated that reductions in pH and dissolved oxygen concentrations and high levels of suspended solids were likely responsible for the observed effects. Thus, some "non conventional" toxins were implicated as exerting toxicity. The full toxicity of this unique effluent would not have been detected using conventional acute toxicity tests. The consistantly-observed changes in water chemistry parameters indicated effluent equillibration was occurring. Highest levels of aqueous aluminum were observed at 6.3 percent effluent. Thus, alum sludge effluents may serve as a source of toxic aluminum due to equillibration processes, especially under lower pH conditions.

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