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Acute and Chronic Effects of Alum to Midge Larva (Diptera: Chironomidae)

David S. Lamb[1] and Gary C. Bailey[2]

[1]*Michael Kennedy Consulting Engineers, W. 1720 4th Avenue, Spokane, WA 99204; and* [2]*Department of Civil and Environmental Engineering, Washington State University, Pullman, WA 99164*

The use of aluminum sulfate (alum) to precipitate phosphorus in highly eutrophic lakes is increasing (PETERSON 1979). As this use increases, the need to study the effects of alum on the biota also increases. Possible adverse effects of alum on lake organisms may be due to chemical toxicity from dissolved aluminum compounds or to physical inhibition of movement, feeding, or reproduction from the precipitated aluminum hydroxide floc. Lethal and sublethal effects of alum on a wide range of fish and aquatic invertebrates described in the literature are reviewed by BURROWS (1977). There is, however, a lack of information as to the effects of alum on benthic insects.

Aluminum chemistry in natural water is extremely complex. Aluminum combines readily with a number of elements and the resulting compounds may appear as both solid and dissolved forms. Therefore, special attention must be paid to other ions found in the water to which alum is applied and especially how these ions interact with the Al^{+3} ion and the $Al(OH)_{3(s)}$ polymer.

The objective of the present study is to determine the acute and chronic effects of alum to *Tanytarsus dissimilis*. *T. dissimilis* is a representative of the Chironomidae, the family of organisms which occupies a significant portion of the benthic invertebrate community of lakes and which are important fish food organisms.

MATERIALS AND METHODS

Test water was obtained from Liberty Lake, Washington, three to four weeks before the start of each bioassay and filtered (.45 μm) as soon as possible. Alum stock solutions were prepared using standard, reagent grade aluminum sulfate ($Al_2(SO_4)_3 \cdot 18\ H_2O$) crystals and stored in stoppered, acidwashed Erlenmeyer flasks.

Twenty-four hours after solution preparation a procedure was started to acquire a pH of 7.8 in the acute test solutions and 6.8 in the chronic test solutions. A lower pH was necessary in the chronic test to be able to hold it relatively constant over the test period. The pH was adjusted by adding sodium hydroxide (NaOH) to each solution and Control, every second day for the first six days after preparation, and every four days thereafter, until the pH stabilized at the desired level.

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The larval test organisms were obtained from stock culture tanks fifteen to eighteen hours prior to the start of each test and measured to get the instar desired. The larvae were held in Petri dishes containing clean lake water and algae until the start of each test.

The tests were conducted in 50 mL acid-washed Pyrex beakers containing 30 mL of solution. Five larvae were transferred randomly to each beaker as soon as the solutions were added and a pH reading was taken. For the chronic tests a substrate of algae was established in each beaker by allowing 40 mL of a culture of *Selenastrum capricornutum* to settle and then decanting off the unwanted media. All tests were conducted at 20°C with a 14-10 hour light-dark interval.

At the beginning of each test, specific conductance, carbon dioxide (CO_2), and methyl orange alkalinity were measured in the stock solutions. At the same time a sample of each stock solution was filtered (.45 µm) and analysed for calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), silicate (SiO_2), sulfate (SO_4), chloride (Cl), ammonia-nitrogen (NH_3-N), nitrite nitrogen (NO_2-N), phosphate phosphorus (PO_4-P), and total phosphorus (t-P) (APHA 1975). At the conclusion of each test, the beaker contents were measured for pH and then filtered (.1 µm) and acidified for dissolved aluminum analysis (PERKIN-ELMER 1977). The dissolved oxygen (D.O.) was checked in the test beakers during Tests #1, #4 and #5.

A computer model was also used to determine the distribution of aluminum (Al) and the chemical agents responsible for the observed effects. This program, called REDEQL2 (MCDUFF & MOREL 1973) uses the Newton-Raphson Iterative Method to calculate equilibrium speciation given the actual water chemical characteristics.

Acute Tests

The solutions tested in the three acute tests contained alum concentrations of 80, 160, 240, 320, 400, 480, 560, 720, and 960 mg/L. (Tests #1 and #2 used all these doses; Test #3 used all except 480, 560 and 720 mg/L). Duplicate beakers were utilized for each dose tested. Second instar larvae were used in Test #1 and third instars were used in Tests #2 and #3 for easier observation and handling. These tests followed "Standard Methods" (APHA 1975) and were run for 96 hours.

Chronic Tests

Solutions tested in the chronic tests (numbers 4 and 5) included 10, 80, 240, 480, and 960 mg/L of alum. Test #4 contained doses of 240 and 960 mg/L with five and ten replicates respectively. Test #5 contained five replicates each of 10, 80, 240, and 480 mg/L of alum. Second instar larvae were used for these tests. These tests ran for 55 days with observations at 24 hours and a minimum of once a week thereafter. The larvae were periodically transferred to beakers containing fresh algae and alum solution when deterioration (yellowing) of the substrate was observed.

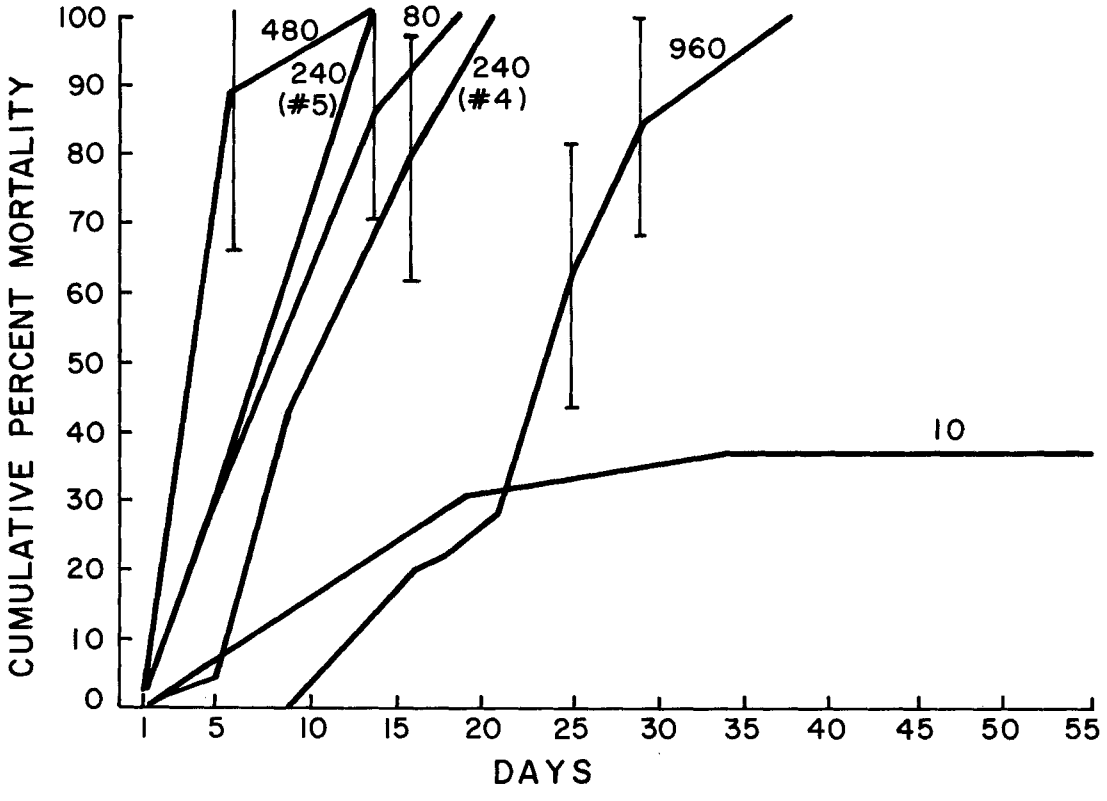

Figure 1. Cumulative percent mortality with 95% confidence intervals (when >50%) for chronic bioassays with *Tanytarsus dissimilis* in alum solutions (alum doses in mg/l).

The confidence intervals for the cumulative percent mortality (>50%) were calculated using the variance among replicates for a given dose.

RESULTS AND DISCUSSION

Acute Tests

The results of acute bioassays (Tests #1, #2, and #3) indicated that there was no apparent effect of alum on either second or third instar *T. dissimilis* at alum doses between 80 and 960 mg/L. Throughout the 96 hours the larvae, including Controls, were generally active and they exhibited typical movements and food searching.

Due to the polymeric, coagulant nature of Al, a white to grey precipitate (floc) was formed in all alum solutions. In the test beakers this floc layer varied from a patchy distribution 1 to 2 mm thick to a consistent 3 to 4 mm thick layer. Many larvae built or extended the tubes they inhabit with the floc material. A microscopic examination revealed that floc was also ingested by the larvae.

The D.O. measured at the end of 96 hours in Test #1 was 5.5 ± .2 mg/L. The mean pH was observed to drop in all acute tests over the 96-hour period from 7.71 (±.11) to 6.85 (±.15). Dissolved Al was < .1µg/L at all alum concentrations.

Of the additional parameters tested in the stock solutions only Na, SO_4, and specific conductance were seen to vary over the range of alum doses. The variation in Na concentrations was from a background of 11.2 ± 2.7 mg/L (acute test Control solutions) to 118.8 mg/L in the 960 mg/L alum solution (Test #2). Sulfate increased approximately 30 mg/L with each additional 80 mg/L of alum, from a background of 3.0 to a high of 410 mg/L (Test #1). Specific conductance was 73 ± 14 µmhos/cm in the Controls and 840 µmhos/cm in the 960 mg/L solution with increases proportional to the Na concentration.

The following chemical concentrations found in the Control solutions are typical of concentrations measured in the alum solutions: Ca, 5.0 ± .5 mg/L; Mg, 1.2 ± .1 mg/L; K, 1.1 ± .3 mg/L; Cl, 1.4 ± .4 mg/L; CO_2, <1 mg/L; bicarbonate (HCO_3), 30 ± 5 mg/L as $CaCO_3$; NH_3-N, .019 ± .008 mg/L; NO_2-N, <.01 mg/L; and NO_3-N, .070 ± .009 mg/L. Phosphate and total phosphorus in the Controls were .008 ± .007 mg/L and .014 ± .006 mg/L respectively while levels in all alum solutions were <.001 mg/L (PO_4-P) and <.005 mg/L (t-P). SiO_2 values in the Controls were 4.3 ± .8 mg/L and .7 ± .02 mg/L in all alum solutions.

Chronic Tests

Mortalities in the chronic assays were recorded in all alum doses tested but with widely different rates of dieoff (Figure 1). The time to reach 50% mortality is shortest with 480 mg/L at about 4 days. The mortality time for 80 mg/L and 240 mg/L is not significantly different (α = .05) and occurs between 8 and 10.5 days. At 960 mg/L it took more than 23 days for 50% of the larvae

to die. The 10 mg/L dose shows less than 37% motality at 55 days when the test was terminated. There appears to be no difference in mortality between 10 and 960 mg/L until after about 35 days. Combined Control mortality (Test #4 and #5) was 5.4%.

As with the 96-hour assays the larvae were generally active; they also built tubes and were observed to feed on the algae substrate. Prior to death however the larvae were often found in an "impaired" state characterized by little or no response to prodding.

The floc layer in the chronic test beakers appeared slightly more dense than in the acute beakers due to algae being trapped within it. This layer was also somewhat thicker but was piled up around the beaker edges in the 80, 240, and 480 mg/L test beakers. The 10 mg/L beakers contained very little floc in small clumps scattered over the beaker bottom.

The D.O. in the chronic beakers ½ hour before the lights came on was measured at 4.8 ± .2 mg/L. The pH in the stock alum solutions was adjusted to a lower level in the chronic tests (6.8) as compared to acute tests (7.8) which resulted in an overall (initial, mid-test and final for all doses) average of 6.63 with a standard deviation of .32. Soluable aluminum concentrations were <.1 µg/L except in the 240 mg/L beakers of Test #5 which measured .139 ± .021 mg Al/L.

Available chemical and physical data for Tests #4 and #5 solutions include: Cl, 2.3 ± .3 mg/L; HCO_3, 30 ± 1.6 mg/L as $CaCO_3$, CO_2, <1 mg/L; NH_3-N, .038 ± .02 mg/L; NO_3-N, .59 ± .44 mg/L; and NO_2-N. <.01 mg/L. The PO_4-P and t-P levels determined in the Test #4 Control were .010 and .011 mg/L respectively. The conductivity was 60 µmhos/cm in the Control and 55, 123, 235, 400, and 615 µmhos/cm in solutions of 10, 80, 240, 480 and 960 mg/L of alum respectively.

There appears to be some chemical toxicity at alum concentrations of 80, 240, and 480 mg/L, the effect being most evident at 480 mg/L. Secondly, the heavy alum floc at 960 mg/L appears to cause a stress by impeding movements and perhaps feeding to an extent that mortalities occur after an extended time. Additionally there seems to be a physiological stress due to some aspect of our test conditions which causes an overall lengthening of larval development time. The accepted life cycle time for this species is 14 days at 20°C (NEBEKER 1973). In the present study no larvae pupated in 55 days, however, two Control larvae in Test #4 were determined to in the fourth instar (near maturation) after 78 days.

The results of the chemical analysis performed on the toxicant solutions, particularly for dissolved Al, show no variation to account for the observed chemical toxicity. As a result, a chemical equilibrium computer model was utilized to determine a theoretical distribution of aluminum and other constituents in these solutions.

Baseline water quality data for the computer inputs was taken from Test #1. Values for Al, SO_4 and Na were calculated from the alum doses and total base added during the pH adjustment. (Alum doses of 10, 80, 240, 480 and 960 mg/L correspond

to 0.8, 6.5, 19.4, 38.9 and 77.7 mg Al/L respectively.) The pH input value was 6.8.

The results of this mathematical analysis indicated that aluminum is found in seven forms (including Al^{+3}) with solid forms predominating at all alum doses. At an alum concentration of 10 mg/L, 85.6% of the Al was bound with silicate in a compound of the general formula $Al_2(SiO_3)_2(OH)_{2(s)}$. Other forms present in order of decreasing concentration were $(Al(OH)_{3(s)}$, $Al(OH)_4^-$, $Al(OH)^{+2}$, $Al(SO_4)^+$, and $Al(SO_4)_2^-$. The most prevalent dissolved species, the aluminate ion $Al(OH)_4^-$, was found at a concentration of .19 µg/L with the four other dissolved species adding only 7.3×10^{-4} µg/L. At 80 mg/L alum, 87.7% of the Al was found as $Al(OH)_{3(s)}$ and only 12.1% as $Al_2(SiO_3)_2(OH)_{2(s)}$. The concentrations of $Al(OH)_4^-$, $Al(OH)^{+2}$ and Al^{+3} remained about the same with $Al(SO_4)^+$ and $Al(SO_4)_2^-$ increasing slightly. These patterns continued without fluctuation through all remaining doses. At 960 mg/L, $Al(OH)_{3(s)}$ represented 99.0% of the Al in the solution with the total dissolved species representing 1% (∿.21 µg/L).

The computer output indicates that after the silicate has been removed from solution (precipitated) the solid hydroxide controls the distribution of Al. This has in fact been substantiated experimentally (ROBERTSON & HEM 1969, HEM et al. 1973). It is unlikely that these solids are the cause of the chemical toxicity noted.

Even though the calculated concentrations of many components of these solutions came out very close to actual levels, there are limitations to the accuracy of these predictions as evidenced by the difference between calculated and measured dissolved Al concentrations (.19 and <.1 µg/L) respectively). These differences are apparently due to both chemical and physical interactions for which reaction constants are not known. Even though these interactions may be relatively subtle and not change the overall distribution of Al, they still may lead to changes in the character of solid (floc) or dissolved species and thus affect toxicity.

Due to the polymeric, positively charged character of the floc (HEM & ROBERTSON 1967), negatively charged ions and particles may become adsorbed and removed from solution. As noted previously algae became trapped during alum solution addition to chronic test beakers. Other substances removed include phosphate ion (HSU 1975), colloidal solids, clay particles and organic compounds (CLARK et al. 1977). This adsorption could serve to concentrate potentially toxic materials directly above lake bottom organisms.

Many naturally occurring organic compounds increase the concentration of dissolved Al by forming "organoaluminum" complexes (LIND and HEM 1975). No analyses were performed for organics in the test solutions, however, it appears that some interaction between the alum and the algae or an algae by-product was responsible for the toxicity because of the discrepancy between the acute and chronic tests. Algae release a wide range of organic compounds (HELLEBUST 1974) including growth-inhibiting substances which may affect other organisms. High release rates of extracellular products of algal photosynthesis are related CO_2 limitation, high population density and light intensity

(WETZEL 1975). All of these could be factors associated with the settled algal substrate in the chronic tests.

We also considered the possible toxic effects or stress effects of D.O., pH, SO_4, depth of water, crowding, and nutrition but these were found in our tests not be critical factors.

IMPLICATIONS FOR LAKE TREATMENTS

Any attempts to extrapolate information indicated in this study to impacts of in-lake alum treatments on the biota must consider both chemical and physical effects. Careful consideration must also be given to the limnological character of the water to be treated in determining the dose required. Examples (as reviewed by DUNST et al. 1974) include 18 mg Al/L at Horseshoe Lake, Wisconsin (added as granular $Al_2(SO_4)_3$), 12 mg Al/L at Snake Lake, Wisconsin (as alumnate and liquid alum), 7.3 mg Al/L in Pickerel Lake, Wisconsin (as liquid alum), and 50 mg Al/L in Langsjon, Sweden (as granular $Al_2(SO_4)_3$). Considerable variation was seen in two Spokane County, Washington lakes as well. Medical Lake is a hard water (total hardness 120 mg/L as $CaCO_3$), sodium bicarbonate (730 mg/L total alkalinity as $CaCO_3$) lake which received 150 mg/L alum (13.5 mg Al/L) (GASPERINO & SOLTERO 1978). Liberty Lake is an extremely soft water lake (total hardness averaging 23 mg/L as $CaCO_3$) with limited buffer capacity (total alkalinity 14-30 mg/L as $CaCO_3$) which received 10 mg/L as granular $Al_2(SO_4)_3$ resulting in an in-lake Al concentration of about 0.8 mg Al/L. (FUNK et al. 1975).

While some of these lake treatment doses are within the range causing toxicity in these bioassays (80 to 960 mg/L) there are other factors which would tend to mitigate toxic effects. The predominant hardness causing ions for instance, can compete with Al^{+3} for both organic and inorganic ligands and decrease the tendency to form soluble complexes (STUMM & MORGAN 1970). If an algal bi-product particular to *Selenastrum* is in fact causing or contributing to mortality it is unlikely that this would be found in significant enough amounts in a diverse lake system to be a problem.

The physical toxicity, however, could be a factor regardless of dose. The Medical Lake treatment resulted in a floc layer about 14 cm thick with 0.6 to 1.3 cm being deposited at Liberty Lake. Since both of these are greater than the depth in the 960 mg/L test beakers, mortalities might be expected. A heavy floc layer may not be a problem for already established larvae with a normal development time, but a substantial floc layer could inhibit pupae from reaching the surface and the deposited eggs from reaching the sediments. Thus, the time (season) when an alum treatment occurs may be an important factor. A late fall treatment would find most benthic insects dormant or at low levels of activity. Also, by the time these organisms were returning to activity the floc layer may have settled.

Considering the effects of the test organism response in these assays and the lake characteristics which tend to reduce mortality it seems unlikely that a well planned alum treatment

would result in significant mortality in benthic insect populations.

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