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# Arsenic Toxicity Changes in the Presence of Sediment

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Arsenic has been widely used in herbicides resulting in high soil and sediment concentrations in some areas. Arsenite has been reported to be more toxic than arsenate (Fowler 1977); with *Daphnia magna* average LC50's of 4.4 and 7.4 mg/L, respectively (U.S. EPA 1984). *D. magna* has been a commonly used indicator of aquatic toxicity and standardized methods have been developed for acute toxicity testing (U.S. EPA 1985). Arsenic is quite similar chemically to phosphorus and sulfur, thus it produces toxic effects, in part, by replacing these elements in essential metabolic processes. Procaryotic testing allows evaluation of effects on the critical processes on which they are actively involved enzymatically, e.g., carbon (cellulose decomposition), nitrogen (nitrification, nitrate reduction), phosphorus, and sulfur cycling (sulfate reduction).

Phosphatase is produced by organisms when phosphate becomes unavailable (Stewart and Wetzel, 1982). These monoesterase enzymes play a key role in the phosphorus cycle. Phosphatases have been used in toxicity assessments of organic and metal impacts (Burton and Lanza 1985; Sayler et al. 1979). Arsenate depresses activity (Flint and Hopton 1977) to a greater degree than arsenite (Burton 1984).

The effect of sediments on ameliorating metal toxicity to *Daphnia* has not been reported. However, arsenic and other metalloids/metal are known to concentrate in sediment and adsorb to particulates (Turner and Rudd 1983; Clement and Faust 1981; Ferguson and Gavis 1972). This study investigated the effect of sediments on standard arsenite LC50 determinations with *D. magna* and alkaline phosphatase activity (APA).

## MATERIALS AND METHODS

Tests were conducted with a control and 5 concentrations of arsenite. Each treatment during a test was in triplicate 200 mL

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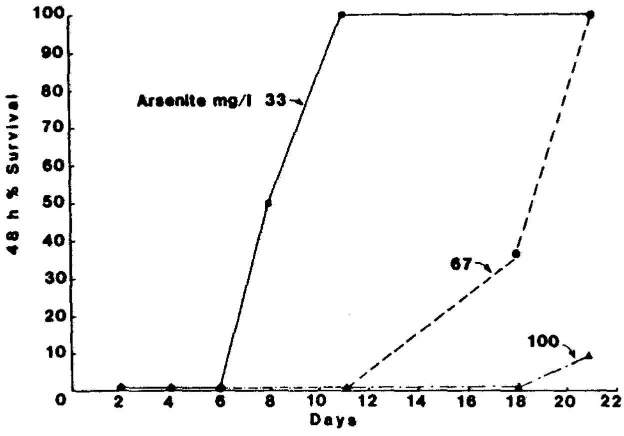


Figure 1. *Daphnia magna* percent survival over 11 consecutive 48-h tests, in the presence of Lake Lavon sediment amended with arsenite at 33, 67, and 100 mg/L (nominal).

beakers. The range of 6 treatments were conducted in beakers containing 200 mL U.S. Environmental Protection Agency reconstituted hard water (APHA 1981), and in beakers containing 150 mL EPA water and 50 mL Lake Lavon sediment. Before adding *D. magna*, EPA water was gently added to 50 mL sediment and allowed to settle for 24 h. Arsenic acid (Sigma) was dissolved in EPA water and added to the overlying water 24 h before *D. magna* exposure. Arsenic concentrations represent nominal values. At 24 h, the water column was gently bubbled with air and the dissolved oxygen (DO) raised to 8.0 mg/L. Parameters which were monitored through the studies included: 1) turbidity, 2) dissolved oxygen, 3) pH, 4) temperature, 5) *D. magna* survival, 6) *D. magna* dry weight, and 7) alkaline phosphatase activity (APA). At the beginning and end of each 48-h test, turbidity (Hach Turbidimeter Model 2100A and DO (Yellow Springs Instrument Model 54A oxygen meter) were measured. Water and sediment pH (Orion 201 meter) were measured at time 0 and at test termination. Tests were conducted 20 C.

Five to 13 new neonate *D. magna* (UTD culture), less than 24-h in age, were added to each beaker every 48-h by a random number procedure. Counts of live and dead *D. magna* were made at 24-h intervals. At 48-h, live *D. magna* were removed and their dry weight determined by drying at 60 C for 24-h and weighed on a Cahn model 25 electrobalance. *D. magna* dry weights were also measured at the initiation of each 48-h test. This allowed assessment of any changes in *D. magna* weight during treatment exposure. The 48-h tests were repeated until changes in survival stabilized. In Test 1 there were 5 consecutive 48-h exposure tests and in Test 2 there were 9 48-h exposure periods over a 50 day period.

APA was determined by the method of Sayler *et al* (1979). In

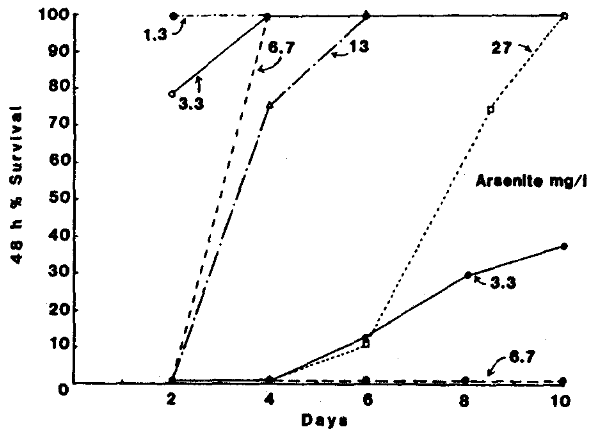

Figure 2. *Daphnia magna* percent survival over 5 consecutive 48-h tests with and without Lake Lavon sediment and arsenite amendments. Closed figures = water treatments without sediment.

this assay, the substrate p-nitrophenyl-phosphate (NPP) (Sigma) is cleaved by bacterial alkaline phosphatase to leave p-nitrophenol (NP), which is a yellow colored compound that can be measured spectrophotometrically. Two to three replicate assay tubes were incubated for 1 hour at 30 C. Assay tubes consisted of 2 mL sediment, 4 mL Tris buffer (pH 8.6) and 1 mL NPP. Reactions were terminated with addition of 1 mL of 1 N NaOH. Blanks consisted of the same mixture, with NPP deleted. Tubes were then centrifuged at 2,575 x g for 10 minutes. NP formation was measured on a Bausch and Lomb Spectronic 20 at 418 nm absorbance. Dry weight determinations of sediment test aliquots were run with each assay and NP ug converted to NP ug/g dry weight (APA) using a standard curve. Percent survival and LC50's for the *D. magna* were calculated using a computerized probit analysis (Finney 1971).

## RESULTS AND DISCUSSION

*D. magna* 48-h LC50 tests conducted in beakers with and without sediment allowed concurrent comparisons to APA and also clearly showed effects of sediment on arsenite toxicity. Two tests conducted for extended periods, at different ranges of arsenite, revealed similar patterns. Survival in sediment beakers containing arsenite increased over time (Table 1, Figures 1,2). In Test 2, 67 mg/L arsenite caused 100% mortality, in repetitive 48-h exposures, until day 20. The percentage of organisms surviving the 48-h exposure period increased at day 20, to 37%. For the 48-h exposure period ending at day 28, the rate of survival was 100%. A similar trend was observed at other concentrations. Degree of survival was a function of arsenite concentration through time. However, percent survival in arsenite beakers containing only water changed only slightly at the lowest concentrations. In Test 1, 48-h percent survival in

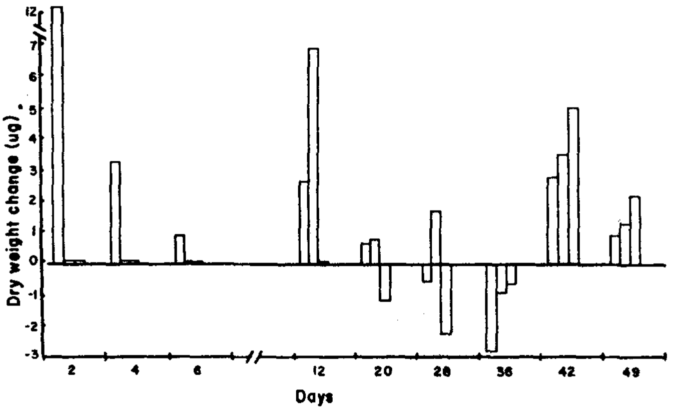


Figure 3. Test 1. *Daphnia magna* dry weight change during repeated 48-h tests, with and without Lake Lavon sediment and arsenite amendments. First bar = water control, second bar = sediment control, third bar = 1.3 mg/L As+3, fourth bar = 3.3 mg/L As+3.

Table 1. *D. magna* survival in water/sediment beakers during exposure to 5 arsenite concentrations. Lavon sediment.

| Percent survival (water[a]/sediment[b]) | | | | | | |
|---|---|---|---|---|---|---|
| TEST 1 | | | | | | |
| Day | 1.3[c] | 3.4[c] | 6.8[c] | 13.5[c] | 27.0[c] | 48-h LC50 |
| 2 | 100/100 | 0/78 | 0/0 | 0/0 | 0/0 | 2.1/4.1 |
| 4 | " | 0/100 | 0/100 | 0/76 | " | 2.1/16.4 |
| 6 | " | 17/100 | " | 0/100 | 0/6 | 2.4/19.7 |
| 8 | " | 33/100 | " | " | 0/75 | 2.8/- |
| 10 | " | 39/100 | " | " | 0/100 | 2.9/- |
| TEST 2 | | | | | | |
| Day | 1.3[c] | 33.5[c] | 67.0[c] | 100.0[c] | 133.0[c] | |
| 2 | 100/100 | 0/0 | 0/0 | 0/0 | 0/0 | 6.6/6.6 |
| 4 | " | " | " | " | " | 6.6/10.6 |
| 6 | " | 0/50 | " | " | " | 6.6/33.0 |
| 12 | " | 0/100 | 0/0 | " | " | 6.6/47.0 |
| 20 | " | " | 0/37 | " | " | 6.6/59.9 |
| 28 | " | " | 0/100 | 0/10 | " | 6.6/85.6 |
| 36 | " | " | " | 0/25 | " | 6.6/89.9 |
| 42 | " | " | " | 0/95 | " | 6.6/113.2 |
| 49 | " | " | " | 0/75 | " | 6.6/107.8 |

a. Survival in beakers containing EPA water only.
b. Survival in beakers containing both sediment and EPA water.
c. Nominal arsenite concentration in the water column, in mg/L.

water at 3.4 mg/L arsenite increased from 0 to 17% at day 6 and by day 10 was 39%. Arsenite in the water column apparently became adsorbed to sediment through time, becoming less available to *D. magna*. Turbidity caused by adding EPA water to sediment in

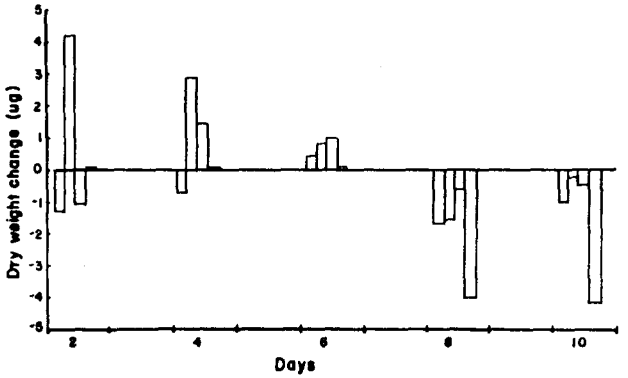


Figure 4. Test 2. *Daphnia magna* dry weight change during repeated 48-h tests in the presence of Lake Lasvon sediment, with and without arsenite amendments at 33 and 67 mg/L. First bar = control, second bar = 33 mg/L As+3, third bar = 67 mg/L As+3.

Table 2. Nitrophenol production (APA) in Lake Lavon sediment, with and without arsenite amendments, during *D. magna* bioassay test 1.

| Treatment | | Time | NP(ug/g)[a] | SD |
|---|---|---|---|---|
| | Control | Day 0 | 48.5 | 0.4 |
| As+3 | 3.4 ppm | | 39.5 | 0.7 |
| | 6.8 | | 37.6 | 1.6 |
| | 13.5 | | 38.7 | 0.8 |
| | 27.0 | | 37.9 | 1.5 |
| | Control | Day 4 | 40.6 | 0.3 |
| As+3 | 3.4 ppm | | 38.7 | 0.5 |
| | 6.8 | | 39.0 | 0.3 |
| | 13.5 | | 38.2 | 0.2 |
| | 27.0 | | 36.7 | 0.1 |
| | Control | Day 8 | 35.1 | 0.1 |
| As+3 | 1.3 ppm | | 34.2 | 1.0 |
| | 3.4 | | 34.2 | 0.9 |
| | 6.8 | | 34.1 | 0.0 |
| | 13.5 | | 33.4 | 0.8 |
| | 27.0 | | 31.6 | 0.5 |
| | Control | Day 11 | 35.0 | 0.3 |
| As+3 | 1.3 ppm | | 35.4 | 0.3 |
| | 3.4 | | 34.8 | 0.3 |
| | 6.8 | | 34.5 | 1.0 |
| | 13.5 | | 34.6 | 0.2 |
| | 27.0 | | 33.2 | 0.6 |

a. n = 2 on each treatment (nitrophenol ug/g dry weight sediment) test beakers cleared quickly. Turbidity was an average of 49 ntu at time zero and reduced to a nondetectable level after 2 days in both tests. The lack of mortality in controls suggested turbidity was not a major negative factor in survival but may have had a important positive effect in reducing water arsenic concentrations. The role of sediment in ameliorating metals in overlying water due to adsorption processes has been shown in several studies (Hunt 1983; Turner and Rudd 1983). A similar toxicity reduction phenomena has been shown to occur when food was added to *D. magna* toxicity tests (U.S. EPA 1984). *Daphnia* spp. have been shown in laboratory tests, to grow larger when fed sediments with organic matter than sediments without organic matter (Arruda *et al* 1983). Another factor which may have contributed to decreasing toxicity is oxidation of arsenite to the less toxic arsenate species. Survival in beakers without sediment changed very little over time, as

Table 3. Nitrophenol production (APA) in Lake Lavon sediment, with and without arsenite amendments, during *D. magna* bioassay test 1.

| Treatment | | Time | NP(ug/g)[a] | SD |
|---|---|---|---|---|
| | Control | 0 h | 45.5 | 0.4 |
| As+3 | 1.3 ppm | | 46.8 | 0.7 |
| | 33.5 | | 44.5 | 0.2 |
| | 67.0 | | 45.3 | 0.3 |
| | 100.0 | | 46.0 | 0.0 |
| | 133.0 | | 45.1 | 0.7 |
| | Control | 1 day | 44.8 | 0.2 |
| As+3 | 1.3 ppm | | 45.6 | 1.7 |
| | 33.5 | | 43.0 | 1.0 |
| | 67.0 | | 44.6 | 0.2 |
| | 100.0 | | 44.4 | 0.6 |
| | 133.0 | | 44.3 | 0.4 |
| | Control | 3 days | 46.1 | 0.4 |
| As+3 | 1.3 ppm | | 47.0 | 0.1 |
| | 33.5 | | 43.1 | 0.8 |
| | 67.0 | | 40.9 | 0.0 |
| | 100.0 | | 41.6 | 0.4 |
| | 133.0 | | 39.5 | 0.3 |
| | Control | 6 days | 43.8 | 0.5 |
| As+3 | 1.3 ppm | | 42.9 | 0.0 |
| | 33.5 | | 40.6 | 1.1 |
| | 67.0 | | 41.7 | 0.3 |
| | 100.0 | | 41.0 | 0.2 |
| | 133.0 | | 38.4 | 1.1 |
| | Control | 50 days | 47.4 | 0.0 |
| As+3 | 1.3 ppm | | 35.3 | 0.1 |
| | 33.5 | | 34.8 | 0.1 |
| | 67.0 | | 32.5 | 0.1 |
| | 100.0 | | 31.9 | 0.8 |
| | 133.1 | | 26.4 | 0.2 |

a. n = 2 on each treatment (nitrophenol ug/g dry weight sediment)

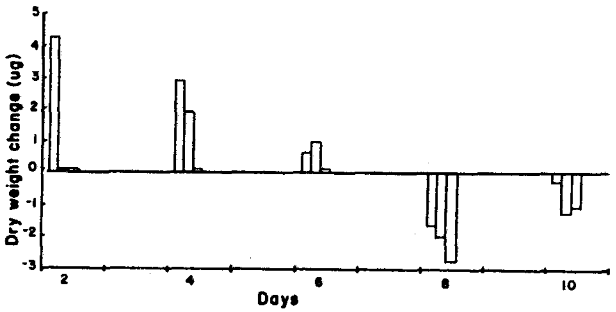


Figure 5. Test 1. *Daphnia magna* dry weight change during repeated 48-h tests in the presence of Lake Lavon sediment, with and without arsenite amendments at 6.7 and 27 mg/L. First bar = control, second bar = 6.7 mg/L As+3, third bar = 27 mg/L As+3.

arsenic apparently remained in the water column where it continued to exert toxic effects on *D. magna*.

Initially, when EPA water was added to sediment test beakers, there was a D.O. sag to approximately 2 mg/L during the first 24 h. Beakers were then bubbled with air, without resuspending sediment, and the D.O. level raised to 8.0 mg/L. *D. magna* were not added during the D.O. sag period. During the experiments D.O. remained between 5.2 and 7.3 mg/L in sediment beakers. D.O. in water beakers remained between 8.1 and 8.6 mg/L. As with turbidity, lack of mortality in controls suggested no acute D.O. effects. The pH of EPA water at time zero was 7.9 and fluctuated between 7.6 and 7.9 in the water beakers during the tests. In sediment beakers pH dropped slightly to 7.2 - 7.5, with no apparent effect on survival.

Dry weight analyses of *D. magna* also showed sediment effects (Fig. 3-5). *D. magna* increased in weight over a 48-h period in sediment beakers, possibly a result of higher bacteria levels. The slight turbidity during initial periods of exposure may have contributed particle-bound bacteria to the filter feeding *Daphnia*, thus acting as a food source. Over time, dry weight increases in sediment beakers diminished to levels similar to water controls. However, at the end of Test 2 dry weights of daphnids in beakers containing sediment increased slightly. This may have resulted from delayed bacterial growth as a large number of dead *D. magna* remained on the sediment surface after 4 weeks of repetitive testing and may have served as a bacterial substrate. Dry weight increases were also inhibited by exposures to high arsenite concentrations.

APA analyses of sediment during *D. magna* tests revealed findings similar to previous enzyme studies (Burton 1984) (Tables 2,3). APA slightly decreased in activity over time and was affected by arsenite after 24-h. This delay may have been a result of arsenite concentrations increasing in the sediment as it left the water column or several hours of arsenite exposure was required before an effect level was reached. Alkaline phosphatase was shown to be a principle enzyme released by *D. magna* (Boavida and Heath 1984) which may have contributed to stable levels. This phenomenon is a function of sediment type and other variables (Burton 1984). It is also possible the organics, e.g., fecal pellets, excreted by the daphnids may have affected enzyme activities and microbial populations. The simple short-term enzyme assay used in this investigation did not closely compare to the *D. magna* toxicity pattern. The most sensitive assay is not necessarily the most important when considering varied ecosystem effects.

This series of tests with sediment, arsenite, *D. magna*, and microbial activity in combination is significant for several reasons. Using sediment in the LC50 test better represents *in situ* conditions than traditional LC50 tests with water only. Natural aquatic systems contain varying degrees of suspended solids and also contain sediment, therefore daphnids are in the presence of suspended solids and sediment when exposed to toxicants. These results show the presence of sediments significantly increase *D. magna* survival through time when initially exposed to arsenite.

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