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# Thiocyanate, calcium and sulfate as causes of toxicity to *Ceriodaphnia dubia* in a hard rock mining effluent

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ABSTRACT

A series of Toxicity Identification Evaluations (TIEs) to identify the cause(s) of observed toxicity to *Ceriodaphnia dubia* have been conducted on a hard rock mining effluent. Characteristic of hard rock mining discharges, the effluent has elevated ($\sim$3000 mg $l^{-1}$) total dissolved solids (TDS) composed primarily of $Ca^{2+}$ and $SO_4^{2-}$. The effluent typically exhibits 6–12 toxic units (TUs) when tested with *C. dubia*. Phase I and II toxicity identification evaluations (TIEs) indicated $Ca^{2+}$ and $SO_4^{2-}$ contributed only $\sim$4 TUs of toxicity, but this was likely an underestimate due to problems with simulating the supersaturated $CaSO_4$ concentrations in the effluent. Treatment of the effluent with $BaCO_3$ to precipitate $Ca^{2+}$ and $SO_4^{2-}$ revealed that these ions contribute $\sim$6 TUs of the observed toxicity, but the remaining source(s) of toxicity (up to 6 TUs) remained unidentified. Subsequent investigations identified thiocyanate ($SCN^-$) in the effluent at 100–150 μM. Toxicity tests reveal that *C. dubia* are sensitive to $SCN^-$ with an estimated IC25 of 8.3 μM for reproduction in moderately hard water suggesting between 12 and 18 TUs of toxicity in the effluent. Additional experiments demonstrated that $SCN^-$ toxicity is reduced in the high TDS matrix of the mining effluent. Testing of a mock effluent simulating the major ion and $SCN^-$ concentrations resulted in 10.4 TUs, suggesting that $Ca^{2+}$, $SO_4^{2-}$ and $SCN^-$ are the three toxicants present in this effluent. This research suggests $SCN^-$ may be a more common cause of toxicity in mining effluents than is generally recognized.



## 1. Introduction

This paper describes the procedures and results of a toxicity identification evaluation (TIE) performed on a lead–zinc mine with onsite milling operations. Typical of lead–zinc mining and milling operations, this facility crushes ore and subjects it to a chemical flotation process to extract Pb and Zn, with the remaining tails being deposited in an impoundment. All mine drainage water and mining impacted water are also collected in the tailings impoundment. Mining activities accelerate the naturally occurring oxidation of sulfide minerals such as pyrite ($FeS_2$) and sphalerite (ZnS), which results in the mine drainage water containing high levels of dissolved metal sulfates. Typical of many hard rock mining operations, the mine utilizes a lime ($Ca(OH)_2$) treatment plant for the removal of heavy metal contamination in the tailings impoundment water. The treatment plant removes the dissolved metals from solution and replaces them with $Ca^{2+}$ resulting in an effluent with high concentrations of $CaSO_4$ but relatively low metal concentrations (Table 1).

The mine is required to conduct whole effluent toxicity (WET) testing as part of it NPDES permit. During periods of discharge, the mine conducts monthly chronic WET tests with *Ceriodaphnia dubia* and *Pimephales promelas* in accordance with standard protocols (USEPA, 2002). The mine has sporadically failed to meeting the permit specified WET limit of 10.3% effluent for *C. dubia* but is always in compliance for *P. promelas* (Fig. 1). As a result, under the National Pollution Discharges Elimination System (NPDES) permit, the mine was required to initiate a TIE to identify the cause(s) of observed toxicity.

Toxicity identification evaluations are a series of experimental manipulations used to either remove or render biologically unavailable various classes of toxicants that might occur in an effluent. The toxicity of the manipulated effluent is then compared to the baseline toxicity of the effluent. A reduction in toxicity resulting from the experimental manipulations relative to baseline suggests that the targeted class of toxicants is contributing to observed toxicity. Subsequent experiments can then be conducted to identify the specific toxicant(s) within that class that are causing toxicity. The USEPA has developed a formalized

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**Table 1**
Mean effluent water quality (1998–2003).

| Parameter | Concentration (μM) |
|---|---|
| Bicarbonate | 600 |
| Cadmium | 0.010 |
| Calcium | 16,400 |
| Chromium | 0.015 |
| Copper | 0.033 |
| Lead | 0.008 |
| Magnesium | 1600 |
| Nickel | 0.204 |
| Potassium | 1000 |
| Selenium | 0.033 |
| Sodium | 2600 |
| Sulfate | 19,200 |
| Zinc | 0.853 |

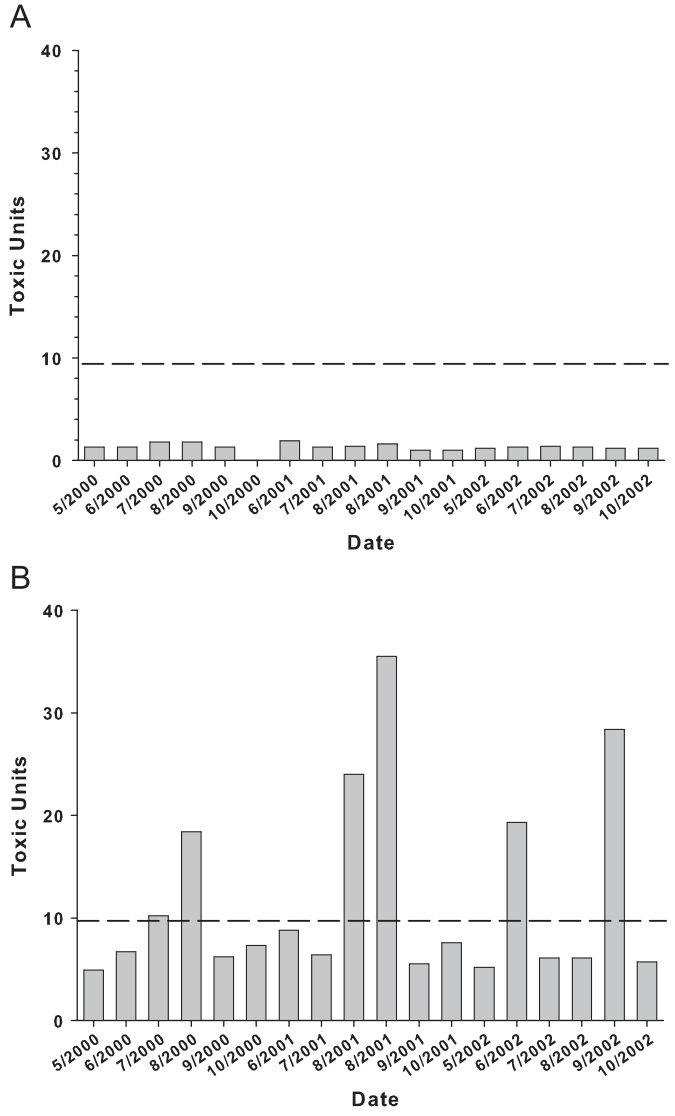


**Fig. 1.** Toxicity of mine effluent to (A) *Pimephales promelas* and (B) *Ceriodaphnia dubia* in standard 7-d bioassays over time. Dashed line represents NPDES permit.

series of procedures for conducting these types of studies (USEPA, 1991; USEPA, 1993). This paper describes the procedures used and results obtained for a TIE performed on the above described mining effluent.

## 2. Methods and materials

### 2.1. General

All testing was conducted in an environmental chamber maintained at $25 \pm 1$ °C. *Ceriodaphnia dubia* neonates were obtained from in-house cultures. Culture water and dilution water for the Phase I and Phase II TIE, which were performed at the Parametrix Environmental Laboratory in Seattle, WA, was USEPA moderately hard water (1.14 mM $Na^+$; 0.35 mM $Ca^{2+}$; 0.50 mM $Mg^{2+}$; 0.11 $K^+$; 0.11 mM $Cl^-$; 0.85 mM $SO_4^{2-}$; 1.14 mM $HCO_3^-$; pH: 7.8 and hardness: 84 mg $l^{-1}$ as $CaCO_3$) while all subsequent experiments were conducted at the University of Miami with dechlorinated Miami tap water diluted by one-third with deionized water (0.75 mM $Na^+$; 0.30 mM $Ca^{2+}$; 0.10 mM $Mg^{2+}$; 0.05 mM $K^+$; 1.00 mM $Cl^-$; 0.08 mM $SO_4^{2-}$; 0.80 mM $HCO_3^-$; pH: 7.9 and hardness: 40 mg $l^{-1}$ as $CaCO_3$).

All testing with *C. dubia* was conducted in general accordance with the methods described in USEPA (2002). The only difference in test methods was that for the first 2 days of exposure, neonates for a given treatment were maintained in a single 250 mL test chamber containing 150 mL of test solution in order to minimize the level of effort required to perform daily water changes. Beginning on day 3 when reproduction was characterized, daphnids were transferred to individual 30 mL test chambers containing 15 mL of test solution as per standard protocols. A preliminary test demonstrated that this modified method resulted in comparable IC25s when compared to the standard 7-d test method consistent with previous studies on a wide range of toxicants (Oris et al., 1991). All tests were conducted with 10 replicates per treatment except for the $BaCO_3$ treated effluent (see section 2.4 for further description), which used only 5 replicates due to volume limitations.

### 2.2. Phase I TIE

Initial experiments generally followed standard Phase I TIE procedures as outlined in USEPA (1992). Briefly, a baseline bioassay was performed to determine the IC25 of the unmanipulated effluent. Based on the results from the baseline test, subsequent Phase I TIE manipulations were performed on 50% and 100% effluent. The specific effluent manipulations were aeration to remove volatile or sublatable toxicants, filtration through a 0.45 μM filter to remove particulate bound toxicants, filtration through a solid phase C-18 column to remove non-polar organics, addition of $Na_2S_2O_3$ to eliminate reducible toxicants such as chlorine and chelate some metals, addition of $Na_2EDTA$ to chelate cationic metals, and adjustment of the effluent to pH 6 and 8.5 to evaluate changes in toxicity associated with pH-dependent toxicants. Given the high ionic strength of the effluent an additional treatment was added in which the effluent was passed through a cation/anion exchange column (Burgess et al., 1997).

### 2.3. Phase II TIE

As presented later, Phase I TIE results suggested that the primary source of toxicity was likely related to the high ionic strength of the effluent. Phase II TIE experiments focused on testing this hypothesis. For each test, the IC25 was determined and compared to the results from a baseline test on the mine effluent. First, a test was performed in which the ionic composition ($Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, $Cl^-$, $HCO_3^-$, $SO_4^{2-}$) of the effluent was simulated by adding reagent grade salts (Sigma Chemicals, St. Louis, Mo.) to deionized water to determine whether only the major ions were contributing to observed effluent toxicity. Another test was performed in which the ionic composition of the effluent was simulated, but $Mg^{2+}$ was substituted for all but 1 mM of the $Ca^{2+}$ present in the effluent in order to test whether $Ca^{2+}$ was the principal cause of toxicity. While the relative toxicity of $Ca^{2+}$ and $Mg^{2+}$ was unknown, we expected there would be some difference in their toxicity and so anticipated observing a change in simulated effluent toxicity (either more or less toxic) as a result. A third experiment was performed in which the effluent was again passed through the cation/anion exchange column, which effectively removed nearly all major ions and was toxic (see Results), and then reagent grade salts were added to the post-column effluent at the same concentrations used to make USEPA moderately hard water (USEPA, 2002). The final experiment in this series involved spiking the effluent with $NaHCO_3$ to increase the alkalinity from 11 to 35 mg $l^{-1}$ to test whether the low alkalinity of the effluent was contributing to observed toxicity.

### 2.4. Barium carbonate experiments

Results from the Phase II TIE suggested that elevated TDS, particularly $Ca^{2+}$ and $SO_4^{2-}$ were contributing significantly to effluent toxicity but perhaps not the sole toxicants. However, efforts to fully simulate the ionic composition of the effluent, particularly $Ca^{2+}$, were unsuccessful due to solubility limitations. Given this, we conducted several additional experiments in an attempt to generate a supersaturated $CaSO_4$ solution and to selectively remove $Ca^{2+}$ and $SO_4^{2-}$ from the effluent to determine the effect of this removal on toxicity.

In an initial experiment, we attempted to create a simulated effluent with supersaturated $CaSO_4$ concentrations by adding 25 mM $CaSO_4$ to deionized water that had been heated to 60 °C to increase solubility and then slowly (5 °C $h^{-1}$) cooling the solution back to room temperature. The remaining salts needed to simulate the effluent ionic composition were then added to this solution and a standard 7-d bioassay performed.

In a second experiment, an amount of $BaCO_3$ equal to the $Ca^{2+}$ concentration (25 mM) was added to the effluent to selectively precipitate out $Ca^{2+}$ (as $CaCO_3$) and $SO_4^{2-}$ (as $BaSO_4$). This solution was mixed for 1 h and then allowed to settle overnight. The resulting supernatant was alkaline (pH 9.0) and highly buffered. This solution was slowly adjusted to pH 7.9 over the course of a day by gradual addition of HCl prior to toxicity testing. The ionic composition of the post-treated effluent was determined including measurement of any residual $Ba^{2+}$. A standard 7-d bioassay with *C. dubia* was then conducted on the effluent before and after $BaCO_3$ treatment.

A third test was conducted to determine the chronic toxicity of $Ba^{2+}$ to *C. dubia* and assess whether any residual $Ba^{2+}$ present in the effluent after $BaCO_3$ treatment could be contributing to observed post-treatment toxicity. Nominal test concentrations of 50, 100, 250, 500 and 1000 μM $Ba^{2+}$ were used in a standard 7-d toxicity test with *C. dubia*. All test concentrations were analytically verified.

Finally, we evaluated whether any ionic imbalance in the effluent after treatment with $BaCO_3$ might be contributing to observed toxicity. To accomplish this, reagent grade salts were added to deionized water to simulate an ionic composition comparable to the mine effluent after $BaCO_3$ treatment. This simulated effluent was then subjected to a standard 7-d bioassay with *C. dubia* at concentrations of 0, 20, 40, 60, 80, and 100% effluent.

### 2.5. Sodium thiocyanate experiments

During the above experiments, analysis of the effluent anion composition by ion chromatography revealed an unidentified peak in the chromatogram. Discussions with environmental staff at the mine suggested that this peak might be thiocyanate ($SCN^-$), which is present in the effluent at 100–150 μM. Analyzing $SCN^-$ spiked samples, it was determined the peak was not $SCN^-$, but the elevated concentrations prompted an investigation into $SCN^-$ toxicity in the effluent. The identity of the unknown peak was never determined.

Two experiments were conducted to evaluate $SCN^-$ toxicity. In the first experiment, $SCN^-$ (as NaSCN) toxicity to *C. dubia* was determined in a standard 7-d toxicity test. In the second experiment, the ionic composition of the effluent was simulated and NaSCN was spiked into the simulated effluent at a nominal concentration of 150 μM (the maximum concentration measured in the mine effluent) and toxicity determined in a standard 7-d bioassay with *C. dubia*.

### 2.6. Analytical chemistry

Cations and anions were measured using atomic absorption (Varian SpectrAA 220FS) and ion chromatography (DIONEX DX-120), with the exception of bicarbonate, which was measured using a total $CO_2$ analyzer (Corning 965). Hardness was determined from measured $Ca^{2+}$ and $Mg^{2+}$ concentrations. Total dissolved solids were determined as the sum of measured ion concentrations. A colorimetric method (APHA method 4500-CNM) was micro-modified and used to determine $SCN^-$ concentrations (APHA, 1995).

### 2.7. Data analysis

The IC25 is the standard statistical endpoint used under the NPDES permitting system in the United States and so was used as the statistical endpoint throughout this study. The IC25 (and 95% confidence limits) were estimated using linear regression techniques (linear interpolation or probit analysis, as appropriate to the data). All analyses were performed on measured test concentrations. In TIEs, the contribution of multiple toxicants to the overall toxicity is often accounted for in toxic units (TUs), which is defined as 100 divided by the statistical endpoint of interest (IC25 in this case). For example, an effluent with an IC25 of 10% would have 10 TUs. This method was used to account for the contribution of different effluent components in the following results and discussion.

## 3. Results

### 3.1. Water quality

Test temperature was maintained at 26 ± 1 °C in all experiments performed. Measured dissolved oxygen ranged between 7.2 and 8.2 mg $l^{-1}$ across all tests and pH between 7.5 and 8.0 except in tests where pH was intentionally manipulated to a different value.

### 3.2. Phase I TIE results

In the baseline test, the mean number of neonates per female was 18.8, 7.9 and 0 in the control, 50% and 100% effluent, respectively. None of the standard TIE treatments were effective at reducing toxicity in 100% effluent, although a small reduction in toxicity was observed for the cation/anion exchange treatment with 2.2 neonates per female produced (Fig. 2). At 50% effluent, the C-18 SPE column had a small, but significant ($p < 0.05$), reduction in toxicity with 13.4 neonates per female, while the cation/anion exchange treatment eliminated all toxicity (Fig. 2). Chemical analysis of the effluent after cation/anion exchange revealed a significant reduction in potentially toxic major ions as well as several potentially toxic metals (Table 2).

### 3.3. Phase II TIE results

Attempts to simulate and modify the ionic composition of the effluent were of limited success particularly with respect to $Ca^{2+}$ and $SO_4^{2-}$ (Table 3). Under normal conditions, the solubility of $CaSO_4$ is approximately 14 mM (Merck, 1996), significantly lower than the 18–25 mM observed in the mine effluent. This supersaturated condition is achieved in the Pb/Zn extraction and subsequent wastewater treatment processes by supersaturated addition of $Ca(OH)_2$ and the oxidation of high concentrations of metal-bound sulfide in the effluent to $SO_4^{2-}$, a process not readily simulated in the laboratory. Consequently, the simulated effluent had lower concentrations for $CaSO_4$ than typically observed in the effluent (Table 3).

The estimated IC25 (95% CI) in the baseline effluent was 22.5% (4.4–33.4%), while both the simulated effluent and simulated effluent with $Mg^{2+}$ substituted for $Ca^{2+}$ were significantly less toxic, with IC25s of 68.9% and 64.7% effluent, respectively (Fig. 3). When salts, at concentrations used to make moderately hard freshwater (USEPA, 2002), were added back to effluent treated by cation/anion exchange, no toxicity was observed with an estimated IC25 > 100% effluent, a significant reduction in

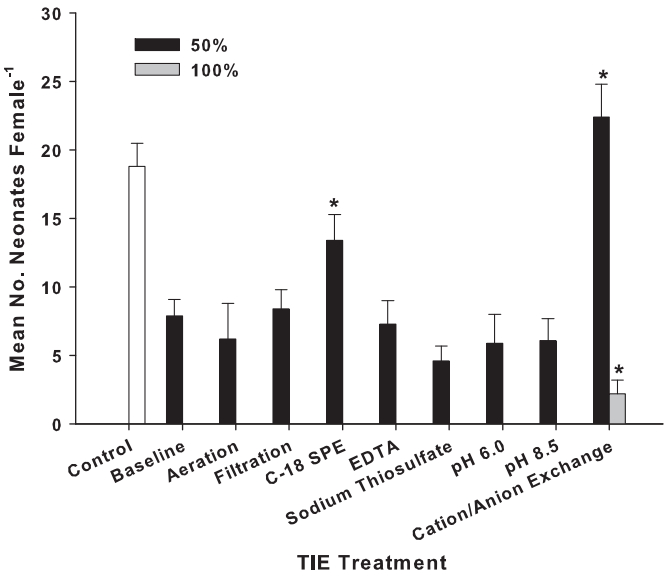


**Fig. 2.** Results of Phase I TIE showing mean number of neonates produced per female in different TIE treatments relative to that produced in control water and baseline (untreated) effluent. Data for 50% and 100% effluents presented. Note, no neonates were produced in 100% effluent in any of the TIE treatments except cation/anion exchange. *indicates significantly increased number of neonates relative to those produced in the corresponding baseline effluent concentrations.

toxicity compared to effluent just treated with cation/anion exchange (Fig. 2). Addition of $NaHCO_3$ to the effluent to increase alkalinity had no effect on the effluent toxicity (Fig. 3).

### 3.4. Barium carbonate experiments

Attempts to create a supersaturated $CaSO_4$ solution were of limited success. Final concentrations in the simulated effluent were 15.1 $Ca^{2+}$ and 16.7 mM $SO_4^{2-}$, 1.4 and 3.7 mM higher than was achieved in the simulated effluent during Phase II testing (Table 4). Treatment of the effluent with $BaCO_3$ effectively reduced $Ca^{2+}$ and $SO_4^{2-}$ concentrations from 25.1 and 20.8 mM to 0.16 and 0.38 mM, respectively. The residual $Ba^{2+}$ concentration was 4 μM. However, this treatment also resulted in an elevated $CO_3^{2-}$ concentration and a correspondingly high pH of 9.0. Acidification of this effluent using HCl to pH 7.9 resulted in a final $Cl^-$ concentration of 6.54 mM (Table 4).

Assessment of baseline effluent toxicity resulted in an IC25 (for reproduction) of 17.8% effluent while the simulated effluent had an estimated IC25 of 24.5% effluent. $BaCO_3$ treated effluent exhibited an IC25 of 9.7% effluent and the toxicity of the simulated $BaCO_3$ treated effluent was comparable (overlapping 95% confidence intervals) with an IC25 of 12.8% effluent. Although the estimated IC25s were similar for the actual and simulated $BaCO_3$ treated effluents, there was a marked departure in the dose–response relationship for both survival and reproduction. For survival, the $BaCO_3$ treated effluent had a similar dose–response relationship to the baseline test across the range of concentrations tested while in the simulated $BaCO_3$ treated effluent, no significant mortality was observed at any concentration tested. For reproduction, a significantly higher reproductive output was observed at $\geq$40% simulated $BaCO_3$ treated effluent compared to the actual $BaCO_3$ treated effluent (Fig. 4). The toxicity test performed on $BaCO_3$ in standard laboratory water resulted in a reproductive IC25 of 73 μM (Fig. 5), significantly higher than the residual $Ba^{2+}$ concentration in the $BaCO_3$ treated effluent (4 μM).

### 3.5. NaSCN experiments

The toxicity test of $SCN^-$ spiked into laboratory water resulted in an estimated IC25 for *C. dubia* reproduction of 8.3 μM (Fig. 6a), significantly lower than the 100–150 μM $SCN^-$ typically measured in the effluent. In a second toxicity test, a mock effluent simulating the effluent ionic composition with 150 μM $SCN^-$ added was performed. The resulting estimated IC25 for this test was 9.6% effluent equivalent to 15 μM $SCN^-$ (Fig. 6b).

## 4. Discussion

### 4.1. Phase I and II TIE

Results from the Phase I TIE revealed only the C-18 SPE and cation/anion exchange treatments significantly reduced effluent toxicity (Fig. 2). While statistically significant, the C-18 SPE treatment only resulted in a modest reduction in toxicity compared to the cation/anion exchange treatment, which eliminated toxicity at 50% effluent and was the only treatment in which some reproduction occurred in 100% effluent. In addition to non-polar organics, C-18 columns also remove metals such as $Ag^+$ and $Cu^{2+}$ that bind strongly to sulfhydryl groups but are unlikely

**Table 2**
Chemical analysis of 100% effluent before and after cation/anion exchange.

| Parameter | Untreated effluent | Post-treatment effluent |
|---|---|---|
| Cadmium (nM) | <44 | <44 |
| Calcium (mM) | 12.73 | 0.08 |
| Chloride (mM) | 0.310 | <0.280 |
| Copper (nM) | 409 | 536 |
| Lead (nM) | 68 | <14 |
| Magnesium (mM) | 1.563 | 0.030 |
| Potassium (mM) | 0.358 | <0.005 |
| Silver (nM) | <93 | <93 |
| Sodium (mM) | 0.913 | 0.061 |
| Sulfate (mM) | 18.750 | <0.010 |
| Zinc (nM) | 3.520 | 612 |
| pH | 7.9 | 6.3 |

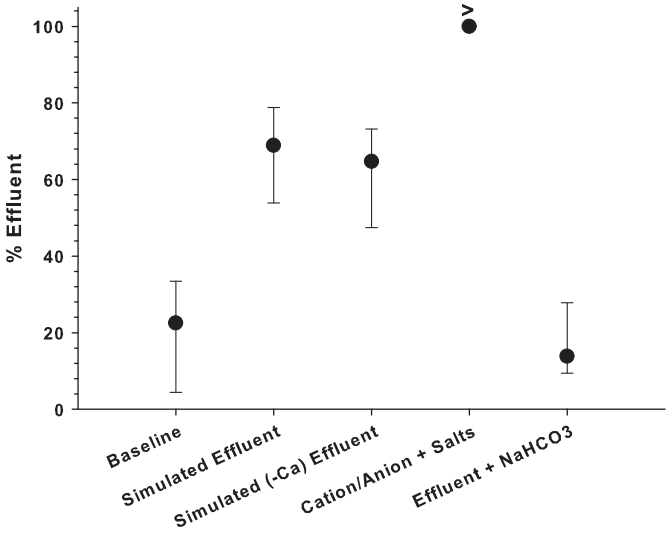


**Fig. 3.** Phase II TIE results. Measured IC25 (95% CI) of baseline effluent and different simulated waters in standard 7-d bioassays with *Ceriodaphnia dubia*. > =IC25 > 100% effluent for the cation/anion exchange+salts treatment.

**Table 3**
Ionic composition of Phase II TIE test waters (mM).

| Parameter | Baseline effluent | Simulated effluent | Simulated (-Ca) effluent | Cation/anion+salts | Effluent+$NaHCO_3$ |
|---|---|---|---|---|---|
| $Ca^{2+}$ | 25.1 | 13.8 | 1.1 | 0.42 | 25.1 |
| $K^+$ | 1.1 | 0.66 | 0.82 | 0.05 | 1.1 |
| $Mg^{2+}$ | 2.3 | 1.2 | 12.4 | 0.55 | 2.3 |
| $Na^+$ | 3.5 | 3.3 | 3.1 | 1.2 | 3.9 |
| $Cl^-$ | 0.68 | 0.98 | 0.98 | 1.06 | 0.68 |
| $HCO_3^-$ | 0.21 | 0.27 | 0.23 | 0.84 | 0.53 |
| $SO_4^{2-}$ | 20.8 | 13.0 | 13.2 | 0.11 | 20.8 |
| pH | 7.9 | 7.8 | 7.8 | 7.9 | 8.0 |

**Table 4**
Ionic composition of test waters in $BaCO_3$ experiments (mM).

| Parameter | Baseline effluent | Simulated effluent | $BaCO_3$ treated effluent | Acidified $BaCO_3$ treated effluent |
|---|---|---|---|---|
| $Ca^{2+}$ | 25.1 | 15.2 | 0.16 | 0.09 |
| $K^+$ | 1.1 | 0.82 | 0.68 | 0.91 |
| $Mg^{2+}$ | 2.3 | 1.6 | 2.0 | 2.2 |
| $Na^+$ | 3.5 | 3.2 | 3.4 | 3.7 |
| $Cl^-$ | 0.68 | 1.4 | 0.66 | 6.54 |
| $HCO_3^-$ | 0.21 | 0.68 | 4.74 | 0.54 |
| $CO_3^{2-}$ | 0.01 | 0.05 | 1.95 | 0.02 |
| $SO_4^{2-}$ | 20.8 | 16.7 | 0.38 | 0.46 |
| $Ba^{2+}$ (μM) | NM | NM | 4.0 | 5.7 |
| pH | 7.9 | 8.0 | 9.0 | 7.9 |

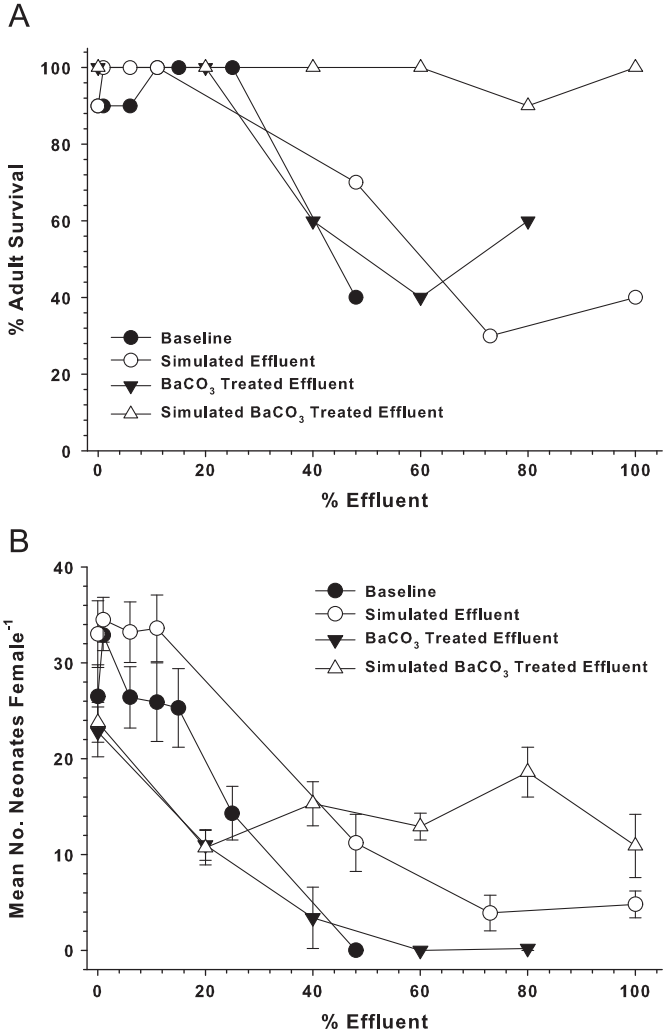


**Fig. 4.** (A) Effects of baseline effluent, simulated effluent, $BaCO_3$ treated effluent, and simulated $BaCO_3$ treated effluent on *C. dubia* survival in a standard 7-d test. (B) Effects of baseline effluent, simulated effluent, $BaCO_3$ treated effluent, and simulated $BaCO_3$ treated effluent on *C. dubia* reproduction in a standard 7-d test. Resulting IC25s for reproduction were 17.8, 24.5, 9.7 and 12.8% effluent.

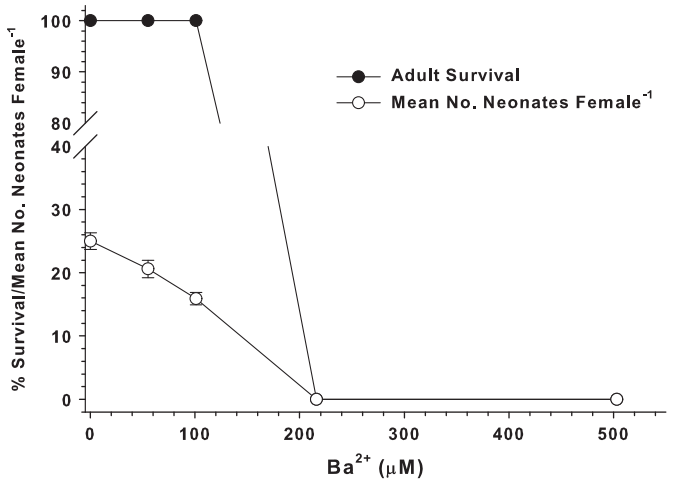


**Fig. 5.** Toxicity of $Ba^{2+}$ (as $BaCO_3$) to *C. dubia* in a standard 7-d toxicity test. Estimated IC25 for reproduction = 73 μM.

to bind to $Ca^{2+}$ and $Mg^{2+}$ which have a low affinity for these groups (USEPA, 1992). Given the strong reduction in toxicity using the anion/cation exchange treatment, the modest toxicity reduction observed in the C-18 column treatment was not pursued further. Analysis of the post-anion/cation exchange effluent indicated that the column was extremely effective at removing both major ions and trace metals (Table 2) and that column breakthrough was an unlikely cause of observed toxicity. Rather, we hypothesized that the limited reproduction in 100% effluent for this treatment was the result of excessive removal of essential ions (e.g., $Ca^{2+}$, $Na^+$, $HCO_3^-$) rather than residual toxicity in the effluent. The elimination of toxicity in 50% effluent in this treatment was the result of the lab dilution water providing sufficient ions to support normal survival and reproduction in *C. dubia* (USEPA, 2002). Consequently, Phase I results appeared generally consistent with the hypothesis that elevated total dissolved solids (TDS), particularly $Ca^{2+}$ and $SO_4^{2-}$, were likely causing most or all of the observed toxicity to *C. dubia.* The relative insensitivity of *P. promelas* to the effluent (Fig. 1a) was also consistent with this hypothesis as this species is considerably less sensitive than *C. dubia* to TDS toxicity (Mount et al., 1997).

The Phase II TIE focused on the hypothesis that elevated $Ca^{2+}$ and $SO_4^{2-}$ were the most likely causes of toxicity. Efforts to fully test this hypothesis were complicated by solubility issues with $CaSO_4$ and we were unable to fully simulate the ionic composition of the effluent. The simulated effluent was significantly less toxic (1.5 TUs) than the actual effluent (6–12 TUs) as might be expected, given $CaSO_4$ concentrations in the simulated effluent were approximately half those measured in the actual effluent. Substituting $Mg^{2+}$ for most of the $Ca^{2+}$ in the simulated effluent had no effect on toxicity. However, given that $Ca^{2+}$ was already only half of that observed in the effluent, this did not provide clear evidence regarding the relative contributions of $Ca^{2+}$ and $SO_4^{2-}$ to

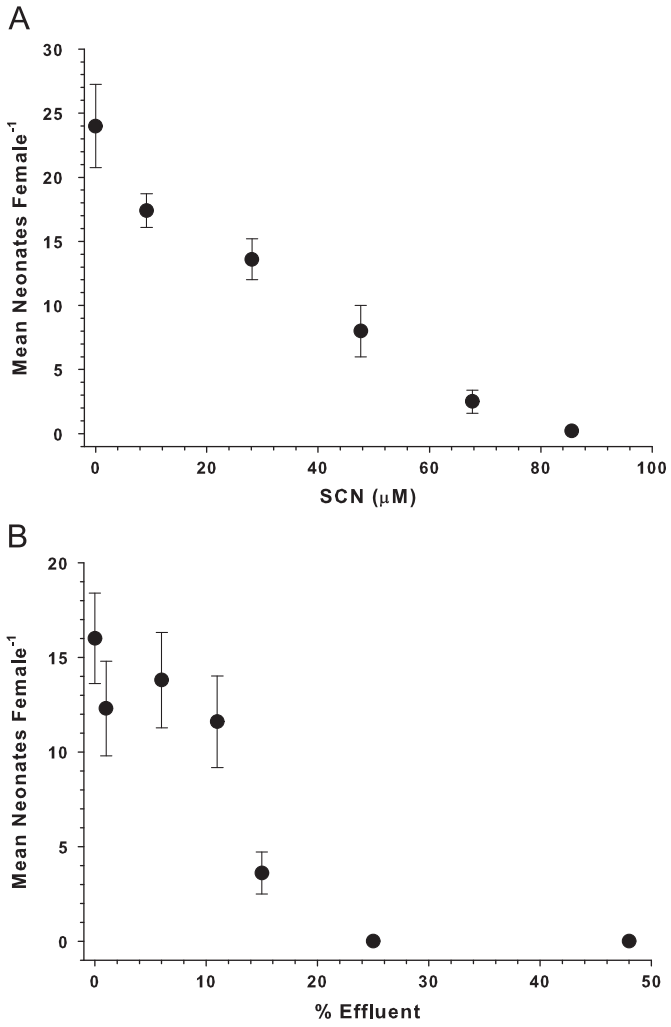


**Fig. 6.** (A) Toxicity of SCN (as NaSCN) to *C. dubia* in a standard 7-d toxicity test. Estimated IC25 for reproduction = 8.3 μM. (B) Toxicity of a mock effluent simulating the effluent ionic composition plus 150 μM SCN. Estimated IC25 = 9.6% effluent.

observed toxicity. Addition of reagent grade salts back to cation/anion exchange treated effluent eliminated all toxicity confirming the hypothesis that this treatment is effective at removing all toxicity and that toxicity observed in this treatment during Phase I was an artifact due to ion depletion. Finally, addition of $NaHCO_3$ to the effluent had no effect on toxicity eliminating the possibility that low effluent alkalinity might be contributing to toxicity.

### 4.2. $BaCO_3$ experiments

At the completion of the Phase II TIE, both the analytical chemistry and TIE manipulations generally supported the hypothesis that elevated $Ca^{2+}$ and $SO_4^{2-}$ were possibly the sole toxicants present in the effluent. However, problems with $CaSO_4$ solubility precluded definitive testing of this hypothesis. To address this, attempts were made to create a supersaturated $CaSO_4$ solution and a series of experiments were performed to selectively remove $CaSO_4$ from the effluent using $BaCO_3$.

We were only able to increase the $CaSO_4$ concentration in the simulated effluent by ~3 mM (15–16 mM total) compared to the Phase II TIE testing, but this significantly increased the simulated effluent toxicity from 1.5 TUs up to 4 TUs. While this was still considerably lower than the 6–12 TUs typically observed in the effluent, $CaSO_4$ concentrations were also still 5–10 mM lower than typically observed in the effluent. Treatment of the effluent with $BaCO_3$ effectively reduced $Ca^{2+}$ and $SO_4^{2-}$ in the effluent to $<0.5$ mM each, concentrations that are not toxic to *C. dubia* (Mount et al., 1997). However, despite this reduction in $CaSO_4$ concentration, effluent toxicity remained effectively the same (10.4 TUs) or slightly higher when compared to untreated effluent (Fig. 4). Measured $Ba^{2+}$ concentrations in the post-treatment effluent were 4–6 μM, while the measured IC25 for $Ba^{2+}$ was 73 μM (Fig. 5), providing strong evidence that excess $Ba^{2+}$ in the post-treatment effluent was not causing toxicity.

We also simulated the ionic composition of the $BaCO_3$ treated effluent using reagent grade salts added to deionized water in order to evaluate the potential effect of ion imbalance in this water on *C. dubia*. Based on previous studies (Mount et al., 1997), we hypothesized the high $Cl^-$ concentration resulting from titrating the $BaCO_3$ treated effluent back to pH 7.9 using HCl might be toxic (Table 4). The IC25 of this simulated effluent was very similar to the IC25 for the $BaCO_3$ treated effluent, suggesting that the toxicity observed in the treated effluent was not from unidentified toxicants, but rather from an ionic imbalance (most likely high $Cl^-$) created in the treatment process.

Although the IC25s for the $BaCO_3$ treated effluent and simulated $BaCO_3$ treated effluent were very similar, careful inspection of the dose–response for these two tests (Fig. 4) revealed that the simulated effluent was in reality less toxic than the actual $BaCO_3$ treated effluent. For example, at 60% effluent, the post-treatment effluent showed a complete inhibition of reproduction and 60% mortality while the simulated post-treatment effluent exhibited only a 54% reduction in reproductive output and no mortality.

Given these differences in dose response, it seemed likely that one or more additional toxicant(s) besides $CaSO_4$ was present in the effluent as the artifactual toxicity introduced by acidifying the $BaCO_3$ treated effluent did not account for all of the observed toxicity. Based on the TIE studies conducted to date, we hypothesized that it was likely to be an inorganic substance (due to high stability of effluent toxicity) that was not subject to changes in toxicity as a function of ambient pH over the range of 6–8.5 (based on the graduated pH test in the Phase I TIE). This hypothesis was further supported by the results of the Phase II cation/anion exchange experiment where no toxicity was observed in treated effluent, indicating that all toxicants present were likely ions.

### 4.3. NaSCN experiments

In analyzing the effluent ionic composition during the $BaCO_3$ experiments, a significant unidentified peak was observed in the ion chromatography analysis. It was suggested that this unidentified peak might be $SCN^-$, as it was routinely measured in the effluent at concentrations in the 100–150 μM rang. However, a spiking study adding known amounts of $SCN^-$ to samples analyzed via anion chromatography demonstrated that the unidentified peak was not $SCN^-$. The inability to confirm that the unidentified peak was $SCN^-$ does not exclude the possibility of $SCN^-$-toxicity. While the toxicity of $SCN^-$ to *C. dubia* has not been characterized, Watson and Maly (1987) estimated a 96-h LC50 of 55 μM for *Daphnia magna* while Bhunia et al. (2000) estimated a 96-h LC50 of 219 μM $SCN^-$ for the cladoceran *Moina micruru*. Hence, the $SCN^-$ concentration in the effluent was within the range where effects might be observed for acute toxicity, with chronic (i.e., reproductive) toxicity likely occurring at even lower concentrations. Further, the toxicity of $SCN^-$ was unlikely to be modified by any of the TIE manipulations with the

exception the cation/anion exchange treatment, consistent with experimental results. On this background, investigation of $SCN^-$ was initiated.

Results from a 7-d toxicity test with $SCN^-$ revealed that *C. dubia* reproduction was quite sensitive with an IC25 of 8.3 μM. While this result suggested that $SCN^-$ could indeed be the missing toxicant, consideration of the data in terms of toxic units (TUs) was problematic. The IC25 suggested $SCN^-$ was contributing between 12 and 18 TUs (assuming 100–150 μM $SCN^-$ in the effluent) while it had already been established that elevated $CaSO_4$ contributed $\geq 4$ TUs. Assuming additivity, the effluent would be predicted to have $\geq 16$–23 TUs, while typically only 6–12 TUs were observed. Given this, we hypothesized that the elevated $CaSO_4$ in the effluent might actually be exerting a protective effect against $SCN^-$ toxicity. To test this hypothesis we performed a 7-d toxicity test simulating the effluent ionic composition with the inclusion of 150 μM $SCN^-$. The resulting IC25 was 9.6% effluent (10.4 TUs) suggesting our hypothesis was correct and that the combination of elevated $CaSO_4$ and $SCN^-$ accounted for all the observed effluent toxicity.

The exact mechanism by which the ionic composition of the effluent protects against $SCN^-$ toxicity is not clear. Thiocyanate is a well known inhibitor of $Cl^-/HCO_3^-$ exchangers in fish (De Renzis, 1975) and it has been postulated that daphnids utilize a similar system (Bianchini and Wood, 2008). In chronic exposures, $SCN^-$ is also known to disrupt thyroid function (Lanno and Dixon, 1994; Lanno and Dixon, 1996). Given that daphnids lack the equivalent of a thyroid hormone and that reproduction does not appear to be affected by thyroxine levels (Kashian and Dodson, 2004), this pathway which is important in fish, is unlikely to be important in daphnids. The $Cl^-$ concentration in laboratory water and the effluent are very similar so differences in $Cl^-$ gradient do not explain the results. It is possible that the elevated $Ca^{2+}$ concentrations in the effluent reduce diffusive $Cl^-$ loss and therefore reduce the effects of inhibition of $Cl^-$ uptake. Alternatively, $SCN^-$ may be affecting reproduction directly by an undetermined mechanism.

## 5. Conclusions

Results from this study highlight the difficulties in conclusively demonstrating the causes of toxicity in high TDS effluents as has been previously described (Goodfellow et al., 2000) although we believe it was achieved in this study. Toxicity associated with TDS is typically recalcitrant to standard TIE manipulations and spiking studies are often confounded by solubility issues. Further, for effluents with metals or other ions present at elevated concentrations, the interactions (both additive and antagonistic) between ions can be difficult to unravel.

Importantly, this study is the first to demonstrate the chronic sensitivity of *C. dubia* to $SCN^-$. Using the data from the 7-d chronic test on $SCN^-$ in laboratory water, a 96-h LC50 of $>86$ mM $SCN^-$ was observed. This is comparable to other available acute data for daphnids, suggesting *C. dubia* is not unusually sensitive to $SCN^-$, rather it is the reproductive endpoint for cladocerans that is sensitive to this toxicant. Historically, $SCN^-$ has not been considered a toxicant of concern in mining wastewater as it is often at concentrations that are not acutely toxic to daphnids and fish (Boening and Chew, 1999; Smith and Mudder, 1991). However, given that $SCN^-$ is present at concentrations we determined to effect daphnid reproduction and that $SCN^-$ appears to be unaffected by any of the standard TIE methods, we believe it may actually be a relatively common, but previously unidentified contributor to observed chronic toxicity in mining effluents.

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