Toxicity Identification Evaluation 2008 - 2011 Study for the Mesabi Nugget Pits

Mesabi Nugget Phase I Project

Prepared for
Mesabi Nugget, LLC

June 2011

A new age of iron.

BARR

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June 2011

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Executive Summary

Whole effluent toxicity (WET) testing has been conducted since 2006 with outfall SD001 water (same as Area 1 Pit water--the water supply and part of the water treatment system for the Phase I project) at the Mesabi Nugget Phase I project, the operation of the first commercial scale demonstration plant of the ITMK3 iron nugget technology. After a year and a half of quarterly WET testing, it was apparent that water from SD001 was chronically toxic to the test species *Ceriodaphnia dubia*, (*C. dubia*) however, toxicity was intermittent. Mesabi Nugget began to evaluate the cause of the intermittent toxicity in late 2007 and reported these initial findings in early 2008.

Concurrent with Phase I project development, a second phase of the project – Phase II – began environmental review and permitting in 2008. Phase II consists of reopening two existing iron ore mines, and construction of crushing and concentrating facilities to produce iron ore concentrate for the Phase I project and for potential sale elsewhere. The two existing mines, Area 2WX and Area 6 Pits, were mined as recently as 2001, and have since filled with water. Because of the WET testing issues at SD001, WET testing was initiated in late 2008 with Area 2WX and 6 Pits water at the request of the Minnesota Pollution Control Agency. Testing with these waters (as well as Area 1 Pit) is ongoing. Results provided in this report demonstrate that chronic toxicity (with *C. dubia*) at Area 2WX and 1 Pits is intermittent, and while Area 6 Pit water is consistently toxic to *C. dubia,* the degree of toxicity is highly variable however.

In conformance with the requirements of NPDES permit MN0067687 and US EPA guidance, Mesabi Nugget and Mesabi Mining embarked on a Toxicity Identification Evaluation or TIE for Area 1, 2WX, and 6 Pits. TIE tests were conducted from 2008 through early 2011. TIE testing followed two approaches: (1) remove or add different constituents or control different parameters such as pH to identify the parameters responsible for toxicity, and (2) conduct a series of standard chronic WET tests with *C. dubia* paired with analysis of several water quality constituents. TIE tests conducted included: (1) ion exchange to remove negatively charged constituents, (2) lime softening to remove alkalinity, (3) EDTA addition to bind metals, (4) calcium addition to rebalance the ratio of calcium to magnesium ions in pit water, (5) selenium addition to alleviate micronutrient deficiency with respect to this metal, (6) use of carbon dioxide headspace during WET testing to prevent pH rise during the test, and (7) addition of organic carbon because the pits have very low total organic carbon which is hypothesized to be supportive of aquatic life. Most of these treatments did not reduce toxicity, however, selenium and organic carbon addition significantly reduced toxicity. From these tests it

became clearer that toxicity for the three pits is to a large extent a result of what is "missing" from the pit waters rather than that which is in the pits. Published toxicity metrics (IC25) for sulfate and bicarbonate (the dominant ions in the three pits) suggest that Area 6 Pit water could be toxic because of high concentrations of these constituents in this pit, however, sulfate and bicarbonate in Area 1 and 2WX Pit waters are far below toxicity metrics (e.g., dissolved solids are not likely the cause of toxicity for Area 1 and 2WX Pit waters). It is also notable that the concentration of major ions in the pits (calcium, magnesium, sulfate, and bicarbonate) do not change significantly during the course of a year, however, toxicity can change dramatically. During the course of this TIE investigation, it has been observed that toxicity appears to change when the pits mix or when there is a change in weather, temperature, or season. It is hypothesized that subtle physical changes in the pits affect pit chemistry.

Chronic *C. dubia* WET testing conducted with Area 1, 2WX, and 6 water collected every 2 weeks from June 2010 through the present has provided insights into the relative contribution of major ions, trace constituents, and physical/chemical parameters on toxicity. For Area 6 Pit water, 44 percent (r^2=0.44) of the WET testing variability is due to the major anions and cations, while 97 percent (r^2=0.97) of variability can be explained when trace constituents such as nickel, selenium, silica, molybdenum, and manganese are added to the logistic-toxicity models. This indicates that over half of the toxicity variability in Area 6 Pit is due to deficient trace metal concentrations (note that the overall high dissolved solids content of Area 6 Pit is likely responsible for the baseline level of toxicity in this pit water while the trace metal concentration appears to be able to move this baseline level either up or down). It is hypothesized that trace metals improve WET test results because trace metals are vital micronutrients for *C. dubia*. Similar results were found for Area 1 and 2WX pits. Logistic-regression models for the Saint Louis River showed that higher trace metal concentrations in the river water also improved WET testing results (i.e., *C. dubia* reproduction).

Based upon selenium addition tests, TOC addition tests, and the logistic regression equations developed for Area 1, 2WX, and 6 Pit waters, it can be concluded that a lack of micronutrients appears to be responsible for poor WET test results (e.g., reduced young production) for Area 1 and 2WX Pit waters. It is also possible that elevated levels of sulfate and alkalinity aggravate micronutrient deficiencies by blocking or chemically interfering with micronutrient uptake. Changes in pit geochemistry, reflected in pH and other parameters, also may make nutrients less available at certain times of the year. WET test results for the Area 6 are likely a combination of high sulfate and bicarbonate and micronutrient deficiencies.

In conformance with the requirements of NPDES permit MN0067687 and US EPA guidance, Mesabi Nugget and Mesabi Mining propose to embark upon a Toxicity Reduction Evaluation or TRE. This TRE may potentially include: (1) laboratory experiments whereby micronutrients (copper, zinc, selenium, molybdenum, nickel, and phosphorus) are added to pit waters to quantify which micronutrients improve WET testing results and what levels of micronutrient additions are needed to pass WET tests, (2) in light of the improved understanding of toxicity, reexamine the benefits of constructed wetlands, partial lime softening, reactive barriers, and reverse osmosis on WET, and (3) evaluate combinations of treatment, micronutrient addition, mixtures of the three pits waters, and discharge options that will lead to passing WET tests.

1.0 Introduction

For the Phase I nugget plant, chronic whole effluent toxicity (WET) testing with water from outfall SD001 is required as a condition of Mesabi Nugget NPDES permit MN0067687 (issued on July 29, 2005). WET tests were conducted with test species *Ceriodaphnia dubia* and fathead minnows. Growth and survival of minnows in SD001 water (same as Area 1 Pit water) was not reduced in WET tests conducted for compliance purposes. However, reproduction of *C. dubia* was notably reduced compared to controls (Partridge River water sampled at Allen Junction, Minnesota served as the control water). An initial investigation was conducted by Mesabi Nugget and a memorandum of findings was provided to the MPCA on January 9, 2008 (Mesabi Nugget, 2008). A toxicity identification evaluation (TIE) study for SD001 (Area 1 Pit) was initiated in October, 2008.

As part of baseline work for the Mesabi Nugget Phase II project Environmental Impact Statement, WET testing was also conducted with Area 2WX and Area 6 Pit water. Water from Area 2WX Pit was found to be intermittently toxic while water from the Area 6 Pit was consistently toxic. WET testing results for Area 6 and Area 2WX Pits were used in conjunction with TIE tests (using Area 1 Pit water) to begin to identify the cause of toxicity for these pits. Test findings were reported to the MPCA in March 2009 (Barr Engineering, 2009) and in July 2009 (Barr Engineering, 2009a). WET testing for Area 6, 2WX, and 1 Pits continued through the summer of 2009. Cations, anions, metals, and other basic chemical and physical measurements were taken concurrently for summer 2009 WET tests. Although bicarbonate concentration was best correlated with differences in toxicity between pits, WET testing results were highly variable for each pit even though bicarbonate was very stable (i.e., for each individual pit toxicity changed while bicarbonate did not). Principle component analysis of this data also provided some evidence that trace metals and other minor constituents in the pit waters were correlated with WET testing results. These test results were provided in a December 4, 2009 memorandum to Mesabi Nugget (Barr Engineering, 2009b).

Toxicity identification evaluation activities continued in 2010 and early 2011. Linking pit water chemistry to WET testing outcomes (e.g., finding the toxicant) remained the focus of this study. Because the revised Mesabi Nugget Phase II Water Management Plan (Bar Engineering, 2011) includes the potential construction of a pipeline to the Saint Louis River, an additional goal of the program included identification of pit water dilution (measured as effluent percentage of total receiving water flow) necessary to pass WET tests. These WET tests would be used to determine acceptable limits for discharges from the proposed Phase II mining and concentrating project. WET testing results prior to 2010 suggested that there may be a seasonal effect on WET testing results. Testing in 2010 and 2011 was also structured to better quantify the seasonality of WET testing results and to determine whether discharge limits could be developed for each season.

This report presents the results of WET testing and TIE activities from June 2010 through February 2011. TIE testing activities conducted in 2008 and 2009 are also discussed with more detail provided for results that have furthered understanding of WET properties for Area 1, 6, and 2WX Pit waters.

2.0 Pit Water Chemistry

2.1 General Chemistry

Area 1, 2WX, and 6 Pits as well as potential receiving waters (Partridge and Saint Louis Rivers) have been monitored as part of the Phase I or Phase II project. Some of this data has been compiled to demonstrate the similarities and differences between the pit waters as well as the differences and similarities between the pit and potential receiving waters.

Table 1 shows the average surface water chemistry of Area 1, 2WX, and 6 Pits for the May 2008 through August 2009 monitoring period. Table 2 shows average surface water chemistry of these pits and the Saint Louis River for WET test samples collected in 2010 and 2011. There are some notable concentration differences between the pits as well as the receiving waters. For specific conductance and the major ions sulfate, calcium, and magnesium; concentrations are notably higher for Area 6 Pit. The ranking for these constituents from highest to lowest concentration is Area 6 Pit>Area 1 Pit>Area 2WX Pit>Partridge River>Saint Louis River. It is notable that alkalinity is largely the same for Area 1 and 2WX Pit and is higher for Area 6 Pit, but alkalinity is not the primary reason that dissolved solids are much higher for the Area 6 Pit. Sulfate levels in Area 6 Pit are approximately 1,000 milligrams per liter while in Area 1 Pit sulfate is around 360 milligrams per liter, and in Area 2WX, sulfate is around 110 milligrams per liter. Partridge River has a higher concentration of sulfate than Area 2WX Pit while the Saint Louis River has about half the sulfate of Area 2WX Pit. Area 6 Pit has slightly higher levels of some metals, including sodium, manganese, molybdenum, nickel, and selenium. Concentrations of these metals are very low in comparison to levels that are considered toxic. It is also noteworthy that metals (e.g., copper, selenium, manganese, nickel, and cobalt) in the Partridge and Saint Louis Rivers are often equal to or higher than levels in the Area 1, 2WX, or 6 Pits.

2.2 Physical/Chemical

In addition to differences in chemical composition, the pits and the receiving rivers have different physical/chemical properties. For example, the pH of Area 1, 2WX, and 6 Pits are similar (pH is approximately 8.4), but the pH of the Partridge and Saint Louis Rivers is notably lower (pH is approximately 7.5). The surface temperature of the Area 6 Pit is lower than Area 1 and 2WX Pits. Dissolved oxygen levels area largely similar for the three pits and the receiving waters.

For each pit, there is also a difference between surface and bottom waters. For example, moving from the surface to the bottom of the Area 6 Pit, dissolved oxygen goes down, pH goes down, and specific conductance goes up (see Figures 1 and 2). Because of the bathymetry and higher dissolved solids content of bottom waters, the pits are strongly stratified. When the pits mix in the fall and the spring, the entire water volume of the pits does not mix—only the top 10 to 15 meters of the pits appears to mix. This makes the pits net sinks for several constituents, including nutrients. The result is that nutrients are low in the surface waters of the pits and the pits have low productivity.

2.3 Trends

The chemistry of the pits changed during the duration of the 2010 (started in June) and early 2011 WET testing season. For example, calcium, alkalinity and manganese were high in Area 1 Pit in June, declined during the summer, and increased in later summer through winter (Figure 3). These constituents are affected by changes in pH and redox which also fluctuate by season (e.g., calcium and magnesium carbonate solubility changes with pH). pH in Area 1 Pit started low in June, rose in mid-summer and declined through the fall and winter (Figure 4). Other parameters less sensitive to pH (sulfate, chloride, and potassium) increased in concentration from June through winter (Figure 5). Some of the trace metals such as molybdenum and nickel varied from June 2010 through January 2011 and it appears that pit water mixing in mid-summer and the fall may have effected concentrations (Figure 6). Although these trends are notable, the results of WET tests (discussed in Section 3.0) do not appear to be driven by the seasonal trend of any individual parameter. Concentrations of chemical constituents did not move in unison during different seasons of the testing period, hence, the cumulative effect of different chemicals was constantly changing throughout the testing period (see Section 4.0).

The chemistry of Area 2WX Pit water also changed seasonally from June 2010 through January 2011. Alkalinity, calcium, and manganese were higher in the spring, decreased in the summer and rose in the fall with pit mixing (Figure 7). pH was lower in the spring, rose during mid-summer, dropped in the fall when the pit mixed, and then rose in the late fall and winter (Figure 8). Other constituents such as sulfate, potassium and chloride did not change much by season or throughout the year (Figure 9). It appears that molybdenum and nickel concentrations changed with pH changes in the spring and summer but also with pit mixing in the fall (Figure 10). The chemistry of the Saint Louis River also experienced seasonal changes with concentration of most constituents as well as pH peaking in the summer (Figures 11, 12, 13, and 14).

3.0 Seasonal Evaluation of Pit Toxicity

The total concentration of major ions (e.g., bicarbonate as alkalinity, chloride, potassium, calcium, magnesium, and sulfate) are very different for Area 1, 2WX Pit, and 6 Pits (Table 1). Area 6 Pit has the highest concentration of anions and cations, followed by Area 1 and Area 2WX Pits. Results of chronic WET tests indicate that Area 6 Pit is the most toxic (the test endpoint in this case is the number of *C. dubia* young propagated per adult female), followed by Area 1 Pit, and then Area 2WX Pit (Table 3). A simple comparison of average toxicity of these pits and pit ion concentration indicates that the pit with the highest ion concentration (e.g., Area 6 Pit) is also the most toxic. Area 1 and Area 2WX Pits have similar ion composition and concentrations except Area 1 Pit water which has much higher sulfate levels than Area 2WX Pit water. Sulfate is the dominant ion in Area 6 Pit while bicarbonate is the dominant ion in Area 2WX Pit water.

It is notable that although there is some change, the concentration of major ions in each individual pit does not change greatly during a given season or year (see Figures 3, 5, 7, 9, 11, and 13). However, chronic whole effluent toxicity varies significantly. For example, Figure 15 shows all of the chronic *C. dubia* testing data that has been collected for all three pits (testing began as early as August 2006). This figure demonstrates that the WET testing results can be highly variable and that test results can change on a seasonal, monthly, and even weekly timescale. Because it appeared that test results were correlated with season and to more thoroughly document seasonal changes in toxicity, WET tests were conducted with Area 1, 2WX, and 6 Pit waters every two weeks, starting in June 2010 and continuing through the present. This data, which is provided in Figure 15, is also separated out and presented in Figure 16 to better examine seasonal toxicity. However, it can be seen in Figure 16c that it does not appear that pit toxicity follows a clear seasonal pattern (data from June 2010 through February 2011). Figure 16a shows that test variability is not simply an artifact of the WET test and variability of this method. This variability is also not affected by the test endpoint used to evaluate the results (see IC25 results in Figure 16c). Note that Area 6 Pit data is not shown here because testing began in August 2010 and the limited data set could not be used to examine seasonal effects.

4.0 Pit Toxicity and Chemical Composition

Traditionally, a TIE study is designed to find a given toxicant or a few toxicants that may vary in effluent and hence can be identified as the cause of variable WET testing results. Early on in this TIE study it was clear that the basis for the observed WET testing results for Area 1, 2WX and 6 Pit waters was much more subtle. In 2006, 2007, and 2008, it appeared that the month or season in which WET testing was conducted corresponded to whether a WET test passed (passing is IC25>100 percent, IC25 is the effluent concentration at which there is a 25 percent decline in young production). It was presumed that there was some seasonal effect on WET testing results. Pit mixing, changes in water temperature or dissolved oxygen levels were hypothesized to correspond in some manner with WET testing results and season. Because of the subtle nature of pit toxicity, concurrent chemical measurements were taken for each chronic WET test conducted from June 2010 through February 2011 (testing is still ongoing). These concurrent chemical measurements were compared to the WET testing results to quantify the relationship between chemistry and toxicity.

4.1 Area 1 Pit

Results of WET tests conducted with Area 1 Pit water collected from June through December 2010 were analyzed using logistic regression. Logistic regression was used to quantify the effect of pit chemistry on WET testing results, measured as the “number of young produced per female adult.” The dependent variable is the “number of young produced per female adult” and the independent variable is the concentration of constituents measured in water collected for WET tests and some field measurements (such as dissolved oxygen, field pH, and temperature) taken concurrently with water sampling. Logistic regression is S-shaped (Figure 17) and is well suited to toxicity data. For example, small increases of a given constituent typically do not elicit a toxic response until reaching a critical level. Once this critical level is reached, the toxicological response is typically steep and exponential. However, once the toxicological response is complete (e.g., all organisms are dead or not reproducing); increased dose cannot elicit an increased toxicological effect. Overall, the S-shape of the logistic regression provides a lower and upper boundary to the WET testing results.

Logistic models were built for Area 1 Pit WET test results to identify constituents or constituent groups that had the greatest effect on WET test results. This was achieved by building several models with different constituent groups. For Area 1, these groups included: (1) the major cations and anions, (2) cations and anions plus trace constituents and metals, (3) cations, anions, trace constituents and metals, plus total organic carbon, dissolved oxygen, field measured pH, field temperature, and boron, and (4) all constitutes in #3 plus specific conductance. The graphs in Figure 18 show the results of the Area 1 Pit logistic modeling effort. Changes in concentrations of anions and cations accounted for 47 percent of the WET testing variability (reproduction per female adult ranged from 7 to 25). When trace metals and silica were added to the model, 67 percent of the observed WET test variability could be explained. When physical-chemical parameters such a field temperature, field pH and dissolved oxygen were added to the model, 85 percent of the WET testing variability could be explained. This indicates that changes in WET test results, which can be very large (reproduction per female adult ranged from 7 to 25), are not simply random events but are the result of subtle changes in pit chemistry. It is notable that pit temperature, dissolved oxygen, and pH can have an effect on WET testing results. It is very likely that these parameters affect the solubility of major cations and anions (e.g., calcium and carbonate) but also affect the speciation, complexation, and availability of trace constituents such as selenium, nickel, manganese, molybdenum, copper, and zinc.

4.2 Area 2WX Pit

Logistic models were also built for Area 2WX Pit WET test results to identify constituents or constituent groups that had the greatest effect on WET test results. This was achieved by building several models with different constituent groups. For Area 2WX, these groups included: (1) the major cations and anions, (2) cations and anions plus trace constituents and metals, (3) cations, anions, trace constituents and metals, plus total organic carbon, dissolved oxygen, field measured pH, temperature, and boron, and (4) all constitutes in #3 plus specific conductance. Changes in the concentration of anions and cations were only weakly correlated (r^2=0.27) with young production (Figure 19). When trace constituents and metals were added to the model, 79 percent of the observed WET test variability could be explained. This indicates that changes in trace metals concentrations, likely acting as micronutrients (see Section 6.0), were the primary cause of WET testing variability observed for Area 2WX Pit water (young production per adult female ranged from 10 to 27). With the addition of physical/chemical parameters and specific conductance to the logistic regression model, 97 percent of WET testing variability could be explained.

4.3 Area 6 Pit

Logistic models were also built for Area 6 Pit WET. Because fewer data were available for Pit 6, models could not make use of all the measured parameters but were limited to: (1) the major cations and anions, and (2) cations and anions plus trace constituents and metals. Cations and anions were able to explain 44 percent (e.g., r^2=0.44) of the WET testing variability (Figure 20). When trace constituents and metals were added to the model, 97 percent (r^2=0.97) of the observed WET test variability could be explained. These findings are similar to Area 1 and Area 2WX Pits.

4.4 St. Louis River

In accordance with the Mesabi Nugget Phase II Water Management Plan (Barr, 2011), the St. Louis River is a potential receiving water. St. Louis River water also served as the control water for WET tests conducted from June through December 2010. The basic presumption for WET testing is that an effluent, whether treated or untreated, should have toxicological properties that are not significantly different from the receiving water. However, a question that needs to be asked is "What are the properties of a natural water that allow for the development of abundant and healthy aquatic life?" With respect to WET testing, the more relevant question is "What are the properties of a natural water that determine the average number of young produced by *C. dubia* in chronic WET tests?" This question is relevant because *C. dubia* young production in 100 percent St. Louis River water was fairly variable (19 to 31), and in some cases the young production in Area 1 and 2WX Pit was higher than the St. Louis River.

Logistic models developed for WET tests with St. Louis River water show that changes in anions and cation levels account for 60 percent of WET variability, while anions, cations, trace metals, pH and temperature account for approximately 99 percent of WET variability (Figure 21). The logistic model consists of coefficients that are either negative or positive with respect to the number of young produced per adult. Negative coefficients reduce young production and positive coefficients increase young production. Table 4 shows which model parameters are positive or negative. Only alkalinity and calcium are negative. All trace metals were positive. Field pH and temperature were positive. In 66 percent of the models for the pits and St. Louis River, selenium, nickel, silicon, and molybdenum were positively correlated with young production. It is notable that metals were higher in the St. Louis River compared to Area 1 and 2WX Pits, suggesting that metals were not toxicants but rather were acting as micronutrients for these pits. Compared to St. Louis River water, metals were higher in Area 6 Pit water with the exception of copper which was over three times higher in the St. Louis River.

4.5 Common Waters Model

The logistic regression models of the pits and St. Louis River largely indicate that the concentration of cations and trace metals determine young production in chronic *C. dubia* WET tests. Chloride, sodium, sulfate and alkalinity are negatively correlated to young production while trace metals, pH, and temperature are positively correlated to young production. A unified logistic model, consisting of the pit and St. Louis River WET test results was developed to further evaluate the effect of these constituents on WET test results and to better understand commonalities between Area 1, 2WX, and 6 Pits, and the St. Louis River.

It can be seen in Figure 22a that alkalinity cannot be used as a reliable predictor of pit toxicity. It is clear the pits with the highest alkalinity has the highest toxicity and that the St. Louis River has low alkalinity and low toxicity, however, young production can be the same for the St. Louis River, Area 1 and 2WX Pits despite very different alkalinity. If additional cations and anions are added to the model, predictive ability is improved (Figure 22b). If trace metals are added to the model, the model is greatly improved, the pits and St. Louis River begin to merge and appear as one data set. This suggests that the some of the same chemical mechanisms that affect young production in St. Louis River water also affect young production in pit waters.

5.0 TIE Tests Prior to June 2010

From December 2008 through May 2009, a series of pit water TIE treatments were conducted and included:

- ion exchange to remove negatively charge constituents,
- lime softening to remove alkalinity,
- EDTA to bind metals,
- calcium addition to rebalance the ratio of calcium to magnesium ions in pit water,
- selenium addition to alleviate micronutrient deficiency with respect to this metal,
- use of carbon dioxide headspace during WET testing to prevent pH rise during the test, and
- addition of organic carbon because the pits have very low total organic carbon which is hypothesized to be supportive of aquatic life.

The ion exchange, lime softening, and calcium addition experiments did not provide any further understanding of the pit toxicity mechanism. Lime softening of Area 6 Pit water, which removes alkalinity, did not improve *C. dubia* young production in 7 of 9 (improvement was not significant for test with increased *C. dubia* young production) tests. EDTA addition to Area 6 Pit water did not reduce toxicity. The results of the selenium addition, carbon dioxide headspace, and TOC addition experiments, however, provided some additional understanding of potential toxicity mechanisms.

For effluents with high alkalinity, it has been observed that pH rise during WET testing can cause both acute and chronic toxicity. To determine if pH control could improve *C. dubia* reproduction and possibly lead to passing WET tests, WET tests were conducted with Area 1 and 6 Pit waters under a carbon dioxide headspace. The use of a carbon dioxide headspace improved young production but only marginally (Figure 23). For Area 1 and Area 6 Pits, pH rises by approximately 0.5 units during WET testing. With a carbon dioxide headspace, pH rise is limited to between 0.1 to 0.2 pH units. These pit waters, which act more like natural waters, are likely in equilibrium with carbon dioxide in the atmosphere, and hence pH rise is not as significant as treated industrial effluent that has supersaturated levels of carbon dioxide.

The US EPA "chronic manual," which provides WET testing guidelines and procedures (US EPA, 2002), recommends the addition of sodium selenate (Na_2SeO_4) to *C. dubia* cultures to a final selenium concentration of 2 µg/L to satisfy the biochemical needs of *C. dubia*. Because selenium is a micronutrient and the concentration of selenium in the pit waters is typically less than 1.0 µg/L, it was hypothesized that low selenium may have contributed to reduced *C. dubia* reproduction in past WET tests. To test this hypothesis, a range of selenium concentrations in the form of sodium selenate were added to Area 6 and Area 1 Pit water (Figures 24 and 25). Chronic *C. dubia* WET tests with Area 6 Pit water spiked with sodium selenate from 5 to 100 µg/L (as selenium) improved young production from 7 young per adult female with no selenium to 14 young per adult female with the addition of 10 µg/L selenium (Figure 24). No additional young production was stimulated with selenium between 20 to 100 µg/L. Selenium addition to Area 1 Pit water at 10 µg/L did not notably improve young production with water collected in February 2009 (see Figure 25); however, young production without selenium addition was already high for this test leaving little room for improvement. The WET test with Area 1 Pit water collected in May 2009 also showed little improvement with selenium addition despite the lower young production. Overall, it is clear that selenium addition to pit waters can improve young production.

Similar to selenium, total organic carbon (TOC) is very low in Area 1, 2WX, and 6 pit waters. To examine whether low TOC affects WET testing results, TOC was added to pit water prior to WET testing. The source of the organic carbon was natural organic matter extracted from the Suwannee River in Georgia (International Humics Society, University of Minnesota, Saint Paul). To a TOC concentration of 15 mg/L, organic carbon was added to Area 6 and 1 Pit water collected in May 2009. A full dilution series test was conducted with the TOC-spiked pit waters. The St. Louis River served as the control and the diluent. The addition to TOC to the pit waters clearly reduced toxicity and improved young production notably (Figure 26). It is possible that the properties of natural organic matter alone provide a more suitable habitat for zooplankton (e.g., *C. dubia*) survival and reproduction, however, the organic carbon source used for this experiment carried some trace metals and other ions. The addition of the natural organic matter to a final TOC concentration of 15 mg/L increased the concentrations of the following metals and trace constituents in the pit water: iron (86 ug/L), silicon (540 ug/L), copper (0.46 ug/L), molybdenum (0.32 ug/L), and zinc (6.5 ug/L). It is possible that the addition of the natural organic matter improved *C. dubia* reproduction because of these trace ions, however, the effect of these trace ions needs to confirmed with additional testing before a definitive conclusion can be provided.

6.0 Working Toward a Mechanistic Understanding of Toxicity

6.1 Sulfate and Bicarbonate Toxicity

According to the logistic regression models for the pits and St. Louis River, alkalinity, sulfate, chloride, and sodium are most often negatively correlated with *C. dubia* young production (see Table 4). The following discussion of anion and cation toxicity focuses on alkalinity and sulfate largely because chloride and sodium are well below levels that should be toxic, and other constituents such as calcium, magnesium, and potassium were shown to be most often positively correlated with *C. dubia* young production (Table 4).

Both TIE studies conducted by Barr and published literature are discussed below to evaluate the potential for sulfate and bicarbonate toxicity for Area 1, 2WX, and 6 Pits. The TIE studies have been conducted on industrial effluents that consisted of nearly pure mixtures of sodium sulfate and mixtures of calcium, magnesium, and carbonate. Chronic whole effluent toxicity tests were conducted on the sodium sulfate dominated effluent, producing an average IC25 of 1,006 mg/L as sulfate. The IC25 with respect to TDS for this effluent was 1,491 mg/L. A study by Lasier and Harden, 2010, provided IC25 (for *C. dubia*) estimates for sulfate that ranged from 496 to 1060 mg/L. Elphick et al., 2011, provided sulfate IC25 values of 622 and 1,174 mg/L for *C. dubia* cultured in moderately hard and hard water. Hall and Borton, 2009, provided an average sulfate IC25 value of 595 mg/L for *C. dubia*. These data suggest that sulfate levels in Area 6 Pit could be a major contributor to Area 6 Pit toxicity given that sulfate averaged 1,229 mg/L from June 2010 through January 2011 in Area 6 Pit. The chronic no observed effect level (NOEC) for sulfate for the sodium sulfate dominated effluent was 606 mg/L, suggesting that direct sulfate toxicity for Area 1 Pit (399 mg/L sulfate) and 2WX Pits (115 mg/L sulfate) is unlikely. Note that it is difficult to separate out the direct toxic effect of sulfate, presumably due to the effect of excessive ionic strength, from other effects such as the inhibition of trace metal uptake by *C. dubia* (discussed below).

For an industrial effluent primarily consisting of calcium, magnesium, bicarbonate, and some sulfate (270 mg/L), the IC25 (for *C. dubia*) of the effluent expressed as alkalinity was approximately 700 mg/L. A study by Lasier and Harden, 2010, provided IC25 (for *C. dubia*) estimates for bicarbonate that ranged from 621 to 780 mg/L. Alkalinity is around 300 mg/L for Area 1 and 2WX Pits and around 500 mg/L for Area 6 Pit (see Table 1).

The pit chemistry and published toxicity and TIE data suggest that the levels of alkalinity and sulfate in Area 6 Pit could be the primary cause of observed chronic *C. dubia* toxicity. It seems less likely that sulfate and alkalinity in Area 1 and 2WX Pits are the primary cause of observed WET test failures.

6.2 Importance of Trace Metal Deficiency

The logistic regression models of the pits and receiving water suggest that changes in the concentration and availability of trace metals, used by *C. dubia* as micronutrients, has the largest and most consistent effect on *C. dubia* reproduction (the endpoint used to determine whether a WET test is a failure or is passing) in the pit and receiving waters. A literature review of animal and zooplankton micronutrients indicates that nickel, molybdenum, copper, zinc, and selenium function as critical micronutrients and perform important metabolic functions (see http://www.nickelinstitute.org/index.cfm?ci_id=13020&la_id=1. http://www.gustrength.com/nutrition: selenium-an-essential-micronutrient , Kisker et al., 1997; Lam and Wang, 2008). The study by Lam and Wang is particularly relevant because it demonstrated that copper, zinc, and selenium deficiency can significantly reduce zooplankton reproduction. This study also documented morphological defects with selenium deficiency. The deficiency led to degradation of the zooplankton antennae which led to swimming impairment, eventual loss of mobility, and hence an inability to feed properly which eventually led to death. This study also noted that the zooplankton used in this study (*Daphnia magna*) were not able to regulate the uptake of selenium but were able to actively retain zinc and hence were able to compensate for periods of low zinc availability.

Among the many potential reasons for the micronutrient deficiencies of the pits, four have been identified and include: (1) limited watershed, steep pit bathymetry, and strong stratification causes the pits to be net sinks for micronutrients and this leads to low micronutrient levels of in the surface waters, (2) sulfate, calcium, magnesium, and bicarbonate are high in the pits and may block micronutrient uptake by *C. dubia*, (3) organic carbon is low in the pits and may be less available to facilitate chelated uptake of micronutrients, and (4) micronutrient availability may be affected by seasonal changes in speciation and binding by pit constituents such as carbonate.

Trace metals and nutrients inputs to Area 1, 2WX, and Pit 6 are limited to areas directly adjacent to the pits, groundwater, precipitation, and dry deposition. Because of limited inputs and the tendency of these pits to be net sinks for trace metals and nutrients such as phosphorus, metals and nutrients are low in the pit surface waters. Comparison of metals and nutrients in the Partridge River (a potential surrogate to the St. Louis River) to Area 1 and 2WX Pit waters indicates that copper, selenium, iron, zinc, nickel, and phosphorus are higher in Saint Louis River water. It is notable, however, that compared to the St. Louis River, Area 6 Pit has higher levels of metals except for iron, copper and the trace nutrient phosphorus. For Area 1 and 2WX Pits, trace metal deficiencies may be more a function of concentration in the water column whereas deficiency for Area 6 Pit may be a function of interference by high sulfate or other pit constituents. Area 6 Pit, however, has much lower levels of copper and phosphorus compared to the Partridge River (a potential surrogate to the St. Louis River).

A study by Ogle and Knight, 1996, demonstrated that zooplankton (*Daphnia magna*) exposed to high sulfate waters were not able to uptake as much selenium compared to waters with low sulfate. It is possible that the similarity of the three dimensional structure of sulfate (see Figure 27) and selenium in oxidized form (selenate) causes a physical inhibition or blockage of selenium uptake. Molybdate, and other pit constituents such as ortho-phosphate, also have the same general three dimensional tetrahedral structure as sulfate.

6.3 Chemical Speciation

Based upon the logistic regression models described in Section 4.0, changes in pit and receiving water constituent concentrations correspond to observed changes in WET test results (i.e., young production per female adult). Other chemical-physical parameters also appear to affect WET testing results (e.g., higher field pH as well as higher field temperature appear to improve WET test results). Metals, presumably acting as micronutrients, can associate with other chemicals present in the pits. Copper, zinc, molybdenum, selenium as selenate, and nickel, for example, may form dissolved "aqueous" complexes with sulfate, chloride, carbonate, and hydroxide. A geochemical model called Geochemists Workbench was used to estimate the degree of metal complexation and to identify the chemicals in each complex. It appears that the metals in Area 1, 2WX, and 6 Pits are primarily forming complexes with sulfate, chloride, and hydroxide. With the exception of iron, metals do not appear to form aqueous complexes with carbonate. The fraction of a given metal in the complexed (e.g., $CuSO_4$) versus free (e.g., copper as Cu^{++}) form also does not appear to correlate with WET testing results. It seems reasonable to assume that the degree of metal complexation affects metal availability, but it is not clear if a complexed metal is more or less available to *C. dubia*. This simple analysis and the results of the logistic regression analysis suggest that the concentration of metals rather than the speciation (i.e., the degree of complexation) is more clearly related to the outcome of chronic *C. dubia* WET tests.

7.0 Conclusions and Discussion

According to the US EPA guidance document "Technical Support Document for Water Quality-based Toxics Control," whole effluent toxicity is "a useful parameter for assessing and protecting against impacts upon water quality and designated uses caused by the aggregate toxic effect of the discharge of pollutants." This implies that there are pollutants in a discharge and WET test failures are due to the pollutants. Unlike water quality criteria that are applied uniformly to dischargers, the outcome of a WET test is highly dependent upon the quality of the receiving water because the receiving water is used as the "control" water in WET testing. Chronic toxicity for an effluent is defined as a significant difference (for chronic toxicity, the statistic is IC25) between growth or reproduction of an organism in the control water versus the effluent. If the receiving water (e.g. the control water) is of particularly high quality, an effluent may be judged as "toxic" even though it does not have high levels of toxic pollutants. For example, synthetic laboratory water, which is developed from distilled water or groundwater conditioned with salts, does not contain any pollutants. However, if synthetic laboratory water is used as a surrogate effluent, the synthetic water could be classified as toxic. To test this hypothesis, synthetic laboratory water was diluted with St. Louis River water to determine how the laboratory water would compare to Area 1 and 2WX Pit water (water collected in October 2010). It can be seen in Figure 28 that synthetic laboratory water was similar to Area 2WX Pit water and was almost classified as toxic. However, the synthetic laboratory water clearly does not have pollutants in it. For this test, young production in Area 1 and Area 2WX Pit water were similar to but slightly lower than the synthetic control water (e.g., if synthetic laboratory water was the control water, the pit waters would not be classified as toxic). The implication is that an effluent in Southern Minnesota, for example, may be classified as "passing" while the same effluent in Northern Minnesota may be "failing." The other implication is that a WET test failure may not be due to pollutants in an effluent, but because of the superior natural properties of a chemically and biologically balanced receiving water.

It appears that the overall level of toxicity observed in Area 6 Pit water is the result of high alkalinity and high sulfate. Another way to word this is: the maximum potential level of *C. dubia* reproduction in Area 6 Pit water is limited by high levels of alkalinity and sulfate in Area 6 Pit waters. Most of the WET testing variability observed in the past, however, appears to be due to fluctuating levels trace metal micronutrients. It is presumed that the trace metal micronutrient levels in Area 6 Pit water are not high enough to satisfy the micronutrient needs of *C. dubia*. Yet, it has not been determined what the maximum potential reproduction level is for *C. dubia* in Area 6 Pit water if the micronutrient deficiency is alleviated. Evidence to support this finding includes published literature and other TIE studies that indicate that the alkalinity and sulfate levels in Area 6 Pit are above or closely approach chronic toxicity thresholds. However, it is not clear what the toxic mechanism is for sulfate and alkalinity at the levels found in Area 6 Pit. It is possible that these constituents affect the chemistry in a way that prevents micronutrient (e.g., potential micronutrients include selenium, copper, zinc, iron, phosphate, and molybdenum) uptake by *C. dubia*. Ogle and Knight, 1996 demonstrated that high sulfate inhibits selenium uptake by *Daphnia magna*. Because of the similar shape of sulfate and selenate, it is possible that high sulfate physically blocks selenium uptake by zooplankton. It is notable that other molecules such as molybdate and phosphate also “look” similar to sulfate. It is also possible that levels of alkalinity and sulfate stress the osmoregulatory functions of *C. dubia*.

Levels of sulfate and alkalinity in Area 1 and 2WX Pit are below chronic toxicity thresholds for sulfate and alkalinity, hence, it is appears unlikely that sulfate and alkalinity are the primary cause of chronic WET failures for these pits. Based upon selenium addition tests, TOC addition tests, and the logistic regression equations developed for Area 1 and 2WX Pit waters, a lack of micronutrients is likely responsible for low young production during certain times of the year. It is also possible that elevated levels of sulfate and alkalinity also aggravate micronutrient uptake by blocking or chemically interfering with micronutrient uptake.

The regression models for Area 1, 2WX, and 6 Pits indicate that micronutrient deficiencies in the pit waters, and possibly inhibited micronutrient uptake by sulfate and alkalinity, likely play a significant role in WET testing outcomes. It has been demonstrated that selenium is deficient in the pit waters and addition of selenium can improve young production. Other micronutrients, such as molybdenum, zinc, copper, phosphate, chromium, and cobalt may be deficient in the pit water. It is possible that the IC25 could be increased and/or toxicity eliminated with the addition of these micronutrients to Area 1 and 2WX Pit waters. The effect of these micronutrients needs to be experimentally quantified with further controlled laboratory testing.

8.0 References

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Tables

Table 1. Average chemistry of water used for WET tests conducted from June 2010 through January 2011.

Parameter	Units	Area 1 Pit		Area 2WX Pit		Area 6 Pit		St. Louis River
		Surface	Below Thermocline	Surface	Below Thermocline	Surface	Below Thermocline	
Alkalinity, total	mg/L	325	356	305	318	521	647	64.1
Carbon, total organic	mg/L	1.8	1.6	1.6	1.6	1.4	1.4	18.9
Chloride	mg/L	11	11	14	14	10	10	3.8
Sulfate	mg/L	399	404	115	116	1229	1396	66.3
Dissolved oxygen	mg/L	8.4	7.1	8.5	9.3	8.4	0.4	8.6
Field pH	su	8.39	8.0	8.48	8.3	8.44	7.5	7.5
Specific Conductance	umhos@ 25oC	1257	1271	803	822	2663	3169	328
Temperature,	oC	15.2	8.6	15.5	9.2	11.9	8.3	13.6
Turbidity	NTU	0.2	0.0	0.0	0.0	0.0	0.0	1.7
Boron	ug/L	126	132	111	114	202	219	52.6
Calcium	mg/L	39	47	24	28	41	64	20.5
Iron	ug/L	25	25	25	25	25	25	966
Magnesium	mg/L	154	160	84	85	404	473	22
Manganese	ug/L	65	626	5.3	4.2	590	3546	164
Molybdenum	ug/L	2.1	1.8	1.6	1.6	11.5	13.4	1.3
Nickel	ug/L	1.3	1.4	0.8	1.0	1.8	3.6	1.9
Potassium	mg/L	13	14	9	10	22	24	1.9
Selenium	ug/L	0.25	0.25	0.25	0.24	0.25	0.20	0.46
Silicon	mg/L	4.0	4.2	3.3	3.3	5.1	5.6	0.5
Sodium	mg/L	22	20	29	30	62	66	3.9

Table 2. Average surface water chemistry of Area 1 Pit, Area 2WX Pit, Area 6 Pit, Partridge River, and Saint Louis River water collected primarily as part of baseline surface water monitoring for Mesabi Nugget.

Parameter	Units	Area 1 Pit(1)	Area 2WX(1)	Area 6 Pit(1)	Partridge River(1)	St. Louis River(2)
Alkalinity, bicarbonate as CaCO3	mg/L	326	309	501	117	no data
Alkalinity, total as CaCO3	mg/L	328	313	465	110	53
Bromide	mg/L	0.082	0.032	0.62	0.05	no data
Chemical Oxygen Demand	mg/L	10.0	5.0	6.6	55.8	no data
Chloride	mg/L	9.92	14.16	9.36	4.73	3
Fluoride	mg/L	0.12	0.12	0.12	0.19	no data
Hardness, total	mg/L	733	409	1511	289	no data
Nitrate + Nitrite as N	mg/L	0.05	0.11	0.050	0.10	no data
Nitrogen, ammonia as N	mg/L	0.116	0.05	0.050	0.15	no data
Phosphorus total	mg/L	0.0032	0.0035	0.005	0.015	no data
Solids, total dissolved	mg/L	792	456	1853	370	no data
Solids, total suspended	mg/L	1.2	1.3	1.9	1.2	no data
Sulfate	mg/L	363	114	1025	162	54
Carbon, total organic	mg/L	1.8	1.6	1.8	19.7	no data
pH, standard units	su	8.35	8.53	8.31	7.61	7.61
Specific Conductance umhos@ 25^{o}C	umhos/cm	1179	726	1925	599	237
Dissolved oxygen	mg/L	9.9	10.5	9.9	9.1	7.6
Aluminum	ug/L	12.5	12.5	12.5	113	no data
Antimony	ug/L	0.13	0.25	0.16	0.1	no data
Arsenic	ug/L	0.9	3.14	1.20	1.0	no data
Barium	ug/L	1.7	3.5	1.57	16	no data
Beryllium	ug/L	0.093	0.100	0.10	0.09	no data
Boron	ug/L	122	116	164	99	41
Cadmium	ug/L	0.15	0.10	0.09	0.09	no data
Calcium	mg/L	37	28	44	29	17
Chromium	ug/L	0.57	0.50	0.57	0.55	no data
Cobalt	ug/L	0.31	0.35	0.64	0.45	no data
Copper	ug/L	0.56	0.35	0.97	3.40	no data
Iron	ug/L	25	47	29	1032	1119
Lead	ug/L	0.26	0.25	0.27	0.30	no data
Magnesium	mg/L	156	82	330	53	16
Manganese	ug/L	68.8	7.6	272	255	215
Molybdenum	ug/L	1.74	1.74	11.37	1.67	1.1
Nickel	ug/L	1.88	1.03	4.34	3.6	2.1
Potassium	mg/L	12.8	9.7	21.1	4.6	1.3
Selenium	ug/L	0.40	0.20	0.87	0.55	0.60
Silver	ug/L	0.049	0.100	0.07	0.05	no data
Sodium	ug/L	14.3	29.3	57.8	9.7	4.8
Strontium	ug/L	103	70	179	173	no data
Thallium	ug/L	0.18	0.20	0.23	0.2	no data
Titanium	ug/L	5.0	5.0	5.0	5.0	no data
Zinc	ug/L	3.0	3.0	3.7	3.5	no data

(1) Monitoring data for samples collected from May 2008 through August 2009.

(2) Monitoring data for samples collected on June 1 and June 15, 2009.

Table 3. Average young production per female in WET tests conducted with pit, river, and laboratory water.

Water Source	Average Young Production Per Female
Pit 1[(1)]	15.1
Pit 2WX[(1)]	16.7
Pit 6[(2)]	7.0
St. Louis River[(3)]	24.2
Laboratory Water[(2)]	20.0

(1) Testing from October 2008 through February 2011
(2) Testing from August 2006 through February 2011
(3) Testing from June 2010 through February 2011

Table 4. Direction of effect of constituent concentration on young production. A "+" indicates that young production is positively correlated to constituent concentration. A "-" indicates that young production is negatively correlated to constituent concentration.

Constituent Group	**Constituent**	**Water Source**			
		Area 1 Pit	**Area 2WX Pit**	**Area 6 Pit**	**St. Louis River**
Anions and Cations	Alkalinity	—	+	—	—
	Chloride	—	—	—	Not in Model
	Sulfate	—	+	—	Not in Model
	Calcium	—	+	+	—
	Magnesium	+	—	+	+
	Sodium	—	—	—	+
	Potassium	—	+	+	Not in Model
	Selenium	+	—	+	+
	Nickel	—	+	+	+
	Silicon	+	+	—	+
	Molybdenum	+	+	—	+
	Manganese	+	—	—	Not in Model
Organic Carbon	Total Organic Carbon	+	—	Not in Model	Not in Model
Physical Chemical	Dissolved Oxygen	—	+	Not in Model	Not in Model
	Field pH	+	+	Not in Model	+
	Field Temperature	—	+	Not in Model	+
Other	Boron	+	+	Not in Model	Not in Model
	Specific Conductance	—	+	Not in Model	Not in Model

Figures

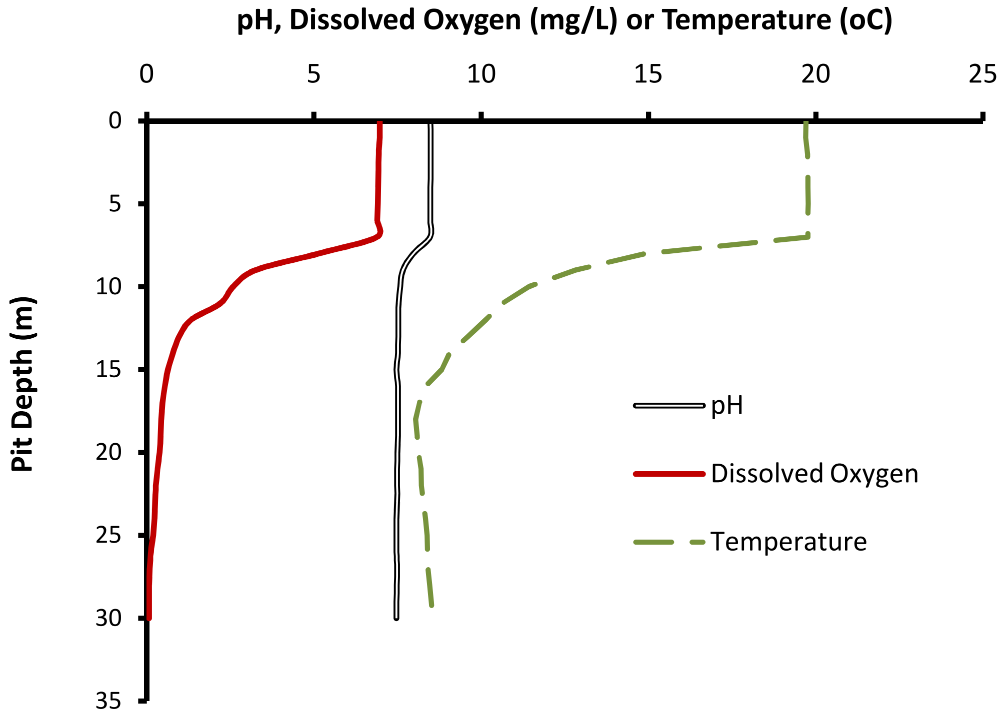


Figure 1. Area 6 Pit pH, dissolved oxygen, and temperature by depth on August 17, 2010.

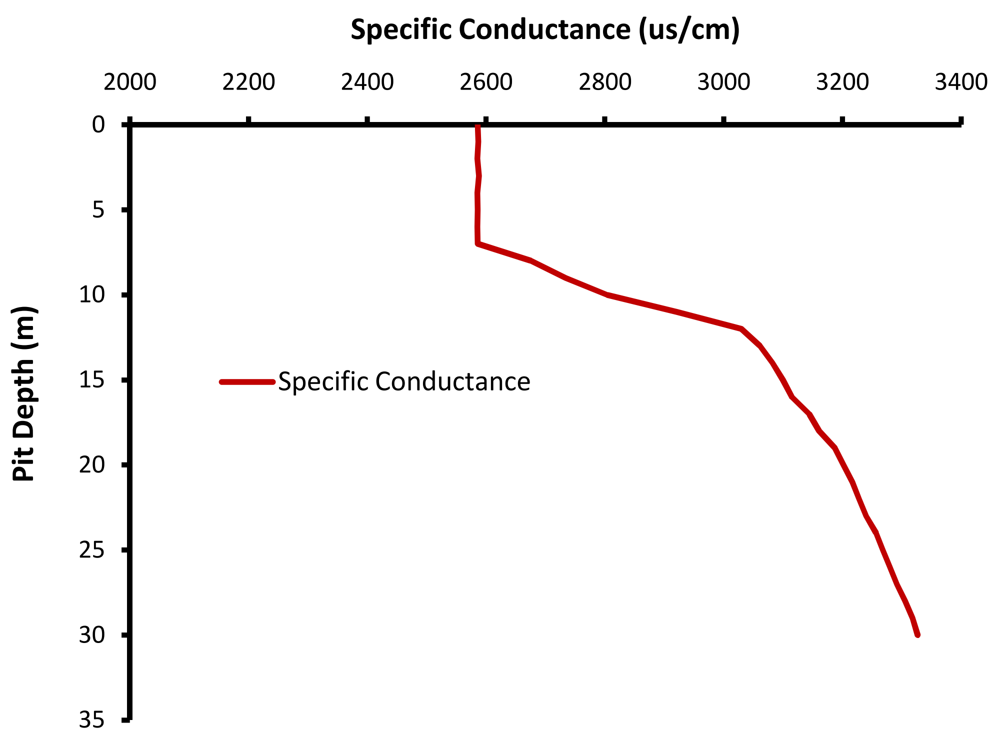


Figure 2. Area 6 Pit specific conductance by depth on August 17, 2010.

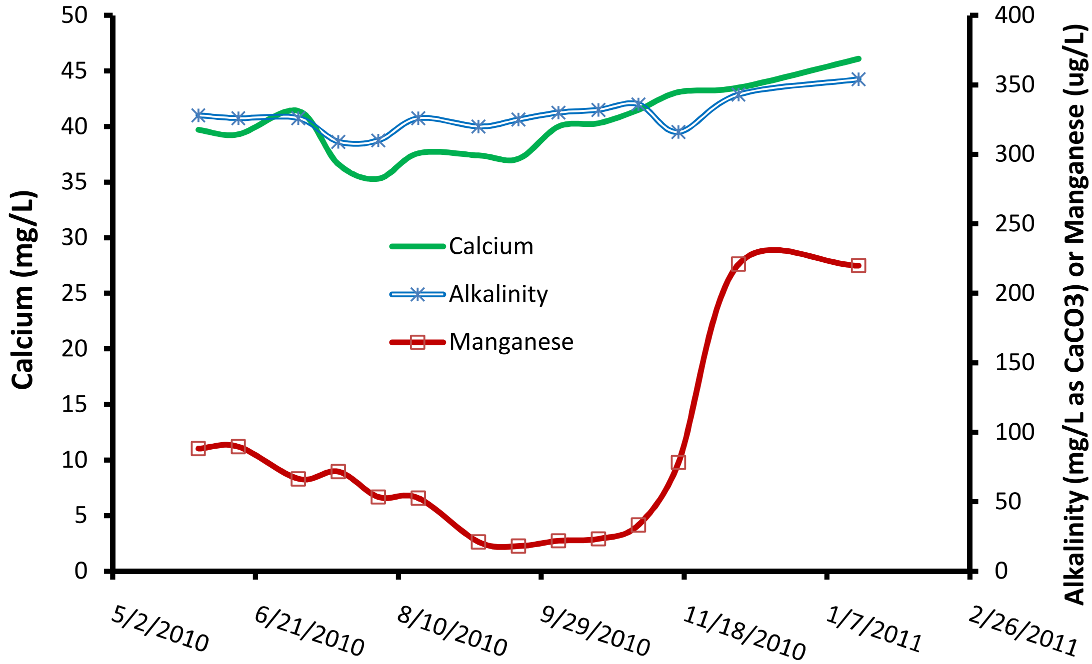


Figure 3. Seasonal changes in Area 1 Pit surface waters for calcium, alkalinity, and manganese.

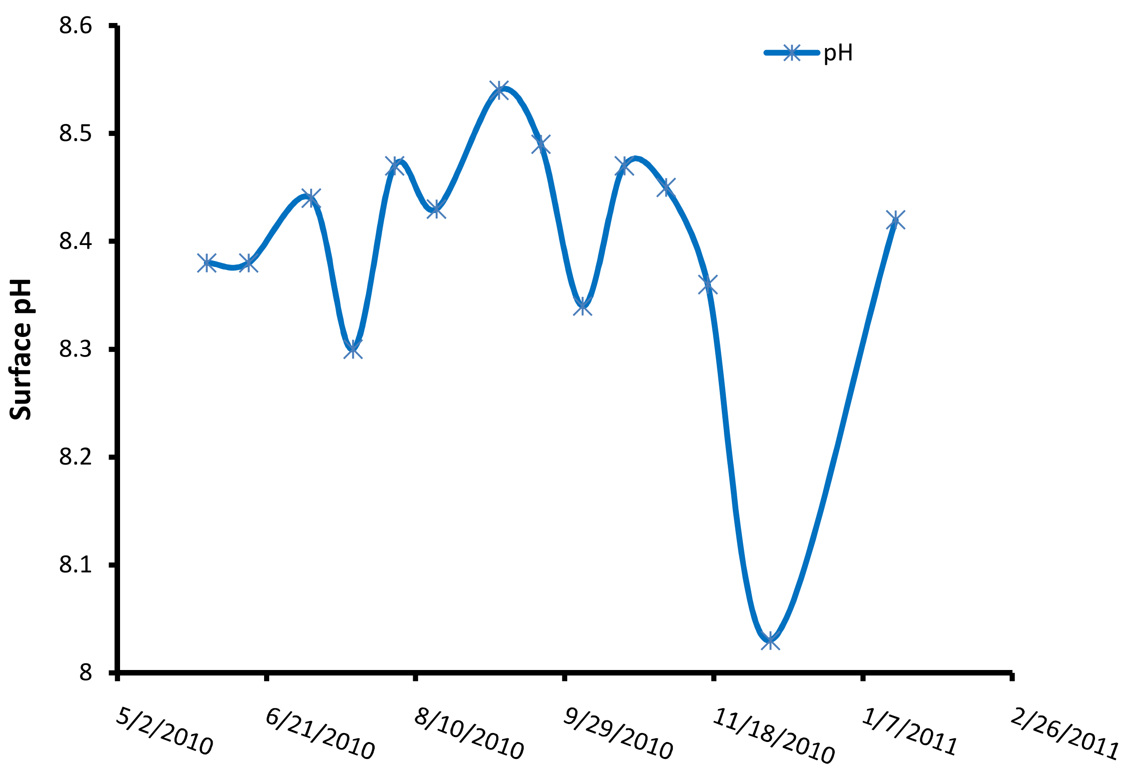


Figure 4. Seasonal changes in Area 1 Pit surface waters for pH.

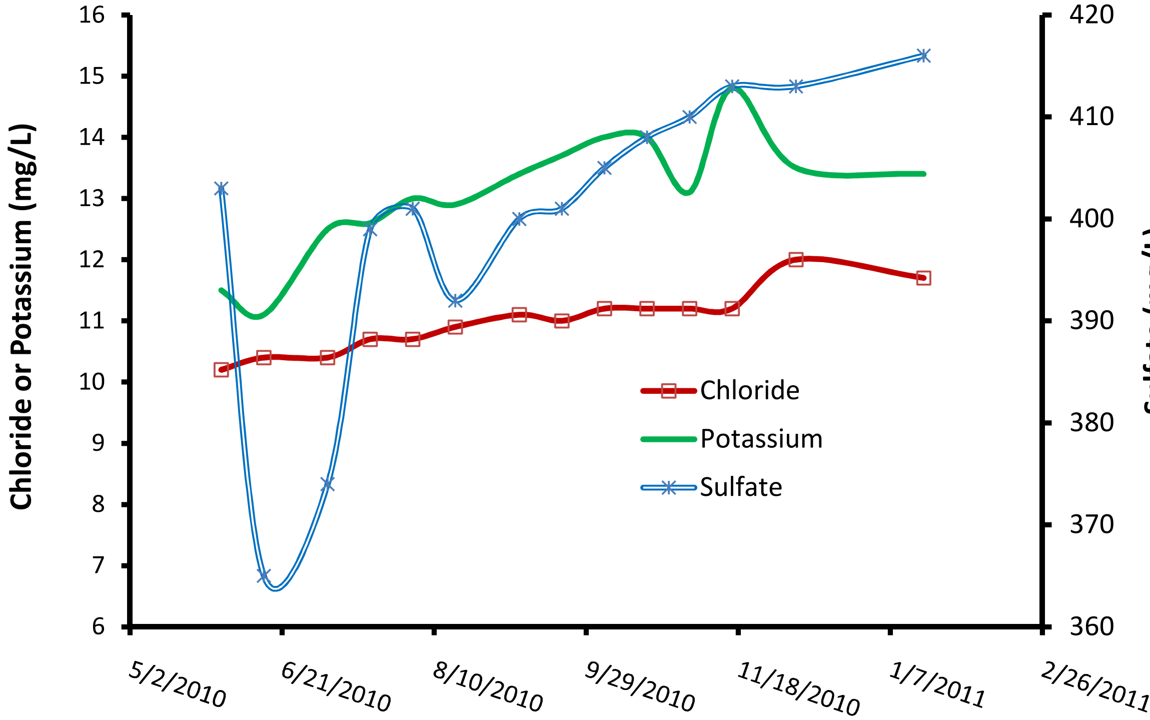


Figure 5. Seasonal changes in Area 1 Pit surface waters for chloride, potassium and sulfate.

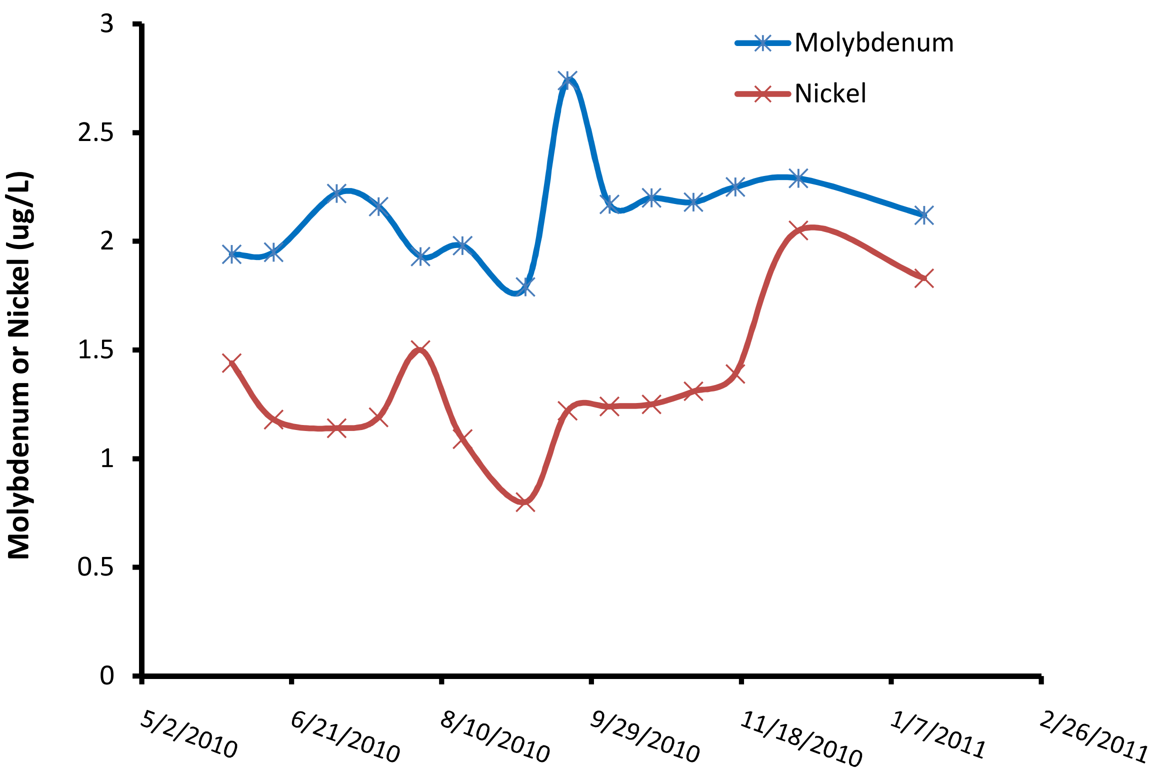


Figure 6. Seasonal changes in Area 1 Pit surface waters for molybdenum and nickel.

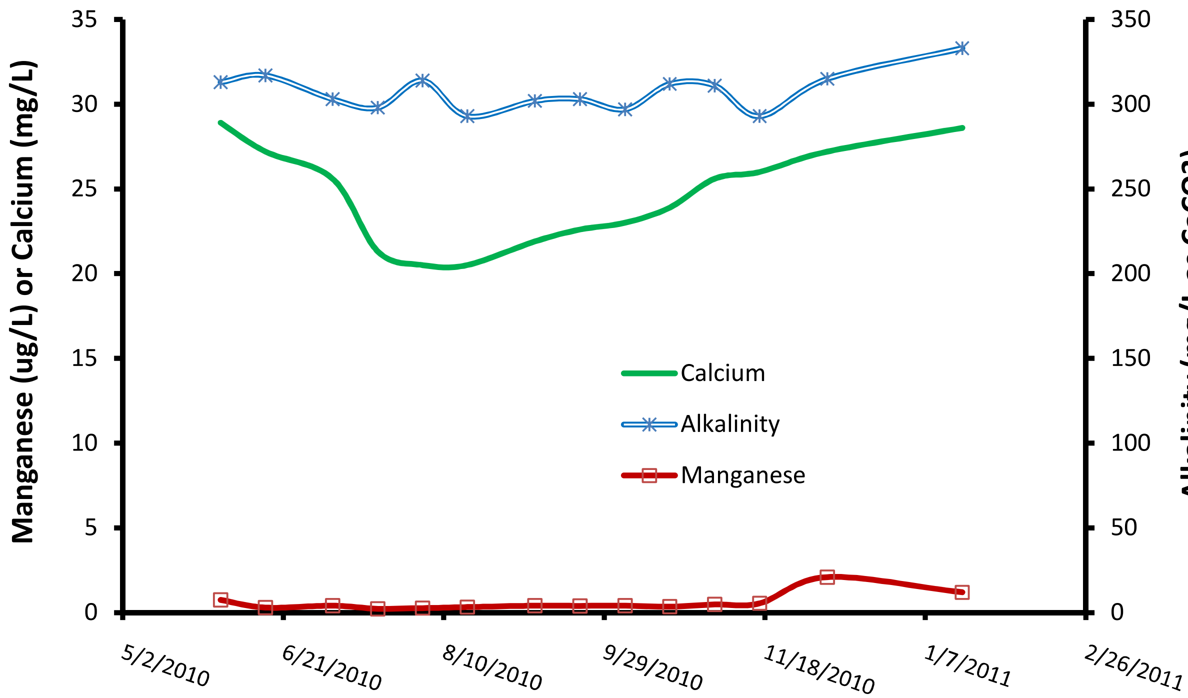


Figure 7. Seasonal changes in Area 2WX Pit surface waters for calcium, alkalinity, and manganese.

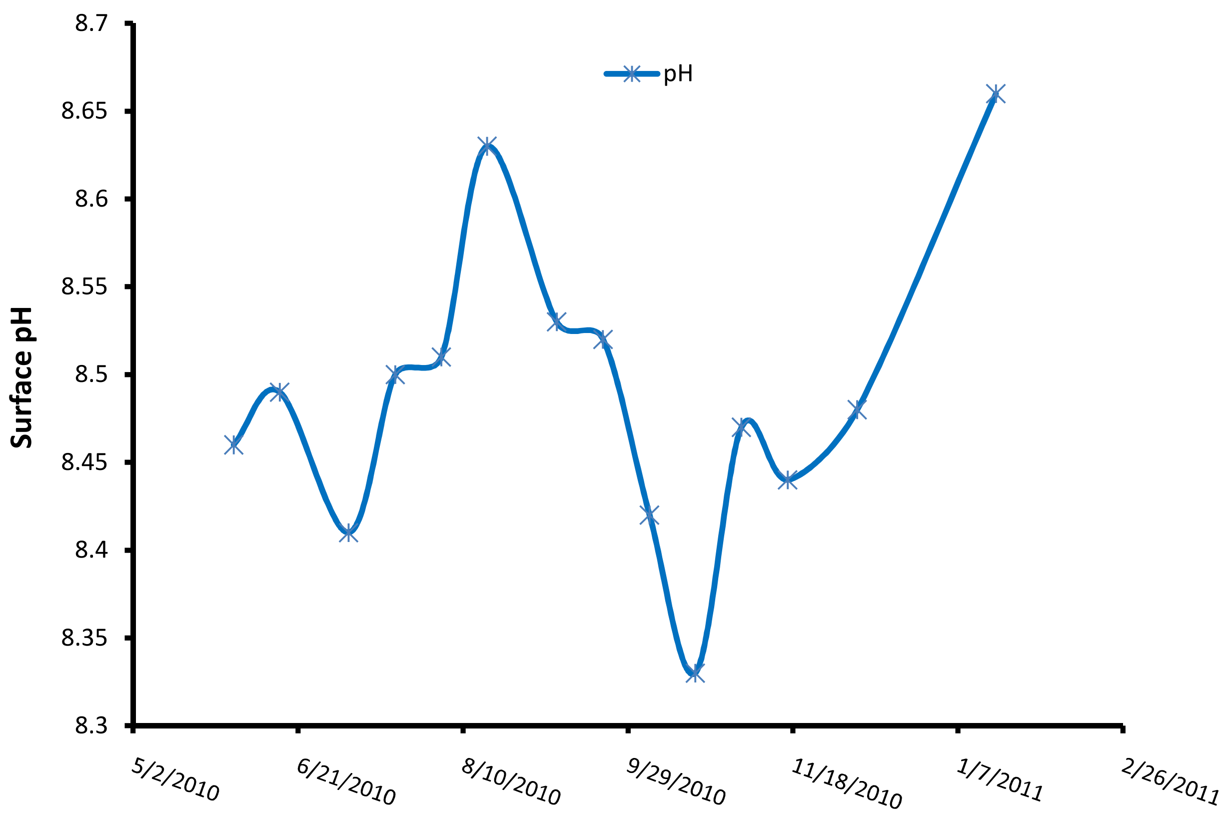


Figure 8. Seasonal changes in Area 2WX Pit surface waters for pH.

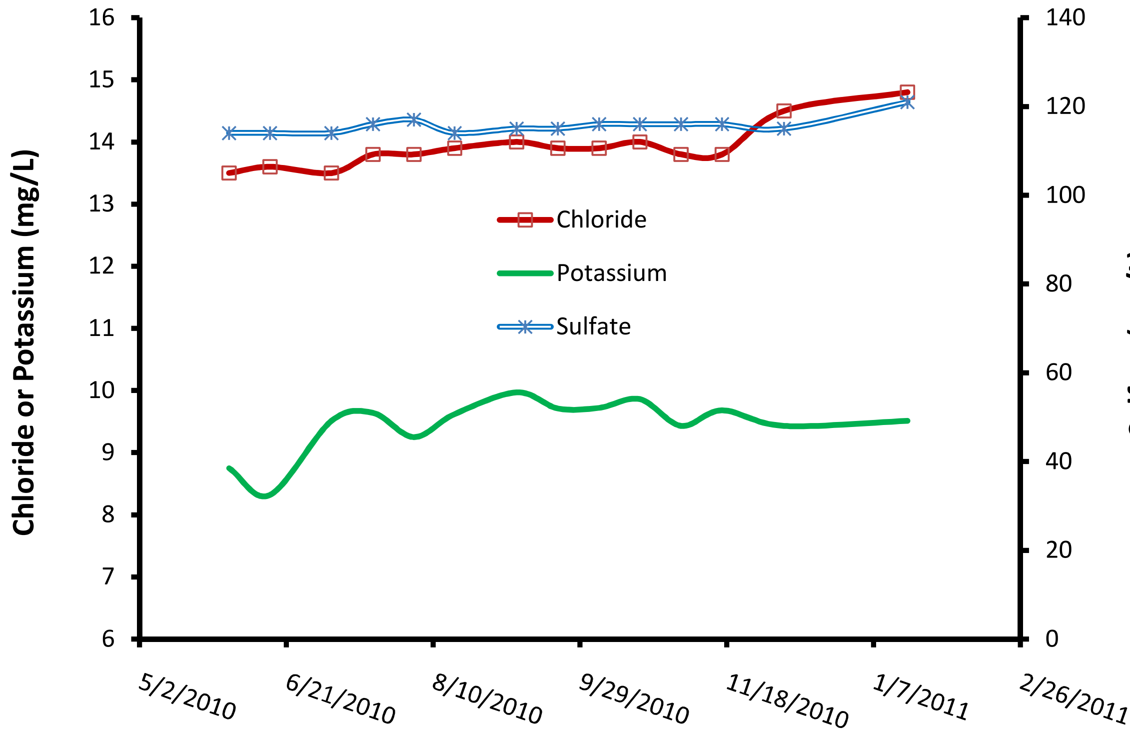


Figure 9. Seasonal changes in Area 1 Pit surface waters for chloride, potassium, and sulfate.

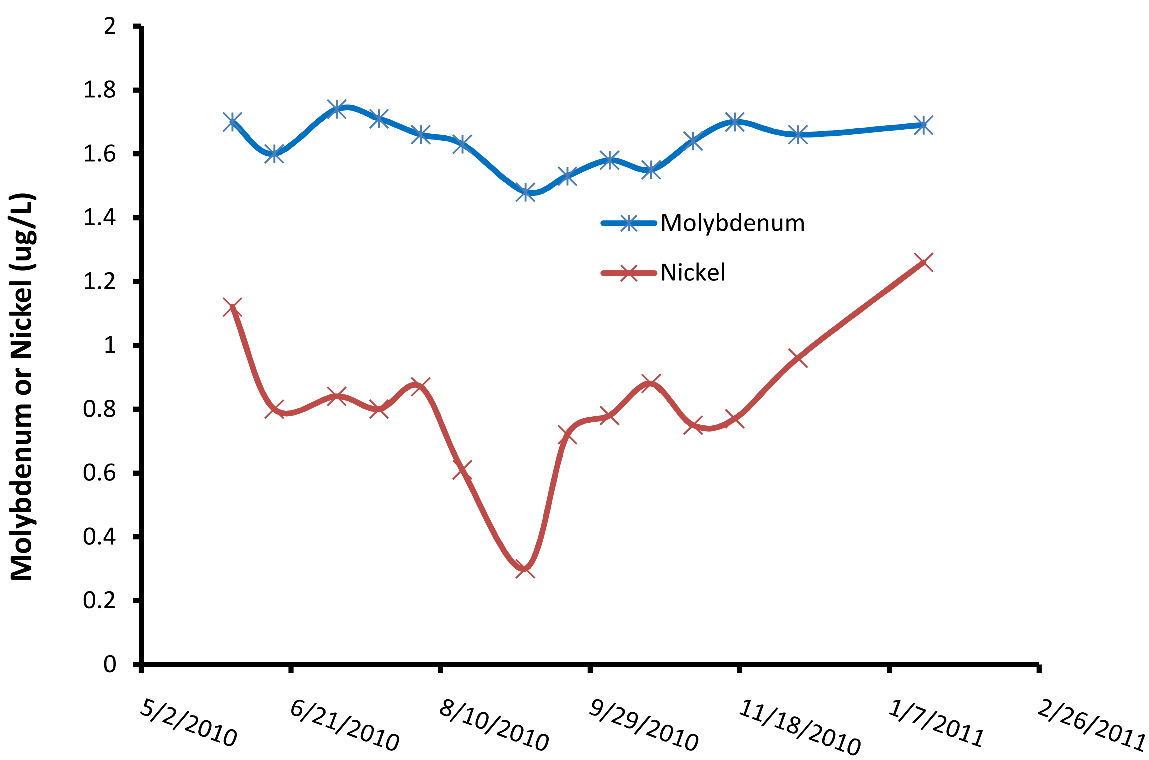


Figure 10. Seasonal changes in Area 1 Pit surface waters for molybdenum and nickel.

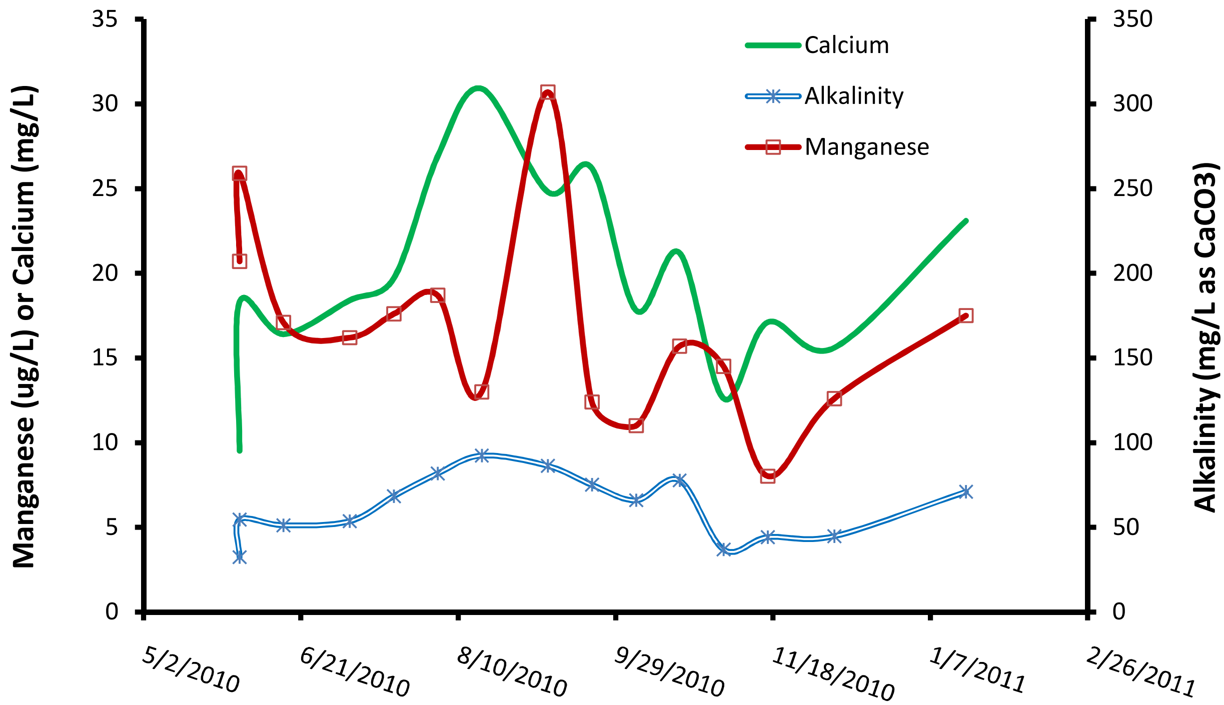


Figure 11. Seasonal changes in Saint Louis River surface waters for calcium, alkalinity, and manganese.

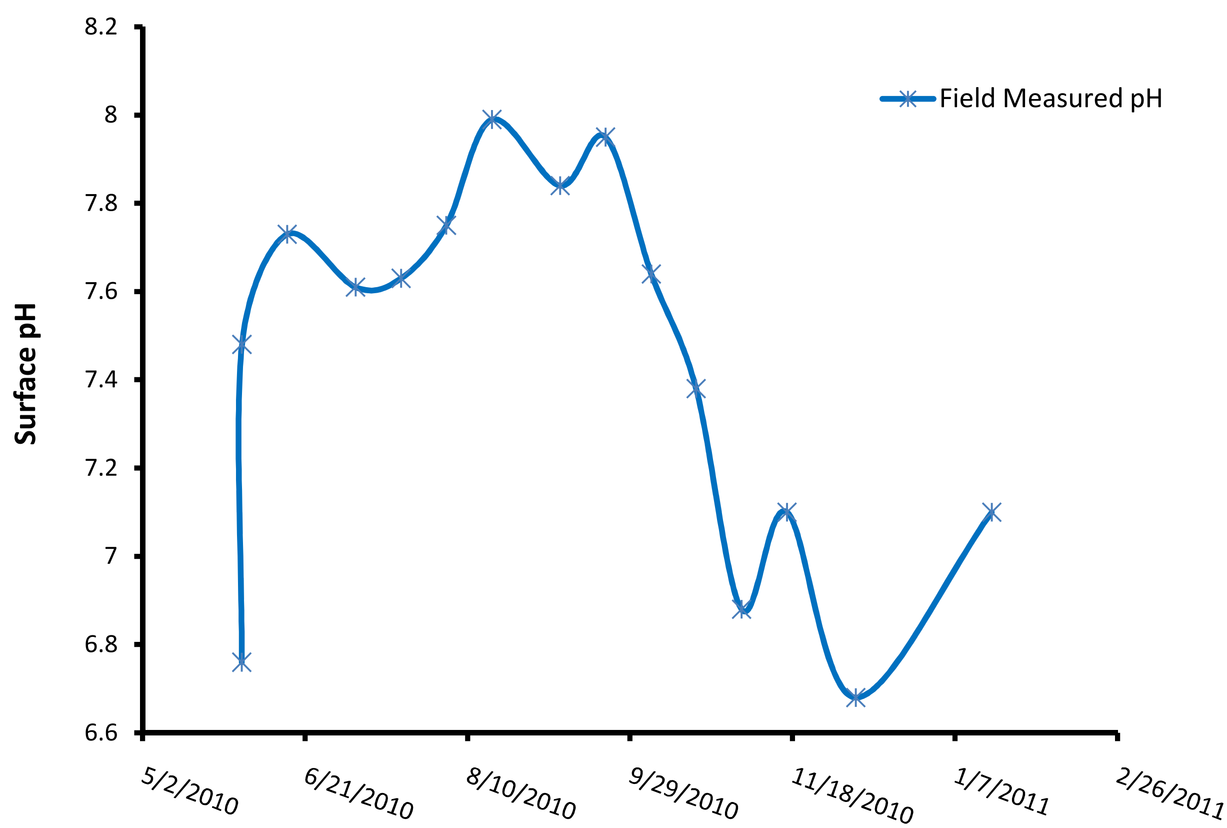


Figure 12. Seasonal changes in Saint Louis River surface water pH.

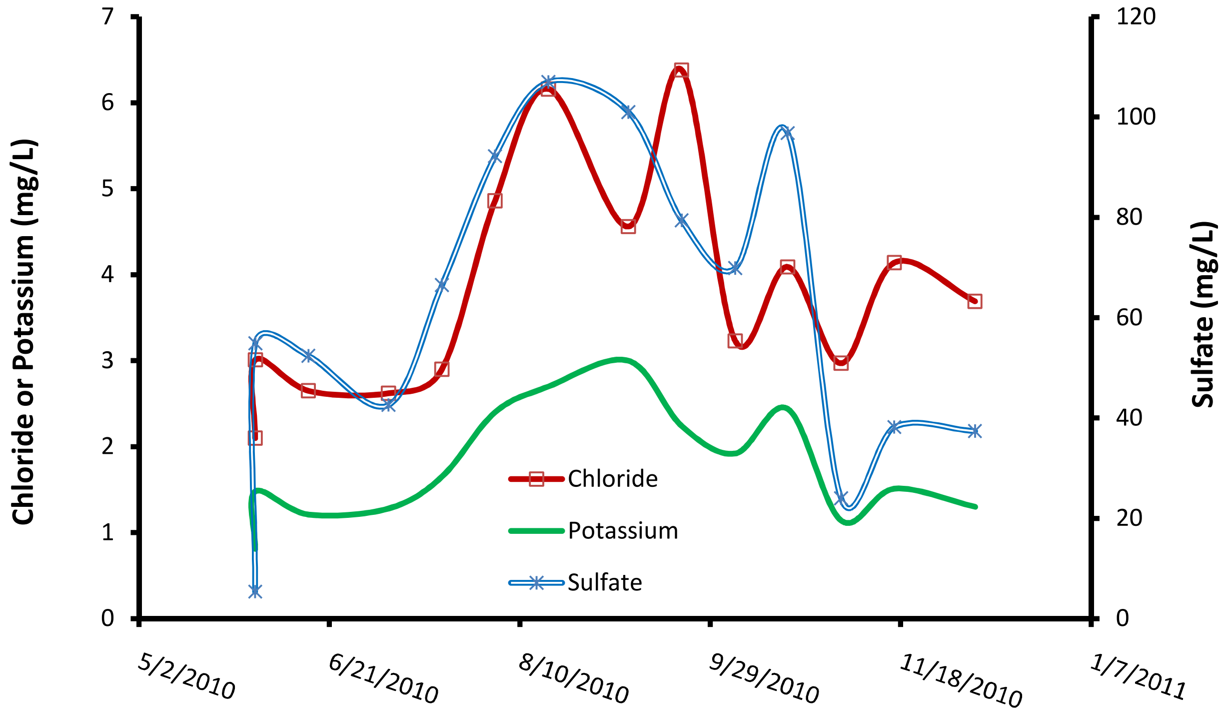


Figure 13. Seasonal changes in St. Louis River surface waters for chloride, potassium, and sulfate.

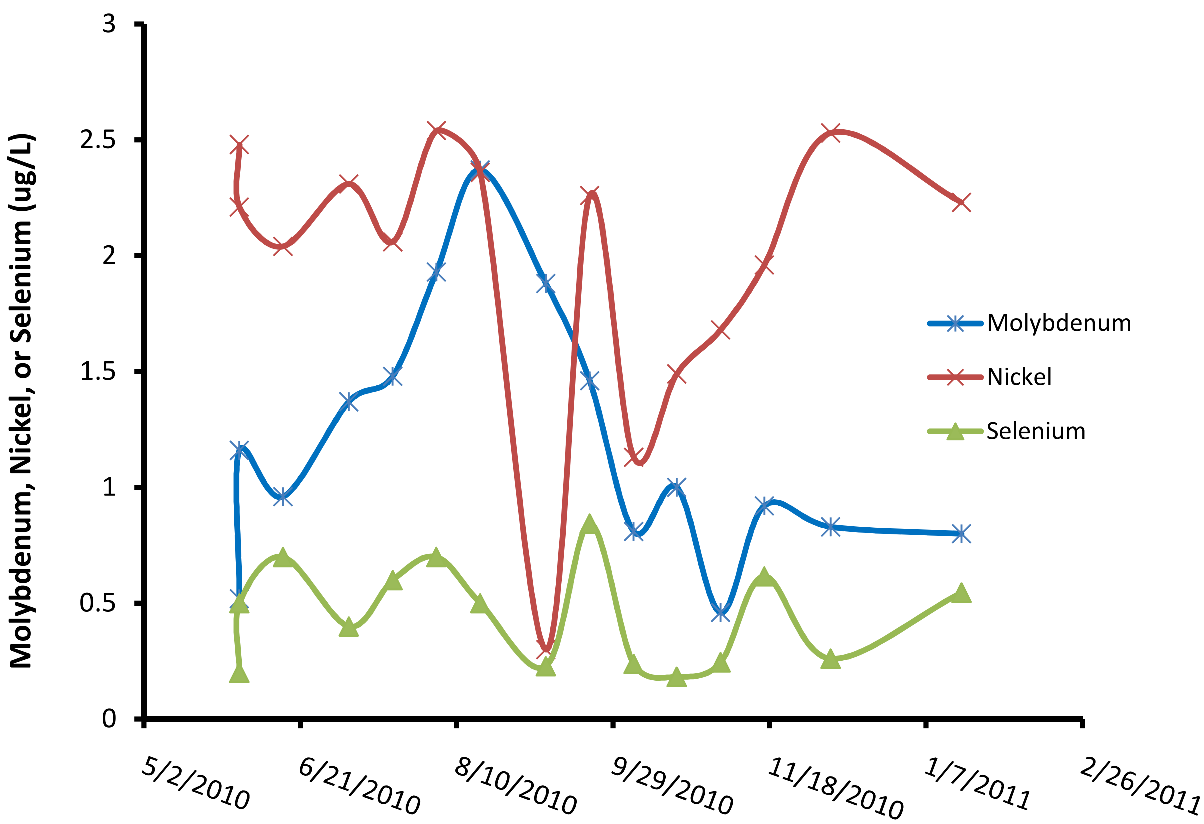


Figure 14. Seasonal changes in St. Louis River surface waters for molybdenum, nickel, and selenium.

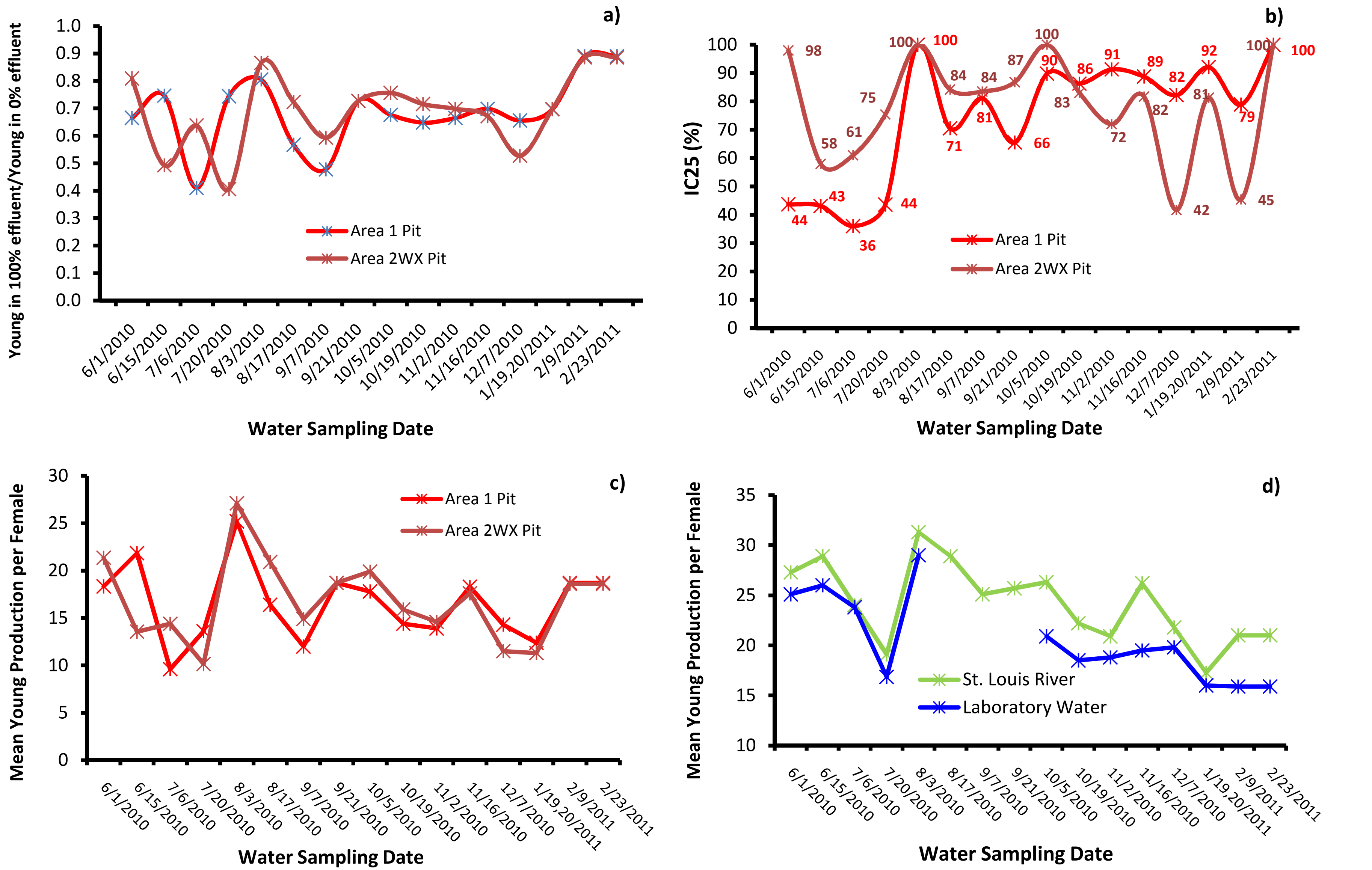


Figure 15. Results of Area 1 and Area 2WX Pit whole effluent toxicity tests conducted from June 2010 through February 2011. Results are presented as young production in undiluted pit water divided by young production in the recieiving water (a), IC25--concentration of pit water that causes a 25 percent reduction in young production compared to the control water (b), young production per female in undiluted (100 percent) pit water (c), and young production in St. Louis River and laboratory water (d).

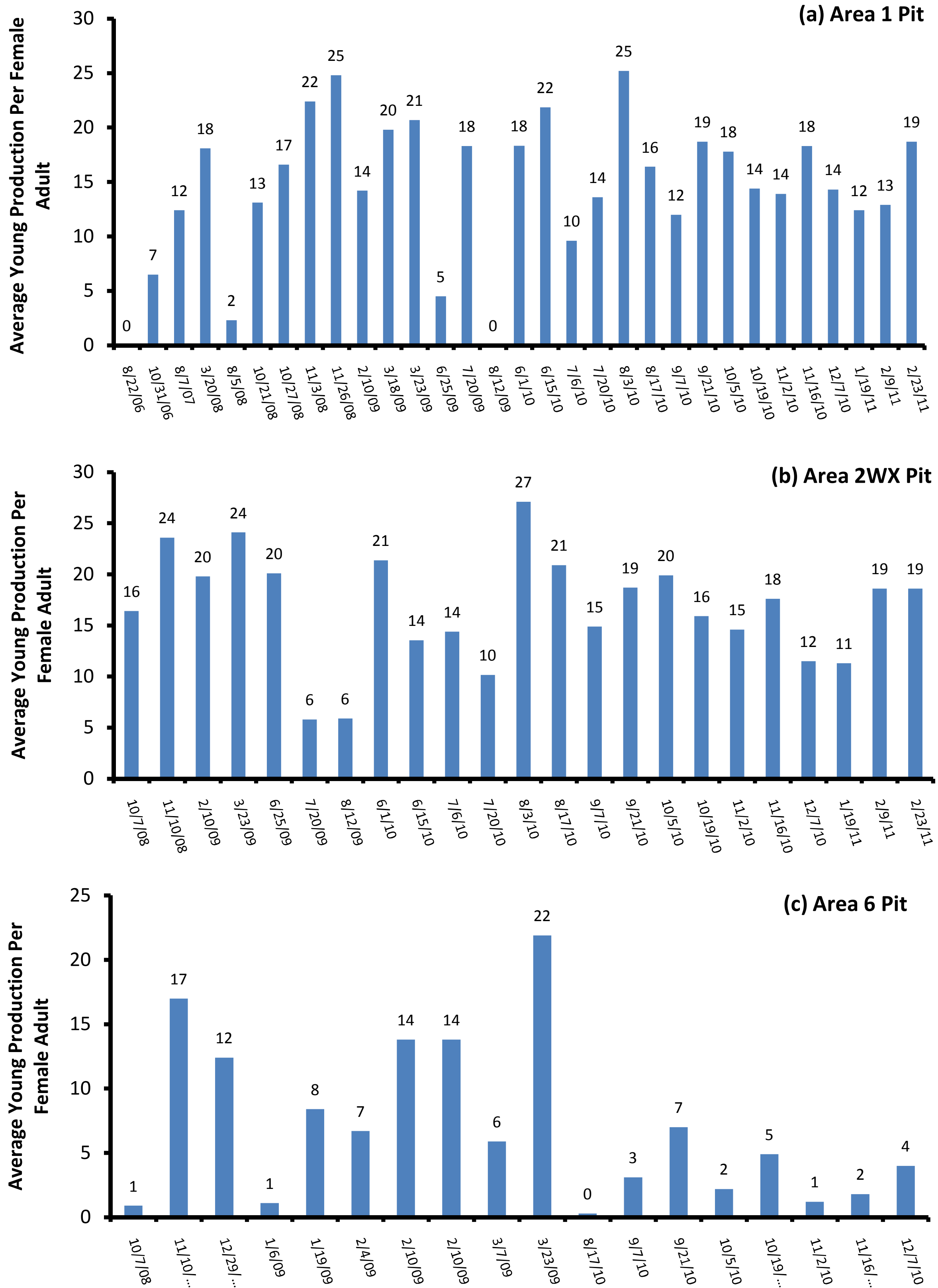


Figure 16. Demonstration of WET testing variability with pit waters for tests conducted as early as August 2006 through February 2011. Data presented are average young production for chronic *C. dubia* tests with 100 percent (a) Area 1 Pit, (b) Area 2WX Pit, and (c) Area 6 Pit water.

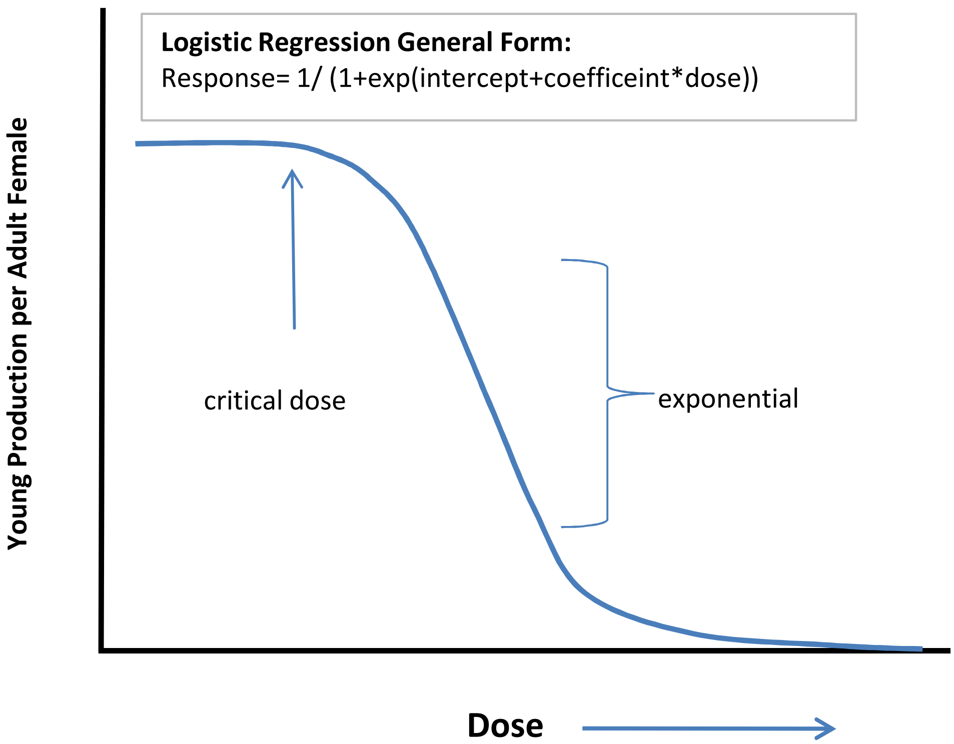


Figure 17. Characteristic shape of a dose-response curve for aquatic toxicity.

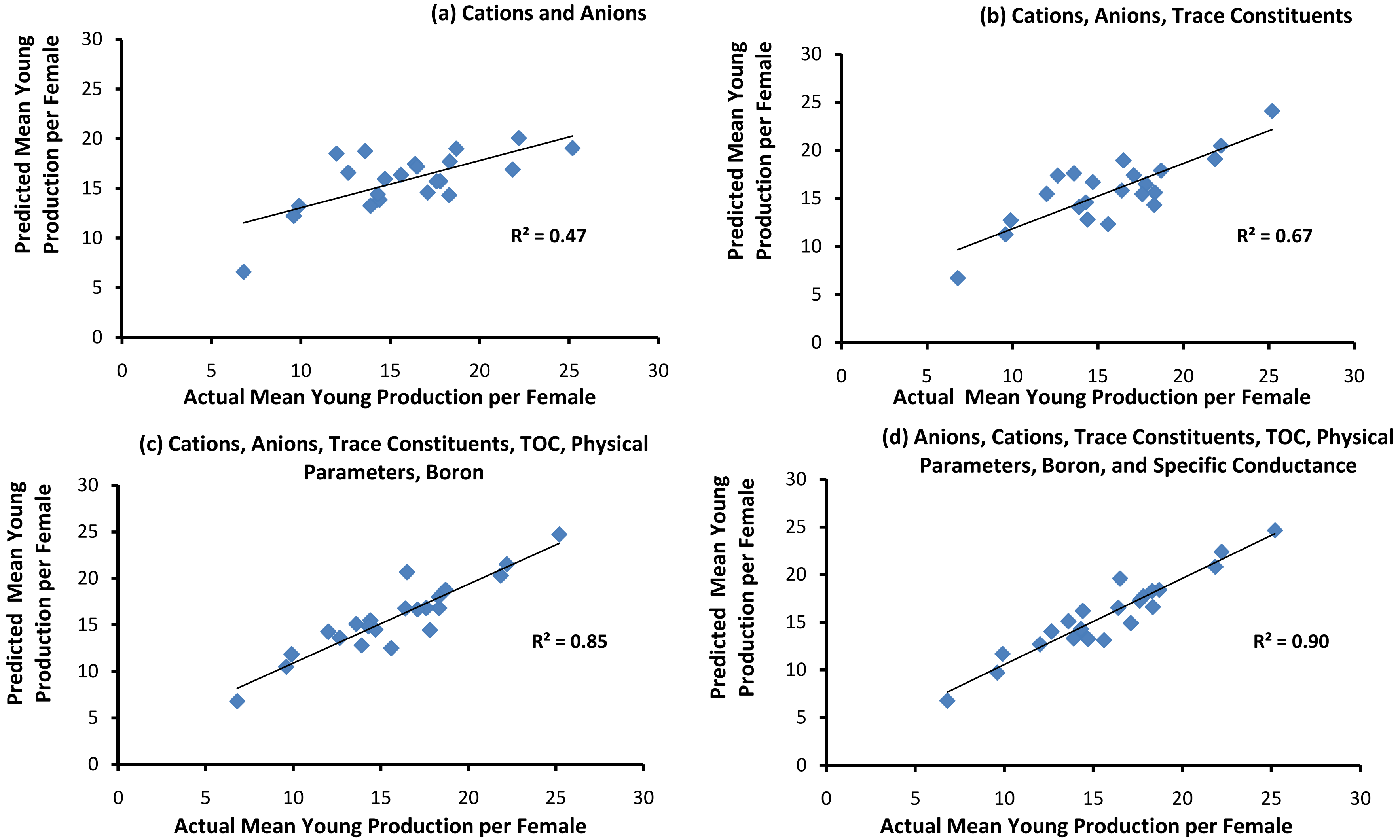


Figure 18. Predictive model results using the chemical composition and physical attributes of 100 percent Area 1 Pit water used in chronic *C. dubia* WET tests. Figures show the actual mean young production per female against the predicted mean young production per famale for four logistic models with: (a) alkalinity, chloride, sulfate, magnesium, sodium, potassium, (b) constituents in "a" plus selenium, nickel, silica, molbdenum, and manganese, (c) constituents in "a" plus "b" plus total orangnaic carbon, dissolved oxygen, field pH, temperature, and boron, and (d) constituents in "a" plus "b" plus "c" plus specific condutance.

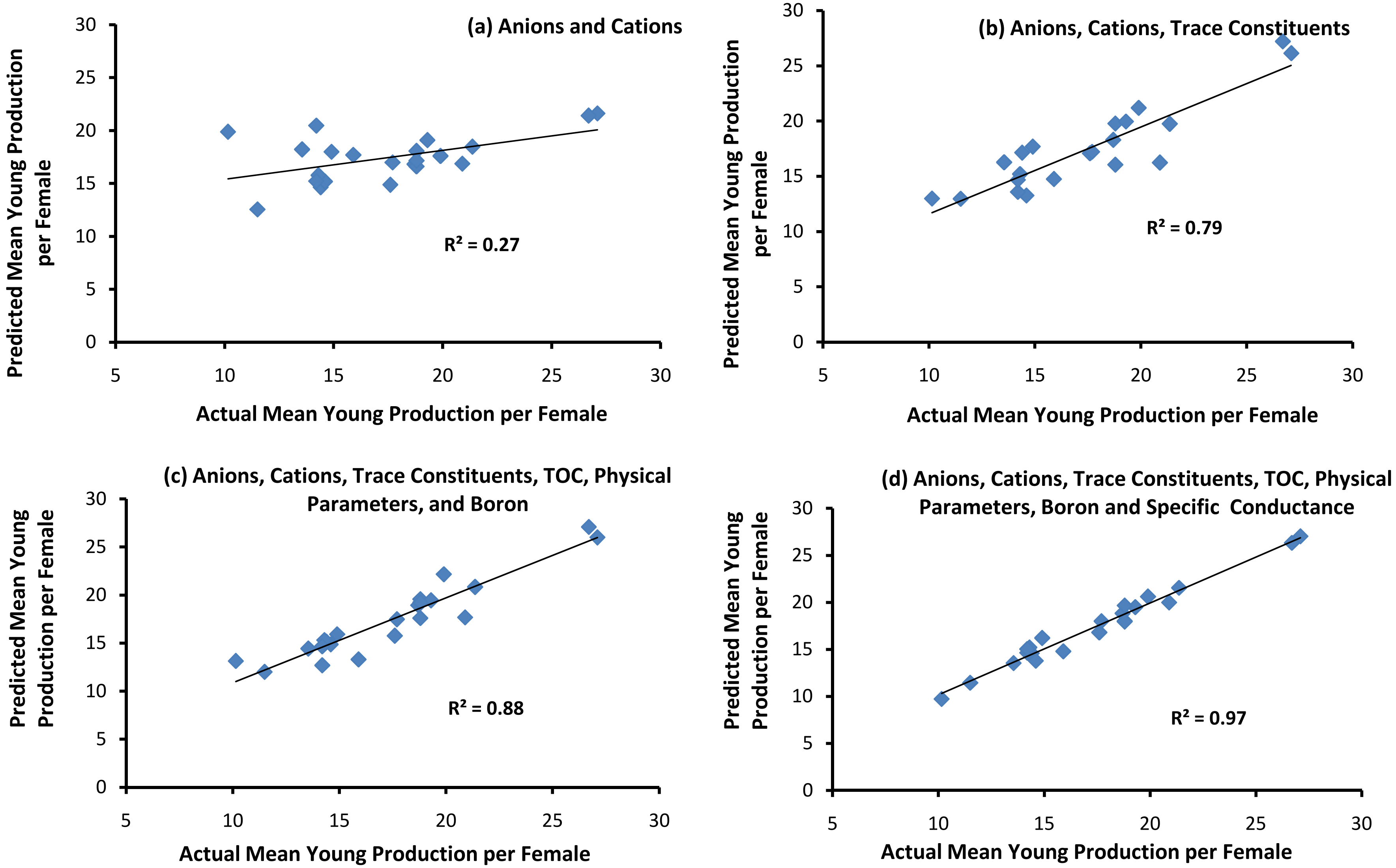


Figure 19. Predictive model results using the chemical composition and physical attributes of 100 percent Area 2WX water used in chronic *C. dubia* WET tests. Figures show the actual mean young production per female against the predicted mean young production per famale for four logistic models with: (a) alkalinity, chloride, sulfate, magnesium, sodium, and potassium, (b) constituents in "a" plus selenium, nickel, silica, molbdenum, and manganese, (c) constituents in "a" plus "b" plus total orangnaic carbon, dissolved oxygen, field pH, temperature, and boron, and (d) constituents in "a" plus "b" plus "c" plus specific condutance.

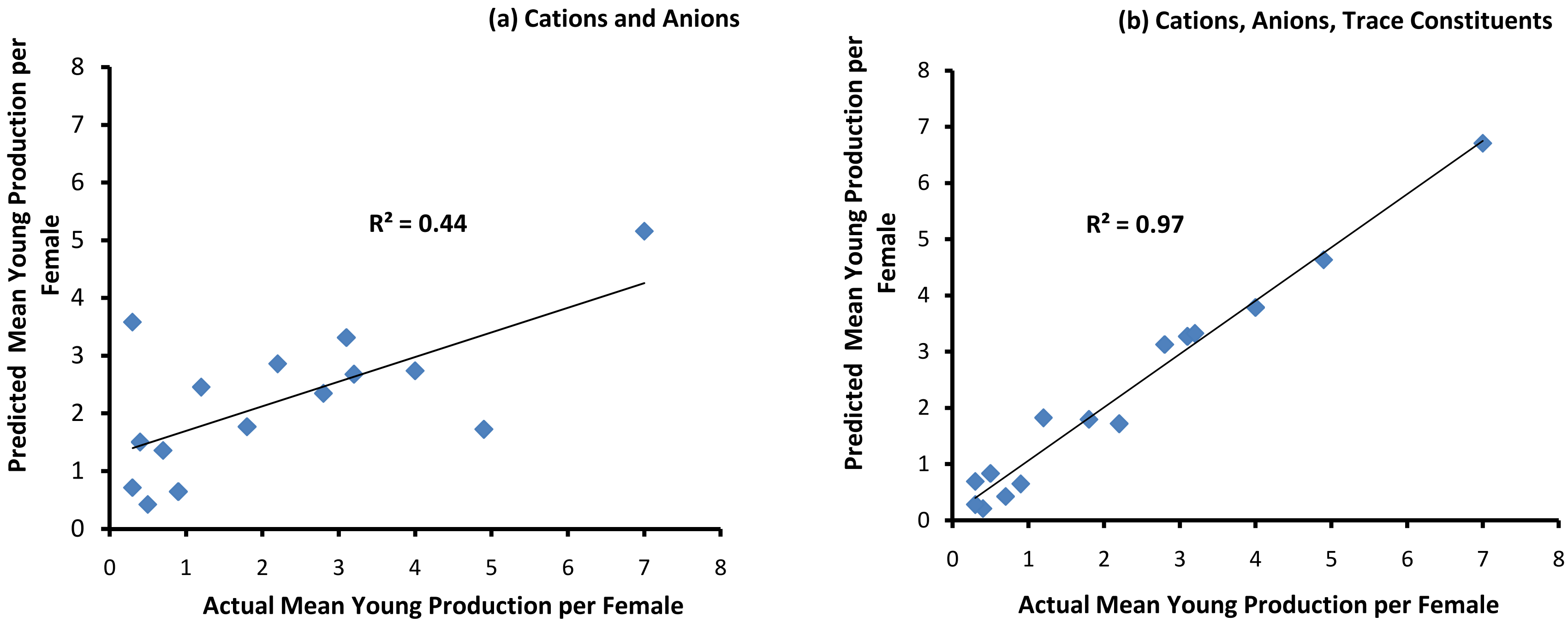


Figure 20. Predictive model results using the chemical composition and physical attributes of 100 percent Area 6 water used in chronic *C. dubia* WET tests. Figures show the actual mean young production per female against the predicted mean young production per famale for two logistic models with: (a) alkalinity, chloride, sulfate, magnesium, sodium, and potassium, (b) constituents in "a" plus selenium, nickel, silica, molbdenum, and manganese.

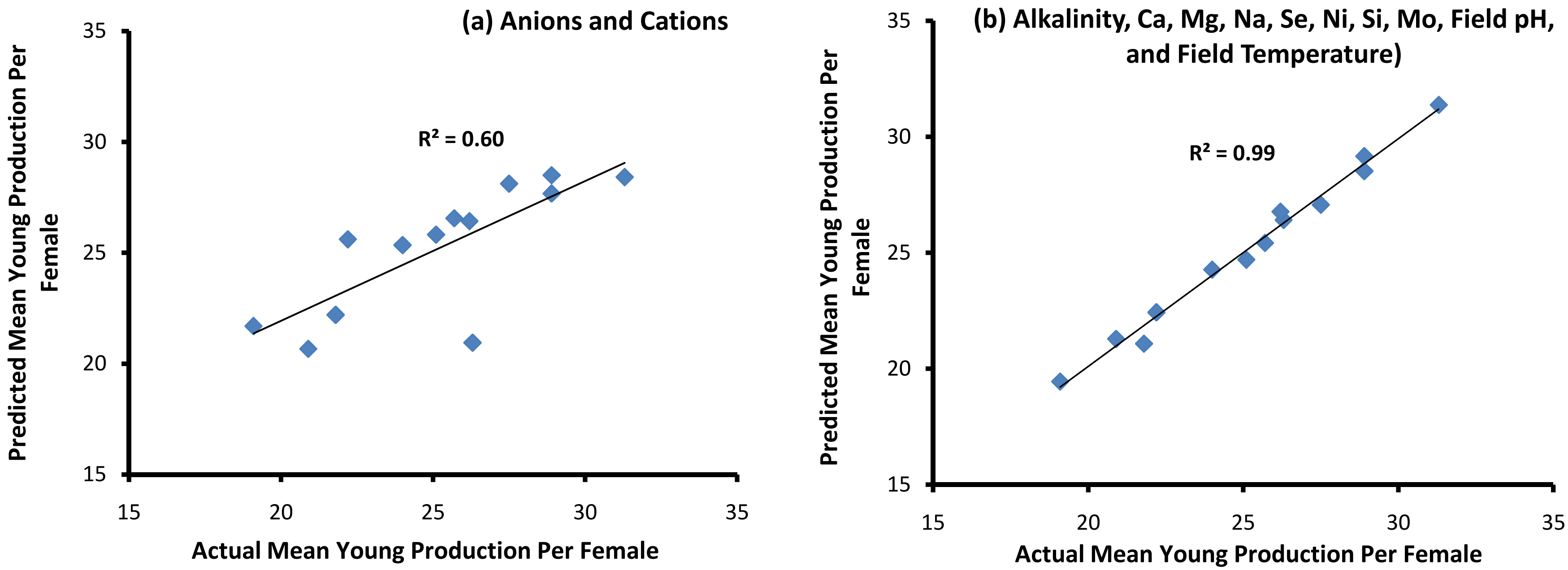


Figure 21. Predictive model results using the chemical composition and physical attributes of 100 percent St. Louis River water used in chronic *C. dubia* WET tests. Figures show the actual mean young production per female against the predicted mean young production per famale for two logistic models with: (a) alkalinity, chloride, sulfate, magnesium, sodium, and potassium, (b) alkalinity, calcium, magnesium, sodium, selenium, nickel, silica, molbdenum, filed measured pH, and temperature.

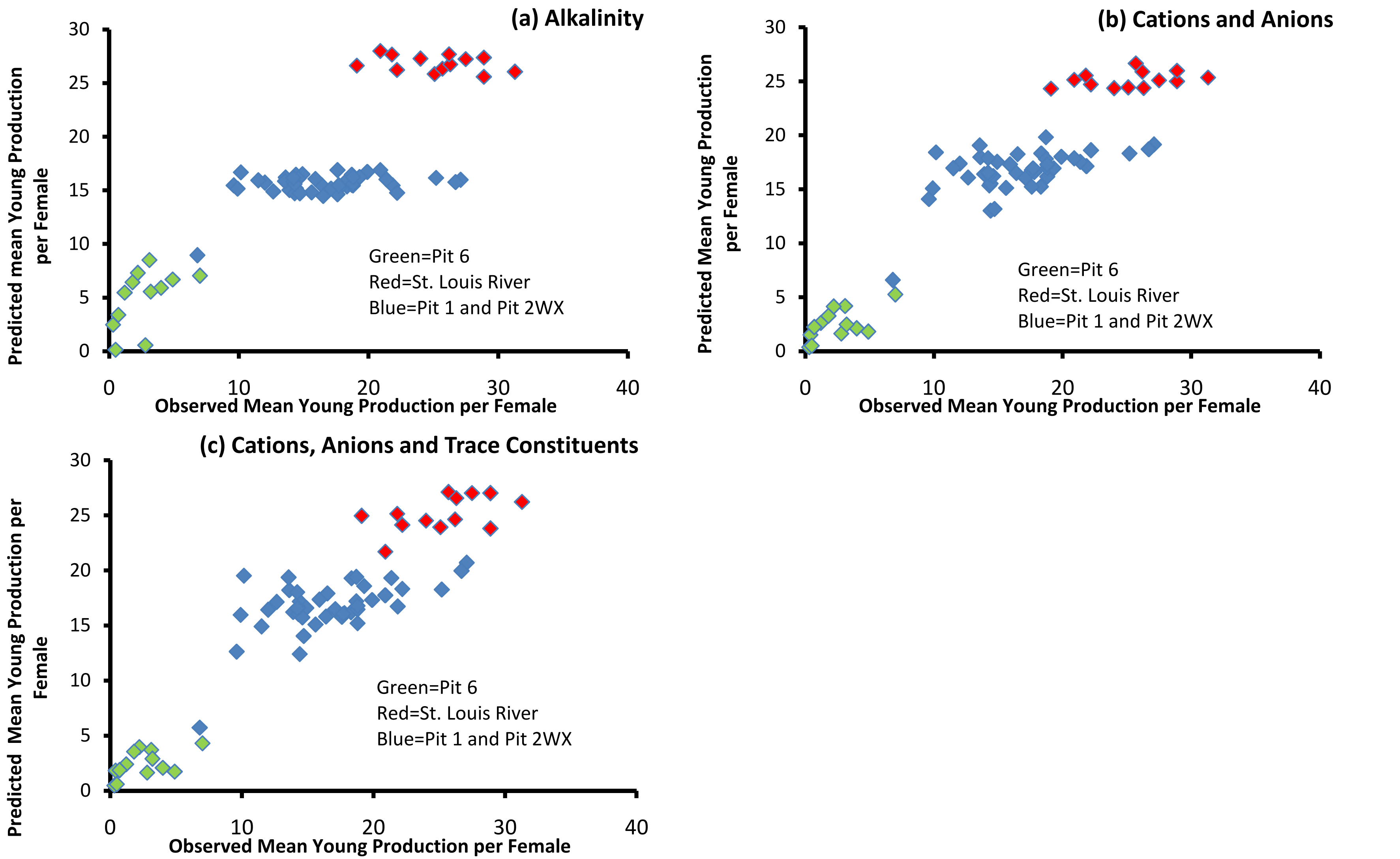


Figure 22. Predictive model results using the chemical composition and physical attributes of 100 percent Area 1, 2WX, and 6 Pits, and St. Louis River water used in chronic *C. dubia* WET tests. Figures show the actual mean young production per female against the predicted mean young production per famale for two logistic models with: (a) alkalinity, (b) alkalinity, chloride, sulfate, magnesium, sodium, and potassium, and (c) alkalinity, chloride, sulfate, magnesium, calcium, sodium, potassium, selenium, nickel, silica, molbdenum, and manganese.

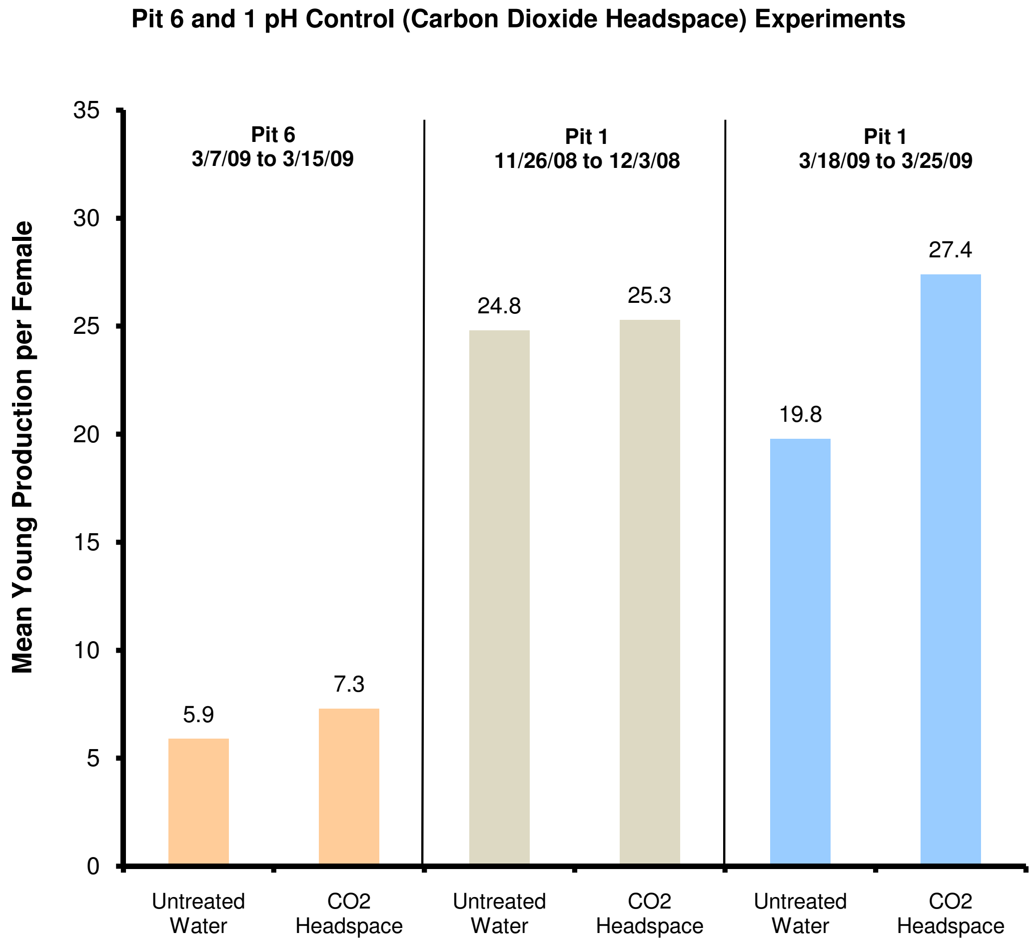


Figure 23. Effect of pH control with the use of a carbon dioxide head space on C. dubia young production. WET tests conducted with Area 1 and Area 6 Pit water.

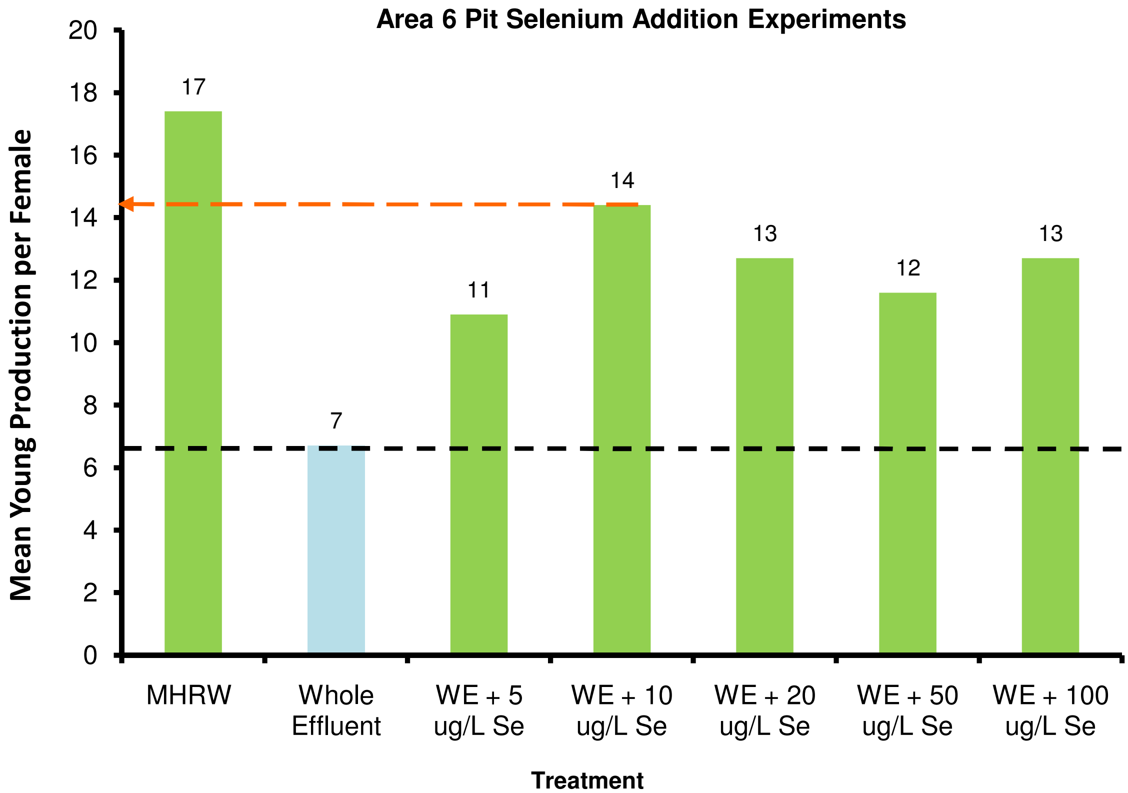
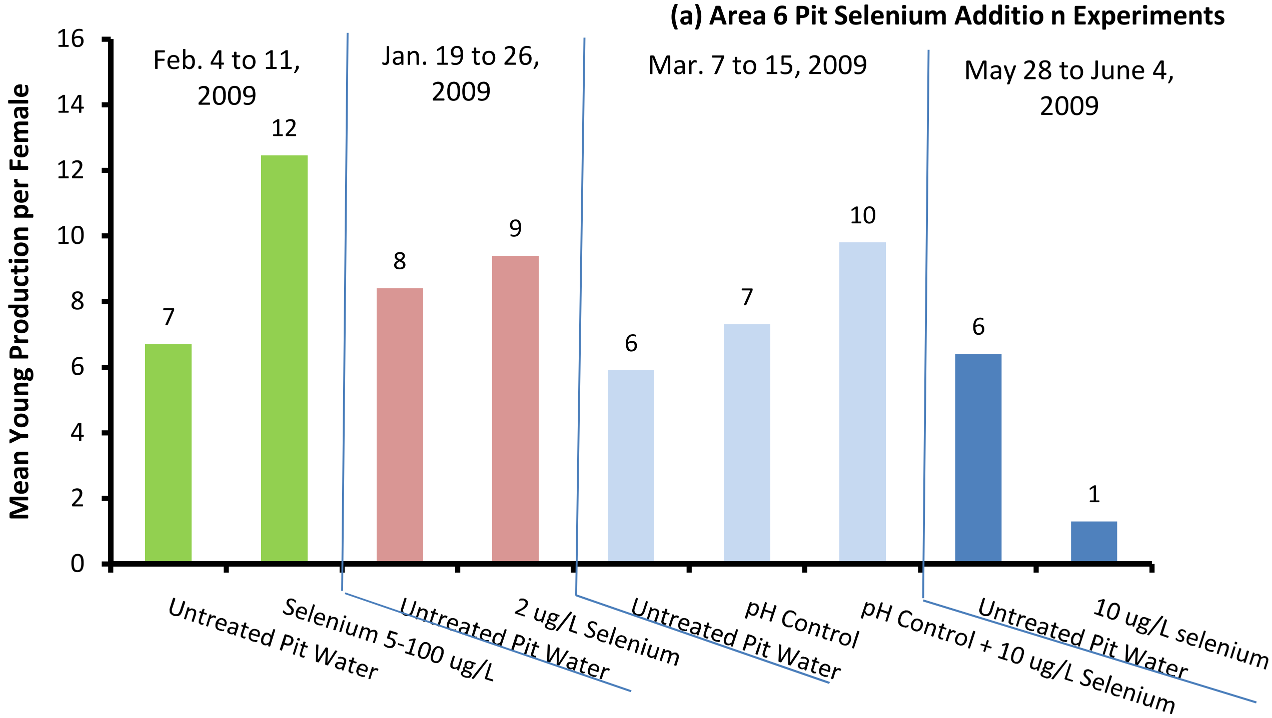


Figure 24. Effect of selenium addition to Area 6 Pit water (collected December 29, 2008) on young production per *C. dubia* female. MHRW is moderately hard reconstituted water which is commonly referred to as laboratory water and is often used as a control water.



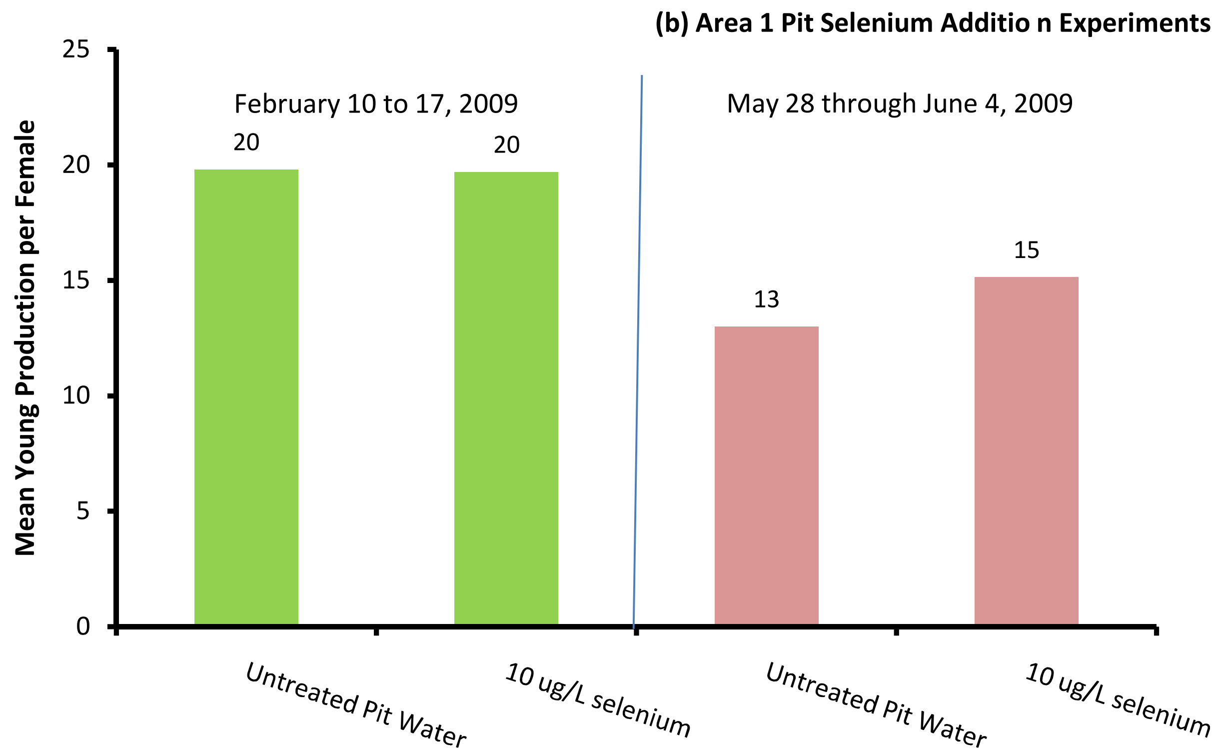


Figure 25. *C. dubia* mean young production per female with selenium addition to (a) Pit 6 and (b) Pit 1 water.

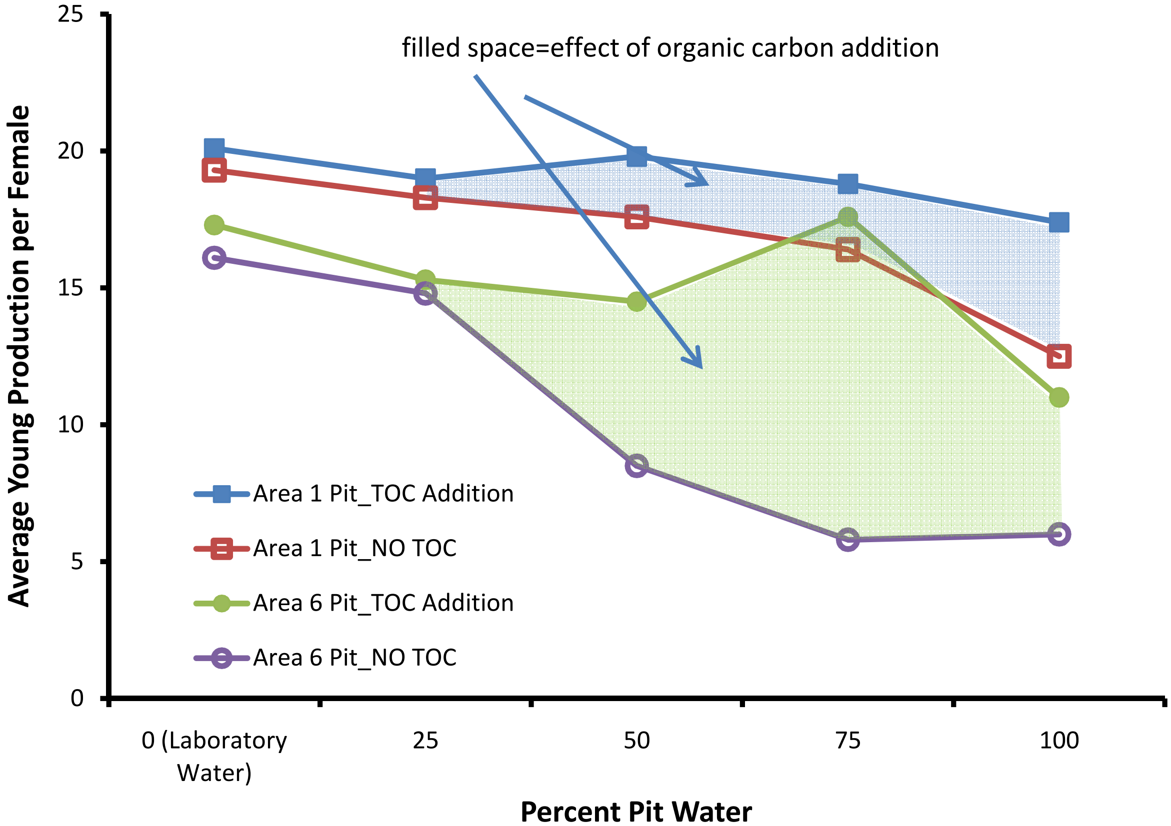


Figure 26. Average young production per female in chronic *C. dubia* WET tests with Area 1 and 6 Pit water with and without the addition of natural organic matter. Natural organic matter was from Suwannee River water in Georgia.

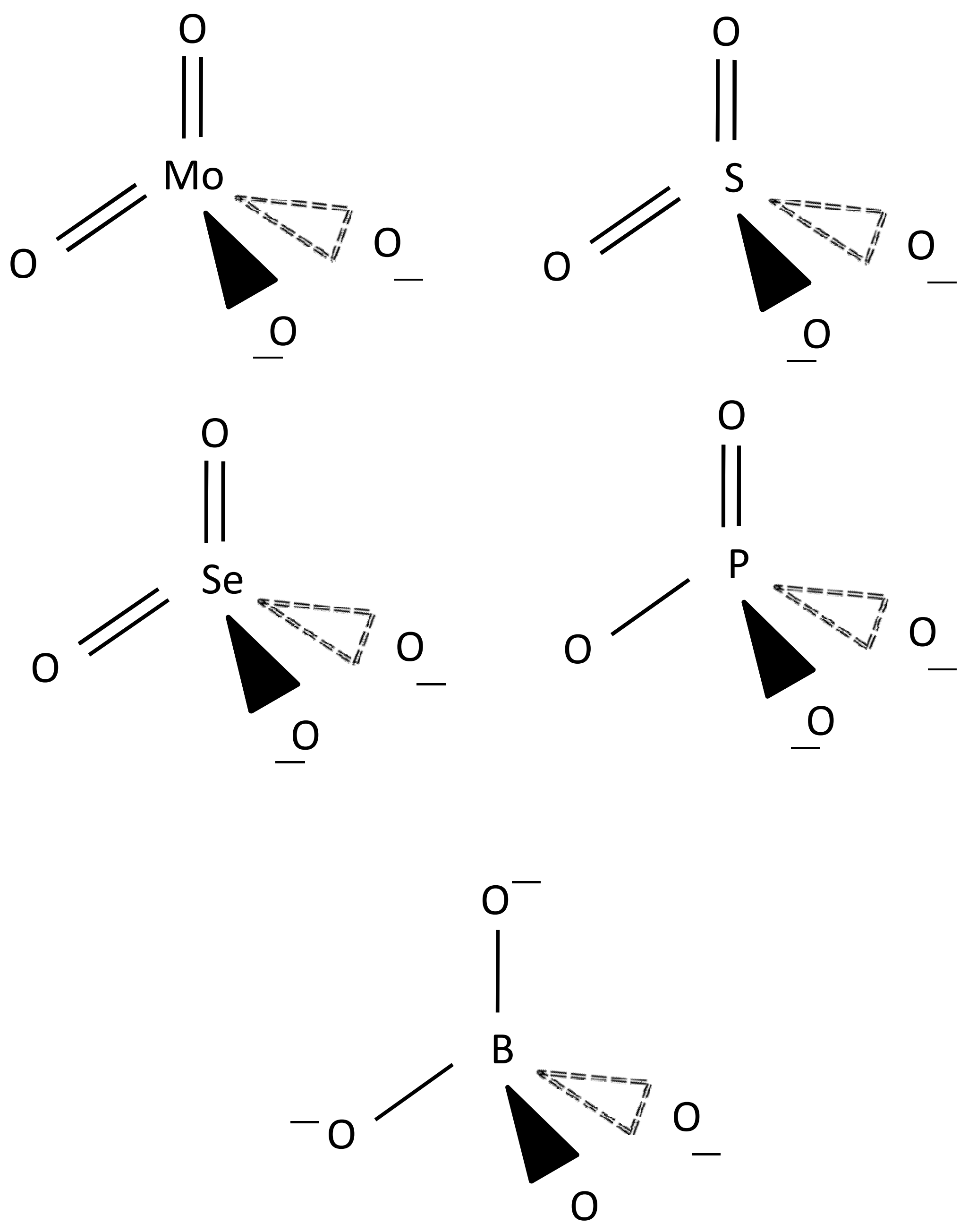


Figure 27. Three dimensional shapes of molybdate, sulfate, selenate, phosphate and borate.

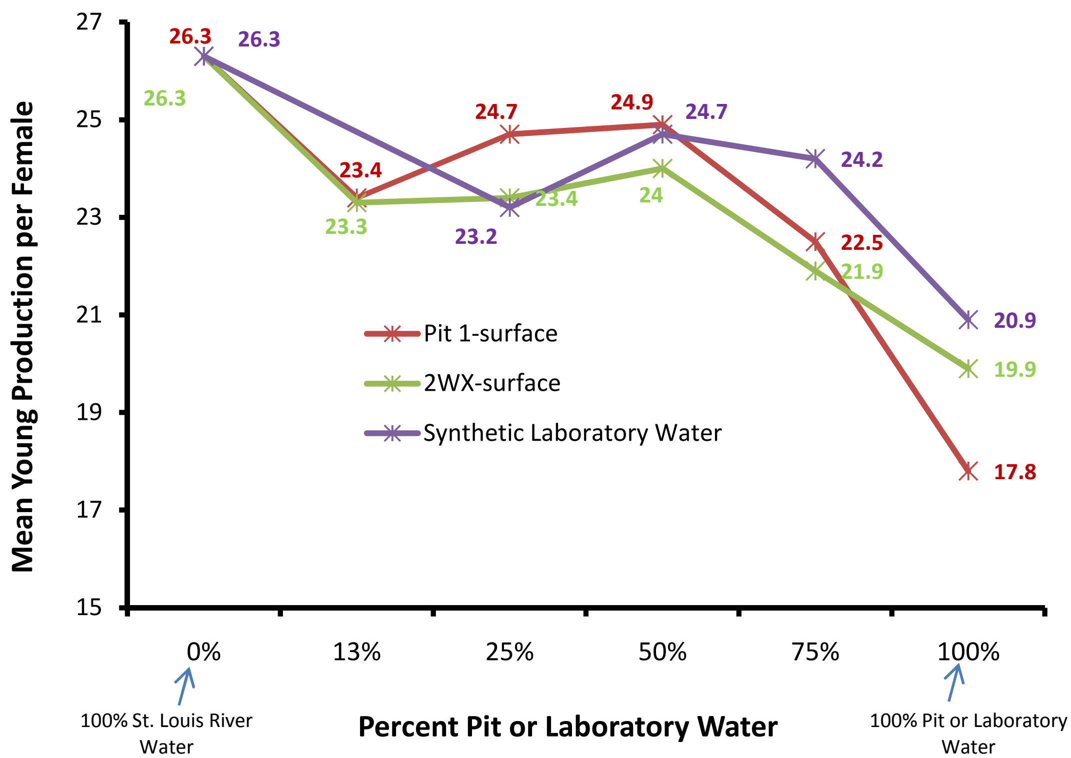


Figure 28. Results of toxicity tests conducted with Area 1, Area 2WX, and synthetic laboratory water diluted with St. Louis River water. Pit and river waters were collected on October 5, 2010.