

LEACHATE AND ENZYME BIOASSAY TOXICITY ASSESSMENT TESTS AT THE TIP TOP MINE, A marginally IMPACTED SITE¹

Jessica Moehle, James Ranville, LaDonna Choate, Thomas Wildeman, and Philippe Ross

Abstract: Over the past three years, a decision tree has been developed to rank mine waste sites for potential environmental impacts. This approach relies on simple leach tests to determine the chemical composition and toxicity of water in contact with mining wastes. When the pH of the leachate solutions is less than 5, the toxicity of the water is certain. However, when the pH of the leachate solutions is greater than 5, lower concentrations of toxic metals make toxicity assessment uncertain and a simple “in-vitro” test is necessary. These methods were used to evaluate a mine site that is marginally impacted. The Tip Top Mine in Gamble Gulch, Colorado is a high mountain site where the stream upstream of the mine is pristine and downstream of the influx of acid rock drainage, the aquatic ecosystem is marginally impacted. Aquatic toxicity assessments, made using a microbial enzyme bioassay, were conducted to determine the impact of contaminants on the stream. All tests show that the stream water upstream of the adit inflow is unimpacted. However, the stream downstream of the inflow shows concentrations of Al, Cu and Zn that are only slightly higher than acute aquatic toxicity limits. Leaching tests on stream sediment samples taken at the adit entrance show concentrations of contaminants that are also higher than toxicity limits. Simple enzyme bioassay tests, using metals sensitive bacteria, were conducted to establish the toxic response of the sediment leachate. The preliminary results show that leachate water upstream of the adit is not toxic and downstream, the leachate solution is marginally toxic. Duplicate leach tests and enzyme bioassay tests were conducted to determine the reproducibility of these approaches.

Additional Key Words: enzyme bioassay, metal contamination, mine-wastes, contaminated soils and sediments, toxicity testing

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Introduction

Recently, a simple deionized water leach and enzyme bioassay toxicity method has been developed for the assessment of mine-waste dumps (Wildeman, et al., 2003; Hageman and Briggs, 2000). The leach method has been particularly effective for characterizing marginally impacted sites (Bazin, et al. 2003). Regions with many mine-waste dumps have applied such methods to determine prioritization of site cleanup (Hageman, 2004; Heflin et al., 2004). Previously, scientists from the US Geological Survey (USGS) and the Colorado School of Mines (CSM) have compared this method with other methods to evaluate accuracy of sampling, preparation, and analytical methods (Hageman et al., 2005). In addition, for mining impacted sites where in-vitro toxicity tests are necessary, simple enzyme bioassay tests are being studied to see how well they correlate with the traditional tests that use *Ceriodaphnia dubia*. This study used MetPlate™ as a bioassay. *E. coli* is the test species and this bioassay is fairly straightforward and easy to perform. The site chosen for this study was the Tip Top Mine in the Perigo mining area located at the headwaters of Gamble Gulch in Colorado. In July of 2004, water, stream sediment, and the mine-waste dumps at the Tip Top mine were sampled. Leachate tests were conducted on the sediments and waste dump samples. The leachate solutions were then subjected to a bioassay in order to determine the toxicity of the sample.

The Tip Top Mine is an abandoned metal mining site in Gamble Gulch, a perennial stream in the Boulder Creek watershed. The spatial relationship among the sampling locations is shown schematically in Fig. 1. Perennial inflow from the adit is the major source of contamination in the stream. Downstream from the adit, other point and non-point abandoned metal mining operations cause additional contamination to the stream. In addition to the adit water, there are two mine-waste dumps on the site from which contaminated water could possibly flow into the gulch.

Over the last decade, efforts have been made to reduce the amount of water seeping into the mine tunnels and this has met with some success. The pH of the water has been raised from 3.3 to 3.7 and dissolved iron has dropped from 42 to 3.8 mg/L. Nevertheless, adit water exceeds the Colorado cold water aquatic limits for Al, Cd, Cu, Fe, Mn, and Zn (Table 1). In the stream downstream of the mine, Al, Cu, and Zn remain slightly higher than the aquatic limits.

Based on the one July 2004 visit, the mine site fits the criteria of being the first definite place on Gamble Gulch (Fig. 2 and 3) where the water is toxic to aquatic organisms. The initial phase of the study sought to answer these questions:

- Is the adit water the main source of toxicity to the stream?
- Are contaminants, potentially leached from the sediments upstream of and downstream of the stream, contributing to the toxicity?
- Could water potentially draining from the mine-waste dumps on the site contribute to the aquatic toxicity found in the stream?

This paper gives the results from this sampling event and answers the above questions.

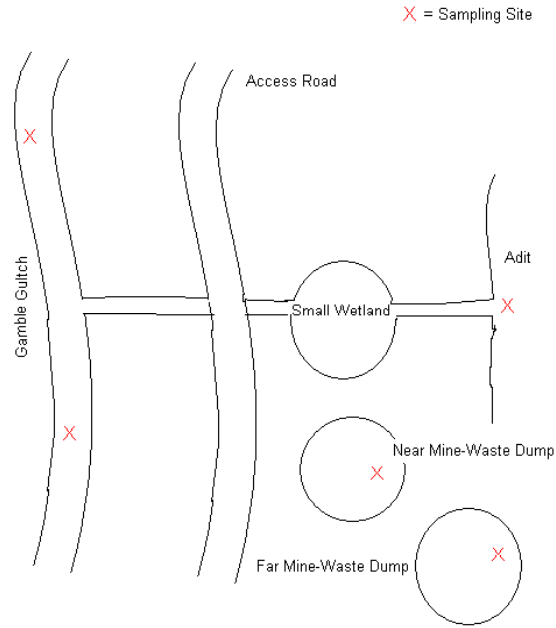


Figure 1. A plan view of the Tip Top mine site showing the spatial relationship among the sampling sites. The distances are not to scale.

Table 1. Concentrations of contaminants in mg/L in the waters at the Tip Top site and values of aquatic stream standards. B.D.L. indicates a value found lower than detection limits. The shaded regions indicate concentrations that are higher than the Colorado water limits.

Tip Top and Gamble Gulch Waters							
(mg/L)							
Element	Det. Limits	Stream Stds.		UPSTREAM FROM ADIT	ADIT WATER	ROAD WATER	DOWNSTREAM FROM ADIT
		Aquatic	Toxic				
Ag	0.0014	0.00015	5	B.D.L	B.D.L	B.D.L	B.D.L
Al	0.0173	0.1		B.D.L	5.4	4.9	0.77
As	0.0610	0.05	5	B.D.L	B.D.L	B.D.L	B.D.L
Be	0.0001	0.6		B.D.L	0.002	0.002	0.000
Cd	0.0015	0.005	1	B.D.L	0.011	0.011	0.003
Cr	0.0039	0.125	10	B.D.L	B.D.L	B.D.L	B.D.L
Cu	0.0015	0.01		0.004	0.199	0.185	0.046
Fe	0.0044	1		0.156	3.8	1.4	0.24
Mn	0.0005	1		0.001	2.7	2.6	0.72
Ni	0.0035	0.2		B.D.L	0.055	0.059	0.016
Pb	0.0137	0.05	5	B.D.L	B.D.L	B.D.L	B.D.L
Se	0.0504	0.05	1	B.D.L	B.D.L	B.D.L	B.D.L
Zn	0.0013	0.1		0.048	1.65	1.66	0.48



Figure 2. Gamble Gulch upstream of the inflow from Tip Top adit. Note that the streambed is filled with organic litter and small cobbles that are free of precipitates.



Figure 3. Gamble Gulch downstream of the inflow from the Tip Top adit. Note that the streambed is completely covered with Al and Fe oxyhydroxide precipitates.

Sampling and Analytical Methods

In this study three methods for assessing a mine site were utilized. These include: 1.) composite sampling; 2.) mine waste leaching procedures using deionized water; and 3.) microbial bioassays of leachates for metal toxicity.

Sampling Methods

During the July 2004 visit, composite samples of the stream sediment were collected upstream and downstream of the point of adit inflow. The method for taking a composite sample developed by Smith et al. (2000) calls for dividing the site into 30 sections of roughly equal area and then taking a sample from each section. In the actual securing of a composite sample, these roughly 30 equal sections are modified by what portions of the streambed and waste dump are accessible. The study by Hageman et al. (2005) provides evidence that the elemental concentrations from leachate tests on duplicate composite samples will be within a factor of four. Water samples were taken from the gulch approximately 200 m upstream and downstream of the adit inflow. In addition, the water flowing from the mine was sampled at the adit and at a point before the water flows through a culvert under a road about 100 m from the adit and 200 m from the gulch.

Analytical Methods

For the water samples pH and Eh was determined using a portable millivolt meter (Orion) with pH values considered accurate to ± 0.1 units. Alkalinity was determined in samples having a pH greater than 4.0 by titration to the bromocresol green endpoint (pH about 4.5) using an alkalinity kit (HACH).

The composite mine-waste dump samples were split and portions were used to perform leaching tests that are integral to the Assessment Decision Tree that has been devised for mine-waste dump (Wildeman et al., 2003). The three leachate tests are described below.

Colorado Division of Minerals and Geology (CDMG) Test This test by Herron et al. (2001) of the Colorado Division of Minerals and Geology uses a volume basis to determine the potential for metal release from soils when exposed to surface water. The procedure is as follows: 150 mL of whole sediment sample are placed into an 800 mL plastic beaker and 300 mL of deionized water is added. The sample is stirred vigorously for 15 seconds and then the beaker is covered with Parafilm. The contents are allowed to settle for 90 minutes. After this time, approximately 10 mL of leachate is filtered with a 0.45 μm filter, acidified with HNO_3 acid and analyzed using ICP-AES. Also after 90 minutes, the pH, Eh, ionic conductivity, and alkalinity are measured on the leachate.

United States Geological Survey (USGS) Field Leach Test This USGS test uses a mass basis to determine the potential for metal release from soils when exposed to natural waters (Hageman and Briggs, 2000). Fifty g of <10 mesh sediment sample are massed into a 1 L Nalgene® bottle. Approximately 1 L deionized water is added slowly so that no dust would be lost. The bottle is capped and vigorously hand shaken for 5 minutes. The contents are then allowed to settle for 10 minutes. The leachate is then filtered with a 0.45 μm filter, acidified with HNO_3 acid and analyzed using ICP-AES. The pH of the sample is also measured after 10 minutes.

Toxicity Characteristic Leaching Procedure (TCLP) This test is a modified version of Method 1311 developed by the U. S. Environmental Protection Agency (USEPA, 1992). The test as originally conceived by the EPA was to test metals mobility in landfills. Here, the test

determines the mobility of metal in the presence of mildly acidic waters. It also closely approximates the carbonate mobility step that is performed in sequential leaching studies (Tessier et al., 1979). An extraction fluid is prepared by adding 5.7 mL of concentrated glacial acetic acid to 500 mL of water. Then 64.3 mL of 1 N NaOH is added to the solution and the solution was brought to a volume of 1 L using deionized water. The pH of this solution should be 4.93 ± 0.05 . A volume of 40 mL of this extraction fluid is added to 2.00 g of < 80 mesh sediment sample in a 125 mL Nalgene® bottle. The bottle is then agitated end over end using a rotary tumbler for 24 hours. The leachate is then filtered with a 0.45 µm filter, acidified with HNO₃ acid, and analyzed using ICP-AES.

The CDMG and USGS test both use deionized water; however the most notable difference in the two tests is the use of volume versus mass. The USGS test uses a 20:1 mass ratio of water to solid, which is the ratio used for the regulatory EPA extraction tests (USEPA, 2001). The volume ratio for the CDMG test is 2:1 of water to solid, which would normally be less than a 2:1 mass ratio. Also, in the USGS test, the water is in contact with the solid for a total of 10 minutes, whereas the water is in contact with the solid for 90 minutes in the CDMG test. These procedural differences cause the CDMG test to leach more ions from the solid than the USGS test. For the modified TCLP test, there is a question of whether the acetate extraction solution should be at a pH of 3 or 5. Because this is a not a strict regulatory procedure it was decided to use a pH of 5 because the results would possibly produce a difference from the other two tests. Also, most sequential extraction procedures use an acetate solution buffered to a pH of 5 as one of the steps (Tessier et al., 1979). The primary objective of this study was to evaluate these method differences and how they relate to a bioassay.

Elemental Analyses Water samples and the leachate solutions were analyzed for elemental concentrations using ICP-AES by the CSM laboratory (Golden, CO). Approximately 10 mL of filtered sample, acidified with nitric acid, is required. The samples are then analyzed on a Perkin Elmer Optima 3000 ICP-AES for the following 31 elements: Ag, Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Se, Si, Sn, Sr, Ti, V, and Zn. All concentration results are given in mg/L. During the ICP-AES analysis, an internal standard of Sc is used to correct for variations in sample uptake and plasma conditions. Also, concentration check standards are analyzed in the beginning and after every 20 samples to monitor the stability of all analytical conditions. Results on collocated water samples show the relative standard deviation of a concentration value is about 5 % as long as the concentration is 10 times the limit of detection.

Bioassay A bioassay is a toxicity test that uses a biological specimen. Bioassays can be helpful and employed to determine the bioavailability (the availability of a compound to an organism through mechanisms such as ingestion or absorption) of the toxic metals present in a sample. Metals are introduced to aquatic systems through the very nature of the mining procedures, such as the use of large amounts of process water, the exposure of large quantities of waste material to the weathering environment, and the fact that mines are often close to surface waters.

The bioassay used in this study was the MetPLATE™ test. MetPLATE™ is a microbioassay that uses a mutant *Escherichia coli* strain sensitive to toxic metals and the property of selective inhibition of the activity of hydrolase enzyme B-galactosidase (Bitton, Jung & Koopman et al. 1994). It utilizes inhibition of the biosynthesis of an enzyme in *Escherichia coli* and is a rapid assay that is not sensitive to toxic organic compounds (Bitton, Jung & Koopman et al. 1994). In general, when compared to *Daphnia* bioassays, the sensitivity of this enzyme test to metals is of

the same order of magnitude or lower (Apartin & Ronco et al. 2001). The procedure involves serial 50:50 dilutions of sample in order to determine the relationship between sample concentration and toxic effect.

In the first step of the procedure, 0.9 mL of each sample solution is placed in a clean centrifuge tube with a cap. The bacterial reagent (freeze-dried *E. coli*) of the MetPLATE kit is rehydrated with 5.0 mL of the diluent (moderately hard water), shaken and allowed to incubate at room temperature for 15 minutes. During this time, 0.9 mL of the positive control (toxic CuSO_4 solution), is placed in a centrifuge tube. Then 0.1 mL of the reconstituted bacteria is added to the control and all of the sample tubes. All the tubes are incubated for 90 minutes at 35°C. After this incubation, 0.2 mL aliquots of all tubes were placed, into the 96-well microplate via the pre-planned order of samples and blanks. The chromogenic substrate (color indicator) is then rehydrated with 10 mL of buffer (enzyme substrate) and shaken for 30 seconds. Then 0.1 mL of the reconstituted substrate is added to the desired wells of the microplate with a multi-pipettor. The microplate is finally incubated for an hour or until an intense purple color is developed. The intensity of the resulting purple gives an indication of enzyme activity and is inversely proportional to the sample toxicity. Absorbance is measured at 575 nm using a microplate reader (Multi-scan with Pathlength Correction).

Results

The ICP-AES results for waters collected from the site are shown in Fig. 4. The results for the leachates are shown in Fig. 5-7. A standardized format is used in which results are plotted on a logarithmic concentration axis. This format, called Element Concentration Pattern Graphs (ECPG) allows the relative differences in concentrations among samples to be better observed even when some of the values are relatively low. The x-axis order of elements is as follows:

1. Na, K, and S: These elements are readily soluble elements and should correlate best among the samples assuming that the sulfur species in the water is primarily sulfate.
2. Ca, Mg, and Sr: Carbonate minerals could control the concentrations of these elements if these were present in the sediment/water system.
3. Pb, Cu, Zn and Ni: Either sulfide minerals or carbonate minerals could control the concentrations of these elements if these were present in the sediment/water system.
4. Fe, Mn, and Al: Oxide minerals could control the concentrations of these elements if these sedimentary minerals were present in the sediment/water system.

The EPCG's of solutions from leachate tests conducted on three of the composite samples taken from the site are presented as follows:

- Sediment from Gamble Gulch upstream of the inflow of the Tip Top mine water (Fig. 5).
- Sediment from Gamble Gulch downstream of the inflow of the Tip Top mine water (Fig. 6).
- Composite sample of the surface of mine-waste dump labeled as the far waste pile in Fig. 1 (Fig. 7).

Discussion

Comparison of the Waters at the Tip Top Site

In order to compare the samples, copper and zinc concentrations will be examined in this section. In water upstream of the adit inflow, Cu and Zn concentrations are lower than the stream standard, at 0.004 mg/L Cu and 0.048 mg/L Zn respectively (Table 1). Downstream of the adit, the stream flow is diluted and is marginally toxic based on concentrations of 0.046 mg/L Cu and 0.48 mg/L Zn. The adit has trace-metal concentrations that are higher than the stream standards, the concentrations of Cu and Zn being 0.199 mg/L and 1.65 mg/L respectively.

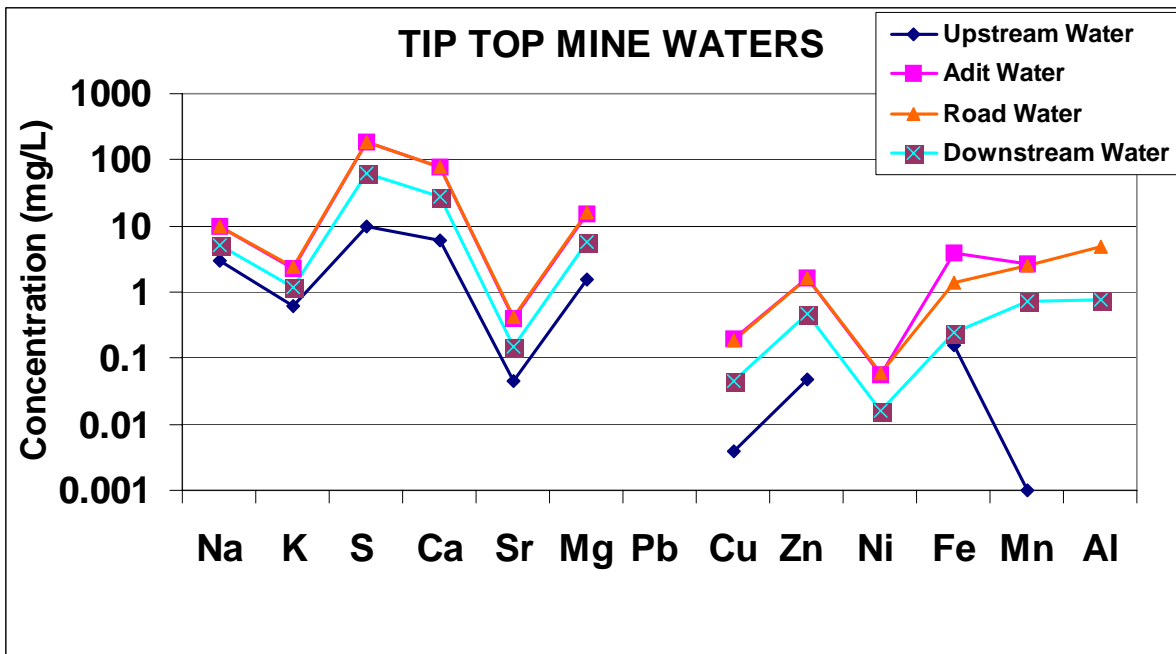


Figure 4. Results for water samples collected at the Tip Top mine.

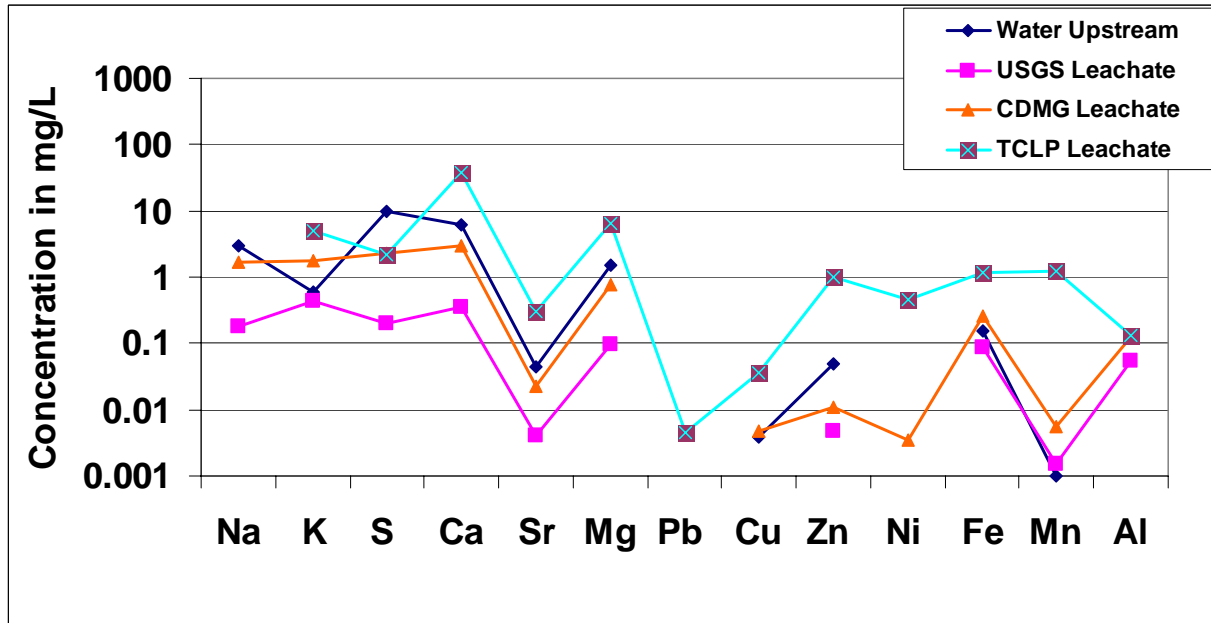


Figure 5. Results for stream water and leachates of stream sediment collected upstream of the inflow of the Tip Top adit.

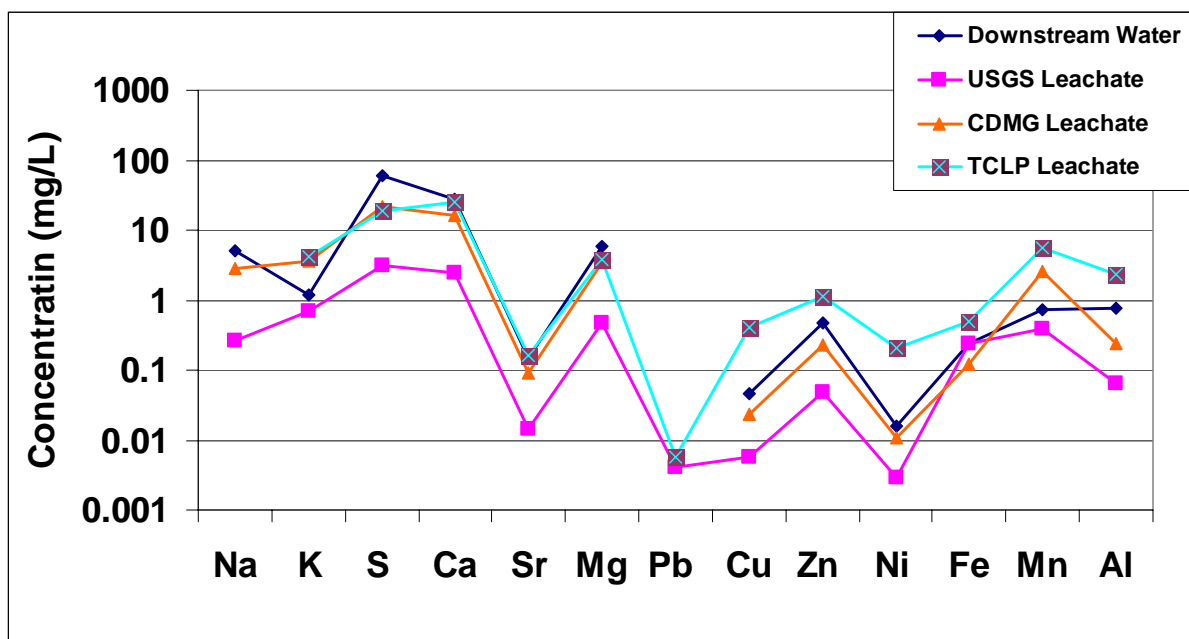


Figure 6. Results for water and leachates of stream sediment collected downstream of the inflow of the Tip Top adit.

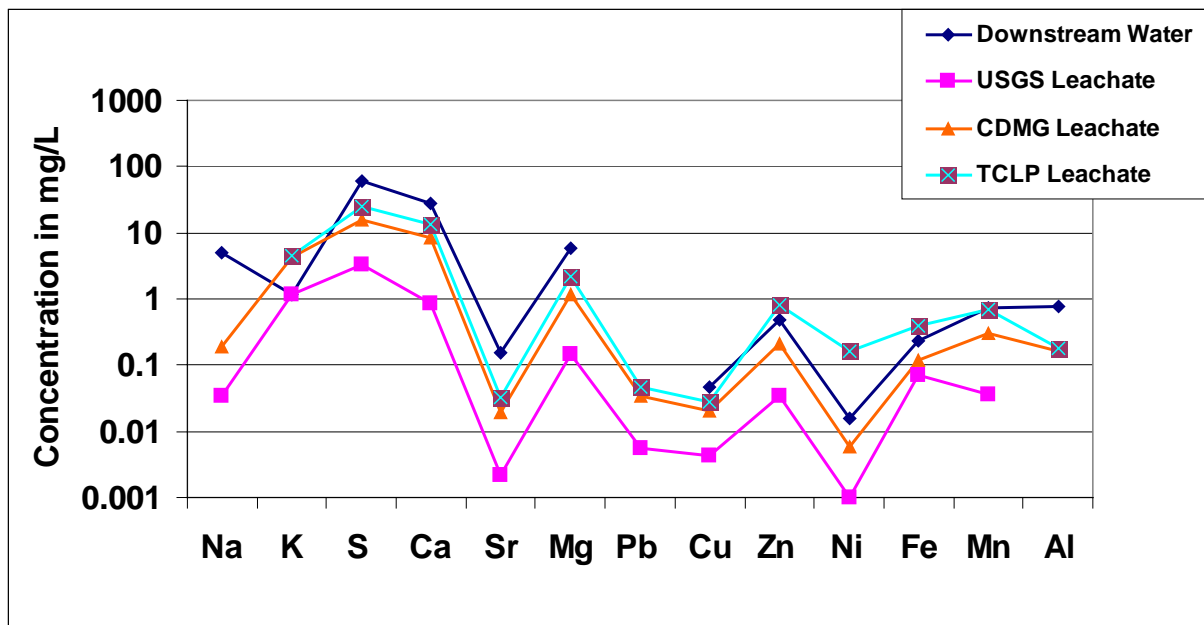


Figure 7. Results from leachates of a mine waste pile (labeled far-waste pile in Figure 1).

The conclusion that the adit is a source of contamination is reasonable when the stream itself is physically examined. There is a point in the stream where the clear water from upstream the adit and the deep orange water from the adit merge and flow downstream.

A second potential source of contamination to the area is a small water source that runs along the road and has high levels of copper and zinc as well. Concentrations of 0.185 mg/L Cu and 1.66 mg/L Zn indicate that this runoff may be coming from the adit and thus providing another point of contamination downstream. The sampling visit was in mid-July, and by this time, spring runoff was complete and the stream was at low-flow. In a study of nearby North Clear Creek, Harvey et al. (2003) found that dissolved metals concentrations are significantly lowered during spring runoff conditions due to dilution. Assuming the concentrations of the metals in the adit water remain constant, but stream flow is increased during runoff, concentrations of Al, Cu, and Zn (which were higher than aquatic toxicity levels in July 2004) likely comply with standards during high flows.

Results of the Leachate Tests on Sediments

Three different leachate tests were conducted to determine the range of possible concentrations when water interacts with sediment and mine-waste samples. As expected, the USGS test extracted fewer metals than the CDMG test (Fig. 5 and 6). Note in Fig. 5 that the metals extracted by the CDMG and USGS tests from sediment taken from upstream of the adit inflow have concentrations generally higher than concentrations in the gulch water. On the other hand, gulch water from downstream of the adit inflow has metal concentrations at or higher than the metal concentrations extracted by the USGS and CDMG tests. Thus, aquatic toxicity is primarily attributed to the adit water and not from metals leached from the sediment.

The modified TCLP test releases concentrations of toxic metals that are significantly higher than what is in the water or is leached from the sediments by the other tests (Fig. 5 and 6). The

large release of metals by the TCLP was also noted in a project that studied sediment and soil from a mine site in Brazil (Wildeman et al., 2004). This release of metals is attributed to complexation by the acetate and the reduced pH in the TCLP solution. Because the sediment upstream of the adit inflow also shows a trend, it is believed that some of these metals are due to the geochemical baseline of natural soil and sediment.



Figure 7. A view facing down gradient from the top of the mine-waste furthest from the adit. Note that vegetation is growing on the dump and that there is no kill zone at the base of the dump.

Results of the Leachate Tests on the Mine-Waste Dumps

The leachate results for mine-waste dump closest to the adit had in general no aquatic toxicity based on comparison to the stream standards. For the mine-waste dump farthest from the adit the CDMG leachate test extracted 0.021 mg/L of Cu and 0.21 mg/L of Zn. These concentrations are marginally toxic, but lower than the concentrations of Cu and Zn that are in the stream downstream of adit inflow. As viewed from the top of this mine dump, plants are growing on the slopes of the dumps and there is no vegetative kill zone at the base of the dump (Fig. 7). Both of these conditions are signs that the dump is not impacting the environment to any great extent (Wildeman et al., 2003). These results lead to the conclusion that metals leaching from the mine-waste dumps are not affecting Gamble Gulch.

Reproducibility of CDMG

The CDMG leachate test is volume based and it was thought that the reproducibility of the test might be poor. In order to determine if this method was reproducible, three samples were obtained using the methods described above and the ICP-AES results were compared. Figures 9, 10, 11, and 12 are the ECP graphs of each of the four samples tested (upstream the adit, downstream of the adit, the near mine-waste dump, and the far mine-waste dump). There appears to be some variation in the metal concentrations found in the leachates using the CDMG method. However, for the most part the variation is within the reported factor of four predicted by Hageman et al 2005. As illustrated in the Fig. 9, 10, 11, 12 the elements from the oxide minerals (Fe, Al, and Mn) vary the most among the replicates. This variability may account for some of the variation in the Cu and Zn concentrations seen throughout the replicate analysis.

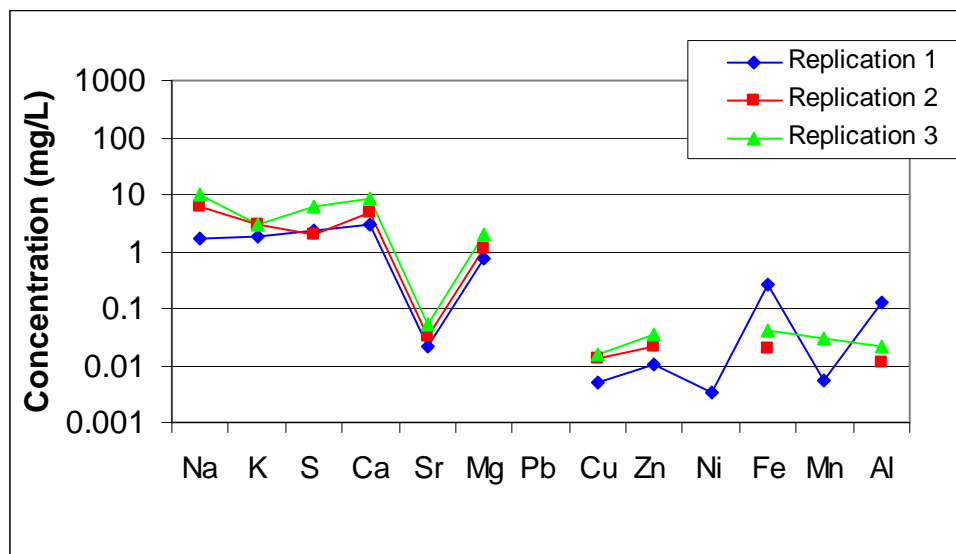


Figure 9. Results from stream sediment upstream of the inflow of the Tip Top adit water CDMG leach.

Bioassay Results

The solutions from the leachate tests were used in simple in-vitro toxicity tests that use an enzyme to monitor a biological response to the toxic metals present in the leachate. Currently, it appears that MetPLATETM enzyme tests using the bacteria, *Escherichia coli* are quite sensitive to contaminant metals. The TCLP leachate solution with acetic acid was hypothesized to have adverse effects on the test organism. The USGS test almost always yields lower concentrations of metals and so using this solution could generate a false negative where the in-vitro test would show no toxicity (although toxicity may be present). Therefore, the CDMG leachate test was used in the enzyme testing because it almost always generates higher concentrations of metals and does not contain acetic acid. Results from this and previous studies have shown that, in situations of marginal toxicity, the metal concentrations from the CDMG test are closer to the actual mine water that is found on the site (Wildeman et al., 2003; Bazin et al., 2003; Wildeman et al., 2004) as shown in Fig. 6 and 7.

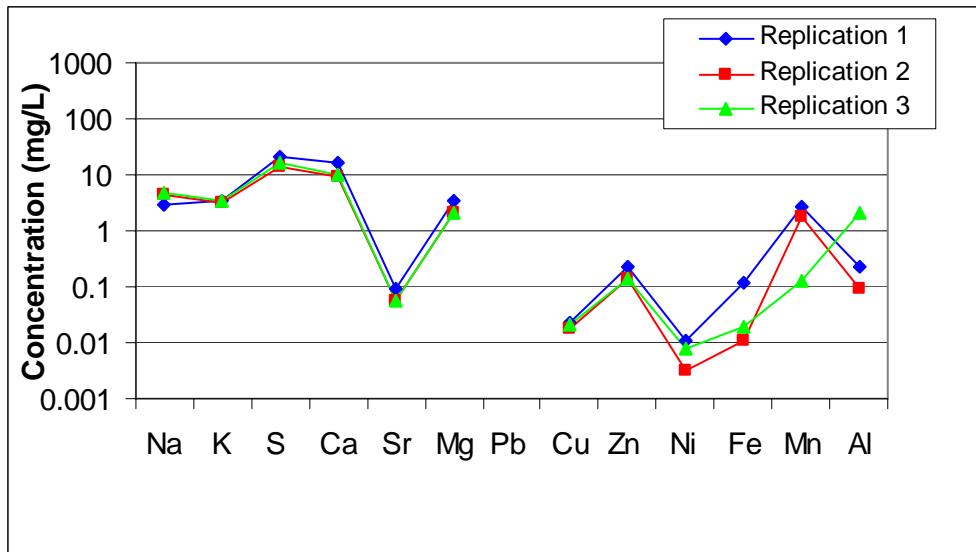


Figure 10. Results from stream sediment downstream of the inflow of the Tip Top adit water CDMG leach.

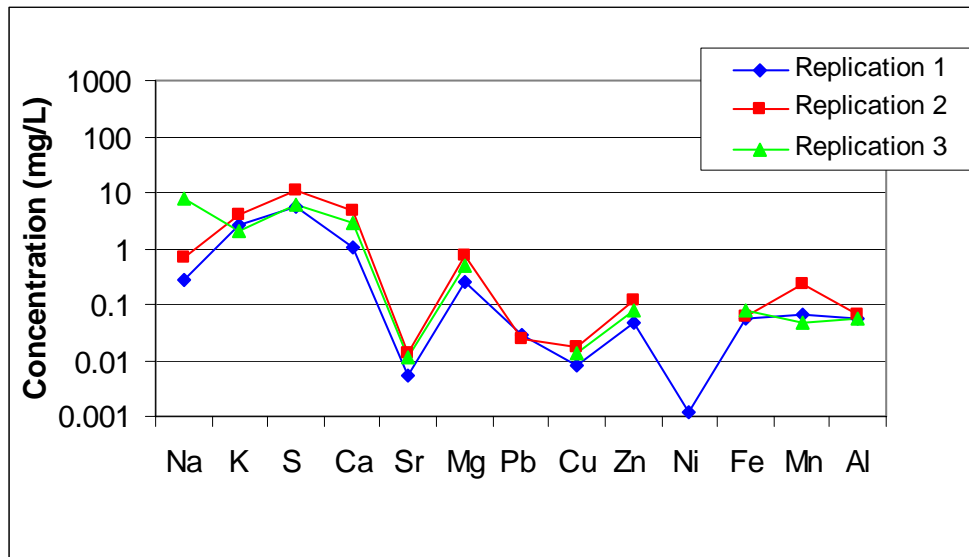


Figure 11. Results from mine-waste nearest the adit for the CDMG leach.

To examine the use of the CDMG leachates to test aquatic toxicity, two leachate samples of the four sediments and soils were subjected to MetPLATE™ tests. The inhibition of the enzyme following serial dilution of sample is illustrated in Fig. 13. The use of various sample dilutions and controls is illustrated Table 2. These controls are used to detect if there is a background absorbance in the sample; if the bacteria and the sample react chemically; or if the sample and the chromogenic substrate react chemically.

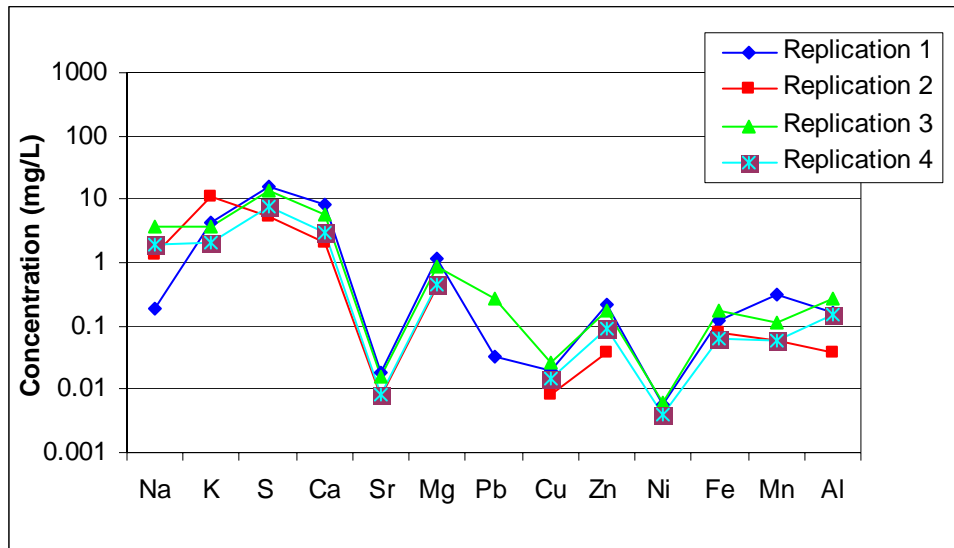


Figure 12. Results from mine-waste dump furthest from the adit in the CDMG leach.

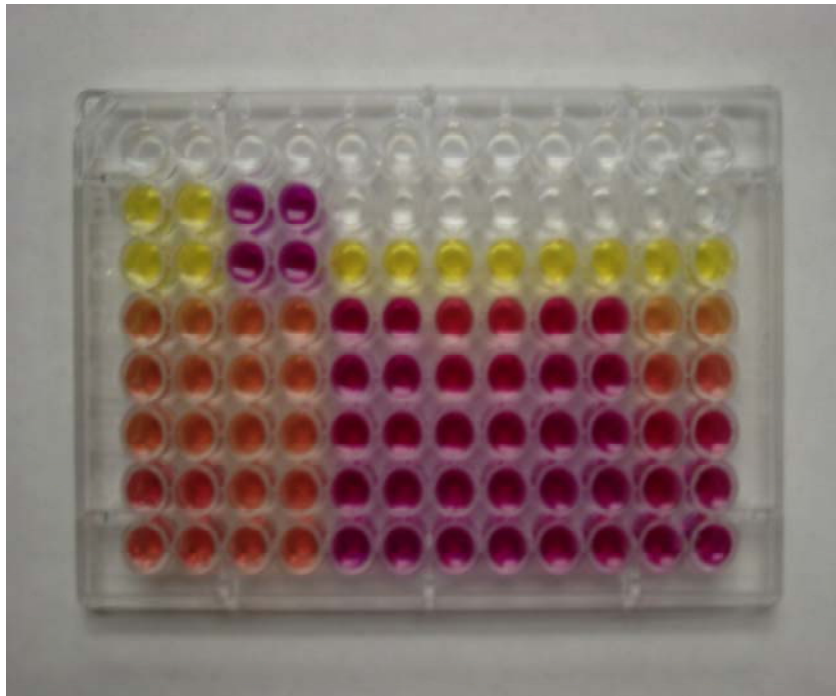


Figure 13: Photograph of second MetPLATE™ response for leachate samples after incubation

In the first MetPLATE™ test, leachates from replicate 2 (Fig. 5-7) were examined. In Fig. 14 the x-axis is the strength of the leachate in percent of the original concentration, and the y-axis is the absorbance at the wavelength of 575 nm. A low absorbance indicates that the bacterial enzyme is being inhibited. No inhibition for leachates of the sediment upstream the adit

Table 2: Placement of the samples in the wells for the above MetPlate™. The first row indicates the sample used. Chromophore and bacteria was added to the sample (B/C). No bacteria and no chromophore was added to the sample (NB/NC). Only chromophore was added (NB/C). Only bacteria was added (B/NC). The percentages represent the dilution factor of the original samples.

	1	2	3	4	5	6	7	8	9	10	11	12
Copper (ug/L)	Mine-waste Dump 2 +Cu	Mine-waste Dump 2 +Cu	Mine-waste Dump 2 +Zn	Mine-waste Dump 2 +Zn	Sediment Upstream of the Adit	Sediment Upstream of the Adit	Sediment Downstream of Adit	Sediment Downstream of Adit	Mine-waste Dump 1	Mine-waste Dump 1	Mine-waste Dump 2	Mine-waste Dump 2
A: background No chromophore	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
B: background Mixed C/NC B/NB	+ Control B/C	+ Control B/C	+ Control NB/NC	+ Control NB/NC	100% NB/NC	100% NB/NC	100% NB/NC	100% NB/NC	100% NB/NC	100% NB/NC	100% NB/NC	100% NB/NC
C: background Mixed C/NC B/NB	+ Control B/NC	+ Control B/NC	+ Control NB/C	+ Control NB/C	100% NB/C	100% NB/C	100% NB/C	100% NB/C	100% NB/C	100% NB/C	100% NB/C	100% NB/C
D	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
E	50%	50%	50%	50%	50%	50%	50%	50%	50%	50%	50%	50%
F	25%	25%	25%	25%	25%	25%	25%	25%	25%	25%	25%	25%
G	12.5%	12.5%	12.5%	12.5%	12.5%	12.5%	12.5%	12.5%	12.5%	12.5%	12.5%	12.5%
H	6.25%	6.25%	6.25%	6.25%	6.25%	6.25%	6.25%	6.25%	6.25%	6.25%	6.25%	6.25%

and mine-waste dump furthest from the adit was indicated by the high level of absorbance. In contrast, sediment downstream of the adit showed a marginal amount of inhibition of the enzyme. The EC50, defined as the effective concentration of sample concentration where the enzyme activity has been inhibited by 50%. The EC50 is obtained by comparison of the negative (no toxicity) and positive (complete toxicity) controls. The EC 50 is the concentration of sample at which the absorbance is half way between the two controls. The mine-waste dump nearest the adit had an absorbance below the EC50, showing there was a definite toxic response for the leachate obtained from this mine-waste dump.

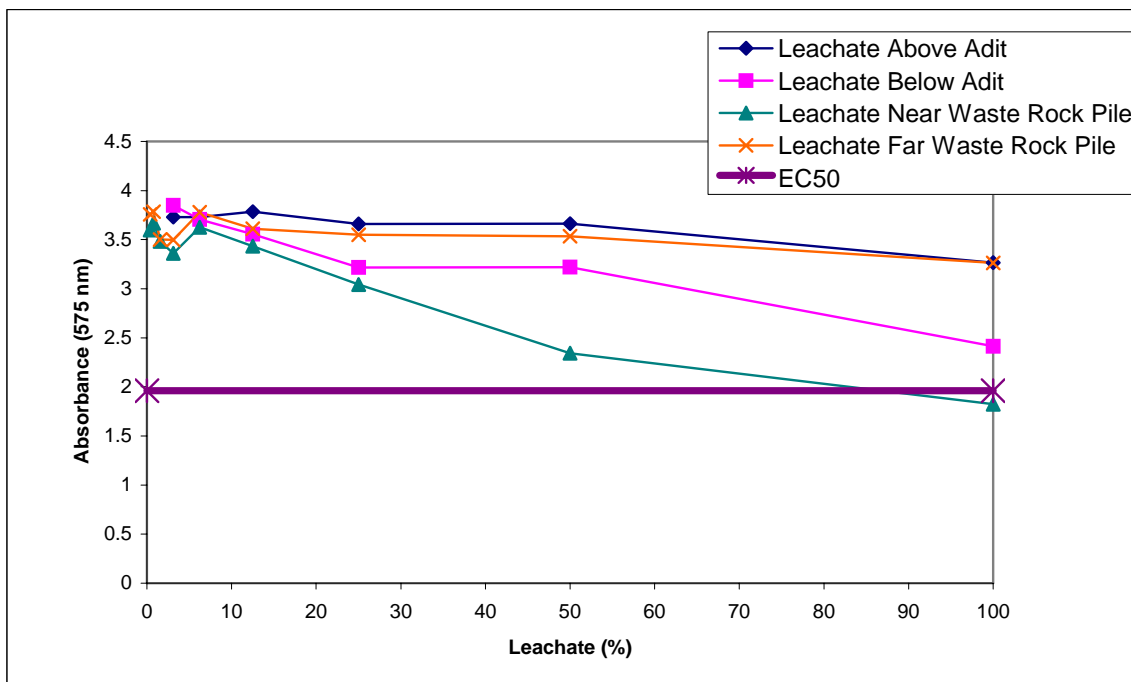


Figure 14: MetPLATE™ response for first replicate of leachate samples.

The second MetPLATE™ analysis was performed using the third replicate leachate (Fig. 5-7) run with solution from four sediment samples, as well as two samples spiked with known concentrations of Cu and Zn. Leachate solutions from mine waste dump furthest from the adit were spiked with Cu and Zn in order to determine if one of the metals has a greater affect on the inhibition of the bacterial enzymatic activity. The results (Fig. 15) indicate that for the 100% sample there was again no inhibition for sediment upstream of the adit, a marginal inhibition of activity for the near mine waste dump, and toxic responses for the far mine waste dump, sediment downstream of the adit and the two metal- spiked leachates. The change in response for the near and far mine-waste dumps between tests may be due to the fact that the pH's of the leachate solutions for the near and far dumps were different between tests. For the first trial, the leachate solution for the near waste dump had a pH of 3.55 and the leachate solution for the far waste dump had a pH of 4.07. However, for the second trial the near waste dump had a higher pH than seen in the first trial, 4.37 and the far waste dump's pH lowered to 3.77. The variation seen may have to do with the lack of a buffer system for the pH range being studied in this particular aquatic system. Another possibility for a change in the response of the MetPLATE™ could be that the sediment and mine-waste samples were not analyzed

immediately after collection nor were they stored at 4 °C which would have minimized the effect of enzyme activity on the leaching results (Brohon et al. 1999). Therefore the variation seen may also be a manifestation of changes in soil conditions by enzymatic activity causing a higher concentration of metals to be available for leaching.

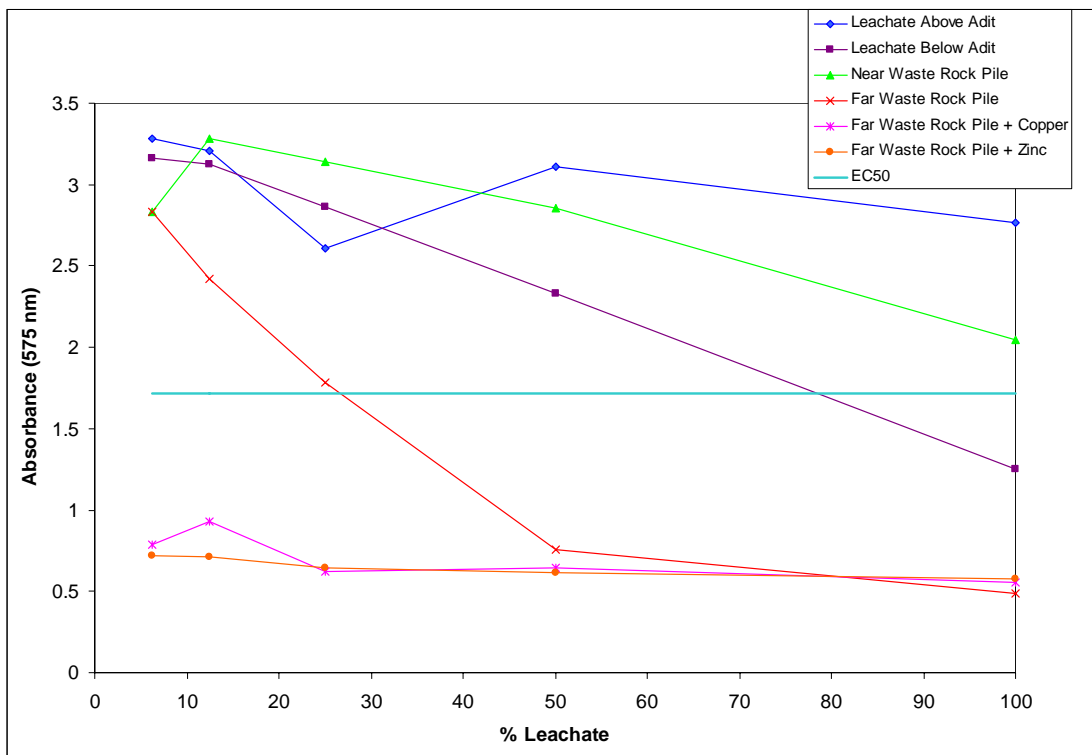


Figure 15: MetPLATE™ response for second replicate of leachate samples

Conclusions

Toxic metals contaminate gamble Gulch, the stream running beside the Tip Top Mine site. The primary source of metal contamination is entering the stream from the adit. MetPLATE™ enzyme tests of the leachate solutions are able to determine whether a sediment or soil is heavily impacted or not impacted. This was proven for sediment collected upstream of the adit, which was non-toxic, and the metal- spiked solutions of the far mine-waste dump leachate, which were toxic (seen in Fig. 15). The toxic response for marginally toxic samples, however, is difficult to determine by the leachate tests and the MetPLATE™ enzyme tests. The sediment downstream of the adit can be classified as moderately toxic by the ICP-AES results but differed in the MetPLATE™ results. For the second leachate test the sample was found to be non-toxic while the third leachate test produced a toxic response in the bioassay tests (Fig. 15). Concentrations of metals leached from the sediment downstream of the adit, however, do not account for the levels of metal found within the stream.

In the case of far mine waste dump, the leachate contained higher concentrations of copper and zinc and produced a toxic response for the MetPLATE™ in the second replicate while the third replicate showed lower levels of copper and zinc and showed no toxicity for the bioassay (Fig. 11). Again there was a disagreement between replicate analyses of the far mine-waste dump. The third leachate test proved to have higher concentrations of Zn and Cu than the second leachate test and produced a toxic response while the second test did not. This change in concentration may have to do with a change in pH. The third leachate test for the far mine-waste dump had a lower pH than the second leachate test. This would allow more of the oxide forming minerals to be dissolved in solution, thus leaching Cu and Zn into the solution as well. A higher level of dissolved oxide minerals was observed in the third leachate test than in the second leachate test (Fig. 12). Once again, the metal concentrations found in the far and near mine-waste dumps cannot account for the high levels of metals found in the water downstream of the adit input indicating that the main source of contamination is the actual adit input itself.

Based on these results it appears that the MetPLATE™ test is very sensitive to the results of the CDMG leachate test. For very toxic or totally no toxic the results are consistent. For marginally toxic samples, the variation in the CDMG leachate compositions means that it is currently difficult to obtain unambiguous leachate tests or MetPLATE™ enzyme tests to evaluate the toxicity of the system. Further work on improving reproducibility of the leachate tests is warranted to accurately assess a marginally impacted system.

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