Copper Removal from the Water Column

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Introduction

European regulatory requirements have created a need to define whether or not metals are removed from freshwaters and in a timely manner, reducing their potential for adverse ecological effects. Discussions among industry and academic scientists of the Environmental Toxicity Advisory Panel (ETAP) suggested research in this area as a priority. Metals Environmental Research Associations (MERA) decided to fund a research effort to begin addressing the issue, of which this project is one component funded by the International Copper Association. The overall goals of this research project were to

1) Initiate research to demonstrate substrate-associated Cu removal from the water column using a range of freshwater substrate types;
2) Develop and optimize a Transformation/Dissolution Protocol Extended (TDP-E) based on the OCED 29 method; and
3) Initiate research to better demonstrate that, once Cu is removed from the water column, it is not released from substrates during remobilization in an ecologically significant manner.

Specific project aims were to:

1) Select from a variety of substrates a suitable substrate for conducting metal removal studies;
2) Demonstrate a new TDP-E method and its ability to remove Cu from the water column removal using various low binding potential (LBP) substrates (TDP-E part 1);
3) Evaluate remobilization of Cu after removal in TDP-E testing (E-TDP part 2); and
4) Begin studies on the role of pH, Eh and Kd in the TDP-E and mesocosm systems (OECD 308) to determine their role in irreversibility.

Following discussions with scientists from MERA, a series of experiments were designed to evaluate their influence of Cu removal from the water column. These initial tests included the following evaluations: Comparisons of three substrates, introduced as dry or wet and pre-incubated wet or non-incubated; The pH response to the introduction of CO_2 in the TDP-E; Water removal efficiency of bedded substrate (OECD 308 and TDP-E); and Cu response to follow-up remobilization in the TDP-E.

Methods: Optimization of the Transformation/Dissolution Protocol Extended

The effect of varying selected experimental parameters in the TDP-E protocol was evaluated. An overview of all tests that were conducted is given in Table 1. Unless otherwise stipulated, all tests were conducted according to the following "basic protocol:"

Basic protocol, Part 1

1. Flasks are filled with 1 mg/L dissolved metal solution (prepared from a soluble salt of the relevant metal) in 1 L 10x dilute OECD 203 medium. Procedural blanks containing only 10x dilute OECD 203 medium are included.
    a. Two replicates per treatment
2. Prior to experiment, all solutions are bubbled with 0.5% CO_2 – balance air for 24 h to maintain pH 6.
3. Prior to substrate addition, a background (-1 h) sample is taken from each flask. This serves as starting value of metal solution (i.e. to calculate % removal throughout experiment)
4. Upon initiation of the test, 10 grams of substrate are added to the flasks. The flasks are subsequently homogenized by shaking for 1 minute at 100 r.p.m on an orbital shaker.
5. The solutions are allowed to react for 96 h or 28 days in static conditions. Bubbling with 0.5% CO_2 – balance air is maintained throughout the experiment. Samples are collected filtered through 0.2 micron filter and analyzed after 2, 6, 24, 48, 96, 168, 336 and 672 h for dissolved metal concentration. pH, dissolved oxygen and temperature are measured at each sampling interval.
6. To maintain sufficient volume, fresh 10x dilute OECD 203 medium was added to each flask

Remobilization, Part 2

1. After completion of the basic protocol, part 1, the flasks are agitated vigorously, on an orbital shaker at 150 rpm for 1 hour, in order to mimic a remobilization event (Figure 1)
2. The solutions are bubbled with 0.5% CO_2 – balance air for 4 d under static conditions
3. The solutions are sampled periodically at 0, 2, 6, 24, and 96 h after completion of the resuspension event. Samples are analyzed for dissolved Cu and Fe concentrations; pH, dissolved oxygen concentration, and temperature

Figure 1: Buffalo replicate post 1 h remobilization

Table 1: Overview of experiments

Experiment nr.	Factors considered	Notes	Figure nr.	Table nr.
1	basic protocol substrate loading (1g vs 10g)	substrate used: Raisin; Buffalo	3	3
2	basic protocol, substrate loading (10 g vs 100 g Raisin)	substrate used: Raisin, Buffalo, CANMET duration only 96h	4	4
3	basic protocol, substrate loading (10 g vs 100 g)	substrate used: Raisin incubated or Raisin non-incubated	5	5, 8
4	basic protocol, dual metal exposure (Ni & Cu)	substrate used: Raisin Incubated, Raisin Non-Incubated, Buffalo, CANMET duration 28 d	6, 7	6, 7
5	remobilization	duration 96h + 1 h remobilization	8, 9, 10, 11	9
6	mixing time	duration only 24h substrate used: Raisin	12, 13	10
7	various ionic strengths	substrate Raisin, duration only 96h	14, 15, 16	
8	modified OECD 308 sulfide amendments	duration 28 d	17	11

9	modified OECD 308 substrate loading	substrate wet Raisin (84 g & 42 g) duration 96 h	18	12, 13

Basic protocol modifications

For the following experiments, selected elements of the basic protocol were modified as follows:

A 28 d test (experiment #4) was also conducted with 10x diluted OECD solution spiked with 1 mg/L of copper chloride and 1 mg/L of nickel chloride together, utilizing CANMET LBP, Raisin Non-Incubated, Raisin Incubated, and Buffalo substrates (10 g treatments). All substrates are from riverine environments, with CANMET, Raisin Non-Incubated and Raisin Incubated are field collected (Ontario and Michigan) while Buffalo is a NIST certified reference sediment. Percent copper removal was calculated based on an initial -1h time sample for results in Tables 3 - 13. As in basic protocol, prior to substrate addition, a background (-1 h) sample is taken from each flask.

"Raisin Non-Incubated" substrates were represented by dried Raisin substrate, while "Raisin Incubated" substrate was prepared as follows: 1) weigh dried Raisin substrate in a Pyrex bottle, 2) slightly wet substrate with deionized water (< 3 mL), 3) purge headspace with nitrogen, and 4) seal bottle and hold at room temperature for at least 7 days. These substrates were utilized in experiment numbers 4, 5, 6, and 7.

A remobilization experiment (#5) was conducted at the end of a 96 h exposure by placing test flasks on an orbital shaker at 150 rpm for 1 h. Water samples were collected for dissolved Fe and Cu at various times, post-shaking.

Another study (#6) investigated the role of early mixing periods on copper removal. Treatments included Raisin substrate with no mixing, 1 minute, 1 h, and 2 h mixing periods with sampling over 24 h.

Osmotic effect tests (#7) were conducted to investigate a mechanistic process for Cu removal. The ionic strength was adjusted by KCl addition to OECD 203 solution to achieve 0.01 M, 0.1 M, and 1.0 M solutions. Given the rapid rate of outer sphere complexation of Cu, samples were collected at times 0, 1, 3, 5, 10, and 20 minutes.

Methods: Mimicking anoxic conditions using a modified OECD 308 test method

A modified OECD 308 test (#8) was designed using Septa jars (250 mL) loaded with wet Raisin substrate (42 g or 84 g) and 10 x diluted OECD 203 solution (240 mL or 120 mL, respectively) and 1week incubation at room temperature. After 1 week, Cu salt solution was spiked into each jar (1 mg Cu/L target) and water sampling commenced. The pH was adjusted via introduction of 0.5% CO_2 through the top cap.

Another modified OECD 308 test (#9) was conducted with sulfide amendments (FeS and FeSO4) and organic matter treatments. Wet raisin substrate (84 g) was amended with 1 g sulfide compound and/or organic matter and allowed to incubate for 28 d at room temperature. After 28 d equilibration, Cu salt solution was spiked into each jar (1 mg Cu/L target) and water sampling commenced. Pre- and post-experiment samples were collected for routine analyses and simultaneously extracted metals and acid volatile sulfides.

Figure 2: Modified OECD 308 Septa jar design. Wet Raisin substrate (84 g and 42 g) added to each jar with 1week incubation at room temperature in 10x diluted OECD 203 solution.

Results and Discussion

The ability of different substrates to influence Cu removal from the water column was evaluated with a focus on evaluating the influence of a variety of methodological parameters on metal removal. The substrates and pre-treatment testing was conducted using Buffalo, CANMET, and Raisin substrates. Table 2 summarizes substrate chemistry and characteristics. Buffalo substrate is a NIST certified substrate and several factors (total organic carbon, copper substrate values, and texture) were not reported. Texturally, Raisin and CANMET are classified as sand suggesting they are LBP substrates. The CANMET and Buffalo substrates were provided by CanmetMINING. The Raisin substrate was collected from the Raisin River near Ann Arbor, Michigan and has been used as a sediment toxicity and contaminant control and reference sample for several years by our laboratory.

Overall, the experiments demonstrated Cu is rapidly removed from the water column under a wide variety of conditions using the T/DP-E and modified OECD 308 methods. Removal rates varied depending on testing conditions and substrate type, but most resulted in rapid removal (over 70%) within 96 hrs.

The pH and amount of substrate loading were important drivers for Cu removal. As pH increased Cu was removed faster from the water column, likely due to increased complexation to substrate particles and formation of carbonate and hydroxide complexes. There was greater pH drift above the target pH of 6 with substrates containing more binding sites, such as with increased organic carbon and Fe concentrations. Given this phenomenon, the least pH increase occurred using the CANMET substrate. Also, as substrate loading was increased, Cu was complexed faster and removed from the water column.

Experiments 1, 2, and 3

Experiments 1 and 3 deviated from the basic T/DP-E protocol by adjusting substrate loading using 1, 10 and 100 g treatments. Experiment 2 (Figure 4) included a 100 g loading Raisin treatment. Experiment 1 (Figure 3) evaluated Buffalo and Raisin substrates while Experiment 2 tested CANMET, and Experiment 3 investigated Incubated and Non-incubated Raisin substrates.

Experiment 3 focused on several treatments of Raisin substrate (dry (or non-incubated) = dried at 60° C for > 48 h; incubated = dry + wetted with Milli-Q water + nitrogen purged and undisturbed for 1 week).

CANMET low binding potential (LBP) and Buffalo substrates were also evaluated. Results indicated Buffalo substrate removed Cu more rapidly compared to Raisin substrates (Figure 5 and Table 5). Additionally, substrate loading was adjusted for 100 g treatment of each substrate type as it resulted in faster Cu removal.

Buffalo 10 g and Raisin 100 g treatments responded similarly in Experiment 2, with pH drift occurring. The pH was difficult to control given variability of flow rates through the manifold and gas depletion at high flow rates, no matter which substrates were used. Buffalo 10 g and Raisin 100 g treatments exhibited greater pH jumps to 7.42 and 7.91, respectively (Table 4). Larger sediment loading accentuated upward pH drift, which is not allowed in the T/DP. Cu removal was not largely affected by substrate type, but highly affected by the amount of substrate loading.

Seventy percent removal was calculated based on an initial -1h time sample. Buffalo 10 g treatment achieved 70% removal almost immediately after substrate addition with Buffalo 1 g meeting the targeted 70% removal at 2 h (Table 3). In Experiment 2, CANMET substrate 70% removal occurred between 24 and 96 h, while others were within 2 hours. It is apparent that CANMET had the LBP of any of the three test substrates due to the slower removal of Cu from the water column. Buffalo and Raisin 100 g treatments performed similarly (Table 4); likewise, Experiment 3 treatments of Raisin incubated 10 g, incubated 100 g, and non-incubated 100 g all achieved 70% removal within 2 hours. The non-incubated Raisin 10 g treatment did not reach 70% removal until 96 h. This suggests that when some substrates are wetted and held at room temperature for a week, diagenesis begins and additional binding ligands are formed both through microbial and abiotic processes.

Substrate mass and pH had an important role in Cu removal as evidenced in 1 g vs 10 g vs 100 g treatments, regardless of the substrate type. Greater pH jumps were also noted in 100 g treatments. The 100 g treatments are most efficient at removing Cu, followed by 10 g and 1 g, respectively; however, all removed Cu within 96 h. It is not surprising 100 g treatments were most effective at metal removal, however, their associated pH increase also assisted in Cu removal. The pH drift could not be controlled effectively using 0.5% CO_2 bubbling.

The acid volatile sulfide/simultaneously extracted metal ratio (AVS/SEM) is a model that predicts when sediments are not toxic. A one-to-one (or greater) relationship of AVS to SEM suggests that no free (thus toxic) metal will be released into surrounding waters. These results are shown for Raisin (pre/post exposure, incubated vs non-incubated substrates) in Table 8. SEM elements analyzed include Ni, Cu, Fe, Mn, Cd, Zn and Cr. Cd values were reported as non-detects by ICP-OES. AVS concentrations in Raisin substrates, both pre/post exposure and incubated/non-incubated values, were low with slightly elevated SEM values, resulting in potential for toxicity. Pre-exposure, Non-Incubated substrate had no sulfide, however, Post-Exposure, Non-Incubated substrates had slightly elevated levels of sulfide. This would be expected as sulfide will oxidize in the presence of air. Non-Incubated treatments were only tested with dried Raisin substrate and the AVS test is typically conducted on wet substrates. The drying resulted in low to non-detectable AVS, thus creating aartifact that prevents the use of the SEM-AVS based bioavailability model.

Experiment 4

Results from a 28 d test with Buffalo, CANMET, Raisin Incubated, and Raisin Non-Incubated exposed to Ni and Cu are presented in Figure 6, Figure 7, Table 6, and Table 7. All test substrates achieved 70% removal of both metals in 28 d. The pH was stable during T/DP-E tests using Buffalo and CANMET substrates, with maximums of 6.28 and 6.14, respectively. Both Raisin treatments resulted in pH increases at 24 hrs (Incubated = 7.21 and non-incubated = 7.03).

Based on results from the 28 d test with combined Ni and Cu, it appeared these metals may compete for binding sites in all substrate types, thereby reducing their removal rates from the water column when limited amounts of substrate are used. Only the Raisin treatments achieved 70% removal of Ni in 96 h,

while all four treatments achieved 70% removal of copper within 96 h. All treatments achieved 70% removal of Ni within 28 d.

Experiment 5

Results of a 96 h exposure with 1 h remobilization at 150 rpm are presented in Figure 8, Figure 9, Figure 10, Figure 11, and Table 9. All treatments achieved 70% copper removal. No significant flux of Cu or dissolved Fe was detected post remobilization (Table 9) suggesting it is irreversibly bound in ambient waters with a pH of 6 or greater.

Intense mixing at 150 rpm for 1 h did not cause Cu remobilization into overlying water. Slightly elevated dissolved Fe was noted in the Buffalo treatment post remobilization but dropped at the 96 h sample period post remobilization. Texturally, Buffalo substrate was less dense ("fluffy") compared to Raisin and CANMET, both of which are sandy. This may have played a role in Fe flux during mobilization as Buffalo more readily mixed into the water column compared to other treatments, as illustrated in Figure 11. The pH drift post remobilization occurred with all treatments with the exception of CANMET. This suggests CANMET may be a suitable choice for LBP substrate testing. At the time of remobilization, all treatments achieved 70% removal of Cu and no significant flux was noted, suggesting strong, irreversible binding to Cu.

Experiment 6

A 24 h study with various initial experiment mixing periods showed prolonged mixing resulted in more rapid Cu removal, but it also increased pH (Figure 12). Visually, two hours after an initial mixing period, the 2 h mixing test flask appears more turbid as compared to 1 h mixing period (Figure 13). Further testing could evaluate other substrates. Two h and 1 h treatments achieved 70% removal of Cu within 24 h (Table 10).

Experiment 7

Figures 14 – 16 show results from the evaluation of the effect of ionic strength on Cu removal. Seventy percent removal by 96 h was achieved by almost all treatments except for CANMET LBP 1.0 M and 0.1 M ionic strength treatments. The source of discrepancy between initial (-1 h) samples in each exposure are unknown. These results suggest ionic strength is not part of a removal mechanism for Cu.

Experiment 8

Testing of the OECD 308 protocol for metal removal provided results similar to the primary findings of the T/DP-E and validated results of Ni removal by Kent State University (Costello et al., 2017). Wet Raisin 84 g and 42 g treatments in a modified OECD 308 study performed similarly. Both treatments removed 70% of copper within 1 h (Figure 17 and Table 11). The pH was consistent throughout, ranging from 5.98 – 6.14. pH proved more consistent through Experiment 8, likely due to nature of the design (i.e. bedded sediment). The metal removal data show Cu was not largely affected by the substrate loading rate. This is not surprising given the large amount of substrate in the flask bottom, providing an excess of binding sites.

Experiment 9

Results from modified OECD 308 28 d sulfide and organic matter amendment tests are shown in Figure 18 and Table 12. The pH was stable throughout, ranging from 5.86 – 6.18. Sulfide or organic matter ('+P' treatments) amendments did not affect the substrate's ability to remove Cu, as all treatments reached 70% removal or greater within 24 h. Dissolved Fe results were interesting in early sampling periods of FeSO4 treatment, with a spike of 23 mg/L at time 0 sampling, but Fe values steadily dropped to 0.225 mg/L at 24 h sample period. After 24 h, all Fe results were below detection limit.

In this experiment, each treatment is 84 g wet Raisin substrate with the addition of Fe amendment and/or organic matter. SEM-AVS values pre- and post-experiment (Table 13) indicate a potential for toxicity in

Reference (84 g wet Raisin substrate) and Raisin + FeS treatments. This prediction did not change over the 28 d period, suggesting AVS concentrations in the flask substrates are stable.

Conclusions and Recommendations

Evaluations of the new T/DP-E demonstrated its usefulness as a hazard classification test for waterborne metals. The importance of substrate type, loading rate, and pH in the removal of Cu was established. A low binding substrate, such as CANMET is appropriate for hazard classification and does not result in upwards pH drift. A small amount of substrate loading is required to provide adequate metal ligands for complexation and rapid removal from the water column within minutes to hours. Ionic strength is not important and the ten-fold dilution of OECD reconstituted water should be used, as in the T/DP. In addition, the irreversible removal of Cu from the water column was demonstrating at a pH of 6 or greater when Cu bound substrates are vigorously mixed for 1 to 2 hrs. These findings are an additional line-of-evidence demonstrating the rapid removal of Cu from the water column, following the findings of Costello et al (2017) using a modified OECD 308 approach, and a host of other peer-reviewed studies as cited in the Executive Summary (Burton et al., 2018).

We recommend that future research focus on identifying the key substrate parameters that allow for a variety of substrates to be used in hazard classification testing. This will eliminate the need for using the CANMET sediment which is only available in limited quantities and unique to Canada and its' CanmetMINING agency. In addition, further research may be useful to define speciation changes in metals removed during the T/DP-E and their irreversibility.

Tables and Figures

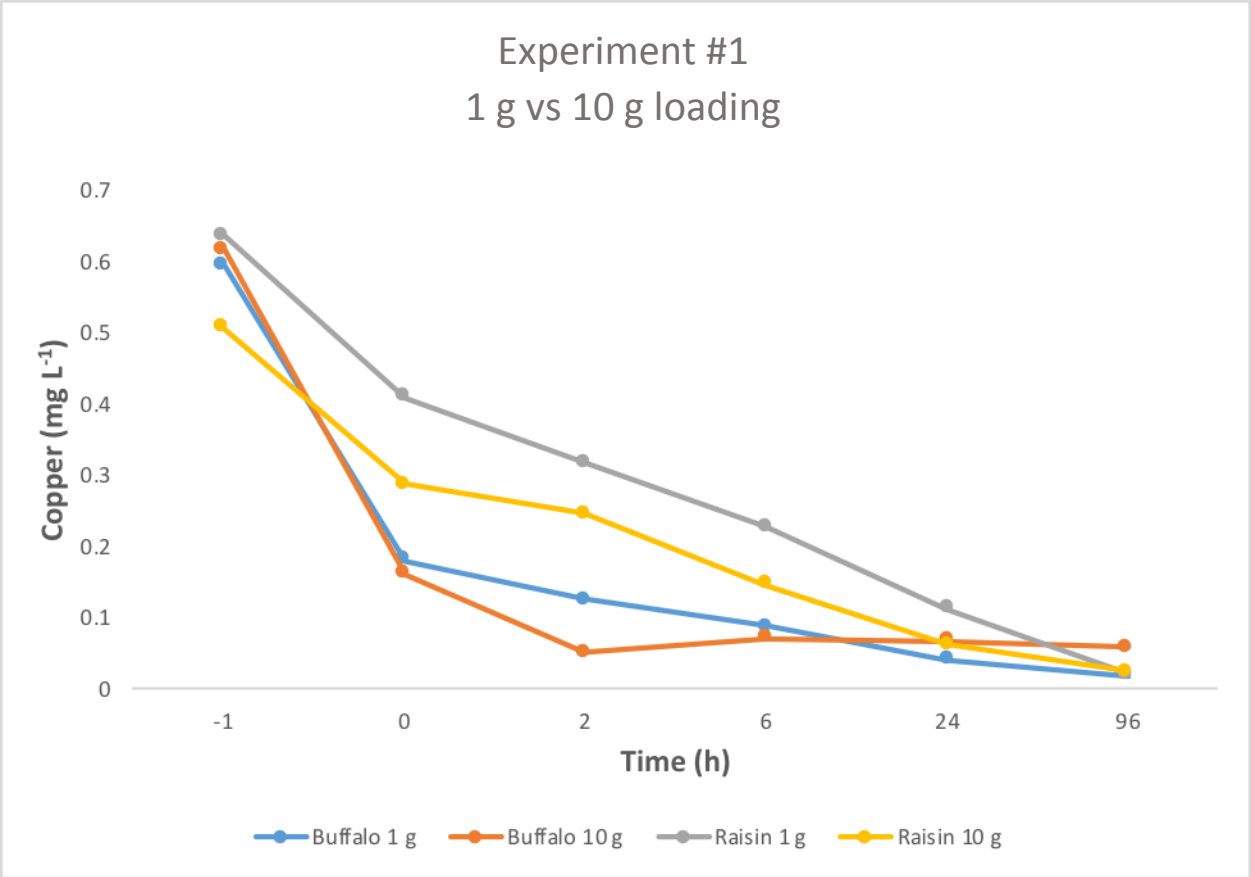

Figure 3: Experiment 1. Buffalo vs Raisin, 1 g vs 10 g exposure. Sediment added to flasks, placed on shaker table for 5 minutes, then substrate is allowed to settle for 5 minutes prior to Time 0 sample.

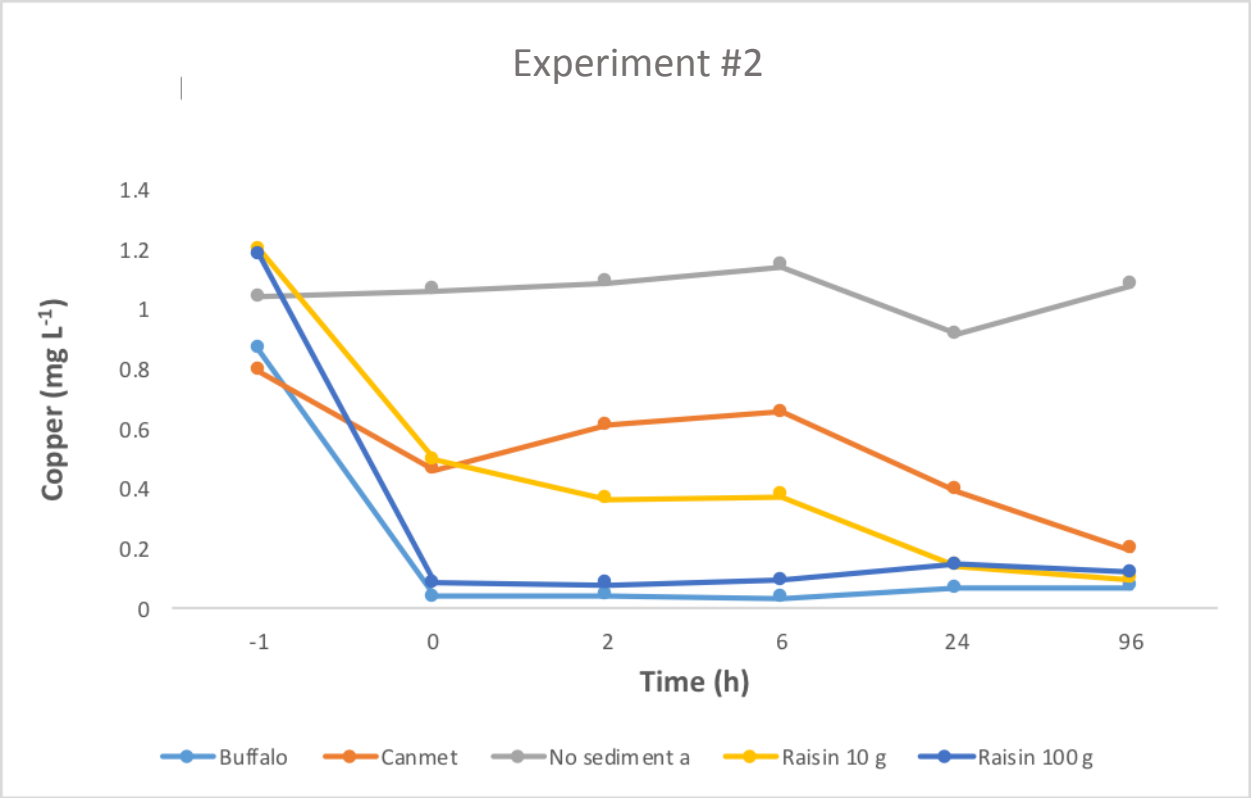

Figure 4: Experiment 2. Buffalo 10 g vs CANMET LBP 10 g vs Raisin 10 g vs Raisin 100 g.

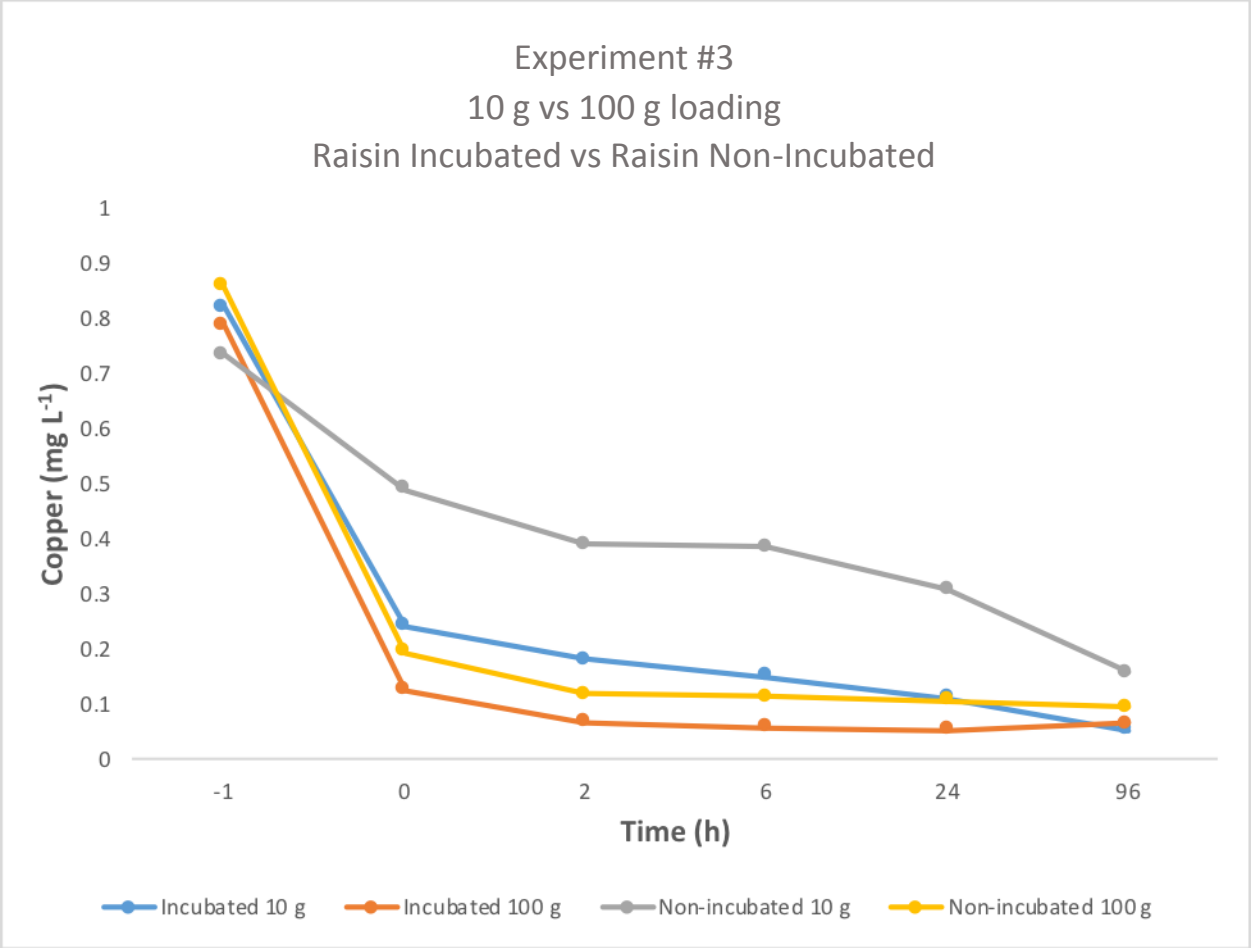

Figure 5: Experiment 3. Raisin substrate incubated vs non-incubated, 10 g vs 100 g exposure.

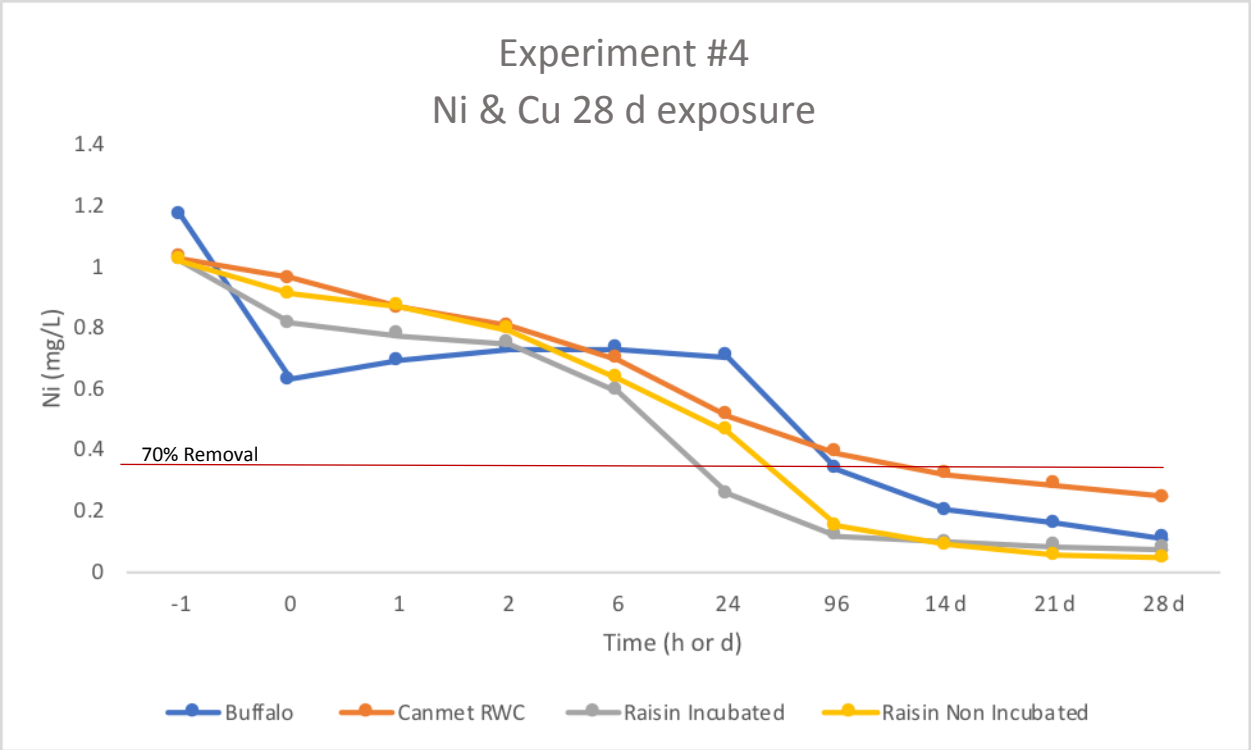

Figure 6: Experiment 4. Ni and Cu 28 d exposure; Ni only shown here. Buffalo time 0 h and 2 h lower than subsequent sampling. Substrate adhered to the side of flasks and with 10 x OECD 203 addition following each sampling time (to replenish volume) and some loss of substrate during each sampling which may account for lower values. Raisin non-incubated and Raisin Incubated treatments achieved 70% removal of nickel within 96 h.

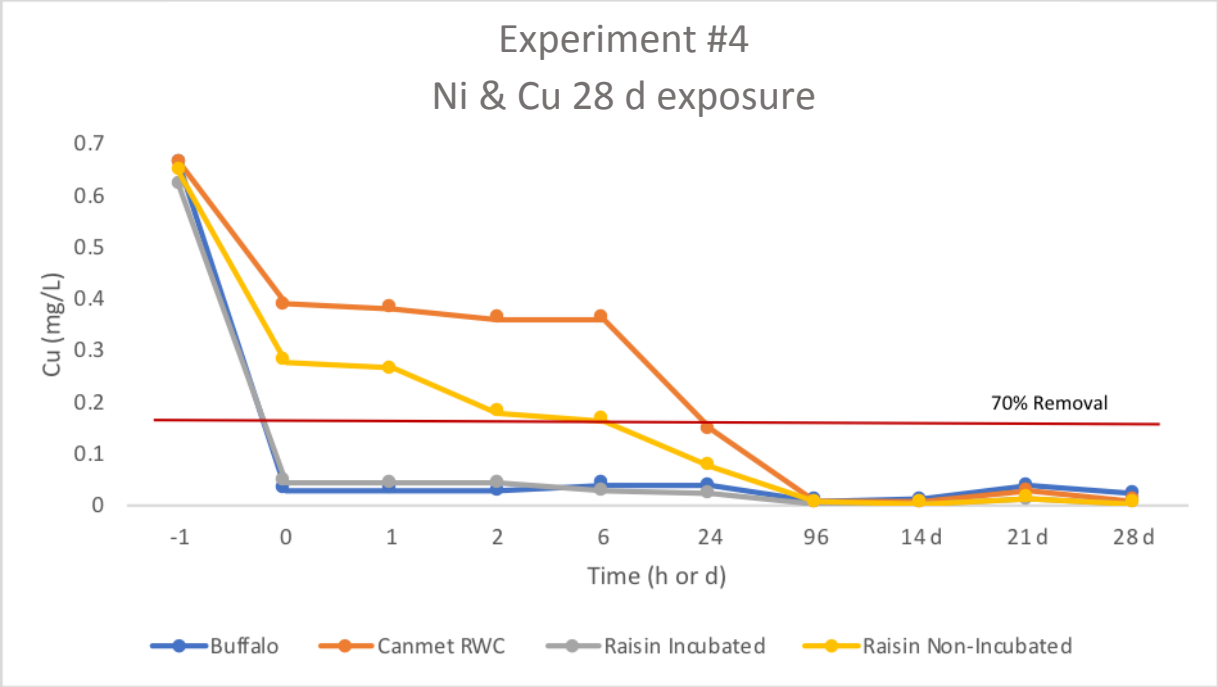

Figure 7: Experiment 4. Ni and Cu 28 d exposure; Cu only shown here. All treatments achieved 70% removal within 96 h.

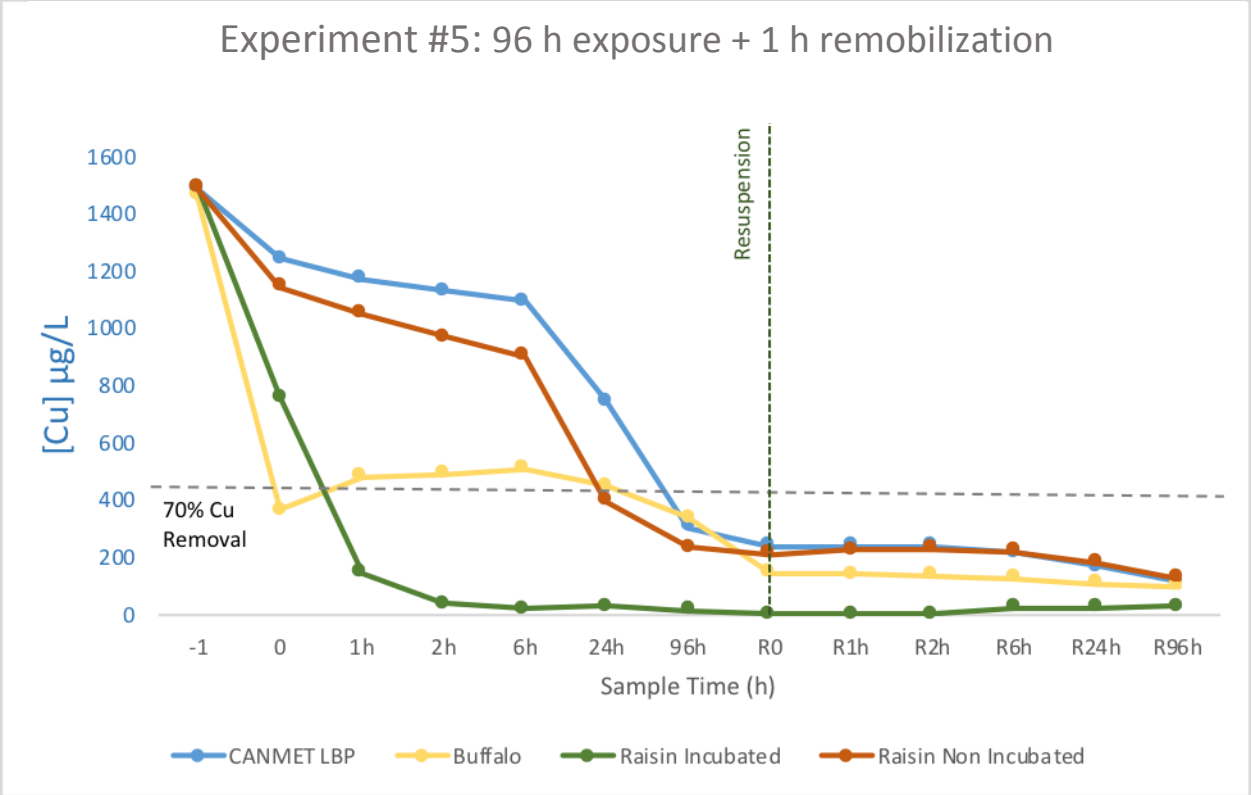

Figure 8: Experiment 5. 96 h exposure with 1 h remobilization at 150 rpm on orbital shaker table. No flux of Cu was detected post remobilization. All treatments achieved 70% removal within time constraints of experiment.

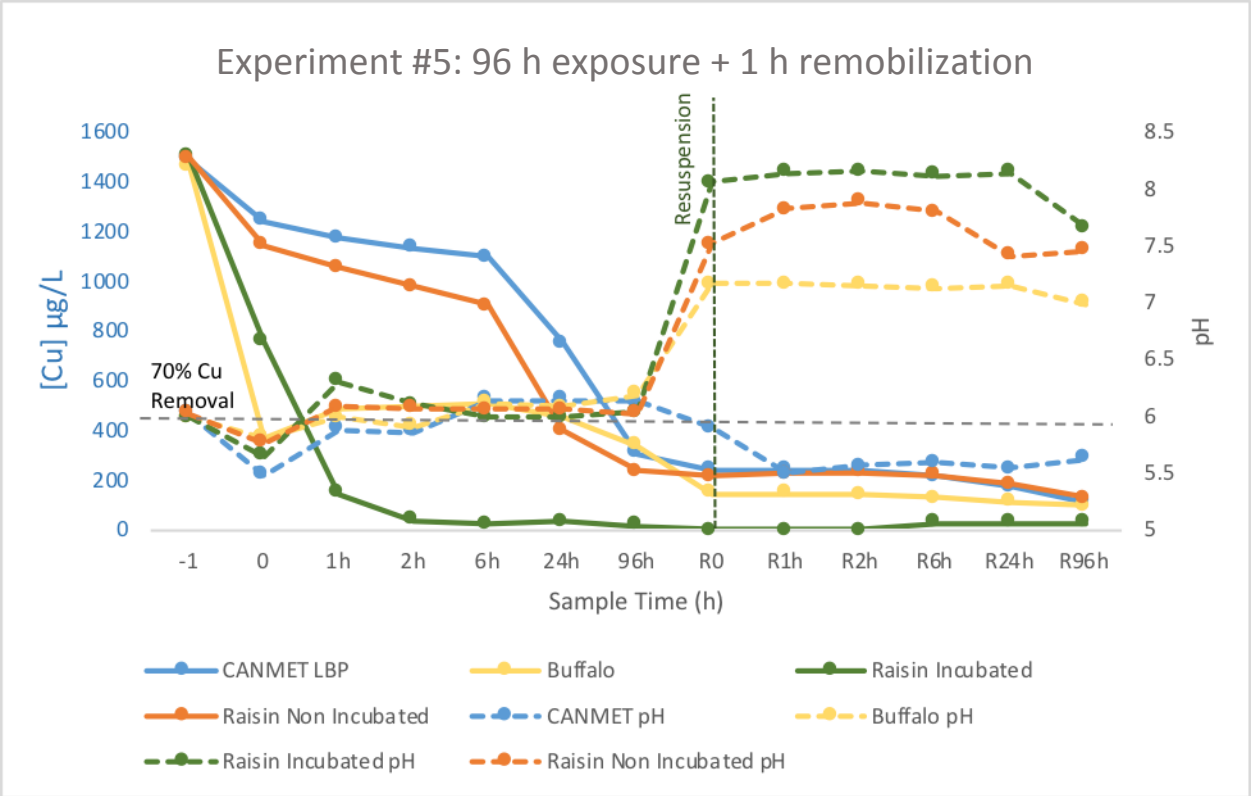

Figure 9: Experiment 5. 96 h exposure with 1 h remobilization at 150 rpm on orbital shaker table with pH overlay. pH ranged from 5.48 – 8.15 but remained stable in control flask (pH 5.96 – 6.2, not shown on graph).

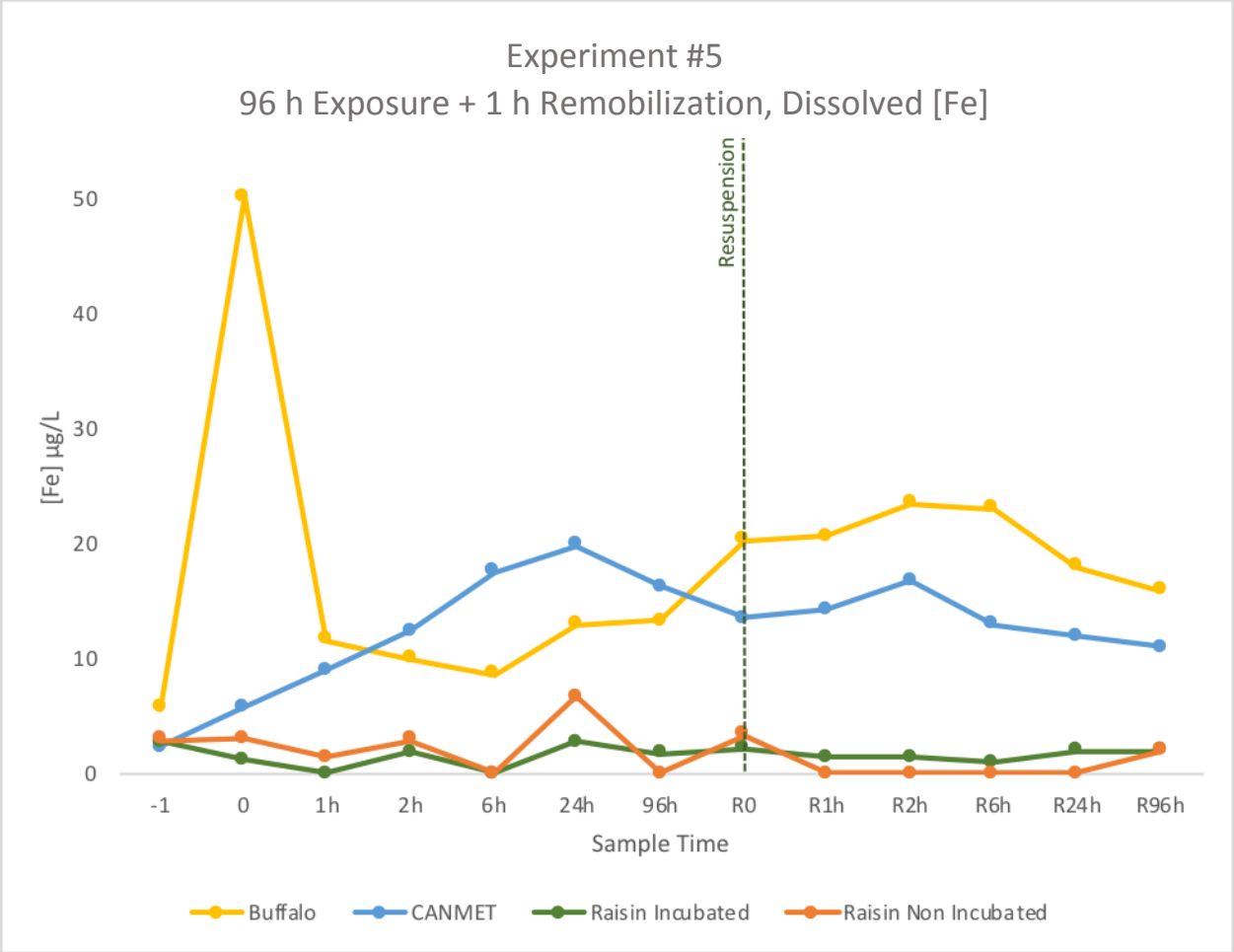

Figure 10: Experiment 5. 96 h exposure with 1 h remobilization at 150 rpm on orbital shaker table dissolved Fe results. No significant Fe flux was noted post remobilization.

Figure 11: Experiment 5. 1 h remobilization in progress. Note turbid water column in Buffalo replicates compared to other treatments.

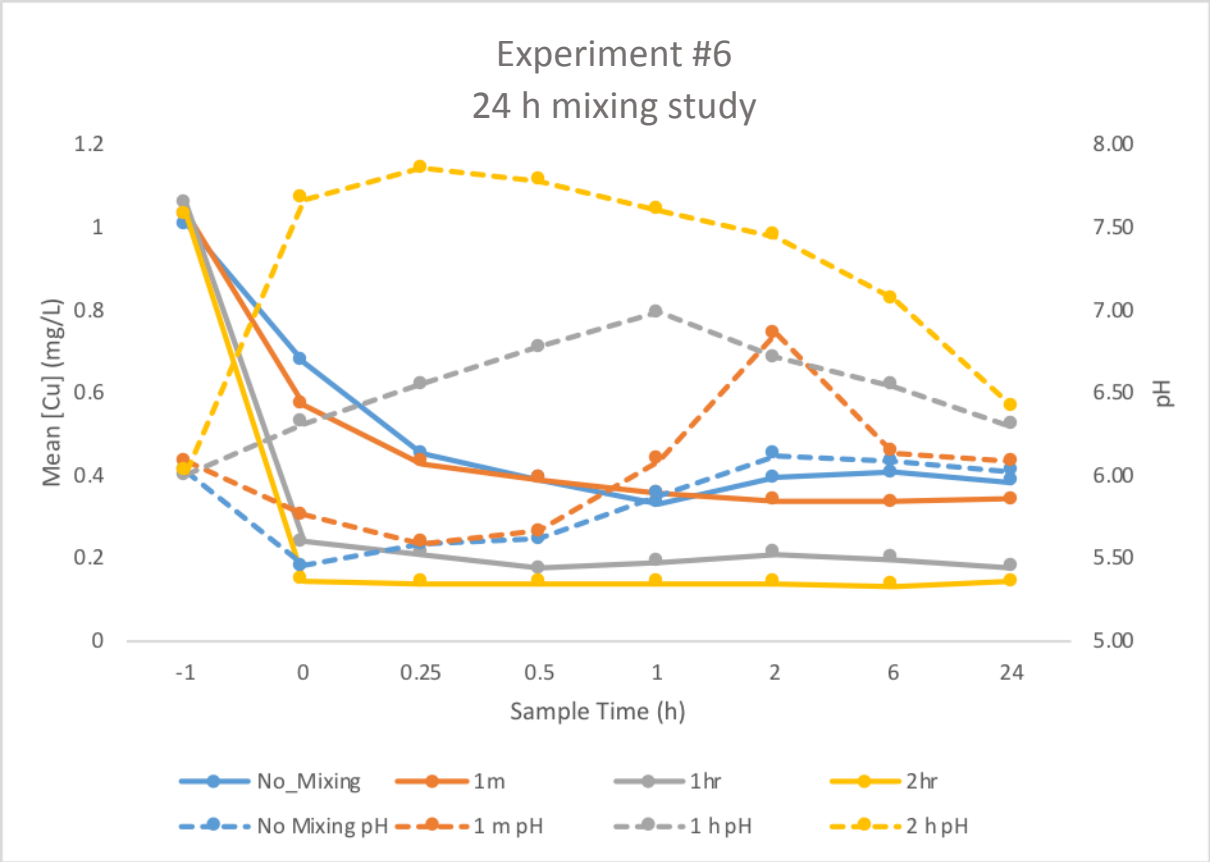

Figure 12: Experiment 6. 24 h mixing study with Raisin substrate and pH. pH varied most in 2 h mixing treatment. 1 h and 2 h mixing treatments achieved 70% removal of Cu within 24 h.

Figure 13: Experiment 6. 1 h and 2 h replicates from 24 h mixing study at sample time 2 h post mixing.

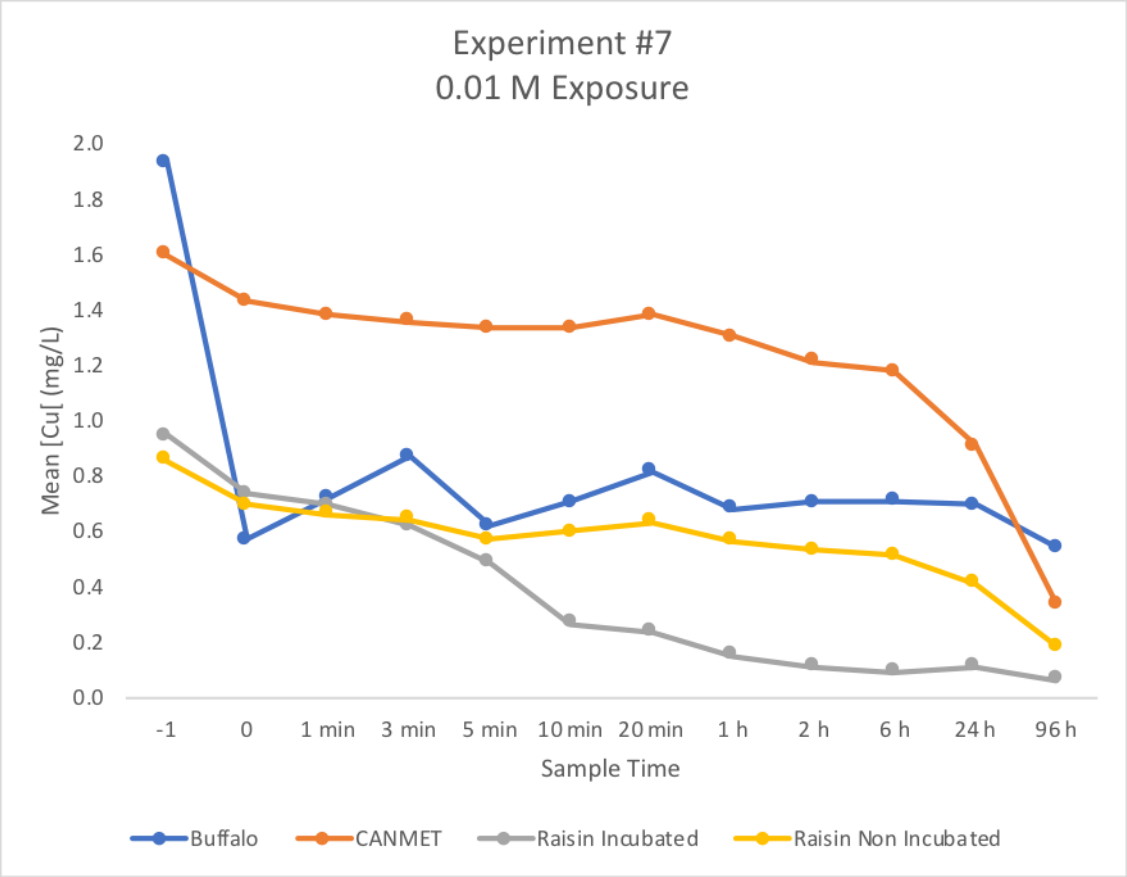

Figure 14: Experiment 7. Osmotic strength test at 0.01 M. All treatments achieved 70% removal of Cu.

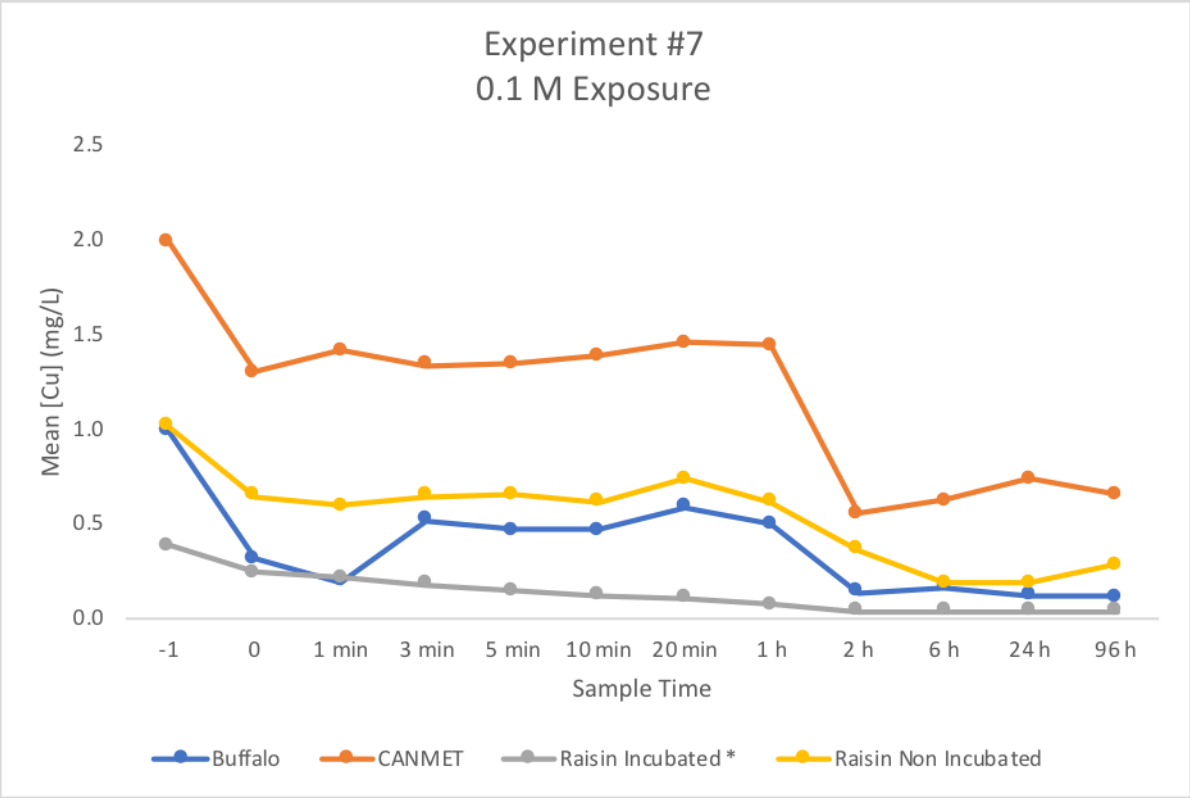

Figure 15: Experiment 7. Osmotic strength test at 0.1 M. CANMET did not achieve 70% removal within 96 h. Note wide discrepancy of -1 (background) values of metal solution prior to sediment addition.

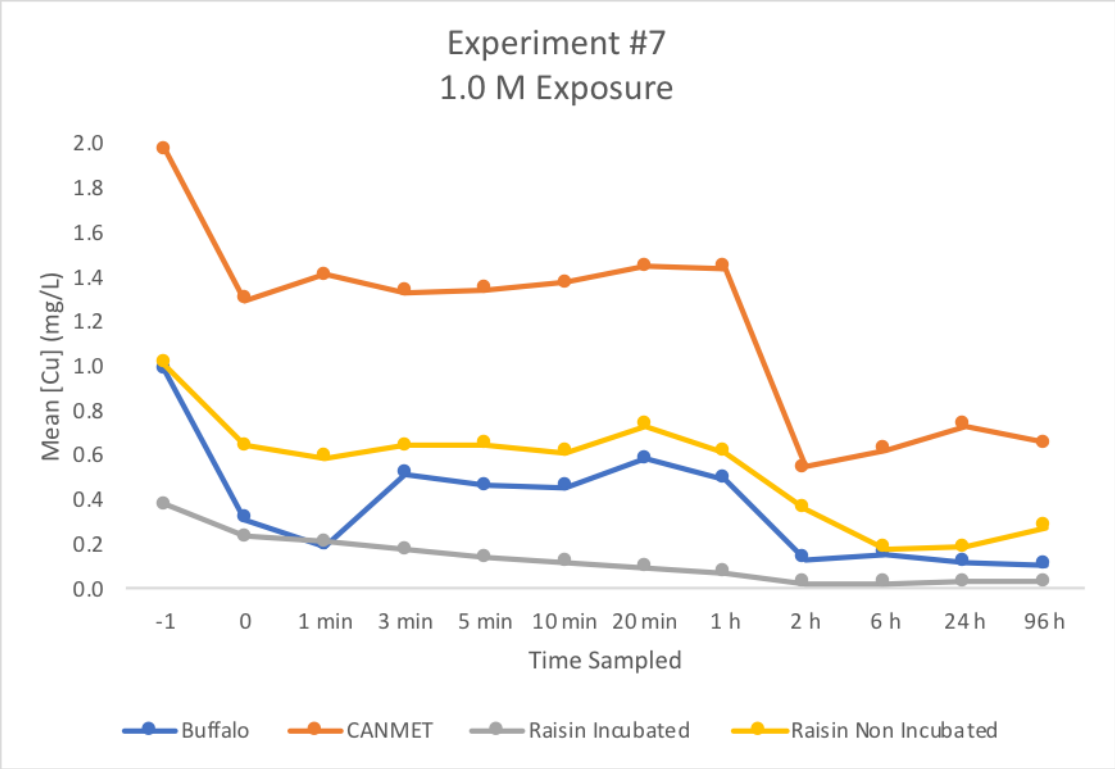

Figure 16: Experiment 7. Osmotic strength test at 1.0 M. CANMET did not achieve 70% removal within 96 h.

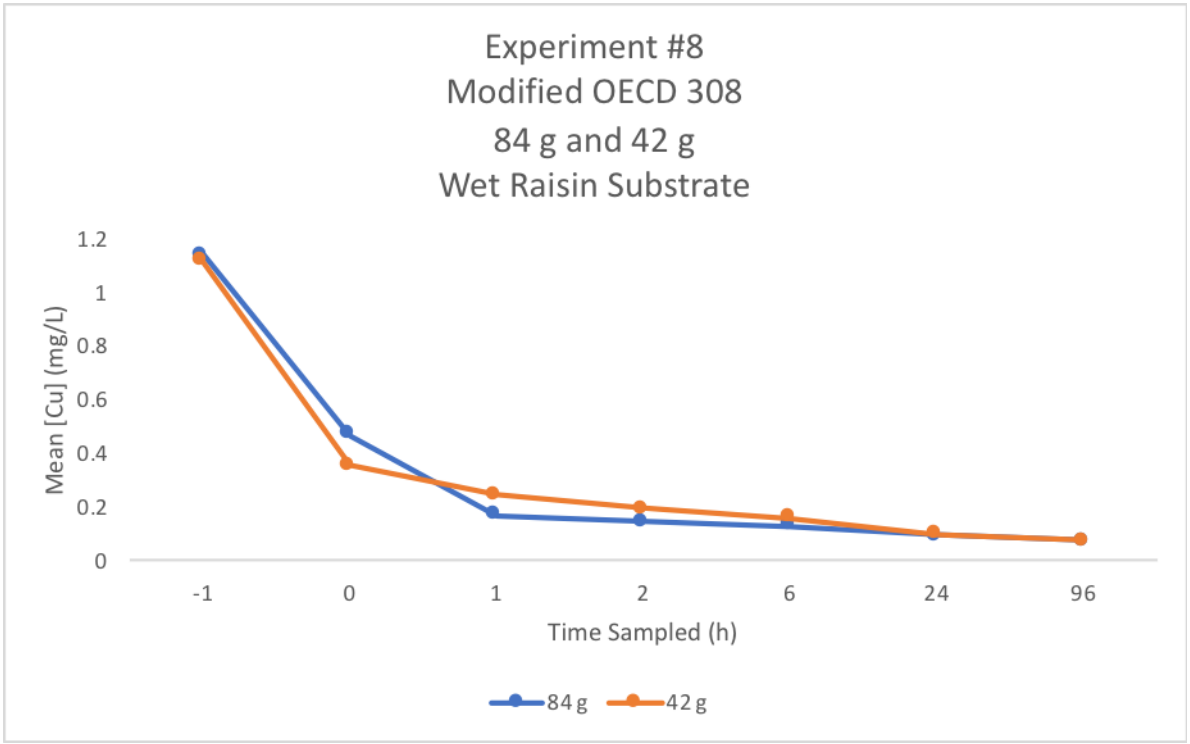

Figure 17: Experiment 8. Modified OECD 308 test. 84 g and 42 g treatments behaved similarly to one another, achieving 70% removal of Cu within 1 h.

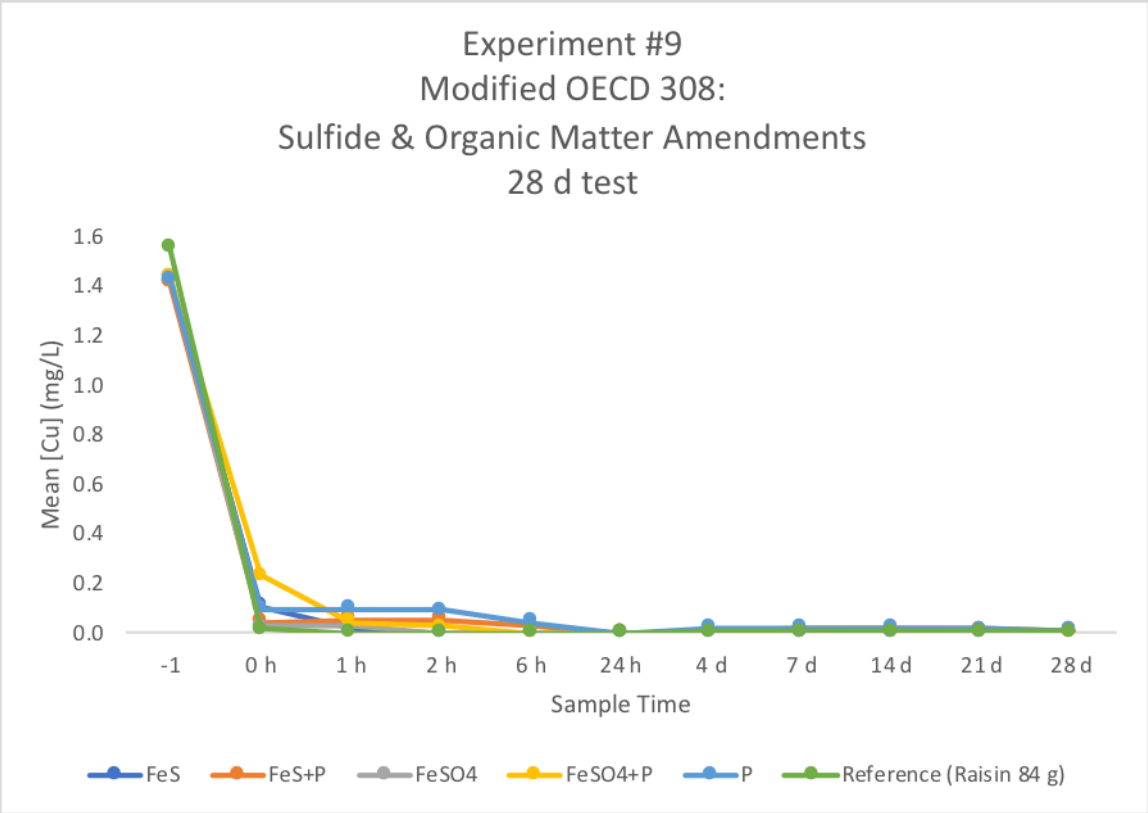

Figure 18: Experiment 9. 28 d modified OECD 308 test with sulfide and organic matter amendments. All treatments achieved at least 70% or more Cu removal within 24 h.

Table 2: Summary of substrate chemistry. TOC, Cu, or texture data not provided by NIST.

	TOC (% dry)	Total Fe (%)	Mn (µg/g)	Ni (µg/g)	Cu (µg/g)	Zn (µg/g)	Pb (µg/g)	Texture
Raisin	1.87	0.908	454	11.7	6.67	30.7	3.58	Sand
CANMET	1.1	1.56	506	34	18	54.7	13.5	Sand
Buffalo	--	3.97	544	42.9	--	408	150	--

Table 3:Experiment 1. Sediment loading test Buffalo 1 g, 10g vs Raisin 1 g and Raisin 10 g. All treatments achieved 70% Cu removal within 24 h.

Treatment	Time (h)	Mean Cu (mg/L)	% Cu Removed	Mean pH
Buffalo 1 g	-1	0.596	0.00	6.30
Buffalo 1 g	0	0.181	69.63	6.73
Buffalo 1 g	2	0.126	78.86	6.79
Buffalo 1 g	6	0.088	85.23	6.83
Buffalo 1 g	24	0.041	93.12	6.72
Buffalo 1 g	96	0.02	96.64	6.30
Buffalo 10 g	-1	0.618	0.00	6.23
Buffalo 10 g	0	0.162	73.79	6.65
Buffalo 10 g	2	0.052	91.59	6.67
Buffalo 10 g	6	0.071	88.51	6.69
Buffalo 10 g	24	0.069	88.83	6.51
Buffalo 10 g	96	0.058	90.61	7.19
Raisin 1 g	-1	0.636	0.00	6.14
Raisin 1 g	0	0.41	35.53	6.71
Raisin 1 g	2	0.317	50.16	6.76
Raisin 1 g	6	0.227	64.31	6.83
Raisin 1 g	24	0.114	82.08	6.65
Raisin 1 g	96	0.021	96.70	6.84
Raisin 10 g	-1	0.507	0.00	6.24
Raisin 10 g	0	0.288	43.20	6.49
Raisin 10 g	2	0.247	51.28	6.64
Raisin 10 g	6	0.146	71.20	6.95
Raisin 10 g	24	0.063	87.57	6.66
Raisin 10 g	96	0.025	95.07	7.03

Table 4: Experiment 2. 96 h exposure. All treatments achieved 70% Cu removal within 96 h.

Treatment	Time (h)	Mean Cu (mg/L)	% Cu Removed	Mean pH
Buffalo	-1	0.8705	0	5.9
Buffalo	0	0.043	95.06031	6.89
Buffalo	2	0.0445	94.888	6.74
Buffalo	6	0.039	95.51982	6.71
Buffalo	24	0.0695	92.01608	6.68
Buffalo	96	0.074	91.49914	6.51
CANMET LBP	-1	0.7975	0	6.01
CANMET LBP	0	0.4675	41.37931	6.28
CANMET LBP	2	0.6145	22.94671	6.29
CANMET LBP	6	0.6575	17.55486	6.17
CANMET LBP	24	0.3945	50.53292	6.11
CANMET LBP	96	0.199	75.04702	6.07
No substrate	-1	1.0415	N/A	5.89
No substrate	0	1.0665		5.91
No substrate	2	1.093		5.98
No substrate	6	1.1475		6.02
No substrate	24	0.918		6.01
No substrate	96	1.0845		5.99
Raisin 10 g	-1	1.2015	0	6.14
Raisin 10 g	0	0.496	58.71827	6.72
Raisin 10 g	2	0.369	69.28839	6.76
Raisin 10 g	6	0.3775	68.58094	6.9
Raisin 10 g	24	0.145	87.93175	6.83
Raisin 10 g	96	0.1	91.67707	6.55
Raisin 100 g	-1	1.1875	0	6.2
Raisin 100 g	0	0.0855	92.8	6.62
Raisin 100 g	2	0.0805	93.22105	6.75
Raisin 100 g	6	0.0945	92.04211	6.89
Raisin 100 g	24	0.149	87.45263	6.45
Raisin 100 g	96	0.1225	89.68421	6.34

Table 5: Experiment 3. Incubated 10 g, 100 g vs Non-Incubated 10 g, 100 g loading. All treatments achieved 70% removal within 96 h.

Treatment	Time (h)	Mean Cu (mg/L)	% Cu Removed	Mean pH
Incubated 10 g	-1	0.8225	0.00	6.13
Incubated 10 g	0	0.2425	70.52	7.08
Incubated 10 g	2	0.1815	77.93	6.89
Incubated 10 g	6	0.151	81.64	6.85
Incubated 10 g	24	0.111	86.50	7.16
Incubated 10 g	96	0.0535	93.50	7.81
Incubated 100 g	-1	0.7875	0.00	6.14
Incubated 100 g	0	0.1265	83.94	7.85
Incubated 100 g	2	0.068	91.37	7.8
Incubated 100 g	6	0.058	92.63	7.74
Incubated 100 g	24	0.055	93.02	7.55
Incubated 100 g	96	0.067	91.49	7.01
Non-incubated 10 g	-1	0.734	0.00	6.11
Non-incubated 10 g	0	0.491	33.11	6.81
Non-incubated 10 g	2	0.3905	46.80	6.86
Non-incubated 10 g	6	0.385	47.55	6.88
Non-incubated 10 g	24	0.3095	57.83	6.9
Non-incubated 10 g	96	0.157	78.61	7.48
Non-incubated 100 g	-1	0.8585	0.00	6.08
Non-incubated 100 g	0	0.1955	77.23	6.96
Non-incubated 100 g	2	0.12	86.02	7.34
Non-incubated 100 g	6	0.115	86.60	7.15
Non-incubated 100 g	24	0.1065	87.59	7.095
Non-incubated 100 g	96	0.095	88.93	6.975

Table 6: Experiment 4. 28 d Ni and Cu test values (Ni only below) with % Ni removed calculated

Treatment	Time (h)	Mean Ni (mg/L)	% Ni Removed	Mean pH
Buffalo	-1	1.166	0.00	6.02
Buffalo	0	0.63	45.97	6.28
Buffalo	1	0.69	40.82	6.26
Buffalo	2	0.73	37.39	6.21
Buffalo	6	0.73	37.39	6.18
Buffalo	24	0.704	39.62	6.22
Buffalo	96	0.334	71.36	6.18
Buffalo	14 d	0.199	82.93	6.11
Buffalo	21 d	0.1555	86.66	6.08
Buffalo	28 d	0.106	90.91	6.08
Canmet	-1	1.029	0.00	6.02
Canmet	0	0.961	6.61	5.88
Canmet	1	0.8655	15.89	5.91
Canmet	2	0.8055	21.72	5.89
Canmet	6	0.6945	32.51	6.14
Canmet	24	0.5085	50.58	6.14
Canmet	96	0.39	62.10	6.12
Canmet	14 d	0.3185	69.05	6.08
Canmet	21 d	0.283	72.55	6.11
Canmet	28 d	0.2445	76.24	6.04
Raisin Incubated	-1	1.019	0.00	6.2
Raisin Incubated	0	0.8125	20.26	6.59
Raisin Incubated	1	0.7745	23.99	6.58
Raisin Incubated	2	0.7465	26.74	6.66
Raisin Incubated	6	0.5875	42.35	6.46
Raisin Incubated	24	0.252	75.27	7.21
Raisin Incubated	96	0.115	88.71	6.84
Raisin Incubated	14 d	0.094	90.78	6.79
Raisin Incubated	21 d	0.08	92.15	6.7
Raisin Incubated	28 d	0.073	92.84	6.6
Raisin Non-Incubated	-1	1.0205	0.00	6.24
Raisin Non-Incubated	0	0.909	10.93	6.49
Raisin Non-Incubated	1	0.8715	14.60	6.64
Raisin Non-Incubated	2	0.789	22.68	6.95
Raisin Non-Incubated	6	0.634	37.87	6.66
Raisin Non-Incubated	24	0.4585	55.07	7.03
Raisin Non-Incubated	96	0.1465	85.64	6.79
Raisin Non-Incubated	14 d	0.086	91.57	6.88
Raisin Non-Incubated	21 d	0.0525	94.86	6.88
Raisin Non-Incubated	28 d	0.0435	95.74	6.8

Table 7: Expt 4. 28 d Ni and Cu 28 d (Cu only below) with % Cu removed

Treatment	Time (h)	Mean Cu [mg/L]	% Cu Removed	Mean pH
Buffalo	-1	0.661	0.00	6.02
Buffalo	0	0.0295	95.54	6.28
Buffalo	1	0.0295	95.54	6.26
Buffalo	2	0.0275	95.84	6.21
Buffalo	6	0.0385	94.18	6.18
Buffalo	24	0.0375	94.33	6.22
Buffalo	96	0.008	98.79	6.18
Buffalo	14 d	0.0105	98.41	6.11
Buffalo	21 d	0.0365	94.48	6.08
Buffalo	28 d	0.021	96.82	6.08
Canmet	-1	0.664	0.00	6.02
Canmet	0	0.389	41.42	5.88
Canmet	1	0.38	42.77	5.91
Canmet	2	0.36	45.78	5.89
Canmet	6	0.36	45.78	6.14
Canmet	24	0.1465	77.94	6.14
Canmet	96	0.0065	99.02	6.12
Canmet	14 d	0.006	99.10	6.08
Canmet	21 d	0.026	96.08	6.11
Canmet	28 d	0.0085	98.72	6.04
Raisin Incubated	-1	0.619	0.00	6.2
Raisin Incubated	0	0.045	92.73	6.59
Raisin Incubated	1	0.0415	93.30	6.58
Raisin Incubated	2	0.0425	93.13	6.66
Raisin Incubated	6	0.027	95.64	6.46
Raisin Incubated	24	0.022	96.45	7.21
Raisin Incubated	96	0.003	99.52	6.84
Raisin Incubated	14 d	0.003	99.52	6.79
Raisin Incubated	21 d	0.01	98.38	6.7
Raisin Incubated	28 d	0.0045	99.27	6.6
Raisin Non-Incubated	-1	0.646	0.00	6.24
Raisin Non-Incubated	0	0.278	56.97	6.49
Raisin Non-Incubated	1	0.265	58.98	6.64
Raisin Non-Incubated	2	0.178	72.45	6.95
Raisin Non-Incubated	6	0.163	74.77	6.66
Raisin Non-Incubated	24	0.0755	88.31	7.03
Raisin Non-Incubated	96	0.0055	99.15	6.79

Raisin Non-Incubated	14 d	0.0035	99.46	6.88
Raisin Non-Incubated	21 d	0.0125	98.07	6.88
Raisin Non-Incubated	28 d	0.0035	99.46	6.8

Table 8: AVS/SEM values for Raisin pre- and post- exposure, Incubated vs Non-Incubated

Treatment	[S2-] (µmol/g)	SEM (umol/g)	SEM-AVS	Toxicity Predicted?
Pre-exposure Non-Incubated	0.00	1.20	1.20	y
Post-exposure Non-Incubated	0.02	0.39	0.38	y
Pre-exposure Incubated	0.04	0.07	0.04	y
Post-exposure Incubated	0.02	1.06	1.04	y

substrates.

Table 9: Experiment 5. 96 h + 1 h remobilization dissolved Cu, Fe, and pH values

Treatment	Sample Time (h)	[Cu] ug/L	[Fe] ug/L	pH	% Cu Removed
CANMET LBP	-1	1486.69	2.36	6.02	0.00
CANMET LBP	0	1243.00	5.77	5.48	16.39
CANMET LBP	1h	1171.68	8.94	5.88	21.19
CANMET LBP	2h	1130.67	12.44	5.85	23.95
CANMET LBP	6h	1095.31	17.55	6.14	26.33
CANMET LBP	24h	749.10	19.79	6.14	49.61
CANMET LBP	96h	305.63	16.28	6.12	79.44
CANMET LBP	R0	239.12	13.50	5.89	83.92
CANMET LBP	R1h	239.44	14.18	5.49	83.89
CANMET LBP	R2h	238.69	16.79	5.56	83.94
CANMET LBP	R6h	216.00	13.00	5.59	85.47
CANMET LBP	R24h	169.00	12.00	5.54	88.63
CANMET LBP	R96h	113.00	11.00	5.62	92.40
Buffalo	-1	1462.02	5.65	5.98	0.00
Buffalo	0	367.57	50.19	5.81	74.86
Buffalo	1h	484.12	11.58	5.98	66.89
Buffalo	2h	493.00	10.05	5.9	66.28
Buffalo	6h	509.47	8.68	6.11	65.15
Buffalo	24h	448.51	13.01	6.08	69.32
Buffalo	96h	336.42	13.30	6.18	76.99
Buffalo	R0	145.78	20.29	7.16	90.03
Buffalo	R1h	141.48	20.61	7.16	90.32
Buffalo	R2h	137.78	23.54	7.15	90.58
Buffalo	R6h	129.00	23.00	7.13	91.18

Buffalo	R24h	110.37	18.00	7.15	92.45
Buffalo	R96h	98.00	16.00	6.98	93.30
Raisin Incubated	-1	1497.15	2.74	5.98	0.00
Raisin Incubated	0	755.35	1.15	5.64	49.55
Raisin Incubated	1h	144.80	0.00	6.3	90.33
Raisin Incubated	2h	38.89	1.90	6.1	97.40
Raisin Incubated	6h	22.11	0.00	5.98	98.52
Raisin Incubated	24h	28.58	2.74	5.98	98.09
Raisin Incubated	96h	14.05	1.78	6.03	99.06
Raisin Incubated	R0	0.00	2.17	8.06	100.00
Raisin Incubated	R1h	0.00	1.42	8.14	100.00
Raisin Incubated	R2h	0.00	1.40	8.15	100.00
Raisin Incubated	R6h	26.00	1.00	8.11	98.26
Raisin Incubated	R24h	26.30	2.00	8.14	98.24
Raisin Incubated	R96h	27.00	2.00	7.65	98.20
Raisin Non incubated	-1	1491.96	2.89	6.02	0.00
Raisin Non incubated	0	1144.41	2.97	5.76	21.72
Raisin Non incubated	1h	1049.59	1.44	6.08	28.21
Raisin Non incubated	2h	974.30	2.89	6.07	33.36
Raisin Non incubated	6h	902.31	0.00	6.05	38.28
Raisin Non incubated	24h	396.06	6.66	6.05	72.91
Raisin Non incubated	96h	233.65	0.00	6.02	84.02
Raisin Non incubated	R0	211.52	3.34	7.51	85.53
Raisin Non incubated	R1h	224.09	0.00	7.82	84.67
Raisin Non incubated	R2h	228.70	0.00	7.88	84.36
Raisin Non incubated	R6h	221.00	0.00	7.79	84.88
Raisin Non incubated	R24h	183.00	0.00	7.41	87.48
Raisin Non incubated	R96h	129.00	2.00	7.45	91.18

Table 10: Experiment 6. 24 h mixing study results

Treatment	Sample_Time_h	Mean [Cu] (mg/L)	% Cu Removed	pH
No Mixing	-1	1.007	0.0	6.02
No_Mixing	0	0.674	33.0	5.45
No_Mixing	15 min	0.451	55.2	5.58
No_Mixing	30 min	0.390	61.3	5.61
No_Mixing	1 h	0.332	67.0	5.88
No_Mixing	2 h	0.393	61.0	6.12
No_Mixing	6 h	0.405	59.8	6.08
No_Mixing	24 h	0.382	62.1	6.02
1m	-1	1.033	0.0	6.08
1m	0	0.569	44.9	5.76
1m	15 min	0.428	58.5	5.59
1m	30 min	0.391	62.1	5.66
1m	1 h	0.356	65.5	6.09
1m	2 h	0.339	67.2	6.85
1m	6 h	0.335	67.6	6.14
1m	24 h	0.341	67.0	6.08
1hr	-1	1.053	0.0	6.00
1hr	0	0.238	77.4	6.31
1hr	15 min	0.209	80.2	6.55
1hr	30 min	0.174	83.4	6.77
1hr	1 h	0.190	82.0	6.98
1hr	2 h	0.210	80.0	6.71
1hr	6 h	0.197	81.3	6.54
1hr	24 h	0.177	83.2	6.30
2hr	-1	1.029	0.0	6.02
2hr	0	0.145	85.9	7.67
2hr	15 min	0.137	86.7	7.85
2hr	30 min	0.139	86.5	7.78
2hr	1 h	0.139	86.5	7.6
2hr	2 h	0.137	86.7	7.44
2hr	6 h	0.133	87.1	7.06
2hr	24 h	0.142	86.2	6.42

Table 11: Experiment 8. Modified OECD 308 Wet Raisin 84 g and 42 g 96 h exposure

Treatment	Sample time	Mean Cu (mg/L)	% Cu Removed
84 g	-1	1.142	0
84 g	0	0.467	59.09
84 g	1	0.171	84.99
84 g	2	0.145	87.29
84 g	6	0.131	88.54
84 g	24	0.097	91.52
84 g	96	0.077	93.25
42 g	-1	1.114	0
42 g	0	0.353	68.35
42 g	1	0.244	78.11
42 g	2	0.195	82.51
42 g	6	0.162	85.45
42 g	24	0.101	90.94
42 g	96	0.077	93.06

Table 12: Experiment 9. 28 d modified OECD 308 sulfide and organic matter amendments test

Treatment	Time (hours)	Cu (mg/L)	Fe (mg/L)	% Cu Removed
FeS	-1	1.417	0	0.00
FeS	0 h	0.109	0.006	92.31
FeS	1 h	0.022	0	98.44
FeS	2 h	0	0	100.00
FeS	6 h	0	0	100.00
FeS	24 h	0	0	100.00
FeS	4 d	0.001	0	99.93
FeS	7 d	0	0	100.00
FeS	14 d	0.002	0	99.87
FeS	21 d	0.002	0	99.84
FeS	28 d	0	0	100.00
FeS+P	-1	1.410	0.000	0.00
FeS+P	0 h	0.043	0.425	96.96
FeS+P	1 h	0.051	0.426	96.37
FeS+P	2 h	0.052	0.419	96.31
FeS+P	6 h	0.025	0.281	98.22
FeS+P	24 h	0	0	100.00
FeS+P	4 d	0.011	0	99.20
FeS+P	7 d	0.014	0	99.03
FeS+P	14 d	0.017	0	98.82
FeS+P	21 d	0.018	0	98.71
FeS+P	28 d	0.009	0	99.35
FeSO4	-1	1.441	0.006	0.00
FeSO4	0 h	0.028	23.740	98.03
FeSO4	1 h	0.032	20.595	97.76
FeSO4	2 h	0	18.964	100.00
FeSO4	6 h	0	6.828	100.00
FeSO4	24 h	0	0.225	100.00
FeSO4	4 d	0	0	100.00
FeSO4	7 d	0	0	100.00
FeSO4	14 d	0	0	100.00
FeSO4	21 d	0	0	100.00
FeSO4	28 d	0	0	100.00
FeSO4+P	-1	1.434	0.000	0.00
FeSO4+P	0 h	0.232	0.983	83.81
FeSO4+P	1 h	0.042	0.228	97.05
FeSO4+P	2 h	0.024	0.154	98.30
FeSO4+P	6 h	0	0.220	100.00
FeSO4+P	24 h	0	0.373	100.00

FeSO4+P	4 d	0.010	0	99.28
FeSO4+P	7 d	0.016	0	98.89
FeSO4+P	14 d	0.015	0	98.93
FeSO4+P	21 d	0.012	0	99.17
FeSO4+P	28 d	0.005	0	99.66
P	-1	1.431	0.000	0.00
P	0 h	0.099	0.147	93.06
P	1 h	0.097	0.141	93.21
P	2 h	0.094	0.143	93.45
P	6 h	0.042	0.089	97.08
P	24 h	0.000	0	100.00
P	4 d	0.019	0	98.66
P	7 d	0.023	0	98.41
P	14 d	0.022	0	98.47
P	21 d	0.018	0	98.74
P	28 d	0.009	0	99.34
Reference (Raisin 84 g)	-1	1.550	0.000	0.00
Reference (Raisin 84 g)	0 h	0.018	0.017	98.84
Reference (Raisin 84 g)	1 h	0	0.017	100.00
Reference (Raisin 84 g)	2 h	0	0.014	100.00
Reference (Raisin 84 g)	6 h	0	0.007	100.00
Reference (Raisin 84 g)	24 h	0	0	100.00
Reference (Raisin 84 g)	4 d	0.005	0	99.65
Reference (Raisin 84 g)	7 d	0.006	0	99.64
Reference (Raisin 84 g)	14 d	0.006	0	99.60
Reference (Raisin 84 g)	21 d	0.006	0	99.63
Reference (Raisin 84 g)	28 d	0.004	0	99.77

Table 13: Experiment 9 SEM-AVS values

Treatment	Pre_Post	SEM-AVS Mean	SEM:AVS/ (fOC) Mean	Toxicity predicted?
Reference (Raisin 84 g)	Pre	0.16	16.37	y
Reference (Raisin 84 g)	Post	0.19	18.81	y
FeS	Pre	1.39	12.60	y
FeS	Post	0.40	7.97	y
FeS + P	Pre	-14.48	-111.39	n
FeS + P	Post	-13.66	-227.62	n
FeSO4	Pre	-0.40	-10.05	n
FeSO4	Post	-9.52	-271.87	n
FeSO4 + P	Pre	-12.95	-129.49	n
FeSO4 + P	Post	-11.11	-100.96	n
P	Pre	-1.04	-6.92	n
P	Post	-0.65	-8.14	n