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Colloidal and truly dissolved metal(loid)s in wastewater lagoons and their removal with floating treatment wetlands

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COLLOIDAL AND TRULY DISSOLVED METAL(LOID)S IN WASTEWATER LAGOONS AND THEIR REMOVAL WITH FLOATING TREATMENT WETLANDS

By

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ABSTRACT

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Climate change is predicted to cause continuing declines in late-season streamflow, thus increasing the relative contribution of wastewater effluent to surface water flows. Wastewater effluent represents a critical point source of metal and metalloid contamination to aquatic ecosystems and wastewater lagoons are the most common wastewater treatment system in the rural United States. Although the fraction of total wastewater metals and metalloids in "dissolved" forms (defined here as < 450 nm) likely drives the potential for negative effects on receiving waters, this broad operational definition lumps truly dissolved solutes (<1 nm) with small colloids and nanomaterials (1-450 nm; hereafter colloids). This size distinction may be important as colloidal particles and truly dissolved solutes differ in their interactions with aquatic organisms and likely would require different strategies for their removal from wastewater. One potential tool for improving metal(loid) removal in wastewater lagoons is floating treatment wetlands, which consist of hydroponically grown plants on floating mats. This study examined the distribution of metal(loid)s between truly dissolved and small colloidal size fractions in six wastewater lagoon systems. Additionally, the efficacy of floating treatment wetlands in removing metal(loids) and influencing the distribution of contaminants among truly dissolved and small colloidal size ranges was examined. In this survey of six lagoons, it was found that iron, lead, copper, manganese, and zinc were most abundant as small colloidal particles while aluminum, arsenic, and chromium were found mostly as truly dissolved solutes. The floating treatment wetlands were especially effective at removing those metal(loid)s that were abundant in colloidal forms, suggesting a potential role for floating treatment wetlands in enhancing wastewater lagoon efficiency for some metal(loid) contaminants.

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1. Introduction

Droughts are expected to become more frequent and more extreme due to anthropogenic climate change (IPCC, 2014; Peterson et al., 2013) which in turn, is expected to both decrease late-season stream flows and increase the relative importance of wastewater effluent to surface water (Brooks et al., 2006; Chang & Bonnette, 2016; Drury et al., 2013; Lee & Rasmussen, 2006; Naidoo & Olaniran, 2013). This shift has already been observed in arid and semi-arid regions around the world, where streams are becoming increasingly dependent on wastewater effluent to maintain base flows (Jin et al., 2017; Mimikou et al., 2000; van Vliet et al., 2013). Although wastewater effluent has the potential to decrease stress on freshwater organisms by maintaining flow in the face of drought, effluent also serves as a stressor by increasing the concentrations of nutrients, biological oxygen demand (BOD), metals, and metalloids (Holeton et al., 2011; Pottinger et al., 2013; Wakelin et al., 2008).

Wastewater lagoons are a common form of wastewater treatment in the United States and around the world. Today, there are over 7,000 facultative lagoons used across the United States, occurring primarily in rural areas (U.S. EPA, 2012), including 105 municipal lagoon systems in Montana. These lagoons are bound by state regulations such as water quality-based effluent limits (WQBELs), and must comply with state water quality standards under the umbrella of the federal Clean Water Act (CWA) standards (U.S. EPA, 2016). Wastewater lagoons can be effective at meeting WQBELs when the size of the lagoons are appropriate for the level of inputs (Massoud et al., 2009).

While wastewater lagoons can be effective at reducing BOD, nutrients, and pathogens, they are not necessarily intended to remove metals and metalloids (hereafter metal(loid)s). This may lead to the potential persistence of elevated metal(loid) concentrations in effluent (Karvelas et al., 2003). It may be further expected that lagoons in mining impacted watersheds may have elevated levels of metal(loid)s due to higher background levels of metals as a result of mining disturbances. While the metals Zn, Cu, Ni, Co, and Cr are essential for metabolic and physiological processes in aquatic organisms, when in excess they can be toxic like the non-essential metals Ag, Cd, Pb, and Hg, and the metalloid As (Fashola et al., 2016). Elevated metal(loid) inputs into aquatic systems can thus have deleterious effects on aquatic organisms with high concentrations potentially causing mortality, while chronic sublethal exposures may affect growth, morphology, and behavior (Fashola et al., 2016). The stress from metal contamination can be tolerated by only a subset of species often resulting in decreased biodiversity and shortened food webs (Hogsden & Harding, 2013).

When discussing the forms of metals moving through ecosystems, historically, metal(loid) contaminants were defined as either (1) particles large enough to be retained by a filter and assumed to have low bioavailability, or (2) a "dissolved" fraction operationally defined as passing through a filter and assumed to have higher bioavailability (Hochella et al., 2008). However, much of what passes through standard filters (*e.g.,* 450 nm) may not be dissolved solutes, but may instead exist as particles in the nanoparticle (1-100 nm) or small colloidal particle (100-450 nm) size ranges (Hassellov & von der Kammer, 2008) These small particles remain in suspension because their rate of molecular diffusion is greater than their settling velocity (Nystrand e al., 2012; Pugh et al., 1983; Schwab et al., 2015; Stumm & Morgan, 1996). It may be important to split rather than lump 'truly dissolved' solutes (<1nm) from small colloidal particles and nanomaterials (hereafter small colloids, 1-450 nm), and compare these to the 'suspended particulate matter' (SPM), which consists of large colloids (450-1000 nm) and larger particles (>1000 nm; Nystrand et al., 2012; Yang et al., 2015).

Thinking about the distribution of contaminants among these different sizes may help to understand the behavior, biogeochemical cycling, and transport of trace metals in aquatic systems (Auffan et al., 2009; Baalousha et al., 2011) including wastewater lagoons. On one end of the size continuum, truly dissolved contaminants are thought to be mobile, readily taken up by organisms, and thus have the potential to be highly toxic (Nystrand et al., 2012; Schwab et al., 2015). On the other end of the size continuum, suspended particulate matter may be less toxic as it has a lower surface area per unit volume, which contributes to lower solubility, reactivity, and a decreased ability to sorb and release contaminants (Auffan et al., 2009). It is also expected that larger SPM is more prone to sediment out of suspension as its settling velocity exceeds its molecular diffusion velocity (Auffan et al., 2009). Additionally, SPM is less readily ingested by most aquatic organisms with the exception of filter feeders (Nystrand et al., 2012; Sigg et al., 2000). Sitting between SPM and truly dissolved solutes, particles in the small colloidal fraction can be highly reactive (Weltens et al., 2000) because the surface area to volume ratio increases as particle size decreases (Auffan et al., 2009) and this high reactivity makes them capable of concentrating contaminants (Yang et al., 2015). Contaminants in this size range can enter aquatic food webs through several mechanisms including direct uptake, passive uptake by diffusion, and uptake through ingestion of other organisms and their internal or sorbed contaminants (Hogsden & Harding, 2013; Schwab et al., 2015; Weltens et al., 2000). Thus, for certain organisms, the small colloidal fraction may be more bioavailable than even the dissolved fraction, while for others it may be less (Nystrand et al., 2012).

Despite the potential importance of this colloidal fraction, much about its fate and transport remains unknown. This distinction between small colloidal particles and truly dissolved solutes may be important as they likely differ in their interactions with aquatic organisms and would require different strategies for their removal from wastewater. Understanding the distribution of metals across size fractions is an important first step in understanding how these metal(loid)s behave in wastewater lagoons. It could also inform how to best manage lagoons for metal(loid) removal, and inform the food web implications for receiving waters of colloid metal(loid)s in effluent.

Given that effluent with elevated metals can have ecological impacts across all levels of the food web, and given the high cost of replacing and maintaining lagoon systems with higher efficiency wastewater treatment systems, there is a need for lower-cost approaches to improving wastewater lagoon efficiency. One relatively new approach for increasing lagoon efficiency is the addition of floating treatment wetlands (FTWs), which consist of buoyant mats planted with an assemblage of plants. As the plant roots extend into the water column, they create a large surface area through which nutrients and metals can be taken up and sequestered by plants and the periphyton—the collection of algae, bacteria, and fungi that colonize the roots (Faulwetter et al., 2011; Hubbard et al., 2004; Tanner & Headley, 2011). The roots also serve as a source of labile carbon which can fuel the growth of planktonic and attached heterotrophic microbes and stimulate the removal of excess nitrogen (N), phosphorous (P), and metals (Shahid et al., 2018), which may then be delivered to the sediment as the periphyton sloughs off the roots (Borne et al., 2013; Tanner & Headley, 2011).

In a range of lab and field trials FTWs have been tested for their efficacy in stormwater and wastewater experiments. In those studies focused on stormwater management, FTWs have proven to have the potential to remove Nitrogen (N), Phosphorus (P), and metals including Cu, Pb, Zn, through accumulation, sorption, and precipitation (Figure 1; Borne et al., 2013; Ladislas et al., 2015; Stewart et al., 2008; Van de Moortel et al., 2010). Studies examining FTWs in wastewater lagoons have largely focused on their ability to remove N and P, but few studies have focused on their ability to remove metal(loid)s in wastewater lagoons where concentrations are likely to be higher. Finally, we did not find studies exploring the effect of FTWs on the distribution of contaminants among size fractions.

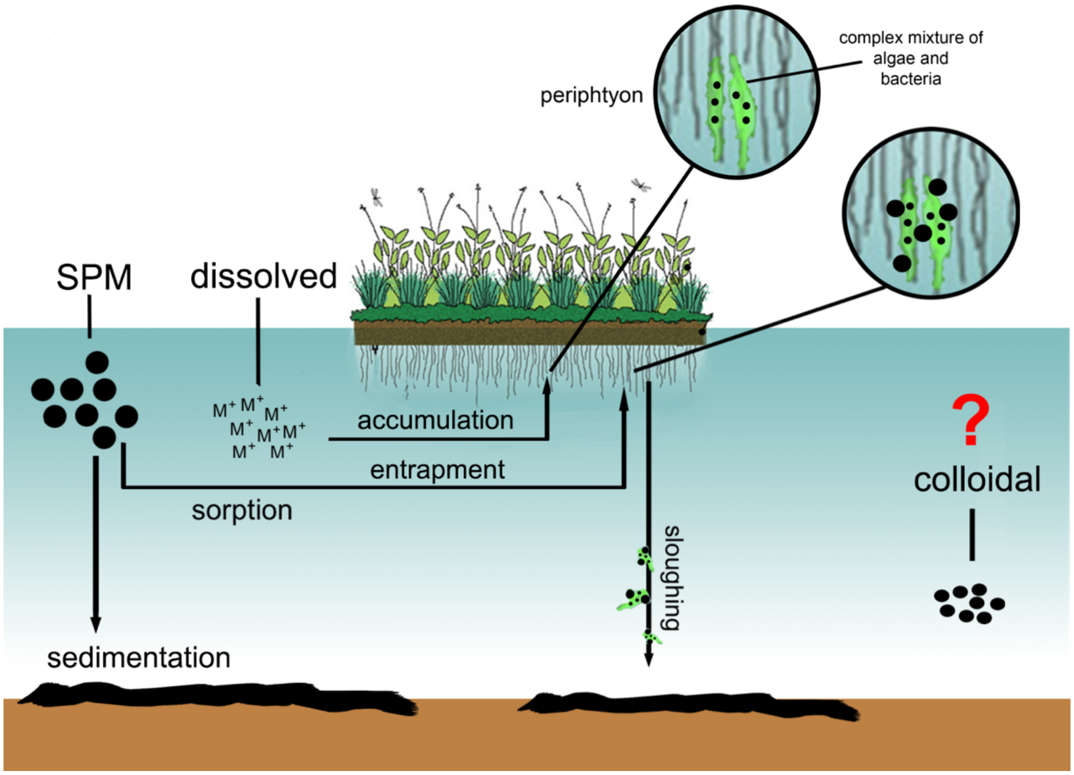

Figure 1. Conceptual model of how metal(loid) particles move through a system with FTWs.

The goals of this study were to: 1) characterize the concentration of metal(loid)s and their distribution among size fractions in wastewater lagoons; 2) investigate the effect of FTWs on metal(loid) concentration; 3) understand the effect of FTWs on the size distribution of metal(loid)s; and 4) determine if FTWs have a similar effect on metal(loid)s under high and low concentrations. To examine the concentration and size distribution of metal(loid)s in wastewater lagoons, a field survey of six different wastewater lagoon systems was conducted. To examine the effects of FTWs on metal(loid) concentration, distribution among size fractions, and efficacy of FTWs at low and high concentration, a mesocosm experiment using wastewater lagoon water from one of the systems included in our field survey was conducted.

2. Materials and methods

2.1 Field Sites

To characterize the total metal(loid) concentration and the distribution of metal(loid)s between small colloids and truly dissolved solutes in wastewater lagoons, we selected six wastewater treatment lagoons to sample which are located in a mining-influenced watershed of western Montana. The six different lagoon systems differed in their size, the number of lagoons in series, and whether or not they were aerated. We sampled all of the lagoons at each facility, though we only report data on the terminal lagoons. All systems either discharge directly into the adjacent river or indirectly through infiltration into the alluvial aquifer.

To examine the ability of FTWs to remove metal(loid)s from wastewater under low and high wastewater concentrations, we conducted a mesocosm experiment with and without FTWs at the Missoula Wastewater Treatment Facility (Missoula, MT). Twelve mesocosms were established; six mesocosms had FTWs and six did not (Figure 1-A in the appendix). Mesocosms consisted of 300-gallon stock tanks (Rubbermaid, Atlanta, USA) with liners made of 12 mil black/white Dura Skrim polyethylene sheeting (Americover, Escondido, USA). All mesocosms were filled with groundwater that was passed through a carbon block filter to remove dissolved and particulate matter (CFB-PLUS20BB, Pentek, Pittsburgh, USA). FTWs were 50.8 x 99.06 x 16.51 cm, and were sized to give 20% coverage of the mesocosms. Seeds and bareroot emergent macrophytes were selected and planted into the FTWs. Transplanted bareroot species were: *Sium suave, Equisetum hymale, Juncus arcticus, Carex aquatilis, and Schoenoplectus acutus* (Fourth Corner Nurseries, Bellingham, USA). The FTWs were seeded with equal amounts of

Calamagrostis canadensis, Mentha arvensis, and *Helianthus anuus* (Prairie Moon Nursery, Winona, USA; Figure 2-A in the appendix). The planting medium consisted of a mix of 1/3 rockwool and 2/3 peat in each pre drilled 3 inch deep planting hole. FTWs were established for 2.5 months (Figure 3-A in the appendix) with daily watering and with weekly cycling of water between all mesocosms in order to achieve similar water chemistry between all mesocosms.

To test the efficacy of FTWs in removing metal(loid)s under high or low concentrations of these contaminants, 2000 gallons of water was collected and hauled in a septic pump truck from one of the field sampling sites with known heightened levels of metal(loid)s. Six high concentration mesocosms and six low concentration mesocosms were established, with three of each concentration (high or low) and each cover type (FTW or open). Mesocosms were first drawn down to either 93 gallons or 195 gallons and then received either 195 gallons or 93 gallons of wastewater for the high and low concentration mesocosms, respectively.

2.2 Field Site Procedures

Field sampling at wastewater lagoons was carried out over a three day period in July 2017. At each pond, water samples for metal(loid) concentration were collected from three separate locations along the shoreline of the lagoon (50 mL). Samples were collected from the middle of the water column using a telescoping water sample dipper (Bel-Art, Wayne, USA) and stored in a cooler on ice until processing. At each replicate location, water was characterized for dissolved oxygen, pH, conductivity, and temperature using a YSI Professional Series Probe (YSI, Yellow Springs, USA).

Mesocosm sampling occurred over the course of five weeks during August and September 2017. To account for rapid changes, water samples were collected four times on day one, once per day for the next three days, and every five days thereafter for the remainder of the five-week experiment. All samples were kept on ice until processing (< 1 day). Given the possible role of environmental conditions on driving metal(loid) biogeochemistry, environmental parameters (pH, conductivity, temperature, and dissolved oxygen) were measured at each sampling time point using a YSI Probe. At the end of the mesocosm experiment, benthic organic matter was collected from all twelve mesocosms, dried at 60 degrees Celsius, and then pulverized.

2.3 Filtration of Water Samples

Water samples were split into three fractions: unfiltered; filtered (<450 nm; small colloids, nanoparticles, and truly dissolved solutes); and ultrafiltered (< 1 nm; truly dissolved). Tower filtration was used for the < 450 nm fraction and centrifugal filtration with 1 kDa ultrafiltration centrifuge filters (Microsep, Pall Corporation, Port Washington, USA) was used to obtain the <1 nm fraction. A small volume of sample water was filtered through both the 450 nm and 1 kDa filter and discarded prior to collecting sample filtrate in order to allow the most representative samples through the filters. Using the whole water and two filtrates, the 1-450 nm (colloidal), and >450 (suspended particular matter; SPM) size fractions were calculated. After filtration was completed, samples were acidified to 1% concentrated nitric acid for preservation.

2.4 Laboratory Analysis

Preserved water samples and benthic OM were quantified for a suite of 13 major and trace elements using an inductively coupled plasma mass spectrometer (ICP-MS; Agilent7500cx, Santa Clara, USA). Detection limits for ICP-MS water sample analysis are located in Table 1. Analytes that were below the detection limit were set to ½ the detection limit (Clark, 1998). For those metal(loid)s where all of the samples had concentrations at or below the detection limit (V, Se, Co, Ni in both the field collection and mesocosm experiment; Cr in the mesocosm experiment), their data were removed from analysis.

Table 1. Limits of detection of field lagoon and mesocosm water samples for inductively coupled plasma mass spectrometry (ICP-MS) analysis.

		Al	V	Cr	Fe	Mn	Co	Ni	Cu	Zn	As	Se	Cd	Pb
Limit of Detection (µg/L)	Field Lagoon	0.81	0.01	0.04	7.35	0.05	0.13	0.02	1.3	0.39	0.01	0.12	0.01	0.06
	Mesocosm	7.25	0.04	0.3	34.68	0.13	0.76	0.26	0.57	3.18	0.11	0.66	0.03	0.03

2.5 Data Processing and Statistical Analyses

In both the field survey and mesocosm study, truly dissolved (< 1nm) metal(loid) concentrations were taken directly from the ultrafiltered samples, while colloidal (1-450 nm) and SPM (> 450 nm) metal(loid) concentrations were calculated. The SPM fraction was calculated as the difference between the unfiltered and <450 nm filtered samples, while the colloidal fraction was calculated as the difference between the < 450 nm and < 1 nm filtrates. In calculating these differences, there were several instances where the difference would yield negative numbers. In those cases where the concentration of an element was 15% higher in the smaller size class than in the larger size class, that sample was removed from further analysis for that element in that fraction. For samples that were 0 to 15% higher in the smaller size class than in the larger, concentrations were set to be equivalent between the two size classes. All analyses were conducted in R version 3.1.2 (R Core Team, 2015).

For the field survey of wastewater lagoons, the SPM, colloidal, and truly dissolved fractions were averaged across samples from all three replicate sampling locations from each terminal lagoon (Rmisc R Package). To quantify the percentage of colloidal metal(loid)s within the traditionally defined dissolved (<450 nm) fraction we used the equation:

$$\%\,Colloidal = \frac{1\text{–}450\ nm}{<450\ nm} \times 100$$

To determine the effect of FTWs on metal(loid) concentrations the natural log transformed response ratio was calculated:

$$\ln RR = \ln \frac{\overline{X}_{Open}}{\overline{X}_{FTW}}$$

where RR is the response ratio, $\overline{X}_{open}$ is the mean concentration in open mesocosms and $\overline{X}_{FTW}$ is the mean concentration in FTW mesocosms calculated for each analyte as measured at each time point for each treatment type (high, low). Uncertainty was calculated using 95% confidence intervals for each point.

$$\text{Error (E)} = ln\,RR \times \sqrt{\left(\frac{(SD_{Open}*1.96)}{\overline{Open}}\right)^2 + \left(\frac{(SD_{FTW}*1.96)}{\overline{FTW}}\right)^2}$$

where $\overline{open}$ is the mean concentration in open mesocosms, $\overline{FTW}$ is the mean concentration in FTW mesocosms, and SD_{Open}, and SD_{FTW} are the standard deviation for Open and FTW mesocosms, respectively. All of these values were previously calculated using summary statistics (Rmisc R Package). Upper and lower bounds of the 95% confidence intervals were calculated by:

$$CI\ upper = lnRR + E;\ CI\ lower = lnRR - E$$

To quantify the percent of metals removed during the mesocosm experiment, all metal(loid) concentrations were first converted to masses at each timepoint by multiplying the concentration of each element by the measured water volume at the start of the experiment adjusted for evaporation. Changes in water volume were accounted for by assuming that the only changes in conductivity were due to evaporation and precipitation. The percent removal was then calculated as:

$$\% \, Removed = 100 - (\frac{Mass_{final}}{Mass_{initial}} * 100)$$

To quantify the effect of FTWs on metal(loid) concentrations, generalized linear mixed effect models (lme4 R Package) were fit to each response variable. A model was fit to each response variable including the fixed effects of cover (FTW, open), treatment (high, low), size fraction (truly dissolved, colloidal, and SPM), nested within time (day of experiment). Generalized linear mixed effect modeling (GLMM) was used to include a random effect (mesocosm number) to account for mesocosm level differences in the model. Parameters were analyzed using Gamma distribution and log link. Models were tested by analysis of variance (ANOVA) and chi squared results to determine the effect of independent variables and interaction terms. To determine at what levels differences occurred for our fixed effects, post hoc comparisons were performed using Tukey HSD within lsmeans (lsmeans R package), with $\alpha = 0.05$ as the threshold for significance tests.

3. Results

3.1 Field Lagoons

3.1.1 Environmental Data

Lagoon waters represented a range of pH, conductivity, temperature and dissolved oxygen conditions (Table 2). Site 3 had the highest temperature at 19.7 while site 6 had the lowest temperature recorded at 7.1. Dissolved oxygen (% saturation) was greatest at site 1 at 109.6 with site 6 at the lowest recorded levels of 77.5. Specific conductivity was found to be lowest at site 4 (181.7 µS/cm) while site 5 had the highest at 645.3 µS/cm. The pH of site 3 was the lowest at 7.8 while site 2 was recorded at the highest of 10.5.

Table 2. Details information of field lagoons. Physicochemical data recorded is recorded here as the mean of replicates from the terminal pond.

Site	Aeration	No. lagoons in series	Temperature (Celsius)	DO (% Sat)	Specific Conductivity	pH
1	Aerated	3	8.2	109.6	293.9	8.56
2	Non-Aerated	3	14.5	88.2	442	10.5
3	Aerated	2	19.7	83.6	273.3	7.8
4	Non-Aerated	2	15.3	89.2	181.7	9.1
5	Non-Aerated	1	14.8	82.7	645.3	8.4
6	Non-Aerated	1	7.1	77.5	292.7	9.7

3.1.2 Metal(loid) characterization

The concentration of metal(loid)s varied widely across the terminal lagoons at the six treatment systems sampled (Figure 2A), with all elements having a colloidal component. Mean concentrations were as high as 231 µg/L for Fe, down to 0.9 µg/L for Pb. Coefficients of variation ranged from a high of 2.09 for Al down to 0.66 for As. For the distribution of the eight focal elements measurable in the < 450 nm fraction, most either had median values that were largely colloidal (25 to 50% colloidal), or mostly colloidal (>50 %; Figure 2B). The percent colloidal varied by element with Fe and Pb appearing predominantly in the colloidal fraction at >75% and >60%, respectively. The metals Cu, Mn, and Zn were intermediate in their percent colloidal, ranging from 30% to 60% colloidal. The distribution of Al and Cr had much lower amounts in the colloidal fraction, ranging from 15% to 30%, while arsenic had the lowest colloidal fraction with a median of 10%.

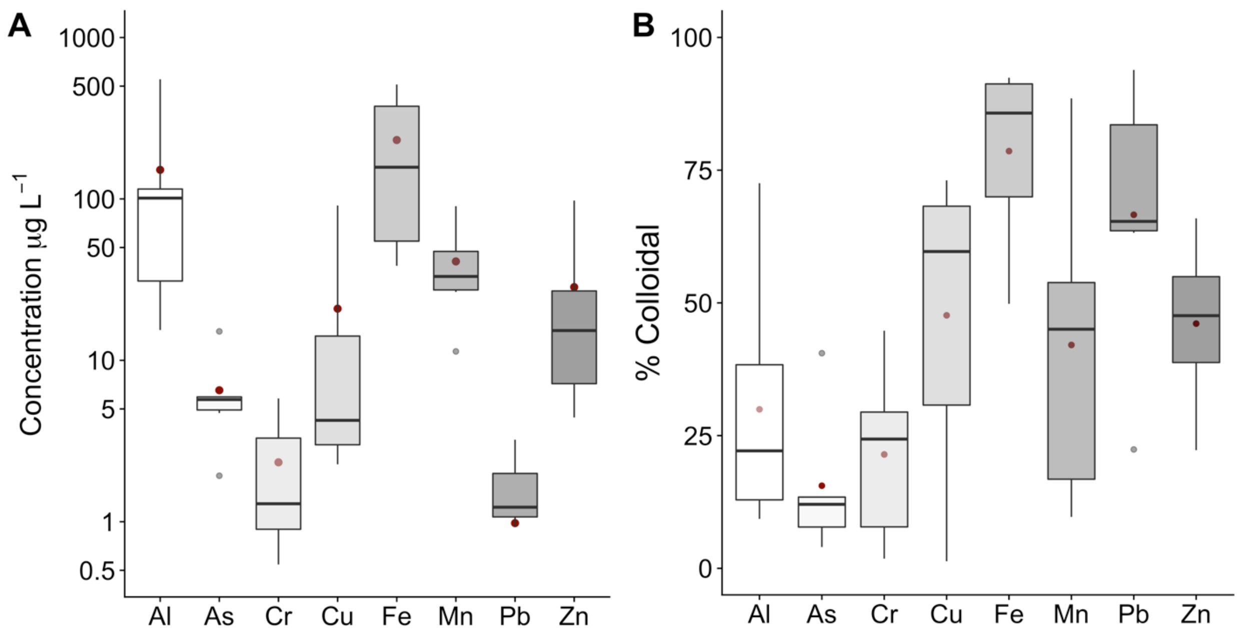

Figure 2. Boxplot represent the first quartile, median, and third quartile, red dot represents the mean, whiskers represent minimum and maximum values. A) Comparison of metal(loid) concentration across all terminal lagoons. B) Comparison of percent colloidal of all elements in the terminal lagoons of all field sites. N=6 for all elements except n=5 for Cr and n=4 for As.

Interestingly, of the four environmental variables recorded, surprisingly few were found to be correlated to the percent colloidal (Table 3). The elements Al, Cr, Fe, and Zn were not significantly correlated with any of the environmental variables (i.e., pH, DO, specific conductivity, or temperature). Of the eight metal(loid)s analyzed, only Pb was found to be correlated ($p < 0.0001$) with pH. Temperature was found to be weakly correlated with only Al ($p < 0.05$) and Zn ($p < 0.05$). The specific conductivity was correlated with Cu ($p < 0.0001$), Mn ($p < 0.05$), and Pb ($p < 0.0001$). Specific conductivity was found to be the most significant of the environmental variables in determining the percent colloidal. However, it was only significant for colloidal Cu ($p < 0.001$), Mn ($p < 0.05$), and Pb ($p < 0.05$; Figure 3). Dissolved oxygen was significantly related to percent colloidal Pb (0.05) and As ($p < 0.001$).

*Table 3. Significance (p value) for each environmental variable, tested using generalized linear mixed effect models for each element. Only displaying data where significance was determined. Significance level is annotated as '***' (significant at 0.0001) and'.' (significant at 0.10).*

Environmental Variable	Metal(loid)	Significance (p value)
Specific Conductivity (uS/cm)	Cu	<0.0001 ***
	Mn	0.061 .
	Pb	0.074 .
pH	Pb	<0.0001 ***
Dissolved Oxygen (% Sat)	As	0.068 .
Temperature (Celsius)	Al	0.066 .
	Zn	0.079 .

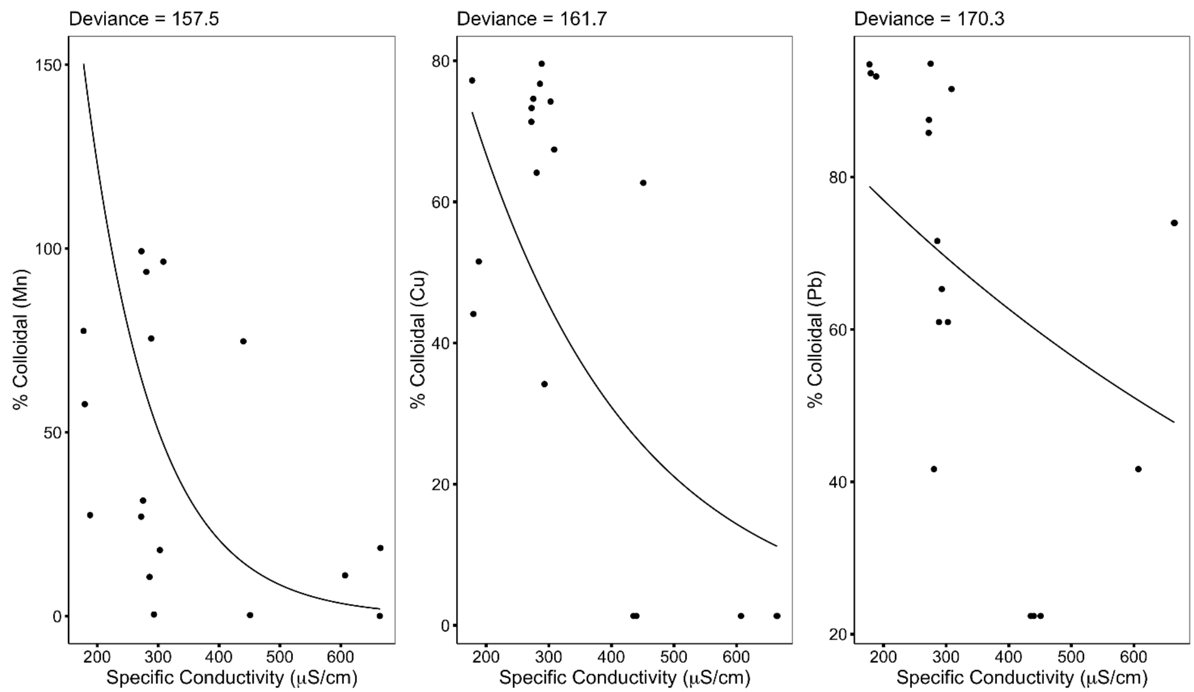

Figure 3. The percent colloidal for Mn, Cu, and Pb plotted against specific conductivity. Line represents the model fit of only fixed variables from generalized linear mixed effect model. Deviance represents the model fit.

3.2 Mesocosms

3.2.1 Patterns in pH, temperature, conductivity, and dissolved oxygen

Open mesocosms generally had significantly higher pH values than FTW mesocosms ($p < 0.001$) for most of the duration of this experiment for both concentration treatments (Figure 4D). In the high concentration treatment, pH levels started out similar under both cover types, while in the low concentration treatment, even at 0.1 Days the pH values were slightly higher in the open mesocosms. While pH remained relatively constant and circum-neutral in FTW mesocosms—with means ranging from 7.3 [7.2 to 7.5] to 7.7 [7.6 to 7.8] in high concentration mesocosms and from 7.5 [7.4 to 7.7] to 8.2 [8.0 to 8.3] in low concentration mesocosms—pH in open mesocosms steadily rose during the course of the first ten days of the experiment. Open mesocosms reached a maximum pH by Day 10 of 8.5 [8.4 to 8.7] in the high treatment and 9.5 [9.3 to 9.6] in the low treatment (Figure 4D). From Day 10 onward, the general temporal patterns in open mesocosms continued to be similar in both low and high treatments as pH declined until reaching 7.4[7.3 to 7.5] at Day 18 (high) and 8.2 [8.1 to 8.4] at Day 24 (low) before again rising until the end of the experiment at Day 33.

In the high concentration treatments, neither temperature (Figure 4A) nor specific conductivity (Figure 4B) were significantly different between open and FTW mesocosms; however, in the low concentration treatment, mesocosms with FTWs were found to have significantly higher temperatures ($p < 0.001$) between Days 10-15 and again at Day 28, while specific conductivity was significantly different from Day 3 through to the end of the experiment. While the temperature differences were significant, the magnitude was < 1 °C. For specific conductivity, the differences were up to 83 μS/cm.

Dissolved oxygen (DO) was significantly lower ($p < 0.001$) in the presence of FTWs for both high and low concentration treatments (Figure 3C). In both FTW and open mesocosms receiving either high or low concentration treatments, there was an initial rapid decline in DO during the first hours of the experiment, followed by fluctuating levels throughout the experiment. Mean DO ranged from 9.8 [8.1 to 11.9] to 38.3 [31 to 46] % saturation in the high treatment FTW mesocosms, from 17.6 [14 to 20] to 57 [46 to 69] in the low treatment FTW mesocosms, 11.6 [9.5 to 14] to 67.8 [55 to 82] % in the high open mesocosms, and 68.7 [55 to 82] to 113.3 [93.3 to 137.6] % in the low open mesocosms.

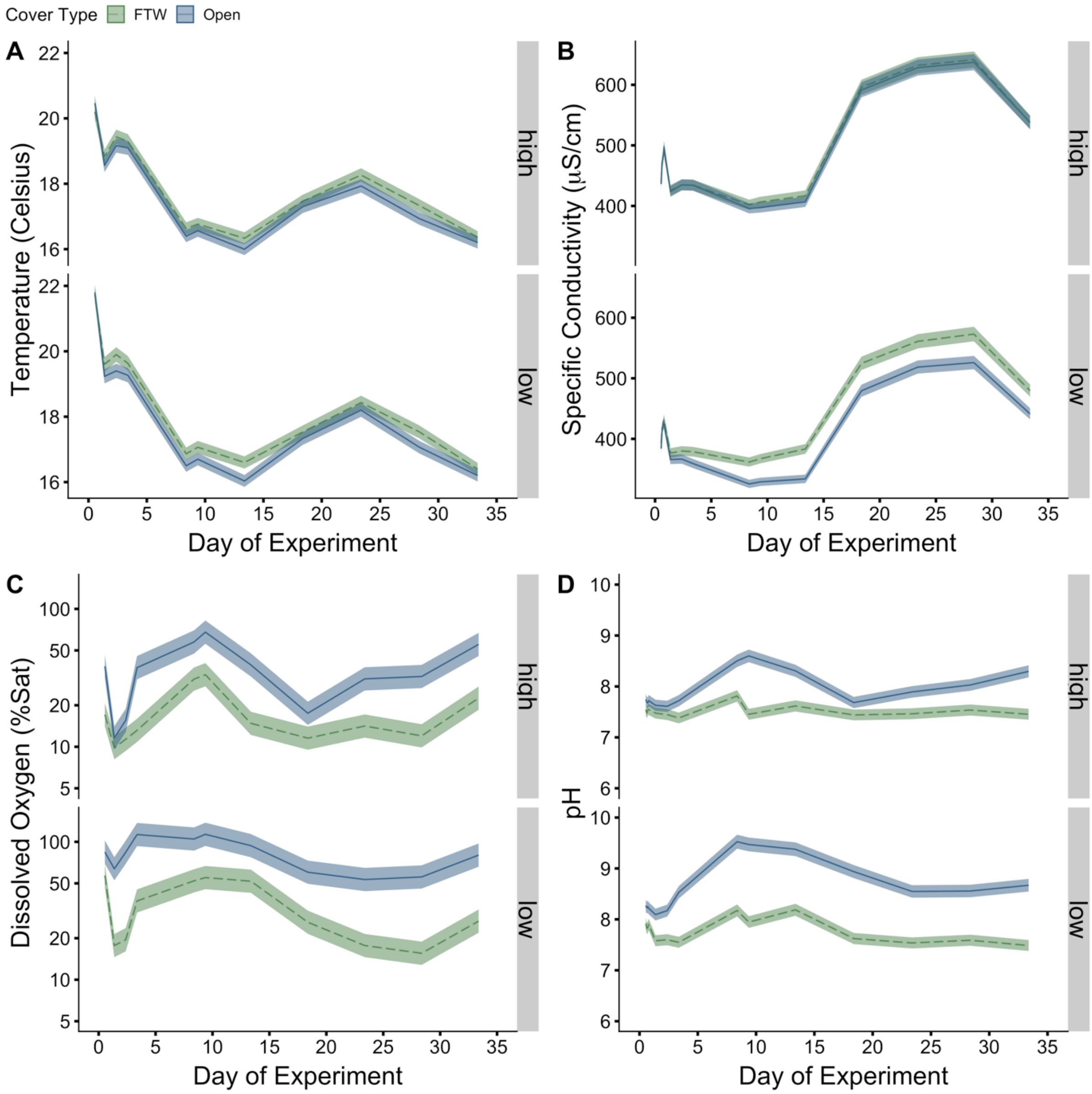

Figure 4. Environmental data from mesocosm experiment. The different treatments (high,low) are displayed in panels on the right, blue and green represent cover open and FTW cover type. The shading around the lines represent the 95% confidence intervals. Dissolved oxygen y-axis is logged.

3.2.2 Effect of FTW's on total metal(loid) concentration over time

In the high concentration treatment (1/3 groundwater and 2/3 lagoon water), mesocosms with FTWs had lower concentrations of all metal(loid)s compared to open mesocosms. This can be seen clearly in the natural log response ratio (lnRR), where positive values indicate higher concentrations in open mesocosms, negative values indicate higher concentrations in FTW mesocosms, and non-overlap of error bars and the origin indicates a significant difference between open and FTW (Figure 5A). At the beginning of the experiment, all metal(loid)s were at similar concentrations in FTW and open mesocosms, but as early as Day 2 there was divergence for Cu, Pb, Fe, and Zn, which all have lower concentrations in mesocosms with FTWs. While As was the least affected by the presence of FTWs, it still had significantly lower concentrations in the presence of FTWs.

In the low concentration treatment (2/3 groundwater, 1/3 lagoon water), the extent of the differences between FTW and open cover types was muted in comparison to the high concentration treatment (Figure 5B). By Day 2, FTW mesocosms had significantly lower Cu, Pb, and Fe concentrations. Unlike in the high concentration treatment, this effect was not consistent throughout the experiment, with Pb, Cu, and Fe all moving between being significantly lower in mesocosms with FTWs than the open mesocosms, and with As lower in FTW mesocosms only on Day 33. Interestingly, Zn is significantly higher in the FTW mesocosms on Days 8, 13, and 18.

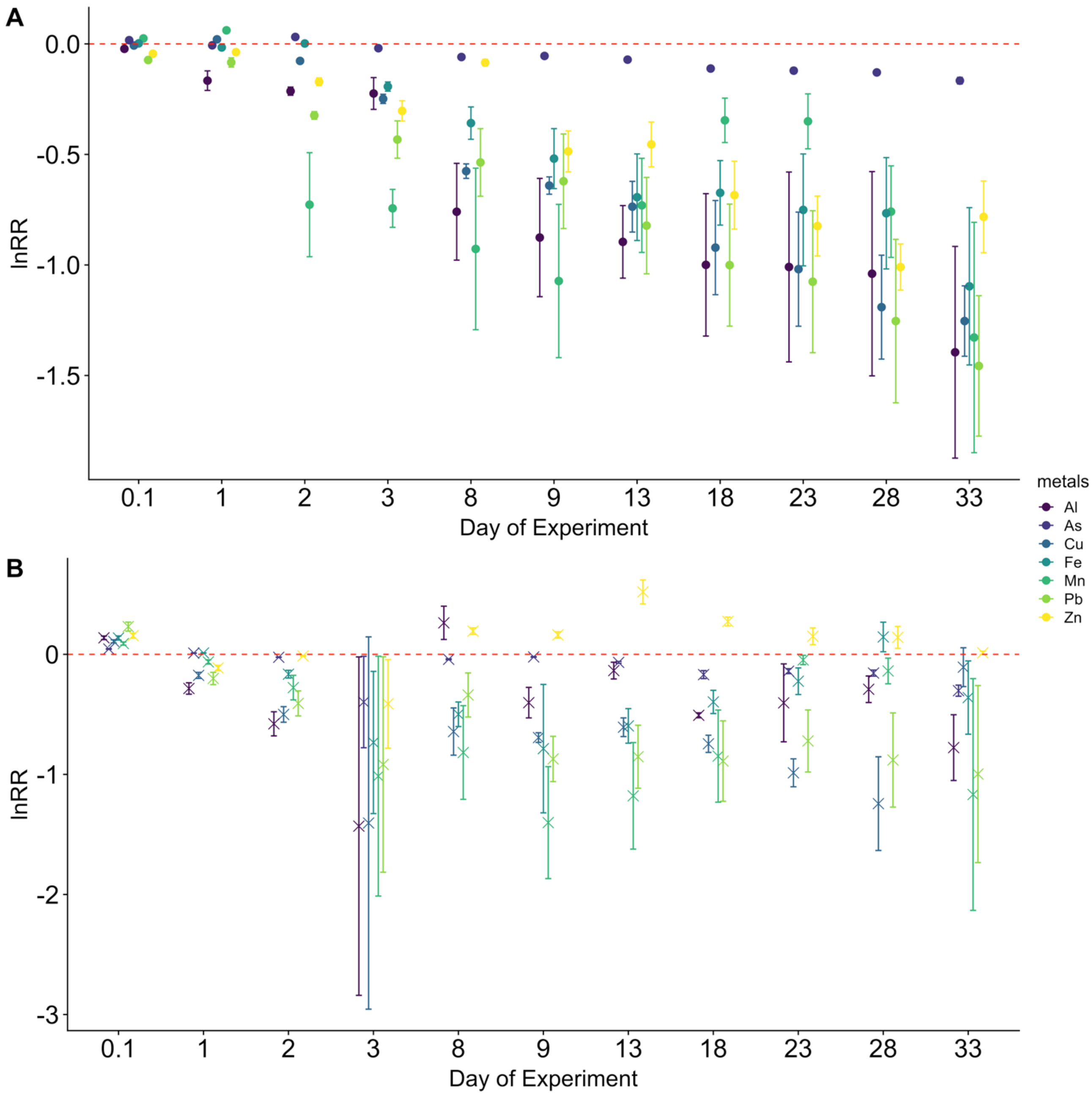

Figure 5. Natural log of the Response Ratio (Open/FTW). Error bars represent 95% confidence intervals. A) High concentration wastewater represented by solid dots B) Low concentration treatments represented by X.

3.2.3 Distribution of elements among size fractions in the presence and absence of FTWs

The distribution of metal(loid)s among size fractions changed over time, and differed by element though patterns were generally similar for both high and low concentration treatments. For Pb and Fe (Figure 6), as well as for Cu and Mn (Figure 7), the SPM and the small colloid fractions were dominant. In contrast, Zn (Figure 8A) was roughly equally divided among fractions and As was highest in the truly dissolved fraction (Figure 8B). For Pb, Cu, Zn, Mn, and Fe, there was an initial rapid decrease in SPM which coincided with a rapid increase in the colloidal fraction for both FTW and open mesocosms. This was followed by either a stable or declining concentration in the colloidal fraction in FTW mesocosms. In open mesocosms, in contrast, the colloidal metal(loid) concentrations continued to increase for all elements with the exception of As. The patterns for SPM were similar for Pb and Fe, in that open systems had significantly higher concentrations ($p < 0.001$- $p < 0.01$) while FTWs had lower. Differences were less clear for SPM metal(loids) under low concentration treatments. Arsenic showed high concentrations in the truly dissolved fraction while indicating no difference in the concentration under the presence of FTWs.

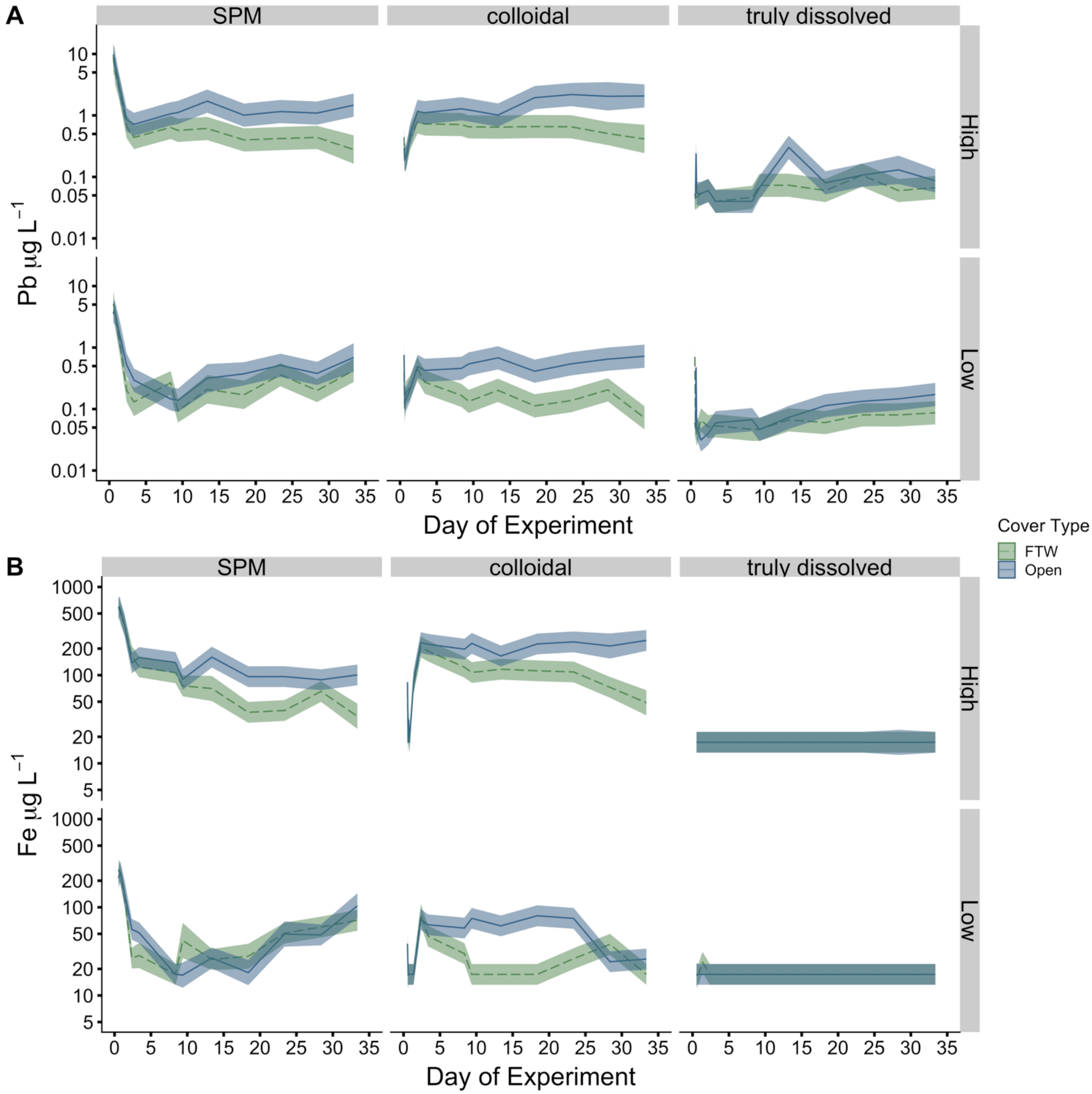

Figure 6. Concentrations of Pb (A) and Fe (B) under two cover types with green representing mesocosms with FTW and blue representing mesocosms left open. The figure is faceted along the top by the three size fractions: >450 nm (SPM), 1-450 nm (colloidal), <1 nm (truly dissolved). High and low indicate high concentration wastewater treatment or low concentration wastewater treatment. The shading around the line represents the 95% confidence interval.

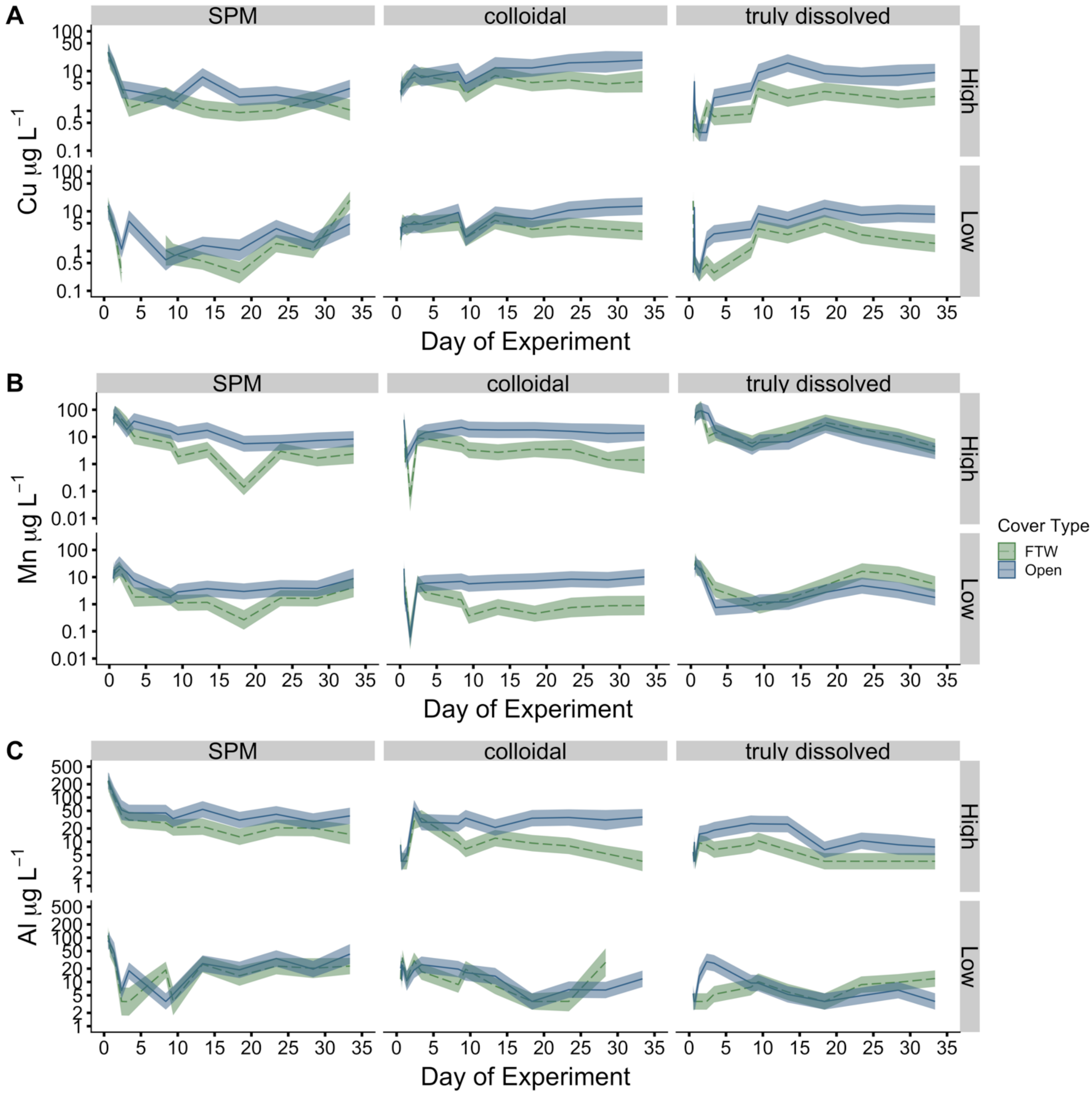

Figure 7. Temporal trends in concentrations of Cu (A), Mn (B), and Al (C) under two cover types with green representing mesocosms with FTW and blue representing mesocosms left open. The figure is faceted along the top by the three size fractions: >450 nm (SPM), 1-450 nm (colloidal), <1 nm (truly dissolved). High and low indicate high concentration wastewater treatment or low concentration wastewater treatment. The shading around the line represents the 95% confidence interval. There is no time point for Cu in the SPM size at time point 5 and for Al in the colloidal at time point 33 as they were lost in censoring.

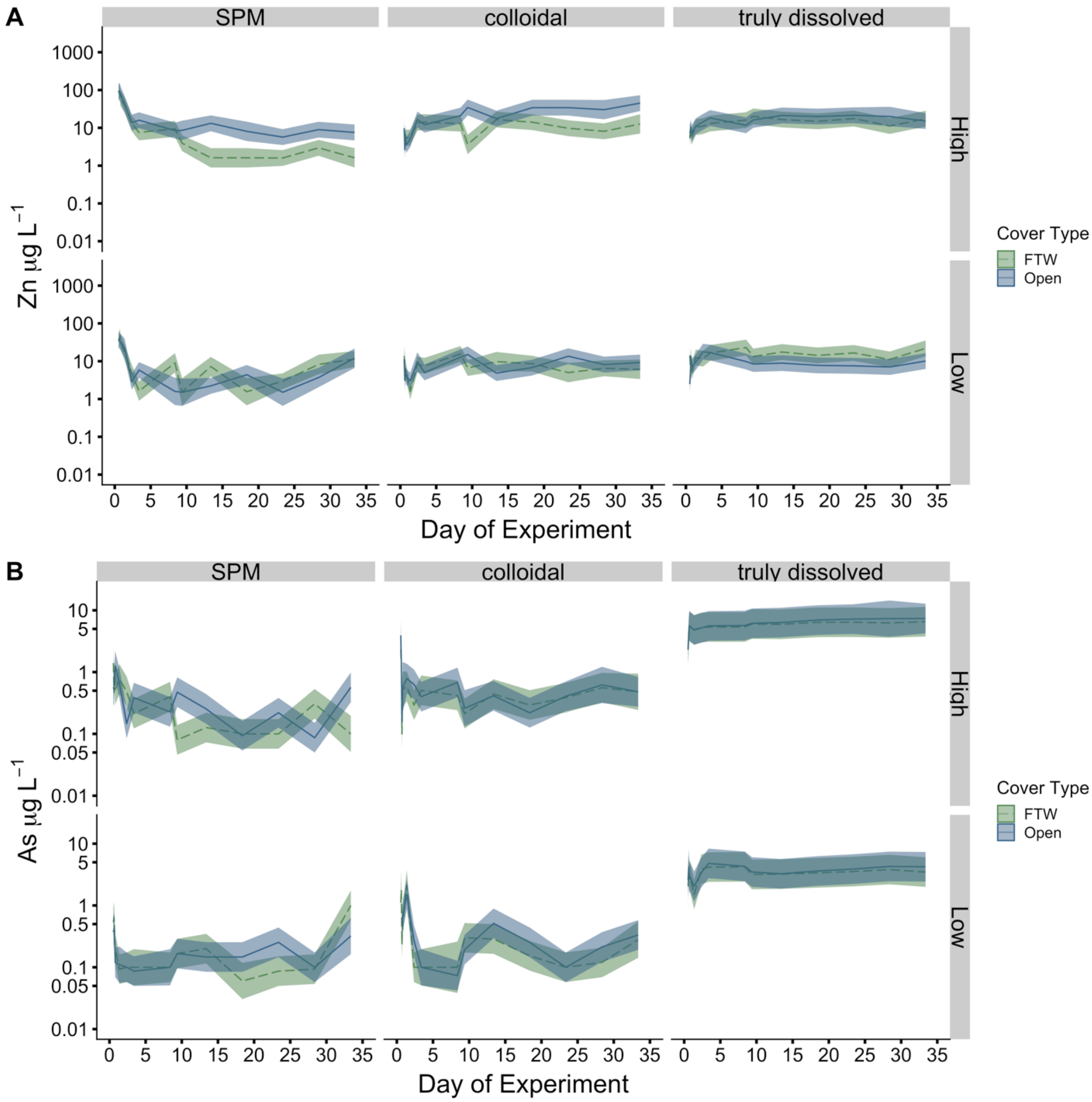

Figure 8. Temporal trends in concentration of As and Zn under two cover types with green representing mesocosms with FTW and blue representing mesocosms left open. The figure is faceted along the top by the three size fractions: >450 nm (SPM), 1-450 nm (colloidal), <1 nm (truly dissolved). High and low indicate high concentration wastewater treatment or low concentration wastewater treatment. The shading around the line represents the 95% confidence interval.

3.2.4 Influence of FTW's on metal(loid) distribution among size fractions and benthic organic matter

In the high concentration treatment, FTWs generally led to a decrease in the concentration of metal(loid)s in the water column and an increase in concentrations in benthic organic matter; in the low concentration treatment, the differences were more variable but the trends were similar. The effects of FTWs on SPM in the high concentration treatment was strongest for Al, Pb, and Zn (Figure 9), while all other metals have 95% confidence intervals crossing the zero line. The effect of FTWs was most distinct on the colloidal size fraction with a strong effect on all metals, but not on the metalloid As. The most marked difference was for Al, though Cu, Fe, Mn, Pb, and Zn all had similar declines in the FTW mesocosms as compared to the controls. In the truly dissolved fraction, FTWs had only a modest effect on truly dissolved Cu and to some extent Mn. Interestingly, there was a corresponding increase in the concentrations of metal(loid)s in the benthic organic matter of the mesocosms with FTWs (Figure 9). While there were some effects of FTWs in the low concentration treatment, and while the trends were similar to the high concentration treatment, the results were much more variable and only occasionally were they significant (Figure 10).

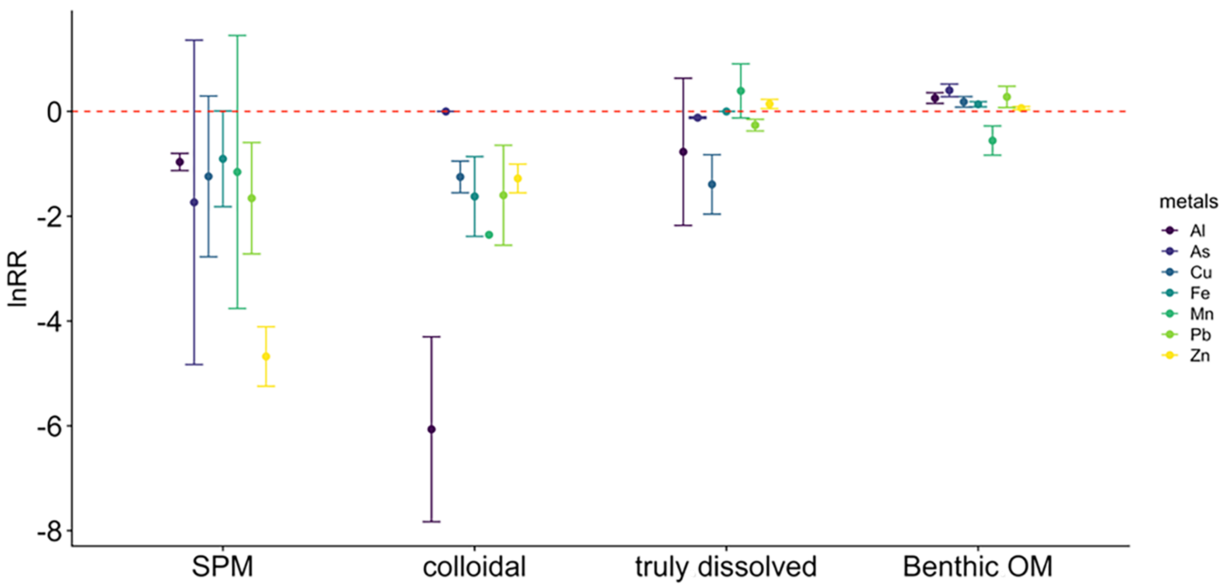

Figure 9. Natural log of the Response Ratio (Open/FTW) for truly dissolved (<1 nm), colloidal (1-450 nm), suspended particulate matter (>450 nm), and the benthic organic matter from final timepoint (Day 33) of the experiment under high treatment. Error bars represent 95% confidence intervals.

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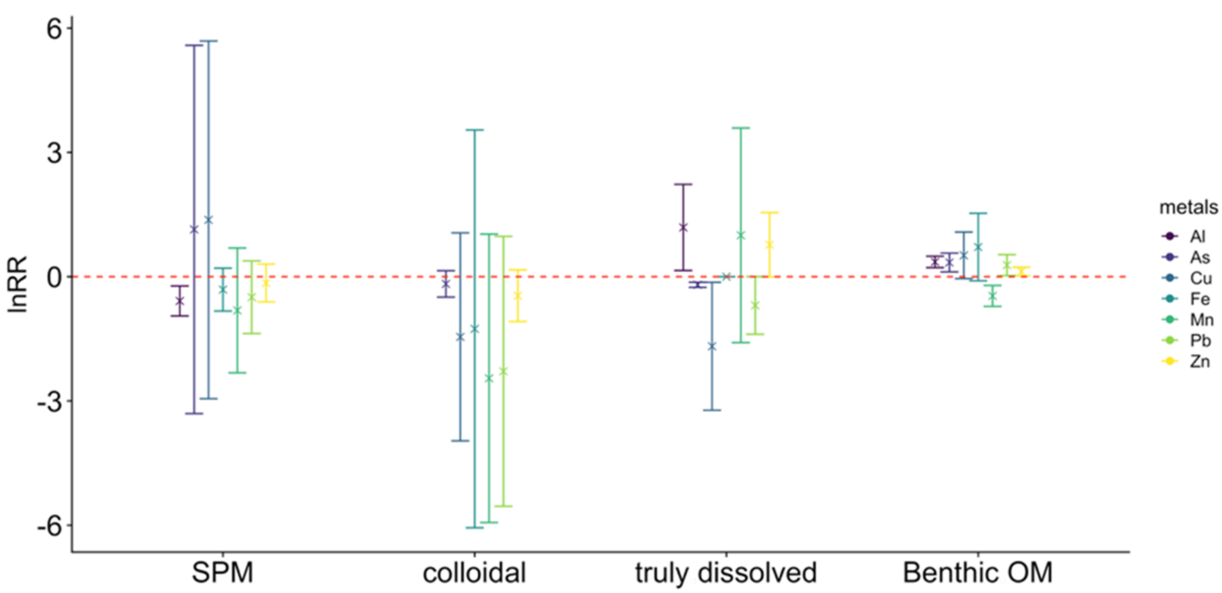

Figure 10. Natural log of the Response Ratio (Open/FTW) for truly dissolved (<1 nm), colloidal (1-450 nm), suspended particulate matter (>450 nm), and the benthic organic matter from final timepoint (Day 33) of the experiment under low treatment. Error bars represent 95% confidence intervals.

3.2.5 Percent metal(loid) removal under high and low treatment

The percent mass metal(loid) removal varied by element (Figure 11) and treatment. Mesocosms with FTWs, were shown to have no significant difference in percent Pb removal under high and low treatments. The metalloid As was the only analyte which had a greater removal under low treatment. All other metals in mesocosms with FTWs had a greater percent removal under high treatment. In open systems, there was no difference in percent removal between high and low treatment for As, Fe, Pb, or Zn. While Al, Cu, and Mn showed greater removal under high treatment systems in open cover types.

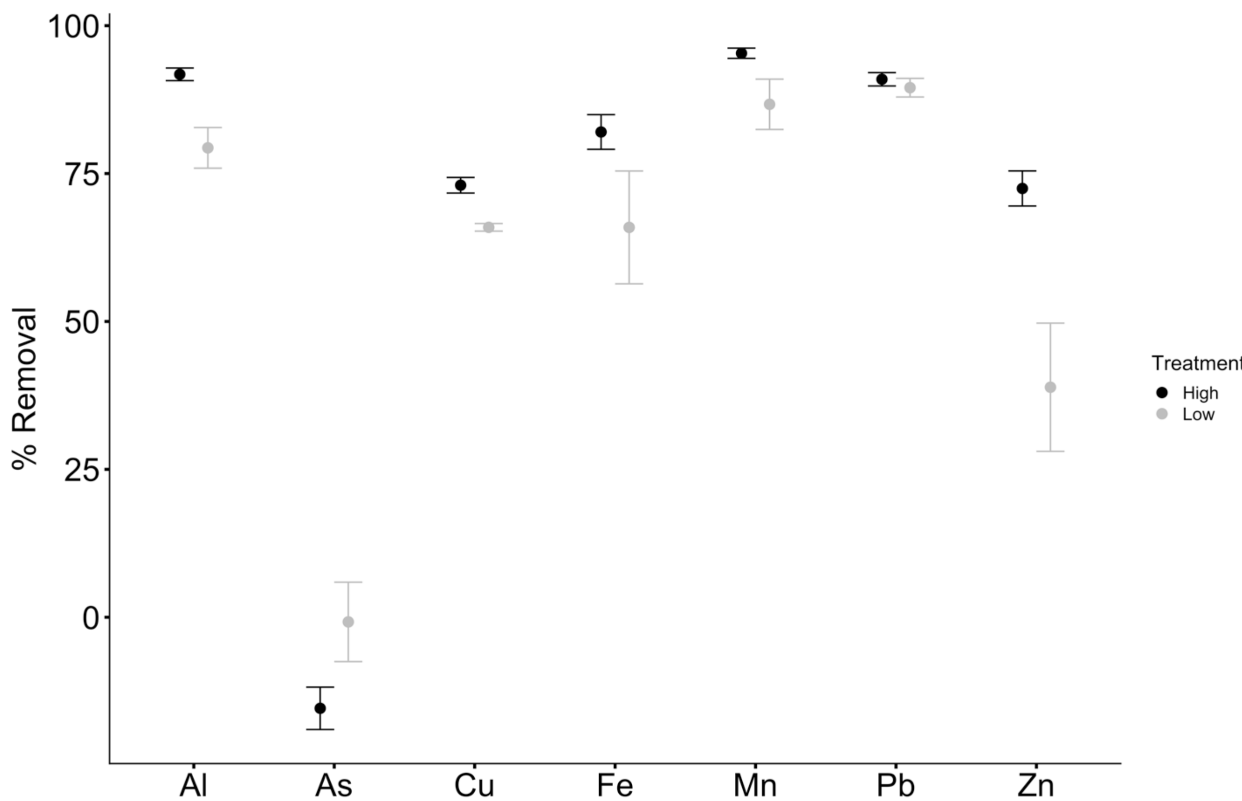

Figure 11. Percent metal(loid) mass removal. Black points represent high treatment and grey points represent low treatment. Error bars represent the standard error.

4. Discussion

This study characterized the percentage of "dissolved" metal(loid)s that was colloidal in six wastewater lagoons in a mine-waste contaminated watershed, quantified the effect of FTWs on total metal(loid) concentration, and identified patterns of metal(loid) size distribution in the presence and absence of FTWs. Results from this study provide added insights into the prevalence and biogeochemistry of colloidal metal(loid)s in wastewater lagoons and suggest FTWs may be useful in removing metal(loid)s, particularly in the colloidal size fraction.

4.1 Colloidal particles are an important form of metal(loid)s in lagoons

The percentage of metal(loid)s found in the colloidal fraction (1-450 nm; Figure 2B) was >25% of what passes through a 450 nm filter for seven out of the eight elements we examined, and was up to 75% in the case of Fe. While As was consistently below 25% colloidal, it still had a median of 10% colloidal and the highest sample was 20% colloidal. These data are in marked contrast to the assumption inherent within the historical operational definition of dissolved, that suggests elements in this fraction are largely free ions or ions bound by low molecular weight organic matter (Buffle and Leppard, 1995; Hoffmann et al., 1981). The findings presented here from wastewater lagoons are consistent with those reported in studies of metal(loid) colloids in freshwater systems (Kimball et al., 1995; Trostle et al., 2016; Yang et al., 2015).

There are several factors that are commonly thought to drive the speciation and partitioning of metal(loid)s including redox potential, ionic strength, pH, and dissolved organic matter concentration (Zhang et al., 2018). Given that the lagoons sampled were fairly well oxygenated, we expected the redox potential to be positive and likely above 0.4 mV. For elements such as Pb and Fe, if we assumed that speciation had equilibrated based on the pH and Eh of the lagoons, we would expect both of these metals to exist as insoluble metal hydroxides or carbonates. Depending on the size of the particles formed, organic coatings they acquired, and the nature of the steric and/or electrostatic forces that may have stabilized or destabilized them, the resulting particles could have stayed in suspension or aggregate and form larger SPM and precipitate; based on our observed patterns, they are likely forming small stable particles that are remaining in suspension.

While the divalent metals Cu, Mn, Zn, and trivalent Al were also all associated with the colloidal fraction, they also had sizeable concentrations in the dissolved fraction. Based solely on their expected partitioning between dissolved phases and solid phases in the pH range observed in the lagoons (7.1 to 10.7), we would expect all of these to tend towards solid oxide or hydroxide forms in the lagoons though it may be that these elements were not at equilibrium with regards to the Eh or pH of the surface water at the time of sampling. Alternatively, it may be that the higher percentage of these elements in the truly dissolved fraction represents ions chelated by low molecular weight organic matter.

It is important to note that for all of these elements, while we know the proportion that was found in the colloidal size range and truly dissolved, we can only speculate as to the forms they were in. Although it is reasonable to assume some component of the colloidal metals were found as metal/metal-oxide colloidal-scale particles, it is also possible that some of them were sorbed on the surface of other metal/metal-oxide particles as has been observed for silver on TiO_2 (Kim et al., 2012) or as has been observed for the metalloid As on ferrihydrite (Yang et al., 2015). For elements like Cu, which have a known high affinity for chelation in organic matter, it may be that they were in the colloidal fraction as metal ions bound by ligand exchange to high molecular weight organic matter (Cabaniss, 1988; Karthikeyan & Elliot, 1999).

Our data provide evidence that conductivity may play a factor in the percent colloidal Cu, Mn, and Pb, but neither pH or temperature were found to have strong relationships with percent colloidal for any of these metals. As specific conductivity increased between different field lagoons, the concentration of Cu, Mn, and Pb declined. This observation is consistent with published patterns showing that increased ionic strength, which is correlated to specific conductivity, leads to declining colloidal stability. This phenomenon is driven by the fact that, as ionic strength increases, it can weaken the electrostatic repulsion that can be essential for promoting colloidal stability (El Badwy et al., 2010). This reduction of electrostatic repulsion could lead to aggregation and sedimentation. It was surprising to not see relationships between pH or temperature with percent colloidal, given the role that pH can play on surface charge, and that lower temperatures can drive increased aggregation. This suggests that colloidal stability in these systems is likely driven more strongly by other parameters, such as dissolved organic matter or even the activity of aquatic organisms which may regenerate colloids from aggregates thereby promoting their persistence in the water column.

4.2 Floating treatment wetlands decreased DO and pH

In mesocosms with FTWs, both DO and pH were lower than they were in open mesocosms regardless of the treatment concentration (Figure 4C and 4D). The driver of both of these was likely a difference in the balance between primary productivity and respiration in the mesocosms with FTWs compared to those without. In the presence of FTWs, DO was likely lower due to increased heterotrophic respiration from the added presence of root biomass and its associated periphyton. The FTWs, while only occupying 20% of the surface area of the mesocosms, would have decreased light entering the mesocosms and thus suppressed productivity by the phytoplankton, which would have also contributed to lower DO. This decrease in DO should also be accompanied by a decreased pH due to an accumulation of carbonic acid associated with increased respiration; this is indeed the pattern we observe and which has been observed in other studies on FTWs (Borne et al., 2013b; Van de Moortel et al., 2010; White & Cousins, 2013). These proposed mechanisms are consistent with those presented in Pedersen et al., 2013, though Neori et al., 2000 and Headley and Tanner, 2012 posits that decreased pH could also be from organic acids released from the roots and chemical reactions that occur in the enhanced treatment zone beneath the FTW.

Based on previous FTW research, we expected to see differences in temperature, but we only detected minimal differences (Figure 4A). This could be due to our use of 20% coverage which may not have been enough to affect a noticeable difference in temperature. Additionally, given that our mesocosms were aboveground, there was interception of solar radiation by the sides of the mesocosms as well as the surface of the water, further reducing any likely differences. Finally, the lack of a difference could also be an artifact of our 9:30 am water collection and physicochemical measurement time. It may be that later in the day we would have seen more striking differences.

4.3 Total metal(loid)s removed varied by element

In accordance with our expectations, we found the presence of FTWs was associated with a decrease in metal(loid) total concentrations, with the greatest decreases for Al, Cu, Fe, Mn, Pb, and Zn with little effect on As concentrations (Figure 5A, B). The effect was most distinct in high concentration mesocosms, and those metal(loid)s which were most effectively removed in the presence of FTWs were the same metal(loid)s that showed a significant decline in the colloidal concentration over time compared to open mesocosms. This suggests that the decrease in total concentration of metal(loid)s may have been driven by the more efficient removal of colloidal metal(loid)s by FTWs.

4.4 Distribution of metal(loid)s among size fractions varied temporally

Consistent with our expectations, we saw rapid sedimentation of materials in the SPM fraction in the initial days of our experiment, presumably due to the elevated settling velocity of the larger particles in this size fraction (Figures 6-8). Interestingly, as SPM concentrations were dropping, we also saw an increase in the colloidal fraction for most elements (Al, Cu, Fe, Mn, Pb). This suggests that redistribution of elements among size fractions is a dynamic process taking place on relatively short timescales. This may be driven by disruption of aggregates in the SPM fraction by the activity of zooplankton and benthic macroinvertebrates. It may also have been biogeochemical processes driving dissolution of metal(loid)s in SPM which could then form colloids. Though we saw an increase in the colloidal fraction as SPM declined, we did not see a similar increase in the truly dissolved fraction during times when the colloidal fraction declined.

Following this initial period, mesocosms with FTWs began to have lower concentrations of metal(loid)s than open mesocosms, especially in the high treatment and in the colloidal size fraction (Figures 6-8). FTWs may facilitate the removal of colloidal metal(loid) particles through sorption of the particles to the periphyton growing on the roots of the FTW plants. This is consistent with the mechanism proposed in studies examining the role of FTWs in reducing turbidity in stormwater ponds. In one such study, FTWs were found to remove 2-3 fold more fine clays (<400 nm) than open water controls (Tanner and Headley, 2011). In terms of the longer term fate of these sorbed metals, it has been hypothesized that they are trapped in the periphyton initially, but that the periphyton likely sloughs off and transports the sorbed particles to the sediment (Borne et al., 2013; Tanner and Headley, 2011). Moreover, it has also been suggested that the increased supply of organic compounds (labile carbon) from FTW plants may stimulate coagulation and flocculation, causing these larger particles to settle to the sediment layer.

Some insights into this difference between FTW mesocosms and open mesocosms can be gleaned from research into the fate, transport, and impacts of silver nanoparticles (AgNPs) in aquatic systems. When studied in aquatic mesocosms with dense submersed macrophyte vegetation, AgNPs were rapidly removed from the water column (Colman et al., 2014). In contrast, a whole lake AgNP addition experiment in the Experimental Lakes Area (ELA) in Ontario found persistence of AgNPs and no signs of agglomeration (Furtado et al., 2015). It may be that in systems with an increased amount of surface area be it submersed macrophytes or the roots of plants growing in FTWs, the increased surface area in the system may facilitate greater sorption, leading to enhanced removal from the water column.

4.5 Metal(loid)s in benthic organic matter

The results from this experiment show a greater contribution of metal(loid)s in the benthic layer of mesocosms containing FTWs. This result suggests that metal(loid)s were being removed from the water column to the benthic layer more effectively in systems with FTWs vs. open. We propose that this increase in metal(loid) concentrations in the benthic layer of FTW systems may be driven in part or in total by the entrapment of colloids and SPM in periphyton, which then sloughs off the roots and accumulates in the benthic layer (Figure 12). There was very little difference in the truly dissolved concentrations (Figure 9), suggesting that accumulation of dissolved solutes in the periphyton or uptake by plant roots may be less important for removal from the water column than might be expected.

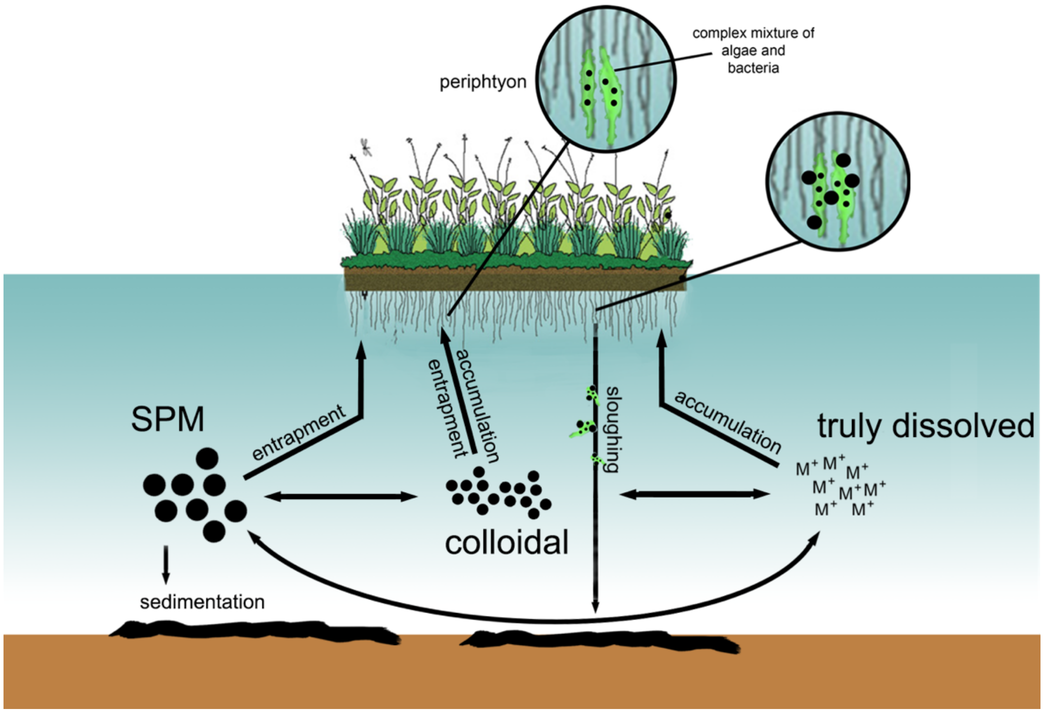

Figure 12. Conceptual model displaying the observed pathways of metals in this FTW mesocosm experiment.

5. Conclusion

Combining a field survey and mesocosm experiment we were able to show that colloidal metal(loid) particles are an important form to consider in wastewater lagoons and that FTWs are effective in removing colloidal metal(loids) from the water column. This study also suggests that colloidal metal(loid)s are likely an important form to consider in stream ecosystems receiving wastewater effluent, especially when considering the increasing contribution of wastewater effluent to streams in arid and semi-arid regions around the world. Colloidal metal(loid)s may be transported and move through aquatic food webs differently than particulate or dissolved forms. Understanding the distribution of elements among colloidal and truly dissolved fractions may also be important in optimizing mechanisms to remove metal(loid) contaminants from wastewater prior to discharge into streams.

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Appendix

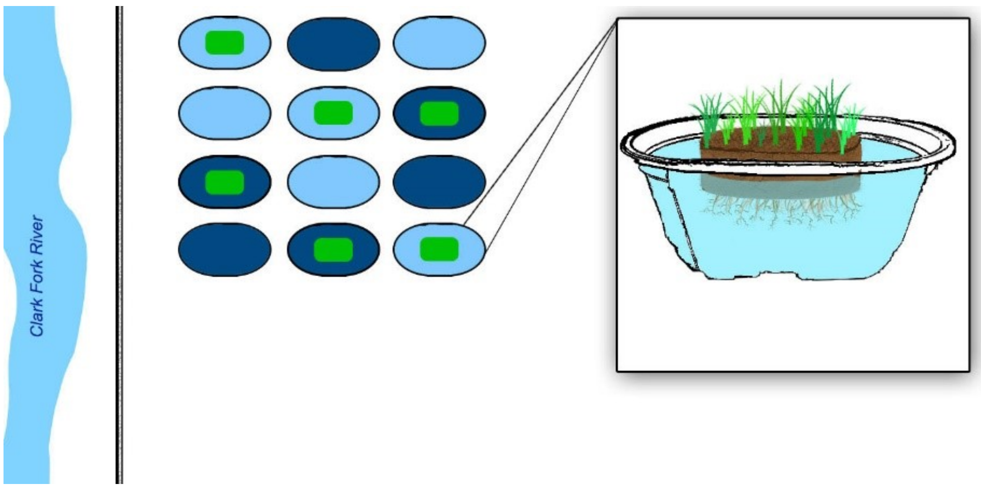

Figure 1-A. Experimental design for mesocosm experiment. Light blue mesocosms represent low concentration wastewater treatments and dark blue mesocosms represent high concentration wastewater treatments. Green rectangles represent presence of FTW.

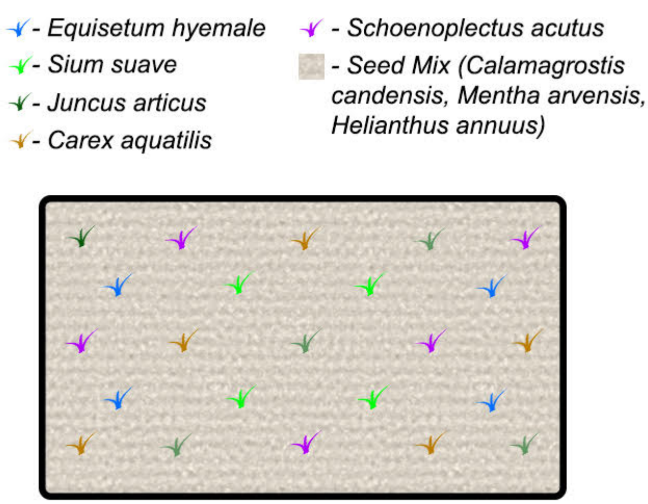

Figure 2-A. Schematic of planting design for all FTWs in Experiment 2.


Figure 3-A. Photograph of FTW in mesocosm.