Impacts of flue gas desulfurization (FGD) gypsum in hypereutrophic waters

by

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 5, 2023

Keywords: Gypsum (calcium sulfate), cyanobacteria, soluble reactive phosphorus, trace metals, aquaculture, hardness

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Abstract

Flue gas desulfurization (FGD) gypsum is a by-product of carbon-based energy sources that has been beneficially used as a component of concrete mixes and wallboard as well as in land-based agriculture as a soil amendment. In recent decades, FGD gypsum has started to be used in natural waters as a way to increase hardness, control phosphorus availability, and potentially mitigate eutrophication all due to the increase in calcium cations upon dissolution. These impacts have the potential for FGD gypsum to be used as an alternative management tool in hypereutrophic waters, such as aquaculture ponds. This research investigated the impacts of a much larger range of FGD gypsum concentrations than previously tested to determine if there were any negative impacts as concentrations increase and to ultimately find an optimal dose. Furthermore, this research aimed to test the optimal dose in a real-world whole pond setting at an active catfish aquaculture pond to determine its overall impacts and success in being used as a management tool in hypereutrophic waters. The results of this research found that as the concentration of FGD gypsum increases (upwards of 1,000 mg/L), you will start to see more negative impacts, such as a decrease in zooplankton biomass, a temporary increase in cyanobacteria, and select trace metal increases. A moderate dose, such as 500 mg/L, can control for algal growth while limiting these side effects. When used in hypereutrophic catfish ponds, this 500 mg/L dose can end up interacting with the nutrient-rich sediment causing a release of soluble reactive phosphorus and a subsequent spike in cyanobacterial abundance. Although FGD gypsum ultimately turned out to not provide the expected results seen in previous studies when used in hypereutrophic fishponds, it did not cause any trace metal contamination and could still potentially be used as a source of hardness with improved application methods.

Acknowledgments

This research would not have been possible without the help, guidance, and support of so many people over the past two years. Thank you to my advisor, Dr. Alan Wilson, and the other members of my committee, Dr. Kevin Wang and Dr. Allen Torbert, for their mentorship and assistance in all aspects of this research. They provided guidance in both the initial design and implementation of these experiments as well as helping me brainstorm ideas and answering all my questions when things turned out (very) different from what we expected. I am also very grateful for the USDA-ARS for their funding support of this research and for giving me the opportunity to travel and attend a conference to present my results.

An enormous thank you to all my friends and lab mates in the WilsonLab. This research was only as successful as it was thanks to you all. You made hot, sweaty, and sometimes muddy field days incredibly fun (and, of course, thank you to Taco Bell for fueling us afterwards), were essential in processing all my samples in the lab on time and helped me stay sane through some incredibly tough and stressful days. Matt, Kate, Peyton, Michael, Ashley, and Jade, I am so grateful for each and every one of you and know you will do amazing things with your own research in the years to come! A big thank you to Matt Gladfelter for being there for me from day one and teaching me all the ins and outs of our lab and to Suzie Tenison for being the kindest and most supportive friend and lab mate that I could have started this journey with (I'm so proud of you!) I am also incredibly lucky to have made so many amazing friends, both in the Fisheries school and in so many other departments, during my time here. You all ensured we still had fun throughout all the craziness. A special thank you to Thomas Moorman, my very first friend here who always made sure I was doing okay, brought me lemon cookies, and made Alabama feel like my second home. I am eternally grateful for your kindness and support.

Thank you to my friends and mentors from my time with the Chesapeake Conservation Corps (Tara Drennan) and Hood College's Center for Coastal and Watershed Studies (Dr. Drew Ferrier, Dr. Kevin Sellner, Claire Hudson, and Susan Bolton Simonson) for introducing me to the world of water quality and all things algae. My career path changed for the better thanks to you all.

Last but not least, I am so grateful for my family and friends back home for being there for me from 1,000 miles away. All your texts, calls, facetimes, zoom workouts, care packages, and more made being so far from home a little bit easier. I am so lucky to have such an amazing support system and could not have done this without you all.

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Positive and negative impacts of flue gas desulfurization (FGD) gypsum on water quality

Abstract

Flue gas desulfurization (FGD) gypsum, a byproduct of carbon-based energy sources, has typically been incorporated as a component of concrete mixes and wallboard and beneficially used as an agricultural amendment to enhance crop production and improve the quality of runoff. These various uses for the byproduct aid in reducing the amount that is ultimately landfilled. Limited studies have investigated its benefits when used directly in aquatic settings, such as ponds and lakes, to increase hardness and potentially mitigate eutrophication. A 36-day field mesocosm experiment tested a larger range of FGD gypsum concentrations (500 to 2,000 mg/L) than those previously tested in the literature to investigate its desired and potentially undesired impacts on water quality and the algal community. High FGD gypsum concentrations, 1,000 and 2,000 mg/L, were found to have more undesired impacts than the 500 mg/L treatment, including an initial spike in cyanobacteria, a decrease in total zooplankton abundance, and an increase in certain trace metals in the highest treatment. Ultimately, the 500 mg/L FGD gypsum treatment was found to have fewer undesired impacts while still resulting in significant desired effects, including those on hardness and pH, as well as moderate reductions in algal abundance. This experiment provides a better understanding of the effects of FGD gypsum when directly used in an aquatic setting, determines an optimal dose for future field experiments, and helps provide the groundwork for developing an upper threshold on FGD gypsum so as to not have the negative effects outweigh the positive.

Introduction

Flue gas desulfurization (FGD) gypsum is a synthetic form of gypsum created as a by-product of carbon-based energy sources (typically coal combustion) (Chen and Dick, 2011; Chen et al., 2014). Limestone forced oxidation scrubbers remove sulfur dioxide from the flue gas stream of sulfur-heavy energy sources, which produces calcium sulfate as a final product (Chen and Dick, 2011). While natural forms of gypsum have been traditionally used in agricultural production for hundreds of years dating back to the 18th century, FGD gypsum has taken over as the main source in recent decades due to availability and cost efficiency (Watts and Dick, 2014). The many uses of FGD gypsum in agricultural production include the reclamation of alkali soils, reduction of phosphorus runoff into nearby waters, promotion of soil aggregation, and to act as a source of inexpensive and readily available calcium and sulfate nutrients for crops (Chen et al., 2008; Chen and Dick, 2011; Chen et al., 2014; Torbert and Watts, 2014; Watts and Dick, 2014). Although limited in number, some studies have also discovered the ability of natural gypsum and FGD gypsum to be used in aquatic settings as well, most often in eutrophic and hypereutrophic ponds and lakes. In these aquatic environments, gypsum has been used to increase calcium hardness, adsorb or precipitate phosphorus, reduce pH and algal biomass, flocculate clay particles, and remove off-flavor compounds (Salonen and Varjo, 2000; Seo and Boyd, 2001; Whangchai et al., 2016, Wu and Boyd, 1990). Once in water, FGD gypsum can release calcium cations and sulfate anions. Most benefits result from the surplus of calcium cations added to the water column, which can have both direct and indirect effects on the physical, chemical, and biological properties in the water column. For example, the increase in calcium cations directly increases total hardness, reduces pH through calcium carbonate precipitation, reduces phosphorus through the precipitation of calcium phosphorus compounds (such as hydroxyapatite), and flocculates clay particles and algal cells through the interaction of surface charges (Salonen and Varjo, 2000; Whangchai et al., 2016; Wu and Boyd, 1990). It can also have continued and delayed effects, such as the control of algal abundance due to the reduction of biologically available phosphorus, which in turn can also reduce pH (due to the removal of carbon dioxide during photosynthesis) (Wu and Boyd, 1990; Wurts and Durborow, 1992).

While studies have investigated gypsum application in eutrophic and hypereutrophic waters for the previously stated benefits, there have been gaps in determining if higher concentrations of FGD gypsum can achieve even greater benefits, or concurrently whether they can induce potentially undesired or even harmful effects. Previous studies which state that gypsum is "safe for human and aquatic life" have not extended beyond a maximum concentration of 600 mg/L (Whangchai et al., 2016) and have not quantified potential negative impacts, such as trace metal contamination. The oxidation process by which FGD gypsum is formed aims to remove undesirable chemical contaminants. However, while the purity of FGD gypsum (~99.6%) is higher than mined gypsum (~87.1%), heavy metal contamination could still be a potential risk (Dontsova et al., 2005). Torbert and Watts (2014) did not detect any trace metals in water runoff from grass plots treated with FGD gypsum. However, the potential trace metal contamination of FGD gypsum being used directly in an aquatic setting has not been well-studied.

The purpose of this 36-day field experiment was to thoroughly investigate both the desired and potentially undesired effects of FGD gypsum on water quality. Specifically, we aimed to test a larger range of FGD gypsum concentrations (500 – 2,000 mg/L) than previously used in the literature to determine whether there is an optimal dose that can balanced the desired effects with any potential undesired effects. Therefore, we conducted a field mesocosm experiment to test immediate and delayed impacts of FGD gypsum on water quality, the algal community, and trace metal concentrations in the water column.

Materials and methods

Experimental design

The 36-day mesocosm experiment was conducted from 7 September 2022 through 13 October 2022 in pond S-1 located at the Auburn University E.W. Shell Fisheries Center in Auburn, Alabama, U.S.A (32°40'55.2"N 85°30'36.6"W). The 1,600 L mesocosm enclosures were made from clear polyethylene plastic that were sealed at the bottom, open at the top, and suspended from PVC frames that were attached to a floating dock in the middle of the pond (Buley et al., 2021; Chislock et al., 2019; Gladfelter et al., 2022). Five days before the start of the experiment, the enclosures were filled with the surrounding pond water to an average depth of 1.5 meters and fertilized with sodium phosphate (NaH_2PO_4) and potassium nitrate (KNO_3) to increase ambient total phosphorus and nitrogen concentrations by 20%. Each enclosure was designated a treatment (control, 500 mg/L of FGD gypsum, 1,000 mg/L of FGD gypsum, or 2,000 mg/L of FGD gypsum) with four replicates of each, creating a randomized block design. FGD gypsum was sourced from a power plant in central Alabama.

On day 0, a pre-treatment sample was collected from each enclosure. The enclosures were mixed by dropping a Secchi disk to the bottom and pulling it up ten times. A rigid tube sampler was used to collect an integrated water sample from each enclosure to a depth of 1 meter twice. These samples were combined in a bucket and stored in a plastic cubitainer for transfer back to the lab for analyses. The various gypsum concentrations were then mixed into the assigned enclosures. To do so, a bucket was filled with 15 liters of water that was pulled from the target enclosure. The assigned FGD gypsum concentration was added to the extracted water and mixed with an electric paint mixer for 60 seconds to create a homogenous mixture and promote dissolution of the gypsum. This slurry was then poured back into the assigned enclosure, and the entire enclosure was hand-mixed with a PVC pipe in circular motions for another 60 seconds. Water samples following FGD gypsum addition were collected on days 8, 15, 22, 29, and 36 using the previously mentioned tube sampler method. A mid-experiment fertilization occurred on day 15 following sample collection in which another sodium phosphate and potassium nitrate mixture at a 10% level of the ambient total phosphorus and nitrogen levels was added to each enclosure.

Hardness, alkalinity, and pH

Water samples from the enclosures were analyzed for total hardness in mg/L of $CaCO_3$ using the Hach 5B Hardness Test Kit and total alkalinity in mg/L of $CaCO_3$ using the Gran titration method (Wetzel and Likens, 1991). The pH was measured at the enclosures *in-situ* using a Hydrolab YSI multisonde submerged a quarter meter below the surface.

Algal pigments

Algal pigments, including chlorophyll-*a* and phycocyanin, were measured and used as proxies for total phytoplankton or cyanobacterial abundance, respectively (Kasinak et al.,2015; Sartory and Grobbelaar, 1984). For both pigments, samples were filtered through a Pall A/E glass fiber filter to collect algal cells. Filters were stored in black plastic containers at -10° C until pigments were extracted. For chlorophyll-*a* extraction, filters were submerged in 90% aqueous ethanol and stored for 23 hours in the dark at 4° C before allowing the extracts to warm to room temperature (~23° C) for one hour (Sartory and Grobbelaar, 1984). For phycocyanin extraction, filters were submerged and ground in 20 mL of 50 mM phosphate buffer with a

Teflon pestle and stored for 4 hours at room temperature before centrifuging and filtering extract with 0.2 µm inline filter (Kasinak et al., 2015). Extracted pigments were then quantified fluorometrically using a Turner Designs Trilogy benchtop fluorometer. Green algae chlorophyll was differentiated from total phytoplankton and cyanobacterial chlorophyll using a separate benchtop bbe PhycoProbe (bbe Moldaenke). This fluorometry-based tool can separate algal classes beyond the phytoplankton vs. cyanobacteria determination that is possible with pigment extraction.

Zooplankton

Zooplankton biomass was quantified through day 15 to determine if the initial FGD gypsum mixing method, amount of FGD gypsum material added, or immediate flocculation mechanism had any impacts on zooplankton at the beginning of the experiment. To collect zooplankton, 2 L of water were passed through a 100 µm sieve and any collected organisms were transferred to a 20 mL glass vial and preserved with 95% ethanol. The zooplankton samples were counted using a 1 mL Sedgwick-Rafter chamber and a Nikon Eclipse 50*i* compound microscope at 40x magnification (Belfiore et al., 2021; Yang et al., 2018), and all organisms were identified to the genus level by referencing *Pennak's Freshwater Invertebrates of the United States: Porifera to Crustacea* (Smith, 2001). Samples were either counted in their entirety or subsampled if the chamber could not fit all organisms. Zooplankton length was measured in each sample and converted to biomass using the appropriate length-weight regression (Culver et al., 2011). For statistical analyses, the genus-level data were grouped into major taxa (i.e., Cladocera, Copepoda, Rotifera, and Ostracoda).

Nutrients

Total nitrogen (TN), total phosphorus (TP), and soluble reactive phosphorus (SRP) were measured for each sampling date. Both TP and SRP were measured to determine if FGD gypsum influences the biologically available dissolved forms of phosphorus (SRP) when compared to the overall phosphorus found in the system (TP). The TN and TP were measured from whole water samples, while SRP was measured from samples filtered through a Pall A/E glass fiber filter. The TN and TP were measured spectrophotometrically using the persulfate digestion method (Gross and Boyd, 1998). The SRP was measured colorimetrically with a modified single solution method according to Murphy and Riley (1962). All measurements were conducted using a PerkinElmer UV/VIS Lambda 365 spectrophotometer (PerkinElmer, Waltham, MA).

Trace heavy metals

Trace heavy metals were quantified before FGD gypsum addition, and four hours, eight days, and 22 days after FGD gypsum addition. Whole water samples collected with the integrated tube sampler were transferred to a clean, plastic sterile bottle and preserved with nitric acid (HNO_3) before analysis for arsenic, cadmium, copper, lead, and selenium using inductively coupled plasma-atomic emission spectrometry (ICP-AES) and for mercury using cold vapor atomic absorption (CVAA) spectrometer (Campisano et al., 2017).

Statistical analysis

Statistical analyses were performed in R version 4.1.3. A repeated-measures analysis of variance (RM-ANOVA) using a restricted maximum likelihood (REML) method to estimate variance was used in the *nlme* package to evaluate differences in the response variables among treatments over time (Pinheiro et al., 2020). The response variable data were first transformed using either a log or square root transformation; whichever method brought the data to a normal distribution. If there were any significant differences ($p<0.05$) observed in the model (either treatment, time, or the treatment-time interaction), a *post hoc* test in the *emmeans* package was used to conduct pair-wise multiple comparisons with a Tukey correction (Lenth et al., 2020). Depending on the response variable and the overall trend of the data, either the differences among treatments at specific timepoints within the experiment or the average differences among treatments across the entire experiment were compared.

Results

Hardness, alkalinity, and pH

Across the entire experiment, there was a significant increase in average hardness for the FGD gypsum-treated enclosures when compared to the control enclosures (RM-ANOVA, $F_{3,72}$ = 815.11, p<0.0001; Figure 1A). All enclosures began with an average hardness range between 34-43 $CaCO_3$ mg/L. After adding the FGD gypsum, all treatments increased in hardness by the next sampling day (day 8) to sequentially increasing degrees as relating to the treatment concentration increases. By day 8, the 500 mg/L treatment increased approximately 10x to an average hardness of 346 $CaCO_3$ mg/L (p<0.0001), the 1,000 mg/L treatment increased approximately 15x to an average hardness of 530 $CaCO_3$ mg/L (p<0.0001), and the 2,000 mg/L treatment increased approximately 20x to an average hardness of 761 $CaCO_3$ mg/L (p<0.0001) when compared to the control average (51 $CaCO_3$ mg/L). All concentrations remain elevated throughout the duration of the 36-day experiment when compared to the control (Figure 1A).

Across the entire experiment, there was a significant decrease in average alkalinity for the FGD gypsum-treated enclosures when compared to the control enclosures (RM-ANOVA, $F_{3,72}$ = 224.19, p<0.0001; Figure 1B). All enclosures began with an average alkalinity of approximately 29 $CaCO_3$ mg/L. After FGD gypsum addition, all treatments decreased in average alkalinity by the next sampling day (day 8) to a range between 17-22 $CaCO_3$ mg/L compared to the control (28 $CaCO_3$ mg/L) and remained significantly less through the duration of the 36-day experiment ($p<0.0001$).

The FGD gypsum had an overall, significant treatment effect on pH (RM-ANOVA, $F_{3,72} = 42.5$, $p<0.0001$; Figure 1C) but these effects did not become apparent until day 15. All enclosures began with an average pH of 8.5-8.7. From day 15 onwards, the average pH of the control increased and remained elevated throughout the rest of the experiment (9.3-9.6 depending on the sampling date), while all treatments remained significantly less on average ($p<0.0001$). Although the 500 mg/L treatment did not maintain as low of a pH during days 22 and 29 like the higher FGD gypsum treatments (9.3 and 9.2 compared to 8.6-9.0), by the end of the experiment (day 36) all pHs in the FGD gypsum treatments were significantly lower than the control with an average 7.8-8.0 compared to the control average of 9.3 ($p<0.0001$).

Algal abundance via pigment concentration

Chlorophyll-a

Chlorophyll-*a* concentration was utilized as a proxy for total phytoplankton abundance. Overall, there was not an experiment-wide treatment effect on chlorophyll-*a* concentration (RM-ANOVA, $F_{3,12} = 1.45$, $p=0.2783$, Figure 2A). However, there was a significant treatment-time interaction (RM-ANOVA, $F_{15,60} = 12.32$, $p<0.0001$), indicating significant treatment effects during different time points within the experiment. All enclosures began with an average chlorophyll-*a* concentration of 58-64 μg/L. On day 8 after FGD gypsum addition, chlorophyll-*a* concentration in the 1,000 mg/L and 2,000 mg/L treatments had significantly increased to an average of 87 and 98 μg/L, respectively, when compared to the control (average 56 μg/L) ($p=0.0465$, $p=0.0106$). However, chlorophyll-*a* concentration quickly decreased by the next sampling date (day 15). After the mid-experiment fertilization, the trends flipped as the control and 500 mg/L treatment increased and remained elevated through the rest of the 36-day experiment when compared to the 1,000 mg/L and 2,000 mg/L treatments. The chlorophyll-*a* concentration of the control increased to an average of 98 μg/L on day 22 and by the end of the experiment leveled out to an average of 70 μg/L. These concentrations were significantly higher than the 2,000 mg/L treatment on days 22 through 36 ($p \leq 0.0356$) and significantly higher than the 1,000 mg/L treatment on days 29 through 36 ($p \leq 0.0185$). The chlorophyll-*a* concentration of the 500 mg/L treatment increased to an average of 89 μg/L on day 22 and by the end of the experiment leveled out to an average of 59 μg/L. These concentrations were significantly higher than the 2,000 mg/L treatment on days 29 through 36 ($p \leq 0.0158$) and significantly higher than the 1,000 mg/L treatment on day 29 ($p=0.0367$).

Phycocyanin

Phycocyanin concentration was utilized as a proxy for cyanobacterial abundance. Overall, there was a marginal experiment-wide treatment effect on phycocyanin concentration (RM-ANOVA, $F_{3,12} = 2.80$, $p=0.0856$; Figure 2B). However, there was a significant treatment-time interaction (RM-ANOVA, $F_{15,60} = 4.78$, $p<0.0001$). All enclosures began with an average phycocyanin concentration of 24-33 μg/L. On day 8 after FGD gypsum addition, phycocyanin concentration in the 1,000 mg/L and 2,000 mg/L treatments doubled and tripled, respectively, when compared to the control ($p=0.0067$, $p=0.0007$). The 2,000 mg/L treatment was also significantly higher than the 500 mg/L treatment ($p=0.0283$). This increase in phycocyanin on day 8, which indicates an increase in cyanobacteria, most likely explains the increase in chlorophyll-*a* as well, since cyanobacteria also produce chlorophyll-*a* as a pigment. After this peak, phycocyanin levels in those higher treatments began to drop back down by day 15 and stabilized near the concentration observed in the control through the end of the experiment.

Green algae

Since green algal concentration was quantified via the bbe PhycoProbe's internal chlorophyll fluorescence analysis as opposed to in-lab pigment extraction, the green algal abundances could be quantified separately from cyanobacteria and the total phytoplankton abundance. Across the entire experiment, there was a significant increase in average green algal concentration in the control when compared to the treatments (RM-ANOVA, $F_{3,12} = 25.97$, $p<0.0001$; Figure 2C). However, there was also a significant treatment-time interaction (RM-ANOVA, $F_{15,60} = 17.92$, $p<0.0001$) in which the treatment effect did not become apparent until day 22 – the sample date following the mid-experiment fertilization. All enclosures began with an average green algal concentration of 26-28 µg/L chlorophyll-*a*. From day 22 through the end of the experiment, the control spiked after fertilization and remained significantly elevated at levels roughly 2-3x that of the concentrations found in the treatments ($p\leq0.0033$). Additionally, the 2,000 mg/L treatment had significantly lower green algal levels than the 500 mg/L treatment ($p\leq0.0193$) from day 22 onwards and significantly lower green algal levels than the 1,000 mg/L treatment from day 29 onwards ($p\leq0.0236$). These results show that the FGD gypsum did not have an immediate impact on green algae concentration once added but did prevent exponential growth after the mid-experiment fertilization.

Zooplankton biomass

Total zooplankton biomass

The total zooplankton biomass consisted of the sum of dry biomass measured for the following taxa: Cladocera, Copepoda, Rotifera, and Ostracoda. Zooplankton were measured and quantified through day 15 to determine if they were negatively impacted by the initial turbulence or flocculation during FGD gypsum addition, which could explain the brief spike in cyanobacterial abundance on day 8. Across those 15 days, there was an overall negative treatment effect on zooplankton biomass (RM-ANOVA, $F_{3,12} = 5.34$, p=0.0143; Figure 3) as well as a treatment-time interaction (RM-ANOVA, $F_{9,35} = 5.83$, p=0.0001). All enclosures began with an average zooplankton dry biomass concentration around 1,856-2,200 µg/L. Although total zooplankton biomass was collected immediately following FGD gypsum application (approximately 4 hours after), significant effects were not evident until day 8 when the 2,000 mg/L treatment contained roughly 70% less biomass than the control (p=0.0099). By day 15, both the 2,000 mg/L and 1,000 mg/L treatments had significantly less zooplankton biomass than the control ($p \leq 0.0396$), while the 2,000 mg/L treatment was also significantly less than the 500 mg/L treatment (p=0.0004). It is evident that the FGD gypsum negatively impacted zooplankton survival, especially for the higher concentration treatments.

Cladocera biomass

Cladocerans were the taxa of zooplankton most impacted by the FGD gypsum addition. Across the 15 days, there was a significant overall negative treatment effect on cladoceran biomass (RM-ANOVA, $F_{3,47} = 21.02$, $p<0.0001$; Figure 4A). There was also a significant treatment-time interaction (RM-ANOVA, $F_{9,47} = 6.47$, $p<0.0001$) in which the effects did not become apparent until day 8. All enclosures began with an average cladoceran biomass concentration of 793-1,141 µg/L. The 2,000 mg/L treatment had a 99% reduction in cladocerans by day 8 and a complete extirpation by day 15. These values were significantly lower than the control and 500 mg/L treatment ($p \leq 0.0010$). The 1,000 mg/L treatment had a 94% reduction in cladoceran biomass by day 8 but by day 15 had slightly increased back to only an 87% reduction of the initial concentration. These results were significantly less than the control on day 8 and 15 as well as significantly less than the 500 mg/L treatment on day 15 ($p \leq 0.0300$). Although the 500 mg/L treatment did have a 70% reduction in cladoceran biomass by day 8, it never significantly differed from the control ($p > 0.05$) and the biomass concentration had increased by day 15. Again, the higher FGD gypsum treatments seem to have the largest negative impact on cladoceran survival in the enclosures.

Copepoda biomass

Although copepods were not as severely impacted by the FGD gypsum as cladocerans, there was still a marginal treatment effect on copepod biomass (RM-ANOVA, $F_{3,47} = 2.65$, $p=0.0596$; Figure 4B). Specifically, the 2,000 mg/L treatment was the only treatment to have significant effects on the biomass. There was also a significant treatment-time interaction (RM-ANOVA, $F_{9,47} = 2.66$, $p=0.0139$) in which these effects did not become apparent until day 15. All enclosures began with an average copepod biomass concentration of 884-1,254 µg/L. By day 15, the 2,000 mg/L treatment saw an 80% reduction in copepod biomass, which was significantly less than both the control and 500 mg/L treatment ($p \leq 0.0104$). Neither the 500 mg/L or 1,000 mg/L treatments had reductions in biomass concentration that were significantly different from the control ($p > 0.05$).

Ostracoda and Rotifera biomass

Across the 15 days, there was neither a significant treatment effect or treatment-time interaction for either ostracod or rotifer biomass (Figure 4C, Figure 4D). By day 15, there was a nearly complete reduction in rotifer biomass in all enclosures, including the control. While there may have been a marginal treatment effect on ostracod biomass (RM-ANOVA, $F_{3,47} = 2.54$, $p=0.0678$), the control was increasing in ostracod biomass by day 15 while the higher FGD gypsum treatments had stabilized at a lower concentration. The large variation among treatments and days are most likely masking this effect. The ostracods identified and measured in these enclosures varied greatly in size, which ultimately impacted the overall variation between treatments.

Nutrients – total nitrogen (TN), total phosphorus (TP), and soluble reactive phosphorus (SRP)

Flue gas desulfurization gypsum did not have a treatment effect on TN across the entire experiment (RM-ANOVA, $F_{3,12} = 0.84$, $p=0.4955$; Figure 5A). All enclosures began with an average TN concentration of 1,229-1,324 µg/L. Although TN concentrations fluctuated over time throughout the experiment (RM-ANOVA, $F_{5.60} = 25.97$, $p<0.0001$), the treatments did not differ from one another and ended with an average TN concentration of 1,395-1,563 µg/L.

In contrast, FGD gypsum did have an overall treatment effect on TP across the entire experiment (RM-ANOVA, $F_{3,12} = 10.05$, $p=0.0014$; Figure 5B), but these effects only became apparent on or after day 15. All enclosures began with an average TP concentration of 106-112 µg/L and, from day 15 onwards, the control began to slightly increase in TP and remain elevated through the end of the experiment (117-138 µg/L). Although there were fluctuations in TP over time depending on the sampling date, the higher FGD gypsum treatments on average stayed consistently lower with TP levels about 15-29% (for the 1,000 mg/L treatment) and 21-34% (for the 2,000 mg/L treatment) lower than the control ($p\leq0.0178$). The 2,000 mg/L treatment was also on average significantly lower than the 500 mg/L treatment ($p=0.0347$).

Since TP is comprised of both dissolved and particulate forms of inorganic and organic phosphorus in the enclosures, these results cannot completely differentiate whether the observed FGD gypsum effects were due to nutrient availability differences or phytoplankton abundance differences. However, SRP, the dissolved form of phosphorus available to phytoplankton, was very low in all enclosures throughout the entire experiment (always remaining below 15 µg/L) and FGD gypsum was determined to not have a treatment effect on it across the entire experiment (RM-ANOVA, $F_{3,72} = 1.20$, p= 0.3159, Figure 5C). Although SRP fluctuated slightly over the course of the experiment, the treatments never differed from one another or from the control. Thus, the differences in TP can be mostly attributed to changes in particulate phosphorus.

Trace heavy metals

Before FGD gypsum addition, most heavy metal concentrations were below detection limit in all enclosures (Table 1). Low levels of copper were measured in some of the enclosures, but no treatment group had all enclosures with detectable levels. After FGD gypsum addition, selenium was the only metal measured above the detection limit within all enclosures of the 2,000 mg/L treatment at an average concentration of 13.88-15.58 µg/L (method detection limit = 11.10 µg/L) depending on the sampling date. Selenium was also measured in some of the enclosures from the 500 mg/L and 1,000 mg/L treatments. However, it was not detected in all enclosures within each treatment, thus leading to a skewed treatment average. Mercury was briefly detected in the 2,000 mg/L treatment four hours after FGD gypsum addition. Again, this metal was not detected in all enclosures within the treatment and led to a skewed treatment average. By the following sampling date, mercury was no longer detected. All other metals, including arsenic, cadmium, and lead, were below the detection limit for the duration of the experiment.

Discussion

This experiment assessed the use of FGD gypsum at varying concentrations in an aquatic setting to determine the positive and negative impacts it may have on water quality parameters, including the phytoplankton and zooplankton communities. Specifically, we tested a larger range of FGD gypsum concentrations than previously reported (Salonen and Varjo, 2000; Seo and Boyd, 2001; Whangchai et al., 2016, Wu and Boyd, 1990) to determine if there is an optimal concentration to be used in a real-world setting. Additionally, since there is a potential risk of trace metal contamination with the use of any byproduct from a fossil fuel energy source, we also investigated the amount of trace metals that enter the water column after the dissolution of FGD gypsum.

Overall, our results found that the higher FGD gypsum concentrations, including 1,000 mg/L and 2,000 mg/L, produced the most extreme results for both the desired and undesired outcomes. Flue gas desulfurization gypsum increases hardness due to the addition of calcium cations in the water that can influence overall total hardness (Whangchai et al., 2016; Wu and Boyd, 1990). While total hardness for each treatment significantly increased compared to the control, it increased stepwise by roughly 200 mg/L $CaCO_3$ for each increase in the FGD gypsum treatment concentration (Figure 1A). As expected, flue gas desulfurization gypsum additions resulted in decreased alkalinity for all the treatments when compared to the control (Figure 1B). This is again due to the increase in calcium cations in the water column leading to the precipitation of calcium carbonate (Mandal and Boyd, 1980). Although effects on pH were not significant until after day 15, which also followed the mid-experiment fertilization, the clear treatment effect between the control and treatments was most likely due to the spike in phytoplankton (specifically green algae) seen in the control during the second half of the experiment (Figure 1C). While lower concentrations of carbonate ions may play a small role in reducing pH, most of the delayed difference can be attributed to an increase in photosynthesis in the control that ultimately removes carbonic acid from the water (Axelsson, 1988; Wurts and Durborow, 1992,). Wu and Boyd (1990) reported a similar delayed effect on pH that did not become apparent until phytoplankton abundance differed between their control and treatment as well. This desired FGD gypsum effect on pH could be especially important for use in aquaculture ponds as ammonia becomes more toxic to aquatic life as pH increases (Thurston et al., 1981).

The impact that FGD gypsum had on the overall algal abundance can be broken down into total phytoplankton, cyanobacteria, and green algae in the first half and second half of the experiment. The immediate spike in total phytoplankton for the treatments on day 8 can be attributed to an increase in cyanobacteria (Figure 2A, Figure 2B). While this was an initially surprising result as FGD gypsum dissolution would not add any significant amounts of nutrients necessary for phytoplankton growth to the water column, further investigation into other potential causes revealed that zooplankton abundance changes may have been the cause. The overall decrease in zooplankton abundance in the treatments by day 8, especially for the higher treatments, are mostly attributed to the decrease in cladocerans (Figure 3, Figure 4A). This decrease could be due to several reasons including the initial mixing of FGD gypsum into the enclosures harming the animals, the flocculation and settling of particles and undissolved FGD gypsum also settling the less mobile zooplankton, or zooplankton feeding on undissolved FGD gypsum that could ultimately kill the cladocerans or clog their filtering appendages. Similar zooplankton abundance and composition trends were also reported in another study using chitosan modified kaolinite (CMK) as a flocculant in which there was an initial and temporary decrease in total zooplankton due to a decline in cladocerans when compared to the copepods

(Tang et al., 2018). Cladocerans are filter-feeding zooplankton that play a role in controlling phytoplankton abundance, including cyanobacteria according to more recent studies (Belfiore et al., 2021; Chislock et al., 2019; Guo and Xie, 2006). Thus, this initial and sudden decrease in cladocerans could have allowed for a sharp proliferation in cyanobacteria. However, this increase in cyanobacteria was short-lived and abundances decreased by day 15, most likely due to a limit in biologically available nutrients as the enclosures only had an SRP range of 8-15 µg/L.

By the second half of the experiment, we observed an increase in total phytoplankton in the control and 500 mg/L treatment following the mid-experiment fertilization (Figure 2A). Most of this increase can be attributed to an increase in green algae (Figure 2C). At this point in the experiment, the enclosures had settled out from the initial mixing that created a turbulent and clouded water column in the higher treatments. However, as evident from the still-increased hardness concentrations in the treatments by mid-experiment, there were still plenty of free calcium ions available to control phosphorus availability for algal growth after the fertilization. Hardness concentrations decreased slightly in the treatments following this fertilization and green algal growth took off in the control, which had consistently maintained low hardness (and thus free calcium ion) concentrations. These phytoplankton abundance results in the second half of the experiment were similar to the results from previous field studies using some form of gypsum (Salonen and Varjo, 2000; Seo and Boyd, 2001; Whangchai et al., 2016; Wu and Boyd, 1990). Although the 500 mg/L treatment saw more of an increase in green algae than the higher treatments, it was still significantly less than the control. Ultimately, this concentration seems to be the best choice in controlling algal abundance as it did not cause the initial large spike in cyanobacteria or delayed spike in green algae after fertilization, nor did it have a negative impact on zooplankton abundance.

Over the course of the entire experiment, the only nutrient that FGD gypsum had an impact on was TP (Figure 5B). While most previous studies found that gypsum reduced SRP concentration through calcium precipitation (Seo and Boyd, 2001; Whangchai et al., 2016, Wu and Boyd, 1990), this study did not find any significant effects on SRP (Figure 5C). This result could be due in part to the low levels of SRP initially found in the enclosures at the beginning of the experiment potentially masking any discernable changes among the treatments. The treatments do temporarily have lower SRP concentrations than the control on the first sampling day following FGD gypsum addition, but the differences and the variation do not produce any statistically significant effects. However, the FGD gypsum did reduce TP when compared to the control from day 15 onwards, especially for the higher concentration treatments. Thus, FGD gypsum was able to control the particulate forms of phosphorus, most likely through the delayed reduction of algal abundance.

Although the use of FGD gypsum in agricultural settings initially created potential concerns surrounding trace heavy metal contamination, the U.S. Environmental Protection Agency released their official contamination risk report in March 2023 entitled “Beneficial Use Evaluation: Flue Gas Desulfurization Gypsum as an Agricultural Amendment”. The report concluded that FGD gypsum is safe in regard to the risk of heavy metal contamination, yet the use of FGD gypsum directly in aquatic settings was not one of the practices examined (U.S. EPA , 2023). The conclusion of the EPA report is supported by numerous studies that have found the trace heavy metal levels to be below the detection limits or below established standards in various experimental setups that analyzed concentrations in soil, agricultural field runoff, or plant/animal biomass (Chen et al., 2014; Torbert and Watts, 2014; Watts and Dick, 2014; Zhao et al., 2018). Our results were somewhat similar in that five of the six trace metals measured were below the detection limits for all treatments. The only metal fully detected was selenium, which was only found in the highest concentration treatment (2,000 mg/L). The 13-15 µg/L range found in the 2,000 mg/L enclosures is higher than the 1.5 µg/L threshold for aquatic life chronic exposure in lentic systems (U.S. EPA, 2016). It is important to note that the lower detection limit for the external lab's method is 11.10 µg/L. Thus, there could potentially still be selenium concentrations in the other treatments between the U.S. EPA's 1.5 µg/L threshold and the 11.10 µg/L detection limit. While selenium is an essential element in small amounts, it can create issues at higher concentrations for fish and aquatic invertebrates through bioaccumulation, causing reproductive impairments, reduced growth, and even mortality (U.S. EPA, 2016; Hamilton, 2003). Although the elevated concentrations remained through day 22, the structure of the enclosed mesocosms does not allow for any dispersal and sedimentation that would occur in a natural lake or pond. Ultimately, a 2,000 mg/L treatment of FGD gypsum is on the extreme upper end of feasible concentration ranges to use in aquatic settings both regarding necessity and cost, and the potential for selenium contamination only further discourages the need for a treatment that high.

Conclusions

As a byproduct of carbon-based energy sources, FGD gypsum was originally destined to remain in landfills indefinitely. Fortunately, the agricultural community has found beneficial uses for FGD gypsum to improve crop production and reduce runoff impacts. Now that the potential of FGD gypsum in aquatic settings to increase hardness, reduce pH, and ameliorate the downsides of eutrophication has been discovered, FGD gypsum has another potential beneficial use opportunity. This experiment tested a large range of FGD gypsum concentrations in natural waters to determine its positive and negative impacts on water quality, including the algal and zooplankton communities. While FGD gypsum is a generally stable compound deemed safe for aquatic life, we found that the higher concentrations produced more undesired results, such as the initial spike in cyanobacteria, reduction in zooplankton, and certain trace metal contamination, compared to the moderate 500 mg/L concentration. Ultimately, the 500 mg/L concentration was the optimal dose as it minimized the unintended results while still producing moderate, desired results by the end of the experiment, such as an increase in hardness, reduction of pH, and control of algal abundance. Further experiments would be beneficial in determining the varying impacts of FGD gypsum in different waters varying in physicochemical properties at more specific concentrations between 500 and 1,000 mg/L to determine if there is an even more efficient dose.

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Figure Legends

Figure 1. Average total hardness (A), alkalinity (B), and pH (C) for each treatment (± 1 SE) over time. The vertical dashed line represents the mid-experiment fertilization on day 15. Treatments sharing the same letter at a specific time are not significantly different ($p \geq 0.05$) and treatments with different letters are significantly different ($p < 0.05$). Hardness increased in FGD gypsum treatments and remained elevated, alkalinity decreased in FGD gypsum treatments and remained lower, and pH increased in the control and remained elevated from day 15 onwards.

Figure 2. Average chlorophyll-*a* (A), phycocyanin (B), and green algae (C) for each treatment (± 1 SE) over time. The vertical dashed line represents the mid-experiment fertilization on day 15. Treatments sharing the same letter at a specific time are not significantly different ($p \geq 0.05$) and treatments with different letters are significantly different ($p < 0.05$). Chlorophyll-*a* and phycocyanin spiked on day 8 in the higher FGD gypsum treatments, while green algae spiked in the control following the mid-experiment fertilization after day 15.

Figure 3. Average total zooplankton biomass for each treatment (± 1 SE) between day 0 and day 15. Treatments sharing the same letter at a specific time are not significantly different ($p \geq 0.05$) and treatments with different letters are significantly different ($p < 0.05$). The inset graph represents the change between the start and hour 4. Zooplankton biomass in the higher FGD gypsum treatments decreased when compared to the control.

Figure 4. Average cladoceran biomass (A), copepod biomass (B), rotifer biomass (C), and ostracod biomass (D) for each treatment (± 1 SE) between day 0 and day 15. Treatments sharing

the same letter at a specific time are not significantly different ($p \geq 0.05$) and treatments with different letters are significantly different ($p < 0.05$). Cladoceran biomass in the higher FGD gypsum treatments decreased compared to the control and 500 mg/L treatment. Copepod biomass in the 2,000 mg/L treatment decreased compared to the control and 500 mg/L treatment as well.

Figure 5. Average total nitrogen (A) and total phosphorus (B) and soluble reactive phosphorus (C) for each treatment (± 1 SE) over time. The vertical dashed line represents the mid-experiment fertilization on day 15. Treatments sharing the same letter at a specific time are not significantly different ($p \geq 0.05$) and treatments with different letters are significantly different ($p < 0.05$). There were no significant changes in total nitrogen and soluble reactive phosphorus between treatments, but total phosphorus decreased in the higher FGD gypsum treatments when compared to the control and 500 mg/L treatment and remained lower through the end of the experiment.

Figure 1

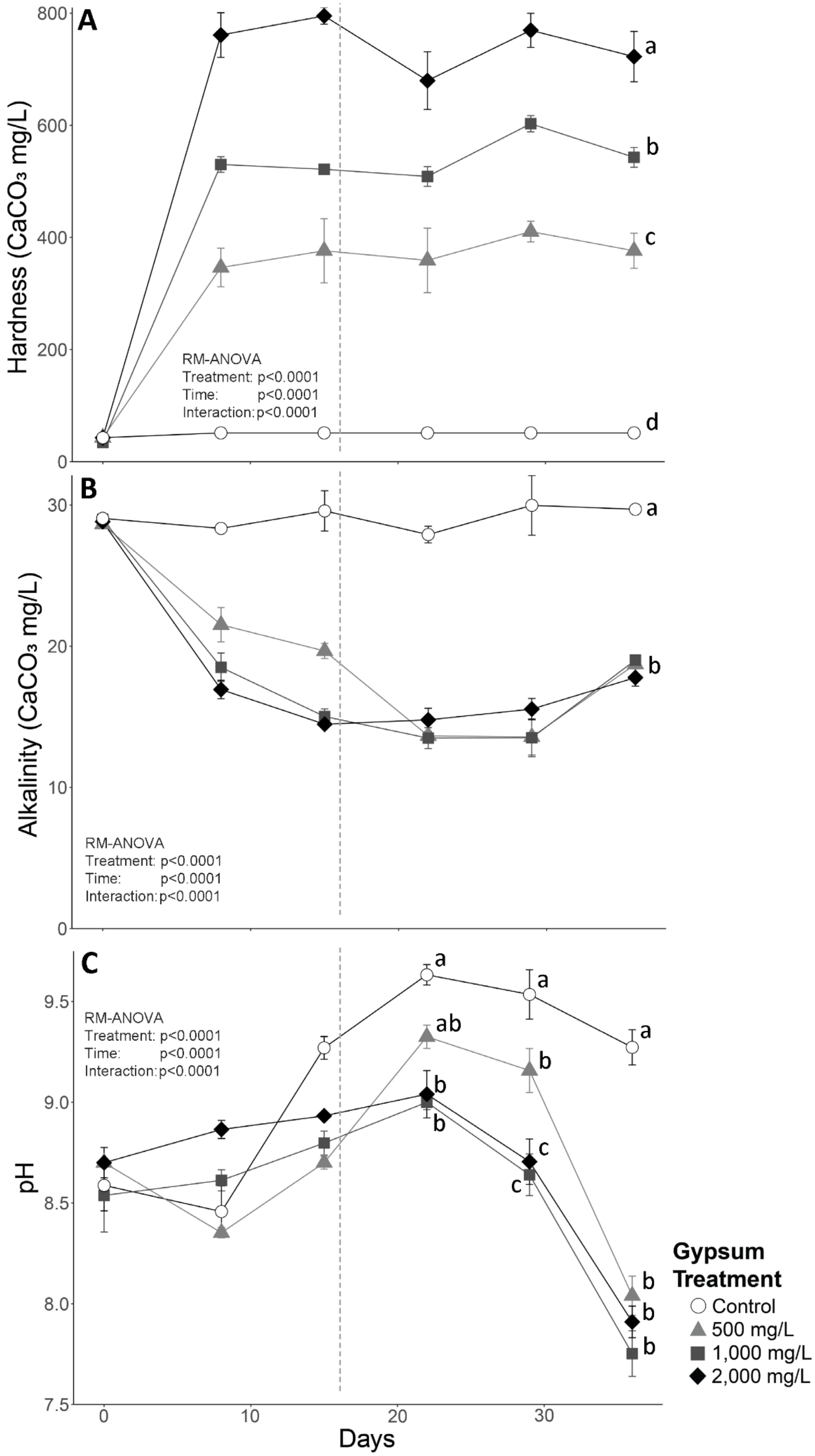

A
B
C
Hardness (CaCO₃ mg/L)
Alkalinity (CaCO₃ mg/L)
pH
Days
RM-ANOVA
Treatment: p<0.0001
Time: p<0.0001
Interaction: p<0.0001
Gypsum Treatment
Control
500 mg/L
1,000 mg/L
2,000 mg/L

Figure 2

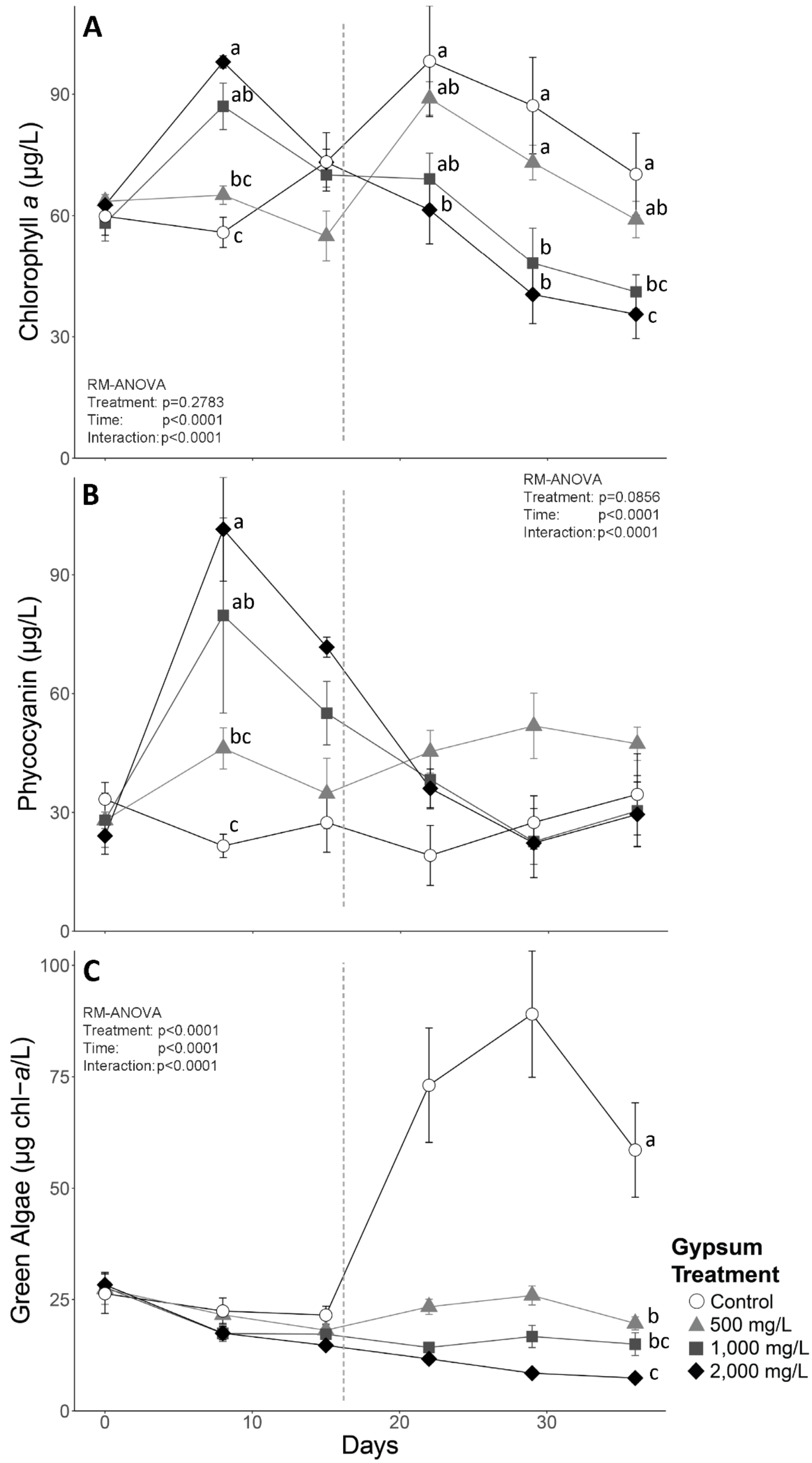
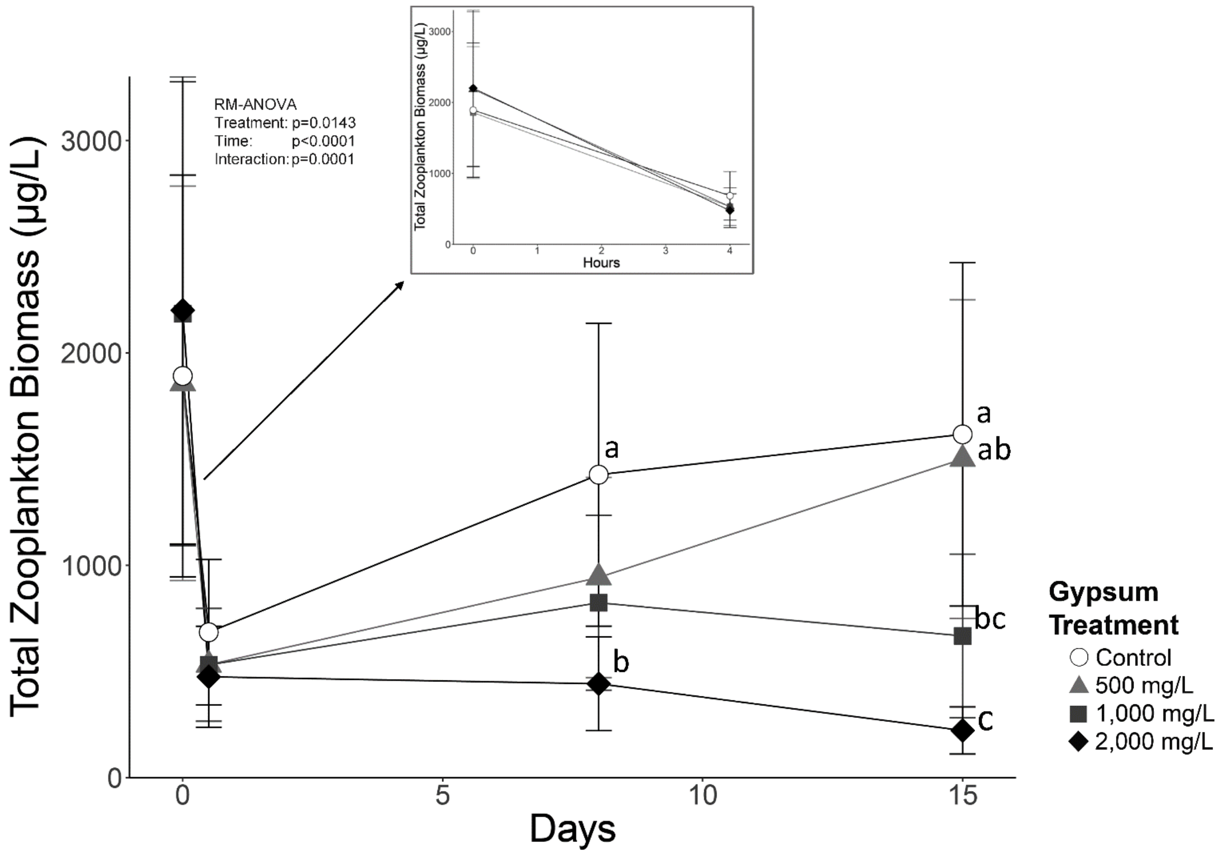
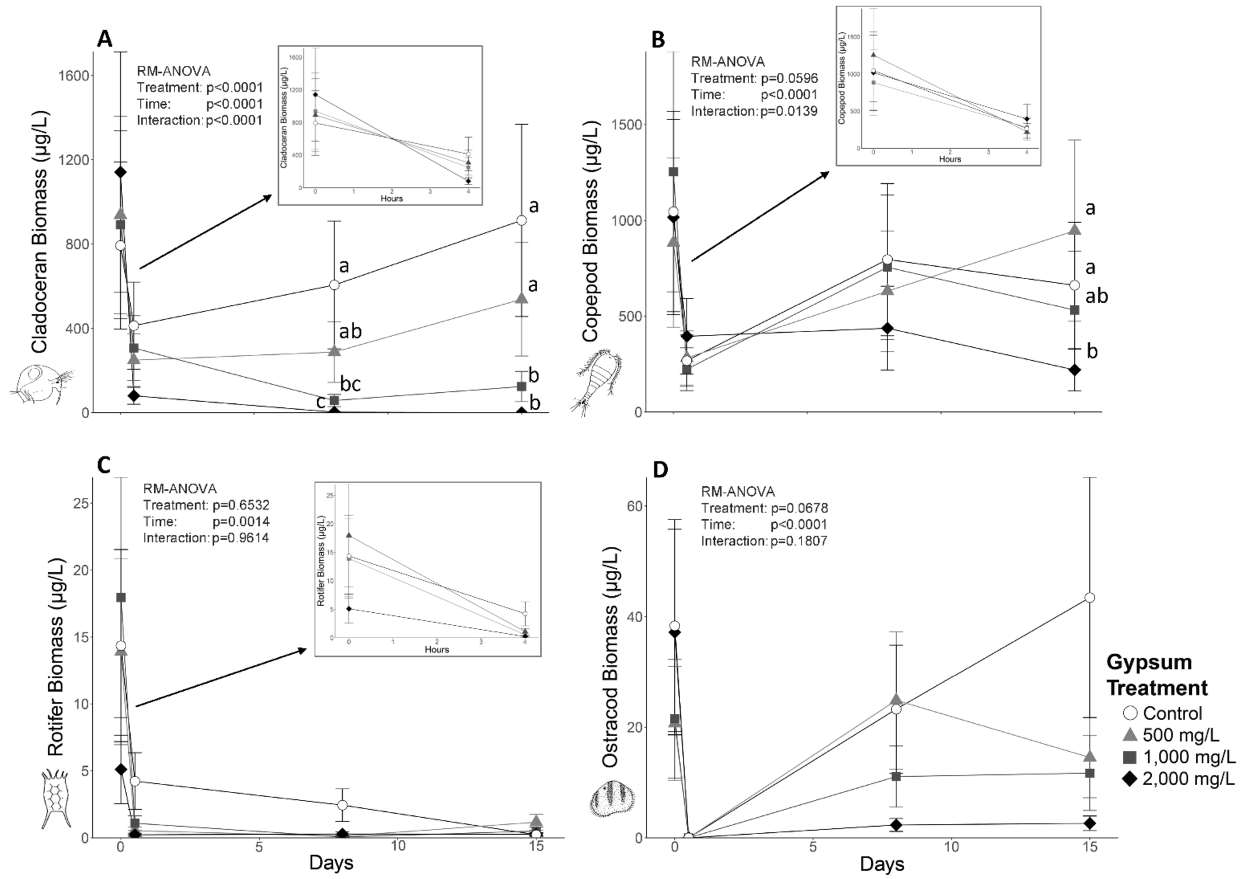

A
RM-ANOVA
Treatment: p=0.2783
Time: p<0.0001
Interaction: p<0.0001
Chlorophyll a (µg/L)
B
RM-ANOVA
Treatment: p=0.0856
Time: p<0.0001
Interaction: p<0.0001
Phycocyanin (µg/L)
C
RM-ANOVA
Treatment: p<0.0001
Time: p<0.0001
Interaction: p<0.0001
Green Algae (µg chl−a/L)
Days
Gypsum Treatment
Control
500 mg/L
1,000 mg/L
2,000 mg/L

Figure 3



RM-ANOVA
Treatment: p=0.0143
Time: p<0.0001
Interaction: p=0.0001
Total Zooplankton Biomass (µg/L)
Hours
Days
a
a
ab
b
bc
c
Gypsum Treatment
Control
500 mg/L
1,000 mg/L
2,000 mg/L

Figure 4



A
RM-ANOVA
Treatment: p<0.0001
Time: p<0.0001
Interaction: p<0.0001
Cladoceran Biomass (µg/L)
B
RM-ANOVA
Treatment: p=0.0596
Time: p<0.0001
Interaction: p=0.0139
Copepod Biomass (µg/L)
C
RM-ANOVA
Treatment: p=0.6532
Time: p=0.0014
Interaction: p=0.9614
Rotifer Biomass (µg/L)
D
RM-ANOVA
Treatment: p=0.0678
Time: p<0.0001
Interaction: p=0.1807
Ostracod Biomass (µg/L)
Days
Hours
Gypsum Treatment
Control
500 mg/L
1,000 mg/L
2,000 mg/L

Figure 5

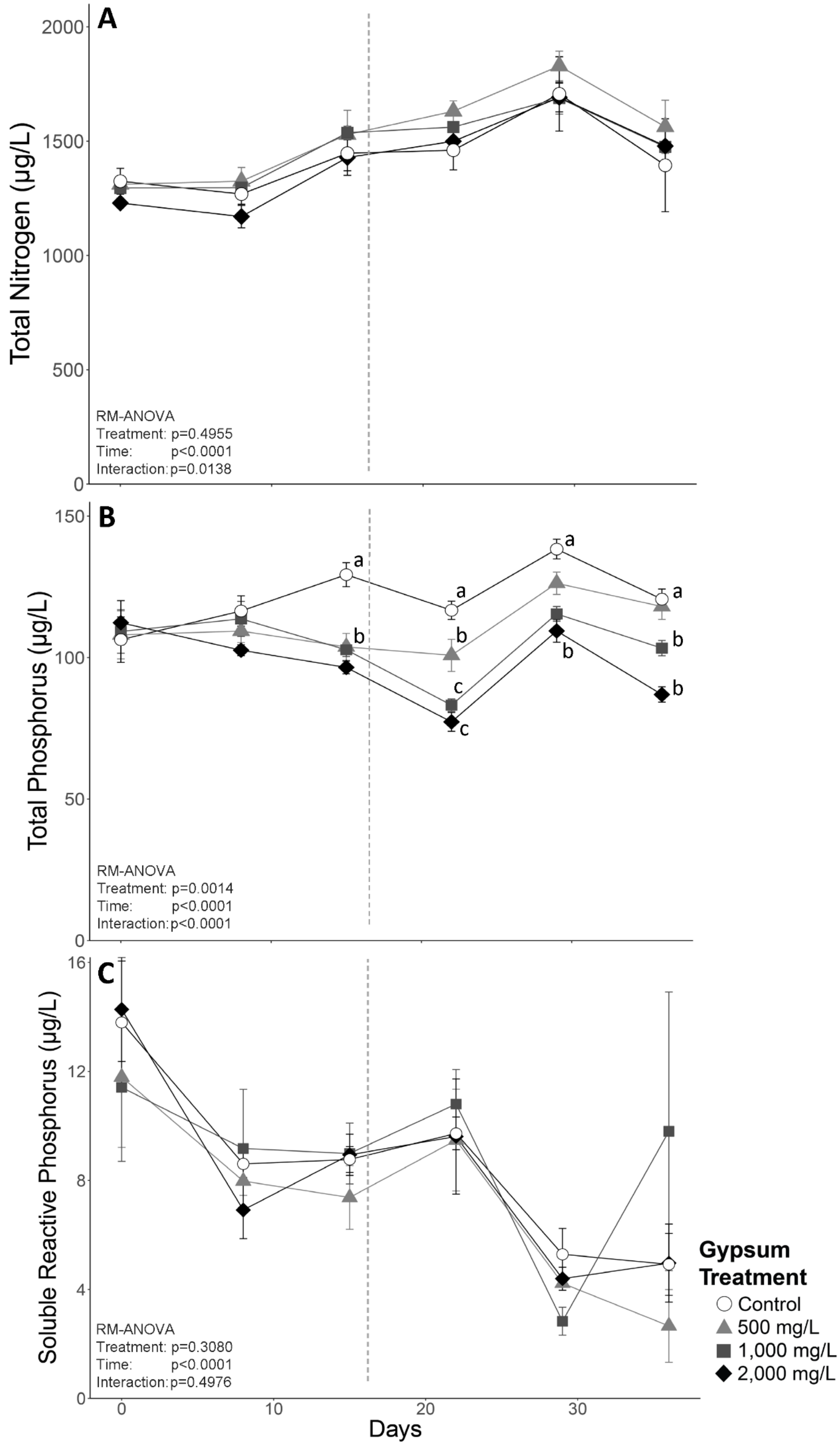

A
Total Nitrogen (µg/L)
2000
1500
1000
500
0
RM-ANOVA
Treatment: p=0.4955
Time: p<0.0001
Interaction: p=0.0138
B
Total Phosphorus (µg/L)
150
100
50
0
a
b
c
RM-ANOVA
Treatment: p=0.0014
Time: p<0.0001
Interaction: p<0.0001
C
Soluble Reactive Phosphorus (µg/L)
16
12
8
4
0
RM-ANOVA
Treatment: p=0.3080
Time: p<0.0001
Interaction: p=0.4976
0
10
20
30
Days
Gypsum Treatment
Control
500 mg/L
1,000 mg/L
2,000 mg/L

Table Legends

Table 1. Average trace metal concentrations in each treatment at day 0, hour 4, day 8, and day 22. BDL = below detection limit. An asterisk (*) indicates that not all replicates in a treatment were above the detection limit. The only detectable trace metal found in all replicates was selenium, which was found at levels between 13.9 and 15.6 μg/L in the 2,000 mg/L treatment.

Table 1

		Before gypsum				4 hours after gypsum				8 days after gypsum				22 days after gypsum			
Metal (ug/L)	**Detection Limit (µg/L)**	**Control**	**500 mg/L**	**1,000 mg/L**	**2,000 mg/L**	**Control**	**500 mg/L**	**1,000 mg/L**	**2,000 mg/L**	**Control**	**500 mg/L**	**1,000 mg/L**	**2,000 mg/L**	**Control**	**500 mg/L**	**1,000 mg/L**	**2,000 mg/L**
Arsenic	18.40	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Cadmium	3.80	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Copper	2.40	2.80*	3.10*	BDL	2.70*	BDL	BDL	BDL	BDL	2.50*	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Lead	14.60	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Mercury	1.00	BDL	BDL	BDL	BDL	BDL	BDL	BDL	1.30*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Selenium	11.10	BDL	BDL	BDL	BDL	BDL	11.90*	BDL	**15.58**	BDL	BDL	11.50*	**13.88**	BDL	BDL	11.20*	**14.35**

Chapter 2

Impacts of flue gas desulfurization (FGD) gypsum on water quality and the algal community in catfish aquaculture ponds

Abstract

Catfish aquaculture ponds are at high risk of experiencing excessive algal growth, especially cyanobacteria, that can lead to negative water quality issues due to their consistent input through regular feedings and internal cycling of nutrients. Conventional algaecides used in aquaculture, such as copper sulfate, may be effective in the short-term but can potentially lead to developed resistance in phytoplankton over time or harmful effects on non-target species. An alternative nutrient management strategy, which has traditionally been used in land-based agriculture, is via flue gas desulfurization (FGD) gypsum, a form of calcium sulfate that is created as a by-product of carbon-based energy sources. A six-month field experiment was conducted on active catfish aquaculture ponds at a farm in west Alabama to test the ability of FGD gypsum to manage excessive algal blooms throughout the growing season while also investigating its impacts on other water quality parameters. In stark contrast to previous results from FGD gypsum application in eutrophic waters, this study discovered that FGD gypsum-treated ponds experienced a large increase in soluble reactive phosphorus (SRP) that led to a spike in phytoplankton abundance, specifically cyanobacteria. Supplementary microcosm experiments aided in determining that FGD gypsum can pull legacy phosphorus out of the nutrient-rich, flocculant sediment found in aquaculture ponds. While these results were unexpected and ultimately undesired, we did discover that the use of FGD gypsum at the 500 mg/L concentration does not produce any trace metal contamination in the water column or in the fish tissue. Overall, this experiment discovered new results that FGD gypsum can produce

when used in hypereutrophic waters with nutrient-rich sediments and provides the foundation for future research directions to determine the specific mechanisms behind the interaction of FGD gypsum with the sediment.

Introduction

Excess nutrient loading from anthropogenic sources can lead to situations of freshwater eutrophication. This is especially true in aquaculture ponds that require consistent input of feed to support the cultured species as well as regular output of high-nutrient waste from these cultured species re-entering the same system (Tucker and Schrader, 2020; Zhong et al., 2011). Eutrophication caused by excessive nutrients can trigger increased growth of phytoplankton. While a healthy, productive system requires a certain amount of phytoplankton, in many situations excessive growth can cause reduced water clarity, low dissolved oxygen mediated through microbial decomposition, and a high potential for fish kills (Anderson et al., 2002). In other situations, excess nutrients can lead to the proliferation of cyanobacteria, commonly known as blue-green algae, that have the potential to release toxins causing further issues for fish health (Drobac et al., 2016; Tanvir et al., 2021; Xie et al., 2005).

The catfish aquaculture industry in the United States is located predominantly in the southeast, where the warm climate (in combination with the shallow, nutrient-rich pond systems) can lead to severe cyanobacterial blooms during the growing season and beyond (Belfiore et al., 2021). Thus far, only a few algaecides are approved for use in catfish aquaculture (including copper sulfate and diuron) and, while effective in the short-term, can promote induce long-term algal resistance and/or harm non-target organisms (Buley et al., 2021; García-Villada et al., 2004; Mischke et al., 2009). Alternative control methods that can regulate algal levels in the long-term with minimal side effects on other organisms are the ideal management strategy. One of these potential alternative algal control methods is the use of flue gas desulfurization (FGD) gypsum, which is a synthetic form of gypsum ($CaSO_4$) created as a by-product of carbon-based energy sources (typically coal combustion) (Chen and Dick, 2011; Chen et al., 2014). Limestone forced oxidation scrubbers remove sulfur dioxide from the flue gas stream of sulfur-heavy energy sources, which produces calcium sulfate as a final product (Chen and Dick, 2011). Various forms of gypsum have been traditionally used in land-based agricultural production, including the reclamation of alkali soils, reduction of phosphorus runoff into nearby waters, promotion of soil aggregation, and to act as a source of inexpensive and readily available calcium and sulfate (Chen et al., 2008; Chen and Dick, 2011; Chen et al., 2014; Torbert and Watts, 2014; Watts and Dick, 2014). A limited number of studies have also discovered the ability of gypsum to be used in aquatic settings as well, most often in eutrophic and hypereutrophic systems. In these environments, gypsum can release calcium cations and sulfate anions, resulting in numerous direct and indirect effects on various water quality parameters. These effects range in intensity and duration but may include an increase in calcium hardness, the adsorption or precipitation of phosphorus, the reduction of pH and algal biomass, the flocculation of clay particles, and the removal of off-flavor compounds (Salonen and Varjo, 2000; Seo and Boyd, 2001; Whangchai et al., 2016, Wu and Boyd, 1990).

In addition to calcium and sulfate, synthetic FGD gypsum also contains varying levels of trace metals. The formation of FGD gypsum via the multi-step oxidation process aims to remove undesirable contaminants, but this process is not absolute. While the purity of FGD gypsum is higher than mined gypsum, approximately 99.6% compared to 87.1% respectively, heavy metal contamination is still a potential risk (Dontsova et al., 2005). In March 2023, the U.S. Environmental Protection Agency released their official contamination risk report entitled "Beneficial Use Evaluation: Flue Gas Desulfurization Gypsum as an Agricultural Amendment". While the report concluded that FGD gypsum is safe in regard to potential heavy metal contamination, its potential use in aquaculture ponds was not examined (U.S. EPA, 2023). Our prior pond mesocosm study investigating FGD gypsum ranges between 500 mg/L and 2,000 mg/L only found limited trace metal contamination in the water column at the highest concentration treatment (Zinnert et al., In preparation). However, the power plant sources of the FGD gypsum may manufacture products of varying purity that could have a range of potential contamination risk.

Although previous studies have investigated the use of gypsum in eutrophic and hypereutrophic waters for various reasons, none have taken place in active catfish aquaculture ponds. Thus, the purpose of this six-month field experiment was to determine the water quality impacts of synthetic FGD gypsum use in hypereutrophic catfish aquaculture ponds, especially focusing on its potential ability to mitigate nuisance cyanobacterial blooms. By conducting this experiment from May through November 2022, we were able to track changes in the ponds over the course of the entire growing season to understand both the effectiveness and duration of the impacts of FGD gypsum. Additionally, we also aimed to understand whether the use of FGD gypsum in food-fish production ponds carried any potential risk regarding water column or fish tissue trace metal contamination.

Materials and methods

Experimental design

To test the effects of FGD gypsum in hypereutrophic aquaculture ponds, a six-month whole pond experiment was conducted from May to November in 2022. In late spring 2022, eleven aquaculture ponds from a catfish farm in west Alabama were selected for the experiment based on water quality parameters collected in mid-May to ensure a normal distribution of quantifiable characteristics. Six ponds were selected for the control and five ponds were selected for the gypsum treatment, which received 500 mg/L of FGD gypsum in each pond. The treatment group originally had six ponds to equal the control group, but one pond had to be removed from the experiment due to necessary renovations by the farm owner. Throughout the duration of the six-month experiment, there was no intentional water exchange between ponds, but they were actively treated with regulated chemical treatments at the discretion of the farm owner as the ponds were always in production. Ponds were sampled once in mid-June before FGD gypsum addition, and FGD gypsum was added to treatment ponds by the farm owner on 14 June 2022. The FGD gypsum material was pushed into the ponds from one corner and allowed to naturally disperse via wind, turbulence, and active pond aerators. After FGD gypsum addition, ponds were sampled once per week through June, every other week through August, and then every six weeks through November. To collect water samples, a rigid tube sampler was used from the shore to collect 1 L of pond water from four sides of the pond, which were combined in a bucket and stored in a plastic cubitainer for transfer back to the lab for further analyses.

Laboratory analyses

Hardness, alkalinity, algal pigments, nutrients, and trace metals were measured following each sampling date using the water samples collected from the ponds. Hardness was measured as total hardness in mg/L of $CaCO_3$ using the Hach 5B Hardness Test Kit. Alkalinity was measured as total alkalinity in mg/L of $CaCO_3$ using the Gran titration method (Wetzel and Likens 1991).

Algal pigments, including chlorophyll-*a* and phycocyanin, were quantified and used as indicators of algal abundance. Chlorophyll-*a* concentration is used as a proxy for total phytoplankton abundance and phycocyanin as a proxy for the portion of phytoplankton that is specifically cyanobacteria. For both pigment analyses, samples were filtered through a Pall A/E glass fiber filter to collect algal cells and stored in black plastic containers at -10 ºC until pigments were extracted. For chlorophyll-*a* extraction, filters were submerged in 90% aqueous ethanol and stored for 23 hours in the dark at 4 ºC before allowing the extracts to warm to room temperature (~23 ºC) for one hour (Sartory and Grobbelaar, 1984). For phycocyanin extraction, filters were submerged and ground in 20 mL of 50 mM phosphate buffer with a Teflon pestle and stored for 4 hours at room temperature before centrifuging and filtering extract with 0.2 µm inline filter (Kasinak et al. 2015). The extracted pigments were then quantified using the appropriate module in a Turner Designs Trilogy benchtop fluorometer. Green algal chlorophyll was differentiated from total phytoplankton and cyanobacterial chlorophyll using a separate benchtop bbe PhycoProbe (bbe Moldaenke). This fluorometry-based tool can separate algal classes beyond the phytoplankton vs. cyanobacteria determination that is possible with pigment extraction.

Nutrients, including total nitrogen (TN), total phosphorus (TP), and soluble reactive phosphorus (SRP) were measured following each sampling date. Total nitrogen and TP were quantified from whole water samples and measured spectrophotometrically after persulfate digestion (Gross and Boyd, 1998). Soluble reactive phosphorus was quantified by first filtering samples through a Pall A/E glass fiber filter and then measuring colorimetrically with a modified single solution method according to Murphy and Riley (1962). All analyses measured final concentrations using a PerkinElmer Lambda 365 UV/VIS spectrophotometer.

Trace metals in the water column were measured before FGD gypsum addition and on weeks 1, 2, 3, and 21 following FGD gypsum addition. Water samples were collected with the integrated tube sampler and transferred to a sterile bottle and preserved with nitric acid (HNO_3) before analysis for arsenic, cadmium, copper, lead, and selenium using inductively coupled plasma-atomic emission spectrometry (ICP-AES) or mercury using cold vapor atomic absorption (CVAA) spectrometer (Campisano et al., 2017).

Trace metals in fish tissue were measured once approximately 9 months after FGD gypsum addition. Fish were harvested by the farmer from both the control and treatment ponds used in the experiment. Tissue samples from the harvested fish were analyzed using inductively coupled plasma-mass spectrometry (ICP-MS) following pressure digestion for arsenic, mercury, lead, and cadmium (Julshamn et al., 2007) or following microwave digestion with nitric acid and hydrogen peroxide for selenium (Pacquette and Thompson, 2015).

Supplementary sediment experiments

After the completion of the six-month whole pond experiment, two subsequent lab-based microcosm experiments were conducted to further investigate FGD gypsum's potential ability to pull SRP out of the pond sediments. For both experiments, sediment was collected from an active catfish pond located at the same farm used in the field experiment that did not previously receive FGD gypsum. The sediment was collected with a stainless-steel dredge sampler and stored in capped buckets.

The first experiment aimed to test the ability of FGD gypsum to extract SRP out of the pond sediments. Two-gallon plastic buckets were filled with pond sediment to a depth of 5 centimeters and filled with 5 L of hypereutrophic pond water that had been filtered through a 0.35 µm filter to remove most phytoplankton and other particulates. The FGD gypsum treatments included 0 (control), 250, 500, 1,000, and 2,000 mg/L with three replicates of each. The FGD gypsum was mixed into each bucket and stirred for 10 seconds with as minimal disruption to the sediment as possible. Water samples were collected and analyzed for SRP before FGD gypsum addition, 1 day after FGD gypsum addition, and then every 7 days until the end of the experiment on day 56. For the duration of the experiment, the buckets were under a 12-hour light/dark cycle at ~23 °C, mixed daily for 10 seconds, and positions rotated twice a week.

The second experiment specifically aimed to test if it is the sulfate ions produced during FGD gypsum dissolution that promote the release of SRP from the sediment. Two-gallon buckets were filled with the same pond sediment to a depth of 3 centimeters and filled with 4 L of hypereutrophic pond water that had been filtered through a 0.35 µm filter to remove most phytoplankton and other particulates. Potassium sulfate (K_2SO_4) was used as the sulfate source as it is a neutral salt that readily dissolves. The potassium sulfate treatments included 0 (control), 500, and 1,000 mg/L with three replicates of each. As previously stated, the potassium sulfate was mixed into each bucket and stirred for 10 seconds. Water samples were collected and analyzed for SRP before potassium sulfate addition, 1 day after potassium sulfate addition, and then every 7 days until the end of the experiment on day 56. The buckets followed the same light cycle, temperature, mixing protocol, and rotation schedule as the previous experiment.

Statistical analyses

Statistical analyses were performed in R version 4.1.3. A repeated-measures analysis of variance (RM-ANOVA) using a restricted maximum likelihood (REML) method to estimate variance was used in the *nlme* package to evaluate differences in the response variables among treatments over time (Pinheiro et al., 2020). The response variable data were first transformed using either a log or square root transformation; whichever method brought the data to a normal distribution. If there were any significant differences ($p<0.05$) observed in the model (either treatment, time, or the treatment-time interaction), a *post hoc* test in the *emmeans* package was used to conduct pair-wise multiple comparisons with a Tukey correction (Lenth et al., 2020). Depending on the response variable and the overall trend of the data, either the differences between the treatment and control at specific timepoints within the experiment or the average difference between the treatment and control across the entire experiment were compared.

Results

Hardness and alkalinity

As expected, across the entire six-month whole pond experiment there was a significant increase in total hardness for ponds treated with FGD gypsum when compared to the control (RM-ANOVA, $F_{1,9}$= 22.69, p=0.001, Figure 1A). Before FGD gypsum addition, all ponds began with an average hardness range of 111-120 mg/L $CaCO_3$. After FGD gypsum addition, treated ponds saw an approximately 105% increase in hardness by the following week, while control ponds remained stable. Although hardness values for both the treated and control ponds did vary depending on the sampling date, the hardness of the treated ponds remained significantly higher than the control ponds through the entire experiment (p=0.0011).

Although there was no treatment effect on alkalinity across the entire experiment (RM-ANOVA, $F_{1,9}$= 1.479, p=0.2598, Figure 1B), there was a significant treatment-time interaction (RM-ANOVA, $F_{9,82}$= 11.25, p<0.0001), indicating significant treatment effects at different time points within the experiment. All ponds began with an average alkalinity range of 119-125 mg/L $CaCO_3$. After FGD gypsum addition, treated ponds quickly decreased in alkalinity by approximately 30%. While these results were significant when compared to the control, the decreased alkalinity in the treated ponds was significantly lower for only three consecutive sampling dates following FGD gypsum addition (four weeks in total) ($p \leq 0.0392$). Six weeks after FGD gypsum addition, the treated ponds were no longer significantly different from the control ponds.

Total nitrogen (TN), total phosphorus (TP), and soluble reactive phosphorus (SRP)

FGD gypsum had no significant effects on total nitrogen (TN) across the entire experiment (RM-ANOVA, $F_{1,9}= 0.02$, $p=0.8995$, Figure 2A). Although there was a significant treatment-time interaction (RM-ANOVA, $F_{9,82}= 2.60$, $p=0.0108$) and there appeared to be an increase in the treated ponds during the August sampling dates, no time points were significant. Overall, TN did significantly increase from the beginning of the experiment in May with approximately 4 mg/L and leveling out in November with approximately 6 mg/L (RM-ANOVA, $F_{9,82}=32.90$, $p<0.0001$).

While FGD gypsum did not have an overall treatment effect on total phosphorus (TP) (RM-ANOVA, $F_{1,9}= 3.28$, $p=0.1034$, Figure 2B), there was a significant treatment-time interaction (RM-ANOVA, $F_{9,82}= 5.41$, $p<0.0001$) in which the treated ponds were significantly higher than the control ponds throughout the middle of the experiment (weeks 8 to 12 after FGD gypsum addition). All ponds began with an average TP of approximately 0.8 mg/L. In the two weeks following FGD gypsum addition, all ponds (treated and control) trended downwards. Four weeks after FGD gypsum addition, the treated ponds began to increase in TP while control ponds remained relatively stable. By week 8, the treated ponds had roughly double the amount of TP as the control ponds ($p=0.0057$) and remained significantly higher through week 12 ($p \leq 0.0203$). By the end of the experiment, treated ponds had decreased back to approximately 1 mg/L, which was still marginally higher than the control ($p=0.0501$).

Soluble reactive phosphorous (SRP), the dissolved biologically available form of phosphorus, was determined to have more complicated results than TP. FGD gypsum did not produce an overall treatment effect on SRP (RM-ANOVA, $F_{1,9}= 0.42$ $p=0.5347$, Figure 3). However, there was a significant treatment-time interaction (RM-ANOVA, $F_{9,82}= 3.83$, $p=0.004$), in which the impacts of FGD gypsum can be differentiated visually at separate points within the experiment. All ponds began with an average SRP range of 0.14-0.15 mg/L. One week after FGD gypsum addition, the treated ponds decreased to 29% of the starting concentration. However, these results were not statistically significant in part due to the variation between individual ponds. After this initial dip in SRP, concentrations increased and stabilized until week 6, when SRP in the treated ponds began to increase. From week 6 through week 12, SRP in the treated ponds continued to linearly increase, reaching a maximum at week 12 with concentrations 4.7x the amount found in the control. However, only marginal statistical significance was found for these time points within the experiment ($p=0.0607$). The FGD gypsum treatment did have one outlier pond, which consistently had low, stable SRP concentrations that were anywhere from 2% to 11% of the concentrations found in the other treated ponds (Figure 4). When conducting statistical analyses with the outlier pond removed, significant differences between the treatment and control were found for week 6 ($p=0.0486$) through week 12 ($p=0.0078$). By the end of the experiment in November, the elevated SRP concentrations in the treatment had decreased back down to similar concentrations as the control.

Phytoplankton dynamics

Chlorophyll-*a* concentration was utilized as a proxy for total phytoplankton abundance. FGD gypsum did not have a treatment effect on chlorophyll-*a* over the course of the entire experiment (RM-ANOVA, $F_{1,9}$=0.46, p=0.5155, Figure 5A), nor did it have a significant treatment-time interaction (RM-ANOVA, $F_{9,82}$=0.56, p=0.8255). Chlorophyll-*a* concentration did change over time (RM-ANOVA, $F_{9,82}$=7.05, p<0.0001) but never exceeding the 250-500 µg/L concentration range measured throughout the entire experiment.

Phycocyanin concentration was utilized as a proxy for cyanobacterial abundance. FGD gypsum did not have an overall treatment effect across the entire experiment (RM-ANOVA, $F_{1,9}$= 1.51, p=0.2498, Figure 5B), but it did have a significant treatment-time interaction (RM-ANOVA, $F_{9,82}$= 4.17, p=0.0002). All ponds began with an average concentration range of 389-616 µg/L. By week 4 after FGD gypsum addition, the treated ponds had significantly increased to phycocyanin concentrations that were roughly 3x the amount of the control ponds (an average of 1,125 µg/L vs. 365 µg/L, respectively) (p=0.0453). These significantly elevated phycocyanin concentrations for the treated ponds remained through week 8 (p≤0.0170) until dropping back down by week 10. By the end of the experiment in November, both the control and treatment had stabilized at average concentrations of 143 to 190 µg/L.

FGD gypsum did not have an overall treatment effect on green algal abundance (RM-ANOVA, $F_{1,9}$= 1.40, p=0.2667, Figure 5C), but did have a marginally significant treatment-time interaction (RM-ANOVA, $F_{9,82}$=1.83, p=0.0742). Although we did not find strong statistical significance, we still found marginal increases in green algae in the control when compared to the treatment in the weeks following FGD gypsum addition. All ponds began with an average green algae concentration range of 180-183 µg/L. From week 4 through week 8, the treated ponds had slightly decreased and stabilized, while the control ponds increased in concentration by approximately 47-70% depending on the sampling date. Although our results were just below passing the 95% confidence level threshold for statistical significance, the green algae levels and their fluctuations still provide relevant and practical information.

Trace metals in water column

Copper was the only trace metal measured in all pond replicates above the detection limit in both the control and treatment with ranges of approximately 8-20 μg/L in the control and 7-14 μg/L in the treatment (Table 1). However, the measured levels in the FGD gypsum-treated ponds never surpassed the levels measured in the control ponds at each timepoint. Although mercury was detected in three of the individual ponds before FGD gypsum addition, it was not detected in all the replicates within the control or treatment and those detected values were near the detection limit. Arsenic, cadmium, lead, and selenium concentrations were all below detection limit for both the control and treatment at all timepoints before and after FGD gypsum.

Trace metals in fish tissue

Arsenic and selenium were the only trace metals detected in the fish tissues collected from the fish in the experimental pond (Table 2). However, the concentrations found from fish in the treated ponds were not significantly higher than those found in the control ponds. The average arsenic concentration in the treated ponds was 0.033 mg/kg, which was not different from the control pond's average concentration of 0.031 mg/kg ($p=0.41$). The average selenium concentration in the treated ponds was 0.128 mg/kg, which was not different from the control pond's average concentration of 0.121 mg/kg ($p=0.26$). Cadmium, lead, and mercury were below detection limit for all tissue samples in both the control and treatment.

Supplementary sediment experiment

For the first experiment in which FGD gypsum was added to the microcosm buckets, there was a slight increase in SRP in the lowest concentration treatment (250 mg/L) by day 21 when compared to the control that continued until the end of the 56-day experiment (Figure 6A). Although all other treatments remained lower and hovered near the control for most of the experiment, by day 49 all FGD gypsum treatments saw a quick increase in SRP and remained at elevated levels for the last two weeks of the experiment. While only one treatment (1,000 mg/L) showed marginal statistical significance ($p=0.0892$) by the end of the experiment, the visual difference in SRP concentrations seen between the treatments and the control provide practical information for comparison with the second experiment.

For the second experiment in which potassium sulfate was added to the microcosm buckets, SRP levels remained low in the treatments and the control until after day 21 (Figure 6B). From day 21 onward, SRP levels began to increase in all microcosm buckets with the control seeing the largest increase by day 35. These levels lowered by the last two weeks of the experiment while the treatments begin to increase. However, the differences between the treatments and control were minute when compared to the final differences between the treatments and the control in the first experiment. While this experiment did not produce any statistically significant results at any time point ($p \geq 0.1548$), the comparison with the results of the more interesting FGD gypsum sediment experiment provides practical information for future directions.

Discussion

This experiment investigated the use of FGD gypsum in aquaculture ponds as a way to control algal abundance during the productive and hypereutrophic growing season. Based on a previously tested range of FGD gypsum concentrations, the optimal dose was selected and utilized to assess its impact on the algal community and various water quality parameters in a real-world farm pond setting as opposed to a controlled microcosm or mesocosm environment (Zinnert et al., In preparation). In addition, since FGD gypsum is a by-product of fossil fuel energy sources and contains trace amounts of heavy metals, this study also determined whether FGD gypsum in aquaculture ponds increases concentrations of those trace metals in the water column or can potentially bioaccumulate in the tissue of fish reared in those ponds.

Overall, this experiment produced both expected results for certain water quality parameters based on the previous literature testing gypsum as well as highly unexpected results for others. The increase in hardness and decrease in alkalinity following FGD gypsum addition was as expected (Zinnert et al., In preparation). The dissolution of FGD gypsum adds free calcium ions to the water column, which directly increases hardness concentrations (Whangchai et al., 2016; Wu and Boyd, 1990; Wurts and Durborow, 1992). Calcium is essential for fish health and biological processes, such as bone and scale formation, successful development and hatching of eggs, and retention of salts for nerve, heart, and muscle function (Boyd et al., 2016; Chatakondi and Torrans, 2012; Hossain and Yoshimatsu, 2014; Wurts and Durborow, 1992). Thus, FGD gypsum can be used as a soluble source of calcium to increase hardness beyond levels that may be achieved with agricultural limestone (Wu and Boyd, 1990). The observed temporary decrease in alkalinity is most likely due to the precipitation of calcium carbonate following the increase in calcium ions from FGD gypsum dissolution (Mandal and Boyd, 1980). Although it is a significant decrease when compared to the control ponds, the resulting alkalinities in the treated ponds (~80 mg/L $CaCO_3$) are still considered to be a moderate target level to support food-fish ponds (Boyd and Tucker, 2012; Boyd et al., 2016).

Based on previous studies using some form of gypsum in eutrophic or hypereutrophic waterbodies, we expected to see a reduction of phosphorus in the water column (Seo and Boyd, 2001; Whangchai et al., 2016; Wu and Boyd, 1990). While we did see a brief and marginal reduction in phosphorus one week after FGD gypsum addition in the treated ponds, the effects quickly diminished and both TP and SRP concentrations consistently increased in the treated ponds between weeks 4 and 12. This delayed and dramatic increase in phosphorus concentrations in the treated ponds was unexpected, especially given that the FGD gypsum product does not contain any quantifiable levels of phosphorus that may contribute to phosphorus concentrations in the water column during immediate or gradual dissolution (Chen and Dick, 2011). This is supported by our previous mesocosm study in which FGD gypsum treatments ranging from 500 to 2,000 mg/L never increased TP or SRP concentrations in the water column (Zinnert et al., In preparation). However, our previous mesocosm study only utilized pond water and did not contain any sediment. Thus, the significant increase in phosphorus discovered in this experiment is most likely due to the release of legacy phosphorus in the pond sediment (i.e., internal loading). As catfish aquaculture ponds are highly productive and constantly receiving inputs of phosphorus from fertilization, feeding, phytoplankton decomposition, and fish excretion, their sediments can adsorb and store high levels of phosphorus (Boyd, 1985; Hargreaves and Tucker, 2003).

Based on the results of the supplemental sediment experiment, it is evident that FGD gypsum can release dissolved phosphorus out of the sediment and into the water column. However, the exact mechanism creating this upward diffusion of phosphorus remains elusive, especially when comparing to previous results from the literature. Studies conducted by Salonen and Varjo (2000) and Varjo et al. (2003) found that gypsum could be used to prevent phosphorus release from sediments in eutrophic lakes. While this is the opposite result from what we discovered, these studies differed in that they 1) were conducted in natural lake systems (albeit still moderately shallow) as opposed to hypereutrophic fish production ponds, 2) utilized an iron-gypsum compound, or 3) specifically applied the gypsum in a way to create a 1 cm thick layer on the sediment surface. Furthermore, our hypothesis that the increased sulfate from FGD gypsum dissolution could instead be the cause of the phosphorus release was also proven inconclusive based on the results of the supplemental sediment experiment. Previous studies have documented that high sulfate levels in surface waters can lead to an increase in phosphorus release from aquatic sediments due to enhanced mineralization of organic matter or through the reduction of available iron that can make insoluble phosphorus compounds (Caraco et al., 1993; Jin et al., 2013). However, our potassium sulfate supplemental experiment did not produce the same treatment spike in SRP as the FGD gypsum supplemental experiment. Thus, the release of phosphorus must be due to either the increase in calcium or the interactions of any undissolved gypsum with the sediment, yet the exact mechanism remains unclear.

Although the original goal of this study was to see how effective FGD gypsum was at controlling harmful algal blooms, we discovered the opposite to be true. The large spike in cyanobacteria in the treated ponds can be seen occurring roughly around the same time as the SRP increase. As dissolved forms of phosphorus (i.e., SRP) can be directly used by algae for proliferation (Bormans et al., 2016), elevated SRP levels can support greater algal biomass. Thus, the large increase in SRP that is released from the sediments in the treated ponds is causing this unexpected yet significant elevation in cyanobacterial abundance. The high cyanobacterial abundance in the treated ponds may also explain why the green algal levels only increase in the control ponds during the growing season. In those FGD gypsum-treated, cyanobacteriadominated ponds, the green algae still have plenty of SRP available to use but may be outcompeted for other necessary resources, such as light, by bloom-forming cyanobacteria (Huisman et al., 1999; Roeselers et al., 2007).

Fortunately, the selected dose of FGD gypsum (500 mg/L) did not contribute to any increase in heavy metals in the water column nor any significant increases in fish tissue. While the only detected metal throughout the experiment was copper, the concentrations in the treated ponds never surpassed those of the control ponds (even in the early weeks following FGD gypsum addition). Copper is typically found in aquaculture ponds due to the use of copper sulfate as an algaecide (Jacob et al., 2016; McNevin and Boyd, 2004), so these background concentrations without any treatment effect are unsurprising. Although arsenic and selenium, the two metals detected in the fish tissue, can be of concern for human consumption of seafood (Avigliano et al., 2019), the FGD gypsum treated ponds did not see a significant increase in the concentrations of these metals when compared to the control. Overall, FGD gypsum does not contribute to trace metal contamination in the water column or fish tissue and should be considered safe for use in food-fish production ponds.

Conclusions

Due to the constant input of nutrients in catfish aquaculture ponds, algal growth can occur beyond a balanced level and create numerous issues related to water quality and fish health. Finding alternatives to conventional algaecides could provide new management tools that may be successful in the long-term and do not have negative impacts on non-target species. Although limited in the current literature, previous use of FGD gypsum in eutrophic freshwater systems had been promising in its ability to reduce soluble phosphorus and control algal growth.

However, this experiment aimed to test its effectiveness to do the same in hypereutrophic catfish ponds over the course of the entire growing season and discovered the opposite results. In these ponds with phosphorus-rich, flocculant sediment, the FGD gypsum caused a release of soluble phosphorus from the sediment and subsequently creating a spike in cyanobacterial growth. While the exact mechanisms as to why this phosphorus release is occurring are still unknown, it is clear that FGD gypsum acts differently in food-fish ponds from how it does in more natural systems. As FGD gypsum has been shown to not cause any trace metal contamination to the water column or fish tissue and thus provides a potentially safe alternative to other chemicals, further studies are needed to elucidate the exact interaction of FGD gypsum with the nutrient-rich sediment. These finer details could provide more insight as to whether there are alternative application methods, doses, or environmental requirements that would eventually allow for the beneficial use of this product in the catfish aquaculture industry.

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Figure Legends

Figure 1. Average total hardness (A) and alkalinity (B) for the treatment and control (± 1 SE) over time. The checkered arrow indicates when FGD gypsum was added to the treatment. An asterisk (*) above a sampling date indicates statistical significance ($p<0.05$) at that specific timepoint if there was not an overall treatment effect. Hardness increased in the FGD gypsum treatment and remained elevated while alkalinity decreased in the FGD gypsum treatment for approximately 4 weeks before returning to levels similar to the control.

Figure 2. Average total nitrogen (A) and total phosphorus (B) for the treatment and control (± 1 SE) over time. The checkered arrow indicates when FGD gypsum was added to the treatment. An asterisk (*) above a sampling date indicates statistical significance ($p<0.05$) at that specific timepoint. Total nitrogen increased throughout the growing season, but the treatment never significantly differed from the control. Total phosphorus slightly decreased following FGD gypsum addition but subsequently increased in the treatment and was significantly higher than the control between weeks 8 and 12 after being treated.

Figure 3. Average SRP for the treatment and control (± 1 SE) over time. The checkered arrow indicates when FGD gypsum was added to the treatment. A pound symbol (#) above a sampling date indicates statistical significance ($p<0.05$) at that specific timepoint after removing the outlier pond. The inset graph shows more detail of the date FGD gypsum was added and the two weeks following. Soluble reactive phosphorus slightly decreases in the treatment but then spikes and becomes significantly higher than the control between weeks 6 and 12 after being treated.

Figure 4. Soluble reactive phosphorus concentrations for the five ponds each treated with 500 mg/L FGD gypsum over time. Pond 3 remains consistently low as an outlier throughout the entire experiment while the other four ponds follow similar trends.

Figure 5. Average chlorophyll-*a* (A), phycocyanin (B), and green algae concentrations (C) for the treatment and control (± 1 SE) over time. The checkered arrow indicates when FGD gypsum was added to the treatment. An asterisk (*) above a sampling date indicates statistical significance ($p<0.05$) at that specific timepoint. Chlorophyll-*a* fluctuated throughout the experiment, but the treatment never significantly differed from the control. Phycocyanin spiked in the treatment and remained significantly higher than the control between weeks 4 and 8 after being treated with FGD gypsum. Green algae never significantly differed between the treatment and control, but there was a marginal increase in the control between weeks 4 and 8.

Figure 6. Average SRP for the FGD gypsum supplemental sediment experiment (A) and the potassium sulfate supplemental sediment experiment (B) (± 1 SE) over time. Although there was not any statistical significance due to large variation, there is still an increase in SRP from the microcosms treated with FGD gypsum as opposed to those treated with potassium sulfate.

Figure 1

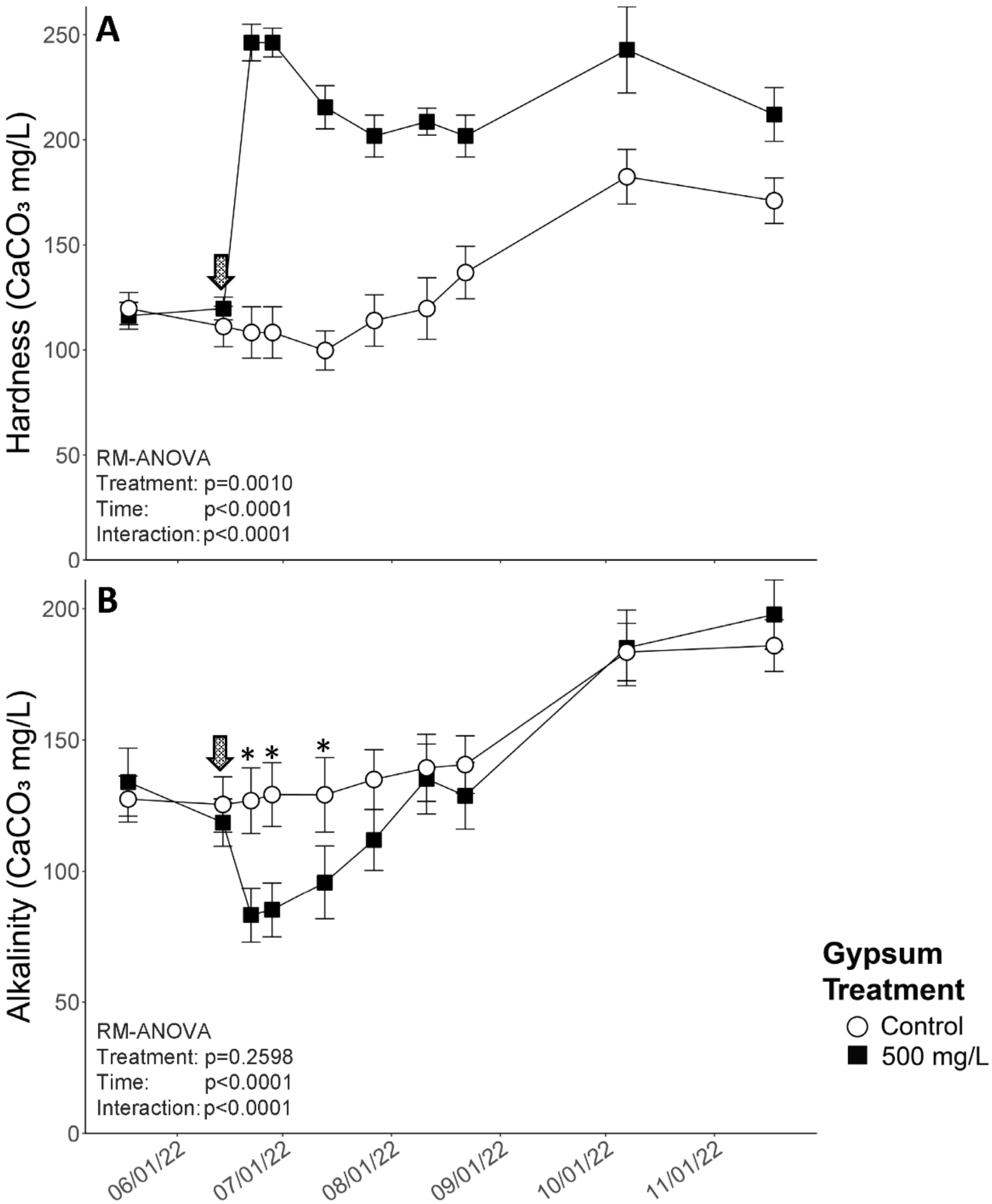

A
B
Hardness (CaCO₃ mg/L)
Alkalinity (CaCO₃ mg/L)
RM-ANOVA
Treatment: p=0.0010
Time: p<0.0001
Interaction: p<0.0001
RM-ANOVA
Treatment: p=0.2598
Time: p<0.0001
Interaction: p<0.0001
06/01/22
07/01/22
08/01/22
09/01/22
10/01/22
11/01/22
Gypsum Treatment
Control
500 mg/L

Figure 2

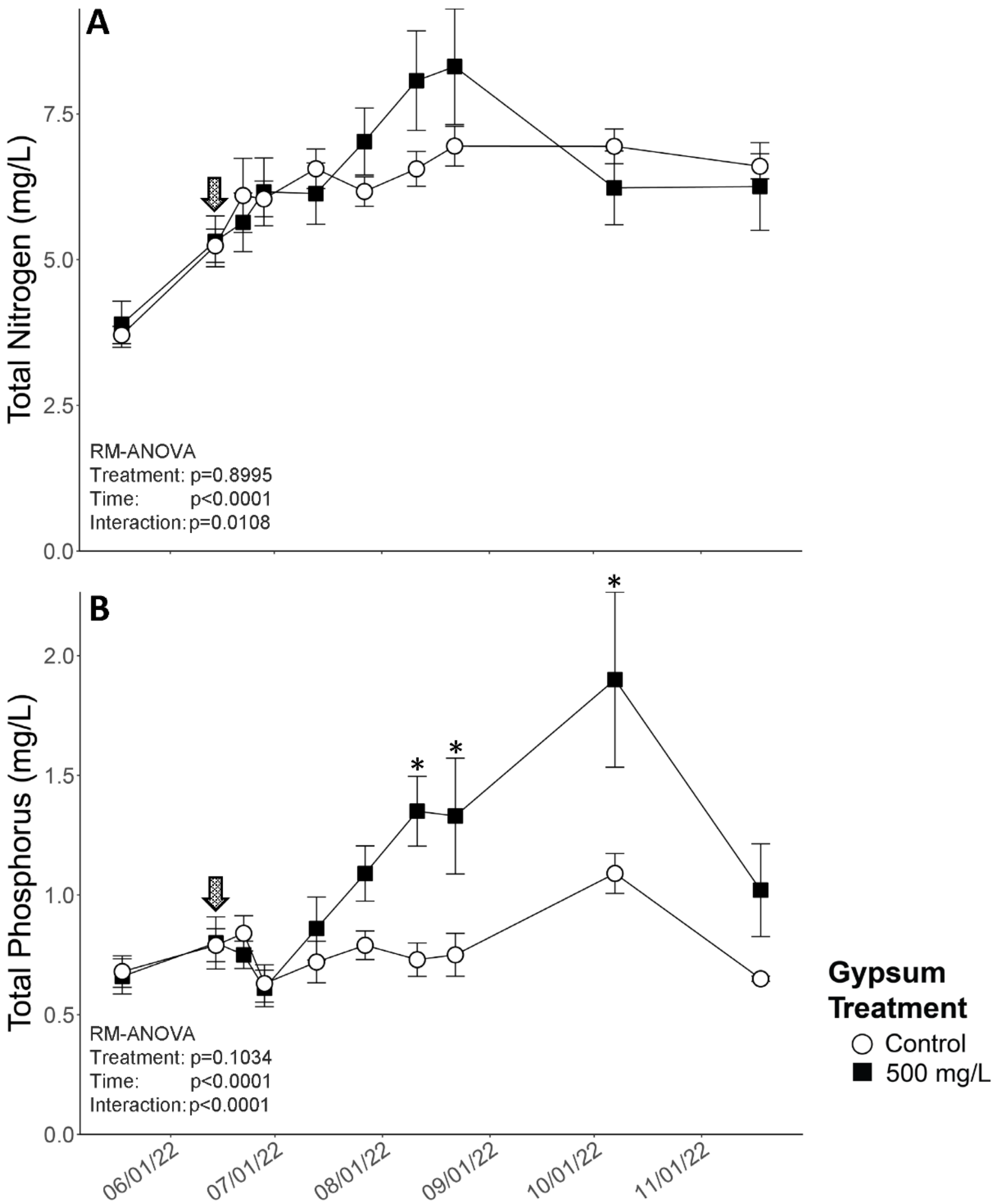

A
Total Nitrogen (mg/L)
7.5
5.0
2.5
0.0
RM-ANOVA
Treatment: p=0.8995
Time: p<0.0001
Interaction: p=0.0108
B
Total Phosphorus (mg/L)
2.0
1.5
1.0
0.5
0.0
*
*
*
RM-ANOVA
Treatment: p=0.1034
Time: p<0.0001
Interaction: p<0.0001
06/01/22
07/01/22
08/01/22
09/01/22
10/01/22
11/01/22
Gypsum Treatment
Control
500 mg/L

Figure 3

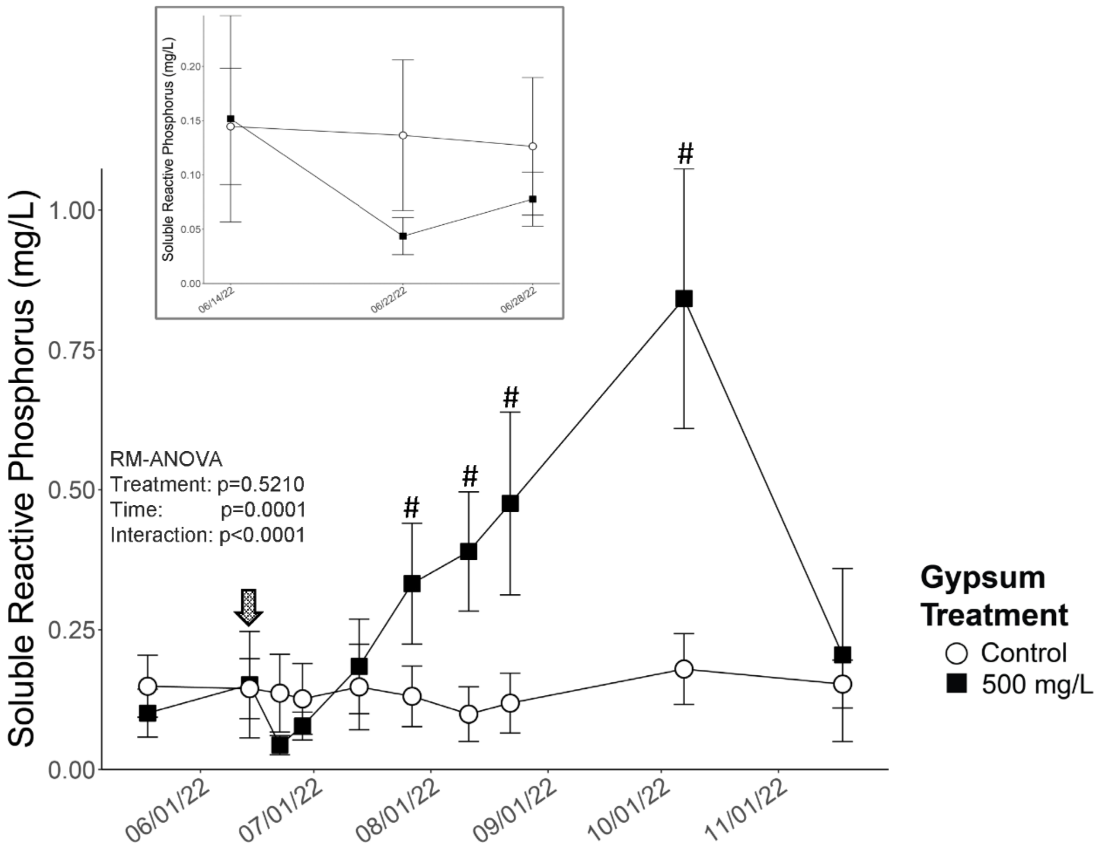

Soluble Reactive Phosphorus (mg/L)
0.00
0.25
0.50
0.75
1.00
06/01/22
07/01/22
08/01/22
09/01/22
10/01/22
11/01/22
RM-ANOVA
Treatment: p=0.5210
Time: p=0.0001
Interaction: p<0.0001
#
#
#
#
Gypsum Treatment
Control
500 mg/L

Figure 4

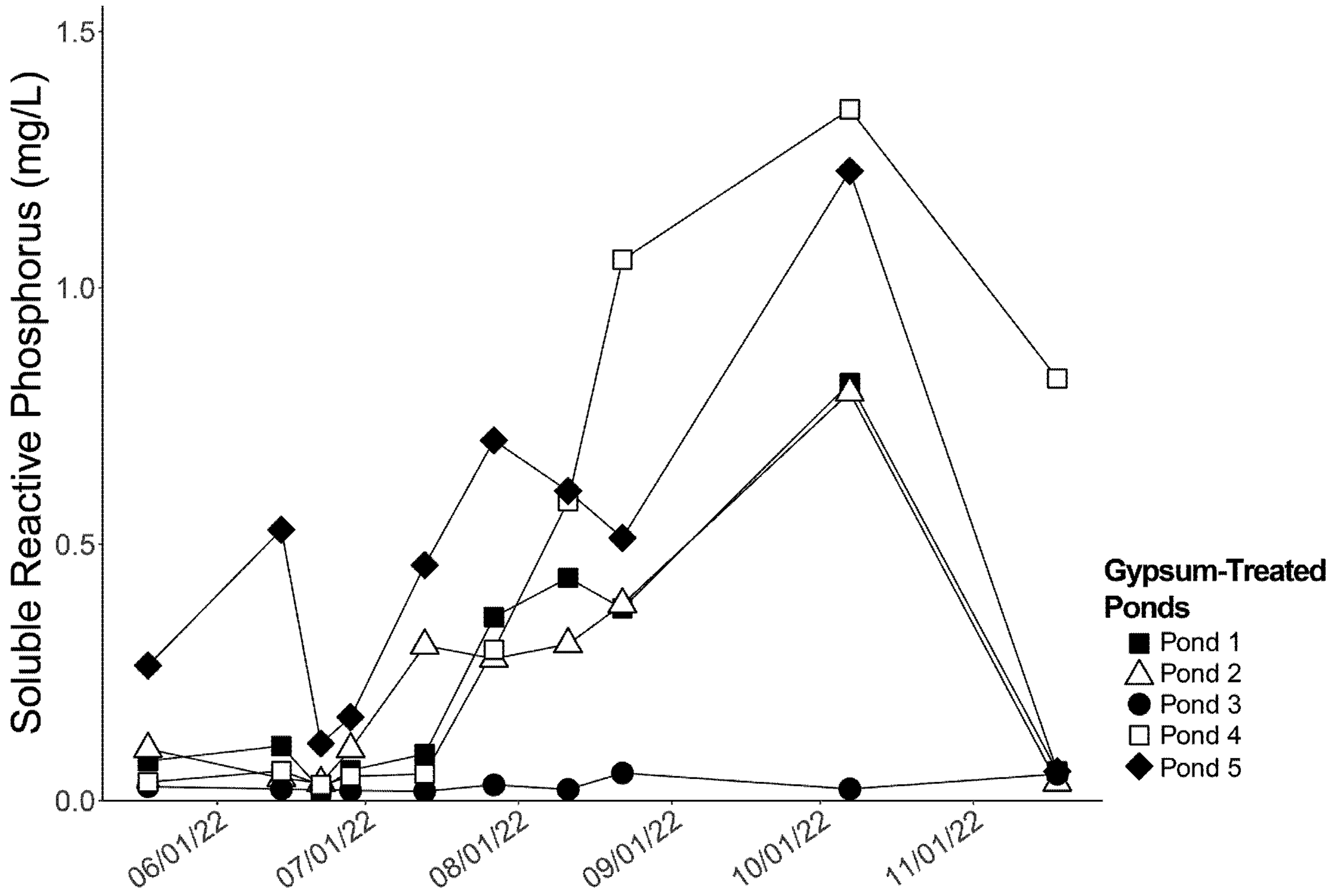
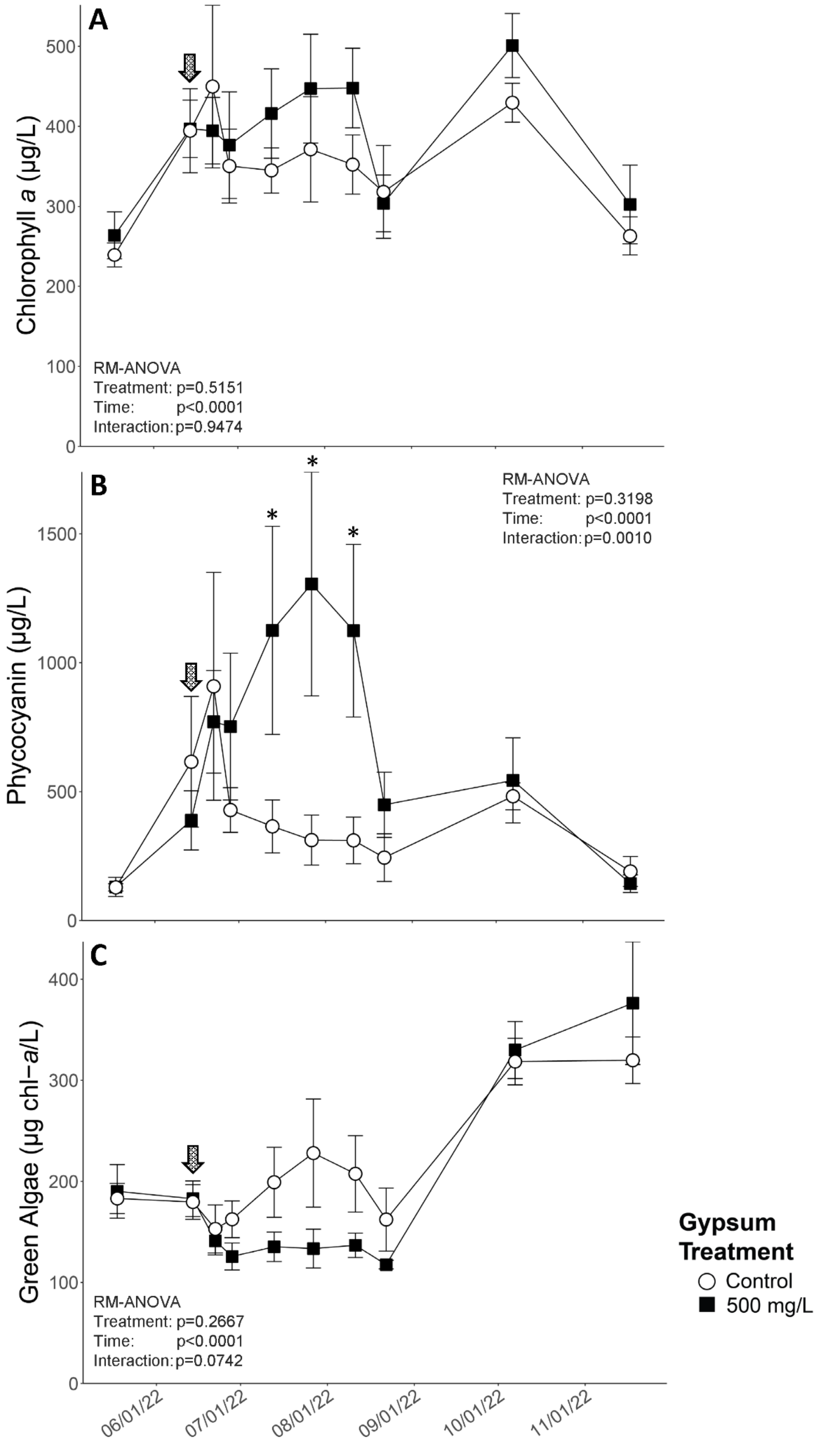
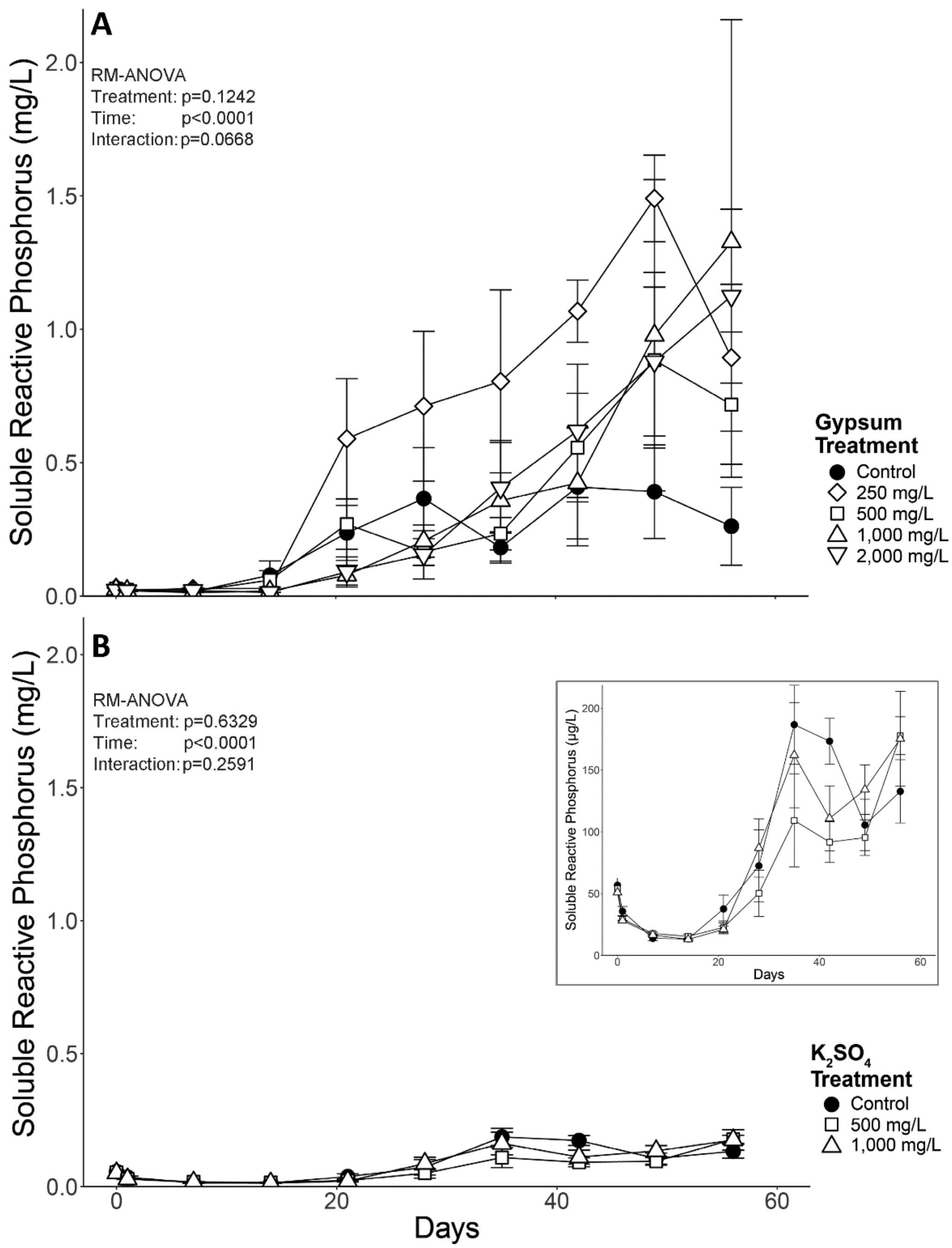

Soluble Reactive Phosphorus (mg/L)
1.5
1.0
0.5
0.0
06/01/22
07/01/22
08/01/22
09/01/22
10/01/22
11/01/22
Gypsum-Treated Ponds
Pond 1
Pond 2
Pond 3
Pond 4
Pond 5

Figure 5



A
Chlorophyll a (µg/L)
RM-ANOVA
Treatment: p=0.5151
Time: p<0.0001
Interaction: p=0.9474
B
Phycocyanin (µg/L)
RM-ANOVA
Treatment: p=0.3198
Time: p<0.0001
Interaction: p=0.0010
C
Green Algae (µg chl-a/L)
RM-ANOVA
Treatment: p=0.2667
Time: p<0.0001
Interaction: p=0.0742
06/01/22
07/01/22
08/01/22
09/01/22
10/01/22
11/01/22
Gypsum Treatment
Control
500 mg/L

Figure 6



A
RM-ANOVA
Treatment: p=0.1242
Time: p<0.0001
Interaction: p=0.0668
Soluble Reactive Phosphorus (mg/L)
Gypsum Treatment
Control
250 mg/L
500 mg/L
1,000 mg/L
2,000 mg/L
B
RM-ANOVA
Treatment: p=0.6329
Time: p<0.0001
Interaction: p=0.2591
Soluble Reactive Phosphorus (µg/L)
K2SO4 Treatment
Control
500 mg/L
1,000 mg/L
Days

Table Legends

Table 1. Average trace metal concentrations found in the water column for the treatment and control before FGD gypsum and weeks 1, 2, 3, and 21 after FGD gypsum addition. BDL = below detection limit. An asterisk (*) indicates that not all replicates in a treatment were above the detection limit. The only detectable trace metal found in all replicates was copper, but the treatment never had higher levels than the control.

Table 2. Average trace metal concentrations found in the tissue of fish reared in the treatment and control ponds. The only detectable trace metals were arsenic and selenium, but concentrations in the treatment were never significantly higher than the control.

Table 1

		Before gypsum		1 week after gypsum		2 weeks after gypsum		3 weeks after gypsum		21 weeks after gypsum	
Metal (µg/L)	Detection Limit (µg/L)	Control	500 mg/L	Control	500 mg/L	Control	500 mg/L	Control	500 mg/L	Control	500 mg/L
Arsenic	18.40	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Cadmium	3.80	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Copper	2.40	14.12*	11.95*	16.35	12.92	15.95	13.94	19.68	12.12	7.62	6.94
Lead	14.60	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Mercury	1.00	1.50*	1.50*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Selenium	11.10	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Table 2

Metal (mg/kg)	Control	500 mg/L
Arsenic	0.031	0.033
Cadmium	BDL	BDL
Lead	BDL	BDL
Mercury	BDL	BDL
Selenium	0.121	0.128