Lee County
Natural
Resources

Final Report

Water Quality and Treatment C-43 Mesocosm Using Combined Wetlands and Engineered Treatment Technologies

DEP Grant Agreement Number INV10

This Project and the preparation of this report has been funded wholly by the Florida Department of Environmental Protection (DEP) through Innovative Technologies Grant Agreement No. INV10. The total cost of the Project was $180,000.

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Executive Summary

On April 22, 2021 the State of Florida Department of Environmental Protection (DEP) and the Lee Board of County Commissioners (County) entered into DEP Grant Agreement Number INV10, a state-funded Innovative Technology grant.

The purpose of the Water Quality and Treatment C-43 Mesocosm Using Combined Wetlands and Engineered Treatment Technologies project was to test enhanced, engineered treatment of water from the freshwater portion of the Caloosahatchee River (C-43) to increase plant uptake of nutrients. The specific goal was to increase the rate of nitrogen removal above what has been found using solely plant communities.

The project was located on an existing South Florida Water Management District-owned study site in Glades County on a segment of the freshwater Caloosahatchee River (C-43).

The primary objectives of the project were:

1) To review existing research to gain understanding of plant nitrogen uptake and how that might be enhanced
2) To construct three “pretreatment” technology trains connected to tanks (mesocosms) containing wetland vegetation
3) To conduct comprehensive water quality monitoring that would help determine the effectiveness of each treatment train technology
4) To develop a report outlining work completed and key results

To accomplish these objectives, Lee County entered into a research agreement with Florida Gulf Coast University to complete the work. Comprehensive water quality monitoring for this project included collecting treatment train samples at 16 sampling points for 8 sampling events. Samples were analyzed for nutrients, field parameters, etc. Results from the study indicate improved reduction of nutrients, bacteria, and algae in all three tested treatment trains.

1.0 Introduction

1.1 Background

Lee County is bisected by the Caloosahatchee River Estuary (CRE) which is impacted by red tides (Karenia brevis), blue green algae (often Microcystis spp.), and macroalgal harmful algal blooms (HABs). The CRE terminates into the Gulf of Mexico, which also is subjected to red tides in this region that can be aggravated by anthropogenic inputs in the nearshore environment. The CRE watershed is designated as critical smalltooth sawfish (Pristis pectinata) habitat, has a transient Florida manatee (Trichechus manatus) population, supports commercial fisheries such as blue crab (Callinectes sapidus) and stone crab (Menippe mercenaria), is experiencing declining coverage of seagrass and oyster reefs, and is important for recreational anglers and fishing guides. Aside from these environmental concerns, there are economic issues. HABs that are widely reported on can negatively affect real estate values and tourism. Additionally, Lee County and associated municipalities have spent hundreds of thousands of dollars in efforts to mitigate HABs on technologies including the installation of bubble curtains, peroxide treatments, and physical removal of algal biomass. Finally, HABs can represent a significant health issue for humans, pets, and wildlife.

The FDEP first adopted the Caloosahatchee Estuary Basin Management Action Plan (BMAP) in November 2012 to implement a total nitrogen (TN) Total Maximum Daily Load (TMDL) in the Caloosahatchee River and Estuary downstream of the Franklin Lock and Dam (S-79). In November 2017, the DEP and local stakeholders completed the first 5-Year Review to evaluate implementation at the end of the first phase and make recommendations for future phases of the BMAP. The information gathered as part of the 5-Year Review was used to develop an updated BMAP for the Caloosahatchee River and Estuary Watershed. In addition, in January 2019, Executive Order 19-12 (Item C) included a requirement to update and secure all restoration plans, within one year, for waterbodies impacting south Florida communities, including the Caloosahatchee River and Estuary BMAP. The updated Caloosahatchee River and Estuary BMAP was adopted in January 2020. The BMAP requires Lee County and other stakeholders to identify pollutant sources and implement projects and activities to achieve allocated nutrient reductions within the watershed.

Accordingly, Lee County pursued a DEP Innovative Technologies Grant to further evaluate innovative technologies to combat algal blooms and nutrient enrichment.

The South Florida Water Management District (SFWMD) developed a wetland demonstration testing facility in Glades County to research how best to reduce nitrogen from Caloosahatchee River surface waters. This site, named the C-43 Water Quality Treatment and Testing Project, holds 12 tanks (mesocosms) containing wetland vegetation that were used to conduct a water quality assessment of nutrient removal from Caloosahatchee River water. The SFWMD gave permission to Lee County to use 6 of the 12 existing mesocosm tanks to conduct innovative technologies research.

Thanks to this opportunity, the Lee County Department of Natural Resources, in partnership with Florida Gulf Coast University (FGCU), undertook undertake additional research on water quality using the existing SFWMD mesocosms to test enhanced, engineered treatment of the water to increase the plant uptake of nutrients, specifically nitrogen.

1.2 Project Location

The Caloosahatchee River (C-43 Canal) extends from structure S-77 at Lake Okeechobee westward through two additional water control structures - S-78 and S-79 - where it transitions to the Caloosahatchee River Estuary (tidal Caloosahatchee). The property on which the project was located lies west of Moore Haven, on the south side of the C-43 Canal (the freshwater portion of the Caloosahatchee River), east of LaBelle and upstream of the S-78 water management structure, in Section 26, Township 42, Range 30, in Glades County, Florida (Figure 1). The project is located at Latitude (decimal degrees): 26.786426; Longitude (decimal degrees): -81.288696.

The Project is located on a portion of a 1,700 acre property - formerly owned and managed as agricultural land by the Boma family – that was purchased for the purpose of constructing a water quality project to benefit the Caloosahatchee watershed. The SFWMD purchased the property, named the *C-43 Water Quality Treatment and Testing Project*, in 2007 with a $10 million contribution toward its purchase from the Lee Board of County Commissioners.

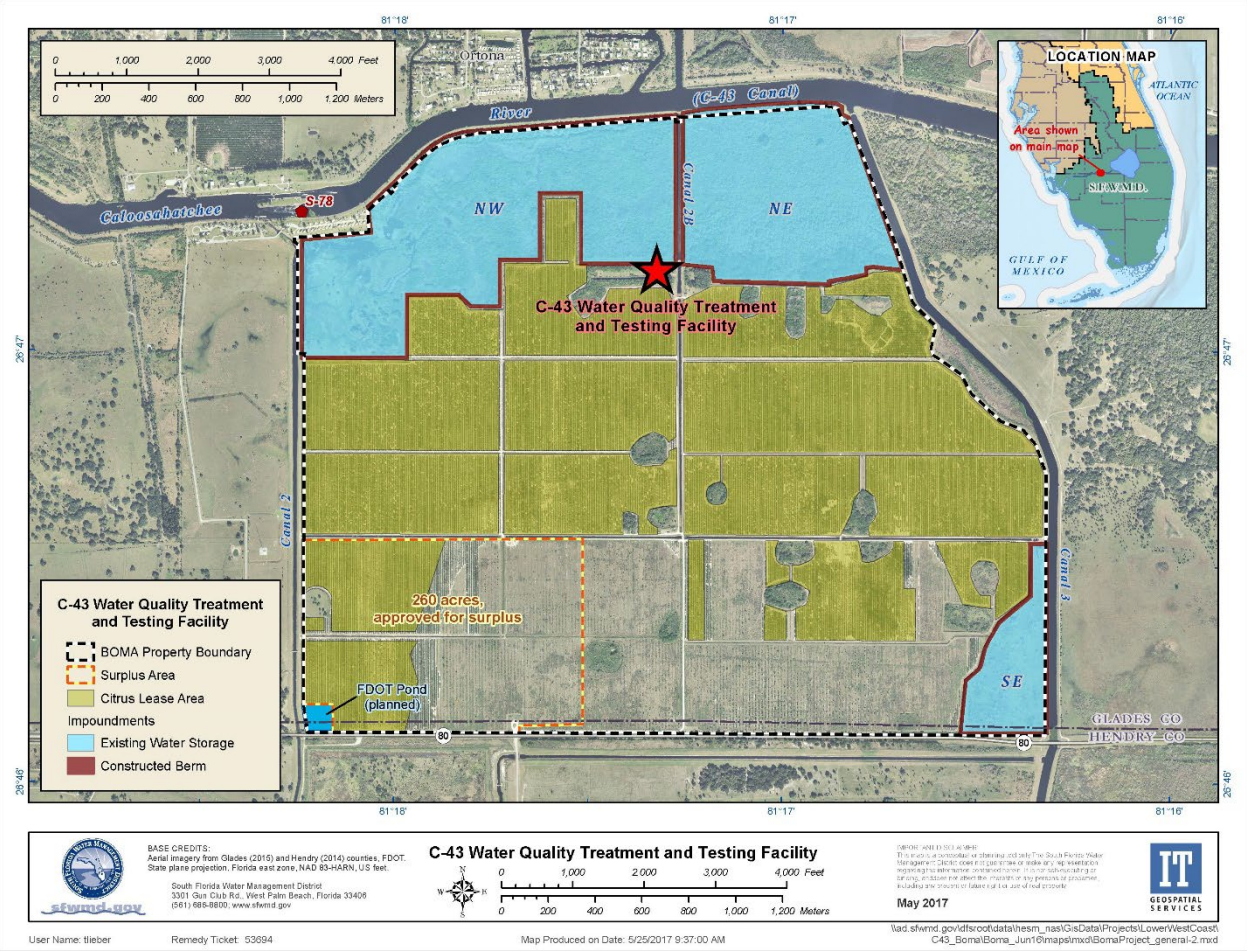

Figure 1. *Project location map*

1.3 Project Description

The Florida Gulf Coast University (FGCU) team conducted research using the mesocosm tanks to test engineered treatment methods that could improve the plant uptake of nitrogen. Because natural plant communities tend to more effectively remove reduced forms of nitrogen from the water compared to oxidized forms and organic nitrogen, it was the intent of the project to apply technology to the feed water from the Caloosahatchee River that would reduce the concentration of organic nitrogen and convert at least part of the nitrogen to ammonium. The specific goal was to increase the rate of nitrogen removal above what has been found using solely the plant communities. Engineering technologies that have the potential to be scaled up to treat very large quantities of surface water to reduce nitrogen loading and harmful algal blooms in the Caloosahatchee River and Estuary were applied.

Lee County and the FGCU team investigated the water treatment processes by running three concurrent experiments. Water treatment was conducted on the feed water on two of the three trains; one train was used as a control. Each train consisted of two tanks, one containing a submergent wetland community and the other containing an emergent wetland community, with or without pretreatment of the feed water (Figure 2). Entry of river water into the first train was preceded by slow sand filtration of the water. The second train water treatment scheme included UV treatment of the raw water prior to entry into the slow sand filter. The third train did not include any pretreatment of the feed water and was used as a control. All treatment train processes involved water flowing through first emergent, then submergent vegetation tubs as the final stage. Figure 3 below shows the complete site mesocosm tank layout; the project utilized 6 of the 12 tanks on site.

The three project treatment train configurations were (Figure 2):

1. The reference, or control, train process in which water flows from the river into Tub 8 (emergent) then through the Tub 1 (submergent).
2. A sand filter treatment train process, in which water flows from the river, through the sand filter, then on to Tub 10 (emergent), and Tub 7 (submergent).
3. A sand filter plus UV treatment train process, in which water flows from the river through the sand filter, then through the UV treatment, then on to Tub 9 (emergent), then Tub 2 (submergent)

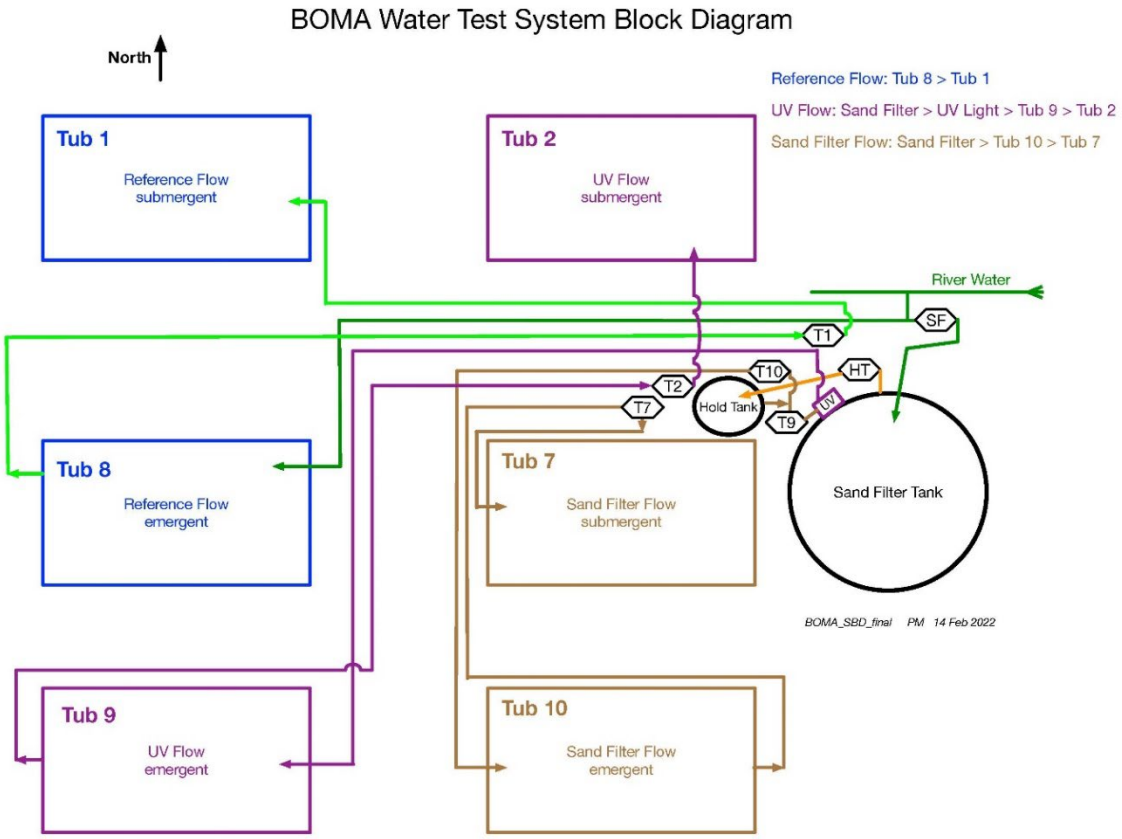

Figure 2. Project configuration

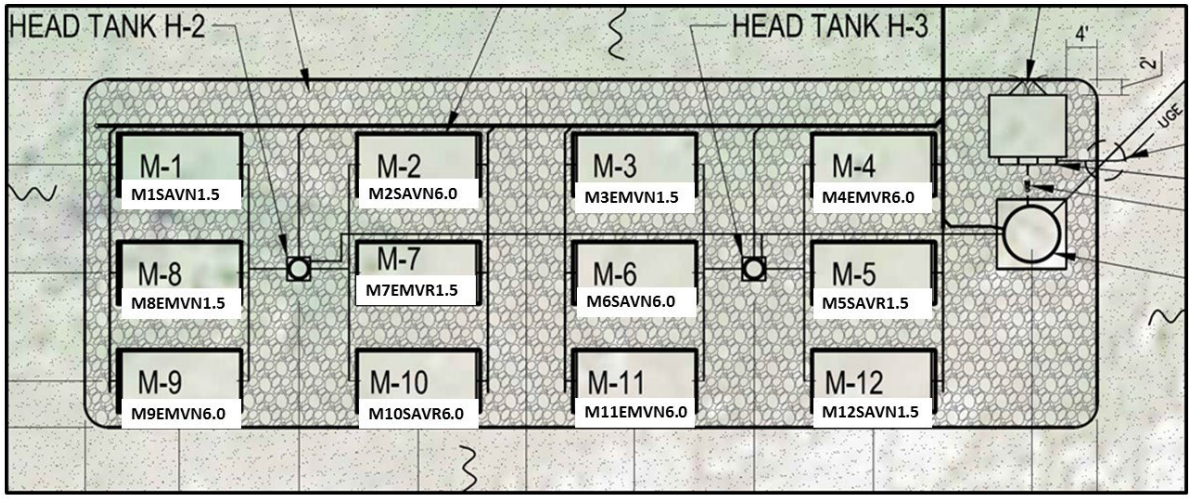

Figure 3. Mesocosm arrangement/ tank layout. The project utilized tanks 1, 2, 7, 8, 9 and 10.

1.4 Project Timeline

State of Florida Department of Environmental Protection Grant Agreement Number INV10 was fully executed on April 15, 2021 with an expiration date of February 28, 2023. Figure 4 is a graphical representation of the chronological sequence of project activities.

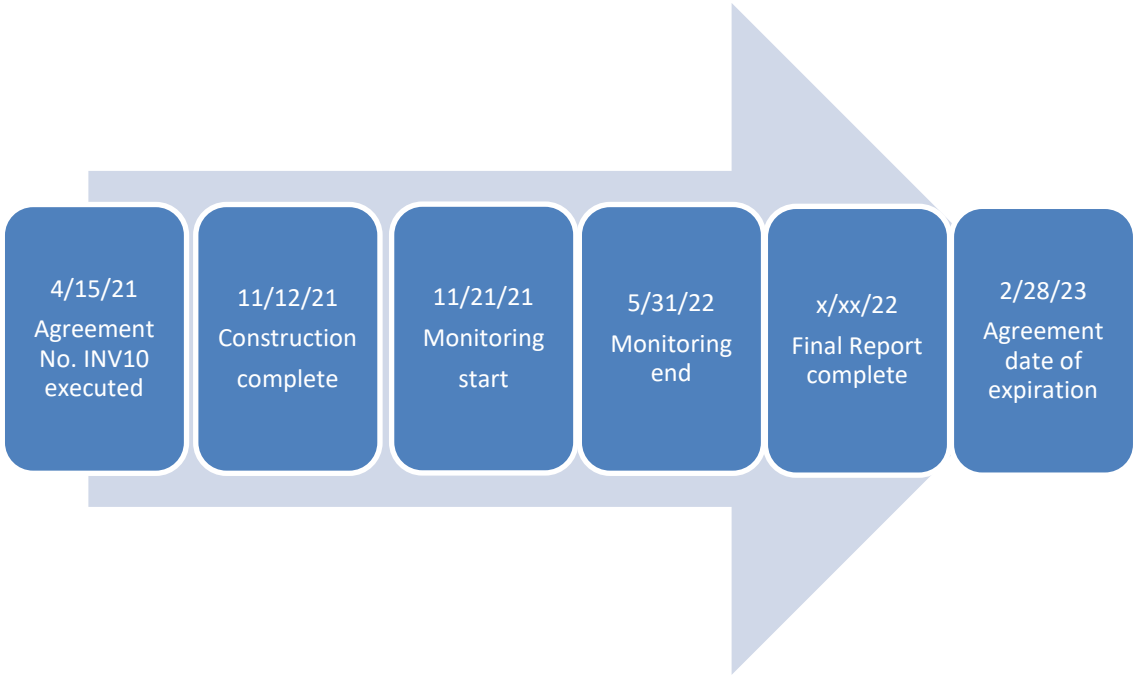

Figure 4. *Project timeline*

1.5 Grant award amount and anticipated benefits

FDEP executed grant agreement #INV10 with Lee County on April 22, 2021. The Florida Department of Environmental Protection Innovative Technologies Grant award amount was $180,000.00. No match funding was provided.

Extensive research in total phosphorus (TP) removal from storm and surface waters using wetland treatment systems has been conducted. However, studies about the mechanisms for total nitrogen (TN) removal via wetland treatment systems have been limited. The project was designed to build upon research that was completed by the SFWMD at the mesocosm wetland demonstration testing facility. That research focused on detailed data review and evaluation of alternative treatment processes of natural treatment options, including constructed wetlands

dominated by either emergent vegetation (EMV) or submerged aquatic vegetation (SAV). The SFWMD phased research demonstration, comprising wetland mesocosms and bioassays, followed by test cells and field-scale cells, was envisioned by the agency to facilitate and establish the basis for design of a future, full-scale constructed treatment facility.

The results from this project will be shared with the DEP, the SFWMD, and other stakeholders. The results from the project can be used to assess which type of treatment or best management practice might be best implemented by stakeholders responsible for TN reduction in their watersheds. Because one of the principles of the project was to test technologies that could be scaled up, the results of the study may be used to plan projects that would improve water quality through use of the tested technologies.

2.0 Project Budget and Schedule

2.1 Summary – Total Project Costs

- Total contractual services expenditures from Lee County to Florida Gulf Coast University were **$180,000.00**.
- The total amount of grant funding provided by a Florida Department of Environmental Protection Innovative Technologies Grant was **$180,000.00** (Table 1).
- The project incurred no changes to the original grant-funded portion of the budget.
- No local match funding was provided

Table 1. *Grant Funding*

Task No.	Category	Grant Funding Budget Amount
1.	Contractual Services	$1,000.00
2.	Contractual Services	$40,000.00
3.	Contractual Services	$138,000.00
4.	Contractual Services	$1,000
	Total:	$180,000.00
	Percentage Match:	0%

2.1.1 Other Project Costs

The South Florida Water Management District also incurred expenses to support the project. The estimated costs of site maintenance from November 2021 through June 2022 are as follows:

Expenses Description	Date	Cost
Tetra Tech Maintenance Dive	Oct-21	6,954.48
Tetra Tech Support for Pump Operations	Feb-22	2,158.80
Electrical Supplies	Jun-22	187.47
Fence Rental (8 months)		1,670.77
Approximate Electrical (8 months)		800.00
Total SFWMD Project Costs		$11,771.52

2.2 Project Schedule

Following is discussion of the project schedule versus actual completion, including changes required to the schedule, unexpected site conditions and adjustments, significant unexpected delays and corrections, and/or other significant deviations from the original project plan.

State of Florida Department of Environmental Protection Grant Agreement Number INV10 was executed on April 15, 2021 with an expiration date of February 28, 2023. A slight adjustment within the schedule was necessary (Task 1b, QAPP end date – see below), but the project as a whole was completed on the original grant project schedule.

The grant parties executed two change orders to the original agreement:

1. Change Order No. 1, executed 5/18/2021: added Task 1a (Draft Quality Assurance Plan), with a Task End Date of 6/30/2021, and changed the Task End Date for Task 1b (Final Quality Assurance Project Plan) from 5/31/2021 to 6/30/2021.

2. Change Order No. 2, executed 8/18/2022: corrected Task End Dates for Task 3, Monitoring, and Task 4, Final Report, to reflect a typographical error (changed dates from 2021 to 2022).

Lee County submitted one QAPP Addendum on 5/5/2022. The purpose of the Addendum was to update the list of sampling locations and sampling frequency.

Table 1. Project Schedule: grant agreement task end date vs. actual task end date

Task/Deliverable	Task or Deliverable Title	Task Start Date	Task End Date	Actual Task End Date
1a	Draft Quality Assurance Plan	07/01/2020	06/30/2021	Submitted 5/21/2021
1b	Final Quality Assurance Project Plan	07/01/2020	07/31/2021	Approval received 7/28/2021
2	Construction of Project	07/01/2020	12/31/2021	11/12/2021
3	Monitoring	07/01/2020	12/31/2022	5/31/2022
4	Final Report	07/01/2020	12/31/2022	1/30/2023

Other notable schedule milestones:

- Lee County entered into an Agreement for Water Quality and Treatment Research with FGCU: this agreement was fully executed on May 18, 2021.

- Lee County entered into a Revocable Right of Entry/License Agreement with the South Florida Water Management District: the resolution was passed and adopted by the SFWMD Governing Board on June 10, 2021. The agreement granted permission for Lee County and FGCU staff to enter the premises from June 10, 2021 to December 2022 for project purposes.

2.3 Project Plan and Schedule Challenges

The project team experienced several unexpected circumstances over the course of the project and made adjustments accordingly. The project team made no significant deviations from the original project plan or schedule despite the need for unanticipated maintenance and repairs to the systems. Following is a description of some of the unexpected events that occurred during the project.

Inability to procure construction items due to supply chain issues caused a delay in acquisition of certain materials for construction, but the team was able to adapt and avoid significant delays. For example, a black hold tank was unavailable so FGCU purchased a white tank and painted it black.

On or around December 23, 2021, the power went out at the project site. Investigation by a SFWMD electrician revealed that an electrical component (the breaker panel) was damaged. Typically the SFWMD can acquire such a part overnight, but supply chain issues caused a significant delay in delivery of the part. On January 25, 2022 the SFWMD electrician installed the new panel, but was unable to prime the pump that moves water from the Caloosahatchee to the mesocosms. On January 28, 2022, a Tetra Tech employee, under contract with the SFWMD, was able to get the water supply pump running. After the pump was functional again, a member of the FGCU project team filled the tanks, adjusted the water flow, and performed some vegetation management on tanks 1, 2, and 7 (removing any filamentous algae). The system became fully operational again on January 28. Due to the system being inoperable from December 23, 2021 through January 28, 2022, the team was unable to collect samples in the month of January 2022.

On March 13, the system reported a low holding tank alarm, so an FGCU Research Assistant went to the site, cleaned the sand filter that had become clogged, removed approximately 15 gallons of schmutzdecke, and added new sand. He also maintained the mesocosms (vegetation tanks) by removing any filamentous algae. The Research Assistant suggested that maintenance of the system would be required/should be completed every two weeks.

On April 15 FGCU staff cleaned the filter and replaced the slow sand filter pump, which had failed. On April 24 the filter was clogged again. Because the system was demanding maintenance every two weeks, Lee County sent an email request to the DEP asking if the project team could sample every two weeks to coincide with the frequency of needed system maintenance. On Monday, April 25 the County received DEP approval to begin sampling every two weeks.

June 2022 was full of unexpected events. On June 9 FGCU staff informed the County that the main river pump that supplies water to the treatment train system had failed; FGCU went to the

project site and attempted to reset the pump without success. Tom Missimer, Executive-in-Residence and Professor (member of FGCU research team) and an FGCU student cleaned the sand filter and added some sand while they were onsite. Tom Missimer contacted SFWMD staff to report the pump failure and discuss pump repair.

The following day, June 10, SFWMD staff determined that the three-phase electric supply had an issue, and contacted Glades Electric (the electric utility in that area) to request a repair. Glades Electric was able to get the electric service back online; the three-phase connector had become unhooked at the pole due to wind or lightning. After that was repaired, SFWMD staff still could not restart the pump.

On June 13 a SFWMD electrician went to the project site and discovered burned-up electrical components, the thermal units, on June 14 the SFWMD electrician ordered the parts that he thought would repair the pump, and on June 17 the SFWMD electrician installed the new thermal units and they immediately "blew." He completed additional troubleshooting and determined that the pump had other burned-up parts that were causing it to short out. At that point SFWMD staff determined that the pump was irreparable.

Because there was no money budgeted for a pump replacement, and eight sampling events had been completed, the project was brought to a close. On June 17 Lee County notified the DEP grant manager, Nick Daigle, that the project would end and no additional sampling events would be conducted.

3.0 Project Activities

3.1 Summary of completed study activities

Results from previous research on the topic of nitrogen reduction was reviewed to determine what innovative technology configurations might produce results that were more successful. It is with this in mind that the project design was established.

The South Florida Water Management District acquired all permits for the original research conducted on the mesocosm project site; no additional permits were required for this project. The following is a list of all project site permits, issue dates and issuing authorities. All permits

were issued to the South Florida Water Management District.

- Construction Permit issued by Glades County Building Department – Building/Electrical, May 27, 2016
- Barron Water Control District General Permit issued June 30, 2014
- Jacksonville District US Army Corps of Engineers, in accordance with 33 U.S.C. 408, January 23, 2015
- South Florida Water Management District, Environmental Resource Permit Minor Modification, July 8, 2015

Tank preparation – an important first step in the project construction phase of the project - began in July 2021. FGCU staff completed several activities to prepare the mesocosm tanks for the project. Muck and undesirable vegetation left from previous research projects in the mesocosms was removed from the tanks. Vegetation that was removed included rooted emergent and submergent vegetation and unrooted algae (mainly Chara). Plant composition and the amount of muck was highly variable between the tanks, so preparing them to ensure their similarity for dependable results was an important step in the process. Once the tanks were cleaned, the team replanted vegetation that was removed from the tanks in the cleaning process. These plants were cleaned, trimmed and prepared for re-planting. Each mesocosm slated for emergent vegetation received *Typha domingensis* (cattail) and *Schoenoplectus californicus* (giant bulrush) transplants. Each mesocosm slated for submergent vegetation received *Vallisneria americana* (tape or eel grass), planted every 10-15 cm.

Project construction that involved tanks, piping, electronics, etc. was completed in early November 2021. FGCU staff completed all construction activities.

The monitoring phase of the project was planned as not to exceed a period of 12 months after construction; eight sample events were completed between November 2021 and May 2022. A two-month pretesting and start-up period was built into the schedule to allow the system to stabilize and to ensure that the system was operating correctly prior to sample collection, but the system steadied sooner than anticipated and the two-month start-up period was not necessary. Samples were collected during eight sampling events at 16 locations (Figure 4), and evaluated by Sanders Laboratory and FGCU. Sampling results and analysis may be found in the Appendix A *Report to Lee County Department of Natural Resources, Water Quality and Treatment Research at the C-43 Mesocosm Site, Hendry County, Florida Using Combined*

Wetlands and Engineered Treatment Technologies. A spreadsheet showing all sampling results may be found as ***Appendix B*** *–Boma Water Quality Master Table – Final.*

Sampling events were completed on the following dates:

1. November 21, 2021
2. December 21, 2021
3. February 21, 2022
4. March 23, 2022
5. April 18, 2022
6. May 4, 2022
7. May 18, 2022
8. May 31, 2022

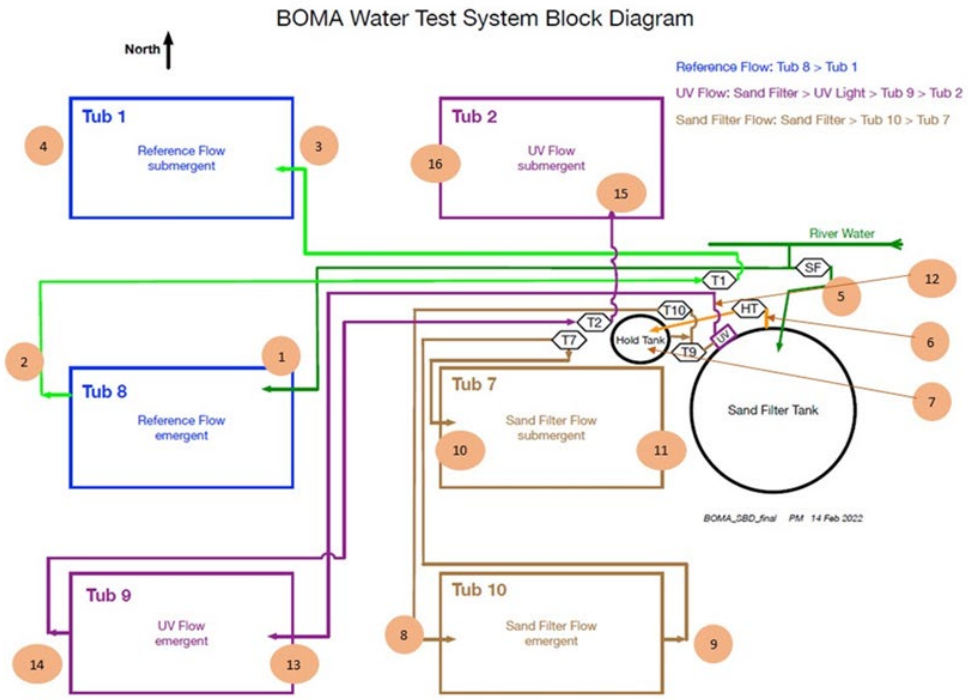

Figure 5. System schematic and sampling locations

Following is a brief description of each sampling location shown in Figure 5:

1. Control inflow to Typha tub
2. Control outflow from the Typha tub
3. Control inflow to the tape grass tub
4. Control outflow from the tape grass tub
5. Sand filter inflow

6. Sand filter outflow
7. Sand filter-secondary holding tank
8. Sand filter inflow to Typha tub
9. Sand filter ouflow from Typha tub
10. Sand filter inflow to tape grass tub
11. Sand filter outflow from tape grass tub
12. Sand filter + UV discharge
13. Sand filter + UV inflow to Typha tub
14. Sand filter + UV ouflow from Typha tub
15. Sand filter + UV inflow to tape grass tub
16. Sand filter + UV outflow from tape grass tub

Routine maintenance of the project involved cleaned the sand filter on a regular basis. This involved removing schmutzdecke, and added new sand (Figure 6). Schmutzdecke is a visible biological skin that builds up in the top layer of the sand in the filter. It is a sticky layer or "muck" often referred to as the micro-flora skin or Schmutzdecke. Schmutzdecke is a German word meaning "dirty layer" (Huisman & Wood, 1974). The Schmutzdecke is often made of autotrophic bacteria, fungus, algae, protozoans, and a number of water living larvae plus the metals iron, manganese and silicon, but it makeup depends on the incoming water characteristics and the habitat in the certain filter (Huisman & Wood, 1974). The Schmutzdecke removes nitrogen and phosphate and releases oxygen. The fine sand grains leads to slow filtration which means that the water stays a long time above and in the filter, this gives the biological skin plenty of time to purify the water (Österdahl, 2015).

Figure 6. *Comparison of clean sand and Schmutzdecke removed during maintenance of sand filter (3/23/22)*

Mesocosm maintenance also involved the removal of any filamentous algae, to reduce competition with desirable vegetation such as *Vallisneria americana* in the tanks.

All planned project activities were completed, and Lee County does not intend to pursue any additional phases of the research.

3.2 Project Photographs

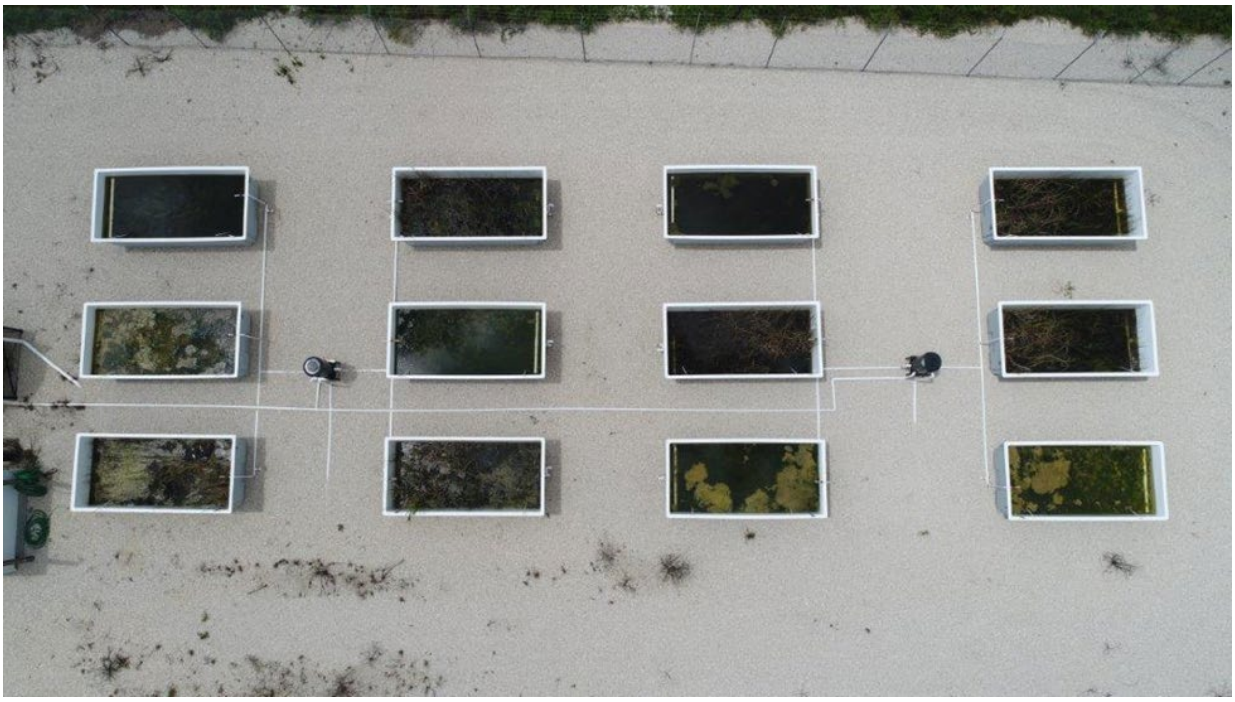

***Figure 7**. Project site prior to construction (2-2-21)*

***Figure 8.** Photo of project site during construction (9-13-21)*

***Figure 9**. Photo of project site after construction (11-21-21)*

***Figure 10.** Tank 1 vegetation (2/21/22)*

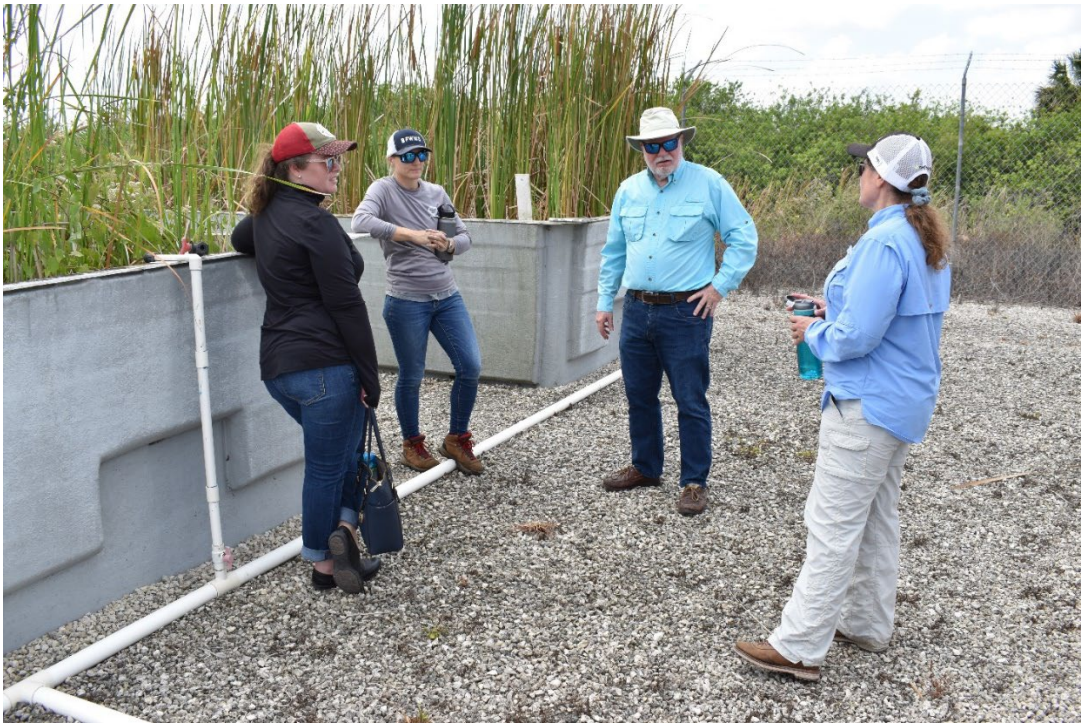

Figure 11. *Project team members from FGCU and SFWMD (5/27/21).*

Figure 12. *Drone photo of mesocosms (7/12/21)*

Figure 13*. Serge Thomas, FGCU maintaining mesocosm vegetation (9/13/21)*

Figure 14. *Caloosahatchee River (C-43) pump intake maintenance (10/15/21).*

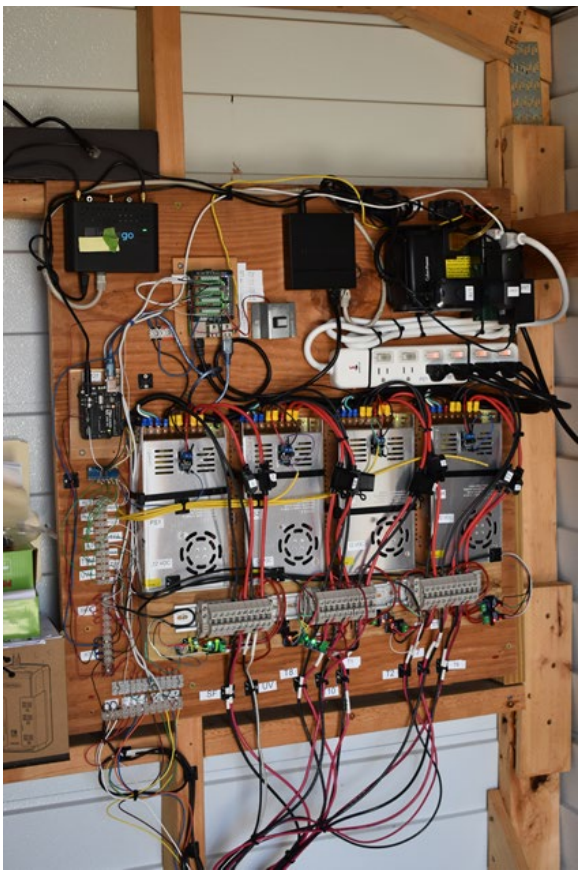

***Figure 15**. Sand filter (3/23/22)*

4.0. Discussion

Despite several project challenges such as equipment failure, sufficient data was collected to be able to ascertain the effectiveness of the water treatment configurations.

A key finding from the project is that all three treatment trains tested showed significant improvements in water quality for the treated water. In each treatment configuration process, the last step –water flowing through vegetation - provided the most significant removal of macro-nutrients. The slow sand filter did remove a large quantity of organic carbon, and the UV treatment did reduce the concentration of bacteria and algae. A comprehensive analysis of the project may be found in the Appendix A *Report to Lee County Department of Natural Resources, Water Quality and Treatment Research at the C-43 Mesocosm Site, Hendry County, Florida Using Combined Wetlands and Engineered Treatment Technologies.*

Thorough and thoughtfully planned data collection allowed a comprehensive and critical evaluation of how each of the water treatment processes worked.

5.0 Conclusion

The information gleaned from this study will assist watershed managers in identifying effective projects and abatement strategies to improve water quality in the Caloosahatchee River and Estuary. Projects designed to more effectively remove nutrients from the Caloosahatchee River are needed to moderate the potential for harmful algal blooms to occur. The technology arrangements tested for this project were successful in more effectively reducing nutrients from Caloosahatchee River water. Additionally, the three types of treatment trains testing in a mesocosm setting are able to be scaled up for larger applications.

Results from the project will add to the body of knowledge regarding evaluation of new approaches to large-scale management of water resources. The project technologies hold potential to further the objectives of preventing or mitigating harmful algal blooms.

Appendix A

Report to Lee County Department of Natural Resources: Water Quality and Treatment Research at the C-43 Mesocosm Site, Hendry County, Florida Using Combined Wetlands and Engineered Treatment Technologies

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Cooperative Research Agreement with the Lee Board of County Commissioners Funded by a Florida Department of Environmental Protection Innovative Technologies Grant

January 2023

AUTHORS

Thomas M. Missimer, Ph.D., Co-PI | Florida Gulf Coast University
Ashley Danley-Thomson, Ph.D., P.E., Co-PI | Florida Gulf Coast University
Serge Thomas, Ph.D. | Florida Gulf Coast University
Seneshaw Tsegaye, Ph.D. | Florida Gulf Coast University
Peter R. Michael, MSE | Florida Gulf Coast University

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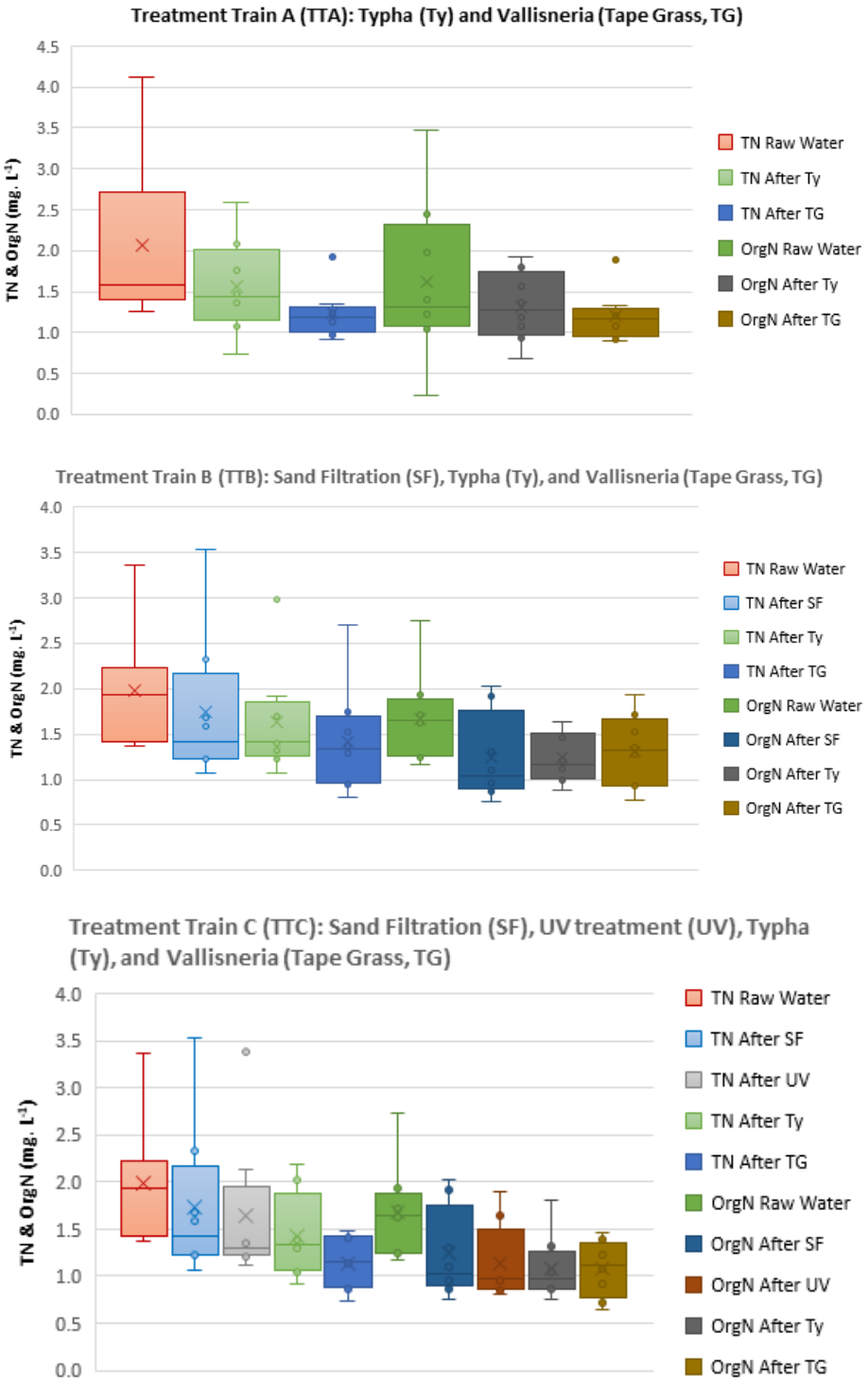
EXECUTIVE SUMMARY

Through a partnership between the Lee County Department of Natural Resources, the South Florida Water Management District, the Florida Department of Environmental Protection, and Florida Gulf Coast University, an investigation was conducted to test an innovative technology that could reduce nutrients in water bodies and prevent or mitigate harmful algae blooms. A primary objective of the project was to evaluate the removal of organic nitrogen by a comparison between a strictly vegetation treatment train and two treatment trains that include engineered enhancements that could be scaled up to large capacities. At the Boma site in Hendry County three water treatment trains were assessed in terms of effectiveness at removal of nutrients and biomass that would affect the water quality in the river and other waterbodies in the Caloosahatchee basin. Since best management practices can include slow sand filters, ultraviolet treatment, and treatment wetlands, the treatment methods used in these trains were: A. passage of the raw water from the river through an emergent vegetation tub (*Typha sp*. or cattail) and a submergent vegetation tank (*Vallisneria sp.* or tape grass), B. passage of the raw water from the river through a slow sand filter followed by emergent vegetation tank (*Typha sp*.) and a submergent vegetation tub (*Vallisneria sp.*), and C. passage of the raw river water through a slow sand filter and UV treatment followed by an emergent vegetation tub (*Typha sp*.) and a submergent vegetation tub (*Vallisneria sp.*).

Detailed water quality data were collected from the three trains and at other locations on the site to assess the effectiveness of each treatment train and compare them to ascertain which one was most effective. A statistical analysis of the results of comparing the water quality entering each train with that leaving each train showed that all three systems significantly improved water quality, but there was no statistically significant difference between them. In each case, the last process, submergent vegetation, provided the most significant removal of macro-nutrients. The slow sand filter did remove a large quantity of organic carbon amounting to 8 to 12 kg for each cleaning of the filter top. The UV treatment did reduce the concentration of bacteria and algae.

Impact of the slow sand filter showed that anoxia could not be reached within the filter to help reduce the organic nitrogen because the filter bed would require a greater thickness and the overall contact time would need to be raised to greater than 8 hours. Use of the UV to help break down the organic carbon was limited because of the high turbidity of the water, the low contact time, and the low intensity of the lamp.

The submergent vegetation tub was particularly effective at nutrient removal because of growth of algae other than the planted *Vallisneria* sp. Rapid growth of the algae *Cladophora* sp. and some other species required that the tubs be cleaned each time the sand filter was cleaned. It should be noted that the *Cladophora* sp. and some of the other algal species float to a significant degree. The purposeful growth of *Cladophora* sp. should be considered with harvesting and possible use of the fiber as a source of cellulose. In addition, an engineered enhanced vegetative treatment process should be considered for the stored water just prior to placement back into the river.



Executive Summary Figure. Total and Organic Nitrogen concentrations throughout the processes of each treatment train

Introduction

Background

Southwest Florida continues to make progress in meeting the goals of the Water Quality Restoration Program, which seeks to control the concentrations and loads of certain constituents including nutrients, as nutrients can lead to and exacerbate algae blooms. Through a partnership between the Lee County Department of Natural Resources, the South Florida Water Management District, the Florida Department of Environmental Protection, and Florida Gulf Coast University, an investigation was conducted to test an innovative technology that could reduce nutrients in water bodies and prevent or mitigate harmful algae blooms.

In particular, this project sought to evaluate the removal of organic nitrogen, in addition to other water quality parameters, by a comparison between a strictly vegetation treatment train and two treatment trains that include engineered enhancements that could be scaled up to large capacities. Since best management practices can include slow sand filters, ultraviolet treatment, and treatment wetlands, at the Boma site in Hendry County three water treatment trains were assessed in terms of effectiveness at removal of nutrients and biomass that would affect the water quality in the river and other waterbodies in the Caloosahatchee basin.

The South Florida Water Management District (District) is in the process of designing and constructing several large-scale water storage reservoirs located on the south side of the Caloosahatchee River. It is the purpose of these reservoirs to be used for wet season diversion of excess discharge from the river with release back into the river during the dry season to help balance freshwater discharges into the Caloosahatchee River Estuary. While the plan has considerable environmental merit in terms of managing freshwater discharge and water quality to maintain or enhance the biological functions of the estuary, the issue of water quality must be addressed (Scarlatos, 1988; Doering et al., 2002; Barnes, 2005; Tolley et al., 2005; Liu et al., 2009; Agobian, 2010; Andresen, 2011; Buzzelli et al., 2013; Qiu and Wan, 2013; Buzzelli et al., 2014a, 2014b; Cook, 2014; Volety et al., 2014; Chen et al., 2015; Palmer et al., 2016; Ross, 2016; Sun et al, 2016; Volety et al., 2016; Armstrong et al., 2019; Rumbold and Doering, 2020; McFarland et al., 2022). Additional stormwater collection systems in the Caloosahatchee River Basin also may require removal of nutrients and organic carbon. Therefore, the research conducted applies not only to the reservoirs but to other water bodies within the Basin.

The quality of water in the Caloosahatchee River during the highest discharge periods of the summer months is commonly laden with excess concentrations of nutrients, turbidity, color, and associated harmful algal blooms. Once the river water is pumped into the reservoirs, the high nutrient content along with high water temperature and a stagnant setting will exacerbate the issue of algal blooms in the reservoir along with other aquatic plant growth (Jeppesen et al., 2007; Paerl and Huisman, 2008; Gebrehiwot et al. 2017; Liu et al, 2021; Lee et al., 2022). Therefore, treatment of the reservoir water and other stormwater collection bodies within the Caloosahatchee River Basin will be required before it can be discharged back into the river to meet the goals of the estuary freshwater management plan.

A number of other methods have been applied to shallow lakes for control of algal blooms, other aquatic vegetation, and high organic carbon concentrations (Lürling and Mucci, 2020). Chemical methods have been applied using copper-based algaecides (Bishop et al., 2018) or herbicides including glyphosate or 3-(3,4-dichlorophenyl)-1,1dimethylurea (Duran) (Jančula and

Maršálek, 2011; Matthijs et al, 2016). However, residues of these compounds could be discharged into the river during water recovery cycles. The addition of a coagulant plus a ballast compound has been suggested to remove algae and other organic compounds from the water column (Noya et al., 2017). Adding a clay flocculant to eutrophic waters so that the clay sequesters both nutrients and organic material in such a way that the resulting floc settles to the bottom of the water body has also been used successfully (Chen et al. 2018). If the clay is bentonite, which is chemically inert, the combination of the clay and the organic material tends to harden on the bottom and cannot be re-suspended by wind mixing. In this case, the bottom can be periodically dredged. This technique has been used in southwest Florida in lakes used to manage stormwater.

Another means of water treatment is to use vegetation to uptake the nutrients and filter the turbidity and particulate organic carbon, similar to the stormwater treatment areas in the Everglades (South Florida Water Management District, 2022). In order to access the potential for development of a "natural" stormwater treatment strategy, the South Florida Water Management District (SFWMD) developed a research site in Hendry County, located upstream of the S-78 water management structure on the south side of the Caloosahatchee River (Figure 1). The site has 12 tanks with dimensions of about 7m x 3.5m x 1.5 m containing wetland vegetation that was used to conduct a water quality assessment of nutrient removal from Caloosahatchee River water that was directed to flow through the wetland cells (Cornwell et al., 2019). There were three key findings of the research: (1) no single plant community appears to control nitrogen removal (denitrification), (2) the sediments in the tanks represent a net sink for nitrogen and phosphorus, and (3) the average denitrification was 14.4 ± 23.0 mg N/m^2/day with the highest rate occurring in June at 24.3 ± 29.7 mg N/m^2/day and the lowest rate occurring in December at 10.9 ± 11.4 mg N/m^2/day. They concluded that denitrification was significant in the mesocosms.

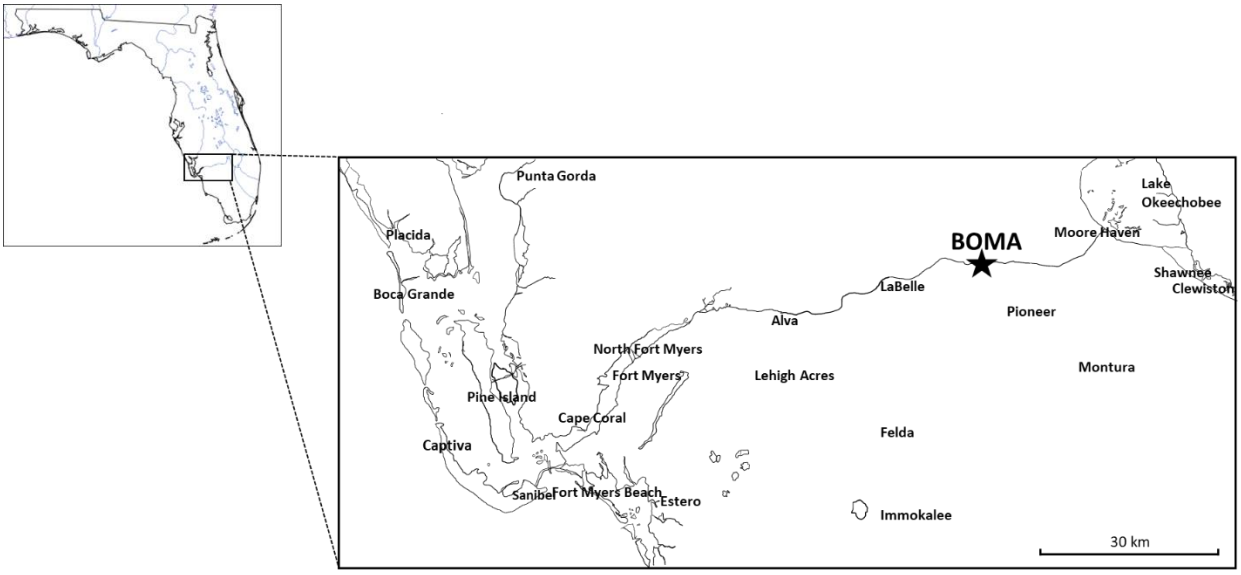


Figure 1. Site location map.

Project Goal and Objectives

Another option to control the quality of water in stormwater storage facilities is to develop a biological treatment process using plants with enhanced nutrient removal facilitated by engineered water treatment. The goal would be to convert the nitrogen in the surface water from nitrate/nitrite to ammonium and to reduce the concentration of organic nitrogen. An investigation of this process was conducted at the C-43 mesocosm site using 6 of the 12 tanks remaining from past research. The primary objective of the research is to ascertain if well-known engineering processes that have high potential for upscaling can be used to facilitate the uptake of nutrients in plants. If this process is feasible, it could be developed on the sites of reservoirs and stormwater ponds, leave less residuals for disposal, and operate at a lesser cost.

Methodology

Source water for the project

The water supply for the project comes indirectly from the Caloosahatchee River via a canal. A pump on the canal contains a screen to strain floating aquatic vegetation. The pump is located about 2,200 feet from the site and is maintained by the South Florida Water Management District. The water-supply pump feeds an onsite storage tank from which the water is pumped into various parts of the test apparatus.

Figure 2. Primary water supply tank.

Original proposed design of the project

The original design of the project included three water treatment trains. Water treatment was proposed on the feed water on two of the three trains, and one train was to be used as a control. Each train consisted of two tanks containing different wetland communities with the first tank being emergent plants (primarily *Typha* sp.) and the second tank contained

submergent vegetation (primarily *Vallisneria sp.* tape grass) (Figure 3). Entry of river water into the first train was taken directly from the supply tank without treatment and was fed by gravity into the two wetland tanks. The second train was designed to be treated by slow sand filtration of the water before entering two wetland tanks. The third train water treatment scheme included slow sand filtration followed by UV treatment and then wetland treatment in two tanks.

The reasoning behind the design was that the slow sand filtration would remove a large part of the particulate organic carbon and algae and create anoxia in the tank to help convert nitrate and nitrite to ammonium. In addition, the UV following slow sand filtration would help break down some of the organic nitrogen to smaller size molecules that could be taken up by the aquatic plants in the tanks and therefore, enhance the nutrient removal.

The duration of the experimental treatment processes was designed for a period not to exceed 12 months after the installation, and pretesting for a period of two months. The deliverables from the research were monthly data summaries and a final report to be delivered within 60 days after the research was completed.

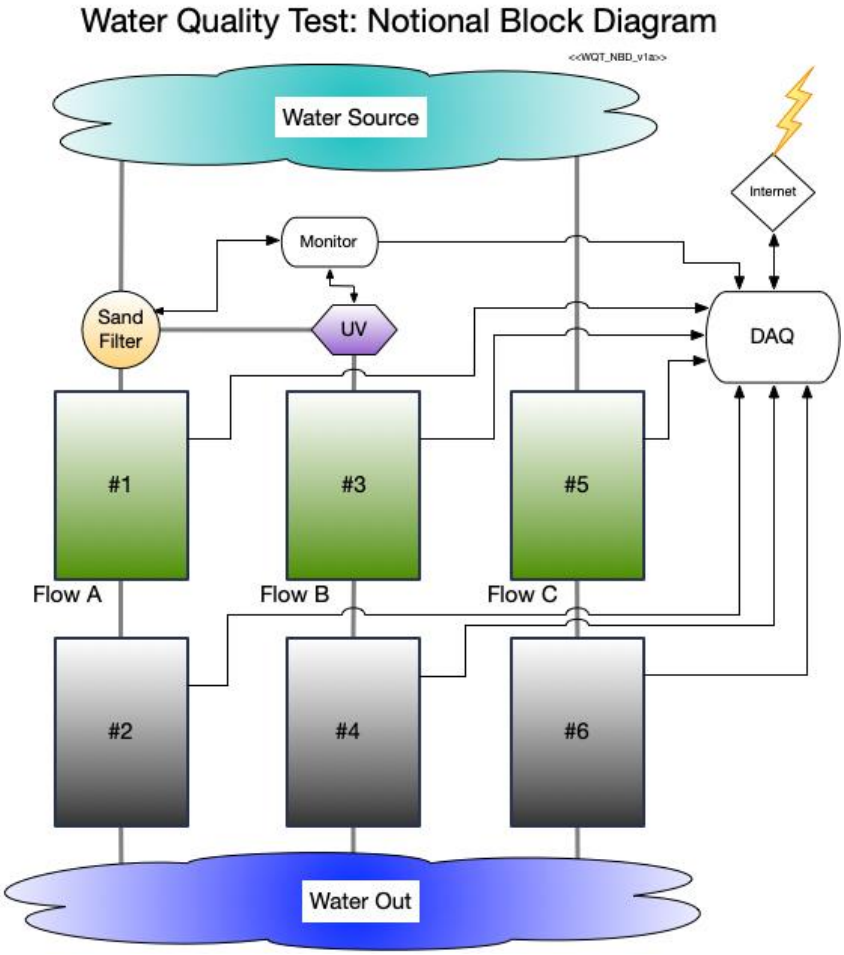


Figure 3. Schematic diagram of the initial project design.

Modified construction of the project and controls

Site conditions and a series of health events triggered some design changes to the project. The COVID19 epidemic caused major cost increases in construction materials and sharp price increases in pumps and electronic components. Thus, a more efficient design was developed and constructed to lie close to the original budget. This section describes the design and concept of operation of the power and monitoring subsystem for the Boma water quality project. The subsystem provides the means to pump and track the movement of the Caloosahatchee River source water.

System Water Flow

Water from the Caloosahatchee River is pumped via a pipe to a main supply tank in the Boma test facility. The original Boma facility (see image below) consisted of the river water pump, the main supply tank, two holding tanks, and twelve test tubs. The experiment used the existing main supply tank, six tubs and added a new sand filter tank and a filtered water holding tank. Three parallel water paths were created, referenced, filtered, and UV-treated to compare water quality treatment. Two tubs were assigned to each path, the first with emergent plants and the second with submergent plants.

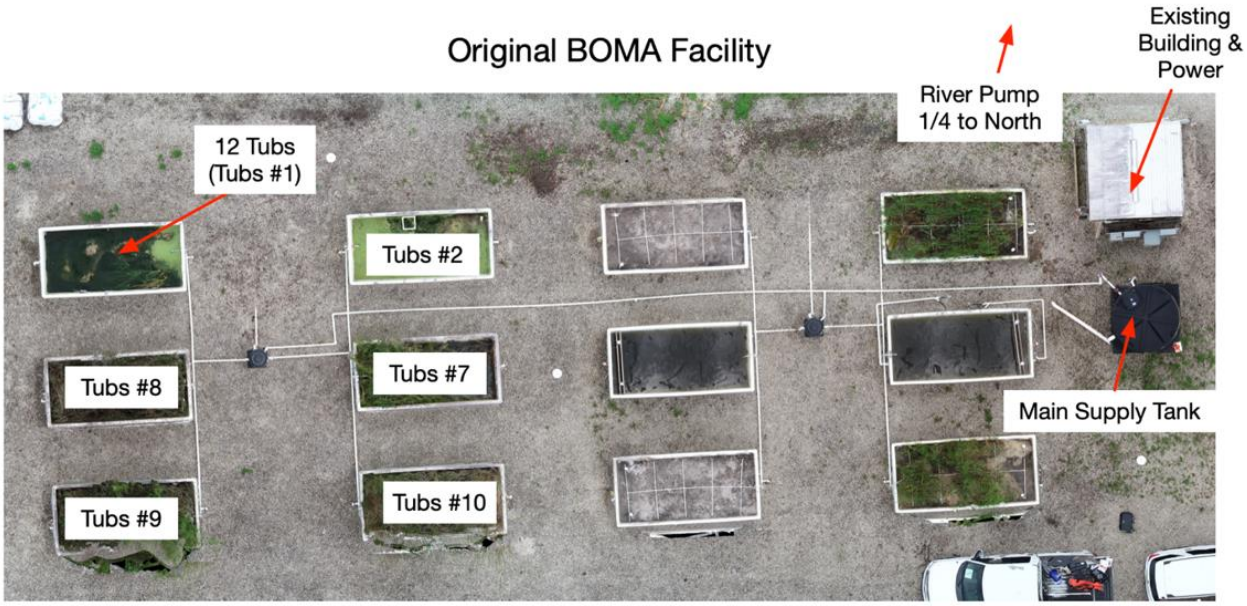


Figure 4. Layout of the site showing the main supply tank, the storage building with the power supply, and the tubs containing the vegetation.

Experimental Layout

The three experimental paths utilized the following tanks and tubs: the reference path used gravity fed from the main supply tank to Tub 8 containing emergent plants. Pump T1 then pumped water from Tub 8 to the submergent plant Tub 1. Pumps from the emergent plant tubs to the submergent tubs were required because the water level in the submergent tubs was

higher than in the emergent tubs. Both the filtered and UV paths utilized river water filtered by the new slow sand filter tank. Pump SF moved river water from the main supply tank to the top of the slow sand filter tank. Since the sand filter tank did not have enough gravity head, pump HT moved water from the base of the sand into the filtered water holding tank.

The filtered water tank used gravity to feed water directly to Tub 10 for the filtered water flow. For the UV path, gravity fed the water through a UV light and then to Tub 9. The UV light consists of a 12 VDC powered Blackcomb LB5-06 rated for 23 liters per minute with a 22-watt bulb. The UV light was set to run continuously. Two pumps moved the water from Tub 10 to pump T7 to Tub 7 to for the filtered flow, and from Tub 9 to pump T2 to Tub 2 for the UV flow (Figure 5).

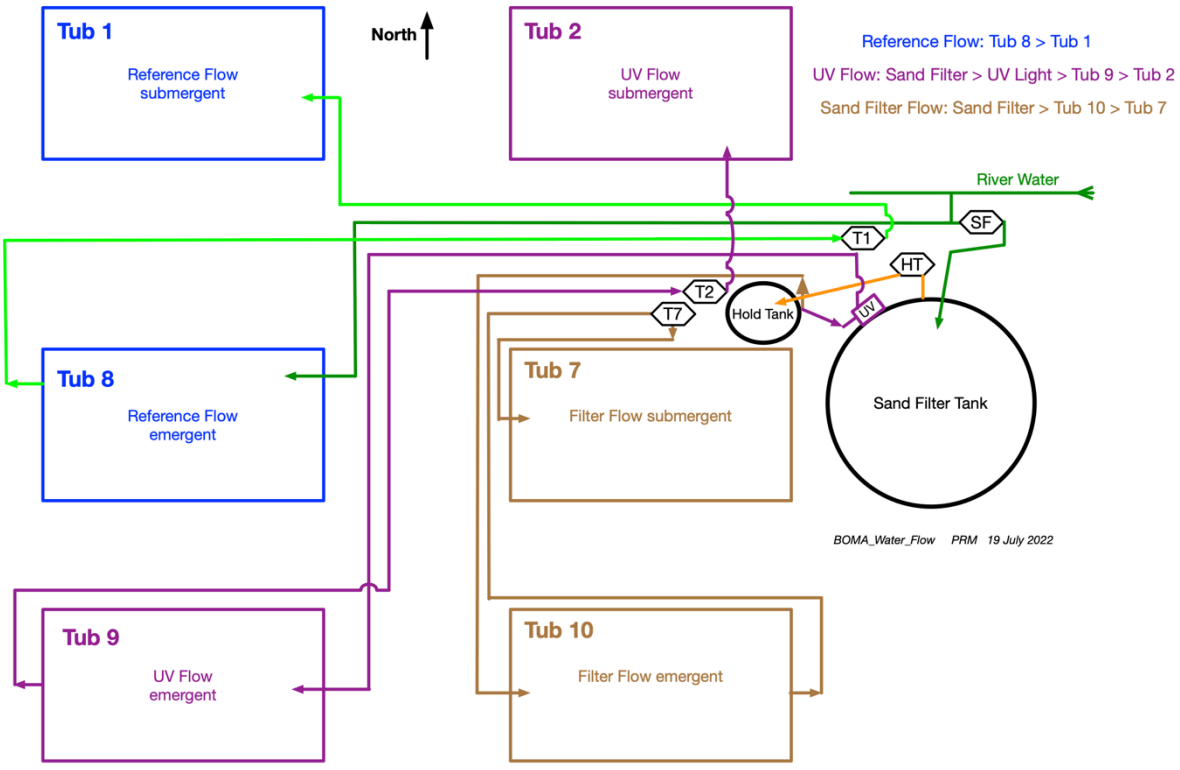


Figure 5. Water flow and pumps.

Standard Operation

Under normal conditions, the system operated as follows: the main river water pump ran continuously and filled the main holding tank. Any excess water was drained out to an adjacent canal. The reference path used gravity to feed water into Tub 8. Pump T1, running continuously, moved water from Tub 8 to Tub 1. Any excess water was drained to the adjacent canal. Pump SF moved water from the main holding tank to the top of the sand filter tank. The river water percolated through the sand filter and then was moved by pump HT to the top of the filtered water holding tank. Gravity fed water from this tank directly to Tub 10 and through the UV light to Tub 9. Pumps T7 and T2 moved water from Tub 10 to Tub 7 and from Tub 9 to Tub 2. Any excess water drained to the adjacent canal. The monitoring system continuously

checked that this operation was OK and reported any voltage, current, and water level problems.

Figure 6 shows the sand filter, filtered water holding tank, and the five pumps: SF, HT, T1, T2, and T7. The white pipes had water, and the grey were for electrical power and monitoring subsystems.

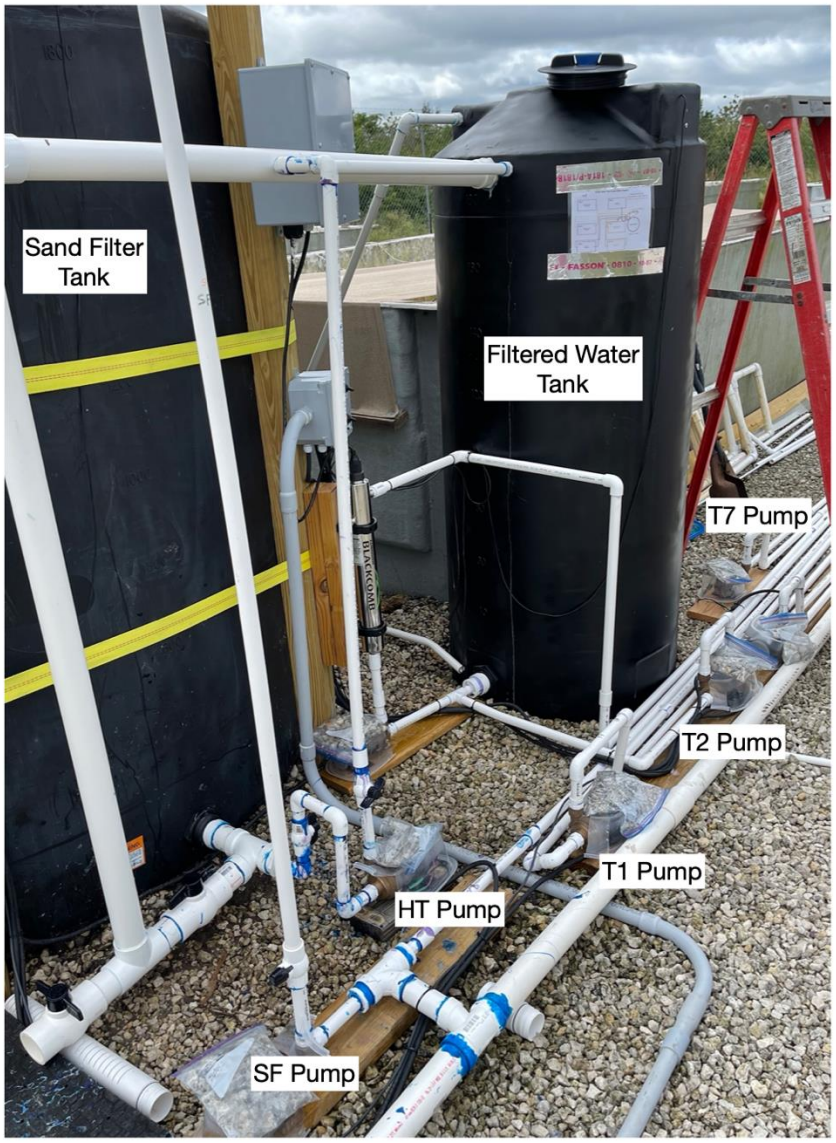


Figure 6. Slow sand filter, UV unit, filtered water tank, and pumps with associated piping and electrical conduits.

Power and controls

To enable a safe and cost-effective system, pumps operating on 12 VDC provided the required pump and UV light power. The field site contained only the power wiring, control

switches, pumps, and UV light. The supply power and power monitoring systems were installed in the existing site-building. Figure 7 shows the tanks, two field control boxes, and the UV light system.

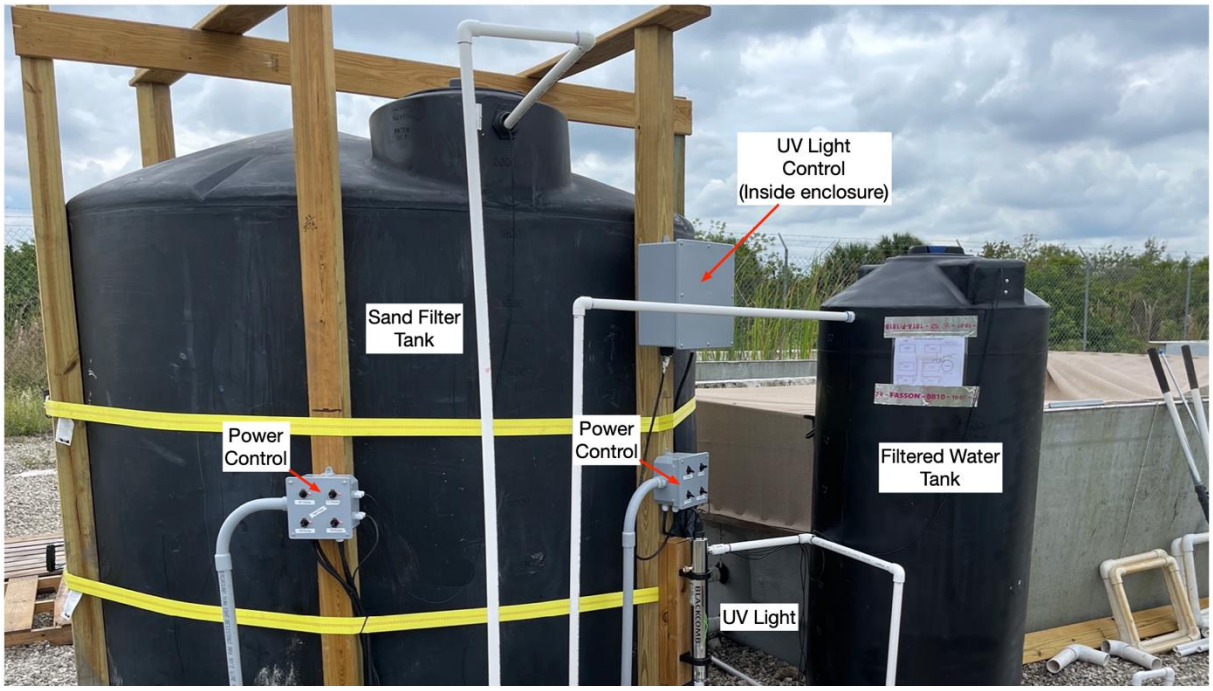


Figure 7. Slow sand filter, filtered water tank, and UV system with the power boxes and electrical conduit.

The power control system provided a method to check power availability, control pump, and UV light. All control systems were installed in water-resistant enclosures, with cable penetrations protected by gland fittings. The UV control module and one of the control boxes are shown in Figure 8.

Figure 8. UV control and power control and indicator enclosure.

Monitoring Status

The monitoring system provided status on available power and water levels. Sensors included source AC power, 12 VDC power supply voltages, current flow to the pumps and UV light, and float switches to indicate the water level in the tanks and tubs. Since the water supply contained numerous particles and the water flow was small (≈ 1 gallon per minute from emergent to submergent tubs), standard flow meters would not work. Instead of expensive flow meters, low-cost float level sensors provided the water status. An example of the float switch and mounting system is shown in Figure 9. A summary of the monitoring system is given in Table 1.

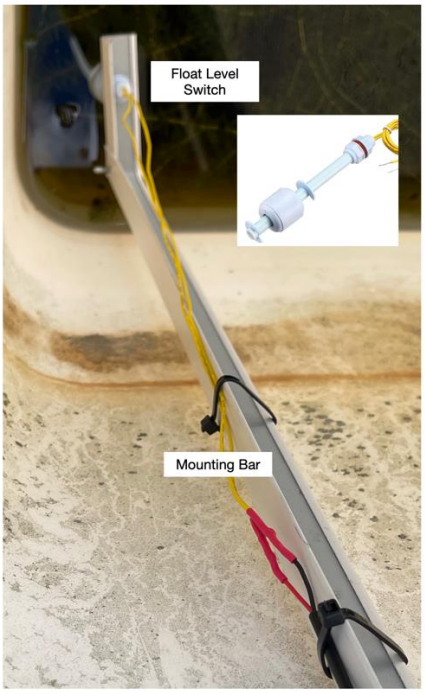


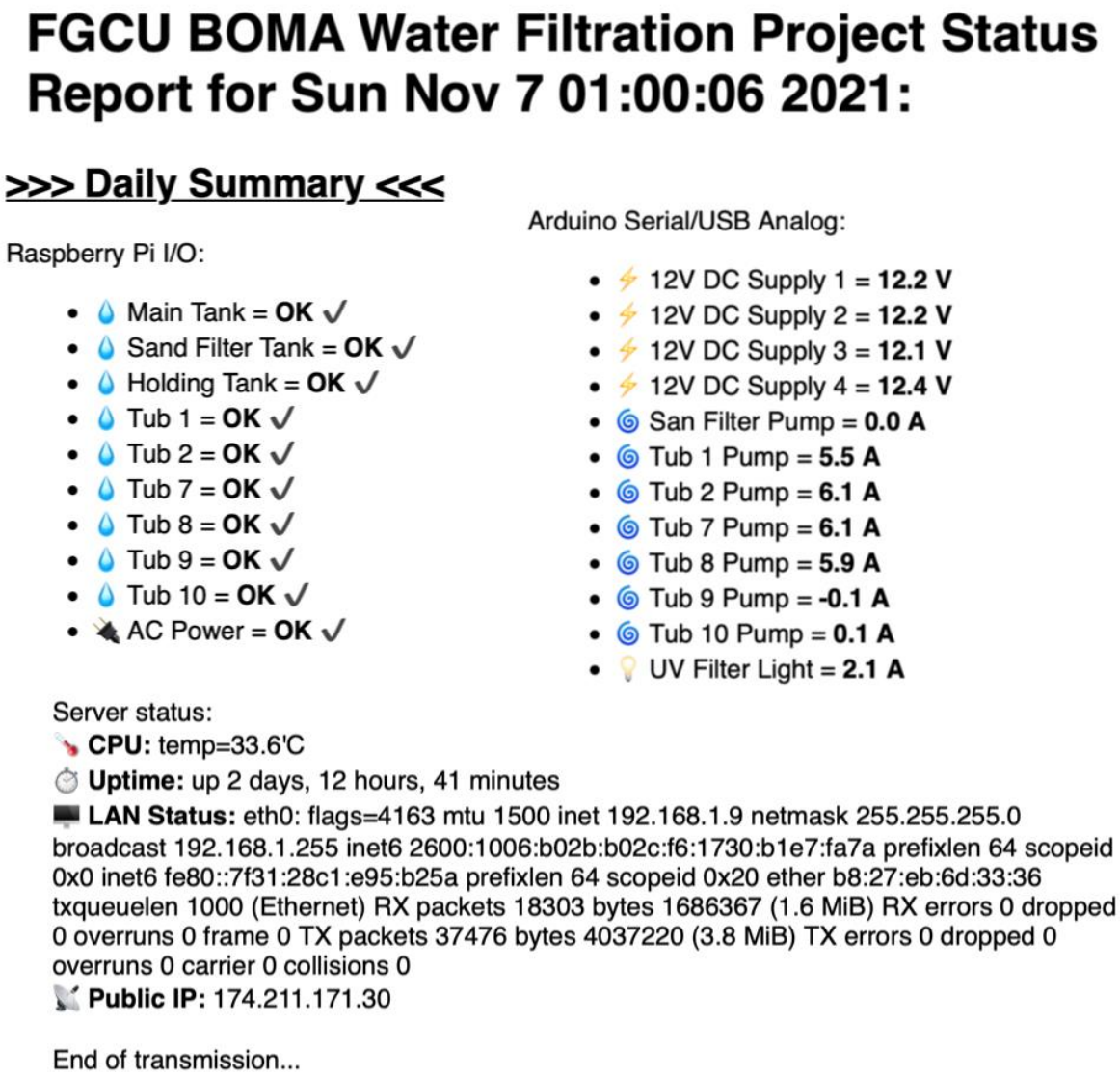
Figure 9. Water level float switch.

Table 1. Summary of monitoring indicators.

Monitored Data	Type	Comment	Failure Indication
Binary Inputs			
Main Supply Tank	Float Switch	River water available	Loss of pumping from the river
Sand Filter Tank	Float Switch	Sand filter feed water status	Pump SF failure
Filtered Water Tank	Float Switch	Filtered water status	Pump HT failure, clogged Sand Filter
Tub #1	Float Switch	Low water level in tub	Pump for T1
Tub #2	Float Switch	Low water level in tub	Pump for T2
Tub #7	Float Switch	Low water level in tub	Pump for T7
Tub #8	Float Switch	Low water level in tub	Main holding tank level
Tub #9	Float Switch	Low water level in tub	Low filtered water tank level
Tub #10	Float Switch	Low water level in tub	Low filtered water tank level
Input AC Power	Relay	AC input power	Loss of AC power to the facility
Analog Inputs			
Power Supply #1	Voltage	Status of 12VDC power	Power supply failure
Power Supply #2	Voltage	Status of 12VDC power	Power supply failure
Power Supply #3	Voltage	Status of 12VDC power	Power supply failure
Power Supply #4	Voltage	Status of 12VDC power	Power supply failure
UV Light	Current	System operational	UV light bulb failure
Current to SF pump	Current	System operational	Pump failure
Current HT pump	Current	System operational	Pump failure
Current to pump T1	Current	System operational	Pump failure
Current to pump T2	Current	System operational	Pump failure
Current to pump T7	Current	System operational	Pump failure

The Arduino microcontroller collected and digitized the analog inputs. The Raspberry Pi computer collected the digital status information and analog data transferred from the Arduino. Once the data were assembled, a daily status email was sent to enable remote monitoring of the Boma system. The email consists of three general parts. The first is a header stating the Project name and ending in the day of week, month, day, the time, and the year. The second part is the Daily Summary, with an easy to identify check for OK and a red X for a problem. The third part lists the server data acquisition system status including temperature,

uptime, and other technical performance information. Of concern is the >>>> Daily Summary <<<< part. An internet connection was installed at the site to allow the reporting of the system to the project team. If there was an AC power failure, an immediate email was sent. Since the monitoring system was powered by an uninterruptable power supply (UPS), the monitoring system continued to operate (for a short time) even with a power failure. Two example emails are shown in Figures 10 (the full email) and 11 (the Daily Summary part only). The first shows a good status, the second one with problems.



FGCU BOMA Water Filtration Project Status Report for Sun Nov 7 01:00:06 2021:

>>> Daily Summary <<<

Raspberry Pi I/O:

- 💧 Main Tank = **OK** ✓
- 💧 Sand Filter Tank = **OK** ✓
- 💧 Holding Tank = **OK** ✓
- 💧 Tub 1 = **OK** ✓
- 💧 Tub 2 = **OK** ✓
- 💧 Tub 7 = **OK** ✓
- 💧 Tub 8 = **OK** ✓
- 💧 Tub 9 = **OK** ✓
- 💧 Tub 10 = **OK** ✓
- 🔌 AC Power = **OK** ✓

Arduino Serial/USB Analog:

- ⚡ 12V DC Supply 1 = **12.2 V**
- ⚡ 12V DC Supply 2 = **12.2 V**
- ⚡ 12V DC Supply 3 = **12.1 V**
- ⚡ 12V DC Supply 4 = **12.4 V**
- 🌀 San Filter Pump = **0.0 A**
- 🌀 Tub 1 Pump = **5.5 A**
- 🌀 Tub 2 Pump = **6.1 A**
- 🌀 Tub 7 Pump = **6.1 A**
- 🌀 Tub 8 Pump = **5.9 A**
- 🌀 Tub 9 Pump = **-0.1 A**
- 🌀 Tub 10 Pump = **0.1 A**
- 💡 UV Filter Light = **2.1 A**

Server status:
🍓 **CPU:** temp=33.6'C
⏱ **Uptime:** up 2 days, 12 hours, 41 minutes
🖥 **LAN Status:** eth0: flags=4163 mtu 1500 inet 192.168.1.9 netmask 255.255.255.0 broadcast 192.168.1.255 inet6 2600:1006:b02b:b02c:f6:1730:b1e7:fa7a prefixlen 64 scopeid 0x0 inet6 fe80::7f31:28c1:e95:b25a prefixlen 64 scopeid 0x20 ether b8:27:eb:6d:33:36 txqueuelen 1000 (Ethernet) RX packets 18303 bytes 1686367 (1.6 MiB) RX errors 0 dropped 0 overruns 0 frame 0 TX packets 37476 bytes 4037220 (3.8 MiB) TX errors 0 dropped 0 overruns 0 carrier 0 collisions 0
📡 **Public IP:** 174.211.171.30

End of transmission...

Figure 10. Example of control system email showing accetable operational staus.

Figure 11. Example of a control email showing operational issues in six locations.

The email shown in Figure 11 was the result of the failure of the main river water pump. With no input water, the main tank water ran low. With no water available, the follow-on system also ran low. The email notifications were very helpful in identifying issues and enabling quicker repairs, especially indications of the sand filter system clogging. With a clogged sand filter, the filtered water holding tank did not fill correctly. Since Tubs 9 and 10 were gravity fed from the sand filter, they also reported low (Figure 12).

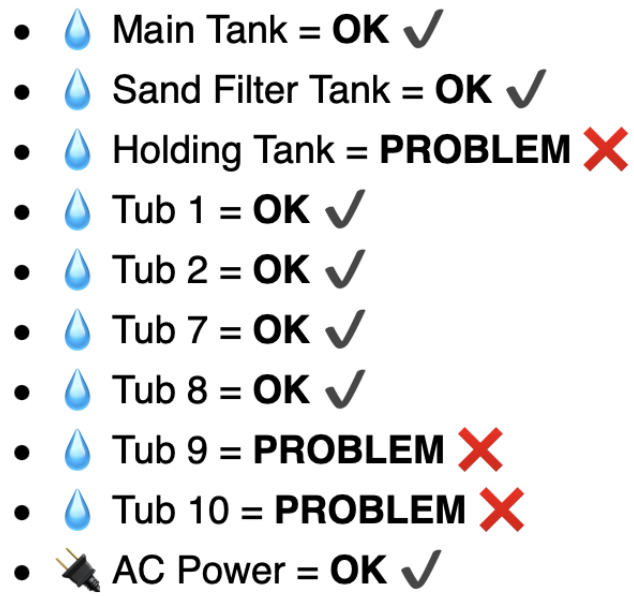


Figure 12. Monitoring system email showing the slow sand filter clogging issue which required cleaning (removal of top 4" of the sand filter to enable water to effectively flow through the filter post-cleaning).

Monitoring and Power System

The monitoring and power systems were installed in the existing building. The system was connected to existing AC power and provided the 12 VDC required for the field devices and a collection point for status monitoring. An image of the panel and labels for the major components are shown in Figure 13. The entire control system was sophisticated and allowed efficient operations of the experimental apparatus.

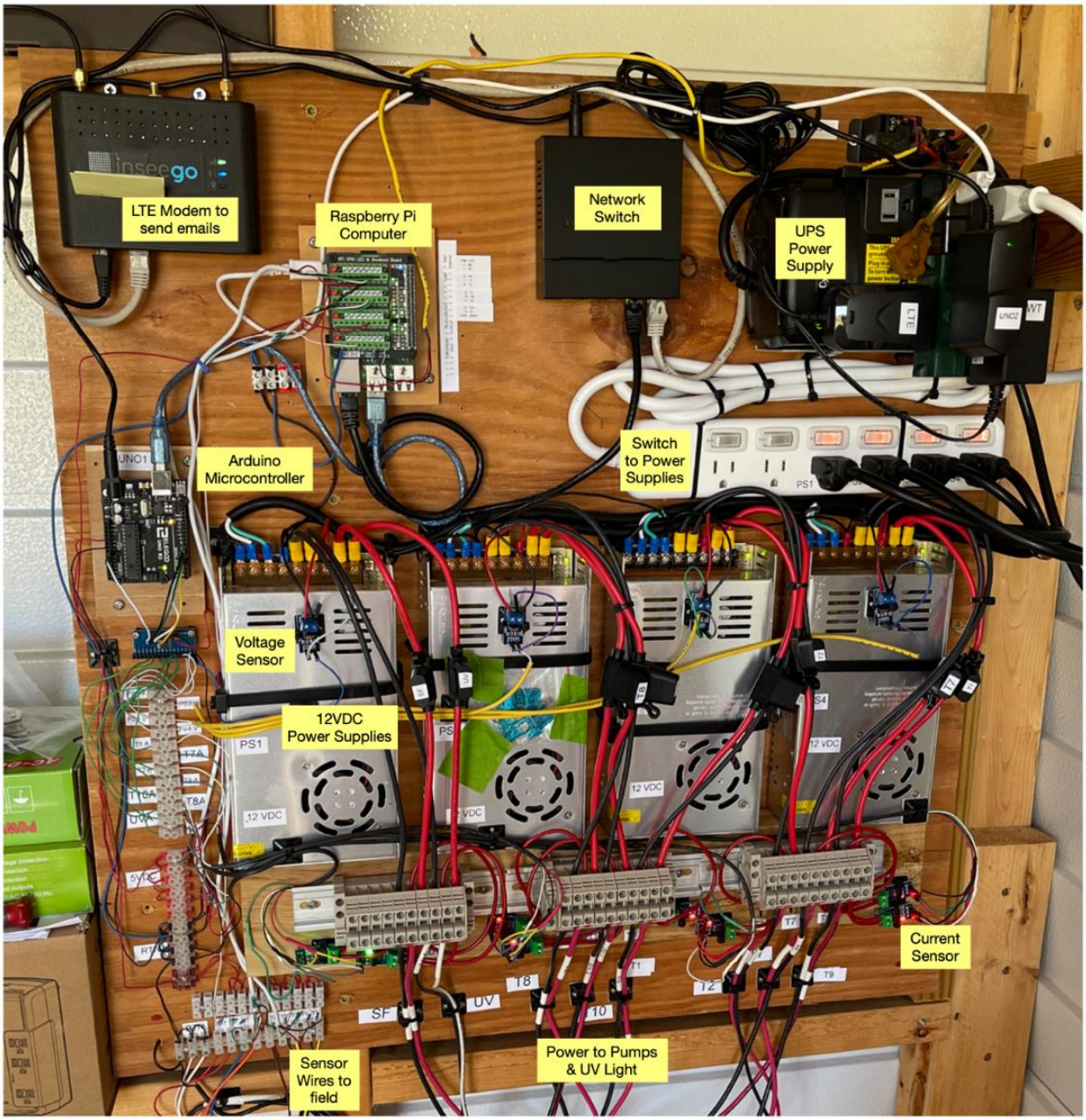


Figure 13. Control panel and monitoring system.

Design and Operation of the Slow Sand Filter

The original design contained two small slow sand filters. This design was found to be economically inefficient and was replaced by a single slow sand filter with a substantially larger volume of graded sand. The dimensions of the 2500-gallon slow sand filter tank were approximately 102- inches in diameter and 79-in inches in height. The tank was modified by adding a 30-inch hole in the center of the top and a discharge hole 2 inches in diameter at the base. Approximately 23,000 lbs. of sand were placed into the tank in graded layers (Figure 14). As shown in Figure 7, it was necessary to stabilize the tank by installing a series of 4 x 4-inch posts that were cemented into the ground. Straps were placed around the structure to prevent the tank walls from splitting. The top of the posts were interconnected to allow ease of entry of a person into the tank during cleaning.

The slow sand filter was constructed based on the standard design used in potable water treatment facilities (Huisman and Wood, 1974; Crittenden et al, 2005). The basal layer of gravel was 1 ft thick and consisted of 1/8-in x 1/4-in (3.175 mm x 6.35 mm) gravel. The gravel base was constructed by placement of an initial 3-inch layer. Then, a network of 2- inch diameter, schedule 40, machine slotted PVC pipes were placed atop the gravel layer. The ends of the screen were capped, and the screen extended to a 2-inch diameter, schedule 40 PVC outflow pipe. A special fitting was used to seal the discharge line from the tank to prevent leakage. An additional 9 inches of gravel was placed above the collection screen. The approximate flow rate through the slow sand flow rate was about three GPM to produce a contact time of about five hours. A spillover at the top of the filter maintained one foot of driving head.

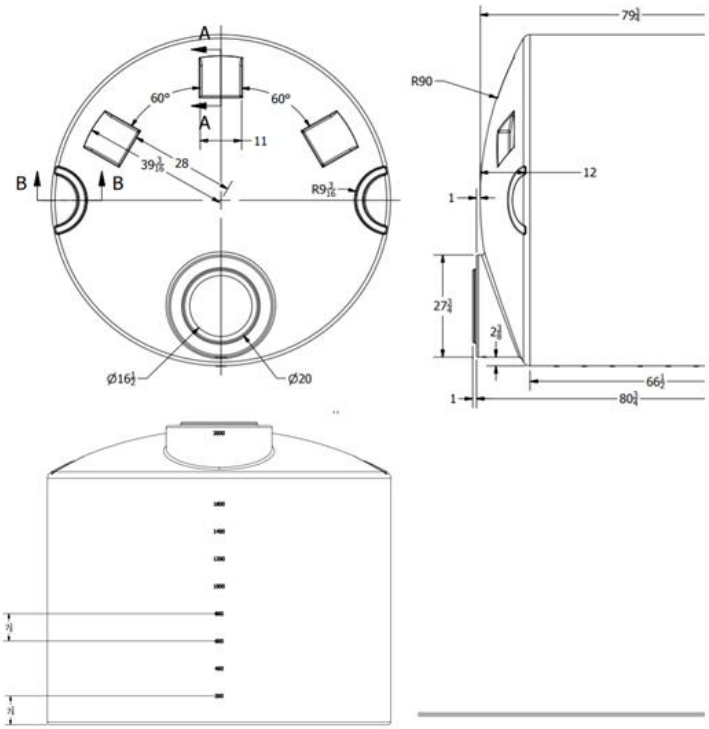


Figure 14. Slow sand filter design (dimensions in inches).

A series of four sand layers were placed above the gravel with sufficient grading to prevent the layers of sands from plugging the intake screen. The first layer was 1.19-2.38 mm sand with a thickness of 6 inches. The next layer upward was a 6-inch layer of sand with a size range of 0.85-1.68 mm. This layer was followed by 12- inches of sand with a size range of 0.59-1.19 mm. The top and primary sand filtration layer was 24- inches thick and had a size range from 0.42-0.50 mm. The space between the top of the sand filter and the inflow pipe provided the driving head to operate the filter by gravity. The inflow pipe contained a number of "spokes" containing holes to allow the water to flow into the filter evenly during startup. A spillover pipe was also installed to maintain the head in the tank at a constant number. The hole in the top of the tank was sufficiently large to allow manual cleaning of the tank when the control system alerted that the sand filter was clogged.

An organic and particulate layer formed at the water sand interface, which is termed the *schmutzdecke*. A considerable amount of biochemical water treatment occurred in this layer, which tended to become a few cm thick. When the rate of water flow through the *schmutzdecke* becomes too slow, the filter had to be cleaned by removing this organic layer and replacing it with clean sand of the same size. The automated telemetry system provided an indication of when cleaning was necessary (see control section). The duration of operation before cleaning was dependent on the quality of the source water being filtered. In major water treatment plants using rivers or reservoirs, the cleaning time typically ranges from 1 to 3 months. The Caloosahatchee River water quality contains an extremely large quantity of organic material and turbidity, which caused cleaning in the early operational stages (test stage) to occur every 20 to 23 days. After a month and one half of operation, it was necessary to clean the filter every 12 to 15 days.

Water Quality Sample Collection

The water quality sampling scheme was developed to assess the veracity of the water treatment technologies employed in comparison to the baseline system. Within the operating system, 16 locations were established to adequately monitor water quality to allow full technology evaluation. The sample locations are given in Table 2. There was some purposeful redundancy in the sampling because some organic material can accumulate within the plumbing system and could cause some variation in both the inflow water and in the transport of water between the wetland treatment tubs.

Table 2. Locations of the water quality samples collected with form used quality control assurance and tracking.

Station No.	Description	Sample No.	Sample Date	Sample Time
1	Control inflow to *Typha* tub			
2	Control outflow from *Typha* tub			
3	Control inflow to *Vallisneria* tub			
4	Control outflow from the *Vallisneria* tub			
5	Slow sand filter inflow			
6	Slow sand filter outflow			
7	Slow sand filter secondary-holding tank			
8	Slow sand filter inflow to *Typha* tub			
9	Slow sand filter outflow from *Typha* tub			
10	Slow sand filter inflow to *Vallisneria* tub			
11	Slow sand filter outflow from *Vallisneria tub*			
12	Slow sand filter + UV treatment discharge			
13	Slow sand filter + UV treatment inflow to *Typha* tub			
14	Slow sand filter + UV treatment outflow from *Typha* tub			
15	Slow sand filter + UV treatment inflow to *Vallisneria* tub			
16	Slow sand filter + UV treatment outflow from *Vallisneria*			

The water quality of the inflow water from the Caloosahatchee River was measured at two locations, which are stations 1 and 5. Effects of the vegetation treatment on the Caloosahatchee River water as a control can be evaluated by comparison of the data from stations 1 and 4. The treatment provided in the control train for solely the *Typha* tub can be evaluated by comparing data from stations 1 and 2 and the tape grass tub by comparing data from stations 3 and 4. Variation in the water quality caused by growth in the pipe between the two vegetation tubs can be observed by comparing data from stations 3 and 4.

The impact on water quality from slow sand filtration can be assessed by comparing the data from stations 5 and 6. The full impact of slow sand filtration and vegetation treatment can be compared by assessing water quality changes between stations 5 and 11. Any water quality changes occurring in the holding tank (used for hydraulic flow balance) can be evaluated by comparing data from stations 6 and 7. Note that the holding tank was painted black to inhibit aquatic plant and biofilm growth. Any water quality changes induced to pipe transport between the holding tank and the slow sand filter *Typha* tub can be evaluated by assessing changes between stations 7 and 8. The effectiveness of water treatment by the slow sand filter *Typha* tub can be evaluated by assessing changes between stations 8 and 9. Any impacts of water quality of the pipe connecting slow sand filter water between the T*ypha* and *Vallisneria* tub can be evaluated by comparing data from stations 9 and 10. The treatment effects of the slow sand *Vallisneria* tub can be evaluated by comparing data from stations 10 and 11.

The combined slow sand filtration and UV treatment with vegetation treatment can be evaluated by comparing data from stations 5 and 16. The impacts of any connection pipe organic shedding between the combined slow sand filter and UV discharge and the *Typha* tub can be evaluated by comparing the data from stations 12 and 13. The impacts of the T*ypha* tub treatment for the slow sand filter and UV treatment can be evaluated using a comparison between stations 13 and 14. Any pipe impacts to water quality between the wetland plant tubs for slow sand filter and UV treated water can be evaluated by comparing the data from stations 14 and 15. The treatment provided for the slow sand filter and UV treatment by the *Vallisneria* tub can be evaluated by comparing the data from stations 5 and 15.

Water Quality Measurements and Laboratory Methods

A series of chemical parameters were measured in the field using meters during each of the sampling events, while water samples were collected for transportation to the laboratory for chemical analyses. The sampling methods followed the filed QAPP as approved by Lee County and the Florida Department of Environmental Protection (FDEP). The FDEP required that the laboratory doing the primary analytical work on the samples was NELAC certified. Therefore, the samples were analyzed by Sanders Laboratories and their subcontractor Pace Analytical. These laboratories are certified and approved by the FDEP and have filed QAPP documents with the department. They follow the Standard Operating Procedures (SOPs) required by the FDEP.

The only analytical procedure performed at the Florida Gulf Coast University Emergent Technologies Institute laboratory was the quantification of the bacteria in the water using a flow cytometer. The SOP and description of the analytical methods used is described based on the research work of Harvey et al. (2020). While these samples were analyzed for seawater, the procedure is generally the same.

The method from Harvey et at. (2020) is: *Flow cytometry has become the standard method to quantify microbial cell numbers in lieu of conventional fluorescence microscopy because it can quickly analyze the number, size, viability, and the physiology of cells with a combination of various fluorescent dyes (Hammes et al., 2012). SYBR Green nucleic acid stain is the predominant staining solution for use with the flow cytometer to analyze bacteria cell concentrations in water samples and has been done with samples from Saudi Arabia and other global locations (Dehwah and Missimer, 2016). Similar methods have been used to quantify bacterial counts in samples from Lake Zurich, Switzerland (Hammes et al., 2008) and the Chungcheong province in Korea (Park et al., 2018).*

Water samples used for microbial cell counts were put into 50 mL centrifuge tubes with 2% volume of 30% formaldehyde solution and placed at -80°C until analysis. Bacterial and algal cells were measured using an Accuri C6 plus flow cytometer. Lasers were used to excite unstained autofluorescent cells of phototrophs (mainly picocyanobacteria) and stained bacterial cells. Laser wavelengths were set at 488 nm for blue, green emission collected in the FL1 channel (533 +/- 30nm) and red fluorescence in the FL3 channel (>670nm) [35,36]. The flow cytometer was calibrated using 2 drops of BD™ CS&T RUO Beads (beads consist of equal quantities of 3-µm bright, 3-µm mid, and 2-µm dim polystyrene beads in phosphate buffered saline (PBS) with bovine serum albumin (BSA) and 0.1% sodium azide) in 500 µL of ultrapure water. The frozen water samples were thawed in a beaker of warm water for approximately 10 minutes before performing the analyses. For the bacterial counts, 50 mL of each water sample was pipetted

into 10 mL tubes and placed in 35°C water to incubate for 10 minutes. The samples were then stained with 5 µL of SYBR Green II RNA gel staining solution, vortexed, and placed back into the 35°C water to incubate for another 10 minutes (Alshahri et al., 2017; Van der Merwe et al., 2014). After the second incubation, each sample was vortexed and measured on the flow cytometer individually. The system settings used were as follows: the run limit was set to 50 µL, fluidics on medium (35 µL /min; core size 16 um), and the threshold was set to 600 in the FL1 channel for the total bacterial cell counts [36]. For unstained autofluorescent counting of autotrophs, 500 µL of from each sample was pipetted into a 10 mL tube and incubated for 10 minutes at 35° C and then processed with a run limit set to 50 µL, fluidics was set on 'medium' (35uL/min; core size 16 µm) and a threshold of 900 for red fluorescence in the FL3 channel was used (Van der Merwe et al., 2014). Each time three vials were analyzed as triplicates.

Pre-Sampling Planting and Establishment of the Mesocosm Vegetation

When the project was started, the vegetation in the three tubs that utilized emergent vegetation (i.e., tubs 8, 9 and 10) already contained *Typha domingensis* (cattail) as well as some *Schoenoplectus californicus* (giant bullrush) and some other sedges and grasses such as the invasive plant *Panicum repens* (torpedo grass, Figure 15).

Figure 15. Top: Vegetative state of the tubs prior to being cleaned (07/02/2021). Photo taken with an aerial drone (DJI™ phantom 4 Pro). Bottom left: cleanup of the phytodetritus and sediment. Bottom right: tub after being cleaned.

All tubs also contained a significant amount of phytodetritus, some of which had turned into a dark organic sediment overlying a layer about a foot thick of sand sourced from the property.

From 07/12/2021 to 07/15/2021, the tanks were cleaned of their vegetation as well as of their phytodetritus and sediment (Figure 15). This was accomplished by first unrooting the vegetation by hand and hand tools whilst taking care of preserving the roots of *T. domingensis* and *S. californicus*. The least severely damaged individuals of those two species were kept in a horse trough filled with water until they could be replanted (Figure 16). The heads of *T. domingensis* were chopped off with a machete as special attention was taken to limit the

damage of the rhizomes (Figure 16). The removal of the phytodetritus and the sediment was done after the tanks were drained overnight. These materials were allowed to dewater and cake on top of the sand, so that they could be removed out of each tub. On average, about 6 cart loads (approximately 60 cubic feet) were removed from each tub (about 7-8 inches of sediment + detritus accumulation in each tub). The planting then occurred from 07/16/2021 to 07/17/2021 (Figure 16) by splitting the amount of *T. domingensis* (about 195 individuals with about 65 transplants per tub) and *S. californicus* (about 300 individuals with 100 transplants per tub) amongst the three tubs. Plants were thus at about 10-12 inches from one another to achieve a plant density of about one plant per square foot. The water level in these tubs was then set at about a foot above the surface of the sand substrate and the plants were randomly planted as to occupy the entire surface of each tub. The plants were then allowed to grow for approximately four months using untreated river water flowing through the tubs in and out.

The three tubs selected for the submerged vegetation (tubs 1, 2 and 7) contained either emergent rooted vegetation as aforementioned in tubs 8, 9 and 10 (albeit with more undesirable vegetation) or a mixture of mostly macroalgae (*Chara* sp., muskgrass or stonewort) with some invasive *Hydrilla verticillata* (waterthyme) as well as a mixture of filamentous green algae with the dominant alga being *Cladophora* sp. These tanks were cleaned similarly to the other tanks, and planting of *Vallisneria americana* (tape grass or eel grass) was accomplished between 07/16/2021 and 07/17/2021. These shoots originated from a donor detention pond in Cape Coral, Florida, and they were left acclimating in tanks under an 80% canopy at the FGCU Buckingham property. Plants were planted by hand in the sandy substratum at every 5-6 inches (about 4 plants per square foot) so that each tub was planted with about 400 plants. The water level was set in the submergent tubs at about 3 ft above the soil. The tape grass was fed with river water for about four months before treatment was initiated.

For all the tubs, before the experimental treatment system was turned on, all tanks were inspected and almost all undesirable algae and vegetation was removed. The environmental conditions in the tanks were in very good condition at the start of the experiment (Figure 16).

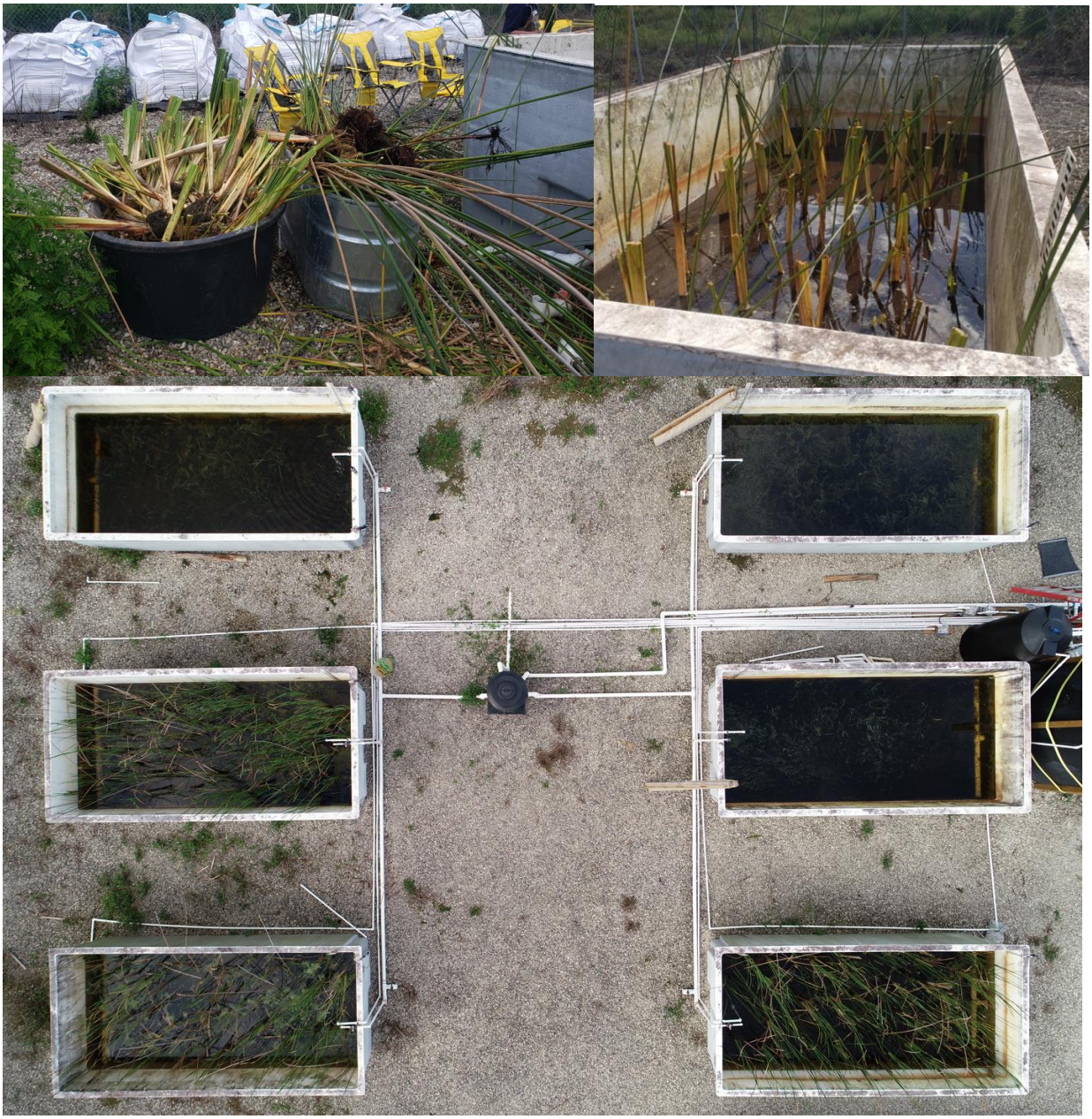


Figure 16. Top: plants being set aside before being sorted and planted (left) and after being planted (right). Bottom: Vegetative state of the tanks the day the experiment started (11/21/2021). Photo taken with an aerial drone (DJI™ phantom 4 Pro).

Monitoring of the Mesocosm vegetation

The vegetation was monitored during each sampling of water quality and during cleaning of the slow sand filter. Floating vegetation (i.e., *Lemna minor* (duckweed) and the fern *Azolla* sp.) was netted out of all tubs after the first event only, and it was conducted in all submerged vegetation tubs for all other events. The tubs with submerged vegetation also were cleaned from encroaching green filamentous algae (mainly *Cladophora* sp.) using nets and by gentle raking. This green alga grew in abundance as metaphyton (a floating mat) as well as epiphyton

(attached on the *V. americana*) within all tanks and especially in the control tank. It interfered with the light source in the submergent vegetation tanks. During the months of March through the end of the experiment, the tubs were covered with a tan shade cloth (light blocking of approximately 50%) to limit the green filamentous algae. Additionally, some sparse stands of *H. verticillata* appeared, but those were left in place as submergent plants.

Drone surveys using a DJI™ phantom 4 pro were conducted during all of the water sampling events, and photographs of each tub were taken over time to show the condition of the vegetation. To cut through the glare of the water surface in the tubs, the camera of the drone was covered with a polarized lens. These photographs were taken after the water was sampled and were ideally taken when the sun was at its zenithal position. However, for some events, these photographs were taken in the middle of the afternoon with less ideal lighting. Special care was taken to have the drone positioned on the apex of each tub with the drone stationary at about 10 feet above it. At the office, each photograph was rotated to orient it horizontally, and it was cropped to show the border of each tub only. Attempts to enumerate *V. americana* were in vain as the water was tannic and only the plants close to the surface could be accounted for. Passing the hand above the bottom of each tank confirmed that many *V. americana* could not be accounted for using the drone. This issue was, however, not a real problem for accounting the emergent vegetations, which could be well accounted at the beginning of the experiment as well as at the end especially in the absence of wind. For this enumeration, photographs were contrasted in PowerPoint and then marked with a digital pen. For *T. domingensis*, a shoot with several leaves would count as an individual whilst for *S. californicus*, each stem was counted as long as they were not visually too clustered spatially. This would not replace an actual count in situ, but this surrogate method gave a fair and pretty consistent assessment of the plants' growth (Figures 17 and 18). Notes accounting for the extent of floating vegetation and algae as well as the number inflorescences for *T. domingensis* were also recorded.

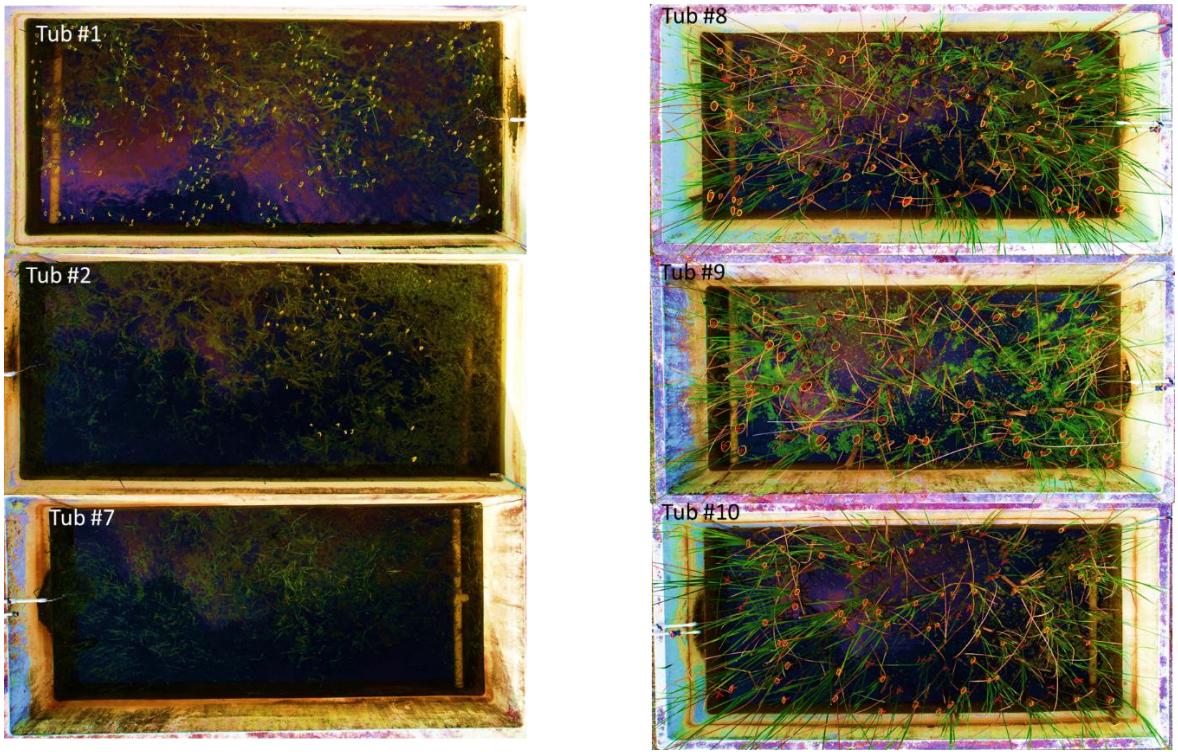


Figure 17. Enumeration of emergent and submerged vegetation by boosting the contrast of the aerial pictures (11/21/2021). This was done for all eight events. Note that only part of the submerged vegetation is visible so that the accounting of those was aborted. Markings were made using a stylus and a digitizing tablet.

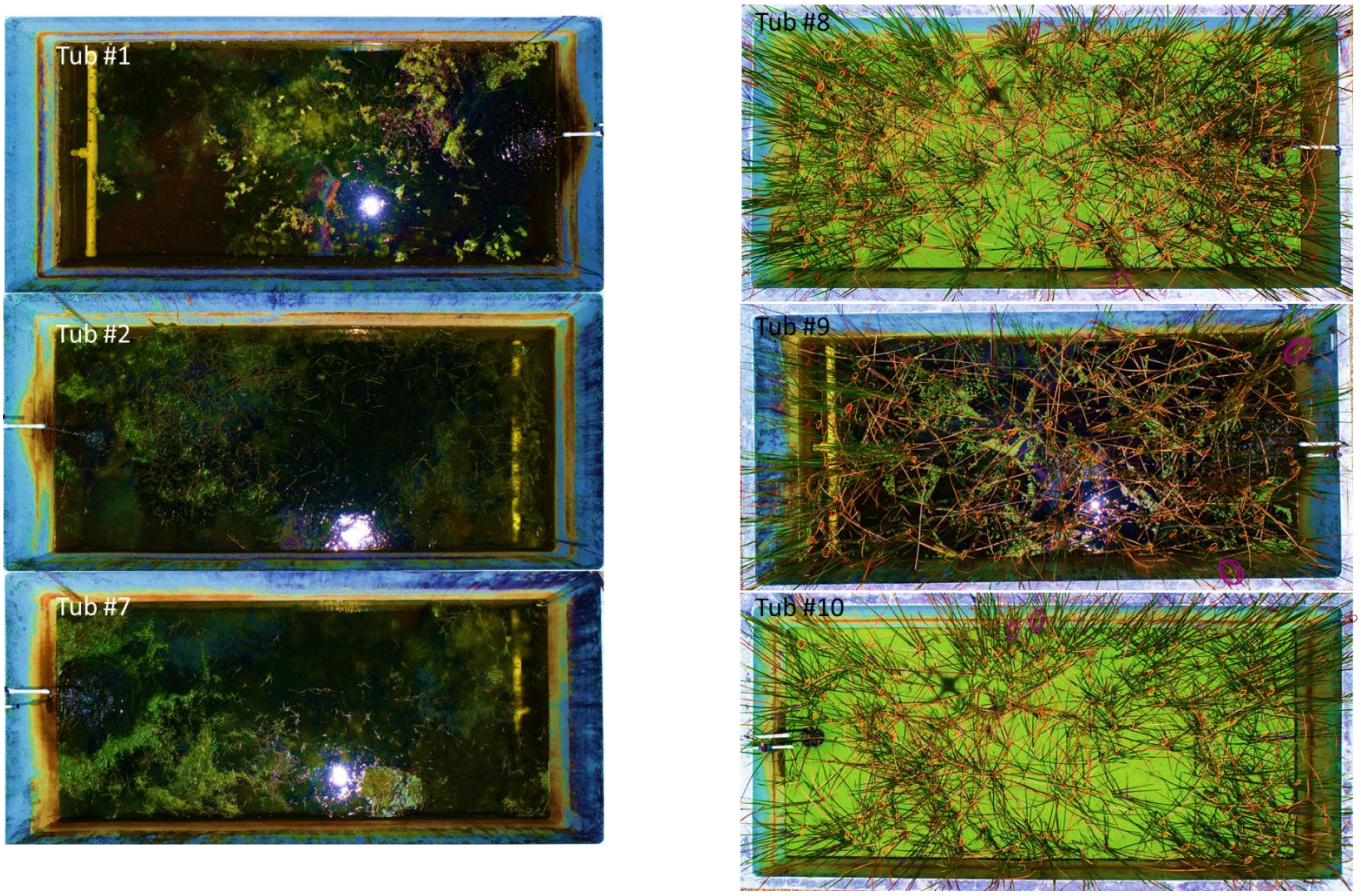


Figure 18. Photographs of the tubs on 05/18/2022. Note the absence of floating vegetation in Tub 9 (UV treatment) in comparison with Tub 8 (reference) and 10 (filter only). Also noteworthy is the collapse of the submerged vegetation in all tubs, but very pronounced in Tub 1 (reference) and the remaining floating filamentous green algae in all the tubs.

Operational issues

There were two operational issues that created great difficulty and impacted the project. First, water from the river was supplied by a pump and pipeline maintained and operated by the South Florida Water Management District. The main supply pump failed during the Christmas holiday season after the second sampling event, which occurred on December 21, 2021. The District had to order special parts, and the pump was not operational until about February 14, 2022. A second pump failure occurred on about June 7, 2002. In this case, lightning struck the transformer and destroyed the wiring to the pump and the pump. There were no funds in the District maintenance budget to buy a new pump and to reconstruct the system to allow the next four sampling events, which was two months of operation. This issue caused curtailment of the project.

The second major operational problem was the rapid rate of the slow sand filter clogging. The original project labor budget was based on 12 cleanings during the life of the project based on a very conservative estimate made from data collected at numerous slow sand filtration plants operated around the world. The initial clogging period was between 20 and 23 days

based on the river water quality. Unfortunately, the clogging period reduced to 13 to 15 days and caused greatly increased labor costs. A request to Lee County and the FDEP was made to decrease the sampling period to 14 days rather than monthly due to the losses based on labor and materials overruns. If the project were to be completed over the full 12 months, additional filter sand would have had to be purchased in addition to the labor costs.

While the operational difficulties limited the number of sampling events to eight from the 12 desired, the sampling events occurred in a full range of climatic and seasonal conditions that occurred onsite and in the Caloosahatchee River. This project shows the necessity of adaptive management when investigating natural systems with engineering enhancements.

Statistical methods

It is essential to perform a statistical analysis at a certain meaningful abstraction level to find interesting patterns and to determine whether the result of a data set is statistically significant (Chen et al., 2002). Three treatment trains that are considered in this study include (1) Treatment Train A (TTA)-Control: Raw Water/Vegetation Tank 1 out (Typha)/Vegetation Tank 2 Out (Vallisneria), (2) Treatment Train B (TTB): Raw Water/Sand Filter In/Sand Filter Out/Vegetation Tank 1 Out (Typha)/ Vegetation Tank 2 Out (Vallisneria), (3) Treatment Train C (TTC): Raw Water/After Sand Filtration/After UV/ Vegetation Tank 1 out (Typha)/ Vegetation Tank 2 Out (Vallisneria). Raw water quality parameters were measured for each treatment train.

The inflow to TTA was directly connected to the control *Typha* tub, while the inflow for the TTB and TTC was connected to Slow Sand Filtration System. The Shapiro-Wilk test was performed on the data to assess for normality. The results showed that data was normally distributed ($p > 0.05$), so parametric statistical analyses, such as t-tests and ANOVA, are required. If the data were non-normal, a non-parametric test, such as Kruskal-Wallis would be required.

A two-sample t-test was performed to compare water quality parameters in the inflow to TTA and TTB/C. In addition, a two-sample t-test was performed to compare water quality parameters within each treatment train. The two-sample t-test is used to determine if the means of two groups are equal. A one-way ANOVA was performed to compare three treatment trains for 12 key water quality parameters. A one-way ANOVA compares the means of two or more independent groups in order to determine whether there is statistical evidence that the associated group means are significantly different

Results

Evaluation of the Effectiveness of the Treatment Technologies

To evaluate the effectiveness of the various process and treatment trains, temporal graphs showing measured concentrations and box plots were used for the water quality parameters.

Sand filter treatment effectiveness independent of the vegetation tubs

Data were collected from Stations 5 and 6 to evaluate the treatment provided by the slow sand filter. Station 5 was the raw water river water from the main storage tank onsite. The samples were collected from the inflow to the sand filter at the top. Station 6 was located at the sand filter outflow at the outflow valve.

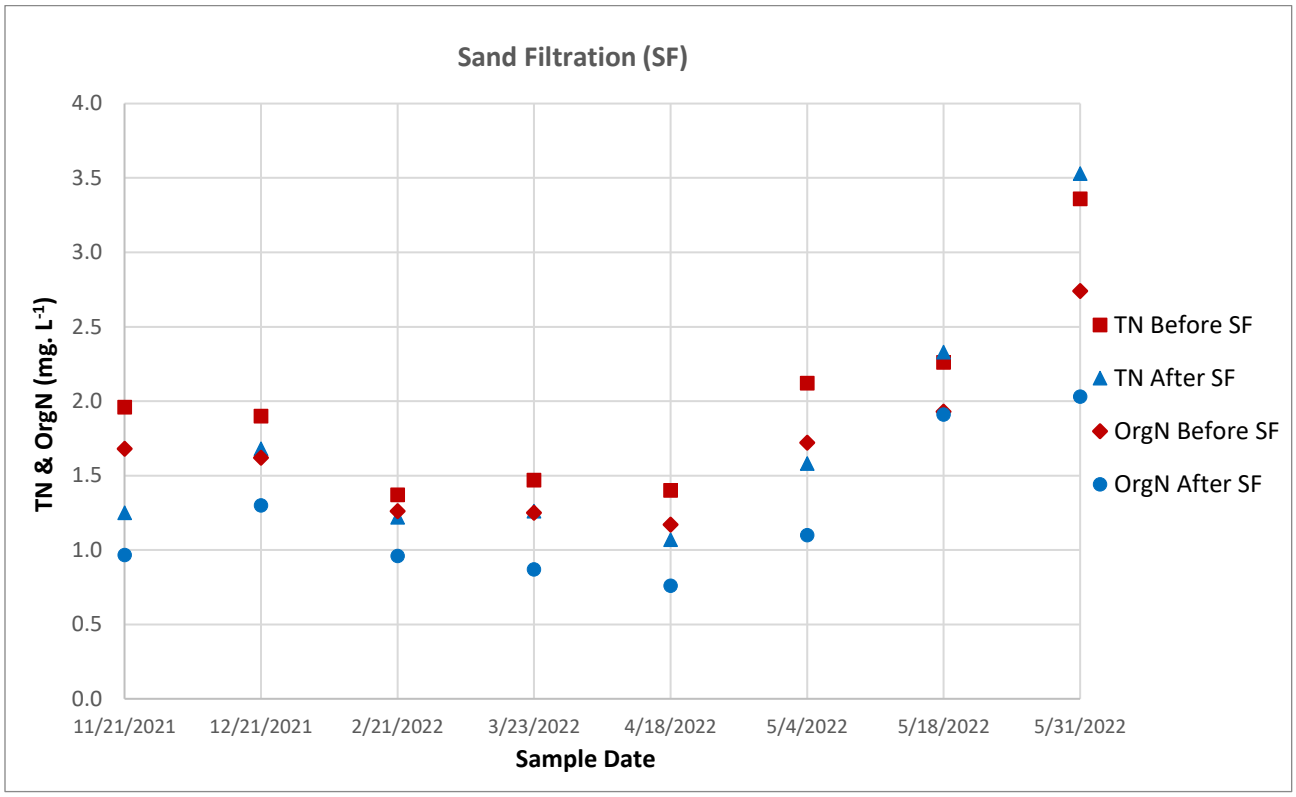


Figure 19. Plot of the total nitrogen and organic nitrogen before and after the sand filtration.

Figure 19 shows that in most cases the total nitrogen was lower after sand filtration, and there was also a reduction in the organic nitrogen. The box plots in Figure 20 show the same trend with a slight lowering of total nitrogen and organic nitrogen provided by sand filtration. It should be noted that some of the outlier measurements do impact the box plot full ranges in concentrations.

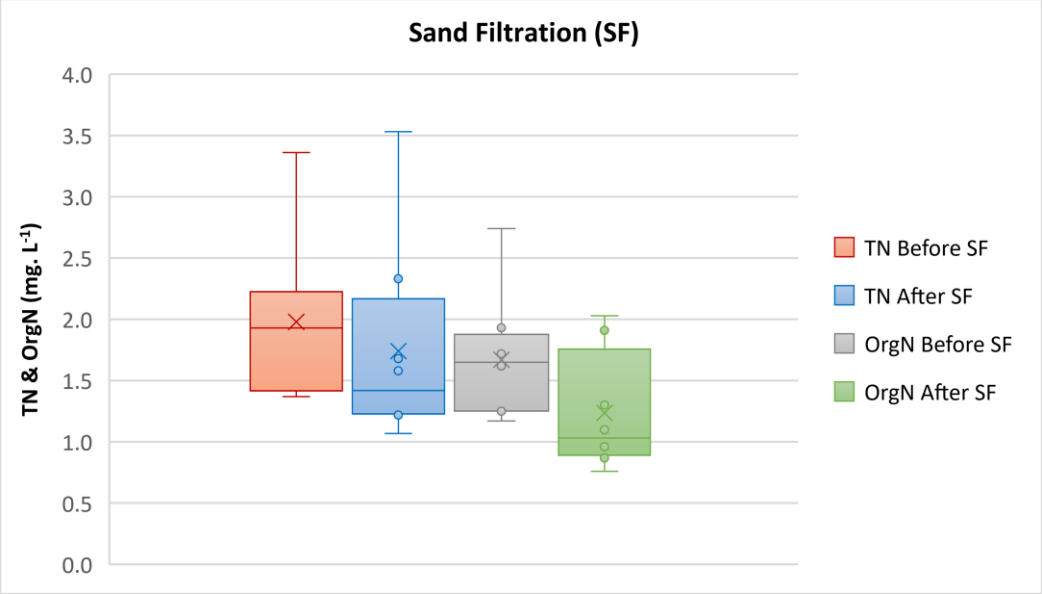


Figure 20. Box plots of total and organic nitrogen before and after sand filtration.

Figures 21 and 22 show that nitrate and nitrite concentrations increased during sand filtration, but ammonia decreased. There is considerable scatter in the data obtained. Based on the reductions in total and organic nitrogen, it appears that the increase in nitrate and nitrite concentration occurred based on breakdown of ammonium, suggesting there may have been nitritation and nitratration occurring within the sand matrix.

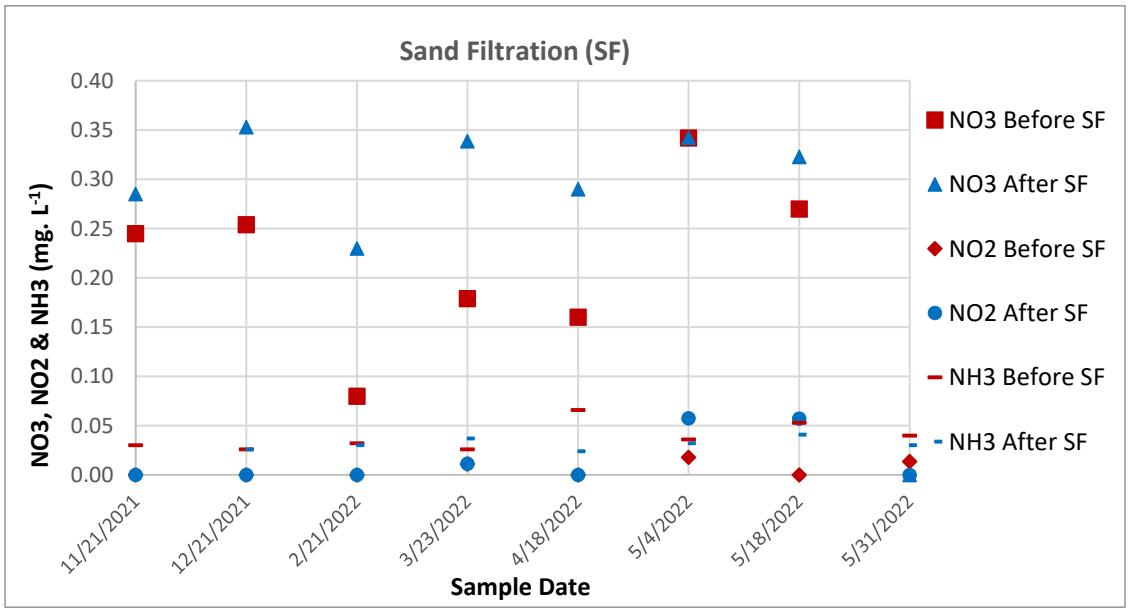


Figure 21. Temporal changes in nitrate, nitrite, and ammonia concentrations before and after sand filtration.

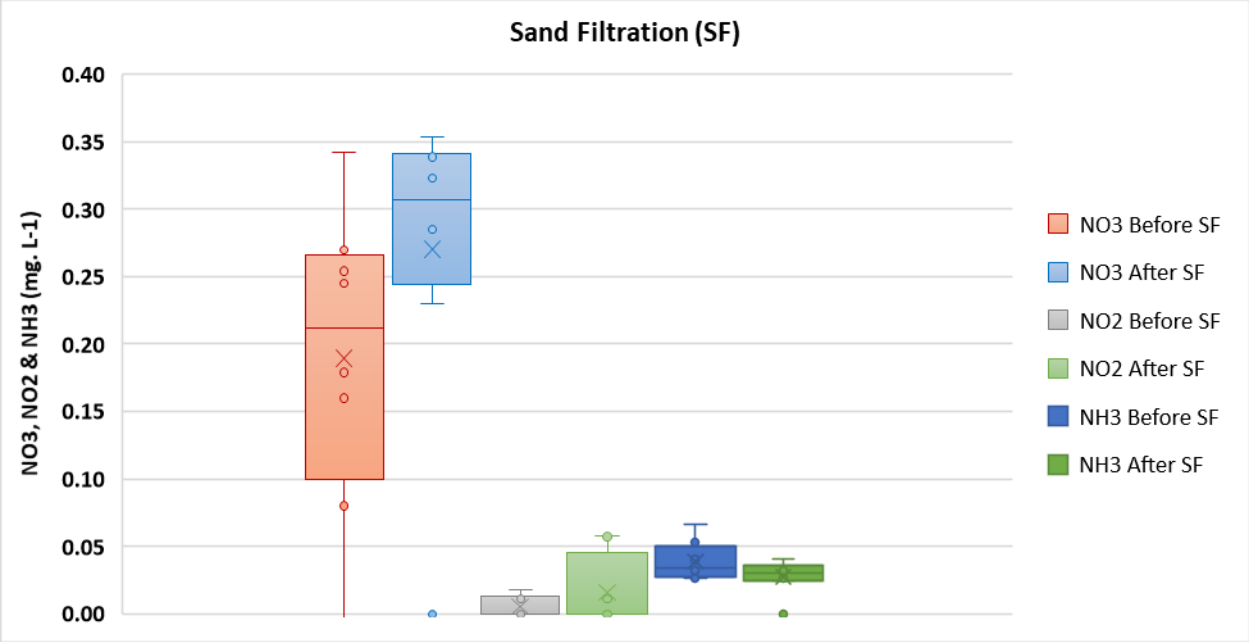


Figure 22. Box plots of nitrate, nitrite, and ammonia before and after sand filtration.

Changes in the concentrations of total phosphorus and orthophosphate are show in Figures 23 and 24. In the temporal plot, there is considerable scatter in the data (Figure 23). The box plots in Figure 24 show an increase in the concentration of both total phosphorus and orthophosphate during sand filtration. There were outlier measurements that somewhat impacted the analysis.

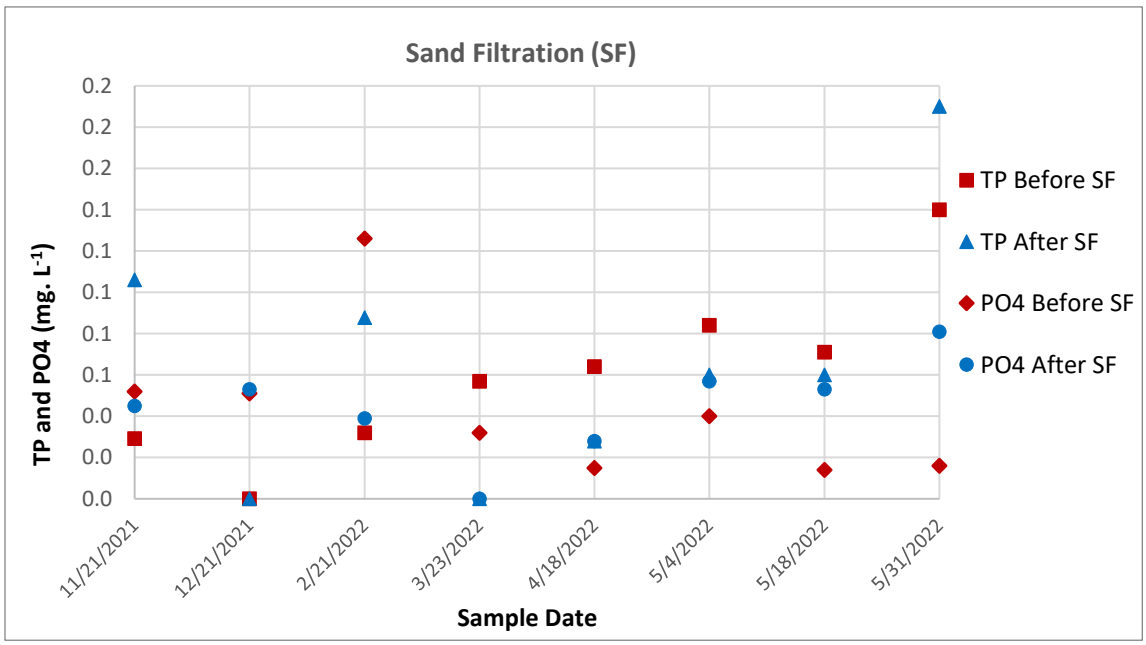


Figure 23. Temporal concentrations of total phosphorus and orthophosphate before and after slow sand filtration.

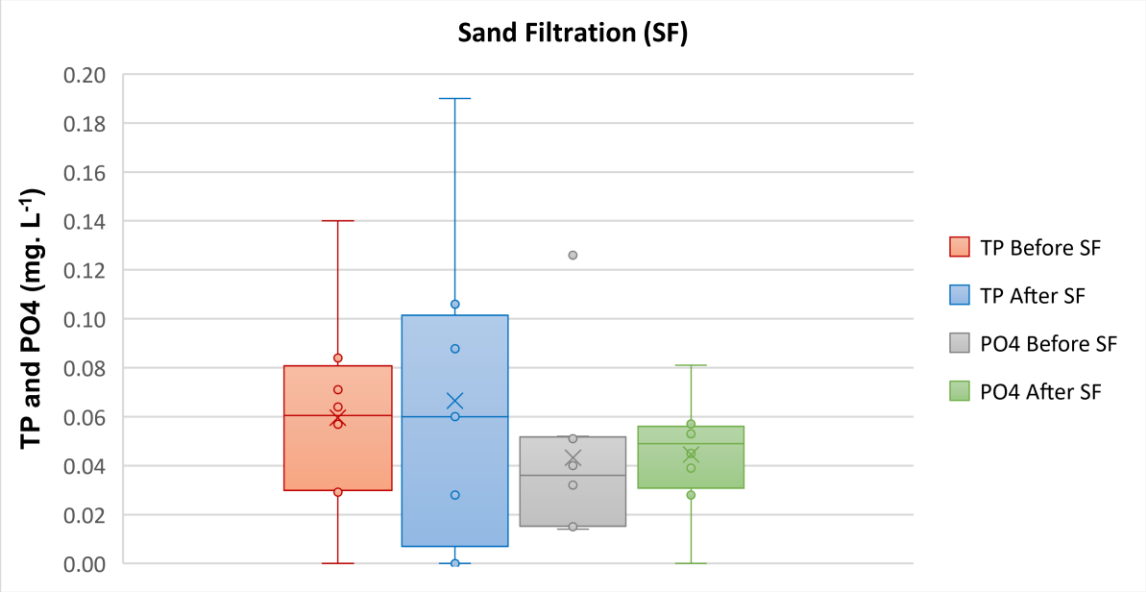


Figure 24. Box plots of total phosphorus and orthophosphate before and after slow sand filtration.

Both total and dissolved organic carbon concentrations decreased during slow sand filtration (Figures 25 and 26). The scale of the temporal changes in Figure 25 does not clearly show the reductions, but the box plots clearly show it (Figure 26).

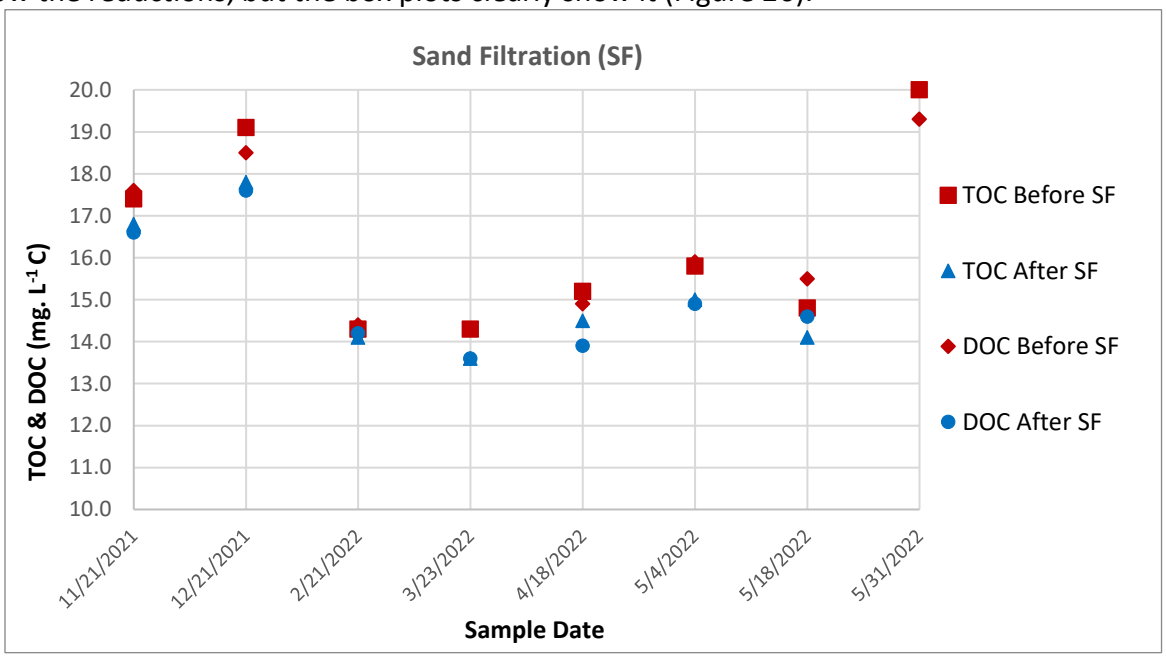


Figure 25. Temporal plots of total and dissolved organic carbon before and after sand filtration.

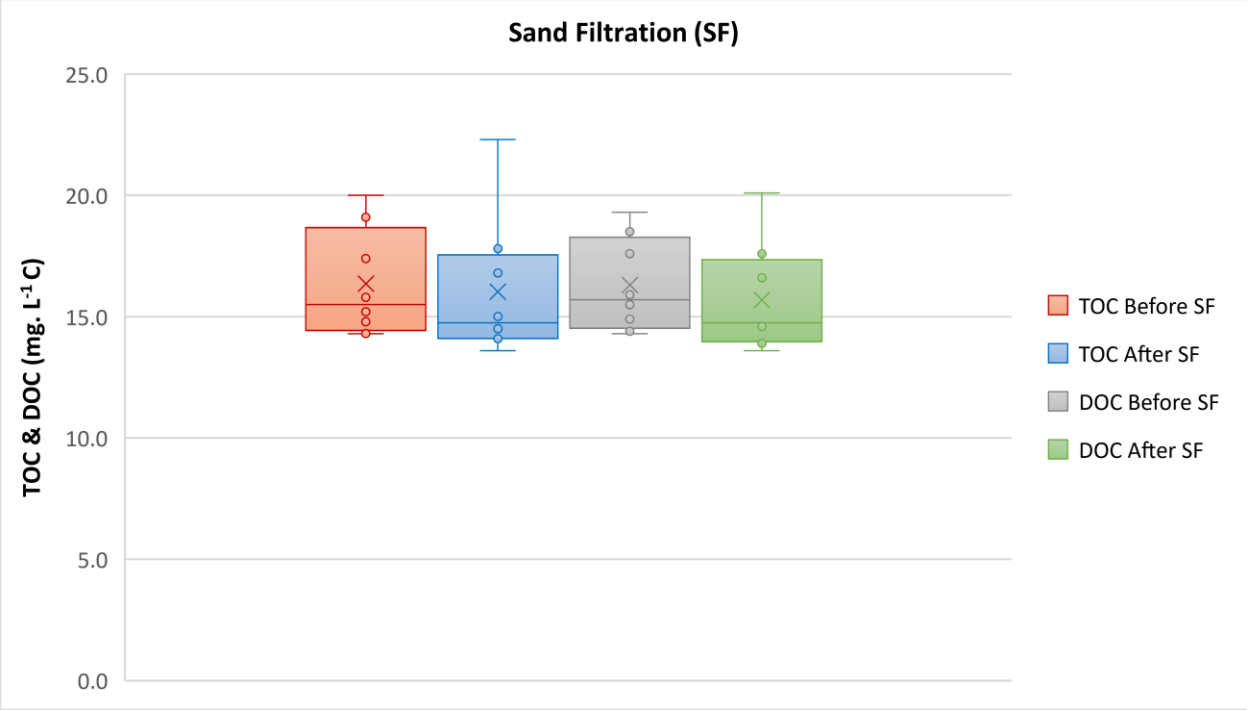


Figure 26. Box plots of the total and dissolved organic carbon before and after sand filtration.

The concentrations changes in chlorophyll A before and after slow sand filtration are shown in Figures 27 and 28. In this case, both laboratory and field measurements were made and showed differing results. The field instrument data showed a slight decrease in chlorophyll A concentration, while the laboratory data exhibit a major reduction. The laboratory data are supported by observations during operation where the top of the filter required removal of an organic crust every 13 to 23 days. Much of this material was organic debris and living algal and bacterial material.

As expected, both the temporal and box plot data show substantial reductions in the concentration of total bacteria, algae, and cyanobacteria during slow sand filtration (Figures 29 and 30). It is particularly interesting that some concentrations of total bacteria, algae, and cyanobacteria did break through the sand filtration. The percentage of breakthrough was total bacteria > algae > cyanobacteria.

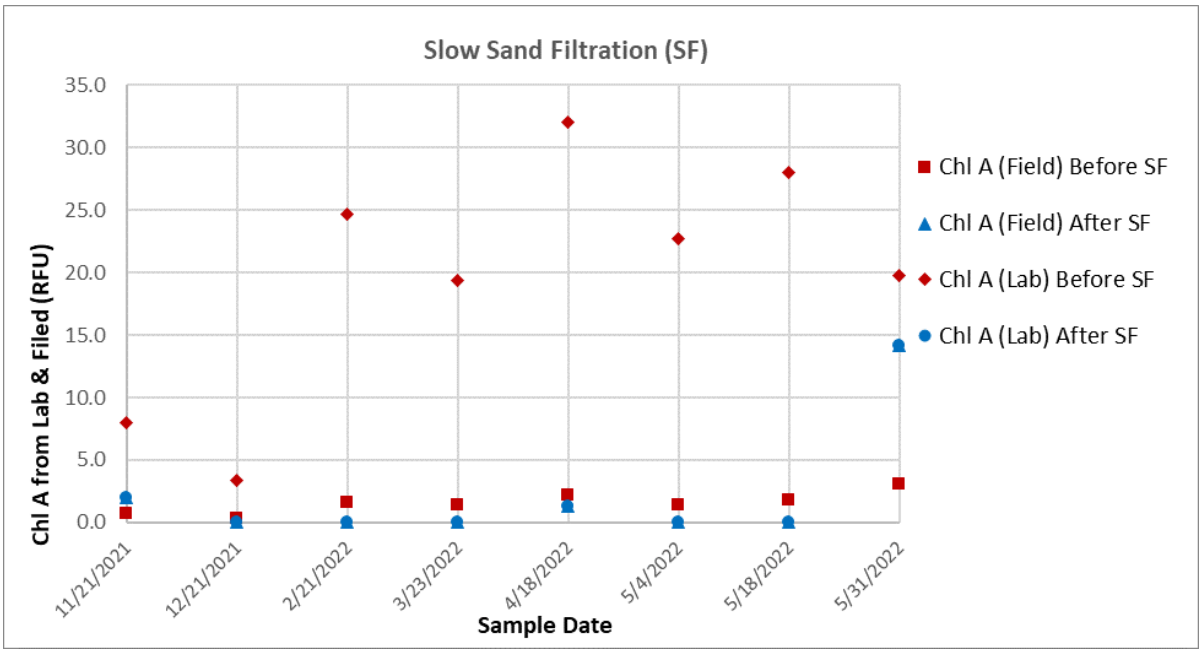


Figure 27. Chlorophyll A measured in the field and laboratory before and after slow sand filtration.

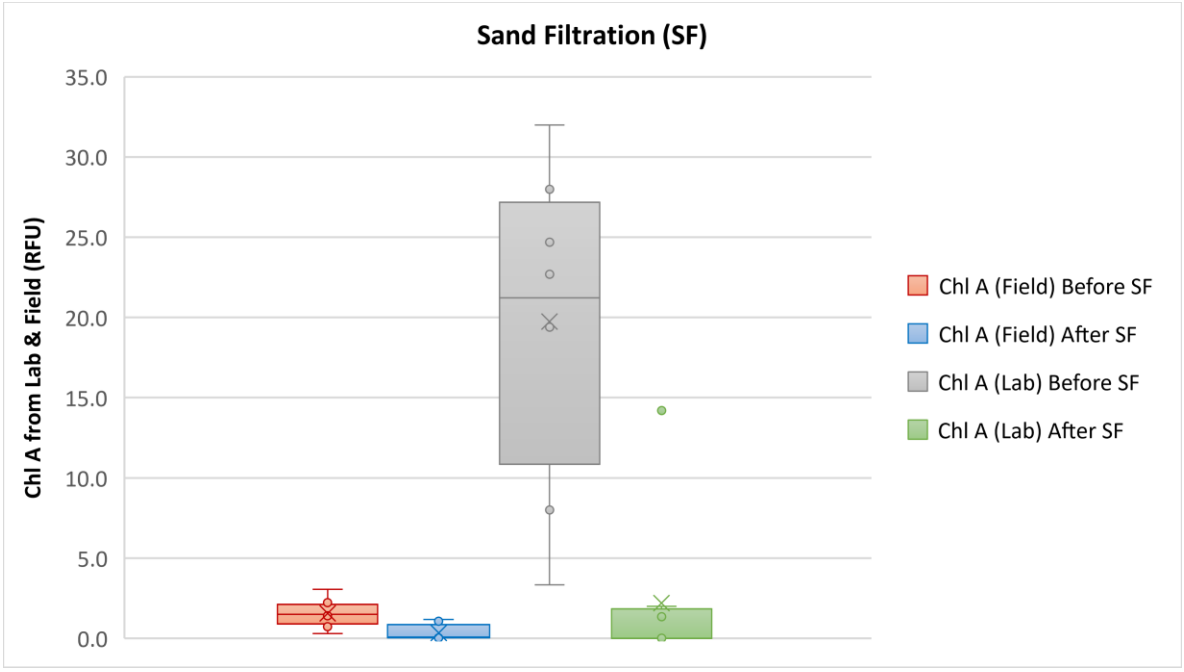


Figure 28. Box plot of chlorophyll A measured in the field (meter) and in the laboratory before and after slow sand filtration.

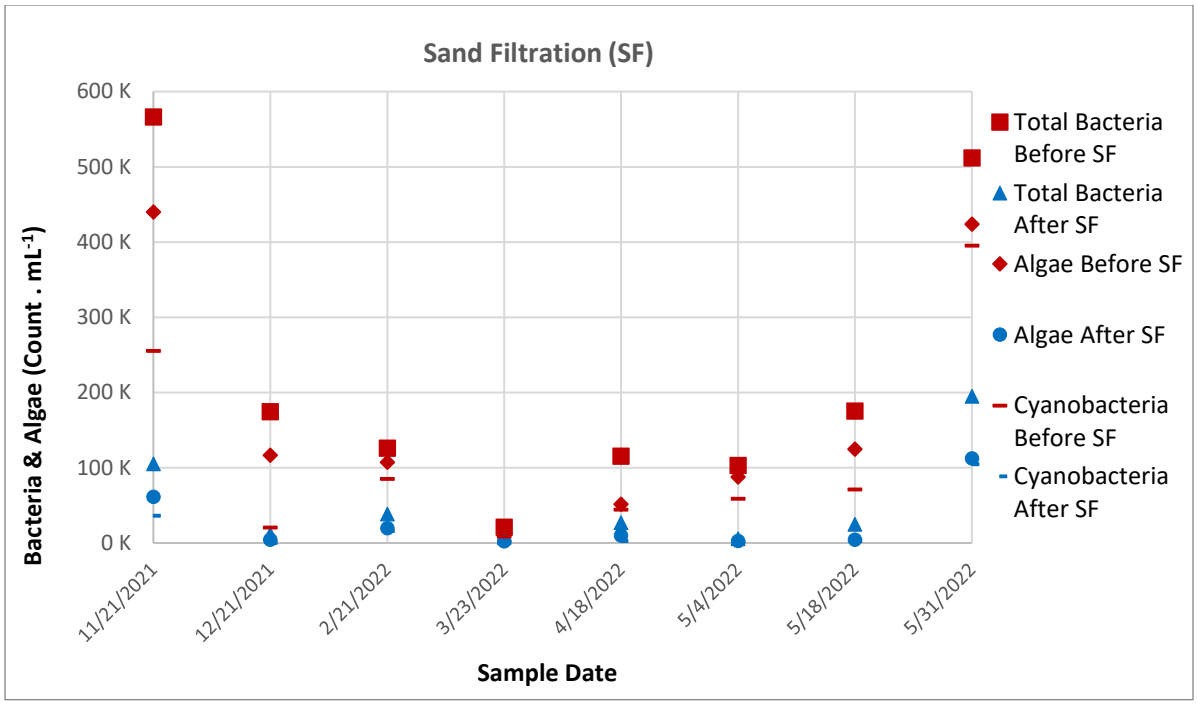


Figure 29. Temporal concentrations of total bacteria, algae, and cyanobacteria before and after slow sand filtration.

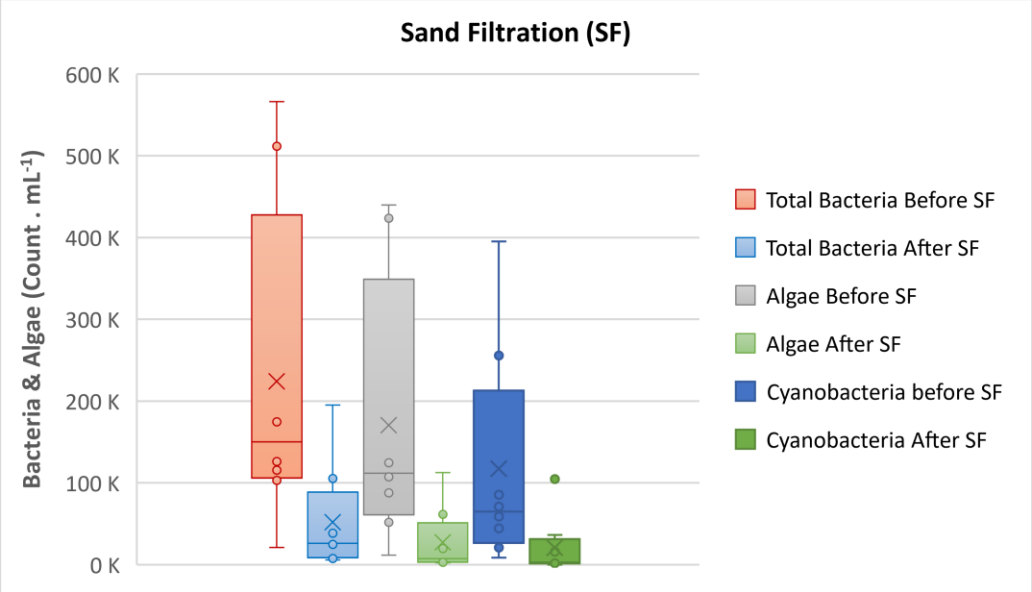


Figure 30. Box plot of total bacteria, algae, and cyanobacteria before and after slow sand filtration.

Temporal plots of actual conductivity, specific conductivity, total dissolved solids (TDS), and turbidity are shown in Figure 31. Box plots of actual and specific conductivity are shown in Figure 32 with box plots of TDS and turbidity shown Figure 33. The temporal data and box plots of the conductivity and the TDS have some temporal scatter, but the box plots show that little variation occurred through the sand filter, which was expected. Turbidity was greatly reduced

by the sand filtration, which was supported by the required number of cleanings of the filter (Figure 33).

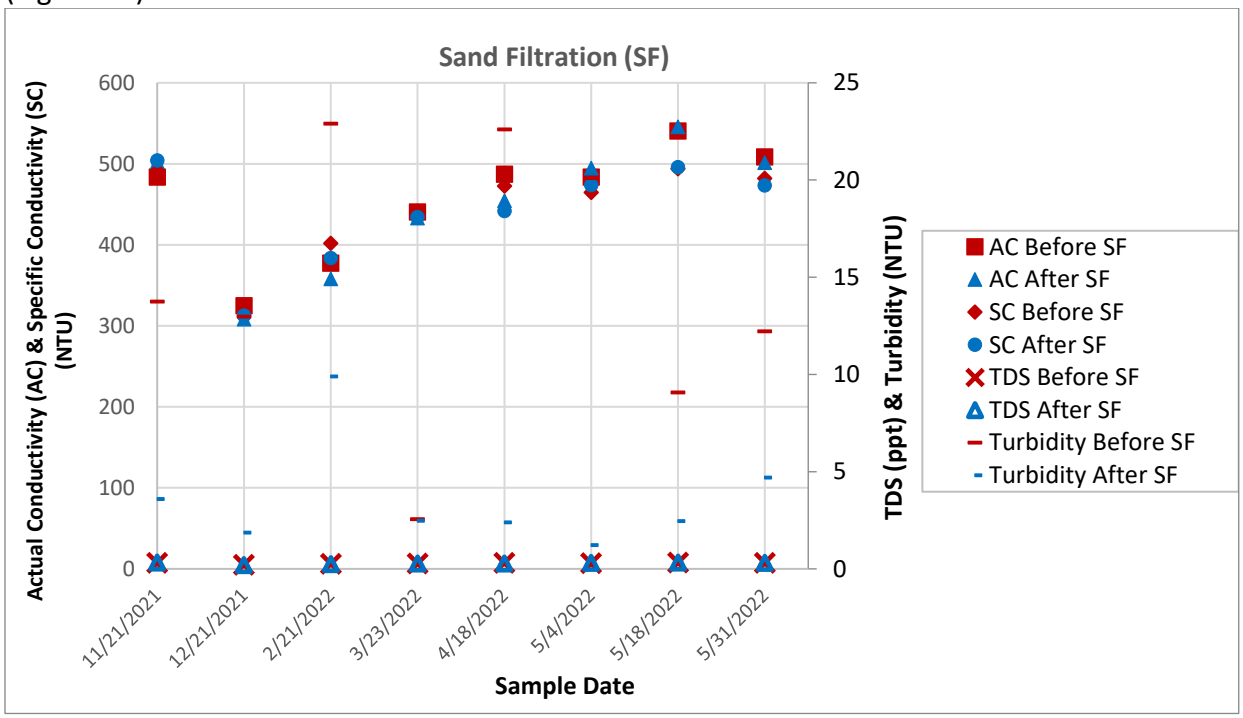


Figure 31. Temporal variation in the actual conductivity, specific conductivity, TDS, and turbidity before and after slow sand filtration.

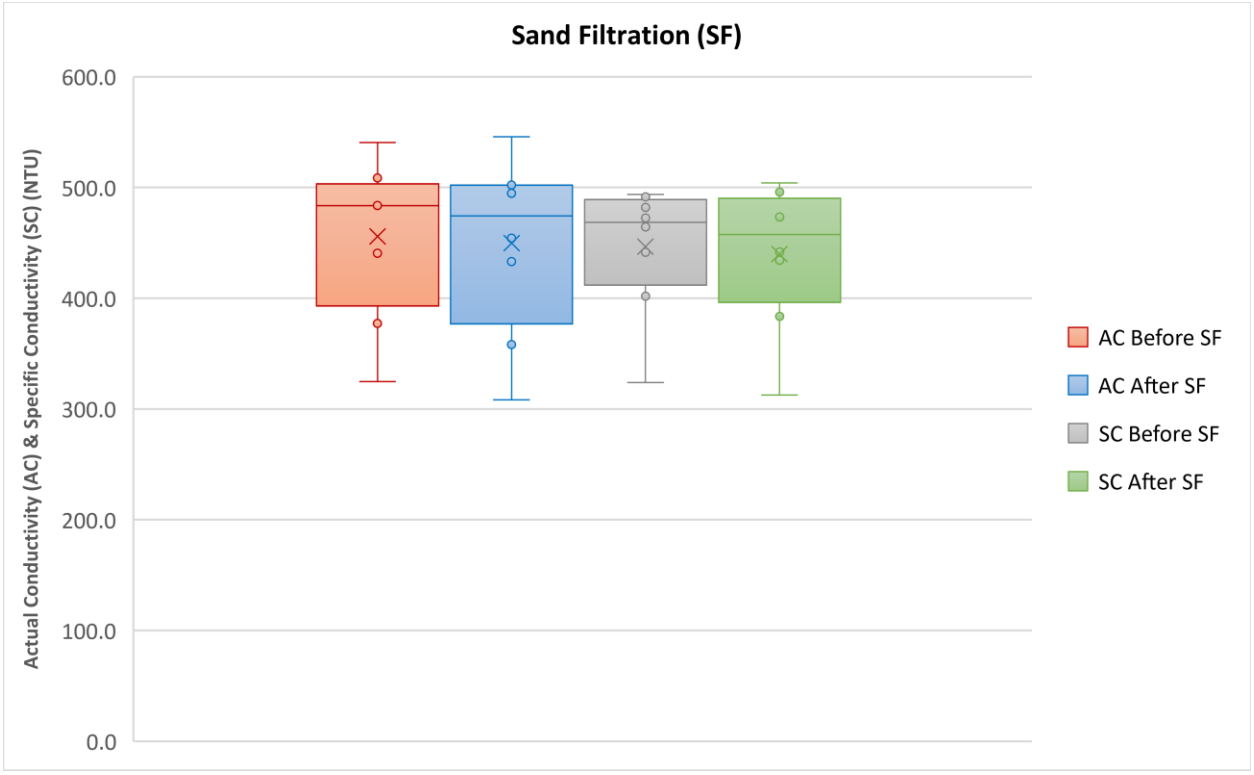


Figure 32. Box plots of actual and specific conductivity before and after sand filtration.

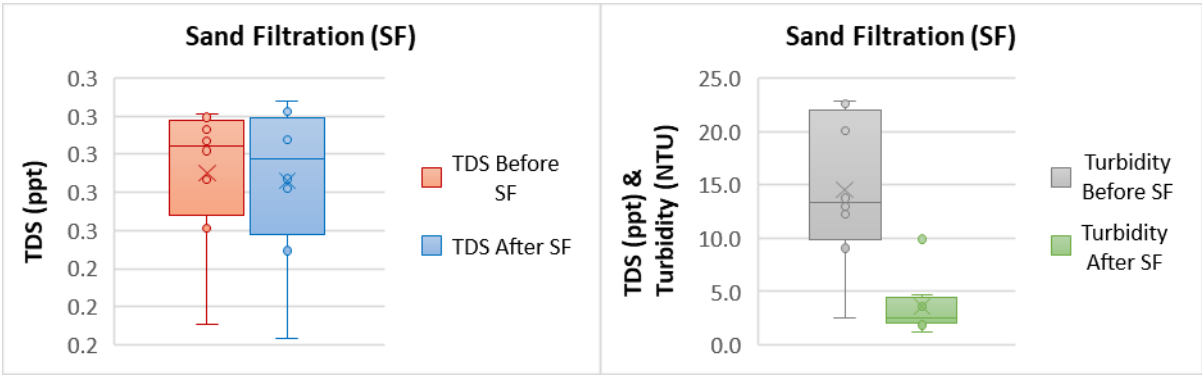


Figure 33. Variation in TDS and turbidity before and after sand filtration.

Temporal and box plot variations of real oxygen concentration and real oxygen saturation showed that oxygen tended to reduce across the sand filter (Figures 34 and 35). Reduction in dissolved oxygen was expected based on the very high concentration of biochemically active organic matter in the water. The dissolved oxygen influx ranged from about 2.8 to 7.1 mg/L in the inflow water with the fluctuation in temperature and wind mixing during the sampling period.

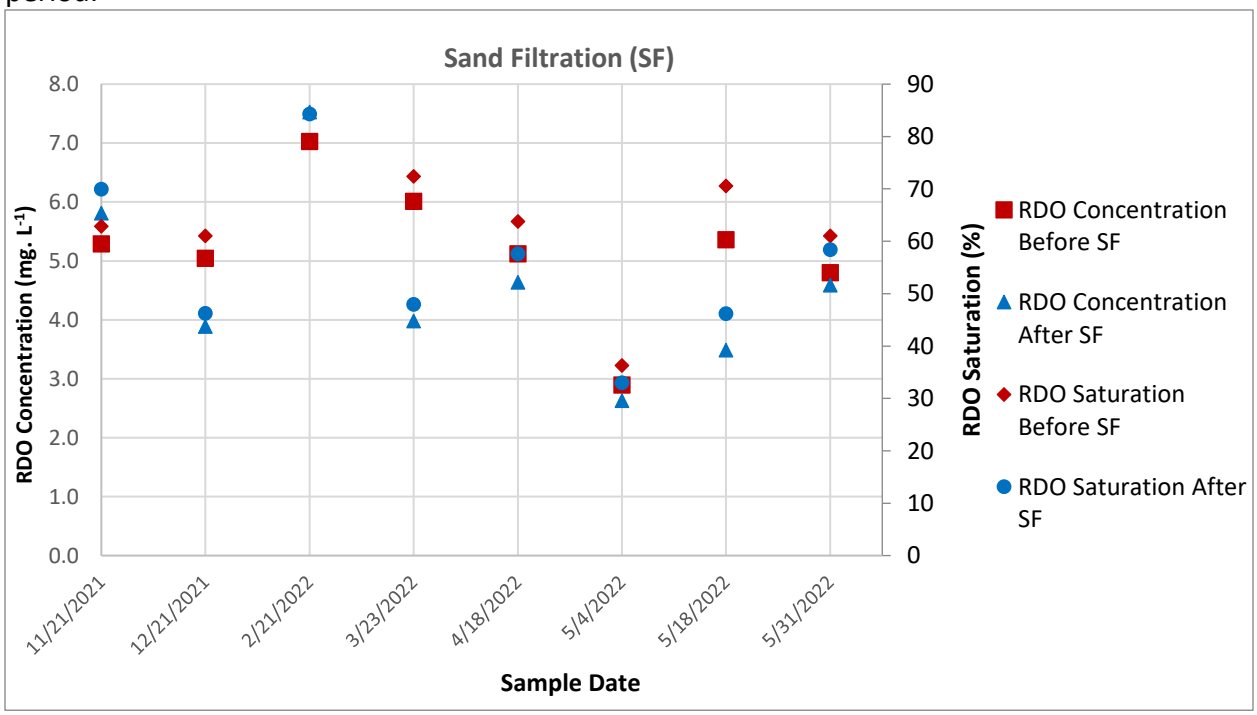


Figure 34. Real dissolved oxygen concentration and saturation before and after sand filtration.

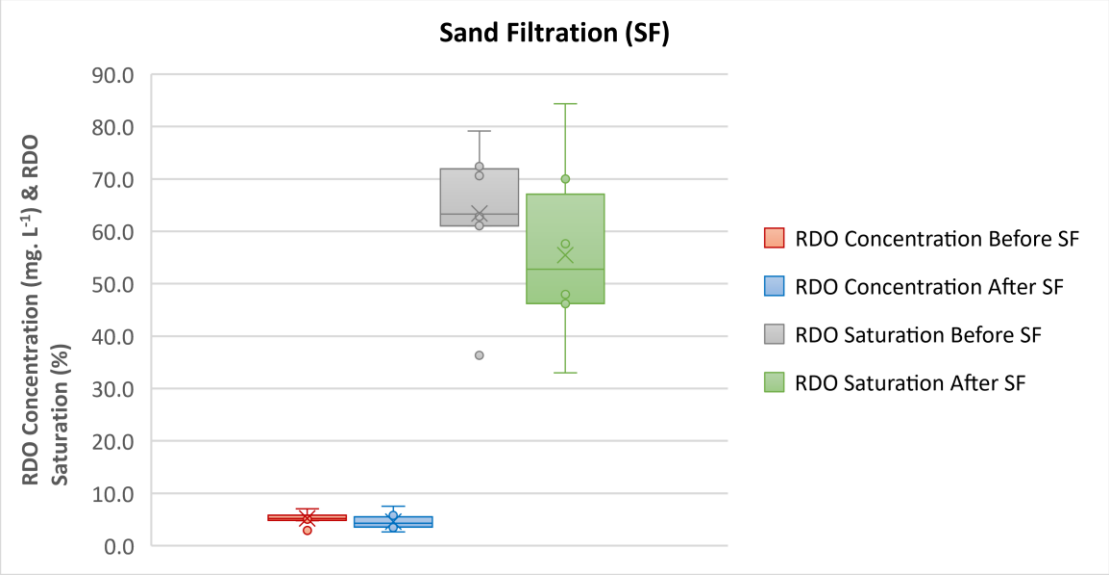


Figure 35. Box plot of real dissolved oxygen concentration and real dissolved oxygen saturation (temperature dependent).

Water temperature and saturation are nearly constant across the sand filter (Figures 36 and 37).

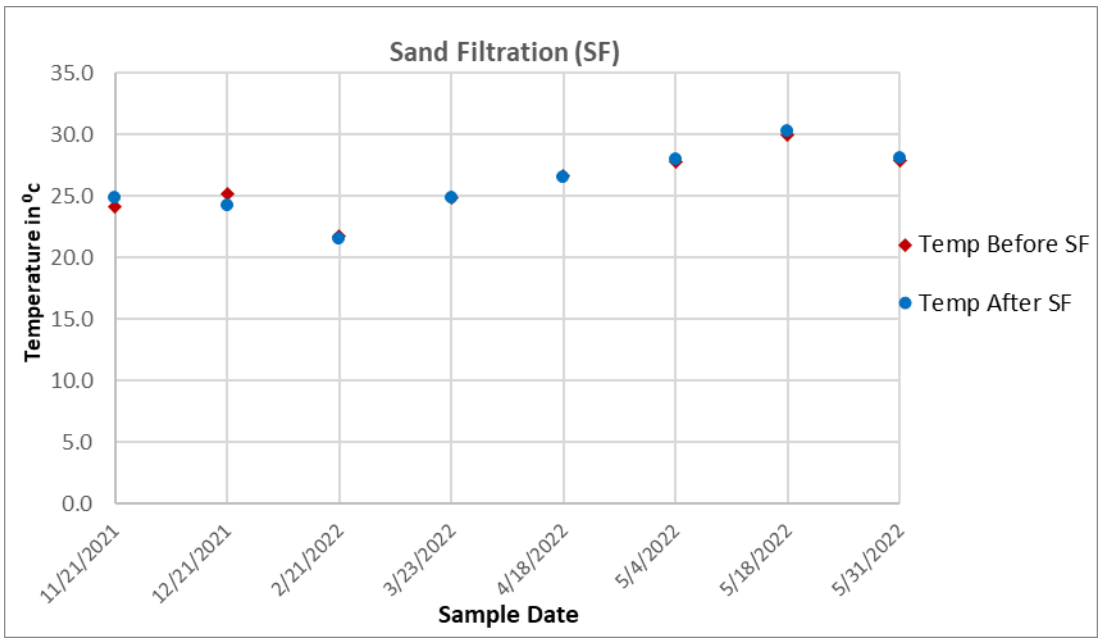


Figure 36. Water temperature before and after sand filtration.

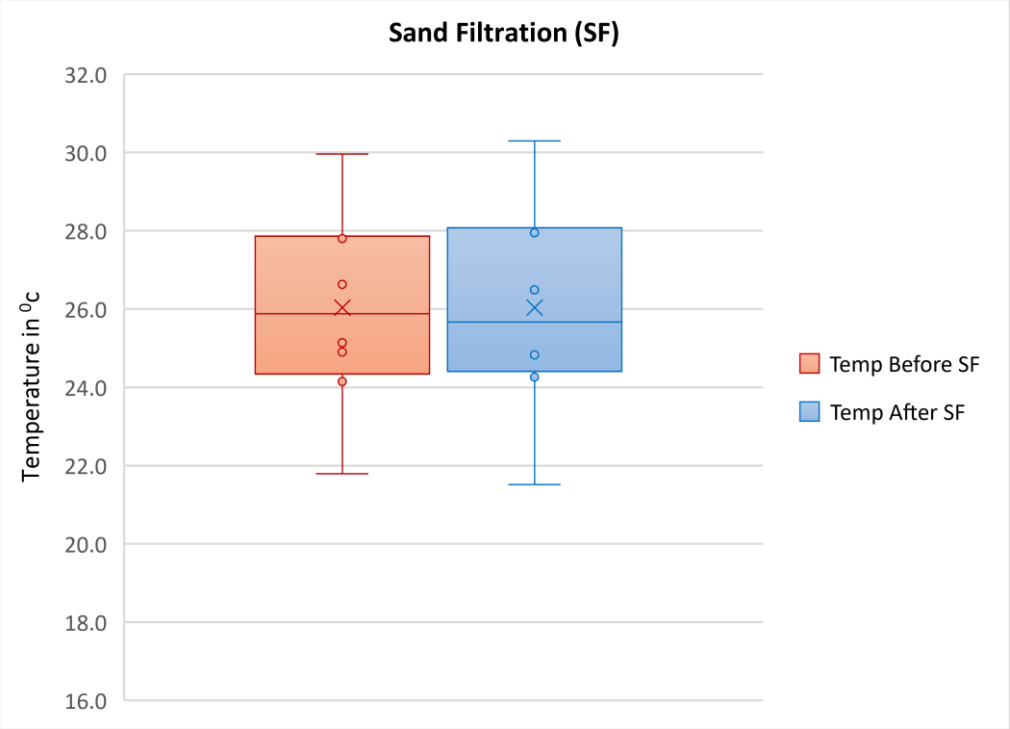


Figure 37. Box plots of water temperature before and after sand filtration.

Temporal variations in oxidation-reduction potential (ORP) and pH measured before and after sand filtration show minimal variation in most of the measurements, but some outlier values were obtained (Figure 38). The box plot for these data show that little real variation occurs across the sand filter (Figure 39).

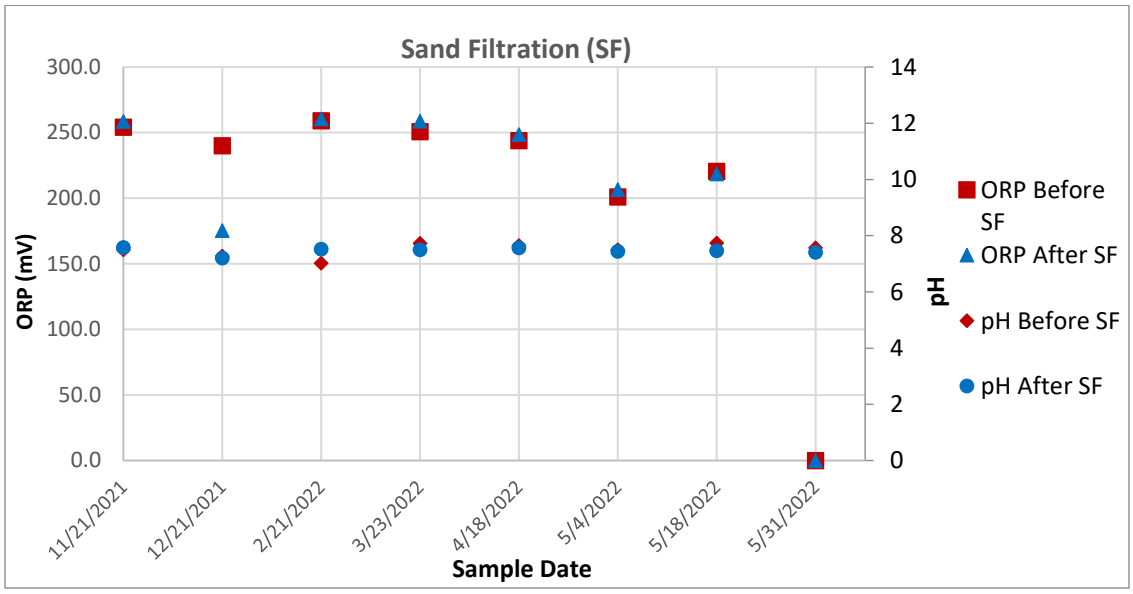


Figure 38. Temporal ORP and pH variation before and after sand filtration.

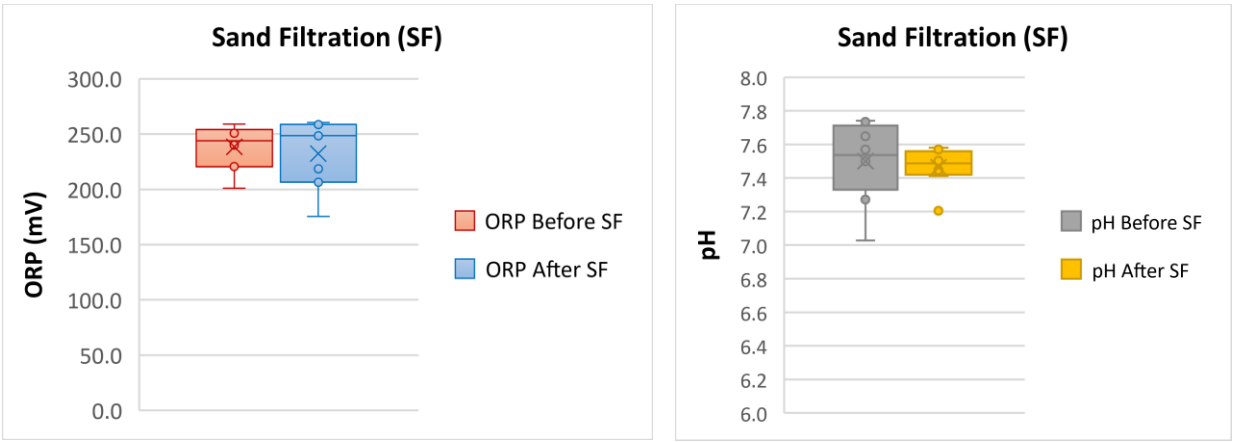


Figure 39. Box plots of ORP and pH before and after slow sand filtration.

Treatment of organic nitrogen using UV treatment independent of slow sand filtration and the vegetation tubs

Temporal variation in the total and organic nitrogen before and after UV treatment shows considerable scatter, and in many cases little variation (Figure 40). Box plots for the total nitrogen and organic nitrogen before and after UV treatment confirm that the UV process is not effectively breaking down the organic nitrogen (Figure 41).

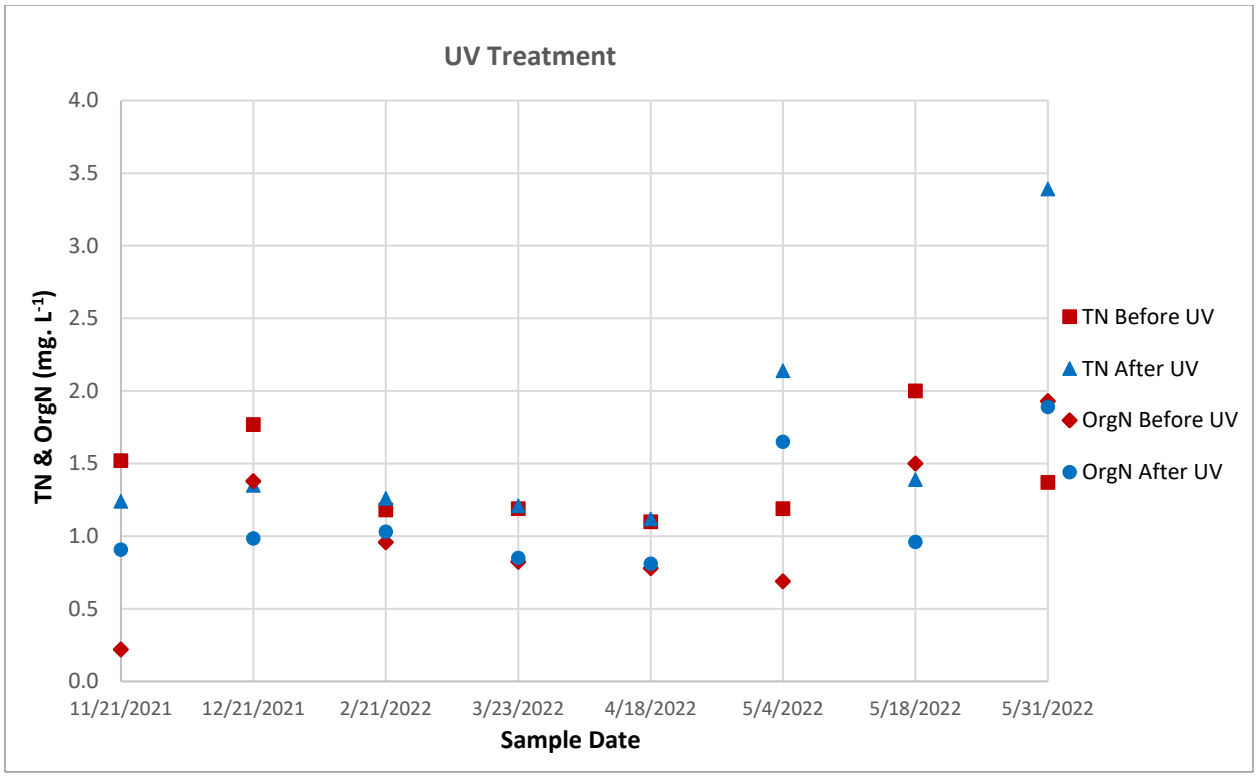


Figure 40. Temporal variation in the concentrations of total and organic nitrogen before and after UV treatment.

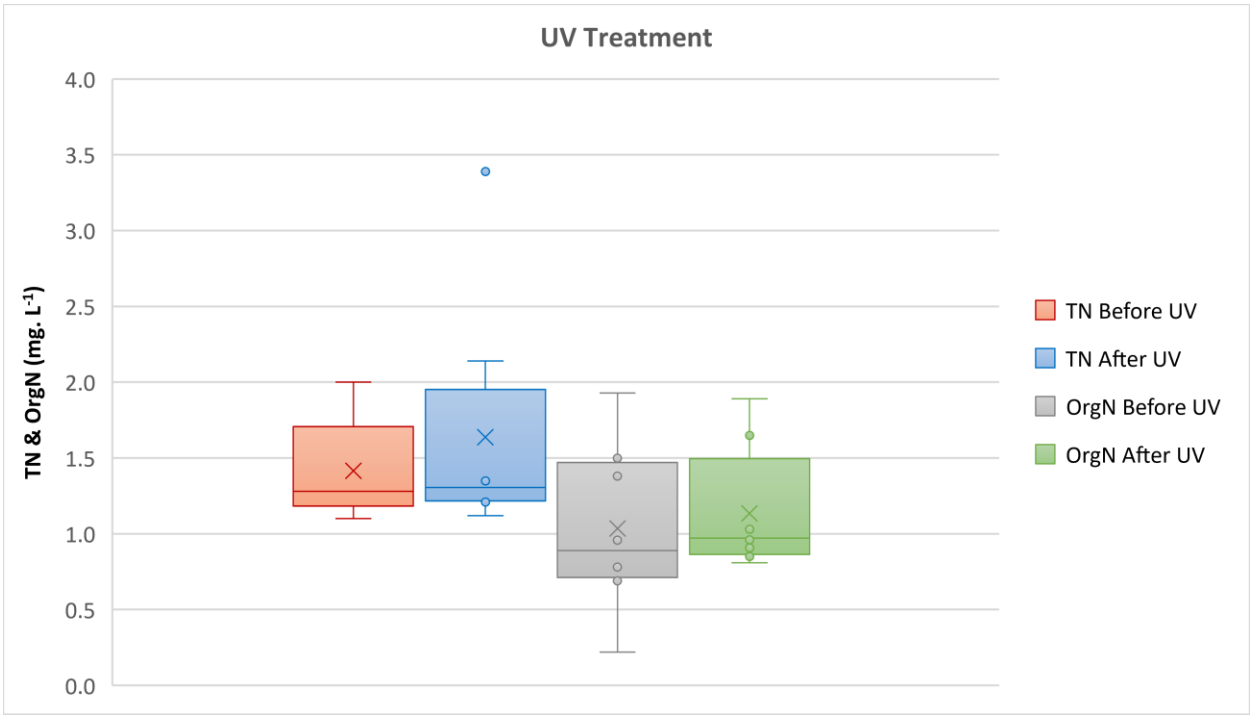


Figure 41. Box plot for variation of total and organic nitrogen before and after UV treatment.

UV treatment had little impact on the other parameters measured during the investigation. Plots of all parameters are given in Appendix A. One surprise was that the UV had little impact on the concentrations of total bacteria, algae, and cyanobacteria. A stronger UV light combined with a longer contact time could have led to different outcomes.

Details of Effectiveness of Water Treatment of the Emergent and Submergent Vegetation in Train A (control train)

The changes in water quality and other parameters were measured to assess the effectiveness of the aggregated emergent (Typha) and submergent (*Vallisneria*) vegetation in water treatment. This was achieved by comparing the data between stations 1 and 4. This is the base case condition or control wherein the raw water from the river enters the first tank at station 1 (Typha) and leaves the last tank (*Vallisneria* sp., tape grass) at station 4.

The temporal and box plot data for total and organic nitrogen show major reductions in concentrations from the vegetation. There is some scatter in the data, but the overall pattern is distinctive.

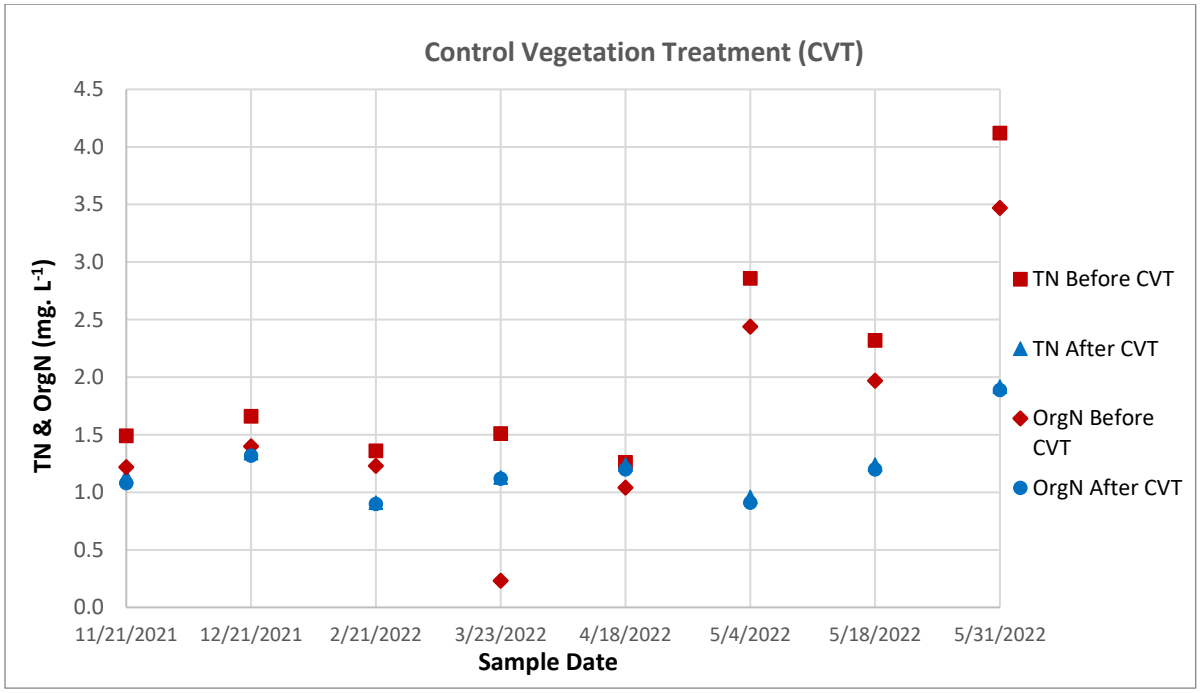


Figure 42. Temporal concentrations total and organic nitrogen in the control before and after passage through both vegetation tanks in the control train.

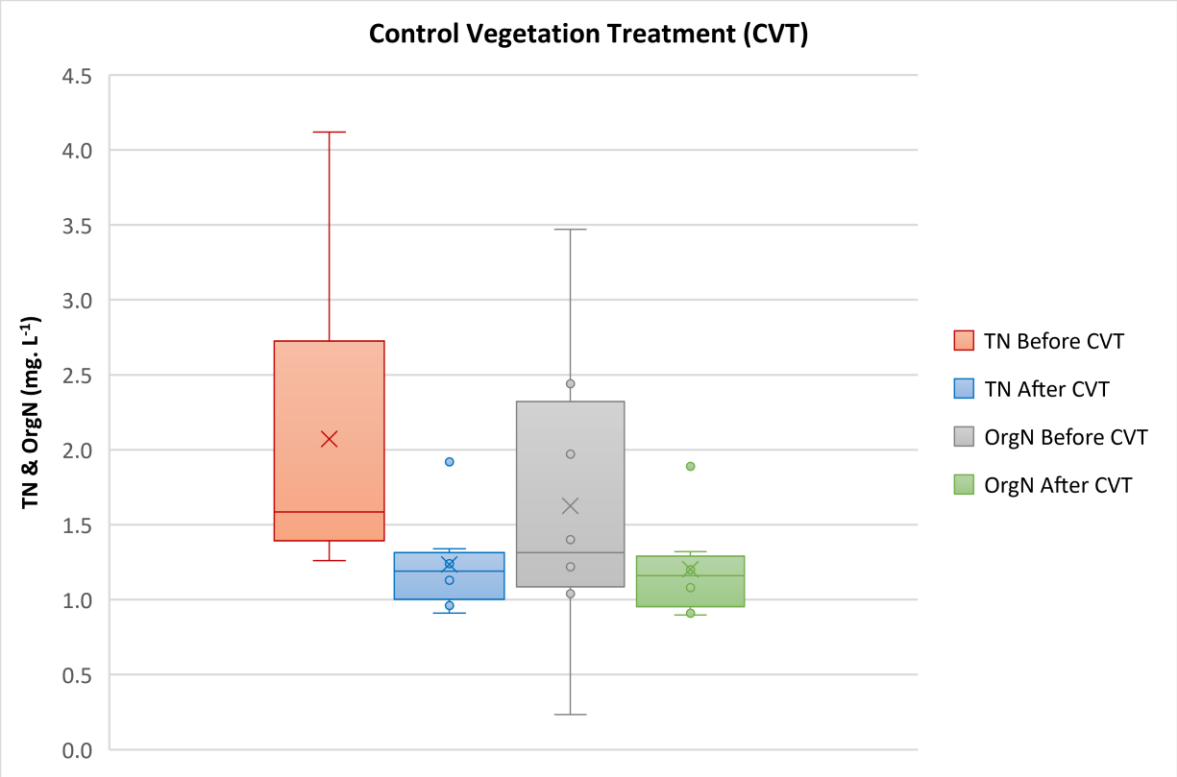


Figure 43. Box plots of total and organic nitrogen concentrations before and after passage through both vegetation tanks in the control train.

Temporal and box plot concentrations of nitrate and ammonia show major reductions between the river water at station 1 and the discharge from tank 4. The concentration of nitrite was so small that any real change in concentration was not significant. In many sample events, nitrite concentrations were below detection limits.

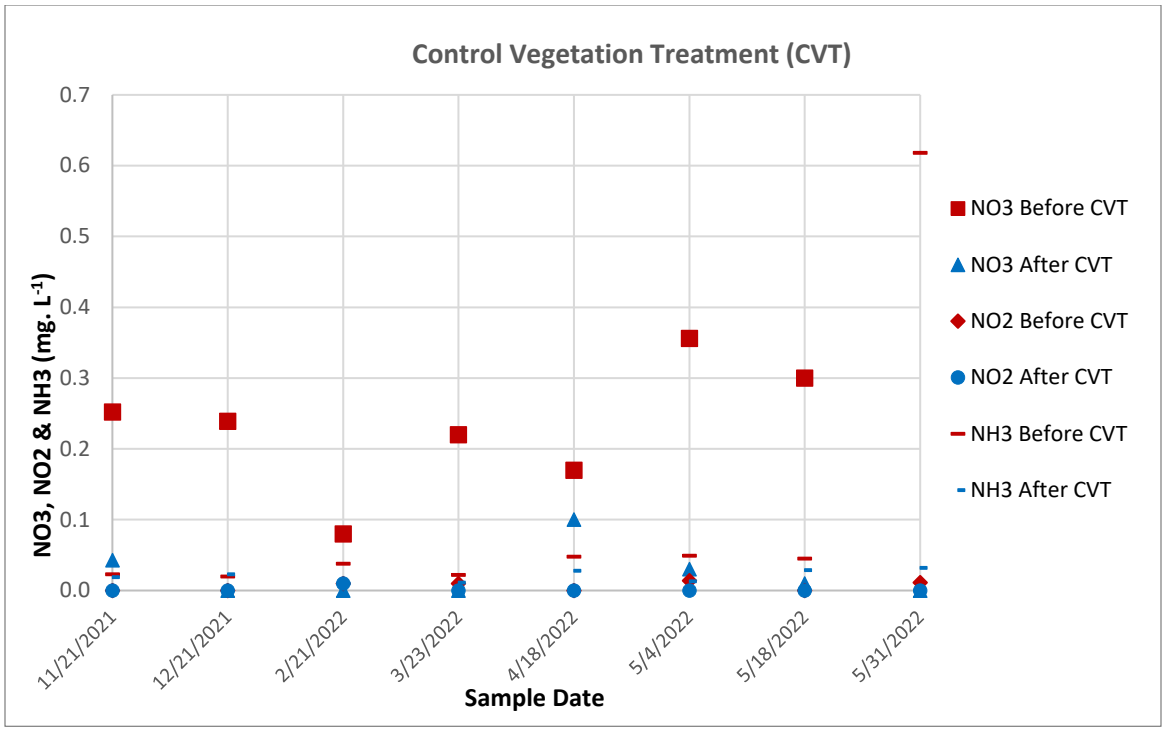


Figure 44. Temporal concentrations of nitrate, nitrite, and ammonia before and after vegetation treatment in the control with no other treatment.

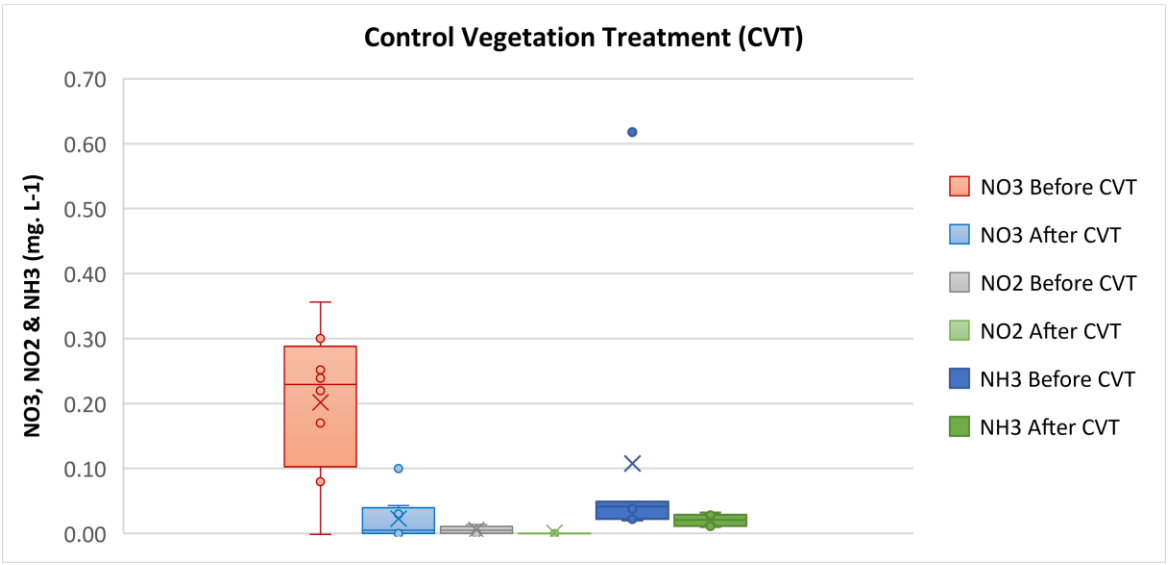


Figure 45. Box plots of the nitrate, nitrite, and ammonia concentrations before and after vegetation treatment in the control train with no other treatment.

Similar to the nitrate and ammonia removal, the vegetation treatment in the control train showed high removal of both total phosphorus and orthophosphate (Figures 46 and 47). If the single outlier concentration was removed from the phosphate data, the percentage of removal would be even higher.

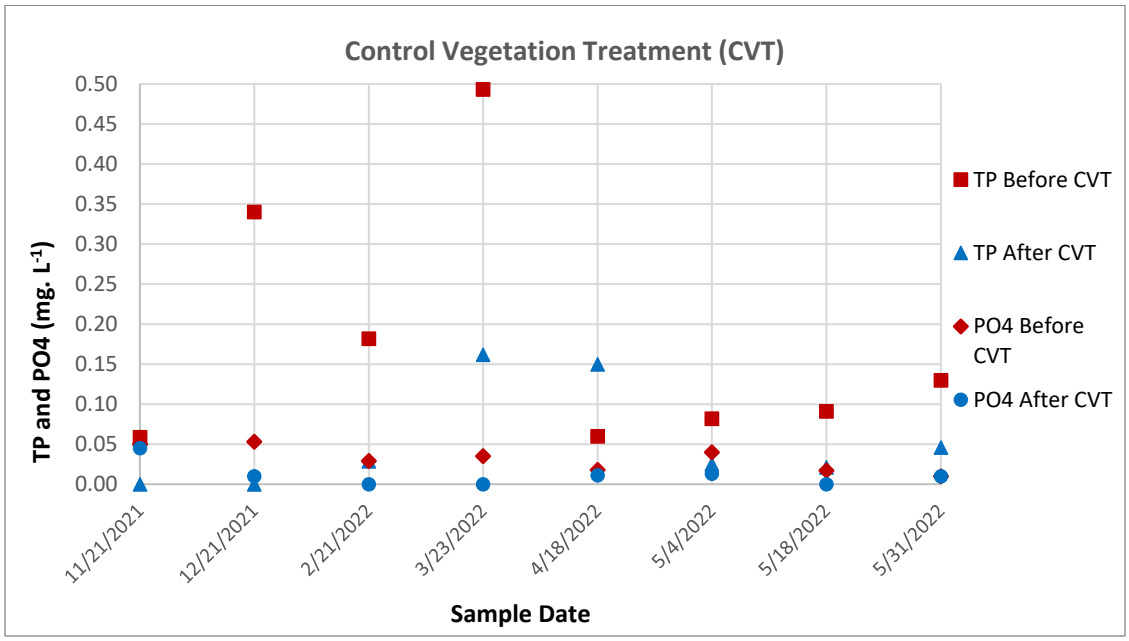


Figure 46. Temporal concentrations of total phosphorus and orthophosphate before and after vegetation treatment in the control train.

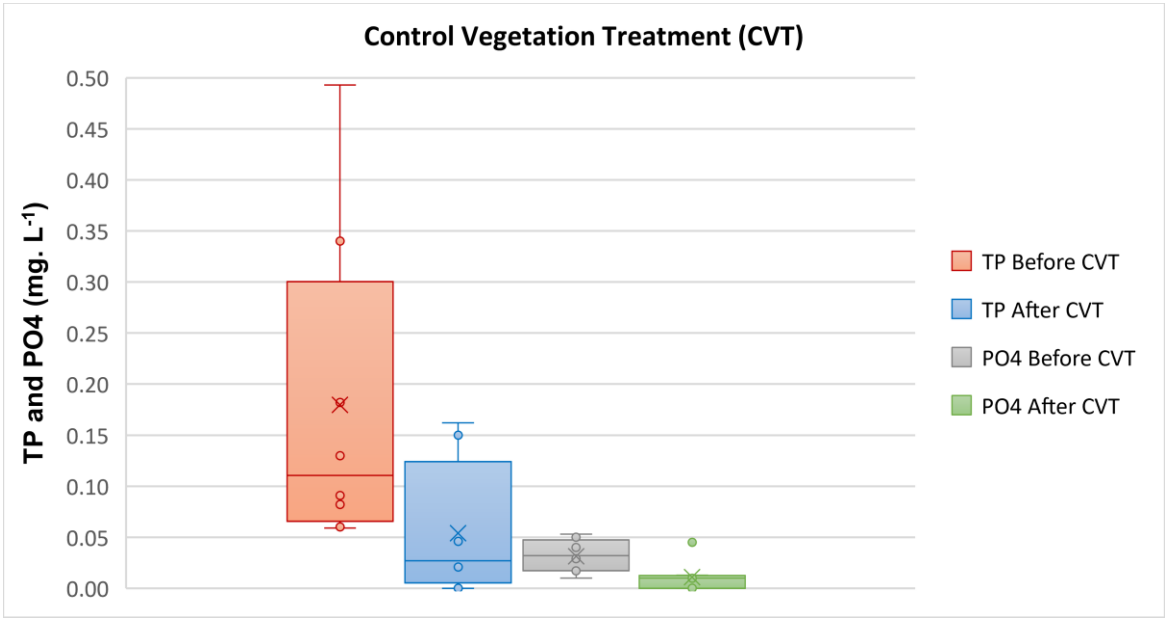


Figure 47. Box plot of the total phosphorus and orthophosphate concentration before and after vegetation treatment in the control train.

Measured concentrations of TOC and DOC before and after vegetation treatment show some reductions in each case (Figures 48 and 49). However, the statistical significance of these changes is reported in a later part of the report. There are some significant outliers in the data, which may influence the changes in concentration (Figure 49).

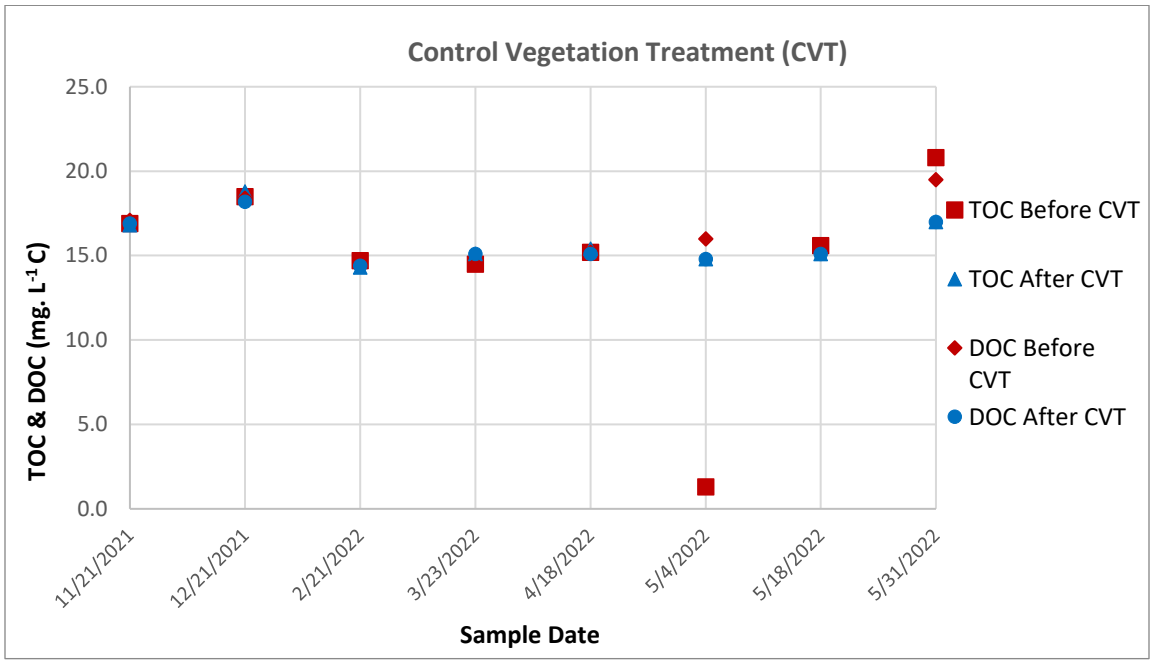


Figure 48. Temporal TOC and DOC concentrations before and after vegetation treatment in the control train.

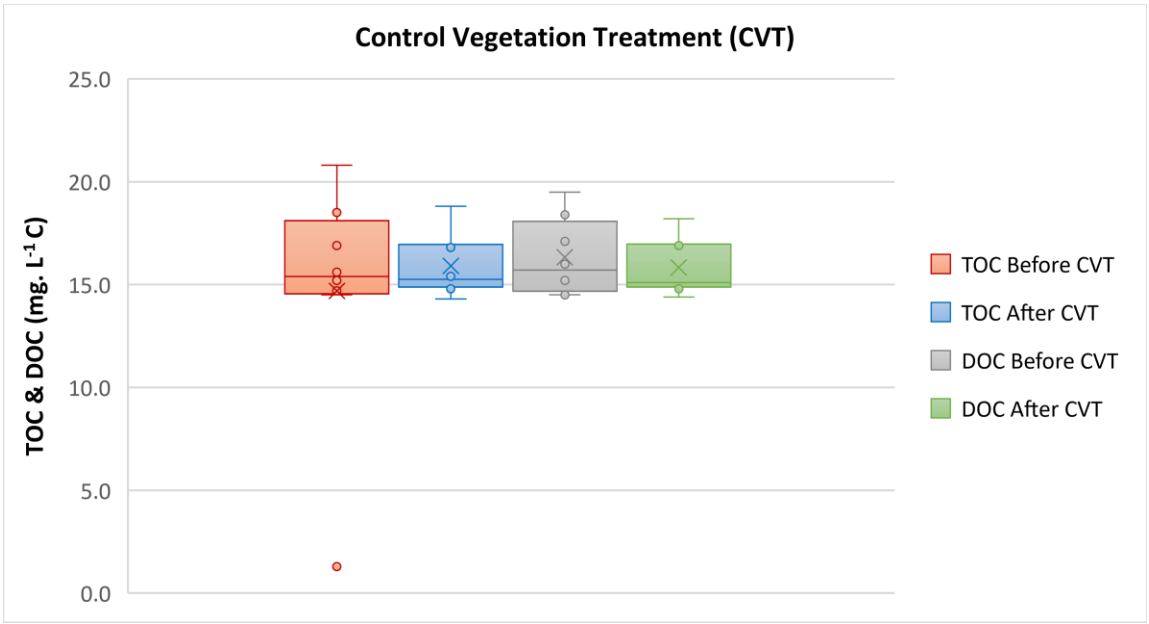


Figure 49. Box plots of the TOC and DOC concentrations before and after vegetation treatment in the control train.

The vegetation treatment produced significant reductions in chlorophyll A in both the field measurements (meter) and in the laboratory measurements (Figures 50 and 51). Some scatter in the temporal data can be observed (Figure 50), but the box plot clearly illustrates the reduction (Figure 51).

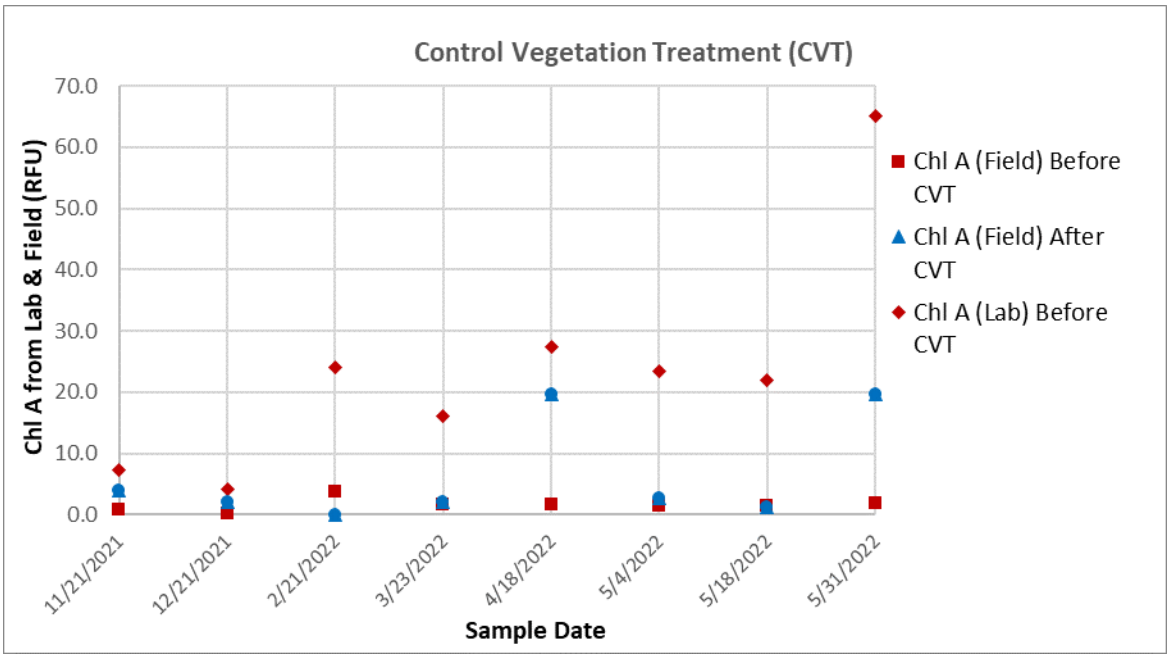


Figure 50. Temporal changes in concentration of chlorophyll A measured by field meter and laboratory analyses before and after vegetation treatment in the control train.

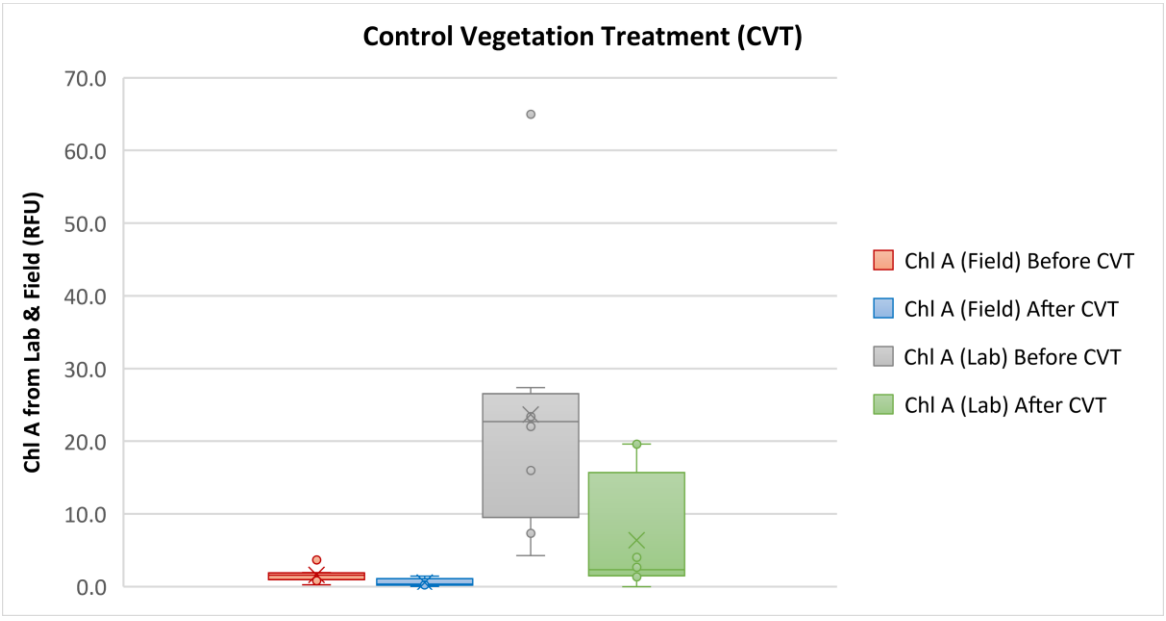


Figure 51. Box plot of chlorophyll A measured by field meter and in the laboratory before and after vegetation treatment in the control train.

The temporal and box plots of the total bacteria, algae, and cyanobacteria before and after the vegetation treatment shows reductions in all three parameters (Figures 52 and 53). There are some outliers in the data as clearly shown in the box plots (Figure 53).

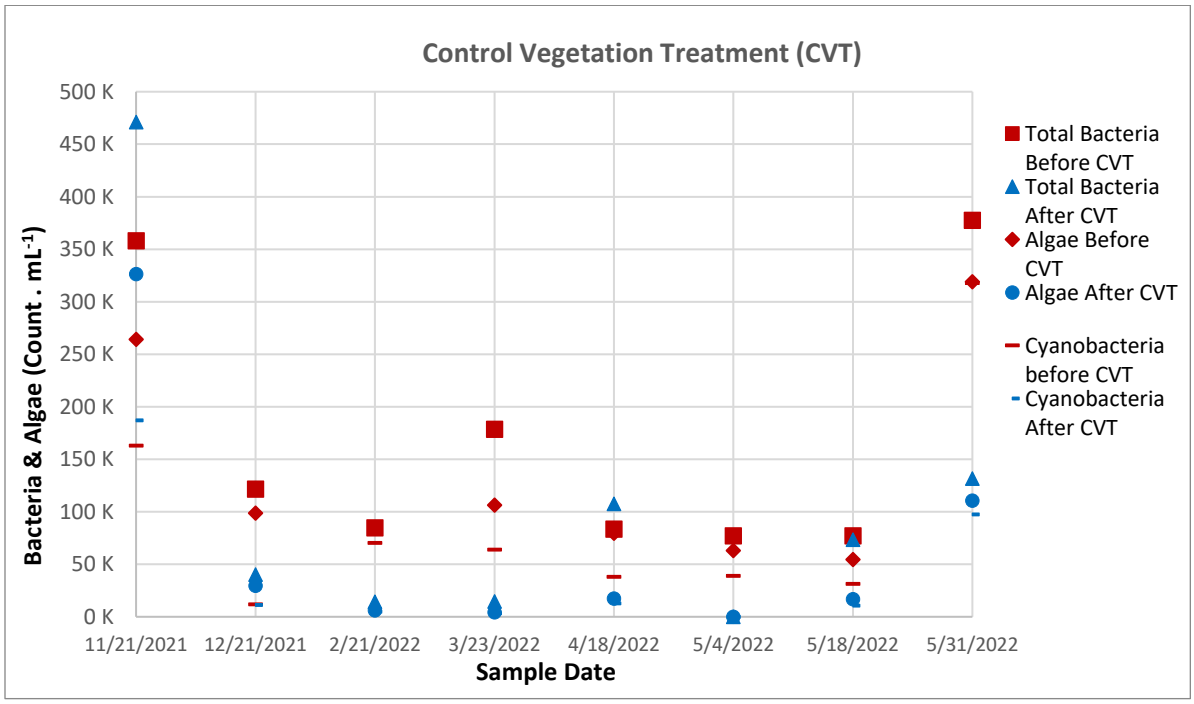


Figure 52. Temporal variations in total bacteria, algae, and cyanobacteria concentrations before and after vegetation treatment in the control train.

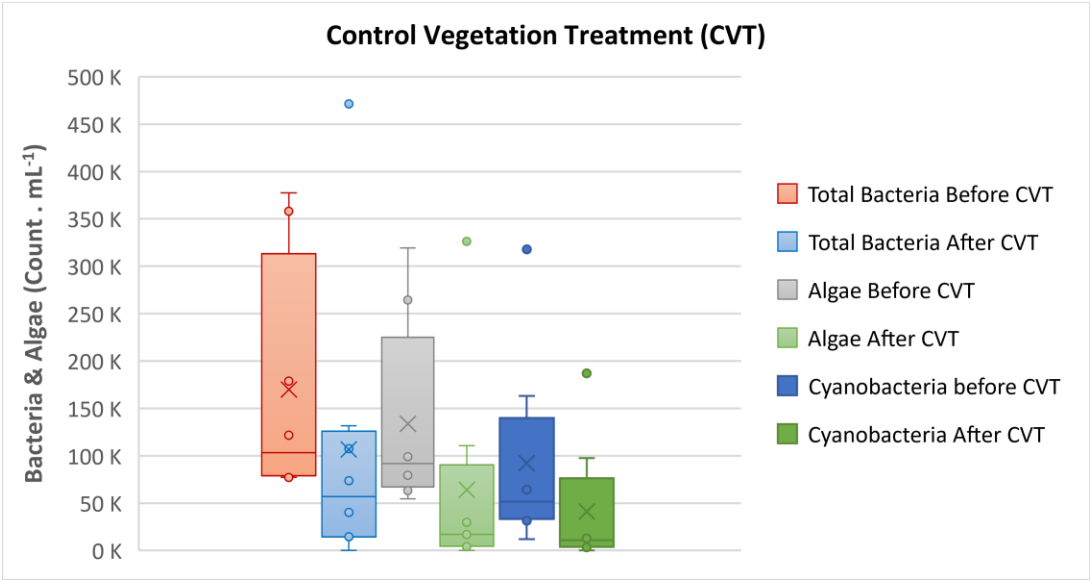


Figure 53. Box plot of total bacteria, algae, and cyanobacteria concentration before and after vegetation treatment in the control chain.

Evaluation of changes in conductivity, TDS, and turbidity in the control vegetation train before and after treatment showed that actual and specific conductivity and TDS did not change significantly (Figures 54 and 55). The scatter in the meter data did produce some variation based on observations of the box plot. The turbidity was reduced significantly based on the box plot of before and after vegetation treatment (Figure 55).

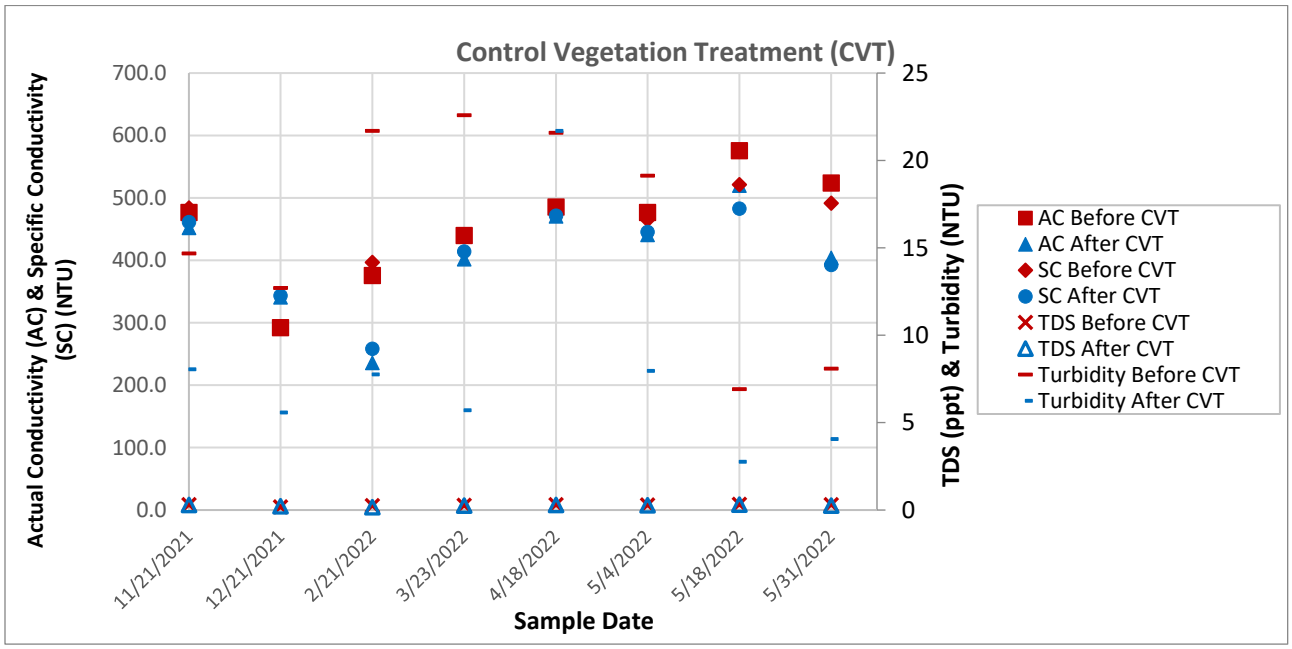


Figure 54. Temporal changes in the actual conductivity, specific conductivity, TDS, and turbidity before and after vegetation treatment in control train.

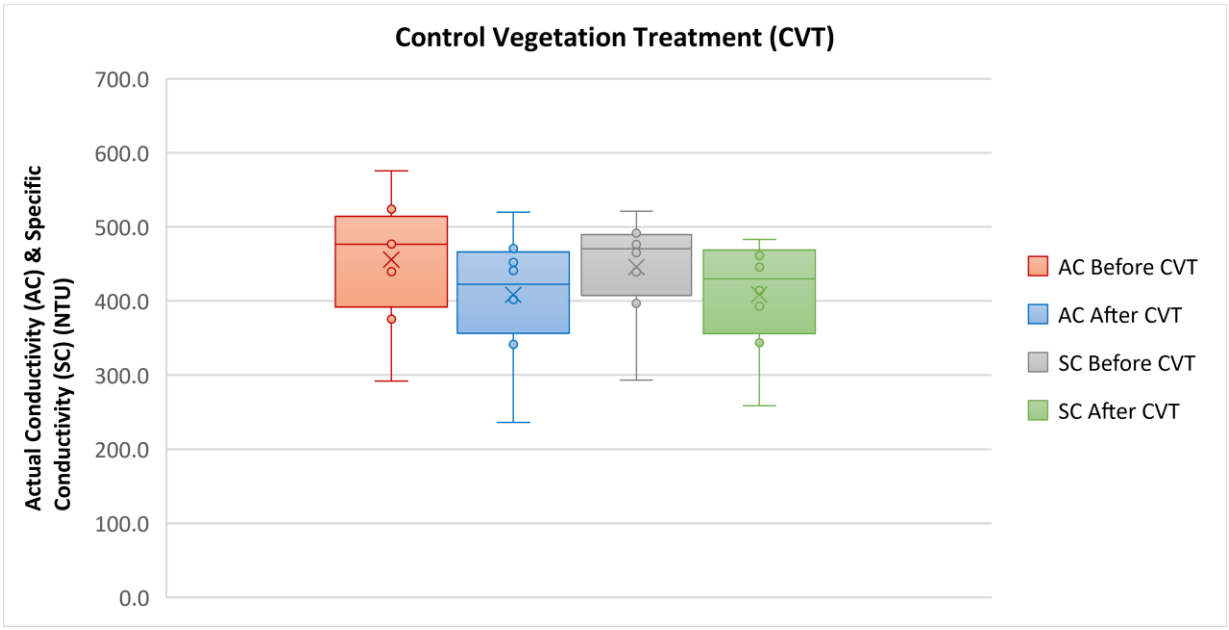


Figure 55. Box plot of actual and specific conductivity before and after vegetation treatment in the control chain.

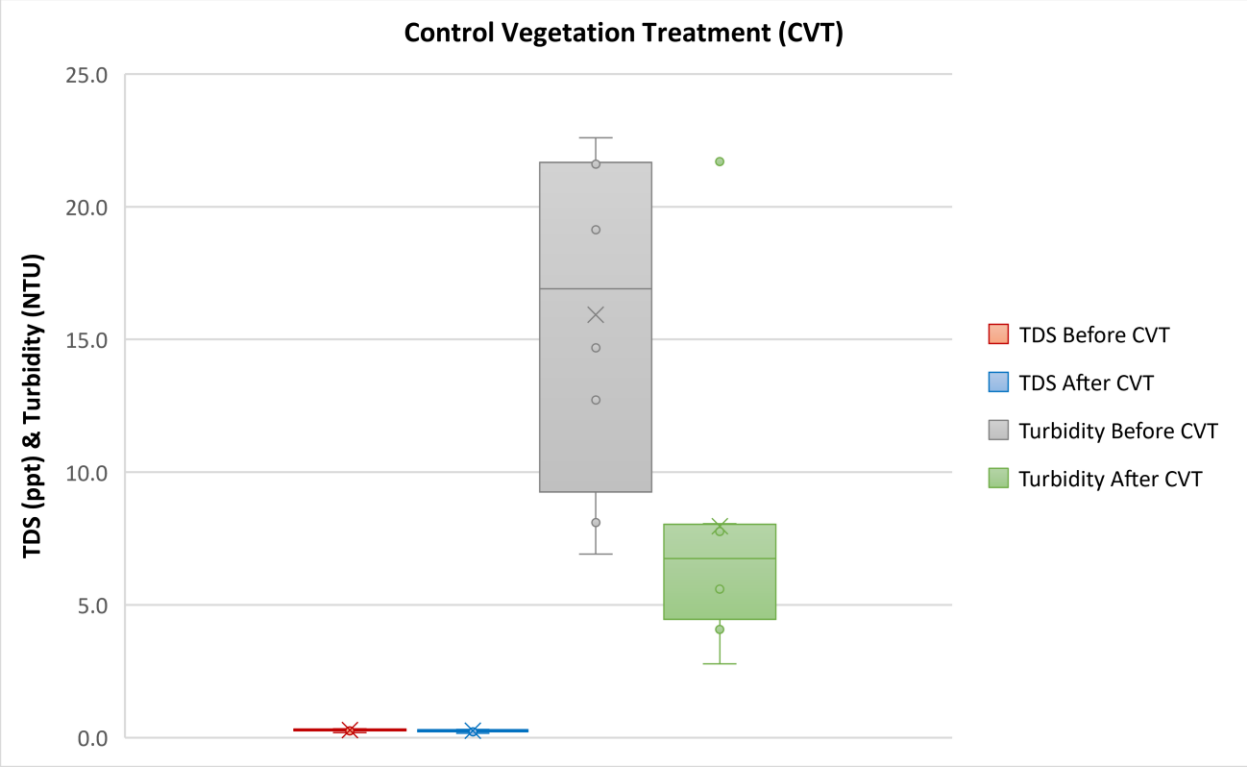


Figure 56. Box plot of TDS concentration and turbidity after vegetation treatment in the control train.

Based on the meter data collected in the field, the dissolved oxygen concentration and saturation increased during vegetation treatment (Figures 57 and 58). The saturation changes were dramatic from about 59% to 99% (Figure 58).

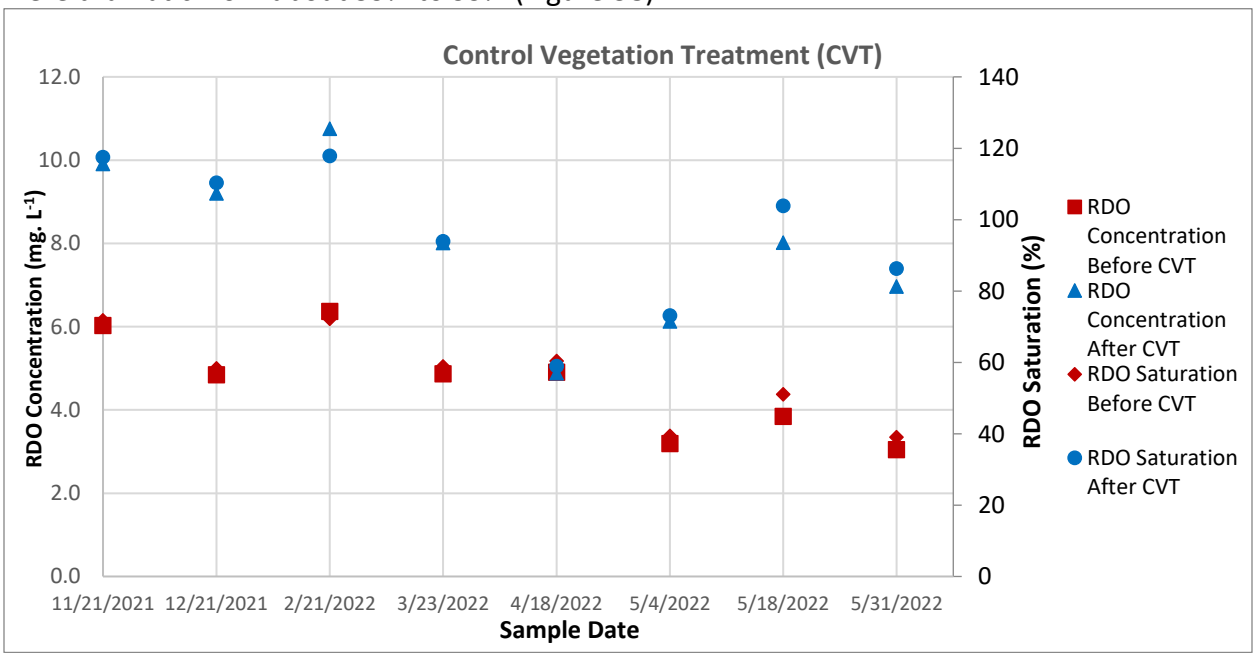


Figure 57. Temporal plots of oxygen concentration and saturation before and after vegetation treatment in the control train.

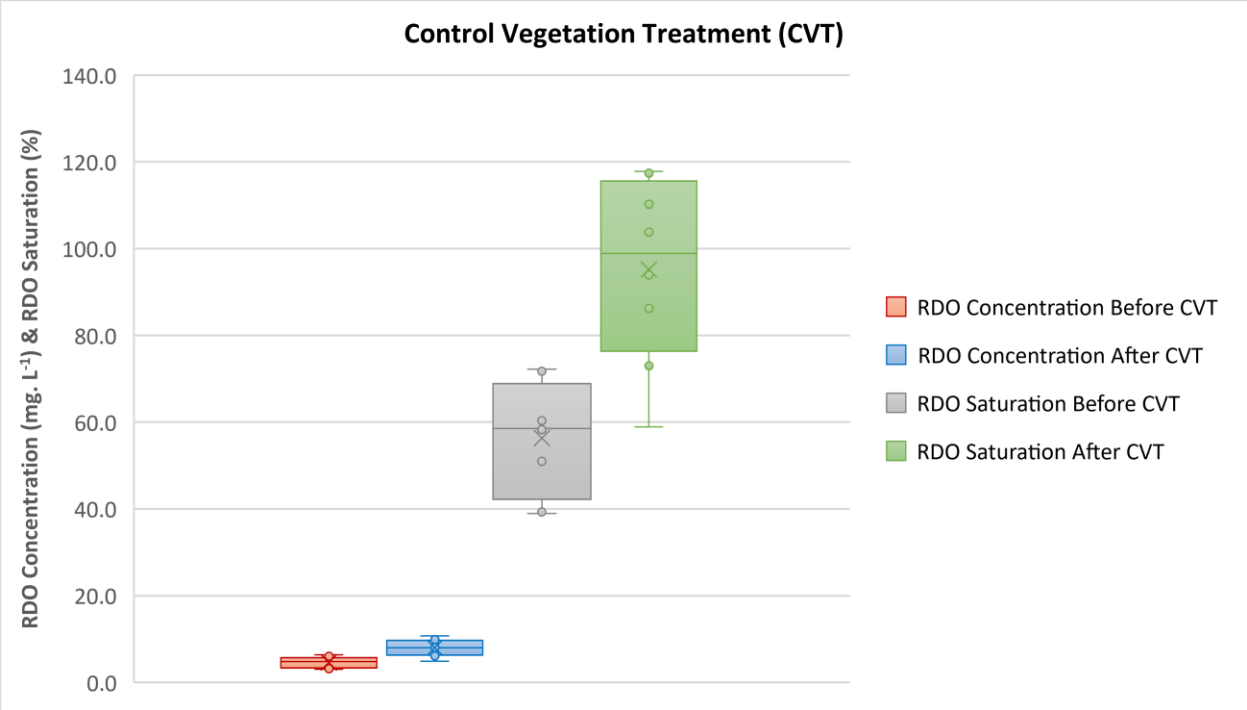


Figure 58. Box plot of dissolved oxygen concentration and saturation before and after vegetation in the control train.

A minor water temperature reduction was observed before and after vegetation treatment in the control chain (Figures 59 and 60). The change was a few tenths of a degree C, which is not believed to have significance.

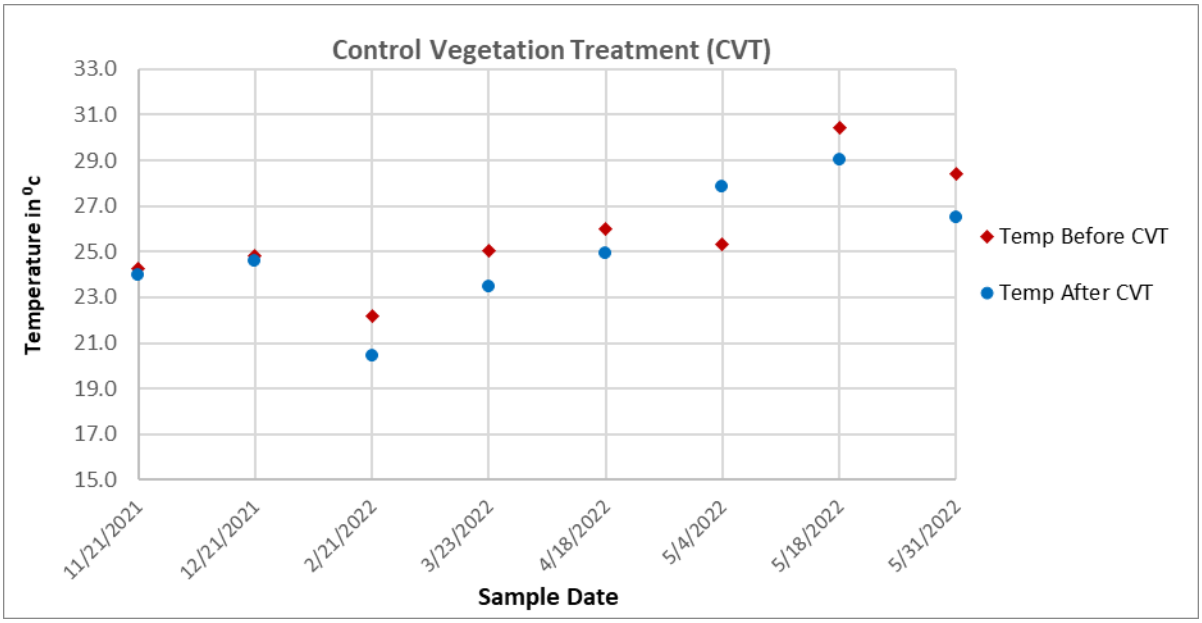


Figure 59. Temporal plot of temperature before and after vegetation treatment in the control train.

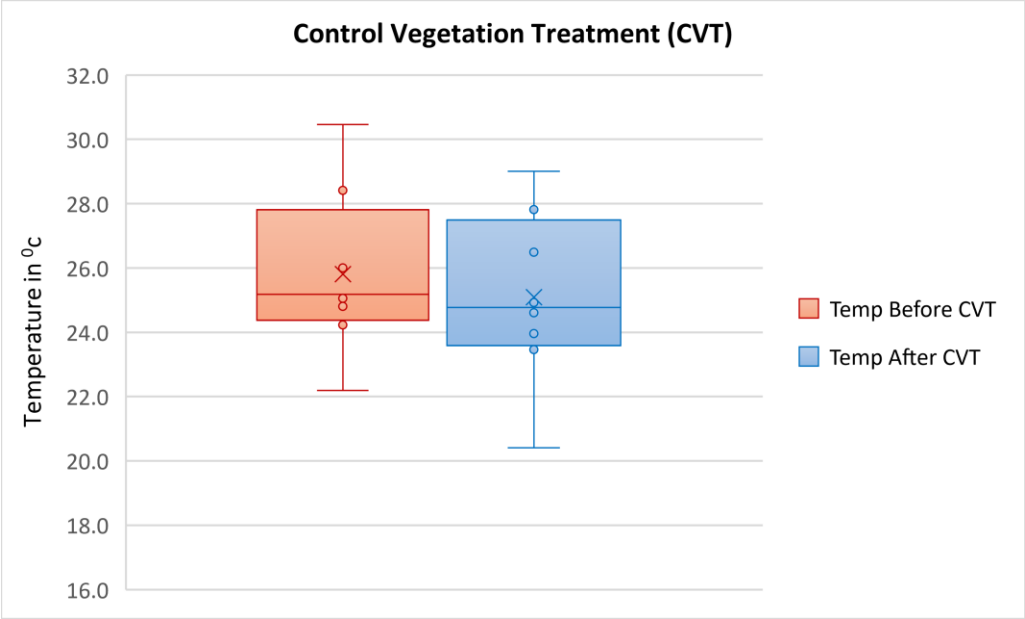


Figure 60. Box plot of temperature changes before and after vegetation treatment in the control train.

Oxidation-reduction potential declined during the vegetation treatment in the control chain (Figures 61 and 62). In contrast, the pH significantly increased during the vegetation treatment, which is best illustrated in the box plot (Figure 62).

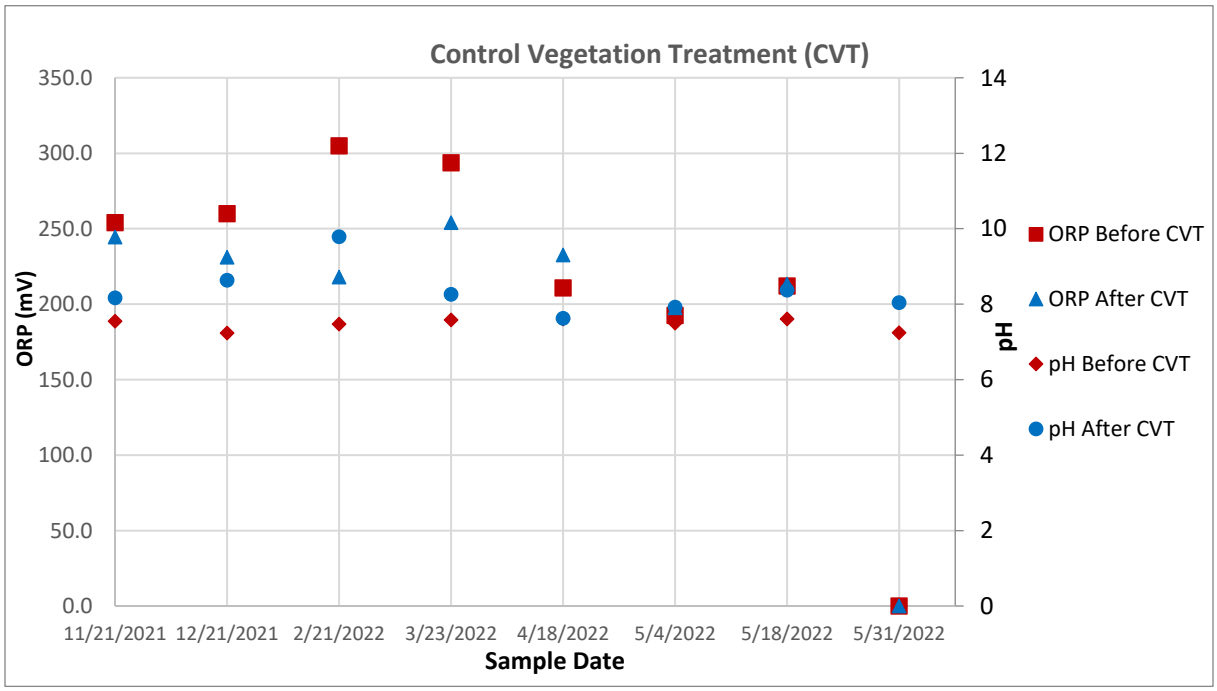


Figure 61. Temporal field measurements of ORP and pH before and after vegetation treatment in the control train.

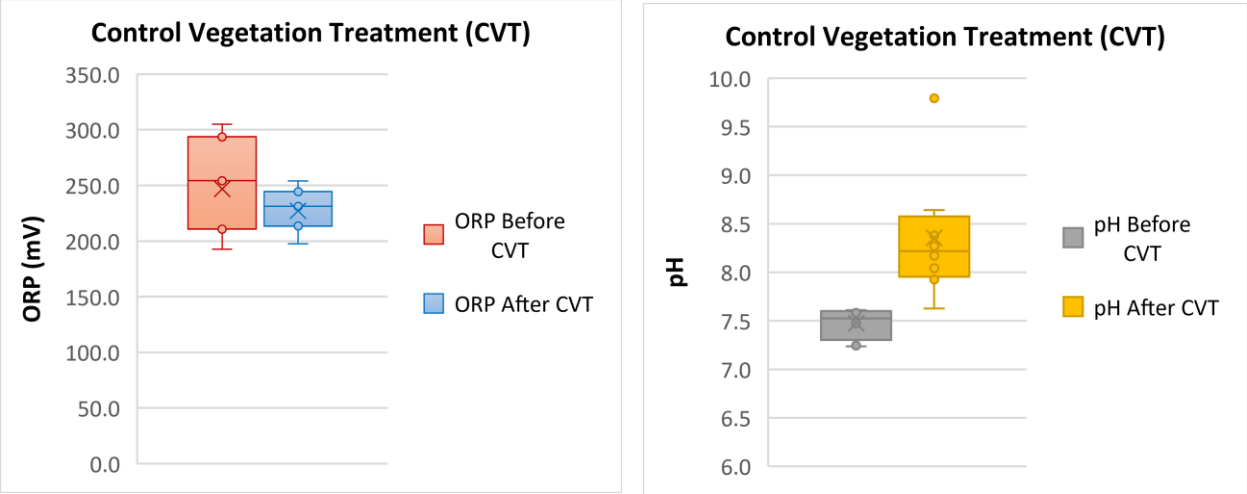


Figure 62. Box plots of ORP and pH before and after vegetation treatment in the control train.

Full Treatment Train A Analysis (Control): Raw Water/Vegetation Tank 1 out (Typha)/Vegetation Tank 2 Out (Vallisneria sp., Tape Grass)

The control case is based on influx of raw water with only vegetation treatment. Box plots were used to assess the changes in various parameters during treatment. These analyses were used in comparison to trains B and C to assess the overall effectiveness of the engineered solution versus solely vegetation.

Impacts of the vegetation treatment types on concentrations of total and organic nitrogen are shown in Figure 63. Total nitrogen based on the median values were reduced by both the *Typha* and *Vallisneria* tubs. While the concentration changes were relatively small, the percent reduction from beginning to end was nearly 20%. The organic nitrogen also was reduced during both vegetation treatment processes. Again, the concentration changes were small, and the percentage reduction was lower.

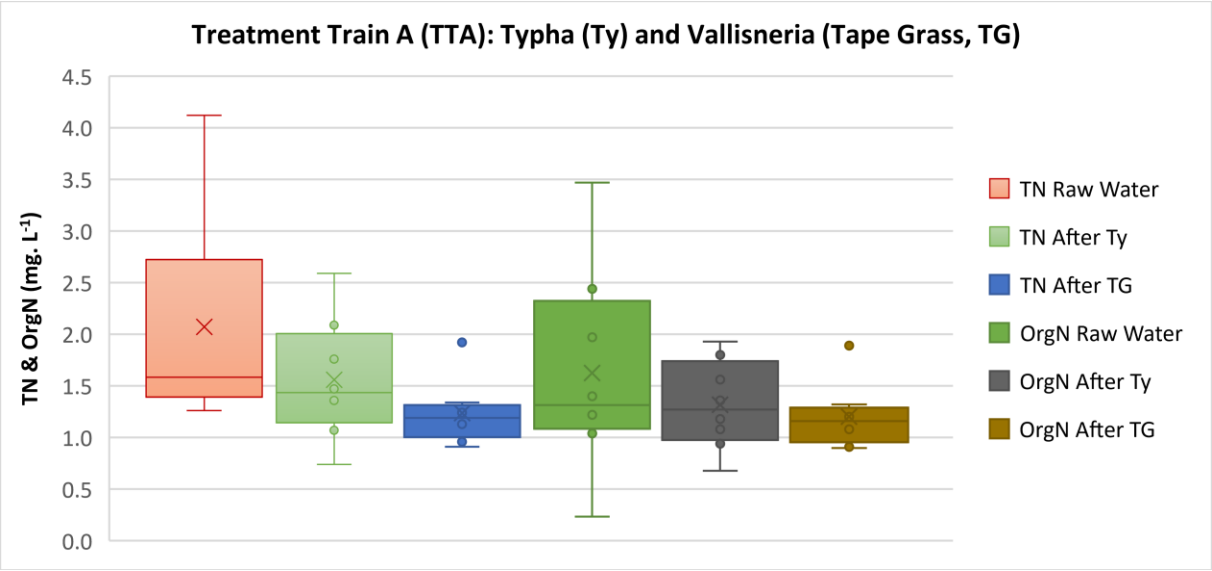


Figure 63. Box plots of changes in total and organic nitrogen in train A (control).

Changes in concentration of nitrate, nitrite, and ammonia in train A are shown in Figure 64. Both the *Typha* and the *Vallisneria* treatment stages produced significant lowering of concentrations with the mean discharge being close to zero. The treatment train had little impact on nitrite, which is considered to be minor due to its low concentration. The vegetation tubs again produced a lowering of ammonia.

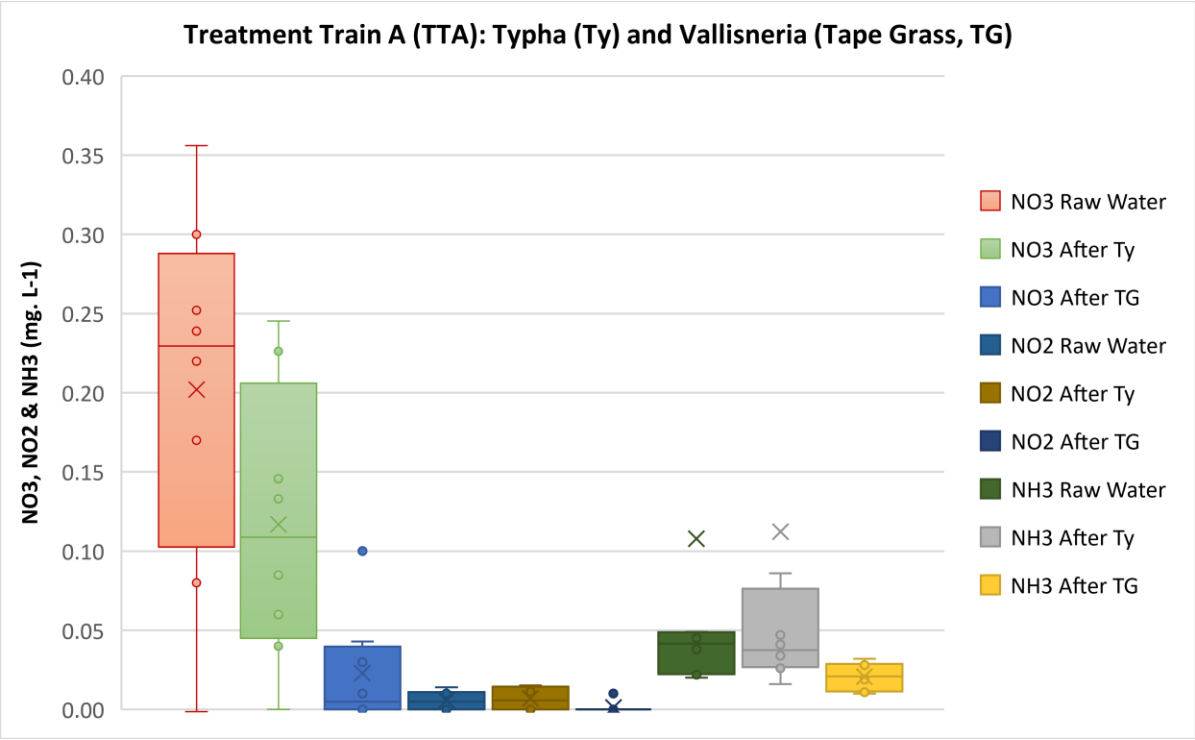


Figure 64. Box plots of nitrate, nitrite, and ammonia in treatment train A (control).

The pattern of total phosphorus and orthophosphate removal by the vegetation tubs was similar to that exhibited by the nitrogen parameters (Figure 65). However, the total phosphorus concentration reduction by the *Typha* tub was greater than the *Vallisneria* tub. With regard to the phosphate, the *Typha* and *Vallisneria* vegetation tubs performed about equally.

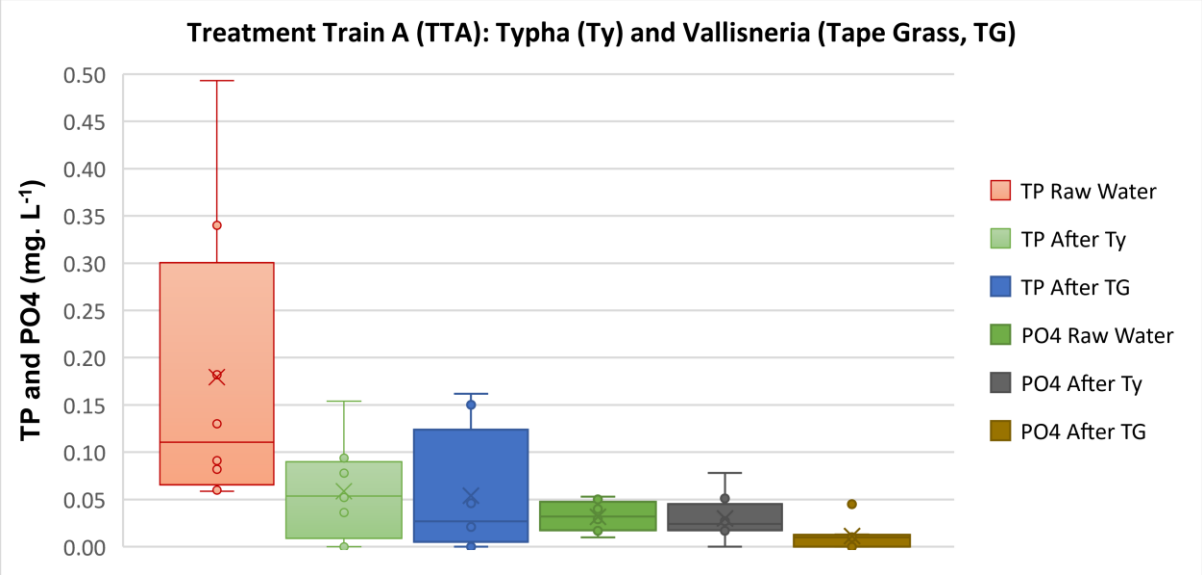


Figure 65. Box plots of total phosphorus and orthophosphate concentration changes in train A (control).

The control train using the two vegetation types produced little change on the concentrations of total and organic carbon (Figure 66). In both the total and dissolved organic carbon, the concentrations at the discharge were slightly lower compared to the raw water.

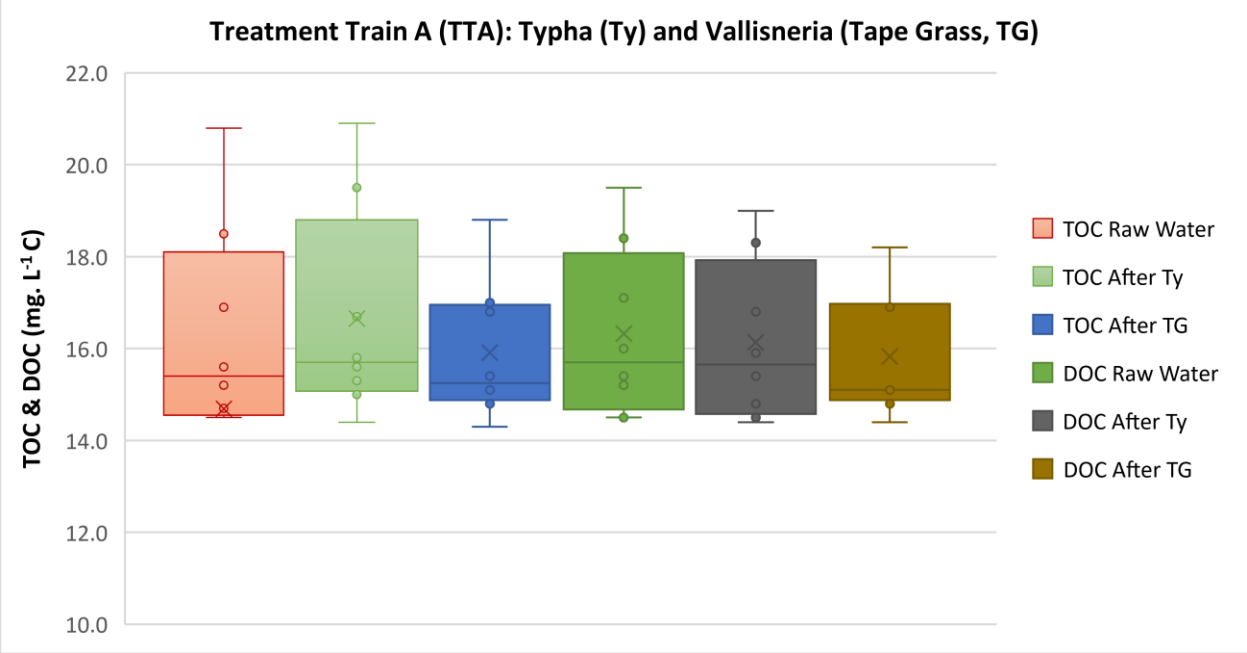


Figure 66. Box plots of concentrations of total and dissolved carbon in treatment train A (control).

In both the field measurements and the laboratory analyses of chlorophyll A, the vegetation tubs lowered the concentrations (Figure 67). There was considerable scatter in the data that

produced large boxes, but comparison of the mean values illustrates the magnitude of the removal. A comparison of the mean value of the raw water to the discharge water shows a reduction of nearly 90%.

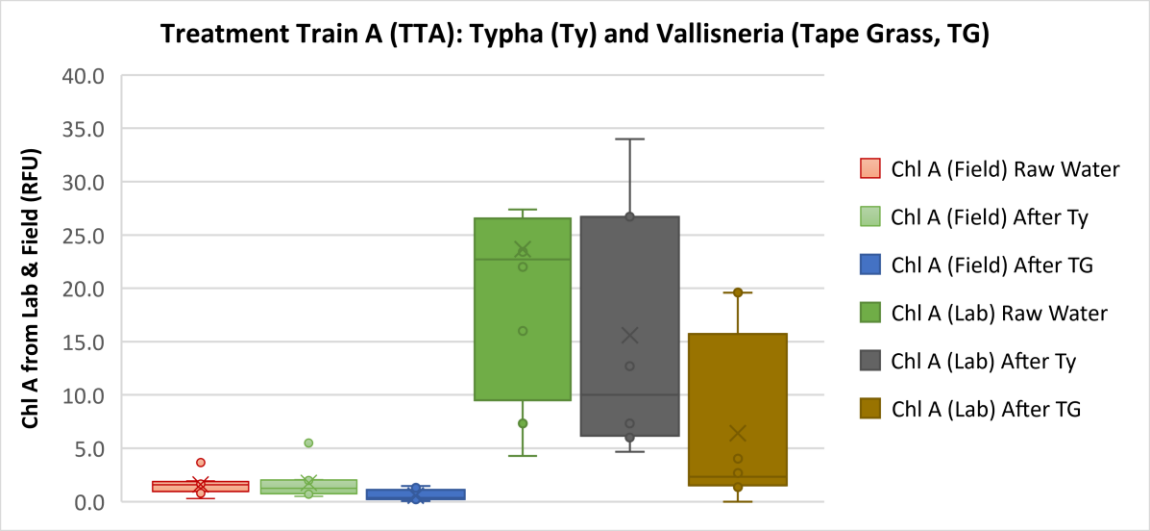


Figure 67. Box plots of field instrument measured and laboratory analyzed chlorophyll A in treatment train A (control).

Train A vegetative treatment produced concentration reductions in algae and cyanobacteria (Figure 68).

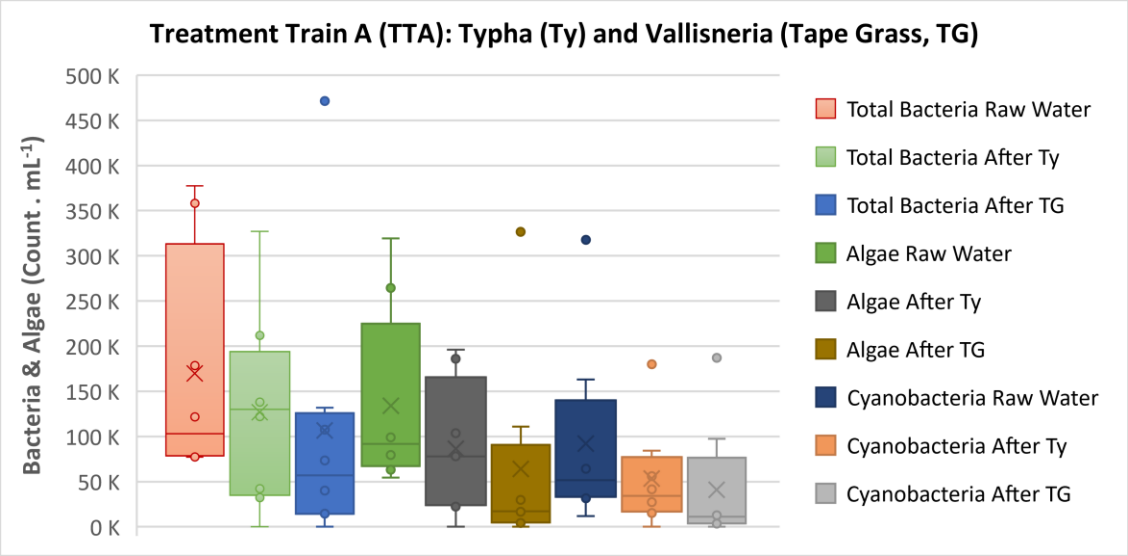


Figure 68. Box plots of total bacteria, algae, and cyanobacteria in treatment train A (control).

The conductivity values and TDS concentrations were essentially unchanged through treatment train A as expected (Figures 69 and 70). The variation in these values were within the scatter based on the field instrument error and some temperature variations. However,

considerable reduction in the turbidity was achieved based on the mean value comparisons (Figure 70). It appears that the largest reduction occurred in the *Vallisneria* tub.

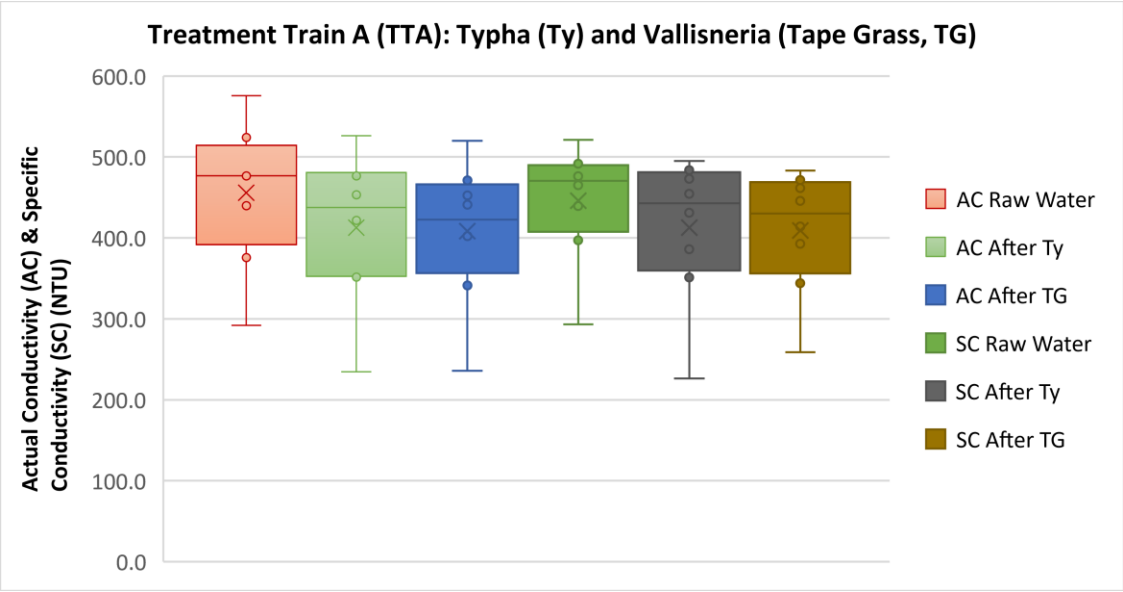


Figure 69. Box plots showing measurements of actual and specific conductivity measured by field instrument in treatment train A (control).

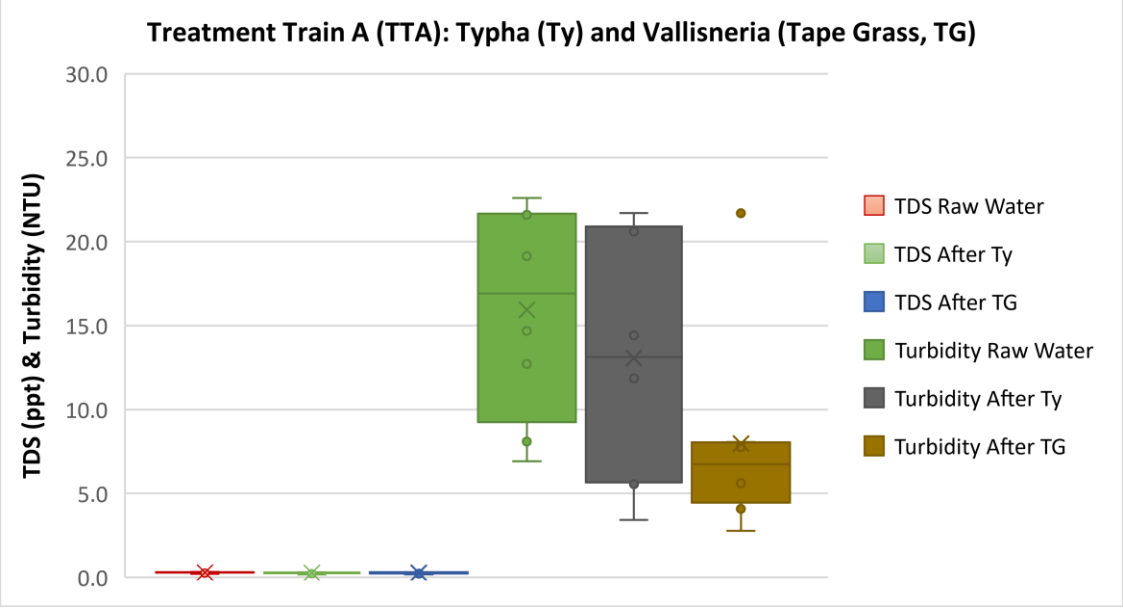


Figure 70. Box plots of TDS concentrations and turbidity measured by field instrument in treatment train A (control).

Dissolved oxygen concentration and saturation show an interesting relation in treatment train A. The mean dissolved oxygen concentration and saturation values are similar in the raw water and the discharge water, but increase significantly within the *Vallisneria* tub (Figure 71).

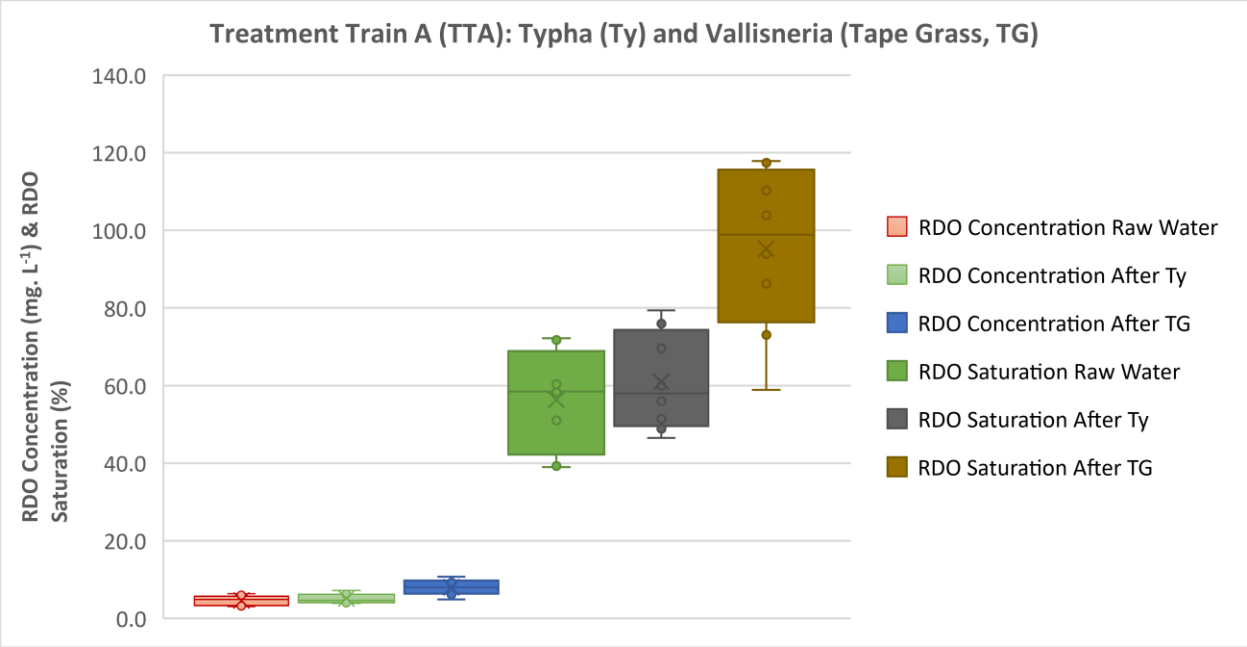


Figure 71. Box plots of field measured dissolved oxygen and saturation in treatment train A (control).

The range in temperature is very low at a few tenths of a degree C within treatment train A (Figure 72). There is a slight decline in mean temperature from the raw water to the *Typha* tub to the Vallisneria tub but is not likely significant.

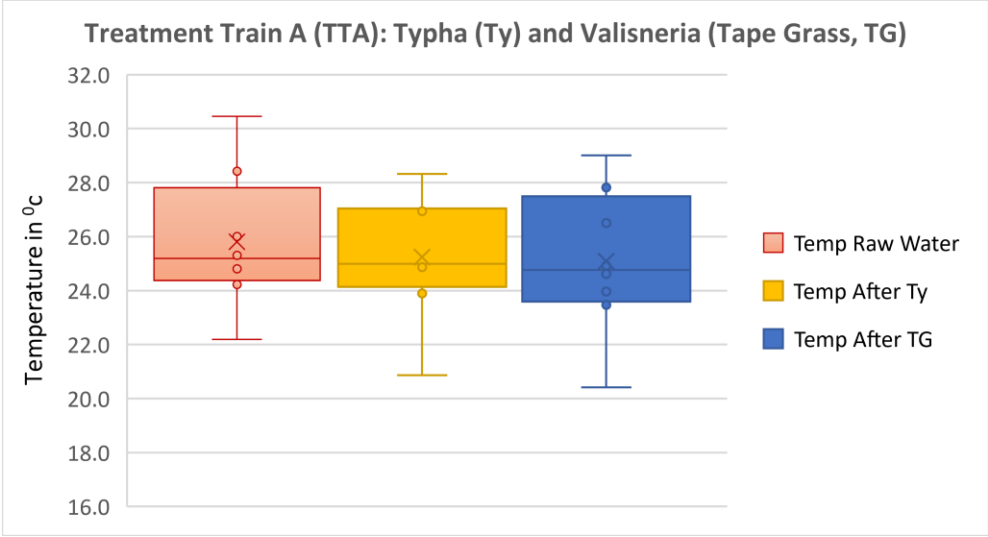


Figure 72. Box plot of temperature in treatment train A (control).

The oxidation-reduction potential (ORP) is lower in the vegetation treatment tubs compared to the raw water (Figure 73). The change between the raw water and the discharge of the *Typha* tub is small but is greater between the *Typha* and *Vallisneria* tubs.

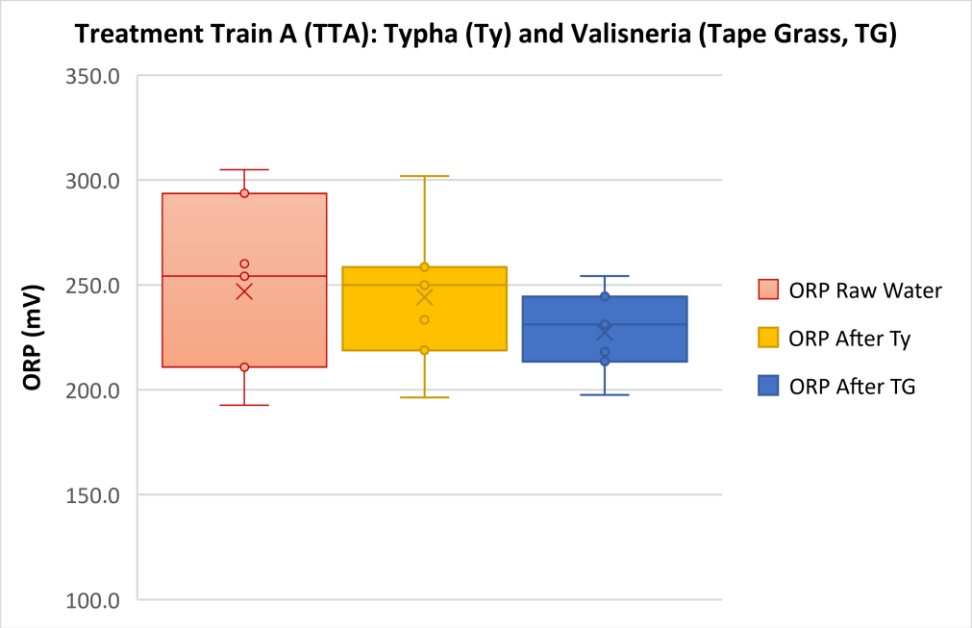


Figure 73. Box plot of oxidation-reduction potential in treatment train A (control).

There is an interesting pattern in pH variation. The pH is close to 7.5 in the raw water and in the *Typha* tub (Figure 74). However, the mean rises to about 8.2 in the Vallisneria tub. Photosynthesis raises pH due to the biogeochemistry reactions which produce increasing amounts of OH^- (Prins et al., 1980).

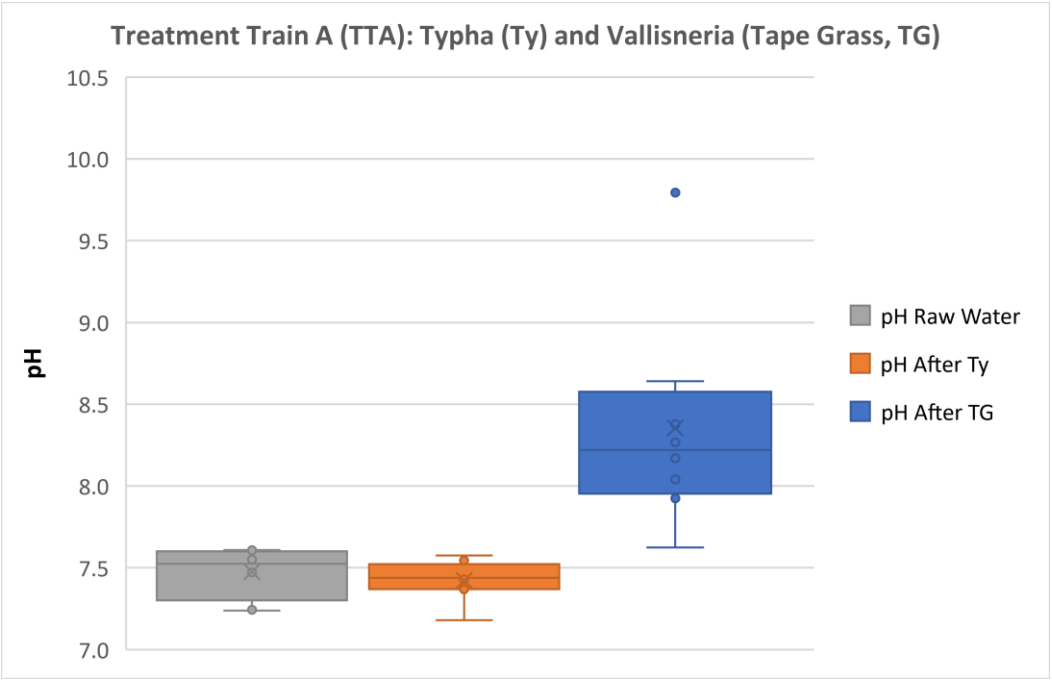


Figure 74. Box plot of pH in treatment train A (control).

Full Treatment Train B Analyses: Raw Water/Sand Filter In/Sand Filter Out/Vegetation Tank 1 Out (Typha)/ Vegetation Tank 2 Out (*Vallisneria****)***

Train B contained the first engineered enhancement of the vegetative treatment of the river water, which was slow sand filtration. The approximate flow rate through the slow sand flow rate was about three GPM to produce a contact time of about five hours. A spillover at the top of the filter maintained one foot of driving head. Box plots were used to value the treatment efficiency of the system.

The total nitrogen concentration was significantly reduced by slow sand filtration (Figure 75). No change occurred in the *Typh*a tub and a small decrease was observed in the *Vallisneria* tub. Based on a comparison of the mean values, the sand filter reduced the organic nitrogen concentration by about 40%. The two vegetation tubs added some organic nitrogen back into the water, but overall, the exit concentration was lower than the inflow concentration.

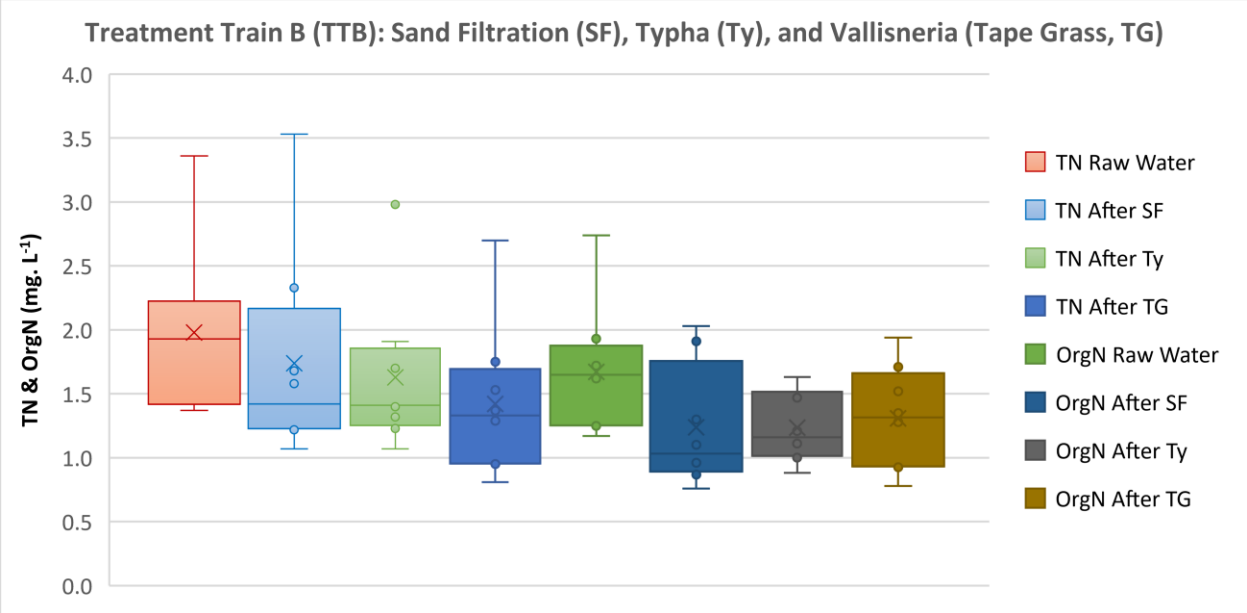


Figure 75. Box plot of the total and organic nitrogen concentrations in treatment train B.

The nitrate and ammonia concentrations followed a similar pattern with an increase from the raw water to the sand filter discharge to some reduction in the *Typha* tub to a very strong reduction in the *Vallisneria* tub (Figure 76). The mean value of nitrate was close to zero at the final discharge and the mean ammonia value was about 0.2 mg/L. The nitrite values are not meaningful based on their very low concentrations with many values falling below detection limits.

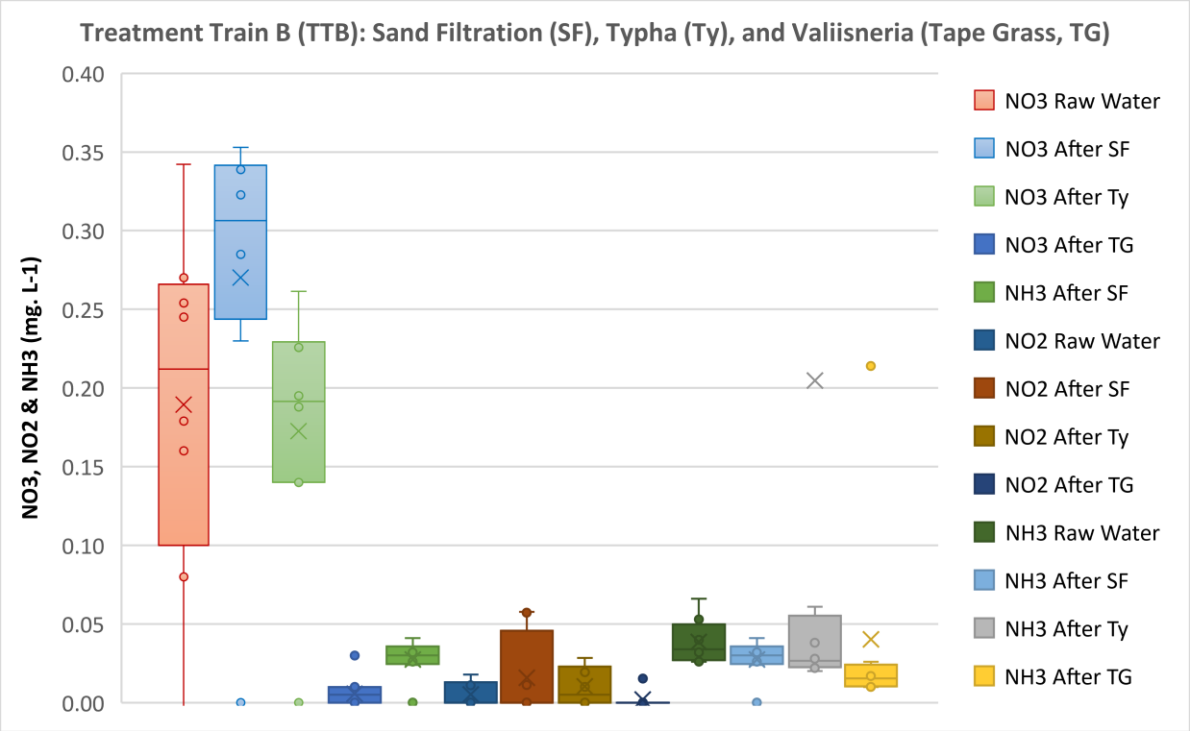


Figure 76. Box plots of nitrate, nitrite, and ammonia concentrations in treatment train B.

Sand filtration had no significant impact on total phosphorus concentration, but the vegetation treatment was quite significant (Figure 77). Total phosphorus removed by the *Typha* and *Vallisneria* tubs was about equal, resulting in the discharge concentration of <0.1 mg/L. The pattern of changes in orthophosphate concentrations was different. The sand filter discharge showed a higher concentration compared to the raw water, while the *Typha* tub shows a lower value and the *Vallisneria* tub significantly lowered the concentration.

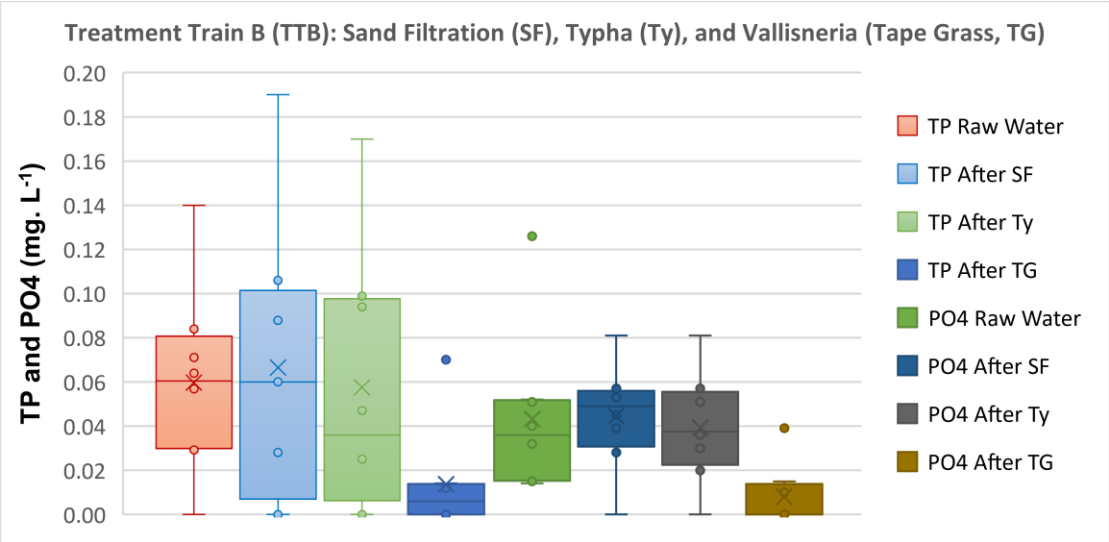


Figure 77. Box plots of total phosphorus and orthophosphate concentrations in treatment train B.

Both TOC and DOC were reduced to a degree by slow sand filtration, but only between 1 and 2 mg/L, which is a small part of the mass (Figure 78). The two vegetation treatment tubs had little impact on the concentration.

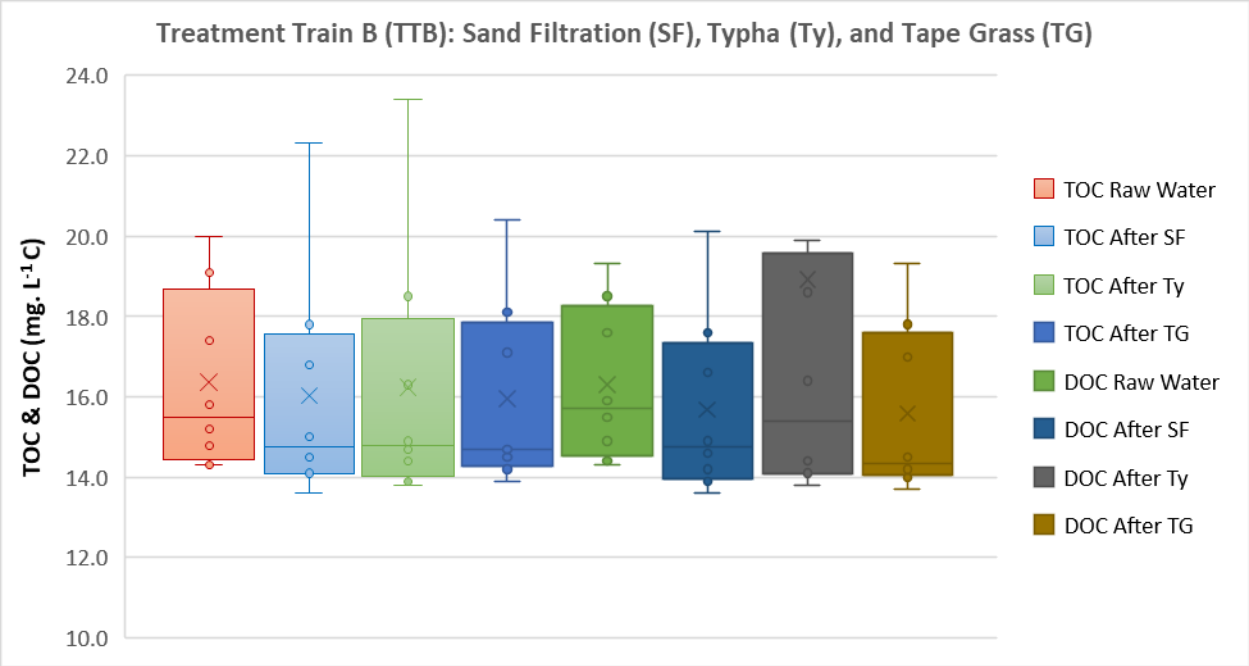


Figure 78. Box plots of TOC and DOC concentrations in treatment train B.

Chlorophyll A was effectively removed by slow sand filtration as demonstrated in both the field meter and laboratory analyzed measurements (Figure 79). However, some chlorophyll A was added back into the water by the *Typha* and *Vallisneria* vegetation tubs. The laboratory-analyzed chlorophyll A values (Figure 79) appear to add back rather large amounts, but a close look at the mean values shows that the outlier values greatly impact the box size.

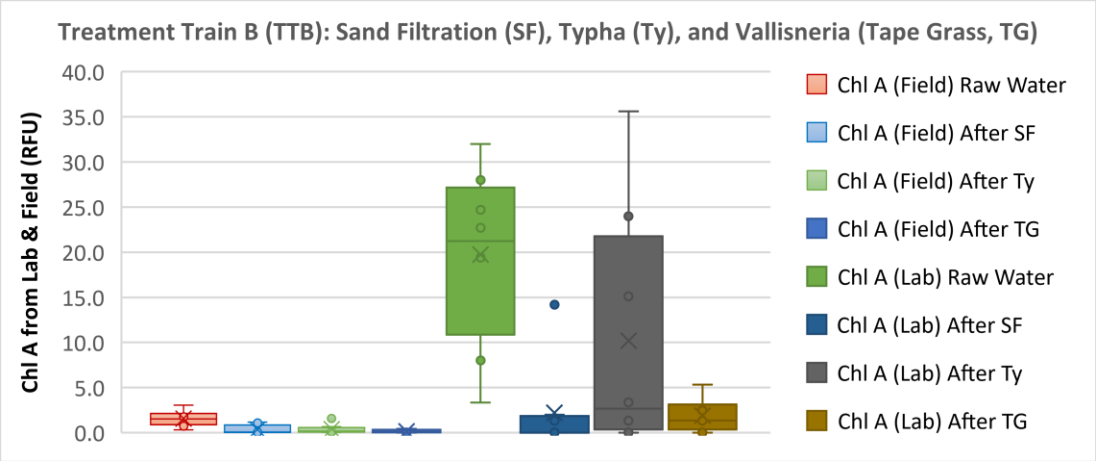


Figure 79. Box plots of meter-measured and laboratory analyzed chlorophyll A in treatment train B.

Total bacteria, algae, and cyanobacteria were effectively removed by slow sand filtration (Figure 80). A slight increase in all parameters occurred in the *Typha* tub and a slight reduction

followed in the *Vallisneria* tub. A comparison of the inflow to outflow shows the overall treatment for all three was effective.

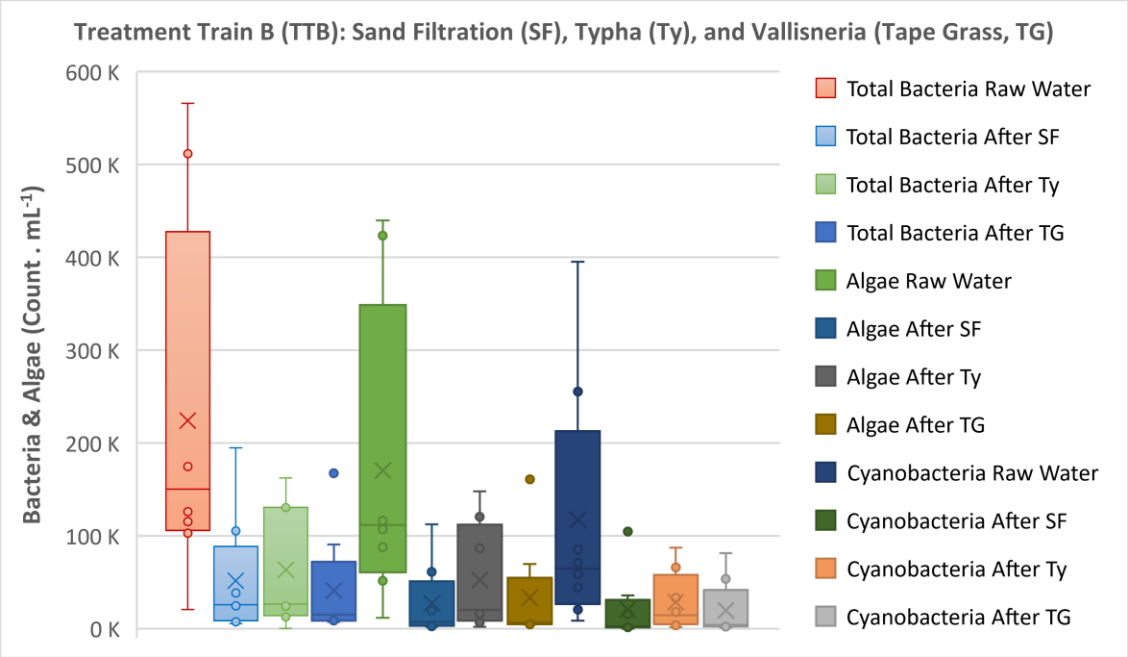


Figure 80. Box plots of total bacteria, algae, and cyanobacteria abundance in treatment train B.

The processes in treatment train B did not significantly affect the conductivity values nor the TDS concentrations (Figure 81 and 82). The variations observed were likely caused by instrument drift and small changes in temperature. Turbidity removal was quite effective in the sand filter, but some turbidity was added in the *Typha* tub discharge. Turbidity values were reduced in the discharge of the *Vallisneria* tub. By comparison of the mean values, inflow turbidity was reduced by about 80% in the treatment train.

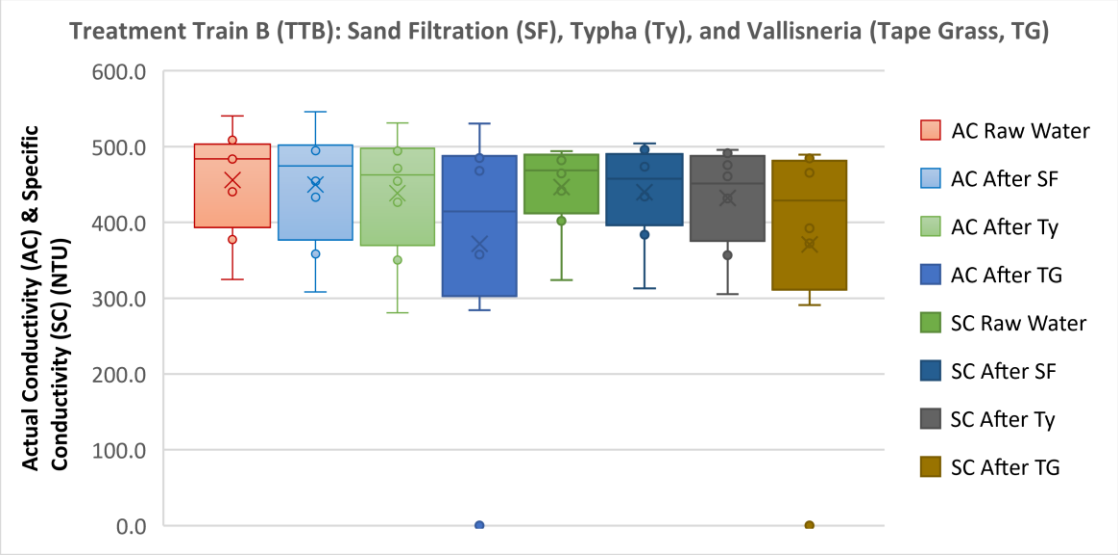


Figure 81. Box plots of actual conductivity and specific conductivity values in treatment train B.

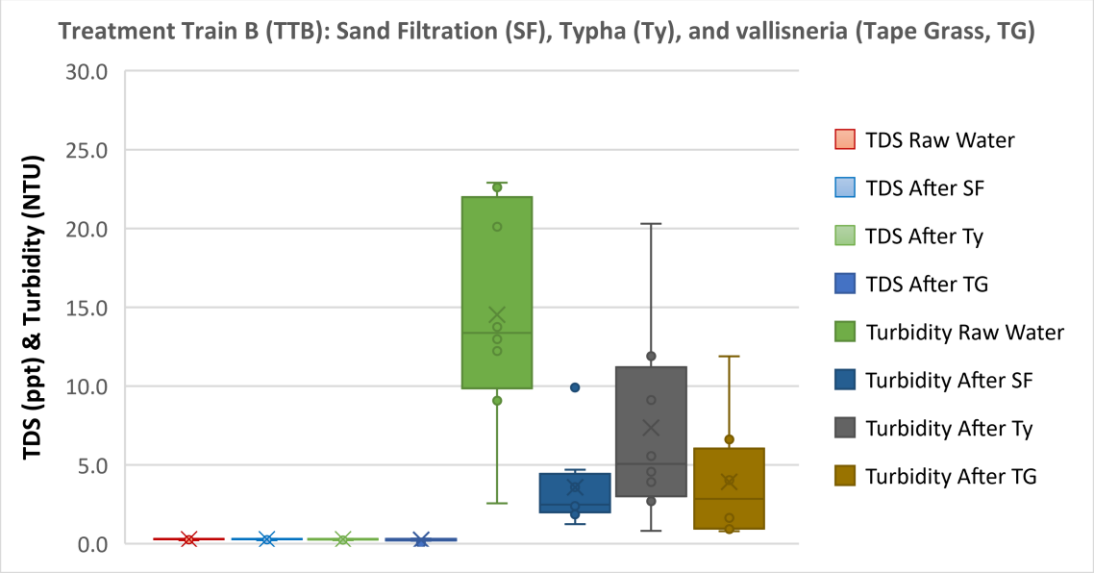


Figure 82. Box plots of TDS concentration and turbidity values in treatment train B.

Dissolved oxygen concentrations and saturation decreased in the sand filter (Figure 83). Then, both concentration of the oxygen and the saturation percentage increased in both vegetation tubs. The most extreme increase occurred in the *Vallisneria* tub where the mean value was very close to saturation and many of the temporal values were above saturation.

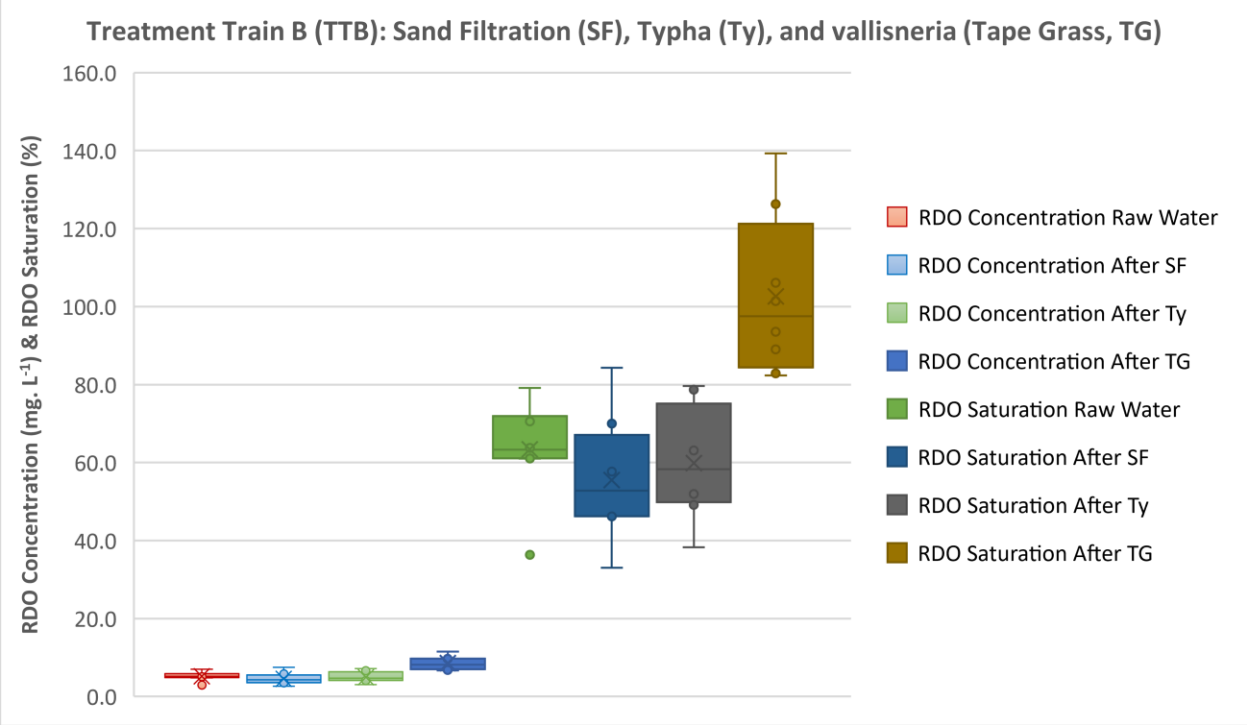


Figure 83. Box plots of dissolved oxygen concentration and saturation percentage in treatment train B.

Changes in water temperature in treatment train B were only a few tenths of a degree C (Figure 84). It appeared to have cooled slightly in the sand filter, and both the vegetation tubs.

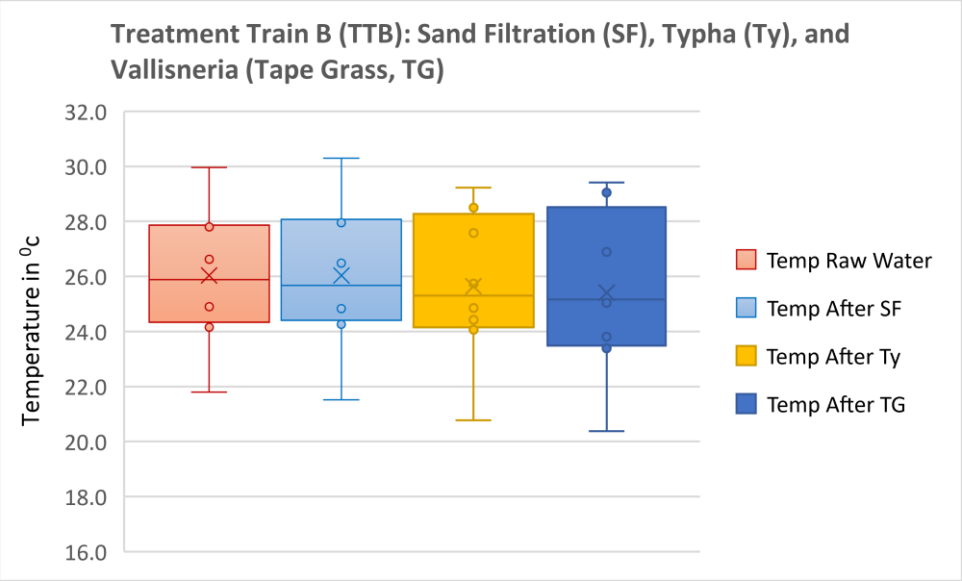


Figure 84. Box plots of water temperature in treatment train B.

The median of the oxidation-reduction potential (ORP) increased slightly through the sand filter and subsequently decreased slightly in the *Typha* tub (Figure 85). It decreased further in the *Vallisneria* tub to a greater degree compared to the lowering in the *Typha* tub.

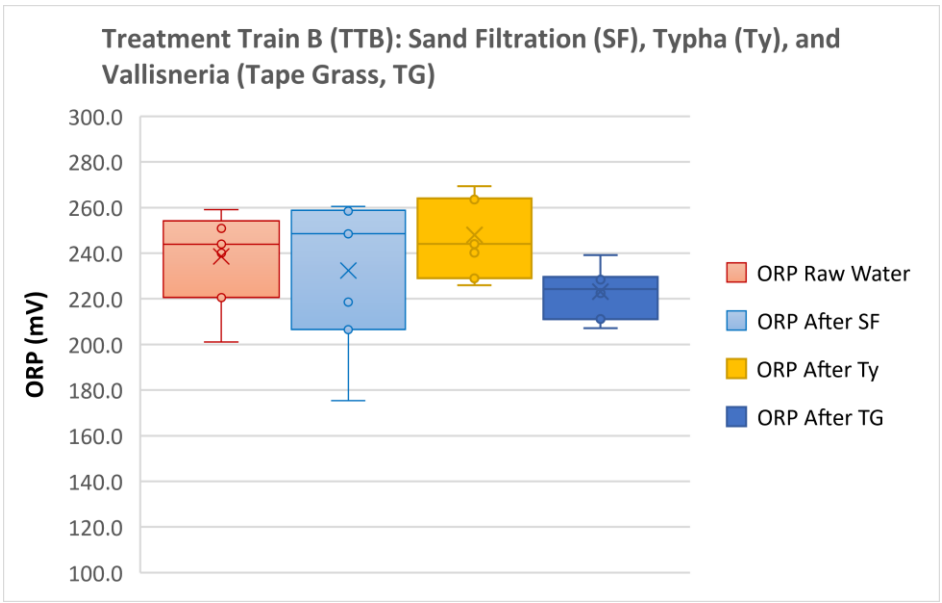


Figure 85. Box plots of variation in field-measured oxidation-reduction potential in treatment train B.

Field measurements of pH show that sand filtration did not change it very much, and pH stayed near 7.5 or slightly alkaline (Figure 86). There was a slight lowering of the mean in the *Typha* tub to about 7.4, and then a substantial increase in the *Vallisneria* tub to a mean near 8.7. Photosynthesis raises pH due to the biogeochemistry reactions which produce increasing amounts of OH- (Prins et al., 1980).

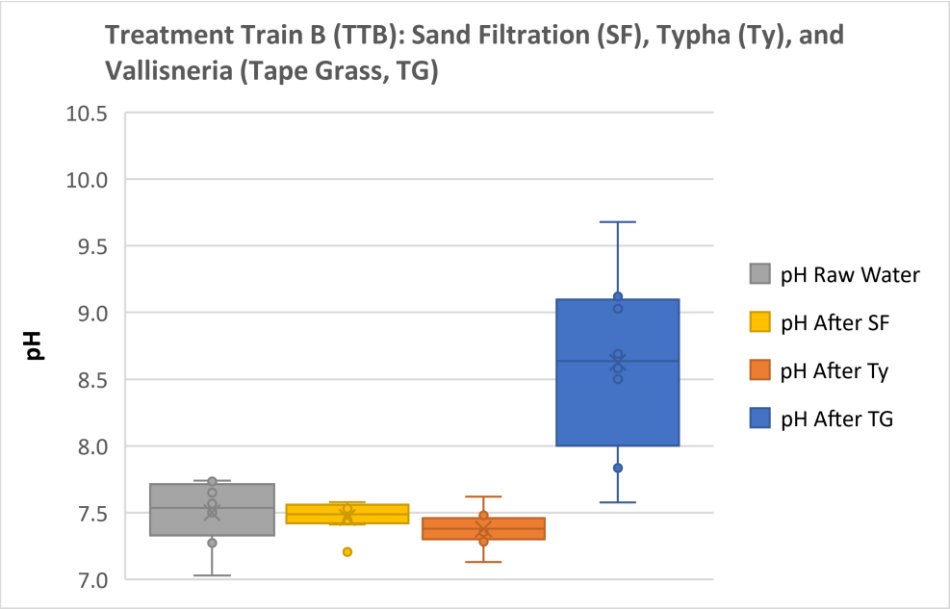


Figure 86. Box plots of pH changes in treatment train B.

Full Treatment Train C Analyses: Raw Water/After Sand Filtration/After UV/ Vegetation Tank 1 out (Typha)/ Vegetation Tank 2 Out (Vallisneria)

Evaluation of the water treatment achieved for each process was done by using box plots of the full dataset. In train C, the sequence of the full process is raw water/after slow sand filtration/after UV treatment/after emergent vegetation tub (*Typha*) and at the discharge of the submergent vegetation tub (*Vallisneria*).

One of the primary objectives of the study was to evaluate whether the full process train would reduce organic nitrogen concentrations, particularly the UV exposure which had the potential of breaking down the organic nitrogen molecules. Figure 87 shows total and organic nitrogen through the entire process. The mean after the *Typha* tub appears to have risen slightly, which may be a function of a single outlier point. The largest reduction in organic nitrogen was achieved in the sand filtration process with minor changes in the UV and vegetation treatment processes (Figure 86).

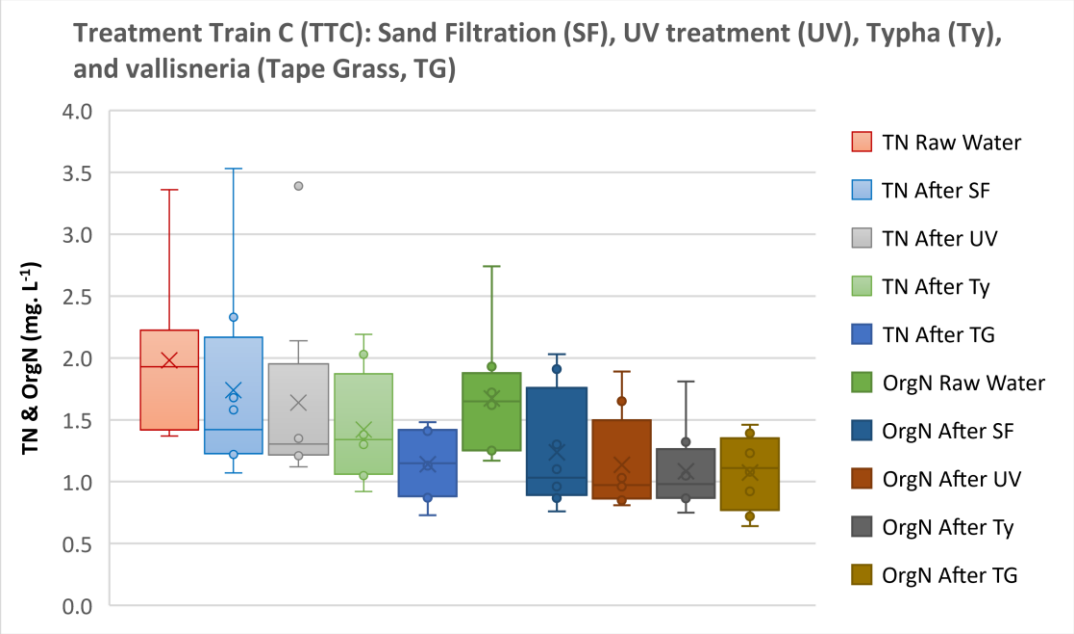


Figure 87. Comparison of effectiveness of the full process train C on concentrations of total and organic nitrogen.

Process train C had mixed results on nitrate, nitrite, and ammonia reduction (Figure 88). Total nitrogen concentration during the sand filtration process actually increased nitrate above the raw water. The UV process had no impacts. The two vegetation treatment tanks show significant uptake of nitrate with the last tank (*Vallisneria* tub) being the most significant. Nitrite is not a significant parameter and occurs at low concentrations. The ammonia concentrations show a reduction during sand filtration and UV with a slight increase after the *Typha* tub, and a final lowering in the *Vallisneria* tank.

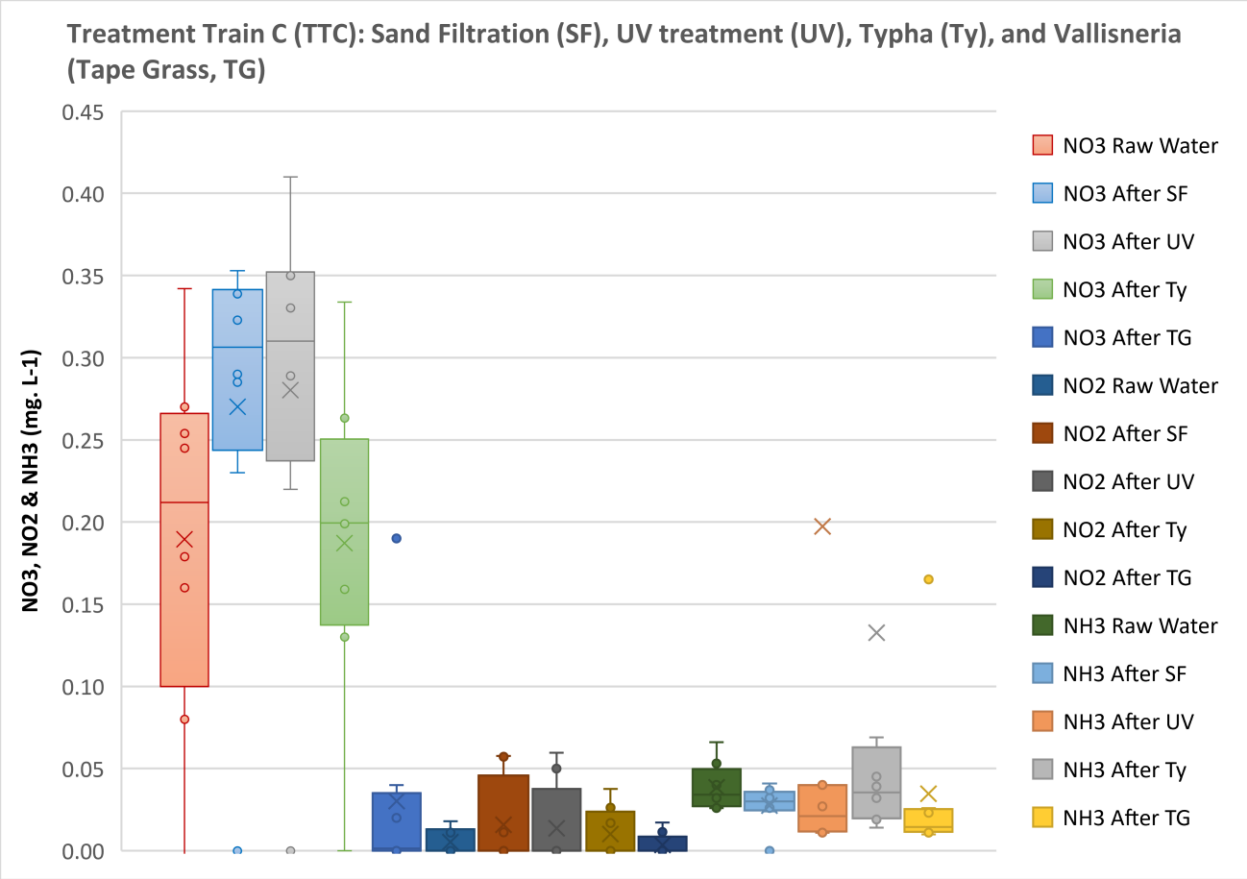


Figure 88. Treatment effectiveness of the various process in train C for reduction in nitrogen concentration.

Removal of total phosphorus and orthophosphate showed a similar pattern (Figure 88). Total phosphorus concentration remained rather constant through the first four processes and showed a significant drop in the last process, which was the *Vallisneria* tub. Orthophosphate increased slightly during sand filtration, stayed constant through UV treatment, declined slightly in the *Typha* tub, and most significantly in the *Vallisneria* tub. A comparison of the initial raw water mean concentrations of total phosphorus and orthophosphate at about 0.06 and 0.02 mg/L respectively to the final means of about 0.02 and 0.015 mg/L respectively show some removal.

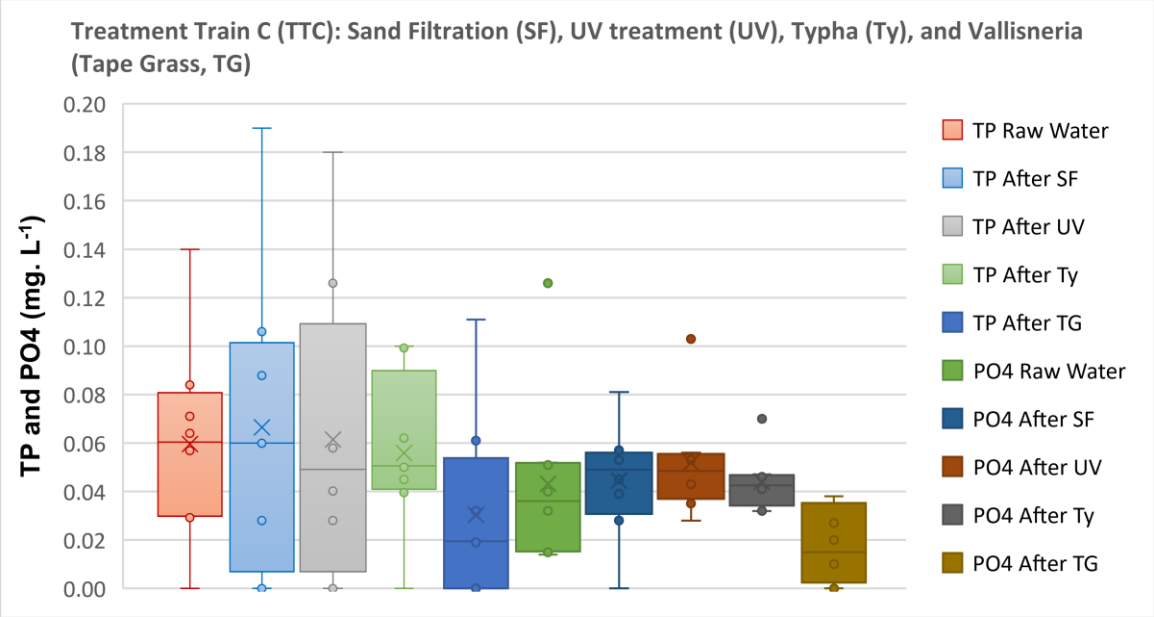


Figure 89. Box plots of changes in total phosphorus and orthophosphate concentrations during the processes in train C.

Based on the box plot comparisons of TOC and DOC, concentrations through treatment train C show a narrow range of mean values indicative of minimal treatment (Figure 90). The slow sand filtration did remove some TOC and DOC, but the UV and vegetation treatment processes did not greatly change the concentrations, and the vegetation treatment tanks actually slightly increased concentrations.

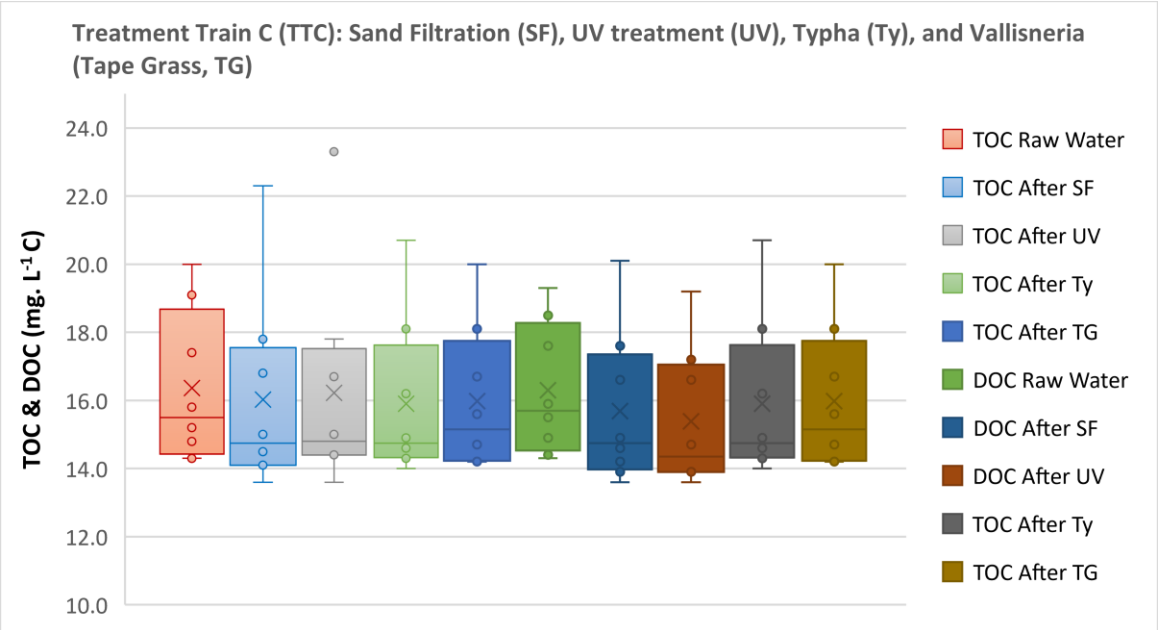


Figure 90. Box plot of changes in TOC and DOC through treatment train C.

Changes in the field meter and laboratory-analyzed values for chlorophyll A show dramatic reduction from the raw water through the sand filtration process (Figure 91). The UV process contributed to a minor reduction. The chlorophyll A values increased in the vegetation treatment processes. However, the reduction of the means in the laboratory chlorophyll A data from above 21 RFU to near 2 RFU is a significant reduction.

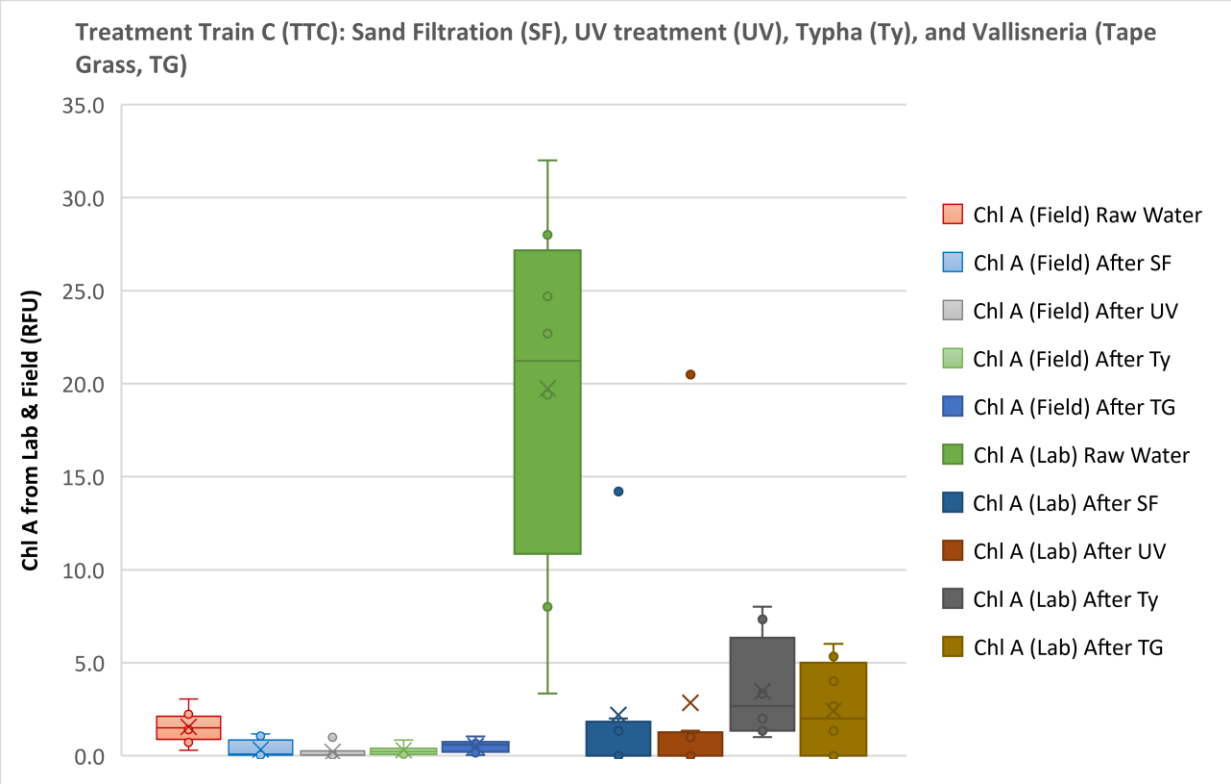


Figure 91. Changes in the field measured and laboratory analyzed values of chlorophyll A in treatment train C.

Concentrations of total bacteria, algae, and cyanobacteria in treatment train C showed that the sand filtration was very effective at removal of all microbes (Figure 92). Additional concentration reduction does occur in the UV process. The two vegetation tubs did not significantly change the counts.

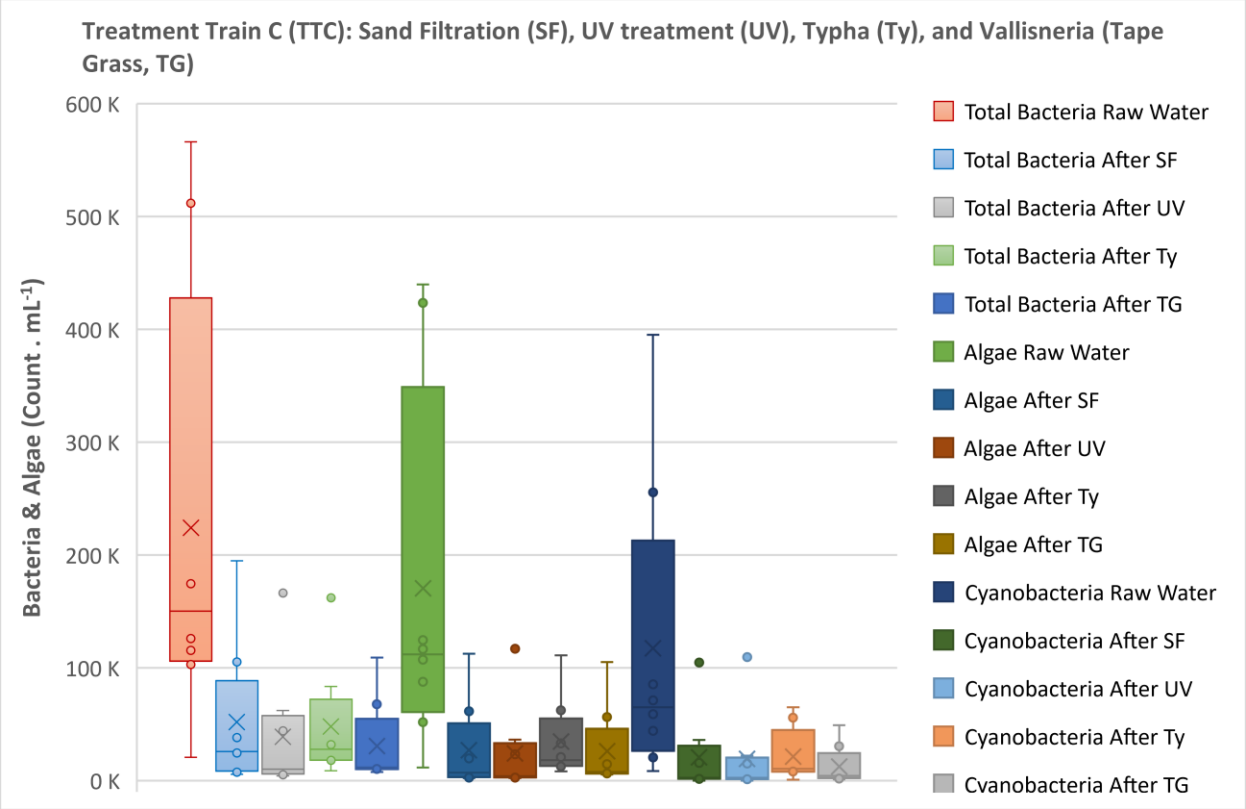


Figure 92. Box plots showing the effectiveness of the treatment processes in train C.

As expected, treatment occurring in train C did not have significant impacts on the conductivity values and TDS concentrations (Figures 93 and 94). The turbidity showed some rather odd trends in that the sand filtration which removed most of the turbidity, but in each subsequent process it increased until at the final discharge it was above the raw water. We believe that this was caused by buildup of organic material in the tank discharge pipes and is not a true analysis of the turbidity removal. The median value after treatment is still below the raw water mean and 25th percentile, indicating an upward skew from outliers.

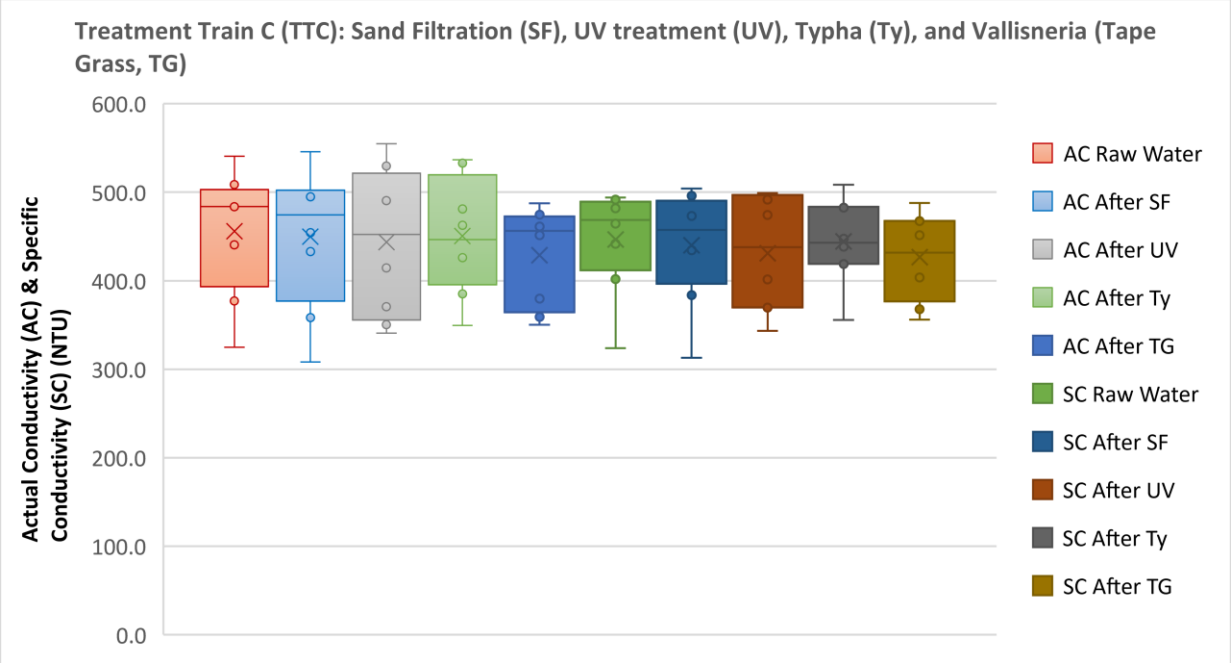


Figure 93. Box plots of the conductivity data across all train C treatment processes.

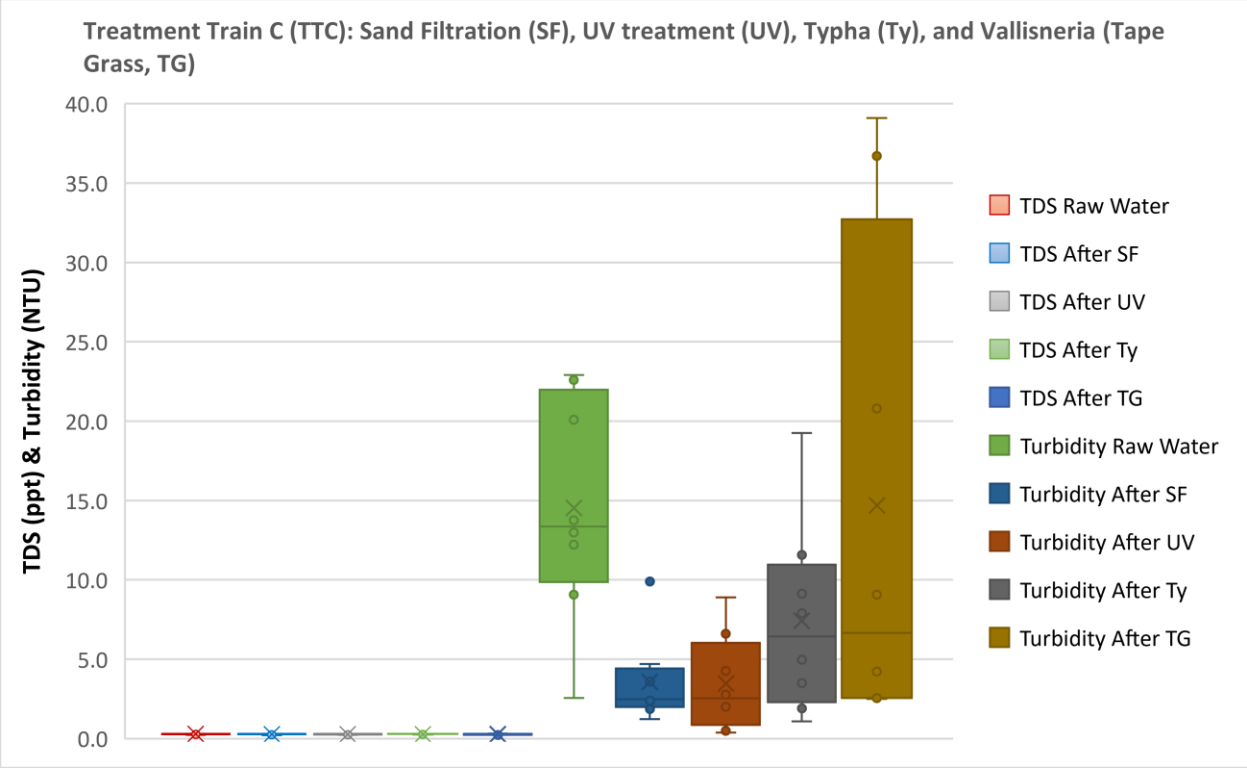


Figure 94. Box plots of the TDS concentrations and turbidity through the train C processes.

Field measurements of the dissolved oxygen concentration and percentage of saturation show that the sand filter lowered the values as expected (Figure 95). The UV did not have any significant effect, but each vegetation treatment tub added oxygen, with the *Vallisneria* tub increasing it to above saturation.

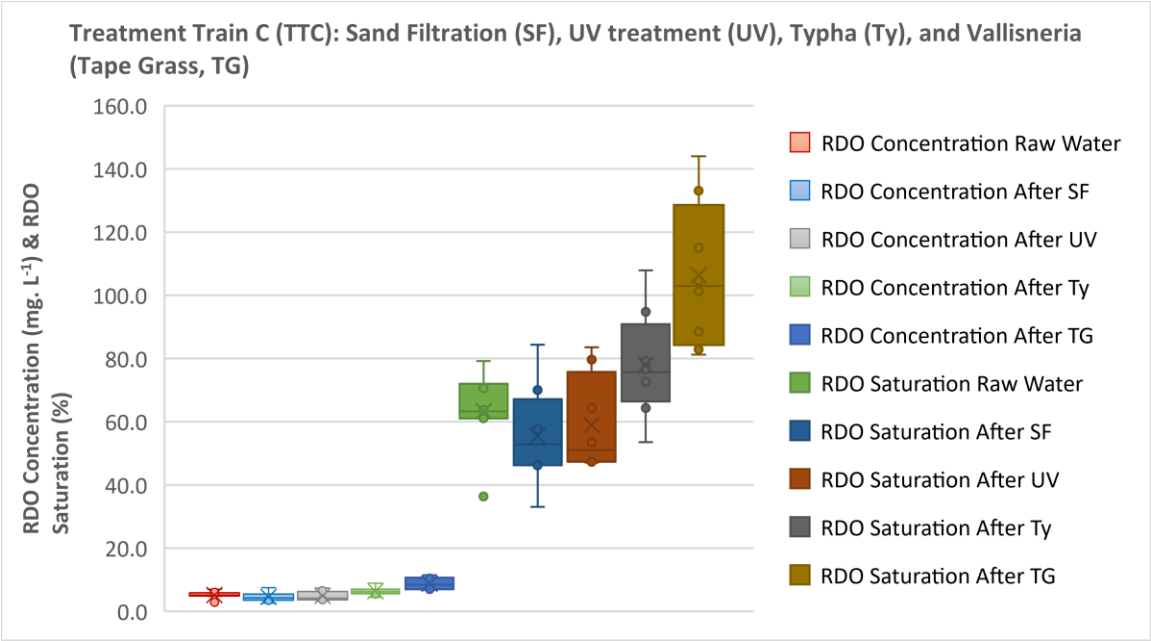


Figure 95. Box plots of dissolved oxygen concentration and saturation in treatment train C.

The water temperature varied little (<1 ^{o}C) through treatment train C (Figure 96). Comparison of the mean values shows some cooling in the vegetation tanks, but not of significance.

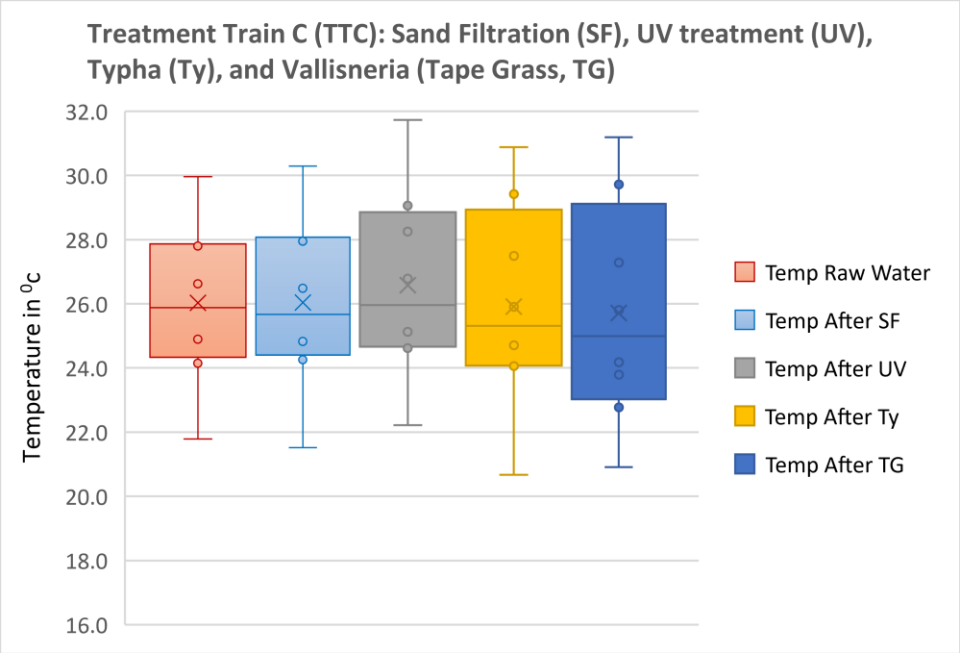


Figure 96. Variation of water temperature through train C treatment process.

The oxidation-reduction potential (ORP) varied through treatment train C (Figure 97). The raw water and sand filter discharge were nearly equal followed by a minor reduction after UV treatment. It increased in the *Typha* tub and then reduced significantly in the *Vallisneria* tub at the end.

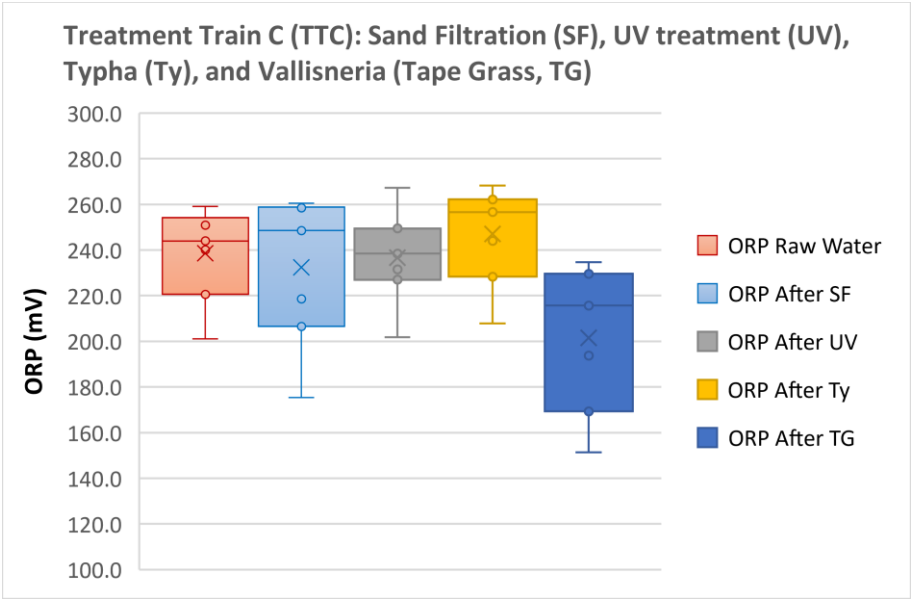


Figure 97. Box plot showing the oxidation-reduction potential variation in treatment train C.

The pH stayed in a very narrow range centered near 7.5 from the raw water through sand filtration and UV treatment (Figure 98). It increased slightly in the *Typha* tub and then increased greatly in *Vallisneria* tub. This shows the obvious impacts of photosynthesis as photosynthesis raises pH due to the biogeochemistry reactions which produce increasing amounts of OH- (Prins et al., 1980).

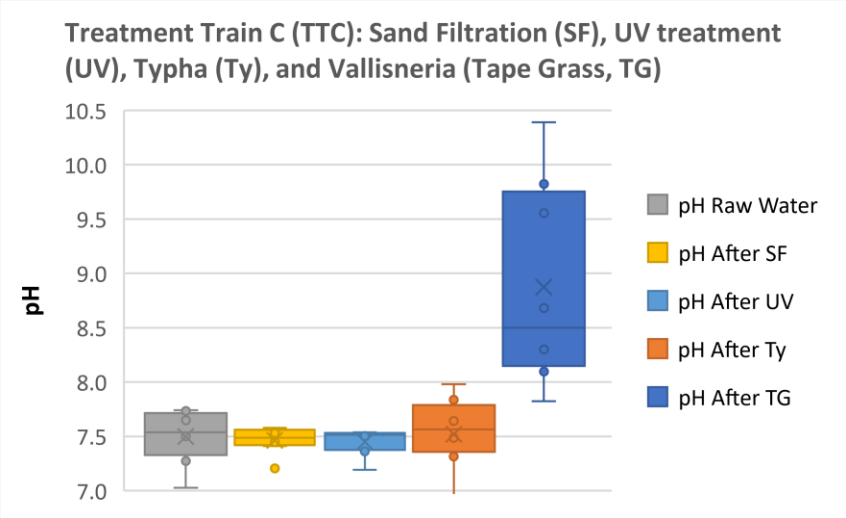


Figure 98. Box plots of the changes in pH in treatment train C.

Impacts of the Holding Tank on Water Quality

It was necessary to install a holding tank after the slow sand filter treatment to provide a gravity flow balance in part of the system. Although the tank was painted black to reduce biochemical activity within the temporarily stored water, inevitably some had to occur. A detailed series of graphics are given in Appendix B to show the details of the changes in water quality observed. Since the tank is really not a significant part of the water treatment system, it is not discussed in detail. Based on the sampling of before and after water quality data collected, the holding tank had the following impacts on water quality: 1) slight decreases in total and organic nitrogen concentration occurred, 2) no significant changes occurred to nitrate, nitrite, and ammonia concentrations, 3) total phosphorus concentration increased slightly (likely not statistically significant) and orthophosphate concentrated stayed the same, 4) TOC and DOC concentrations showed no change, 5) measurements of chlorophyll A by field instrument and laboratory analysis showed no significant variation, 6) total bacteria concentration declined slightly and algae and cyanobacteria concentration showed little change, 7) conductivity values and TDS concentrations were unchanged, 8) turbidity showed a very minor increase, 9) the dissolved oxygen concentration declined slightly and the saturation increased from about 5 to 45%, 10) water temperature remained constant, 11) the mean oxidation-reduction potential declined from about 250 to 220 mV, and the pH mean declined from about 7.48 to 7.38.

Impacts of the Piping System on Water Quality at Various Locations

The piping that connects the various processes on the site is schedule 40-, one- and two-inch diameter, white colored PVC pipe. It was observed during sampling that if the connecting piping was stepped on or jarred, the water would become turbid at the entry point into a treatment process. There are two possible explanations for the internal biofilm issue which are: 1) organic matter has formed a biofilm on the inside of the pipe based on the high organic composition of the water, the possible charge of the pipe, and the low flow rate that prevents scouring, and 2) there is some light penetration through the pipe that promotes initial organic biofilm growth with enhancement from the raw river water. The issue may also be a combination of both issues. It should be noted that the high concentrations of total bacteria, algae, and cyanobacteria in the water make it likely that transparent exopolymer particles (TEP) are also abundant in the raw water. TEP is composed of acidic polysaccharides, which are gels and quite sticky. This substance could form the base of the biofilm to promote growth in thickness and at the same time provide food for some of the living bacteria.

The section of pipe chosen for analysis connected the holding tank to the *Typha* tub or connected sampling stations 7 and 8. To show the impact of the biofilm within the transmission pipe, a number of affected parameters are shown with an evaluation of the impact on water quality.

Data collected show that there are small increases in the total and organic nitrogen added in the pipeline between the two points (Figures 99 and 100). The total nitrogen mean is affected by an outlier (high concentration), so the change is probably less than indicated by comparison of the means.

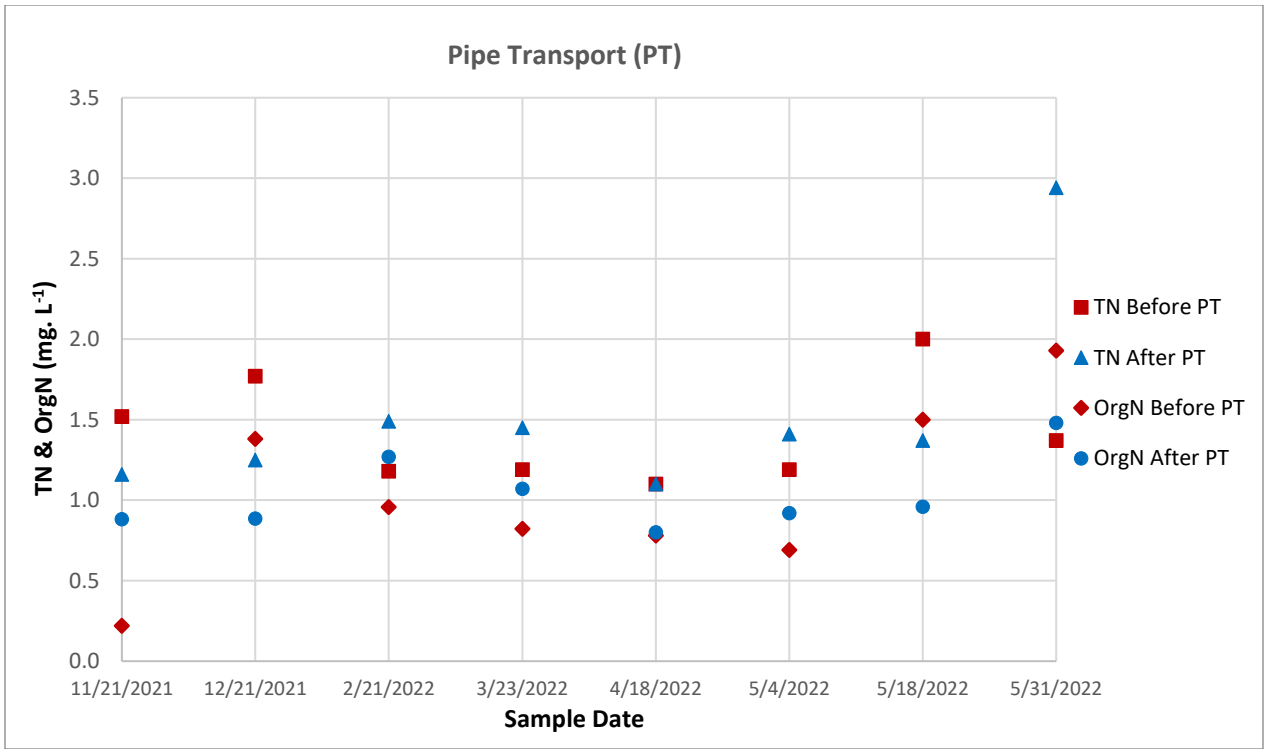


Figure 99. Temporal changes in concentration of total and organic nitrogen from travel through a pipeline from station 7 to 8.

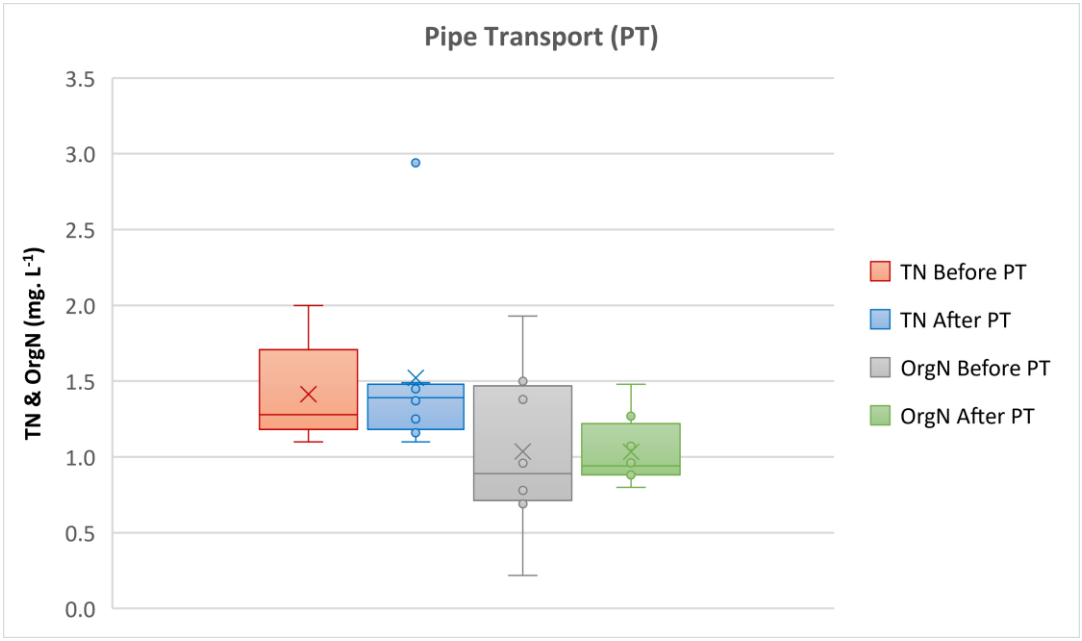


Figure 100. Box plots showing changes in total and organic nitrogen in the pipeline connecting stations 7 and 8.

Changes in nitrate, nitrite, and ammonia concentrations in the pipeline were minimal (Figures 101 and 102). A small reduction in nitrate mean can be observed in Figure 102.

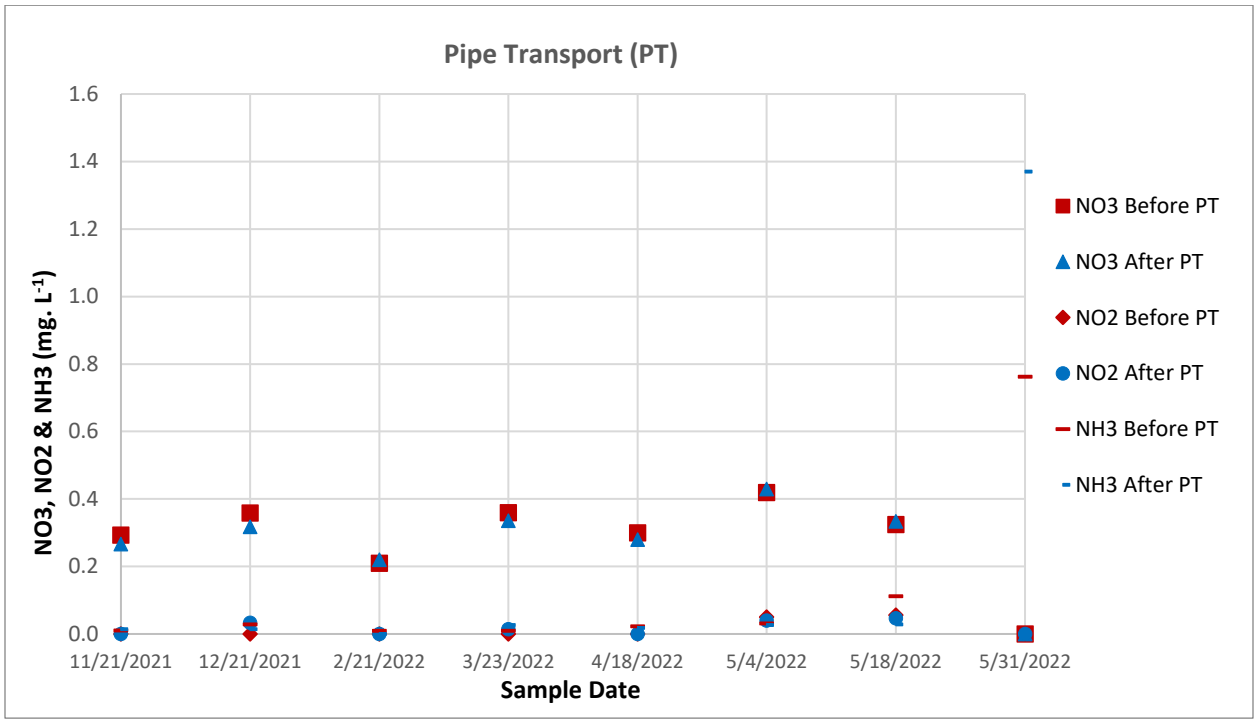


Figure 101. Temporal changes in nitrate, nitrite, and ammonia concentration in the pipeline connecting station 7 and 8.

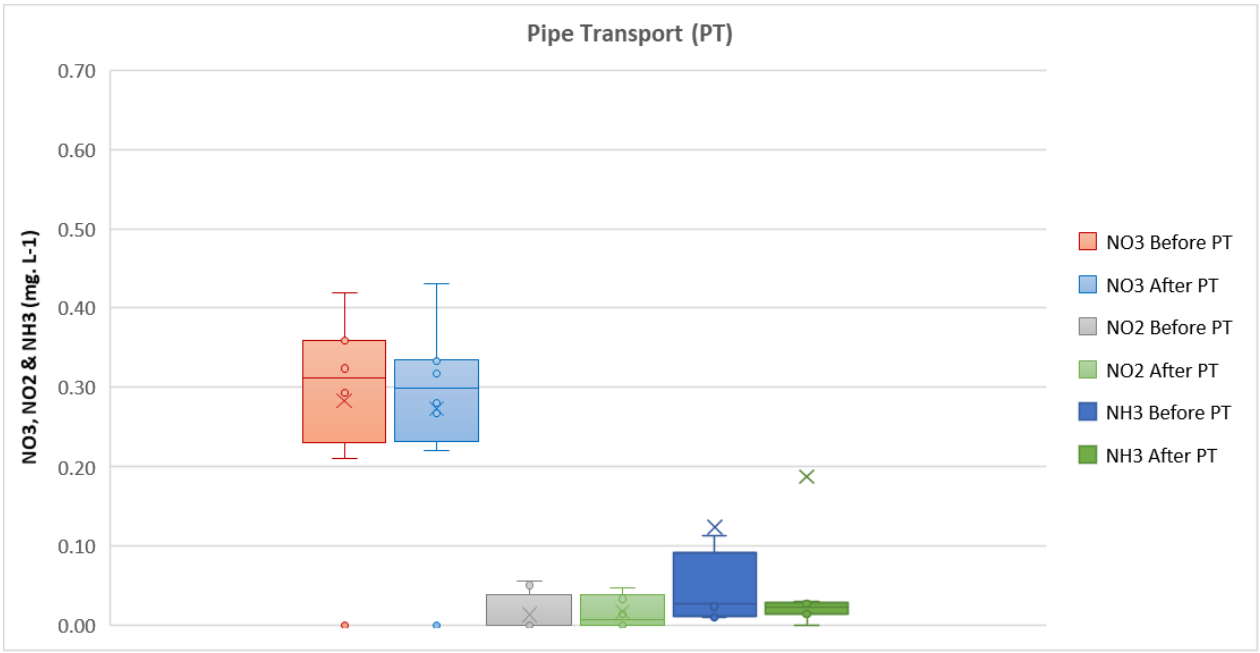


Figure 102. Box plot of the concentration changes in nitrate, nitrite, and ammonia in the pipeline between station 7 and 8.

Concentration of the total phosphorus was lowered to a significant degree in this segment of pipeline (Figure 103). The orthophosphate concentration showed no significant changes.

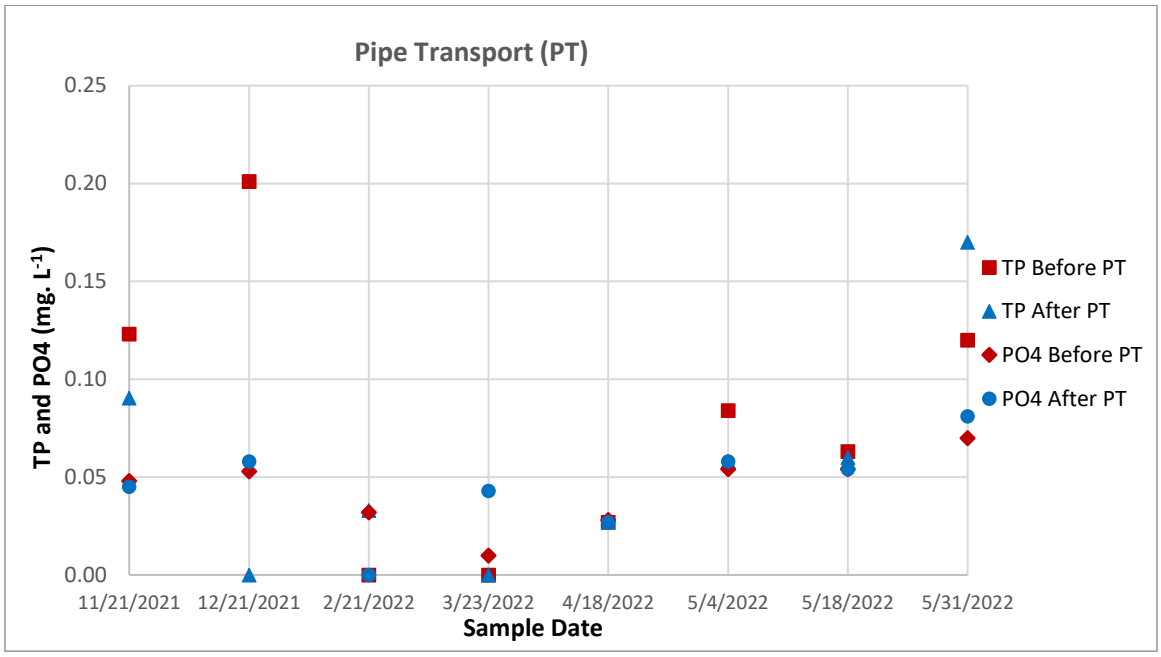


Figure 103. Temporal changes in the concentration of total phosphorus and orthophosphate in the pipeline connecting stations 7 and 8.

There were no significant changes in the concentrations of total and dissolved carbon and the graphics for this comparison are in Appendix C. Water transport in the pipeline between stations 7 and 8 also did significantly affect the chlorophyll A values and total bacteria, algae, and cyanobacteria concentrations based on comparisons of the mean values (graphs in Appendix C).

Transport of the water through the pipeline did not affect the real conductivity and specific conductivity values and the TDS concentrations (Figures 104-106). However, there was a slight decrease in the turbidity (Figure 106).

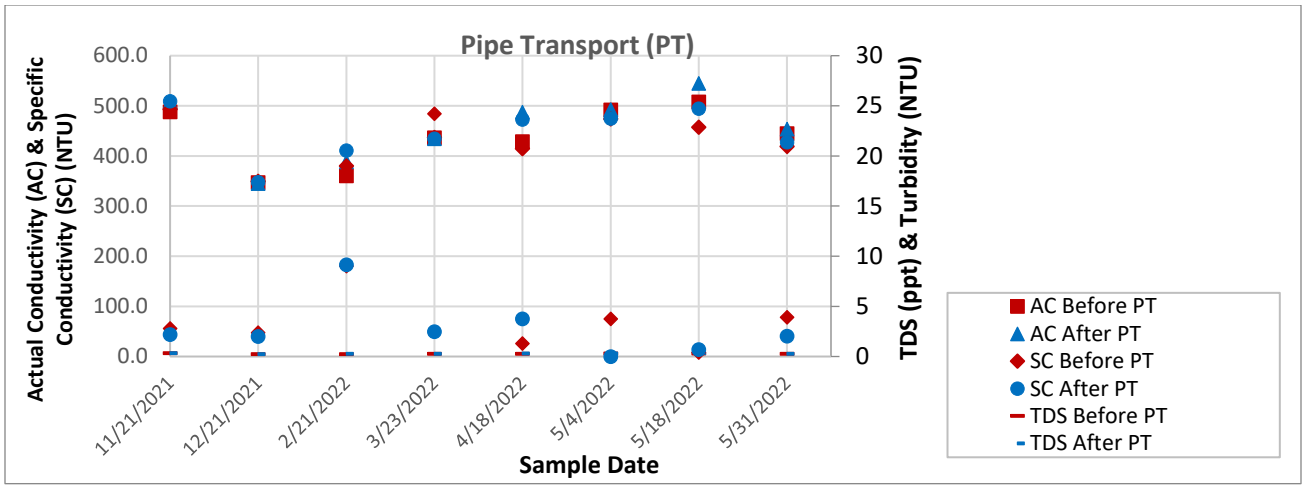


Figure 104. Temporal changes in real conductivity, specific conductivity, TDS concentration, and turbidity in the pipeline from stations 7 to 8.

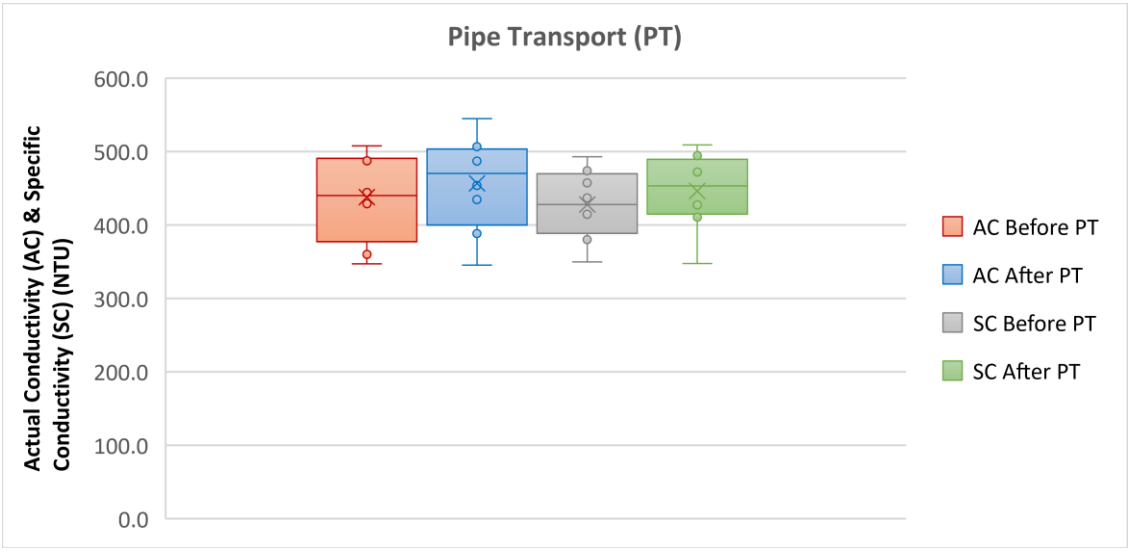


Figure 105. Box plot of the real conductivity and specific conductivity in the pipeline between stations 7 and 8.

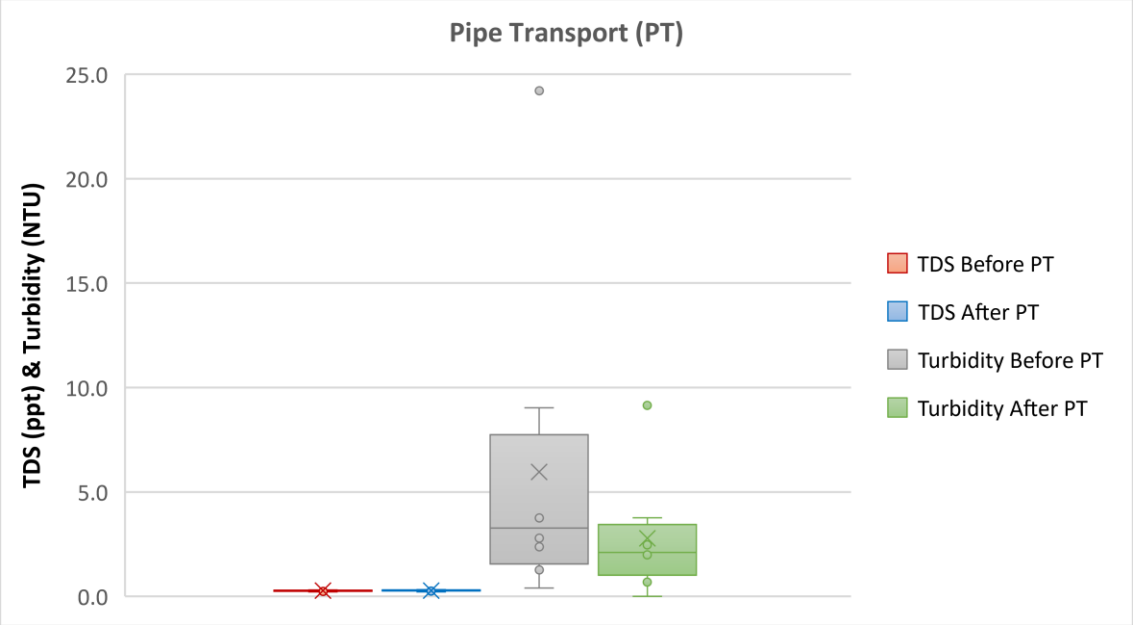


Figure 106. Box plots of the TDS concentration and turbidity changes in the pipeline between stations 7 and 8.

The dissolved oxygen concentration and percentage of saturation increased in the pipeline between stations 7 and 8 (Figures 107 and 108). Water temperature did not change significantly between stations 7 and 8 (graphs in Appendix C).

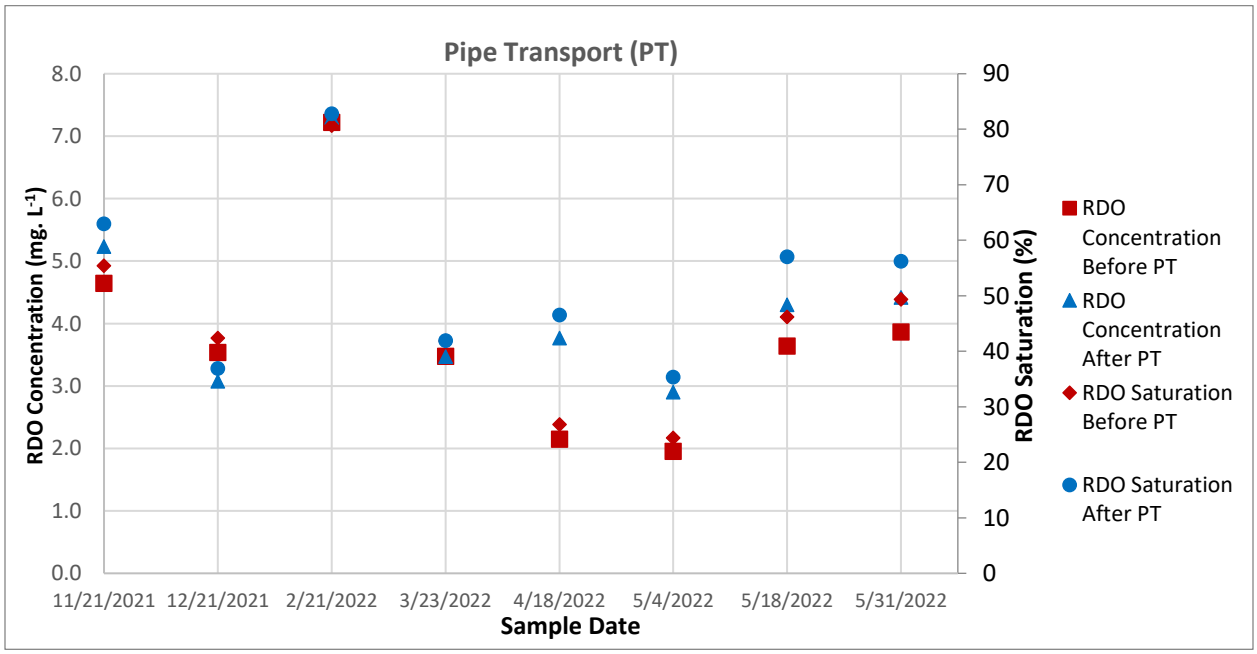


Figure 107. Dissolved oxygen concentration and percentage of saturation in the pipeline between stations 7 and 8.

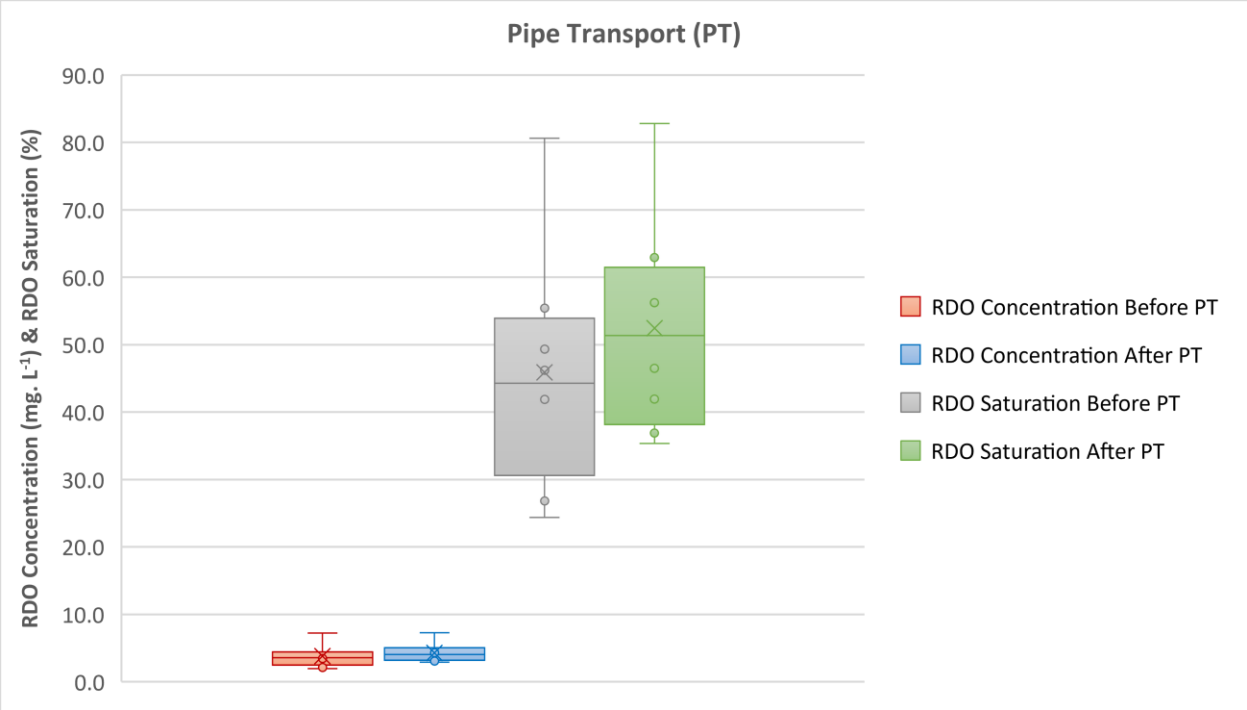


Figure 108. Box plot of dissolved oxygen concentration and saturation percentage changes in the pipeline between stations 7 and 8.

The oxidation-reduction potential rose significantly, and the pH rose slightly through the pipeline connecting stations 7 and 8 (Figures 109 and 110).

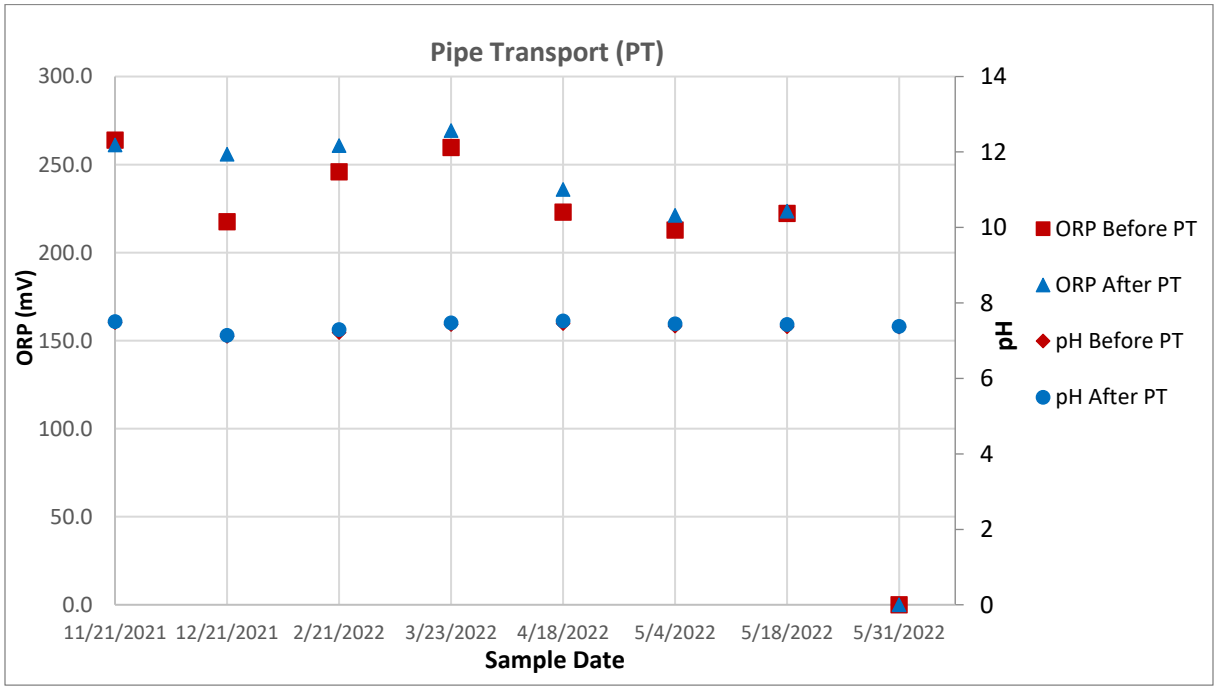


Figure 109. Temporal changes in reduction-reduction potential and pH during pipeline transport between stations 7 and 8.

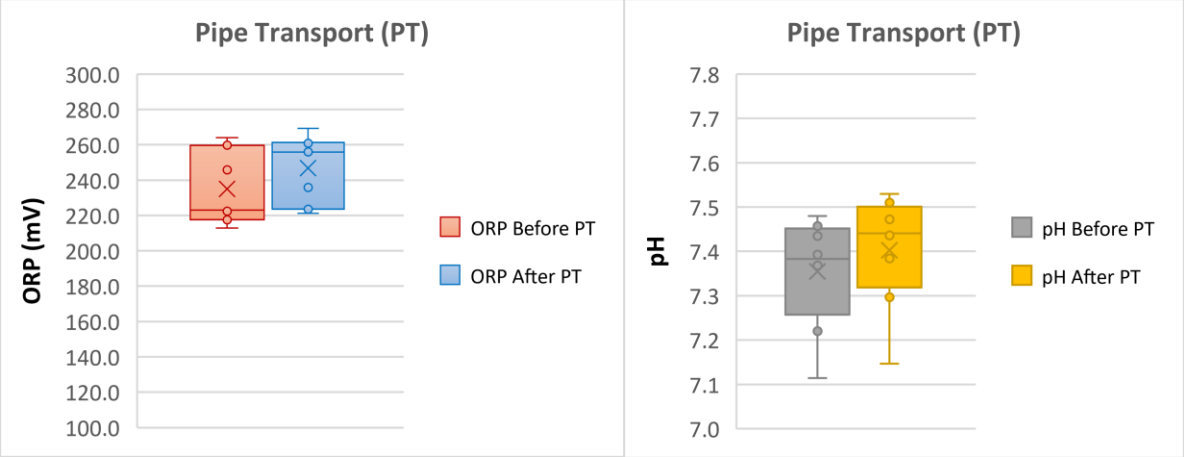


Figure 110. Box plots of oxidation-reduction potential and pH changes in the pipeline between station 7 and 8.

Changes in the Vegetation with Time and Treatment Activities

Even though great care was taken to evenly plant the emergent vegetation, after four months of acclimation and growth, each tank started with a slightly different number of plants ranging from 61 (tub 9, UV treatment) to 104 (tub 8, reference treatment, Figure 111). This equates to a loss of plants ranging from 50% to about 15%. Emergent plants overall grew slightly in number (1.8 times in average) during this experiment growing from their rhizomes (asexual vegetative multiplication). This growth seems to have slowed down at the end of the experiment. Not accounted for numerically, *T. domingensis* also grew taller and expanded laterally as its foliage grew. Foliage; however, looked browner and less expansive as it was in the middle of the dry season. Although it was not possible to determine from the photographs when *S. californicus* started to reproduce sexually, it was found that *T. domingensis* began reproducing as early as 02/21/2022 (event 3) starting in tub 10 (filter treatment) and was present in all tubs the next event (03/23/2022) with tub 8 (reference) having the most inflorescences (seven in total). In comparison, tubs 9 (UV treatment) and 10 (filter treatment) reached similar values a month later (04/18/2022). The number of inflorescences in all tubs declined rapidly thereafter (Figure 111). The amount of floating vegetation in the tubs (mixture of *L. minor* and the fern *Azolla* sp. predominantly) started in tub 9 (UV treatment), but then receded quickly whilst it grew very thick in the two other tubs and persisted until the end of the experiment. It is not known why this floating vegetation disappeared in tub 9 (Figure 18). Because *S. californicus* was harder to decipher on the photographs, less assertions can be made for that species. This plant followed the same growth dynamics as *T. domingensis,* but the results for this species have to be taken with caution at the end of the experiment as the stems of *S. californicus* were hard to distinguish from those of *T. domingensis,* which gained in height and foliage.

V. americana growth dynamics were difficult to track using the monitoring method chosen. However, raking tub 1 (reference treatment) was incommensurably more intense in this tub, which lacked floating vegetation, but had thick metaphyton and epiphyton (both mainly from the alga *Cladophora* sp.) on the leaves of *V. americana*. This severely pulled the plant up as well

as blocked its photosynthesis so that tub 1 lost tremendous amounts of plants by the end of the experiment. Tubs 2 (UV treatment) and 7 (filter treatment) were more successful at growing healthy stands of *V. americana,* but those were also covered with epiphytes and a thick blanket of both floating plants and filamentous algae. At the end of the experiment, *V. americana* was still present, but was visually less abundant than at the beginning of the experiment.

Overall, the sand filter water in conjunction or not with the UV treatment lowered the ability of *T. domingensis* to grow and especially reproduce as well as in the reference treatment. This effect was even more pronounced for *V. americana,* which is severely impacted from the growth of especially *Cladophora* sp. that it is able to grow faster than the plant. However, even with filtered water, *V. americana* would still likely lose the competition against this filamentous green alga. The nutrients levels in the filtered water are high enough to promote the growth of microphytes (here *Cladophora* sp.) compared to macrophytes.

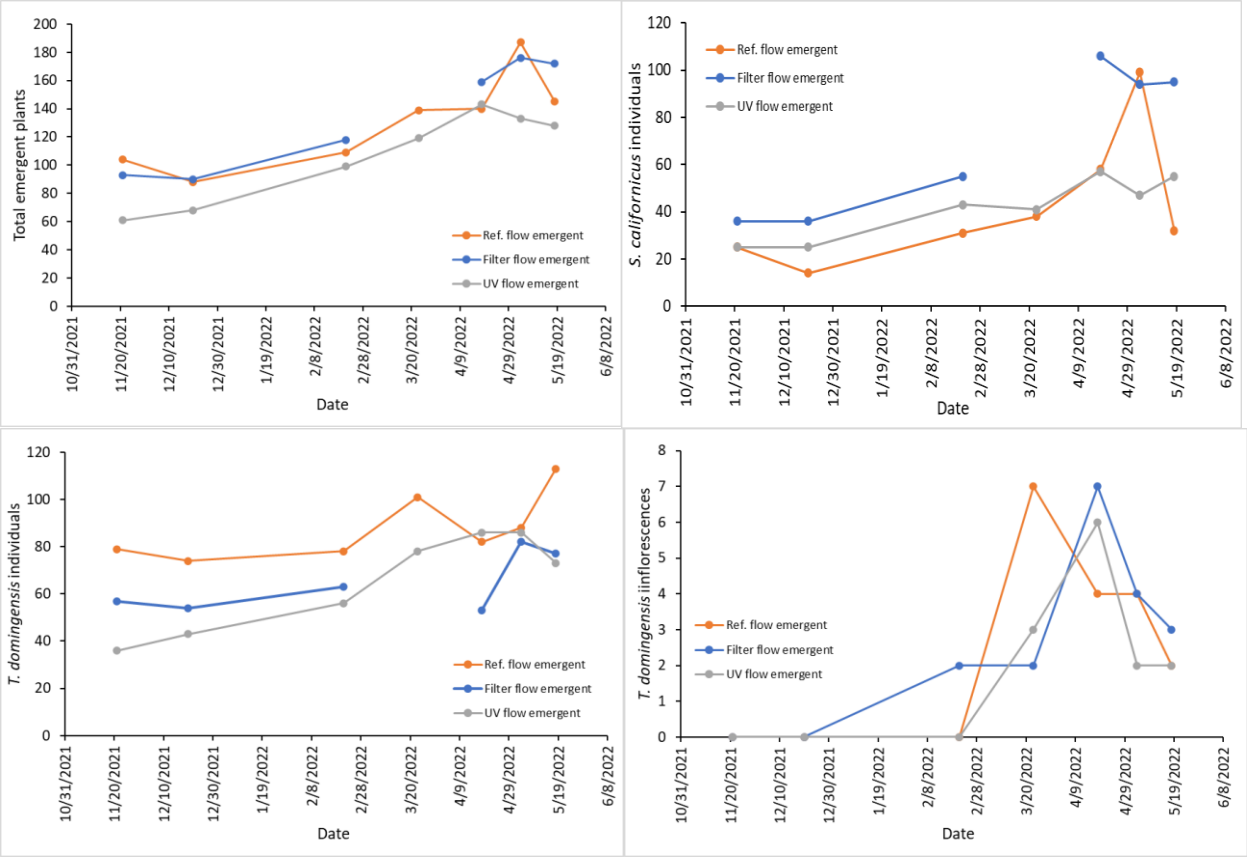


Figure 111. Growth dynamics of emergent plants in tubs #8 (reference treatment), #9 (UV treatment) and #10 (filter treatment). Top left: change in total number of plants, top right: change in S. californicus, bottom left: change of T. domingensis and bottom left: change in T domingensis inflorescences. Note: the missing datum for tub10 is due to a corrupted photograph. This missing datum is not present for the bottom right graph since inflorescences were visible by zooming on the photograph encapsulating all six tubs.

Biomass Removed During Cleaning of the Schmutzdecke on the Surface of the Slow Sand Filter

Based on observations made in the field while cleaning the surface of the slow sand filter, it is estimated that between 8 and 12 kg of organic matter were removed during each cleaning. The amount of organic carbon removed was based on the time between cleaning and the TOC concentration in the river water. Approximately 15 cleaning events occurred during the project duration, thereby removing between 120 and 180 kg of organic carbon. The flow rate through the slow sand filter was about 11.4 L/min. This illustrates how poor the quality of river water is in terms of treatment difficulty.

Statistical Analysis of the Data

The raw water quality parameters were measured for each of the treatment trains. The inflow to TTA is directly connected to the control *Typha* tub while the inflow for the TTB and TTC is connected to Slow Sand Filtration System. A two-sample t-test was performed to compare raw water quality parameters in the inflow to TTA and TTB/C. The results in Table 3 shows there was not a significant difference in raw water quality between inflow to TTA and TTB/C. In addition, a two-sample t-test was performed to compare water quality parameters within each treatment train. The result shows there was a significant improvement in water quality, however, there is small improvement shown in NO2, NH3, and OrgN. A one-way ANOVA was performed to investigate the difference in treatment trains for 12 key water quality parameters. The difference between the outflow and inflow water quality is used to analyze the statistical difference between the three treatment trains. The one-way ANOVA revealed that the differences between the means of the treatment trains for most of the water quality parameters are not statistically significant (Table 3).

Table 3. ANOVA and t-Test results for comparison of measurement parameters

Due to 508 compliance requirements, Table 3 was removed from this document. To access the full document, which does not meet 508 compliance standards, please reach out to InnTech_HAB@FloridaDEP.gov

Discussion

Raw Water Quality from the Caloosahatchee River

The water quality is quite poor in terms of TOC and DOC load. This conclusion is based on the high concentrations found in the raw water, the 8 to 12 kg of organic debris removed during each cleaning of the slow sand filter, and the presence of cyanobacteria, green algae, fungi, and diatoms in the water. A sample of the schmutzdecke (organic detritus) on November 17, 2021 showed the presence of the microbes (Figures 112 to 113). Nematodes and fungi were also found in the debris along with amorphous organic material (Figure 116).

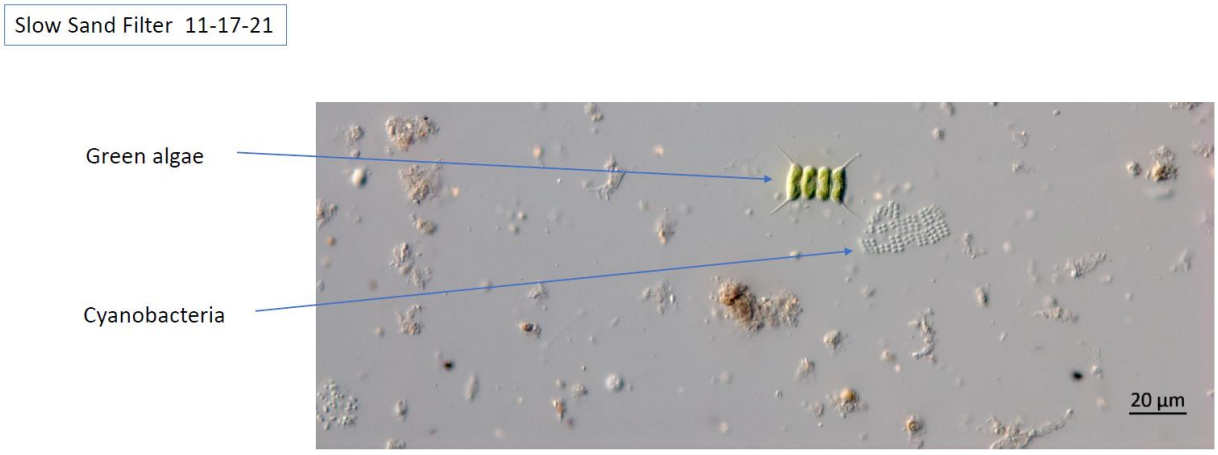


Figure 112. Photographs of cyanobacteria and green algae in the organic debris removed from the slow sand filter (photograph provided by Barry Rosen).

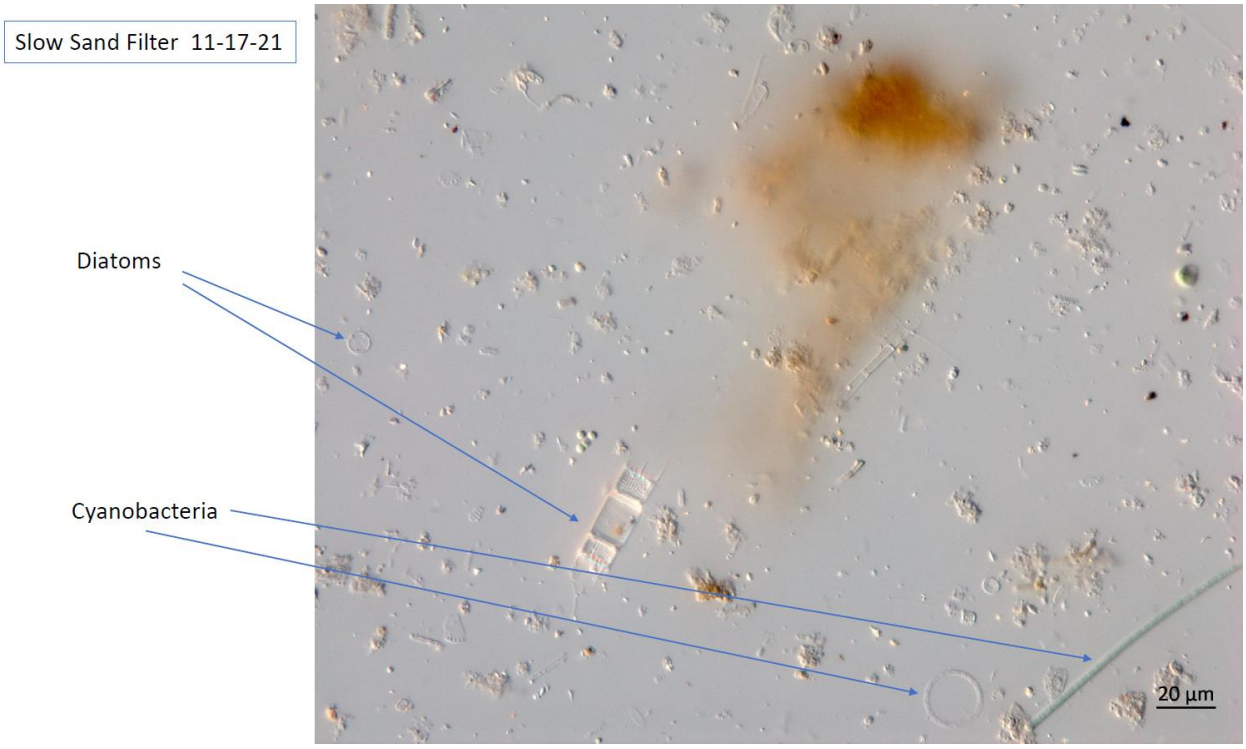


Figure 113. Cyanobacteria and diatoms in the organic debris removed from the slow sand filter (photograph provided by Barry Rosen).

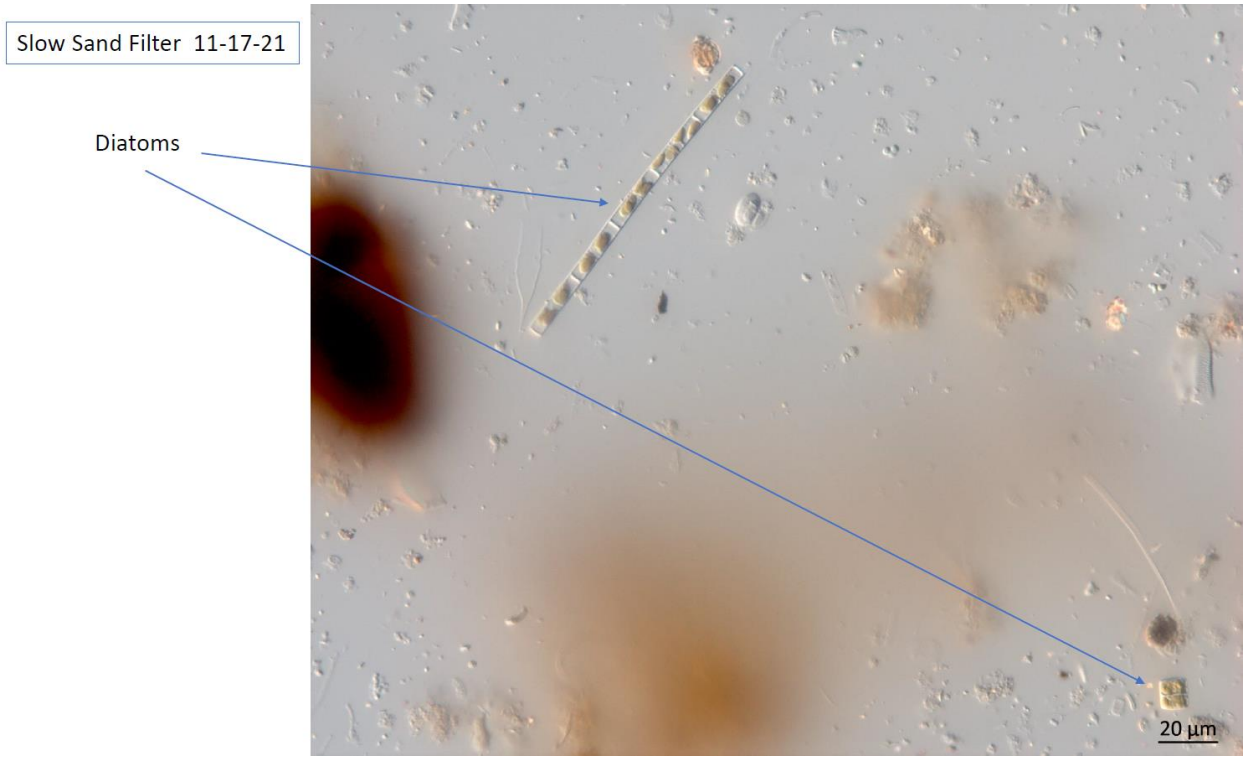


Figure 114. Diatoms in the organic debris from the slow sand filter (photograph provided by Barry Rosen).

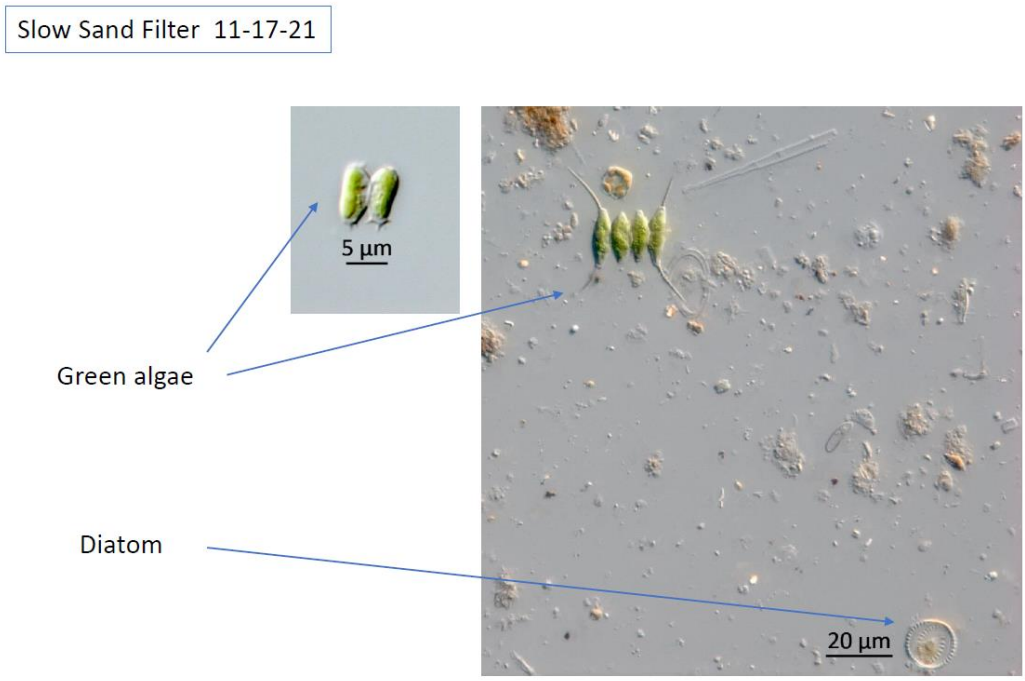


Figure 115. Green algae and a diatom found in the organic debris from the slow sand filter (photograph from Barry Rosen).

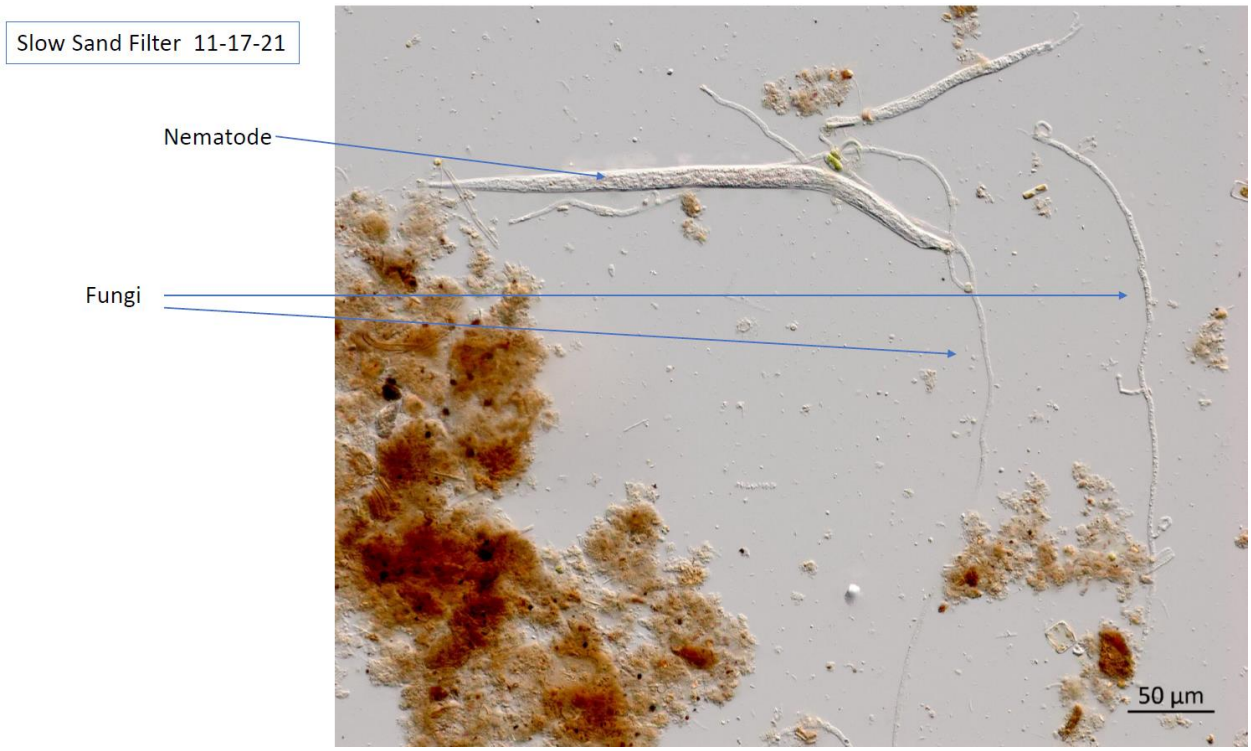


Figure 116. Fungi and a nematode with amorphous organic debris from in the organic debris (lower left) removed from the slow sand filter (photograph from Barry Rosen).

Samples of the raw water during the project were collected from two locations on the site; at the entrance to Train A (control) and at the inflow to the slow sand filter. The reason for the duplicate sampling was to ascertain if any differences in water quality occur based on the highly heterogeneous nature of the raw water and the interior biofilm coating of the piping system. The statistical analysis between the raw water samples showed that it was not significant, but there were some observed differences in some parameters.

Overall Assessment of the Effectiveness of the Two Treatment Technologies (Trains B and C) Verses only Vegetative Treatment (Control Train A)

As shown in Table 3, the statistical analysis showed that a comparison of the raw water to that coming out of each treatment train produced no statistical difference. In each case total nitrogen, organic nitrogen, nitrate, nitrite, total phosphorus, and orthophosphate were reduced during treatment (Figures 117, 118 and 119). There were, however, some differences and special circumstances that need to be discussed based on the how the systems operated.

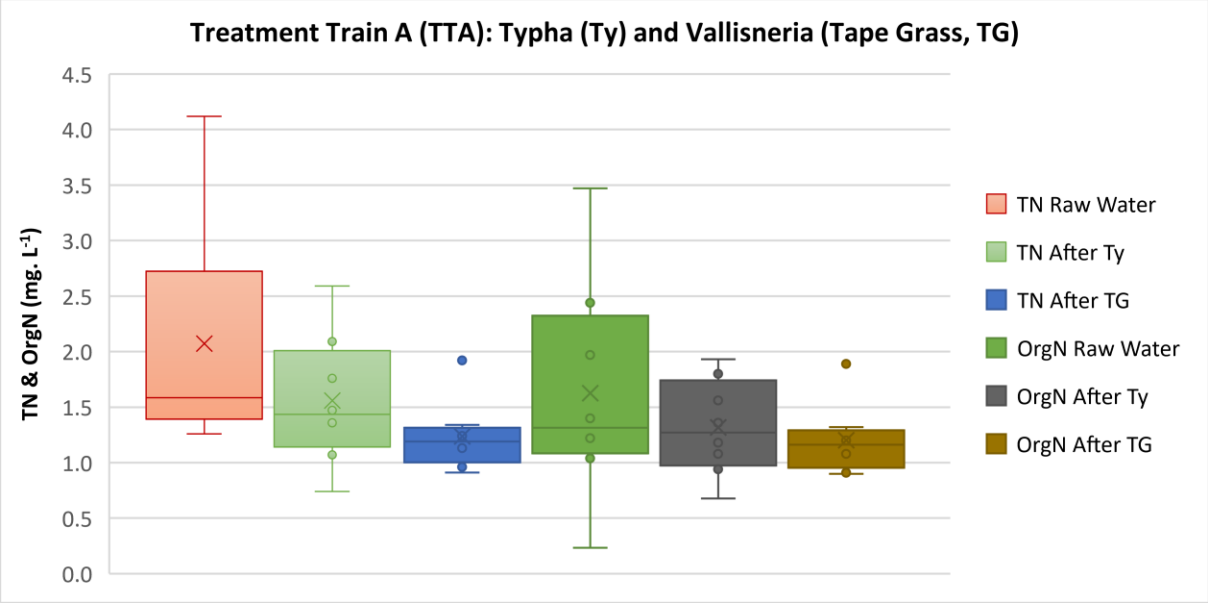


Figure 117. Box diagram of the changes in concentrations in total and organic nitrogen in Train A (vegetation only control).

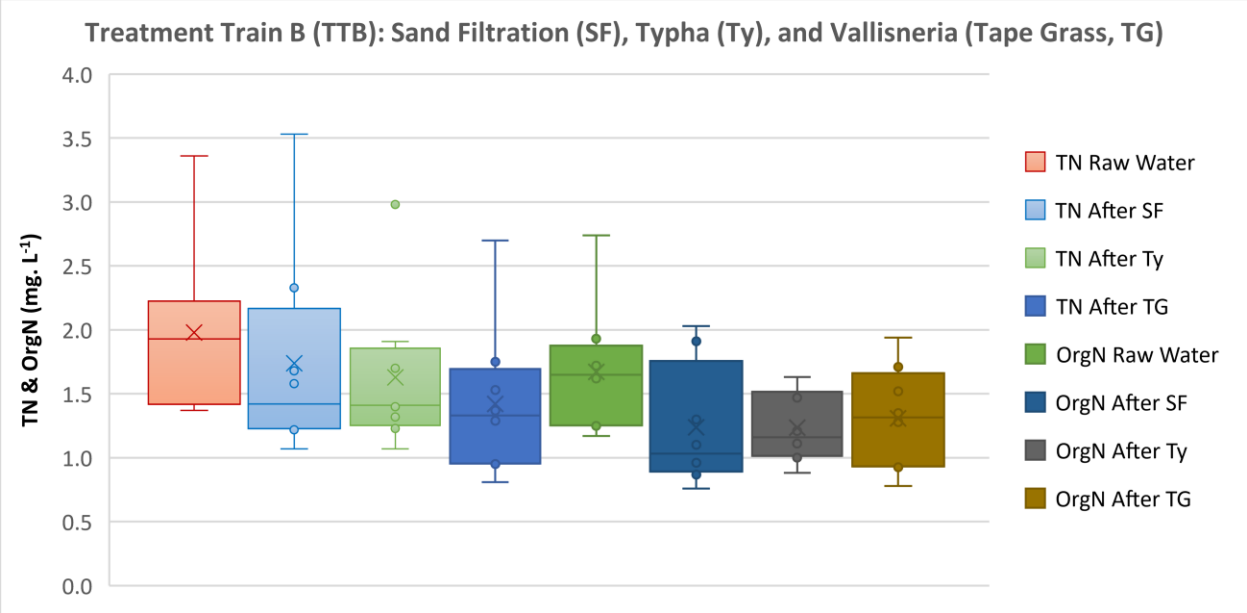


Figure 118. Box diagram of the changes in concentrations in total and organic nitrogen in Train B (slow sand filtration + vegetation).

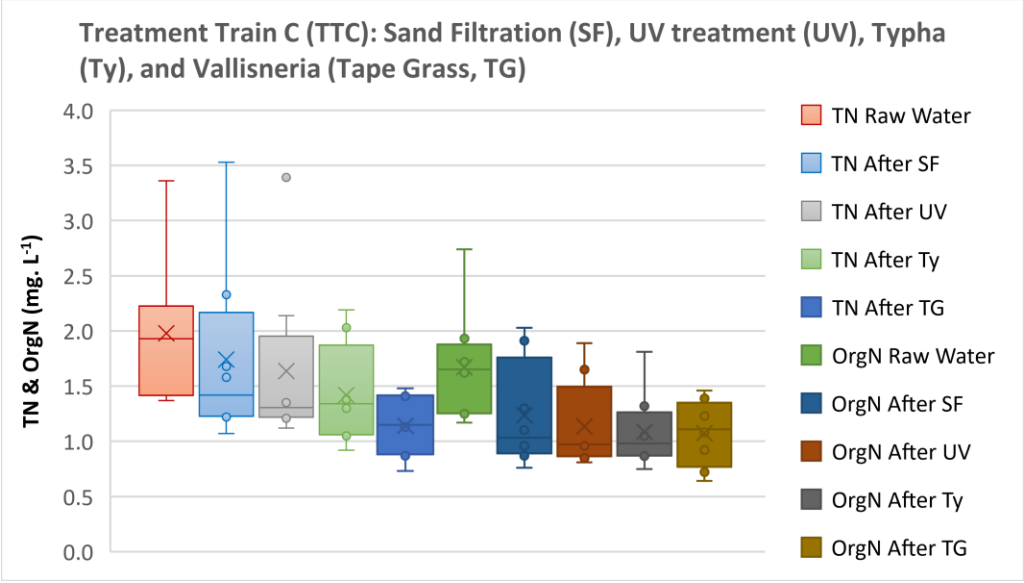


Figure 119. Box diagram of the changes in concentration in total and organic nitrogen in Train C (slow sand filtration + UV + vegetation).

Based on a comparison of the three treatment trains, the slow sand filter and UV both removed some organic nitrogen. It was postulated that the slow sand filter would be somewhat effective in creating reducing conditions at its base, which would convert some of the nitrogen to ammonia. This was not as effective as possible based on the rather low retention time in the filter. The high turbidity and color of the water also impacted the effectiveness of the UV in breaking down some of the organic nitrogen.

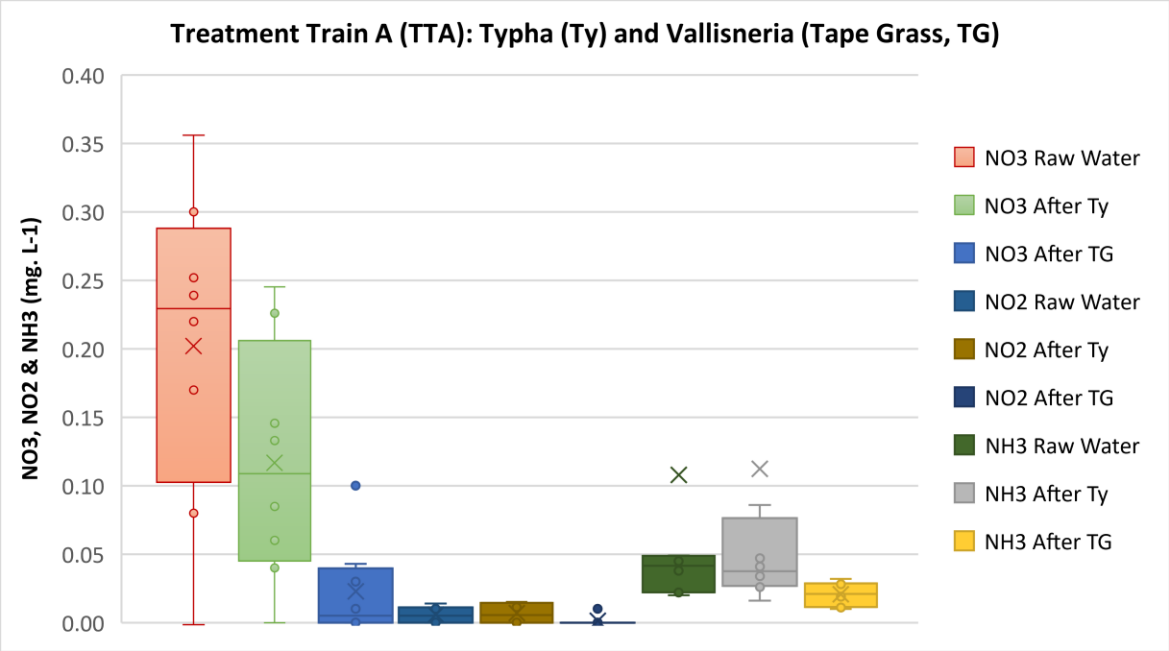


Figure 120. Box plot of the variation in concentration of nitrate, nitrite, and ammonia in treatment train A (vegetation only, control).

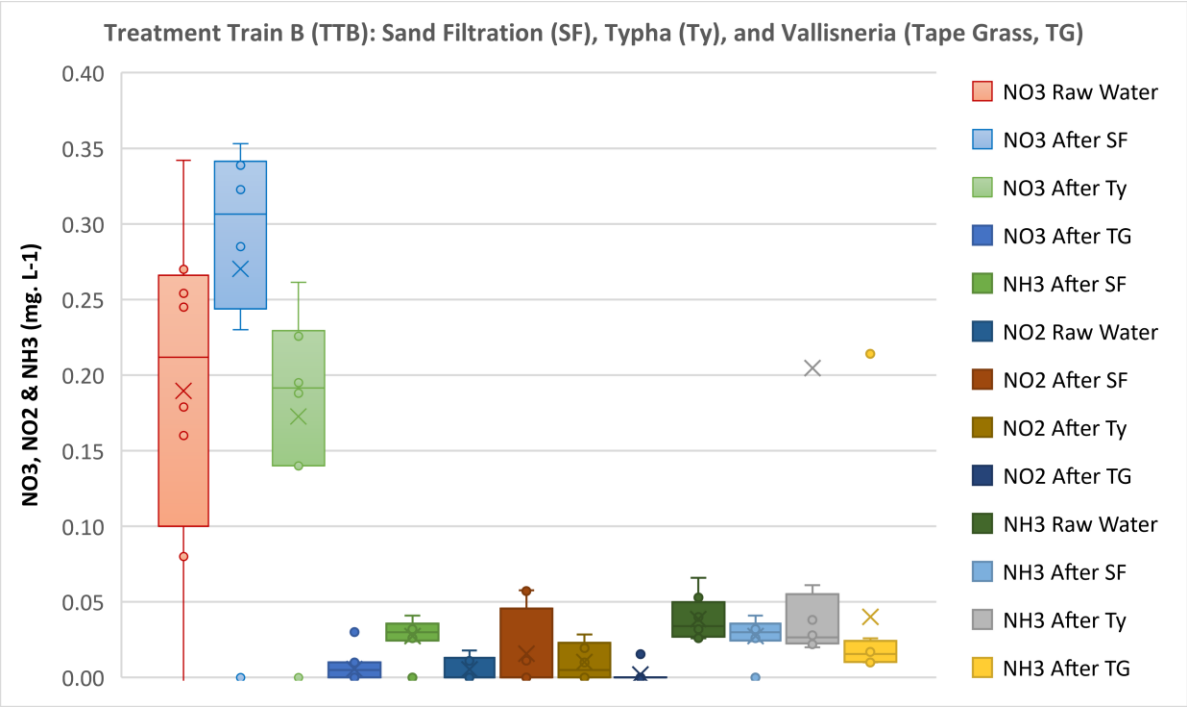


Figure 121. Box plot of the variation in concentration of nitrate, nitrite, and ammonia in treatment train A (slow sand filtration + vegetation).

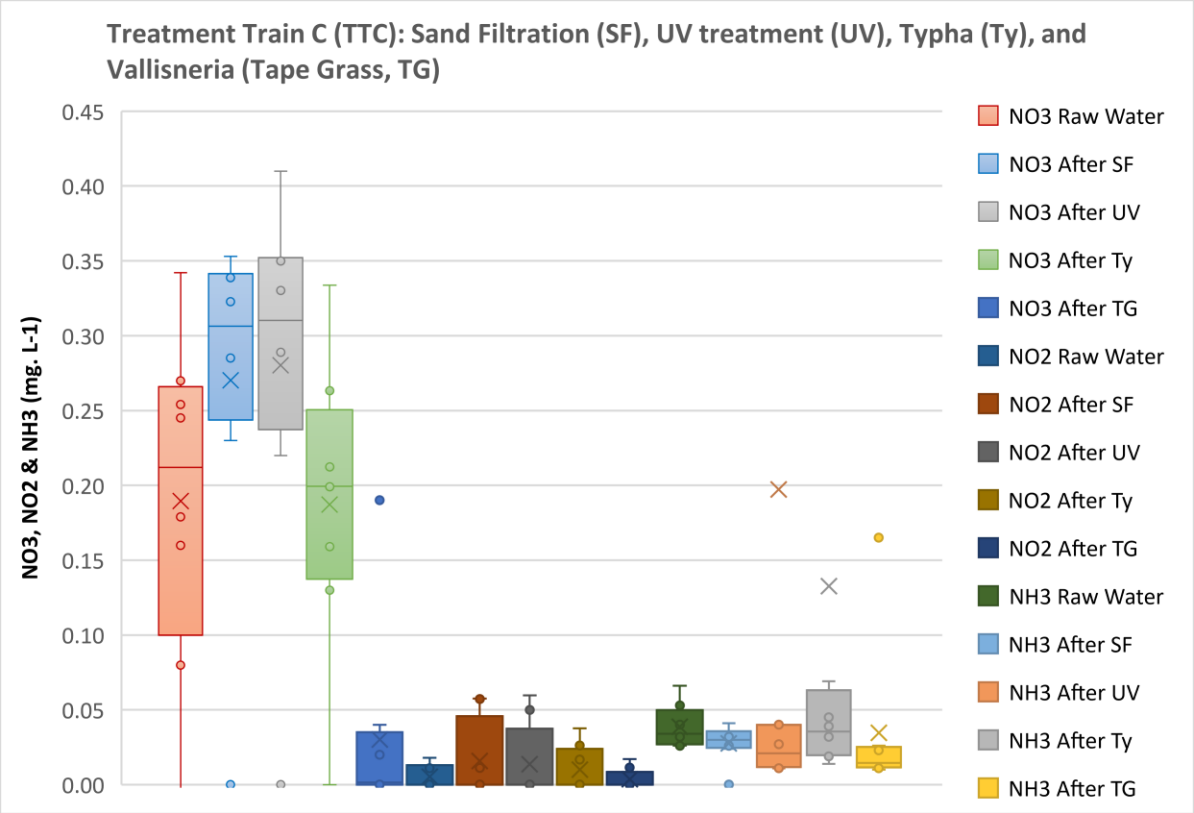


Figure 122. Box plot of the variation in concentration of nitrate, nitrite, and ammonia in treatment train c (slow sand filtration + UV + vegetation).

Removal of nitrate in all three trains was most effective in the *Vallisneria* tub or the last treatment process. In the vegetation only treatment train A, this last tank contained a variety of vegetative types, not just tape grass. Other fast-growing vegetation was recruited from the river water and aided the removal of the nitrogen nutrients. However, it was necessary to harvest a large algae species (e.g., *Cladophora* sp.) to maintain the tape grass in a living state. The fast-growing algae species also had to be cleaned to a lesser degree in the tape grass tanks of trains B and C.

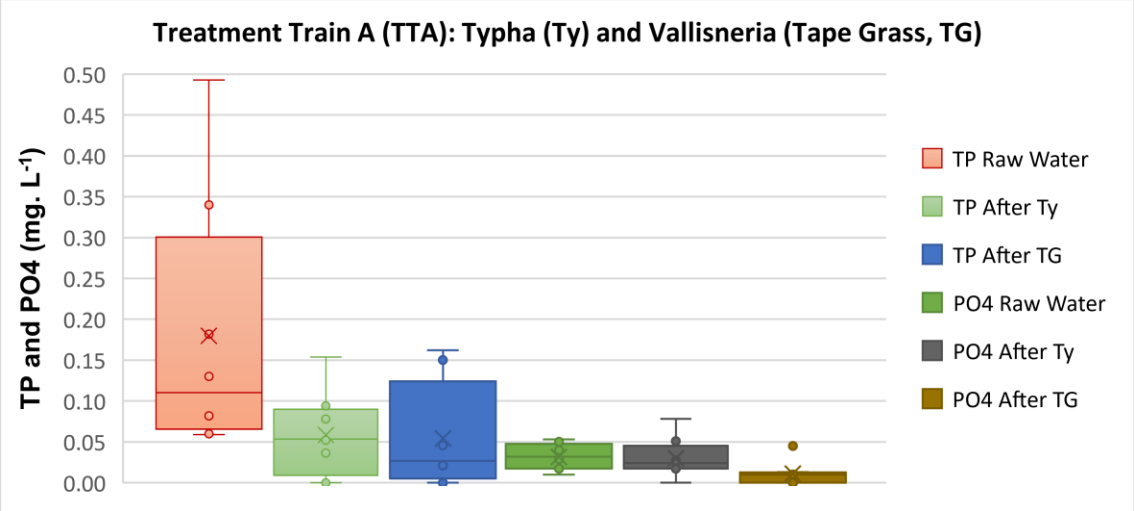


Figure 123. Box plot showing the changes in total phosphorus and phosphate in treatment train A (vegetation only, control).

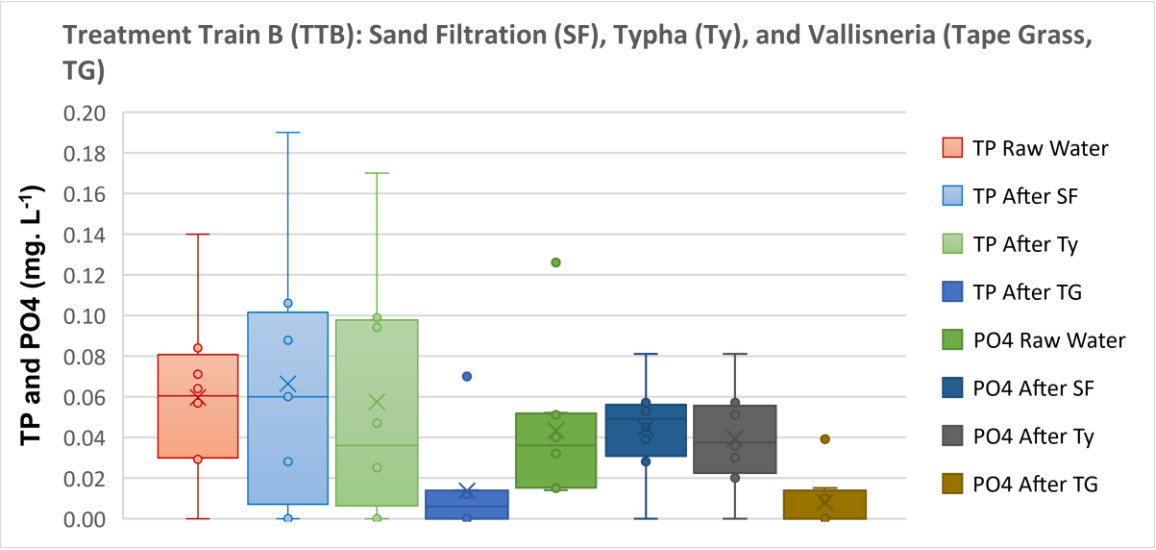


Figure 124. Box plot showing the changes in total phosphorus and phosphate in treatment train B (slow sand filtration + vegetation).

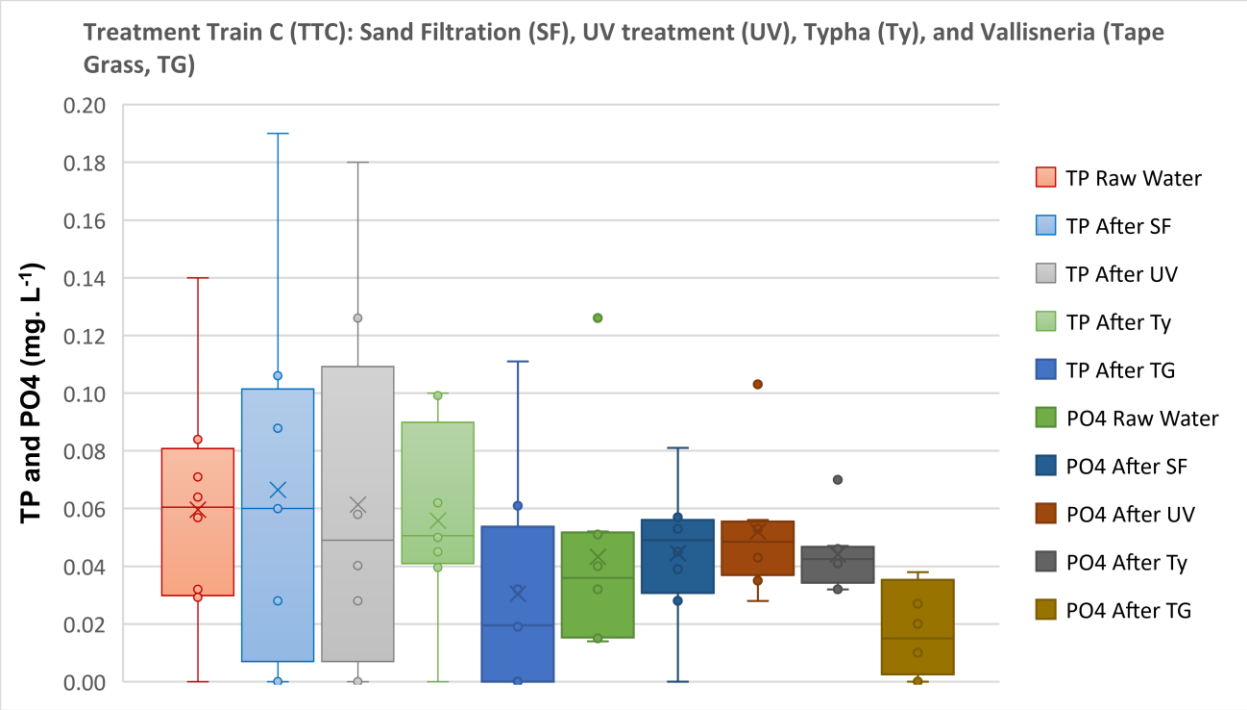


Figure 125. Box plot showing the changes in total phosphorus and phosphate in treatment train C (slow sand filtration + UV + vegetation).

All three treatment trains were effective at reducing concentrations of total phosphorus and orthophosphate (based on mean values). With regard to total phosphorus, train B was most effective with a concentration reduction to less than 0.10 mg/L, while train C lowered the concentration to below 0.20 mg/L and train A to below 0.25 mg/L. Orthophosphate was also effectively lowered, but train A was most effective with a reduction to below 0.01 mg/L, while trains B and C lowered it to less than 0.02 mg/L. Despite the statistical analyses, there are some differences in how the trains were effective in removal of specific analytes.

The slow sand filtration process effectively removed particulate biomass including algae, bacteria, turbidity, and reduced chlorophyll A. The UV did also reduce the total bacteria, algae, and cyanobacteria concentrations.

Is UV Treatment Effective in Reducing the Concentration of Organic Nitrogen?

UV treatment did lower the concentration to a very limited degree but was not effective due to the high color and turbidity of the raw water, the flow rate, and the limited power of the UV lamp. Upscaling of the process could help it to be more effective by increasing the UV power and reducing the flow rate.

Water Treatment and Impacts on Vegetation Growth in the Mesocosms

Based on the mass of floating organic material, mostly the algae *Cladophora* sp., the engineered treatment did reduce the amount of growth in the train B and C *Vallisneria* tubs compared to the train A *Vallisneria* tub. It should be noted that the mass of *Cladophora sp.* was mostly floating. If harvested, this material could be composted or used as a natural fiber.

Lessons Learned: What Experimental Design Changes Could Be Used to Improve the Engineered Treatment?

If the processes would be up-scaled to provide a greater degree of treatment at very high volumes, the slow sand filtration process would need to have a thicker media bed, perhaps six to eight feet and the flow rate would need to be sufficiently low to increase the retention time to eight hours or longer. This design would aid in creating reducing conditions within the filter and would encourage the conversion of more organic nitrogen into soluble nutrients, such as ammonia and orthophosphate, which are taken up rapidly in the vegetation treatment tanks. In addition, the cleaning of the large-scale sand filter tanks would need to be accomplished using an automated process, such as used in many existing slow sand filter, potable water treatment facilities.

If a UV process was to be implemented as part of an engineered process train, the flow hydraulics would need to provide a longer contact time with the raw water (tray design) and would have to be coordinated with the slow sand filtration retention time to provide water with a lower turbidity. In addition, the power of the UV light source would need to be increased.

Could the Engineered System Function to Lessen Algal Blooms in the Storage Reservoirs or Any Stormwater Storage Facilities Occurring in the Calooshatchee River Basin Over Critical Times?

Perhaps the treatment process could be implemented in any stormwater facility before the water is returned to the Caloosahatchee River rather than when the raw water is pumped into the reservoir. This would provide the river with better water quality. In addition, the algal blooms could be allowed to occur in the reservoir to allow the floating algae to aid in the treatment process. The algae could then be harvested as part of the treatment process.

Is There Some Commercial Value for Harvesting Cellulose or Fiber from the Green Algae Cladophora sp. to Offset Water Quality Treatment in the Reservoir Lakes?

One of the important observations made during this research was the incredible growth rate of *Cladophora sp.* in the submergent vegetation tanks, particularly in train A (control). After this plant was harvested to maintain the health of the *Vallisneria*, it was found that if left in the sunlight for several weeks to dry, it produced a fiber similar to hemp. The fiber appears to be strong and could be harvested for commercial use. This issue has been explored by Mihranyan (2011). Extraction of the cellulose fiber appears to be easier than the hemp extraction process. This could have commercial value that could be used to offset the treatment of the reservoir water.

Conclusions

The research objectives of the Boma project were achieved despite the challenging times causing supply chain disruptions, cost increases, pump system failures caused by lightning damage, and part failures. Despite the reduced number of samples collected, the sampling events were representative of all seasonal climatic conditions, and did allow detailed analysis of the three treatment trains originally suggested for evaluation.

It was found that all three treatment trains were effective at removal of nutrients and organic biomass from poor water quality in the Caloosahatchee River water. There were no statistical differences among the three treatment process trains, which were: A. emergent vegetation (Typha) with submergent vegetation (*Vallisneria*), B. low sand filtration with emergent (Typha) and submergent (*Vallisneria*) vegetation, and C. slow sand filtration, UV, and emergent (Typha) and submergent (*Vallisneria*) vegetation.

The detailed data collected allowed a more thorough understanding of how these treatment processes work in the field under pilot-scale operation. The recruitment of the filamentous algae *Cladophora sp.* from the river water was an important observation because not only did it aid in the treatment performance of the submergent vegetation tub, it also provided insight into operational difficulties for future operation of the reservoirs and other stormwater retention areas in the Caloosahatchee River Basin. The rapidly growing and floating algae will provide a serious challenge to future reservoir water quality management that will make coagulation with alum unlikely as a successful method to reduce nutrient concentration and biomass. The presence of the *Cladophora sp.* may also provide an opportunity for harvest of the plant for use as a commercial source of cellulose fiber.

This research also suggests that new approaches need to be evaluated in large-scale management of water quality. A combination of using vegetation for water quality treatment with some engineered enhancements still needs to be assessed and investigated with some design improvements.

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Appendix

Appendix A. UV treatment impacts on parameters other than total and organic nitrogen

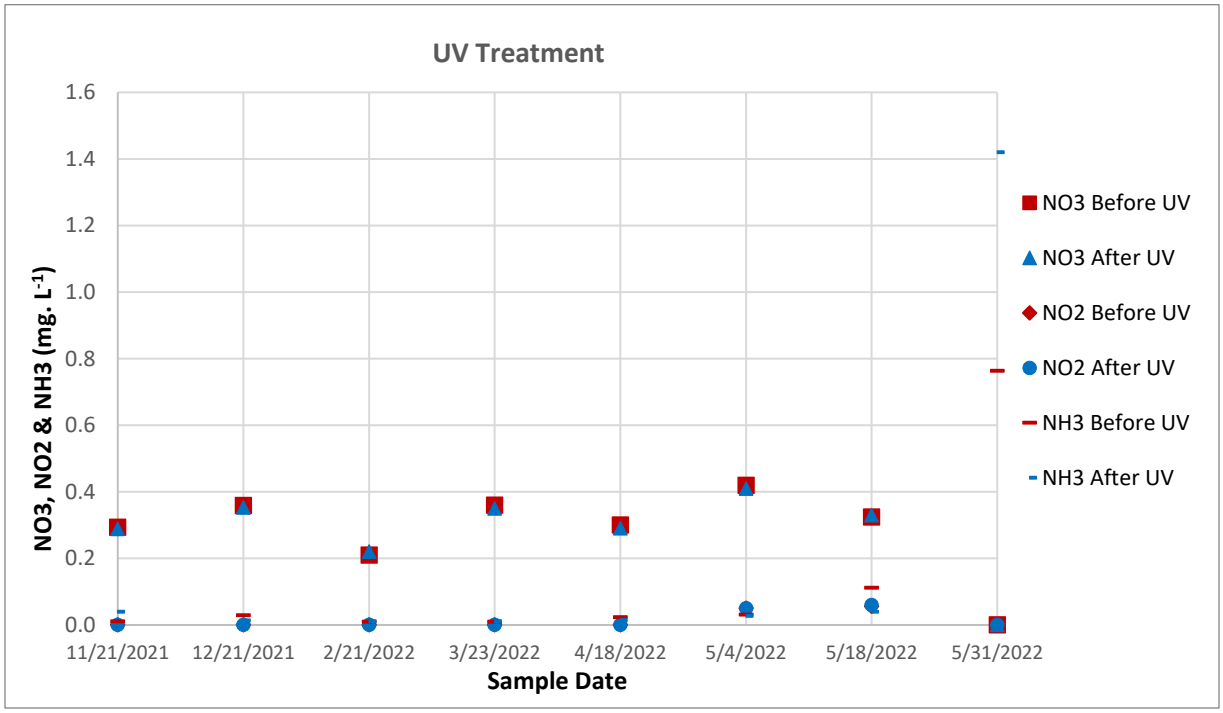

Figure A- 1. Temporal concentrations of nitrate, nitrite, and ammonia before and after UV treatment.

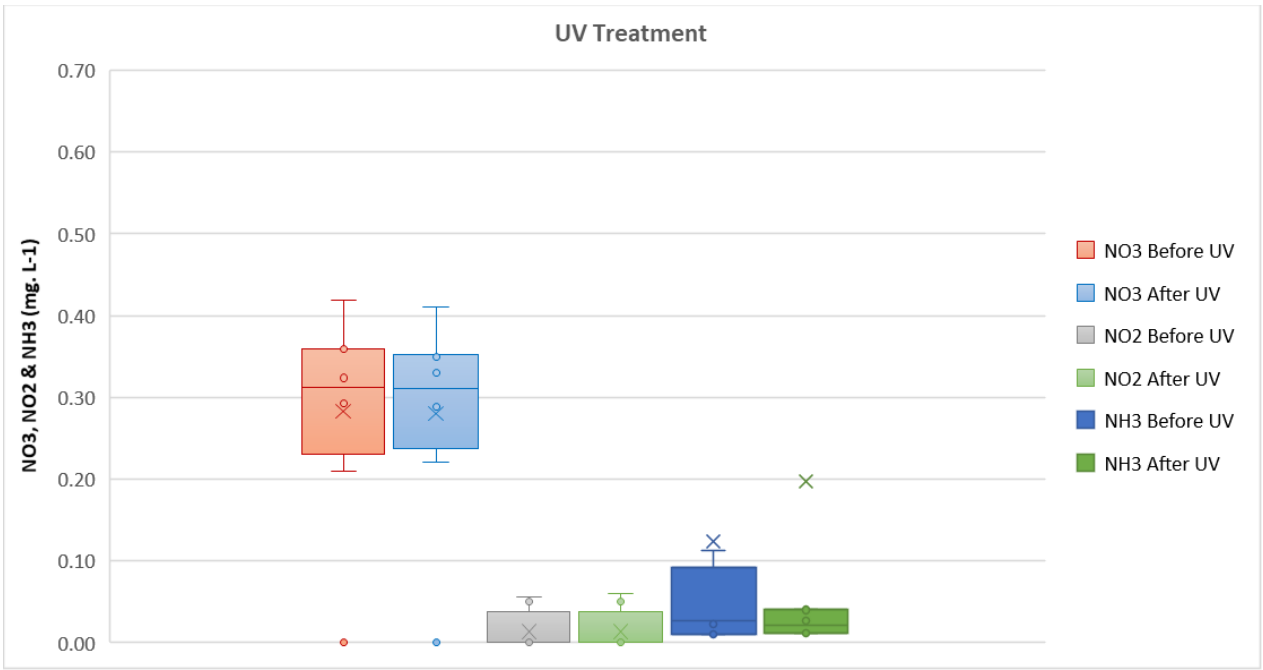

Figure A- 2. Box plot of nitrate, nitrite, and ammonia before and after UV treatment.

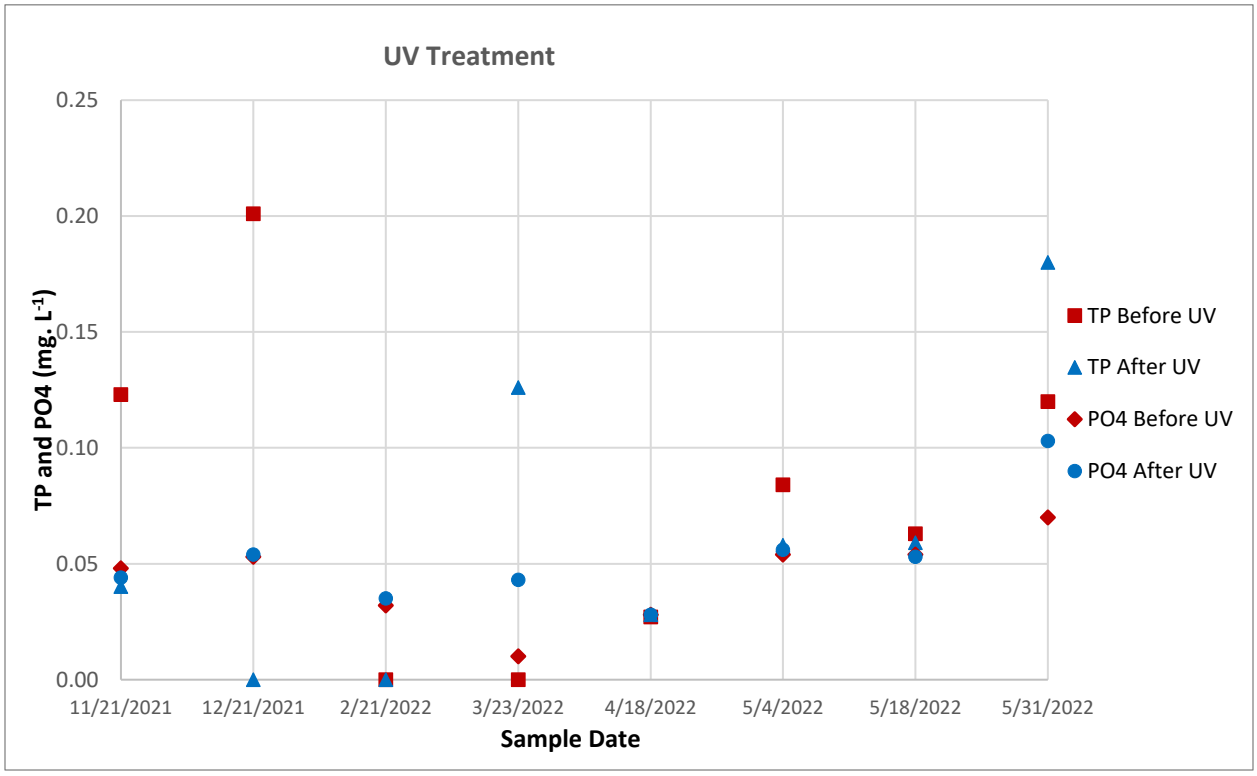

Figure A- 3. Temporal concentrations of total phosphorus and orthophosphate before and after UV treatment.

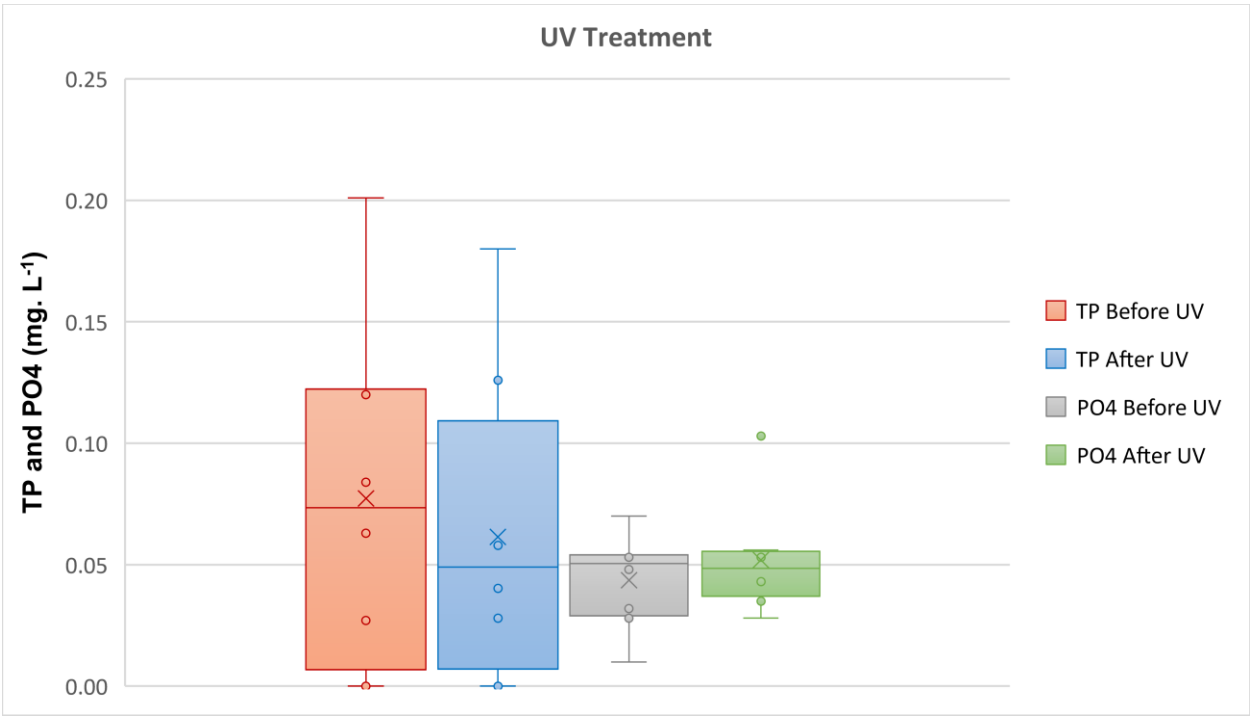

Figure A- 4. Box plots of total phosphorus and orthophosphate concentrations before and after UV treatment.

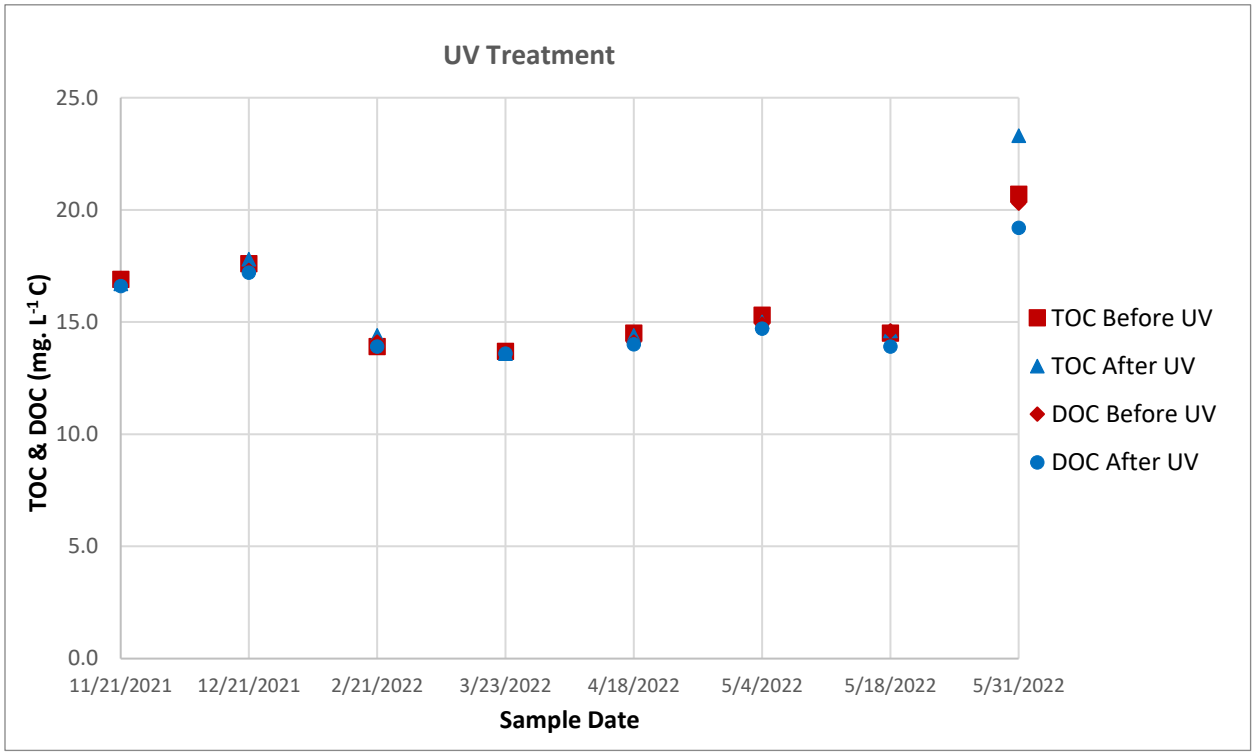

Figure A- 5. Temporal concentrations of total organic and dissolved carbon before and after UV treatment.

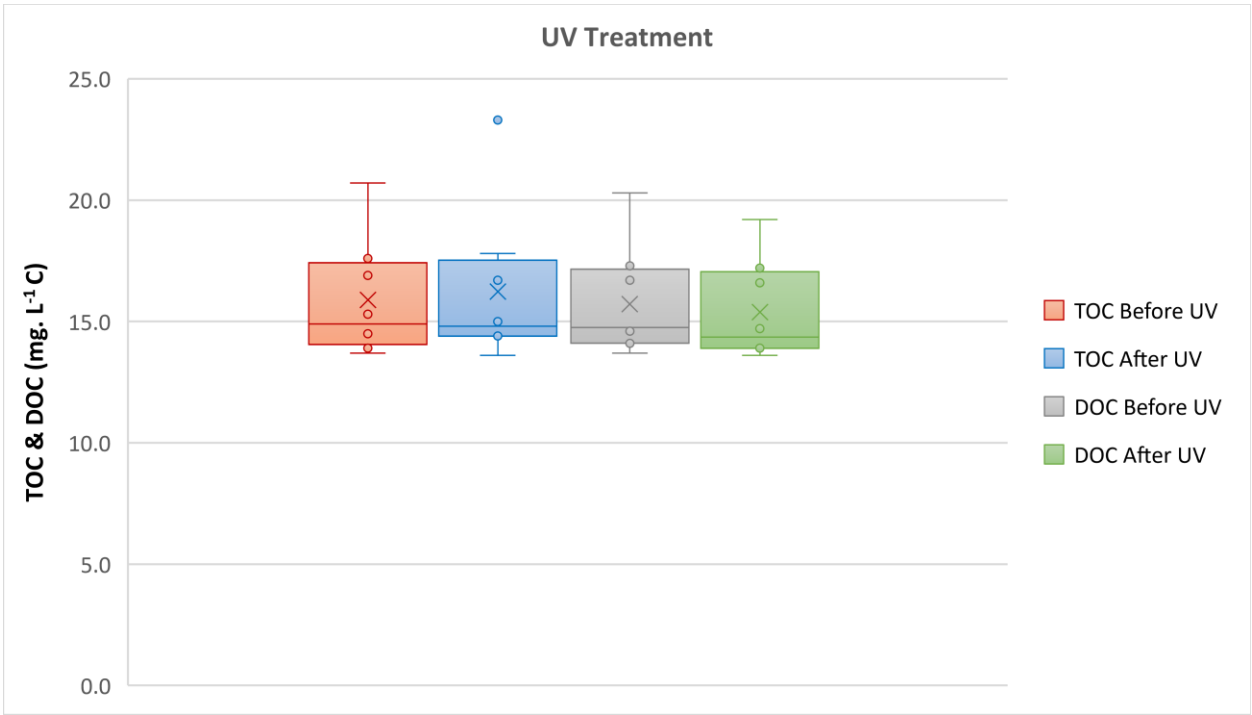

Figure A- 6. Box plots of total and organic carbon concentrations before and after UV treatment.

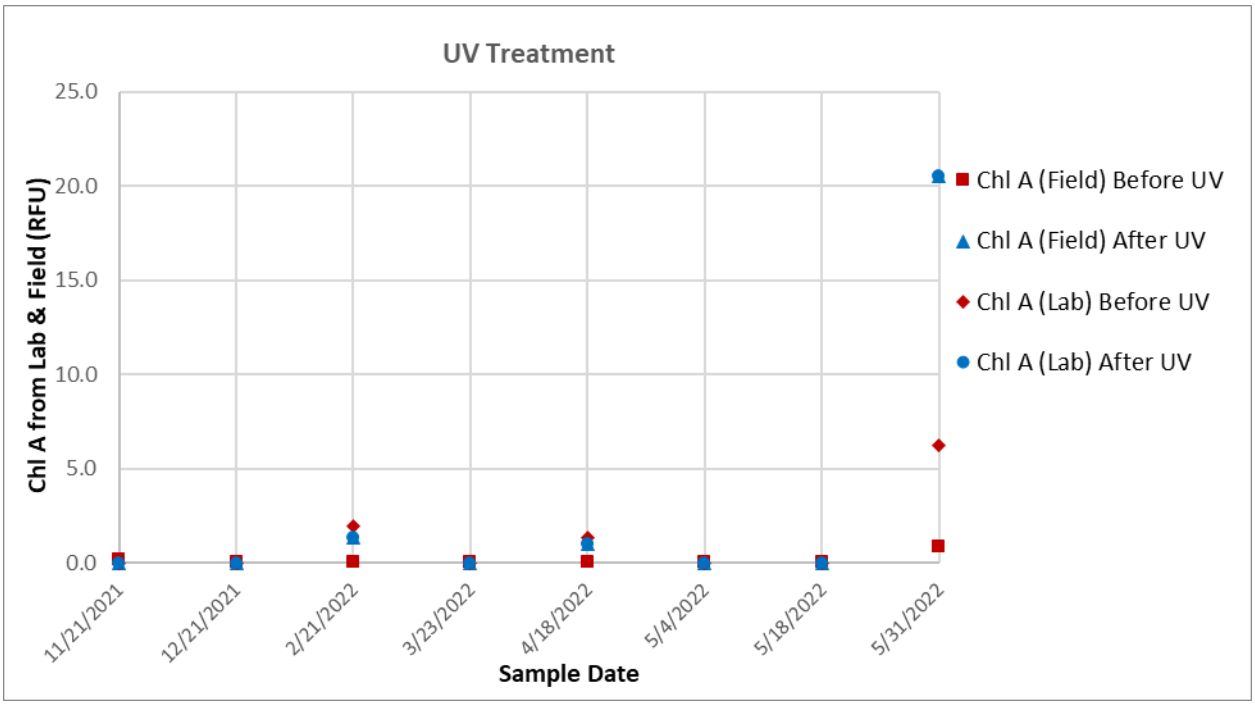

Figure A- 7. Temporal concentrations of field and laboratory measured chlorophyll A before and after UV treatment.

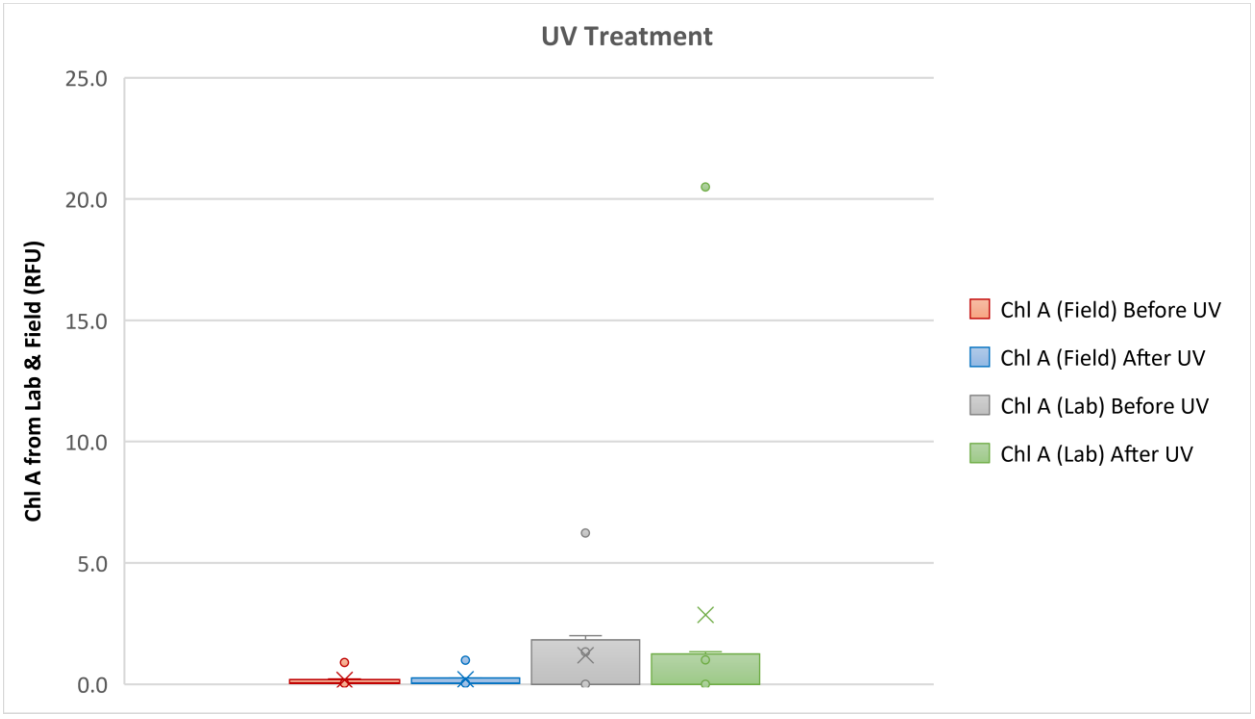

Figure A- 8. Box plots of field and laboratory concentrations measured chlorophyll A before and after UV treatment.

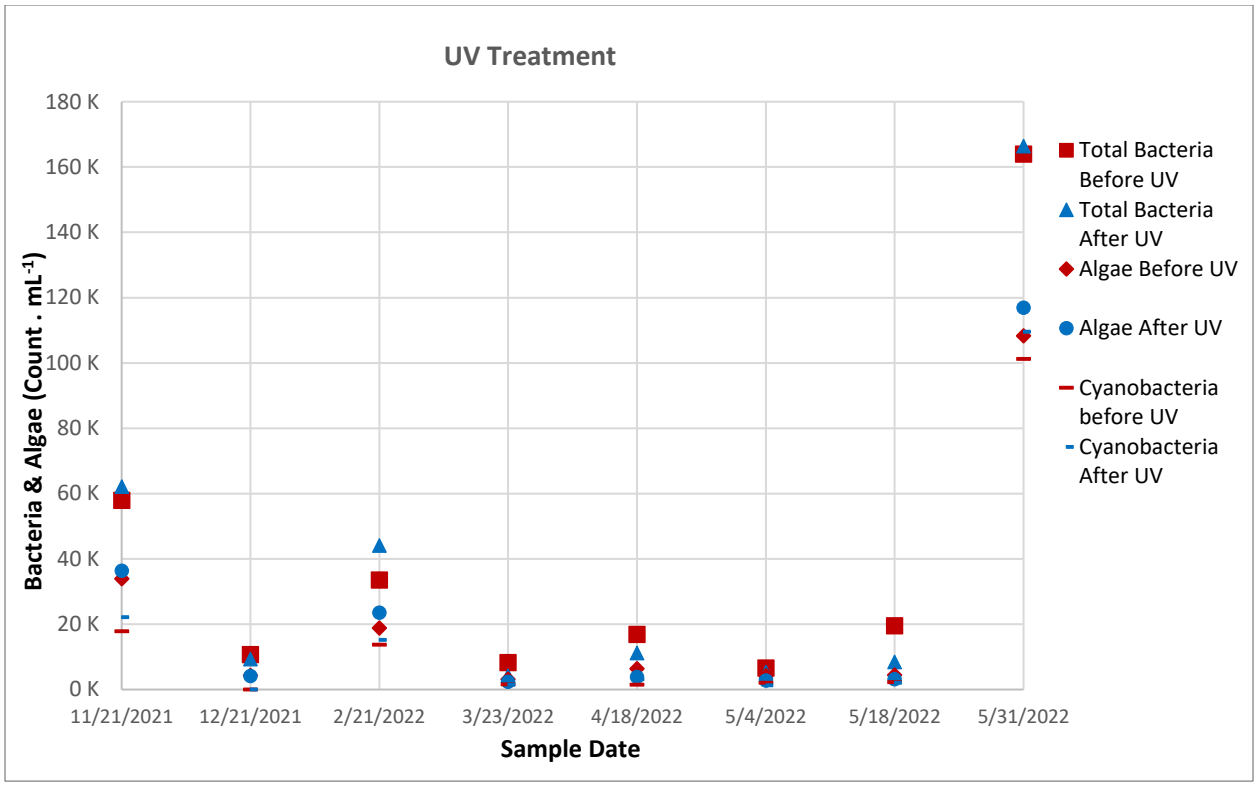

Figure A- 9. Temporal concentrations of total bacteria, algae, and cyanobacteria before and after UV treatment.

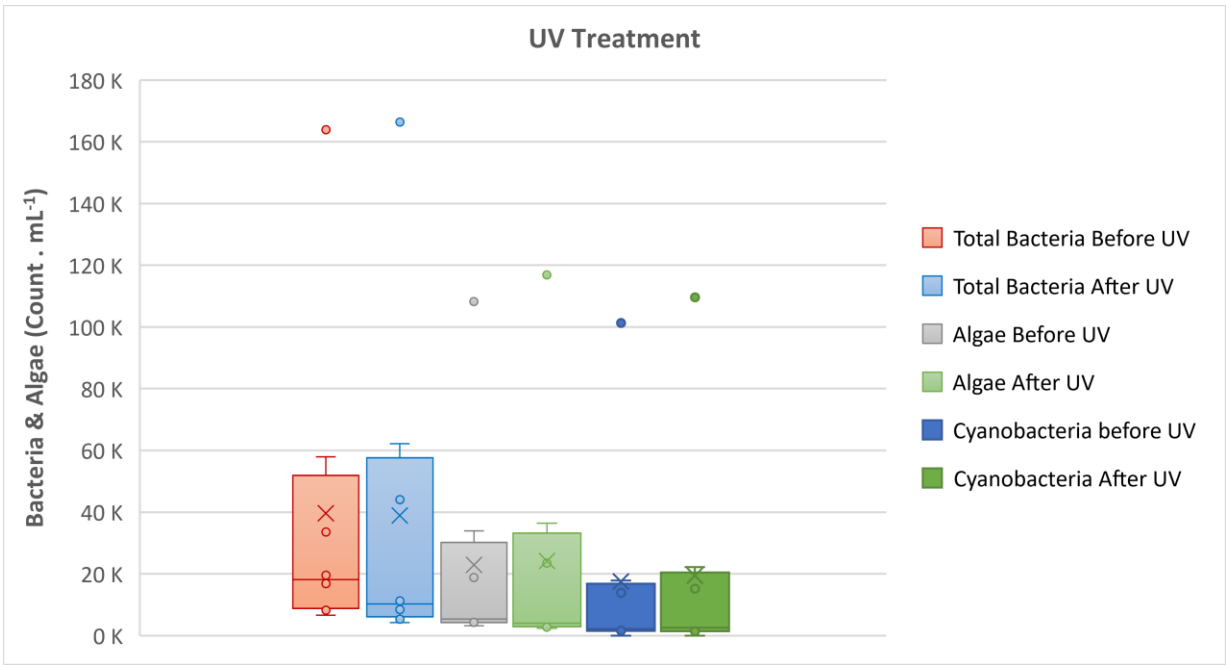

Figure A- 10. Box plots of total bacteria, algae, and cyanobacteria concentrations before and after UV treatment.

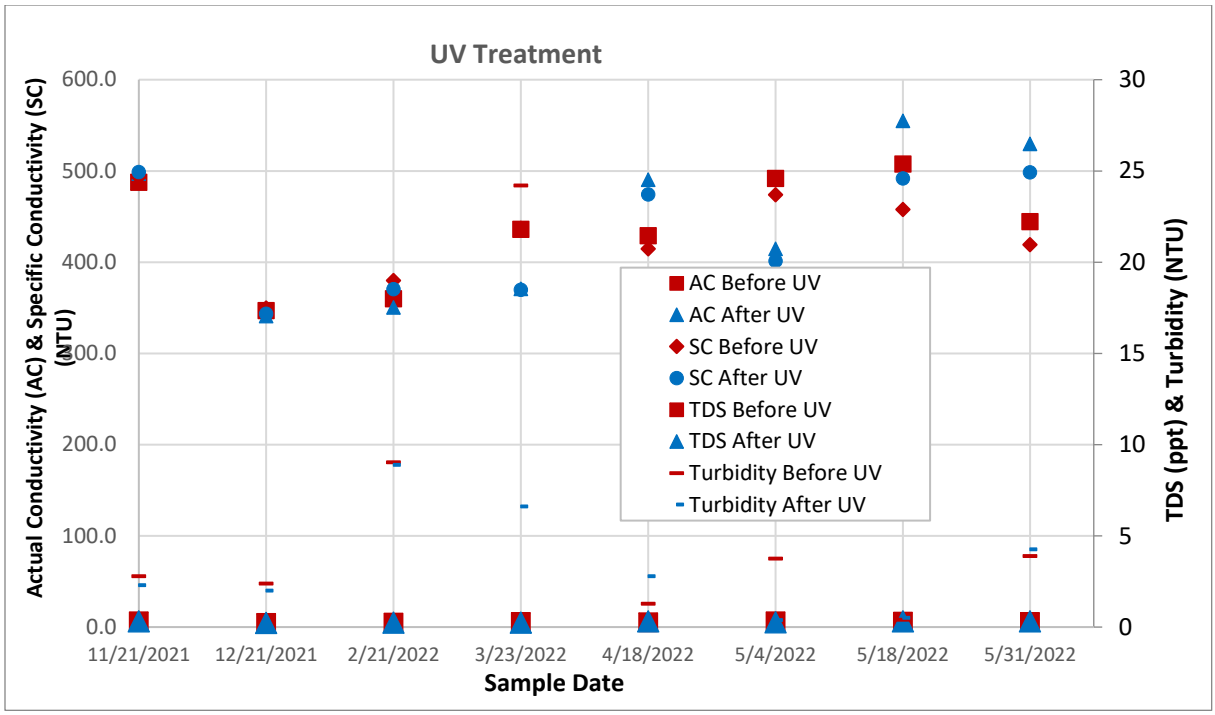

Figure A- 11. Temporal measurements of actual conductivity, specific conductivity, TDS, and turbidity before and after UV treatment.

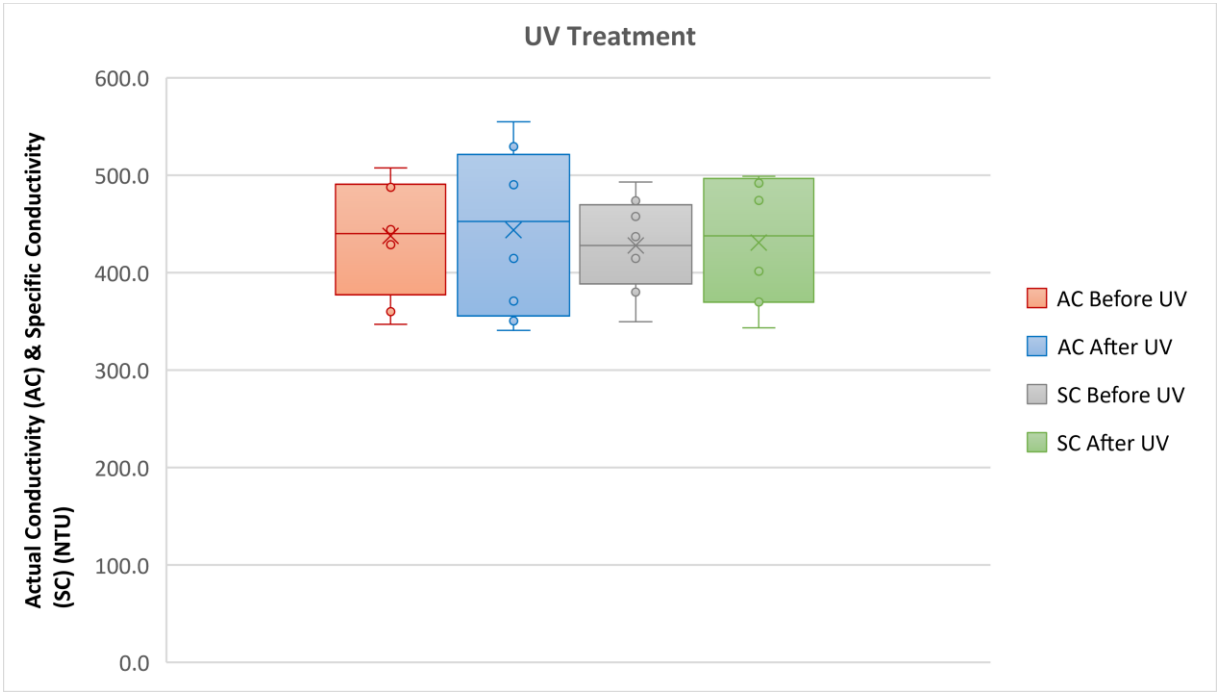

Figure A- 12. Box plots of actual and specific conductivity measurements before and after UV treatment.

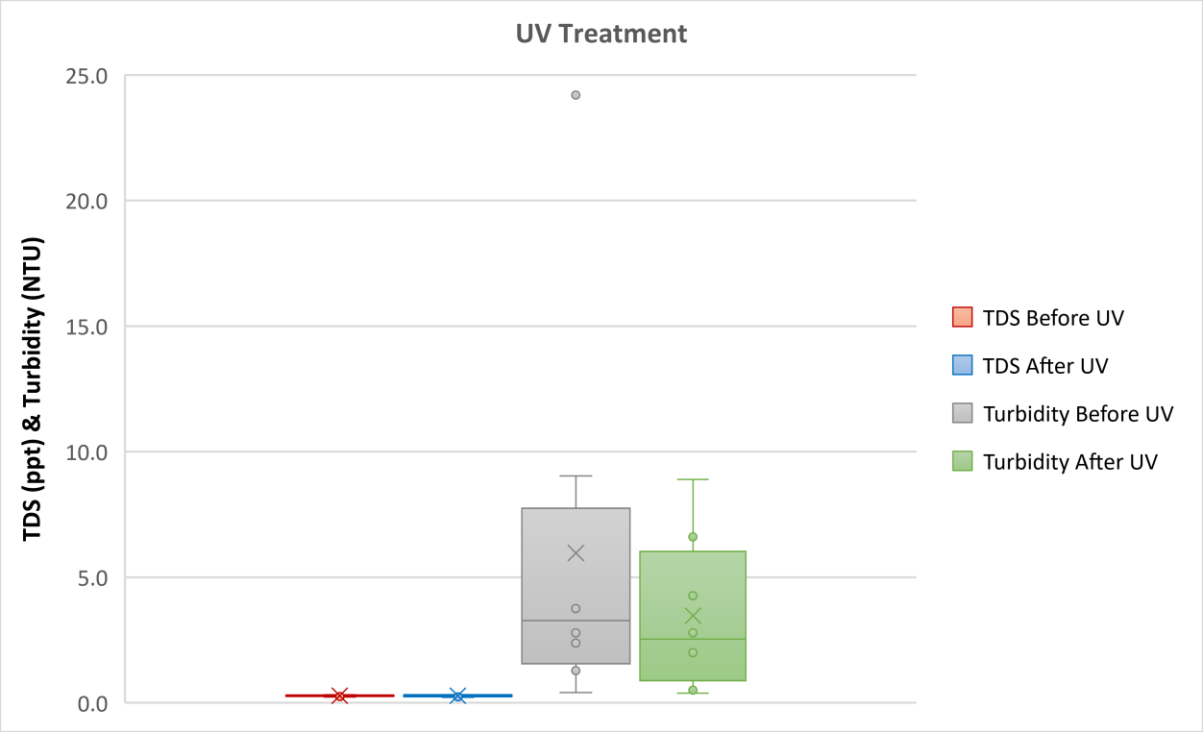

Figure A- 13. Box plots of TDS concentration and turbidity measurements before and after UV treatment.

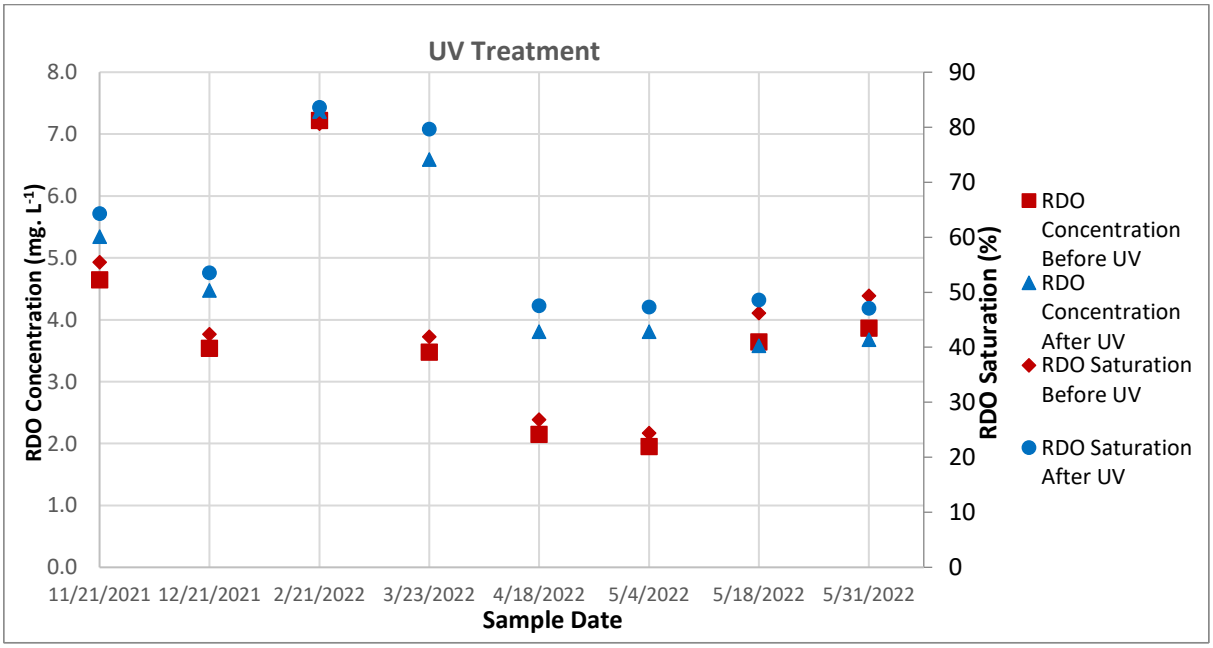

Figure A- 14. Temporal concentrations of dissolved oxygen and saturation before and after UV treatment.

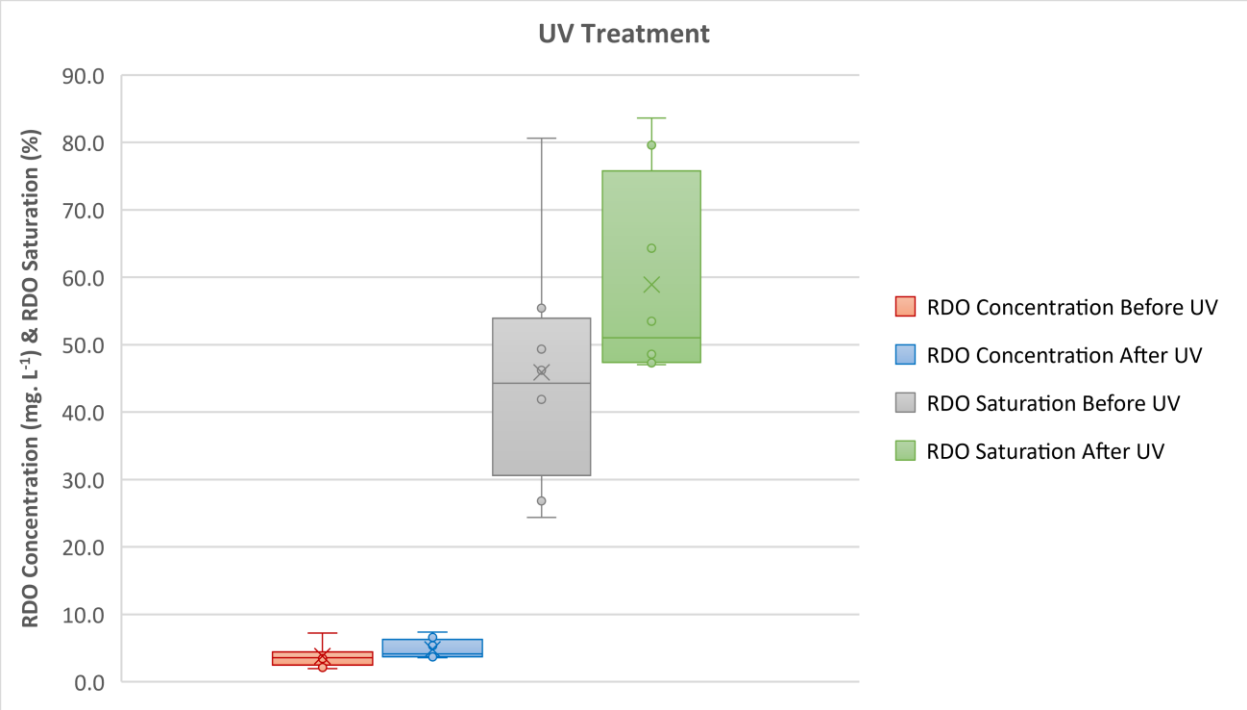

Figure A- 15. Box plots of dissolved oxygen concentration and saturation before and after UV treatment.

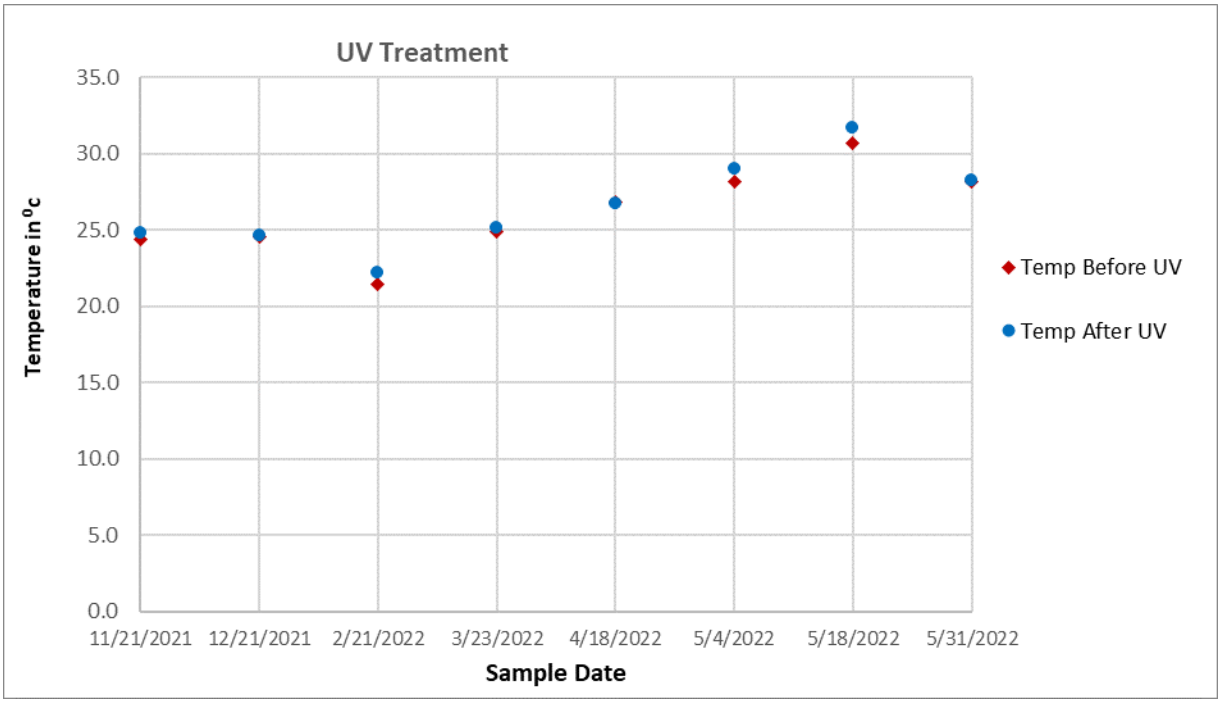

Figure A- 16. Temporal variation in temperature before and after UV treatment.

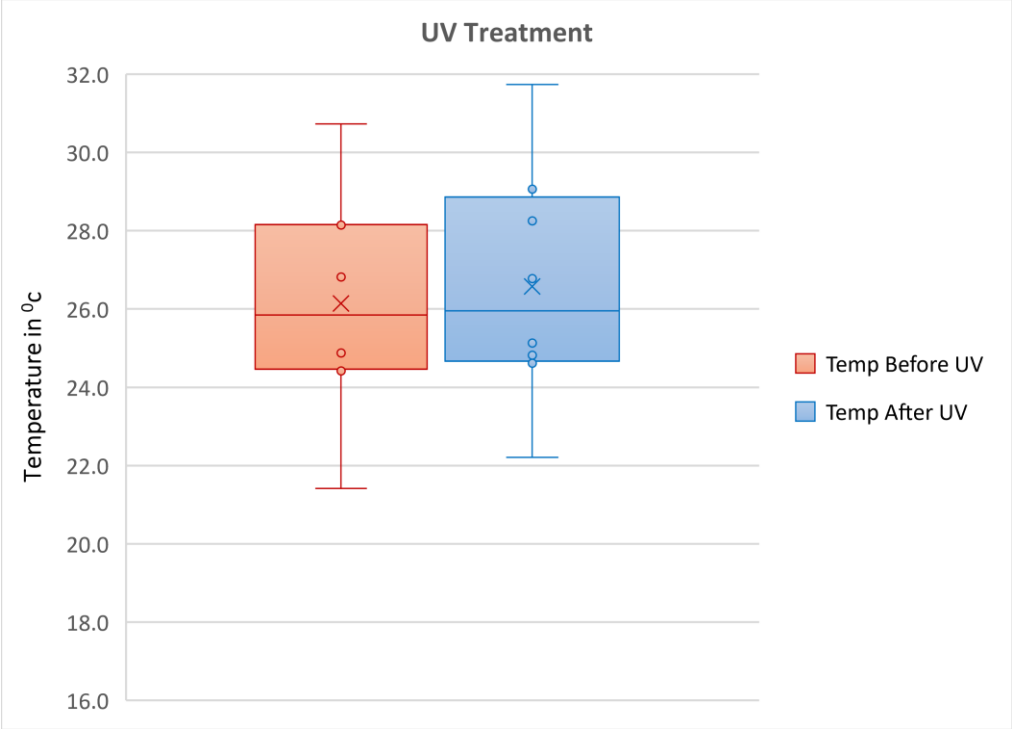

Figure A- 17. Box plot of temperature before and after UV treatment.

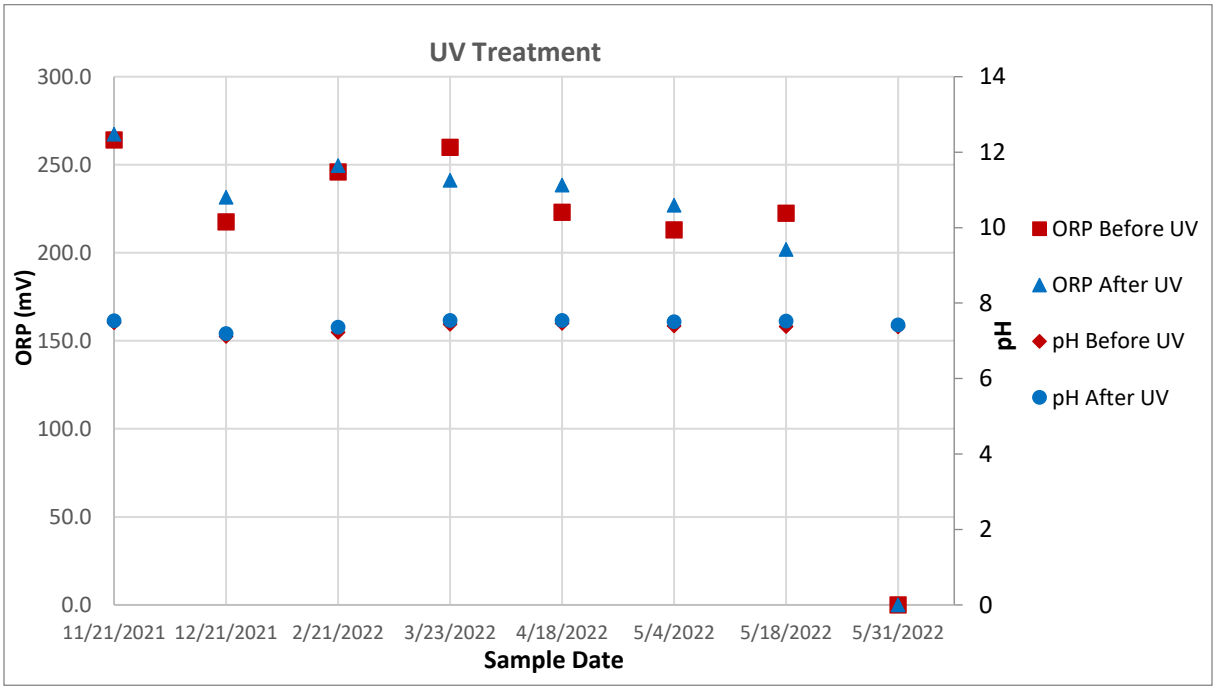

Figure A- 18.Temporal measurements of oxidation-reduction potential and pH before and after UV treatment.

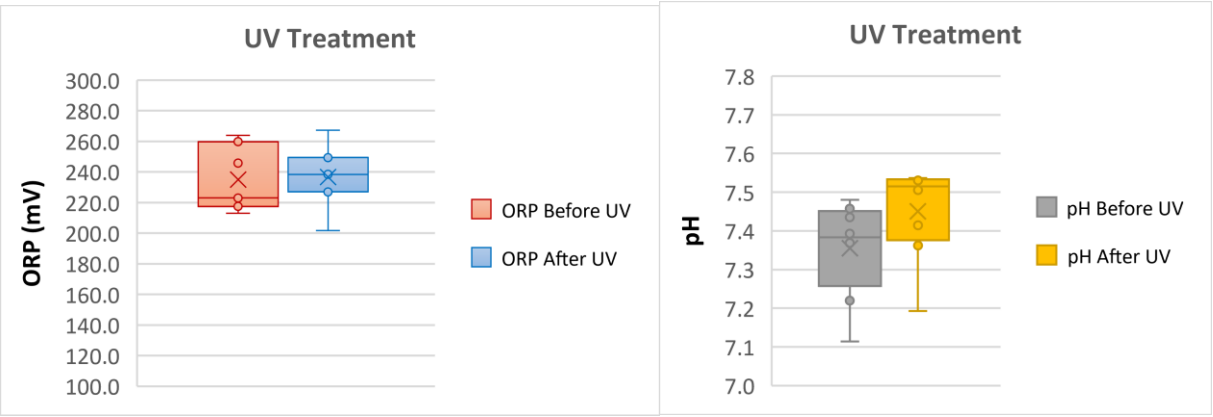

Figure A- 19. Box plots of oxidation-reduction potential before and after UV treatment.

Appendix B. Water quality graphs associated with the impacts analysis of the holding tank

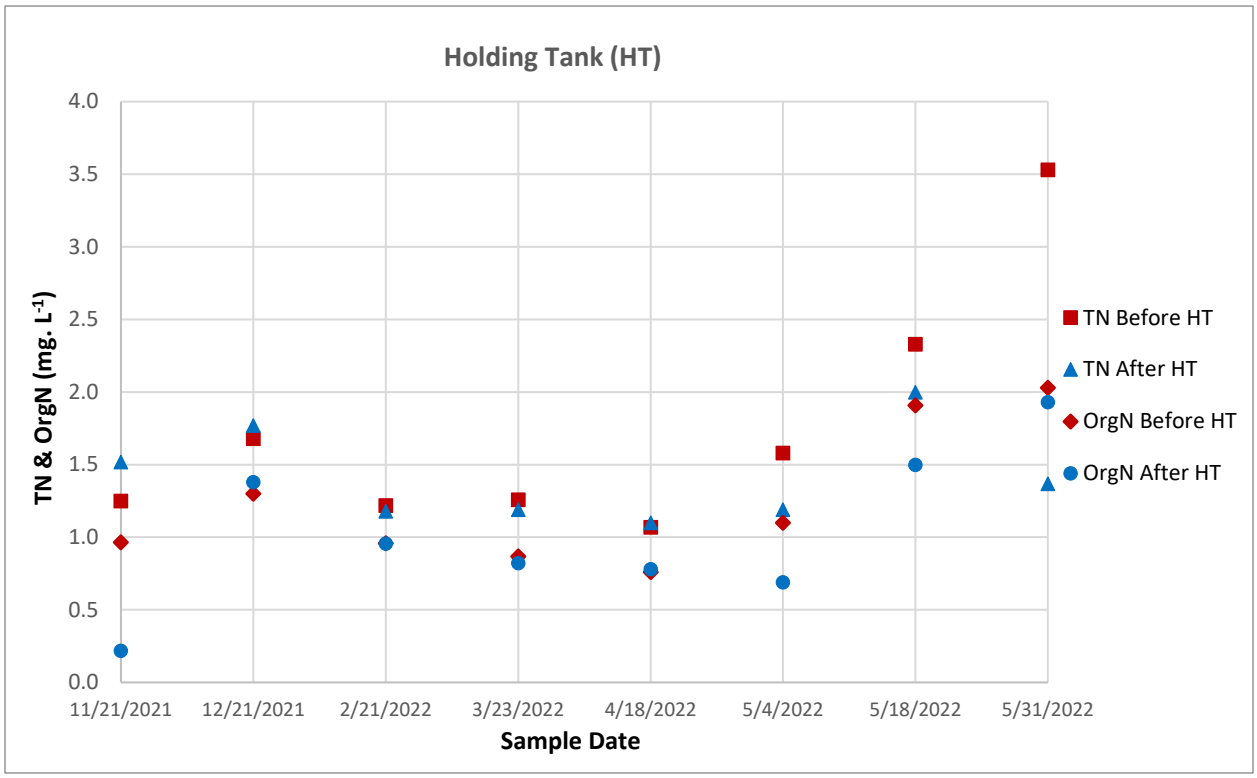

Figure B- 1. Temporal variation in total and organic nitrogen concentrations before and after the holding tank.

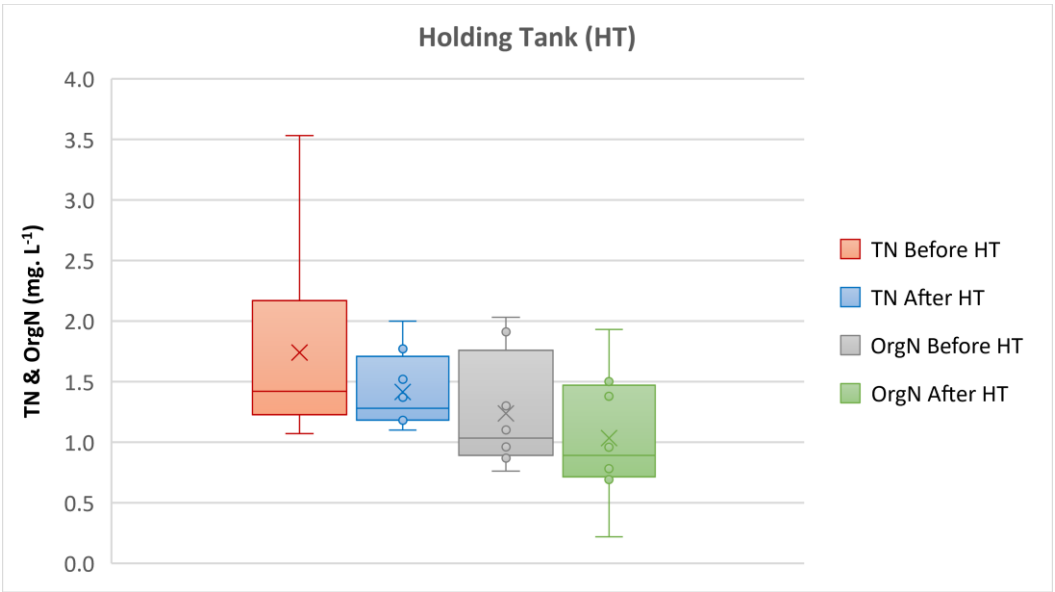

Figure B- 2. Box plot of total and organic nitrogen concentrations before and after the holding tank.

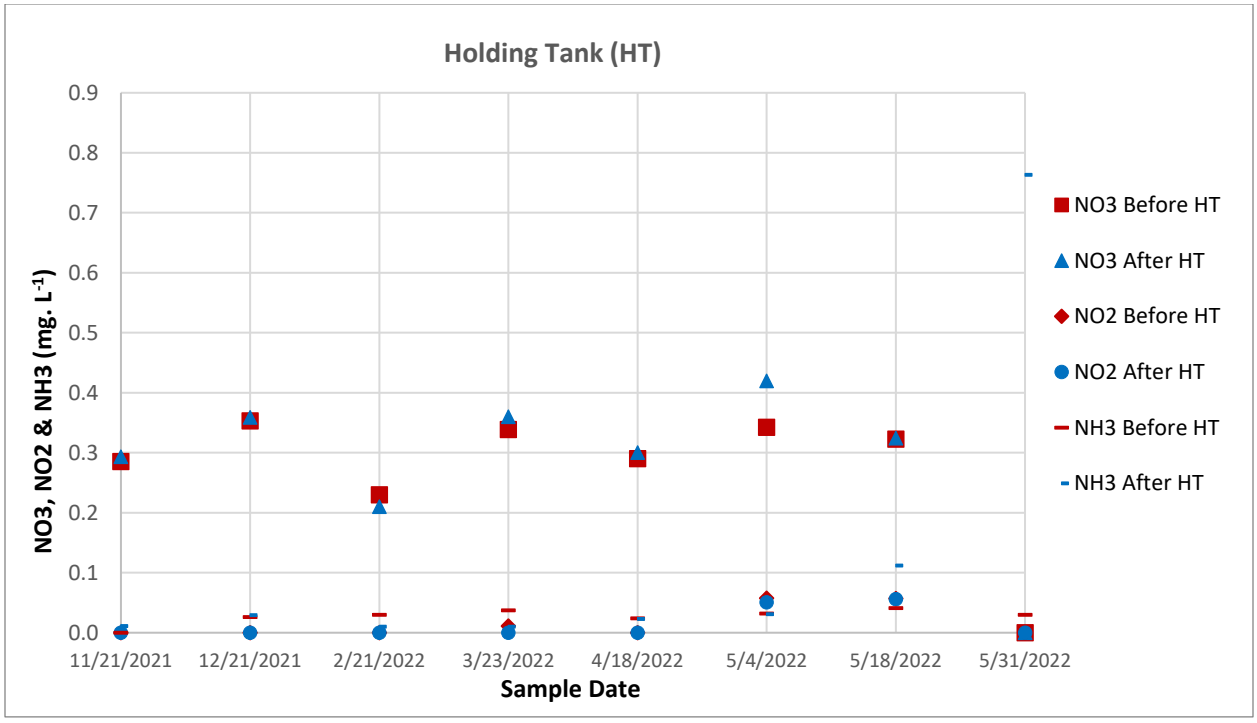

Figure B- 3. Temporal concentrations of nitrate, nitrite, and ammonia before and after the holding tank.

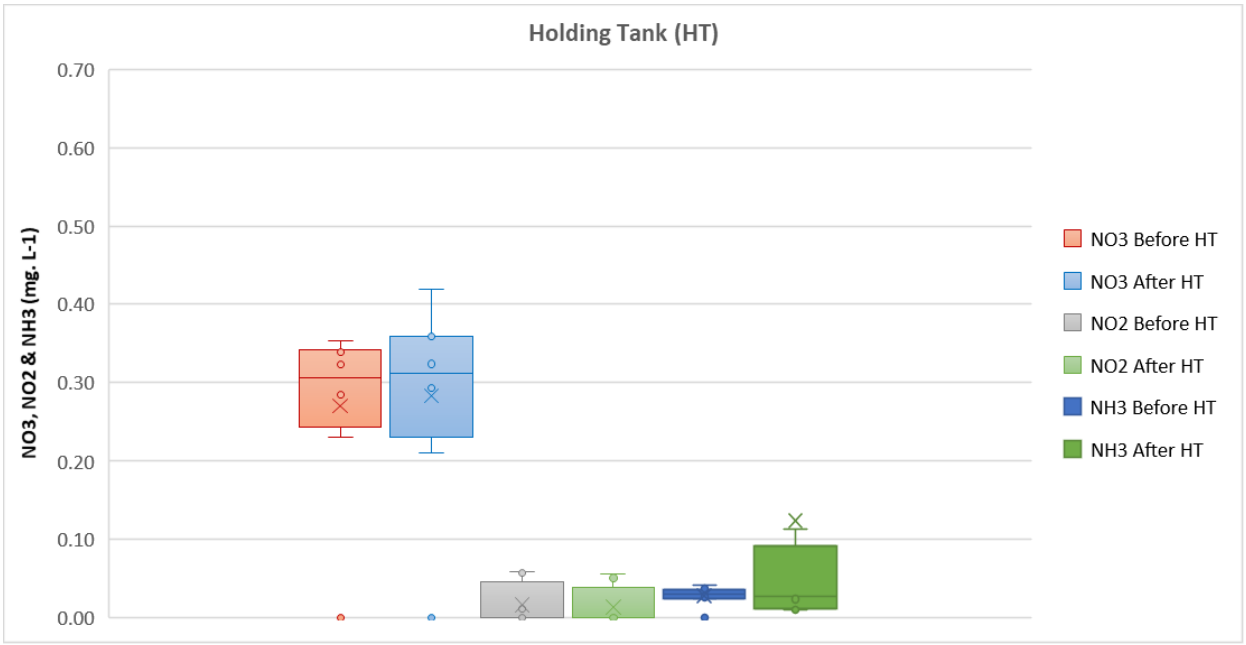

Figure B- 4. Box plot of nitrate, nitrite, and ammonia concentration before and after the holding tank.

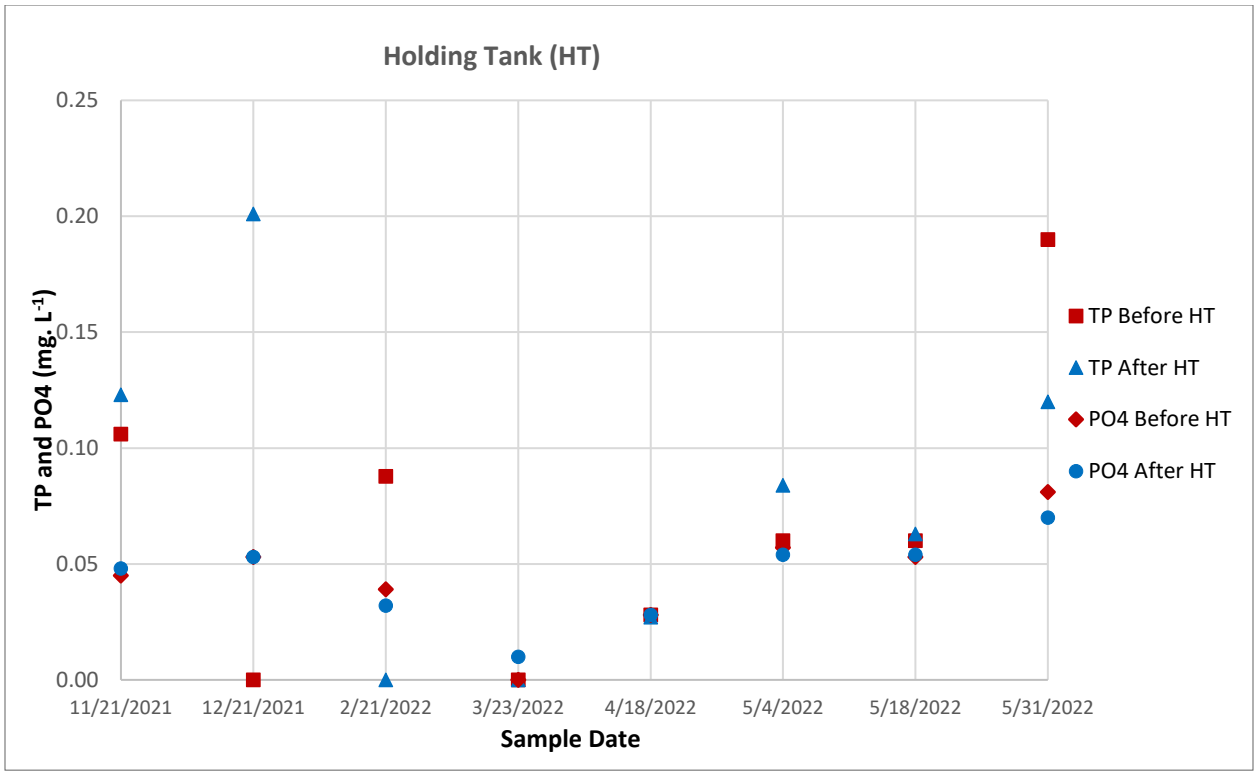

Figure B- 5. Temporal concentrations of total phosphorus and orthophosphate before and after the holding tank.

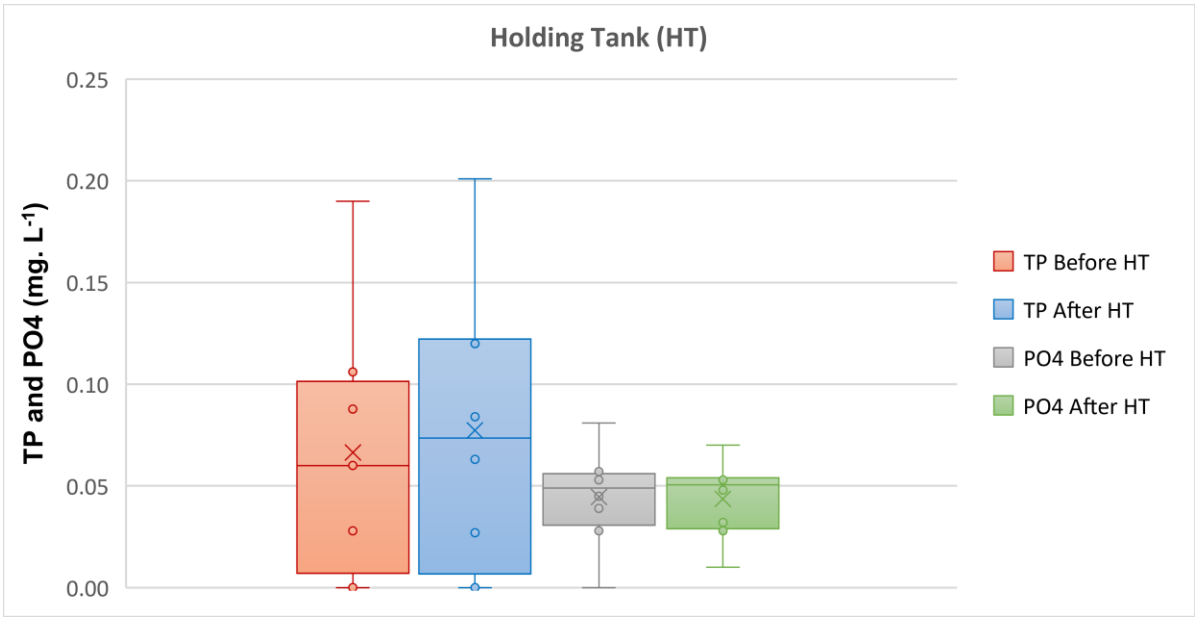

Figure B- 6. Box diagram of total phosphorus and orthophosphate concentrations before and after the holding tank.

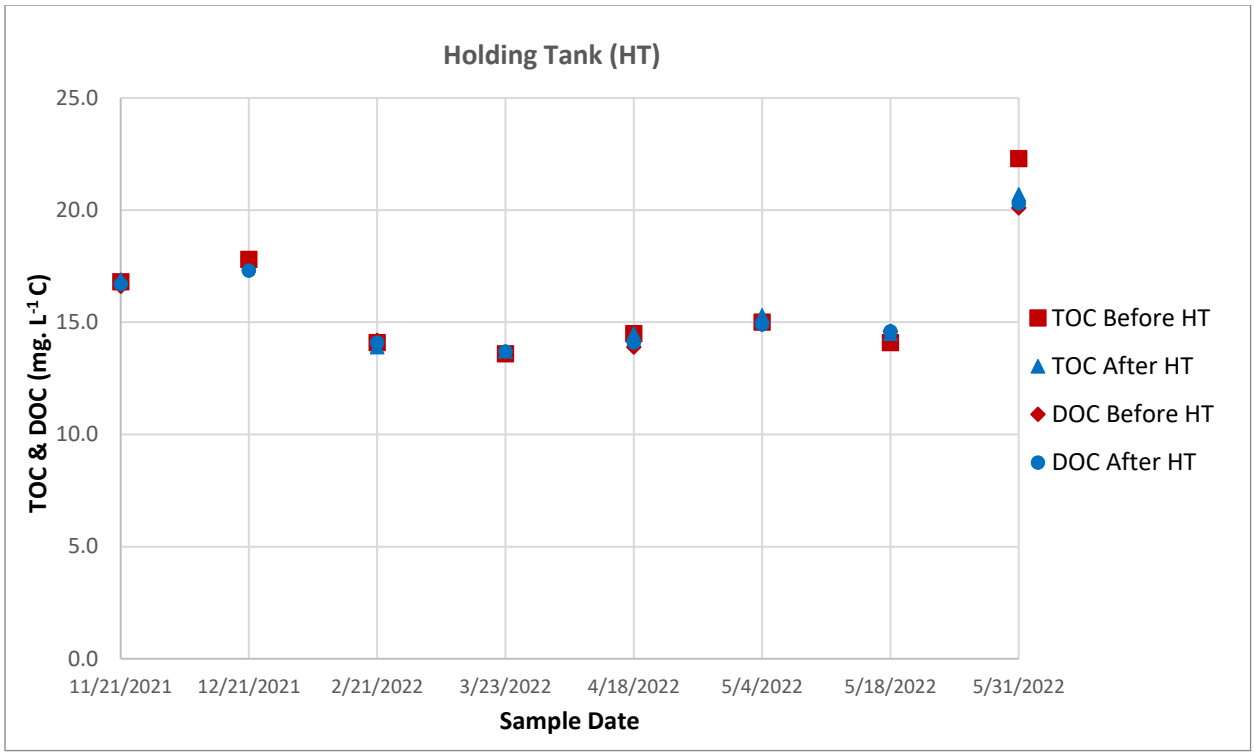

Figure B- 7. Temporal concentrations of TOC and DOC before and after the holding tank.

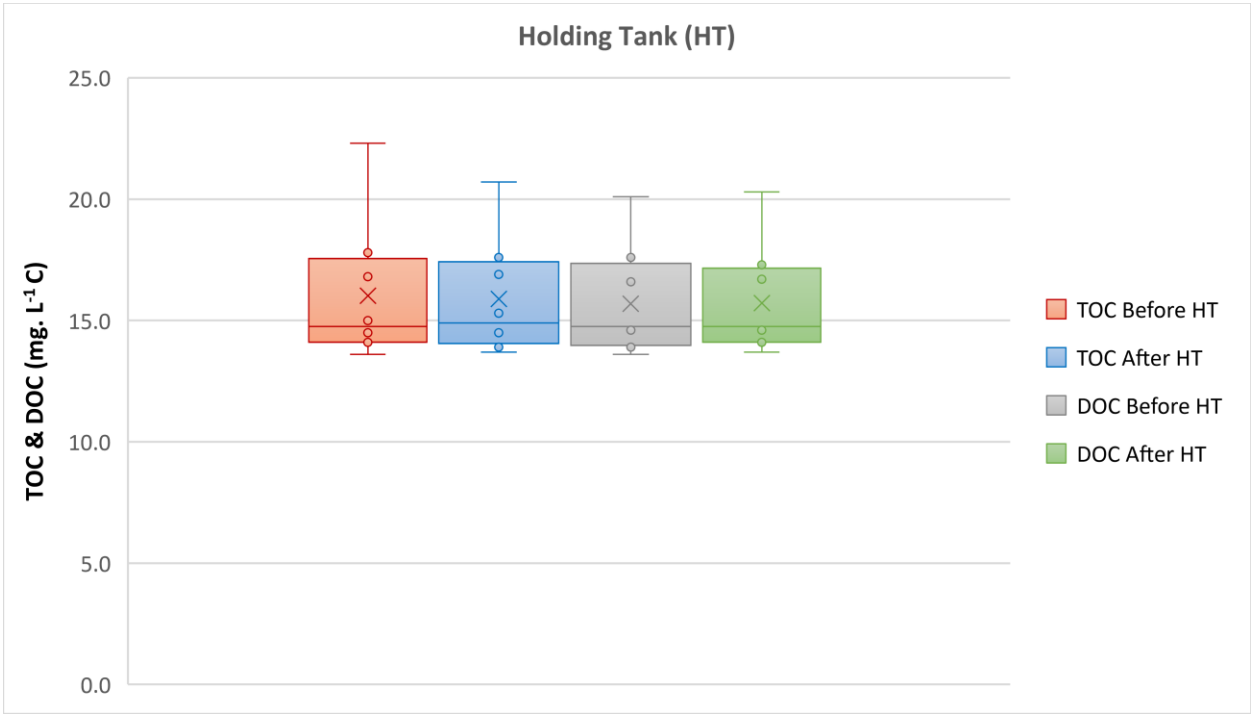

Figure B- 8. Box diagram of TOC and DOC before and after the holding tank.

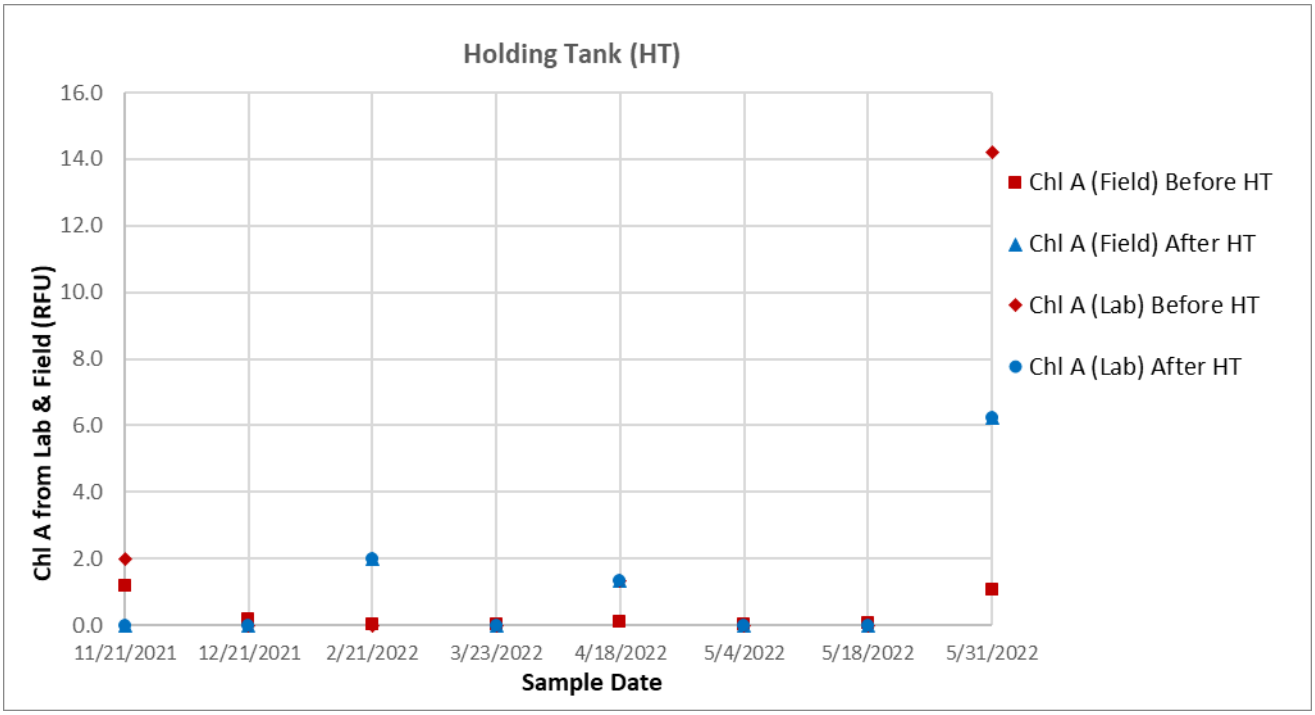

Figure B- 9. Temporal diagram of field meter measurement and laboratory analysis of chlorophyll A before and after the holding tank.

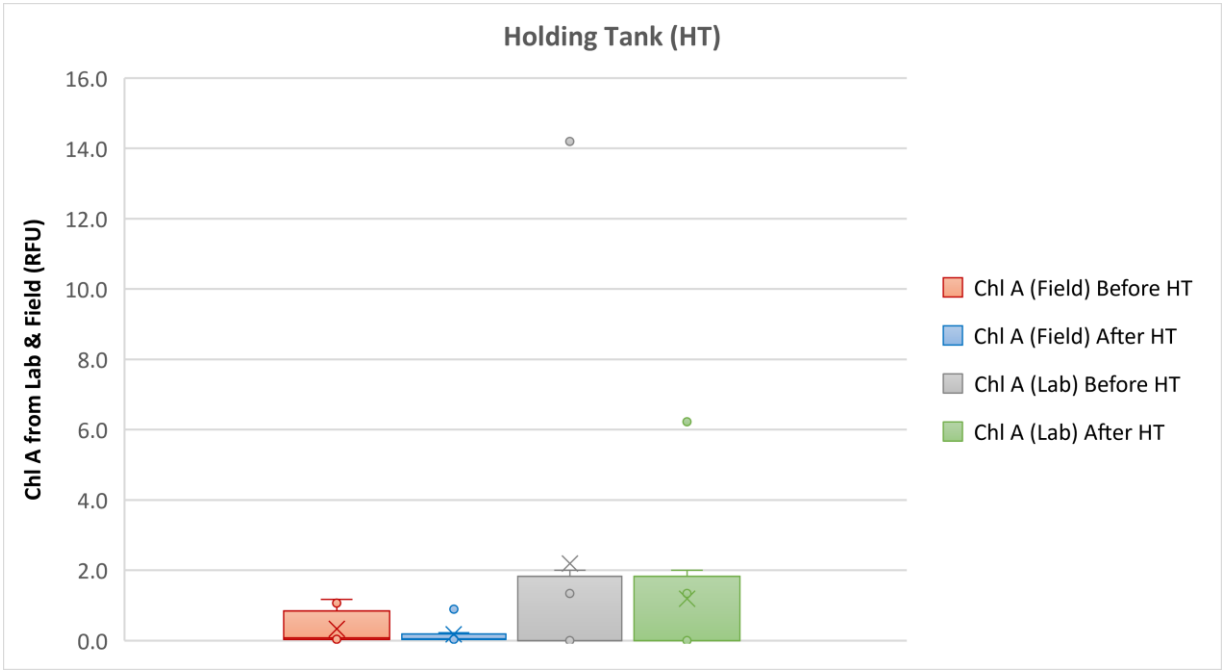

Figure B- 10. Box diagram of field measured and laboratory analyzed chlorophyll A before and after the holding tank.

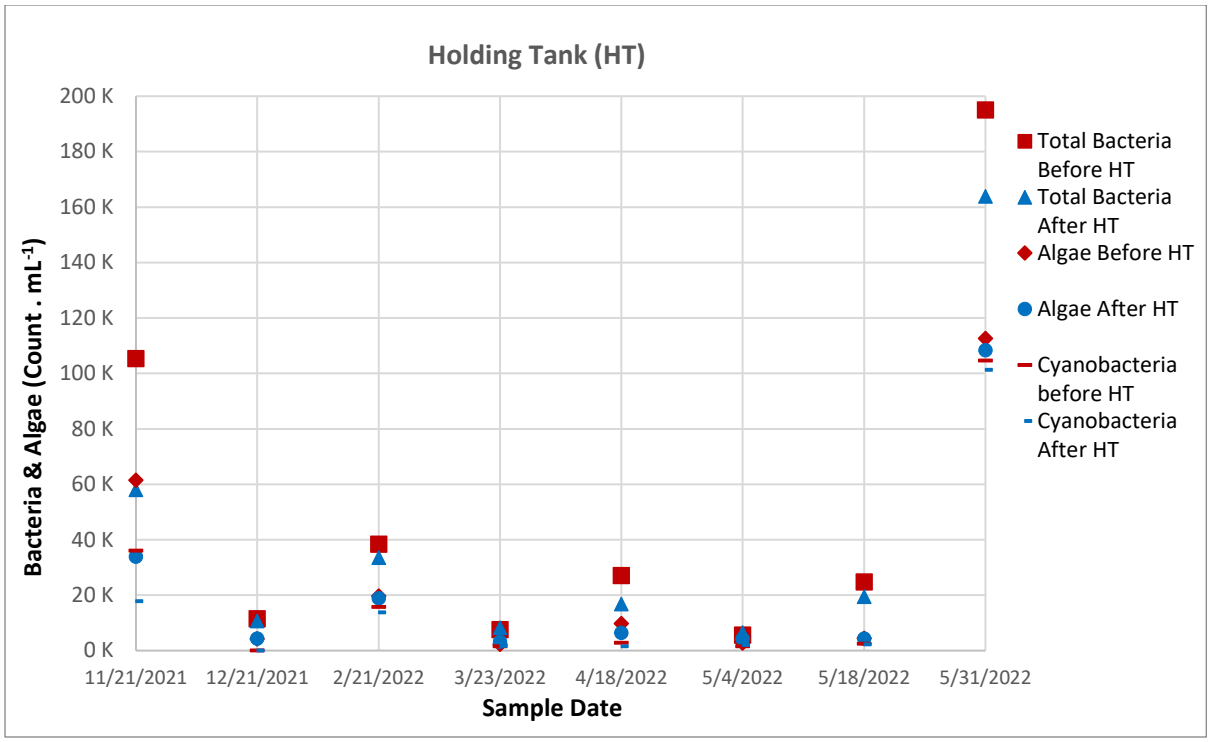

Figure B- 11. Temporal concentrations of total bacteria, algae, and cyanobacteria counts before and after the holding tank.

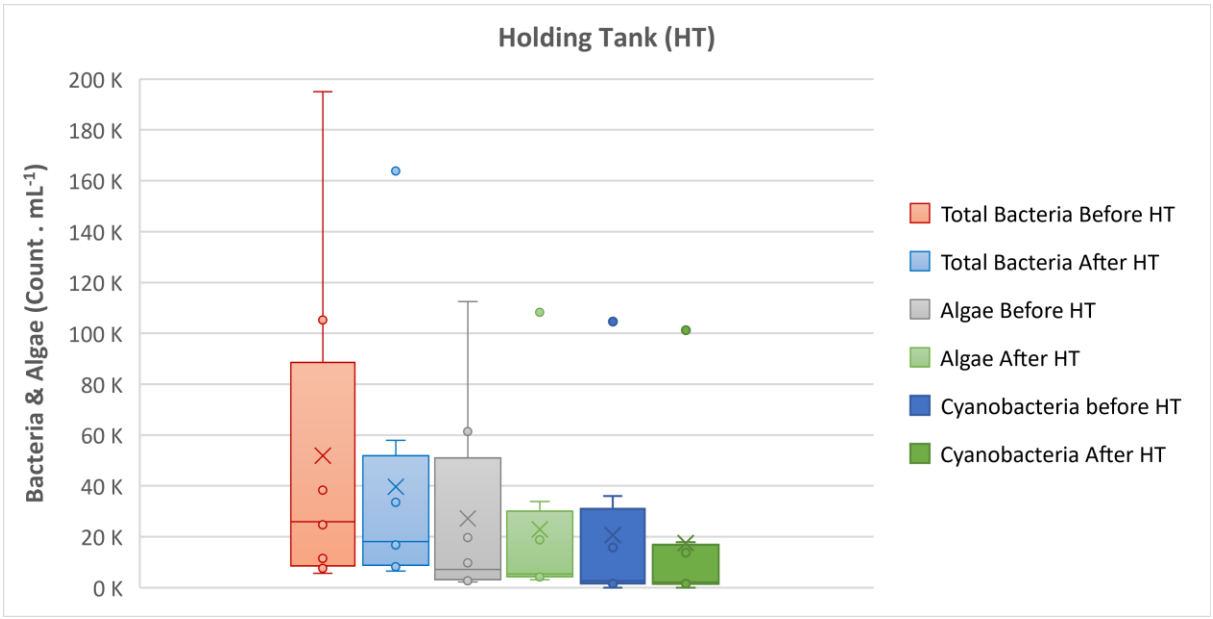

Figure B- 12. Box diagram of the total bacteria, algae, and cyanobacteria counts before and after the holding tank.

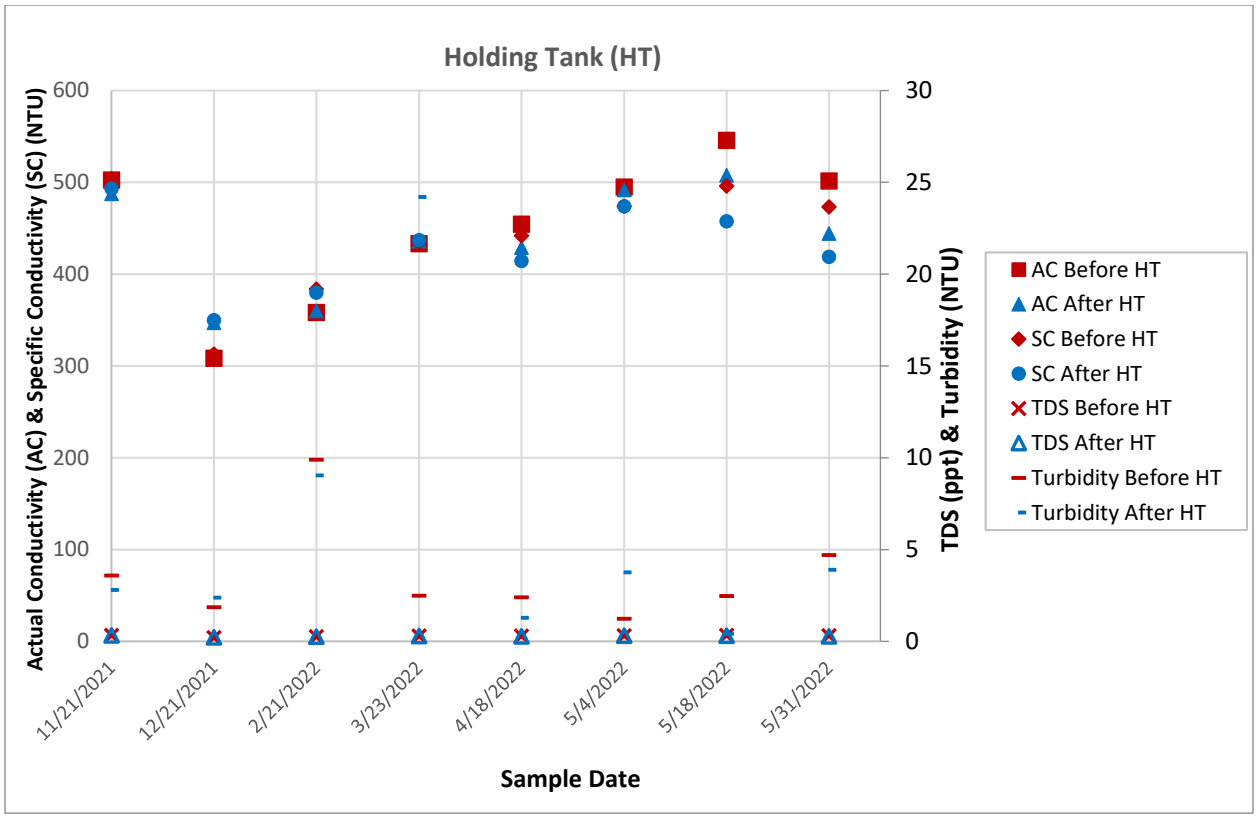

Figure B- 13. Temporal measurements of actual conductivity, specific conductivity, TDS concentrations, and turbidity before and after the holding tank.

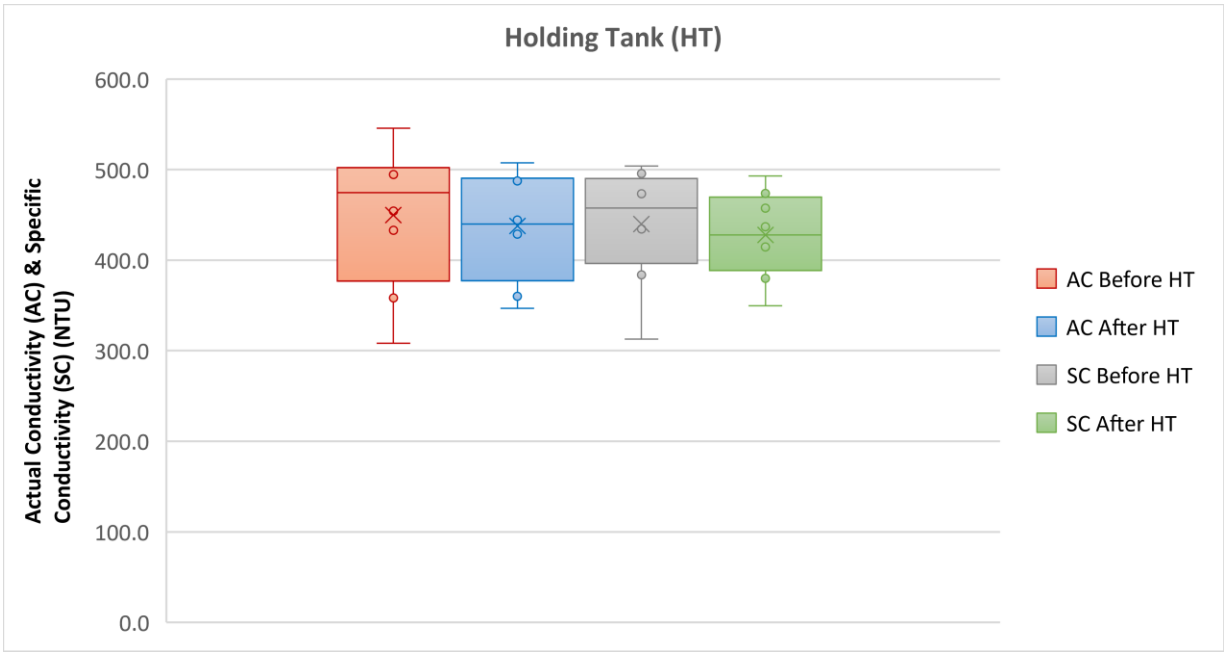

Figure B- 14. Box diagram of the actual condctivity and specific conductivity before and after the holding tank.

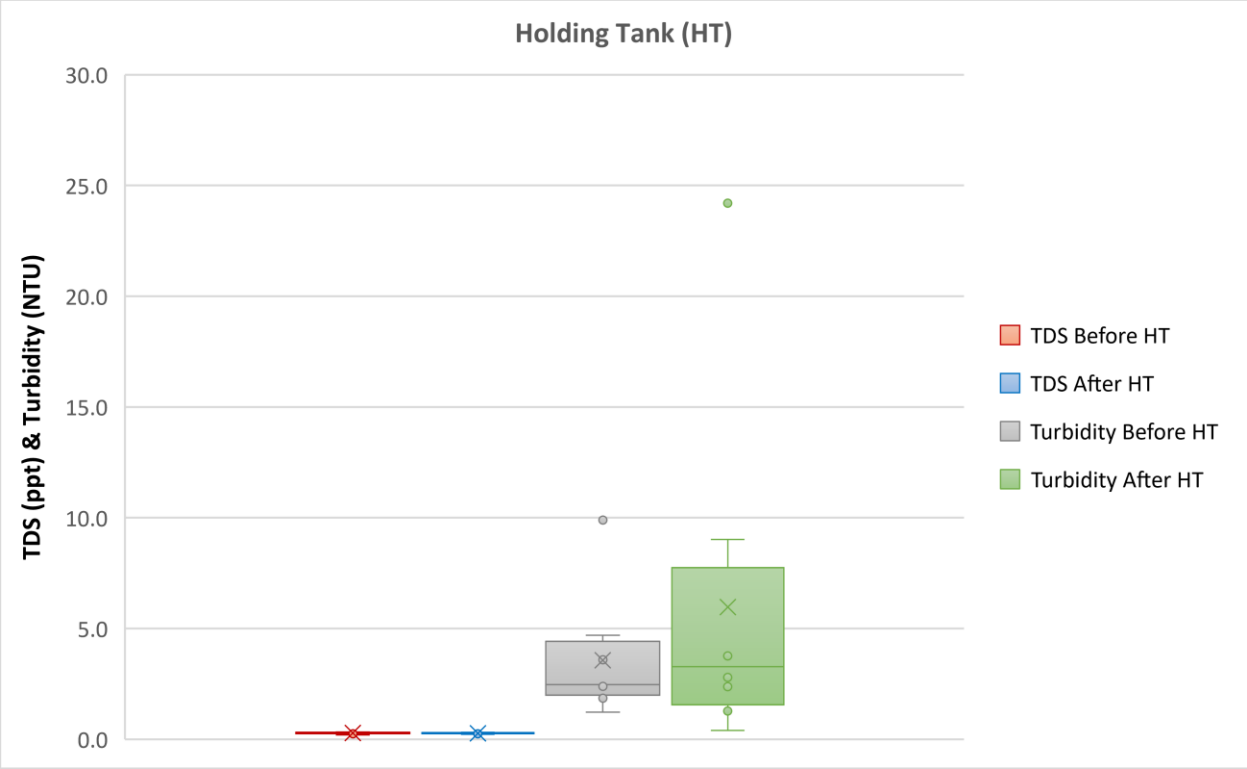

Figure B- 15. Box diagram of the TDS concentration and turbidity before and after the holding tank.

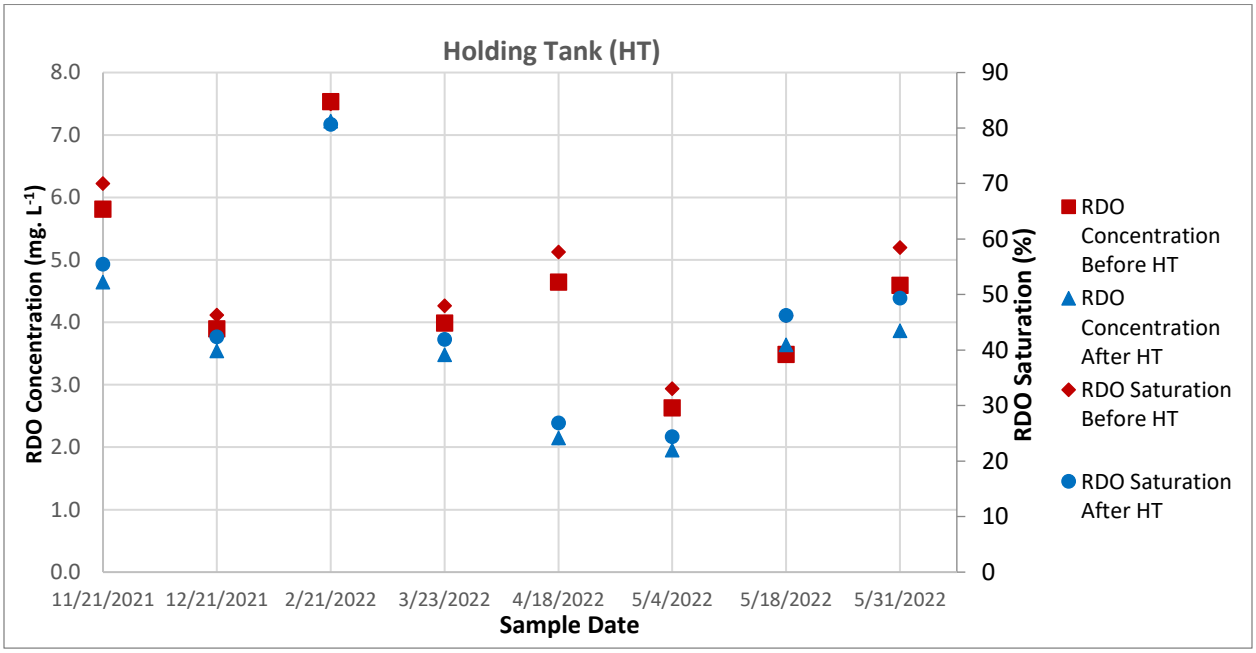

Figure B- 16. Temporal concentration of dissolved oxygen and saturation percent before and after the holding tank.

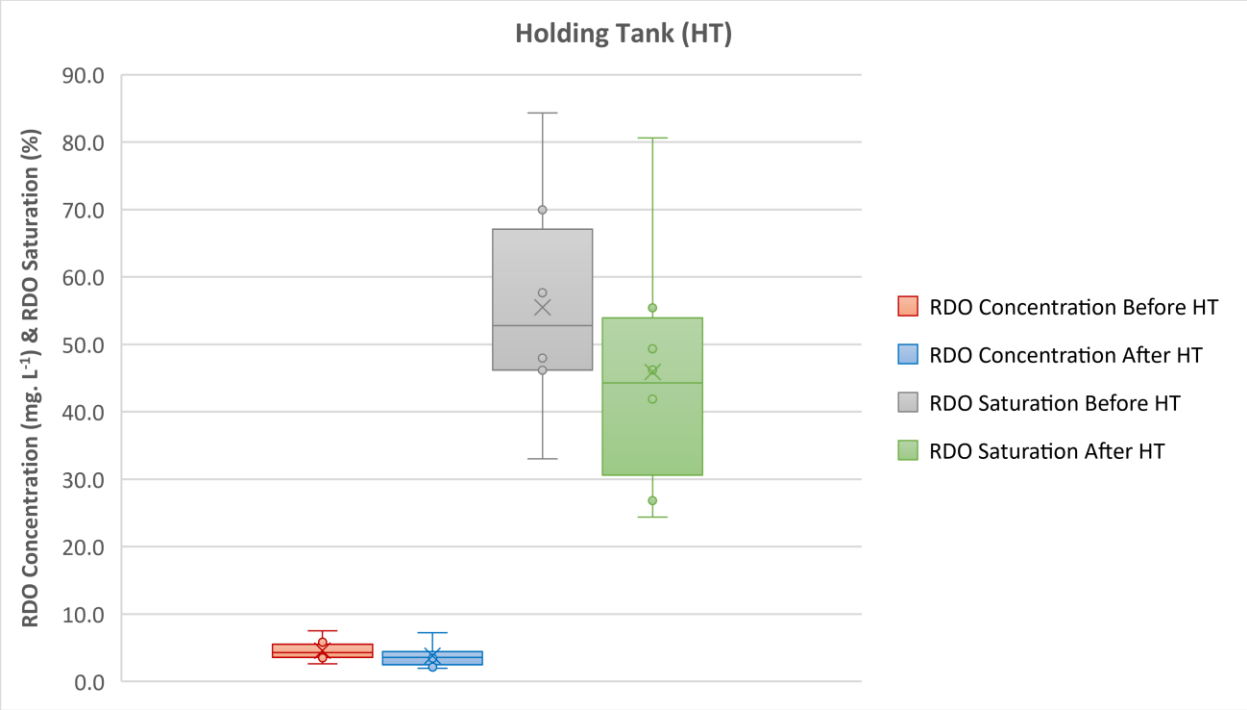

Figure B- 17. Box diagram of oxygen concentration and satuation percent before and after the holding tank.

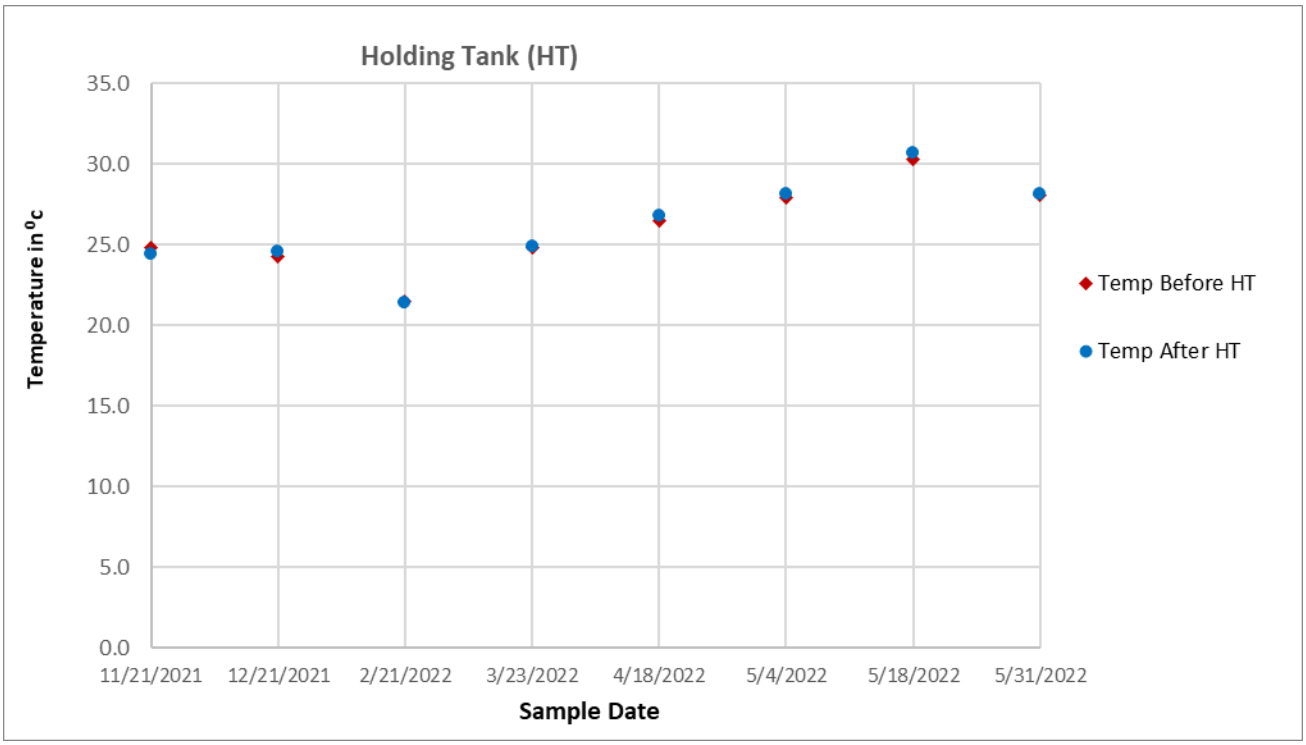

Figure B- 18. Temporal measurements of water temperature before and after the holding tank.

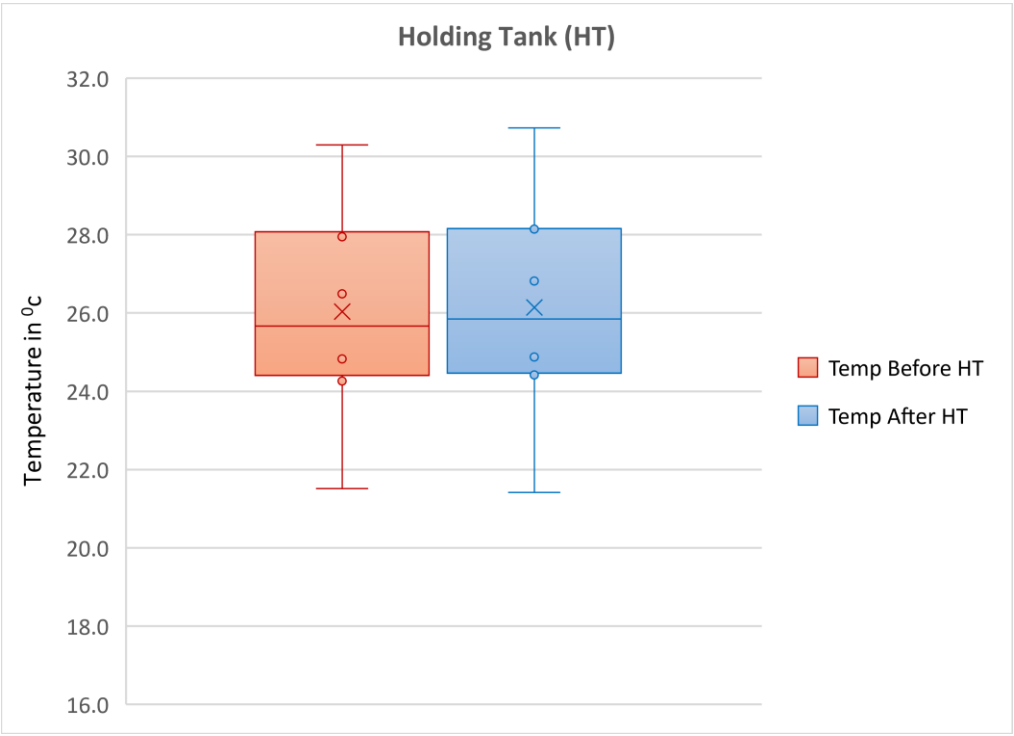

Figure B- 19. Box diagram of water temperature measurments before and after the holding tank.

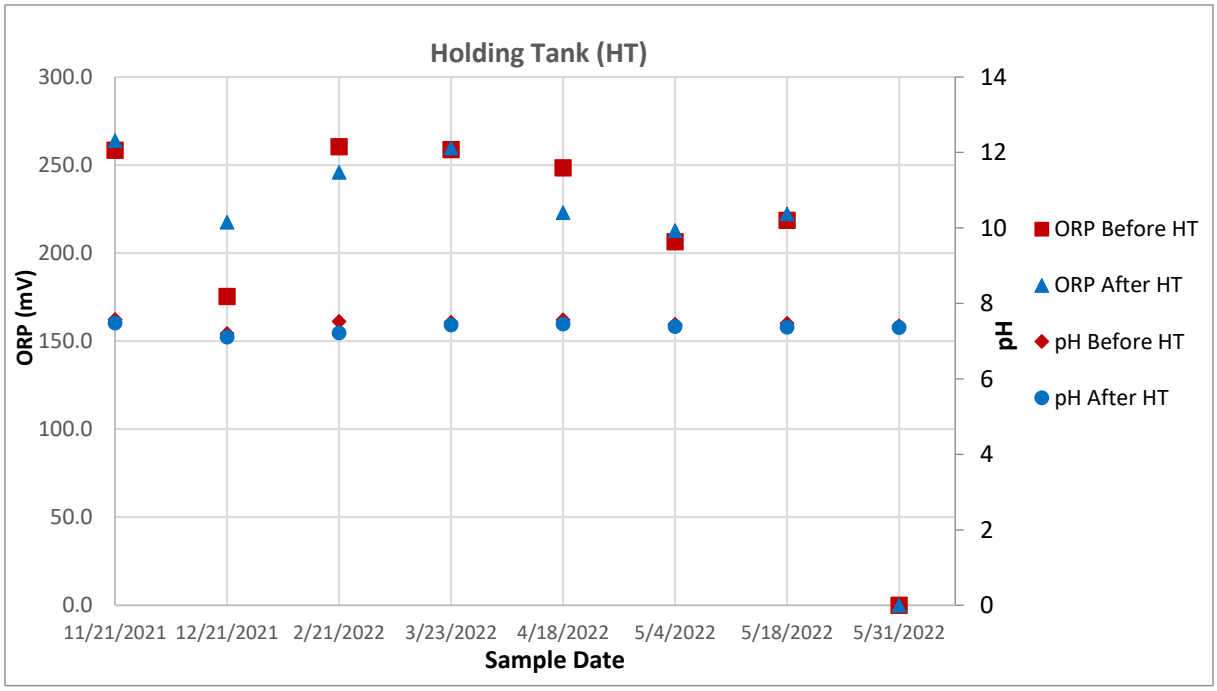

Figure B- 20. Temporal measurements of oxidation-reduction potential and pH before and after the holding tank.

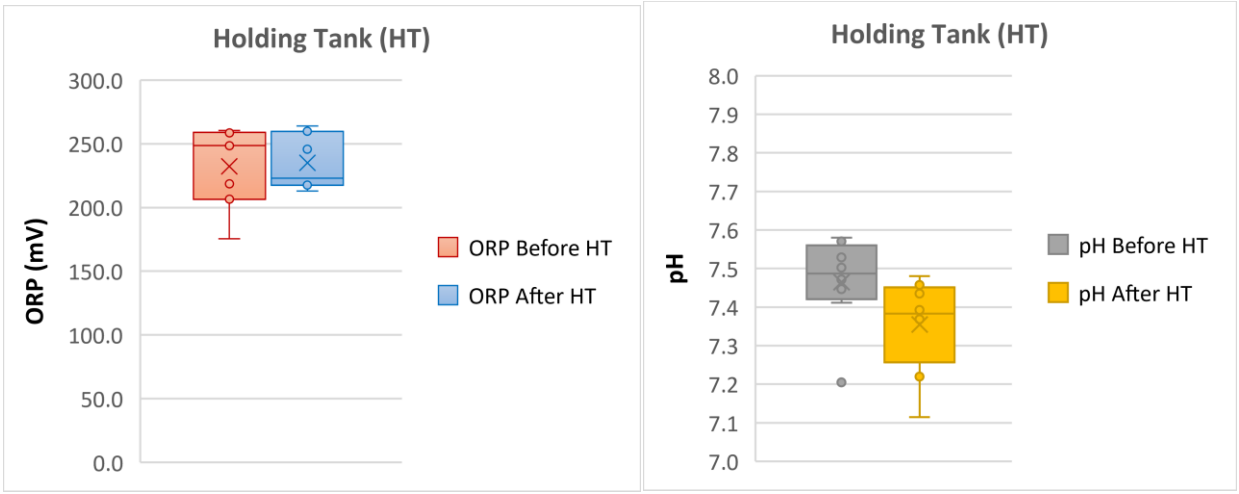

Figure B- 21. Box plots of oxidation-reduction potential and pH before and after the holding tank.

Appendix C. Selected water quality graphs associated with the impacts analysis of parameter changes in the pipeline between stations 7 and 8.

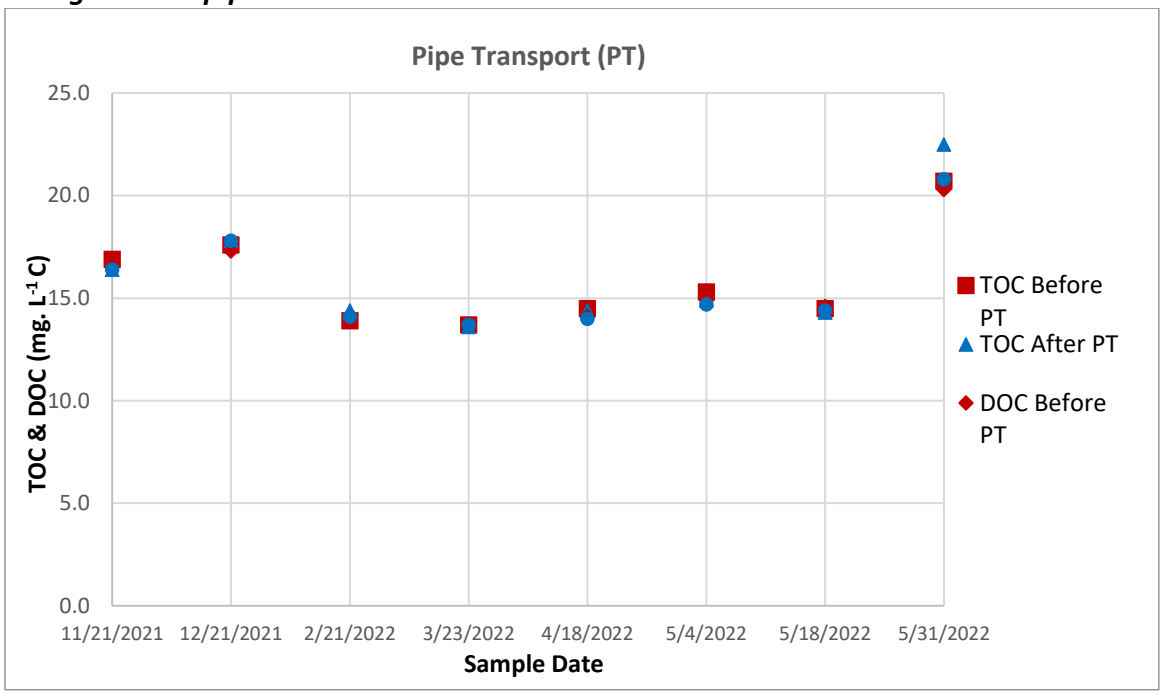

Figure C- 1. Temporal changes in concentration of TOC and DOC in the pipeline bewteen stations 7 and 8.

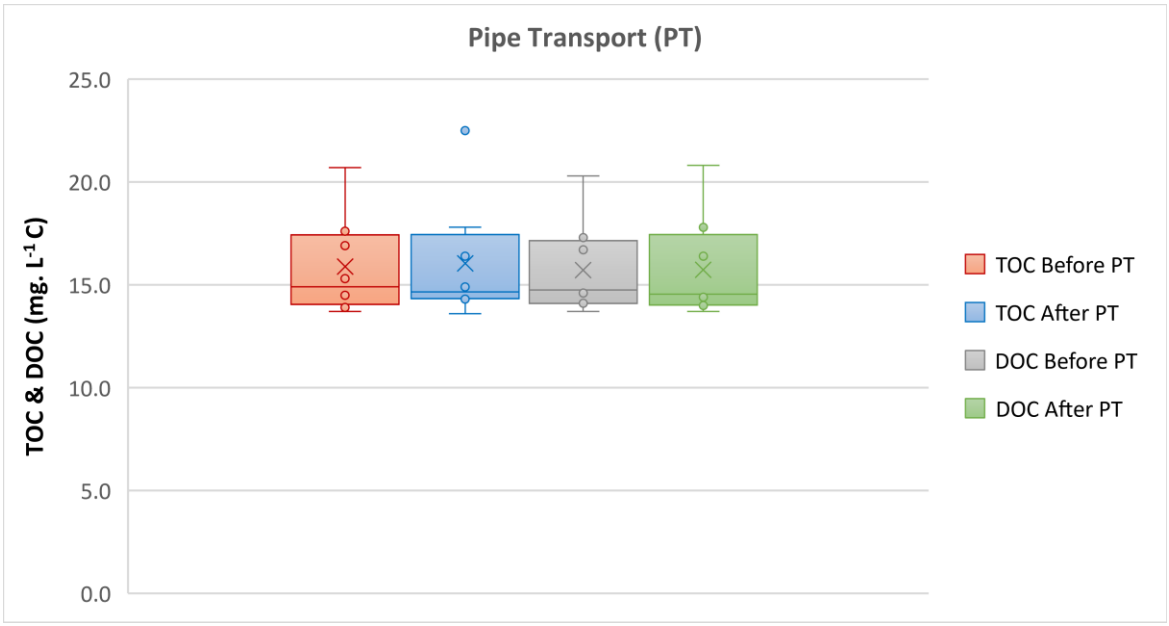

Figure C- 2. Box plot of the changes in concentration of TOC and DOC in the pipeline between stations 7 and 8.

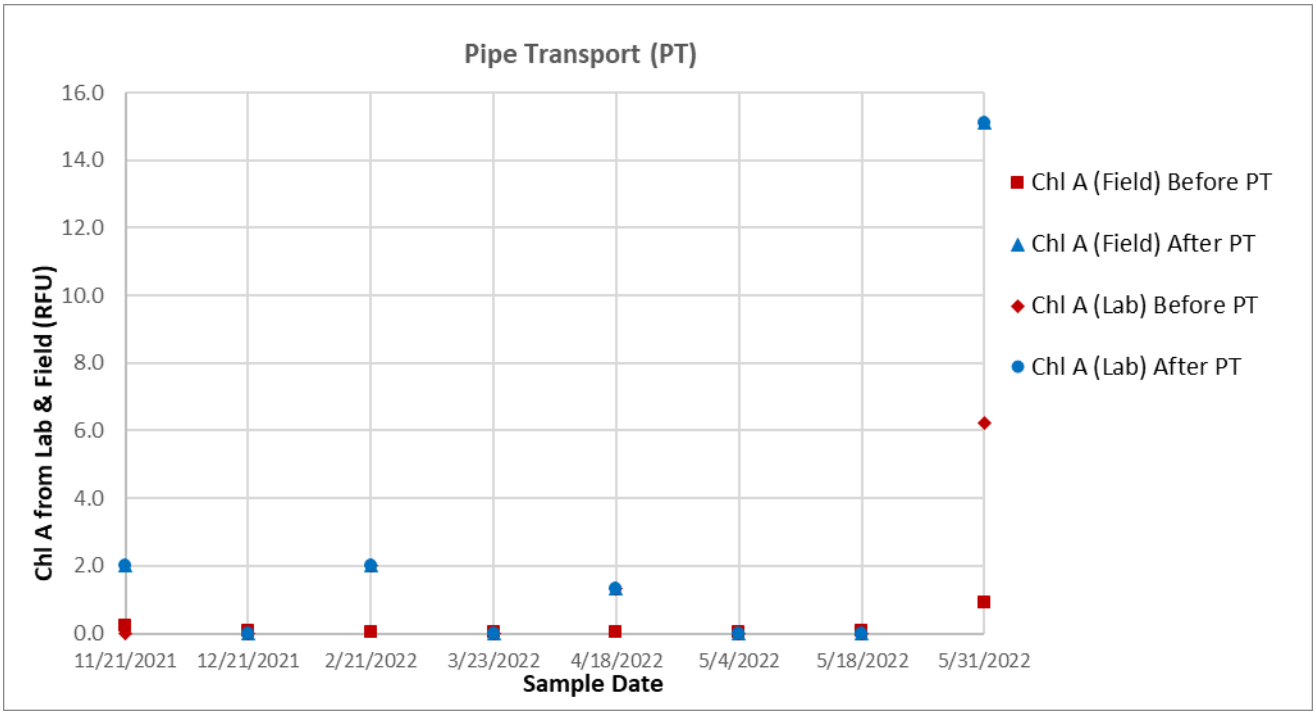

Figure C- 3. Temporal changes in the field meter measured and laboratory analyzed chlorophyll A in the pipeline connecting stations 7 and 8.

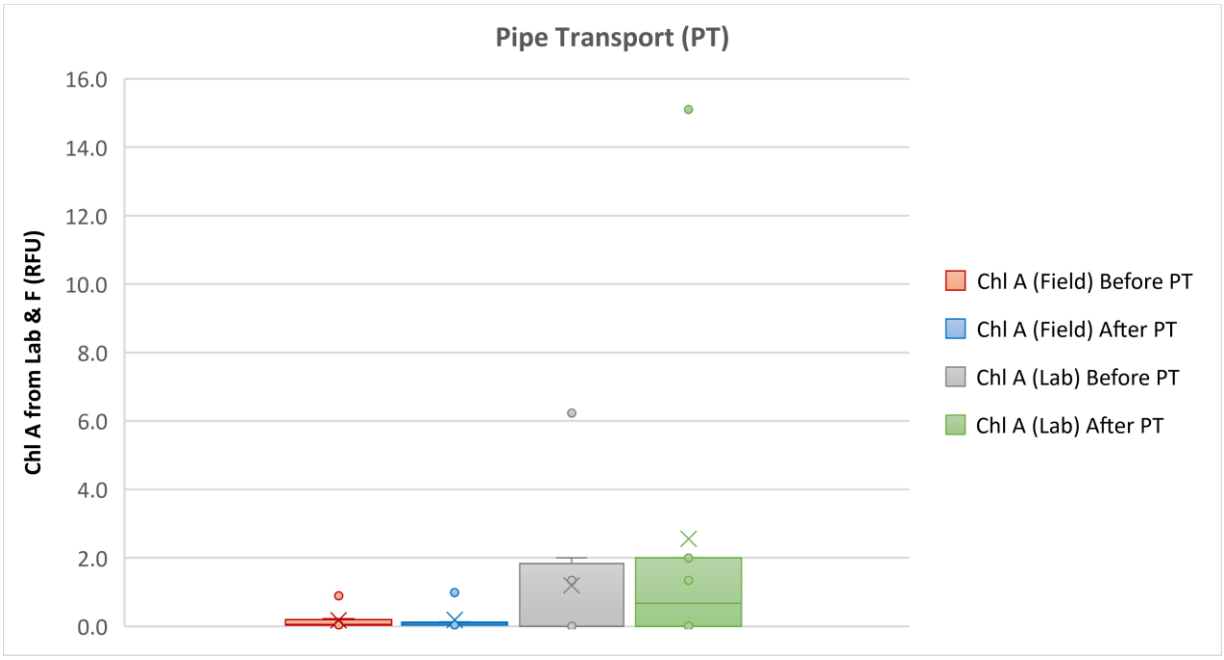

Figure C- 4. Box plot of chlorophyll A values measured using a field meter and analyzed in the laboratory in the pipeline between stations 7 and 8.

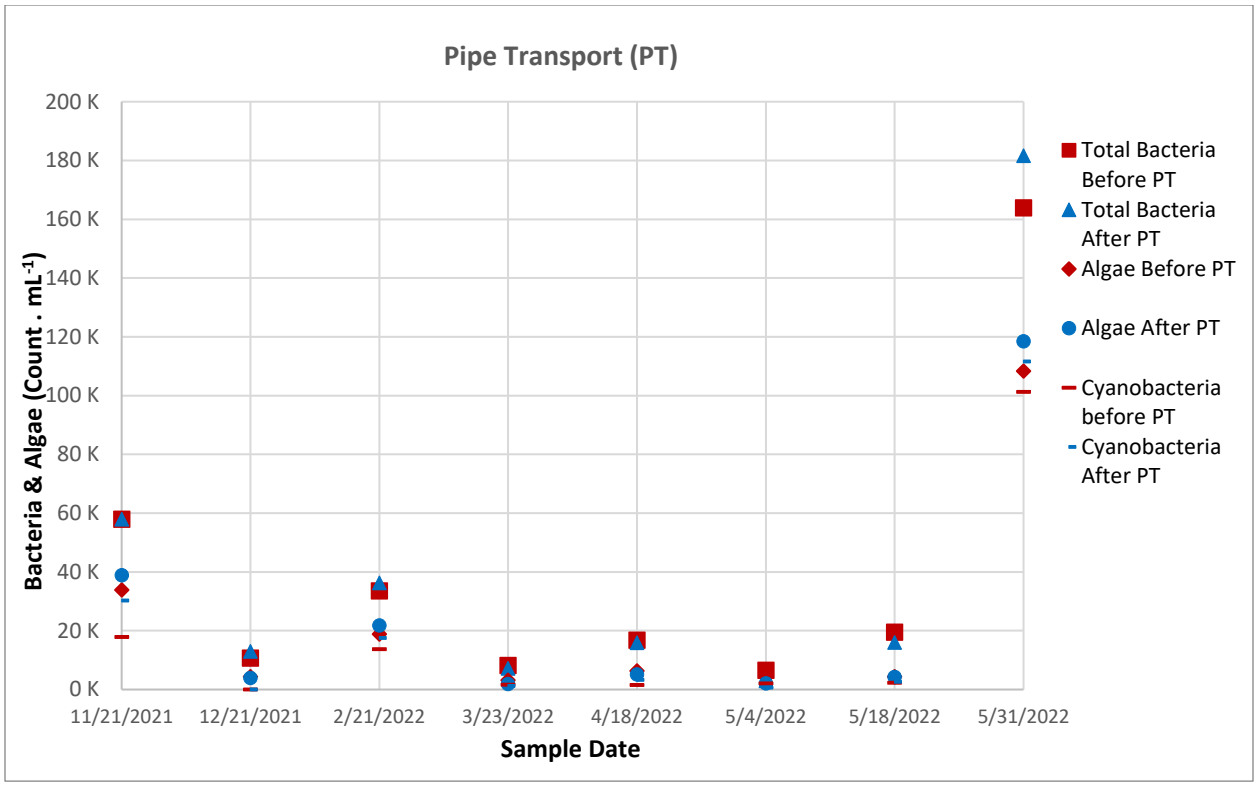

Figure C- 5. Temporal concentrations of total bacteria, algae, and cyanobacteria in the pipeline between stations 7 and 8.

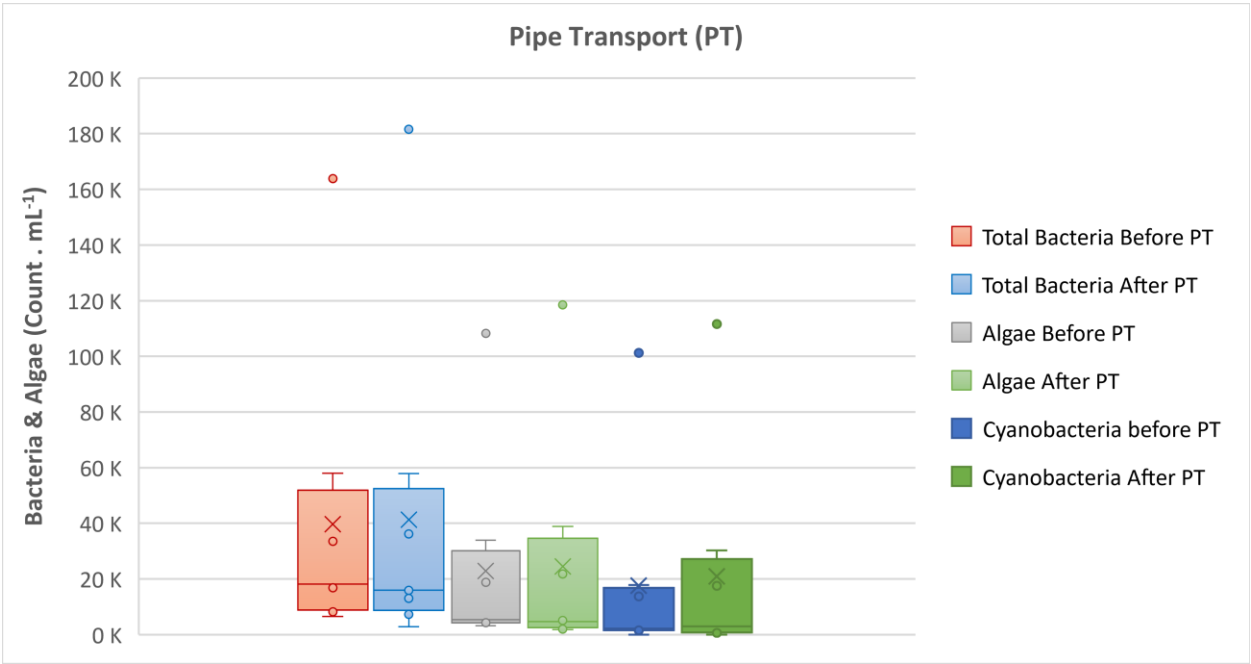

Figure C- 6. Box plots of total bacteria, algae, and cyanobacteria concentrations in the pipeline between stations 7 and 8.

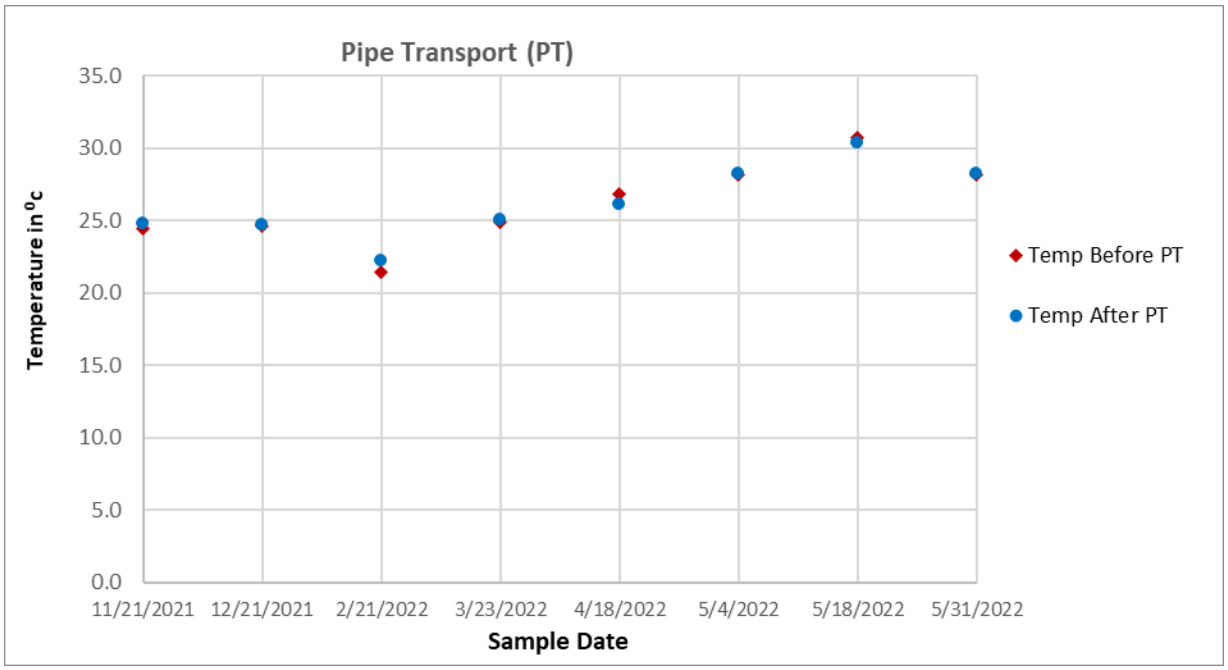

Figure C- 7. Temporal changes in temperature in the pipeline between stations 7 and 8.

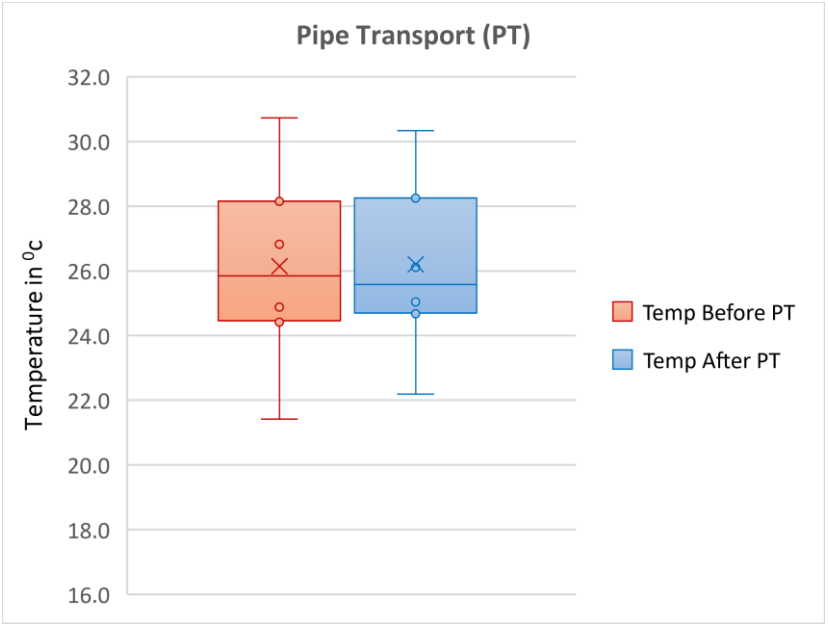

Figure C- 8. Box plot of temperature changes in the pipeline between stations 7 and 8.

Appendix D. A two-sample t-test to compare raw water quality parameters in TTA and TTB/C

Table D- 1. t-Test results for raw water turbidity in TTA and TTB/C

	Turbidity Raw Water TTA	*Turbidity Raw Water TTB&C*
Mean	15.9325	14.5225
Variance	39.2145	49.6637
Observations	8	8
Pooled Variance	44.4391	
Hypothesized Mean Difference	0.0000	
df	14.0000	
t Stat	0.4230	
P(T<=t) one-tail	0.3394	
t Critical one-tail	1.7613	
P(T<=t) two-tail	0.6787	
t Critical two-tail	2.1448	

Table D- 2. t-Test results for raw water TN in TTA and TTB/C

	TN Raw Water TTA	*TN Raw Water TTB&C*
Mean	2.0725	1.9800
Variance	0.9811	0.4254
Observations	8	8
Pooled Variance	0.7032	
Hypothesized Mean Difference	0.0000	
df	14.0000	
t Stat	0.2206	
P(T<=t) one-tail	0.4143	
t Critical one-tail	1.7613	
P(T<=t) two-tail	0.8286	
t Critical two-tail	2.1448	

Table D- 3. t-Test results for raw water NO2 in TTA and TTB/C

	NO2 Raw Water TTA	*NO2 Raw Water TTB&C*
Mean	0.0057	0.0053
Variance	0.0000	0.0001
Observations	8	8
Pooled Variance	0.0000	
Hypothesized Mean Difference	0.0000	
df	14.0000	
t Stat	0.1012	
P(T<=t) one-tail	0.4604	
t Critical one-tail	1.7613	
P(T<=t) two-tail	0.9208	
t Critical two-tail	2.1448	

Table D- 4. t-Test results for raw water NO3 in TTA and TTB/C

	NO3 Raw Water TTA	*NO3 Raw Water TTB&C*
Mean	0.2020	0.1896
Variance	0.0135	0.0130
Observations	8	8
Pooled Variance	0.0133	
Hypothesized Mean Difference	0.0000	
df	14.0000	
t Stat	0.2151	
P(T<=t) one-tail	0.4164	
t Critical one-tail	1.7613	
P(T<=t) two-tail	0.8328	
t Critical two-tail	2.1448	

Table D- 5. t-Test results for raw water NH3 in TTA and TTB/C

	NH3 Raw Water TTA	*NH3 Raw Water TTB&C*
Mean	0.1079	0.0386
Variance	0.0426	0.0002
Observations	8	8
Pooled Variance	0.0214	
Hypothesized Mean Difference	0.0000	
df	14.0000	
t Stat	0.9464	
P(T<=t) one-tail	0.1800	
t Critical one-tail	1.7613	
P(T<=t) two-tail	0.3600	
t Critical two-tail	2.1448	

Table D- 6. t-Test results for raw water OrgN in TTA and TTB/C

	OrgN Raw Water TTA	*OrgN Raw Water TTB&C*
Mean	1.6254	1.6713
Variance	0.9768	0.2589
Observations	8	8
Pooled Variance	0.6179	
Hypothesized Mean Difference	0.0000	
df	14.0000	
t Stat	-0.1167	
P(T<=t) one-tail	0.4544	
t Critical one-tail	1.7613	
P(T<=t) two-tail	0.9087	
t Critical two-tail	2.1448	

Table D- 7. t-Test results for raw water TP in TTA and TTB/C

	TP Raw Water TTA	*TP Raw Water TTB&C*
Mean	0.1796	0.0596
Variance	0.0247	0.0018
Observations	8	8
Pooled Variance	0.0132	
Hypothesized Mean Difference	0.0000	
df	14.0000	
t Stat	2.0867	
P(T<=t) one-tail	0.0278	
t Critical one-tail	1.7613	
P(T<=t) two-tail	0.0557	
t Critical two-tail	2.1448	

Table D- 8. t-Test results for raw water PO4 in TTA and TTB/C

	PO4 Raw Water TTA	*PO4 Raw Water TTB&C*
Mean	0.0315	0.0433
Variance	0.0003	0.0014
Observations	8	8
Pooled Variance	0.0008	
Hypothesized Mean Difference	0.0000	
df	14.0000	
t Stat	-0.8284	
P(T<=t) one-tail	0.2107	
t Critical one-tail	1.7613	
P(T<=t) two-tail	0.4214	
t Critical two-tail	2.1448	

Table D- 9. t-Test results for raw water Chl A (filed) in TTA and TTB/C

	Chl A (Field) Raw Water	*Chl A (Field) Raw Water*
Mean	1.6167	1.5602
Variance	0.9751	0.7204
Observations	8	8
Pooled Variance	0.8477	
Hypothesized Mean Difference	0	
df	14	
t Stat	0.1228	
P(T<=t) one-tail	0.4520	
t Critical one-tail	1.7613	
P(T<=t) two-tail	0.9040	
t Critical two-tail	2.1448	

Table D- 10. t-Test results for raw water Chl A (lab) in TTA and TTB/C

	Chl A (Lab) Raw Water TTA	*PO4 Raw Water TTB&C*
Mean	19.7363	0.0433
Variance	94.0810	0.0014
Observations	8	8
Pooled Variance	47.0412	
Hypothesized Mean Difference	0	
df	14	
t Stat	5.7425	
P(T<=t) one-tail	0.0000	
t Critical one-tail	1.7613	
P(T<=t) two-tail	0.0001	
t Critical two-tail	2.1448	

Table D- 11. t-Test results for raw water total bacterial in TTA and TTB/C

	Total Bacteria Raw Water TTA	*Total Bacteria Raw Water TTB&C*
Mean	169860.0000	224177.5000
Variance	16131125371.4286	40287492850.0000
Observations	8	8
Pooled Variance	28209309110.7143	
Hypothesized Mean Difference	0	
df	14	
t Stat	-0.6468	
P(T<=t) one-tail	0.2641	
t Critical one-tail	1.7613	
P(T<=t) two-tail	0.5282	
t Critical two-tail	2.1448	

Table D- 12. t-Test results for raw water algae in TTA and TTB/C

	Algae Raw Water TTA	*Algae Raw Water TTB&C*
Mean	133857.5000	170365.0000
Variance	10004609078.5714	27400380428.5714
Observations	8	8
Pooled Variance	18702494753.5714	
Hypothesized Mean Difference	0.0000	
df	14.0000	
t Stat	-0.5339	
P(T<=t) one-tail	0.3009	
t Critical one-tail	1.7613	
P(T<=t) two-tail	0.6018	
t Critical two-tail	2.1448	

Table D- 13. t-Test results for raw water cyanobacteria in TTA and TTB/C

	Cyanobacteria Raw Water TTA	*Cyanobacteria Raw Water TTB&C*
Mean	92065.0000	117450.0000
Variance	10427764085.7143	18493823314.2857
Observations	8	8
Pooled Variance	14460793700.0000	
Hypothesized Mean Difference	0.0000	
df	14.0000	
t Stat	-0.4222	
P(T<=t) one-tail	0.3396	
t Critical one-tail	1.7613	
P(T<=t) two-tail	0.6793	
t Critical two-tail	2.1448	

Appendix E. A two-sample t-test to compare water quality improvements in TTA

Table E- 1. t-Test results for turbidity in TTA

	Turbidity Raw Water	*Turbidity After TG*
Mean	15.933	7.96
Variance	39.214	34.473
Observations	8	8
Pooled Variance	36.844	
Hypothesized Mean Difference	0	
df	14	
t Stat	2.627	
P(T<=t) one-tail	0.010	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.020	
t Critical two-tail	2.145	

Table E- 2. t-Test results for TN in TTA

	TN Raw Water	*TN After TG*
Mean	2.073	1.235
Variance	0.981	0.097
Observations	8	8
Pooled Variance	0.539	
Hypothesized Mean Difference	0	
df	14	
t Stat	2.281	
P(T<=t) one-tail	0.019	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.039	
t Critical two-tail	2.145	

Table E- 3. t-Test results for NO2 in TTA

	NO2 Raw Water	*NO2 After TG*
Mean	0.006	0.00125
Variance	0.000	0.000
Observations	8	8
Pooled Variance	0.000	
Hypothesized Mean Difference	0	
df	14	
t Stat	1.755	
P(T<=t) one-tail	0.051	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.101	
t Critical two-tail	2.145	

Table E- 4. t-Test results for NO3 in TTA

	NO3 Raw Water	*NO3 After TG*
Mean	0.202	0.022875
Variance	0.014	0.001
Observations	8	8
Pooled Variance	0.007	
Hypothesized Mean Difference	0	
df	14	
t Stat	4.166	
P(T<=t) one-tail	<0.001	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.001	
t Critical two-tail	2.145	

Table E- 5. t-Test results for NH3 in TTA

	NH3 Raw Water	*NH3 After TG*
Mean	0.108	0.020625
Variance	0.043	0.000
Observations	8	8
Pooled Variance	0.021	
Hypothesized Mean Difference	0	
df	14	
t Stat	1.194	
P(T<=t) one-tail	0.126	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.252	
t Critical two-tail	2.145	

Table E- 6. t-Test results for OrgN in TTA

	OrgN Raw Water	*OrgN After TG*
Mean	1.625	1.20225
Variance	0.977	0.098
Observations	8	8
Pooled Variance	0.537	
Hypothesized Mean Difference	0	
df	14	
t Stat	1.154	
P(T<=t) one-tail	0.134	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.268	
t Critical two-tail	2.145	

Table E- 7. t-Test results for TP in TTA

	TP Raw Water	*TP After TG*
Mean	0.180	0.0540875
Variance	0.025	0.004
Observations	8	8
Pooled Variance	0.014	
Hypothesized Mean Difference	0	
df	14	
t Stat	2.090	
P(T<=t) one-tail	0.028	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.055	
t Critical two-tail	2.145	

Table E- 8. t-Test results for PO4 in TTA

	PO4 Raw Water	*PO4 After TG*
Mean	0.032	0.011125
Variance	0.000	0.000
Observations	8	8
Pooled Variance	0.000	
Hypothesized Mean Difference	0	
df	14	
t Stat	2.664	
P(T<=t) one-tail	0.009	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.019	
t Critical two-tail	2.145	

Table E- 9. t-Test results for Chl A (filed) in TTA

	Chl A (Field) Raw Water	*Chl A (Field) After TG*
Mean	1.617	0.549543504
Variance	0.975	0.277
Observations	8	8
Pooled Variance	0.626	
Hypothesized Mean Difference	0	
df	14	
t Stat	2.697	
P(T<=t) one-tail	0.009	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.017	

Table E- 10. t-Test results for Chl A (lab) in TTA

	Chl A (Lab) Raw Water	*Chl A (Lab) After TG*
Mean	23.676	6.4025
Variance	346.701	67.627
Observations	8	8
Pooled Variance	207.164	
Hypothesized Mean Difference	0	
df	14	
t Stat	2.400	
P(T<=t) one-tail	0.015	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.031	
t Critical two-tail	2.145	

Table E- 11. t-Test results for total bacterial in TTA

	Total Bacteria Raw Water	*Total Bacteria After TG*
Mean	169860	121957
Variance	16131125371	25737836590
Observations	8	7
Pooled Variance	20564992088	
Hypothesized Mean Difference	0	
df	13	
t Stat	0.645	
P(T<=t) one-tail	0.265	
t Critical one-tail	1.771	
P(T<=t) two-tail	0.530	
t Critical two-tail	2.160	

Table E- 12. t-Test results for algae in TTA

	Algae Raw Water	*Algae After TG*
Mean	133858	73083
Variance	10004609079	13830257924
Observations	8	7
Pooled Variance	11770293161	
Hypothesized Mean Difference	0	
df	13	
t Stat	1.082	
P(T<=t) one-tail	0.149	
t Critical one-tail	1.771	
P(T<=t) two-tail	0.299	
t Critical two-tail	2.160	

Table E- 13. t-Test results for cyanobacteria in TTA

	Cyanobacteria Raw Water	*Cyanobacteria After TG*
Mean	92065	46854
Variance	10427764086	4931275562
Observations	8	7
Pooled Variance	7890923229	
Hypothesized Mean Difference	0	
df	13	
t Stat	0.983	
P(T<=t) one-tail	0.172	
t Critical one-tail	1.771	
P(T<=t) two-tail	0.343	
t Critical two-tail	2.160	

Appendix F. A two-sample t-test to compare water quality improvements in TTA

Table F- 1. t-Test results for turbidity in TTB

	Turbidity Raw Water	*Turbidity After TG*
Mean	14.523	3.9125
Variance	49.664	14.723
Observations	8	8
Pooled Variance	32.193	
Hypothesized Mean Difference	0	
df	14	
t Stat	3.740	
P(T<=t) one-tail	0.001	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.002	
t Critical two-tail	2.145	

Table F- 2. t-Test results for TN in TTB

	TN Raw Water	*TN After TG*
Mean	1.980	1.42125
Variance	0.425	0.368
Observations	8	8
Pooled Variance	0.397	
Hypothesized Mean Difference	0	
df	14	
t Stat	1.775	
P(T<=t) one-tail	0.049	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.098	
t Critical two-tail	2.145	

Table F- 3. t-Test results for NO2 in TTB

	NO2 Raw Water	*NO2 After TG*
Mean	0.005	0.001925
Variance	0.000	0.000
Observations	8	8
Pooled Variance	0.000	
Hypothesized Mean Difference	0	
df	14	
t Stat	1.031	
P(T<=t) one-tail	0.160	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.320	
t Critical two-tail	2.145	

Table F- 4. t-Test results for NO3 in TTB

	NO3 Raw Water	*NO3 After TG*
Mean	0.190	0.005575
Variance	0.013	0.000
Observations	8	8
Pooled Variance	0.007	
Hypothesized Mean Difference	0	
df	14	
t Stat	4.529	
P(T<=t) one-tail	<0.001	
t Critical one-tail	1.761	
P(T<=t) two-tail	<0.001	
t Critical two-tail	2.145	

Table F- 5. t-Test results for NH3 in TTB

	NH3 Raw Water	*NH3 After TG*
Mean	0.039	0.040125
Variance	0.000	0.005
Observations	8	8
Pooled Variance	0.003	
Hypothesized Mean Difference	0	
df	14	
t Stat	-0.059	
P(T<=t) one-tail	0.477	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.954	
t Critical two-tail	2.145	

Table F- 6. t-Test results for OrgN in TTB

	OrgN Raw Water	*OrgN After TG*
Mean	1.671	1.306875
Variance	0.259	0.166
Observations	8	8
Pooled Variance	0.212	
Hypothesized Mean Difference	0	
df	14	
t Stat	1.581	
P(T<=t) one-tail	0.068	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.136	
t Critical two-tail	2.145	

Table F- 7. t-Test results for TP in TTB

	TP Raw Water	*TP After TG*
Mean	0.060	0.013625
Variance	0.002	0.001
Observations	8	8
Pooled Variance	0.001	
Hypothesized Mean Difference	0	
df	14	
t Stat	2.691	
P(T<=t) one-tail	0.009	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.018	
t Critical two-tail	2.145	

Table F- 8. t-Test results for PO4 in TTB

	PO4 Raw Water	*PO4 After TG*
Mean	0.043	0.009142857
Variance	0.001	0.000
Observations	8	7
Pooled Variance	0.001	
Hypothesized Mean Difference	0	
df	13	
t Stat	2.289	
P(T<=t) one-tail	0.020	
t Critical one-tail	1.771	
P(T<=t) two-tail	0.039	
t Critical two-tail	2.160	

Table F- 9. t-Test results for Chl A (filed) in TTB

	Chl A (Field) After TG	*PO4 After TG*
Mean	0.134	0.009142857
Variance	0.029	0.000
Observations	8	7
Pooled Variance	0.016	
Hypothesized Mean Difference	0	
df	13	
t Stat	1.937	
P(T<=t) one-tail	0.037	
t Critical one-tail	1.771	
P(T<=t) two-tail	0.075	
t Critical two-tail	2.160	

Table F- 10.t-Test results for Chl A (lab) in TTB

	Chl A (Lab) Raw Water	*Chl A (Lab) After TG*
Mean	19.736	1.89625
Variance	94.081	3.199
Observations	8	8
Pooled Variance	48.640	
Hypothesized Mean Difference	0	
df	14	
t Stat	5.116	
P(T<=t) one-tail	<0.001	
t Critical one-tail	1.761	
P(T<=t) two-tail	<0.001	
t Critical two-tail	2.145	

Table F- 11. t-Test results for total bacterial in TTB

	Total Bacteria Raw Water	*Total Bacteria After TG*
Mean	224178	41248
Variance	40287492850	3383321136
Observations	8	8
Pooled Variance	21835406993	
Hypothesized Mean Difference	0	
df	14	
t Stat	2.476	
P(T<=t) one-tail	0.013	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.027	
t Critical two-tail	2.145	

Table F- 12. t-Test results for algae in TTB

	Algae Raw Water	*Algae After TG*
Mean	170365	33660
Variance	27400380429	3136962743
Observations	8	8
Pooled Variance	15268671586	
Hypothesized Mean Difference	0	
df	14	
t Stat	2.213	
P(T<=t) one-tail	0.022	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.044	
t Critical two-tail	2.145	

Table F- 13. t-Test results for cyanobacteria in TTB

	Cyanobacteria Raw Water	*Cyanobacteria After TG*
Mean	117450	19550
Variance	18493823314	935262943
Observations	8	8
Pooled Variance	9714543129	
Hypothesized Mean Difference	0	
df	14	
t Stat	1.987	
P(T<=t) one-tail	0.033	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.067	
t Critical two-tail	2.145	

Appendix G. A two-sample t-test to compare water quality improvements in TTC

Table G- 1. t-Test results for turbidity in TTC

	Turbidity Raw Water	*Turbidity After TG*
Mean	14.523	14.695
Variance	49.664	242.931
Observations	8	8
Pooled Variance	146.297	
Hypothesized Mean Difference	0	
df	14	
t Stat	-0.029	
P(T<=t) one-tail	0.489	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.978	
t Critical two-tail	2.145	

Table G- 2. t-Test results for TN in TTC

	TN Raw Water	*TN After TG*
Mean	1.980	1.14125
Variance	0.425	0.080
Observations	8	8
Pooled Variance	0.253	
Hypothesized Mean Difference	0	
df	14	
t Stat	3.338	
P(T<=t) one-tail	0.002	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.005	
t Critical two-tail	2.145	

Table G- 3. t-Test results for NO2 in TTC

	NO2 Raw Water	*NO2 After TG*
Mean	0.005	0.0035625
Variance	0.000	0.000
Observations	8	8
Pooled Variance	0.000	
Hypothesized Mean Difference	0	
df	14	
t Stat	0.491	
P(T<=t) one-tail	0.316	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.631	
t Critical two-tail	2.145	

Table G- 4. t-Test results for NO3 in TTC

	NO3 Raw Water	*NO3 After TG*
Mean	0.190	0.0301875
Variance	0.013	0.004
Observations	8	8
Pooled Variance	0.009	
Hypothesized Mean Difference	0	
df	14	
t Stat	3.412	
P(T<=t) one-tail	0.002	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.004	
t Critical two-tail	2.145	

Table G- 5. t-Test results for NH3 in TTC

	NH3 Raw Water	*NH3 After TG*
Mean	0.039	0.034625
Variance	0.000	0.003
Observations	8	8
Pooled Variance	0.002	
Hypothesized Mean Difference	0	
df	14	
t Stat	0.206	
P(T<=t) one-tail	0.420	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.840	
t Critical two-tail	2.145	

Table G- 6. t-Test results for OrgN in TTC

	OrgN Raw Water	*OrgN After TG*
Mean	1.671	1.072625
Variance	0.259	0.088
Observations	8	8
Pooled Variance	0.173	
Hypothesized Mean Difference	0	
df	14	
t Stat	2.876	
P(T<=t) one-tail	0.006	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.012	
t Critical two-tail	2.145	

Table G- 7. t-Test results for TP in TTC

	TP Raw Water	*TP After TG*
Mean	0.060	0.030375
Variance	0.002	0.001
Observations	8	8
Pooled Variance	0.002	
Hypothesized Mean Difference	0	
df	14	
t Stat	1.447	
P(T<=t) one-tail	0.085	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.170	
t Critical two-tail	2.144786688	

Table G- 8. t-Test results for PO4 in TTC

	PO4 Raw Water	*PO4 After TG*
Mean	0.043	0.680625
Variance	0.001	3.545
Observations	8	8
Pooled Variance	1.773	
Hypothesized Mean Difference	0	
df	14	
t Stat	-0.957	
P(T<=t) one-tail	0.177	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.355	
t Critical two-tail	2.145	

Table G- 9. t-Test results for Chl A (filed) in TTC

	Chl A (Field) Raw Water	*Chl A (Field) After TG*
Mean	1.560	0.5292694
Variance	0.720	0.110
Observations	8	8
Pooled Variance	0.415	
Hypothesized Mean Difference	0	
df	14	
t Stat	3.200	
P(T<=t) one-tail	0.003	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.006	
t Critical two-tail	2.145	

Table G- 10. t-Test results for Chl A (lab) in TTC

	Chl A (Lab) Raw Water	*Chl A (Lab) After TG*
Mean	19.736	2.42125
Variance	94.081	6.106
Observations	8	8
Pooled Variance	50.093	
Hypothesized Mean Difference	0	
df	14	
t Stat	4.893	
P(T<=t) one-tail	<0.001	
t Critical one-tail	1.761	
P(T<=t) two-tail	<0.001	
t Critical two-tail	2.145	

Table G- 11. t-Test results for total bacterial in TTC

	Total Bacteria Raw Water	*Total Bacteria After TG*
Mean	224178	30600
Variance	40287492850	1398508571
Observations	8	8
Pooled Variance	20843000711	
Hypothesized Mean Difference	0	
df	14	
t Stat	2.682	
P(T<=t) one-tail	0.009	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.018	
t Critical two-tail	2.145	

Table G- 12. t-Test results for algae in TTC

	Algae Raw Water	*Algae After TG*
Mean	170365	26180
Variance	27400380429	1313061714
Observations	8	8
Pooled Variance	14356721071	
Hypothesized Mean Difference	0	
df	14	
t Stat	2.407	
P(T<=t) one-tail	0.015	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.030	
t Critical two-tail	2.145	

Table G- 13. t-Test results for cyanobacteria in TTC

	Cyanobacteria Raw Water	*Cyanobacteria After TG*
Mean	117450	12575
Variance	18493823314	308683171
Observations	8	8
Pooled Variance	9401253243	
Hypothesized Mean Difference	0	
df	14	
t Stat	2.163	
P(T<=t) one-tail	0.024	
t Critical one-tail	1.761	
P(T<=t) two-tail	0.048	
t Critical two-tail	2.145	

Appendix H. A one-way ANOVA result for the treatment trains (TTA, TTA and TTC)

Table H- 1. Anova results comparing turbidity between the treatment trains

Groups	*Count*	*Sum*	*Average*	*Variance*
Turbidity TTA	8	63.780	7.973	31.857
Turbidity TTB	8	84.880	10.610	71.104
Turbidity TTC	8	-1.380	-0.173	294.447

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	505.493	2	252.746	1.908	0.173	3.467
Within Groups	2781.854	21	132.469			
Total	3287.347	23				

Table H- 2. Anova results comparing TN between the treatment trains

Groups	*Count*	*Sum*	*Average*	*Variance*
TN TTA	8	6.700	0.838	0.654
TN TTB	8	4.470	0.559	0.211
TN TTC	8	6.710	0.839	0.261

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.416	2	0.208	0.555	0.582	3.467

Within Groups	7.878	21	0.375			
Total	8.294	23				

Table H- 3. Anova results comparing NO2 between the treatment trains

Groups	*Count*	*Sum*	*Average*	*Variance*		
NO2 TTA	8	0.035	0.004	0.000		
NO2 TTB	8	0.027	0.003	0.000		
NO2 TTC	8	0.014	0.002	0.000		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.000	2	1E-05	0.179	0.837	3.467
Within Groups	0.002	21	8E-05			
Total	0.002	23				

Table H- 4. Anova results comparing NO3 between the treatment trains

Groups	*Count*	*Sum*	*Average*	*Variance*		
NO3 TTA	8	1.433	0.179	0.013		
NO3 TTB	8	1.472	0.184	0.012		
NO3 TTC	8	1.275	0.159	0.019		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.003	2	0.001	0.092	0.913	3.467
Within Groups	0.311	21	0.015			
Total	0.314	23				

Table H- 5. Anova results comparing NH3 between the treatment trains

Groups	*Count*	*Sum*	*Average*	*Variance*		
NH3 TTA	8	0.698	0.087	0.041		
NH3 TTB	8	-0.012	-0.002	0.005		
NH3 TTC	8	0.032	0.004	0.003		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.03957	2	0.020	1.219	0.316	3.467
Within Groups	0.34089	21	0.016			
Total	0.38046	23				

Table H- 6. Anova results comparing OrgN between the treatment trains

Groups	*Count*	*Sum*	*Average*	*Variance*
OrgN TTA	8	3.385	0.423	0.707
OrgN TTB	8	2.915	0.364	0.202
OrgN TTC	8	4.789	0.599	0.180

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.238	2	0.119	0.328	0.724	3.467
Within Groups	7.615	21	0.363			
Total	7.853	23				

Table H- 7. Anova results comparing TP between the treatment trains

Groups	*Count*	*Sum*	*Average*	*Variance*
TP TTA	8	1.004	0.126	0.021
TP TTB	8	0.368	0.046	0.001
TP TTC	8	0.234	0.029	0.003

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.042	2	0.021	2.598	0.098	3.467
Within Groups	0.171	21	0.008			
Total	0.213	23				

Table H- 8. Anova results comparing PO4 between the treatment trains

Groups	*Count*	*Sum*	*Average*	*Variance*
PO4 TTA	8	0.163	0.020	0.000
PO4 TTB	8	0.282	0.035	0.002
PO4 TTC	8	-5.099	-0.637	3.591

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.361	2	1.180	0.986	0.390	3.467
Within Groups	25.151	21	1.198			
Total	27.512	23				

Table H- 9. Anova results comparing Chl A (field) between the treatment trains

Groups (Filed)	*Count*	*Sum*	*Average*	*Variance*
Chl A TTA	8	8.537	1.067	1.821
Chl A TTB	8	11.410	1.426	0.653
Chl A TTC	8	8.247	1.031	0.633

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.764	2	0.382	0.369	0.696	3.467
Within Groups	21.742	21	1.035			
Total	22.506	23				

Table H- 10. Anova results comparing Chl A (lab) between the treatment trains

Groups (Lab)	*Count*	*Sum*	*Average*	*Variance*
Chl A TTA	8	138.190	17.274	197.107
Chl A TTB	8	142.720	17.840	91.434
Chl A TTC	8	138.520	17.315	90.724

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.595	2	0.797	0.006	0.994	3.467
Within Groups	2655	21	126.422			
Total	2656	23				

Table H- 11. Anova results comparing total bacteria between the treatment trains

Groups	*Count*	*Sum*	*Average*	*Variance*
Total Bacteria TTA	7	428080	61154.3	1.4E+10
Total Bacteria TTB	8	1E+06	182930	2.2E+10
Total Bacteria TTC	8	2E+06	193578	2.7E+10

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7.9E+10	2	4E+10	1.835	0.185	3.493
Within Groups	4.3E+11	20	2.2E+10			
Total	5.1E+11	22				

Table H- 12. Anova results comparing algae between the treatment trains

Groups	*Count*	*Sum*	*Average*	*Variance*
Algae TTA	7	496180	70882.9	6.5E+09
Algae TTB	8	1E+06	136705	1.4E+10
Algae TTC	8	1E+06	144185	1.8E+10

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.4E+10	2	1.2E+10	0.913	0.418	3.493
Within Groups	2.6E+11	20	1.3E+10			
Total	2.8E+11	22				

Table H- 13. Anova results comparing cyanobacteria between the treatment trains

Groups	*Count*	*Sum*	*Average*	*Variance*
Cyanobacteria TTA	7	387780	55397.1	6.3E+09
Cyanobacteria TTB	8	783200	97900	1.2E+10
Cyanobacteria TTC	8	839000	104875	1.4E+10

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1E+10	2	5.2E+09	0.471	0.631	3.493
Within Groups	2.2E+11	20	1.1E+10			
Total	2.3E+11	22				

Appendix B

INV10 Water Quality Master Table – Final