Investigation of covers for suppression of cyanobacteria in water treatment settling basins.

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ABSTRACT

During the summer months, there is concern about algae and Cyanobacteria growth within water treatment facilities. Algae growth in settling basins can lead to toxins produced by certain Cyanobacteria as well as off-flavors due to geosmin and MIB production. Algal biomass also contributes to suspended solids which can lead to more rapid filter fouling. While water treatment plants have a variety of options for managing algae populations, we have investigated the use of Xton Inc. polypropylene covers to prevent algae and Cyanobacterial production within simulated settling basins. It was determined that the Xton Inc. covers prevented an algal bloom in mesocosms, likely due to a combination of blocking light and preventing insects from entering the mesocosms. It was found that insect populations led to increased levels of phosphate in the water, the key limiting nutrient for algae growth in freshwater systems.

INTRODUCTION

Algae growth in water treatment facilities is a significant challenge faced by water utilities. This is a problem because algae growth in settling basins can lead to a) potential toxin production by Cyanobacteria, specifically *Microcystis aerigunosa* (Bláha et. al, 2009) and b) off-flavors due to geosmin and MIB production (Mallevialle and Suffet, 1987). Harmful algal blooms due to toxic Cyanobacteria have become an increasingly global concern due to the threat posed to human health and economic damages (Graham et. al, 2016). This is therefore an area of intense public interest resulting in a unique market opportunity for companies developing strategies to control algal blooms. While methods for controlling algal blooms in source water has received the most attention to date, there are also concerns among utilities about algal growth within the treatment facility itself.

Over the years, water treatment facilities have implemented different methods to help control algae growth within their systems. Some of these methods include chemical treatments, aeration, mixing, and ultrasonic sound waves. The most common method used among treatment facilities is chemical intervention by treating the water with a variety of additives (i.e. – algaecides) which have been proven to be effective with quick results. However, chemical additives can be expensive, impact the environment, and require frequent dosing for long term effect (Jančula & Maršálek, 2011). Consequently, facility managers are searching for new and less expensive methods to control algal populations.

North Columbus Water Resource Facility (NCWRF) of Columbus, Georgia initially installed Xton Inc. polypropylene covers to minimize algal growth. Algae growth within the system led to frequent filter fouling. There was also concern about growth of toxic Cyanobacteria within the settling basins. Prior to installing Xton Inc. covers, algae populations in North Columbus were controlled solely with chlorine dioxide. Currently, chlorine dioxide, covers, and a chemical additive called EarthTec are used for algae control. EarthTec, a proprietary formula containing copper sulfate, was first used starting in the middle of summer, 2017. It is advertised to kill algae and also destroy dissolved organic matter (DOM). Plant operators believe the covers purchased from Xton Inc. are effective in preventing algae growth. However, no formal study has been conducted to verify these benefits.

Given that water treatment plants have a variety of options at their disposal for managing algae populations, the objective of this study was to conduct a comparison test among the options currently employed at the North Columbus Water Treatment Plant for suppression of algae growth. We hypothesized that covers would reduce algal growth in settling basins primarily by blocking light.

MATERIALS AND METHODS

Experimental

A mesocosm study was performed at North Columbus Water Resource Facility in which Xton Inc. covers and EarthTec were tested for their effectiveness in preventing algae growth. Chlorine dioxide was not tested given that its anti-algal properties are already well-established. Mesocosms were set up in an effort to simulate conditions in settling basins. This experiment consisted of triplicate mesocosms for controls (no treatment), EarthTec treated water, a single layer cover, and a double layer cover (Fig. 1). Each week, mesocosms were sampled and partially flushed with fresh water from Lake Oliver (the treatment plant's supply). This fresh water was treated with alum in the same concentrations used by the water treatment plant, creating a small, parallel version of their settling basins. Likewise, the EarthTec dose matched that of the treatment plant at 19 mg/L. Each week, two samples were collected from each mesocosm. The first sample (80 ml) was used for measurement of optical density and chlorophyll *a*. The second sample (40 ml) was used for DNA extraction. Samples were centrifuged in 50 ml tubes at 5000 rpm for 5 minutes to form pellets of cell material for subsequent analyses. The supernatant was also retained for soluble nutrient analyses by ion chromatography.

Figure 1. Photo of experimental setup on site of North Columbus Water Resource Facility.

Analysis

Chlorophyll *a*, optical density, and nutrient data were collected weekly from the mesocosms. Samples with high Chl a readings had their DNA extracted and analyzed by quantitative PCR in order to detect the presence of Cyanobacterial genes and a gene associated with the production of microcystin toxin (*mycD*). Anion chromatography was performed on samples to analyze nutrient levels.

Chlorophyll a extraction and optical density measurements

Light absorbance of the water samples were measured by a spectrophotometer at optical density (OD) 550 and 680 nm as a proxy for suspended matter (e.g. cells) in the water. High ratios of OD 680 to 550 are also indicative of high chlorophyll levels in the water (Wang et al. 2018). In addition, chlorophyll *a* was extracted and quantified based on the Standard Method 10200 (AWWA 1999). 1.5 ml of 90:10 acetone/magnesium carbonate solution was added to the cell pellets for chlorophyll extraction. Subsequently, 0.5 ml of 0.5 mm zirconia silica beads were added to the suspension and homogenization was carried out for 20 seconds at 6 m/s. The disrupted suspensions were immediately transferred to 15 ml tubes on ice and the total suspension volume was brought to 5 ml through the addition of 90:10 acetone/magnesium carbonate solution. The tubes were incubated at 4 °C in the dark for a minimum of 2 hours to further extract pigments. The disrupted suspension was then centrifuged at 2800 rcf for 5 minutes and the supernatant was transferred to a 1-cm quartz cuvette. Absorbance was measured in a spectrophotometer at OD 750 nm and 664 nm. Dilution with acetone was performed if the readings at OD 664 nm were above 1.0. The extract in the cuvette was acidified using 0.1M HCl to convert chlorophyll a to pheophytin a and the absorbance was measured at OD 750 nm and 665 nm. The readings at 750 nm were subtracted from the OD 664 and 665 readings to correct for background. The corrected values were used to calculate chlorophyll *a* and pheophytin *a* concentrations as follows (AWWA 1999):

$$Chlorophyll\ a, \left(\frac{ug}{L}\right) = \frac{26.7\ (OD664 - OD665)V_1}{V_2 L}$$

$$Pheophytin\ a, \left(\frac{ug}{L}\right) = \frac{26.7\ [1.7(OD665) - OD664]V_1}{V_2 L}$$

Where:

V_1 = volume of acetone extract, L.
V_2 = volume of water sample, L.
L = light path of the cuvette, cm.
OD664, OD665 = optical densities of 90% acetone extract before and after acidification, respectively.

Anion chromatography

Anions were measured by using a conductivity detector on a Shimadzu Prominence High Pressure Liquid Chromatography instrument. Collected water samples were filtered through a 0.2 µm filter into a HPLC vial and 20 µm of sample was injected into the column. Anions were separated on a Dionex AS22 column (4x250 mm) coupled to an AS500 self-regenerating

suppressor supplied with 26 mA. The column flow rate was 1 ml/min of 1.5 mM sodium bicarbonate and 4.5 mM sodium carbonate in NanoPure water. The column oven was set to 28 ºC. LC Solutions software (Shimadzu) was used to perform peak integration.

Growth of Microcystis 2667 for qPCR positive control

Microcystis 2667 was used as a positive control for Cyanobacterial 16S and *mycD* genes in the qPCR assay. Bold 3N medium was used to grow *Microcystis aeruginosa* (UTEX 2667). Triplicate 500 ml culture bottles with 300 ml Bold 3N medium were inoculated with 5 ml of concentrated UTEX 2667. Stir bars were used to mix cultures at 300 rpm and air was supplied at 150 ml/min. Light was supplied by T5 fluorescent bulbs at 10,000 lux operating on a 14:10 light/dark cycle. Optical density at 550 and 680 nm was measured every three days using a spectrophotometer. Dry weight was determined at the end of the culture period (7 days) by filtration and gravimetric measurement. Culture samples (1 ml) were obtained for DNA extraction.

DNA extraction

Cell pellets were obtained from mesocosm water samples for DNA extraction. The cell pellet was resuspended in 1 ml dH_2O and 200 ul of this suspension was used for DNA extraction using the FastDNA Spin Kit (MP Biomedicals) per the manufacturer's instructions. In all cases, the kit's CLS-Y buffer was used for extraction. DNA extraction was also performed on the Microcystis 2667 for the qPCR positive control.

qPCR amplification

qPCR was used to obtain relative abundance of genes associated with Cyanobacteria. qPCR was performed using primers sets described by Rinta-Kanto (Rinta-Kanto et al., 2005). PerfeCTa Syber Green FastMix master mix was used (Quanta Bio) with primer concentrations of 100 nM. A 20 µl reaction volume was used. The qPCR program for the Cyanobacterial 16S rDNA assay consisted of 2.5 min activation at 95 ºC followed by 50 cycles at 95 ºC for 15 s, 53 ºC for 15 s, and 72 ºC for 30 s. The qPCR program for the *myc*D assay was identical to that of the 16S rDNA except that 52 ºC was used as the annealing temperature.

Table 1. Table showing primer sequences with estimated TM (at 0.25 uM primer loading) with specified annealing temperatures.

Primer	Target Gene	Sequence (5'-3')	Tm (0.10 uM)	Annealing Temp (°C)
mcyDF2	mcyD	GGTTCGCCTGGTCAAAGTAA	53.9	52
mcyDR2	mcyD	CCTCGCTAAAGAAGGGTTGA	53.2	52
CYAN108F	Cyan 16S	ACGGGTGAGTAACRCGTRA	54.8	53
CYAN377R	Cyan 16S	CCATGGCGGAAAATTCCCC	55.6	53

RESULTS AND DISCUSSION

Chlorophyll a and optical density

Figure 2A shows the average chlorophyll *a* concentrations throughout the experiment for each mesocosm type. Chlorophyll measurements within the control and EarthTec mesocosms increased for the first three weeks, while the chlorophyll readings for the covered mesocosms and raw water remained low or undetectable. For the rest of the experiment, the chlorophyll *a* concentrations for all the mesocosms, including the Control and EarthTec, were low or undetectable.

Figure 2B shows the average optical density at 550 nm throughout the experiment for each mesocosm type. Optical density for the first three weeks also increased, which supports the chlorophyll *a* data. There was also an increase in OD 550 nm around week 6 which did not correspond to an increase in chlorophyll. This increase was also observed in the raw lake water, indicating that there was a higher level of background turbidity that was not caused by growth of algae.

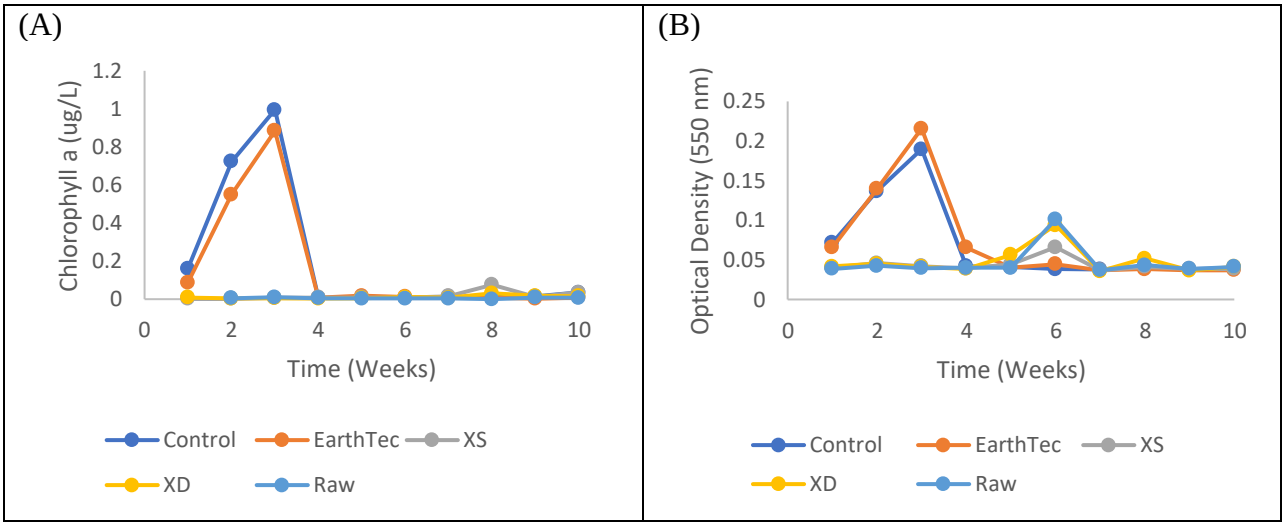

Figure 2. Time course plot of (A) chlorophyll *a* and (B) optical density. Control mesocosms had no covers or treatments, EarthTec received 19 mg/L of EarthTec's proprietary mixture, XS are single layer Xton covers, XD are double layer Xton covers, and Raw refers to the lake water.

Chlorophyll *a* readings suggest that the Xton covers were effective at controlling algal populations. In contrast, EarthTec did not have a significant effect on preventing algal growth in the mesocosms. In the second and third weeks of the experiment, the uncovered mesocosms experienced a large increase in both chlorophyll *a* and optical density, indicating the occurrence of an algal bloom in the mesocosms (Fig. 3). This result was expected given the role of the covers in blocking light penetration. No difference was observed between single and double layers of covers.

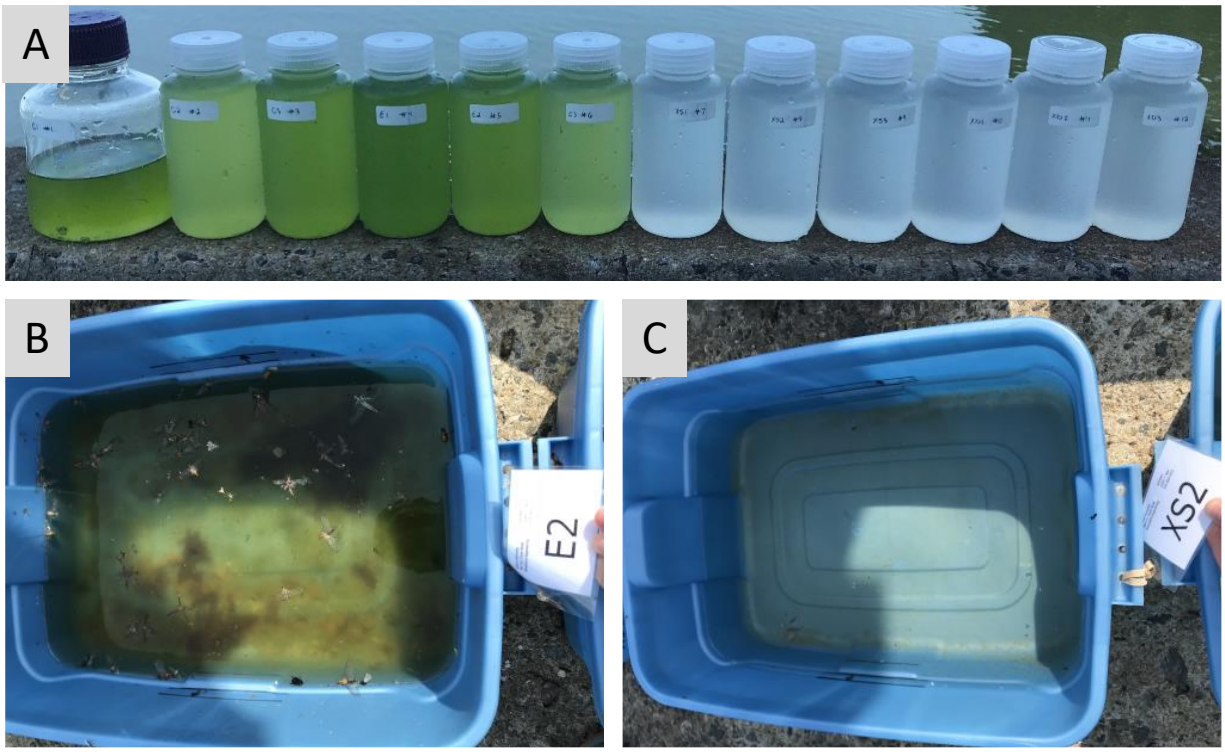

Figure 3. Photos of samples and mesocosms during algal bloom event. (A) Photo of samples collected from week 3 of experiment. Samples on the left were from uncovered mesocosms and samples on the right are from covered mesocosms. (B) Photo of an uncovered mesocosm and (C) a covered mesocosm during week 3.

In week 8, there was a second, smaller spike in chlorophyll *a* in the mesocosms. Interestingly, this spike occurred only in covered mesocosms. In these cases, biomass was observed growing in a film on the side of the mesocosm walls (Fig. 4). Moreover, this biomass primarily grew on walls that were exposed to morning and evening sun, indicating that light was penetrating the walls. However, this film did not appear in uncovered mesocosms, suggesting that these organisms were adapted to a low-light environment. This effect is unlikely to occur in a real settling basin given that the walls do not allow for even low levels of light transmission. However, this result suggests the need to ensure that there are no gaps between the covers and the walls of the settling basin.

Figure 4. Photo during week 8 sampling of single layer covered mesocosm showing biofilm on side wall.

Despite the presence of algal blooms in the mesocosms, chlorophyll measurements of the raw lake water showed no sign of an algal bloom, however. This suggests that conditions in the mesocosm (absent covers) were particularly favorable for algae growth. Previous studies show that algal blooms typically occur with the combination of warm temperatures, excess nutrients, and shallow water with good light penetration (Wang et al., 2017). All of these factors were present in the experimental mesocosms during the observed algal bloom. A large number of insects (particularly mayflies) were observed around the lake, including in the mesocosms during weeks 2 and 3 (Fig. 3D). Given that the algal blooms coincided with this event, we investigated if it was related to nutrients brought into the mesocosms by insects.

Nutrient profiles in mesocosms

Anion chromatography showed that phosphate levels were undetectable in nearly all water samples, including raw lake water. However, the uncovered mesocosms had positive phosphate readings, whereas the covered ones did not (Fig. 5A). This suggests that the presence of the insects in the mesocosms resulted in the release of phosphorus nutrients e.g. due to feces or death and decomposition of insects. Given that phosphate is a limiting nutrient in freshwater systems (Schindler, 1977), it is likely that the covers prevented insects from entering the mesocosm and thereby indirectly prevented phosphate addition to the mesocosms. This in turn was likely a major contributor in preventing algae growth. This secondary effect of the covers was not expected but shows how the covers can not only block light but also prevent insect populations from gaining access to settling basin water.

Anion chromatography showed that the uncovered mesocosms had a significant reduction in the nitrate levels compared to the covered mesocosms and raw water (Fig. 5B). The reduction in nitrate levels is a direct result of the algal bloom that occurred during weeks 2 and 3. Nitrate also declined around week 8 in the covered mesocosms, which corresponded to a second, smaller spike in chlorophyll *a* due to the biofilm growth.

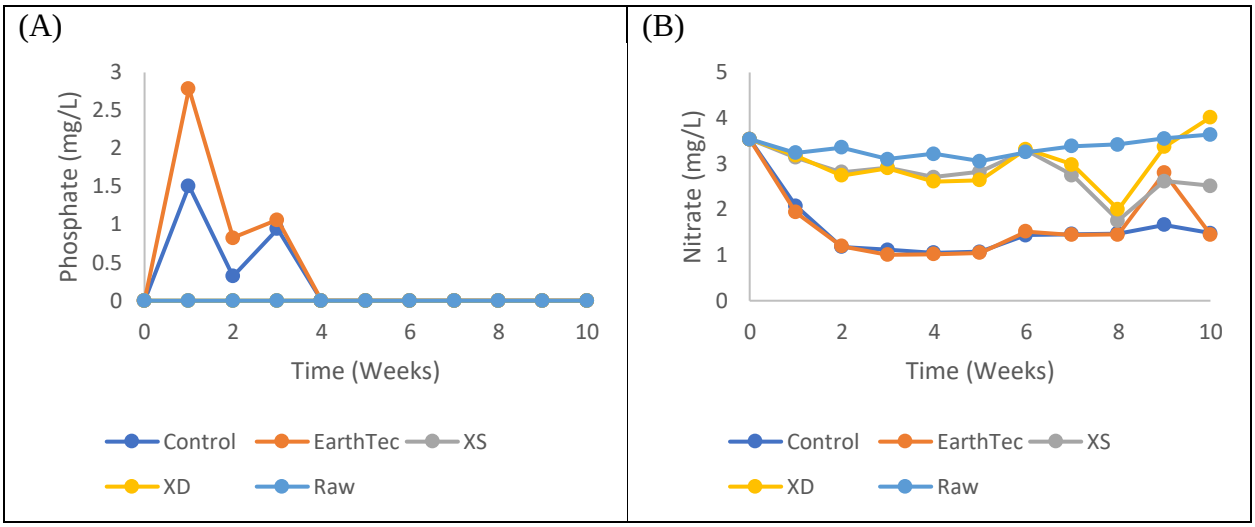

Figure 5. Time course plot of (A) nitrate and (B) phosphate concentrations in the mesocosms and raw water. Phosphate likely stimulated the algal bloom which caused subsequent decline in nitrate in the water.

qPCR amplification of cyanobacterial genes

Given concerns about Cyanobacteria and potential toxin production in settling basins, a limited set of qPCR assays was carried out to determine the presence of total Cyanobacteria and a microcystin toxin gene (*mycD*) in the mesocosms during the bloom period (week 3). Cyanobacterial genes were detected in some of the mesocosms during the algal bloom period (Fig. 6A). The *mycD* toxin gene was undetectable in all of the mesocosms during the algal bloom period (Fig. 6B). This indicates that the covers can prevent cyanobacteria growth but that undetectable levels of *mycD*-producing toxic cyanobacteria were present in this study period.

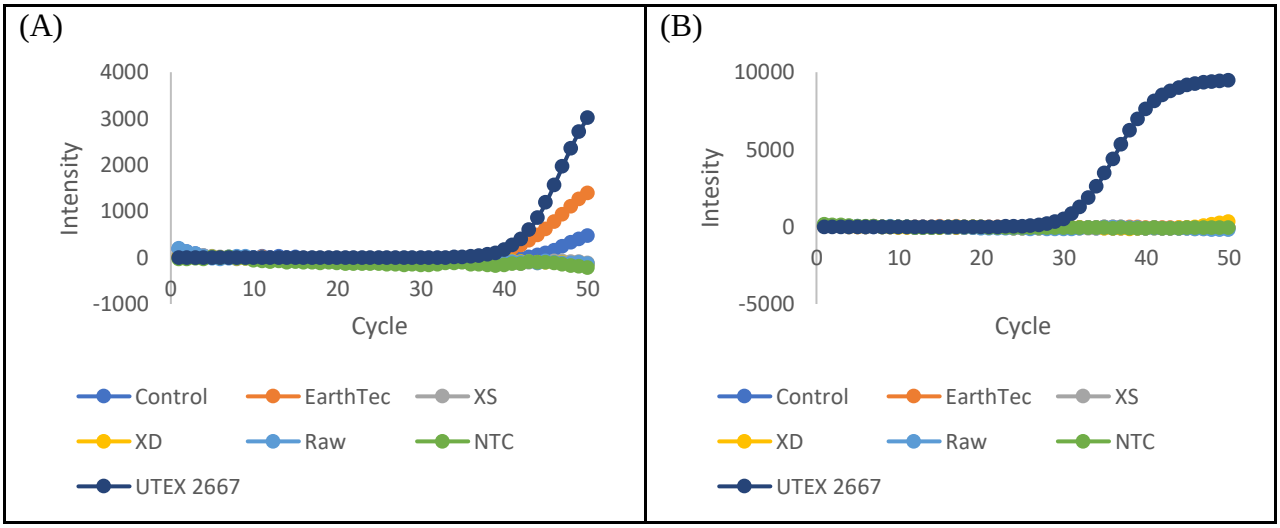

Figure 6. qPCR results on week 3 sampling using (A) CYANO and (B) *mycD* primer.

CONCLUSIONS

Given that water treatment plants have a variety of options at their disposal for managing algae populations, the objective of this study was to conduct a comparison test among the options currently employed at the North Columbus Water Treatment Plant. As hypothesized, the Xton Inc. covers prevented algal growth in mesocosms. However, the effectiveness of the covers appeared to be derived largely from preventing insects from entering the mesocosms in addition to any light-blocking effects. An insect event coincided with a spike in phosphate levels in uncovered mesocosms which induced an algal bloom. There were no significant differences observed between single layer and double layer covers in terms of preventing algae growth. Cyanobacteria genes were detected in uncovered mesocosms but not in covered mesocosms, underscoring the effectiveness of the covers in preventing Cyanobacterial growth. Under the conditions tested, EarthTec appeared to have little effect on algae growth.

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