Roodeplaat Dam Treatment Report

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1. Highlight

Roodeplaat Dam (Gauteng Province, South Africa) was treated with *Lake Guard*™ Oxy against a severe bloom of *Microcystis* sp. The treatment reduced the total biomass of the cyanobacterium by 3-4 orders of magnitude, or 99.9-99.99% decrease, over the course of several days (Fig. 1).

The overall dose of the *Lake Guard*™ Oxy of 2.27 ton/km^2, equals to a theoretical concentration of 0.057 ppm H_2O_2. This level is well below the lethal dose essential to directly kill cyanobacteria and thus cannot account for this dramatic reduction in the cyanobacterial biomass. Rather, as expected, the well-targeted, prolonged oxidative stress induced by the treatment, has activated a biological cell death signal within the cyanobacterial population. This signaling cascade accounted for the bulk collapse of the cyanobacterial population in the aquatic environment, which further initiated the self-healing process in the water body. Of note, during and after treatment, no impact on fauna or flora was observed. These results and conclusions are consistent with the results obtained in thousands of Lake Guard™ treatments all over the world.

2. Background

2.1. Roodeplaat Dam:

Roodeplaat Dam is a 4.39 square km multipurpose dam, situated North-East of Pretoria, under the jurisdiction of Tshwane Municipality, Gauteng, South Africa. Although originally designed for irrigation, it has become a major source of potable water for Magalies Water, which serves the heavily populated provinces of Gauteng, Limpopo and North West. Over the years, the dam has gained popularity for recreational purposes and to this day is regarded as the best rowing venue in South Africa and the only one that meets international standards.

Toxic cyanobacterial blooms in Roodeplaat Dam have been recorded since the 1970s (Pieterse & Toerien, 1978). They are associated with the influx of sewage effluent that runs into the Dam from at least two different sources. Cyanobacterial infections in the lake are a cause for concern to all the dam's stakeholders, when water does not meet minimal quality standards for drinking or recreation, affecting the health and livelihoods of its surrounding communities. The impact of blooms on the ecological fabric is devastating.

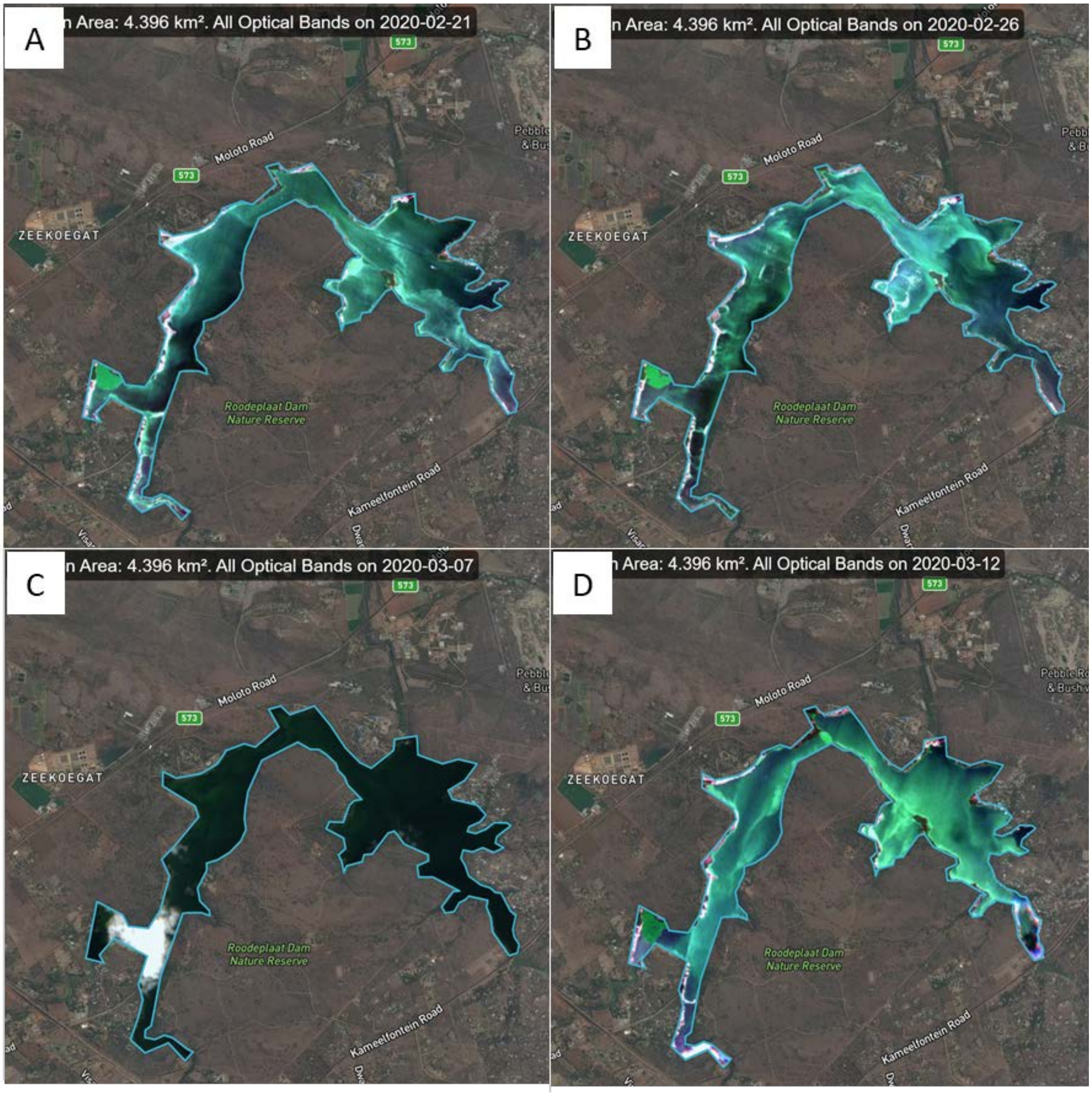

Fig. 1. Images of Roodeplaat Dam taken by the satellite *Sentinel-2,* between 07-08 AM for (A) Feb. 21, 2020; (B) Feb. 26, 2020 - the morning of the first day of treatment; (C) March 7, 2020, 10 days post-treatment (Satellite imagery for March 2 was not available due to heavy clouds blocking the view of the dam); (D) March 12, 2020. Bright blue-green colors are the visible appearance of cyanobacteria scum on the water surface; white color within the polygon represents patches of clouds. Data was generated by SkyWatch (www.skywatch.com)

To date, there have been no feasible means to treat cyanobacterial blooms in the dam, which resulted in the increase of the yearly cyanobacterial inoculum in the water, that kept intensifying in every subsequent bloom-cycle. This phenomenon is not unique to Roodeplaat Dam, but common to many water bodies around the world where cyanobacterial blooms increase in biomass and frequency year over year (Ho et al., 2019). Consequently, as detailed hereunder, Roodeplaat Dam is and has been subjected, for many years, to an ongoing severe toxic bloom.

2.2. Treatment Objectives:

In early February 2020, BlueGreen Water Technologies (BlueGreen) was approached by members of the World Masters Regatta 2023 Organizing Committee in South Africa, requesting a proposal for the treatment of Roodeplaat Dam with the intention to combat the heavy cyanobacterial bloom present in the lake ahead of the Regatta Junior Championships scheduled for March 6-7, 2020.

2.3. Treatment: *BlueGreen*'s *Lake Guard™* Technology

BlueGreen is currently commercializing two products, the *Lake Guard*™ Blue and the *Lake Guard*™ Oxy. Both products are floating, time-releasing formulations of market-approved algaecides. The products are commercially available in Israel, the USA, China, and South Africa. The *Lake Guard*™ products are certified internationally by the NSF/ANSI/CAN-60 standard for treatment in drinking water and are approved for use in all water sources by the US EPA. *Lake Guard*™ Oxy is also approved for treatment in drinking water by the Israeli Ministry of Health. Both products have been successfully applied in thousands of commercial applications around the world with outstanding results.*Lake Guard*™ Blue is composed of 95% (w/w) copper sulfate pentahydrate. *Lake Guard*™ Oxy is based on 98% (w/w) sodium percarbonate that releases hydrogen peroxide (H_2O_2) as its active ingredient.

The *Lake Guard*™ technology turns a generic, market-approved compound into a potent algaecide that selectively targets cyanobacteria in water bodies. Its novel physical properties allow the *Lake Guard*™ to 'home-in' on cyanobacterial aggregations on the surface of the water, following their movement patterns in real-time while releasing the active ingredient to interact, primarily, with bacterial cell matter, without being wasted elsewhere. As will be demonstrated hereunder, this "silver bullet" trait turns the *Lake Guard*™ into an optimal solution whilst applying the lowest algaecidal concentration possible. Furthermore, this well-timed, targeted application of an algaecide, triggers a biological cell-death cascade specific to cyanobacteria, causing a population-wide collapse, while allowing non-toxic algal species to re-populate the water column and serve as natural competitors inhibiting future cyanobacterial resurgence.

The application of the *Lake Guard™* is simple, requires no special equipment or preparation and can be done modularly with no size or shape limitation. This comes in stark contrast to existing methodologies that require large quantities, are hazardous and labor-intensive, and involve a high-cost full-lake shock treatment.

More importantly, *Lake Guard™* can and should be used preventatively, during the early stages of an evolving cyanobacterial outbreak, using even lower algicide doses. Such preventative protocol "pulls the rug" from under the formation of cyanobacterial pandemic, increases species' diversity by promoting the growth of non-toxic eukaryotic algae that redistribute nutrients in the water, reseting and rebalancing the overall ecosystem. This healthy balance in the aquatic environment is sustained for extended periods and naturally prevents cyanobacteria from reclaiming dominance over longer intervals.

3. Emergency Treatment at Roodeplaat Dam

3.1. Setup

Roodeplaat Dam has a surface area of 4.39 km^2 and a volume of ~44,000,000 m^3 at full supply capacity. The crescent-shaped lake stretches over 8 km with multiple 'fingers' extending to different directions. Its catchment area is 668 km^2 and consists of grasslands, shrub-covered ridges, and bushvelds.

3.2. Monitoring and Sampling Methods:

3.2.1. **YSI ProDSS probe**: Temperature, dissolved oxygen (DO), pH, chlorophyll-b (Chl-b is a proxy to determine the total biomass of eukaryotic algae in the water), phycocyanin (PC, is a proxy to determine the total biomass of cyanobacteria), electrical conductivity in the water (SPC, µsiemens/cm) and Oxidation Reduction Potential (ORP, mV).

3.2.2. **Satellite imaging** to determine the spatial distribution of cyanobacteria over the water surface. Data from Sentinel 2 satellite was generated and analyzed by SkyWatch (www.skywatch.com) for the dates Feb. 21, Feb. 26, Mar. 07 and March 12. The images on all dates were acquired between 07:00-08:00 am.

3.2.3. **YSI 9300 photometer:** measures hydrogen peroxide (H_2O_2) concentration and alkalinity.

3.2.4. **Microscopy:** A qualitative sampling of the microorganisms in the aquatic environment. Samples were collected from the water surface by 100 µm phytoplankton net.

The in-situ measurements were taken daily, for 10 days, during the morning hours, from various sampling points around the lake as detailed in Fig. 2. A total of 889 samples were collected and analyzed. The total surface area was methodically divided into 3 main sectors:
Regions A-D (~1.5 km^2), Regions E-F (~1.2 km^2) and Region G (~250,000 m^2). Main cyanobacterial infection zone was concentrated between Regions B to E.

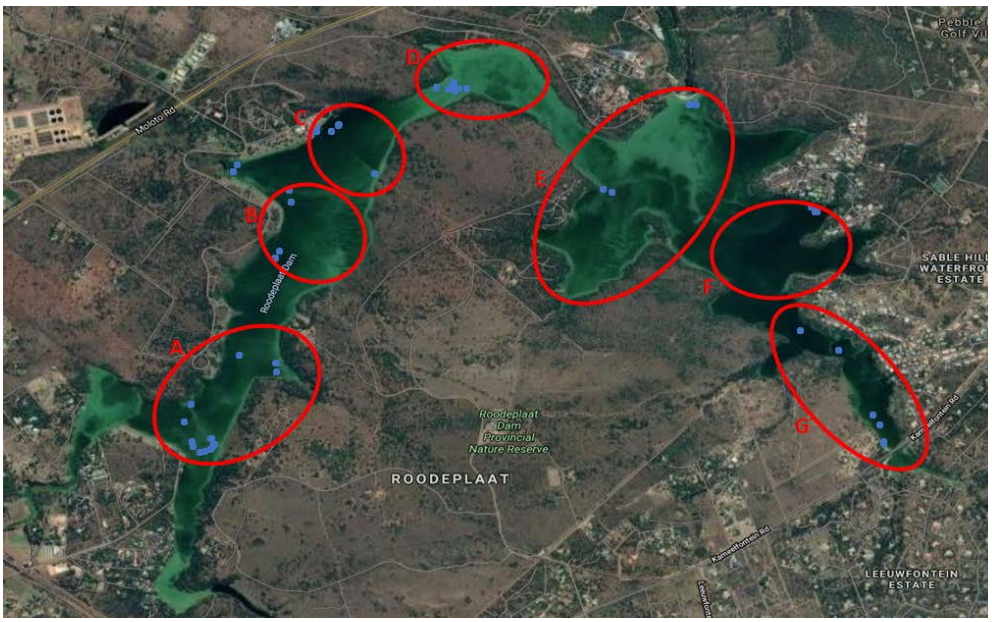

Fig. 2. A generic aerial photo of Roodeplaat Dam. Blue dots represent the GPS coordinates where samplings were withdrawn. Reference regions are circled and marked in red (A-G).

3.3. Pre-treatment Survey and Treatment Considerations:

Albeit a short notice, BlueGreen's scientific team completed an entire lake survey within a few days (starting on Feb. 23, 2020), leading to the following findings:

- An extreme cyanobacterial bloom mostly *Microcystis botris* (99.0%) and *Pseudanabaena mucicola* (~0.5%), Fig. 3. Total cyanobacterial cell density ranged between 10^8-10^9 cell/ml with visible scum that covered ~80% of the entire dam’s surface (Figs. 1A-B);
- Extremely high concentrations of cyanobacterial scum trapped within water hyacinth (*Eichhornia crassipes*) that covered ~10% of the water surface;
- Physical and other barriers, e.g. hyacinth, prevented access to ~25% of the water surface;
- Inconsistent wind patterns that changed every few hours.
- Sewage effluent was spilling into the dam at 2 locations around Regions A and B.

Given the severity and the type of the cyanobacteria responsible for this specific outbreak, an absolute minimum dose of 20 tons of Lake Guard™ Oxy was surmised to achieve the treatment's primary objective: reducing the cyanobacterial load to safe levels prior to the Regatta Championships.
Due to funding limitations and a tight deadline, *BlueGreen* secured a total of 9.4 tons of the Lake Guard™ Oxy, less than 50% of the required estimate.

3.4. Treatment:

Roodeplaat Dam was treated using a total of 9.4 tons of the Lake Guard™ Oxy in the week of Feb. 24th, 2020. Application rates are reported in tons (1 metric ton equals to 2,204 lb).

3.5. Application:

Supported by South Africa's Department of Water & Sanitation, Department of Environment Forestry & Fisheries, and assisted by Sable Hills, Gys Pitzer and Hugo Maree's watercraft, the *BlueGreen* team applied the treatment from a moving boat during the morning hours, as follows:

- Feb. 26, 2020: Regions A-G, a total of 2.0 ton of *Lake Guard*^{TM} Oxy or ~0.5 ton/km^2 (~0.045 ppm Lake Guard™ Oxy or 0.011 ppm H_2O_2);
- Feb 27, 2020. Regions B-E, a total of 2.4 ton of *Lake Guard*^{TM} Oxy (~ 0. 054 ppm Lake Guard™ Oxy or 0.013 ppm H_2O_2);
- Feb 28, 2020. Regions B-E, a total of 5 ton of *Lake Guard*^{TM} Oxy (~0.113 ppm Lake Guard™ Oxy or 0.028 ppm H_2O_2).

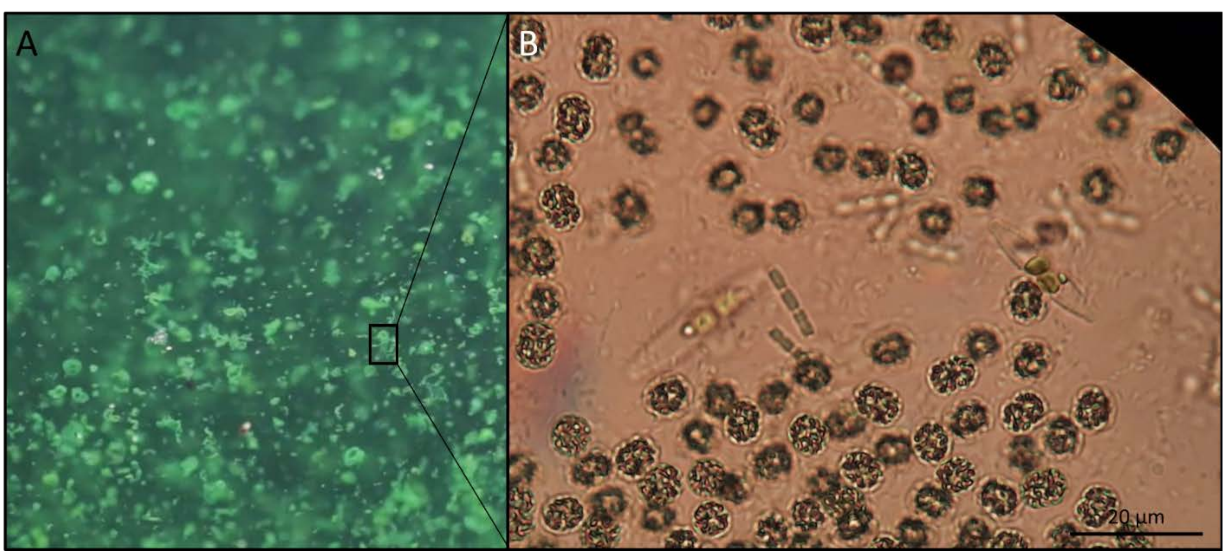

Fig 3. Microscope images of (A) 5X enlargement of a water sample from the dam, and (B) 400X magnification of a typical sample. The phytoplankton community is dominated by *Microcystis botris*, round, uneven cells, which account for ~99.0% of the total phytoplankton population. *Pseudanabaena mucicola,* short, filamentous cyanobacteria, with 3-4 cells in each filament, accounted for ~0.5% of the total phytoplankton population. Other phytoplankton species were observed at very low density, e.g. the two *Diatom* sp. seen in the middle of the figure.

Results and Discussion:

A daily treatment dosage of 0.5-1.0 ton *Lake Guard*™ Oxy/km^2 was applied in the lake over three consecutive days. Each treatment took 2-3 hours to apply without affecting the ongoing rowing or fishing activities in the lake. The *Lake Guard™* particles moved with the currents and winds as projected, together with the cyanobacterial scums. Lysis of cyanobacteria cells and the clearing of scum could be clearly observed within a short time after the treatment in different areas of the lake (Fig. 4), including deep within condensed hyacinth concentrations (Fig. 5).

The daily applications were designed to trigger and amplify the auto-catalytic-cell-death within the cyanobacterial population and to fine-tune its effect in real-time, under the dam's severe bloom condition. Consistent with past experience (e.g. HERE, HERE, HERE, and HERE) and in line with many peer-reviewed publications (e.g. Barrington *et al*., 2013; Daniel *et al*., 2019; Spoof *et al*., 2020; Zhou *et al.*, 2020), applying oxidative stress against a population of cyanobacteria over time triggers a suicidal signaling cascade within the general cyanobacteria population that propagates throughout the water column to naïve planktonic cells. This phenomenon begins immediately after treatment when affected cyanobacteria show clear signs of stress. The ultimate reduction in cyanobacterial biomass paves the way for competing eukaryotic algae to occupy the vacant ecological niche and further inhibit the remaining cyanobacterial cells with their own arsenal of allelochemicals. The growing beneficial green algae population tie-up important nutrients away from cyanobacteria (e.g. Phosphorus and Nitrogen), and increase the biodiversity in the aquatic ecosystem, a sign of a healthier water body.

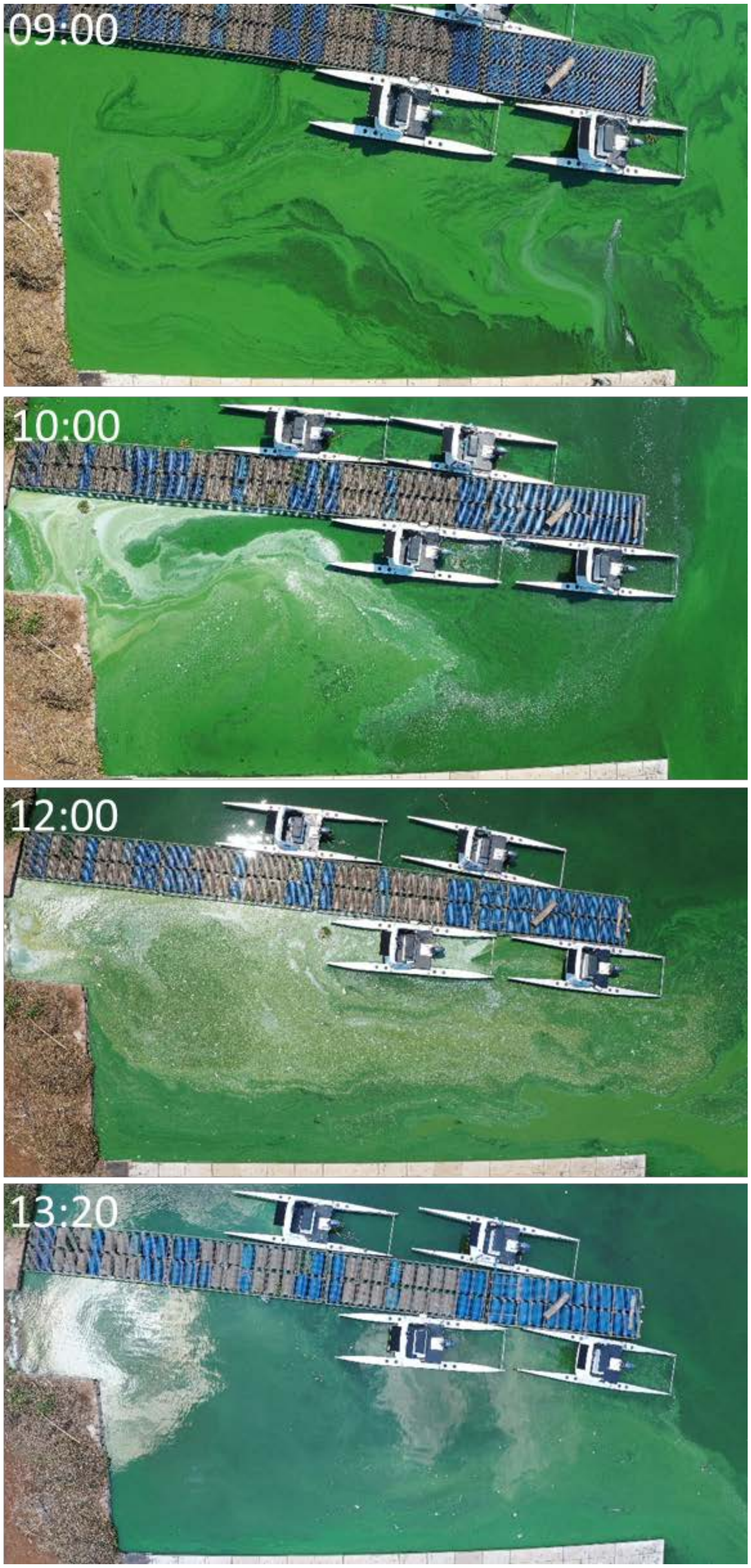

Fig. 4. Time-lapse photographs taken on February 27, 2020 by a drone above the boat dock (Region C). The time of photographing is shown in the upper left corner of each picture. The first picture is time zero and shows the *Microcystis* scum pushed towards the deck by the wind and currents. *Lake Guard™* Oxy was broadcasted far from the dock, then drifted together with the wind and currents to concentrate alongside the cyanobacterial aggregates. A few hours later, clear changes to the scum's color and consistency can be seen concurrent with a reduction in biomass due to the effect of the time-released H_2O_2 molecules.

Fig. 5. Dead/dying cyanobacterial cells (gray-brown smears) that continued accumulating on the water surface at different locations in the dam days after the application of Lake Guard™ Oxy has ended: (A) Region D; (B) Region E and (C) Region C.

Notably, the ability to achieve such a positive response in the water column is dose-dependent. Even when ignoring the physical and biological (hyacinth) barriers to treatment, the enormous concentration of cyanobacteria in Roodeplaat Dam prior to treatment (10^8-10^9 cell/ml) meant that reducing 99.9% of *Microcystis* sp. biomass, or decreasing the cyanobacterial cell density by 3 orders of magnitude, would translate to remaining cyanobacterial cell density in the range of 10^5-10^6 cells/ml (still 10-100 folds higher than the WHO recommendation) – at a time when the overall cyanobacterial biomass has a potential to multiply every few hours. In other words, the criteria for tipping the balance in favor of eukaryotic algae dictates that cyanobacterial biomass be sufficiently reduced for the beneficial algae to succeed them. As explained below, this is a dose-dependent process.

At the backdrop of these heavy challenges, it was decided to increase the oxidative stress in specific areas with relatively higher cyanobacterial concentrations in order to amplify the anticipated biological signaling cascade of programmed cell death that complements the treatment.

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The overall effect of the treatment on the lake was overwhelming. 10 days after the first treatment, on the target day of the Regatta Junior Championships (March 7, 2020), the overall cyanobacterial biomass in Roodeplaat Dam was dramatically reduced relative to pre-treatment levels, as clearly visible in the satellite imagery (Fig. 1C). This landslide outcome is the result of the biological chain reactions that were put in motion by the treatment, as demonstrated as well in the *in-situ* measurements taken in the lake, in real-time (Fig. 6). These measurements reported various parameters about the phytoplankton population in the dam. Phycocyanin (PC) and chlorophyll-b (Chl-b) are direct proxies for the amount of pigment in the water. They correlate to the biomass of cyanobacteria and total algae, respectively, in the lake. The large rise in PC and Chl-b levels shortly after treatment (Figs. 6A-B) is an artifact caused by the release of pigments from masses of dead cyanobacterial cells, as documented in multiple cases with *Lake Guard™* treatments.

Two complementing physiological trends confirm the reduction of the photosynthetic activity in the water: pH and dissolved oxygen (DO) levels. The pH continuously dropped from a pre-treatment of 9.64 to 8.1 two days after the last treatment (Fig. 6C). This is attributed to the declining photosynthetic consumption of CO_2 and faster respiration by the bacteria consuming the organic carbon released by lysing cyanobacterial cells. The rising DO levels in the days before the treatment, from 50% to over 150% (Fig. 6D) is due to the rising photosynthetic O_2 evolution with the intensifying cyanobacterial bloom (see also the the satellite imagery, Fig. 1A-B). Two days after the last treatment, DO levels were back at 50%, due to reduced photosynthetic activity and the degradation process of cyanobacterial biomass by heterotrophic bacteria.

In other words, the minor, yet well targeted oxidative stress applied against cyanobacteria over the 3-day treatment has generated a demonstrable biological effect on cyanobacteria throughout the entire dam.

As expected, H_2O_2 concentrations in the water column before, during and after the treatment (0.01-0.1 ppm) were very low, within the instrument's standard error range. Given the very low *Lake Guard™* Oxy applied and its slow release (and H_2O_2 cleavage capabilities of the phytoplankton) it does not have any measurable effects on the overall H_2O_2 concentrations in the water. Water temperature ranged between 23-27°C, which was immaterial to the treatment. Water alkalinity did not change during the treatment and remained at the range of 80-90 mg/l $CaCl_2$. Other water parameters such as the water ORP state (Fig. 6E) and conductivity, SPC (Fig. 6F), did not change during the treatment indicating that the effect on the cyanobacterial populations was primarily biological. Of note, throughout the treatment with the Lake Guard™ Oxy and thereafter, no fish-kill or any other impact on flora or fauna was observed. These results are also consistent with *BlueGreen*'s previous work (e.g. HERE, HERE and HERE).

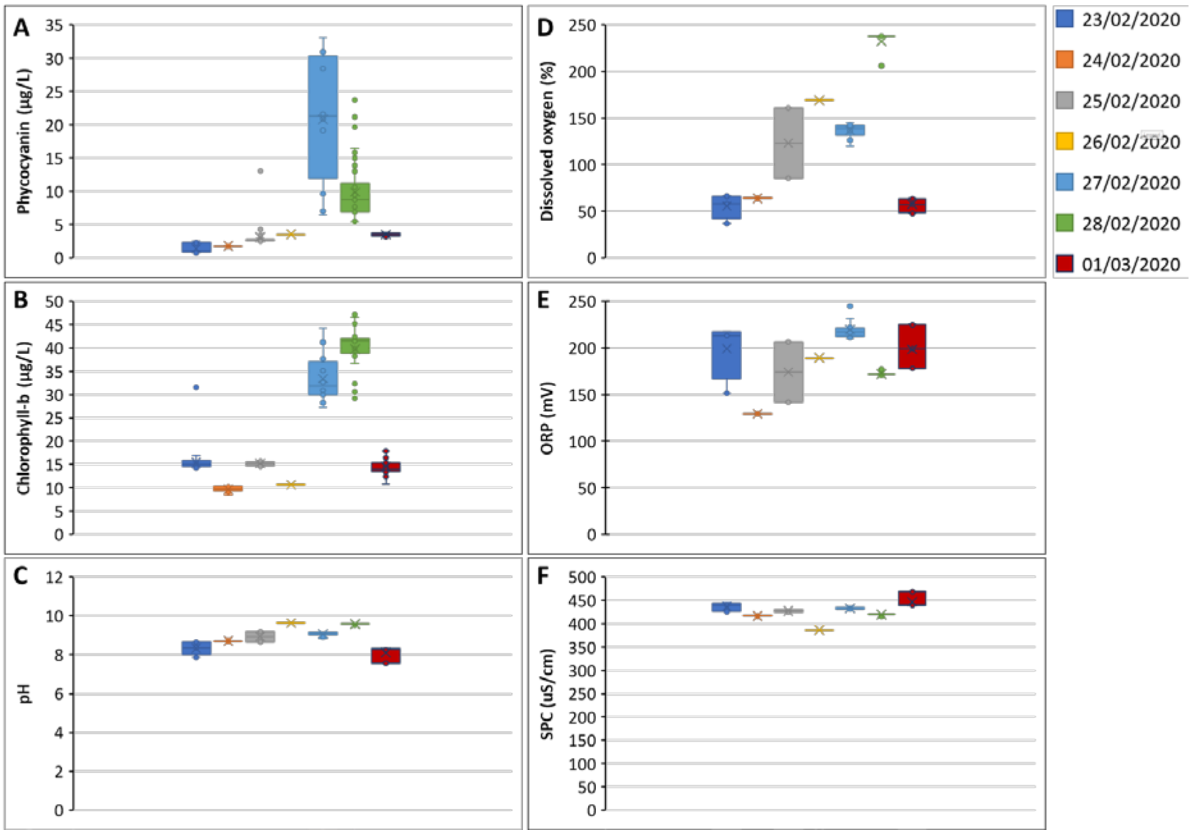

Fig. 6. The changes in biomass and photosynthetic activities evident from in-situ readings taken from multiple sampling points along the lake (avg of 15 measures per sampling point): PC (A) and Chl-b (B) readings dramatically increase after the application of the first treatment on February 26, which had the lowest dose rate, an indication of the release of pigment into the water from dead/dying cells. These correspond with a post-treatment drop in pH (C) and DO (D), that sharply decreased with the declining photosynthetic activity in the water. No meaningful change in the chemistry of the water as indicated by ORP (E) and conductivity levels (F), a hallmark of the *Lake Guard™* treatment.

In summary, the *in-situ* results (Fig. 6) are in agreement with the satellite images (Fig. 1), and consistent with previous *Lake Guard*^{TM} treatments. They all demonstrate how a targeted treatment with the *Lake Guard*^{TM} Oxy activates a potent signaling mechanism within the cyanobacterial population and resulting in their dramatic collapse. Moreover, the effect of the biological signal was not limited to treated areas only (Figs. 4 & 5) but extended to the entire lake. Comparing the condition of the lake before and after treatment (Figs. 1B & 1C), clearly shows that the coverage and the intensity of the cyanobacterial infection dramatically decreased including areas that were out of reach to the *Lake Guard™* Oxy particles. These areas include patches covered with hyacinth, areas closed by booms, or areas, that for logistical reasons, were not earmarked for treatment whatsoever.

It is worth noting that the effect of the treatment was short-lasting. The actual treatment dose of less than 50% of the minimum dose recommendation was not sufficient. As significant as the impact of the treatment was on the cyanobacterial population in the lake, ~99.9% reduction, it did not achieve its main biological outcome – to allow the beneficial eukaryotic algae to reach a critical mass and tip the biological balance in their favor. Instead, the remaining *Microcystis* cells in the dam, that had high enough cell density, recovered within days of treatment, and started multiplying at an exponential rate, to, ultimately, recover to their original nubmers.

A model of the dynamics of the cyanobacterial bloom in Roodeplaat Dam and the effect of the *Lake Guard*^{TM} Oxy treatment is shown in Figure 7.

The growth curve of cyanobacteria starts flat during winter time when it is defined by a low replication rate due to suboptimal conditions associated with temperature as well as other microorganisms in the water that compete over the same resources and interfere with each other's quest for dominance. Later, during springtime, when temperatures rise, the *Microcystis* cell-density starts to increase steadily (Fig. 7 "lag phase" yellow box "A"). As the weather gets warmer and the days longer, the doubling time of cyanobacterial cells shortens as they move into a "logarithmic phase" (Fig. 7 orange box "B"). In other words, the total *Microcystis* biomass in the dam can double every few hours without interruption. This curve reaches a plateau (Fig. 7 white area "stationary phase"), mainly when resources become scarce and conditions less favorable. During wintertime, most cyanobacterial species slow growth only to resurge again, starting from a higher cell density than the year before when conditions are favorable, priming for a more substantial bloom season.

The eukaryotic algae (Fig. 7, green curve), on the other hand, recovered in biomass and diversity immediatelly after the first treatment in Roodeplaat Dam (Fig. 7 Arrow "C"). However, they could not reach a critical mass that would have allowed them to colonize the ecological niche outcompeting the cyanobacteria (Fig. 7 Arrow "E"). The surviving *Microcystis* cells, still at a relatively high cell-density (Fig. 7 blue curve), resumed their exponential growth rate and thus regained dominance over the ecological niche.

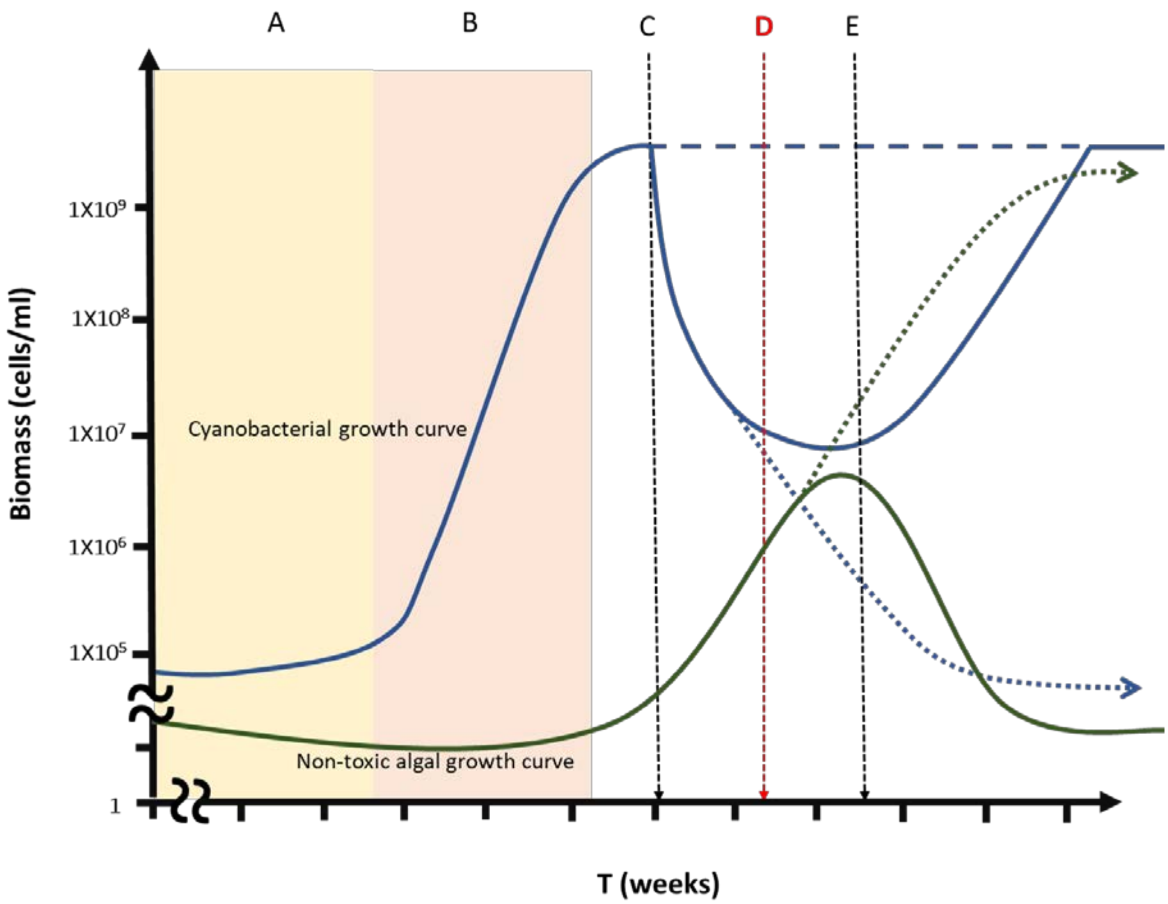

Fig. 7. A model of the growth curves of a cyanobacterial bloom (blue line) vs. a non-toxic green algae (green line) in the water at Roodeplaat Dam before (boxes "A" and "B"), during (Arrow "C"), and after treatment. The model also hypothesizes the outcome of a potential additional treatment (Arrow "D") on the composition of the phytoplankton in the water (dotted blue arrow for cyanobacteria and dotted green arrow for the non-toxic algal species).

Had the necessary follow-up treatment of 2-5 ton/km^2 been applied, it would have reduced the cyanobacterial biomass to its target lag phase (below 10,000 cells/ml. Fig. 7. Arrow "D" and blue dotted line) allowing non-toxic algae (Fig. 7. green dotted line) to thrive and occupy the vacant ecological niche, further tipping the balance in their favor and inhibiting cyanobacterial resurgence.

This risk of cyanobacterial resurgence is highlighted in *BlueGreen*'s standard recommendation of starting the treatment at the earliest stages of the bloom formation, and continue monitoring and applying targeted, preventative treatment against any cyanobacterial resurgence in order to maintain a bloom-free lake.

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