AlgaEnviro

# Overview of Case Studies Using *Diatomix* in Raw Water Storage Dams, Lagoon STPs, Wastewater Effluent Storage Dams and Wetlands - 2019

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*Diatomix* is a liquid nano-silica nutrient mixture containing the micronutrients required for the growth of diatom microalgae. The micronutrients in *Diatomix* are adsorbed onto amorphous silica structures that are in the nano-scale of 5 – 20 nm in diameter. As only diatoms have a requirement to take up silica, they are the only algae that benefit from the micronutrient boost from taking up *Diatomix*. With a micronutrient boost, diatoms can successfully out-compete the other algae for the main nutrients, nitrogen and phosphorus (N and P).With less inorganic nitrogen and phosphorus available in the water column, blue-green algae (cyanobacteria), macroalgae, e.g.filamentous algae, and larger water weeds, e.g.*Cabomba*, *Hyacinth* and *Salvinia* are starved of N and P and therefore .die off.

Another significant benefit, particularly in wastewater treatment, is that bacterial activity is enhanced due to the increased dissolved oxygen content as a result of the increased diatom growth. This increase in dissolved oxygen and bacterial activity assists in bringing down the biochemical oxygen demand (BOD) in the wastewater. *Diatomix* has successfully been used in Australia and New Zealand over the last five and a half years, with impressive results, some case studies show a reduction of blue-green algae populations by >99%, as well as significantly reduced water weed problems

This report provides an overview of several separate case studies at raw water storages, wastewater treatment lagoons and effluent storage dams. The volume of *Diatomix* dosed during these case studies was dependent upon the mass of nitrogen and phosphorus in the lagoon, dam system or influent. *Diatomix* is not a supplementary supply of silica and reactive silica does need to be present.

Each site had a slightly different approach to the method or timing of dosing and there have been a range of responses that have provided valuable insights into the use of *Diatomix* and the most notable trends are presented here.

## Case Study 1

Case Study 1 was two raw water storage dams on a farm, 3.3 and 1.4 hectares in size respectively. The Blue green algae (BGA) counts were variable to begin with; Dam 1 went from 70,900 cells per mL down to 4,700 and then returned to 46,600 before dosing started. Dam 2 ranged from 2,700 BGA cells/mL to 117,000 BGA cells/mL before the *Diatomix* dosing started on the 26th of March 2017.

*Once Diatomix* dosing started, in both dams the BGA counts reduced to average values of 1,885 BGA cells/mL and 2,025 BGA cells/mL respectively. Over the December period in Dam 1, dosing was overlooked for a couple of weeks due to the holidays. When a bloom was observed in Dam 1 at the end of December, dosing was re-instated and BGA numbers dropped again, while diatom numbers increased. Unfortunately, the client selected not to continue regular monitoring and chose to manage the dosing 'by eye' from this point. This suited them but was less helpful in regards to this case study.

These results are presented in Figure 1 and Figure 2.

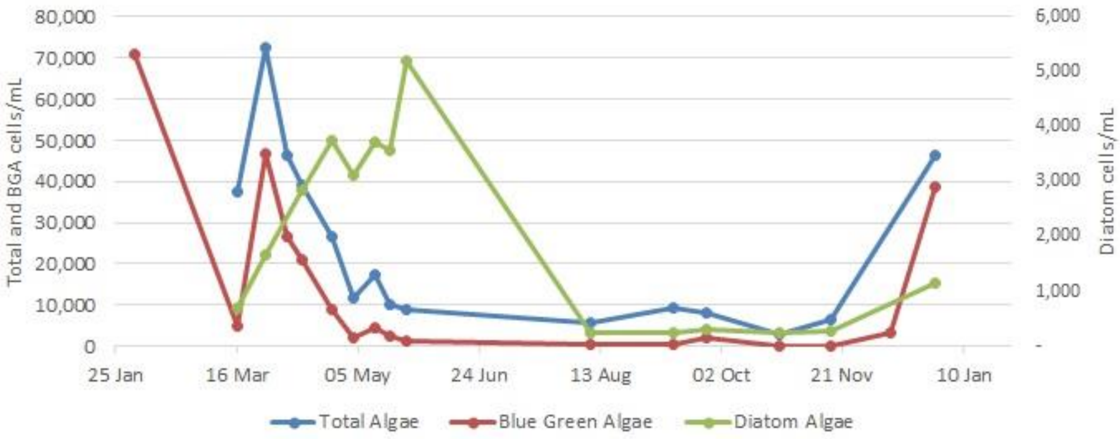


**Figure 1 – Dam 1 – Raw water storage treated with *Diatomix* to reduce BGA cell counts. This 3.35 Ha dam required 0.6 litres of *Diatomix* per week.**

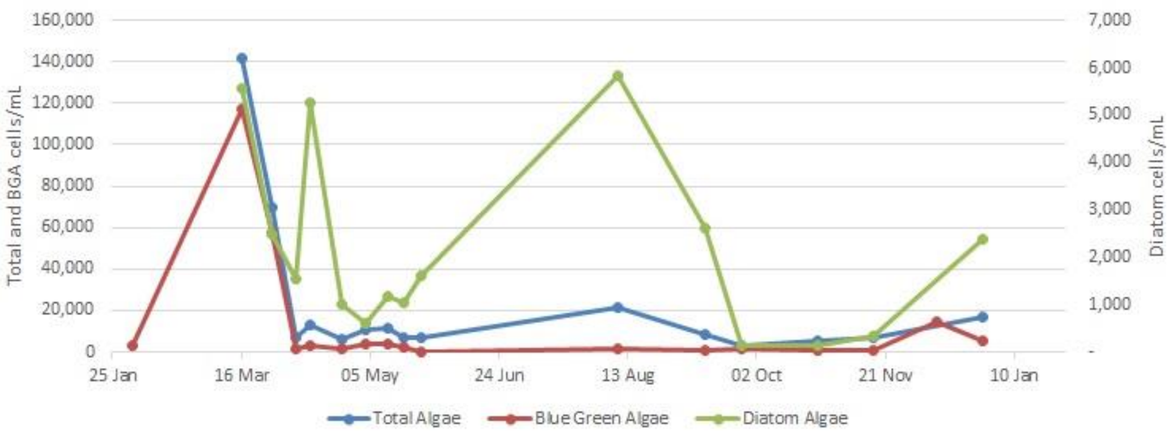


**Figure 2 – Dam 2 – Raw water storage treated with *Diatomix* to reduce BGA cell counts.**

As the diatom bloom established and the diatoms made use of the available nutrients the BGA did not bloom again in such high numbers. The growth of diatoms does not however appear to be extensive in the results. This is because many diatoms that grow when *Diatomix* is dosed grow on the biofilms and dam bottom (benthic area), so when the 'suspended algae' are sampled, algae growing in biofilms are not represented and this makes it impossible to clearly demonstrate that the observed changes in suspended algae are being driven by changing diatom populations.

As the diatoms are at the bottom of the food chain, many diatoms are eaten and the inorganic N and P taken up by diatoms is then changed into organic N and P in the animal ecosystem (zooplankton, insects and invertebrates, fish, eels, turtles, birds). This is one of the long-term benefits of *Diatomix* use, stable, healthy ecosystems tend not to be susceptible to BGA blooms.

This client moved to long-term dosing of their water storages so as to ensure the ongoing health of the drinking water supplies for their farm, and the importance of regular dosing was observed when Dam 1 dosing was overlooked for a couple of weeks in December 17.

## Case Study 2

Case Study 2 was at a Lagoon based Sewage Treatment Plant (STP) that has an *Imhoff Tank* for solids separation, followed by a nitrification-denitrification step, settling and effluent release into the lagoon.

Two months of background sampling was completed prior to the case study starting. The dosing of *Diatomix* was sufficient to address both the background Nitrogen (N) concentration in the lagoon as well as the mass of N entering the lagoon from the STP. From the start of this period the silica concentration began to trend downward, showing a consistent uptake of silica through diatom activity (data not shown). The Total Cell count began to stabilise with the use of *Diatomix*, where the total cells reduced from an average of 1.5 million cells per mL prior to *Diatomix* use to a value of 36,000 cells per mL during *Diatomix* use, a reduction of 96%. Over the ensuing winter period, without *Diatomix*, there was a very low Total and BGA cell count. This may have partially been affected by the initial reduction of Total cell count through *Diatomix* use, but as there were no control ponds, this is not clear.

Prior to the first *Diatomix* dosing, the proportion of BGA in the Total Cell count was greater than 96%. During the first period of dosing this proportion did not reduce, but during Summer dosing from January 2016 the proportion reduced to 77%. This is a useful, but not immense, impact in regards to the proportion of BGA present, but there was also an 85% reduction in BGA Cell count, so the BGA reduced from an average of 1,530,000 BGA cells per mL to 227,000 cells per mL during summer when *Diatomix* was dosed.

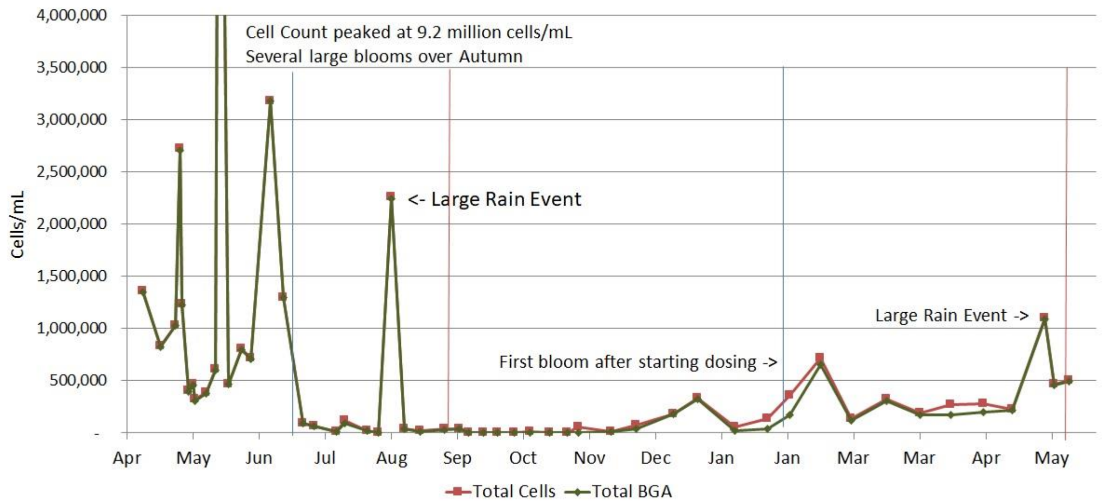


**Figure 3 - The characteristics of the Case study 2 Lagoon during two periods of dosing *Diatomix* at two concentrations. (Blue line shows dosing start and red line dosing stopped). The large rain events are highlighted as they were associated with the spike in cell counts which were quickly reduced and stable conditions were re-established.**

There was a reasonably large rain event during the first dosing regimen and this increased flow into the STP Lagoon caused corresponding spikes in the N and P measurements. This increase in nutrients was associated with an increase in cell count as well, but the cell count reduced quickly afterwards and remained low. This on-going stability of algal counts, outside of rain events, was not observed prior to *Diatomix* dosing as can be seen in the initial sampling period up to the 15th of June in Figure 3.

The Total Cell count reduction would in many situations be a concern as the growth of algae is required to reduce the concentration of nutrients in the water. *Diatomix* dosing reduced the Total cell count to as low as 2,400 cells/mL and yet the nutrient levels have remained stable and low. This is because the nutrients are being taken up by the increased activity of diatom algae in the benthic zone, and so despite a decrease in the Total 'Suspended' Cell Count observed from the sampling, the management of nutrients through diatom algae continues in the benthic zone.

An additional benefit of having the suspended algal cell count reduce so dramatically is that there is a greater penetration of ultra-violet light from the sun into the lagoon water which improves effluent disinfection. The diatom algae that are growing in the periphyton and benthic zone get more light when there is a lower suspended algae population and with diatom algae growth in the benthic zone the dissolved oxygen (DO) concentration is very likely to be higher in this area. This carries forward to the fact that aerobic bacteria would be able to degrade more sludge if DO in the benthic zone was higher, thus reducing sludge accumulation.

The difference in the state of the lagoon can be seen in Figure 4, the image on the left was prior to the case study beginning and the right-hand image is during the second dosing period. The most notable aspects are the loss of the 'blue-green' colour in the water and how the algal density has reduced as the lagoon bottom can be seen to a greater depth.

**Figure 4 - A before and after comparison of the same lagoon during Case study 2. The 'Blue-green' colouring on the left has gone and the lagoon bottom can be seen to a greater depth on the right.**

As graphs of algal speciation can be very difficult to view, this data is available upon request from AlgaEnviro.

The diatoms are a good food source for the zooplankton and invertebrates in the lagoon and nutrients taken up by diatoms from the effluent would be transported to the food chain where they are more stably retained in the long term, with a greater percentage of the nutrients being exported through insect larvae hatching and flying away, small fish being fished out by birds, etc. Where there is a need for greater or more regular nitrogen and phosphorus removal from the water, then a 6 monthly or yearly fish capture and removal could occur with netting or electro-fishing.

## Case study 3

The third case study site consisted of an *Imhoff tank* and a series of three lagoons. *Diatomix* was initially dosed into the third lagoon after six weeks of background monitoring. Nearer to the end of the study the dosing in the third lagoon was temporarily stopped to see how the algal assemblage and the nutrient profiles would change. At the same time that the Lagoon 3 dosing was stopped (Figure 5), Lagoon 1 started to be dosed until the end of the study (Figure 6).

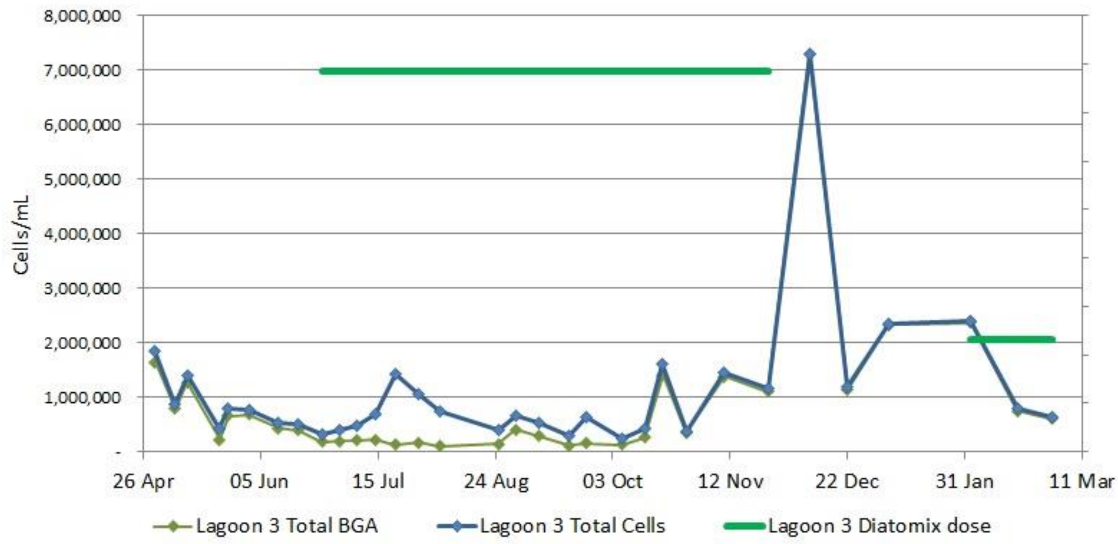


**Figure 5 – Case Study 3 - Lagoon 3 - Total Cell Count and Blue-Green Algae counts over an 11-month period as well as dosing level of *Diatomix* into the lagoon – (millilitres per square metre of pond area). When *Diatomix* dosing was suspended in November (2015) BGA cell counts bloomed immediately and remained high until *Diatomix* dosing was re-instated in February when they dropped down again.**

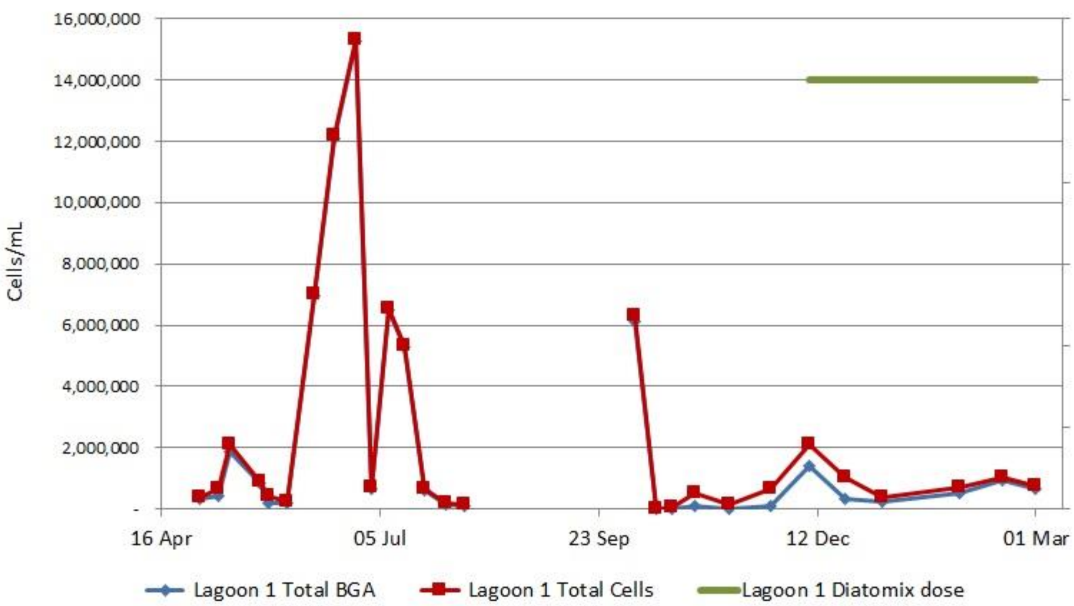


**Figure 6 – Case Study 3 - Lagoon 1 - Total Cell Count and Blue-Green Algae counts over an 11-month period as well as dosing level of *Diatomix* into the pond – (millilitres per square metre of pond area). Dosing started in December until March, the existing bloom was reduced and suppressed for the remaining period.**

The proportion of the Total Cell Count that was BGA was an average of 95% in Lagoon 1 prior to dosing with *Diatomix* and this reduced to 69% during the 4 months of *Diatomix* dosing. In Lagoon 3 this proportion was initially 86%, while during the dosing of *Diatomix* in Lagoon 3 the average value reduced to 54%.

## Case study 4

Case Study 4 was at a Barramundi aquaculture farm in Northern Queensland. The issues at the farm were the need to keep ammonia levels low, due to toxicity, and also reduce excessive weed growth in the farm storage lagoons. The storage lagoons would normally be mechanically harvested every six weeks to ensure the weeds did not spread out too far from the edges of the lagoon. After dosing with *Diatomix* the frequency of weed removal was done for maintenance once a year. Figure 7 illustrates the storage lagoon just before the mechanical weeding and under normal conditions it would be expected to look the same after 6 weeks. With *Diatomix* dosing the photo on the right was taken 22 weeks after the mechanical weeding. As can be seen, weed growth is no longer problematic.

**Figure 7 - Case Study 4 - Weed growth in a Barramundi farm water storage lagoon. Normally the lagoon required mechanical harvesting every 5 weeks. With *Diatomix* dosing, the photo on the right was taken 22 weeks after *Diatomix* dosing.**

Due to the nature of a fish farm, there is always a high concentration of ammonia and nitrate entering the storage lagoon from fish waste and uneaten feed. By bio-manipulating the diatom population with *Diatomix* it is possible to keep the nitrogen uptake going toward diatom algae rather than the weeds. As seen in Figure 8 the nitrate levels fluctuate regularly. The diatoms respond to influxes of nitrogen and bring the concentration down quickly. *Diatomix* use has become part of the operating procedure for water management at this site.

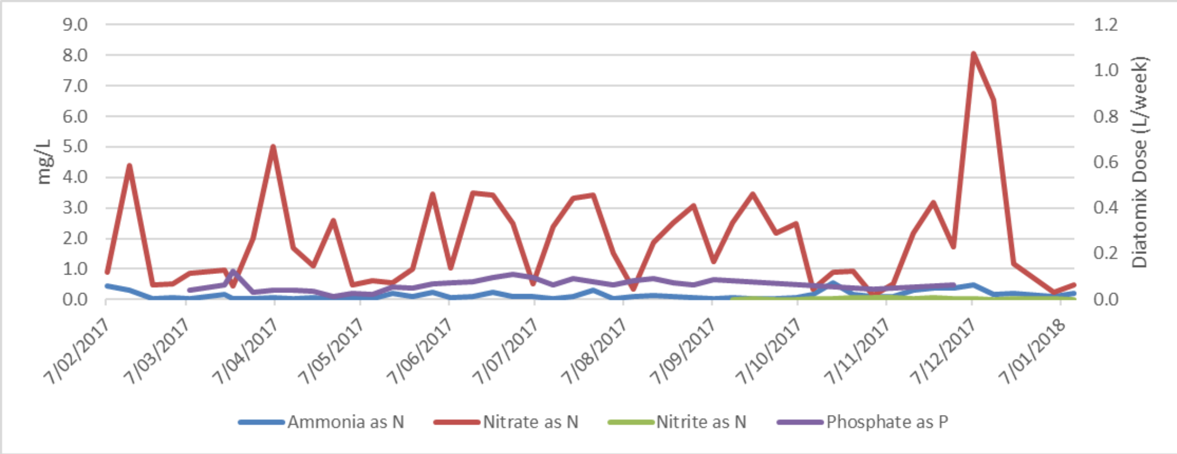


**Figure 8 – The concentration of nitrogen and phosphorus in a barramundi farm storage lagoon. *Diatomix* consistently keeps ammonia levels down and the lack of weed growth is evidence that the fluctuating nitrate levels are not taken up by the weeds.**

## Case study 5

Case study 5 was slightly different in how it was approached. The site also had a long history of high BGA blooms each year from spring through to autumn. There was no background data on this case study in regards to cell counts, as the situation at the lagoon was, whilst not desirable, considered to be 'situation normal', with high summer BGA. After they became aware of a potential remedy for this problem, they contacted AlgaEnviro.

Sampling started once *Diatomix* dosing was initiated. As can be seen in Figure 9, the Total Cell Count began to reduce quickly once *Diatomix* was dosed. Arguably this could have been caused by the normal 'crash' of an intense bloom, but this was not followed up by a BGA recovery and a subsequent high bloom. The 'bloom, crash, bloom' behaviour seen in Figure 3 and Figure 6 prior to the introduction of *Diatomix* did not occur in this instance.

Once diatoms are able to re-establish in a lagoon system, the population is more stable and their growth reduces and holds back the BGA populations. This is further demonstrated in the final month of the first trial in this case study (up to May 2016), where the proportion of BGA that made up the Total Cell Count reduced from an average of 93% early in the dosing down to an average of 47% in the month of May, with the last sample date being an average of 22% BGA in the Total Cell Count. During this Case Study, the BGA cell count reduced by 99.4% from the first sample to the last.

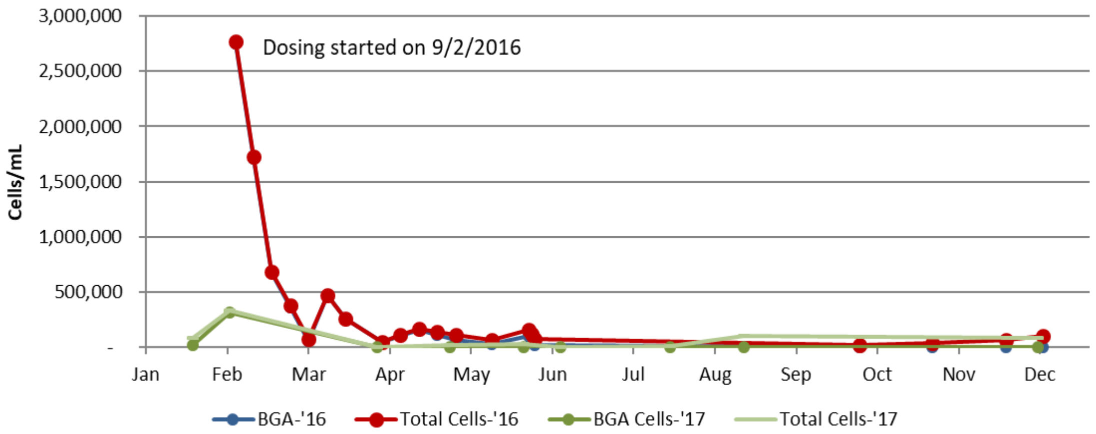


**Figure 9 - Case Study 5 – Single Lagoon - Total Cell Count and Blue-Green Algae counts over a 96-week period with two dosing periods. Dosing started in February '16 and went through to June '16. The dominating BGA bloom reduced and then the BGA count began to reduce further, with other algae then being seen in the Total Cell Count. Dosing began again in October 2016 and since then there have been 7 samples showing 0 cells/mL BGA, and the highest BGA Cell Count in the last 14 months was 311,000 cells/mL. Due to long periods between sampling it is not clear how long that spike actually lasted.**

**Figure 10 – The state of the Lagoon at the start of Case Study 5 and 14 months later**

Since October 2016 this site has instigated a dosing plan, with dosing of *Diatomix* being managed through the use of the automated, solar-powered, *Diatomixer* system available from AlgaEnviro. The clarity is markedly better than has been seen in the last year.

In the 20 months prior to dosing *Diatomix*, the average nitrate and Total Nitrogen concentrations at this site were 0.96 mg $NO_3^-$/L and 6.56 mg TN/L respectively. After *Diatomix* dosing began at this lagoon, the average values have reduced to 0.81 mg $NO_3^-$/L (15% reduction) and 4.72 mg TN/ L (28% reduction) respectively. The site also ran two aerators (5.5 kWh) for about 20 hours per day, intended to increase the dissolved oxygen and improve lagoon stability. Four months after dosing began these aerators were turned off and have not been used again. The power cost savings at this site are greater than the annual cost of *Diatomix* dosing.

**Figure 11 – The state of the Lagoon after 6 weeks of dosing with *Diatomix*.**

## Case study 6

In a standard wetland system, the treatment process relies on the wetland to remove nutrients by being taken up by plants. At some point when removal efficiency has dropped below a set level, the wetland would normally be reset. One consequence of the removal efficiency reducing over time is an increase in the number of aquatic weeds and macro-algae due to higher levels of nutrient remaining dissolve in the water. As many of these weeds and algae grow in a 'bloom and bust' pattern there are often periods of high nutrient release from the site, when rotting biomass releases these nutrients and this means the receiving waters receive eutrophied water.

A recent trial in a wetland system, treating STP effluent was performed to determine if *Diatomix* treatment could alter the fate of the inorganic nutrients. The intention of this study was to assess if the use of *Diatomix* would stabilise the algal community, reducing the number of periods where nutrient levels were high, and also reduce the presence of aquatic weeds and macroalgae. An additional aspect of this trial was to determine if the food webs would improve and increase biodiversity, given the assumption that *Diatomix* enhances the diatom growth and diatoms make up a large portion of the base of many food webs.

The control of weeds such as Azolla and macroalgae, e.g. filamentous algae were apparent soon after the trial began. These were observed both visually from the reduction of these plants (Figure 12 and Figure 13) as well as from spikes in the total nitrogen and phosphorus as the biomass died back and degraded. Once the greater majority of the biomass had died back and degraded, the Total Nitrogen levels could be seen to have stabilised and the reduction of water weed and macroalgae was also apparent.

**Figure 12 – The release point of a wetland cell (Cell 2) treated with *Diatomix*. The top picture is Day 0 of treatment and the bottom picture is 100 days later. The reduction of *Azolla spp.* and filamentous macroalgae is very apparent.**

**Figure 13 – The release point of the wetland cell (Cell 3) that is fed Cells 1 and 2. Cell 1 was the untreated Control cell and Cell 2 was also treated with *Diatomix*. The top picture is Day 0 of treatment and the bottom picture is 100 days later.**

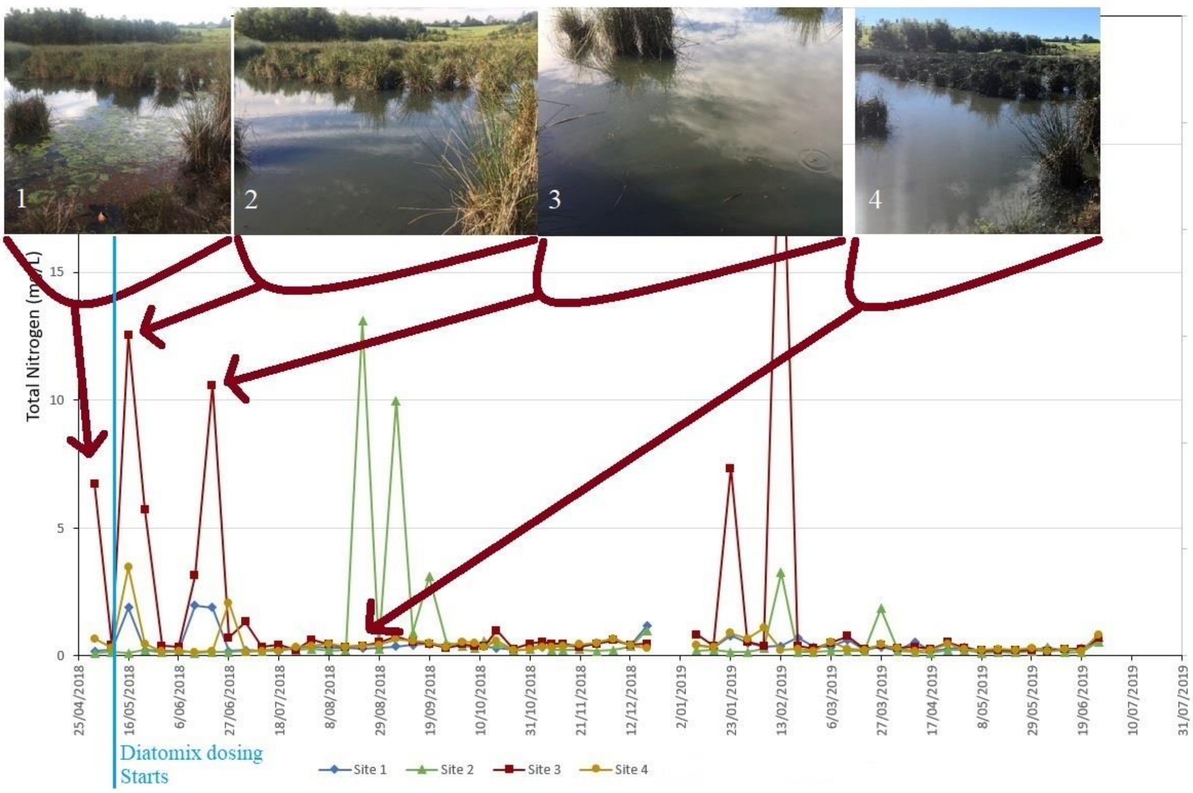


**Figure 14 – Prior to *Diatomix* dosing, Total Nitrogen at site 3 was 7mg/L, and weed and macroalgae was present (Inset Photo 1). After dosing started the first two spikes of Total Nitrogen were associated with higher microalgae counts (Inset Photo 2 and 3) as the weed and macroalgae had died back and was degrading. 100 days after dosing started, Total Nitrogen was significantly lower and the water was free of weed, macroalgae and microalgae (Inset Photo 4).**

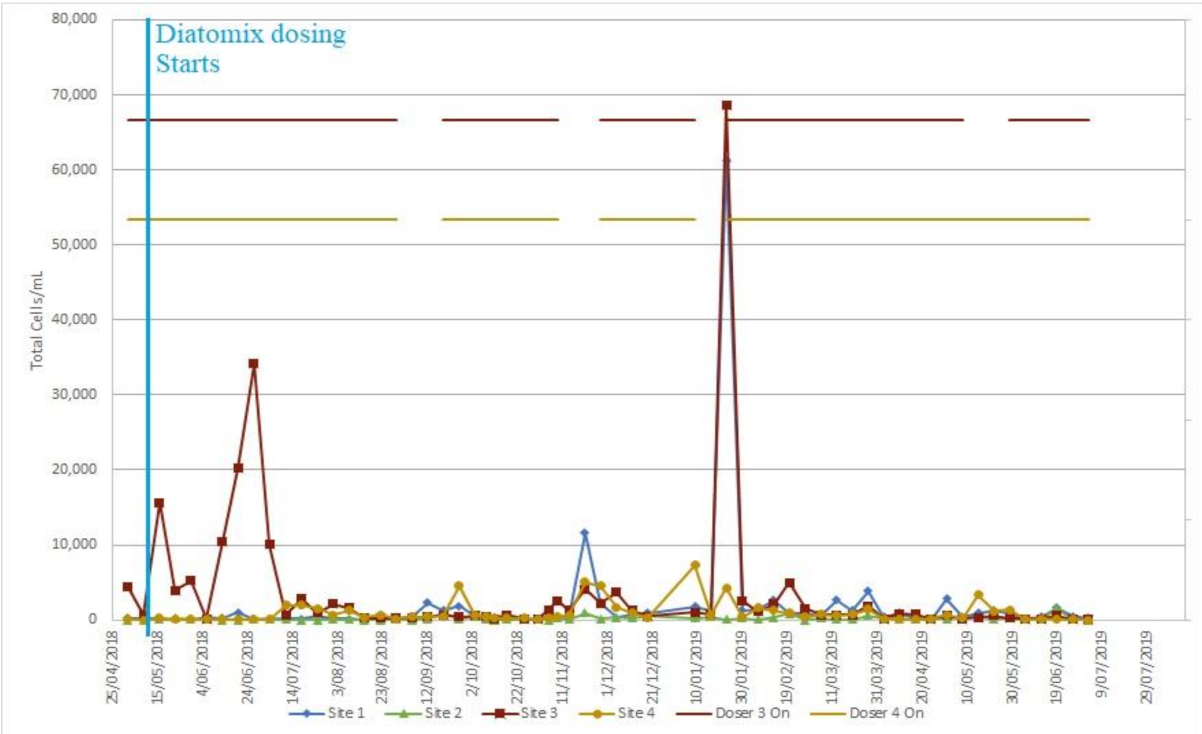


**Figure 15 – Total algae (Cells/mL) at the 4 test sites in the wetland trial. Sixty days after dosing had begun cell counts were generally low. A spike in Cells/mL at Site 3 may be associated with a period when the *Diatomix* dosers were not working, although Site 1 (a Control wetland cell) also spiked at that time.**

The change in the state of the wetland cells is more visually apparent than the nutrient and microalgal data reveals. Because weeds and macroalgae can use nutrient at similar levels to microalgae, the small changes in the nutrient profile may imply there has been limited improvement. As well as this, a wetland system is designed for macrophyte plants take up nutrients and this should limit microalgae growth. The sampling regime did not monitor the presence of weeds and macroalgae, which is why the photographs through this study tend to highlight the major changes at this site. At the time of preparing this report, the results of a biodiversity study that occurred during this Case Study have yet to be released, but will be added into this report when it is available.

## Summary

The six case studies presented here cover a range of scenarios in regard to the conditions of a raw water storage, aquaculture ponds, STP lagoons or STP effluent treated in lagoons or wetlands. Each case study has a notable change in the performance of the site. These changes have been in relation to reductions in the total cell counts, the percentage of the blue-green algae present in the water or reductions in macroalgae and weed growth within the waterbodies treated.

At the sites with high Total Cell counts and high blue-green algae counts, in all case studies a marked reduction has been demonstrated between the starting cell count (or the average cell count) during the period leading up to *Diatomix* being dosed. Case study 5 has clearly demonstrated a reduction and long-term stabilisation in the algae and blue-green algae counts.

The continued dosing of *Diatomix* will lead to improved performance as the waterbody establishes a new biota and ecosystem that is not seen during blue-green algae or macroalgae/weed dominance. The biodiversity study that will soon be available for Case Study 6 is expected to demonstrate these changes.

The data demonstrates that *Diatomix* reduces blue-green algae counts, and the photo records of Case Studies 4 and 6, and many other not presented here, demonstrate the reduction of macroalgae and weed (Azolla, duckweed, Salvinia) growth. When tested, the data also shows that nutrient levels reduce, and it is this reduction in ammonia, nitrate and phosphate that lowers the growth of BGA and or macro weeds. *Diatomix* is being used in raw water storages, drinking water storages, aquaculture farms for prawns and barramundi, stormwater lakes and dams, as well as for sewage treatment systems. Because of the increased activity of diatoms, especially benthic diatoms, induced by the addition of *Diatomix* there have been many positive changes to the water quality.

Prolonged use of *Diatomix* has demonstrated the long-term benefits to reducing nutrient levels and unwanted blue-green algae, macroalgae and weed growth in a simple and effective way.