BiOWiSH™ TECHNOLOGIES

Case Study

BiOWiSH™ Aqua

BiOWiSH™ Aqua Achieves Rapid Bioremediation

Improves Water Quality in 2 Hectare Ornamental Lake

Executive Summary

Findings

BiOWiSH™ Aqua achieved rapid bioremediation to significantly improve the water quality of Ranisangar Chaupati Lake over the course of our 8-week study. At the beginning of our case study, Ranisangar Chaupati Lake was graded on the Water Quality Index (WQI) as bad water quality and on the Trophic State Index (TSI) as highly eutrophic. BiOWiSH™ Aqua was added to the lake as an all-natural, environmentally safe protocol to improve the water quality. This resulted in overall improvement in the WQI from 36.6 (Bad quality) to 57.5 (Moderate quality) and the trophic status of the lake improved from hypereutrophic to eutrophic state.

The Dosing Process

The overall dosing investment was no more than 2 hours per week. First, the lake was arbitrarily divided into 11 sections to help with the dosing process. 22 samples were taken 5 times throughout the 8-week study. These samples were analyzed and included in this case study under the Results section. When the data was compiled, we saw improvements in all areas of testing.

Objective

The main objective behind the project was to improve the overall water quality of Ranisagar Chaupati Lake through the implementation of an all-natural environmentally safe protocol using BiOWiSH™ Aqua.

Background

The state of Chhattisgarh in central India was created in the year 2000 as a result of the Madhya Pradesh Reorganization Act. It is the 10th largest state in India (by surface) and with a population of over 25 million people it is one of the fastest developing states in India. The state is dotted with numerous lakes, ponds and other water bodies that serve as a major tourist attraction. Most of the pilgrimage sites in Chhattisgarh are located in the vicinity of a lake or a pond. Human activities in the vicinity of the water bodies have caused deterioration of water quality. The Ranisagar Chaupati – a highly eutrophic lake, located in Rajnandgaon district of the state Chhattisgarh – was suggested by the local municipal corporation as a location to demonstrate BiOWiSH™ Aqua's capacity to improve water quality through an all-natural bioremediation protocol.

Fig. 1 *Google-map of the area showing the study area - Ranisagar Chaupati lake, the park, surrounding lakes and nearby communities.*

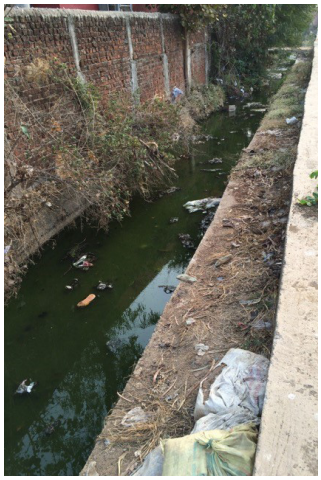


Fig. 2 *Raw sewage in nallah surrounding Chaupati pond.*

Fig. 3 *Nallah overflow into Chaupati side pond.*

The Ranisagar Chaupati Lake situated at 21°5'28"N 81°1'22"E in Rajnandgaon district is surrounded by garden which is used for public recreation and is likely to receive a higher nutrient load than any other lake situated in its vicinity. The lake has a surface area of 20100 m2 (2.01 ha) and an average initial water depth of 1.3 m. Stagnant bodies of water such as lakes or ponds are typically more affected by surrounding human activities than flowing surface water (like streams or rivers). Ranisagar Chaupati is used for public recreation, but the pond has been receiving discharges of domestic sewage and gray waters from an open canal which collects from surrounding settlements (nallah) for over a decade.

BiOWiSH™ continues to work in different settings around the world in an effort to demonstrate the power of its all natural bioremediation protocols as a means to improve water quality and provide a long term solution with zero capital expenditure.

Solution

Ranisagar Chaupati is basically a small and shallow lake which continuously received inflow of domestic sewage and grey waters from the nearby households over the past decade. An eight-week bio remediation protocol using BiOWiSH™ Aqua was implemented starting December 28th, 2015. Before starting the study, all of the pond's inlets and outlets were closed and floating aquatic plants and debris were removed.

Samples drawn in December 13th, 2015 revealed the following starting water quality:

Parameters	Unit	Lake condition before bioremediation with BiOWiSH™ Aqua*	Bathing Water Standards as per MoEF**
PH		9.3	6.5 - 8.5
DO	mg/l	3.6	5 or more
COD	mg/l	88.8	<10
BOD	mg/l	34.7	3 or less
TSS	mg/l	58.5	<10

Table 1 *Table explaining initial sampling*
**Results for samples drawn on December 13th, 2015*
***Standards for Bathing water as per MoEF- Ministry of Environment, Forest and Climate Change (Government of India)*

Dosing

The lake was arbitrarily divided into 11 sections to facilitate dosing.

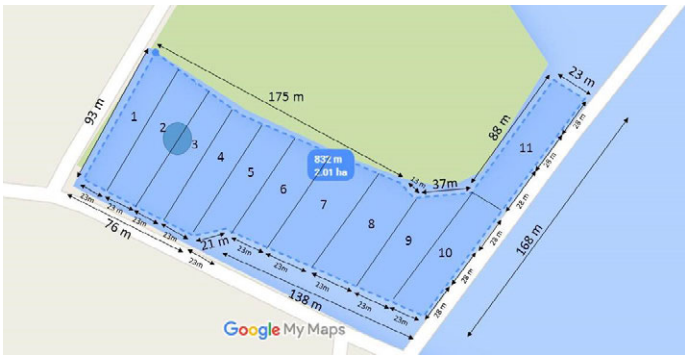


Fig. 4 *Lake divided into 11 sections*

Date	Description
December 28th	1st dosing (Shock Dose)
January 7th	2nd dosing - Maintenance Dose
January 14th	3rd dosing - Maintenance Dose
January 21st	4th dosing - Maintenance Dose
February 4th	5th dosing - Maintenance Dose
February 11th	6th dosing – Maintenance Dose
February 18th	7th dosing – Maintenance Dose
February 25th	8th dosing – Maintenance Dose

Table 2 *Summary of dosing events*

An initial shock dose of 56 kg was sprayed on December 28. Lower maintenance doses of 14 kg (aiming to maintain a 0.5 ppm activity for the calculated water volume) were added once a week.

BiOWiSH™ Aqua is a water soluble powder and ships ready to dose. For each pond section, the required amount of product was dissolved in pond water and sprayed on the surface using a portable pump. The entire dosing event for the 2 ha pond took no longer than two hours each week.

Fig. 5 *Team getting ready for dosing*

Fig. 6 *Product used: BiOWiSH™ Aqua*

Fig. 7 *NEERI team dosing BiOWiSH™ Aqua in Ranisagar Choupathi Lake*

It is important to point out that the bioremediation program using BiOWiSH™ Aqua was set to last 16 weeks. However, this report only reflects data collected during the initial 8 weeks of the study. After closing all inlets to the pond, water loss rate due to ground seepage and evaporation was seen to be far greater than expected. On March 1st (eight weeks into the study) several thousand cubic meters of water were pumped from the neighboring Ranisagar lake, so for the purpose of this study, we are only presenting the data collected prior to pumping.

Sampling

CSIR – NEERI: The National Environmental Engineering Research Institute (NEERI) established in Nagpur in 1958, was created and funded by the Government of India (GoI) reporting to the Ministry of Science and Technology (India) of Central Government. NEERI is a pioneer laboratory in the field of environmental science and engineering and part of Council of Scientific and Industrial Research (CSIR) having five zonal laboratories at Mumbai, Kolkata, Hyderabad, Chennai and Delhi.

NEERI played a major role in auditing the entire project. Since the bioremediation project for Ranisagar Chaupathy lake was approved by the State Government of Chhattisgarh, it was important that the sampling,

chain of custody and water quality analysis be done by a reputable GoI certified lab. NEERI's chief scientists played a key role during the entire project and all of the data used for this report can be found on their published final report for the project.

NEERI's sampling protocol included 22 sampling locations distributed across the pond's area. Grab samples were collected from these locations on the dates shown in the table below. Surface water samples were collected directly without any filtration in acid-rinsed polypropylene bottles, depth water samples were collected approximately 1 m from the surface using depth sampler and transferred to clean polypropylene bottles and sludge samples from the bottom of the lake were collected using a van-veen sediment sampler and then transferred into zip-lock polyethylene bags. Similarly, samples for phytoplankton and zooplankton were collected separately and preserved immediately on-site, while samples for chlorophyll a estimation were collected in acid-rinsed amber colored polypropylene bottles and kept away from direct sunlight until analyzed. Separate samples for fecal coliform analysis were collected in sterile polypropylene bottles. Sample pH, temperature and dissolved oxygen were immediately determined on-site, while the remaining samples were shipped for analysis at CSIR-NEERI at Nagpur after adequate preservation of the collected samples.

Amongst the 5 sampling events conducted, 2 sampling events (2nd and 4th monitoring) were conducted to analyze 13 major parameters (pH, temperature, DO, COD, BOD, ammonia, nitrate, TKN, phosphate, suspended solids, total dissolved solids, fecal coliform and chlorophyll a) and 3 sampling events (1st, 3rd and 5th monitoring) were conducted to analyze 33 parameters for complete analysis of the lake including water characteristics, sediment characteristics, aquatic diversity and physio-chemical parameters inclusive of above mentioned 13 parameters.

Date	Description
December 13th	1st sampling before dosing (lesser parameters)
January 13th	2nd sampling (full parameters)
January 27th	3rd sampling (lesser parameters)
February 10th	4th sampling (lesser parameters)
February 24th	5th sampling (full parameters)

Table 3 *Summary of sampling events*

Fig. 8 *Satellite Image of Ranisagar Chowpathy Lake*

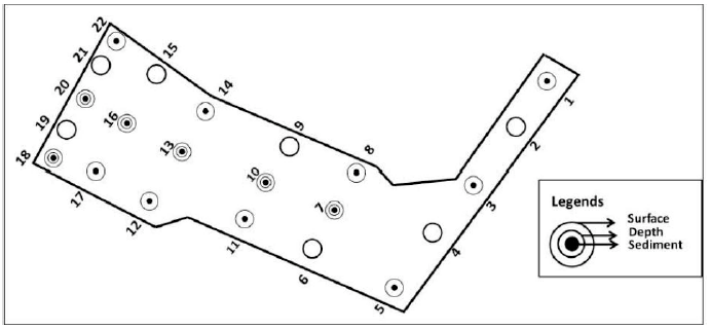
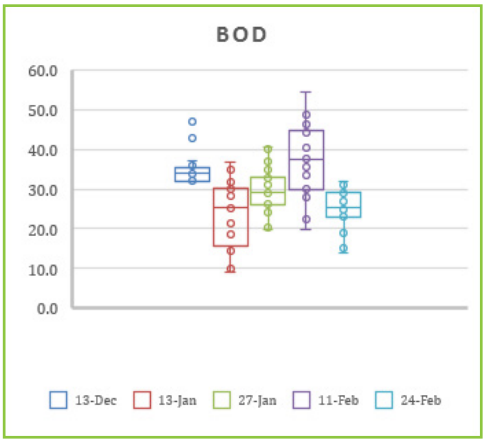


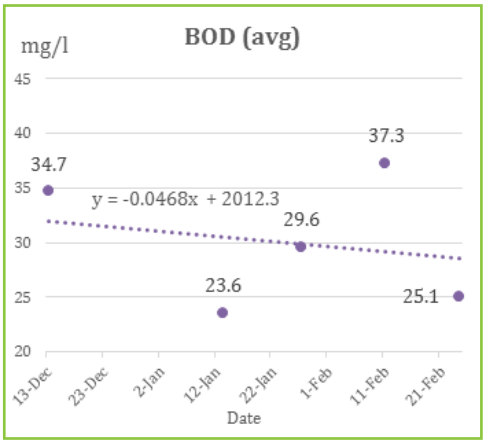
Fig. 9 *Schematics of sampling location in Ranisagar Lake*

Results

The graphs below show the 22 surface water samples for each sampling event plotted in a "box graph" style. The top and bottom lines for the box represent upper and lower quartiles, while the center line shows the mean (arithmetic average for the 22 samples). The bars extending from the boxes show: Quartile +/- 1.5 * (Inter Quartile Range)

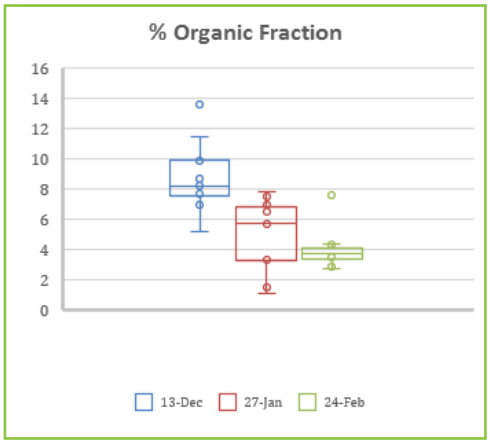


Graph 1 *Five point summaries of data collected during the study period for BOD*

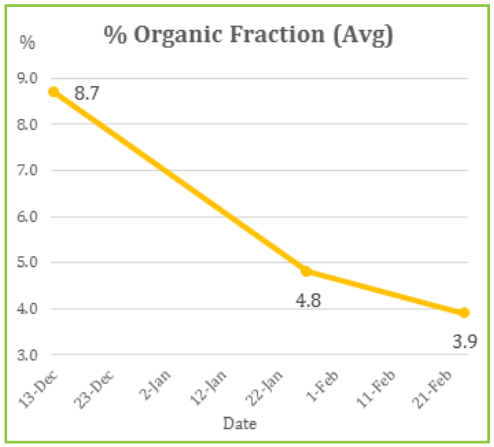


Graph 2 *Average values of 22 sampling points for BOD*

The fluctuations in BOD can be attributed to the rapid degradation of organic matter in the system. This increased biological activity in the sediment may have resulted in changing release of organic components which contributed to varying BOD readings. Higher temperature and low water depth also contributed to the increase in BOD after the 2nd monitoring. It is important to show that the BOD trend line in Graph 2 (adjusting to a linear fit) exhibits a negative slope.



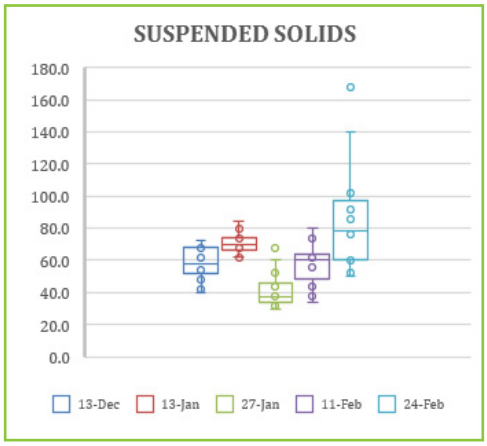
Graph 3 *Three point summaries of data collected during the study period for % Organic Fraction*



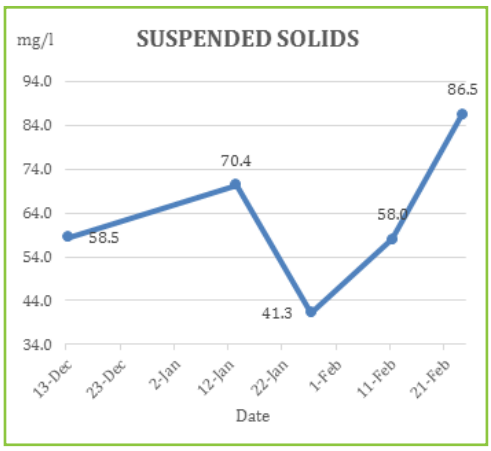
Graph 4 *Average values of 13 sampling points for % Organic Fraction*

Sludge in sewage treatment lagoons are likely to contain over 50% organic fraction. Old material which is allowed to mineralize for years may have 10 to 15% organic fractions. Chaupathi pond sludge was found to have an initial 8.7% organic fraction, with very high levels of Fe and Mn – indicating iron rich sandy soils and a very advanced stage of mineralization, a very poor indication for bioremediation. Despite this, a rapid action of the biology in BiOWiSH™ could be seen on the sludge by week 4. A 45% drop in sludge organic fraction was seen 4 weeks into the program.

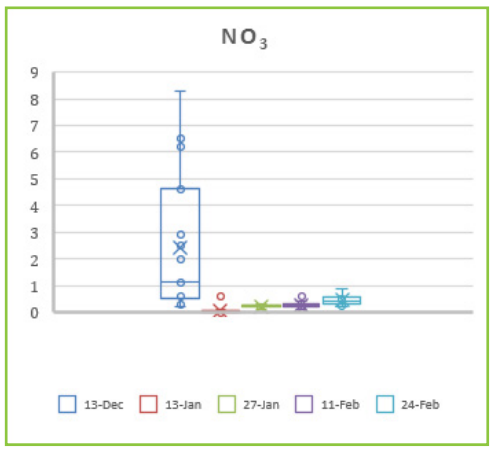
Increased biological activity is also reflected in the TSS spike seen below. Achieving lower BOD and sludge organic fraction values in 8 weeks shows how rapidly BiOWiSH™ microorganisms can catalyze biological action in water systems.



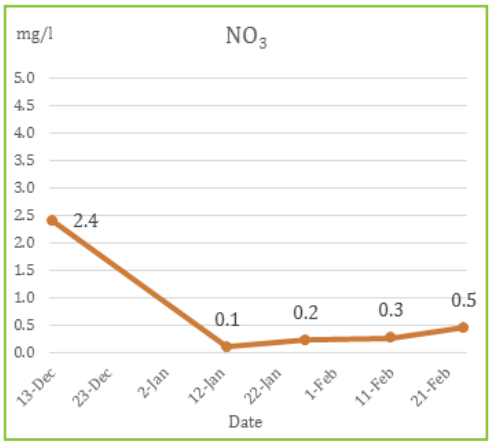
Graph 5 *Five point summaries of data collected during the study period for Suspended Solids*



Graph 6 *Average values of 22 sampling points for Suspended Solids*

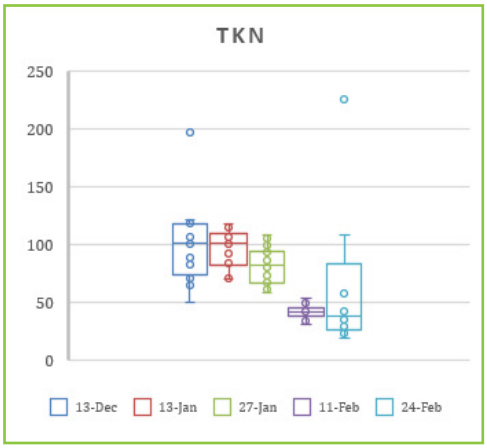


Graph 7 *Five point summaries of data collected during the study period for NO_3*



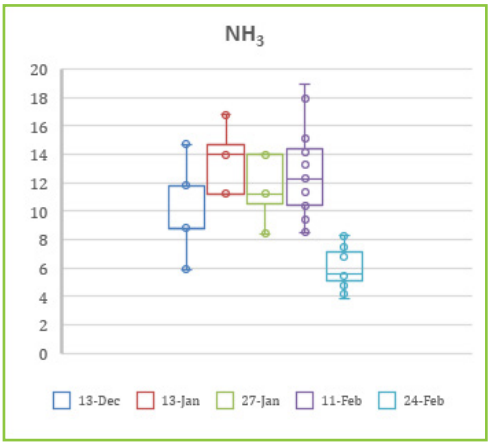
Graph 8 *Average values of 22 sampling points for NO_3*

BiOWiSH™ Aqua contains a consortium of microorganisms which can facilitate diverse nitrogen management metabolic pathway in water bodies. The microbial consortium in BiOWiSH™ Aqua is able to promote ammonia (NH_3) oxidation directly to gaseous forms of nitrogen through Heterotrophic Nitrification and Denitrification (HND). This results in lower ammonia nitrogen concentrations while avoiding peak formations of nitrite and nitrate (which may be toxic to aquatic life). The different oxidative/reductive pathways in HND by-pass the use of dissolved oxygen as an electron acceptor in aerobic nitrification, resulting in higher DO levels for the pond (as seen on Graph 14).

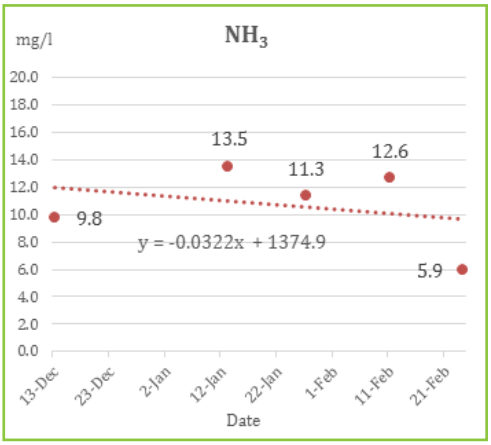


Graph 9 *Five point summaries of data collected during the study period for TKN*

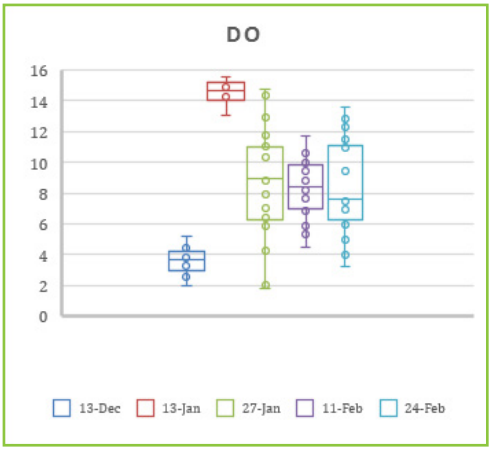
Graph 10 *Average values of 22 sampling points for TKN*



Graph 11 *Five point summaries of data collected during the study period for NH_3*



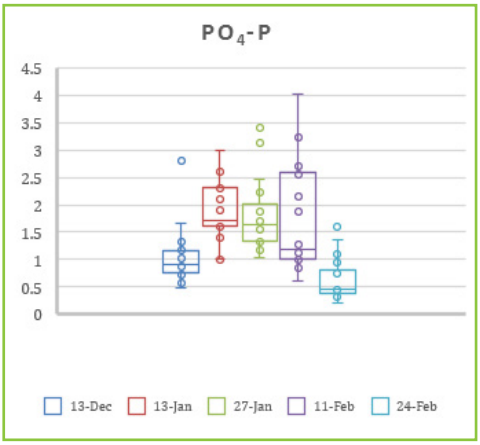
Graph 12 *Average values of 22 sampling points for NH_3*



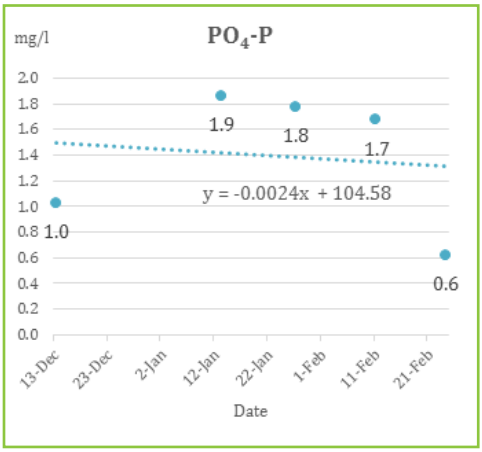
Graph 13 *Five point summaries of data collected during the study period for DO*

Graph 14 *Average values of 22 sampling points for DO*

We see an increase in phosphate concentrations which may be explained by the increased release of nutrients from the lake sediment via aggressive decomposition of organic fraction. A sharp drop in phosphate concentration could be observed after the 5th monitoring, indicating that the slower rate of organic degradation in the sludge allows for more effective phosphate settling.

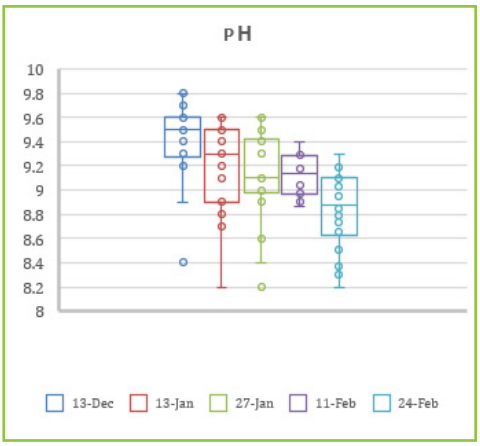


Graph 15 *Five point summaries of data collected during the study period for PO_4-P*

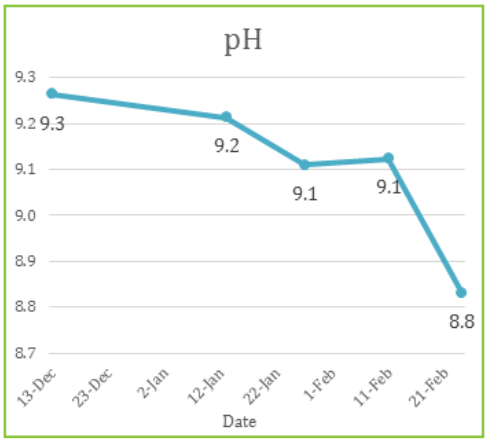


Graph 16 *Average values of 22 sampling points for PO_4-P*

During the study, the pH of the lake decreased consistently from an average value of 9.3 to 8.8. Decrease in pH indicates improved biological activity. BiOWiSH™ Aqua showed great effect on restoring the pH values in the lake water.



Graph 17 *Five point summaries of data collected during the study period for pH*



Graph 18 *Average values of 22 sampling points for pH*

Water Quality Index (WQI):

WQI, developed by National Sanitation Foundation (NSF-WQI), was used to quantify the change in the quality of water in response to dosing of BiOWiSH™ Aqua. NSF-WQI categorizes water quality into five categories: 0-25 Very Bad, 25-50 Bad, 50-70 Moderate, 70-90 Good and 90-100 Excellent. In this method, nine different physio-chemical parameters are used to determine the WQI (dissolved oxygen, fecal coliform, pH, BOD, temperature, total phosphate, nitrate, turbidity and total solids).

(http://bcn.boulder.co.us/basin/watershed/wqi_nsf.html).

The overall changes in the WQI of the lake during 1st monitoring to 5th monitoring is depicted as follows:

Sampling	1st	2nd	3rd	4th	5th
NSF-WQI	36.6	46.6	55.1	56.1	57.5
Water Quality	Bad	Bad	Moderate	Moderate	Moderate

Table 4 *Changes in the WQI of lake during 1st monitoring to 5th monitoring*

Trophic State Index (TSI):

Trophic State Index (TSI) classifies the trophic status of a lake or any water body using a measurable value. The most commonly used numerical classification method of the trophic status of a lake was that developed by Robert Carlson in the year 1977 and is known as Carson's Trophic State Index (TSI). Carson's TSI uses three dependent variables which have direct correlation with the trophic status of the lake, namely, algal biomass, transparency of the lake water (which enables light penetration), and the nutrient concentration to classify the trophic status of the lake into hyperoligotrophic, oligotrophic, mesotrophic, eutrophic and hypereutrophic conditions.

TSI Values	Trophic Status
<30	Hyperoligotrophic
30-40	Oligotrophic
40-50	Mesotrophic
50-70	Eutrophic
>70	Hypereutrophic

Table 5 *Classification of lakes based on TSI Values*

Based on the TSI values, Ranisagar Chowpathy lake was classified as hypereutrophic before starting the bioremediation project with BiOWiSH™ Aqua. The water quality improved significantly after dosing with BiOWiSH™ Aqua and resulted the trophic status of the lake to shift from hypereutrophic to eutrophic state in 8 weeks.

Discussion

The dosing of BiOWiSH™ Aqua in Ranisagar Chaupati Lake has remarkably improved water quality. This resulted in overall improvement in the water quality index (WQI) from 36.6 (Bad quality) to 57.5 (Moderate quality) and the trophic status of the lake improved from hypereutrophic to eutrophic state.

- The concentration of chlorophyll a, an algal pigment that imparts green color to the water, decreased by 40%. Decrease in concentration of Chlorophyll a was due to significant change in the population density (size) and dynamics of phytoplankton (algal) species. 21% reduction was observed in the phytoplankton population after dosing with BiOWiSH™ Aqua.
- The reduction in population of phytoplankton was reflected in reduction in pH of the lake water from an average value of 9.3 to 8.8. Very high pH in lake bodies results due to high photosynthetic activity.
- BiOWiSH™ Aqua also caused significant reduction in the concentrations of organic ammonia (represented as TKN), inorganic ammonia and nitrate by 65%, 40% and 79%, respectively.
- The organic carbon concentration of lake water, as represented in terms of BOD concentrations also showed an average reduction of 29% within two months of BiOWiSH™ Aqua dosing.

Parameters	Before Dosing (13th Dec 2015)	After Dosing (24th Feb 2016)	Observations	Remarks
pH	9.3	8.8	Decreased by 5%	Improved
Dissolved Oxygen	3.6	8.4	Increased by 133%	Improved
Chlorophyll a	0.30	0.18	Decreased by 40%	Improved
TKN (as N)	98.7	34.5	Decreased by 65%	Improved
Ammonia (as N)	9.8	5.9	Decreased by 40%	Improved
Nitrate (as NO_3)	2.4	0.5	Decreased by 79%	Improved
Phosphate (as P)	1.0	0.6	Decreased by 40%	Improved
BOD (3d, 27°C)	35	25	Decreased by 29%	Improved
Sediment Organics (%)	8.7	2.4	Decreased by 72%	Improved
Phytoplankton (Density/L)	7.7×10^6	6.1×10^6	Decreased by 21%	Improved
Palmer's Pollution Index (PPI)	19	10	Decreased by 47%	Improved
Zooplankton (Density/L)	81333	81	Decreased by 99.9%	Improved

Table 6 *Effect of Bio augmentation with BiOWiSH™ Aqua on several parameters before and after dosing*

- Similarly, the lake sediment also showed a 72% reduction in organic fraction during this period.
- Improvement in the water quality is also reflected by overall decrease in the numbers of pollution indicating algal species in lake water as represented by Palmer's Pollution Index (PPI). The PPI of the lake decreased by 47% indicating improvement of lake water quality in terms of reduced nutrient concentration.

Continuing a long term bioremediation program with BiOWiSH™ Aqua will help to sustain these improvements in a cost-effective way.

Contact

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Biological help for the human race

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