



INNOECOFOOD - Eco-innovative technologies for improved nutrition, sustainable production and marketing of agroecological food products in Africa

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Characterization of spirulina strains from local lakes

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1. Summary

As part of WP4, Task 4.1, field visits for algae sampling were conducted in all four ECOHUB partner countries: Kenya (Oct 14–21, 2024), Tanzania (Dec 14–21, 2024), Ghana (Nov 27–Dec 1, 2024, and Mar 6–9, 2025), and Egypt (Feb 16–20, 2025). Samples were collected from various local lakes and Spirulina farms in each country, including Lake Simbi, Lake Nakuru, Lake Oloiden, Lake Elementaita, Lake Bogoria, Keriko Secondary School Spirulina Farm and the IIMSAM (Intergovernmental Institution for the use of Micro-algae Spirulina Against Malnutrition) Spirulina farm from Kenya, Lake Big Momella, Lake Small Momella, Lake Natron and Lake Manyara from Tanzania, Lake Volta, CSIR-WRI, Bio-Green Agro Farm, and Fish&Feed Farm from Ghana, and Amria Farm for Microalgae and the Lake Elhamra (Red) from Egypt. All algal samples from sources were labeled accordingly, and transported to BMAEU laboratories for characterization.

At each lake, samples were collected from three different spots, with three samples per spot. Around 1.5 L of lake water and 1 L of farm tap water were collected for quality and toxicity analysis. After the filtration, all water samples were filtered and analyzed on-site for the 7 different parameters including pH, nitrate (NO₃⁻), sodium (Na⁺), calcium (Ca⁺²), potassium (K⁺), salt (NaCl), conductivity and temperature using mobile device. All water samples were also analyzed for ammonium (NH₄), dissolved oxygen (DO), total suspended solids (TSS), phosphate (PO₄), sulfate (SO₄), magnesium (Mg), aluminum (Al), copper (Cu), zinc (Zn), arsenic (As), iron (Fe), cadmium (Cd), cobalt (Co), lead (Pb), chromium (Cr), nickel (Ni), and manganese (Mn) by an accredited institutional laboratory. Each parameter was measured in triplicate, with results reported as averages. Due to distinct national standards, local project partners are responsible for interpreting the data.

Through the studies on isolation and selection of new strain, 11 Spirulina strains were phenotypically isolated and monocultured—five from Kenya, five from Tanzania, and one from Egypt. No strains were found in Ghana. Light and confocal laser scanning microscopy (CLSM) were used for morphological characterization. The samples showed strong autofluorescence, enabling high-resolution imaging without staining. However, phenotypic analysis alone was insufficient for species-level identification, prompting further molecular analysis.

Molecular identification using five genetic markers—16S rRNA, 23S rRNA, *cpcBA* intergenic spacer, sulfolipid biosynthesis genes (*sqdX*, *sqdB*), and the RuBisCO large subunit gene (*cbbL*)—enabled robust species classification and identification. *Arthrospira platensis* and *Limnospira fusiformis* were consistently detected, confirming successful isolation of Spirulina strains. The Keriko Farm isolate was confidently classified as *Arthrospira platensis* or a related *Limnospira* species, and similar identifications were made for samples from Lake Bogoria and IIMSAM Farm. Although the *sqdB* gene could not be amplified in the Amria Farm isolate—possibly due to a mutation or primer mismatch—other markers confirmed it as Spirulina. The CYA106-F/CYA781Rb primer pair provided useful species-level resolution only for the Keriko sample.

Currently, the isolated and identified new strains are being cultivated and their optimum growth conditions and performance are being assessed.

2. Objective

The objective of the deliverable was to characterize the diverse isolated spirulina strains collected in local lakes in each ECOHUB partner countries (Kenya, Ghana, Tanzania and Egypt) in Africa from the phenotypic and molecular levels.

3. Characterization of spirulina strains from local lakes

3.1 Sampling in Kenya

3.1.1 Summary Report for Kenya Field Work and Sample Collection

BMAEU staff has visited **Kenya** for the field and sampling work, where one of the ECOHUB centers in Africa are located, **between 14-21 October 2024**. We carried out the project work together with the project partner researcher **Dr. Mary Opiyo from Kenya- Marine and Fisheries Research Institute (KMFRI)** and **Dr. Judith Nyunja from Kenya- Wildlife Research and Training Institute (Kenya- Wildlife Research and Training Institute)**. During the fieldwork, samples of lake water and Spirulina were collected particularly from alkaline lakes in Kenya, namely **Lake Simbi, Lake Nakuru, Lake Oloiden, Lake Elementaita and Lake Bogoria**. We were accompanied by officials and security units from the National Parks where the lakes are located, and guided by local people who lives in the sampling area. Then we visited the Spirulina farm established by the teachers and students of **Keriko Secondary School in Kericho city** and took samples of the water used in the culture and the Spirulina grown. Finally, **the IIMSAM (Intergovernmental Institution for the use of Micro-algae Spirulina Against Malnutrition) Spirulina farm** located near Kisumu city was visited, where samples of water used in the culture and Spirulina grown were taken. All water samples were measured for parameters such as temperature, pH, salinity, conductivity, nitrate, potassium, calcium, sodium, and geographical, flora and fauna information were noted. Finally, the samples were properly preserved and brought to Turkey in a way that would preserve the vitality of the organisms in them. This 8-days task was completed safely, effectively and efficiently. We visited the research institute of our project partner KMFRI in Kisumu. Here, some samples from the field were filtered and measured.



KMFRI Research Institute in Kisumu

3.1.2 Sampling Data from the visit to Kenya (14-21 October 2024)

During the fieldwork, lake water and Spirulina samples were collected from three different spots of each local lakes including **Lake Simbi, Lake Nakuru, Lake Oloiden, Lake Elementaita and Lake Bogoria**. Also, spirulina culture samples and tap water samples were taken from the local, private Spirulina farms; **Keriko Secondary School Spirulina farm** and **the IIMSAM (Intergovernmental Institution for the use of Micro-algae Spirulina Against Malnutrition) Spirulina farm** in Kenya. Local people guided us in reaching the sampling points and collecting the samples.

On October 16th, Lake Oloiden and Lake Elementaita were visited for sampling. Lake Oloiden is a small alkaline lake in the Great Rift Valley of Kenya. It is located near Lake Naivasha, a larger and better-

known freshwater lake in the region. Lake Oloiden is known for its alkaline properties. Because it is separated from Lake Naivasha, its salinity can be higher than Lake Naivasha. Therefore, it has its own flocks of flamingos and hippos, which can be quite numerous. Although this lake is normally a separate lake from its immediate neighbor, Naivasha Lake, due to rising water levels, the isolation has occasionally been broken, causing the water of Naivasha Lake, a freshwater lake, to mix with the water of Oloiden Lake, an alkaline lake, and diluting the alkalinity of Oloiden Lake's water. Dr. Judith, a local researcher from the National Wildlife Research and Training Institute, reported that they had previously isolated spirulina samples from Naivasha Lake, but no samples had ever been taken from Oloiden Lake. Therefore, sampling was not done for Naivasha Lake.



Lake Naivasha



Lake Oloiden



Lake Elementaita

NASA - World Wind, Satellite images of Lake Naivasha, Lake Elementaita, and Lake Oloiden in Kenya. Permission details; These images are in the public domain because it is a screenshot from NASA's globe software World Wind using a public domain layer, such as Blue Marble, MODIS, Landsat, SRTM, USGS or GLOBE.



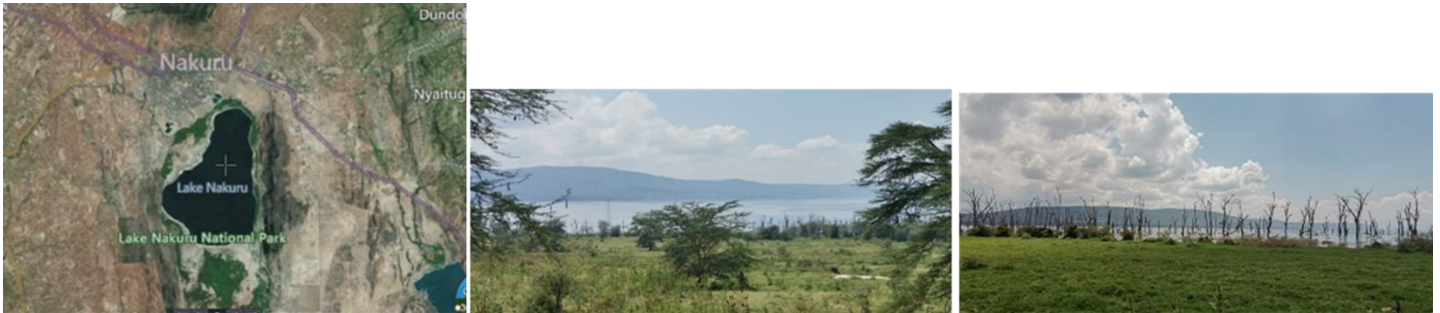
Lake Oloiden

Lake Elementaita is a soda lake (high alkalinity, high biodiversity) located between Lake Naivasha and Lake Nakuru. Today the lake is a protected area due to its bird life and has been named as a UNESCO heritage site together with Lake Nakuru and Lake Bogoria. It seems that the rainwater diluted the water of the lake Elementaita and increased the water level. Therefore, the alkaline characteristic of the lake has decreased. It is home to pelicans and other birds. Especially the digestive wastes left by pelicans are visibly dense at the water's edge. Since sampling was carried out from 3 different spots very close to each other and permitted due to safety precautions, a common coordinate was used for these three spots.



Lake Elementaita

On October 17th, Lake Nakuru and Keriko High School Farm were visited for sampling. The Lake Nakuru's abundance of algae attracts a vast quantity of flamingos that famously lined the shore. Millions of flamingos nesting along the shores. The surface of the shallow lake is often hardly recognizable due to the continually shifting mass of pink. It is reported that about 10,000 years ago, Lake Nakuru, together with neighboring Lake Elementaita and Lake Naivasha formed one single, deep freshwater lake that eventually dried up, leaving the three lakes as remnants. It is also reported that water levels in Lake Nakuru have since varied considerably, with the lake almost drying up several times over the past 50 years. In 2013, levels again increased rapidly, leading to the migration of many flamingos to Lake Bogoria in search of food supply., leading a dramatic change for algae growth in the lake.



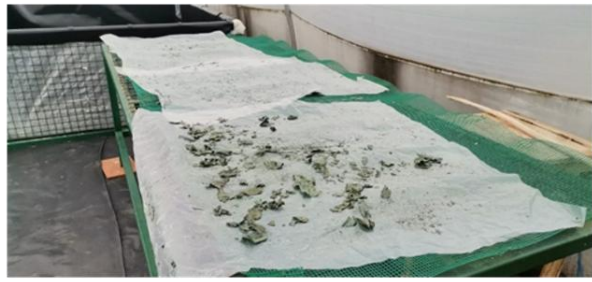
Lake Nakuru

NASA - World Wind, Satellite image of Lake Nakuru in Kenya. Permission details; This image is in the public domain because it is a screenshot from NASA's globe software World Wind using a public domain layer, such as Blue Marble, MODIS, Landsat, SRTM, USGS or GLOBE.

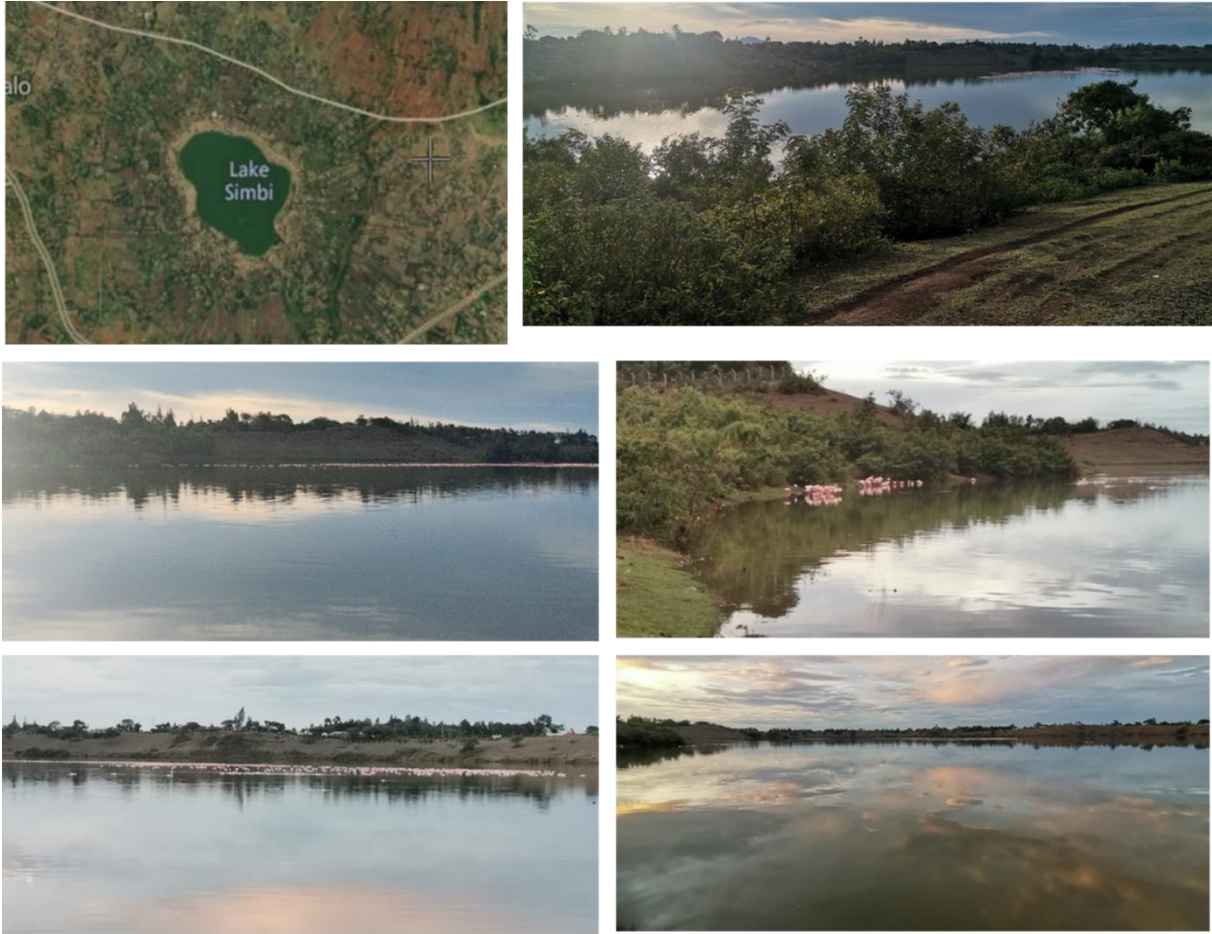
Keriko Secondary School Spirulina farm. This farm was founded by Benjamin Buluku, a teacher at Keriko High School, and is still actively producing spirulina, and the spirulina powders are served for breakfast to children in primary schools in the region. Benjamin Buluku runs the farm with his students and has knowledge and experience in spirulina farm establishment, cultivation, harvesting and sustainability.

On October 18th, Lake Simbi was visited for sampling. Lake Simbi is a small alkaline crater lake of volcanic origin with no known inlet and outlet, measuring about one kilometer in radius. Lake Simbi supports a substantial bird population that includes flamingos. Due to its high alkalinity, the lake is not used for drinking and has no fish. Mining of salt by the locals from the lake.

On October 19th, the IIMSAM Farm was visited for sampling. IIMSAM is one of the thirty (30) Inter-Governmental Organizations (IGOs). IIMSAM is an entirely Humanitarian IGO that has been playing a proactive role in providing nutritious Spirulina as aid worldwide. IIMSAM's Flagship Program is named as "Sheikh Zayed Bin Sultan Al Nahyan Spirulina Feeding Centre" in Kisumu, Kenya. IIMSAM is an organization in Kenya that has been in existence since 2006. IIMSAM owns two spirulina tanks, which are currently operated. Dimensions of each pond are 30 X 3 X 0.4 meters (length X wide X depth) = 36 m² volume. It was reported that they harvest a total of 10 kg of spirulina powder per month from both ponds. They also use Zarouk media in the initial cultivation, and Zarouk media with urea after the harvest. It is also noted that there are two different strains, spiral and straight forms of Spirulina, the straight one (light green culture) is from local company called Tiwani (<https://www.tiwanispirulina.com>) and other spiral one (dark green culture) is from Switzerland-France.



Spirulina Farm in Keriko Secondary School



Lake Simbi

ASA - World Wind, Satellite image of Lake Simbi in Kenya. Permission details; This image is in the public domain because it is a screenshot from NASA's globe software World Wind using a public domain layer, such as Blue Marble, MODIS, Landsat, SRTM, USGS or GLOBE.

After the field and sampling work was completed, each of the samples was kept uncovered at room temperature and in sunlight until they were returned to Turkey, in the meantime Zarrouk Media was added to each sample to allow the possible spirulina species to survive.

Three spots were identified (within safety and transportation conditions) and 3 samples were taken from each spot. Separate 500 mL samples of goal water were also taken from each spot to measure water quality. All unfiltered water samples were measured for parameters such as temperature, pH, salinity, conductivity, nitrate, potassium, calcium, potassium, sodium. Also, geographical coordinates, local flora and fauna information was noted, too. GPS coordinates were taken from the link; <https://www.gps-coordinates.net/gps-coordinates-converter> .



Lake Bogoria

NASA - World Wind, Satellite image of Lake Bogoria in Kenya. Permission details; This image is in the public domain because it is a screenshot from NASA's globe software World Wind using a public domain layer, such as Blue Marble, MODIS, Landsat, SRTM, USGS or GLOBE.

On October 20th, Lake Bogoria was visited for sampling. Lake Bogoria (formerly Lake Hannington) is a saline, alkaline lake that lies in a volcanic region in a little north of the equator. Lake Bogoria is periodically home to one of the world's largest populations of lesser flamingos. Lake Bogoria is the only alkaline lake that has minimal lake water fluctuations, and is used as a refuge for flamingos and other animals when other relatively shallow lakes dry out. Lake Bogoria is a soda lake: it has no surface outlet; its waters are strongly alkaline (pH:10.5). High salt concentrations are also given (40‰) and this increases when the total amount of water decreases (overconcentration). Thus, following an extreme draught from March to October 2009, salt saturation reached 48‰. Its waters contain large concentrations of Na^+ , HCO_3^- , CO_3^{2-} et Cl^- ions. Soda lakes are highly productive ecosystems.

3.1.3 Field Work Locations for Sampling, Lakes and Farms visited in Kenya

Lake Oloiden 1. Spot:

Coordinates: 0°49'0"S 36°15'43"E Elevation: 1880 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Oloiden 2. Spot:

Coordinates: 0°49'0"S 36°15'43"E Elevation: 1880 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Oloiden 3. spot:

Coordinates: 0°49'37.72"S 36°17'19.51"E Elevation: 1880 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Elementaita 1. spot:

Coordinates: 0°27'1.9"S 36°15'37.841"E Elevation: 1782 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Elementaita 2. spot:

Coordinates: 0°27'1.9"S 36°15'37.841"E Elevation: 1782 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Elementaita 3. spot:

Coordinates: 0°27'1.9"S 36°15'37.841"E Elevation: 1782 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Nakuru 1. spot:

Coordinates: 0°18'48.92"S 36°5'4.601"E Elevation: 1768 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Nakuru 2. spot:

Coordinates: 0°24'43.12"S 36°6'52.48"E Elevation: 1765 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Nakuru 3. spot:

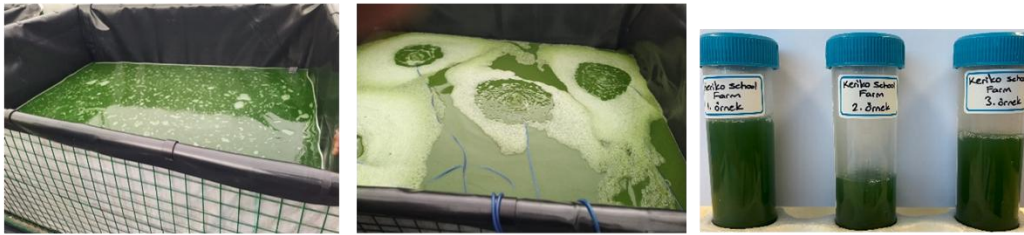
Coordinates: 0°18'48.92"S 36°5'4.601"E Elevation: 1769 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Keriko Secondary School Farm

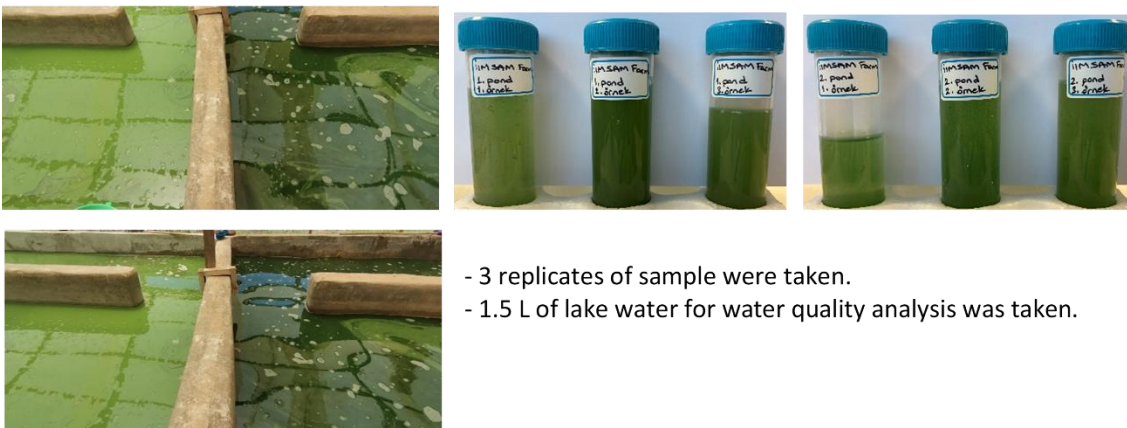
Coordinates: 0°23'39.975"S 36°1'18.556"E Elevation: 2020 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

The IIMSAM Spirulina Farm in Kisumu

Coordinates: 0°6'10.479"S 34°45'15.033"E Elevation: 1170 m



- 3 replicates of sample were taken.
- 1.5 L of lake water for water quality analysis was taken.

Lake Simbi 1. spot:

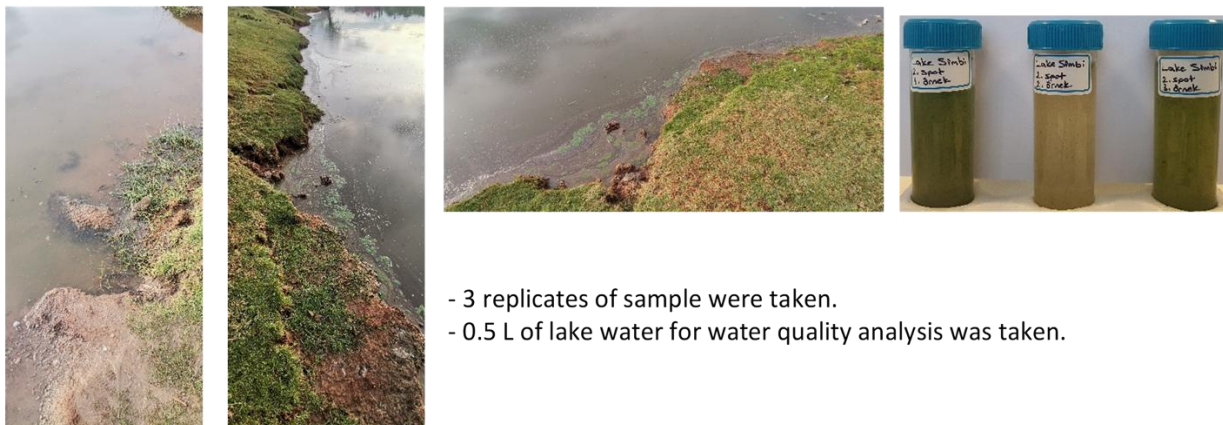
Coordinates: 0°22'13.318"S 35°37'54.44"E Elevation: 1148 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Simbi 2. spot:

Coordinates: 0°22'9.356"S 35°37'55"652E Elevation: 1146 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Simbi 3. spot:

Coordinates: 0°22'16.135"S 35°37'50.043"E Elevation: 1147 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Bogoria 1. spot:

Coordinates: 0°20'22.726"S 36°4'21.132"E Elevation: 995 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Bogoria 2. spot:

Coordinates: 0°20'28.066"S 36°4'8"768E Elevation: 1001 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Bogoria 3. spot:

Coordinates: 0°19'26.09"S 36°4'16.487"E Elevation: 998 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

3.1.4 Results of Water Quality and Toxicity Analysis for Kenya

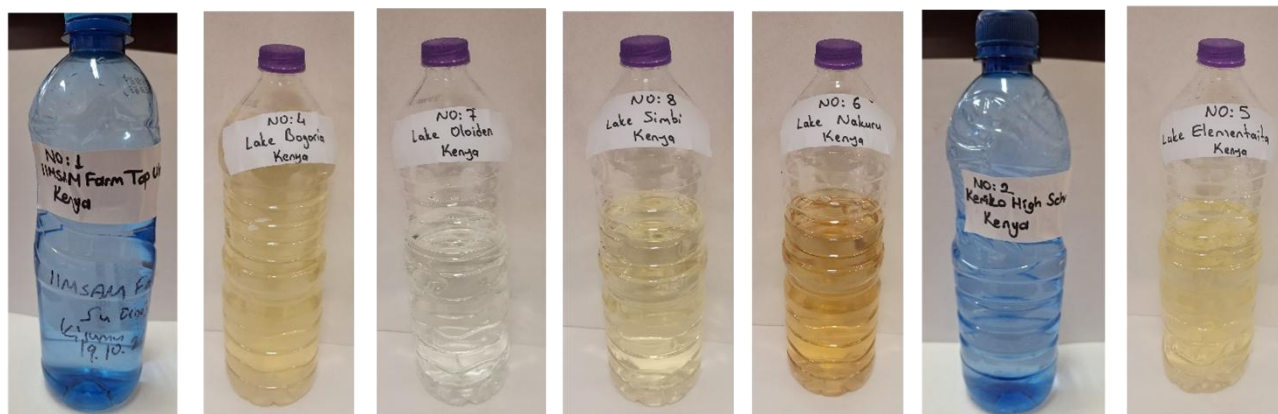
About 1.5 L of lake water samples (0.5 L from each spot) were taken from Lake Simbi, Lake Nakuru, Lake Oloiden, Lake Elementaita and Lake Bogoria, and tap water samples (1.0 L) were taken from the local, private Spirulina farms; Keriko Secondary School Spirulina farm and the IIMSAM (Intergovernmental Institution for the use of Micro-algae Spirulina Against Malnutrition) Spirulina farm for water quality and toxicity analysis.

All water samples from each spot (0.5 L) taken were firstly filtered by AIE Glass Fiber Filter (1 μm pore size, 47 mm) (PALL Corporation), and then filtered again by GF3 grade, glass microfiber filter (47 mm, 0.7 μm pore size) (CHMLAB Group) using water pump (Rocker 400, AC 200 - 240V, 60 Hz, AGHA557, Taiwan) filtration system.

After filtering, all water samples from each spot were analyzed on-site for the 7 different parameters including pH, nitrate (NO_3^-), sodium (Na^+), calcium (Ca^{+2}), potassium (K^+), salt (NaCl), conductivity and temperature using **Horiba 7M LAQUAtwin Kit** (HORIBA, Ltd., Kyoto, Japan).

Lastly, all water samples were also analyzed for ammonium (NH_4), dissolved oxygen (DO), total suspended solids (TSS), phosphate (PO_4), sulfate (SO_4), magnesium (Mg), aluminum (Al), copper (Cu), zinc (Zn), arsenic (As), iron (Fe), cadmium (Cd), cobalt (Co), lead (Pb), chromium (Cr), nickel (Ni), and manganese (Mn) by **Denizli Environmental Quality Laboratory (DENÇEV) of Denizli Municipality- DESKİ General Directorate**, which is nationally and internationally accredited.

The evaluation of the measurement values is left to the project partners of the respective countries, as the reference standards of each country will be different. Each measurement was performed in 3 repetitions and the average values were shared as a result.



Water Samples from Lake and Farms

Results of Water Quality and Toxicity Analysis

Lake Oloiden 1. Spot:

1. Spot Lake Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
9.59	17.66	61.66	14.0	28.66	0.02	444.66	22.57
±0.10	±1.15	±1.15	±0.0	±0.57	±0.00	±1.15	±0.33

Lake Oloiden 2. Spot:

2. Spot Lake Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
9.60	15.66	63.0	15.0	29.0	0.02	438.0	22.10
±0.01	±0.57	±0.00	±0.0	±0.0	±0.00	±4.35	±0.00

Lake Oloiden 3. Spot:

3. Spot Lake Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
9.42	15.0	63.0	16.0	28.66	0.02	475.66	21.17
±0.02	±0.0	±0.00	±6.67	±0.57	±0.0	±5.77	±0.30

Lake Oloiden (Average)

Lake Oloiden Water Analysis by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
9.53	16.11	62.55	15.0	28.77	0.02	452.77	21.94
±0.10	±1.38	±0.76	±1.0	±0.19	±0.0	±20.1	±0.56

Lake Oloiden Water Analysis by DESKI																
NH ₄ mg/L	DO mg/L	TSS mg/L	PO ₄ mg/L	SO ₄ mg/L	Mg mg/L	Al µg/L	Cu µg/L	Zn µg/L	As µg/L	Fe µg/L	Cd µg/L	Co µg/L	Pb µg/L	Cr µg/L	Ni µg/L	Mn µg/L
<0.1	3.96 ±0.05	<10	<0.8	7.98 ±0.02	6.05 ±0.03	44.54 ±0.33	<4	<20	0.60 ±0.01	56.61 ±0.32	<0.5	<0.5	<0.5	<1	1.38 ±0.12	3.77 ±0.17

Abbreviations: Acidity (pH), Nitrate (NO₃⁻), Sodium (Na⁺), Calcium (Ca⁺²), Potassium (K⁺), Ammonium (NH₄), Dissolved Oxygen (DO), Total Suspended Solids (TSS), Phosphate (PO₄), Sulfate (SO₄), Magnesium (Mg), Aluminum (Al), Copper (Cu), Zinc (Zn), Arsenic (As), Iron (Fe), Cadmium (Cd), Cobalt (Co), Lead (Pb), Chromium (Cr), Nickel (Ni), Manganese (Mn), Not Detected (nd)

Lake Elementaita 1. Spot:

1. Spot Lake Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (mS/cm)	Temp. (°C)
10.55 ±0.04	120.0 ±0.0	2800.0 ±0.0	nd	106.66 ±5.77	0.76 ±0.00	11.55 ±0.08	23.7 ±0.20

Lake Elementaita 2. Spot:

2. Spot Lake Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (mS/cm)	Temp. (°C)
10.57 ±0.01	113.33 ±5.77	2800.0 ±0.0	nd	110.0 ±0.0	0.76 ±0.0	11.48 ±0.08	21.80 ±0.10

Lake Elementaita 3. Spot:

3. Spot Lake Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (mS/cm)	Temp. (°C)
10.58 ±0.01	110.0 ±0.0	2800.0 ±0.0	nd	120.0 ±0.0	0.76 ±0.0	11.46 ±0.005	22.00 ±0.00

Lake Elementaita (Average)

Lake Elementaita Water Analysis by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (mS/cm)	Temp. (°C)
10.57 ±0.01	114.44 ±5.09	2800.0 ±0.0	nd	112.22 ±6.93	0.76 ±0.0	11.5 ±0.04	22.50 ±1.20

Lake Elementaita Water Analysis by DESKI																
NH ₄ mg/L	DO mg/L	TSS mg/L	PO ₄ mg/L	SO ₄ mg/L	Mg mg/L	Al µg/L	Cu µg/L	Zn µg/L	As µg/L	Fe µg/L	Cd µg/L	Co µg/L	Pb µg/L	Cr µg/L	Ni µg/L	Mn µg/L
<0.1	4.05 ±0.03	<10	<0.8	0.21 ±0.01	0.34 ±0.02	332.14 ±0.33	<4	<20	9.16 ±0.04	239.88 ±0.12	1.30 ±0.12	<0.5	8.05 ±0.12	8.23 ±0.12	4.81 ±0.06	9.41 ±0.07

Abbreviations: Acidity (pH), Nitrate (NO₃⁻), Sodium (Na⁺), Calcium (Ca⁺²), Potassium (K⁺), Ammonium (NH₄), Dissolved Oxygen (DO), Total Suspended Solids (TSS), Phosphate (PO₄), Sulfate (SO₄), Magnesium (Mg), Aluminum (Al), Copper (Cu), Zinc (Zn), Arsenic (As), Iron (Fe), Cadmium (Cd), Cobalt (Co), Lead (Pb), Chromium (Cr), Nickel (Ni), Manganese (Mn), Not Detected (nd)

Lake Nakuru 1. Spot

1. Spot Lake Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (mS/cm)	Temp. (°C)
10.35 ±0.08	66.00 ±3.00	1300.0 ±0.00	nd	57.33 ±0.57	0.37 ±0.005	5.19 ±0.05	30.2 ±0.20

Lake Nakuru 2. Spot:

2. Spot Lake Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (mS/cm)	Temp. (°C)
10.34 ±0.03	67.0 ±0.0	1400.0 ±0.0	nd	58.33 ±0.57	0.38 ±0.00	5.54 ±0.07	23.0 ±0.00

Lake Nakuru 3. Spot:

3. Spot Lake Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (mS/cm)	Temp. (°C)
10.19 ±0.02	69.0 ±1.73	1300.0 ±0.0	nd	56.0 ±0.0	0.37 ±0.00	5.14 ±0.03	27.2 ±0.60

Lake Nakuru (Average)

Lake Nakuru Water Analysis by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (mS/cm)	Temp. (°C)
10.29 ±0.09	67.33 ±1.52	1333.33 ±57.73	nd	57.22 ±1.17	0.37 ±0.005	5.29 ±0.21	26.8 ±3.20

Lake Nakuru Water Analysis by DESKI																
NH ₄ mg/L	DO mg/L	TSS mg/L	PO ₄ mg/L	SO ₄ mg/L	Mg mg/L	Al µg/L	Cu µg/L	Zn µg/L	As µg/L	Fe µg/L	Cd µg/L	Co µg/L	Pb µg/L	Cr µg/L	Ni µg/L	Mn µg/L
<0.1	3.87 ±0.01	<10	<0.8	52.21 ±0.01	0.75 ±0.01	967.2 ±1.01	<4	20.27 ±0.03	5.93 ±0.01	937.3 ±1.12	0.58 ±0.01	<0.5	3.27 ±0.01	5.65 ±0.01	12.47 ±0.02	75.58 ±0.37

Abbreviations: Acidity (pH), Nitrate (NO₃⁻), Sodium (Na⁺), Calcium (Ca⁺²), Potassium (K⁺), Ammonium (NH₄), Dissolved Oxygen (DO), Total Suspended Solids (TSS), Phosphate (PO₄), Sulfate (SO₄), Magnesium (Mg), Aluminum (Al), Copper (Cu), Zinc (Zn), Arsenic (As), Iron (Fe), Cadmium (Cd), Cobalt (Co), Lead (Pb), Chromium (Cr), Nickel (Ni), Manganese (Mn), Not Detected (nd)

Keriko Secondary School Farm

Keriko Secondary School Farm Tap Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
8.8 ±0.18	19.66 ±0.57	133.33 ±5.77	26.66 ±0.57	21.33 ±0.57	0.04 ±0.0	545.0 ±16.52	27.9 ±0.20

Keriko Secondary School Farm Tap Water Analysis by DESKI																
NH ₄ mg/L	DO mg/L	TSS mg/L	PO ₄ mg/L	SO ₄ mg/L	Mg mg/L	Al µg/L	Cu µg/L	Zn µg/L	As µg/L	Fe µg/L	Cd µg/L	Co µg/L	Pb µg/L	Cr µg/L	Ni µg/L	Mn µg/L
<0.1	3.83 ±0.13	<10	0.84 ±0.04	18.6 ±0.01	1.74 ±0.58	34.66 ±0.33	<4	<20	<0.5	37.6 ±0.12	<0.5	<0.5	<0.5	<1	<1	<1

Abbreviations: Acidity (pH), Nitrate (NO₃⁻), Sodium (Na⁺), Calcium (Ca⁺²), Potassium (K⁺), Ammonium (NH₄), Dissolved Oxygen (DO), Total Suspended Solids (TSS), Phosphate (PO₄), Sulfate (SO₄), Magnesium (Mg), Aluminum (Al), Copper (Cu), Zinc (Zn), Arsenic (As), Iron (Fe), Cadmium (Cd), Cobalt (Co), Lead (Pb), Chromium (Cr), Nickel (Ni), Manganese (Mn), Not Detected (nd)

IIMSAM Spirulina Farm in Kisumu

IIMSAM Spirulina Farm in Kisumu Tap Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (mS/cm)	Temp. (°C)
8.10 ±0.15	10.0 ±1.0	6.66 ±0.57	20.33 ±0.57	4.0 ±0.00	0.01 ±0.00	100.66 ±1.15	27.5 ±0.10

IIMSAM Spirulina Farm in Kisumu Tap Water Analysis by DESKI																
NH ₄ mg/L	DO mg/L	TSS mg/L	PO ₄ mg/L	SO ₄ mg/L	Mg mg/L	Al µg/L	Cu µg/L	Zn µg/L	As µg/L	Fe µg/L	Cd µg/L	Co µg/L	Pb µg/L	Cr µg/L	Ni µg/L	Mn µg/L
0.18 ±0.03	3.95 ±0.13	<10	0.8 ±0.01	28.9 ±0.01	2.11 ±0.58	<10	<4	<20	<0.5	<10	nd	<0.5	<0.5	<1	<1	<1

Abbreviations: Acidity (pH), Nitrate (NO₃⁻), Sodium (Na⁺), Calcium (Ca⁺²), Potassium (K⁺), Ammonium (NH₄), Dissolved Oxygen (DO), Total Suspended Solids (TSS), Phosphate (PO₄), Sulfate (SO₄), Magnesium (Mg), Aluminum (Al), Copper (Cu), Zinc (Zn), Arsenic (As), Iron (Fe), Cadmium (Cd), Cobalt (Co), Lead (Pb), Chromium (Cr), Nickel (Ni), Manganese (Mn), Not Detected (nd)

Lake Simbi 1. Spot

1. Spot Lake Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (mS/cm)	Temp. (°C)
11.1 ±0.04	80.00 ±1.73	3500.0 ±0.0	nd	35.66 ±0.57	0.99 ±0.02	12.57 ±0.15	27.9 ±0.10

Lake Simbi 2. Spot:

2. Spot Lake Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (m/cm)	Temp. (°C)
11.13 ±0.02	78.33 ±0.57	3400.00 ±0.00	nd	38.33 ±0.57	0.97 ±0.01	12.73 ±0.11	27.9 ±0.10

Lake Simbi 3. Spot:

3. Spot Lake Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (mS/cm)	Temp. (°C)
11.17 ±0.02	73.00 ±2.64	3266.6 ±57.7	nd	36.6 ±1.54	0.91 ±0.01	12.65 ±0.05	28.0 ±0.00

Lake Simbi (Average)

Lake Simbi Water Analysis by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (mS/cm)	Temp. (°C)
11.13 ±0.03	77.11 ±3.65	3388.88 ±117.06	nd	36.88 ±1.34	0.95 ±0.03	12.65 ±0.08	27.9 ±0.10

Lake Simbi Water Analysis by DESKI																
NH ₄ mg/L	DO mg/L	TSS mg/L	PO ₄ mg/L	SO ₄ mg/L	Mg mg/L	Al µg/L	Cu µg/L	Zn µg/L	As µg/L	Fe µg/L	Cd µg/L	Co µg/L	Pb µg/L	Cr µg/L	Ni µg/L	Mn µg/L
<0,1	3.74 ±0.03	<10	<0.8	0.15 ±0.01	0.98 ±0.58	198.7 ±9.33	<4	<20	33.12 ±0.02	99.26 ±1.12	<0.5	<0.5	2.20 ±0.01	1.76 ±0.01	13.74 ±0.02	14.99 ±0.07

Abbreviations: Acidity (pH), Nitrate (NO₃), Sodium (Na⁺), Calcium (Ca⁺²), Potassium (K⁺), Ammonium (NH₄), Dissolved Oxygen (DO), Total Suspended Solids (TSS), Phosphate (PO₄), Sulfate (SO₄), Magnesium (Mg), Aluminum (Al), Copper (Cu), Zinc (Zn), Arsenic (As), Iron (Fe), Cadmium (Cd), Cobalt (Co), Lead (Pb), Chromium (Cr), Nickel (Ni), Manganese (Mn), Not Detected (nd)

Lake Bogoria 1. Spot

1. Spot Lake Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (mS/cm)	Temp. (°C)
10.06 ±0.1	86.0 ±1.00	6366.66 ±208.16	nd	106.6 ±11.5	1.53 ±0.05	24.56 ±0.11	30.0 ±0.00

Lake Bogoria 2. Spot:

2. Spot Lake Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (mS/cm)	Temp. (°C)
10.05 ±0.05	84.33 ±2.51	6300.00 ±100.0	nd	94.00 ±4.58	1.6 ±0.01	24.63 ±0.15	30.0 ±0.10

Lake Bogoria 3. Spot:

3. Spot Lake Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (mS/cm)	Temp. (°C)
10.02 ±0.02	86.0 ±2.64	6266.6 ±115.4	nd	89.33 ±2.08	1.56 ±0.05	24.3 ±0.43	30.0 ±0.10

Lake Bogoria (Average)

Lake Bogoria Water Analysis by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (mS/cm)	Temp. (°C)
10.04 ±0.02	85.44 ±0.96	6311.11 ±50.9	nd	97.00 ±8.83	1.56 ±0.03	24.5 ±0.17	30.0 ±0.03

Lake Bogoria Water Analysis by DESKI																
NH ₄ mg/L	DO mg/L	TSS mg/L	PO ₄ mg/L	SO ₄ mg/L	Mg mg/L	Al µg/L	Cu µg/L	Zn µg/L	As µg/L	Fe µg/L	Cd µg/L	Co µg/L	Pb µg/L	Cr µg/L	Ni µg/L	Mn µg/L
<0,1	3.40 ±0.3	<10	<0.8	<0.1	0.93 ±0.02	387.1 ±0.33	7.99 ±0.02	44.33 ±0.06	3.25 ±0.02	80.67 ±0.9	1.36 ±0.03	<0.5	18.76 ±0.01	8.90 ±0.01	5.21 ±0.02	25.41 ±0.07

Abbreviations: Acidity (pH), Nitrate (NO₃⁻), Sodium (Na⁺), Calcium (Ca⁺²), Potassium (K⁺), Ammonium (NH₄), Dissolved Oxygen (DO), Total Suspended Solids (TSS), Phosphate (PO₄), Sulfate (SO₄), Magnesium (Mg), Aluminum (Al), Copper (Cu), Zinc (Zn), Arsenic (As), Iron (Fe), Cadmium (Cd), Cobalt (Co), Lead (Pb), Chromium (Cr), Nickel (Ni), Manganese (Mn), Not Detected (nd)

3.2 Sampling in Tanzania

3.2.1 Summary Report for Tanzania Field Work and Sample Collection

The visit for a field work and sampling was carried out between **14-21 December 2024 to Tanzania**, where one of the ECOHUB centers in Africa is located. We conducted the field work and sampling together with Dr. Deogratias Pius Mulokozi and Israel Zephania Mbwilo, from Tanzania Fish Research Institute (**TAFIRI**) in Tanzania. On the first day of the visit, we had a meeting with Dr. Deogratias Pius Mulokozi about the project activities for spirulina production in Tanzania and the evaluation of the project activities under our joint responsibility.

During the fieldwork, water samples filtered, which may include spirulina strains, were taken from **Great and Small Lake Momella, Lake Natron and Lake Manyara** around the city of Arusha, Tanzania. Water samples from three different spots for each local lake were also taken without filtering for water quality analysis. All unfiltered water samples were measured for parameters such as temperature, pH, salinity, conductivity, nitrate, potassium, calcium, potassium, sodium. Also, geographical coordinates, local flora and fauna information was noted, too. Finally, the samples were properly preserved and brought to Turkey in a way that preserved the vitality of algae species. This 8-day overseas mission in Tanzania was completed in an effective, efficient and healthy manner.



Meeting with Dr. Deogratias Pius Mulokozi about sampling and field work in Tanzania, and the project activities for spirulina production in Tanzania

3.2.2 Sampling Data from the visit to Tanzania (14-21 December 2024)

During the fieldwork, lake water and Spirulina samples were collected from three different spots of each local lakes including Lake Great Momella, Lake Small Momella, Lake Natron and Lake Manyara. Local people guided us in reaching the sampling points and collecting the samples.

On 16 December 2024, Lake Momella in Arusha National Park was visited. Lake Momella is divided into a small and a large Momella, but it was also reported that due to increased rainfall the barrier between the two lakes has been removed and the two lakes have merged. At the time of sample collection, the two lakes were separated by a prominent black patch, so both lakes were sampled. Both are lakes with small groups of flamingos.



Lake Small Momella



Lake Big Momella

On December 17, 2024, Lake Narton was visited. The lake is located at the foot of a still active volcanic mountain called the Mountain of God, this lake is fed by springs from the slopes of the mountain and underground. A water sample was taken from one of the springs. Flamingos are densely populated on the shore and samples were taken from the semi-dry and semi-wet swampy areas along the shore line. The water here is reddish and dark in color and is continuously fed with fresh water from the underground and the foothills of the surrounding volcanic mountain. The water level where sampling is taken place is between 2-10 cm leading the water temperature is higher for spot 1 and 2. For the spot 3, the samples were taken from a water stream coming from the volcanic mountain and containing algae cultures. The water height is between 10-25 cm and the water temperature is lower than in other spots.



Lake Natron

On December 18, 2024, Lake Manyara was visited. This lake is located in the Lake Manyara National Park and is fed by streams originating from the surrounding high hills and mountains, which cause intense and extreme flooding, and the water in the lake was noted to be mud-colored. Samples were taken from semi-watery and semi-arid areas with very low water height (5-20 cm) at the edge of the lake. The water appears muddy due to the mixing of rainwater.

After the field and sampling work was completed, each of the samples was kept uncovered at room temperature and in sunlight until they were returned to Turkey, in the meantime Zaruk Media was added to each sample to allow the possible spirulina species to survive.



Lake Manyara

3 spots were identified (within safety and transportation conditions) and 3 samples were taken from each spot. Separate 500 mL samples of goal water were also taken from each spot to measure water quality. All unfiltered water samples were measured for parameters such as temperature, pH, salinity, conductivity, nitrate, potassium, calcium, potassium, sodium. Also, geographical coordinates, local flora and fauna information was noted, too. GPS coordinates were taken from the link; <https://www.gps-coordinates.net/gps-coordinates-converter> .

3.2.3 Field Work Locations for Sampling, Lakes and Farms visited in Tanzania

Lake Small Momella 1. Spot:

Coordinates: 3°13'19.761"S 36°53'29.461"E Elevation: 1443 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Small Momella 2. Spot:

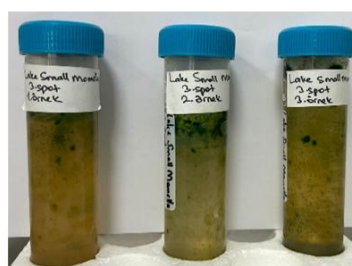
Coordinates: 3°13'11.465"S 36°53'31.451"E Elevation: 1453 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Small Momella 3. spot:

Coordinates: 3°13'6.378"S 36°53'47.765"E Elevation: 1447 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Big Momella 1. spot:

Coordinates: 3°13'4.397"S 36°54'13.384"E

Elevation: 1451 m

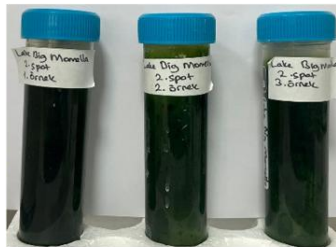


- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Big Momella 2. spot:

Coordinates: 3°13'34.104"S 36°54'31.068"E

Elevation: 1437 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Big Momella 3. spot:

Coordinates: 3°13'48.785"S 36°54'16.433"E

Elevation: 1443 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Natron 1. spot:

Coordinates: 2°35'39.671"S 35°54'47.26"E Elevation: 606 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Natron 2. spot:

Coordinates: 2°35'40.423"S 35°54'59.587"E Elevation: 606 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Natron 3. spot:

Coordinates: 2°35'40.433"S 35°54'59.597"E Elevation: 606 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Manyara 1. spot:

Coordinates: 3°24'5.303"S 35°49'34.849"E Elevation: 964 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Manyara 2. spot:

Coordinates: 3°26'4.625"S 35°48'28.89"E Elevation: 971 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

Lake Manyara 3. spot:

Coordinates: 3°28'27.285"S 35°47'21.724"E Elevation: 968 m



- 3 replicates of sample were taken.
- 0.5 L of lake water for water quality analysis was taken.

3.2.4 Results of Water Quality and Toxicity Analysis for Tanzania

About 1.5 L of lake water samples (0.5 L from each spot) were taken from the Lake Small Momella, Lake Big Momella, Lake Natron and Lake Manyara for water quality and toxicity analysis. All water samples from each spot (0.5 L) taken were firstly filtered by Whatman No 1 filter paper (Cat#1001-125, 11 µm pore size) using water pump filtration system.

After filtering, all water samples from each spot were analyzed on-site for the 7 different parameters including pH, nitrate (NO_3^-), sodium (Na^+), calcium (Ca^{+2}), potassium (K^+), salt (NaCl), conductivity and temperature using **Horiba 7M LAQUAtwin Kit** (HORIBA, Ltd., Kyoto, Japan).

Lastly, all water samples were also analyzed for ammonium (NH_4), dissolved oxygen (DO), total suspended solids (TSS), phosphate (PO_4), sulfate (SO_4), magnesium (Mg), aluminum (Al), copper (Cu), zinc (Zn), arsenic (As), iron (Fe), cadmium (Cd), cobalt (Co), lead (Pb), chromium (Cr), nickel (Ni), and manganese (Mn) by **Denizli Environmental Quality Laboratory (DENÇEV) of Denizli Municipality- DESKİ General Directorate**, which is nationally and internationally accredited.

The evaluation of the measurement values is left to the project partners of the respective countries, as the reference standards of each country will be different. Each measurement was performed in 3 repetitions and the average values were shared as a result.



Water Samples from Lakes

Results of Water Quality and Toxicity Analysis

Lake Small Momella 1. Spot:

1. Spot Lake Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
9.7 ±0.03	25.67 ±1.33	1500.0 ±0.00	nd	210.0 ±0.00	0.43 ±0.01	7.44 ±0.06	26.6 ±0.33

Lake Small Momella 2. Spot:

2. Spot Lake Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
9.71 ±0.01	25.0 ±0.00	1500.0 ±0.00	nd	203.33 ±6.67	0.42 ±0.00	7.48 ±0.01	24.3 ±0.06

Lake Small Momella 3. Spot:

3. Spot Lake Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
9.77 ±0.03	24.67 ±0.33	1500.0 ±0.00	nd	200.0 ±0.00	0.42 ±0.01	7.46 ±0.04	28.1 ±0.20

Lake Small Momella (Average)

Lake Small Momella Water Analysis by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
9.72 ±0.04	25.11 ±0.55	1500.0 ±0.00	nd	204.44 ±5.55	0.42 ±0.01	7.46 ±0.02	26.33 ±1.76

Lake Small Momella Water Analysis by DESKI																
NH ₄ mg/L	DO mg/L	TSS mg/L	PO ₄ mg/L	SO ₄ mg/L	Mg mg/L	Al µg/L	Cu µg/L	Zn µg/L	As µg/L	Fe µg/L	Cd µg/L	Co µg/L	Pb µg/L	Cr µg/L	Ni µg/L	Mn µg/L
<0.1	3.33 ±0.05	<10	<0.8	1.91 ±0.02	2.74 ±0.03	360.09 ±0.33	8.39 ±0.01	24.18 ±0.12	1.36 ±0.01	277.7 ±0.32	<0.5	<0.5	0.60 ±0.01	3.52 ±0.02	14.78 ±0.22	21.30 ±0.47

Abbreviations: Acidity (pH), Nitrate (NO₃⁻), Sodium (Na⁺), Calcium (Ca⁺²), Potassium (K⁺), Ammonium (NH₄), Dissolved Oxygen (DO), Total Suspended Solids (TSS), Phosphate (PO₄), Sulfate (SO₄), Magnesium (Mg), Aluminum (Al), Copper (Cu), Zinc (Zn), Arsenic (As), Iron (Fe), Cadmium (Cd), Cobalt (Co), Lead (Pb), Chromium (Cr), Nickel (Ni), Manganese (Mn), Not Detected (nd)

3.3 Sampling in Ghana (1st visit)

3.3.1 Summary Report for Ghana (1st visit) Field Work and Sample Collection

BMAEU has visited Ghana for the field and sampling work, where one of the ECOHUB centers in Africa are located, between 27 November -1 December 2024. We have carried out the project work together with project partner researcher of CSIR-Food Research Institute and CSIR-Water Research Institute in Ghana; Dr. Etornyo Agbeko. During the fieldwork in Ghana, we were accompanied by PhD students and local staff from the same institute. On the first day of our visit, we had a meeting with Dr. Mike Osei Atweneboana, the director of CSIR-Water Research Institute, and Dr. Esther Whaga and Dr. Aisha Adams Amadu, staff members of the institute, about the field work.

During the fieldwork, lake water and Spirulina samples were collected from different locations in **Lake Volta**. Local people guided us in reaching the sampling points and collecting the samples. We also visited the **CSIR-WRI research facility** in Akosombo town, the **Fish and Feed Farm** in Sogakope town and the **Bio Green Agro Farm** in Agorkpo-Sogakope to collect water and culture samples of Spirulina growing in their ponds. All water samples were measured for parameters such as temperature, pH, salinity, conductivity, nitrate, potassium, calcium, potassium, sodium, and geographical, flora and fauna information were noted. Finally, the samples were properly preserved and brought back to Turkey in a way to preserve the viability of the organisms in them. This 5-day mission abroad in Ghana was completed safely, effectively and efficiently.



Meeting with the Director of CSIR-Water Research Institute, Prof. Dr. Mike Osei Atweneboana, and staff members; Dr. Esther Whaga and Dr. Aisha Adams Amadu



Visit to the Fish and Feed Farm Company

3.3.2 Sampling Data from the visit to Ghana (27 November - 1 December 2024)

During the fieldwork, lake water and Spirulina samples were collected from different locations in **Lake Volta**. Local people guided us in reaching the sampling points and collecting the samples. We also visited

the **CSIR-WRI research facility** in Akosombo town, the **Fish and Feed Farm** in Sogakope town and the **Bio Green Agro Farm** in Agorkpo-Sogakope to collect water and culture samples of Spirulina growing in their ponds. All water samples were measured for parameters such as temperature, pH, salinity, conductivity, nitrate, potassium, calcium, potassium, sodium, and geographical, flora and fauna information were noted.

On November 29, 2024, the CSIR-WRI research facility in Akosombo and Lake Volta spots were visited. In this institute, work is carried out especially in the field of fish farming and there are open and closed ponds for this purpose. There are also several small concrete ponds in a small area within the institute, where algae other water plants from the Lake Volta are cultured. This culture claimed to have spirulina, so sample were taken from these small ponds. Since Lake Volta is an artificial reservoir and contained behind the Akosombo Dam, the alkalinity of the water is very low. Fishing activities are generally carried out in our sampling spot through the lake. The environment of the lake water does not seem to be very suitable for the living conditions of spirulina.



CSIR-WRI Research Facility in Akosombo



On November 30, 2024, the Fish and Feed Farm in Sogakope town and the Bio Green Agro Farm in Agorkpo-Sogakope were visited. The Fish and Feed Farm is a fishery and fish feed-based farm and did not produce spirulina at the time of our visit. However, the farm managers stated that they would like to produce spirulina to feed their fish in the future and for this reason they are trying to cultivate a sample of algae-filled water from Lake Volta in an open pond. The Bio Green Agro Farm operates more as a social organization center of a church than a farm. Here, in a small pond, fish are raised in a culture filled with algae. It is claimed that spirulina is grown here because of the algal growth.

Lake Volta



The Bio Green Agro Farm

The Fish and Feed Farm

3.3.3 Field Work Locations for Sampling, Lakes and Farms visited in Ghana

CSIR-WRI-Pond 1 and Pond 2:

Coordinates: 6°16'57.0"N 0°03'20.0"E Elevation: 28.2 m



- 2 replicates of samples from each pond were taken
- 1 L of tap water for water quality analysis was taken.

Fish and Feeds Farm: (Sogakope City):

Coordinates: 5°50'04.4"N 0°39'36.7"E Elevation: 1 m



- 3 replicates of sample were taken.
- 1.5 L water sample (used in pond) was taken for quality analysis.
- In this farm, algae water samples from Lake Volta were incubated in an area near the fish ponds by the farmers and the algae population was allowed to grow. The water sample (1.5 L) used for this cultivation was taken for the quality analysis.

Bio Green Agro Farm: (Agorkpo-Sogakope):

Coordinates: 6°00'48.1"N 0°35'44.6"E Elevation: 31 m



- 3 replicates of sample were taken.
- 1.5 L water sample (used in pond) was taken for quality analysis.
- The pond in the figure has a dense population of mixed algae. Catfish are also raised in the ponds.

Lake Volta 1. Spot (Marine Area):

Coordinates: 6°17'45.5"N 0°01'37.7"E

Elevation: 77 m



- 3 replicates of sample were taken.
- 1 L of tap water for water quality analysis was taken.
- The area where the samples were taken is muddy, swampy, with dense vegetation and a high fly population.

Lake Volta 2. Spot (Marine Village):

Coordinates: 6°17'43.4"N 0°01'35.2"E

Elevation: 77 m



- 3 replicates of sample were taken.
- Samples were taken and brought to us by local fisherman. Thus, no water sample, no picture of the spot were taken.

Lake Volta 3. Spot (Atimpoku):

Coordinates: 6°13'34.4"N 0°05'33.0"E

Elevation: 16 m



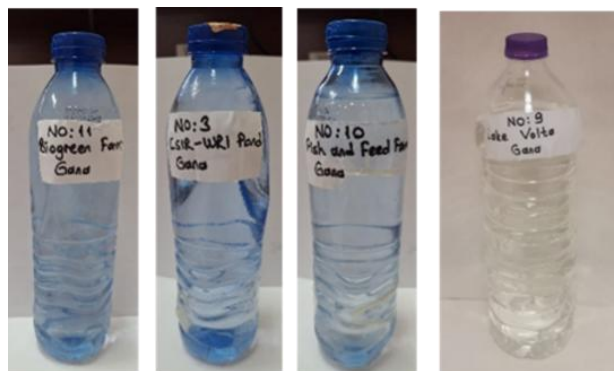
- 3 replicates of sample were taken.
- Lake water sample (0.5 L) was taken

3.3.4 Results of Water Quality and Toxicity Analysis for Ghana

Water samples taken from Lake Volta sampling spots, tap water from the CSIR-WRI Research Facility, the Fish and Feed Farm and the Bio Green Agro Farm for water quality and toxicity analysis. All water samples taken were firstly filtered by Whatman No 1 filter paper (Cat#1001-125, 11 μm pore size) using water pump filtration system. After filtering, all were analyzed on-site for the 7 different parameters including pH, nitrate (NO_3^-), sodium (Na^+), calcium (Ca^{+2}), potassium (K^+), salt (NaCl), conductivity and temperature using **Horiba 7M LAQUAtwin Kit** (HORIBA, Ltd., Kyoto, Japan).

Lastly, all water samples were also analyzed for ammonium (NH₄), dissolved oxygen (DO), total suspended solids (TSS), phosphate (PO₄), sulfate (SO₄), magnesium (Mg), aluminum (Al), copper (Cu), zinc (Zn), arsenic (As), iron (Fe), cadmium (Cd), cobalt (Co), lead (Pb), chromium (Cr), nickel (Ni), and manganese (Mn) by **Denizli Environmental Quality Laboratory (DENÇEV) of Denizli Municipality- DESKİ General Directorate**, which is nationally and internationally accredited.

The evaluation of the measurement values is left to the project partners of the respective countries, as the reference standards of each country will be different. Each measurement was performed in 3 repetitions and the average values were shared as a result.



Water Samples

Results of Water Quality and Toxicity Analysis

CSIR-WRI Tap Water:

CSIR-WRI Tap Water Analysis by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
7.27 ±0.05	6 ±0.00	3 ±0.00	12 ±0.00	nd	nd	68 ±1.00	31.7 ±0.10

CSIR-WRI Tap Water Analysis by DESKI																
NH ₄ mg/L	DO mg/L	TSS mg/L	PO ₄ mg/L	SO ₄ mg/L	Mg mg/L	Al µg/L	Cu µg/L	Zn µg/L	As µg/L	Fe µg/L	Cd µg/L	Co µg/L	Pb µg/L	Cr µg/L	Ni µg/L	Mn µg/L
0.18 ±0.02	3.13 ±0.04	<10	<0.8	1.94 ±0.08	2.86 ±0.10	<10	8.03 ±0.04	<20	<0.5	<10	nd	<0.5	<0.5	<1	<1	8.85 ±0.14

Abbreviations: Acidity (pH), Nitrate (NO₃⁻), Sodium (Na⁺), Calcium (Ca⁺²), Potassium (K⁺), Ammonium (NH₄), Dissolved Oxygen (DO), Total Suspended Solids (TSS), Phosphate (PO₄), Sulfate (SO₄), Magnesium (Mg), Aluminum (Al), Copper (Cu), Zinc (Zn), Arsenic (As), Iron (Fe), Cadmium (Cd), Cobalt (Co), Lead (Pb), Chromium (Cr), Nickel (Ni), Manganese (Mn), Not Detected (nd)

Fish and Feeds Farm Pond Water:

Fish and Feeds Farm Pond Water Analysis by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
7.18 ±0.04	9.33 ±0.57	24.67 ±0.57	10.67 ±0.57	4 ±0.00	0.01 ±0.00	212.67 ±1.52	31.6 ±0.20

Fish and Feeds Farm Pond Water Analysis by DESKI																
NH ₄ mg/L	DO mg/L	TSS mg/L	PO ₄ mg/L	SO ₄ mg/L	Mg mg/L	Al µg/L	Cu µg/L	Zn µg/L	As µg/L	Fe µg/L	Cd µg/L	Co µg/L	Pb µg/L	Cr µg/L	Ni µg/L	Mn µg/L
0.43 ±0.01	3.65 ±0.14	<10	<0.8	16.28 ±0.21	5.55 ±0.09	10.35 ±0.10	<4	29.07 ±0.14	<0.5	12.93 ±0.06	nd ±0.00	<0.5	<0.5	<1	<1	42.08 ±0.24

Abbreviations: Acidity (pH), Nitrate (NO₃⁻), Sodium (Na⁺), Calcium (Ca⁺²), Potassium (K⁺), Ammonium (NH₄), Dissolved Oxygen (DO), Total Suspended Solids (TSS), Phosphate (PO₄), Sulfate (SO₄), Magnesium (Mg), Aluminum (Al), Copper (Cu), Zinc (Zn), Arsenic (As), Iron (Fe), Cadmium (Cd), Cobalt (Co), Lead (Pb), Chromium (Cr), Nickel (Ni), Manganese (Mn), Not Detected (nd)

Bio Green Agro Farm Tap Water:

Bio Green Agro Farm Tap Water Analysis by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
6.96 ±0.05	9 ±0.00	26 ±0.00	25.67 ±0.57	nd ±0.00	0.01 ±0.00	259.67 ±1.52	31.3 ±0.26

Bio Green Agro Farm Tap Water Analysis by DESKI																
NH ₄ mg/L	DO mg/L	TSS mg/L	PO ₄ mg/L	SO ₄ mg/L	Mg mg/L	Al µg/L	Cu µg/L	Zn µg/L	As µg/L	Fe µg/L	Cd µg/L	Co µg/L	Pb µg/L	Cr µg/L	Ni µg/L	Mn µg/L
0.30 ±0.02	2.76 ±0.16	<10	<0.8	0.83 ±0.05	5.66 ±0.09	<10	4.76 ±0.12	40.00 ±0.10	<0.5	12.84 ±0.05	nd ±0.00	<0.5	<0.5	<1	<1	106.42 ±1.05

Abbreviations: Acidity (pH), Nitrate (NO₃⁻), Sodium (Na⁺), Calcium (Ca⁺²), Potassium (K⁺), Ammonium (NH₄), Dissolved Oxygen (DO), Total Suspended Solids (TSS), Phosphate (PO₄), Sulfate (SO₄), Magnesium (Mg), Aluminum (Al), Copper (Cu), Zinc (Zn), Arsenic (As), Iron (Fe), Cadmium (Cd), Cobalt (Co), Lead (Pb), Chromium (Cr), Nickel (Ni), Manganese (Mn), Not Detected (nd)

Lake Volta 1. Spot (Marine Area):

1. Spot (Marine Area) Lake Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
7.34 ±0.10	6 ±0.00	4 ±0.00	9 ±0.00	nd	nd	74.33 ±3.21	31.3 ±0.10

Lake Volta 2. Spot (Marine Village):

2. Spot (Marine Village) Lake Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
7.35 ±0.06	14 ±1.00	19 ±1.00	9 ±1.00	4.33 ±0.57	0.02 ±0.00	62.33 ±7.76	30.7 ±0.17

Lake Volta 3. Spot (Atimpoku):

3. Spot (Atimpoku) Lake Water by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
8.92 ±0.04	9.67 ±0.57	15.67 ±0.57	9.33 ±0.57	4 ±0.00	nd	72.33 ±1.15	28.6 ±0.10

Lake Volta (Average)

Lake Volta Water Analysis by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
7.87 ±0.90	9.89 ±4.00	12.66 ±2.33	9.1 ±0.21	4.16 ±2.41	0.02 ±0.01	69.66 ±2.33	30.2 ±1.41

Lake Volta Water Analysis by DESKI																
NH ₄ mg/L	DO mg/L	TSS mg/L	PO ₄ mg/L	SO ₄ mg/L	Mg mg/L	Al µg/L	Cu µg/L	Zn µg/L	As µg/L	Fe µg/L	Cd µg/L	Co µg/L	Pb µg/L	Cr µg/L	Ni µg/L	Mn µg/L
0.18 ±0.04	3.13 ±0.33	<10	<0.8	1.94 ±0.10	2.86 ±0.58	<10	8.03 ±1.12	<20	<0.5	<10	nd	<0.5	<0.5	<1	<1	8.85 ±1.07

Abbreviations: Acidity (pH), Nitrate (NO₃⁻), Sodium (Na⁺), Calcium (Ca⁺²), Potassium (K⁺), Ammonium (NH₄), Dissolved Oxygen (DO), Total Suspended Solids (TSS), Phosphate (PO₄), Sulfate (SO₄), Magnesium (Mg), Aluminum (Al), Copper (Cu), Zinc (Zn), Arsenic (As), Iron (Fe), Cadmium (Cd), Cobalt (Co), Lead (Pb), Chromium (Cr), Nickel (Ni), Manganese (Mn), Not Detected (nd)

3.4 Sampling in Ghana (2nd visit)

3.4.1 Summary Report for Ghana (2nd visit) Field Work and Sample Collection

A second field and sampling visit was carried out between **6 - 9 March 2025 to Ghana**, where one of the ECOHUB centers in Africa is located. We conducted the project work together with co-researchers Dr. Benjamin Mintah and Dr. Patrick Senam Fatsi from CSIR-Food Research Institute and CSIR-Water Research Institute in Ghana. During the fieldwork in Ghana, we were accompanied by local researchers and workers from the same institute. On the first day of our visit, we visited Prof. Dr. Charles Tortoe, Director of CSIR-Water Research Institute and had a meeting about the project activities in Ghana and the evaluation of the project activities under our joint responsibility.

During the fieldwork, a sample was taken from a small pond in the Mangotsonya area of Accra city, which is usually filled with rainwater and then dries up, containing different types of algae, and recorded as **Accra-1**. This was followed by sampling of algae cultures in small-scale concrete water ponds at the CSIR Water Research Institute, located near Lake Volta. Here the samples from the small ponds from the first visit to Ghana were again sampled, mixed, concentrated in tubes and recorded as **CSIR Pond 1**. At the same location, concentrated samples were also taken from ponds containing cultured microalgae from different sources and recorded as **CSIR-2nd pond** and **CSIR-3rd pond**. Pond CSIR-3 contained large adult catfish. The Institute's tap water sample was also taken again for analysis (1.5 L). Finally, the algae-containing samples and lake water samples previously taken by Dr. Patrick Fatsi from the Nzulezo area of Amanzuri Lake were also recorded as **Amanzuri Lake samples**. All water samples were measured for parameters such as temperature, pH, salinity, conductivity, nitrate, potassium, calcium, potassium, sodium, and geographical, flora and fauna information were noted. Finally, the samples were properly preserved and brought back to the country in a way that preserved the vitality of the organisms in them. This 4-day overseas mission in Ghana was completed safely, effectively and efficiently.



Meeting with the Director of CSIR-Food Research Institute, Prof. Dr. Charles Tortoe, and the researcher, Dr. Benjamin Mintah.

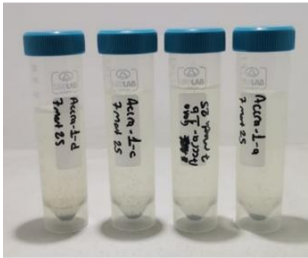
3.4.2 Sampling Data from the visit to Ghana (6 -9 March 2025)

During the fieldwork, a sample was taken from a small pond in the Mangotsonya area of Accra city, which is usually filled with rainwater and then dries up, containing different types of algae, and recorded as **Accra-1**. This was followed by sampling of algae cultures in small-scale concrete water ponds at the CSIR Water Research Institute, located near Lake Volta. Here the samples from the small ponds from the first visit to Ghana were again sampled, mixed, concentrated in tubes and recorded as **CSIR Pond 1**. At the same location, concentrated samples were also taken from ponds containing cultured microalgae from different sources and recorded as **CSIR-2nd pond** and **CSIR-3rd pond**. Pond CSIR-3 contained large adult catfish. The Institute's tap water sample was also taken again for analysis (1.5 L). Finally, the algae-containing samples and lake water samples previously taken by Dr. Patrick Fatsi from Amanzuri Lake (near Nzulezo Village) were also recorded as **Amanzuri Lake samples**. Finally, the samples were properly preserved and brought back to the country in a way that preserved the vitality of the organisms.

3.4.3 Field Work Locations for Sampling, Lakes and Farms visited in Ghana

Accra-City Center:

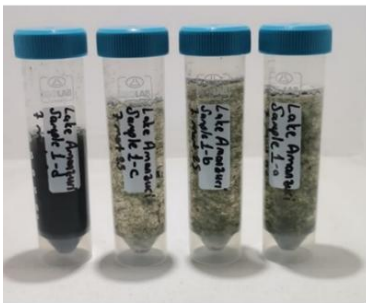
Coordinates: 5°49'57.0"N 0°01'42.4"W Mangotsonya, Accra-Ghana



- 4 replicates of samples were taken
- Water samples for water quality analysis were not taken.

Lake Amanzuri -1. spot (Nzulezo Village):

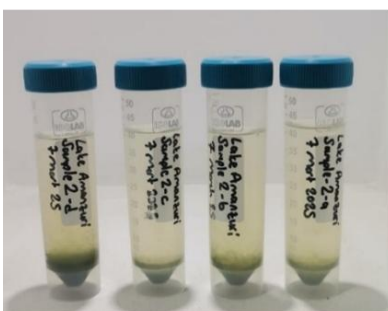
Coordinates: 5°01'16.0"N 2°35'52.8"W Elevation: 9.8 m



- 4 replicates of sample were taken.

Lake Amanzuri -2 spot (Nzulezo Village):

Coordinates: 5°01'17.7"N 2°35'54.6"W Elevation: 9.8 m



- 4 replicates of sample were taken.

CSIR-WRI Akosombo - Pond 1:

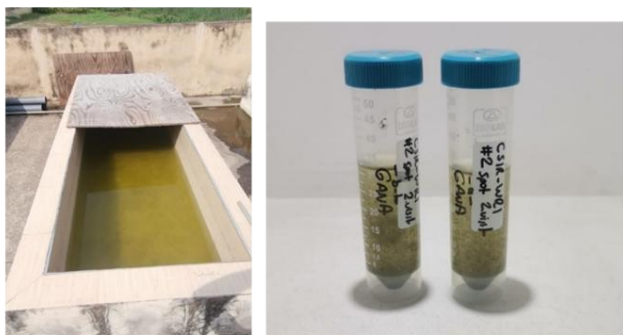
Coordinates: 6°16'57.0"N 0°03'20.0"E Elevation: 28.2 m



- 6 replicates of sample were taken.

CSIR-WRI Akosombo- Pond 2:

Coordinates: 6°16'57.0"N 0°03'20.0"E Elevation: 28.2 m



- 2 replicates of sample were taken

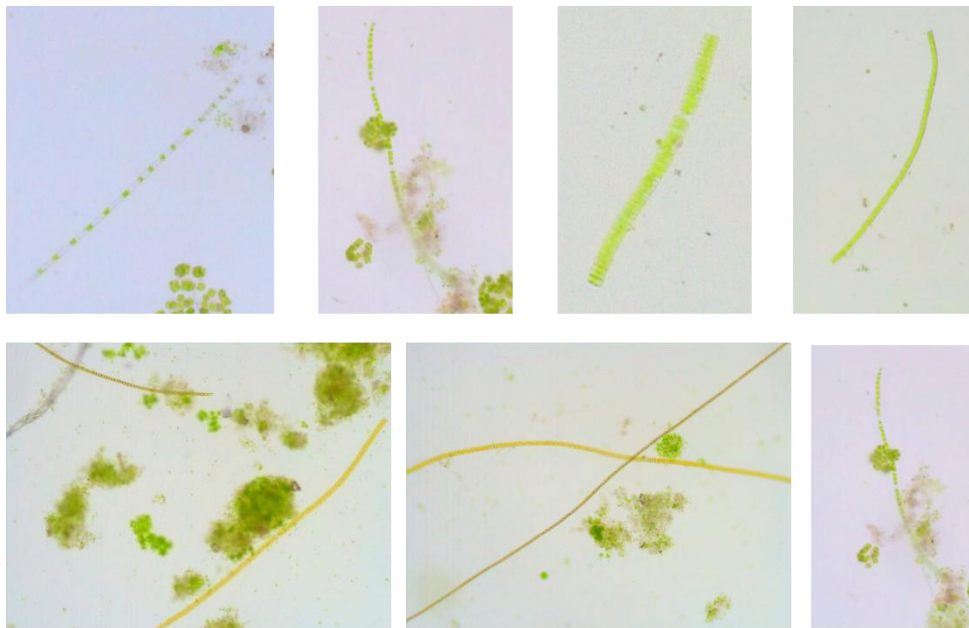
CSIR-WRI Akosombo- Pond 3:

Coordinates: 6°16'57.0"N 0°03'20.0"E Elevation: 28.2 m



- 2 replicates of sample were taken
- Water samples of CSIR-WRI for quality analysis were previously taken during the 1st visit to Ghana.
- The pond in the figure has a dense population of mixed algae. Catfish are also raised in the ponds.

Preliminary microscopic examination of samples at the CSIR-WRI Research Laboratory:



Photos were taken by light-microscopy (10X) in CSIR-WRI

Preliminary microscopic analysis did not reveal any specific spirulina species in the samples. However, samples were transferred to Turkey for a detailed analysis and diagnosis. **It is highly unlikely that a Ghana-specific spirulina species can be isolated from its natural environment, as the minimum conditions required for the survival and reproduction of spirulina species in the local ponds and lakes in Ghana are not sufficient.**

3.4.4 Results of Water Quality and Toxicity Analysis for Ghana

Water samples were taken from Lake Amazuri sampling spots and tap water from the CSIR-WRI Research Facility for water quality and toxicity analysis.

All water samples were firstly filtered by Whatman No 1 filter paper (Cat#1001-125, 11 μm pore size) using water pump filtration system. After filtering, all were analyzed on-site for the 7 different parameters including pH, nitrate (NO_3^-), sodium (Na^+), calcium (Ca^{+2}), potassium (K^+), salt (NaCl), conductivity and temperature using **Horiba 7M LAQUAtwin Kit** (HORIBA, Ltd., Kyoto, Japan).

Lastly, all water samples were also analyzed for ammonium (NH_4), dissolved oxygen (DO), total suspended solids (TSS), phosphate (PO_4), sulfate (SO_4), magnesium (Mg), aluminum (Al), copper (Cu), zinc (Zn), arsenic (As), iron (Fe), cadmium (Cd), cobalt (Co), lead (Pb), chromium (Cr), nickel (Ni), and manganese (Mn) by **Denizli Environmental Quality Laboratory (DENÇEV) of Denizli Municipality- DESKİ General Directorate**, which is nationally and internationally accredited.

The evaluation of the measurement values is left to the project partners of the respective countries, as the reference standards of each country will be different. Each measurement was performed in 3 repetitions and the average values were shared as a result.

** Since there was no Spirulina strain found from the samples of Lake Amanzuri and CSIR-WRI Facilities, the water samples of the lake and the research institute were not analyzed by DESKI, only by Horiba Kit.

Results of Water Quality and Toxicity Analysis

CSIR-WRI-Tap Water:

CSIR-WRI Tap Water Analysis by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
6.90 ±0.12	9.33 ±0.57	4 ±0.00	17.22 ±1.34	nd	nd	76.44 ±1.54	31.9 ±0.10

Lake Amanzuri (Average):

Lake Amanzuri- Lake Water Analysis by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
6.36 ±0.14	9.66 ±0.87	4 ±0.00	nd	4 ±0.00	nd	40.44 ±3.24	30.5 ±0.66

Abbreviations: Acidity (pH), Nitrate (NO₃⁻), Sodium (Na⁺), Calcium (Ca⁺²), Potassium (K⁺), Ammonium (NH₄), Dissolved Oxygen (DO), Total Suspended Solids (TSS), Phosphate (PO₄), Sulfate (SO₄), Magnesium (Mg), Aluminum (Al), Copper (Cu), Zinc (Zn), Arsenic (As), Iron (Fe), Cadmium (Cd), Cobalt (Co), Lead (Pb), Chromium (Cr), Nickel (Ni), Manganese (Mn), Not Detected (nd)

3.5 Sampling in Egypt

3.5.1 Summary Report for Egypt Field Work and Sample Collection

BMAEU was assigned to Egypt, where the ECOHUB centers in Africa are located, for field and sample studies with our project researchers, Bolu Abant İzzet Baysal University faculty members Assoc. Prof. Dr. Ercan Selçuk Ünlü and Assoc. Dr. Murat Telli between **16-20 February 2025**. We had a meeting with our partner in Egypt, Prof. Dr. Refaat Al-Gamal, the director of CLAR Institute and the researchers of the institute to exchange information about our fieldwork and ECOHUB activities in Egypt. We carried out our fieldwork in the presence of our project partners Dr. Yasser Moustafa and Dr. Rahem Abd El-Wahab from CLAR Institute.

One Spirulina farm, which was planned to be visited during the field work, was removed from the visit and sampling list due to repair and maintenance activities. Also, a natural lake that was on our list was removed from the list of sampling sites for safety reasons. Therefore, only the spirulina farm “**Amria Farm for Microalgae**” and the **Elhamra (Red) Lake** with high salt content were sampled. Ali Abd Albaset, a consultant to spirulina farms, accompanied us during the sampling. At the spirulina farm, samples were taken from the tap water used in the culture and the Spirulina grown. We were informed that tap water from the same reservoir is used all over the country. Spirulina sampling and lake water samples were then taken from Lake Elhamra. All water samples were measured for temperature, pH, salinity, salinity, conductivity, nitrate,

potassium, calcium, sodium, and the geographical coordinates and altitudes of the sampling sites were noted. Finally, the samples were properly preserved and brought to our country in a way to preserve the vitality of the organisms in them. This overseas mission, which lasted 5 days including travel, was completed in an effective, efficient and healthy manner.



We had meeting with Prof. Dr. Refaat Al-Gamal, the director of CLAR Institute, and the researchers of the institute, Dr. Yasser Moustafa and Dr. Rahem Abd El-Wahab, to discuss about our fieldwork and ECOHUB activities in Egypt.



One of the facility building of ECOHUB in Egypt is under the construction in the CLAR.



Visiting of the Algal Research and Production Lab of CLAR, and SPARKY Drier bought for the ECOHUB.



Meeting with Dr. Yasser Moustafa, who is responsible for Spirulina Farm in Egypt ECOHUB. Visit to the spirulina farm “Amria Farm for Microalgae” in Egypt

3.5.2 Sampling Data from the visit to Egypt (16 -20 February 2025)

One Spirulina farm, which was planned to be visited during the field work, was removed from the visit and sampling list due to repair and maintenance activities. Also, a natural lake that was on our list was removed from the list of sampling sites for safety reasons. Therefore, only the spirulina farm “**Amria Farm for Microalgae**” and the **Elhamra (Red) Lake** with high salt content were sampled. Ali Abd Albaset, a consultant to spirulina farms, accompanied us during the sampling. At the spirulina farm, samples were taken from the tap water used in the culture and the Spirulina grown. We were informed that tap water from the same reservoir is used all over the country. Spirulina sampling and lake water samples were then taken from Lake Elhamra. All water samples were measured for temperature, pH, salinity, conductivity, nitrate, potassium, calcium, sodium, and the geographical coordinates and altitudes of the sampling sites were noted. Finally, the samples were properly preserved and brought to our country in a way to preserve the vitality of the organisms.

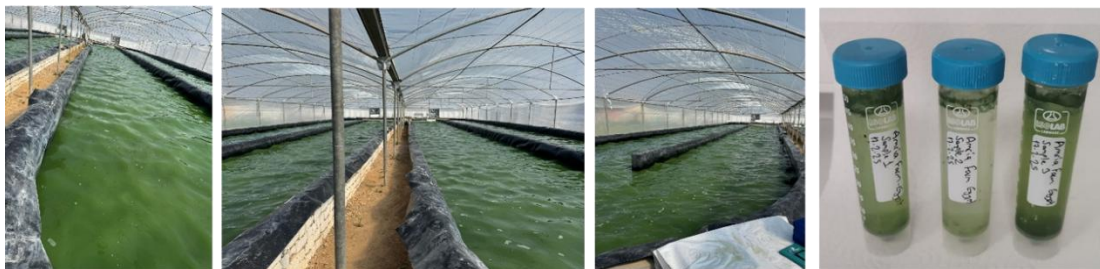
3.5.3 Field Work Locations for Sampling, Lakes and Farms visited in Egypt

All spirulina farms in Egypt cultivate the same native strain, which is from Mansura Governorate. So, there is no restriction or limitation to use, cultivate and trade the Spirulina strain. Even whole genome sequence of the strain is available at GenBank. Dr Yasser will provide the accession number for the strain.

*****In Egypt, all tap water is from same water source, and all Spirulina strain is same strain cultivated through the country.**

Amria Farm (Amria Region, Alexandria Governorate):

Coordinates: 30°59'48.2"N 29°49'13.2"E Elevation: 24.3 m



- 3 replicate of samples from each pond was taken
- 1 L of tap water for water quality analysis was taken.

Lake Elhamra (Red Lake) 1. Spot:

Coordinates: 30°23'47.4"N 30°19'15.2"E Elevation: 10 m



- 3 replicates of sample were taken.
- 500 mL of tap water for water quality analysis was taken.
- The lake water from which the samples were taken is very salty and has a high acidity value, and there are salt deposits at the edge of the lake.

Lake Elhamra (Red Lake) 2. Spot:

Coordinates: 30°23'47.4"N 30°19'15.2"E Elevation: 10 m



- 2 replicates of sample were taken.
- 500 mL of tap water for water quality analysis was taken.
- The lake water from which the samples were taken is very salty and has a high acidity value, and there are salt deposits at the edge of the lake.

Lake Elhamra (Red Lake) 3. Spot:

Coordinates: 30°23'47.4"N 30°19'15.2"E Elevation: 10 m



- 3 replicates of sample were taken.
- 500 mL of tap water for water quality analysis was taken.
- The lake water from which the samples were taken is very salty and has a high acidity value, and there are salt deposits at the edge of the lake.

3.5.4 Results of Water Quality and Toxicity Analysis for Egypt

Water samples taken from Lake Elhamra sampling spots and tap water sample from Amria Farm for water quality and toxicity analysis. All water samples taken were firstly filtered by Whatman No 1 filter paper (Cat#1001-125, 11 μm pore size) using water pump filtration system.

After filtering, all were analyzed on-site for the 7 different parameters including pH, nitrate (NO_3^-), sodium (Na^+), calcium (Ca^{+2}), potassium (K^+), salt (NaCl), conductivity and temperature using **Horiba 7M LAQUAtwin Kit** (HORIBA, Ltd., Kyoto, Japan).

Lastly, all water samples were also analyzed for ammonium (NH_4), dissolved oxygen (DO), total suspended solids (TSS), phosphate (PO_4), sulfate (SO_4), magnesium (Mg), aluminum (Al), copper (Cu), zinc (Zn), arsenic (As), iron (Fe), cadmium (Cd), cobalt (Co), lead (Pb), chromium (Cr), nickel (Ni), and manganese (Mn) by **Denizli Environmental Quality Laboratory (DENÇEV) of Denizli Municipality- DESKİ General Directorate**, which is nationally and internationally accredited.

** Since there was no Spirulina strain found from the samples of Lake Elhamra, the water sample of the lake was not analyzed by DESKİ, only by Horiba Kit.



Water Samples from Lake and Farm

Results of Water Quality and Toxicity Analysis

Lake Elhamra 1. Spot:

Lake Elhamra- 1. Spot Water Analysis by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
8.93 ±0.04	540 ±10.00	nd ±0.00	30.33 ±0.57	300 ±0.00	5.10 ±0.10	54.43 ±1.15	20.5 ±0.10

Lake Elhamra 2. Spot:

Lake Elhamra- 2. Spot Water Analysis by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
9.07 ±0.02	610 ±0.00	nd ±0.00	22 ±1.00	316.66 ±5.77	5.87 ±0.05	57.96 ±0.40	20.45 ±0.15

Lake Elhamra 3. Spot:

Lake Elhamra- 3. Spot Water Analysis by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
9.09 ±0.01	556.66 ±5.77	nd ±0.00	22.66 ±0.57	283.33 ±5.77	5.03 ±0.15	55.60 ±0.10	20.83 ±0.05

Lake Elhamra (Average):

Lake Elhamra- Lake Water Analysis by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ⁺² (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
9.03 ±0.08	568.88 ±36.56	nd ±0.00	25 ±4.63	300 ±16.66	5.30 ±0.49	55.66 ±2.26	20.59 ±0.20

Abbreviations: Acidity (pH), Nitrate (NO₃⁻), Sodium (Na⁺), Calcium (Ca⁺²), Potassium (K⁺), Ammonium (NH₄), Dissolved Oxygen (DO), Total Suspended Solids (TSS), Phosphate (PO₄), Sulfate (SO₄), Magnesium (Mg), Aluminum (Al), Copper (Cu), Zinc (Zn), Arsenic (As), Iron (Fe), Cadmium (Cd), Cobalt (Co), Lead (Pb), Chromium (Cr), Nickel (Ni), Manganese (Mn), Not Detected (nd)

** Since no spirulina was isolated from this extremely salty lake, no toxicity and heavy metal analysis of the water sample taken from the lake was carried out.

Amria Farm Tap Water:

Amria Farm Tap Water Analysis by Horiba 7M LAQUAtwin Kit							
pH	NO ₃ ⁻ (ppm)	Na ⁺ (ppm)	Ca ²⁺ (ppm)	K ⁺ (ppm)	Salt NaCl (%)	Conductivity (µS/cm)	Temp. (°C)
7.75 ±0.10	16.22 ±0.38	70.33 ±0.33	69 ±0.57	8.55 ±0.83	0.02 ±0.00	555.22 ±32.83	24.33 ±0.55

Amria Farm- Tap Water Analysis by DESKI																
NH ₄ mg/L	DO mg/L	TSS mg/L	PO ₄ mg/L	SO ₄ mg/L	Mg mg/L	Al µg/L	Cu µg/L	Zn µg/L	As µg/L	Fe µg/L	Cd µg/L	Co µg/L	Pb µg/L	Cr µg/L	Ni µg/L	Mn µg/L
79.88 ±0.46	5.18 ±0.08	<10	<0.8	63.29 ±0.15	13.68 ±0.58	17.86 ±0.13	7.53 ±0.12	<20	<0.5	<10	<0.5	<0.5	<0.5	1.95 ±0.11	1.01 ±0.06	<1

Abbreviations: Acidity (pH), Nitrate (NO₃⁻), Sodium (Na⁺), Calcium (Ca²⁺), Potassium (K⁺), Ammonium (NH₄), Dissolved Oxygen (DO), Total Suspended Solids (TSS), Phosphate (PO₄), Sulfate (SO₄), Magnesium (Mg), Aluminum (Al), Copper (Cu), Zinc (Zn), Arsenic (As), Iron (Fe), Cadmium (Cd), Cobalt (Co), Lead (Pb), Chromium (Cr), Nickel (Ni), Manganese (Mn), Not Detected (nd)

3.6 Selection, isolation, and purification

All samples from the local lakes and private spirulina farms visited in Kenya, Tanzania, Ghana and Egypt during the field work and sampling is listed and labeled as follow;

Kenya: Lake Simbi, Lake Nakuru, Lake Oloiden, Lake Elementaita, Lake Bogoria, Keriko Secondary School Spirulina Farm and the IIMSAM (Intergovernmental Institution for the use of Micro-algae Spirulina Against Malnutrition) Spirulina farm.

Tanzania: Lake Big Momella, Lake Small Momella, Lake Natron, and Lake Manyara.

Ghana: Lake Volta, CSIR-WRI, Bio-Green Agro Farm, and Fish&Feed Farm.

Egypt: Amria Farm for Microalgae and the Lake Elhamra (Red)

All samples were supplemented with appropriate amounts of water and media to compensate for water evaporation and nutrient loss during sampling and material transport. The 50 mL tubes containing the samples were kept uncapped under white LED light at 70 µmol/m²s⁻¹. All cultures and samples used during isolation and purification were kept at room temperature under the same light intensity.

3.6.1 Selection, isolation, purification, and cultivation studies of Spirulina strains from Kenya samples

Samples in 50 ml tubes were washed several times with Zarrouk medium, inoculated into the liquid medium and diluted into solid medium. After reaching sufficient density, they were washed again and transferred to the horizontal medium shown in the figure and single cells were selected under stereo-microscope. The selected single cells were first cultured in solid medium and then transferred to horizontal medium to reach high density. After reaching sufficient density, they were transferred to 250 mL Erlenmeyer flasks containing 100 mL Zarrouk medium and aerated with 3% CO₂ (v/v) for a certain period of time.

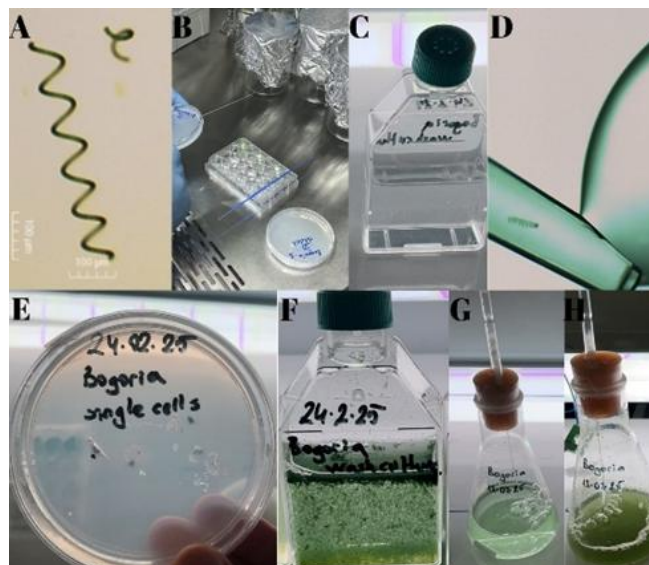


Figure 1. Isolation of individual spirulina cells from samples of **Lake Bogoria**, Kenya (A, D); Cultivation in liquid and solid culture (B, E), horizontal liquid medium (C, F), and cultivation in Erlenmeyer flasks (G-H).

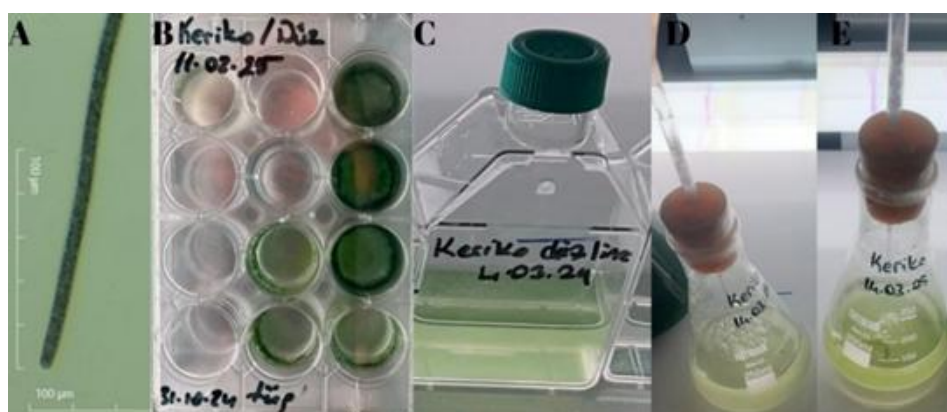


Figure 2. Isolation of individual spirulina cells from samples of **Keriko Secondary School Farm**, Kenya (A); cultivation in a liquid culture (B), horizontal culture medium (C) and Erlenmeyer flasks (D, E).

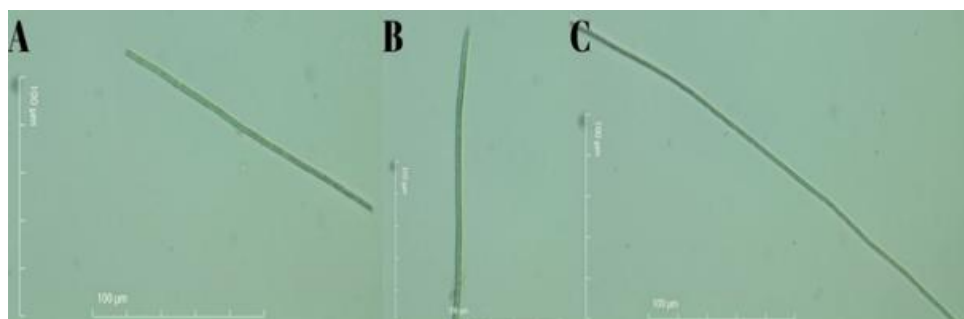


Figure 3. Photographs of the filamentous species observed in the sample of **Lake Elmentiatu**, Kenya taken under the light microscope (A, B, C). The samples were examined under the microscope for the second time and again no species thought to be Spirulina was found. Therefore, the sample was not transferred to the medium.

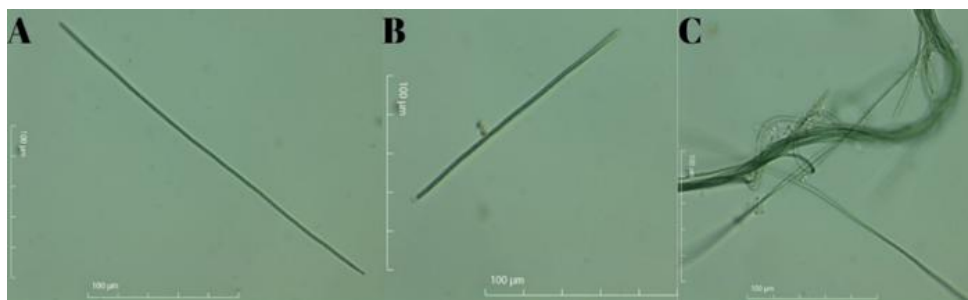


Figure 4. Photographs of the filamentous species observed in the sample of **Lake Simbi**, Kenya taken under the light microscope (A, B, C). The samples were examined under the microscope for the second time and again no species thought to be *Spirulina* was found. Therefore, the sample was not transferred to the medium.

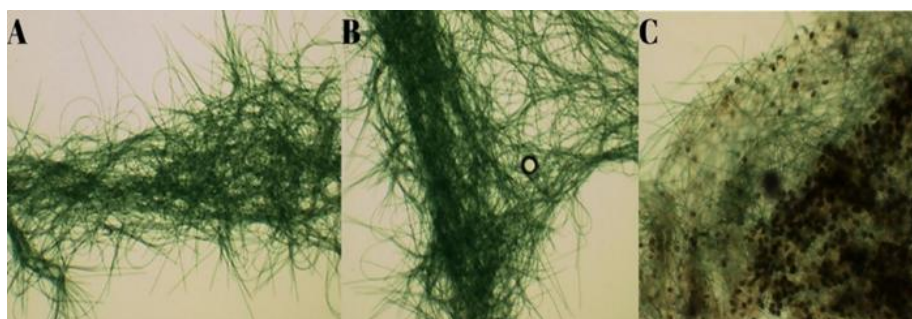


Figure 5. Photographs of the filamentous species observed in the sample of **Lake Oloiden**, Kenya taken under the light microscope (A, B, C). The samples were examined under the microscope for the second time and again no species thought to be *Spirulina* was found. Therefore, the sample was not transferred to the medium.

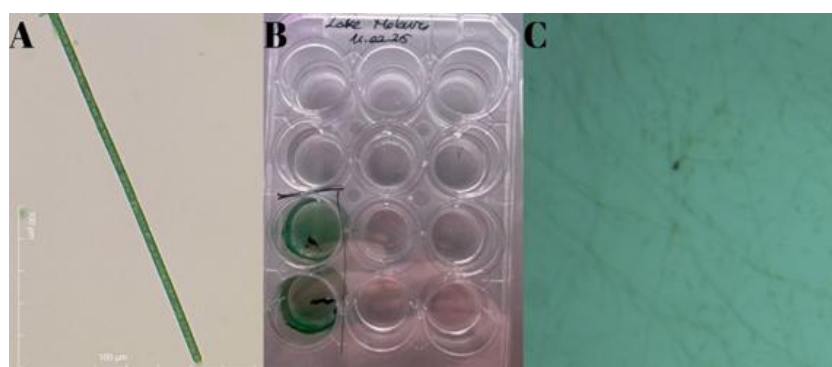


Figure 6. Photographs of the *Spirulina* species observed in the sample of **Lake Nakuru**, Kenya taken under the light microscope (A), isolation and cultivation of the strain (B), and final density of the monoculture of the strain (C).

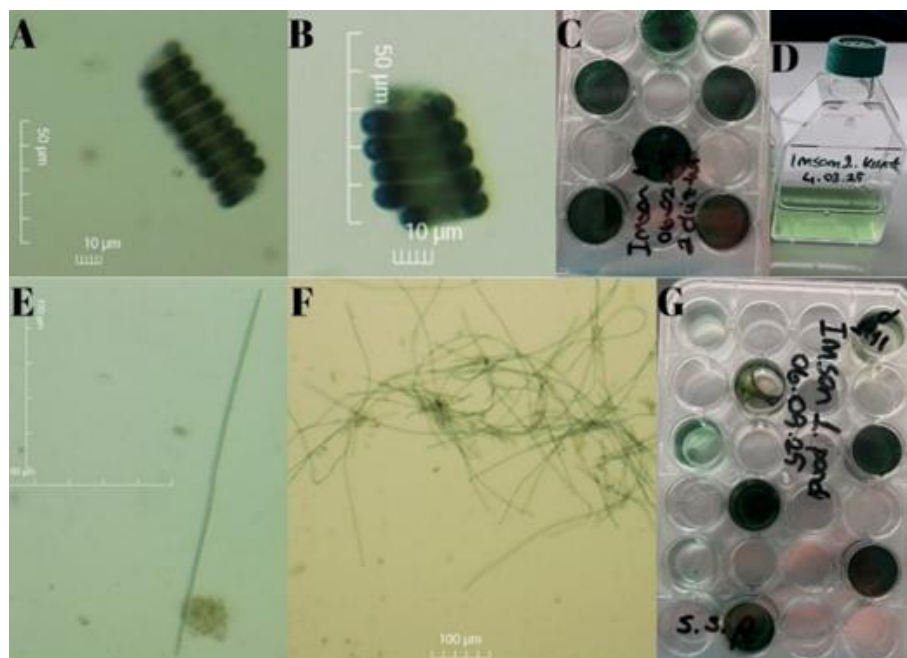


Figure 7. Isolation of spiral and straight strains from the **IIMSAM Farm**, Kenya (A, B, E, F), cultivation in liquid (C, G) and horizontal medium (D).

3.6.2 Selection, isolation, and purification studies of *Spirulina* strains from Tanzania samples

Samples in 50 ml tubes were washed several times with Zarrouk medium, inoculated into the liquid medium and diluted into solid medium. After reaching sufficient density, they were washed again and transferred to the horizontal medium shown in the figure and single cells were selected under stereo-microscope. The selected single cells were first cultured in solid medium and then transferred to horizontal medium to reach high density. After reaching sufficient density, they were transferred to 250 mL Erlenmeyer flasks containing 100 mL Zarrouk medium and aerated with 3% CO₂ (v/v) for a certain period of time.

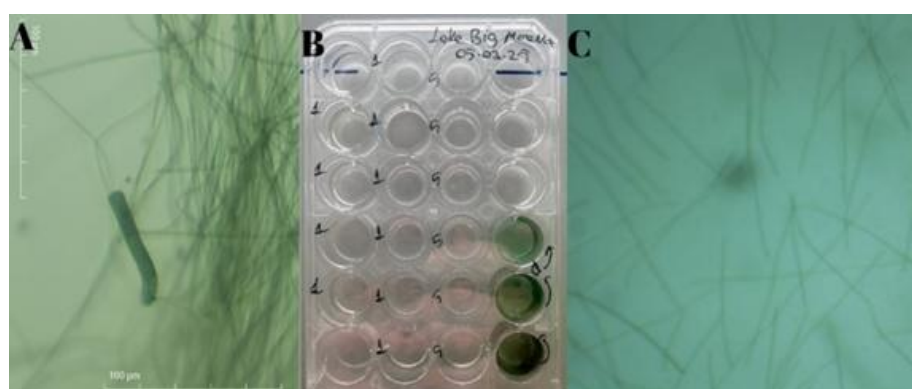


Figure 1. Photographs of the *Spirulina* species observed in the sample of **Lake Big Momella**, Tanzania taken under the light microscope (A), isolation and cultivation of the strain (B), and final density of the monoculture of the strain (C).

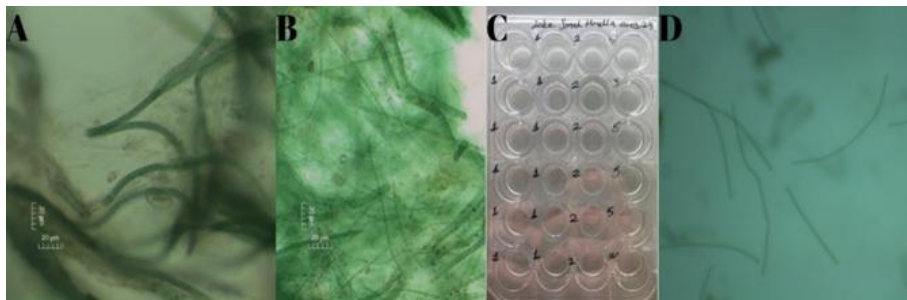


Figure 2. Photographs of the *Spirulina* species observed in the sample of **Lake Small Momella**, Tanzania taken under the light microscope (A), isolation and cultivation of the strain (B), and final density of the monoculture of the strain (C).

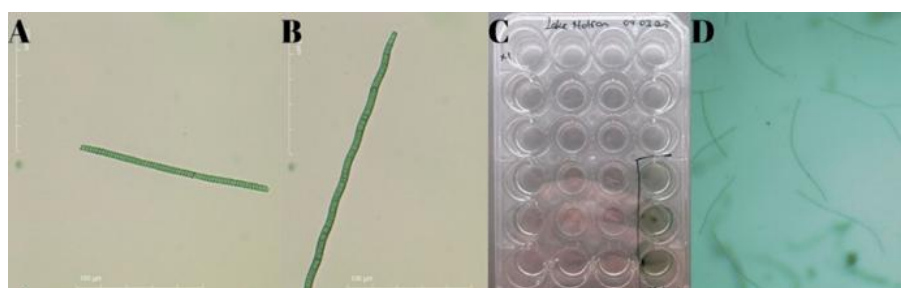


Figure 3. Photographs of the *Spirulina* species observed in the sample of **Lake Natron**, Tanzania taken under the light microscope (A), isolation and cultivation of the strain (B), and final density of the monoculture of the strain (C).

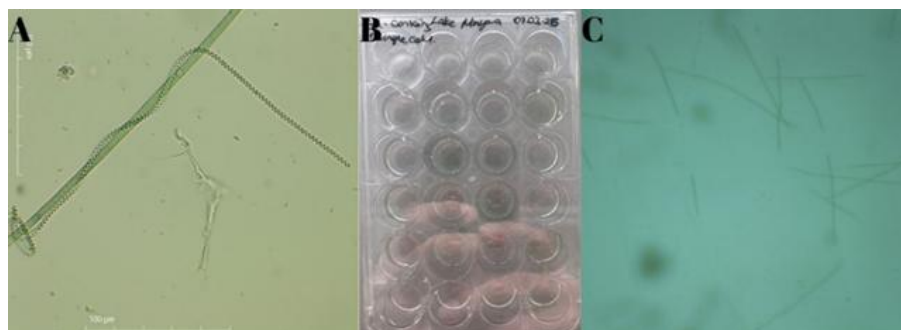


Figure 4. Photographs of the *Spirulina* species observed in the sample of **Lake Manyara**, Tanzania taken under the light microscope (A), isolation and cultivation of the strain (B), and final density of the monoculture of the strain (C).

3.6.3 Selection, isolation, and purification studies of *Spirulina* strains from Ghana samples

In the microscopic analysis of the samples collected from Ghana at the end of the field and sampling studies carried out 2 times, spirulina-like filamentous algae were detected, unfortunately no spirulina species were found. Therefore, cultivation and isolation studies were not carried out for these samples. Microscopic images of these specimens are given below.

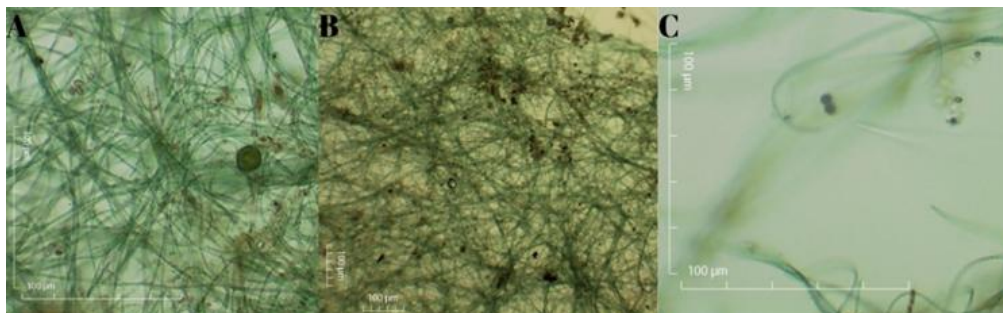


Figure 1. Photographs of the filamentous species observed in the sample of **Lake Volta, Ghana** taken under the light microscope (A, B, C). The samples were examined under the microscope for the second time and no species thought to be *Spirulina* was found. Therefore, the sample was not transferred to the medium.

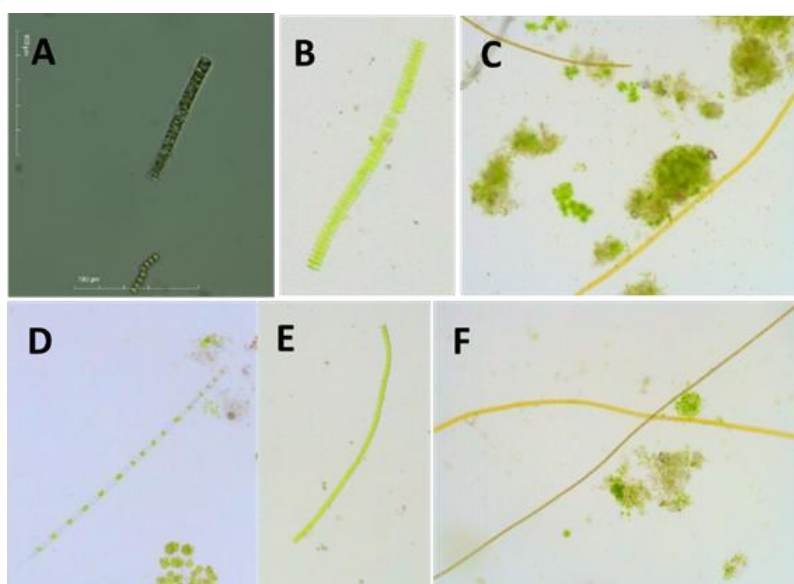


Figure 2. Photographs of the filamentous species observed in the sample of **CSIR-WRI** research facility taken under the light microscope (A, B, C). The samples were examined under the microscope for the second time and no species thought to be *Spirulina* was found. Therefore, the sample was not transferred to the medium.

Sample of **Bio green Agro Farm, Fish&Farm Farm, Lake Amanzuri** and the location called as **Accra from the Accra city center** were examined by the light microscope. Unfortunately, species thought to be *Spirulina* was not found. The two farms in question are not spirulina farms, but have fish ponds specifically for growing fish, and have been observed to use a mixed algae culture in these ponds by bringing in water from the surrounding natural water sources. Therefore, the cultures from these ponds were found to contain other dominant algae instead of spirulina.

3.6.4 Selection, isolation, and purification studies of *Spirulina* strains from Egypt samples

Microscopic examination failed to identify spirulina species in samples from Lake El-Hamra. The high salinity of the lake water does not seem to be suitable for the living conditions of spirulina species. A monoculture was obtained by isolating the spirulina strain from the Amria farm.

The samples in 50 ml tubes were washed several times with Zarrouk medium, inoculated into the liquid medium and diluted into solid medium. After reaching sufficient density, the sample were washed again and transferred to the horizontal medium shown in the figure and single cells were selected under stereo-microscope. The selected single cells were first cultured in solid medium and then transferred to horizontal medium to reach high density. After reaching sufficient density, they were transferred to 250 mL Erlenmeyer flasks containing 100 mL Zarrouk medium and aerated with 3% CO₂ (v/v) for a certain period of time.

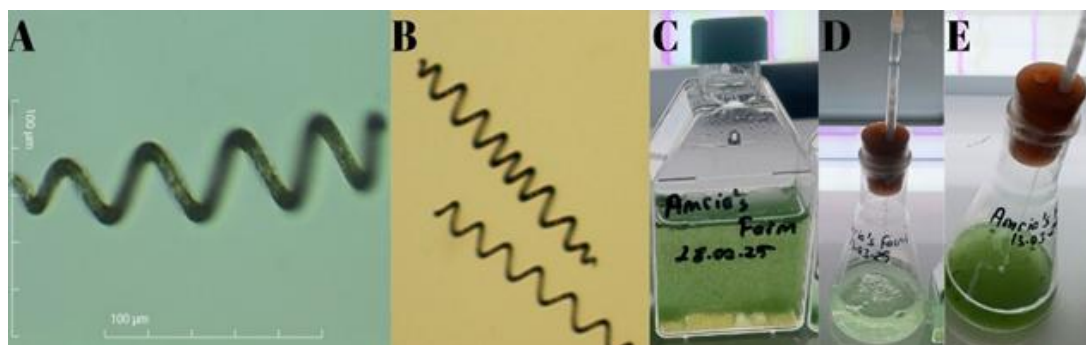


Figure 1. Isolation of individual spirulina cells from samples of **Amria Farm**, Egypt (A, B); cultivation in a horizontal culture medium (C) and Erlenmeyer flasks (D, E).



Figure 2. Photographs of the filamentous species observed in the sample of El-Hamra taken under the light microscope. The sample were examined under the microscope and no species thought to be Spirulina was found. Therefore, the sample was not transferred to the medium

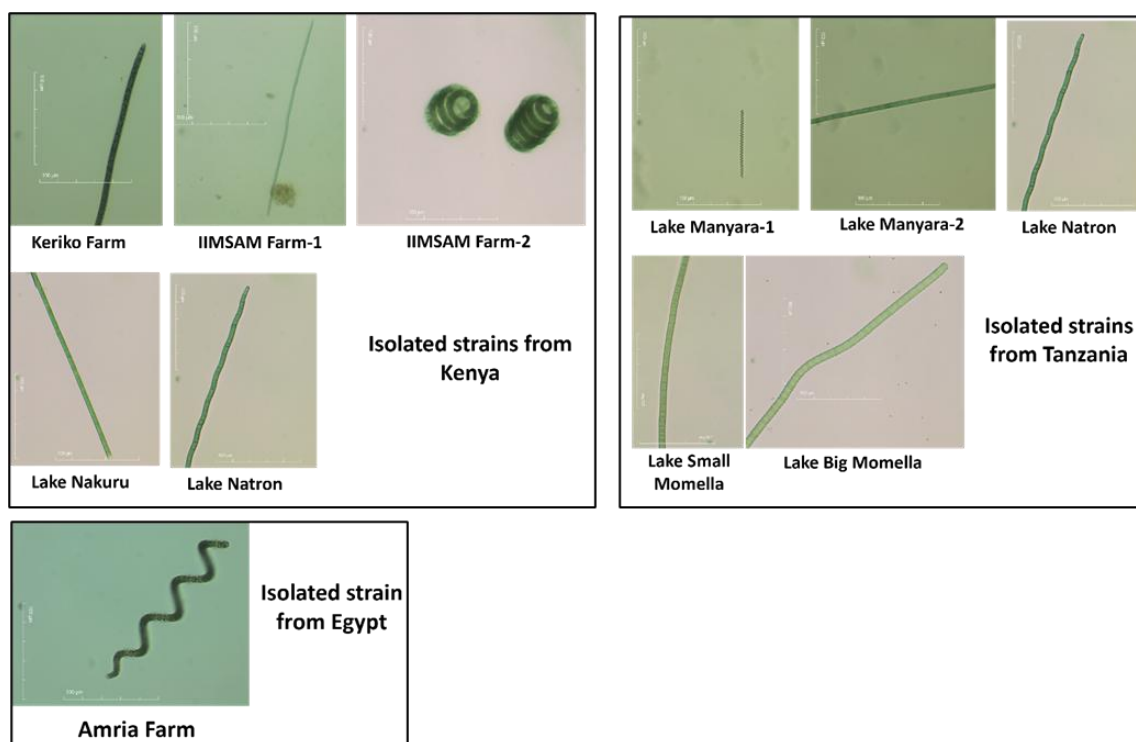
Overall result of selection, isolation, purification and cultivation progress, and Microscopic visualization of isolated Spirulina strains from Kenya, Tanzania, Ghana and Egypt

Through fieldwork, sampling, isolation, purification and cultivation, 11 different Spirulina species have been phenotypically identified, isolated and monocultured to date. These species are summarized in the table below. Light microscopy images of these species are also shared below.

Table 1. Strains isolated, purified and adapted to the Lab conditions

Kenya	Tanzania	Ghana	Egypt
Lake Bogoria: 1 strain	Lake Small Momella: 1 strain	Lake Volta: None	Lake El-Hamra: None
Lake Elmentaita: None	Lake Big Momella: 1 strain	CSIR-WRI: None	Amria Farm: 1 strain
Lake Nakuru: 1 strain	Lake Natron: 1 strain	Lake Amanzuri: None	Total: 1 strain
Lake Oloiden: None	Lake Manyara: 2 strains	Accra: None	
Lake Simbi: Ongoing	Total: 5 strains	Bio-green Farm: None	
Keriko Secondary School Farm: 1 strain		Fish&Farm Farm: None	
IIMSAM Farm: 2 strains		Total: 0 strain	
Total: 5 strains			

Microscopic visualization of isolated strains:



3.7 Phenotypic characterization by Confocal Laser Scanning Microscopy (CLM)

Imaging analyses and protocol development studies were performed on a few isolated spirulina samples obtained from partner countries in Africa. This was accomplished utilizing the Confocal Laser Scanning Microscope (CLSM) at the Central Research Laboratory – ARUM – Advanced Technology Unit of Eskişehir Osmangazi University.

Confocal Microscope was used for this study because to its efficacy in imaging fluorescent specimens. Contrary to a light microscope, this apparatus has been employed to clarify the three-dimensional characteristics of specimens while avoiding blurriness or low-resolution images.

3.7.1 Protocol development for CLM

Isolated spirulina culture samples stored at room temperature were used as material. Ten milliliters glass pipettes were employed to transfer materials onto slides, which were subsequently covered with coverslips. The autofluorescence characteristic of the samples was employed, therefore no special staining procedure was performed.

The images of the prepared samples were subsequently acquired using the ZEISS LSM 800 (Laser Scanning Confocal Microscope) located at ARUM. The microscope system comprises diode laser sources at wavelengths of 405 nm, 488 nm, 561 nm, and 640 nm.

The images were acquired using the LSM 800 microscope (Zeiss) in Airyscan mode, testing various excitation and emission wavelengths available in the system. **The experiments concluded that the spirulina samples produced clearer images at the wavelengths of 505/566 nm (Dy500) and 555/700 nm (RhRex).** Subsequently, the samples were acquired utilizing different magnification lenses (20× and 40× with oil immersion). Three-dimensional data was acquired from the samples utilizing both the snapshot and Z-stack functionalities of the microscope.

Results:

Result 1. The B1 (Bogoria) sample was scanned with a 20× objective and the Dy500 (560nm), RhRex (620nm), and At425 (425nm) wavelengths. Consequently, due to the inability to acquire a distinct reflection at the At425 wavelength, only the Dy500 (560nm) and RhRex (620nm) wavelengths were utilized in additional experiments. While a comprehensive spirulina sample was inaccessible, filamentous structures were acquired, as illustrated in **Figure 1**. The B1 (Bogoria) sample was also scanned with a 40× objective, as illustrated in **Figure 2**. The scanned specimen was recorded as an image.

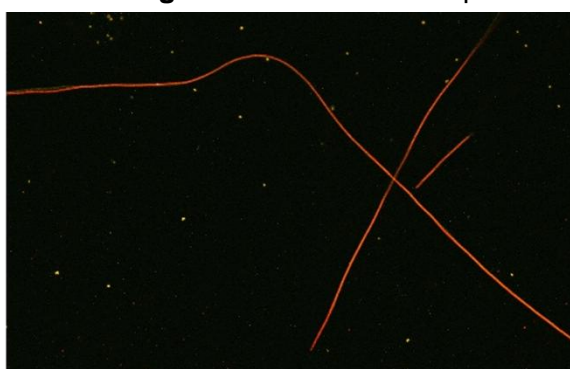


Figure 1. B1 (Bogoria) sample was obtained using a 20× objective.

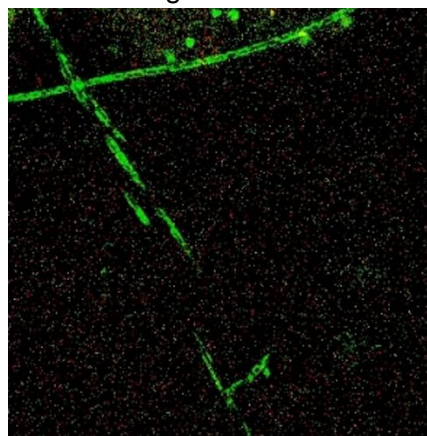


Figure 2. B1 (Bogoria) sample was obtained using a 40× objective

Result 2. The image depicted in **Figure 3** was captured with a 20× objective in the B2 (Bogoria) sample. The optimal reflection was achieved at the RhRex wavelength of 620nm. This image employs the z-stack imaging approach, with image stacks acquired through the 'Color Coded Projection' tool to utilize all

the stacks effectively. **This example is exemplary among the collected photographs due to its better purification and clean background, serving as a guide for future imaging.**

Result 3. The data presented in **Figure 4** was derived from the B3 (Bogoria) sample. The image was acquired from the RhRex (620nm) wavelength with a 40× objective and z-stack features. The sample does not remain purified, containing both spirulina and other filamentous forms. This image may again function as an effective indicator for acquiring improved samples from future specimens.



Figure 3. B2 (Bogoria) sample was obtained using a 20× objective.

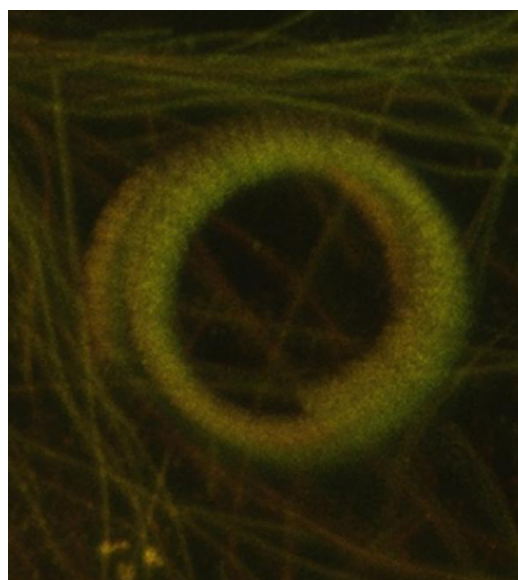


Figure 4. B3 (Bogoria) sample was obtained using a 40× objective

Result 4. The I1 (IIMSAM-2. pond) sample, as illustrated in **Figures 5** and **6**, provided acceptable results. **Figure 5** was captured with a 20× objective at the RhRex (620nm) wavelength. **Figure 6** was captured with a 40× objective at the RhRex (620nm) wavelength, and a 3D image was generated utilizing 'Color Coded Projection'. **These two images provide a valuable reference for future imaging.**

Overall Result:

The samples demonstrate excellent autofluorescence characteristics, enabling promising outcomes through the utilization of suitable objectives and the formation of z-stacks, dependent upon the selection of the aforementioned appropriate wavelengths, thereby eliminating the necessity for diverse staining procedures. This protocol development study showed that under suitable conditions, Spirulina samples can be visualized via a Laser Scanning Confocal Microscope. **Figures 3, 5, and 6** serve as the most notable images of this concept.

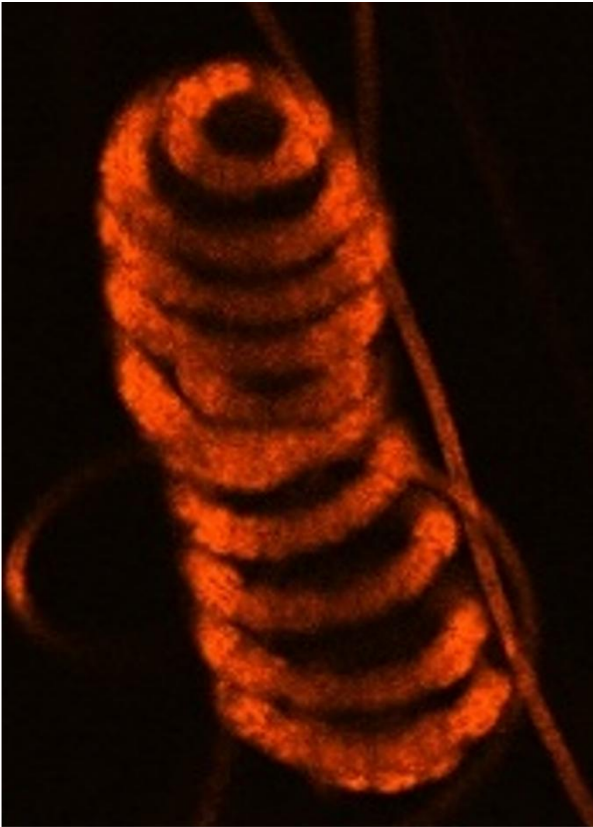
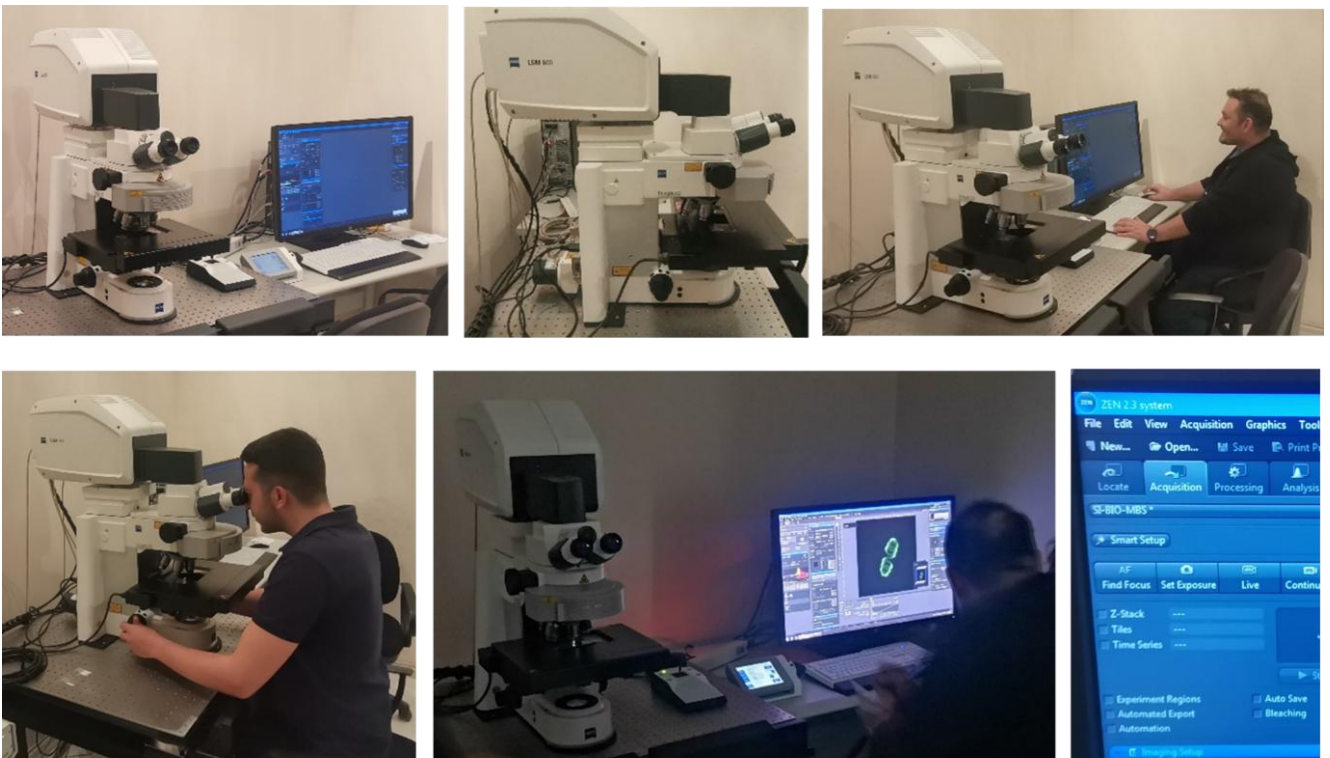


Figure 5. I1 (IIMSAM-2. pond) sample was obtained using a 20x objective.



Figure 6. I1 (IIMSAM-2. pond) sample was obtained using a 40x objective.



CLM studies and observations

3.7.2 CLM analysis of isolated spirulina strains at phenotypic level

The microscopic analysis was conducted at the Advanced Technology Unit of Eskişehir Osmangazi University Central Research Laboratory – ARUM, utilizing the Laser Scanning Confocal Microscope device (CLSM), ZEISS LSM 800.

Materials and Methods:

Isolated and purified *Spirulina* samples for CLSM scans were temporarily labeled based on the name of sample collection site. The spirulina samples analyzed with confocal microscopy were listed and numbered as follows:

1- Lake Natron	2- Amria Farm	3- Lake Manyara 1
4- Keriko High School Farm	5- Lake Bogoria	6- IIMSAM Farm 2 (spiral)
7- IIMSAM Farm 1 (straight)	8- Lake Manyara 2	9- Lake Big Momella
10-Lake Small Momella	11- Lake Nakuru	

To ensure the viability of the spirulina samples till scanning, the mounting media utilized for their purification was employed in the preparations for CLSM. The purified samples were deposited onto the slide using 10 ml glass pipettes and subsequently covered with a coverslip. **No specific staining procedure was employed as the autofluorescence characteristic of the materials was utilized.** The images of the prepared samples were subsequently acquired using the “ZEN 2.3 system” application of ZEISS LSM 800 (Laser Scanning Confocal Microscope) located at ARUM.

The images were acquired using a Zeiss LSM 800 microscope in Airyscan mode, utilizing two excitation/emission wavelengths established during the protocol development studies conducted previously. The compounds were DY-500, with a fluorescence excitation peak at 505 nm and an emission peak at 565 nm, and Rhodamine Red-X, with an excitation peak at 573 nm and an emission peak at 591 nm. The samples were acquired with objectives at different magnifications (20x and 40x with oil immersion). Three-dimensional data were acquired from the samples utilizing both the snapshot feature (when the samples were active and highly mobile) and the Z-stack feature (in regions where the samples stayed comparatively stable) under the microscope. Upon application of the Z-stack, the resultant images were examined externally using the 'Color Coded Projection' or 'Sample Down' choices.

Results:

Result 1. The Lake Natron specimen was examined utilizing 20x and 40x objective lenses. The specimen examined with the 40x objective was generated via the Z-stack feature and the 'sample down' option (**Figure 1**). The samples prominently included photos of spirulina. Due to the samples remaining viable and challenging to preserve, the z-stack function was utilized for 3 minutes, resulting in relatively worthwhile photos. The final photos will be generated from the dynamic samples subsequently.

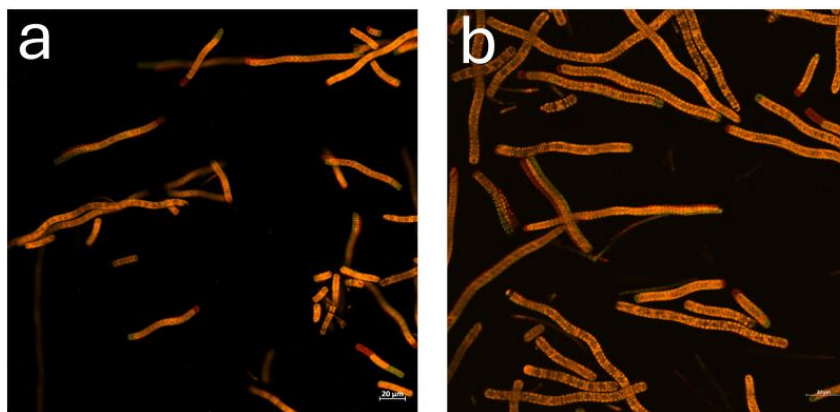


Figure 1. Lake Natron sample. a. 20× objective, b. 40× objective. Scale 20 µm

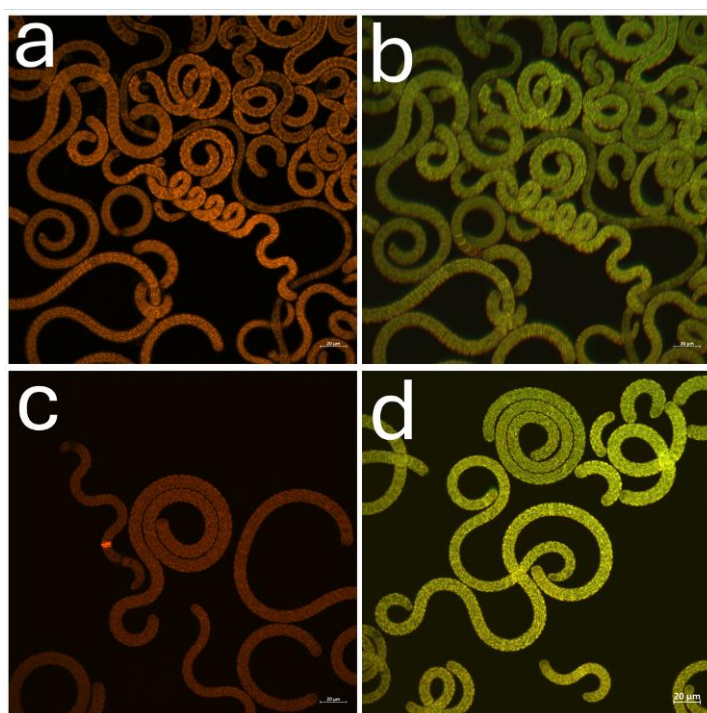


Figure 2. Amria Farm example. a. 40× objective-snapshot, b. 40× objective-Color Coded projection, c. 40× objective-snapshot, d. 40× objective-Color Coded projection. Scale 20 µm.

Result 2. The Amria Farm sample was photographed (**Figure 2**) in multiple modes by concentrating on distinct sections with a 40× objective. **Figure 2a** was captured as a single image, while **Figure 2b** was obtained by a z-stack; nonetheless, blurring occurred due to sample movement. Samples from **Figures 2c** and **2d** were successfully photographed, yielding distinct spirulina images.

Result 3. The Lake Manyara sample was scanned using 40× and 63× objectives. The filamentous structures were clearly visualized in the images (**Figure 3**).

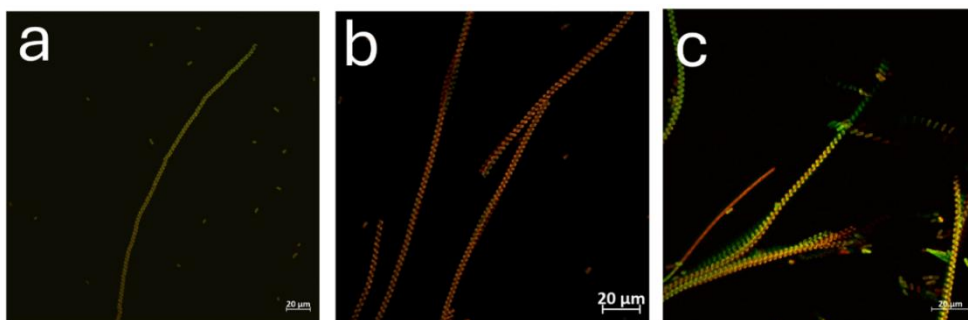


Figure 3. Lake Manyara sample. a. 40× objective-snapshot, b. 40× objective-Color Coded projection, c. 63× objective- Color Coded projection. Scale 20 µm.

Result 4. The Keriko High School Farm sample was scanned using 40x objectives. It was successfully imaged (**Figure 4**).

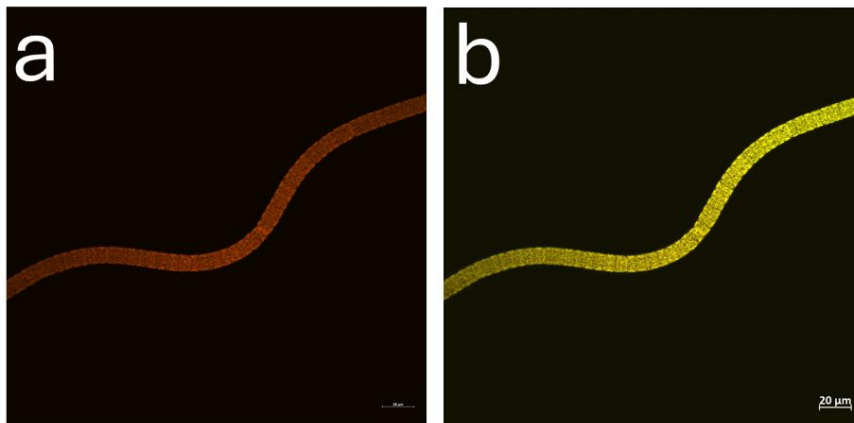


Figure 4. Keriko High School Farm example. a. 40x objective-snapshot, b. 40x objective-Color Coded projection. Scale 20 µm.

Result 5. The Lake Bogoria sample was photographed from various places utilizing 40x lenses (**Figure 5**).

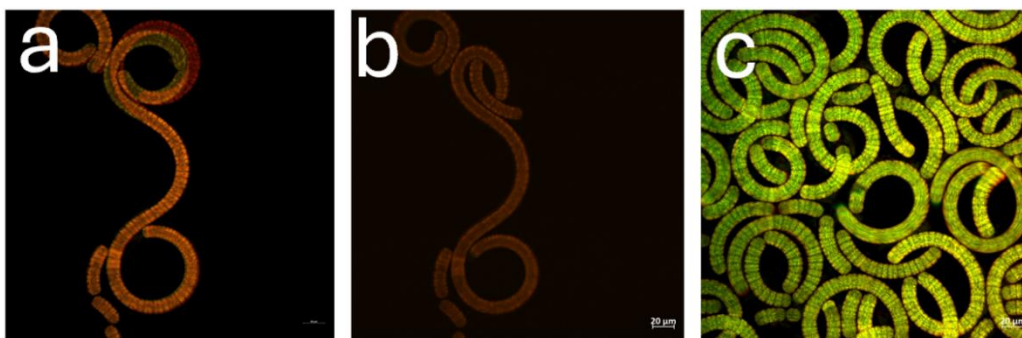


Figure 5. The Lake Bogoria sample. a. 40x objective-snapshot, b. 40x objective- snapshot, c. 40x objective- Color Coded projection. Scale 20 µm.

Result 6. Images of the IIMSAM pond (spiral specimen) sample were acquired from several locations (**Figure 6**).

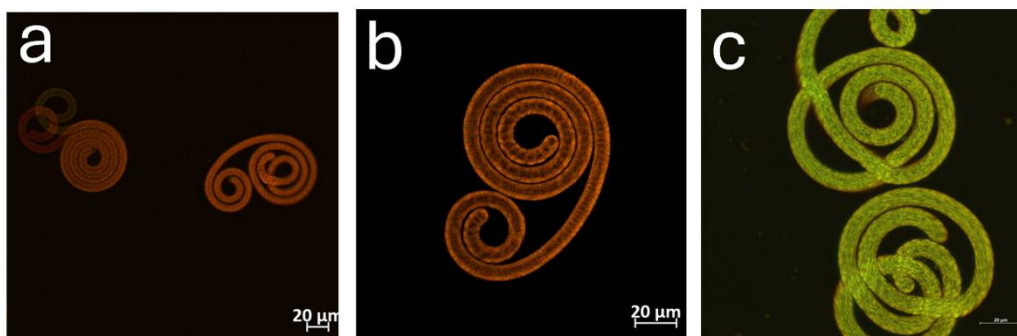


Figure 6. IIMSAM pond (spiral specimen) example. a. 40x objective-snapshot, b. 40x objective- Sample down, c. 40x objective- Color Coded projection. Scale 20 µm.

Result 7. Images of the IIMSAM pond (straight specimen) sample were acquired from several locations (**Figure 7**).

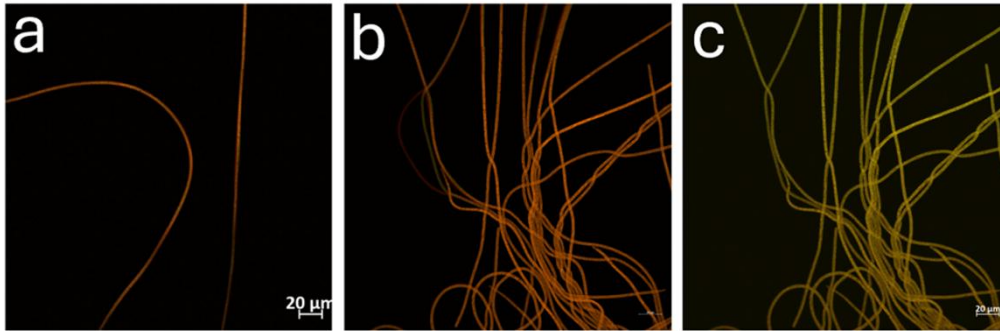


Figure 7. IIMSAM pond (straight specimen) sample. a. 40× objective-snapshot, b. 40× objective-Sample down, c. 40× objective- Color Coded projection. Scale 20 μm.

Result 8. The Lake Manyara- 2 sample images were obtained from different regions (**Figure 8**).

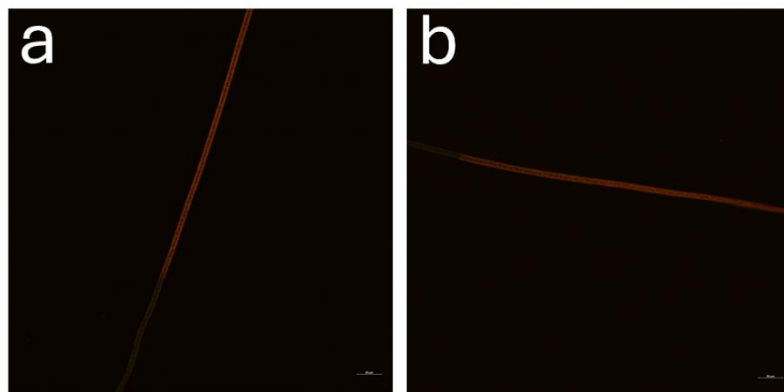


Figure 8. The Lake Manyara-2 sample. a. 40× objective-snapshot, b. 40× objective- Sample down, Scale 20 μm.

Result 9. Images of the Lake Big Momella sample were obtained from different regions (**Figure 9**).

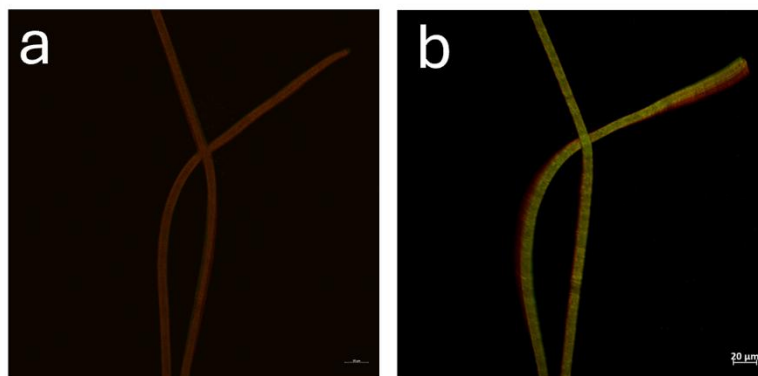


Figure 9. The Lake Big Momella sample. a. 40× objective-snapshot, b. 40× objective- Sample down, Scale 20 μm.

Result 10. Images of the Lake Small Momella sample were obtained from different regions (**Figure 10**).

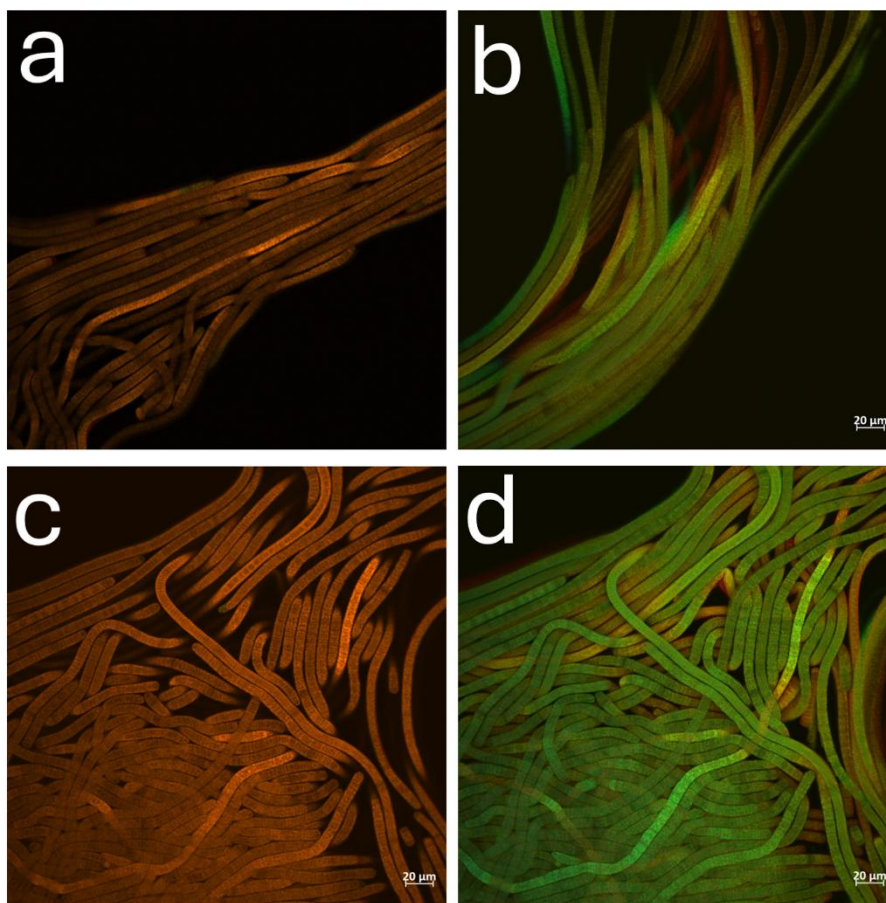


Figure 10. The Lake Small Momella sample. a. 40× objective-snapshot, b. 40× objective- Color Coded Projection, c. 40× objective-Sample Down, d. 40× objective- Color Coded Projection. Scale 20 µm.

Result 11. The Lake Nakuru sample was imaged from different regions (**Figure 11**).

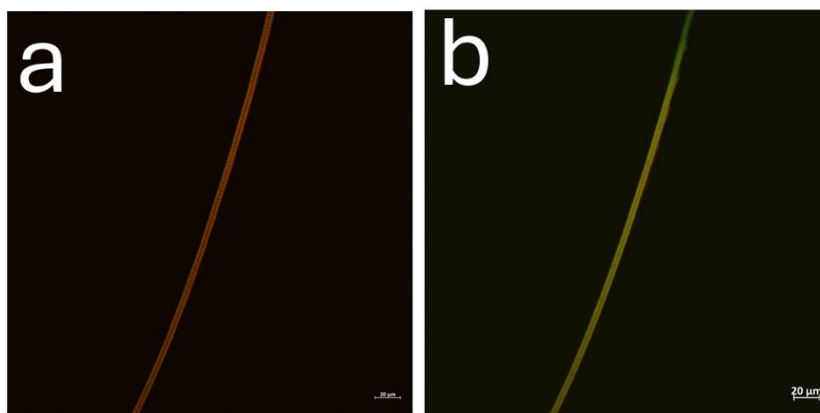


Figure 11. The Lake Nakuru sample. a. 40× objective-snapshot, b. 40× objective- Color Coded Projection. Scale 20 µm.

Overall Result:

Multiple spirulina species sourced purified and isolated were imaged using CLSM, yielding successful findings. All samples exhibited favorable autofluorescence qualities, allowing for imaging without staining. High resolution photographs were obtained from the specimens, but this phenotypic data may not be sufficient to specifically identify and characterize the analyzed specimens in terms of species-level identification. Therefore, further identification analysis with molecular and genetic markers is needed in the further steps.

3.8 Molecular characterization

Due to the high degree of similarity, phenotypic characterization of spirulina species to distinguish them from other similar algae is not sufficient for the identification of species. For this reason, genetic markers, which are also used in the literature, allow for more reliable identification of differences at the DNA level, allowing us to identify new strains.

3.8.1 Optimization of genomic DNA isolation and Polymerase Chain Reaction (PCR) conditions

Before implementation of molecular characterization by genetic markers genomic DNA isolation, gel electrophoresis and PCR conditions need to be optimized in advance.

Isolation of genomic DNA

For the isolation of genomic DNA (gDNA) from 2 weeks-old cultures of *Spirulina platensis* strain FavorPrep™ Plant Genomic DNA Extraction Mini Kit (Favorgen, Taiwan) was used. 140 mg and 85 mg of wet microalgae cultures were centrifuged and washed with PBS. The precipitated cultures were washed with PBS and transferred to sterile microcentrifuge tubes containing acid-washed glass beads (200 µm diameter). 400 µL of FAPG1 Buffer and 8 µL of RNase A solution were added into the tubes. The mixture was homogenized vigorously using Beadbug Microtube homogenizer (Benchmark Scientific, USA) and incubated at room temperature for 2 minutes, followed by incubation at 65°C for 10–20 minutes with gentle inversion every 2–3 minutes. Following the incubation, 130 µL of FAPG2 buffer was added, and the samples were vortexed thoroughly before placing them on ice for 5 minutes. The lysate was transferred to a filter column positioned in a collection tube and centrifuged at 18,000 × g for 3 minutes. The flow-through was discarded, and the washed lysate was transferred to a new sterile microcentrifuge tube. 900 µL FAPG3 buffer was added to the lysate. A new FAPG column was placed into a collection tube, mixture was carefully transferred to the column. The column was centrifuged at 18,000 × g for 1 minute, and the flow-through was discarded. This step was repeated until the entire sample had been processed. The column was then washed by adding 400 µL of W1 Buffer (containing ethanol), followed by centrifugation at 18,000 × g for 30 seconds. The flow-through was discarded, and the column was repositioned into the collection tube. Subsequently, 650 µL of Wash Buffer was added, and the column was centrifuged under the same conditions, twice. To remove residual ethanol, an additional centrifugation step was performed at 18,000 × g for 3 minutes to dry the membrane completely. For the elution of DNA, the FAPG column was placed into a new elution tube, and 100 µL of Elution Buffer at 65 °C was added directly to the center of the membrane. The column was allowed to stand at room temperature for 1 minute. Finally, the column was centrifuged at 18,000 × g for 1 minute to elute the purified DNA. The yield of the extracted DNA was 12 µg for 140 mg microalgae tissue and 7 µg for 85 mg. The extracted DNA samples were stored at –20°C until PCR analysis.

Optimization of PCR condition

In this study we tried to optimize PCR conditions especially for annealing temperature of the primers since a variety of primer sets are going to be used for future identification studies of purified spirulina species. Thus, we adjusted the gradient PCR conditions that the annealing temperatures would cover estimated T_m values of each primer designed. The PCR reactions were carried out using T-Professional Basic gradient thermocycler (Biometra, Germany). **Table 1** shows the contents of each PCR reactions set for 20 µL final volume. **Table 2** shows the details of the primer sets used in the experiments. PCR amplifications were initiated with a denaturation step at 95°C for 3 min, followed by 35 cycles of DNA denaturation at 94°C for 40 s, primer annealing at 55°C, 58°C, 60°C, 62°C or 65°C for 45 s, strand extension at 72°C 1 min, and a final extension step at 72°C for 5 min.

Table 1. Contents of the PCR reaction mixture.

Contents	Volume	Final Concentration
10X Taq Buffer	2.0 µL	1X
dNTP Mix, 10 mM each	0.4 µL	0.2mM
Primer pairs*, 10 µM each	1.0 µL	0.5 µM each
25 mM MgCl ₂	1.2 µL	1.5 mM
Template DNA, 160 ng/µL	0.8 µL	125ng
Taq DNA Polymerase 5 U/µL	0.1 µL	0.5U
Water, nuclease-free	14.5 µL	
Total volume	20.0 µL	

*The list of primer pairs is provided in **Table 2**

Table 2. List of primers tested for PCR optimization of annealing temperature using *Spirulina platensis* gDNA

No	Forward	Reverse	Forward	Reverse	Target	Size (bp)	References
1	CYA106-F	CYA781Ra	CGGACGGGTGAGTAACGCGTGA	GACTACTGGGGTATCTAATCCCAT	16 rRNA	660	(Kormas et al., 2011)
2	CYA106-F	CYA781Rb	CGGACGGGTGAGTAACGCGTGA	GACTACAGGGGTATCTAATCCCTTT	16 rRNA	660	(Kormas et al., 2011; Nübel et al., 1997)
3	CYA359F*	CYA781Ra	GGGGAATYTTCCGCAATGGG	GACTACTGGGGTATCTAATCCCAT	16 rRNA	800	(Nübel et al., 1997)
4	CYA359F*	CYA781Rb	GGGGAATYTTCCGCAATGGG	GACTACAGGGGTATCTAATCCCTTT	16 rRNA	800	(Nübel et al., 1997)
5	27F	809R	AGAGTTTGATCCTGGCTCAG	GCTTCGGCACGGCTCGGGTCGATA	16 rRNA	750	(Jungblut et al., 2010)
6	ILE23F	RP2	ATTAGCTCAGGTGGTTAG	ACGGCTACCTTGTACGACTT	16 rRNA	200	(Strunecký et al., 2012; Weisburg et al., 1991)
7	ILE23F	23SR	ATTAGCTCAGGTGGTTAG	TTTGGCCGCTCTGTGTGCCTAGGTATCC	23S rRNA	400	(Strunecký et al., 2012; Zhang et al., 2022)
8	ILE23F	1492R	ATTAGCTCAGGTGGTTAG	GGTACCTTGTACGACTT	16-23S rRNA ITS	200	(Miller et al., 2013; Strunecký et al., 2012)
9	ILE23F	1492R*	ATTAGCTCAGGTGGTTAG	GGYTACCTTGTACGACTT	16-23S rRNA ITS	200	(Chen et al., 2019; Strunecký et al., 2012)
10	<i>cpc_a1F</i>	<i>cpc_a1R</i>	TCGAAGATCGTTGCTTGAACG	TTAGGTCCTGCATTTGGGTG	<i>cpcBA</i> -IGS*	500	(Kumar et al., 2017)
11	<i>cpc_a1F</i>	CPC1R*	TCGAAGATCGTTGCTTGAACG	AARCGNCTTGRGWATCDGC	<i>cpcBA</i> -IGS*	400	(Choi et al., 2012; Kim et al., 2006)
12	<i>dsqDXF</i> *	<i>sdsqDXR</i> *	GGATYCAYGTKGYBAAYCCDGC	CCNGCBGCCATNGCYTC	<i>sqdX</i> **	600	(Kwei et al., 2011)
13	<i>dsqDBF</i> *	<i>dsqDBR</i> *	GAYGNTAYTYGGNTGG	GGCGTRAAAYTRITRAANAC	<i>sqdB</i> **	900	(Kwei et al., 2011)
14	CPC1F*	CPIR*	GGCKGCTGYTRCGYGACATGGA	GCHGATWCYCAAGNCGYTT	<i>cpcBA</i> -IGS	600	(Kim et al., 2006)
15	IAB-595F*	IAB-1385R*	GAYTTMACTAARGATGAYGA	TCGAACCTGATTCTCTTCCA	<i>cobL</i> ***	800	(Shawer et al., 2023)

Degenerate primer

* *cpcBA*- intergenic space on phycocyanin operon locus

** *Sulfolipid* biosynthesis gene

*** *RuBisCO* large subunit-encoding gene

Results

For the **16S rRNA gene**, strong amplification of 660 bp products were observed for the CYA106F/CYA781Ra (**Figure 1a**) and CYA106F/CYA781Rb (**Figure 1b**) primer sets at all tested temperatures. The most intense bands for both primer sets were observed at 62°C and 65°C. CYA359F/CYA781Ra primer set resulted in a nonspecific band amplification below 400 bp which also affected the overall yield of amplification expected at 400 bp band (**Figure 1c**). A strong amplification was also obtained for CYA359F/CYA781Rb (**Figure 1d**). The band intensity was brightest at 60°C and 62°C. The 27F/809R primer set successfully amplified an 800 bp product with the strongest band appearing at 60°C, while the yield of amplification was decreased slightly at 62°C and 65°C (Fig 1e). ILE23F/RP2 did not yield any product, except some non-specific bands fading with rising temperatures.

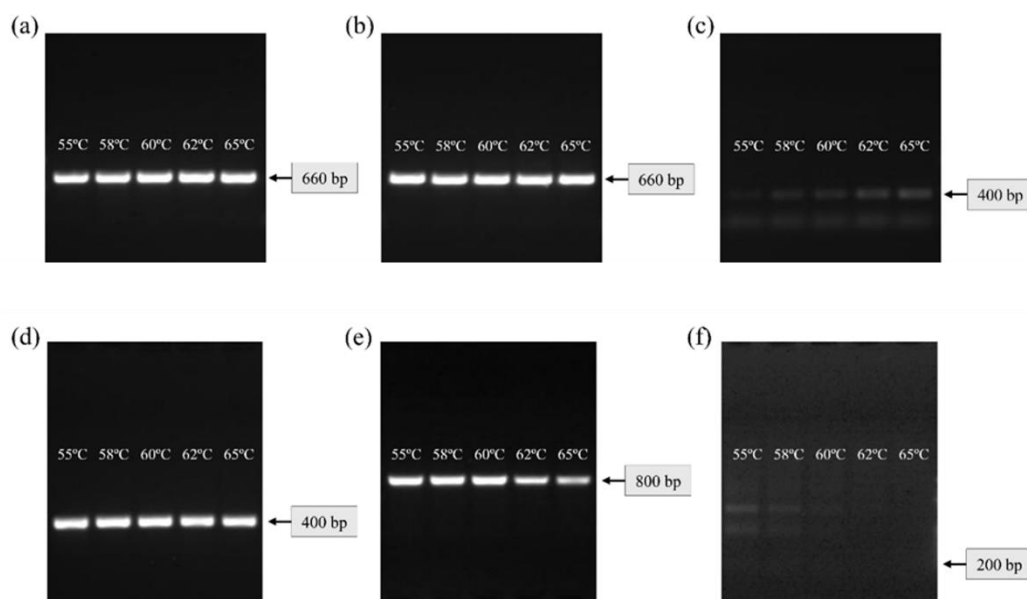


Figure 1. PCR amplification of 16S rRNA gene in *Spirulina platensis* strain. 16S rRNA region was amplified using CYA106F/CYA781Ra (a), CYA106F/CYA781Rb (b), CYA359F/CYA781Ra (c), CYA359F/CYA781Rb (d), 27F/809R (e) and ILE23F/RP2 (f) universal primers. PCR products were run in 1.0% agarose gel prepared in 1XTBA.

Amplification of **23S rRNA region** using ILE23F/23SR primer set produced a distinct 500 bp band which showed best yield at 58°C where the yield drastically decreased with increasing annealing temperatures and slightly lower yield at 55°C compared to that of 58°C (**Figure 2a**). Amplification of 16S-23S rRNA ITS regions using ILE23F/1492R (**Figure 2b**) and ILE23F/1492R_{degenerate} (**Figure 2c**) primer sets did not yield any specific products other than several weak nonspecific products.

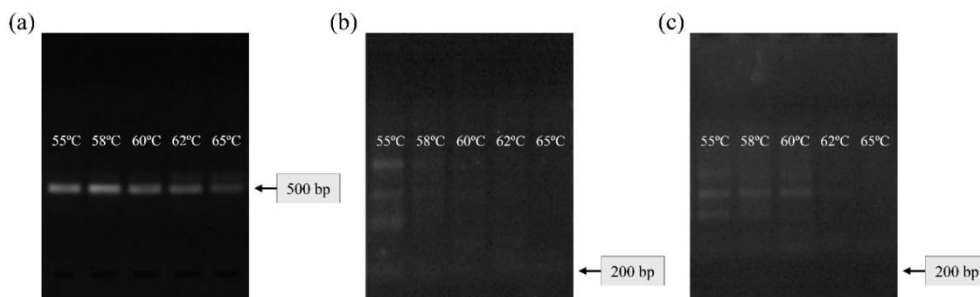


Figure 2. PCR amplification of 23S rRNA and 16S-23S rRNA ITS regions in *Spirulina platensis* strain. 23S rRNA region was amplified using ILE23F/23SR (a) universal primer set. 16S-23S rRNA ITS regions were amplified using ILE23F/1492R (b) and ILE23F/1492R_{degenerate} (c) universal primer sets. PCR products were run in 1.0% agarose gel prepared in 1XTBA.

In the amplification of the **cpcBA-intergenic space** within the phycocyanin operon locus, the cpc_arF/cpc_arR primer set produced a 500 bp fragment, with the most intense band at 65°C (**Figure 3a**). The cpc_arF/CPC1R primer set amplified a 400 bp product, with the best clarity observed at 55°C and decreased with increasing temperatures (**Figure 3b**). A similar pattern was observed for CPC1F/PC1R primer set that amplified a weak 600 bp fragment, but it also resulted with even stronger nonspecific band amplification at about 1100 bp (**Figure 3c**).

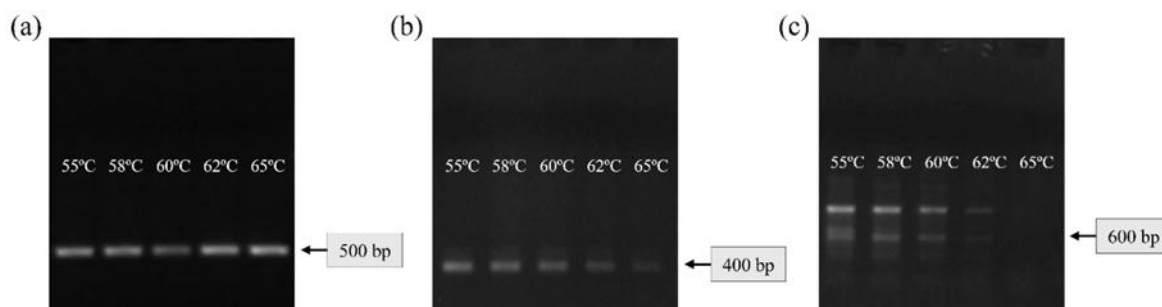


Figure 3. PCR amplification of cpcBA-intergenic space on phycocyanin operon locus in *Spirulina platensis* strain. cpcBA-IGS regions were amplified using cpc_arF/cpc_arR (a), cpc_arF/CPC1R (b) and CPC1F/PC1R (c) primer sets. PCR products were run in 1.0% agarose gel prepared in 1XTBA.

For **sulfolipid biosynthesis genes**, the *sqdX* and *sqdB* primer sets amplified a 600 bp and 900 bp products, with the strongest bands at 55°C (**Figure 4a** and **Figure 4b**). The yields, for both primer sets, decreased with increasing annealing temperatures. In addition, the primer designed to amplify *sqdX* gene resulted with a non-specific band at 1600 bp (**Figure 4a**).

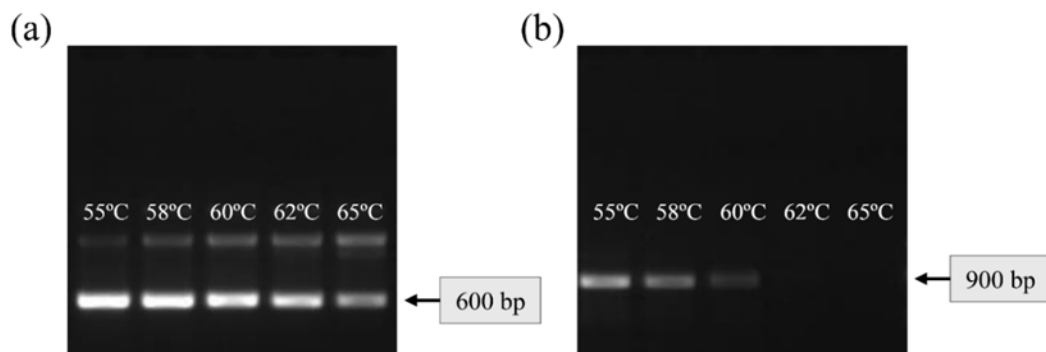


Figure 4. PCR amplification of sulfolipid biosynthesis genes in *Spirulina platensis* strain. *sqdX* and *sqdB* genes were amplified using dsqdXF/dsqdXF and dsqdBF/dsqdBR (b) and primer sets, respectively. PCR products were run in 1.0% agarose gel prepared in 1XTBA.

Amplification of the **RuBisCO large subunit gene (*cbbL*)** using the IAB-595F/IAB-1385R primer set produced an 800 bp fragment (**Figure 5**). The best amplification was observed at 58°C. The band intensity was rapidly decreased, that the weakest band was observed at 60°C and no clear bands were visible at 62°C and 65°C.

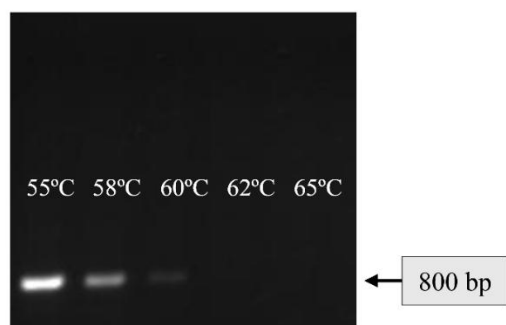


Figure 5. PCR amplification of RuBisCO large subunit-encoding gene in *Spirulina platensis* strain. *cbbL* genes were amplified using IAB-595F/IAB-1385R primer set.

Overall Result:

In conclusion, nine of the designed primer sets were successfully optimized with clear and expected size products. On the other hand, CYA359F/CYA781Ra, ILE23F/RP2, ILE23F/1492R and ILE23F/1492R_{degenerate}, PC1F/PC1R, and dsqdXF/dsqdXF primer pairs failed to either produce a valid PCR product or contain one or more nonspecific bands. Thus, they will be excluded from further experiments. **Table 3** summarizes the optimum annealing temperatures determined by the PCR experiments. Identification of purified spirulina species can be carried out by using one or more of the primer sets given in **Table 3**. The annealing temperature can be adjusted to specific temperature for each primer set's optimum temperature for multiple gene targets using a gradient thermocycler.

Table 3. Recommended annealing temperatures for primer sets

Primer Set		Recommended Annealing Temperatures
Forward	Reverse	
CYA106-F	CYA781Ra	62°C and 65°C
CYA106-F	CYA781Rb	62°C and 65°C
CYA359F#	CYA781Rb	60°C and 62°C
27F	809R	58°C and 60°C
ILE23F	23SR	58°C
cpc_arF	cpc_arR	62°C and 65°C
cpc_arF	CPC1R#	55°C and 58°C
dsqdBF#	dsqdBR#	55°C
IAB-595F#	IAB-1385R#	55°C

Degenerate primer

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3.8.2 Molecular characterization of *Spirulina* strains by genetic markers

Isolation of genomic DNA

FavorPrep™ Plant Genomic DNA Extraction Mini Kit (Favorgen, Taiwan) was used for the isolation of purified samples approximately 2 weeks-old purified cultures from 4 different locations. 90 mg of Keriko Farm culture, 75 mg of Amria Farm, 80 mg of Lake Bogoria culture and 73 mg of IIMSAM Farm culture (spiral form) were centrifuged and washed with PBS. The isolation protocol was followed as previously described in the protocol development studies. The extracted DNA samples were stored at -20°C until PCR analysis.

PCR conditions

The PCR reactions were carried out using T-Professional Basic gradient thermocycler (Biometra, Germany). **Table 1** shows the contents of each PCR reactions set for 50 μL final volume. The gradient PCR conditions to meet optimized annealing temperatures for each primer pair selected for further analysis. **Table 2** shows the primer sets used in the experiments and optimized annealing temperature specific for each primer set. PCR amplifications were initiated with a denaturation step at 95°C for 3 min, followed by 35 cycles of DNA denaturation at 94°C for 40 sec, primer annealing at 54°C , 55°C , 58°C or 62°C for 40 sec, strand extension at 72°C for 1 min 50 sec, and a final extension step at 72°C for 5 min.

Table 1. Contents of the PCR reaction mixture.

Contents	Volume	Final Concentration
10X Taq Buffer	5.0 μL	1X
dNTP Mix, 10 mM each	1.0 μL	0.2 mM
Primer pairs*, 10 μM each	3.0 μL	0.5 μM each
25 mM MgCl_2	2.5 μL	1.5 mM
Template DNA	2.0 μL	~250 ng
Taq DNA Polymerase 5 U/ μL	0.25 μL	0.5U
Water, nuclease-free	36.25 μL	
Total volume	50.0 μL	

*The list of primer pairs is provided in the protocol development studies.

Table 2. Annealing temperatures applied for each primer set during PCR.

Primer Pair	Adjusted Annealing Temperature ($^{\circ}\text{C}$)
CYA106-F/CYA781Rb	62
CYA359F/23SR	58
cpc_arF/cpc_arR	62
dsqdBF/dsqdBR	54
IAB-595F/IAB-595R	55

Gel Electrophoresis of PCR Products and Partial Sequencing

3 μL of PCR products were loaded into 1% agarose gel to confirm the DNA region of interest was well amplified. After the gel run (Cleaver Horizontal Electrophoresis Gel, nanoPAC-300 power supply) the gel was

visualized under ultra violet (UV) light (DNR Bio-Imaging Systems, MiniLumi Imaging Device) to show amplified PCR products shown in the **Figure 1**. Remaining 47 µl of each amplicon was sequenced through Sanger Sequencing technology, and NCBI blastn suite was used for BLAST analysis to identify the closest matches.

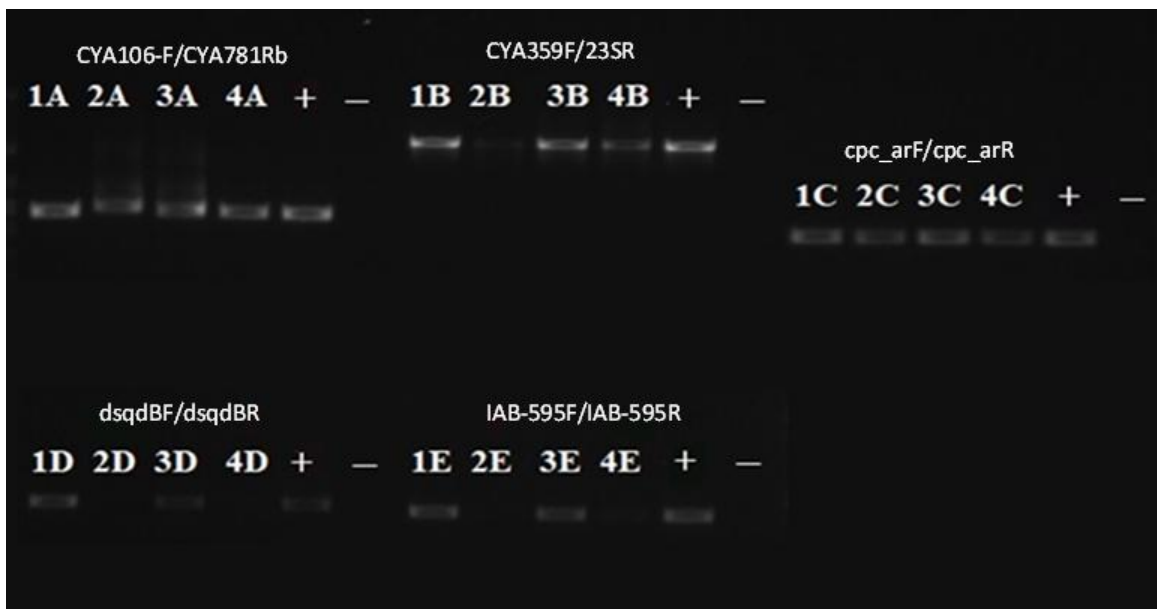


Figure 1. Agarose gel image of PCR reaction. Isolates were represented with numbers (1: Keriko Farm; 2: Amria Farm; 3: Lake Bogoria, and 4: IIMSAM Farm (spiral form). Positive (+) and negative controls (-) were also loaded into the gel. Genomic DNA`s were amplified using five primer sets targeting: A-16S rRNA gene (CYA106-F/CYA781Rb), B-16S-23S rRNA ITS (CYA359F/23SR), C-*cpcBA* intergenic spacer (*cpc_arF/cpc_arR*), D-*sqdB* gene (*dsqdBF/dsqdBR*), E-RuBisCO large subunit gene (IAB-595F/IAB-595R).

Sequencing Results and BLAST Analysis

After obtaining the raw sequences through Sanger sequencing, the sequence data manually cured to remove low quality reads represented with Ns.

Following Sanger sequencing of PCR-amplified products from four isolated strain: **Keriko Farm, Amria Farm, Lake Bogoria, and IIMSAM Farm (spiral form)**, high-quality nucleotide sequences were obtained after confirming the sequences using MEGA12 software. PCR products amplified with five primer sets targeting different genetic loci were analyzed through BLASTn comparisons to determine phylogenetic affiliations.

All five primer sets used in PCR amplification yielded strong, specific bands for the sample from **Keriko Farm**. Sequencing of the **16S rRNA gene (CYA106-F/CYA781Rb)** resulted in a high-confidence alignment (99.67% identity, 100% query cover) with *Arthrospira platensis* MMG-9 and other *Arthrospira* strains. Similarly, the **16S–23S ITS region** showed 95.45% identity to *A. platensis* MMG-9 and related strains. The ***cpcBA* intergenic spacer region** showed 99.60% identity to *Limnospira sp.* PMC 288.06 and *A. platensis* C1, confirming the presence of spirulina. The ***sqdB* gene** was amplified and perfectly matched (100%) to *A. platensis* C1 and *Limnospira fusiformis*. Likewise, the **RuBisCO large subunit gene** also showed 100% identity to *Limnospira fusiformis* KN01, validating the phylogenetic consistency across loci. **Overall, the Keriko Farm isolate was confidently identified as *Arthrospira platensis* or a closely related *Limnospira* species.**

The sequence of **the 16S rRNA** region from **Amria Farm** revealed perfect matches to uncultured bacterial sequences, including Verrucomicrobiales (100% identity). However, **the ITS region** displayed high similarity (98.01%) to *Arthrospira platensis* MMG-9 and GMPA7, confirming the cyanobacterial nature of this isolate. The divergent results compared to results obtained with other primers suggest that the results are affected by low resolution of the loci for species identification. **The cpcBA region** showed 100% identity to *A. platensis* C1 and *Limnospira fusiformis* KN01, aligning with results from other primer sets. In contrast, amplification of **the sqdB gene** failed for the isolate, suggesting possible absence or mutation in the sqdB locus. **The RuBisCO large subunit gene** showed moderate identity (94.13%) with *A. platensis* and *L. fusiformis*, suggesting potential strain-level divergence. These results also suggest the presence of spirulina strain.

The 16S rRNA gene sequence from **Lake Bogoria** culture samples showed relatively low similarity (88.45%) to *Synechocystis minuscula* and uncultured cyanobacteria, suggesting either a divergent *Arthrospira* strain or issues with resolution of the locus. **The ITS region** returned 96.54% identity to *A. platensis* BYN1, and **the cpcBA region** showed 100% identity to both *A. platensis* C1 and *Limnospira fusiformis* KN01, providing better identification predictions. **The sqdB gene** was aligned perfectly (100%) with *Arthrospira* and *Limnospira* sequences, consistent similar to data obtained for the cpcBA region. **The RuBisCO large subunit gene** sequence showed 99.60% identity to *A. platensis* HY and other related species. These results suggest that the culture sample from Bogoria Lake is most closely related to *Arthrospira platensis*.

Amplification of all five gene targets was successful for purified cultures from **IIMSAM Farm (spiral form)**. **The 16S rRNA gene** showed 98.4% identity with *Synechocystis minuscula* SAG, suggesting a false prediction for this culture, as well. **The ITS region** showed 94.2% identity with *A. platensis* RRGK-AP, while **the cpcBA region** returned 100% identity to *A. platensis* C1 and *Limnospira fusiformis* supporting the data. **The sqdB gene** showed lower identity (~89.66%) compared to other isolates, possibly indicating a unique sequence variant. In contrast, **the RuBisCO large subunit gene** returned a 100% identity match with *Limnospira fusiformis* KN01, suggesting a close relationship to known *Arthrospira*–*Limnospira* taxa. **Overall, purified cultures from the IIMSAM Farm (spiral form) belong to *Arthrospira platensis*.**

Overall Result

In conclusion, the application of five genetic markers allowed for the robust identification of cyanobacterial species across different habitats. The consistent presence of *Arthrospira platensis* and *Limnospira fusiformis* across multiple markers supports our findings regarding the success of isolation and purification of spirulina strains. The Keriko Farm isolate was confidently identified as *Arthrospira platensis* or a closely related *Limnospira* species. The overall data suggest that the sample from Lake Bogoria is most closely related to *Arthrospira platensis*. Overall, purified cultures from the IIMSAM Farm (spiral form) belong to *Arthrospira platensis*. The failure to amplify the sqdB gene in Amria Farm samples may suggest either primer mismatch or absence or mutation of the gene. Still, other markers verifies that this isolate can also be considered as Spirulina. Results obtained from CYA106-F/CYA781Rb oligo pair were not satisfying regarding the resolution of the species for the isolates except for Keriko Farm.