Grant No. TG/2012/Tech-D/09

FINAL REPORT - SPIRULINA PROJECT

Date Project Commenced - 26 March 2013

Date 1st Installment of Grant Received - 5 May 2013

Date Completion of Grant Period - 24 March 2014

Date of Submission of Final Report - 30 May 2014

COMPILED AND PRESENTED BY THE TEAM LEADER
Major. General. W J T K FERNANDO
(B.Sc, MIEE,MBIM,MIERE,psc,te)

Spirulina is a blue-green algae. It is a simple, one celled form of algae that thrives in warm, alkaline-fresh water bodies.
Spirulina is being developed as the "Food of the Future" because of its Amazing ability to synthesize high-quality concentrated food more efficiently , Spirulina is 65 to 71% complete protein,with all amino acids in perfect balance. Spirulina is one of the few plant sources of Vitamin B12, usually found in animal tissues. A teaspoon of spirulina supplies 2 ½ times the Recommended Daily Allowance of vitamin B 12 and contains over twice the amount of this vitamin found in an equivalent serving of liver.
Spirulina also provides high concentrations of many other nutrients – amino Acids, chelated minerals, pigmentations, rhamnose sugars (complex natural Plant sugars), trace elements, enzymes – that are in easily assimilable form. Spirulina is one of the cleanest, most naturally sterile foods found in nature

30 May 2014

This Document consists of the Following

1. Final Report at the end of the project
2. Schedule "E" duly completed
3. Schedule "G" duly completed
4. Schedule "H" duly completed
5. Schedule "I" duly completed
6. Test Report
7. Detailed Account for the full project inclusive of expenditure incurred by the Team Leader.
8. Publications.
9. Copy of a letter to NSF re the modifications to the drying system

Maj. Gen. W J T K Fernando
Team Leader
Spirulina Project

Grant Number : TG/2012/Tech-D/09 **Date of Award:** .24 March 2013

Project Title: To Grow The Algae SPIRULINA to Overcome Malnutrition in Children and Pregnant and Lactating Mothers in Villages in Sri Lanka

Date the Project Work Commenced : 26 March 2013 (First part of the first installment of the grant was received on 5 May 2013)

Date of completion of the grant period : 24 March 2014

Date of submission of the final report : 30 May 2014

Total allocation of funds : Rs 1,500,000 (NSF) Rs 654,000 (By General Fernando as Swayang Wattegedara had no Funds to Expend on this project)

Total expenditure at the time of completion of the grant period : Rs 1, 350,000 (NSF), Rs 688,556 (Spent by Maj.Gen Fernando - Team Leader)

A) Details of Team Members

1. Team Member 1, 2, 3, 4, etc.
   a. Major General W J T K Fernando (B Sc, MIEE, MIERE, MBIM, p.s c, t e - **Team Leader** -A chartered Electrical, Electronics and Telecommunications Engineer. Specially interested in finding cheap natural remedies for fighting malnutrition in Children, Pregnant & Lactating Mothers in the villages in Sri Lanka.
   b. Mr. J M S T K Jayalath - **Team Member 2** - Researcher and Operations officer who dedicated most of his time to development of the project. Was the direct liason between the project and ISF. Trained in India
   c. Mr W K A S Dharmasena - **Team Member 3** - Refused to be the Assistant Researcher from the beginning demanding a high salary. Dropped out from the beginning of the project. Subsequently involved in a brawl and was killed.

2. Details of Collaborators

a. Professor S A Kulasooriya, of the Institute of Fundamental Studies in Kandy

b. Dr D N Magana-Arachchi of the Institute of Fundamental Studies in Kandy

Since Maj. General Fernando - the team leader nor the Gandhi Centre did have the technical expertise to grow samples of this algae, they contacted Professor S A Kulasooriya, of the IFS who realizing what this algae could do to reduce malnutrition in children, readily agreed to be a corroborator and help the Gandhi Centre to grow samples of this algae under laboratory conditions after checking the samples for impurities. A memorandum of Understanding was signed between these two parties wherein the Maj. Gen. Fernando and the Gandhi Centre would provide the required chemicals and a small payment towards the technician handling this project and the IFS growing the samples at their laboratory and supervising the growing arrangements and testing the samples grown in Wattegedara.

In addition IFS provided purified samples of the algae to Maj. Gen. Fernando to start growing the algae at their end under the supervision of the IFS.

Further Professor S A Kulasooriya ably assisted by Dr D N Magana-Arachchi of the IFS continues to help and assist Mr Jayalath from Wattegedara who was trained in India in the growing testing and collection processes of the algae spirulina, when he visits IFS weekly.

The project at Wattegedara is now progressing well after several problems due to the lack of water during the severe drought and arrangements have been made to commence manufacturing the Spirulina algae in commercial quantities. Maj. Gen. Fernando will fund this project on a not for profit basis once this algae is grown and ready for marketing in commercial quantities.

Maj. Gen. Fernando and the Gandhi Centre wishes to place on record the expertise, guidance and training facilities provided by both Professor S A Kulasooriya and Dr D N Magana-Arachchi towards this project. This project would not have been possible if not for their unstinted assistance and cooperation.

3. Place where the project was carried out:

At Wattegedara Murutenga in the Team Leaders personal property as the Company did not have any finances to rent out a suitable land. Several villagers were asked to provide the required land to put up the tanks, but they all declined. The Team Leader Major General W J T K Fernando, funded the Grantees component fully as the Company was more or less insolvent. It is the team leaders responsibility to ensure that this project is successfully completed.

4. **Project personnel**

i) Number of personnel employed - Four

ii) Their status after completion of the project – The numbers will be increased to six and will be kept in service as long as the project continues.

5. Final financial statement is attached (Yes) Final Financial Statement attached as Annex "A" to this document.

B) Executive summary of the project

Background

The start of the spiurlina research project in Sri Lanka dates back to 2010 when UNESCO made a revealing statement about malnutrition of children and lactating mothers in Sri Lanka. The Gandhi Centre decided to address this. Arjuna Hulugalle and Upatissa Hulugalle identified spirulina, a powerful nutrient, to address this problem. I got staff and set up this research project in Kanugala, Wattegedara.. A community person was trained in India for this.

Objectives

1. To cultivate the algae spirulina under farm conditions to support the villagersin the Kurunegala District to overcome malnutrition.
2. To market the grown spirulina at an affordable price to the villagers.
3. To provide training and limited employment to village women.

As a trustee of the Gandhi Centre, I accepted the challenge of setting-up the spirulina farm and contributed physical assets to the project. The Spirulina Research project needed heavy investment. To obtain funding I prepared a grant request to the National Science Foundation as the principal signatory for the research project. Since none of the villagers agreed to release land, I identified 15 perches in my property in Kanugala. the NSF approved a grant of Rs.1.5 million and required me to contribute Rs.654,000/- to make it a joint venture. I pledged the partner contribution. The grant was approved in December 2012, effective 2013 April to 2014 March.

Output

Cultivation commenced in May 2013 with the first instalment of the grant. 20

tanks were constructed and the spirulina introduced systematically. Harvesting using the Indian method proved unsatisfactory and we adopted more sterile Swedish methods. These led to a marketable product and the research has successfully shown a process for producing a marketable product.

The production process encountered many problems. The team members pulled in different directions seeking personal payments through grant money. I then had to administer this single handedly, with some help from villagers. Besides personnel issues, there were also growth problems. These were addressed systematically and we now have a highly recommendable cultivation process together with a project that can output of 60 Kgs of Spirulina per month. This project can be replicated in suitable areas in Sri Lanka.

C) Report in detail

1. **Background**

In 2010 when I studied the UN reports on Children's health, I came across the following report re the children in Sri Lanka.

"For a Country that suffers no significant food shortages and provides extensive free maternal and child health services, it is rather paradoxical that malnutrition affects nearly one-third of children and one-quarter of women.

- **Almost one out of five children are born with low birth weight – Around 25% of under five are reported to be under weight, rising as high as 37.4% in some deprived districts.**
- **14% of under fives suffer from acute malnutrition (wasting) when their weight is compared to the weight of a normal child of the same height.**
- **Nearly 58% of infants between 6 & 11 months and 38% children between 12 and 23 months are anemic"**

In my own small way I decided to find a solution to the malnutrition problems in Children & Pregnant and Lactating Mothers in Sri Lanka.

As a trustee of the Gandhi Centre, I discussed with the Board of trustees of this centre who wholeheartedly agreed to my idea and Mr Arjuna Hullugalle, a trustee of the centre said that his brother Mr Upatissa Hulugalle an ardent believer in cultivating spirulina and knowing its benefits said that we should cultivate spirulina. He undertook to fund two personnel for a training program to study the growing procedures of Spirulina in a farm in south India provided, me being a go-getter, undertake the growing of Spirulina to overcome malnutrition in Children and Pregnant & Lactating Mothers in the

Villages in Sri Lanka.

I accepted the challenge and then researched through the internet to study the benefits of spirulina and its cultivating procedures and all other aspects involved. I personally contacted a Swiss organization growing spirulina and obtained their advice and help.

Two trainees with a Scientific background were then trained in India and they brought with them a bottle containing the Spirulina Culture. With our contacts in the Institute of Fundamental Studies (IFS), we handed over the culture to IFS's Professor Ananda Kulasooriya a research scientist to study this culture for contaminants etc. After clearing the culture of the few contaminants the culture was grown under laboratory conditions at the IFS and also at my farm in Wattegedara.

In the meantime and at the request of Prof. Kulasooriya, I submitted a grant request to the National Science Foundation on the 31 January 2012. This grant was approved by NSF on the 17th December 2012. The funding approved was as follows.

Rs 1,500,000 from NSF
Rs 654,000 to be provided by the grantee for this project.

At the time of the grant request, Swayang Wattegedara of which Maj. Gen. W J T K Fernando was the Chairman, had sufficient funds as they were selling Cocopeat to a Japanese Company and therefore put down the name of the Institution as Swayang Wattegedara. However, it was Maj. Gen Fernando's personal effort to prepare the grant request and not by anybody else. Swayang Wattegedara was only a name used in the preparation of this grant request.

By the time the grant was approved, Swayang Wattegedara had lost the Japanese contract. This was the only business that Swayang Wattegedara was doing and therefore had no funds to meet the grantees component of the funds required. Since the effort to get this grant was solely the efforts of Maj.Gen. Fernando, he came forward with his personal funds to meet the grantees component of funds required. He did this till the completion of the project.

We experimented with the cultivation of spirulina for almost two years using glass tanks and a bath tub and perfected the growth of organic spirulina. Our experimentation covered working out practically the amount of fertilizer we require per liter of water used in the tanks and the replenishment fertilizer we required on the weight of the cultivated output of spirulina per day. What we now have is the conditions required to cultivate 100% organic spirulina. Prof.

Kulasooriya tests the spirulina grown by us every month.

At our Spirulina farm in Wattegedara, we recreate the same growing conditions found in nature in order to cultivate true organic spirulina. We use the same fertilizer from nature (phosphorus, nitrogen, and natrium) to fuel our spirulina crops, because that is the natural food for the algae. Our cultivation centers around closed-loop productions in lined tanks with no runoff, and no soil contamination.

The water we use is from a deep well and is free from ground water contamination and far away from pesticide, herbicide and synthetic fertilizer use,

Our organic spirulina is.

a. Very low in heavy metals, yeast and mold
b. Grown without Chilean nitrate, manure, or synthetic minerals
c. Non-GMO and non-irradiated
d. Free from herbicides, pesticides and ground water contamination
e. Gluten-free and produced without chemicals.
f. Grown with only natural herbal fertilizers and pure, ground water

As a further plus, the manufacturing practices and usage of pure vegetarian organic nutrients help ensure that the heavy metals in this spirulina are the lowest possible.

As this is a pioneering project we applied and obtained a grant from the National Science Foundation (NSF) for growing Spirulina to help overcome malnutrition in Children and Lactating & Pregnant Mothers in Sri Lanka. The Data we used is what we worked out during the experimental stage that lasted for almost two years. However, some of the data had to be amended when mass culturing commenced. The project title when we applied for the grant was - **"To grow the alga *Spirulina* to overcome malnutrition of children and pregnant & lactating mothers in villages of Sri Lanka"**

Cultivation process

The cultivation commenced after continuous experimentation for almost two years to perfect the system to be followed and the quantum of fertilizer to be introduced. This is where the Indian method failed and therefore the quantum of fertilizer had to be changed with experimentation. It must be mentioned that with Maj.Gen.Fernando's contacts in Switzerland and with their help and advice the fertilizer to be introduced at the beginning and subsequent replenishment after harvesting was finalized.

Although the project was approved in December 2012, ~~a part of~~ the first part of the grant amounting to Rs 500,000/- was only received on ~~the~~ 5 May 2013. Land for the project was provided by the team leader in his private property Land clearance was done in mid April 2013 whilst awaiting the first part of the grant. On the civil engineering consultants advice, it was decided have a concrete base for the tanks with brick walls around. The brick walls were plastered with cement mixed with waterproofing material. Each tank was 10ft by 5 ft by 2 ft. Twenty such tanks were built with the intention of getting between eighty and one hundred kilograms of dried spirulina per month. Due to incessant rain in the months of June and July the construction of the tanks got delayed. However by the end of July all twenty tanks were constructed. Since it was necessary to prevent any bird dropping and other contaminants getting into the tanks, the tanks were covered with UV protected polythene as shown below. This also prevented rain water and other material blown by the wind, getting into the tanks The tank farm was fully enclosed with a wire fence to prevent unauthorized personnel and stray dogs, cats porcupines, rabbits etc getting in and drinking off the tanks. A drain was dug round the tank farm to prevent rain water getting into the farm area. The tanks were further covered with mosquito netting to prevent insects getting into the tanks.

Outdoor mass culturing of *Spirulina* commenced on August 19th with the inoculation of the 1st tank using a 6L culture grown in a glass container. Daily records of tank water temperature and pH were kept except on Sundays and public holidays. A number of problems were encountered with this initial outdoor culture. Very little growth was observed during the initial few weeks. Presuming that nutrients were inadequate, a load of nutrients of the low cost medium has been added through the ignorance of the field workers and on the instructions of Mr Jayalath. This overloading of nutrients led to the virtual death of most of the algae, but they were saved by the timely intervention of the principal investigator Maj. Gen. Fernando who had given immediate instructions to replenish nutrients approximately equivalent to what is removed during the harvesting of algae. Maj. Gen. Fernando worked out a detailed chart indicating the quantum of nutrients that should be added depending on the harvested wet weight of spirulina and these details were handed over to Mr Jayalath and for him to follow (need to see and need to attached here) these instructions to the letter. This procedure of nutrient additions has gone on, on a trial and error basis and algal growth in the tanks recovered to normal rates by 24th September. Meanwhile two other outdoor tanks have been inoculated and they were all showing satisfactory growth by end of September.Ten outdoor tanks were in operation by end of October and all of them were producing satisfactory harvests of *Spirulina*.

Harvesting

Harvesting had been done using the Indian hand method. This was the only method that Mr Jayalath knew This had several disadvantages.

a. The quantum of harvested spirulina was less than 25% of the grown Spirulina.
b. The possibility of contamination was present due to human hands touching the spirulin in the tanks.
c. The time taken to harvest one tank was over one hour. This meant that the ten tanks would have taken over 10 hours. Further due to the temperature going up the hand harvesters would do a slip-shod job.

To overcome the disadvantage of slow harvesting, Gen. Fernando after discussing with the Swiss authorities decided to introduce mechanization in the harvesting process. This process included the following.

a. An aquarium pump capable of pumping upto a height of 4 ft.
b. A Filter tube 3 Ft long made out of polyester material of 30 to 40 microns, the filter tube had a diameter of 6 inches.
c. The bottom of the filter tube folded twice (!" folding) and clamped with a large crocodile clip.
d. The top of the tube clamped to the outlet pipe of the motor with a ½ inch clamp.
e. The filter supported on top of the spirulina tank using two planks and above the culture level.

To prevent possible contamination all harvesting personnel were provided with,

a. Overcoats
b. Surgical Gloves.
c. Head cover.

Harvesting

Just after harvesting and once the wet spirulina is weighed, replenishment of fertilizer is introduced immediately into the tank from which the spirulina was harvested depending on the wet spirulina extracted from the tank.

After the harvesting is done the wet Spirulina is extracted from the tube into a clean dry cloth wrapped and placed on a flat surface and a 2 kilo weight placed on the cloth with spirulina for all the water to drain out. The spirulina at this stage looks like cheese cake.

Harvested and Water Drained

The cheese cake type of spirulina is now converted into streamers using an industrial type of string hopper machine and spread out on the drying trays. The trays of spirulina are then placed in the dryer operating at between 50 and 60°C and dr1ed for 4 to 6 hrs.

Spirulina When Dried & crushed

Once dry, the spirulina "spaghetti" are gathered on a clean cloth and roughly broken by hand, through the cloth. The fragments thus obtained is conserved in a dry place, away from air and light (e.g. in tins, opaque jars, etc.). or in powder form after grinding and sieving the dry spirulina in opaque glass bottles. In such conditions the nutritional qualities of the spirulina are preserved for almost two years.

We have found a buyer for the limited quantity of Spirulina we produce and as of date we have sold 10 Kilograms of Spirulina at Rs 6,000/- per Kg.

Once we increase production we will extend are selling potential.

We have had discussions with the Industrial Technology Institute (ITI) to produce a biscuit comprising of spirulina and some other ingredients to make it palatable for school children to consume. They are also producing a spirulina and honey mix like a "tala karali" again for childrens consumption.

Plans are going on to start up a similar project in Ambalangoda, Vavuniya and Batticoloa. Gen Fernando is producing a document giving instructions on how to cultivate spirulina as a part of the home garden project.

The main intention of Gen. Fernando who has invested over Rs 600,000/- for this project is to spread it around the country so that children and mothers suffering from malnutrition can overcome this major shortcoming in Sri Lanka. He has no intentions of making profits from the cultivation of spirulina and considers this activity as a national obligation to overcome malnutrition in children and pregnant & lactating mothers in Sri Lanka.

2. Objectives – The three project objectives were :-
   a. To cultivate the algae spirulina under farm conditions to support the villagers in the Kurunegala District to overcome malnutrition.
   b. To market the grown spirulina at an affordable price to the villagers.
   c. To provide training and limited employment to village women.

3. Description of the work (against the proposed project design, work plan etc.)
The Work Plan drawn at the planning stage of the project is as given below

PROJECT WORK PLAN AT COMMENCEMENT

Month

Ser	Activity Description	1st	2^{nd}	3^{rd}	4th	5h	6h	7th	8th	9th	10th	11th	12th
1	Land For Project Identified												
2	Land Levelled & Rolled using Machinery												
3	20 Concrete Tanks 10ft x 5ft x 2 ft constructed on Land Selected												
4	2 x 10ft x 10 ft rooms constructed to house Office/Lab and Chemicals store												
5	Sun Drying Area Constructed												
6	Fence Round the Tank Farm Put Up												
7	6 ft x 4ft Guard Hut Put Up												
8	Water Storage Facility to store 10,000 liters of Water Constructed												
9	Staff Requirement Recruited												
10	Test Equipment Purchased												
11	Monthly Chemical Requirements Purchased												
12	Required Inniculum Grown												
13	Tanks Conditioned and filled with Water												
14	pH Salts and Fertilizer Added to Tanks												
15	Innoculum Introduced into Tanks												
16	Correct Fertilizer Levels in Tanks Maintained												
17	Tanks Covered with Plastic Sheets during Night & Rainy Weather												
18	Grown Spirulina Harvested												
19	Spirulina Sun Dried, Powdered and Packeted												
20	Spirulina Sold to Villagers Through Marketting Campaign												

Comments and Status of Work Plan at the beginning and at the end of the project.

Since part of the first installment of the grant of Rs 500,000/- was received only on the 5th May 2013 the project really got off the ground only on this date. Thus the first month specified in the Work Plan above was May 2013.

a. **Land for the Project** - Inquiries were made from the Directors of Swayang whether they could provide 60 perches of land for the project for the payment of a rental. All of them declined.Inquiries were then made from the villagers to obtain land on payment of a rental. They too declined. As a last resort Maj. Gen. Fernando offered his private land to start up the project without demanding a rental.

b. **Land Leveled & Rolled using Machinery -** Several big trees had to be uprooted using heavy machinery before the ground was leveled and rolled. This aspect of the operation was completed by end May 2013.

c. **Twenty Concrete Tanks 10ft x 5ft x 2 ft constructed on Land Selected**
– This aspect of the project was completed within the specified time period namely by 31st July. The same dimensions as specified in the work plan with a concrete base and brick walls, plastered with water proof material mixed as specified by the consultant was done. No deviation from the original work plan was resorted to.

d. **2 x 10ft x 10 ft rooms constructed to house Office/Lab and Chemicals store –** Since no new buildings were to be constructed as instructed by NSF using the grant funding, an existing building very close to the project was used for this purpose. Partitioning of an 8ft x 8ft space inside the existing building was completed within the specified time period.

e. **Sun Drying Area Constructed -** This was not done as during the experimental stage it was found that when spirulina was sun dried changed colour. Tests carried out in the sundried spirulina showed that some of the vitamins had been lost. The sun drying was done away with and two small temperature controlled dryers locally produced were used. Subsequently a larger dryer designed by the National Engineering Research and Development (NERD) organizations was purchased. This is a deviation from the original work plan. This change was brought to the notice of the NSF on 15 August 2013 and requested approval for such a change. → Not informed as harvesting not yet started at that time

f. **6 ft x 4ft Guard Hut Put Up** - This was not done as NSF did not want any buildings put up using grant funds. The security guard was told to use the verandah of the building used for the office. This is a deviation from the original work plan. why didn't use grantees money if it is essential ?

g **Water Storage Facility to store 10,000 liters of Water Constructed** – This was not constructed as the Team Leader allowed the well in his personal property to be used for the project This is a deviation from the original work plan. → but this is not enough

h. **Staff Requirement Recruited –** Three village females were employed at the time specified in the work plan. The number of employees will be increased to six when we go into full production. The team leader and the team member worked on a honorary basis. – Work plan was strictly followed.

i. **Test Equipment Purchased -** Initially only a few test equipment were purchased with some test equipment being purchased from overseas and paid by credit card. As the project continued all the balance test equipment required were purchased after obtaining overseas quotations as local distributor prices of the same make was much higher. Since Gen. Fernando's son Dr Sanjiva Weerawarana of WSO_2 fame visits the United States every month on business he was requested to purchase some test equipment and was paid by credit card.

j. **Monthly Chemical Requirements Purchased –** The chemical requirements were purchased in stages as required to overcome expiry dates. When required small purchases were done from local shops.

k. **Required Inoculum Grown –**Inoculum required were grown in glass tanks and in a bath tub and for possible emergencies inoculum was grown in the IFS too.

l. **Tanks Conditioned and filled with Water -** Tanks were first cleaned thoroughly with chlorine, then was re filled with water and kept for conditioning for two weeks. The tanks were then emptied and refilled with water. This process took 3 weeks in all.

m. **pH Salts and Fertilizer added to Tanks –** pH salts and the required fertilizer were added to the water in the tanks and kept for two days whilst checking the pH value every two hours. Where the pH values were less than 10, additional pH salts were added to bring the pH value to the correct figure. If the pH value had increased beyond 11 Sodium chloride was added to reduce the pH value to 11. This action was taken over a period of two days.

n. **Inoculum Introduced into Tanks -** After the second day the inoculum grown in the glass tanks were introduced on the basis of 1 litre of inoculum per tank.

o. **Correct Fertilizer Levels in Tanks Maintained –** The correct fertilizer levels in each tank were maintained by checking the growth using a secchi disk and observing the correct pH values. Once this was maintained, and once the white secchi disk when inserted into the tank could not be seen when it was 3 cm deep, it was time for harvesting.

p. **Tanks Covered with Plastic Sheets during Night & Rainy Weather –** This was the original plan but this plan was changed by permanently covering the tanks with UV polythene that allowed 87% penetration of sunlight. This was different from the Indian experience where they covered the tanks only in the night and during wet weather and kept the tanks open during the day allowing bird droppings etc to fall into the tanks. As an additional precautionthe tank tops were covered with mosquito nets to prevent small insects falling into the tanks.

q. **Grown Spirulina Harvested –** Originally the spirulina was harvested by hand, using filtering cloth. Since there was handling of the wet spirulina by the workers, the original plan was modified to harvest using mechanized means. Aquarium pumps were purchase and wearing of coats, hair cover and gloves were introduced. The system used is as indicated in the detailed report given in "C" above.

r. **Spirulina Sun Dried, Powdered and Packeted -** The plan of drying of spirulina in the sun using glass tanks as originally approved by NSF was abandoned as it was found that the spirulina was changing colour and loosing some of its vitamins. Two small dryers were manufactured but it was two small for the job. We then purchased a dryer designed by NERD. This therefore was a deviation from the original plan.

s. **Spirulina Sold to Villagers Through Marketing Campaign -** After testing the powdered spirulina by the IFS the spirulina was consumed by some members of the Gandhi centre, with Maj.Gen. Fernando taking the lead. All reported that there was a big difference in their health conditions. Gen Fernando fed some to te fish in his fish tank. Their growth improved dramatically. However the former Grama Niladhari whose son was the third team member and who withdrew from the project, spread the word in the village that it is dangerous to consume spirulina. Attempts made to counter this wrong concept has not yet been successful. All the output produced is now being sold to a lady in the "Good Market" both in the Colombo Racecourse and in the "Good Market" Battaramulla. It is also noted that foreigners buy this product regularly.

This is a pioneering research project undertaken by both Maj.Gen. W J T K Fernando in association with the Gandhi Center Colombo. The name of Swayang Wattegedara was only used as a name to apply for the Grant. None of the Directors of Swayang or any shareholders gave any form of help. All they did was to ask for money from the Project, which was not possible due to the terms of the Grant. All the funding required towards the two trainees were provided by the Gandhi Centre (the Hulugalle Brothers) and Maj. Gen Fernando was the Grantee who expended all the funds required from his personal Account

4. Objectives achieved to date

Objective 1 -To cultivate the algae spirulina under farm conditions to support the villagers in the Kurunegala District to overcome malnutrition. - Fully Achieved.

This objective has been successfully completed. A massive Awareness campaign has to be carried out in the Kurunegala District and in fact country wide for the villagers to realize the value of this product. However the elite in Colombo, knowing the value of this product is purchasing and consuming the imported capsules and tablets of this product available in pharmacies in Colombo and the suburbs. We have also liaised with ITI and are in the process of developing a "cracker" and something like a "Thala Karali" for the village children.

Objective 2 - To market the grown spirulina at an affordable price to the villagers. Successfully completed.

A market survey was conducted and the results indicated that spirulina is marketed by several pharmacies in Colombo and the suburbs at a price of Rs 80,000/- a kilogram, marketed in 250 milligram capsules and tablets working out to Rs 20 per capsule or tablet. Our spirulina is sold at Rs 6,000/- to Rs 7,000/-per Kilogram. This works out to around Rs 1.50/- per capsule and Rs 1.25 per tablet. This price is well within the purse of the villager.

Objective 3 - To provide training and limited employment to village women. This objective has been achieved

Initially three females were employed to look after the 10 tanks that were functioning. After 3 months one of the females dropped out. Subsequently another was employed to fill the vacancy. Training of these personnel takes almost 6 months. Now that all 20 tanks are functional the number of female employed will be increased to five. Mr Jayalath after he resigned from Swayang Company, has been offered employment in the project and his payments will depend on the output.

5. Discussion results and observations.

Spirulina is a simple one-celled microscopic blue-green algae with the scientific name Arthrospira platensis. Under a microscope, spirulina appears as long, thin, blue-green spiral threads. The odor and taste of spirulina is similar to seaweed.

Spirulina can be found in many freshwater environments, including ponds, lakes, and rivers. It thrives best under pesticide-free conditions with plenty of sunlight and moderate temperature levels, but it is also highly adaptable, surviving even in extreme conditions. More than 25,000 species of algae live everywhere - in water, in soils, on rocks, on plants. They range in size from a single cell to giant kelp over 150 feet long. Macro algae are large like seaweeds. Microalgae are microscopic. Ocean microalgae, called phytoplankton, are the base of the ocean food web.

The spirulina that is grown in Wattegedara under the strict supervision of Gen. Fernando are in tanks which prevents any form of contamination. The pH value being over 10, [and below 11] prevents any other type of algae growing in the tanks. This project was undertaken as a social service project to help overcome malnutrition in children and lactating and pregnant mothers in Sri Lanka. This objective has been achieved with the help of a grant by the National Science Foundation and supported by Gen. Fernando from his personal funds. This being a 1 year pioneering research project that commenced on the 5 May 2013 (The date the first part of the grant as received) was successfully completed on 24 March 2014, well ahead of the 1 year allotted for completion. Sri Lanka's population do not know the value of consuming spirulina on a daily basis, but with a proper awareness program is mounted, malnutrition that is now rampant, can be overcome within a matter of 1 to 2 years. The growing program is now being introduced to the Ambalagoda in the South, Vavuniya in the North and Ampara in the East. A report has been prepared by Gen. Fernando giving the details of how spirulina growing can be done as a home garden project.

The results obtained during the research period has been quite encouraging in spite of the few hiccups experienced. The tanks once we go into production can produce a minimum of 50 Kgs per month after making allowances for no-sunlight days, possible water shortages and worker problems giving an income of Rs 300,000/- per month @ Rs 6,000/- per Kg. This could be achieved after entering the commercial production phase. Expenditure per month can be as follows.

Operations Manager– Rs 75,000/ per month (Basis of Rs 1,500/- per Kg).
6 Workers -Rs 90,000/-Per month (Rs 15,000/-each).Rs 300/- per Kg
Chemicals - Rs 25,000/- per Month
Electricity & Water - Rs 10,000/- per Month
Fuel for dryer(Wood Chips) – Rs 4,000/- per month
Equipment Maintenance – Rs 20,000/- per month
Ground Maintenance - Rs 10,000/- per month
Security - Rs 15,000/- per month
IFS Payment - Rs 30,000/- per month

TOTAL Rs 279,000/- per month

Note – Gen Fernando works as a honorary consultant only.,

The following observations are made after running this project for an year.

1. The workers being village women have to be continuously supervised as their work is slipshod. They tend to avoid daily harvesting.

2. The operations manager has to be a person who has a love for this type of field work. Fortunately we have found a person in the name of Mr Tennakoon to fill this position.

3. If the workers work a little harder the output can easily be increased to

75 Kg, provided that no droughts occur.

4. Slowly but steadily the villagers are getting aware of the value of spirulina after Gen. Fernando spoke to the Children and their parents of the village school in the presence of the Principal and the Staff.

5. It may be possible to increase the selling price of a Kg of Spirulina to Rs 7,000/- per Kg.

6. Output arising from the project.

The physical output of the algae spirulina upto mid May 2014 was almost 11 kgs. This low output was due to several reasons.

1. Due to a lack of a proper dryer some of the harvested spirulina could not be properly dried resulting in the spirulina getting spoilt.
2. The low output was also due to the severe drought that existed from mid October 2013 till mid may 2014.
3. The lack of interest on the part of both Mr Jayalath and the workers blaming the lack of good water, resulted in eight of the tanks getting spoilt. Under normal conditions each tank could produce between 100 to 125 grams per day.

The staff and the administrator was changed and now all 20 tanks are functional. From end June 2014 it is hoped that the physical output will be a minimum of 50 to 60 kgs of spirulina per month. Extra effort will have to be made to market this quantity per month.

7 Deviations to the work plan when compared to the original proposal, if any. Please justify your deviations and state whether you got the prior approval from NSF for such deviation. (state references)

There were 4 deviations from the original work plan.

1.The 10,000 liter water tank was not constructed as that would have costed over Rs 50,000/-. To save money, Gen Fernando agreed for the project to use his well in his property. A question could be raised " if this was built could this have overcome the drought period?". The answer is – No
2.The second deviation was the construction of a 10ft x 10ft guard hut. This was not done as NSF did not agree to spend their money on new building constructions.
3.The third deviation was the construction of a laboratory and a chemical store. This was not done as NSF did not agree to spend their money on new building constructions. However the existing computer centre building was partitioned in its place.
4. The final deviation was regarding the construction of the spirulina drying

area. The plan of drying of spirulina in the sun on a concrete surface was not suitable and representations were made to NSF. The alternative suggested at that stage was the construction of 20 x glass boxes costing Rs 5,000/ a box. NSF agreed to this arrangement after the necessary justification was provided. Subsequently it was found that using glass boxes were also not suitable as it was found that the spirulina was changing colour and loosing some of its vitamins. The cost of setting up a concrete area for drying or producing the 20 glass boxes would have cost Rs 100,000/- for any of these arrangements. Two small dryers were manufactured as an experiment and was found to be successful. However these were two small for the job. We then made representations to the NSF by letter dated 15 August 2013 requesting approval to purchase a dryer designed by NERD. We purchased this dryer for Rs 93,000/- The cost of the 20 glass boxes or constructing a concrete drying area would have cost the same. This therefore was a deviation from the original plan.

8. Whether the work was on schedule? If not, give reasons.

The work on the project was on schedule. In fact the project was completed prior to the completion of 1 year scheduled for the project

9. Major items equipment/consumables & material purchased during the reporting period

a. Major Items of Equipment Purchased.

1. Microscope From Japan - 1
2. pH Meters From US - 3
3. TDS Water Tester from US - 1
4. Electronic Scale - 1
5. Themometers 0-100^{0}C - 5
6. Hydrometer - 1
7. Packeting Machine - 1
8. Dryers (Small) - 2
9. Dryers (Large) - 1
10. Water Pump Fuel Driven - 1

b. Major Items of Consumables Purchased.

1. Sodium bi-carbonate
2. Sodium Chloride (Non Iodized Salt)
3. Urea
4. Potassium Sulphate
5. Magnesium Sulphate
6. Ferrous Sulphate
7. Sodium Hydroxide
8. Phosphoric Acid

9. Hydrochloric Acid

Major Items of Material Purchased.

1. UV Treated Polythene Sheets to cover roof.
2. Chain Link Fencing to enclose tank farm.
3. Steel Gate for tank farm.

10. Problems if any, encountered during the implementation of the project

a. Team Member No 3 –Mr W K S Dharmasena refused to work on the project on a voluntary basis demanding a high salary. The project did not allow payment of salaries to team members. In November 2013 he got involved in a brawl and suffered a major injury. He was unconscious for 6 months and died on the 17 May 2014.

b. Swayang Wattegedara Limited which was the Institution named in the contract for the project did not have funds to input into the project as they had lost the contract with the Japanese Company, they were exporting cocopeat to. The Team Leader Gen Fernando had to step in and provide the full component of the funds that should have been provided by Swayang Wattegedara from his personal funds to bring the project to a successful conclusion. If this was not done the project would have collapsed.

c. A number of growth problems were encountered with the initial outdoor culture. Very little growth was observed during the initial few weeks. Presuming that nutrients were inadequate, a load of nutrients of the low cost medium has been added through the ignorance of the field workers and Mr Jayalath. This overloading of nutrients led to the virtual death of most of the algae, but they were saved by the timely intervention of the principal investigator Maj. Gen. Fernando who had given immediate instructions to replenish nutrients approximately equivalent to what is removed during the harvesting of algae. This procedure of nutrient additions has gone on, and algal growth in the tanks recovered to normal rates by 24th September.

d. A severe drought commenced in the last week of October 2013 resulting in the well supplying water to the project running almost dry. Gen Fernando made arrangement to get water through barrels loaded on a trailer. In spite of this arrangement sufficient water could not be provided for the tanks and by the time the rains came in April 2014, only 2 tanks had been saved. This was a terrible drawback of the project. After doing the necessary cleaning activities under Gen. Fernando's guidance and using the inoculum in these two tanks, initially ten tanks were functional by the 10 May 2014. Water was supplied to the balance 10 tanks and by the 25th May all 20 tanks were functional. Commercial production will commence from June 2014.

have to check where it is going on ?

11. Proposed follow up action regarding output

The output will be sold at Rs 6,000/- or more likely at Rs 7,000/- per kg after a further round of negotiations with the Lady who is purchasing this product for sale in the "Good Market" in Battaramulla and the Race Course. Several others including Baraka have shown interest. Also discussions are going on with ITI to produce a biscuit and a roll similar to a "tala" roll using spirulina to market to school children.

A continuation project has already been started up in Ambalangoda (10 tanks) and should go into production after Prof. Rajapakse returns to the Island in August. A further continuation project will start up in Vavuniya.

Discussions are going on with a Consultant of Mahaveli, to set up individual tanks along the canal line to Kilinochchi.

The intention of Gen. Fernando is to expand country wide with organizations and individuals by providing them the know how on how to grow this product so that the Nation can benefit by the use of this product to overcome malnutrition in Sri Lanka.

D) Comments regarding project implementation, if any

None other than what is given above report.

Signature of the Project Team Leader — 30 May 2014 Date

E) Comments of the Head of the Department/ Institution regarding progress

Not Applicable

F) Recommendation of the Dean of the Faculty and Vice Chancellor or Head of the Institution

Not Applicable

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Signature of the Dean of then Faculty — Date

Not Applicable

Signature of the Vice Chancellor/ Head of the Institution Date

DETAILED ACCOUNT
As At
30 MAY 2014

The Project has the following sub projects with each sub project having a voted expenditure as given below.

Ser	Details of Sub Projects	NSF's Vote	Swayang's Vote
a.	Equipment Hiring Chargers	40,000.00	22,500.00
b.	Design/Engineering/Fabrication	560,000.00	240,000.00
c.	Equipment	60,000.00	31,850.00
d	Consumables & Material	700,000.00	299,990.00
e.	Testing/Trials	70,000.00	30,000.00
f	Other	70,000.00	30,000.00
	TOTALS	**1,500,000.00**	654,340.00

2. Of the above the following funding has been released by NSF through two cheques, namely Rs 500,000/- on 5 May 2013, Rs 630,000/- on 21 May 2013 and Rs 220,000/- in March 2014. A total of Rs 1,350,000/- was released to cover the following sub projects.

Ser	Details of Sub Projects	Funding Released by NSF
a.	Equipment Hiring Chargers	40,000.00
b.	Design/Engineering/Fabrication	560,000.00
c.	Equipment	60,000.00
d	Consumables & Material	550,000.00
e.	Testing/Trials	70,000.00
f	Other	70,000.00
	TOTALS	**1,350,000.00**

scription of Item	Total Budget	NSF Budget	Spent From NSF Account	Swayang Budget	Advanced by Swayang	Gen Fernando - Team Leader	Total Spent
uipment Hiring Charges	62,500.00	40,000.00	40,000.00	22,500.00	0.00	35,000.00	75,000.00
	62,500.00	40,000.00	40,000.00	22,500.00		35,000.00	75,000.00

LN

ion of Item	Total Budget	NSF Budget	NSF Account	Budget	Swayang	Leader	Total Spent
ngineering /Fabrication Costs	800,000.00	560,000.00		240,000.00			
harges - Completion of Surface xture of Plastering Both Inside and Study for suitability of location for nstruction & transport etc.			0.00		0.00	75,000.00	75,000.00
nstruction of Tanks as specified by gn consultand using a 4 inch base,after levelling and stamping tamping machine, 2 ft walls internal ng, use of pudlow and oofing chemical with labour			440,000.00			0.00	440,000.00
Plastering Costs inclusive of @ Rs 6,000/- per tank			120,000.00			0.00	120,000.00
oof stucture for tanks using 2' x 2" Timber, applying wood preservative, concrete bases.			0.00			70,000.00	70,000.00
g the tanks with mosquito netting duit pipes and stiching			0.00			30,000.00	30,000.00
round the tank farm using wooden crete posts and chain link Concrete posts(7) & all required nd labour provided by Contractor. nk Fencing purchased by ransportation from Colombo to dara - Rs 6,000/-			0.00			65,076.00	65,076.00
turing and fixing a steel gate as e to the tank farm.			0.00			8,900.00	8,900.00
g 500 micron Filter Cloth and material (Poonam Sarees) from Equivalent in Sri Lanka Rs						34,443.00	34,443.00
ing and installation of piping sink, taps etc for the tanks ?						15,580.00	15,580.00
e assorted containers required for ng etc of Spirulina						9,708.00	9,708.00
ing Cloth and stitching Coats &						8,500.00	8,500.00
turing 2 Dryers for drying Spirulina						8,500.00	8,500.00
e of six pumps for harvesting						23,000.00	23,000.00
	800,000.00	560,000.00	560,000.00	240,000.00	0.00	348,707.00	908,707.00

MV

tion of Item	Budget	NSF Budge	Account	Budget	Swayang	Fernando	Total Spent
of Consumables	999,990.00	700,000.00		299,990.00			
icals From Glochem			70,860.00				70,860.00
From Lanka Salt			4,576.40				4,576.40
icals from Devi Trading			150,000.00				150,000.00
nicals From Glochem			5,160.00				5,160.00
ort Charges from Colombo to lara to transport Chemicals in			6,000.00				6,000.00
Consignment of Chemicals from			70,860.00				70,860.00
ort Charges from Colombo to lara to transport Chemicals in			6,000.00				6,000.00
ase of Chemicals from Local			39,665.65				39,665.65
pump to pump Water to						50,929.00	50,929.00
ories For Water Pump (Hoses, tc						15,000.00	15,000.00
ase of Chemicals from Local			27,017.95				27,017.95
for drying Spirulina from SPM ng Works, Makevita, Jaela			93,000.00				93,000.00
Consignment of Chemicals from			70,860.00				70,860.00
ort Charges from Colombo to lara to transport Chemicals in			6,000.00				6,000.00
ental Expenses spent by for purchasing chemicals &						8,056.00	8,056.00
ental Expenses spent by for purchasing chemicals &						23,214.00	23,214.00
ying 3 x 100 litres of water lina cultivation during the rought period from er 2013 till 7 April 2014 every y at Rs 12,000/- per month s 66,500/)						66,500.00	66,500.00
ling Expenses of Mr Gnaratne alath for cashing money from ulla						14,500.00	14,500.00
			550,000.00	299,998		178,199.00	728,199.00

scription of Item	Total Budget	NSF Budge	NSF Account	Swayang Budget	by Swayang	Team Leader	Total Spent
t Eqipment	91,850.00	60,000.00		31,850.00			
'urchase of High quality pH :er - ECO inclusive of freight n US by credit card			14,830.00			3,646.00	18,476.00
'urchase of 4 thermometers, ettes,etc						2,320.00	2,320.00
'urchase of Weighing Scale						19,500.00	19,500.00
'urchase of Packet sealing chine						1,950.00	1,950.00
'urchase of Hanna pH Meter			13,670.00			4,434.00	18,104.00
urchase of Water Tester			7,500.00				7,500.00
'urchase of Microscope usive of freight from Japan			24,000.00				24,000.00
'urchase of Grinding Machine						9,800.00	9,800.00
	91,850.00	60,000.00	60,000.00	31,850.00		41,650.00	101,650.00

whres hydrometer - 05
↑
What is purpose of
having hydrometer to
grow spirulina ?

H✓

scription of Item	Total Budget	NSF Budge	NSF Account	Swayang Budget		Team Leader	Total Spent
ner	100,000.00	70,000.00		30,000.00			
Purchase of UV Resistant stic From Hayleys inclusive of livery Charges			58,000.00				58,000.00
Stitching edges of UV Plastic eets using cloth to pass a 1" on pipe, on 2"x 2" wood posts d supports(14.5Ft x 19.5 Ft) ch			6,000.00				6,000.00
Partitioning the Office For Lab						25,000.00	25,000.00
	100,000.00	70,000.00	64,000.00	30,000.00		25,000.00	89,000.00

scription of Item	Total Budget	NSF Budge	Spent From NSF Account	Swayang Budget		Spent by Team Leader	Total Spent
sting/Trials	100,000.00	70,000.00		30,000.00			
yment for Testing Organization			30,000.00				30,000.00
yment for Testing Organization			35,000.00				35,000.00
yment to Terting Technician at F @ Rs 5,000/- per month						60,000.00	60,000.00
	100,000.00	70,000.00	65,000.00	30,000.00		60,000.00	125,000.00

Item	NSF Vote	Swayang Vote	Spent From NSF Account		Spent By Team Leader	Total Spent
ipment Hiring Charges	40,000.00	22,500.00	40,000.00		35,000.00	75,000.00
gn/Engineering/Fabrication	560,000.00	240,000.00	560,000.00		348,707.00	908,707.00
ipment	60,000.00	31,850.00	60,000.00		41,650.00	91,850.00
sumables and Material	700,000.00	299,990.00	550,000.00		178,199.00	728,199.00
ing/Trials	70,000.00	30,000.00	65,000.00		60,000.00	125,000.00
er	70,000.00	30,000.00	64,000.00		25,000.00	89,000.00
Totals	**1,500,000.00**	**654,340.00**	**1,339,000.00**		**688,556.00**	**2,017,756.00**

e 1

om the Rs 1,350,000/- released by NSF towards the Grant Rs 1,339,000 has been spent leaving a balance of Rs 11,000
wever the Bank statement indicates that the Balance is Rs 216,416.03 This amount includes the interest accrued over the period
gether with the Bank guarantee.

?

Correct.

215,434/=

Bank guarantee = Rs. 215,434/=

Where's the Bank statement?

SCHEDULES "E", "G", "H" "I"

Schedule E

Format for Interim and Final Financial Report

The Financial position of grant No. TG/2012/Tech-D/09 as at 24 March 2013 awarded to Maj.Gen. W J T K Fernando(TeamLeader) by National Science Foundation is as follows.

	Funds Received by the Univ/Institution	Total Expenditure Rs	Balance Available Rs
Personal - Consultants	Nil	Nil	Nil
- Technical Asst	Nil	Nil	Nil
- Labourer	Nil	Nil	Nil
- Other	Nil	Nil	Nil
Equipment – Foreign (Items purchased should be stated) - Local	Rs 60,000/- Ph Meters 1 (Rs 14,830) Water Tester(Rs 7,500) Microscope (Rs 24,000) pH Meter 1 (Rs 13,670)	Rs 60,000/-	Nil
Consumables and Material – Foreign - Local	Nil Rs 550,000/-	Nil Rs 550,000/-	Nil
Travel & Subsistence	Nil		
Equipment Hiring Charges	Rs 40,000/-	Rs 40,000/-	Nil
Infrastructure Development (Construction of Tanks to cultivate Spirulina)	Rs 560,000/-	Rs 560,000/-	Nil
Test Trials	Rs 70,000/-	Rs 65,000/-	Rs 5,000/-
Miscellaneous (Other)	Rs 70,000/-	Rs 64,000/-	Rs 6,000/-
TOTAL	**Rs 1,350,000/-**	**Rs 1,339,000/-**	**Rs 11,000/-**

Unspent balance of funds received

Funds Received .... Rs 1,350,000/-
Actual Expenditure .... Rs 1,339,000/-
Balance .... .Rs 11,000/-
Cash Imprest/Cash Balance .... Nil

Balance as at 30 May 2014 .... Rs 11,000/-

Note - For Expenditure Details Please see the attached Annex

Certified Correct

Bursar/Accountant Subash Ranasinghe
Gandhi Centre

Team Leader

Schedule "G"

Equipment Purchased Under The Grant

Grant No. TG/2012/Tech-D/09

Equipment Grant No (Where applicable) Not Applicable

Grantee Maj.Gen. W J T K Fernando

Institution Swayang Wattegedara but did not take part in the project nor funded the project.

No.	Equipment Purchased	Date	Value Rs	Location	Remarks
1	Microscope From Japan		24,000/-	Kanugala Farm	
2	pH Meters From US		28,500/-	Kanugala Farm	
3	TDS Water Tester from US		7,500/-	Kanugala Farm	
4	Electronic Scale		19,500/-	Kanugala Farm	Purchased with grantee funds
5	Packeting Machine		1,950	Kanugala Farm	Purchased with grantee funds
6	Themometers 0-100^{0}C		2,320/-	Kanugala Farm	Purchased with grantee funds
7	Dryer (Large)		93,000/-	Kanugala Farm	- do -
8	Water Pump		50,929		Purchased with grantee funds

Grantee - Maj. Gen. W J T K Fernando
Signature - [signature]
Date 30 May 2014

Schedule "H"

Publications Produced Under the Grant

Grant No – TG/2012/Tech-D/09

Grantee - Maj. Gen. W J T K Fernando

Institution - --

No	Title of Publication	Autho-r	Type of Publication	Expected date	Web	Publisher
1	Spirulina the Super Food	Gen Fernando	Descriptive			News Paper
2	Organic Spirulina Powder	Gen Fernando	Descriptive			News Paper
3	Spirulina – a Study	Gen Fernando	Descriptive			News Paper
4	Cyano Bacteria	Prof. Ananda Kulasooriya	Analysis of Bacteria			Journal

No of Copies of publication attached – Three

Grantee – Maj.Gen. W J T K Fernando

Signature –

Date – 30 May 2014

Schedule I

New Innovation/ Invention

Grant No:……………………..

Grantee: ………………………

Institution:………………………………………………

Part 1: Information on Innovation/Invention						
No	Innovation/Invention(please use additional paper if required)	Date of Invention	Statutory period		Date	Remarks
			1 year	2 year		
1	/					
2						

Part II: Application for Patent Rights							
No	Innovation/Invention(please use additional paper if required)	Elect to retain the patent right		Applied for patent		Date of application for patent rights	Remarks
		Yes	No	Yes	No		
1							
2	/						

No of Copies of publication attached: ……. Spirulina is grown world wide

Grantee: ……………………

Signature: [signature]……………………

Date: 30 May 2014

PUBLICATIONS

SPIRULINA THE SUPER FOOD

A Pioneering Project in Sri Lanka

By

Major General W J T K Fernando

Imagine a plant that can nourish your body by providing most of the protein you need to live, help prevent the annoying sniffling and sneezing of allergies, reinforce your immune system, help you control high blood pressure and cholesterol, and help protect you from cancer. Does such a "super food" exist?

Yes. It's called SPIRULINA. Spirulina is now being grown on a mini commercial basis in Dambadeniya by Swayang Wattegedara Ltd. This project is co-funded by the National Science Foundation and overseen by the Institute of Fundamental Studies Kandy.

Unlike plants you may grow in your garden, this "miracle" plant is a form of blue-green algae that springs from warm, fresh water bodies.

Spirulina History -- Could This Super Food Help End World Hunger?

Spirulina is a simple, one-celled organism that got its name from the Latin word for 'helix' or 'spiral' because of its spring-like physical characteristic. Its scientific name is *Arthrospira platensis*, and it belongs to the cyanobacteria family.

The use of spirulina as a food source dates all the way back to 9th century Chad and it is believed spirulina was used by the Aztecs in 16th century Mexico. Historical records report the harvesting and selling of cakes made from spirulina harvested from Lake Texcoco. It was rediscovered in the 1950's in the same place where it has said to have its origins by a European scientific mission. The spirulina was being harvested and sold in dried flat cakes called "dihé" at the local markets, where natives would use it as a staple for many of their meals.

Spirulina didn't come into commercial production until the 1970s when a French company began the first large-scale spirulina production plant. Within a few years, America and Japan began producing their own spirulina.

Today, these nutrient-rich algae are being used around the world to help treat illness and are being seriously discussed as a sustainable source of food with the potential to end world hunger. Unlike most plants, which need to be cultivated and nurtured, spirulina is a survivor, able to withstand extreme temperature variations and neglect and still thrive. According to studies, spirulina is being successfully used to treat a wide variety of ailments, including those who've been poisoned by arsenic-contaminated water.

How Spirulina Helped Save Millions from Arsenic Poisoning

Most of us take clean, healthy drinking water for granted. Unfortunately, in some countries like Bangladesh, it is a luxury. As I stated in this previous article, much of the Bangladesh water supply is loaded with arsenic and up until the mid-1990s, little could be done to treat dying arsenic poisoning patients.

Bangladeshi researchers conducted a three-month-hospital-based study, where spirulina was given to 33 patients while 17 received placebo doses. 82 percent of those taking spirulina showed tremendous improvement.

An Immune-System Power-Boost -- Spirulina's Impact on Candida and AIDS

According to a study done by the Department of Aquataculture in Taiwan, spirulina shows significant immune-boosting properties. Researchers exposed white shrimp to seawater containing a hot-water extract of spirulina before transferring them to seawater with a pH level of 6.8. The control group was not exposed to spirulina.

The shrimp exposed to the spirulina seawater showed a faster and more promising recovery rate to the high levels of pH than those not given the dose of spirulina first.

Now, let's take a look at what this immune-system boosting power can mean for you:

Candida

If you have an autoimmune disease such as Crohn's disease, chronic fatigue syndrome, Lupus or fibromyalgia, chronic candida yeast can both cause and worsen your symptoms. Spirulina has been shown to encourage and support the growth of healthy bacterial flora in your gut, which can help keep candida overgrowth under control.

HIV and AIDS

Drugs such as AZT used to treat HIV and AIDS patients can actually *cause* the symptoms they are supposed to cure. However, spirulina has been shown to help inactivate the human immunodeficiency virus associated with HIV and AIDS.

Nothing to Sneeze At

If you suffer from seasonal or perennial allergies, you're not alone. Millions of people are allergic to pollen, ragweed, dust, mold, pet dander, and a myriad other environmental contaminants, ensuring the makers of Kleenex will always stay in business.

Unfortunately, many people who have allergic rhinitis treat it with prescription and over-the-counter (OTC) drugs that often do more harm than good. Antihistamines are designed to suppress your immune system, which leads to decreased resistance to disease and dependence on the drug. Certain asthma drugs have been linked to serious side effects as well.

This is where natural methods such as the use of spirulina come in. According to one study, patients treated with spirulina reported relief of symptoms commonly associated with allergic rhinitis, such as nasal discharge and congestion, sneezing and itching, when given spirulina.

Balances Blood Pressure

High blood pressure (hypertension) is a serious health concern that affects millions of Americans today. If you have high blood pressure, you are at increased risk of dying from a heart attack or stroke.

The good news is, following a healthy nutritional plan, getting adequate exercise and applying stress modification techniques such as the Emotional Freedom Technique (EFT) can help normalize blood pressure and get you back on track to optimal health. According to a study done by the Department of Biochemistry in Mexico, 4.5 grams of spirulina given each day was shown to regulate blood pressure among both women and men ages 18-65 years with no other dietary changes made during the six weeks the experiment was run.

Help to Normalize Cholesterol Naturally

Are you on a cholesterol medication? Tens of millions of people take cholesterol-lowering medications every day and, according to "experts," millions more should be taking them, including children.

Cholesterol-lowering medications have also been linked to severe muscle problems such as polyneuropathy (nerve damage in the hands and feet) and rhabdomyolysis (a serious degenerative muscle tissue condition).

Thankfully, there are natural ways to lower your cholesterol. Avoiding fructose and grains, and getting appropriate exercise top the list, but spirulina may also help. According to a study done on elderly male and female patients ages 60-87, those given eight grams of spirulina per day for 16 consecutive weeks showed lower cholesterol levels than those who were given a placebo.

Lowers Stroke Risk

Those with sickle-cell anemia or congenital heart defects are at greater risk for a condition called brain ischemia or cerebral ischemia. Brain ischemia refers to a lack of blood flow to your brain, which causes oxygen deprivation and can lead to a stroke. Just 10 seconds of interrupted blood flow to your brain can cause unconsciousness and lead to serious health consequences.

In a study done at the Institute of Pharmaceutical Technology in India, it was found that a dosage of 180mg/kg of spirulina had a protective effect on the brain and nervous system of rats exposed to high amounts of free radicals, compared to rats not given the spirulina before the experiment. This lab test shows the promising effect of spirulina on stroke prevention.

Helps Reduce Cancer Risk

Cancer is the leading killer of adult Americans under age 70 and in the case of this frightening disease, prevention is worth much more than a pound of cure. It could literally save your life.

My first recommendation in cutting your cancer risk is to eliminate sugar/fructose, grains, and processed foods from your diet. Additives such as fructose feed cancer cells and help them to thrive.

But spirulina may have potential benefits here as well. According to a study done in China, selenium-infused spirulina inhibited the growth of MCF-7 breast cancer cells.

Healthy for Vegetarians -- More Protein than Red Meat

When you think of protein, you probably imagine sitting down to a meal of organic eggs or grass-fed beef or maybe even drinking a whey protein shake.

If you're a vegetarian, you may turn to plant-protein sources such as nuts, beans, lentils, and soy products. Unfortunately, soy is not the health food it claims to be. None of the above-mentioned sources of protein compare to the protein punch delivered by spirulina. Spirulina is 65-71 percent complete protein compared to beef, which is only 22 percent, and lentils, which is only 26 percent.

In addition to being protein-rich, spirulina is an excellent source of vital amino acids and minerals easily assimilated by your body. You would need to consume only two tablespoons of spirulina as a protein substitute for a meal.

Optimal Spirulina -- Types and Dosing

There are many types of spirulina out there so it is important to do your homework before making a purchase. Since spirulina grown in an uncontrolled environment has the potential to become contaminated with heavy metals and other toxins, it is important to choose organic spirulina from a reputable source.

Spirulina comes in capsules, tablets, powders and flakes. The recommended daily dose is typically betweeen three to five grams. You can spread the dose out to twice or three times a day if you like. It is safe to take higher doses, but this is a good place to start. Remember to increase your intake of spring or filtered water when taking spirulina to help it absorb into your system.

An Important Note on Dosing

In addition to being your powerhouse of essential vitamins and minerals, spirulina is a potent detoxifier. For that reason, it is best to start with a small dose and work your way up. Once you see how your body responds, you can then gradually increase your intake.

Potential Adverse Reactions

Spirulina is a safe source of protein, nutrients, vitamins, and minerals that has been used for centuries. Though there are no known side effects associated with spirulina, your body may react to it based on your current state of health. Let's take a look at some of those reactions, what they mean, and what you can do to alleviate them.

The most prominent reactions you may experience are:

- **Slight Fever** -- The high protein content in spirulina increases metabolism, which may elevate body temperature.

- **Dark Green Waste Matter** -- Spirulina can remove accumulated waste product in your colon, which may cause darker stool. Also, spirulina is high in chlorophyll. This will also turn waste matter green.

- **Excessive Passing of Gas** -- This may indicate that your digestive system is not functioning properly or you have an extreme build-up of gas.

- **Feelings of Excitement** -- Your body is converting protein into heat energy, which may cause temporary feelings of restlessness.

- **Breakouts and Itchy Skin** -- This is caused by colon cleansing process and is only temporary.

- **Sleepiness** -- This is caused by the detoxification process and may indicate your body is exhausted and needs better rest.

Remember, your body may go through an adjustment period with spirulina, and your best bet to reduce reaction is to dose gradually to see how your body will react. Increase your water intake, reduce your stress levels, eat according to your nutritional type, and get plenty of rest.

Conclusion

As you can see, spirulina can serve as a potent "super food." Just remember to do your research and arm yourself with knowledge. It is the best way to take control of your health!

SPIRULINA - A STUDY

By

Major General W J T K Fernando

(Some information for this document has been extracted from the Internet)

What is Spirulina

Spirulina is a simple one-celled microscopic blue-green algae with the scientific name Arthrospira platensis. Under a microscope, spirulina appears as long, thin, blue-green spiral threads. The odor and taste of spirulina is similar to seaweed.

Spirulina can be found in many freshwater environments, including ponds, lakes, and rivers. It thrives best under pesticide-free conditions with plenty of sunlight and moderate temperature levels, but it is also highly adaptable, surviving even in extreme conditions. More than 25,000 species of algae live everywhere - in water, in soils, on rocks, on plants. They range in size from a single cell to giant kelp over 150 feet long. Macro algae are large like seaweeds. Microalgae are microscopic. Ocean microalgae, called phytoplankton, are the base of the ocean food web.

The Effects of Spirulina

Spirulina is often deemed the most nutritionally complete of all food supplements, containing a rich supply of many important nutrients, including protein, complex carbohydrates, iron, and vitamins A, K, and B complex. It also has a high supply of carotenoids such as beta carotene and yellow xanthophylls which have antioxidant properties. It is also rich in chlorophyll, fatty and nucleic acids, and lipids. Thus, spirulina has countless uses as a supplement for maintaining good health and for preventing diseases.

Spirulina is the richest beta carotene food, with a full spectrum of ten mixed carotenoids. About half are orange carotenes: alpha, beta and gamma and half are yellow xanthophylls. They work synergistically at different sites in our body to enhance antioxidant protection. Twenty years of research proves eating beta carotene rich fruits and vegetables gives us real anti-cancer protection. Synthetic beta carotene has not always shown these benefits. Research in Israel showed natural beta carotene from algae was far more effective. Natural is better assimilated and contains the key 9-cis isomer, lacking in synthetic. As suspected, natural carotenoids in algae and vegetables have the most antioxidant and anti-cancer power.

Spirulina is an ideal anti-aging food; concentrated nutrient value, easily digested and loaded with antioxidants. Beta carotene is good for healthy eyes and vision. Spirulina beta carotene is ten times more concentrated than carrots.

Iron is essential to build a strong system, yet is the most common mineral deficiency. Spirulina is rich in iron, magnesium and trace minerals, and is easier to absorb than iron supplements.

Spirulina is the highest source of B-12, essential for healthy nerves and tissue, especially for vegetarians.

Healthy Dieting with Spirulina

About 65 to 70% of spirulina's dry weight is protein, which is essential for growth and cell regeneration. It is a good replacement for fatty and cholesterol-rich meat and dairy products in one's diet. Every 10 grams of spirulina can supply up to 70% of the minimum daily requirements for iron, and about three to four times of minimum daily requirements for vitamins A (in the form of beta carotene), B complex, D,and K. By itself, it does not contain vitamin C, but it helps maintain this vitamin's potency.

Spirulina is rich in gamma-linolenic acid or GLA, a compound found in breast milk that helps develop healthier babies. Moreover, with its high digestibility, spirulina has been proven to fight malnutrition in impoverished communities by helping the body absorb nutrients when it has lost its ability to absorb normal forms of food.

Another health benefit of spirulina is that it stimulates beneficial flora like lactobacillus and bifidobacteria in your digestive tract to promote healthy digestion and proper bowel function. It acts as a natural cleanser by eliminating mercury and other deadly toxins commonly ingested by the body.

Spirulina also increases stamina and immunity levels in athletes, and its high protein content helps build muscle mass. At the same time, it can curb hunger that may develop during the most demanding training routines. Thus, it indirectly acts as an effective way to maintain an athlete's ideal body weight.

The Disease Fighter

As well as beta carotene, Spirulina contains other nutrients such as iron, manganese, zinc, copper, selenium, and chromium. These nutrients help fight free radicals, cell-damaging molecules absorbed by the body through pollution, poor diet, injury, or stress. By removing free radicals, the nutrients help the immune system fight cancer and cellular degeneration. In some findings, spirulina has helped reduce oral cancer tumors in laboratory rats, and may thus provide a big medical breakthrough in cancer treatment.

Spirulina's ability to reduce the bad cholesterol LDL in the body helps prevent the onset of cardiovascular diseases, such as hardening of the arteries and strokes. It also helps lower blood pressure. While not clinically proven, spirulina may also protect against allergic reactions and liver infection.

Research confirms Spirulina promotes digestion and bowel function. It suppresses bad bacteria like e-coli and Candida yeast and stimulates beneficial flora like lactobacillus and bifidobacteria. Healthy flora is the foundation of good health and it increases absorption of nutrients from the foods we eat, and helps protect against infection. Spirulina builds healthy lactobacillus, aiding assimilation and elimination and relieving constipation.

Removing Toxins

In 1994, a Russian Patent was awarded for spirulina as a medical food to reduce allergic reactions from radiation sickness. 270 Children of Chernobyl consuming 5 grams a day for 45 days (donated by Earthrise Farms), lowered radio nucleides by 50%, and normalized allergic sensitivities. Today we are subject to an onslaught of toxic chemicals in our air, water, food and drugs. Our bodies need to continually eliminate these accumulated toxins. Spirulina has a completely unique combination of phytonutrients - including chlorophyll, phycocyanin and polysaccharides, that can help cleanse our bodies.

How to Take Spirulina

Spirulina is now commercially available in tablet or powder form. Some health tonics contain spirulina as part of their ingredients. A simple daily regimen for spirulina involves taking a 500 mg tablet four to six times daily.

Spirulina are normally grown in natural settings in Sri Lanka for the first time under carefully controlled conditions to prevent possible contamination from toxic substances. Spirulina given to children in quantities of 250 to 500 mg per day could reduce malnutrition prevalent in children, pregnant women and lactating mothers. Spirulina grown in Wattegedara as a pioneer project is being supervised and Tested very regularly by the Institue of Fundamental Studies in Kandy →?

Proposal to the National Science Foundation (NSF)

A proposal was submitted to the National Science Foundation by Maj. Gen. W J T K Fernando to grow Spirulina as a solution to the Malnutrition existing in very young children, Pregnant Women and Lactating Mothers especially in Sri Lankan Villages, almost over one and a half years ago. A financial grant of Rs 1,500,000/- was approved and awarded by NSF together with a contribution of Rs 654,000/- from Maj. Gen. W J T K Fernando.

The project is now in its final stages of production. The scientific aspects and tests related to this project has been undertaken by the Institute of Fundamental Studies (IFS). Every sample being produced is checked weekly by the IFS →?

Problems that Have been Encountered

One of the major problems the Project has encountered is the inability to convince the villagers that spirulina is the answer to their malnutrition problem. The village mentality is that the food they consume is adequate for their survival. They do not understand that a balanced nutritional diet is required for development of both the body and mind especially in children.

A UNICEF report published recently indicated the following.

"For a Country that suffers no significant food shortages and provides extensive free maternal and child health services, it is rather paradoxical that malnutrition affects nearly one-third of children and one-quarter of women.

- **Almost one out of five children are born with low birth weight – Around 25% of under five are reported to be under weight, rising as high as 37.4% in some deprived districts.**
- **14% of under fives suffers from acute malnutrition (wasting) when their weight is compared to the weight of a normal child of the same height.**
- **Nearly 58% of infants between 6 & 11 months and 38% children between 12 and 23 months are anemic"**

The next problem is to find suitable technical persons – may be with a GCE Advanced level qualification with chemistry and botany as subjects – to undertake these projects if we are to spread it round all the villages in Sri Lanka.

Another problem would be to find the finances to construct and operate at least 2 tanks in each village. However the funding required for such a project would only be around Rs 200,000/- inclusive of chemicals and could be run as a self employment project.

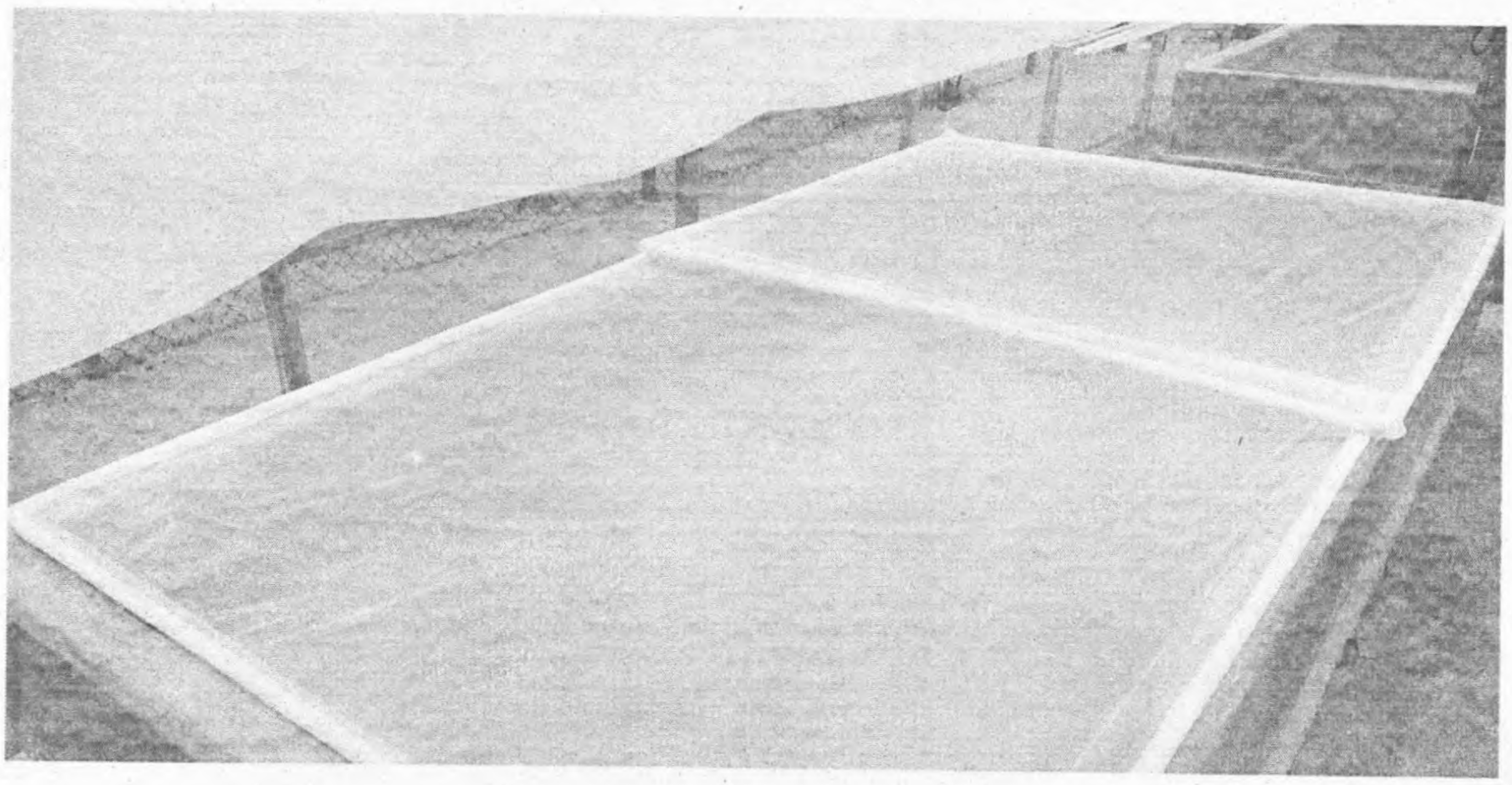
Suggested Solutions

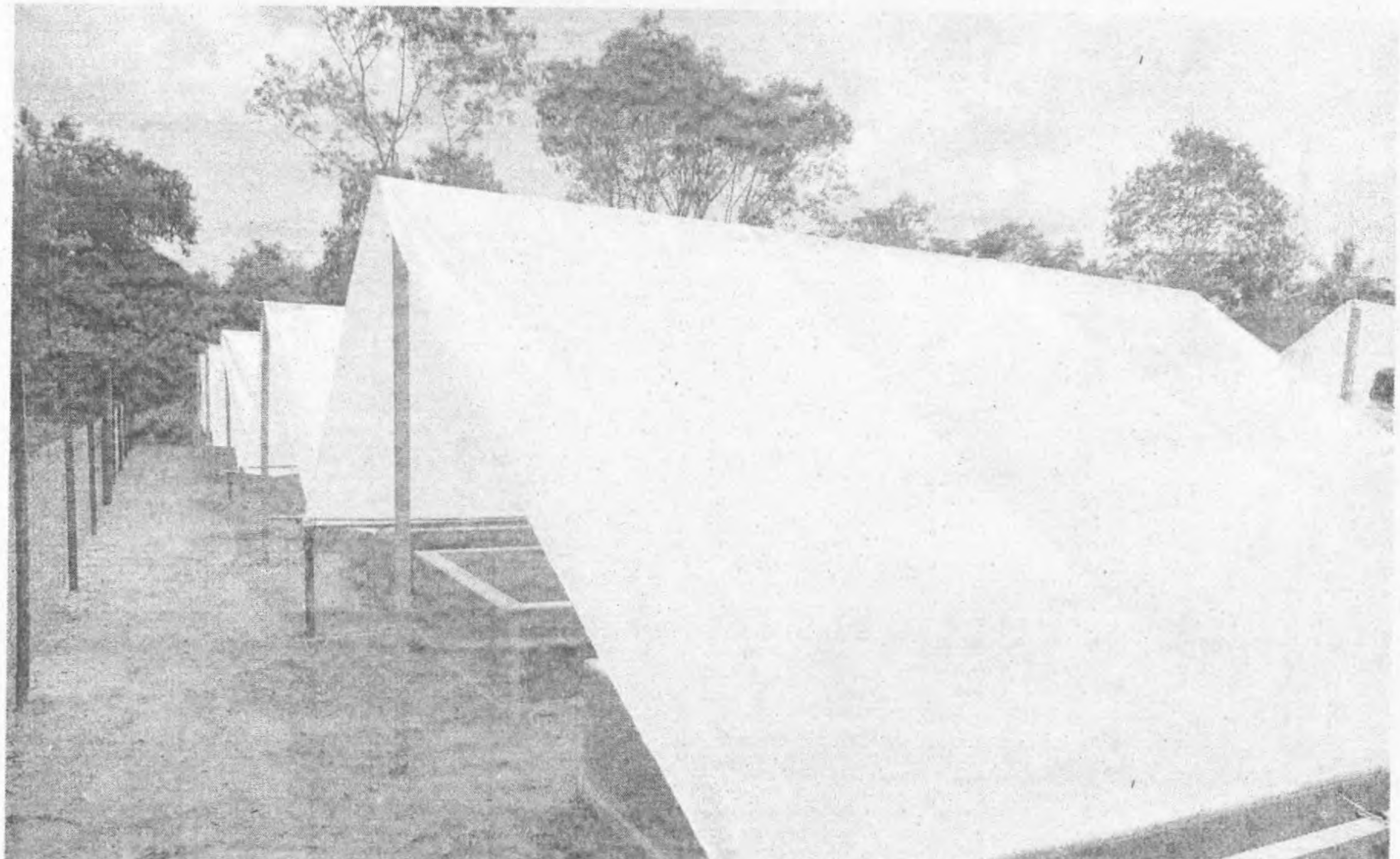
Some possible solutions to solve these problems are:

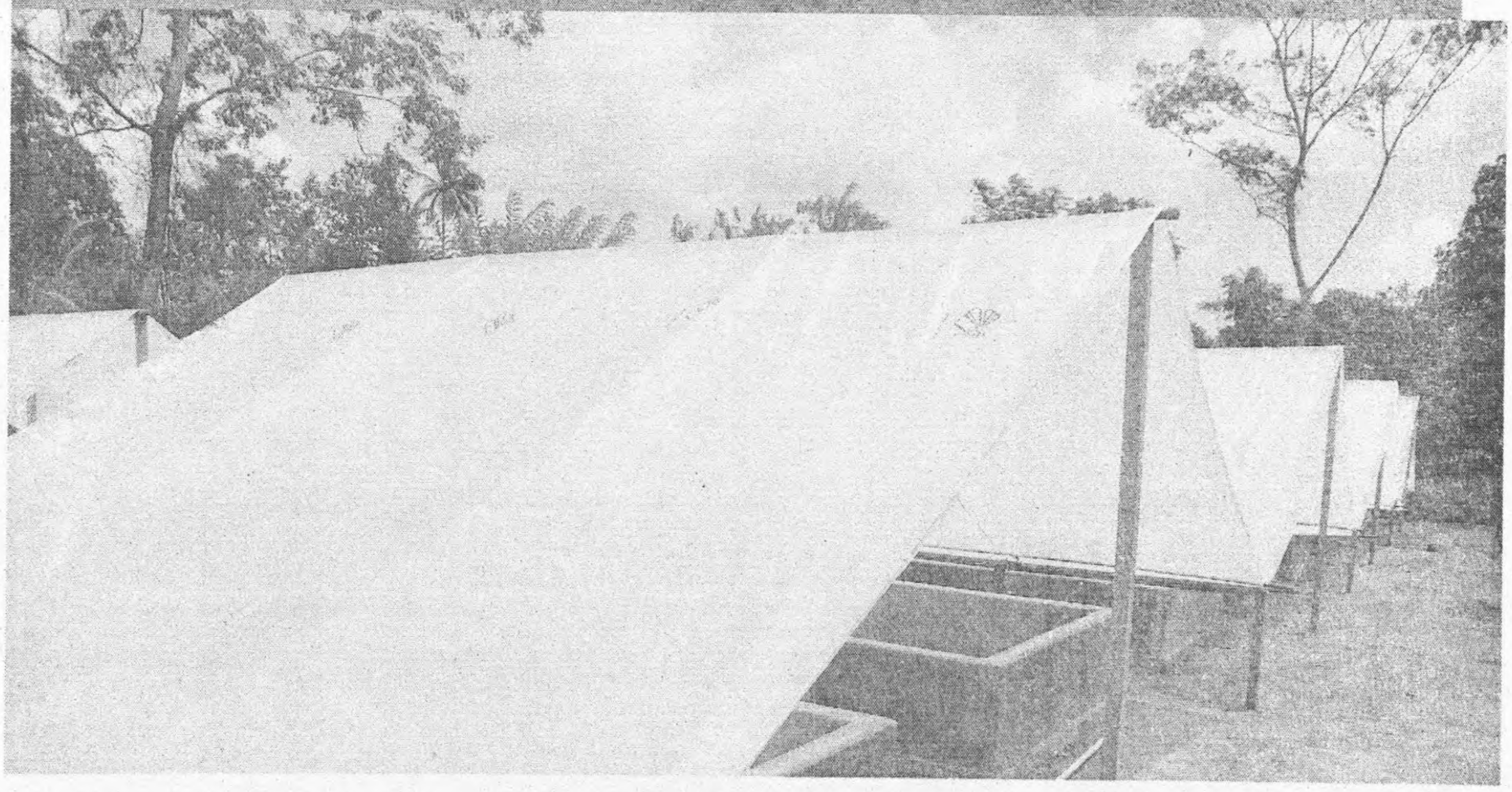
a. Conduct a strong awareness program among villages through the electronic and print media, indicating the benefits and value of consuming spirulina (250mg) per day for children and 500 mg to 1000 mg per day for pregnant and lactating mothers.
b. Conduct a strong awareness activity in the "divinaguma" program and carry out an intensive campaign to popularize the use of spirulina whilst setting this up as a Cottage Industry.
c. Get UNICEF to indicate the value of Spirulina as a combatant for malnutrition among children, pregnant women and lactating mothers.
d. Publicize the beneficial effects of spirulina for enhancement of performance in sports.
e. Use spirulina as a possible reduction on the effects of nuclear radiation of Sri Lankans in case of a nuclear disaster in South India.

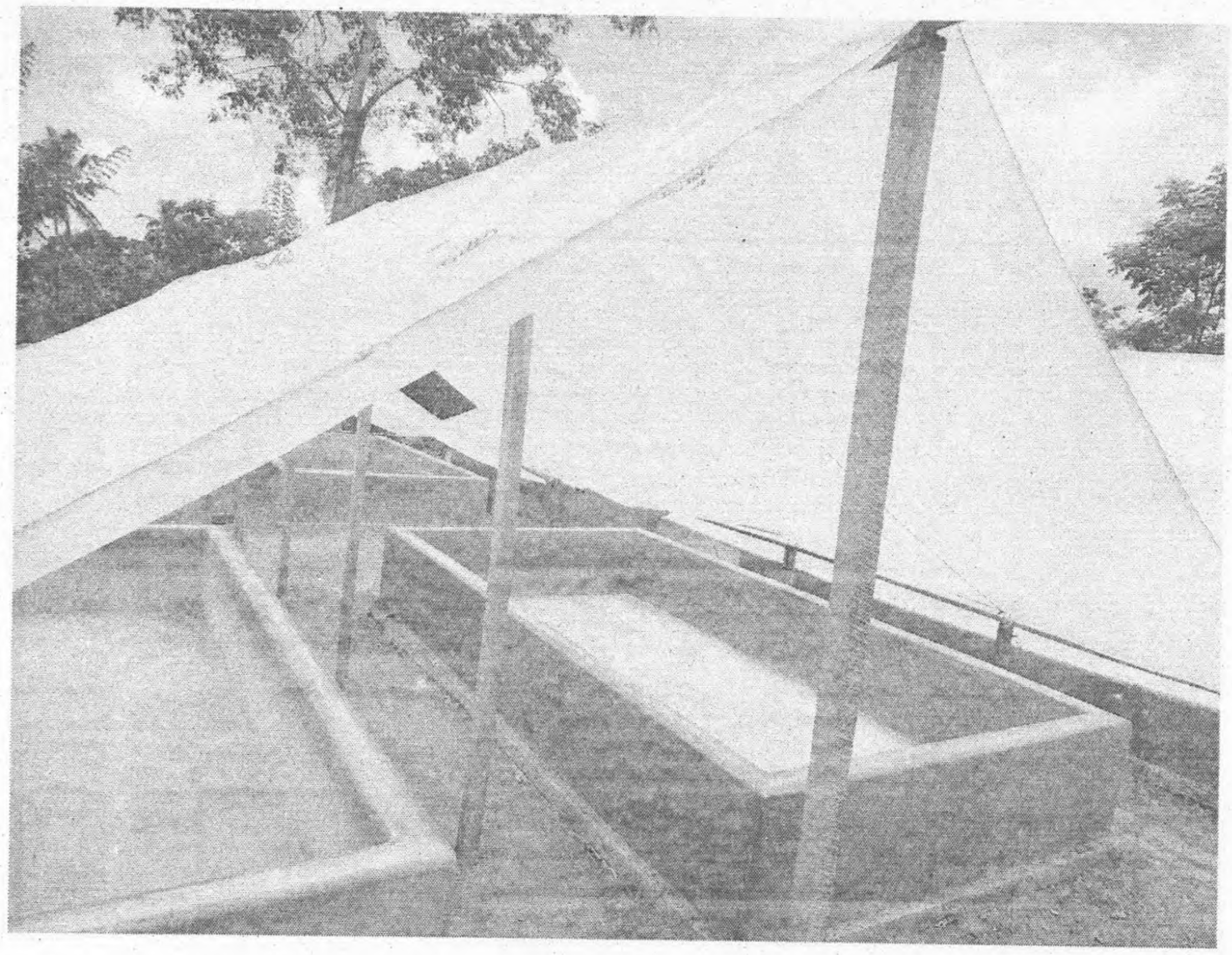
Spirulina farm in Wattegedara

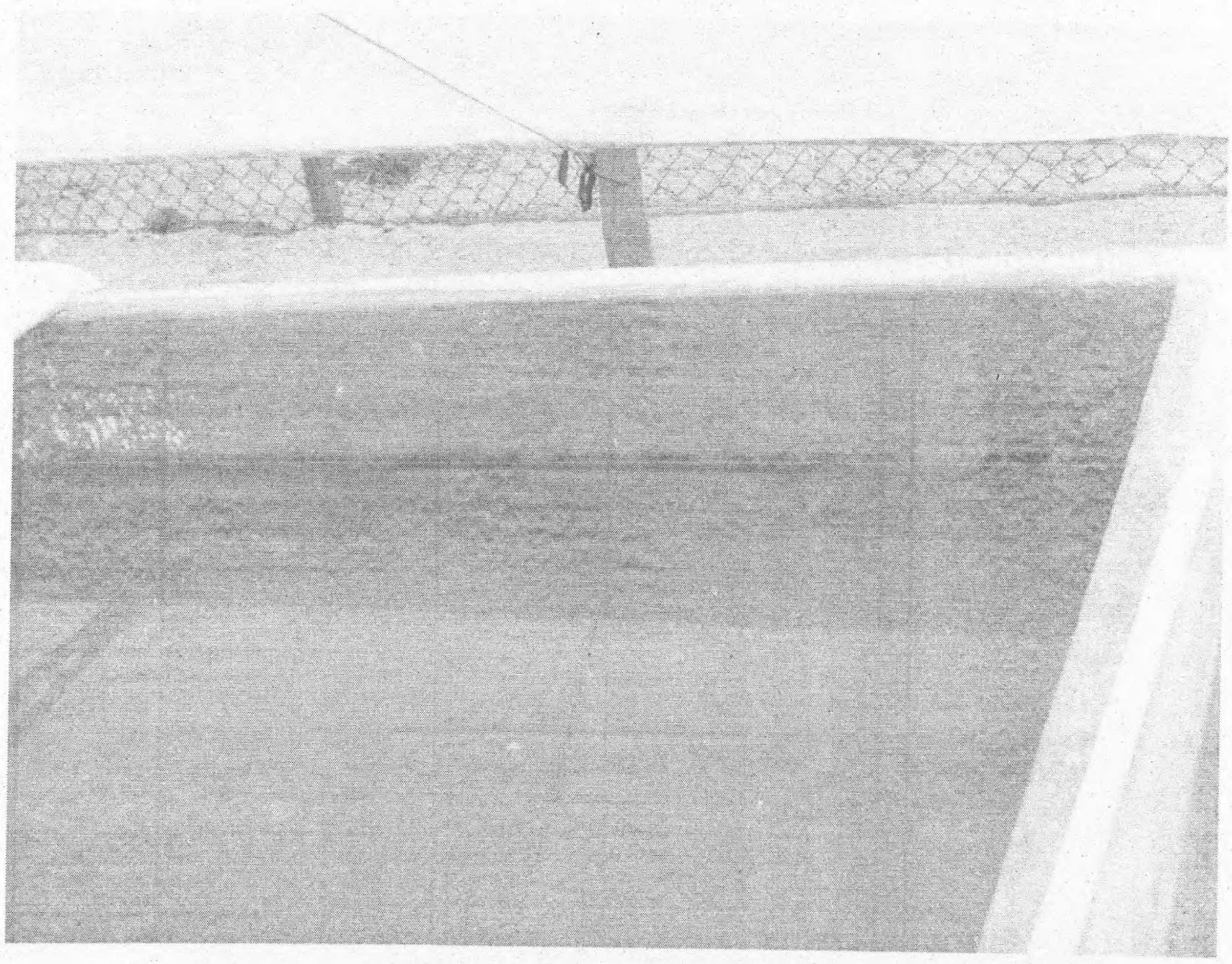
The Spirulina Farm went into test production on the 18 August 2013. Some pictures of the farm is as given below.

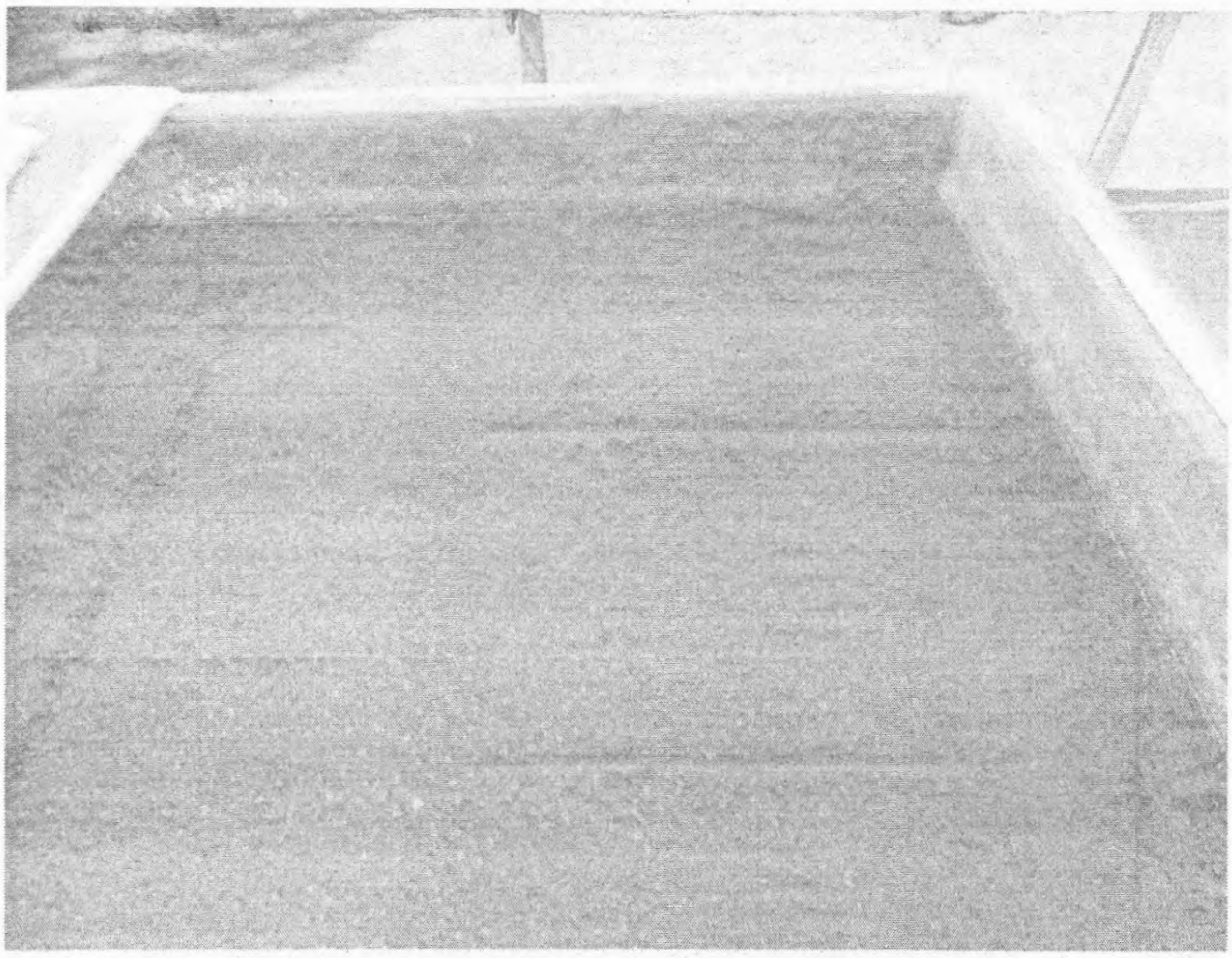










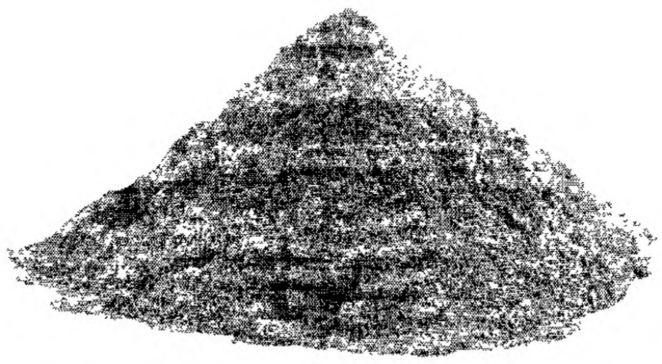


Conclusion

Spirulina is a blue green algae that can be easily grown in Sri Lanka either as a cottage industry or as a commercial crop. Its benefits are tremendous. Swayang Wattegedara supported by a Government grant has commenced growing this algae. If cultivated under scientific conditions and supported by a research organization - Institute of Fundamental Studies, in this case - This project would indeed be a tremendous success.

Major General (Retired) W J T K Fernando
B Sc, MIEE, MIERE, MBIM, psc, te

ORGANIC SPIRULINA POWDER - GROWN IN COVERED TANKS, DRIED AT A MAXIMUM TEMPERATURE OF 50° CENTIGRADE, POWDERED AND BOTTLED



PRODUCT OVERVIEW

Our Organic Spirulina Powder is one of the most complete food sources you will ever find. This amazing green powder has the ability to provide the body with a complete source of protein and most all of the body's amino acid needs. Our Organic Spirulina is rich in Vitamin B12, and contains 3900% more beta-carotene than carrots.

PRODUCT DESCRIPTION

The Ability to Survive the Harshest Conditions
Spirulina (speer-U-lee-nah) gets its name from its microscopic spiral shape, and this one-celled algae transfers its mighty ability to endure harsh conditions to the human body via our Organic Spirulina Powder. Spirulina grows naturally in a hot, alkaline environment, and can survive where no other organism do.

PRODUCT HIGHLIGHTS

- Organic
- Raw
- Vegan
- Gluten-free
- Non-GMO
- High in protein
- High in antioxidants
- 100% pure

The Ultimate Superfood

Spirulina may be the ultimate superfood, providing a complete source of protein with all essential amino acids, the building blocks of protein. Our Organic Spirulina Powder is packed with more protein than soy, making it a great meal supplement for vegans and those with allergies.

In addition to its copper, and selenium content, our Organic Spirulina Powder contains rich amounts of zinc and easily absorbable iron. Spirulina is unique because it is one of the few plant-based sources of Vitamin B12. Would you believe that our Organic Spirulina Powder contains 3900% more beta-carotene than carrots? Adding our Organic Spirulina Powder to juices, shakes, and smoothies gives you an arsenal of nutrients to promote healthy eyes and a strong immune system.

Just three grams of our Organic Spirulina Powder possess more phytonutrients than five servings of fruits and veggies and 300 times more calcium than milk. Our Organic Spirulina Powder is wonderful support for those suffering from osteoporosis and for growing children. It is a low-fat alternative to calcium-fortified beverages. Spirulina has been reported to keep cholesterol levels in check and to lower blood pressure.

Our Organic Spirulina Powder is a powerful antioxidant and anti-inflammatory. It promotes the formation of red blood cells and the growth of beneficial probiotics in the digestive tract. Scientists suggest that consuming spirulina on a daily basis aids in better brain function, memory, and learning abilities. It is also reported to reverse signs of aging.

A Natural Source of Iodine

One of the most unique properties of our Organic Spirulina Powder is that is a natural source of iodine and potassium iodide, which is an essential chemical element for good thyroid function. The thyroid produces hormones that regulate growth and metabolism, and iodine deficient diets have shown to lead to stunted brain development in children, mental slowness, high cholesterol, fatigue, weight gain, depression, and goiter (swelling of the thyroid). Our Organic Spirulina Powder is absorbed more slowly and safely than synthetic sources of iodine. Experts have reported that the iodine in spirulina powder can protect against radioactive iodine from radiation exposure.

A Super Algae

Some say that spirulina has more nutrients per gram than any other food source in the world. This blue-green algae has the characteristics of the green pigment in chlorophyll and the blue pigments in phycocyanin. Spirulina is found mostly in freshwater lakes and ponds, where it grows in an extremely clean and sterile environment because no other organisms can survive the hot, harsh conditions that it thrives in.

Spirulina has been consumed for thousands of years by the Aztec and Mayan people, as well as natives of African and Asian nations where it is still revered as a source of nutrients and medicinal healing.

Our Organic Spirulina Powder is highly digestible, and tastes great when mixed in smoothies and shakes with a little cacao, coconut, fruits, and other greens.

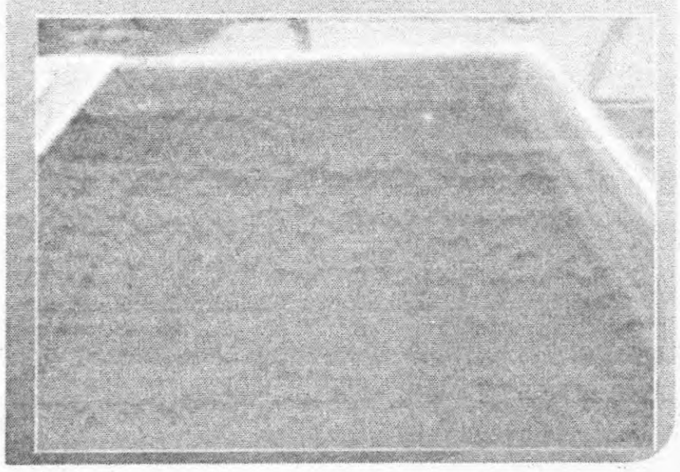
A Quick Word on Pricing

Our spirulina may cost a little more than what you see on the web. This is due to two reasons. First, our spirulina is organic. Second, it's a raw product, meaning that it was dried at low temperatures to preserve the full enzymatic and nutritional value of the food. **Most, if not all, of the cheaper spirulina powders on the market have been spray-dried,** which is a process where very high temperatures are applied to the spirulina as a means of quickly and efficiently drying it into powder. The unfortunate side-effect of this drying process is that it destroys the beneficial enzymes in this amazing product. In short, with spirulina powder, you get what you pay for.

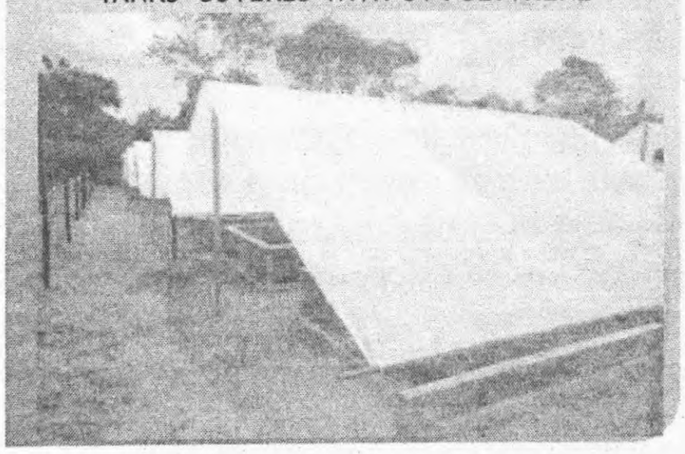
TRULY ORGANIC?

With so much concern about heavy metal contimination in spirulina, most of the organic spirulina available is tank-grown under controlled conditions rather than grown in natural waters. Therefore, our Organic Raw Spirulina Powder is grown in tanks to ensure that it is 100% organic, and not contaminated in any way.

A SPIRULINA TANK



TANKS COVERED WITH UV POLYTHENE



POSSIBLE BENEFITS

While much research is still being conducted, some of the possible health benefits of our Organic Spirulina Powder may include:

- A complete food source
- High in easily absorbable protein
- Contains all 9 essential amino acids
- Rich in vitamin B12
- May be effective in promoting healthy eyes
- May be beneficial for the immune system

- Contains 300% more calcium than whole milk
- May help to promote the growth of probiotics, which help maintain intestinal health
- May help to lower levels of harmful blood fats
- Studies have shown that it contains compounds that may have antiviral and antineoplastic properties
- May be effective in boosting energy and stamina
- Contains a wide array of vitamins and minerals
- Contains 3900% more beta carotene than carrots
- High in readily absorbable iron
- Rich in antioxidants
- May be helpful in maintaining healthy cholesterol levels
- May help to lower high blood pressure
- Contains compounds that may have anti-inflammatory properties
- May be useful in treating chronic fatigue
- Contains 375% more protein than tofu
- May be effective in supporting a healthy cardiovascular system
- May be helpful in promoting the formation of healthy red blood cells
- May help to improve brain function
- May assist in sharpening memory and mental acuity
- May be effective in reversing signs of aging
- Natural source of iodine

SUGGESTED USAGE

The recommended daily usage is 1-2 teaspoons. As with all superfood powders, listen to your body, as you may find that either a smaller or higher intake is more suitable to your body. We recommend that you start with the recommended daily amount, and, if desired, gradually increase or decrease your daily consumption.

This powder blends well with juice, rice or soy milk, and yogurt, or can be added to your favorite smoothie. It can also be mixed into your homemade energy bars. While you can add this powder to a variety of recipes, raw food enthusiasts avoid cooking or heating in order to preserve the food's nutritional value.

STORAGE INSTRUCTIONS

If cared for properly, our Organic Spirulina Powder has a shelf life of two to three years. To preserve the freshness of this product:

- Avoid exposure to heat. The powder will remain fresh if stored at room temperature or below.
- Avoid exposure to direct sunlight.
- Squeeze all of the air out of the bag before sealing.
- Seal the bag tightly after each use.
- Store the powder in a dry place, and avoid all contact with moisture.

PACKAGING

We package all of our superfood powders in glass bottles. which serves as a very effective barrier to light, oxygen, odors, flavors, moisture, and bacteria.

While printed plastic bags with clear windows may look beautiful, studies have shown that the toxins contained in the plastic are absorbed by food that is in direct contact with the plastic. Foods that are raw and all-natural, like superfood powders, are even more susceptible to this type of contamination than processed foods.

COUNTRY OF ORIGIN

Sri Lanka (Tank-Grown)

Major General W J T K Fernando

Ceylon Journal of Science (Bio. Sci.) 40 (2): 71-88, 2011

Cyanobacteria: Pioneers of Planet Earth

S. A. Kulasooriya

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ABSTRACT

Cyanobacteria are among the earliest of inhabitants of Planet Earth and their existence can be traced back to 3.8 billion years. Their oxygenic photosynthesis led to the gradual conversion of the Earth's atmosphere from an anaerobic to an aerobic one. This change enabled the advent of aerobic organisms that eventually underwent rapid evolution and became the dominant, highly diverse members of the current global biodiversity. Cyanobacteria are ubiquitous in their distribution and are found in all the latitudes from Arctic and Anatarctic regions to the Tropical deserts perhaps reflecting their pioneering habitation of the primitive earth. They are also unique in their ability to simultaneously perform oxygenic photosynthesis and oxygen labile nitrogen fixation. Through these processes they make significant contributions to the Carbon and Nitrogen bio-geochemical cycles, particularly in the deep oceans. The ability of these organisms to fix N_2 either independently or in symbiosis with other organisms not only contributes to natural ecosystems but is applied in certain countries particularly for rice cultivation. Their ability to grow in highly polluted environments is also used in the treatment of sewage and industrial effluents. Cyanobacteria are the most efficient among all living organisms in the harvesting of solar energy and are currently looked at as highly attractive candidates for biofuel production. A few species are being used for the production of highly nutritive food supplements. On the negative side, some cyanobacteria form massive growths called 'blooms' in water bodies and many of them produce toxins harmful to fish, digastric animals and are suspected to be responsible for certain human ailments. Having reviewed most of these aspects of cyanobacteria, it is concluded that knowledge on these little known organisms would be invaluable not only for students, scientists and environmentalists but also for industrialists and policy makers.

Key words: *cyanobacteria, blue-green algae, algal toxins, biofertilizers, biofuels*

INTRODUCTION

Cyanobacteria, also known as blue-green algae include a highly diverse group of prokaryotic microorganisms exhibiting oxygenic photosynthesis. Oxygen released by this process gradually changed the original reducing atmosphere of the primitive earth to an oxidizing one (Olsen, 2006) triggering off a dramatic evolution of global biodiversity. The chloroplasts of eukaryotic algae and higher plants have originated from endosymbiotic relationships with cyanobacteria (Martin and Kowallik, 1999 and Raven and Allen, 2003) and this event in the early evolution of life has stimulated the advent of oxygen tolerant flora and fauna capable of aerobic respiration, a highly efficient mechanism of energy utilization. The rapid development of such organisms resulted in the predominance of oxygenic and aerobic species diversity on earth.

Cyanobacteria are genetically highly diverse; they occupy a broad range of habitats across all latitudes perhaps demonstrating the abilities of their pioneering ancestors as the earliest inhabitants of Earth. They are not only widespread in freshwater, marine and terrestrial ecosystems but also found in extreme habitats such as hot springs, hypersaline localities, freezing environments and arid deserts (Fogg *et al.*, 1973). They often live in association with other organisms forming microbial mats, biofilms and benthic communities and such associations are the predominant and sometimes the only life forms found in certain extreme habitats. The fossilized stromatolites believed to have been formed some 3.4 billion years ago (bya) are also microbial communities with cyanobacteria as their autotrophic partners.

Diverse species having different ecological demands exhibit differential adaptations to the conditions at their source locality. The massive communities of *Prochlorococcus* in the oligotrophic deep oceans that live in close association with cyanophages enabling rapid lateral transfer of genomic material opens up

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new vistas on biological adaptation and evolution (Johnson *et al.*, 2006). The endolithic *Chroococcidiopsis* living as the only inhabitant in the extremely inhospitable habitat of the arid Atacama Desert provides a new tool for Astrobiology (Friedmann, 1982 and Wierzchos *et al.*, 2006).

Sequencing of 16S rRNA while confirming the existence of several morphologically uniform and well-defined traditional genera, has also placed similar morphotypes in distant positions in phylogenetic trees. Molecular data therefore provide basic criteria for cyanobacterial taxonomy, but to construct a comprehensive phylogenetic system of cyanobacteria a combination with knowledge on their morphology, physiology and biochemistry is essential (Komarek, 2006).

The ability of certain cyanobacteria to fix atmospheric nitrogen make them unique in their ability to independently secure their carbon and nitrogen requirements (Kulasooriya, 2008). Some of these organisms and symbiotic systems like *Azolla* are used as biofertilizers, particularly in rice production (Venkataraman, 1972 and Azolla Utilization, 1987). They are also applied in oxidation ponds and sewage and sludge treatment plants (Lincoln *et al.*, 1996). Recently a few species have been investigated for biofuel production because their ability to convert solar energy has been found to be the most efficient among all living organisms. Furthermore, their simple genomic structure has enabled the production of biofuel secreting strains through genetic engineering (Lane, 2010). Certain cyanobacteria form blooms in eutrophied water bodies and most of such bloom forming strains produce cyanotoxins that are harmful and sometimes lethal to animals and humans (Carmichael, 1994 and Carmichael *et al.*, 2001). A few species are utilized for the production of highly nutritious food supplements (Kulshreshtha *et al.*, 2008)

This paper reviews the current information on these aspects of cyanobacteria, the roles they may have played in the origin and early evolution of life on earth and their current impacts on global biodiversity.

Role in the oxygenation of the primitive earth's atmosphere

The Planet Earth is believed to have cooled down, solidified and formed oceans and terrestrial habitats some 4.8 to 4.5 (bya). During the next billion years chemical or abiotic evolutionary processes in the primordial soup of the oceans are believed to have given rise to primitive entities capable of self replication. Various scientists have reported on these processes which have resulted in the formation of amino acids (monomers and proteinoid polymers) that integrated to form aggregates such as protobionts and microspheres with semi-permeable membranes. Ribose-nucleic acid (RNA) is believed to have been the original hereditary molecule that grew, split and was passed onto the progeny. These have given rise to the earliest life forms on earth which are believed to be prokaryotic organisms, today represented by the Archea, Eubacteria and the Cyanobacteria. Citing evidence from the Isua super-crustal belt in Western Greenland and similar formations in Akilia Island, Mojzsis *et al.* (1996) proposed life to have existed 3.8 bya. They have also estimated the time that would be taken for a 100 Kbp genome of a primitive heterotroph to develop onto a 7500 Kbp gene of a filamentous cyanobacterium to be 7 million years.

It is most likely that the original cells were heterotrophic utilizing plenty of organic molecules available in the primordial soup together with those produced by other cells. As these food supplies gradually became limiting some of the cells would have developed strategies to use the readily available solar energy by anaerobic photosynthesis. These archaic processes are still retained by a few pigmented bacterial species like *Chlorobium* and *Rhodospirillum* which utilizes energy from the sun to reduce CO_2 and form organic compounds deriving electrons from substrates such as H_2S and FeS exhibiting non-oxygenic photosynthesis.

In a comprehensive mini review Olson (2006) has presented possible pathways on the early evolution of photosynthesis primarily based upon fossil evidence during the Archeon Era. According to him the earliest reductant for CO_2 fixation (even by primitive cyanobacteria) would have been H_2 some 3.8 bya. Fossilized filamentous mats found in the Buck Reef Cherts of South Africa and Evaporites, Stromatolites and micro-fossils observed in the Warrawoona Megasequence in Australia have been attributed to the H_2S driven photosynthesis between 3.5 to 3.4 bya and subsequently Proteobacteria and Protocyanobacteria have utilized Fe^{2+} ions as reductants around 3.0 bya. Evidence of retention of such archaic processes can be observed in certain present day species of cyanobacteria which exhibit light driven CO_2 fixation through cyclic photophosphorylation under anaerobic conditions using electron donors such as H_2S, thiosulphate, or even molecular H_2. Citing evidence from microfossils, stromatolites and chemical biomarkers Olson (2006) suggested that cyanobacteria containing chlorophyll-a capable of O_2 evolving photosynthesis appeared around 2.8 bya, but this process did not have a significant impact on the composition of the atmospheric gases for another 5 million years. The O_2 released to the atmosphere became bound to limestone, iron and other minerals as evidenced from the iron-oxide rich geologic strata observed from those periods. It is also believed that such oxidative reactions of minerals contributed to the 'greening of oceans'. Once most of the minerals got oxidized free O_2 gradually began to accumulate in the atmosphere. Intense solar rays bombarding the earth converted some of the O_2 to O_3 which collected in the upper part of the atmosphere to build up the ozone layer that absorbs the mutagenic UV rays and protects all life forms from unsustainable levels of mutation. Such a build up of a protective O_3 layer would also have enabled the evolution and migration of life forms from the oceans to land.

Towards the end of the Archaeozoic era and beginning of the Proterozoic era the earth began to cool and this led to the reduction of the major greenhouse gases of the atmosphere e.g. water vapour, CO_2 and methane. This cooling also reduced the hygrometric capacity of air and water vapour condensed resulting in continuous torrential rain. These changes of the physical environment led to the proliferation of cyanobacteria in the marine phytoplankton which fixed and stored part of the carbon in the sea and contributed to the reduction of CO_2 in the atmosphere. Release of O_2 to the atmosphere due to oxygenic photosynthesis by cyanobacteria would also have contributed to the reduction of atmospheric methane through oxidation.

Meanwhile the chloroplasts that are today so common in eukaryotic algae and green plants evolved through processes of endosymbioses between cyanobacterial and heterotrophic eukaryotic ancestors. This endosymbiotic hypothesis was originally put forward by Mereschkowsky (1905). Though originally taken with skepticism subsequent electron microscopic, biochemical and molecular biological studies gave credence to this hypothesis and later Martin & Kowallik (1999) translated this paper into English and provided evidence in support of it by molecular analysis of the chloroplast genome of *Arabidopsis thaliana* and comparing it with those of *Nostoc punctiforme*, *Prochlorococcus marinus* and *Synechocystis sp.* PCC 6803 (Martin *et al.*, 2002). Raven and Allen (2003) confirmed these evidences and stated that the complete genome sequences of cyanobacteria and of the higher plant *Arabidopsis thaliana* leave no doubt that the green plant chloroplast originated through endosymbiosis with a cyanobacterium.

The oxygenic photosynthesis of cyanobacteria and chloroplast containing eukaryotes would have accelerated the oxygenation of the atmosphere and this would have had significant impacts on the existing life forms of that time. Oxygen would have been toxic to most of the primitive organisms which were predominantly anaerobic and a large number of them would have died in what was called the 'oxygen catastrophe'. As this process of oxygenation had been very slow and gradual through several millions of years, it enabled the evolution of resistant forms some of which even developed mechanisms to utilize O_2 to secure energy from food through aerobic respiration. This metabolic process was far more efficient than the anaerobic processes of fermentation and the aerobes on earth developed at a dramatic pace driving the anaerobes to near extinction. Today aerobes are the predominant living forms among both flora and fauna thriving in an atmosphere containing 21% of oxygen.

Distribution and associations with other organisms

Cyanobacteria are ubiquitous in their global distribution. They occupy a broad range of habitats across all latitudes, widespread in freshwater, marine and terrestrial ecosystems, also found in extreme habitats such as hot springs, hypersaline localities, freezing environments and arid deserts. Frequently they are the pioneer invaders (often as lichens), of exposed habitats such as bare rocks, recently piled up soil or bare land after natural disasters, exposed walls and partly constructed buildings and similar substrates which offer little or no nutrients. They are also common inhabitants of polluted water bodies, drains and garbage dumps which are generally inhospitable to most other organisms. The versatile abilities of these autotrophic prokaryotes are perhaps a reflection of their pioneering ancestry which at that time dominated the inhospitable primitive earth. Initial colonization of habitats by cyanobacteria if left undisturbed has the potential to develop through progressive autotrophic successions to reach even the final stage of a climax forest under favorable environmental conditions. Such important roles played by these simple prokaryotes justify referring to them as *Pioneers of Planet Earth*.

Among the planktonic cyanobacteria, the ocean dwelling *Prochlorococcus marinus* occupies a unique position. Reported as the tiniest photosynthetic organisms and the most populous autotrophic prokaryotes in the oceans, they are found among the phytoplankton of the oligotrophic deep ocean habitats in dense populations sometimes around 10^5 cells per ml of water. The vast extents of these populations in the oligotrophic deep oceans make a significant contribution to primary production and C-sequestration that ameliorates global warming. Research studies led by Professor Sally W. Chisholm of the Massachusetts Institute of Technology, Boston, USA, have shown the important role played by the massive populations of these minute organisms in carbon sequestration in the vast expanses of the deep open ocean ecosystems. When we realize that over 70% of the earth's surface is covered by oceans it is easy to comprehend the significance of the contribution made by them to global C-sequestration. Another astounding finding of the Massachusetts research team is the close association *Prochlorococcus* cells are having with numerous viruses that facilitate rapid lateral gene transfers among them. With these new findings they were able to get a clear picture of gene diversity and gene flow among these organisms and postulate that such lateral gene transfers endow upon them an intrinsic ability to rapidly adapt to environmental variables such as temperature, predators, light and nutrient changes which these organisms constantly encounter in the deep ocean ecosystems. The team's studies have shown that closely related *Prochlorococcus* strains display an array of physiological differences regulated by the genetic diversity of the constituent cells that provide an extraordinary stability to the overall community which internally adjusts itself to ever changing environmental conditions. These novel observations perhaps warrant the introduction of new terminology to such ecosystems. What is seen here are homogenous populations of *Prochlorococcus marinus* that are "heterogenomic". This dynamic hetero-genomicity due to the continuous lateral gene transfer through closely associated viral vectors enable the homogenous cyanobacterial community to sustain itself in the ever changing environment of the deep ocean. Johnson *et al.* (2006) have categorized these heterogenomic groups as ecotypes and presented a comprehensive report on their niche distribution in ocean scale environmental gradients. Populations of *Procholorococcus* have been categorized into two broad groups. The *High light tolerant group* found on the surface of the Equatorial Pacific, down to 5 m in the Mediterranean Sea, 50 m in the Arabian Sea, 35 m in the Gulf Stream and 90 m in the Sargasso Sea. The *Low light tolerant group* was observed down to 10 m in the North Atlantic, 83 m in the Equatorial Pacific, 120 m in the Sargasso sea and 135 m in the Gulf Stream. Quoting the works of Sally W. Chisholm of the MIT and Robert J. Olsen of the Woods Hole Oceanographic Institute, Nadis (2003) compares this tiny cyanobacterium to the archaic ancestors that originally developed oxygenic photosynthesis on Planet Earth and claims that they account for 50% of the total oceanic photosynthesis. *Prochlorococcus* is also unique among all cyanobacteria as the only genus to possess chlorophyll-b in addition to chlorophyll-a, resembling green algae. In fact this feature led to some debate over its taxonomic position, but all the prokaryotic features that it shares with other cyanobacteria have eventually kept it within this taxonomic group.

Another deep ocean dwelling cyanobacterium of ecological importance is *Trichosdesmium*, a filamentous non-heterocystous cyanobacterium which fixes atmospheric N_2. How this organism has reconciled the O_2 sensitive N_2-fixation while carrying out oxygenic photosynthesis is still an enigma and this will be discussed later under the section on N_2-fixation in cyanobacteria. Marine plankton species of cyanobacteria are not as common as freshwater species but *Trichodesmium* is arguably one of the commonest among them. *T.erythraenum* is more frequently encountered than other species like *T.theibautii*, *T.hilderbrandi* and *T.rubescens*. Drouet (1968) in his revision of the Family Oscillatoriaceae has grouped all these species as *Oscillatoria erythraea* but this did not receive universal acceptance. *Trichodesmium* exists in nature as bundles of filaments visible to the naked eye (Fig. 1). Usually this cynaobacterium is red in colour due to the high content of the accessory red pigment phyco-erythrin but they have been reported to have a range of colours from grey through yellow, green, and purple (Fogg *et al.*, 1973).

It is believed by some that the "Red Sea" got its name due to thick blooms of *T.erythraenum* that frequently formed in it. *Trichodesmium* occurs in tropical oceans where the surface temperature is above 25°C and salinity near 3.5%. It normally occurs as long windrows from a few feet to several miles in length. Wood (1965) has reported a massive bloom of nearly 52,000 km^2 (almost 80% of the area of Sri Lanka). Carpenter and Romans (1991) present *Trichodesmium* as the most important primary producer in the North Atlantic Ocean (ca 165 mgC/m^2/day) which also introduces the largest fraction of new nitrogen to the euphotic zone through fixation (ca 30 mgN/m^2/day). Capone *et al.* (1997) confirmed this report and illustrates in a map its global distribution in all the major oceans along the tropical belt showing its extension to the sub-tropical waters just above and just below the latitudes of 26.5°N and 26.5°S respectively. It is however claimed that its activities are confined to water temperatures above 20°C. Karl *et al.* (1997) presenting results of seven years of time series observations in the North Pacific Ocean Gyre, claimed that N_2 fixation by cyanobacteria accounted for half of the new production and demanded a reassessment of previous bio-geochemical nutrient cycling budgets of one of the world's largest biomes.

Another marine planktic cyanobacterium is *Richelia intracellularis* which lives as an endosymbiont within some large diatoms that are frequently encountered among the phytoplankton of the oligotrophic oceans (Carpenter and Romans, 1991). Although not as common as eukaryotic algae, cyanobacteria are found in the marine intertidal and littoral ecosystems, the common species being *Lyngbya majuscula, Microcoleus ethnoplasts,* (a common mat former), *Spirulina sp.* (a highly nutritious cyanobacterium) and several species of *Oscillatoria*. They are also found in association with marine coralline algae and marine lime stones e.g. *Hyella stella* and *Scytonema endolithicum*.

Nevertheless, the greatest abundance and diversity of cyanobacteria are encountered in freshwaters where they are far more common than their marine counterparts. Several unicellular, colonial and filamentous species have been recorded both as plankton, and benthic micro flora. They are invariably present together with other photosynthetic organisms in mesotrophic waters, the common genera being *Microcystis, Synechococcus, Anacystis, Gloeocapsa, Agmenellum (syn. Merismopedia)* as unicellular and colonial types and *Oscillatoria, Lyngbya, Spirulina, Ananbaena, Aphanizomenon, Nostoc, Cylindrospermopsis, Planktothrix, Calothrix, Rivularia* and *Gleoetrichia* as filamentous types. With pollution and nutrient loading such water bodies become eutrophic and very often certain cyanobacteria that prefer such environments outgrow their counterparts producing explosive growths to form 'algal blooms'. The common bloom forming genera are *Microcystis, Cylindrospermopsis, Anabaena* and *Aphanizomenon*. Some times these blooms could be massive. The author once had the experience of measuring such a bloom in 1991 that formed adjacent to the Kotmale Hydro-Power Reservoir Dam which was 1.5 m deep and extended to more than 2000 m^2 (unpublished personal observation). They foul the water and most of them produce 'algal toxins' that are often lethal to fish and digastric mammals, cause diseases, irritations, allergies and also contribute to liver and kidney ailments among humans. (A more detailed analysis is included in the section on 'toxigenic cyanobacteria'). In the aquatic ecosystems cyanobacteria play a crucial role as oxygenic primary producers as well as nitrogen fixers.

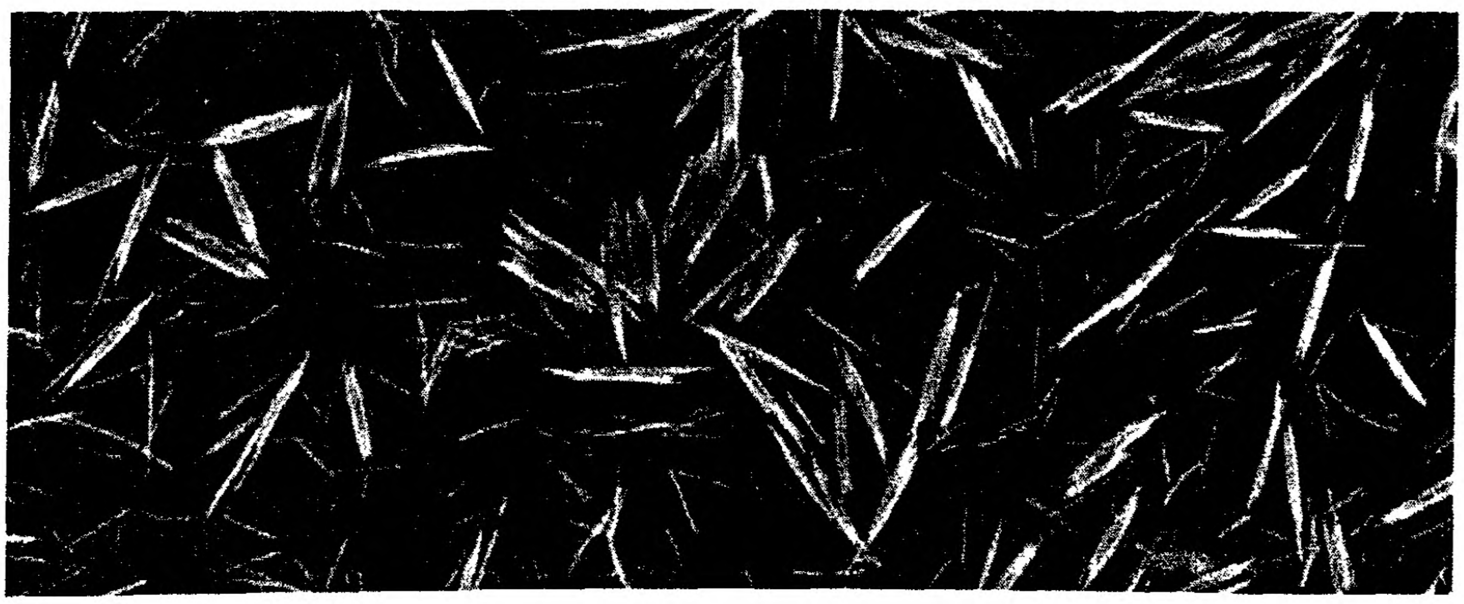


Figure 1. Colonies of *Trichodesmium erythraenum*. (Source: Reproduced from Fogg *et al.*,1973)

Their environmental sensitivity and short life cycles resulting in rapid species turnover are helpful to use them as biological indicators in environmental assessment studies. For example N_2 fixing cyanobacteria have been used to understand the water quality especially high turbidity, low N:P ratio, metal toxicity and nitrogen limitations in the environment. The photosynthetic pigments and resting stages they produce to survive harsh environmental conditions have sometimes remained even for thousands of years. Such remains provide reliable historical information when monitoring data are not available. Recent developments in environmental science and biostatistics help quantify ecological optima and tolerances of such indicator organisms, enable the assessment of past environmental conditions and predict future global scenarios such as climate change (Yatigammana, 2004).

Free living cyanobacteria are found in all terrestrial habitats but are frequently observed in moist environments. They produce luxuriant growth in wetlands, marshy lands and rice fields. Common terrestrial genera are *Aphanocapsa, Gloeocapsa, Merismopedia, Eucapsis* as unicellular and colonial types, *Oscillatoria, Lyngbya, Microcoleus, Spirulina* as undifferentiated filamentous types, *Nostoc, Anabaena, Cylindrospermum, Calothrix, Scytonema, Tolypothrix* as differentiated filamentous forms and *Mastigocladus, Fischerella, Westiella, Westiellopsis* and *Stigonema* as true branched, differentiated filaments This range of morphological diversity extending from simple unicellular forms to multicellular, heterotrichous, true branched filamentous morphotypes bestow upon the cyanobacteria a unique position as the most complex organisms among all the Prokaryota.

Cyanobacteria either by themselves or in symbiosis with fungi as lichens are often the pioneering colonizers of bare soils, rocks and other exposed surfaces with little or no nutrients indicating their ability to adapt to inhospitable environments. Such abilities have sometimes being utilized by soil microbiologists as in the reclamation of alkaline 'Usar Soils' of India by bio-fertilization with *Aulosira fertilissima* (Singh, 1961).

The adaptability of cyanobacteria to extreme environments is exemplified by certain species being extremophiles *i.e.* inhabitants of extreme habitats. They are found in freezing habitats as "green snow", in glaciers and as endolithic members in the sub surface of Arctic and Antartic rocks Friedmann (1982). Friedmann *et al.* (1988) reported cyanobacteria and lichens as cryptoendolithic organisms in the frigid deserts of the Antarctic dry valleys where no other life forms are visible on the surface. They live in between crystals of porous rocks and their activities contribute to the weathering of these rocks. The common genus observed in these freezing habitats is *Chroococcidiopsis*, a unicellular cyanobacterium belonging to the Order Chroococcales. In these habitats temperatures are normally around -20° C and even in the warmest periods do not go over 0° C. On the other hand the extremophile *Chroococcidiopsis* has also been reported as the only life form found in the hyper arid core of the Atacama Desert in South America living as an endolithic organism beneath halite rocks (Weirzchos *et al.*, 2006). Cyanobacteria are also found endolithically in large columnar calcified structures called stromatolites in the Australian tidal flats. Stromatolites are extremely slow growing structures and some of the fossilized stromatolites of Australia believed to be 3.4 billion years of age depict remains of endolithic fossil cyanobacteria. The ability of *Chroococcidiopsis* to grow in such extreme habitats has prompted the NASA scientist E. Imre Friedmann to suggest it as a candidate of choice to be used in its Astrobiology Programs to introduce life to the Red Planet Mars.

Cyanobacteria often live in association with other organisms forming microbial mats, biofilms and benthic communities and such associations are the predominant and sometimes the only life forms found in certain extreme habitats like hot springs. Certain species like *Rickelia intracellularis* lives as endosymbionts in large diatoms. Cyanobacteria are also endosymbiotic in fungi (lichens), bryophytes (*Anthoceros*), pteridophytes (*Azolla*), cycads (coralloid roots), angiosperms (*Gunnera*) and certain marine sponges and corals.

Morphology, taxonomy and molecular biology

Cyanobacteria exhibit the most diverse and complex morphology among all prokaryotic groups, perhaps indicating that they would have been the dominant life forms at some period of the early earth. External gross appearance under aquatic and/or moist conditions is often gelatinous, slimy and occasionally filamentous clusters, with colours ranging from dark green, blue-green, yellow, brown to black, and rarely red (Fig. 2).

This variable colour range is primarily due to their photosynthetic pigments consisting of chlorophyll-a (which all of them possess) together with different concentrations of the accessory phyco-biliprotein pigments, phycocyanin (blue) and phycoerythrin (red). The final external colour of a specimen is largely dependent upon the concentrations of these pigments. Characteristically all cyanobacteria have thin or thick gelatinous sheaths outside their cell walls, their thickness and sometimes their colour contribute to the final appearance of the organism.

Although cyanobacteria perform oxygenic photosynthesis just like eukaryotic green algae and higher plants, they do not store food in the form of starch. Their principal storage product is cyanophycean starch and glycogen together with specialized intracellular storage compounds like lipids, protein containing cyanophycean granules and polyphosphate bodies. Some cyanobacteria particularly the plankton species produce intracellular 'gas vacoules'. These are quite different from typical membrane bound vacoules of eukaryotic cells and are actually groups of gas filled vesicles that collapse under sudden shock and pressure. These vesicles enhance buoyancy and this helps such species to move up and down a water column and occupy a depth that provides the best micro-niche in an aquatic environment.

The somatic diversity of cyanobacteria extends from unicellular forms, colonial assemblages, unbranched filaments to filaments with false and true branching. As these organisms do not show sexual reproduction their taxonomy has been entirely based upon morphology until the mid 20th century (Geitler, 1932, Fritsch, 1942 and Desikachary, 1959). A sample of a brief classification based on morphological diversity is given below:

Group: Algae (Eukaryotic)
Division: Chlorophyta (Green algae)
Class Cyanophyceae or Myxophyceae (Blue-Green algae)

Order Chroococcales – unicellular and colonial members.
Unicells *e.g. Anacystis*, *Synechococcus* and *Chroococcus*,
Loose colonies *e.g. Microcystis*,
Compact colonies *e.g. Gloeocapsa*,
Colonies of definite shape *e.g.* flat plates *Merismopedia (syn. Agmenellum)* and cuboidal colonies *e.g. Eucapsis*.

Order Chaemosiphonales - A small group that grows attached and produces a type of asexual exospores *e.g. Chaemosiphon* and *Dermocarpa*.

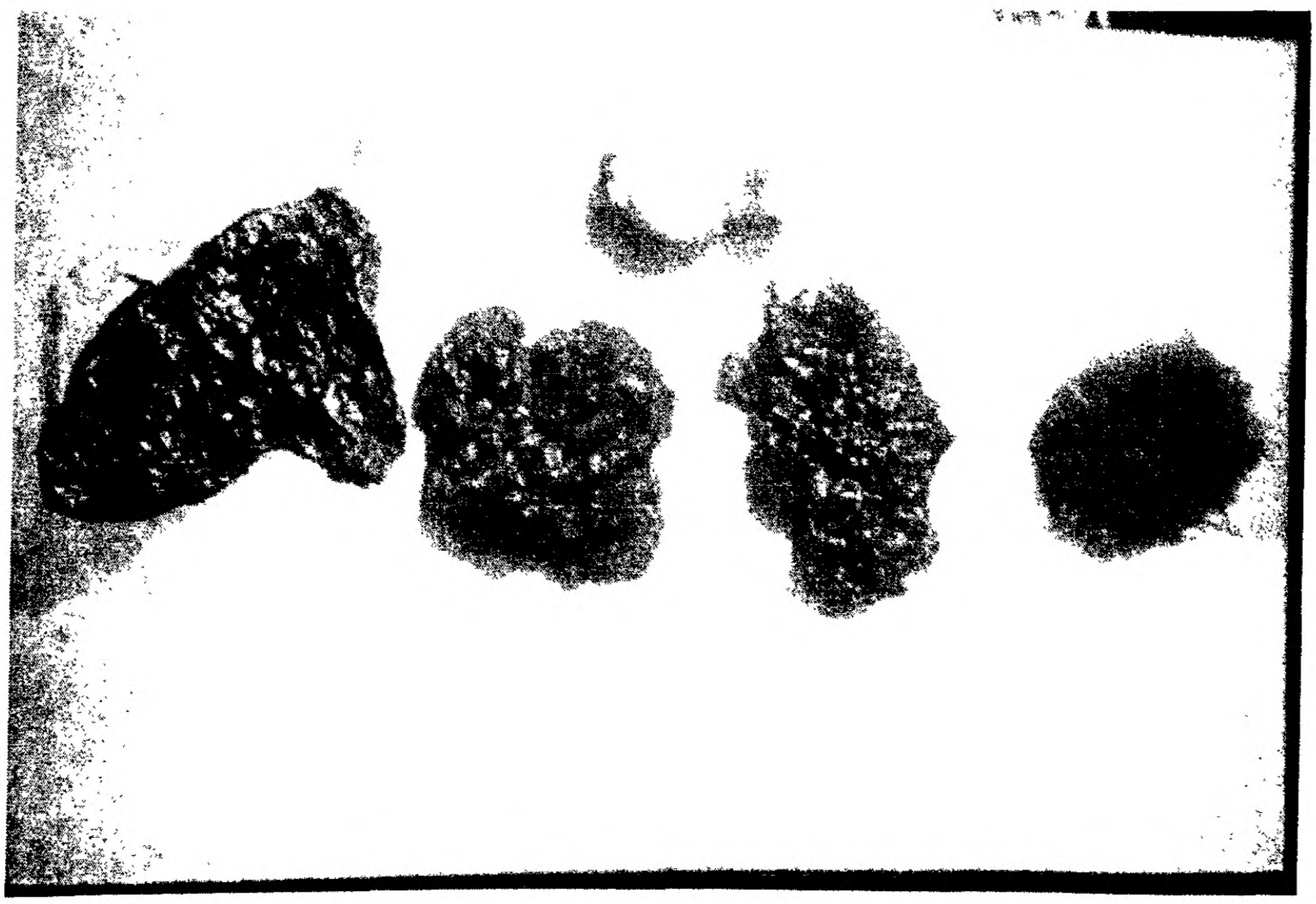


Figure 2. External appearance of some cyanobacteria collected from paddy fields in Sri Lanka.

Order: Oscillatoriales - Unbranched, undifferentiated filaments *e.g. Oscillatoria* and *Spirulina* (thin sheaths), *Lyngbya* (thick sheath), *Microcoleus* (several trichomes within a common sheath).

Order Nostocales - Unbranched filaments exhibiting cell differentiation into vegetative cells, heterocysts and akinetes (spores).
Family Nostocaceae: Simple filaments *e.g. Nostoc, Anabaena, Cylindrospermum* (terminal heterocysts with large basal akinete).
Family Rivulariaceae: Whip-like tapering filaments *e.g Calothrix* (non-colonial with or without spores), and colonial *Rivularia* (non sporing) and *Gloeotrichia* (sporing).
Family Scytonemataceae: Filaments with false branching *e.g. Tolypothrix* (more single branches) and *Scytonema* (more double branches).

Order Stigonematales - Members having true branches.
Family Nostochopsidaceae: Members do not exhibit heterotrichous habit *e.g. Nostochopsis*.
Family Stigonemataceae: Those showing heterotrichous thalli with prostrate and erect systems *e.g.*:
Mastigocladus both prostrate and erect systems uniseriate.
Frischerella prostrate system multiseriate and erect system uniseriate.
Stigonema climax thallus development with both systems multiseriate.

With the advent of electron microscopy and molecular biology these simple classification systems had to be reviewed and revised. Revisions of different groups have been reported by Drouet & Daily (1956) and Drouet (1968, 1973, 1977). The realization of the prokaryotic cell structure of the 'blue-green algae' warranted a radical change in their taxonomy and it was proposed by a leading team of blue-green algologists (at that time) to place the nomenclature of these organisms under the International Code of Nomenclature of Bacteria (Stanier *et al.*, 1978), but this proposal has been questioned (Komarek, 2006). Rippka *et al.* (1979) revised the generic assignments of a number of pure cultures of cyanobacteria. Application of modern molecular biological techniques has brought about radical changes in the taxonomy of cyanobacteria. Sequencing of 16S rRNA while confirming the existence of several morphologically uniform and well-defined traditional genera, has also placed similar morphotypes in distant positions in phylogenetic trees. Molecular data therefore, provide basic criteria for cyanobacterial taxonomy, but to construct a comprehensive phylogenetic system of cyanobacteria a combination with knowledge on their morphology, physiology, biochemistry and ecology is essential. For a comprehensive review of combined molecular and phenotype taxonomic evaluations of cyanobacteria readers are referred to Komarek (2006). In this review he has emphasized the necessity to adopt a 'polyphasic' approach combining the knowledge gained through modern ecological, ultra structural and molecular methods supported by the cultivation of numerous cyanobacterial morphotypes. It is stated that while molecular data provide basic criteria for cyanobacterial taxonomy, a correct phylogenetic system cannot be constructed without combining the data from previous 150 years of studies on cyanobacterial diversity. The limitations of using 'type strains' from standard culture collections for systematic and nomenclatural purposes, are also highlighted in this review.

Nitrogen fixation and its applications

The ability of certain cyanobacteria to fix atmospheric nitrogen make them unique in their being autotrophic in both carbon and nitrogen nutrition. It is also intriguing to find how these prokaryotic organisms (without intracellular organelles) simultaneously perform two incompatible processes of oxygen evolving photosynthesis and oxygen sensitive nitrogen fixation.

Phylogenetic studies have indicated that all nitrogenases have been derived from a common prokaryotic ancestral group that existed prior to the oxygenation of the Earth's atmosphere (Berman-Frank *et al.*, 2003). In this anoxic atmosphere having a predominance of CH_4, CO_2, N_2, and NH_3, ultra-violet radiation would have dissociated NH_3 releasing N_2. Primitive nitrogenases could have initially arisen as respiratory enzymes where N_2 acted as a sink for anaerobic respiration of certain heterotrophs. The slow process of oxygenation over several millions of years primarily due to the oxygenic photosynthesis of cyanobacteria, resulted in the partial pressure of atmospheric oxygen to increase from 4×10^{-6} to >0.03%. This oxygenation was inimical to N_2 fixation and all organisms had to adopt various methods to protect their nitrogenase enzyme from damage by O_2. Gallon *et al.* (1991) has extensively discussed these mechanisms as behavioural adaptations, physical barriers, physiological and biochemical strategies and treated the cyanobacteria as a special group and these have been discussed by Kulasooriya (2008).

Major adaptations among cyanobacteria are structural changes among their cells. The vast majority of cyanobacteria that exhibit aerobic nitrogen fixation possess a type of specialized cell called heterocyst. Compared to vegetative cells heterocysts are generally larger in size, lighter in colour, have thicker cell walls and have thickenings called polar nodules at the points of attachment to adjacent cells. Kulasooriya *et al.* (1972) reported on the differentiation of heterocysts in *Anabaena cylindrica* and its coincidence with the restoration of nitrogenase activity. When cultured in the laboratory in media with combined nitrogen (particularly NH_4 ions), the filaments did not produce heterocysts and they lost their nitrogenase activity. When such induced 'non-heterocystous' filaments were transferred to N-free media, pro-heterocysts reformed within 24 hours. Once these pro-heterocysts matured into heterocysts within the next 24 hours (which could be detected by the formation of polar nodules), nitrogenase activity was restored. It was also found that the cellular C:N ratio was critical for heterocyst formation as well as expression of nitrogenase activity. A mature heterocyst has a thick envelope that consists of an outer polysaccharide fibrous layer, a middle homogenous layer and an inner glycolipid laminated layer (Lang and Fay, 1971). This cell envelope has been shown to be critically important for nitrogen fixation and limit the ingress of O_2 into the heterocysts (Murry *et al.*, 1984 and Murry and Wolk, 1989). Isolated heterocysts showed higher levels of respiration and their absorption spectrum coincided more closely than that of the vegetative cells, with the action spectrum of nitrogenase activity of whole filaments (Fay and Walsby, 1966). Isolated heterocysts contained very little chlorophyll-a and were devoid of phycocyanin and phycoerythrin pigments which are associated with the O_2 evolving photosystem-II of photosynthesis (Thomas, 1970). Using ^{14}C labeled CO_2 Wolk (1970) showed that heterocysts do not fix carbon and Fay and Kulasooriya (1972) demonstrated the prevalence of reducing conditions in heterocysts by the reduction of Triphenyl Tetrazolium Chloride. Eventually Wolk and Wojciuch (1971) demonstrated photo-reduction of acetylene and (Janaki and Wolk, 1982) showed the presence of nitrogenase in isolated heterocysts. Wolk (1982) reviewed and summarized all the available evidences and concluded that the main function of the heterocyst is nitrogen fixation.

Meanwhile reports came in on the detection of nitrogenase activity in certain unicellular as well as non-heterocystous, filamentous species of cyanobacteria. The first report of a unicellular species was that of Wyatt and Silvey (1969) who demonstrated nitrogenase activity in *Gloeocapsa (syn.Gloeothece and Cyanothece)*. These were followed by reports on other unicellular species such as *Synechococcus* and *Aphanothece* (Mitsui *et al.*, 1986), and filamentous non-heterocystous forms like *Microcoleus chnoplastes* (Pearson *et al.*, 1981) and *Oscillatoria sp.* (Gallon *et al.*, 1991). In all these cases it has been found that there was a temporal separation of the two incompatible processes of oxygenic photosynthesis and oxygen sensitive nitrogen fixation along the growth cycle of these organisms. The other reported physiological and biochemical strategies adopted by these organisms have been reviewed in Kulasooriya (2008).

The most enigmatic situation was encountered with the large marine blooms of the non-heterocystous, filamentous cyanobacterium *Trichodesmium erythraenum* which showed very high nitrogenase activity and it was the main contributor to the nitrogen budget of the deep oceans (Karl *et al.*, 1997). Two other species *T. theibautii and T. aureum* have also being identified as nitrogen fixing, non-heterocystous, marine cyanobacteria. Certain studies have demonstrated the presence of some specialized cells called diazocytes in these organisms and it has been suggested that the nitrogenase enzyme is confined to these cells (Jansen *et al.*, 1994, Fredrickson and Bergman, 1995 & 1997, Lin *et al., 1998 and Mulholland and Capone, 2000).* Nitrogen fixation in *Trichodesmium* peaked around midday and varied inversely with photosynthetic O_2 evolution. It has also been suggested that N_2 fixation and photosynthesis in *Trichodesmium* is regulated by circadian rhythms (Chen *et al.*, 1998). Molecular studies on natural populations of *T. theibautii* have shown that nitrogenase is synthesized early morning and *nifH* mRNA is highest before dawn (Wyman *et al.*, 1996). This enzyme remained active until noon when it reached a peak, then declined in the afternoon and got degraded during the night (Capone *et al.*, 1990 and Zer *et al.*, 1993). Twelve *nif* genes cloned and sequenced from *Trichodesmium* are basically similar to those of other cyanobacteria, but show a closer resemblance to 'vegetative cell nitrogenase genes' induced in *Anabaena variabilis* under micro-aerobic conditions. Many other non-heterocystous cyanobacteria such as species of *Oscillatoria, Lyngbya, Microcoleus* and *Plectonema boryanum* have been induced to fix nitrogen under micro-aerobic conditions.

Among other strategies, aggregation of unicellular and non-heterocystous cyanobacteria to form algal mats, colonies, biofilms among themselves and with other microorganisms have been reported to exhibit low levels of nitrogenase activity in marine *Synechococcus* and *Trichodesmium* and terrestrial and freshwater *Oscillatoria, Lyngbya, Microcoleus* and *Plectonema*. Physical barriers such as thick mucilaginous sheaths and capsules have enabled expression of nitrogenase activity in *Gleoetheca* (syn. *Gloeocapsa* and *Cyanothece*). As a biochemical protective mechanism nitrogenase activity has been shown to be regulated by ADP-ribosylation of the Fe-protein (the more O_2 sensitive component of the nitrogenase enzyme complex). Such adaptations originally demonstrated in photosynthetic bacteria have been found to be widespread among cyanobacteria such as *Anabaena CA, Anabaena variabilis, Aphanizomenon flos-aqaue, Oscillatoria limosa, Trichodesmium, Gloeotheca* and *Microcoleus chthnoplasts* as reviewed by Kulasooriya (2008). Continuous synthesis of nitrogenase enzyme has also been demonstrated in certain taxa like *Anabaena CA, Anabaena variabilis, Anabaena flos-aquae and Gloeothece*, but this strategy appears to be of limited distribution.

All these evidences support the idea that although heterocysts are specialized cells for N_2 fixation in cyanobacteria the potential to fix N_2 is present even in the vegetative cells but this is expressed only under reduced oxygen concentrations. These observations have important ecological implications and it is quite likely that the subterranean layers of thick mats of non-heterocystous cyanobacteria may be fixing nitrogen particularly under low light conditions or in the dark. This idea is also compatible with the evolution of the process of N_2-fixation from an archaic reducing Earth's atmosphere through a micro-aerobic atmosphere to the oxygenated atmosphere of present times (Gallon *et al*., 1991 and Berman-Frank *et al*., 2003). In symbiotic associations it is always the cyanobacterial partners that fix nitrogen. They provide nitrogen to the ecosystem in which they live and often the rates of fixation by these systems are higher than those of free-living cyanobacteria, perhaps due to the additional nutrition and micro-aerobic conditions provided by the host. Occasionally cyanobacteria have been reported to be epiphytic on rice plants (Roger *et al*., 1981) and weeds in wetland rice fields (Kulasooriya *et al*.,1981a) and even endophytic in the leaf sheaths of deepwater rice (Kulasooriya *et al*., 1981b) and the nitrogen they fix has been shown to be available to the associated host plants (Watanabe *et al*.1982).

Free living nitrogen fixing cyanobacteria and the aquatic fern Azolla (which has nitrogen fixing *Anabaena azollae* as an endosymbiont), have been used as biofertilizers in rice cultivation. Venkataraman (1972) provides a comprehensive account of the large scale production of cyanobacterial bio-fertilizers in India. Cyanobacteia are initially isolated from rice fields, purified and screened for rapid growth and high N_2-fixation. Selected isolates are then semi-mass cultured in laboratory media and these are used as inoculants for soil based outdoor mass cultures in open ponds or large trays under non-sterile conditions. A sprinkle of P fertilizer, lime and pesticides are usually added to ensure uninhibited rapid growth. After sufficient growth is obtained the cultures are allowed to sun dry and the soil based algal flakes are packed and distributed among farmers to be used as bio-fertilizers. This low cost technology quite suitable for resource poor rural farmers has been successful only in a few areas in India where edaphic conditions are favourable for the growth of cyanobacteria. Reports from certain areas of Myannmar, China and Egypt have also recorded some successes. Roger and Kulasooriya (1980) reviewed most of the literature on the use of cyanobacterial biofertilizers and concluded that their N-input potential ranged from 0 to 80 Kg/ha/season with an average of 25 Kg/ha. Attempts to adopt this technology in Sri Lanka was successful only up to the stage of pot experiments and most of the cyanobacterial fertilizers added to the rice fields could not overcome consumption by the rice field micro-fauna and competition by indigenous micro-flora and failed to colonize the fields (Kulasooriya and Hirimburegama, 1989). Compared to free living cyanobacteria, the application of *Azolla* in rice cultivation has been more successful and widely used particularly in Vietnam, China and certain parts of Philippines. The status of this technology has been well reviewed at a workshop held in Fuzhou in Fujian Province, China and published by the International Rice Research Institute, Philippines in 1987 as '*Azolla Utilization*'. Even in Sri Lanka this technology appeared to be more feasible than free living cyanobacterial biofertlizers. *Azolla* grew well in rice fields at Ambalantota (Fig. 3), Bombuwela, and Peradeniya and showed a potential to replace an equivalence of 55 to 85 KgN/ha of chemical N-fertilizer (Kulasooriya *et al*., 1987). The major

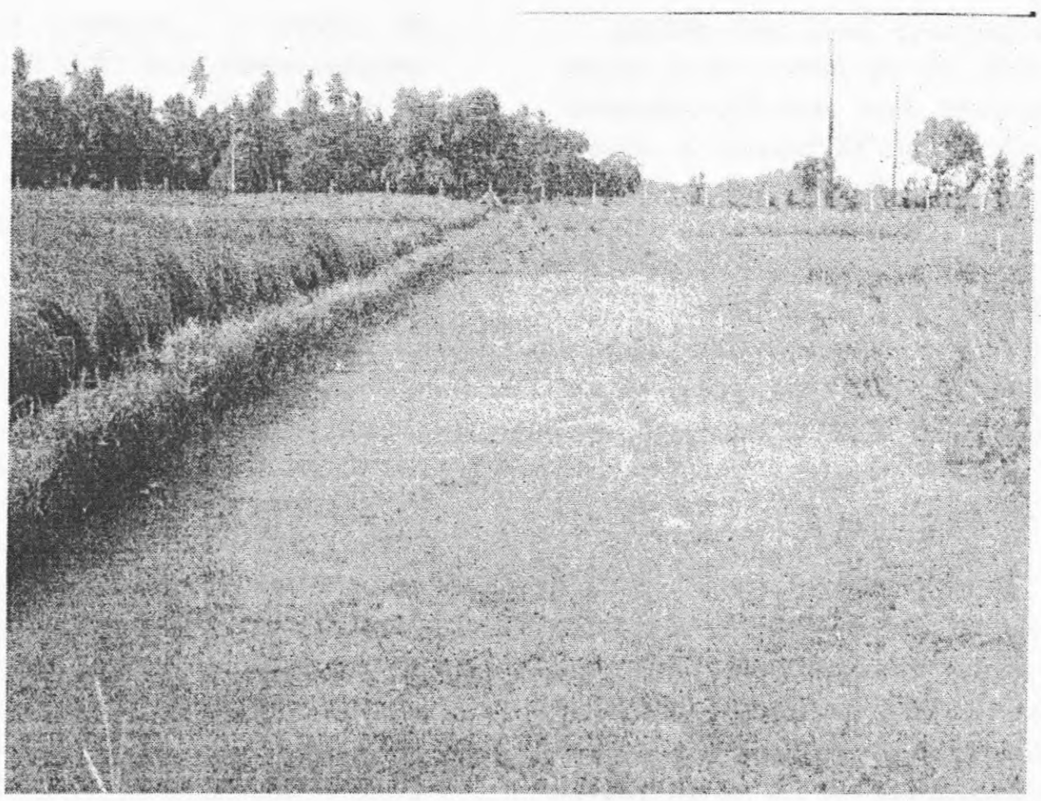


Figure 3. Growth of *Azolla* in a rice field at Ambalantota, Sri Lanka.

constraints for the adoption of this technology by the rice farmers are, handling of fresh bulky material, maintaining fresh cultures during the dry (fallow) periods, the need for frequent P-fertilizer applications and the susceptibility of *Azolla* to pests and pathogens particularly under warm weather. It is therefore evident that the use of N_2-fixing cyanobacteria and their symbiotic systems as biofertilizers for rice have only limited potential for widespread application.

Toxigenic cyanobacteria

Though cyanobacteria make positive contributions to global biodiversity and the environment particularly through carbon and nitrogen fixation they also cause severe problems in fresh water ecosystems and sewage treatment facilities by the production of toxins (Carmichael, 1994). It has already been mentioned that certain species form 'algal blooms' in polluted eutrophic waters (see section on Ecology and Distribution). Bloom forming species notably *Microcystis*, *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis* and *Nodularia* frequently produce cyanobacterial toxins. Many such toxins have been initially named after some of these genera *e.g.* Microcystins from *Microcystis*, Anatoxins from *Anabaena*, Cylindrospermopsin from *Cylindrospermopsis* and Nodularin from *Nodularia*, although the production of such toxins is not confined to these genera but also produced by others. Toxins are not always produced and the mere presence of these genera should be considered only as a potential danger. What factors trigger toxin production is not definitely known although it has been suggested to be a defense mechanism.

In mesotrophic, unpolluted waters the total algal populations are small but their diversity is high with almost equal representations from green algae, cyanobacteria (blue-green algae) and diatoms. When such a water body gets polluted and is subjected to high light and temperature, most of the eukaryotic algae tend to decline and a few blue-green algae (cyanobacteria) particularly those that produce toxins become dominant and sometimes form blooms. This has happened with the ancient irrigation reservoirs of Sri Lanka (Kulasooriya, 2004 and 2005a and b). Surveys conducted by Kulasooriya and others in collaboration with the National Water Supply and Drainage Board from late 1980s to early 1990s were communicated in several seminars and symposia (Kulasooriya, 1997 and 1998, Jayawardana *et al.*, 1998 and Weerakoon *et al.*, 1998) and the general public were made aware by newspaper articles (Kulasooriya, 2005b). Reports from Silva and Samarawickrama (2005) showed the presence of toxigenic cyanobacteria in the Kandy Lake and their dominance in most of the reservoirs of the Mahaweli river basin (Silva and Wijeyaratne, 1999). Jayatissa *et al.* (2006) in a comprehensive study covering 43 freshwater water bodies have shown that most of the irrigation tanks and reservoirs in Sri Lanka including the recent ones constructed under the Mahaweli project, have a predominance of toxin producing cyanobacteria except the reservoirs at

Kalatuwawa and Labugama in which the catchment areas are under strict nature reserves. This is an indication that if pollution is minimized the occurrence of toxin producing cyanobacteria can be reduced. How such conditions can be established and sustained in irrigation tanks (reservoirs) would always remain a dilemma. Jayatissa *et al.* (2006) also reported for the first time the presence of microcystin LR toxin in some of the cyanobacterial samples collected in Sri Lanka. More recent studies have confirmed these results and it is now quite evident that a large number of inland freshwater bodies of Sri Lanka carry heavy populations of toxigenic cyanobacteria with *Cylindrospermopsis* being the dominant genus in a majority of them (Perera *et al.*, 2011a, Perera *et al.*, 2011b, Kulasooriya and Padmasiri, unpublished). The Institute of Fundamental Studies, Sri Lanka, now possesses facilities to detect and quantify cyanobacterial toxins in water samples. A number of samples collected from reservoirs and irrigation tanks from the dry zones of North Central and Wayamba Provinces have shown the presence of such toxins sometimes well over the levels permitted by WHO standards (Dr. D. N. Magana-Arachchi, IFS, personal communication). It is evident from these results that immediate steps should be taken to ameliorate the predominance of toxigenic cyanobacteria in water bodies of Sri Lanka, commencing from those used as sources for water supply schemes. It is also imperative to conduct well formulated scientific studies to verify the claims that toxigenic cyanobacteria are responsible for the prevalence and rapid increase of chronic kidney diseases of unknown etiology in certain areas of Sri Lanka.

Some industrial applications

Cyanobacteria are of common occurrence in waste water treatment plants. In some instances they have been found to be useful as low cost converters of odour causing and pollution creating dairy effluents into micro-algal biomass using sunlight (Lincoln *et al.*, 1996). They were found to be very effective in removing NH_4 ions and other nitrogen rich compounds from such effluents. In other cases toxigenic species have reduced the efficiency of waste water treatment plants (Martins *et al.*, 2011). Toxins produced, particularly microcystins have not been antibacterial but they have adversely affected the protozoan populations. Nonetheless, other secondary metabolites of cyanobacteria have been found to be anti-bacterial and affected the decomposition rates of both aerobic and anaerobic bacteria.

On the other hand, cyanobacteria have been found to be useful in the bioremediation of industrial effluents and extraction of heavy metals. A number of species belonging to the genera of *Synechococcus*, *Cyanothece*, *Oscillatoria*, *Nostoc* and *Nodularia* isolated from pharmaceutical and textile industry effluents were demonstrated to be capable of biodegradation and biosorption of several heavy metals (Dubey *et al.*, 2011). Using lyophilized *Synechococcus sp.* PCC 7942 immobilized in a silica polymer, Gardia-Torresday *et al.* (2011) demonstrated that this cyanobacterium could effectively bind Cu^{2+}, Pb^{2+}, Ni^{2+}, Cd^{2+}, Cr^{3+} & Cr^{4+} and these metals could be recovered by elution with 0.1M HCl. It has even being reported that certain cyanobacteria can be useful in the cleaning of oil spills (Cohen, 2002). He tested species isolated from cyanobacterial mats developing in oil-contaminated sabkhas along the African coasts of the Gulf of Suez and in the pristine Solar Lake, Sinai. He found that axenic cultures of the isolated cyanobacteria by themselves are unable to degrade oil. In association with sulphate reducing and other heterotrophic bacteria from the mats, when exposed to light rapid degradation of oil was observed. The oxygenic photosynthesis of the cyanobacteria stimulated the aerobic decomposition by the associated bacteria. This shows the potential of producing cyanobacterial - eubacterial biofilms as efficient microbial consortia for cleaning of oil spills. Abed (2010) has reported the commencement of such a project in Oman.

Recently a few species of cyanobacteria have been investigated for biofuel production because their ability to convert solar energy to chemical energy has been found to be the most efficient among all living organisms. The efficiency of solar energy conversion in corn and sugarcane is 1–2%, for eukaryotic algae it is around 5% while for cyanobacteria it is 10%. This can be understood if one looks at the photosynthetic pigments of cyanobacteria. In addition to chlorophyll-a these organisms contain phycobilisomes attached to the thylakoid membranes and these act as antennae in harvesting light for photosystem II. Among the pigments contained in the phycobilisomes, Phycoerythrin absorbs energy between the wave lengths of 500 – 600 nm, Phycocyanin absorbs between 550 – 650 nm and Allophycocyanin absorbs between 600 – 675 nm. Together with chlorophyll-a, their light absorption capacity will therefore extend right across the entire spectrum of visible light (Dr. Celia Smith, Department of Botany, University of Hawaii at Manoa, http://www. biologie.uni-hamburg.de/ b-online/ librarywebb/ BOT311/ Cyanobacteria). Furthermore, the simple genomic structure of these organisms has enabled gene sequencing and genetic engineering relatively easier. Professor James C. Liao of the University of California, Los Angeles, has inserted and spliced genes from other organisms to increase the CO_2 fixing ability of Rubisco enzyme of *Synechococcus elongatus*. This transgenic strain produced isobutyraldehyde gas which could be recovered and converted to butanol. Prof. Malcolm R. Brown and Dr. David Nobles of the University of Texas, Austin have inserted a gene from *Acetobacter xylinum* to a cyanobacterium which could secrete alcohol and sugars. Prof. P. Fu and Dr. J. Dexter of the University of Hawaii have transformed *Synechocystis* strain PC 6803 by inserting pyruvate dehydrogenase and alcohol dehydrogenase II genes from *Zymomonas mobilis* to produce ethanol. The biggest break through has been reported by a private company Joule Technologies of Boston, Massachusetts, which has developed Bioreactors containing genetically modified cyanobacteria that could continuously secrete biofuel and ethanol (Lane, 2010). Reports on biofuel production capacities from different sources are given in Table 1.

Table 1. Production capacities of biofuel from different sources.

Source	Gals/ac*	L/ha+
Soybean	50	92
Corn	250	458
Sugarcane	450	825
Oil palm	650	1192
Algae	2000	3668
Joule technologies	15,000	27,512

Source: *Original report, + Converted

The 15,000 gals/ac given by Dr. Dan Roberts, the principal scientist at Joule Technologies, eclipses all other claims. (Mail Online, Science & Technology March 2, 2011).

It is interesting to note that in all these cases the most productive cyanobacteria have been unicellular species. This is perhaps due to their ability to harvest maximum solar energy per cell because the single cells turn and rotate with minimum self shading as they move up and down a bioreactor. Also their simple genomes permit easy genetic engineering compared to more complex counterpart resources. This opens a window of opportunity to conduct fundamental studies in Sri Lanka that could lead us to a commercially viable break through in biofuel production. Most of the other industrial platforms for biofuel production based upon feed stocks derived from higher plants require vast areas of cultivable land. For a small country like Sri Lanka it is a formidable task to allocate such extents of land without compromising on other more important economic uses. Algae based biofuels therefore appear to be an attractive option. Already unicellular cyanobacteria belonging to *Synechococcus*, *Synechocystis*, *Chroococcidiopsis* and *Cyanothece* have been isolated from different localities in Sri Lanka including brackish water and hot springs and partially sequenced (Wanigatunga *et al.*, in preparation). With the commissioning of coal powered electricity generation systems sequestering CO_2 emissions would be a major problem and such waste gases could be harnessed to grow biofuel cyanobacteria. Even the CO_2 emitted by other industries like breweries and cement production factories can be used for algal cultivation.

Cyanobacteria as a food supplement

Among the vast array of cyanobacteria, two species have been used as food supplements of high nutritive value. They are *Spirulina plantensis* (syn. *Arthrospira plantensis*) and *Aphanizomenon flos-aquae*. These species have come from the traditional food habits of ancient civilizations of Aztecs, Mayans and Chinese and have been harvested from natural freshwater lakes such as Lake Chad in Africa, Upper Klamath in North America, Lake Texcoco in Mexico, and Lake Titicaca in Peru, South America. Currently they are also cultivated in large open raceway ponds with paddle wheel stirring under high pH and high temperature to minimize contamination by human pathogenic microorganisms. Such commercial production facilities are today found in the United States, Thailand, India, Taiwan, China, Pakistan, Myanmar and Chile. *Spriulina* is marketed as a source of complete nutrition which no other single organism can offer. Besides having a very high protein content (65 – 71% of dry weight) with all the essential amino acids, it is claimed to contain a wide spectrum of prophylactic and therapeutic nutrients including B-complex vitamins, minerals, proteins gamma linolenic acid and super antioxidants such as beta carotene, Vitamin E, trace elements and a number of unexplored bioactive compounds (Kulshreshtha *et al.*, 2008).

The other species, *Aphanizomenon flos-aquae* as a food supplement (Carmichael *et al*., 2000) is wrought with the fear that certain species of *Aphanizomenon* produce toxins. It has been reported that colony forming species of *Aphanizomenon* do not produce toxins (Carmichael, 1994) and based upon 16s rRNA characteristics certain species have been misidentified (Li *et al*., 2003). However, reports of cylindrospermopsin producing species of *Aphanizomenon flos-aquae* have been reported from Germany (Preussela *et al*., 2006). Thus Food and Drug Administration Agencies as well as commercial enterprises that make *Aphanizomenon* based food supplements have a major responsibility to ensure that their products originate from non toxigenic species.

However, some of the claims of high nutritive value of these cyanobacterial products have been questioned. (Watanabe *et al*., 1999) who reported that *Spirulina* contains a 'psuedo-Vitamin B12' stated that it cannot replace the proper Vitamin B12 requirement, particularly in complete vegetarian diets. The general consensus of opinion appears to be that cyanobacterial based food supplements could provide a high level of nutrition particularly to poor sectors of the population who are malnourished, but they should not be considered as a replacement diet for animal protein sources such as fish, eggs, meat and milk.

Recently *Spirulina* has gained prominence for its therapeutic value against radiation, particularly after the disasters caused by the Chernobyl accident in Belarus, Russia and the tsunami damage to the Fukushima nuclear power plant in Japan. Citing several scientific papers published in the Journal of Applied Phycology Dr. Amha Belay, Vice President of Earthrise Farms in Irvine, California (a large scale *Spirulina* producing commercial venture) has posted review articles on the therapeutic value of this cyanobacterium particularly against effects of radiation (Belay, 2011).

The Institute of Fundamental Studies, Sri Lanka, has commenced a project in collaboration with the Mahatma Gandhi Center, Colombo, to introduce mass culture of *Spirulina* to villages in Sri Lanka as a low cost commercial activity to help alleviate poverty and reduce rural malnutrition Fig. 4 (a), (b) and (c).

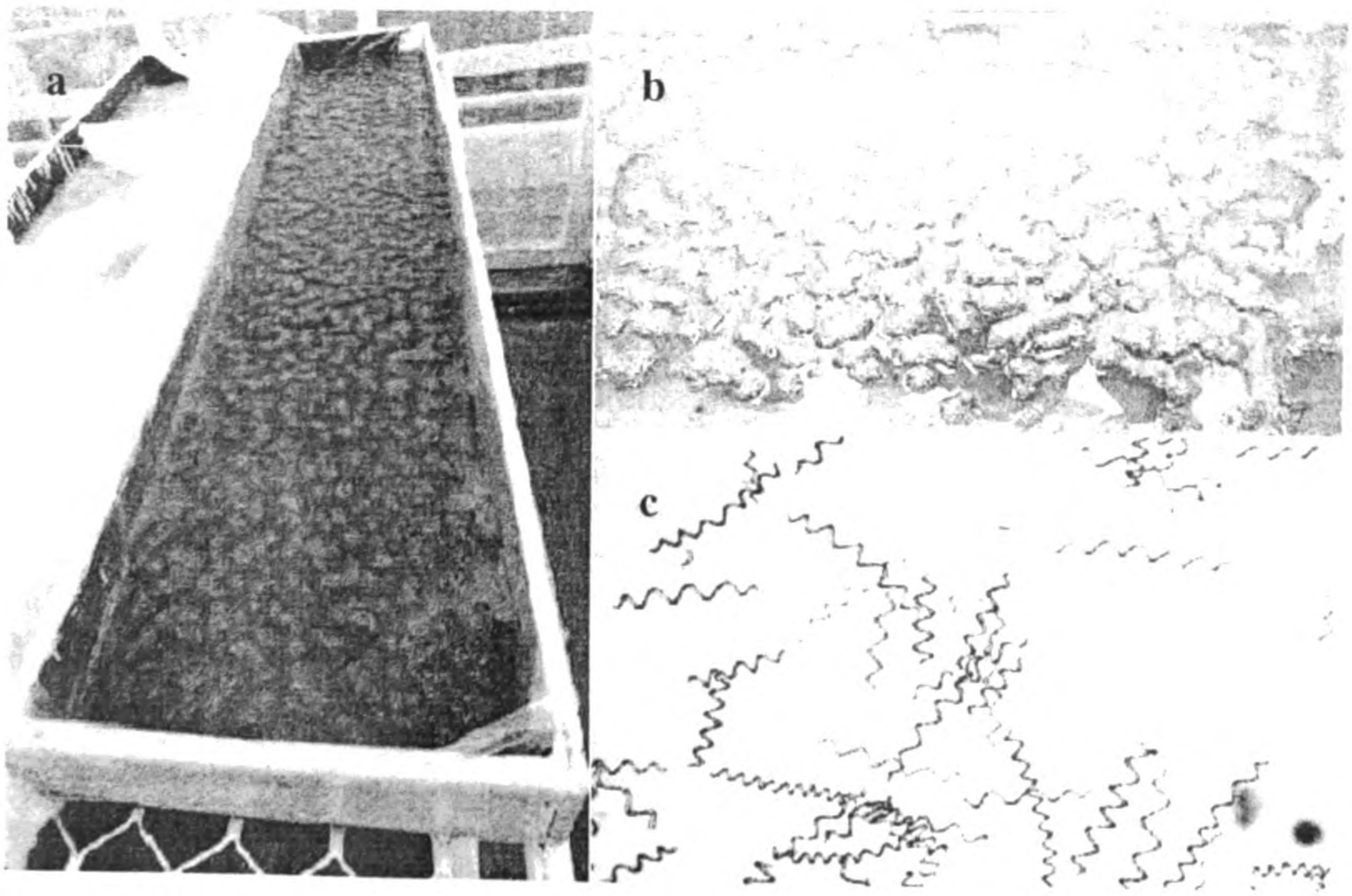


Figure 4. *Spirulina* cultures at the Institute of Fundamental Studies, Sri Lanka. **(a)** Open tray culture, **(b)** Close up of surface and **(c)** Microscopic view (x100).

CONCLUSION

From the foregoing it is evident that although cyanobacteria are microscopic, prokaryotic organisms they are ubiquitous in their distribution and play crucial roles in the sustenance of many ecosystems. Their contributions to the global carbon and nitrogen bio-geochemical cycles are significant. These organisms also possess features that are useful as well as harmful to other living organisms including humans. Some of them appear to be good candidates for biofertilizer and biofuel industries and a few species are gaining recognition as highly nutritive food supplements as well therapeutic agents. Having knowledge about these little known microorganisms is therefore important and useful. If Planet Earth ever faced a global catastrophe resulting in colossal destruction of life, it is most likely that a few cyanobacterial species would survive in some extreme ecological niches and perhaps play a pioneering role in re-establishing life forms in a virtually barren Earth.

REFERENCES

Abed, Raed (2010). Oil bioremediation by cyanobacterial consortia under extreme environmental conditions. Oman Daily Sun (December).

Azolla Utilization (1987). *Proceedings of the Workshop on Azolla Use, Fuzhou, Fujian, China 1985*. The International Rice Research Institute, Los Banos, Philippines. Pp. 296.

Belay Amha (2011) The potential role of *Spirulina* in protection against radiation and its effects. http://www.iherb.com/i/info/pic/Spirulina_RadiationA.pdf and http://www.earthrise.com/pdf/earthrisepdf.

Berman-Frank, I., Lundgren, P. and Fallwoski, P. (2003). Nitrogen fixation and photosynthetic oxygen evolution in Cyanobacteria (Mini review) *Research in Microbiology* **154**: 157 – 164.

Capone, D. G., Zehr, J. P., Paerl, H. W., Bergman, B. and Carpenter, E. J. (1997). *Trichodesmium*, a globally significant marine cyanobacterium. *Science* **276** (May): 1221-1229.

Carmichael, W. W. (1994). The toxins of Cyanobacteria. *Scientific American* **270** (1): 78-86.

Carmichael, W. W., Li., R. And Pereira, P. (2000). Harvesting of *Aphanizomenon flos-aquae* Ralfs ex Born. & Flah.var. *flos-aquae* (cyanobacteria) from Klamath Lake for human dietary use. *J. Appl. Phycol.* **12**: 585-595.

Carmichael, W. W., Azevedo, S. M., An, J. S., Molica, R. J., Jochimsen, E. M., Lau, S., Rinehart, K. L., Shaw, G. R. and Eaglesham, G. K. (2001). Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins. *Environ. Health Perspective* **109** (7): 663 – 668.

Carpenter, E. J. and Romans, K. (1991). Major role of the cyanobacterium *Trichodesmium* in nutrient cycling in the North Atlantic Ocean. *Science* **254** (Nov): 1356 – 1358.

Cohen, Yehuda (2002). Bioremediation of oil by marine microbial mats. *International Microbiology* **5**: 189 – 193.

Desikachary, T. V. (1959). Cyanophyta. Indian Council for Agricultural Research, New Delhi: 686p.

Drouet, F. (1968). Revision of the classification of the Oscillatoriaceae. *Acad. Nat. Sci. Philadelphia, Monograph* **15**: p. 370.

Drouet, F. (1973). *Revision of the Nostocaceae with cylindrical trichomes*. Hafner Press, New York – London: 292p.

Drouet, F. (1977). Revision of the Nostocaceae with constricted trichomes. (*beihefte Zur Nova Hedwigia, Heft* **57**), Publisher: Bisen Singh Mahendra Pal Singh (ISBN 812110503X and 9788211033): 258p.

Drouet, F and Daily, W. A. (1956). Revision of the coccoid Myxophyceae. *Butler Univ. Bot. Stud.* **12**: 1218p.

Dubey, Sanjay Kumar, Dubey, Jaishree, Mehra, Sandeep, Tiwari, Pradeep and Bishwas A. J. (2011). Potential use of cyanobacterial species in bioremediation of industrial effluents. *African Jour. Biotechnol.* **10** (7): 1125 – 1132.

Fay, P. and Walsby, A. E. (1966). Metabolic activities of isolated heterocysts of the blue-green alga *Anabaena cylindrica*. *Nature* **209**: 810 – 812.

Fay, P. and Kulasooriya, S. A. (1972). Tetrazolium reduction and nitrogenase activity in heterocystous blue-green algae. *Arch. Mikrobiol.* **87**: 341 – 352.

Fogg, G. E., Stewart, W. D. P., Fay, P. and Walsby, A. E. (1973). *The Blue-Green Algae*, Academic Press, London & New York: 459 Pp.

Friedmann, Imre E. (1982). Endolithic microorganisms in the Antarctic cold desert. *Science* **215** (4536): 1045 – 1053.

Friedmann, Imre E., Hua, Maosen and Friedmann, Roseli Ocampo (1988). Cryptoendolithic lichen and cyanobacteria communities of the Ross Desert, Antarctica. *Polarforschung* **58** (2/3): 251 – 259.

TEST REPORTS

8 January 2014

Ms Priyanwada Warakagoda
National Science Foundation

NSF Grant: TG/2012/Tech-D/09

TEST REPORT ON SAMPLES OF SPIRULINA CONDUCTED BY THE INSTITUTE OF FUNDAMENTAL STUDIES IN ASSOCIATION WITH THE UNIVERSITY OF PERADENIYA

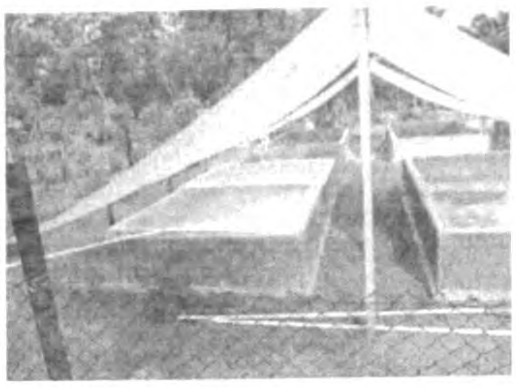
Project title: "To grow the alga *Spirulina* to overcome malnutrition of children and pregnant & lactating mothers in villages of Sri Lanka

Progress Report

Land clearance, preparation leveling, establishment of bunds and drains and construction of 20 tanks (15m x 10m made of brick and mortar and layered with cement concrete) commenced end of March 2013 and was completed in mid July. It took such a long time for the completion of all the tanks due to heavy rains that interrupted construction work several times. Five tanks were filled with water on June 4th and left for consolidation for 15 days. They were cleaned and re-filled with water and kept for a week and this procedure was repeated thrice until July 2nd. Three days later 5L of water from a tank was used in a test run to culture a sample of *Spirulina* to see whether the water is now suitable for algal culturing. As the culture grew successfully, this pre-treatment was adopted to all the subsequent tanks.

Outdoor mass culturing of *Spirulina* commenced on August 19th with the inoculation of the 1st tank using a 6L culture grown in a glass container. Daily records of tank water temperature and pH were kept except on Sundays and public holidays. A number of problems were encountered with this initial outdoor culture. Very little growth was observed during the initial few weeks. Presuming that nutrients were inadequate, a load of nutrients of the low cost medium has been added through the ignorance of the field workers. This overloading of nutrients led to the virtual death of most of the algae, but they were saved by the timely intervention of the principal investigator Maj. Gen. Fernando who had given immediate instructions to replenish nutrients approximately equivalent to what is removed during the harvesting of algae. This procedure of nutrient additions has gone on, on a trial and error basis and algal growth in the tanks recovered to normal rates by 24th September. Meanwhile two other outdoor tanks have been inoculated and they were all showing satisfactory growth by end September. Seven outdoor tanks were in operation by end October and all of them were producing satisfactory harvests of *Spirulina*. Each tank is kept covered by a mosquito net type material to prevent insects, dust particles and leaf litter falling into them. In addition, large, transparent UV polythene 200 guage roofs have been constructed over all the tanks to avoid disturbance by rain. By end November eight tanks were in operation.

The Tank Farm



Tank Covered with mosquito
Netting to prevent insects falling in

While night and day temperatures of the tanks varied from 23°C to 34°C with a mean value of 30.4°C, the pH variation ranged from 9.4 to 12.1 (very occasional) with a mean of 10.4. The original pH of 9.4 increased within the 1st two days after inoculation and remained around 11.3 until the removal of a harvest and the addition of fresh medium. This high pH while stimulating the growth of *Spirulina* has prevented the growth of contaminating algae. Periodic microscopic observations are made on small samples removed from the culture tanks to make sure that cultures remain uncontaminated. By November algal growth in all the tanks has stabilized and Table 1 shows the variation in pH, temperature and fresh biomass yield of the tanks in operation during this period.

Average results of 8 tanks taken over a 30 day period.

Table1: pH, Temperature and Fresh weights recorded from eight tanks

Tank 01	max	Min	average	Total
pH	10.3	9.8	-	-
temp	33	29	-	-
bio mass	1200	175	500	10500g

Tank02	max	Min	average	Total
pH	10.5	9.8	-	-
temp	32	29	-	-
bio mass	1050	220	549	11530g

Tank03	max	Min	average	Total
pH	10.7	10.2	-	-
temp	32	30	-	-
bio mass	1290	240	567.5	13620g

Table 2: Proximate analysis and major nutrients

Proximate analysis & major nutrients	%
DM	93
Crude protein	66.6
Ether Extract	2.01
Crude Fiber	1.37
ASH	8.07
Carbon	44.4
Nitrogen	10.54
Hydrogen	6.9
Phosphorus	0.71

Table 3: Micronutrients and other elements

Minerals	Ppm
Cd	0.025
Cu	0.225
Zn	0.275
Ni	0.095
Fe	9.19
Na	547
Ca	7.65
Mg	12.7
K	157

Consumption

It should however be noted that because of the very high Protein and Iron contents *Spirulina* should not be considered as a food, but only be given in small quantities as a nutritional supplement. It is generally recommended that the maximum daily dose of *Spirulina* for a normal adult should not exceed 10g. *Spirulina* and various preparations made out of it have been commercialized world wide and had been consumed regularly in many countries for the past 40 to 50 years.

Nevertheless to check on the effectiveness of spirulina, prior to *Spirulina* mass cultured at Swayang Wattegedera is commercialized, Gen Fernando has been consuming 1 gram of Spirulina daily and has found that there are considerable health benefits. Furthermore spirulina is available in several big pharmacies in Colombo in both tablet and capsule form but at very high prices, not within the capability of the average income earner.

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Institute of Fundamental Studies

Harvesting

Harvesting had originally been done by hand. This has resulted in the grown content of Spirulina not being obtained. According to Gen. Fernando, he had several discussions with the Swiss spirulina growing authorities, and had accordingly set up a mechanized system using heavy duty aquarium motors with collection of spirulina into bags, using 400 micron filter cloth. This has resulted in the output of spirulina being considerably increased. To prevent any contamination the harvesting staff has been provided with gloves, head cover and coats to be worn at the time of harvesting.

Harvesting Spirulina

Harvested Wet Spirulina

Spirulina After Drying

Analysis of Samples

Proximate analysis of some representative samples of *Spirulina* powder (Table 2) shows that it is very high in proteins and crude fiber in addition to nitrogen and phosphorus. Similarly its micronutrient and other mineral contents (Table 3) also show that it contains almost all the essential minerals. Noteworthy among them is the unusually high content of Fe confirming its importance as an effective source to ameliorate anaemia. Its heavy metal contents fall within the recommended safe levels. (what is recommened safety level)

Tank04	max	Min	average	Total
pH	10.5	10.1	-	-
temp	32	29	-	-
bio mass	2449	140	517.3	15003g

Tank17	max	Min	average	Total
pH	10.6	10.1	-	-
temp	34	29	-	-
bio mass	950	125	557.9	15065g

Tank18	max	Min	average	Total
pH	10.6	9.9	-	-
temp	34	29	-	-
bio mass	1200	160	519.2	12461g

Tank19	max	Min	average	Total
pH	10.6	10	-	-
temp	34	28	-	-
bio mass	1060	240	634.9	15239g

Tank20	max	Min	average	Total
pH	10.6	9.9	-	-
temp	33	28	-	-
bio mass	1120	275	589.7	15334g

Total Fresh Weight	**108.75kg**

This shows that it was possible to harvest about 109Kg of fresh *Spirulina* from these 8 tanks. On the conversion rate of 0.092 Kg dry wt per Kg of fresh wt (calculated by compiling several mean values of fresh and dry wts recorded from different tanks) this harvest works out to 10.03Kg of dry *Spirulina*. On this basis it is possible to obtain 25Kg of dry *Spirulina* per month from the 20 tank farm. The target however, with the new method of harvesting introduced, is to increase the productivity to reach 100Kg of dry *Spirulina* per month.

Ms Madurangi Singapulli
National Science Foundation
Colombo7

15 August 2013

Copy

Dear Ms Madurangi,

NATIONAL SCIENCE FOUNDATION GRANT No. TG/2012-D/09

You may recall my telephone conversation given two days ago regarding the inability to dry the spirulina as specified in our work plan item no. 5. In our experiments conducted we found that drying spirulina exposed to the sun in a drying area destroyed most of the vitamin content as the temperatures went upto as much as 65ºC. We then switched over to a glass tank with the same result. I then discussed the matter with my contacts in Switzerland and on their advice decided to do the drying in a dryer and harvesting using mechanized means. Please see Annex "A" to this letter which gives the details.

As you are aware, this is a pioneering project, and modifications may have to be done to the Work Plan as we proceed in implementing a project.

The cost involved in constructing a drying bed or using glass tanks would involve an expenditure of about Rs 100,000 as indicated in the work plan - Item 5, which we planned to debit under consumables and material.

Could you please get approval for this change from your Board as it does not involve any additional expenditure or change any sub project variations.

Could you please ensure that approval is granted very early, since this is one of the main requirements to make this project a success.

Maj. Gen. W J T K Fernando
Team Leader
Spirulina Project

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