

proceedings of the  
national panel on

# ALGAE AND ITS UTILIZATION

held in april 1976

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of Chemistry Water Sci



Institute of  
PUBLIC HEALTH ENGINEERING AND RESEARCH  
University of Engineering & Technology Lahore Pakistan

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1977

## FOREWORD

The two days National Panel on Algae and its Utilization was organised by the Institute of Public Health Engineering and Research at the University of Engineering and Technology, Lahore. It was held on 13-14 April, 1976. The Scientists and Engineers from research organizations from all over the country participated. In particular the contributions from the Universities of Punjab, Sind, Karachi, Peshawar and Lyallpur, Gordon College Rawalpindi, Department of Fisheries Karachi, Nuclear Institute of Agricultural Biology, Lyallpur, Pakistan Forest Institute Peshawar should be mentioned. The success of the Panel at the national level was made possible because of the long standing need of the research workers in this field for an opportunity in making their findings known to the scientific community. This was so because the panel was first of its kind held in this country. This helped in making the occasion representative of all the sections of the research and scientific community in the country.

The Panel was inaugurated by Professor Peter C.G. Isaac who is a distinguished research worker and an engineer of international repute in the field of public health engineering from England. In his inaugural address he greatly appreciated the efforts of the Institute in bringing together persons from different disciplines on a common platform to discuss a topic which has great significance and application in many fields. The fourteen papers published in this volume represent those selected by the Panel Preparatory and Organizing Committee which places on record their appreciation for the cooperation given by the authors of the papers.

In making recommendations during the course of discussions the need for setting up of algae research centres in the country was highlighted. It was also felt that panels and discussion groups to review the progress made in the field of algae cultivation and its utilization should be held regularly. The Panel recommended that the personnel of the fisheries departments in the country may possess a background in taxonomy and physiology of algae.

A number of people have contributed towards the success of the Panel at the national level. I profited from the help rendered by Professor K.M. Yao WHO Professor of Sanitary Engineering. The members of the Preparatory and Organizing Committee have been a continued source of support and their assistance and efforts have made the Panel a reality. In particular I should mention the name of Syed Mohsin Raza Ali who rendered substantial assistance to the Committee in organising the Panel. Thanks are also due to Khadim Hussain Ziai, Waris Ali and Khurshed Ahmad. The financial assistance rendered in connection with the programme arrangements by Asbestos Cement Industries Limited and Bata Company is gratefully acknowledged.

Lahore  
8 January, 1977

DR. MOHAMMAD NAWAZ TARIQ  
Chairman  
*Preparatory and Organizing Committee*

# PROGRAMME OF THE PANEL

## INAUGURAL SESSION

Tuesday 13 April 1976

- 0900 Recitation from the Holy Quran
- 0910 Keynote Address
- 0930 Inaugural Address
- 0950 Algae as a Source of High Quality Protein
- 1000 Break for Refreshments

## TECHNICAL SESSION I

Chairman of Technical Session

Moinuddin Ahmad  
Director of Fisheries  
Government of Sind  
Karachi

- 1100 Utilization of Algae From Agricultural Aspects
- 1115 Discussion
- 1130 Algae—A Vital Link in Polluted Water Environmental
- 1145 Discussion
- 1200 Freshwater Algae as Food of Fish
- 1215 Discussion
- 1230 Algal Photosynthesis
- 1245 Discussion
- 01 0 Biochemical Investigations of Some Seaweeds of Karachi Coasts
- 0115 Discussion
- 0130 The Economic Importance of Seaweeds in Pakistan
- 0145 Discussion

## TECHNICAL SESSION II

Wednesday 14 April, 1976

Chairman of Technical Session

Dr. Shah Nawaz Arbani  
Professor  
Department of Freshwater Biology & Fisheries,  
University of Sind  
Jamshoro Hyderabad

- 0900 Role of Blue Green Algae in the Economy of Agriculture
- 0915 Discussion
- 0930 Algae as a Source of Food
- 0945 Discussion

- 1000 Mass Cultivation of Algae
- 1015 Discussion
- 1030 Algae in Sewage Oxidation Ponds
- 1045 Discussion
- 1100 Significance of Algae in Sanitary Engineering
- 1115 Discussion
- 1130 Break for Refreshments

#### TECHNICAL SESSION III

##### Chairman of Technical Session

Dr. M. A. F. Faridi,  
Professor of Botany,  
University of Peshawar,  
Peshawar

- 1200 Algae as a Source of Medicine for the Pharmaceutical Industry
- 1215 Discussion
- 1230 Laboratory Cultivation of Blue Green Algae of Saline Soils and Rice Fields
- 1245 Discussion
- 0100 Role of Blue Green Algae in Agriculture
- 0115 Discussion
- 0130 Water Blooms
- 0145 Discussion

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Courtesy: American Centre, Lahore

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- 1930 Place: Hotel Intercontinental, Lahore  
Courtesy: Asbestos Cement Industries Limited, Karachi.

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## KEYNOTE ADDRESS

DR. MOHAMMAD NAWAZ TARIQ

*Director and Professor*

*Institute of Public Health*

*Engineering and Research, Lahore*

Professor Isaac, Ladies and Gentlemen. Engineers and environmental scientists of this Institute have been engaged in research work on pilot scale oxidation ponds since 1972. Their desire to discuss the data so generated prompted this Institute to hold today's National Panel on "Algae and its Utilization". During the planning stage of the Panel we were greatly encouraged to learn that many institutions all over the country are engaged in research work on this subject. Additionally this occasion is significant as it has brought together engineers and scientists belonging to different disciplines on a platform of common interest. This we hope will foster further contacts and exchange of views between them. As far as the theme of today's Panel is concerned the area which have been the subject of investigations and interest by this Institute has been naturally confined to the engineering and related aspects of algae. They include the role of algae in wastewater treatment, water quality management for drinking purposes and its pollution potential. The major beneficial role of algae is the removal of carbon dioxide from water by photosynthesis and the production of oxygen. This is a fundamental factor contributing to the algae-bacteria symbiosis in the working of oxidation ponds which are receiving increasing attention in developing countries as a wastewater treatment device. The amount of oxygen produced by algae during active photosynthesis is many times the amount of carbon dioxide released during the night or on cloudy days when photosynthesis is inhibited or stopped. Two papers being presented on behalf of the Institute highlight the oxidation pond research in relation to algae. An area which demands attention in this respect is the amount of algal cells in the oxidation pond effluent. It is known that the major portion of the suspended solids contained in this effluent consists of algal cells. Their removal is highly desirable before the body of receiving water is reached. It was thought by us that inactivation of algal cells by storage of the effluent in dark conditions may cause their settlement and eventual removal. This is being experimented here. The results are too meagre to be reported.

Concurrent with their beneficial role the engineering problems which arise due to algae are many and varied. Many forms of plankton filamentous algae clog sand filters in water treatment plants, produce undesirable tastes and odours in drinking water and secrete oily substances that interfere with domestic use and manufacturing processes. Some algae cause water to foam during heating as well as cause metal corrosion and the clogging of screens, filters and piping. Algae also coat cooling towers and condensers making these units ineffective.

In making a synoptic reference to the engineering aspects of the subject my purpose is to point out that algae and its utilization is a highly diverse field. Many research workers belonging to different disciplines like biology, botany, agriculture, medicine and engineering have significant information to contribute to the Panel organized here today.

# ALGAE AS A SOURCE OF HIGH QUALITY PROTEIN

DR. M.I.D. CHUGHTAI

*Professor of Biochemistry*

*University of the Punjab, Lahore*

Although no precise requirements for proteins have so far been agreed upon it has been estimated that most people of the world require at least 60—70 gms of proteins per day (1 gm/kg body weight) on an average. It is also desirable that half of this quantity should be of proteins of high biological value. In Pakistan the protein intake is about 50 gms per head per day a quantity which is fairly adequate for intake but proteins of high biological value accounts for only 8 gms which is rather low as at least 15 gms is a bare minimum. Unfortunately dietary proteins cannot be stored in the body like some other nutrients. Insufficient quantity of proteins of correct composition if consumed daily lead to malnutrition, retardation in functioning of normal facilities. The effect may be permanent and substantial particularly in children. The per capita consumption of milk and its products is hardly 100 gms per day in this country. It is about one-fifth of the quantity being consumed in affluent societies. Consequently we should develop a large number of livestock farms to improve the meat and milk supply in the country. It is heartening to note that steps are being taken by the Government in this direction. However it will be long before the country can expect to have sufficient milk and meat supply particularly in view of rapid rise in population. Also marine and fresh water fisheries should be developed to the maximum possible extent to augment protein supply. However, inspite of our best efforts to increase the supply of animal production and fish we will not be able to augment the requirements of proteins of high biological value through these conventional sources. There is thus a great need for developing non-conventional protein supply for which ample resources are available e.g. oil seed cakes (including cotton seed), single cell protein, leaf protein and algae.

At the very outset it must be mentioned that although considerable work has been carried out on unicellular algae in the country, not much work has been undertaken for their production for human consumption or as animal feed.

The unicellular algae have so far received less attention than their importance warrants. Algae could be produced with a protein content of 85% under controlled conditions. The photosynthetic efficiency of algal cultures may be as high as 25% of the light energy compared to crop plants which utilize less than 1%. Consequently it could be possible to meet demands for plant proteins from a small proportion of the acreage used in traditional agricultural production.

Algae could be grown in waterlogged and saline lands which are not suitable for cultivation of conventional crops. A large quantity of algae could be harvested by cultivation in water-logged and saline areas. Some work in this connection has been carried out by us. The investigations show that ground water after the cultivation of algae contains less salt. Such water could be used by mixing it with canal water

for the cultivation of conventional crops. This has economic possibilities as sinking of tubewells no doubt is one of the means of reclamation of waterlogged and saline lands. This could be supplemented by cultivation of algae. In our investigations the protein content of algae grown in water-logged and saline lands ranged from 40 to 50% having high biological value. Additionally some studies have been undertaken. These include the influence of nitrogen source, illumination and temperature on protein content of algae. With urea as a source of nitrogen the protein content ranged from 47 to 60% in the 4 local species studied, the optimum temperature being 25 to 29°C. These studies have revealed that annual per acre production of 20 to 30 tons of algal mass (equivalent to 10 to 15 tons of protein) can be obtained as against the highest protein producers like soyabean which gives a yield not higher than 0.26 ton of protein per acre per year.

Algae is also being used for sewage purification in the form of oxidation ponds; and the effluent of which can be utilized for fish culture and crop irrigation. In this connection I should mention the work being carried out by the IPHER on pilot scale oxidation ponds. The results of this work could be helpful in dealing wastewater disposal problems of similar communities.

We may utilize marine algae for direct human consumption. We have not so far appreciated their economic importance. Some work in this connection has been carried out by the Department of Biochemistry at the University of Karachi.

To intensify our efforts in research in the field of algology it is necessary that a culture collection centre of local algal species and both fresh water and marine be maintained at a suitable research centre in the country.

# UTILIZATION OF ALGAE FROM AGRICULTURAL ASPECTS

DR. SHAH NAWAZ ARBANI

*Professor*

*Department of Fresh Water Biology*

*University of Sind*

*Jamshoro*

In spite of the fact that in many countries of Asia besides *Azotobacter* and few species of autotrophic bacteria like *Rhodospirillum rubrum*, *Chlorobium* sp., *chromatium* sp., there exists another group of micro-organisms, namely, members of Blue Green Algae which play an important role in increasing the soil fertility of rice fields. The importance of these micro-organisms in agriculture have been realised only in recent times.

The members of class Cyanophyceae or Blue Green Algae possess the capacity of fixing atmospheric nitrogen in the soil. They are abundant in the regions devoted to the cultivation of rice. The chief ecological and agricultural importance of the Blue Green Algae depends upon the ability of certain species (Possessing heterocyst) to carry out photosynthesis and nitrogen fixation. These species belonging to nitrogen fixing genera i.e., *Anabaena*, *Nostoc*, *Rivularia*, *Calothrix*, *Gleotrichia*, *Cylindrospermum*, *Aulosira*, *Scytonema* and *Tolypothrix*, serve as an excellent source for utilizing solar energy efficiently for the production of organic matter in the soil. They also contribute considerable amounts of nitrogen to the soil by the fixing atmospheric nitrogen.

On the basis of many scientific investigations it has been found that members of Blue Green Algae are capable of fixing free atmospheric nitrogen and enrich the soil specially the rice fields.

Frank (1889) was the first person to record that Blue Green Algae possess the capacity of fixing atmospheric nitrogen. This is further supported by the investigation of Fogg (1942, 1956, 1962); De (1962, 1939); Venatrkmn (1961); Relwani (1961); De and Mandal (1955); Prasad (1949); De Ali, Singh and Bhattacharya (1956); Watanabe (1959); and Taha (1963).

Howard (1924) and De (1939) have recorded that in India for years the same field is cultivated without addition of manure and it gives best crop. The same is true about Pakistan. Large rice crops are produced in many parts of Pakistan and on the same land year after year without the addition of manure. In Burma also large quantities of paddy have been exported without any reasonable supply of nitrogenous manure.

Some times it is believed that *Azotobacter* and other bacteria are responsible for fixation of nitrogen but the finding of Bose (1938) De and Datta (1952) shows that in India the soils are low in organic matter, nitrogen and phosphorus and mostly water logged and anaerobic during rainy season. These condi-

tions are unfavourable for the growth of Azotobacter for fixation of Nitrogen. Experiments conducted by De (1950, 1952) on paddy soil of Dacca and Faridpur has shown that no nitrogen fixation takes place in dark. If Azotobacter would have been responsible for this, then more nitrogen would have been fixed in dark than in light. It was also proved by their experiments that fixation of nitrogen in waterlogged soil is an algal process, In this context it appear proper to quote Thornton and Miklejohn (1957) that there is a certain irony in the fact that so much is being written about Azotobacter but one really needs to believe that whether this genus is of real practical importance in adding to the soils nitrogen supply for there are many soils in various parts of the world where it is never found, Boswell (1955), Kaila (1954); Miklejohn (1956). (1956).

Taha (1963) has reported that in 1950 that in some regions of Egypt harvest of nonfertilized soils were equal to the soils that were enriched with ammonium sulphate.

In studying the fixation of nitrogen in the rice soils the soil remains alternately dry and waterlogged which can be divided as (1)—(2)—(3)

1. Water-logged period—From transplantation upto harvest time. During this period there are few inches to several feet of water above the soil and which is distinguished by the growth of abundant Algae (Ababaena, Nostoc and species of other genera, found by us and other investigators in various parts of the world).
2. The dry period—which follows in winter after harvest and during this period the conditions remain suitable for micro biological activity.
3. The dedicating period—commencing after winter where the soil temperature reaches upto 50°C.

During the two latter stages also if the fields remain undisturbed, the Algal growth is in a perennating stage. In a dormant stage the dark Blue Algal crusts, can be easily seen which becomes active during rainy season.

The further support to this belief is given by the findings of many scientific investigations. De and Data (1952) has shown no nitrogen fixation takes place during dry period and that the conditions occurring during the said period are not suitable for Azotobacter growth. This clearly shows that Azotobacter is not responsible for nitrogen fixation. Watanabe, Nishigaki and Konishi (1957) observed a fixation corresponding to 20 pounds of nitrogen per acre of soil in pots cropped with rice plants and inoculated with *Tolypthrix tenuis* a powerful nitrogen fixing Algae.

Prasad (1949) analysed the thick algal deposits on the rice lands of south Bihar and concluded that 12.9 pounds of nitrogen per acre added to rice field after harvest and as a result of fixation by algae.

Wills & Green (1948) showed by pot experiments that the amount of nitrogen that was fixed in a soil by Blue Green Algae was enough to support a good crop of rice and leave about 70 pounds per acre in the soil after the crop was harvested.

Allen (1956) has shown that nitrogen fixed by Blue Green Algae is available to the rice plants and that it is possible to grow rice with no nitrogen other than fixed from air by algae growing together with rice plants.

Watanabe, et al (1950) has reported increased growth of rice plants both water and pot culture inoculated with nitrogen fixing algae.

De and Suleman (1950) De and Mandal (1956) have shown that nitrogen fixation was greater in the presence of crops than in its absence. They also observed that addition of phosphate to rice soils whether in soluble form as Potassium phosphate or insoluble form as Calcium phosphate stimulated the fixation of nitrogen by algae. On the other hand Potassium or Magnesium sulphate when applied singly had a depressing effect which was noticeable when the two were used in combination with Phosphate.

Banerji (1933) has described 9 species of Myxophyceae of rice fields from Faridpur. De (1939) have described 40 species of rice fields from Allahabad. Khan (1957) have described 35 species of Blue Green Algae of rice fields from Kashmir and Srinagar.

Okuda and Yamaguchi (1952) has reported 14 species of rice fields near Kyoto University Japan.

Watanabe (1959) have made a detailed survey and have shown that out of 815 samples that he collected from various districts of south and east Asia 16 species of Blue Green algae were found to possess the capacity for fixing atmospheric nitrogen. Their nitrogen fixing capacity was determined.

On course the number of nitrogen fixing species is not very large but it is hoped that future investigations will add more species to the list of the identified nitrogen fixing species.

In Pakistan rice is second food crop but it is unfortunate that the algae of the rice fields has not been worked out. Only one paper of Ali and Sanhu (1972) is available on the Blue Green Algae from saline soil of Punjab. They have identified 106 as non-nitrogen fixers and 29 species as nitrogen fixers.

As it was essential in the first place to determine the distribution and abundance of the Blue Green Algae in the soil of various rice growing regions in our country, a survey was undertaken and collections were made from rice growing areas of Thatta, Hyderabad, Badin, Dadu and Larkana districts of Sind.

A total of 58 species were identified out of which 28 were nitrogen fixers. Out of these 28 species five species i.e., two species of Anabena, two species of Nostoc and one species of cylindrospermum were isolated into pure cultures. More collections will be made this year.

Work is also in progress on classification and isolation of these nitrogen fixing species.

The survey of the remaining districts of Sind is in progress. Attempts will be made to devise economical methods for culturing of these organisms in large quantities and preservation in the form of seedlings in available state.

Our country is rich with algal species. It is very necessary that a National Algal Research Institute and Experimental station may be established to carry out investigations on Ecology, Systematics and Physiology of the nitrogen fixing Blue Green Algae of Pakistan.

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### Discussion

Questions were raised on the nitrogen fixing capacity of blue green algae, their isolation and cultivation.

The author replied that on the basis of many scientific investigations it has been found that members of blue green algae are capable of fixing free atmospheric nitrogen and enrich the soil especially the rice fields. Bacteria also fix nitrogen, he stated, but they do so only in the presence of algae. The author further stated that there are many soils where in absence of bacteria nitrogen fixation takes place. In the presence of rice crop larger amount of nitrogen is fixed than without the presence of rice crops.

To the question of isolation and cultivation of blue green algae the author replied that they had not tackled that problem so far and plan to do so in future.

# ALGAE A VITAL LINK IN POLLUTED WATER ENVIRONMENT

DR. K. M. YAO  
*WHO Professor of Sanitary Engineering,  
Institute of Public Health Engineering  
and Research Lahore.*

## Introduction

Pollution in the sense of modern technology, can be defined as the degradation of the environment due to man's activities. Pollution control is therefore the prevention of the degradation of the environment by man's activities. This latter seemingly harmless definition actually has numerous pitfalls some with serious consequences. For instance, the US government has adopted a water pollution control target of zero-discharge. After the target date set by law no pollutant will be allowed to discharge into natural waters so as to ensure that no degradation occurs to the natural waters. For point sources such as municipal wastewater treatment plants, this is technically attainable, admittedly at very high costs. For nonpoint sources such as agricultural drainage and urban stormwater runoffs the matter becomes quite different and an entirely new approach may have to be developed. The crucial question is how far we really have to go.

Since man is a component of the overall ecosystem of the environment, pollution is in a way inevitable. This note will attempt to undertake a brief examination of the working of the natural processes including that of pollution in the water environment as the basis for general review of the present practice in water pollution control.

## Food Cycle in Polluted Water Environment

Fig. 1 presents the food cycle in a polluted water environment (1). The organic pollutants in sewage are broken down by bacterial action in a number of steps with inorganic compounds such as nitrates as the end products. These end products are then utilized by photosynthesis to form algal cells which provide the major input for food chain leading to food fish for human consumption. Table I indicates the effects of each stage in the cycle on three key environmental parameters oxygen, carbon dioxide and energy. It is generally accepted that depletion of oxygen, production of carbon dioxide or consumption of energy is either a direct factor or a significant cause of degradation of the environment. It is interesting to note that the algal stage is the only stage in the cycle to counterbalance the adverse effects of all other stages on these key parameters. Because of the existence of the algal stage, nature achieves its own delicate design in perfection.

## Modern Wastewater Treatment Technology

The main concept of modern wastewater treatment technology is to "remove" pollutants from the water environment. The treatment processes operate roughly in stages 2, 3 and 4 of Fig. 1. Instead



of going to stage 5 for the completion of the cycle, pollutants are simply removed from further involvement. The problem is therefore transformed from polluted water to solid waste without being actually solved.

Attempts are now being made to encourage the use of land wastewater treatment. This would mean the replacement of the algal stage with a photosynthetic stage of plants and crops. It is however doubtful whether the specific yield in energy and other natural resources of the latter will be comparable to that of algae. An ideal approach appears to be the design of a balanced treatment system incorporating stages 2 to 5 or even 8. Such a treatment system will be much more advanced than the oxidation pond currently in use since the latter is still aimed at the removal of pollutants instead of fulfilling the function of completing the cycle shown in Fig. 1.

### Summary and Conclusions

Algae provides a vital link in the food cycle of a polluted water environment. The natural process of recycling nutrients may lead to a better solution to the increasingly serious problems of pollution and resources depletion.

### Acknowledgement

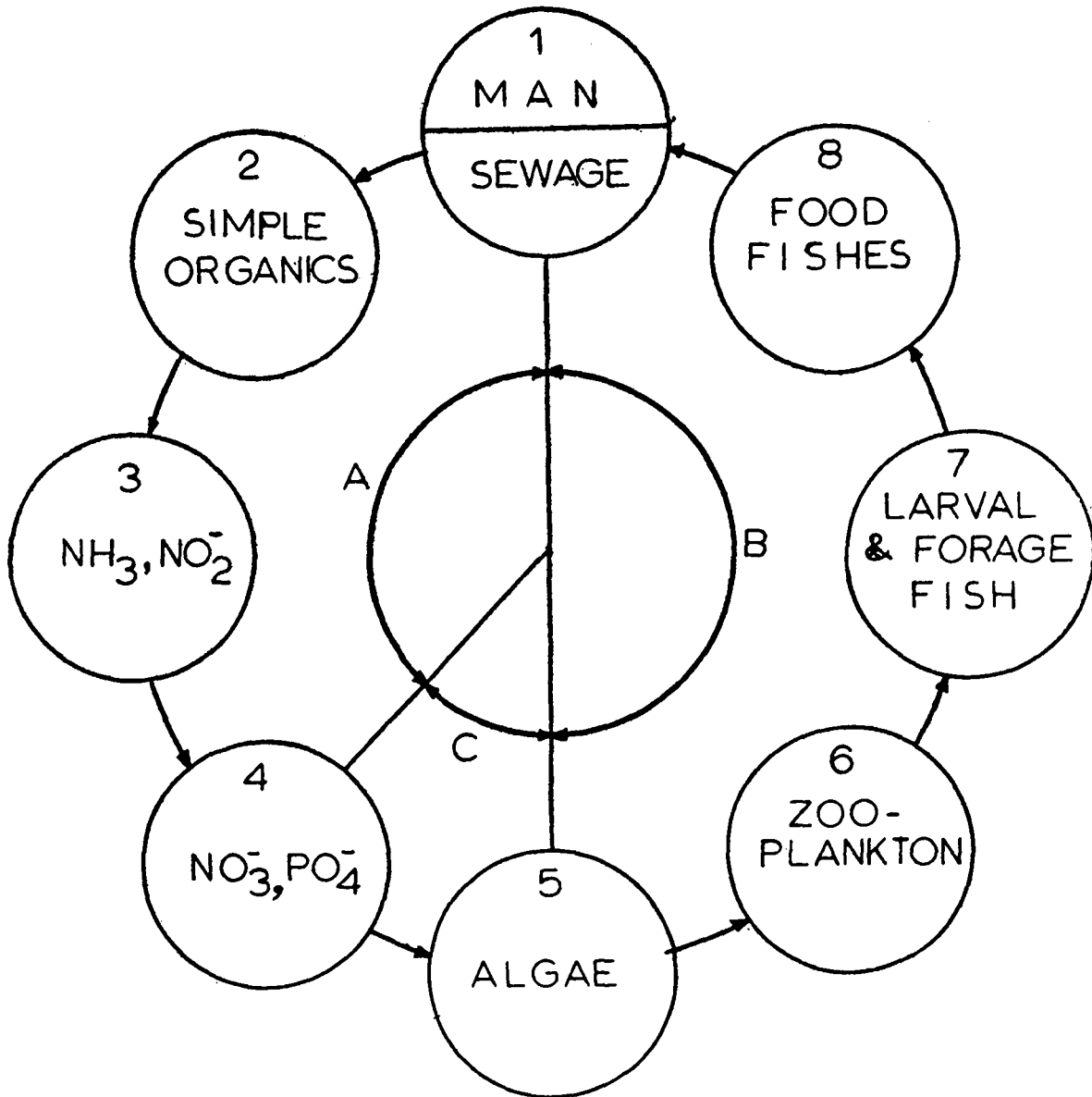
The opinions expressed in the note do not represent the official views of the World Health Organization.

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**TABLE I. Effects of Various Stages on Key Environmental Parameters**

Stage	O <sub>2</sub>	CO <sub>2</sub>	Energy
1	Consumed	Produced	Expended
2	Consumed	Produced	Expended
3	Consumed	Produced	Expended
4	Consumed	..	Expended
5	Produced	Consumed	Gained
6	Consumed	Produced	Expended
7	Consumed	Produced	Expended
8	Consumed	Produced	Expended



A=Bacterial Decomposition  
 B=Biological Activity  
 C=Photosynthesis

FIG. 1. FOOD CYCLE IN POLLUTED WATER ENVIRONMENT

# FRESH WATER ALGAE AS FOOD OF FISH

S. RASHID ALI

Zoology Department,  
Gordon College, Rawalpindi

## Abstract

The aim of this study was to find out the food value of algae for fish. For this purpose the gut contents of 32 different species of fish were studied. It was found that 15 species live on algae and debris, 14 species on algae and aquatic animals and 3 species on animals only. The length of fish investigated varied between 1½ cm. and 14 cm.

## Introduction

This country is facing a shortage of protein yielding animals such as sheep, goats, cattles, poultry and fish. Consequently the prices of mutton, beef, fish and poultry products are very high making them almost beyond the reach of common man. Dr. M. Jameel Qureshi Dean Faculty of Livestock University of Agriculture, Lyallpur in his presidential address to the Agriculture Section of the 16th Annual Science Conference stated that the use of meat and beef as food by the people of Pakistan on an average is only half an ounce per head per day while in Australia it is 10 ounces per head per day. The meagre use of meat and beef and poultry is due to their shortage and high prices. In my opinion we should exploit each and every possible source to increase the production of protein yielding animals. Farming practices of sheep, cattles and poultry are difficult and expensive as compared to fish, prawn and crab culture.

Before starting fish, prawn and crab culture the knowledge of aquatic organisms of water bodies is essential which form the food of these animals. Since 1964 I have been working on the productivity of bottom fauna in relation to the food of fish in different parts of Pakistan. Later on I also studied the presence of algae in the gut-contents of fish.

According to Prescott (1951) algae are said to be "The Pasture of Sea" and it is agreed that algae occupy a basic position in the food cycle of both freshwater and salt water animals. Phytoplanktons are important in the food chain. It is a well-known fact that a luxuriant flora maintains a correspondingly dense population of animals. This relationship was illustrated many times in the lakes of Michigan and Wisconsin. Some of these lakes are highly productive and are excellent for game fish. Algae are directly or indirectly used by fish. Fish either live on algae or algae and animals or on animals which feed on algae. Algae are primary producers and insects their youngs and other animals are secondary producers.

## Material and Methods

The collection of fish was done with the help of drag and cast nets. Small fish were placed directly in formaline while bigger fish were injected with formaline to stop the digestive action in the gut. In the

laboratory fish were dissected for the gut. Gut-contents were studied under dissecting binocular and monocular microscopes.

Results show that 15 species of fish live on algae and debris, 14 on algae and animals and 3 exclusively on aquatic animals. Names of the food organisms are given in Table I.

### Discussion

Out of 32 species of fish investigated, 22 are small and are of little economic importance. Swingle and Smith at Auburn University in a balanced pond maintained a ratio of 1 to 4 lbs, i. e., one lb of piscivorous to 4 lbs of supporting species. Minor Clark in studies of streams of Kentucky found a ratio of approximately 1 to 3. Meehan found in Florida lakes that the ratio of piscivorous to supporting fish was 1 to 2.5. Smaller species of fish can be used as food for piscivorous fish. Among the fish we studied *Callichrous bimaculatus*, *Channa gachua* are piscivorous. It is just possible that some cat fishes like *Rita rita* which is piscivorous fish can be cultured along smaller species. This type study should be done for other species of fish. As far as I know no work has been done on the food of fish and fingerlings of fish, prawns and crabs in Pakistan.

**TABLE FOOD OF FISH**

Serial No.	Scientific Name	Number of Fish dissected	Gut-Contents	
			Algae	Animals
1	2	3	4	5
1.	<i>Chela bacalala</i> (Ham.) (Chal)	5	Navicula & Cocconeis	
2.	<i>Cheia Cachus</i> (Ham.) (Chal)	5	Cosmarium, Plesotaenium, Epithemia, Surirella, Navicula & Pinnularia.	Chironomus larvae
3.	<i>Danio devario</i> (Ham.)	5	Anoneis, Cocconeis, Tabellaria, Cymbella and Gyrosigma	Larvae of Chironomus & Dixia, Corixa
4.	<i>Barilius vagra</i> (Ham.) (Lohari)	10	Copmarium, Microspora, Mougeotia, Synedra, Fragilaria, Nitzschia and Diatoma	Nymphs of mayflies, larvae of Caddisflies & Chironomus, nymphs of Dragon and Damselflies, larvae of Ceratopogomid & Notonecta
5.	<i>Garra gotyla</i> (Gray)	5	Microcystis, Diatoma and Euglena	
6.	<i>Garra modestus</i> (Gray)	5	Amphora, Navicula, Oscillatoria, Fragilaria, Closterium Sigmonde, Diatoma	

1	2	3	4	5
7.	Labeo rohita (Ham.)	5	Diatoma, Navicula, Oscillatoria, Fragilaria, Epithemia and Geminnolla	
8.	Cirrhina mrigala (Ham.)	5	Cymbella, Navicula, Cocconeis, Tabellaria, Synedra, Cosmarium, Spirulina and Chroococcus	
9.	Cirrhine reba (Ham.)	5	Aphanocapsa, Cyanarcus, Oscillatoria, Epithemia, Cymbella, Navicula, Pinnularia and deuris.	
10.	Catla catla (Ham.)	5	Pinnularia, Synedra, Navicula, Fragilaria and Cosmarium	Chironomus larvae and pupae
11.	Puntius sophore (Ham.) (Chiddu)	10	Plourosigma, Mastogolia, Navicula, Tabellaria, Diatoma, Gyrcsigma, Gomphonema and debris	Chironomus larvae
12.	Puntius ticto (Ham.) (Chiddu)	5	Mougeotia, Ulothrix, Closterium, Navicula, Fragilaria and debris	Chironomus larvae, Corixa, Dragonfly nymphs, Caddisfly larvae
13.	Tor putitora (Ham.) (Mahser)	10	Ulothris, Spirogyra, Navicula, Microcystis, Tabellaria, Epithemia Phacus, Synedra, Euglana and Fragilaria.	Planarians, Oligochaetes, Cyclops, Daphnia, Mites Spiders and snail, Chironomus larvae
14.	Aspidoparia morar (Ham.)	5	Microcystis, Fragilaris, Navicula, Synedra & Diatoma	
15.	Nemacheilus botis (Ham.)	10	Fragilaria, Navicula, Cosmarium, and Tabellaria	Chironomus and Ceratopogonia larvae.
16.	Ostoobrama coto (Ham.)		Debris	
17.	Nemachoilus recpicola (Ham.)	5	Synedra, Navicula, Anoneis and Cocconeis	
18.	Wallago attu (Bl. & Schn.)	5	Synedra, Fragilaria and Cocconeis	
19.	Callichrous bimaculatus (Bp.)	5	Fragilaria, Gomphonema, Cocconeis and Cosmarium	
20.	Clupisoma artherionides (Ham) (Ahhel)	5	Glaucozystis, Oscillatoria and Debris	Chironomus larvae

1	2	3	4	5
21	Glyptothorax cavia	5		Bugs & Chironomus larvae
22.	Clyptothorax neziri (Mirza)	5		Trichoptera Chironomus larvae and Corixa
23.	Mystus sp. cavasius (Ham.)			Chironomus larvae abundant
24.	Mystus vitatus		Nitzschia and Cocconeis	Chironomus larvae abundant.
25.	Mystus bleekeri (Day)		Oscillatoria, Stauroneis, Fragilaria and Tabellaria	Chironomus larvae
26.	Channa gachua		Diatoma Cypridae Corixa, Navicula, and Microcystis	
27.	Channa punctatus (Bp.)		Phorimidium, Stephodiscus, Acanthidium, Gomphonema, Navicula, Cymbella, Navicula, Pinnularia and Corixa	Chironomus and Trichoptera larvae and planorbis
28.	Ambassis name (Ham.)		Euglena, Microsystis, Closterium and debris	
29.	Ambassis ranga (Ham.)		Pinnularia, Microcystis, Closterium Pleorococcus and debris	
30.	Colisa fasciata (Bl. & Schn.)		Euglena, Microcystis, Closterium, Fragilaria, Synedra, Amphora, Sphaerella and debris	
31.	Macrognaathus Actulea tum (Bloch.) (Gud.)		Navicula, Gomphonema, Pinnularia Tabellaria, Phanocapse, Sygnema, Synedra and Oscillatoria	Chironomus larvae
32.	Mastacembelus armatus (Lacp)		Microcystis, Fragilaria, Navicula, Synedra	Mayfly nymphs and Bugs

## Discussion

Questions were raised on the species of fish which feed only on animals. To this the author replied that he had identified eight such species of fish which feed only on animals. Regarding blue green algae used as food by fish the author replied that microcystic species has been found in the bodies of many fishes. To another question on fish production the author cited the example of a stream near Rawalpindi. The author was of the view that the productivity of the bottom fauna had decreased due to the wastewater which is coming from a nearby industry.

# ALGAL PHOTOSYNTHESIS

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The conservation of resources namely food, water, oxygen and energy is the keynote of the recent awareness regarding the preservation and protection of human environment. With the fantastic rate of urbanization, population rise and technological advancement all the world over the apprehension is fast deepening that some day we may run out of our basic resources. The scientists and planners are, therefore, already seized of the consciousness of averting that catastrophic eventuality. Scientific effort is now very much oriented towards the maximum conservation of basic resources and exploration of the most economic and fruitful methods of exploitation of solar energy to recoupe the fast dwindling reserves of fossil fuels and conventional agricultural products.

The potentialities of Algal Aquaculture based on sewage and waste effluent substrates have of late received marked recognition in the framework of design and planning of modern cities. The problem of domestic sewage and industrial waste effluents disposal is assuming menacing proportions with the emergence of sprawling cities and heavily populated townships. Algal culture offers itself as a dual purpose solution of the problem in as much as it ensures environmental sanitation by bringing about sewage purification through aerobic breakdown of organic matter oxygen production, regeneration of water and also production of proteinous material which could be used as an animal feed. The marketing of algal proteinaceous material would partly if not completely defray the cost incurred on the massive sewage treatment operations. Very extensive studies have been carried out by several workers (1-7) on the above lines. Golueke and Oswald (8) have come out with a very novel design of a model residential unit with a close circuit system of resources conservation. The underlying principle of this living unit is the attainment of maximum degree of self sustenance and complete harmony with its external environment and energy sources. The system would be sustained by solar energy which is so abundantly available round the year in tropical countries. The proposed cyclic system is based upon the photosynthetic activity of algae which would bring about the reclamation of the waste products of the human beings and animals living in the settlement. The major components of the system are anaerobic digester, algal growth chambers, a sedimentation chamber and a solar energy still.

The proposed residential unit is designed for accommodating a single family of four persons, a cow and fifty chickens. This miniature algal regenerative system could however be extrapolated to fit larger groups and communities. The practical feasibility of the various components has been demonstrated.

The anaerobic digester is meant for the fermentative breakdown of the waste products of the population unit. It is fitted with an inverted dome type cover for the storage of methane gas produced.

This gas, under slight pressure is used as fuel and energy source in the dwelling unit. Carbon dioxide formed by the combustion of methane is transferred to the algal culture unit where it is used as a carbon source.

Photosynthetic algal production unit which is a shallow tank situated on the roof top, is designed on the basis of the waste nitrogen available from the contributing population. In order to ensure greater safety this tank could be covered by means of a transparent plastic sheet. The situation of the algal culture pond on the roof top serves as a buffer and cools the house during the summer. Under suitable conditions of temperature and illumination the daily yield of this reactor would be 1-2.5 kg. dry weight of algal proteins.

A very important biochemical parameter of photosynthetic reactor is the nitrate stripping, and nitrogen to protein conversion efficiency of algae. This parameter which is a function of the different environmental conditions has been studied in detail by us in our studies on the biochemistry of indigenous strains of algae (9). The nitrate to protein conversion efficiencies of the experimental algal strains were found to be 37.2 to 53.0 under an illumination of 10,000 lux. The algal strains were found to possess fairly high nitrate removal efficiencies which ranged from 75.5 to 82.0.

Another vital criterion regarding the suitability of the algal species is their light energy conversion efficiency. In our studies (11) on *Chlorella*, *Scenedesmus* and *Chlamydomonas* the values of light energy conversion efficiencies were found to be in the range of 0.0322 to 0.144 in case of indoor cultures. In the outdoor experiments the corresponding values were found to vary from 0.0175 to 0.0227.

The recent upsurge in the research on algal photosynthesis is mainly due to its great potential as a life support agent in space. The space travels being on the increase, the problem of human dwellings in space with a self sustaining system containing an adequate provision of oxygen, energy, food and water has attracted much attraction of the scientific workers and space research agencies. As a result of a long series of investigations being conducted under the space research organisation of U.S. and the Soviet Union, a very brilliant system, namely, photosynthetic gas exchanger has been evolved. A very comprehensive state of the art review relating to the above studies has been published by Miller *et al* (10). The algal photosynthetic life support system which is designed for prolonged space travels, space laboratories and permanent extraterrestrial bases would carry out food production, oxygen regeneration and carbon dioxide absorption in addition to serving as integrated close-circuit device for organic waste treatment and water reclamation. The well known algal species "*Chlorella*" has been tested for its photosynthetic activity in space and has been found to withstand the environmental conditions of outer space. The above mentioned studies are now in a fairly advanced stage and although the photosynthetic regenerative system is handicapped by a number of biotechnical problems, this remains the most feasible method of ultimate human survival on this planet and in outer space stations.

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# BIOCHEMICAL INVESTIGATIONS ON SOME SEAWEEDS OF KARACHI COAST

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## Abstract

After a thorough survey of the Karachi coast eighteen species were chosen for biochemical and chemical investigations. The samples belong to the three classes of algae: chlorophyceae, pheophyceae and rhodophyceae. Total protein, carbohydrate, fat, crude fibre, ash and moisture contents were determined in these samples. Fat and fatty acids were extracted, separated and estimated using combined technique of solvent extraction, thin layer chromatography, gas liquid chromatography and spectrophotometry.

The sugar composition of the samples were investigated after acid hydrolysis and paper chromatography. The ash obtained from the sample were analysed for sodium, potassium, calcium, magnesium chloride, phosphorus and iron, from the soluble part of ash, and from the insoluble part, the quantity of sand and silicon were determined.

The proteins were extracted, employing various methods and using various extractive medium. These proteins were subjected to polyacrylamide gelelectrophoresis in disc system, S.D.S. system, as well as urea system. The proteins were hydrolysed in 6N HCl for 18 hours and the free amino acids separated and estimated on paper and thin layer chromatography and finally on Beckman C20 amino acid analyzer.

The calorific value of the powdered weed were also determined with the help of bomb calorimeter. The theoretical calculations were also made on the basis of the chemical composition of the sample.

The results of all these parameters were discussed with respect to availability of various components in different species of algae and their probable utility in terms of human and animal feed.

# ECONOMIC IMPORTANCE OF SEAWEEDS IN PAKISTAN

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## Introduction

About two-third of the earth's surface is occupied by oceans and the seas. Their resources are varied and vast and partly comprise of fish, shell fish, other animals, vegetations and the weeds. As compared to terrestrial resources they are under-exploited as yet.

In 1975 the world population was four billion and is increasing at an average rate of 3.0 percent per year. It may exceed 7.0 billion by the end of this century. To produce sufficient food and provide balanced diet for this population is the most important global socio-economic problem today as the food production is not keeping pace with the constant increase in demand.

Similarly in 1975 the population of Pakistan was over 65 million and may exceed 130 million by the year 2000. It has been having a shortfall of over 1.5 million tons of foodgrains per year Malnutrition and under nutrition is common in majority of population. It is one of the most important problems of this country and the Government is making every possible effort to achieve self sufficiency in it and fulfil other requirements of the people.

Experts have forewarned that the land protein resources of animal origin will be virtually exhausted in next 25-30 years and there will be substantial shortfall of conventional food items in the world. This gap can be filled up only by increasing the intake of conventional and non-conventional items of sea food in the daily diet of people and also producing other marine products of utility. The weeds as compared to other aquatic resources much under-exploited in many parts of the world. In Pakistan as yet little has been done.

The weeds being the subject of our today's discussion are the macroscopic marine plants which grow in the littoral zone from high tide mark upto 30 meters depth. They are represented by Thallophytes in the plant kingdom and on the basis of pigment combinations are divided into the following four classes: (i) Myxophyceae—Blue green algae (ii) Chlorophyceae—Green algae (iii) Pheophyceae—Brown algae (iv) Rhodophyceae—Red algae.

A number of plants of the above groups grow in varied abundance in most of the seas depending on fertility, climatic and physiographic condition.

The green algae, as compared to the plants of other groups, are comparatively of little economic value, although some are used as food. The brown and all the red algae are of considerable economic importance largely because of the properties of complex colloidal carbohydrates which occur in their cells. The red algae are most varied and abundant in the tropical zones in which our country is located. The blue green algae are cosmopolitan but they do not include large conspicuous forms. In all the four groups about 40 species mostly brown and red algae are of economic importance.

They play an important role in synthesizing the food in the sea. They also provide protection to smaller fish and other animals in the sea against large carnivorous animals. The snails, many other molluscs, crabs and other crustaceans live upon some parts of the sea weeds.

The human utilization of the weeds started much earlier in the Eastern than in the Western countries. China has been the pioneer country in this respect but the preparation of Agar—Agar had its origin in Japan. It became the first stable weeds product of commerce and is now produced in several countries in large quantity. In the initial stage it gained importance as a "food novelty" and subsequently in the fields of bacteriology and microbiology as a solidifying agent for culture media. Its production was monopolized by Japan from early 18th Century upto 1940. During the second world war its extraction plants were also established in U.S.A. and several European countries. Now a number of other polysaccharides are also being extracted in different countries on commercial scale.

### **Algae As Food**

In natural form the weeds are a poor source of food for man but they are used as "Quality food" and "reclpes". They consist 17 to 60% carbohydrates in the dry weight and also some fats. Their value lies in vitamins and the mineral contents. In some weeds the vitamin C equals to lemon and vitamin B to vegetables and fruits. They also contain the vitamins E, and K in different percentage. In Pakistan there is general shortage of quality food and a wide gap exists in the supply of balanced diet. Their additional supply can play an important role in bridging this gap. They grow in commercial abundance all over 550 miles long coast of the country and cover an approximate area of more than 10,00,000 acres. Like China and Japan they can also be cultivated and produced in much larger quantity in the deltaic zone of Indus river and the bays and backwaters of Baluchistan Coast.

Due to high contents of mineral salts the "Sea Weeds Meal" cannot be used as the only food material for animals. On removing the salts by boiling and other techniques they can be used as a good food supplement like the fish meal for livestock and the poultry. In Norway, Scotland and Ireland the Cattle and the goats graze on seaweeds during low tide. On Baluchistan Coast there is serious shortage of livestock fodder and for poultry feeds some of the weeds can be cultivated to effectively fill the gap. For this purpose possibilities need to be explored to grow *Fucus*, *Rhodymenia*, *Alaria* and other species of *Rhodophyceae*. There is also a need to prepare agar on a large scale to cater for needs in the country. It has been established that the *Laminaria* species contain vitamin B<sub>2</sub>, the *Porphyra*, *Alaria* and *Ulva* species contain vitamins B and C. Almost all the species of red and brown weeds contain sufficient quantity of iodine which can be obtained for varied purposes. They also contain Bromine, Calcium, Magnesium, and the Potassium salts in considerable quantity which can be extracted economically.

The kelp meal and salts are produced commercially in U.S.A. in the form of tablets as nutritional supplements. About 15 varieties of weeds are also eaten in various parts of the world. They are served as soups, sauces, and vegetables. The Japanese *Gracilaria* is used for garnishment, *Laurencia* as hot condiment and *Mesogloia* species as salad.

The *Porphyra tenera* and other species are being farmed over 50,000 acres in different prefectures of Japan and employ more than 5,00,000 persons and there it has become a basis of thriving industry. For example by cultivating this plant in Tokyo Bay alone the fishermen earned over 17.0 million dollars in 1961.

### **Algae As Source of Abstracts**

It has been explained above that the weeds are very important source of agar and its uses are numerous. As an item of food it is used as stabilizer and thickner and an emulsifying agent in the soups. The Carrageenin, a gelatinous abstract, is obtained from the Irish Moss and some species of Gigartina. Similar substances are obtained from other weeds.

Algin is produced extensively from the sea weeds as it occurs in abundance in the form of their cell wall constituents. It is a product of wartime interest for preparing non-flamable camouflage materials. The alginate can be used as laundry starch as it provides more elasticity and toughness to clothes. The alginates are also used for manufacturing oil drilling muds, cartridge primers in certain liquid soaps and shampoos, shoe and car polishes, leather finishes and preparing many other compounds.

They can also serve as stabilizers for many products like ice cream, sherbets, chocolates, milk, cheeses, desserts, puddings, icings, etc.

### **Algae As Source of Industrial Chemicals**

Bromine is fairly wide spread in several species which is extracted in sizeable quantity. They are also used for preparing Acetic acid and acetone for fermentation process. Potash and Iodine can also be obtained as byproducts.

### **Algae As Fertilizers**

Being an important source of potassium compounds several varieties of raw sea weeds have been used as fertilizers in many countries like Japan, China, U.K. Canada, Norway and other countries with coast live. In fertility the sea weeds are comparable to the barnyard manure having, in the dry matter 3 to 17% potassium, 1 to 7% Nitrogen, about 1% Phosphorous (P<sub>2</sub>O<sub>5</sub>) and other trace nutrients. Now they are used for preparing fertilizers in some countries.

In Canada the red algae are used for preparing glue. Its chief sources are Cloiopeltis furcata, Gigartina and few other species. They can also yield carbohydrates, used as stuffing and insulating material and for other novelty uses too.

It will be observed from the brief resume given above that the algae are very useful marine plants. They are being profitably utilized in several countries for varied economic and other purposes. They also grow in sizeable abundance on the coast if favourable conditions exist for cultivating them in sufficient quantity.

Baluchistan Coast being 350 miles long with a number of protected bays and backwaters, sizeable patches of low lands in the intertidal zone and long rocky beds, supports varied types of red and brown algae growth in commercial abundance. It further offers favourable conditions for cultivation to provide an additional source of earnings for the fisherfolk and other persons as their living at present depends on primitive fishing only. On increasing production several plants can be set up for extracting agar agar and other products to make the country self sufficient in it and to improve economy of some people. Similarly the Indus delta having a network of creeks with considerable low lands can be utilized for their cultivation.

It will thus be seen that there are good prospects for increasing sea weeds production and developing its allied industries in the country.

Institute of Marine Biology University of Karachi has surveyed and identified over 30 species of Karachi and Sind Coast. It is a commendable pilot work undertaken the Institute. There is a need to provide further financial and other facilities in this sector for establishing commercial extraction plants.

It is proposed that, for the development of marine plant resources in our country following actions may be taken by the government (a) Acquire accurate estimates of the amount of weeds of economic importance on other coast, (b) Determine the distribution of different weeds species in space and time, (c) Investigate appropriate methods for their collection and cultivation, (d) Study Biology and Biochemistry of important species, (e) Introduce an educational programme to acquaint the public about this practically untouched resource occurring in an area of over 1,000,000 acres of land alongwith their wide range uses to which its products can be put.

# ROLE OF BLUE GREEN ALGAE IN THE ECONOMY OF AGRICULTURE

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## Abstract

Blue green algae have a world-wide distribution in terrestrial habitats. They have been recognised as the first colonizers of barren soils and even volcanic ash which is devoid of all nutrients. Their appearance helps in the establishment of other soil flora and in the accumulation of humus. Their contribution to soil fertility and especially to soils of the rice growing areas of the South-East Asia has been very well documented. According to some authors the fertility of rice fields is exclusively due to the growth of blue-green algae for reclaiming 'Usar' soils of U.P. in India. In Vietnam the age old method of making organic manures has been the symbiotic growth of blue green algae with some aquatic plants. There are references to the effect that growth of blue-green also provides essential materials for the growth of plants. This paper discusses the ecology of blue green algae especially the nitrogen fixing ones in terrestrial habitats and their possible contribution to the economy of agriculture in this area.

## Introduction

It has been stated that algae are the simplest members of the plant kingdom and the blue green algae are the simplest of the algae (Echlin 1966). Although most of the blue green algae are blue green, they are found in a wide range of colours of red, emerald green and near black. In addition to the common pigments-chlorophyll, carotene and xanthophyll the two pigments that are found only in this group of organisms are the blue pigment c-phycoyanin and the red pigment c-phycoerythrin. It is these two pigments that are principally responsible for the group's diverse coloration.

The blue green algae most species of which are filamentous in nature are widely distributed over land and water. Majority of these are aerobic photoautotrophs their life processes requiring only oxygen, light and inorganic substances. Some forms are able to fix atmospheric nitrogen and this perhaps is the most important contribution made by the blue green algae to human affairs.

The importance of blue green algae to agriculture and related fields can be considered under the following heads: (1) Blue green algae as primary colonizers of barren soils, (2) Role of blue green algae as agents of nitrogen fixation, (3) Blue green algae as a source of organic matter and other beneficial substances in soil, (4) Effect of algal growth on fish breeding, (5) Useful algal associations, (6) Negative effects of blue green algae.

## Blue green algae as primary colonizers of barren soil

As reported by Booth (1941) it was Treub who first emphasized the value of blue green algae in 1888 as the primary colonizers on the island of Karakatoa in Indonesia. After the volcanic eruption of 1883 the island had been denuded of all visible plant life. Filamentous blue green algae were the first to appear on the pumice and volcanic ash. Within a few years they had formed a thick gelatinous growth enough to provide a soil rich in organic matter for the growth of higher plants.

The colonization of blue green algae on the new volcanic island Surtsey, Iceland has been described by Henriksson, et al (1972) and Brock (1973). According to Henriksson and Co-workers studies were started by Schwabe in July 1968 only one year after the violent eruption which formed the island had ceased. Different types of algae were detected growing in association with each other and a mass *Funaria hygrometrica* Hedow, only *Anabaena variabilis* Kutz with the potential ability of fixing molecular nitrogen was recorded during the first summer (1968) but next summer three *Nostoc* species with the same ability were detected as well. Studies carried out by Henriksson, et al (1972) on the extent of nitrogen fixation by blue green algae in the early phase of the colonization of this island revealed the fact that algae were in fact fixing molecular nitrogen although the capacity was rather low. The species recorded by them belonged to *Nostoc*, *Anabaena*, *Nodularia* and *Tolypothrix*. However, according to Brock (1973) blue green were quite unimportant as primary colonizers of Surtsey. The algal mats found in a few locations were composed of occoid chloophytes and the primary colonizers of Surtsey were mosses and lichens. He thinks it probable that low temperatures at Surtsey discouraged the growth of blue green algae as was also the Case at Katmai, an area more similar to Surtsey than to Krakatoa. Here again blue green algae were unimportant as pioneers and liverworts dominated. Role of blue green algae agents of nitrogen fixation.

Nitrogen fixing blue green algae appear to be more important for areas where the climate is hot and humid at least during the rice growing season. Soils are neutral to alkaline in reaction and are poor in combined nitrogen (Fritsch and John 1942; Khan 1965; Watanabe 1966; Fogg, et al 1973). There are about one hundred million square kilometers of rice fields in the tropical area and about 95% of this area is in the Indo Pakistan sub continent and the Far East. As most of the world's paddy fields have been supporting the rice crop for years without the addition of artificial fertilizers, many authors have attributed the repletion of rice soil's fertility to the development of algal communities. De (1939) was perhaps the first to suggest that the fertility of rice fields in India was due to the activity of blue green algae on soil and nitrogen fixation by them in paddy fields causing an increase in rice yield have now been reported in many countries of the world. Table I contains some of the data reported to date.

**TABLE I. Nitrogen Gains in Waterlogged Soils containing Blue Green Algae**

Reference	Experimental conditions	Nitrogen gain	
		lb/ac.	period
1	2	3	4
Willis & Green (1948)	.. Louisiana, Crowley Soil, field expt., P.K. fertilizer, planted to rice and unplanted	Upto 70	Growing Season.
	Louisiana, Lake Charles Soil, field expt., P.K. fertilizer, planted to rice.	Upto 82	Growing Season
Prasad, (1949)	.. Rice lands of South Bihar, India	.. 13	After harvest



1	2	3	4
De and Sulaiman, (1950a)	.. India, Faridpur soil, pot culture expt., in light, planted to rice annually for 5 years (gains in soil alone).		
	(1) Water	.. 12	Year
	(2)	.. 74	Year
	India, Tippera soil, pot culture expt., in light planted to rice annually for 5 years (gains in soil alone).		
	(1) Water	.. 16	Year
	(2) Nutrient solution	.. 88	Year
De and Sulaiman, (1950b)	.. India, rice soils in erlenmeyer flasks	.. 600-2600 *	2-4 months
Watanabe, et al.' (1950)	.. Japan, pot culture, soil inoculated with <i>Tolypothrix tenuis</i> .	20	Growing period.
Watanabe, (1962)	.. Japan, Large Cylinders (earthen pipes) in paddy fields, planted with rice.	13.6	3 months
Hosoda and Takata quoted Watanabe, (1966).	Japan, Rice crop inoculated with <i>Tolypothrix tenuis</i> .	14	Crop Season
Okuda & Yamaguchi, (1956)	Japan, various soils in erlenmeyer flasks	160-2800 *	3 months
De and Mandal, (1956)	.. India, rice growing in waterlogged soils in gastight chamber fixation measured by gas analysis.	14 47	Growing period.
Hernandez, (1956)	.. India, lowland rice soils	.. 14-63	Growing Season
Calder, (1959)	.. Uganda swamp soil containing <i>Anabaena</i> , in jars, in open alternatively dried and flooded.	880 *	9 week
Niskigoki & Shioiri, (1959)	.. Japan, laboratory experiments, 3-4 in. layer of soils in illuminated beakers.		
	(1) Virgin river bed soil	.. 430 *	65 days
	(2) Fertilized paddy soil	.. 260 *	65 days
Singh, (1961)	.. India, maize field in Uttar Pradesh covered by <i>Cylindrospermum lichniforme</i> .		
Nawawy, et al (1968)	.. Egypt, 250 ml. conical flasks containing 30 ml. of the nitrogen free medium and inoculated with <i>Calothrix</i> sp.	111 mg/N/l	8 weeks

1	2	3	4
Shtina, (1970)	.. Russia, Unialgal cultures in flasks Synthetic medium.	1.2 to 11.3 mg/100 ml.	60 days
	Sod-podzolic soil	.. 3.6 to 15 mg per 100 g soil	3 months
Subrahmanyam, (1970)	.. India, rice crop in soil	.. 40 kg/ha	Crop Season

\*Amount of nitrogen fixed by blue green algae in laboratory experiments, expressed on an area basis depends to a large extent upon ratio of surface area exposed to light to weight of soil.

Ali and Sandhu (1972) surveyed saline soils of the Punjab and were able to identify 106 species of blue green algae comprising 27 genera and out of these 29 species belonging to 9 different genera appeared to be capable of fixing nitrogen. When some of the isolates were grown in nitrogen free media with and without the addition of micro elements, folidol and wheat straw, it was observed that wheat straw and folidol enhanced the growth of nitrogen fixing blue green algae (un-published results).

#### Blue green Algae as Sources of Organic Matter and Other Beneficial Substances in Soil.

It has already been mentioned that blue green algae are capable of growing under very inhospitable conditions. Singh (1950) observed that while other plants failed to grow on the alkaline "Usar" lands of northern India, blue green algae, consisting of many species, formed a thick stratum on the surface of the soil during the rainy season. The annual addition of organic matter to the soil was of the order of 36.5-59.7 per cent and that the nitrogen 30-38.4 per cent. Field trials in two villages in Benares district showed that after a year of reclamation of "Usar" lands through algal growth, a transplanted paddy crop grown in the subsequent year, produced a yield of 1576-2,000 lb. per acre. In the third year sugarcane gave a yield of 30-35 tons per acre. There was improvement in pH, tilth, exchangeable calcium and water holding capacity of the soil. Some authors have measured the beneficial effects of algal inoculation in terms of increase in the yield of rice without reference to resulting increases in soil nitrogen. Singh (1961) obtained an increased yield of paddy over the control of 368% in posts and 114% under field conditions when inoculated with *Aulosira fertilissima* Rao, Goyal and Venkataraman (1963) showed that inoculation of the same algae increased the paddy yield by 267% and rice by 328% over the controls using sand culture pot experiments. Similarly Aiyer, Salah-ud-din and Venkataraman (1972) showed that soil algae culture containing a mixture of *Tolypothrix tenuis* (Kutz.) J. Schmidt, *Aulosira fertilissima* Ghose, *Cylindrospermum muscicola* Kutz. and *Nostoc* sp., significantly increased the number of productive tillers per unit area by about 30% and also the net profit of the farmer. On the addition of blue green algae Allen (1956) was able to show an increase upto 600% in rice yield in laboratory experiments. Such a finding cannot be readily related to the less favourable conditions which occur in the field but nevertheless they do emphasize the possible benefit to the rice crop. Increase usually between 5 and 30% have also been noted in China (Ley, 1959), Egypt (Nawawy, et al, 1958), Russia, (Perminova, 1964; Shtina, 1965) and India (Subrahmanyam et al, 1965).

Reddy and Giddens (1975) have quoted many authors who had shown nitrogen accumulation in soil crust of grasslands and that algae were responsible for a major portion of the fixation. In their own investigations they were able to show that, in samples of soil crust of 4 year old tall fescuegrass (*Festuca Arundinacea* Schreb), total N and C in the crust was 0.18% and 2.67% respectively, as compared to 0.06%

respectively, in fallow soil. Blue green algae present in the crust were *Anabaena*, *Nostoc*, *Scytonema* and *Oscillatoria*.

Booth (1941) had drawn attention to the soil conservational value of a protective algal crust. Several species of soil algae belonging to the Myxophyceae, constitute an initial stage in plant succession by the formation of a complete algal layer over hundreds of acres of badly eroded land in the south central United States. The rate of infiltration of water into the soil was not slowed down by the algal stratum, soil losses from the plots with algal stratum were greatly reduced as compared with the losses from the bare areas and top inch of soil with algal cover had a higher moisture content as compared with the bare soil. Fletcher and Martin (1948) had also shown, earlier, that invasion of rain crust by microflora consisting of *Oscillatoria*, *Nodularia*, *Microcoleus*, *Nostoc*, several members of *Chroococcaceae* family and *Rhizopus*, *Mucor*, etc. improved infiltration, decreased erosion and aided in the establishment of plant seedlings under vigorous desert conditions. Increases of as high as 300% in organic carbon content and 400% in nitrogen content were reported in the crusts where microbial growth (mainly *Nostoc* spp.) had been extensive. Experiments in India by Marathe (1970) have shown that inoculation of soil with *Symploosa muralis* Kutz, *Chlorogloea fritschii* Mitra and *Hapalosiphon welwitschii* West, increased soil aggregation from 35.7 to 42.8% in the garden soil and from 57.8 to 78% in the sandy soil mixture.

Aiyer, et al (1972) observed that algae could function and stimulate the rice plants even in the presence of high doses of nitrogen. According to Venkataraman and Neelakantan (1967), the additive effect of algae in presence of high levels of nitrogen seemed to be owing to the stimulatory role played by the biologically potent substances produced by these organisms and made available to the crop. Gupta and Kushwaha (1970) studied the effect of presoaking seed and spraying the seedlings of wheat (*Sonora* 64, S.227, S. 308 K. 68 and N.P. 852) after a month with 1% and 2% ether and 2% and 5% water extracts of *Phormidium foveolarum* under field conditions. The application increased the number of tillers, ears, weight of ears, yield of grains and also the total weight of plants in all varieties of wheat studied. There was maximum increase in yield of about 20.8% in *Sonora* 74 64, 20.3% S. 227, 18.5% in S. 308 and 27.7% in K. 58 as a combined effect of pretreatment and single spraying with 2% water extract of *P. foveolarum*. In N.P. 852 pretreatment with 1% and spraying with 2% ether extracts gave a maximum increase in yield of 35.4%.

Obukhova (1961) quoting Fedorova has stated that "the drying blue green after the abatement of water in the rice fields and drying up to the soil, gave good energetic matter for the development of various bacteria including the nitrogen fixing bacteria". Mention should also be made of significant reduction in total sulphides and ferrous iron in the soil due to algal inoculation (Aiyer, et al, 1972). According to authors this is particularly significant for the Kerala region (India) where iron and sulphide toxicity is a common phenomenon. A high oxygen tension in the habitat, arising out of algal photosynthesis seems to facilitate the oxidation of these reduced compounds. Antibiotic production by algae has only been reported by Pattnaik (1970) who observed that an extracellular product of the alga *Phormidium ambiguum* had distinct effect on the inhibition of gram positive and gram negative bacteria and also some fungi. An interesting observation concerning vitamins of the B-group, which have a stimulative action on the development of higher plants, has been made by Odintsova (1970). She has shown the capability of blue green algae for synthesizing the following vitamins of the B-group: meso inositol, biotin, pantothenic acid, thiamine, pyridoxine, nicotinic acid, para-aminobenzoic acid.

#### **Effect of algal growth on fish.**

Obukhova (1961) has emphasized the importance of algae in the regime of rice fields in respect of their effect on fish breeding. According to him there are no contradictory views on this problem. Algae as the sole producers of organic matter in water are such an essential basic food without which the existence of other aquatic organisms including fish is impossible. Again, algae by virtue of their surface floating scum, protect fish from the harmful effect of solar heating up of water in hot summer days. Of no less importance is the ability of algae to produce by photosynthesis, a large amount of oxygen which has a

positive effect not only on the respiration of rice roots but also on the vitality of aerobic organisms including fish.

### Useful Algal Associations

Blue green algae occur in symbiotic association with many other organisms. Some of these are listed in Table 2.

**TABLE 2 : Association of Blue Green Algae with other Plant and Animal Groups**

Group	Genera	Algal Genera
Fungi	Ascomycetes in lichens	Calothrix, Chroococcus, Dichothrix, Gloeocapsa Hyella, Nostoc, Scytonema, Stigonema Nostoc
Filamentous Algae	Phycomycete in Geosiphon	
Diatoms	Enteromorpha	
Bryophytes	Rhizosolenia	
	Anthoceros, Blasia, Cavicularia,	Nostoc or/and Anabaena
Ferns	Azolla	Anabaena azollae
Gymnosperms	Bowenia, Ceratozamia, Cycas Dioon, Encephalartos, Macrozamia, Stangeria, Zamia	Nostoc or/and
Angiosperms	Cunnera, Trifolium	Anabaena, Nostoc (Punctiforme)
Protozoa	Cyanophora paradoxa, Cryptella cyanophora Glaucocystis nostochinearum, Paulinella chromatophora, Peliana cyanea	Various "cyanelles"

Table taken from Fogg, et al (1973).

Usually the associations are not obligatory and the algae can be isolated in culture. This poses the question of what biological advantage it is for these algae which can grow perfectly well in a free living state.

The association of algae with fungi to form lichens is probably the commonest of all symbiotic associations. There are about 17,000 species of lichens and blue green algae occur in about 8% of these (Fogg, 1956; Bond, 1959; Ahmadjian, 1967). The commonest blue green algae are species of Nostoc. Generally the lichens can withstand dry periods longer than what they encounter in their natural habitats. Lichens like blue green algae alone, play an important role in pioneering plant growth on bare rocks. The relation between the fungus and alga is not clearly understood. Presumably the fungus receives carbohy-

drates and perhaps nitrogen compounds from the alga and the alga is able to survive in otherwise inhospitable environments because of the moist medium for growth provided by the fungus.

The well known symbiosis between a fern and a blue green alga, namely the water fern *Azolla Anabaena* association has interested farmers of Vietnam for hundreds of years and an interesting account of this has been given by Obukhova (1961) quoting Zyong Khong Khieva. One place in Tai Vin province was cultivated with rice year after year without mineral fertilizers or local manures, dung, etc. The soil became poorer and poorer and rice yields decreased. Insufficiency of fertilizers particularly sharply affected those soils on which two or three crops were grown in a year. For preventing this the peasants of the La Van village began to use *Azolla pinnata* Rox as a manure for the rice fields. The peasant woman Ba Khen used it for the first time. When other peasants of the village began to use it and it proved beneficial, the feudal lords made *Azolla* their monopoly. Besides this they even forbade the inhabitants of the village La Van to publicise the method of propagation of *Azolla*. This restricted its use on a large scale but now it is widely used in the country and in 19587 there were more than 1000 centres for the propagation of *Azolla*. The peasant woman Ba Khen was even worshipped after her death.

The remarkable property of *Azolla* is that it grows on the surface of water in rice fields and perishes at the time of vigorous bushing out of rice. While dying off, *Azolla* gives up a large quantity of nutrients easily assimilable by rice. The favourable effect of *Azolla* is mainly due to the blue green alga *Anabaena azollae* Strass, a symbiont and a powerful nitrogen fixer which lives in the tissues of its leaves and provides all the nitrogen required for the healthy growth of both the alga and the fern. In all the plots manured with *Azolla*, the rice crop increased by 30-50% and upto 90% on very poor soils. *Azolla* contains  $P_2O_5$  and  $K_2O$  considerably greater than dung. It also improved the structure and tilth of the soil.

### **Negative Effect of Blue green Algae**

Some research workers attribute algae to the category of weeds only. In his detailed work "Rice Gushchen (1938, quoted by Obukhova, 1961) expressed the opinion that algae by profuse growth and formation "biological scum" choked the rice plants. Under certain circumstances fresh water algae like *Microcystis*, *Aphanizomenon* and *Anabaena* can cause death or injury to animals (Echlin, 1966). In South Africa, in 1943 thousands of cattle and sheep were killed along a dam in the Transvaal where the reservoir developed a poisonous bloom of *Microcystis*. The toxic substance was later identified as an alkaloid that affects the liver and central nervous system. The formation of blooms in bodies of water choke the intakes of water supply systems and give the water a disagreeable odour. Lake Erie which once had white beaches and supported a prosperous fishing industry, is now seriously infested with blue green algae. Sewage, industrial wastes and an estimated 80 tons a day of phosphates in water running off from farm lands, have turned parts of the lake into a vast tank for the culture of algae. The algae rob the water of its oxygen, and the lake becomes incapable of supporting fish life. They also wash onto the beaches and cover them with a malodorous green saline.

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# ALGAE AS A SOURCE OF FOOD

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Mankind has always been in search of finding new resources for its nourishment, clothing, living and other needs of its life. The overwhelming increase in human population with limited resources of food has compelled the reserchers to direct their attention towards little green organisms called Algae as new and vast resource of food material.

Algae are very simple, green, multicoloured, even white macroscopic, microscopic, unicellular, multicellular thalus forms. These organisms are marked with differentiation into stems, leaves and roots and are usually found in fresh, stagnant, marine waters or nonaquatic forms which grow in damp soil, on barks of trees and stones.

In China, Japan, Hawaii and Europe Algae have been used since ages as human food. In certain countries seaweeds have been employed as a manure and as a feed for livestock. In Europe several factories have been built to manufacture stock feed from algal resources.

At least forty species of algae are in common use as a food, most of them are green or red in colour. For example an important item of food called Kambu is made in Japan from the large laminariales, especially Laminaria, Alaria and Arthrothamenuis species, many dishes known as "Wakame", "Arame" and "Hajeki" which are prepared from brown seaweed like Undaria pinnatifida, Eisenia bicyclis and Hijekia fusiforme respectively. Similarly Miru is made from codium, a green form of algae.

The nutritive value of algae is high because they have higher contents of carbohydrates, small amount of proteins and fats. The edible Nostoc of China and the Japanese Amanari (Porphyra) have high nitrogen content and an appreciable quantity of carbohydrates and iodine. In general it would appear that the algae foods are of great value to us as a future potential food.

Since second world war much work has been carried on regeneration of algae of commercial importance. This applies particularly to species of Fucus and Laminaria Porphyra, Gelidium and Gloiopeltis in Japan and Agarophyte in all parts of the world. In recent years the algae Chlorella has gained great importance because of its rich proteins, fats and vitamins content and its rapid multiplication in the presence of sunlight. Pilot scale Shlorella farms have already been established in America, Japan, Holland, Germany and Israel which do not require excessive amounts of water.

Iodine from Kelp, (Laminaria), Ecklonia and Eisenia is manufactured in Japan and about 100 tons of iodine is being prepared annually. Japan supplies about 5 to 7% of world requirement of iodine.

In America potash is being extracted from Laminariales e.g. *Macrocystis*, *Nereocystis* and *Alaria*. The Algin and Alginates made from Fucales, Laminariales and *Macrocystis* have become mostly useful for the production of plastics, artificial fibres and stabilizer used in ice creams, syrups. Some of the important food algae are described below.

### **Enteromorpha Lind Ulotrichales, Ulvaceae**

Thallus of the plants are tubular, simple or with more less numerous branches. The species occur in the upper parts of the littoral zone near the shore in quiet waters, attached to small stones and rocks by means of rhizoids. *Enteromorpha* species are capable of enduring a wide range of salinity and therefore often occurs in brackish water or estuaries.

An extensive study of the food value of the Hawaiian *Enteromorpha* species was made by Miller. The edible species are known as limu ele or limu pipilani. As to the vitamin content Miller stated "ele-ele" is a fairly good source of vitamin A and lesser source of vitamin B. Limu is highly appreciated in diet for its flavour and its tendency to prevent constipation. Certain species of *Enteromorpha* are a good source of food for many fishes e.g. *chanos chanos*. Species of *Enteromorpha* are found in Pakistan, India, Malaya, Indonesia and Java. In Philippines certain species are eaten raw as a salad. The Chinese import dried *Enteromorpha* from the Malay peninsula and some of the species are eaten by the Japanese as a supplementary article of diet with boiled rice. *Enteromorpha* species also serve as food for the milkfish in the Philippines.

### **Chnoospora Agardh Ectocarpales**

The members of this small genus of tropical especially pacific algae are characterised by the irregular dichotomously branched thalli bearing numerous tufts of hair, the forks being closer and closer upwards. The aggregated sporangia are spread over the surface and arise from the hairs. The relatively tough, more or less compressed, branches are composed of a central and a cortical tissue. The plants in dried state are dark brown. The tufted plants are attached to stones, rocks and pieces of dead coral in the littoral region. Species *Chnoospora pacifioa* found in India, Pakistan, Durban, Ceylon, Indochina, Indonesia, Java, Japan, Australia and in West Indies are eaten raw like salad.

### **Turbinaria Lamouroux—Fucales, Sargassaceae**

The plants of this genus are attached to the substratum by short branched holdfasts sending up one or a few simple or branched axes. The stiff cylindrical main axis or stripe bears the leaf-like organs. These have the appearances of a triangular shield or disc with a serrate or entire margin, tapering downwards. The air bladders when present are immersed in the leaf like organs near the end. The receptacles grow into dense clusters from the base of the petioles. *Turbinaria ornata* species occurs in Ceylon, Malay, Singapore, Indonesia, Java, and Phillipines. In all tropical and temperate seas from Cape of Good Hope, New Zealand, Chile and China the seaweeds are most frequently eaten raw with lemon juice but some times are cooked with coconut milk. Burkill mentions that the seaweeds are made into a pickle in Eastern Malaysia.

### **Porphyra Agardh—Bangiales, Porphyridiaceae**

The dull purplish thallus of the plants is flat and thin, foliaceous one or two cells thick. The plants are very short stalked and are attached by a very small holdfast expanding above into a soft slippery blade with an entire or lobed margin. *Porphyra* species are found in Indonesia, Phillipines and Hawaii. The different species of *porphyra* furnish a plentiful supply of food to people in quite distant regions of the world. In Scotland Wales and Ireland *Porphyra* species are used as laverbread. It is usually served with bacon for breakfast, made into small flat cakes, and fried crisp in the bacon fat or heated with butter, lemon

juice and served with roast mutton. Before being eaten it can be made crisp over a fire which changes the colour to green. It can be rubbed then be rubbed between the hands and the small pieces are dropped into soups or sauces to give a pleasant flavour. Now-a-days it is often preserved in tins after having been boiled with soyabean sauce. Yendo has given the recipe for making "Sushi" one of the most common seaweed food in Japan. First cold boiled rice is spread over a sheet of asakusanori on the rice are laid pieces of meat or fish. The entire sheet or layer is then rolled like a jelly roll and cut into slices.

### **Agar—Rhodophyceae Red Algae**

Agar is the dried colloidal substance obtained by concentrating a decoction of various red algae specially of Gelidium, Gracilaria and Petrocladium. Agar is obtained from Japan, Newzealand, South Africa and from both the Atlantic and Pacific coasts of USA. The chief carbohydrate in agar agar is a galactaian. Agar agar has long been used as food by the Chinese and Japanese. It is used in the form of jellies and as a thickener for soups and sauces. In Europe and USA. Agar agar is utilized in ice creams, jellies and jams in various industries as a sizing material, emulsifying agent, thickner in the drying and printing of textiles and in the finishing of leather.

### **Conclusion**

From the great advances which have been made in every field during recent years the interest has gradually extended towards products both of vegetable and animal origin. Coastal areas of Pakistan are rich in algal wealth. Some of them are important from economic point of view. As climatic conditions in Pakistan are suitable for algae growth, so attempts must be made for systematic investigations. And this can be done only if we know the food value or their essential ingredients such as proteins or vitamins of those algae which are present in our country and algae which can be introduced from other countries.

There is a great need for the propagation of algae on a commercial scale by establishing algal under water gardens and farms. On account of the presence of big reservoirs of water such as Warsak, Tarbela, Mangla, Rawal Dams and lakes like Safulmaluk, Mancheer it is possible to introduce and propagate fresh water algae on commercial scale in these waters. On the other hand in case of marine algae we have got fortunately large coastal areas which are most suitable for experimental and commercial cultivation of diatoms.

Although algae can be utilized as human food in Pakistan because of the eating habits of the people. However, it is preferable to use it immediately for the preparation of food for livestock, poultry and fishes. It can also serve for the extraction of certain chemicals being imported in Pakistan at present. Use of algae as a manure is common in different countries of the world and most of the forms of marine diatoms occurring in Pakistan can be utilized as a manure in areas adjoining the coastal belt of Pakistan.

Most of the species mentioned in this paper like Laminariales, ulotrichales (Enteromorpha) Chlorella Fucus, Nostoc, Gelidium, Ulva, Sargassum and Tubbinaria can be grown and multiplied in the country's waters. But before bringing this proposal to practical use following suggestions are made for the future development of this natural resource. (1) Complete survey of Algae occurring in coastal belt, rivers, stagnant waters and damp soils be carried out to know the species growing in abundance in these waters. (2) Algal species growing in Pakistan which can be used as a food and for other purposes may be listed alongwith their approximate quantities available for the guidance of industry and trade. (3) Some of the important exotic algal species may be introduced and multiplied artificially near their natural habitats for obtaining the desired materials. (4) Assessment may also be made to judge the cost of cultivation, new income and economic feasibility of growing algae, if the exotic algae are to be grown artificially in Pakistan. Before taking this step some of the firms interested in the utilization of algae may be contacted for the disposal of the produce.

## **Acknowledgement**

The author is thankful to Mr. Altaf Ahmad, Research Officer for going through the article and making useful suggestions.

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# MASS CULTIVATION OF ALGAE

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## Introduction

Nutrition and human health is one of the important problems of the world today particularly in the developing countries where agricultural output cannot cope up with ever-increasing population. This is recognised by the international community and as a result concern on the scarcity of high quality proteins has been expressed. FAO has declared "If the gap between the protein requirement and protein intake continues to widen as at present and with little prospects for improvement then the long term consequences will be disastrous." This gloomy picture has led the scientists throughout the world to explore the possibilities for utilizing the single cell proteins for animal and human consumption. The main purpose of this investigation was the physiology and biochemistry of algae, the reduction of nitrate and nitrite and the purification of the enzymes involved in this process.

## Selection of Algae

The organisms selected for the mass cultivation were micro algae, bacteria, yeast and filamentous fungi. These micro-organisms were selected because of the various technological advantages they possess over the higher plants. The higher plants have very short generation time. In the case of bacteria and yeast it is 0.5 to 2 hours and 1 to 3 hours respectively while algae and filamentous fungi double their biomass in 6 to 8 and 4 to 12 hours respectively. Thus for an ordinary crop 6 to 7 months are required. The protein contents of these microorganisms are very high, most micro organism containing 7 to 12 percent nitrogen which is higher than most of the foodstuff being eaten. The raw material for the cultivation of these algae, fungi and bacteria is available everywhere and in abundance almost in every country and therefore any country can utilize these types of organisms. One of the advantage of these organisms is that the continuous culture of these organisms can be done throughout the year in respect of the climate because artificial climate can be created and the area required by them is very small and the yield per acre will be more than the normal crop. The waste disposal problem is also very low as compared to the other production. In Pakistan plenty of sunshine is available and the temperature is very favourable for the growth of such organisms.

## Mass Cultivation

Research on the mass cultivation of algae had started only recently through the pioneering work of many scientists. These workers explored the possibilities of extracting fats from micro-organisms. Although most of their attempts failed yet this led to a new field. The intensive investigation on the microalgae started soon after the second world war. Results obtained in the United States, Japan, Israel

and Germany have been reviewed by Pervis (1953) and by Tamyia (1957). Species used in the mass cultivation of algae belong to two classes chlorophyceae and cyanophyceae. Although other classes of algae have also been utilized literature on these is rather scanty. Among the chlorophyceae chlorella strain has been widely used. This strain of chlorella is the common work of the biochemist and the physiologist. Ninety nine strains of this species have been studied so far. Some workers have also been able to isolate a high temperature strain of chlorella possessing a special property that it can double its bio-mass in a very short period of two hours at 39°C. It has been suggested that this species can be utilised for the synthetic gas exchange in the case of the space capsule because of its short generation time and thus providing not only food but also oxygen. Mesophilic strain of chlorella is cultivated by various commercial companies in Japan and their product is mainly used as chicken feed. Strains of scenedemus is grown in Germany, Czechoslovakia, Italy, Bulgaria and in some other countries of the world. In United States of America it is utilized as chicken feed. Blue green algae has recently received international recognition. This algae is being eaten in Mexico for quite some time species of spirulina have some very good technological advantages over other micro-algae. It is large having a diameter of nearly 40 micron and a length of nearly 240 micron. The French Petroleum Institute has developed a process of extracting protein from this algae. This algae is also grown in Mexico where they are extracting protein from it for human consumption. Open air culture experiments are going on in Italy, utilizing atmospheric nitrogen for their growth obtaining the yield of seven gm. of algae.

### **Methodology**

The methodology has been divided into two portions namely by clean water process and waste water process. In the case of former process the algae have to be grown on a chemically refined medium whereas in the latter process algae are grown on the sewage waste effluent or any other type of wastewater. In the clean process algae can be cultivated either in open system such as ponds or in the closed system such as by fermentation. In closed system the sterile conditions can be maintained and their products can be utilized for various types of sophisticated work. The one advantage in this system is that algae can be grown autotrophically, mixotrophically and heterotrophically. The cultivation units used vary from one country to another depending upon the choice and the climatic conditions. In most countries oxidation ponds are being used as the cultivation units. Tamyia and his co-worker (1953) designed open air culture units and they were all circular shallow ponds. Closed systems can also be used but certain engineering problems are involved as to how much energy is available and how much energy is reflected. Turbulence mechanism is also one of the factors to be considered because the algae as they grow with age become heavier and settle down. While settling down there will be over-shadowing of some of the culture which will not get sufficient light energy. The turbulent system will facilitate a better illumination.

### **Optical Density**

In the mass culture experiments optical density of the suspension is also considered. Optical density must be correlated with the areal density. Doubling point is another important factor.

### **Light**

Light plays a very important role in the doubling time. Under the laboratory conditions when optimum light is provided and other conditions are optimum then the doubling time of this culture is definitely less than the doubling time of the culture in the open air. Work on scenedemus has been done and it has been observed that the doubling time of the scenedemus is three hours in the case of laboratory culture and it is three days in the case of outdoor culture.

### **Temperature**

Algae can be classified on the basis of their temperature tolerance as triphobic, mesophilic and thermophilic. In mass culture work has been done on mesophilic and thermophilic algae. It has been

shown that the amplitude of the temperature dependence increases strongly with the increase in the optical density. Humidity also plays an important role because in humid climate the temperature of the culture rises. Some algae have a fixing mechanism of the transitory changes in the temperature. The mesophilic algae can be grown under humid tropical conditions.

### **pH**

pH also plays an important role in the mass cultivation of algae. There are certain species of algae which require low pH. Many workers have reported the variation of pH in the species of *Chlorella* and *Scenedesmus*. Variation within the genus is more in the case of *Chlorella* than *Scenedesmus*. Salinity is also one of the important factors in the case of algae which grows in saline culture. In case of *Spirulina* a high pH of 9 to 11 has to be maintained but the salinity has to be maintained at a constant level otherwise the algae will not grow.

### **Nutrient**

Some workers have studied the depletion of nutrients from the media at various intervals of time and the chemical composition of algae. These two factors were correlated as to determine how much of the nutrients are going into the algal species so that the nutrients in the same proportion could be supplemented. Carbon dioxide is also next important factor for the autotrophics grown on algae. The open culture tanks should be built at a place where commercial exhaust gases are available in which large proportion of carbon dioxide is present. So that the algae could be harvested at a cheaper rate. Certain algae has the capacity to utilize organic source and can be grown under dark. After growth of the algae the next step is harvesting and processing.

### **Harvesting and Processing**

Harvesting of algae is very crucial as its size is of the order of ten microns. This type of algae can be screened out by filters. Even if filters are available the algae will clot the filters and will make their use unfit. Therefore this method is not used too often. In this case the cost of operation is also very high compared to other methods. In the United States Flocculation is used. Sometimes certain ion exchange methods are also used. Again the cost of production is too high. In order to decrease the cost of production it is desirable to make use of such algae which can be filtered very easily. Processing of the algae is essential in order to get bacteriologically free product and for making the biomass more digestible for human consumption. The cell walls of algae is usually very stiff and cannot be digested very easily. This stiff cell wall must be broken by some mechanism in order to make them more digestible. Processing is usually done by spray drying and sun drying.

# ALGAE IN SEWAGE OXIDATION PONDS

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## Introduction

The term oxidation pond in its simplest form is applied to a body of water, artificial or natural, employed with the intention of retaining wastewater until it is rendered stable and inoffensive for discharge into receiving water or on land through physical, chemical and biological processes commonly referred to as self purification and involving the action of algae and bacteria under the influence of sunlight (photosynthesis) and air. Organic matter contained in the waste is stabilized and converted in the pond into more stable matter in the form of algal cells which find their way into the effluent.

## Mechanism of Treatment

The sewage is led into the pond with suitable inlet and outlet devices. In the presence of sunlight and sufficient nutrients contained in the incoming sewage a healthy bloom of algae flourishes in such a pond together with a large number of aerobic bacteria and other organisms. As long as algae can provide an excess of oxygen above that required by bacteria, an aerobic environment will be maintained. Under these conditions aerobic bacteria oxidize the organic matter present in the waste resulting in the production of carbon dioxide, ammonia, and water which incidentally happen to be the essential requirements along with sunlight for algal photosynthesis. A mutually beneficial relationship thus exists between algae and bacteria each giving to the other that it needs. The terms symbiosis is used to describe this mutual relationship between algae and bacteria. Hence, it is necessary that in oxidation ponds there must be a balance between the algae and the bacterial population. A part of the organic matter contained in the waste is used by bacteria to make new cells while the rest provides the energy necessary to further the degradation reactions. The algae on the other hand utilize the simpler end products of this bacterial decomposition and with the help of the solar energy synthesize more algal mass simultaneously releasing oxygen. The excess quantity of algae and bacteria produced go out with the effluent.

It was recognised very early that oxidation ponds operated on a bacteria-algae symbiosis in which the bacteria aerobically stabilised the organic matter while the algae grew autotrophically on the stable end products of bacterial metabolism and produced excess oxygen for the use of bacteria. The algae were the microorganisms that seemed to make the difference in the oxidation ponds. The most common algae reported in the oxidation ponds include Euglena (1) (2) (3) (4) (5) Chlamydomonas (2) (3) (5) Chlorogonium (1) (5) Microactinium (2) (4) (5) Ankistrodesmus (2) (3) (4) Scenedesmus (1) (2) (3) (4) (6) Chlorella (1) (2) (3) (4) (5) (6) (7) Oscillatoria (1) (2) (5) (7) Phormidium (7) Navicula (5) (7) Closterium (3) (7) Anaostis (2) (6).



This list of algae is not intended as a complete list of algae that are found in oxidation ponds but rather represents large growth as reported in the literature.

Ludwing et al (8), Merz, et al (9) and Parker (10) reported that *Euglena* and *Chlamydomonas* are the most frequent motile algae in oxidation ponds, *Chlorella* and *Scenedesmus* are small non motile algae present a large surface area to mass ratio so that they remain in suspension with very slight fluid motion. Lakshminarayana (11) reported that most of the time species of *Chlorella* formed the major dominant organisms in the oxidation ponds of India. It was never below 80 percent of the total algal population. Besides *Chlorella* the other organisms were observed in relatively small numbers and their population very rarely exceeded 20 percent of the total population. Oswald, et al (12) reported that the species of *Chlamydomonas* is the least desirable algae in the oxidation ponds for although it can grow with speed. It spreads over ponds surface, shuts out light and accumulates in corners where it decomposes with vile odours. Lackey (13) revealed one of the important facts in his study that there was no variation in the composition of the algal flora of sewage oxidation ponds that can be correlated with geographic locations. Maris (14) during the study of oxidation ponds at Lusaka observed that in winter the concentration of algae increased to over  $1.2 \times 10^6$ /ml. with the dominant flora being *Microactinium*. During summer the total number decreased to less than  $10 \times 10^5$ /ml. both *Euglena* and *Chlorella* being dominant in turns. This decrease in summer appears to be due to windless days and long periods of stratification.

### **Methodology Identification**

Identification of algae were made by a binocular compound microscope. Characteristic markings of each species were compared with standard methods (15) and with *Fresh Water Biology* (16). Whenever difficulty was encountered in the comparison technical key to identification given in these references (15) (16) were consulted. In certain instances comparison with the standard photographs were made by taking pictures of the species by means of a photographic microscope. For motile algae oil immersion objective was used. For rapid swimmers such as *Euglena* Lugol's solution was used. Such a solution would kill the species but leave them recognizable for identification and counting.

### **Algal Growth Measurement**

The total number of algae present as well as the number of each kind in mixed cultures were estimated by counting the cells in a haemocytometer according to a procedure described by Parvis (17).

### **Sampling**

Sampling was done thrice a week (Monday, Wednesday and Friday) from selected points. With abnormal conditions such as the appearance of blooms the frequency of sampling was increased. To measure populations that may change rapidly, daily collections were made and when considered necessary continuously for 24 hours. The samples were collected from appropriate depths. The sampler used was the one described in *Standard Methods* (15). The samples from the sampler were transferred to a wide mouth jar of two litre capacity and examined without lapse of much time. For extended storage the sampling jars were filled to the brim and were preserved by the addition of 40 ml formalin.

### **Experimental Facilities**

The four rectangular oxidation ponds at site are each 150 x 60 feet (46 x 18m) on top and 5 feet (1.5m) deep with side slopes of 1.5 (horizontal) to 1 (vertical). The ponds are lined with bricks on the sides and bottom and are numbered from 1 to 4. During the period of study the ponds received sullage (weak sewage) as influent. They were operated in parallel with a water depth of 4 feet (1.2m). During the period of study the ponds were in proper operating conditions giving varying shades of green colour. The pond effluent

was collected in a ground sump and was pumped to either an earthen pond or crudely formed shallow channels for intermittent irrigation of an experimental field.

## Results

In total forty species of algae were identified from all the four ponds distributed as: eleven species belonging to the group Chlorophyceae (green algae), five species to Bacillariophyceae (diatoms), ten species to Myxophyceae (blue green algae) and the rest (thirteen species) were flagellates (Euglenophyceae, volvocales of Chlorophyceae). Most of the species occurred more than once and in a cycle. Ninety percent of the algae identified were polluted water algae. Examination of these organisms indicated that there were three types of algae present in the ponds the motile algae, the non motile algae, and the filamentous algae. The motile algae had the ability to move and compensate for varying light conditions. *Euglena* and *Chlamydomonas* were the most frequent motile algae identified. *Chlorella* and *Scenedesmus* were small non motile algae that present a large surface area to mass ratio so that they remain suspended with very slight fluid motion. The primary filamentous algae identified were the blue green algae (*Oscillatoria lauterbornii*). The filamentous algae tend to form floating mats that were undesirable in oxidation ponds as they block light transfer, mixing and surface re-aeration. It should be pointed out at this stage that the occurrence of a particular species at a certain time depends upon the range of tolerance for various environmental factors and the optimal requirements of that species at that time.

Table below gives the predominant algal species.

### Predominant Algal Species

Serial No.	Name of Algal Species	Number of Times being Predominant during the Investigation Period			
		Pond 1	Pond 2	Pond 3	Pond 4
1.	<i>Chlorella vulgaris</i>	6	6	4	4
2.	<i>Chlamydomonas reinhardi</i>	4	4	2	2
3.	<i>Oscillatoria lauterbornii</i>	4	4	4	4
4.	<i>Euglena gracilis</i>	3	3	3	3
5.	<i>Ankistrodesmus</i> sp.	2	2	2	2
6.	<i>Scenedesmus quadricauda</i>	1	1	1	1
7.	<i>Lepocindis texta</i>	1	1	1	1

## Discussion

*Chlorella vulgaris* could be regarded as the most common algae of the ponds. Although *Scenedesmus quadricauda* was the first species to appear in the ponds, it soon gave way to *Chlorella vulgaris*.

Coldwell (loc-cit) reported *Euglena gracilis* at Camp Parks in 1946 and in an abstract of another report by Coldwell (loc-cit) *Euglena* was said to be normally predominant but with *Chlorella* and *Pandorina* also present. From studies of polluted streams such as those by Palmer and Lacky (loc-cit) more and better data are available giving further support to the generalization that members of the *Volvocales*, *Chlorococcales*, *Euglenoids* and *Oscillatoria* comprise almost the entire phytoplankton of heavily polluted waters.

The most common algae of the oxidation ponds reported by various workers include: *Chlorella*, *Chlamydomonas*, *Euglena*, *Oscillatoria*, *Scenedesmus*, *Phacus*, *Ankistrodesmus*. Lakshminarayana, et al., (loc-cit) reported that in the oxidation ponds at Nagpur (India) treating domestic sewage *Chlorella* formed the major dominant organism and it was never below 80% of the total population. Besides *Chlorella* other organisms like *Pandorina*, *Phacus*, *Ankistrodesmus* were observed in relatively small numbers.

The predominance of *Chlorella* in the ponds under study could be explained. Not only does this species grow more rapidly in sewage than *Chlamydomonas* or *Euglena*, but it also maintains its maximum growth rate down to lower intensities. The ability to maintain a high growth rate at lower light intensities would give *Chlorella* marked advantage under the turbid conditions that exist in oxidation ponds. It is of interest to note in this connection that *Chlorella pyrenoidosa*, the algae widely used for photosynthesis studies, was originally isolated by Chick (19) in 1903 from sewage, and that she considered this to be the medium par excellence for its development.

Competitions were also observed among various algae of the ponds. The competitions were more marked among the dominant form. The ability for algae to compete successfully is a complex phenomenon. However, it is understood that for a specific combination of environmental equilibrium conditions, the one particular species having physiological characteristics that provide the greatest efficiency in energy and resource utilization will dominate the system. The less efficient species will be gradually eliminated as resources required for their survival are depleted from the environment by the more efficient species. Co-existence at equilibrium conditions therefore could only occur when the more efficient species do not completely remove any one of the resources necessary to another less efficient species. It was observed that during competition the environment of the pond also has an impact on the species taking part in the competition. Only that species will be dominant which has also all its optima for various physical factors satisfied.

*Oscillatoria lauterbornii* was always dominant in the hottest part of the year, *Chlorella* dominant in the beginning of summer. Flagellates usually dominant in the cooler months. The general conclusion drawn from the field studies is that the same small group of algae occurs regularly in oxidation ponds under study. Maximum growth of algae in all the ponds were experienced in summer, moderate in winter and least during rainy season. This was also apparent by the colour of the ponds. Greenest samples were obtained during hot summer months as compared to other seasons (winter and rainy). Factors which must be considered in attempting to explain this include light and temperature which are affected by the seasons. It was observed that the occurrence of a certain species is confined to a particular season for example *Oscillatoria lauterbornii* was predominant only in the hottest part of the season usually in the months of June, and July. *Chlorella* developed under moderate climatic conditions, whereas flagellates like *Euglena*, *Chlamydomonas*, etc., were dominant usually in the winter months.

Hence, it follows that the seasonal variation in the algal species of the ponds also depends upon the response of that species to seasonal changes in the environment, undoubtedly certain species will be more affected by the increase in light temperature of summer months than those of other seasons in accordance with their respective optima for these factors.

## Conclusions

From the foregoing studies the following conclusions are drawn:

1. Forty algal species were identified from the ponds.

2. The following algal species, were found to be predominant: chlorella, chlamydomonas, Euglena, Oscillatoria, Scenedesmus and Ankistrodesmus.

3. Seasonal variation were observed among the algae of the ponds, maximum growth of algae being recorded in summer, moderate in winter and least in rainy seasons.

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## Discussion

It was suggested to the authors that permanent slides of various algal species should be preserved for future references. Some parameters like the effect of pH, dissolved oxygen and temperature on the growth of ponds algae were discussed.

# SIGNIFICANCE OF ALGAE IN SANITARY ENGINEERING

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## Introduction

The purposes of this paper is to present briefly the utilization of algae, specifically, its role and significance in sewage treatment with emphasis on observations and findings on algal species found in the pilot scale oxidation ponds belonging to this Institute, to channelize information about the effect of algae on the water treatment by slow sand filtration, and to mention very briefly the overall problems created by algae when water is used as a resource. It is important to point out that each of the two methods namely, oxidation ponds for wastewater treatment and slow sand filtration for water treatment has a great potential for use in developing countries in particular the rural areas.

## Algae and Sewage Treatment

The stablization of organics in sewage is almost invariably brought about by micro-organisms. Chemical and physical methods such as the use of strong acids or drying and burning are generally too expensive in comparison with the biological treatment. Several types of micro-organisms are frequently involved in this stablization process. The treatment plant operation consists primarily of making conditions ideal for the rapid development of bacteria, molds, protozoa and rotifers since it is through the activities of these micro-organisms that the sewage is changed from an offensive to an in offensive condition.

Recently algae have received more attention for the part they are able to play in sewage treatment. In sewage stablization ponds algae is utilized for the production of oxygen essential to the growth of bacteria and other organisms that breakdown the organic wastes. With algae it is possible to support populations of bacteria so essential for sewage stablization process. Large open shallow basins are required in order that light will be available through the liquid to stimulate algal growth and activity. Certain kinds of algae are able to grow in the presence of high concentration of sewage and these may be stimulated to grow and multiply more rapidly. It appears that only young algal cells will produce large quantities of oxygen, thus making it desirable that the multiplication of additional young cells be kept up as a continuous continuous process at the sewage treatment plant. In oxidation ponds system sewage enters the shallow open basin or lagoon and is allowed to move slowly through it while the algae multiply rapidly and produce the oxygen. The algae which develop in large numbers in sewage stablization ponds are the same kinds that are present in the portions of streams which are polluted with organic wastes.

The massive growth of algae developed in sewage stabilization ponds may eventually become a new source of fertilizers, vitamins, animal feed, and other products of commercial value. Algae also are potentially valuable as efficient users of the nutrient salts resulting from decomposition of sewage so that algae might supplement the land plants grown on farms. Thus the pollution algae may become useful not only as indicators and purifiers but also as producers.

Ecological studies by this Institute as carried out at the pilot scale oxidation pond set up showed that a number of algal species were present in the ponds. During the first and second phase *Euglena gracilis* predominated over other forms of algal species. However in the third phase *Chlorella vulgaris* was the dominant algae, forming more than 50% of the ponds population. It was interesting to note that with the increase in temperature the composition of algal flora changed and green algae were replaced by blue green algae. Another aspect of research on algae is the development of a relationship connecting the different design factors like solar energy utilized in algal growth, pond surface area, efficiency in energy conversion and net weight of algae synthesized daily and the relation of algal growth with the evolution of oxygen by algae.

In brief in stabilization ponds the beneficial use of algae include production of oxygen under conditions whereby it can be used by bacteria to oxidize dissolved organic wastes and produce an effluent high in dissolved oxygen and low in soluble BOD, and production of algae that may be used as fertilizer, livestock feed, and as a source of proteins.

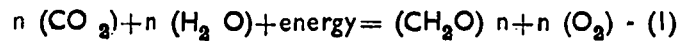
### **Effects of Algae on Filters in Water Treatment**

Biological filtration or slow sand filtration is accomplished by passing raw water through a bed of sand. During its passage the particulate impurities are brought into contact with the surface of sand grains and held in position there. Those impurities which consist of material are retained until eventually removed during the cleaning process while others capable of chemical or biological degradation are converted into simpler forms. The algae that collect and grow on the surface of a slow sand filter as a gelatinous slimy film may be responsible for gradually reducing the flow through the bed. They however perform a useful function in adding oxygen to the water which permits the bacterial decomposition of organic matter within the filter to remain aerobic. Anaerobic activities in the sand bed would tend to render the filtrate less palatable. The slimy mass of algae and other aquatic plants and animals at the surface of a slow sand filter is called the "filter skin". Although they do not, strictly speaking, take part in the mechanism of filtration certain types of algae can have significant effects on the working of a biological filter. These effects may be beneficial or harmful depending on a variety of conditions.

Practically all surface waters contain algae their presence contributing to the natural regenerative processes in streams, rivers and lakes. According to the nature of the water source, its pH, temperature, chemical composition and turbidity, the concentration of nutrients it carries, its depth and velocity of flow, the amount of sunlight it receives and other factors different species of algae will predominate. The algae found in a section of a shallow fast flowing stream, for instance, may not be the same as those prevalent in a deep pool or reservoir fed by that stream. For the same reason the algae predominating in the supernatant water of a filter may markedly differ from those in the raw water source from which it is drawn. Both groups, however, will have an influence on the efficiency of subsequent filtration, the algae in the raw water affecting the DO content at the point of entry to the supernatant reservoir, and the algae in the supernatant water itself producing a number of changes in the chemical quality of the water within the reservoir during the waiting period before it passes downwards into the sand-bed.

As autotrophic organisms, algae need light for their photosynthetic processes and are, therefore, likely to be almost entirely inactive in the supernatant water when the filter structures are covered. Even in uncovered filter reservoirs their growth may be markedly reduced if the raw water is sufficiently turbid to cut off the essential sunlight.

The property of algae most significant to the water purification process is the ability to build up cell material from simple minerals such as water, carbon dioxide, nitrates and phosphates. The carbon cycle may be described by the relationship:

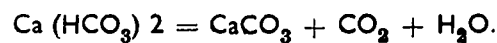


The energy they require for their metabolism is derived from the oxidation of organic matter. The reverse reaction also occurs when algae die and their cell material is liberated to be consumed by the bacteria of the filter-bed:



The relative magnitude of these two reactions govern the growth, constancy and decline of the algal population.

As long as algae are in an active state of growth in spring and summer in temperate climates and longer in tropical areas reaction (1) predominates increasing the oxygen and decreasing the carbon dioxide content. The rise in oxygen content, sometimes to as much as three times the theoretical saturation level, is always an advantage but the lowering of the carbon dioxide content may cause bicarbonates to dissociate into carbonates and carbon dioxide:



The lowering of the bicarbonate content will decrease the temporary hardness, and the insoluble carbonates will precipitate, thus contributing to the clogging of the filter. As the growth of algae continues and their volume increases, the downward movement of the water is hindered, necessitating the periodic removal of the algae from the filter surface.

When the algae are in a steady state, equations (1) and (2) will balance each other. The process described by equation (1), however, requires sunlight, while the degradation of organic matter according to equation (2) continues at all times. The overall effect is thus an increase in oxygen content during day light hours and a corresponding decrease at night. The diurnal variation may be considerable and in severe cases an aerobic conditions may occur during the dark hours.

Algae use organic matter from the raw water to build up cell material, and although when they die an equivalent amount of organic material is liberated, the new material is more easily degradable than the old. There is little difference between a closed and an open filter in the average oxygen content of the effluent. But the oxygen consumption of the open filter is about 10 times that of the covered (15 mg/l compared with 1.5 mg/l). This is due not only to the carbon cycle shown in equations (1) and (2) above, but also to the conversion of unassailable into degradable organic material. The greater oxidative activity means that the chances of harmful organic substances both living and dead being destroyed are correspondingly increased.

Further beneficial effects of algal growth may be found in the contribution that filamentous species make to the formation of an active schmutzdecke, the zoogeal content of which forms a medium for the trapping and proliferation of plankton, diatoms, and other forms of life, thus enhancing straining and absorption. Less suspended matter reaches the filter medium when the schmutzdecke is well established, and this helps to lengthen the interval between successive filter cleanings. In addition, a favourable environment is provided for protozoa and other higher organisms, which feed on bacteria and materially reduce the number of E. coli and pathogens that reach the sand bed. The algae themselves, according to some investigations, produce substances harmful to bacteria, thus reducing their chances of survival.

The presence of heavy algal growths always carries the potential risk that the filter will have to be cleaned too frequently, so that it will go out of service for too great a proportion of time and perhaps require a larger labour force than would otherwise be necessary. In temperate climates risk is accentuated during seasons of rising temperatures, which may cause extensive and sudden algal blooms, and in periods of falling temperatures, when massive mortality may occur. In tropical climates other meteorological or seasonal changes may bring about similar phenomena.

Covering the filters helps to solve this problem: longer and more regular filter runs result, and cleaning may be carried out by day or night and during periods of frost or other inclement weather. The absence of algae in the raw water reservoir of a covered filter may lead to a somewhat reduced filter efficiency, particularly with respect to the reduction of intestinal bacteria, but a compensating benefit may accrue from the exclusion of in-borne contamination or bird droppings. An increase in the rate of chlorination will ensure the hygienic quality of the delivered water. Covering the slow sand filters at one of the treatment plants of the Amsterdam municipal waterworks allowed the average filtration rate under all weather conditions to be raised from 0.1 to 0.3 m/h, while the length of filter runs increased slightly and became decidedly more regular.

Under tropical conditions in which the periods of blooming and dying of algae are less pronounced and ice formation does not occur, filter cleaning normally becomes necessary only at regular and not too frequent intervals, and there is little justification in covering filters. With each filter cleaning, all algal material is removed both dead and living. New algal material is brought in by the raw water, and the reaction according to equation (1) predominates, so causing an increase in the oxygen content, which in turn allows more organic matter to be degraded. At the same time the carbon dioxide cannot decrease, rendering the water less corrosive, and the concentration of nutrient salts and organic material is reduced, lessening the load on the filtration processes. The straining and purifying effects on the schmutzdecke in open filters will contribute to the overall efficiency by maintaining the hygienic quality of the effluent, decreasing filter clogging and prolonging filter runs.

Algae have been responsible for a number of problems relating to the use of water as a resource. The drying off of large concentrations of algae (algal blooms) and their resulting decay on beaches makes some waterways undesirable for the depletions of dissolved oxygen and this in turn interferes with the propagation of many fish species. Most important, algae may reduce the filtration capacity of water treatment plants and cause taste and odour problems.

In short, the ability of algae to produce oxygen makes it of extreme significance in sanitary Engineering. Bacteria in conjunction with algae have been used in the oxidation pond method for sewage treatment, on the other hand, the excess growth and death of some algae in water reservoirs has caused the sanitary engineer many problems in taste and odour control.

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# ALGAE AS A SOURCE OF MEDICINE FOR THE PHARMACEUTICAL INDUSTRY

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Algae are simple aquatic microscopic or macroscopic plants which grow in abundance in freshwater streams, ponds, pools, rivers, stagnant and sea water and moist places. Majority of them contain chlorophyll. Some of the species are reddish and bluish in colour.

From ancient time the Chinese and the Japanese have been utilizing algae as food. The use of agar obtained from *Gelidium* sp, in ice cream, candies, jellies, baked food, cosmetics, textile industry and in the preparation of culture media for bacteriological and mycological work is known to man from very early time. Now a days different countries of the world like Indonesia, Malaysia, India, Singapore and Ceylon are utilizing algae as food in sizable quantities. The marine algae *Caulerpa*, *Chaetomorpha*, *Ulva*, *Padina*, *Sargassum*, *Turbinaria* and *Enteromorpha* are extensively used as a diet because of their high nutritive value. These algae have high carbohydrate content of about 50% with fair amount of protein and fat. Certain types of algae are also rich in vitamins and contain a greater variety of mineral salts than any other food. Beside some marine algae are used as crude drug for different ailments in different parts of the world.

The increased demand of medicines prepared from algae attracted the attention of scientists to utilise the algae as a source of medicine. At present most of the material used for the manufacture of different antibiotics prepared from the algae plants are being imported in this country. Foreign trade statistics of Pakistan show that Alginate and Agar Agar wood are being imported to the tune of rupees ten million and ten thousand respectively. Many antibiotic producing algae grow in abundance in fresh waters, throughout Pakistan. Attention may be given for collection and artificial propagation of few algae which are much valued from medicinal point of view. *Chlorella*, *Pandorina* and *Scenedesmus* are freshwater algae of Pakistan from which antibiotics chlorelline, Pandorine and Scenedesmus are obtained. These antibiotics are used in diseases like pneumonia, influenza, tetanus in man, tetanolysin which causes hemolysis of the red blood corpuscles of horse, goat, sheep, rabbit and other animals and dysentery caused by bacteria *Staphylococcus*, *Clostridium* and *Bacillus* species. Similarly *Acetabularia major* is a marine species and is used as a remedy for stones in bladder. Some other species like *Codium muelleri*, *Enteromorpha compressa* are used for the extraction of ascorbic acid and vitamin A & B respectively.

*Euclima gelatinae* is known for the manufacture of Agar Agar used in different industries like textile, confectionery cosmetics, photographic emulsion in large quantities throughout the world. Agar Agar is a dried hydrophilic colloidal substance obtained from *Gelidium cartilagineum*, *Gracilaria conferta*

voides, *Hypnea musciformis*, *Euchenma gelatinae* and *Eucheuma muricatum*. The commercial Agar Agar contains 16.6% moisture, 2.3% protein, 76.2% nitrogen free extract and 3.9% ash. Agar Agar is used in medicine as a laxative. It is also used in dressing certain types of wounds because of its inhibitory action on blood clotting. Biscuits made from Agar Agar and bran are given to diabetic patients.

*Chondrus crispus* grows in the Atlantic Ocean on submerged rocks at or beyond the low water mark off the coast of New England and Ireland and from the shores of Norway to Gibraltar. Its chief constituents are carragenin, compounds of sodium, magnesium, potassium and calcium with chlorine, iodine, bromine and proteins. It is used as demulcent and nutrient in the form of its mucilage as an emollient, demulcent, emulsifying agent and as a vehicle in various skin lotions. It has an anticoagulant power equal to that of heparin.

*Fucus vesiculosus* grows along coasts and borders of inlets of the North Atlantic Ocean between low and high tide marks. The dried thallus constituting the drug occurs in the entire condition or in species. Its chief constituents are a gelatinous substance termed algin, iodine upto 0.21% in fresh condition, bromine, chloride, mannite and about 0.1% volatile oil. It has been used in obesity because of its iodine content. *Rhodymeria palmata* is a cosmopolitan marine algae which is used as a vermifuge.

After a careful study of the medicinal value of algae certain main objects present themselves from medicinal as well as economic point of view. It would be worthwhile if algae especially those which have medicinal properties are propagated. There are many such algae, e.g., *Chlorella*, *Pandorina*, *Scenedesmus* and *Microcystis* that yield the important and most useful antibiotics which are being imported in Pakistan. Therefore it is suggested that we should propagate the medicinal algae on large scales in dams, rivers and lakes. Production of drug by artificial propagation assure regular and constant supply of raw material to the pharmaceutical industry. Further choice of localities with particular type of climate also enable the cultivator to obtain a product with special desirable qualities.

Pakistan have large coastal area as well as water reservoirs like Tarbela, Mangla, Warsak and Rawal lakes where artificial propagation of algae can be easily done as a by product. Very little work with regard to distribution, availability and efficacy of various medicinal algae of Pakistan has been carried out in this country. In order to achieve success in production of medicinal algae one should take into account the climatic and edaphic factors of the site. Fortunately the climatic conditions of Pakistan are suitable for such a programme.

The problem of cultivation of algae on a large scale requires a thorough and intricate scientific technique somewhat different than agricultural crops. So systematic investigations should be carried out with the co-operation of botanist, agriculturist, chemists and pharmacologist. If the medicinal algae is introduced and propagated in Pakistan on commercial scale pharmaceutical industry would benefit immensely thus helping in saving the foreign exchange being spent at present on the import of finished algal products from developed countries of the world.

## **Discussion**

Questions were raised on the species of algae which contain antibiotics, and the extent to which they were being used in Pakistan by the pharmaceutical industry. The author after naming many species containing antibiotics and available in this country stated that there are extensive possibilities of manufacture of medicines with local species as raw materials. The present state of pharmaceutical industry, she stated, could considerably be improved and expanded in the area of antibiotics based on raw material obtainable from local algal species.

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# LABORATORY CULTIVATION OF BLUE GREEN ALGAE OF SALINE SOILS AND RICE FIELDS

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## **Abstract**

The importance of the successful cultivation of the blue green algae cannot be denied as all the studies like morphology, taxonomy, cytology, physiology and genetics depend mostly on the use of laboratory cultures of the organisms.

Soil samples were collected from different saline soils and rice fields of the Punjab. A small amount of each soil sample was used as inoculum in the following different culture media: Beyerink's, Bristol's, Detmer's, Knop's and Uspenski's. The blue green algae of different types alongwith other algae grew in these media. Eighteen strains of blue green algae in unialgal culture from these laboratory cultures of soil samples collected from different areas of the Punjab were isolated. All these strains are potential nitrogen fixers as they contained heterocysts and are now being maintained in nitrogen free culture media. Simple methods used for isolation of these algae from the collected soils are considered. Factors influencing successful laboratory cultivation of blue green algae will also be presented.

## **Introduction**

The importance of laboratory culture of blue green algae cannot be denied since all the studies like morphology, cytology, ontogeny, pathology, physiology, ecology, genetics are dependent largely on successful laboratory cultivation of the organisms Pringsheim (1946), Prescott (1968). The group of organisms about which we are going to talk is named differently by different workers i.e. blue green algae, Myxophyceae, Schizophyceae, Cyanophyceae, Cyanophyta blue green bacteria and Cyanobacteria due to their peculiar characteristics and confusing taxonomic position Fogg, et al (1973) Smith (1975) Stanier (1975). The laboratory cultivation of blue green algae becomes all the more important because this is the only group of photoautotrophs which have some species capable of using atmospheric elemental nitrogen. In addition to nitrogen fixation the blue green algae possess the capacity of salt tolerance. As there are over 17 million acres of saline soils in Pakistan, the blue green algae appear to be quite important for the fertility of our soils. The warm seasonal conditions during most of the year, sufficient sunlight, neutral to alkaline pH and low organic matter in most of these soils are the conditions which are said to be favourable for the growth of nitrogen fixing blue green algae Watanabe (1966). Keeping these considerations in view we started a survey of blue green algae occurring in different saline soils. It has been reported earlier that we were able to identify more than 106 species of blue green algae belonging to 27 genera and out of these

9 genera constituting species appeared to be nitrogen fixers Ali and Sandhu (1972). During this survey work we tried to isolate nitrogen fixing blue green algae and succeeded in isolating eighteen strains of nitrogen fixers in unialgal culture. The details of laboratory methods used during the laboratory cultivation of these blue green algae and their relative usefulness are being discussed in this paper.

### **Collection of Soil Samples**

To know the various types of blue green algae existing in the saline soils, we collected soil samples from different areas of the Punjab. The samples were taken from highly saline soils, moderately saline soils and reclaimed saline soils. Soil samples were collected in polyethylene bags and for this purpose top six inch soil was taken with the help of a soil sampler. One gram of each soil samples was used as inoculum in different culture media for laboratory culture. The rest of the soil was air dried and passed through 2 mm. sieve and used for soil chemical analysis.

### **Media Preparation and Soil Inoculation**

It is well known that the microscopic observation of soil with water on a slide yields only a few individuals and thus it gives a wrong impression of a poor flora. One of the methods to solve this problem is to take some soil and add it to a suitable culture solution Round (1973). We inoculated one gram of each soil sample into each of the culture media, viz., Beyerink's, Bristol's, Detmer's., Knop's and Uspenki's which were also used by Khan (1957). The glass-ware to be used in media preparation and laboratory culture was thoroughly rinsed in tap and distilled water as soaps and other detergents are considered harmful for the algae. For the purpose of media preparation glass distilled water or deionised water was used since chlorine and brass particles from brass pipes are known to be destructive for most of the algae Bold (1942).

The 500 ml. conical flasks containing 250 ml. of one of the above mentioned culture media were plugged with cotton and autoclaved for 15 minutes at 15 lb/sq. inch. After the sterilization and cooling of the media, one gram of each soil was inoculated into the 5 culture media. All these flasks were shaken manually for a few seconds for the purpose of proper mixing and uniform resetting of the soil particles at the bottom of the flasks. The flasks containing soil culture media, and of course plugged with cotton, were illuminated with a bank of fluorescent tubes and tungston bulbs. The light coming out of the tungston bulb was filtered through water containing copper sulphate to absorb the infra red and red rays so that the illumination somewhat resembled the day light Pringsheim (1946). The temperature around these flasks varied from 25 to 35°C.

### **Observations and Isolation**

After about two weeks of soil inoculation algal growth started only in a few flasks and by the end of the fourth week there was sufficient macroscopic algal growth in most of the flasks. It was observed that the algal growth developed at the bottom on soil surface only in a few flasks. Mostly growth was found on the inner sides of the flasks and on the surface of culture medium as a thin scum in almost all the flasks. The growth comprised a mixture of various types of algae in these culture.

Although algal flora developed in such a mixed culture is useful for preliminary data but for further studies unialgal and ultimately axenic cultures are needed Bold (1966). Since the flasks were not shaken during algal growth, mostly the different types of algae developed in small patches. These small patches of algae differed in shape, colour or some other character and this difference helped in isolation of different strains of blue green algae. There are many methods for the isolation of algae like streaking, mixing, spraying, pipetting, micromanipulation and membrane filter technique Pringshiem (1946) Bowyer and Skerman (1968), McCurdy and Hodgson (1974). A very simple method was devised by us and it requires nothing except a microscope, inoculating needle, microscopic slides and cover slips. These things are easily available in almost all the microbiological laboratories and by this method one is sure enough that he has

selected the desired alga. While observing the algal growth care was taken to pick out algal material from each patch separately to avoid mixing of the different types. During this survey it was found that each patch had only one type or preponderance of a particular alga. When it was found interesting to isolate a particular alga, the following method was used. A sterile cover slip was placed onto a sterile microscopic slide. A small drop of sterile water may be placed on the slide before placing the cover slip over the slide to avoid falling down of the cover slip during isolation. A loopful of algal material was taken out with the help of a sterile nickle chromium wire from the desired patch or desired area of algal growth. This material was placed over the cover slip and examined under the low power of microscope without covering the material by another cover slip. While looking under the microscope the whole material was judged for a single desired type by moving the slide with the help of moveable stage of the microscope. If luckily all the on the cover slip was of only one desired type then the whole cover slip alongwith its algal material was transferred into a sterile culture solution. If the material was a mixture of more types (as it usually is), then another loop from the same desired patch was taken on to a cover slip as before. A few such trials mostly provided us only a single type on the cover slip. If the material was not enough, then one of the cover slips with more material of the desired type was selected and further preparations were made from this material to get a single type. In some cases the slide with more desired material was placed under the microscope, and while looking through the microscope the undesired types present on the cover slip were removed onto the slide with the help of a needle until only one desired type was left on the cover slip. Then the cover slip alongwith its material was inoculated into suitable sterile culture medium. The medium may be supplemented with soil extract of the same soil from which the alga is being isolated. This method is quite simple and we have isolated eighteen strains of nitrogen fixing blue green algae in unialgal culture by this methods. It may also be mentioned that if an illuminated and controlled temperature room is not available then, as suggested by Pringsheim (1946), cultivation and isolation may be performed during the warm season, i.e., from April to September in a cool bright room.

### Maintenance and Mass Culture

The isolated nitrogen fixing blue green algal strains are being maintained on a nitrogen free culture medium to avoid contamination. We are using medium C of Kratz and Myers (1955) to avoid slight modification. The modified medium C is less concentrated in case of macronutrients and is supplemented with microelements of Allen and Arnon (1955). This medium proved better for our isolated strains. One of the reason may be its low concentration as dilute culture media have been considered better by other workers also Pringsheim, 1946; Watanabe et. al., 1961). It has been reported that higher concentrations of some substances cause a longer lag phase and concentrations of phosphates in the range 0.1-0.5% are inhibitory particularly to cells growing on elemental nitrogen Fogg (1965), Fogg et al. (1973). The other reason may be the larger number of microelements included in Allen and Arnon' micronutrient solution, as sometimes the amount of the minor elements especially iron becomes limiting factor due to its precipitation and 0.1-0.2 mg/l of manganese is considered critical for uptake of iron Prescott (1948). The availability of ferrous salts is considered to be of great importance as they also affect the distribution of algae in nature Gromov (1968). The modified medium C does not form any precipitate and this helps in easy availability of the nutrients. The composition of "Modified Medium C" is as follows.

MgSO <sub>4</sub> .2H <sub>2</sub> O	..	0.025 g	Sod. citrate	..	0.165 g
K <sub>2</sub> HPO <sub>4</sub>	..	0.1 g	Fe <sub>2</sub> (SO 43)	..	0.004 g
CaCl <sub>2</sub> .2H <sub>2</sub>	..	0.025 g	Microelements	..	1 ml
KCl	..	0.1 g	Distilled Water to make 1 litre		

The pH of this medium is 7.8 and 1 ml of stock solution of Allen and Arnon's microelements was made 1000 times concentrated to provide quantities of microelements used by them, in only one ml. for one litre of the culture medium. The medium may be supplemented with 1% agar when being used for petri-dishes. Since the algal growth requires many days, to overcome the drying of agar medium, we used 25x250 mm test tubes for normal maintenance of isolated strains. To avoid failure of subculture a heavy inoculum of actively growing alga comprising 100-500 filaments should be used Fogg (1965) Stewart (1969). As suggested by Carr et al., (1973) the old cultures should be stored at room temperature in non direct light instead of a refrigerator.

Mostly it happened that cultures in petridishes were contaminated by other organisms carried by ants and similar insects into the petridishes. To get rid of this problem a small table about 2' x 2' with 8" legs was used. All the four legs of this table were placed in beakers containing water soluble cutting oil. This oil does not dry in short period and it blocked the passage of insects so they could not reach the petridishes placed over this small table.

Sometimes when we needed larger masses of algal material for field inoculation or for other studies, we made use of 2 litre and 5 litre flasks. The culture medium was autoclaved in these flasks with rubber stoppers containing two glass tubings one for incoming and the other for outgoing air. After inoculation, the flasks were illuminated and supplied with a mixture of CO<sub>2</sub> and air in the ratio of 5:95. Since the CO<sub>2</sub> causes a decrease in pH by carbonic acid formation, sodium bicarbonate at the rate of 0.5 g. per litre was added to the culture medium. To get rapid growth of the algae the flasks of the algae the flasks were fixed on to a shaker to avoid settling of algal cells and to give them the chance of equal exposure to light. The shaking also helps in proper diffusion of CO<sub>2</sub> being bubbled through the culture medium. The shaker speed also affects the algal growth as reported by Fogg (1965) in case of *Anabaena cylindrica*. The growth of this organism was doubled when the shaking speed was increased to 90 instead of 65 oscillations per minute and growth stopped at 140 oscillations per minute. As suggested by Prescott (1968), for the supply of CO<sub>2</sub> and air mixture to the algal cultures both large and small flasks may be connected either singly or in series to get maximum benefit of CO<sub>2</sub>. For bubbling of air rubber tubing should not be used as they are damaged easily by autoclaving and they may also provide sulphur compounds and other volatile substances which are harmful to the algae Pringsheim (1946). As proposed by Stewart (1962) the CO<sub>2</sub> + air mixture was passed first through 2% NaHCO<sub>3</sub> to remove oxides of nitrogen, then through 50% H<sub>2</sub>SO<sub>4</sub> to get rid of ammonia. After this it passed through about 2 feet long 2 inches wide column containing cotton wool, glass wool and charcoal to eliminate microbes and ultimately passed through sterile distilled water to moisten the air being supplied to the algal cultures.

### Acknowledgements

We should like to thank Mr. Mohammad Rizwan and Mr. Mohammad Ashraf for media preparation and other technical assistance.

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# ROLE OF BLUE GREEN ALGAE IN AGRICULTURE

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The challenging menace to the world today is the shortage of food and increasing population. If the population goes on increasing at the present rate, the existing food resources will rapidly become insufficient. The possible ways to increase the food production are:

- (a) To bring new lands under cultivation.
- (b) To develop high yielding varieties of crops.
- (c) To use more fertilizers.
- (d) To increase supply of water.

Lester R. Brown, President of Worldwatch Institute pointed out that upto the 1950s the expansion of area under cultivation was the major means of increasing the world's food supply. There are still areas of potential expansion given massive inputs of money and effort. In general however, the most fertile and economical viable farmlands are already under cultivation. In recent years the major means of increasing food supply has been to increase yields per acre of the existing farmlands by adopting high yielding varieties which require more water and fertilizer application.

Each ton of fertilizer applied to an under developed country's grain crops could increase the harvest by ten tons, but due to lack of fertilizers and high cost of their production and transport from developed countries has made fertilizers too expensive for the less developed countries.

Out of the three major fertilizer nutrients (N, P & K) nitrogen requirements are maximum and nature has equipped the atmosphere with 79% nitrogen, which is equivalent to 148,000 tons/acre. The atmospheric nitrogen cannot be utilized by plants directly. It can be utilized only after its conversion to combined inorganic or organic form by fixation either chemically or biologically.

It is the biological fixation which should be given more importance. It needs approximately five rupees per acre fixing as much as 500 lbs N/acre or even more under most favourable environments. The biological nitrogen fixation is of two types: (a) symbiotic nitrogen fixation requires the symbiosis of Rhizobium and legume plant and can fix upto 297 lbs. N/acre/year or more or less depending upon type of legume and environments. In the absence of symbiosis, they cannot fix nitrogen.

In non symbiotic nitrogen fixation *Azotobacter* (aerobic-heterotrophs), *Clostridium* (anaerobic-heterotrophs) and blue green algae (aerobic photoautotrophs) are the major group of microorganism involved. *Azotobacter* can fix upto 40 lbs. N/Acre/year, but on the expense of 2000 lbs. of organic matter, used as carbon and energy source which our soils are already short of. The *Clostridium* is anaerobic, grow slowly and hence not as important.

The most important nitrogen fixer is blue green algae-photoautotrophic it can utilise  $\text{CO}_2$  of the air as C source, energy for growth from sunlight and  $\text{N}_2$  from the atmospheric nitrogen and all the three, i.e., C, N, and energy are never scarce in our country. Water and other nutrient (required in small amounts) it can utilize from soil.

The blue green algae can fix more than 5000 lbs nitrogen/acre/year in the laboratory (Cook, 1967). The nitrogen fixed by blue green algae over a period of six weeks ranged from 13.8 to 144.4 lbs/acre and higher values (upto 70.8 lbs/acre) were achieved when phosphatic fertilizers were added. Now, if we assume that paddy crop is of 3 month's duration, the blue green algae can fix from 27.6 to 141.6 lbs/acre in this period (De and Mandal, 1956). In 1972-73, area under paddy was approximately 3.6 million acres and if we take the amount of nitrogen fixed, it would be 99.36 to 509.76 million lbs. Blue green algae can fix nitrogen in semi arid and desert soils and algae in addition to nitrogen fixation fixed the carbon by assimilating the  $\text{CO}_2$  of the atmosphere and getting the energy from sunlight, and hence enriches the soil with organic matter (Fletcher and Martin, 1948, Fuller et al., 1960 and T Gudiogapuram et al., 1975). Algae is also helpful in checking soil/wind/water erosion by means of binding together of soil particles (Booth, 1941).

Little work has been done in Pakistan on the role of algae and being a very important tool for the agriculturists for the improvement of their lands much emphasis should be given to the algae, particularly blue green algae and the scientific work as regards the role of algae in agriculture should be started on these lines:

1. Large scale programme should be started to inoculate the fields with highly efficient strains.
2. Survey of the existing nitrogen fixing strains and the amount of nitrogen fixed under natural conditions.
3. The efficiency of the isolated strain should be studied for paddy soils, irrigated field and un-irrigated/rained areas.
4. The efficiency of inoculation in combination with phosphatic and potassium fertilizers should be studied.

# WATER BLOOMS

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The growth of an alga in abundance is called "Water-blooms". Algal blooms occur in fresh as well as marine waters. The primary cause of water blooms is the abundance of nitrates and phosphates in still waters. They occur in eutrophic, shallow alkaline lakes or ponds or in seas with abundant supply of phosphates and nitrates. In inland waters bicarbonates or excessive  $\text{CO}_2$  is an essential condition for abundant growth of algae.

It is often only one species that dominates the algal population of the blooming-lakes. After the death or decay of the algae, another may succeed. All bloom forming algae are not poisonous. Luckily only a few of them are toxic especially those which are rich in proteins. The toxic algae can cause death to fish, farm animals and humans. In farm animals convulsions, paralysis, jaundice, constipation, loss of appetite, reduction in milk production, abortion and skin sensitizations are some of the symptoms. In extreme concentrations the end result is the death. Fishes are the targets of the blooms particularly.

The effects of blooms forming algae are two sided, direct and indirect. As regards the direct effect algae produce toxins. Endotoxins and exotoxins both have been isolated.

Among the indirect effects are (1) Human poisoning as a result of eating poison-containing fish, (2) Suffocation due to depletion of oxygen, (3) Increase in bacterial population, (4) Production of  $\text{H}_2\text{S}$  and many odorous substances, (5) Death of aquatic angiosperms and useful algae.

Pyrrhophyta contain some bloom forming species which are responsible for blooms in seas like *Gymnodinium brevis* and *G. Veneticum*, these plants are responsible for the red tide of Gulf of Mexico. *Ceratium hirudinella* is probably the only fresh water toxic algae belonging to the Division.

Many members of Chrysophyta are reported to cause blooms such as *Dinobryon sertularia*, *O. sociale* and *Synnura uvella*, along with some diatoms like *Asterionella gracillia*, *Melosira granulata*, *Stephanodiscus niagrae*, *Coscinodiscus* spp, and *Fragillaria* spp.

The Cyanophyta are particularly notorious as bloom forming algae in inland conditions. The toxic blue green algae include *Anabaena circinalis*, *A. Limnetica*, *A. inaequalis*, *A. nadsonii*, *Aphanizomenon flos aquae*, *Collosphaerium Kuetzingianum*, *Aphanotheca cyanea*, *A. nidulans*, *Nodularia spumigena*, *Lynqbya birgei*, *Gloeotrichia echinulata*, *Microcystis toxica*, *M. aeruginosa* and *Oscillatoria* spp.

The Chlorophyta contain probably only two genera with toxic species, viz., *Scenedesmus* and *Chlorella*, while Euglenophyta have only one genus, *Englena* with a few toxic species.

Microcystis has been particularly investigated for toxicity. Its toxin is non volatile, acidic compounds with qualities of quick absorption. It is a peptide composed only of amino acids and behaves as a neurotoxin. Its components include D-Serine, Lornithine and aspartic and glutonic acids (dicarboxylic acid). So far five acids, viz., aspartic, glutonic, lucine, serine, valine, ornithine and alanine have been isolated from extracts of Microcystis. In humans the effect of Microcystis toxin is the enlargement of liver, prohibition of blood clotting and congestion within spleen, causing disease or death.

In Pakistan almost every village has ponds from which farm animals drink water. These ponds are sometimes near heaps of farmyards manures. Nitrates and Phosphates are washed down from the manure to the ponds and thus ideal conditions for blooms are created. It is not necessary that all these ponds contain toxic algae. The observations so far made indicated that Microcystis aeruginosa is probably of much more importance as a toxic alga in this country, although four species of Euglena have been seen forming blooms but the data so far collected is not conclusive. Indications are that Euglena containing ponds are mostly devoid of fish and water insects.

In a village in Mardan, Microcystis aeruginosa was observed forming a bloom. It is interesting to note that no other living organism was found in this pond. Even the hardy diatoms were absent. The villagers informed, on enquiry, that this pond is not used by them and if perchance any buffalo drinks its water even once it falls ill and abortion or reduction of milk is the end result.

Many methods of the control have been worked out including 1-2 ppm. Copper Sulphate, Chlorine, Sodium arsenate, Potassium Permanganate and Activated Carbon.

### **Discussion**

Questions were asked regarding the control of blooms, their toxicity and their effects on fish. The author explained that copper sulphate application is an economical method for the control of blooms. One to two parts per million of copper sulphate he stated will control the blooms in the pond. The author rejected the idea of using lime powder for the control of blooms because by its use more problems would result. The author explained that there are two types of blooms toxic blooms and non-toxic blooms. Non-toxic blooms are helpful in the cultivation of fish. The author observed that most of the blooms in the Peshawar area are not harmful except microcysticous which creates a harmful bloom.