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Chemical composition of cultures and natural samples of N_2-fixing blue-green algae from rice fields

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Summary. Laboratory cultures, soil cultures, and natural samples of N_2-fixing blue-green algae (BGA) from rice fields were analyzed for dry matter, ash, N, C, P, and a few other constituents.

Results show a very large variability of the composition. Dry matter contents ranged from 0.28% to 13.6% (average 3.3%). Ash contents ranged from 15.6% to 71.3%. Nitrogen contents ranged from 1.9% to 11.8% on an ash-free basis (average 6%). Carbon content was less variable, ranging from 37% to 72% and averaging 43.7%.

A decrease in N and pigment contents, and an increase in reducing sugars, was observed in aging laboratory cultures.

Large differences in composition were observed between field samples and material grown in artificial medium. Soil-grown BGA and field samples were characterized by very high ash contents, N contents lower than those in laboratory cultures, and P deficiency.

Extrapolation from (1) average dry matter, ash, and N contents and (2) records of BGA biomass in rice fields indicates that an algal bloom has a potentiality of about 15–25 kg N per hectare and that a BGA biomass of agronomic significance is visible to the naked eye.

Key words: Blue-green algae – Chemical composition – N_2 – fixation – Rice fields – Cyanobacteria

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Despite the abundance of literature on the role of N_2-fixing blue-green algae (BGA) and on their possible use as a source of nitrogen for rice (Roger and Kulasooriya 1980), little is known about their composition. In books and reviews on BGA, discussions on overall composition, if any, are often very short and the average values either are calculated from limited data (Fogg et al. 1973; Wolk 1973) or are not supported by bibliographic references (Mishustin and Shil'nikova 1971).

A comprehensive knowledge of the composition of BGA would be necessary for the proper evaluation of their agronomic significance.

Fragmentary information is available mainly from physiological studies with laboratory strains grown under artificial conditions. Quantitative data on N, protein, C, carbohydrates, P, and ash contents in N-fixing BGA, collected from the literature, are presented in Table 1. Nitrogen content averages 7% and exhibits a fourfold range of variation (2.8% to 11%). Carbon content is less variable (38%–48%). Carbon: nitrogen ratios range from 4.3 to 7.4. Average values, calculated from a restricted number of data obtained with 14 species in 6 genera, are hardly representative and are presented only to satisfy the reader's intellectual curiosity.

This paper summarizes analyses of N-fixing BGA: (1) grown under artificial conditions in the laboratory as flask or mass cultures in liquid medium, (2) grown on soil in a greenhouse, and (3) collected from natural environments.

Results of a study on the mineralization of some BGA in soil are also presented. Potentiality and implications for utilization of BGA as a N source for rice are discussed.

Table 1. Data on composition of N_2-fixing BGA collected from the literature

Reference	Species	Remarks	N	Carbohydrates	C	C:N	P	Ash
Collyer and Fog (1955)	*Anabaena cylindrica*	11 days old	8.6	32	–	–	–	–
		33 days old	6.9	24	–	–	–	–
Cobb and Myers (1964)	*A. cylindrica*	–	10.4	–	48	4.6	1.8/2.1	6/7
		2 days old	5.0	–	–	–	–	–
Fowden (1954)	*A. cylindrica*	–	5.6	–	–	–	–	–
Choi and Markakis (1981)	*A. flos-aquae*	On N_2, urea or peptone	10.1	–	–	–	–	–
	A. flos-aquae	On nitrate	8.0	–	–	–	–	–
	A. flos-aquae	On nitrite	6.4	–	–	–	–	–
Ke Chang Dang and Nikitina (1980)	*A. variabilis*	Exponential phase	4.8	43	–	–	–	–
	A. variabilis	Lysis phase	3.2	20	–	–	–	–
Healey and Hendzel (1975)	*A. variabilis*	P sufficient	10.5	20	45	4.3	1.56	–
	A. variabilis	P deficient	9.0	43	48	5.3	0.70	–
	A. variabilis	P deficient	5.1	50	38	7.4	0.13	–
Shnyukova et al. (1978)	*Aphanizomenon flos-aquae*	Natural bloom	4.8	13	–	–	0.45	–
		Natural bloom	6.7	–	–	–	0.63	–
Tindal et al. (1977)	*Aphanothece halophitica*	On 1 and 2*M* NaCl	9/11	–	–	–	–	–
		On 3*M* NaCl	10/ 6.9	–	–	–	–	–
De Cano and De Halperin (1978)	*A. stagnina*	Exponential phase	6.7	–	–	–	–	–
				–	–	–	–	–
Williams and Burris (1952)	*Calothrix parietina*	–	3.7	–	–	–	–	–
Kokyrsta and Chekoi (1972)	*Nostoc linckia*	–	–	40/85	–	–	–	–
Williams and Burris (1952)	*N. muscorum*	–	6.0	–	–	–	–	–
Mehta and Vaidya (1978)	*Nostoc* sp.	–	–	48	–	–	–	–
Tirol et al. (1983)	*Nostoc* sp.	–	7.3	–	38	5.3	1.13	–
Harper and Daniel (1935)	*Nodularia spumigea*	–	2.8	–	–	–	0.18	–
	Average		7.0	38	43	5.4	0.96	6.5
	Coefficient of variation		34	53	12	22	74	–

Materials and methods

Conditions of growth

Flask cultures in liquid medium. Regular cultures of our BGA collection maintained in 300-ml Erlenmeyer flasks containing 100 ml GO medium (Rippka et al. 1979) and incubated at the ambient temperature under continuous illumination provided by white cold fluorescent tubes (600 lux) were used. Forty-six samples corresponding to 32 strains of 10 genera were analyzed. Twenty-two cultures were collected before 4 weeks of growth and their dry matter, N, C, reducing sugars, chlorophyll, and phycocyanin contents were measured. Twenty-four other cultures were collected between 4 and 8 weeks of growth and were similarly analyzed.

Mass cultures in liquid medium. Seven strains were grown in 20-l carboys, in GO medium with Na_2CO_3 concentration increased tenfold, continuous bubbling with 0.5% CO_2 in air, continuous stirring, and lighting. Light intensity was increased along the growth period using one to six fluorescent tubes placed vertically 20 cm away from the carboys. Cultures were harvested at the end of the logarithmic phase of growth.

Artificial blooms. In a greenhouse experiment in 0.5-m^2 trays, designed to study the growth of indigenous or inoculated BGA on four submerged soils (acidic, neutral, alkaline, and peat soils), blooms developed at the floodwater surface. Experimental conditions ensured minimum disturbance in the trays. Water was maintained at 5 cm depth with demineralized water and never became muddy. One month after submersion of the soils, floating blooms were harvested, taking care not to disturb the soil.

Soil-based inocula. Algal material was produced in shallow trays, 1 m × 1m, made with a polyethylene film placed on a wooden frame on tables in a greenhouse. Six strains were first grown in 20-l carboys on GO medium. Cultures were harvested shortly after the logarithmic phase of growth and inoculated into the trays. One day before inoculation each 1-m^2 tray received 5 kg sieved dry Maahas soil (aquic Tropudalf, pH 6.9), 10 g superphosphate, 2 g NaCl, 2 ml ethylan (to control grazers), and was flooded to 5 cm with demineralized water. This method of producing soil-based inoculum was

most efficient under dry season conditions in the Philippines (IRRI 1985). After 2 weeks, most of the algal mat that developed at the soil-water interface became detached and floated at the floodwater surface in trays inoculated with *Anabaena*, *Aulosira*, *Nostoc*, and *Tolypothrix*. For *Fischerella* and *Scytonema*, an algal growth was obvious at the soil surface and O_2 bubbles were visible, but the mat neither became detached nor floated. Floating algal mats and the upper layer of the soil colonized by algae were collected and combined in each tray. Being highly contaminated with soils, this material cannot be considered only as BGA. Data have been included because information on the composition of soil-based inocula is lacking and to provide information on the potential biomass of natural algal blooms. Nitrogen content in the ash-free algal material was calculated on the basis of a 40% carbon content in BGA.

Field samples. Floating colonies or algal masses were collected from rice fields in the IRRI farm and the surrounding area using a net, 20 cm in diameter, 1 mm mesh. Material was rinsed with rice field floodwater and organic debris sorted out. Analysis was performed shortly after collection.

Strains. Thirty strains of ten genera have been used for analysis (Table 2). Strains obtained through the courtesy of Dr. Rippka (PCC strains of Pasteur Institute) were axenic. Other strains were unialgal. Two strains of *Oscillatoria*, a genus usually considered non-N_2-fixing in aerobiosis, have also been included.

Methods of analysis. Dry weight and ash were determined on pellets from algal suspension centrifuged at 10 000 rpm for 15 min. Dry weight was measured after 24 h of heating at 80°C in an oven, and ash content by heating the material at 325°C until smoking ceased and then at 480°C overnight. Mineral contents were measured using analytical methods for plants of the IRRI analytical laboratory.

Proteins, carbon, sugars, and pigments were measured using algal suspensions sonicated for 5 min (Sonicator Model W1851, Heat Systems-Ultrasonics, Inc.) in an ice bath. Protein content was measured by the Folin-Ciocalteau phenol method (Lowry et al. 1951) on samples hydrolyzed with NaOH for 1.5 h at 60°C. Bovine serum albumin was used as standard. Carbon was measured by the Walkley and Black method (Black 1965). Sugars were measured by the phenol-sulfuric acid method (Dubois et al. 1956). Chlorophyll *a* and phycocyanin were measured from the absorption spectra of sonicated material according to the equations of Myers and Kratz (1955). Nitrogen in artificial BGA blooms was measured by the standard micro-Kjeldahl method. Data, except dry matter, are expressed on a dry weight basis.

Statistical analysis. Results of analysis of the 46 samples grown in flask cultures were used for cross-correlation study and cluster and multivariate analysis, utilizing the computer facilities and the "CLUSTAN" program of the Agricultural Research Center of the University of the Philippines at Los Bãnos. Dendrograms were drawn from three major components using Ward's method of pooling.

Mineralization study. Water-saturated soil from the IRRI farm (Maahas soil) was passed through a 2-mm sieve. A quantity equivalent to 12.5 g dry weight was placed in a stoppered glass tube 2 cm in diameter and 25 cm in length. Algal and control materials equivalent to 3 mg N (120 ppm dry soil) were placed on the soil and covered with the same quantity of water-saturated soil. Distilled water was added to a height of 4 cm above the soil surface. Tubes

Table 2. Strains and number of samples utilized in the different studies

Strain	Origin	A	B	C	D	Ref. No.
Anabaena variabilis	UPLB, Philippines, Dr. Martinez	1				1
Anabaena sp.	C.A. Dr. Van Baalen		1		1	2
Anabaena sp.	Dr. Newton		1			3
Anabaena sp.	China		1			4
Anabaena sp.	PCC 7120, Dr. Rippka	1	1			5
Anabaena sp.	PCC 7122, Dr. Rippka		1			6
Anabaena sp.	IRRI Greenhouse			1		7
Aphanothece sp.	IRRI farm, Upper MN			1		8
Aulosira fertilissima	Cambridge culture collection	1	1		1	9
Calothrix sp.	PCC 7101, Dr. Rippka		1			10
Calothrix spp. (2)	Upper Banaue, Philippines		1			11–12
Calothrix spp. (3)	Banaue, Philippines		1	6		13–15
Fischerella spp. (2)	Kiangan, Philippines		2			16–17
Fischerella sp.	Banaue, Philippines	1	1	1	1	18
Gloeotrichia sp.	UPLB, Philippines, Dr. Martinez		1			19
Gloeotrichia spp. (2)	Laguna, Philippines		1	4		20–21
Nostoc sp.	Sri Lanka, Dr. S.A. Kulasooriya	1		3	1	22
Nostoc spp. (2)	UPLB, Philippines, Dr. Martinez		2	2		23–24
Nostoc sp.	PCC 73102, Dr. Rippka		1			25
Nostoc sp.	Upper Banaue, Philippines		1	3		26
Nostoc sp.	Banaue, Philippines		1			27
Nostoc sp.	Luisiana, Philippines		1			28
Oscillatoria sp.	PCC 7515, Dr. Rippka		1			29
Oscillatoria sp.	IRRI Farm			1		30
Scytonema sp.	IRRI Farm, Upper MN	1		1	1	31
Tolypothrix tenuis	Dr. I. Watanabe, strain	1	1	1	1	32
Total		7	22	24	6	

A: laboratory mass cultures; B: flask cultures less than 4 weeks old; C: flask cultures older than 4 weeks; D: soil-based inocula. Reference numbers are those used in the Appendix for flask cultures

were covered with "Parafilm" and incubated in darkness at 30°C. At time intervals, triplicate samples were taken and extracted with 200 ml 2*N* KCl for 30 min with a wrist-action shaker. Exchangeable NH_4-N was determined by steam distillation with MgO.

Results and discussion

Laboratory flask cultures

Average values and variability. Pooled numerical data from cultures harvested before and after 4 weeks of growth are given in the Appendix. Average values and an analysis of the variability of the data are presented in Table 3. A graphic summarization of the data is presented in Fig. 1. Pooled data showed that dry matter averaged 3.72% and exhibited large variations [coefficient of variation (CV) = 66%] from 0.28% to 13.1%. Lower values were observed in the mucilaginous genera *Gloeotrichia*, and *Nostoc*. Non-mucilaginous *Calothrix* and *Fischerella* had above-average values. Nitrogen content averaged 5% and exhibited moderate variations (CV = 31%) with values ranging from 3.40 to 8.26. Compared with other genera *Anabaena* had a higher N content (6.7%). Carbon content averaged 41.6% and had the lowest variability (CV = 20%). Carbon: nitrogen ratio averaged 8.09 and ranged from 5 to 13. The two extreme values were observed in the same genus (*Anabaena*). Chlorophyll averaged 0.7% and phycocyanin 5.2%. Both exhibited a wide range of variation as shown by coefficients of variation of 81% for chlorophyll and 67% for phycocyanin.

To some extent Fig. 1 and Table 3 allow a comparison between intergeneric and intrageneric variability of the composition. The average value of the CV of the five genera (average generic CV) is an estimate of intrageneric variability whereas the CV of the mean

Table 3. Average generic values and variability of the contents in major components in N_2-fixing BGA grown in flask cultures

Genera		DM	N	C	C:N	Sugars	Chl *a*	Phyc	Average[a] CV
Anabaena	*n*	6	6	5	5	6	6	6	
	Mean	4.07	6.66	51.1	7.46	30.0	1.61	11.6	
	CV	73	28	20	42	28	46	35	39
Calothrix	*n*	9	9	3	3	9	9	9	
	Mean	6.26	5.38	35.6	6.61	56.4	0.62	3.62	
	CV	13	20	10	24	32	80	23	30
Fischerella	*n*	4	4	3	3	4	4	4	
	Mean	6.48	3.85	35.6	8.09	43.3	0.48	2.40	
	CV	69	42	11	25	26	76	44	42
Gloeotrichia	*n*	6	6	2	2	6	6	6	
	Mean	1.70	4.89	39.5	8.28	64.2	0.61	3.95	
	CV	94	36	10	24	41	67	49	46
Nostoc	*n*	14	14	6	6	14	14	14	
	Mean	2.62	4.83	42.2	9.12	51.0	0.61	5.67	
	CV	61	26	10	34	26	71	38	38
Other genera	*n*	7	7	3	3	7	7	7	
	Mean	3.81	4.10	nd	nd	67.3	0.48	3.40	
	CV			Not taken into account					nd
Pooled data	*n*	46	46	22	22	46	46	46	
	Mean	3.72	4.99	41.6	8.09	53.2	0.71	5.19	
	CV	66	31	20	31	37	81	67	48
	Max.	13.1	8.26	67.3	13.2	114!	2.45	15.5	
	Min.	0.28	1.78	29.7	4.82	19.9	0.08	1.14	
Average generic CV		62	30	12	30	31	68	40	39[b]
CV of the means[c]		48	20	16	12	26	59	67	35[d]

Nitrogen (N), Carbon (C), sugars, chlorophyll *a* (Chl *a*), and phycocyanin (Pcy) are expressed in percentage of dry weight. Dry matter content (DM) is in percentage of fresh weight. Original data are presented in the Appendix.

n: number of data: CV: coefficient of variation as percentage of the mean

[a] Intrageneric CV
[b] Average intrageneric CV
[c] Intergeneric CV
[d] Average intergeneric CV

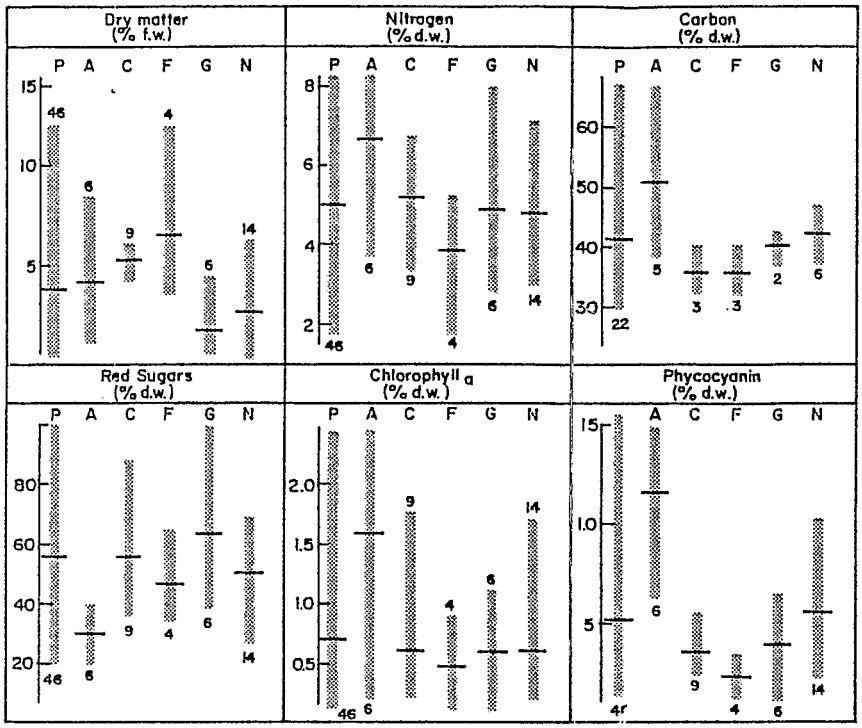

Fig. 1. Graphic summarization of the analysis of N_2-fixing BGA grown in flask cultures. *Vertical bars* show extreme and average values for the pooled data (*P*) and for each genus (*A: Anabaena; C: Calothrix; F: Fischerella; G: Gloeotrichia; N Nostoc*). Number of strains analyzed is indicated on the *top* or *bottom* of each *bar*

values of the five genera is an estimate of the intergeneric variability. For dry matter, N, sugars, and chlorophyll, average intrageneric variability was larger than the intergeneric variability. For carbon and phycocyanin average intrageneric variability was lower than the intergeneric variability (Table 3). This was mostly due to a higher mean value and larger variability of *Anabaena* strains which appeared to be clearly separated from the other genera (Fig. 1). When all variables are considered together (last column, Table 3), it appears that average intrageneric variability (39%) was slightly higher than intergeneric variability (35%).

Effect of the age of the culture. The comparison of cultures less than and older than 4 weeks (Fig. 2) showed a decrease in N and pigment contents and an increase in sugars in older cultures. Results for dry matter are variable but apparently there was a decrease in dry matter content in older mucilagenous strains (*Anabaena, Gloeotrichia, Nostoc*) and an increase in nonmucilagenous strains (*Calothrix, Fischerella*).

Correlations between the constituents. When samples less than 4 weeks old, for which C analysis was performed, are considered (Table 4A), a highly significant positive correlation was observed between pro-

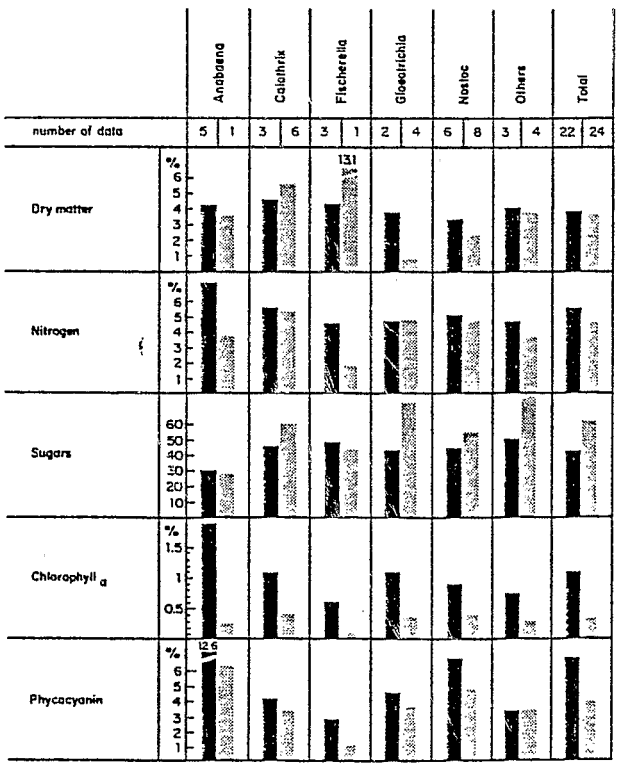

Fig. 2. Influence of age of culture on dry matter, N, sugars/chlorophyll *a*, and phycocyanin contents. The numbers under each strain are the number of data analyzed. *Solid bars;* cultures less than 4 weeks old; *shaded bars:* cultures more than 4 weeks old

Table 4. Coefficients of correlation between the contents in some major components of N_2-fixing BGA

A. 22 cultures less than 4 weeks old					
					$n = 22$
Proteins	+0.31 ns				r 1% = 0.52
Sugars	−0.32 ns	−0.59**			r 5% = 0.41
Chl *a*	−0.02 ns	+0.72**	−0.71**		
Phycocyanin	+0.07 ns	+0.69**	−0.61**	+0.61**	
Carbon	−0.42*	+0.19 ns	−0.23 ns	+0.54**	+0.34 ns
	Dry matter	Proteins	Sugars	Chl *a*	Phycocyanin
B. 24 cultures older than 4 weeks					
					$n = 24$
Proteins	−0.29 ns				r 1% = 0.50
Sugars	−0.15 ns	+0.31 ns			r 5% = 0.40
Chl *a*	−0.26 ns	+0.59**	−0.13 ns		
Phycocyanin	−0.34 ns	+0.60**	−0.02 ns	+0.46*	
	Dry matter	Proteins	Sugars	Chl *a*	
C. Pooled data (A + B)					
					$n = 46$
Proteins	−0.02 ns				r 1% = 0.37
Sugars	−0.13 ns	−0.19 ns			r 1% = 0.37
Chl *a*	−0.01 ns	+0.63**	−0.59**		
Phycocyanin	−0.06 ns	+0.67**	−0.43**	+0.66**	
	Dry matter	Proteins	Sugars	Chl *a*	

*: significant at 5% level; **: significant at 1% level

tein and pigment (chlorophyll and phycocyanin) contents. A highly significant negative correlation was observed between sugar and protein contents, and between sugar and pigment contents. A negative correlation was observed between C content and dry matter but not between sugar content and dry matter. There was no significant correlation between carbon and sugar contents.

Negative correlations observed between sugars and proteins or pigments in young cultures appear to be age related because they became nonsignificant in older cultures (Table 4B).

Pooled data (Table 4C) showed a positive correlation between protein and pigment contents and chlorophyll *a* and phycocyanin contents, and a negative correlation between sugars and pigment contents. No correlation was found between sugars and protein contents.

Dendrogram analysis. Dendrogram analysis of pooled data (Fig. 3) shows a division into two major groups (1–33 and 34–46). The first group contains all strains older than 4 weeks and some strains younger than 4 weeks. The second group comprises strains younger than 4 weeks. Further grouping of the strains shows some tendencies but no rigorous grouping of the species. The following groups can be distinguished: 1–10, dominated by mucilaginous strains older than 4 weeks; 11–22, dominated by nonmucilaginous strains but there is no clear effect of the age of the culture; 23–33, dominated by culture older than 4

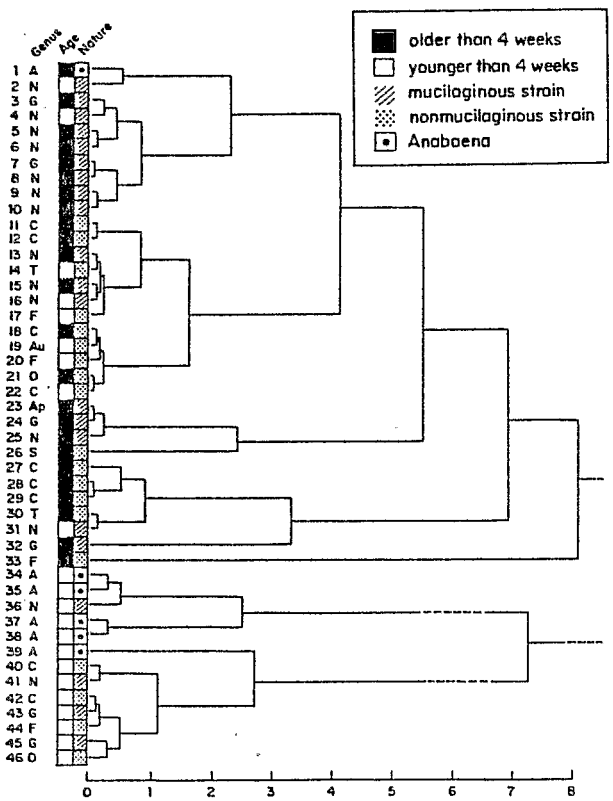

Fig. 3. Dendrogram analysis of the composition of the flask cultures

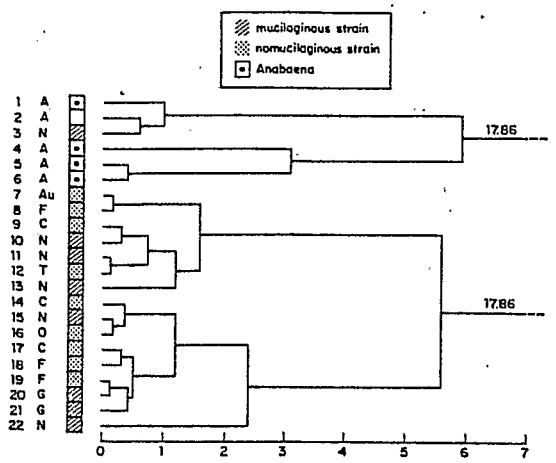

Fig. 4. Dendrogram analysis of the composition of flask cultures less than 4 weeks old

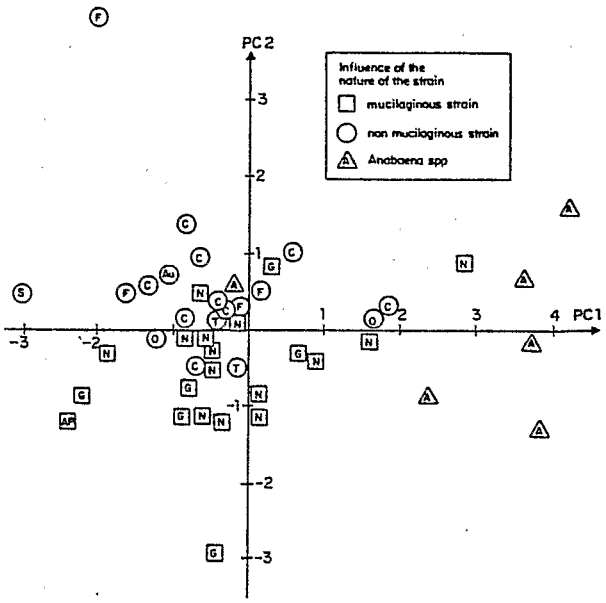

Fig. 5. Principal components analysis of the composition of flask cultures: distribution in scores and nature of the strain. *A: Anabaena; Ap: Aphanathece; Au: Aulosira; C: Calothrix; F: Fischerella; G: Gloeotrichia; N: Nostoc; O: Oscillatoria; S: Scytonema; T: Tolypothrix*

weeks, both mucilaginous and nonmucilaginous strains are included; 34–39, *Anabaena* more than 4 weeks old; 40–46, cultures less than 4 weeks old.

When cultures less than 4 weeks old, for which 5 variables were taken into account for dendrogram establishment (Fig. 4), are considered, only a grouping of *Anabaena* strains is obvious (1–6); other genera are split between the two other subgroups (7–13 and 14–22).

Multivariate analysis. Principal component analysis of the pooled data (Table 5) shows that the first two components represented about 74% of the total variance. The first component had major values of loading on N and pigment contents (positive) and sugar content (negative). The second component had major loading on dry matter content (positive) and sugar content (negative). When the scores of the strains and their nature (Fig. 5) are considered, three overlapping clouds can be distinguished: *Anabaena* strains, mucilaginous strains, and nonmucilaginous strains. When the scores of the strains according to their age (Fig. 6) are considered, a grouping into two overlapping clouds and a larger variability of the composition of young strains compared with old strains is obvious.

Laboratory mass cultures

The six strains used (*Anabaena variabilis, Aulosira fertilissima, Fischerella* sp.,*Scytonema* sp., *Nostoc* sp., and *Tolypothrix tenuis*) constitute a representative sample of the strains frequently recommended for inoculation in rice fields (Venkataraman 1981). When grown under artifical conditions and harvested at the end of the logarithmic phase of growth , these strains had an N content, on an ash-free basis, averaging 8% and exhibiting relatively low variations among strains (CV = 9%) (Table 6). The carbon content averaged 45% (ash-free basis) and had a remarkably low range of variation (CV = 4.6%). The carbon: nitrogen ratio ranged from 4.8 to 6.3 and

Table 5. Principal components analysis of the composition of the flask cultures and loading factors ($n = 46$)

		Dry matter	Nitrogen	Sugars	Chl *a*	Phyco-cyanin	% of variance	Cumulate % of variance
Loading factors	1	0.005	0.488	−0.405	0.557	0.536	52.4	52.4
	2	0.877	−0.225	−0.401	−0.028	−0.135	21.5	73.9
	3	0.472	0.541	−0.675	−0.111	0.129	15.3	89.3
	4	0.044	−0.276	−0.051	−0.552	0.784	6.5	95.8
	5	0.081	−0.585	0.465	0.610	0.250	4.2	100

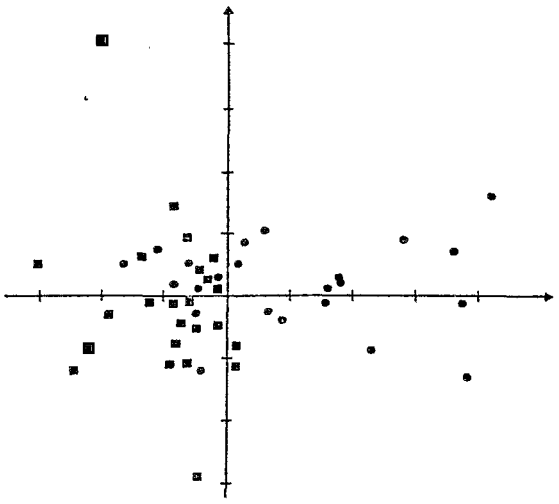

Fig. 6. Principal components analysis of the composition of flask cultures: distribution in scores and age of the strain influence of the age of the culture: ■ – older than 4 weeks; • – younger than 4 weeks

averaged 5.6. Ash content averaged 7.5% and ranged from about 6% to 12%. Average cation contents (P, K, Mg, Ca) were of the same order of magnitude, ranging from 0.55% to 0.65%. Variability was higher than that of C and N. Highest variability was observed for Mg content. Concentrations of oligoelements (Cu, Mn, Zn, Fe, Al, Na) ranged from a few ppm (Cu) to 2000 ppm (Fe). All exhibited a very high degree of variation (CV = 69%) whereas other oligoelements exhibited moderate variation (CV = 23%–29%).

Blooms produced on soil in trays

Algal blooms produced on soil had average contents of 26.7% ash, 6.3% N and 42.8% C on an ash-free basis (Table 7). The lowest variability was observed

Table 6. Composition of mass cultures of BGA harvested at the end of the logarithmic phase of growth. Data are expressed on a dry weight basis

	Ash	N	N ash free	C	C ash free	C:N	P	K	Mg	Ca	Cu	Mn	Zn	Fe	Al	Na
	(%)	(%)	(%)	(%)	(%)		(%)	(%)	(%)	(%)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)
Anabaena variabilis	6.48	7.88	8.42	40.8	43.6	5.18	0.62	0.59	0.54	0.60	18	605	62	1586	156	0.82
Aulosira fertilissima	5.65	7.62	8.08	41.9	44.4	5.50	0.61	0.48	0.48	0.49	34	545	83	1414	188	0.58
Tolypothrix tenuis	6.14	6.92	7.37	41.6	44.3	6.01	0.53	0.47	0.39	0.60	23	505	44	1416	611	0.80
Scytonema sp.	8.85	6.29	6.90	39.7	43.5	6.31	0.40	0.65	0.44	0.68	24	561	59	2085	963	1.48
Fischerella sp.	11.80	7.04	7.98	41.1	46.6	5.84	0.75	0.57	1.09	1.02	22	925	92	2345	1630	0.93
Nostoc sp.	7.50	8.55	9.24	41.1	44.4	4.81	0.66	0.63	0.48	0.67	17	677	42	1845	904	1.04
Anabaena 7120	5.58	7.68	8.13	44.6	49.3	5.81	0.56	0.46	0.45	0.49	31	431	51	1250	664	0.47
Mean	7.42	7.42	8.02	41.5	45.1	5.64	0.59	0.55	0.55	0.65	24	607	66	1705	731	87
CV%	30.2	9.93	9.31	3.6	4.6	9.1	18.6	14.4	43.6	27.7	26.1	26.3	29.7	23.5	69.2	28.6

CV: coefficient of variation as a percentage of the mean

Table 7. Composition of blooms produced in soil trays in a greenhouse

Strain	*Gloeotrichia*	*Aulosira*	*Anabaena*	*Cylindrospermum*	Average	CV (%)
Remarks	Average of two composite samples (2 × 4)	One composite samples (4)	One sample	One sample		
Dry matter (% fresh weight)	0.94	6.41	2.45	6.28	4.02	68
Ash (% dry weight)	27.3	27.5	15.0	37.2	26.75	34
N (% dry weight)	3.01	5.83	6.44	4.13	4.85	32
N (% dry weight, ash free)	4.00	7.34	7.43	6.29	6.26	25
C (% dry weight)	30.14	31.1	39.4	28.8	32.36	15
C (% dry weight, ash free)	40.0	41.9	45.4	43.8	42.8	5
C:N	10.0	5.3	6.1	6.9	7.07	29
P (% dry weight)	0.14	0.35	0.39	0.38	0.31	37
K (% dry weight)	0.32	0.39	0.47	0.19	0.34	35
Mg (% dry weight)	0.43	1.50	0.43	0.31	0.64	79
Ca (% dry weight)	1.53	2.19	2.62	1.85	2.05	23

for C (CV = 5%). Coefficients of variation were 34% for ash and 25% for N.

Monospecific soil-based inocula

Soil-based inocula were characterized by very high ash contents (78.4%–81.3%), obviously due to the soil harvested with the algal material (Table 8). Nitrogen in ash-free algal material, calculated from the N and ash contents in the harvested material and in the soil utilized for multiplication, ranged from 5.9% to 8%. Nitrogen content of the standing biomasses was equivalent to values ranging from 11 to 19 kg N/ha.

Field samples

General results. Analysis of 11 samples of field-grown BGA is presented in Table 9. Ash content was high and averaged 52%. Nitrogen content on an ash-free basis averaged 4.8%. Carbon content on an ash-free basis averaged 40% and exhibited low variability (CV = 7%). The carbon: nitrogen ratio averaged 8.5 and ranged from 6.6 to 11.6. The phosphorus content averaged 0.1% and K 0.3%. Because of the high ash content of the samples, Mg and Ca were high and exhibited large variability.

Detailed analysis of a mucilaginous bloom. Table 10 presents the analysis of a bloom of mucilaginous N_2-fixing strains which has been observed yearly since 1980 in non-N plots of IRRI's farm during the dry season. It consisted of *Aphanothece* as the dominant genus and *Gloeotrichia* as an associated one.

The material was characterized by low dry matter content (1.31%), high ash content (59%), and low N content even on an ash-free basis (3.8%).

Although the colonies were carefully washed and drained, collected material had a high ash content. This indicates that mucilage of the algae absorbs soil

Table 8. Composition and productivity of monospecific soil-based inocula of N_2-fixing BGA

Strain	Soil-algal mat				BGA (ash free)		
	Dry weight (g/m^2)	N (%)	C (%)	Ash (%) (kg/ha)	Dry weight (kg/ha)	Algal N (kg/ha)	% N
Soil before inoculation	–	0.150	1.33	84.4	–	–	–
Anabaena variabilis	313	0.509	3.78	78.5	176.0	15.94	6.32
Aulosira fertilissima	470	0.545	3.92	79.0	278.6	13.24	7.03
Fischerella sp.	273	0.758	4.73	78.4	212.5	13.29	5.88
Nostoc sp.	377	0.563	4.25	79.3	252.1	11.50	6.53
Scytonema sp.	430	0.444	3.24	81.3	188.3	18.98	6.81
Tolypothrix tenuis	356	0.514	3.92	79.8	226.2	16.91	7.96

Table 9. Analysis of field samples of N_2-fixing BGA (all data in percentage dry weight)

Sample	Location	Ash	N	N ash free	C	C ash free	C:N	P	K	Mg	Ca
Aphanothece + *Nostoc* bloom	Nueva Ecija	46.1	2.83	4.89	26.1	45.1	9.2	0.122	0.343	1.22	8.30
Nostoc commune	Luzon	30.7	3.20	4.49	29.7	41.7	9.3	0.050	0.172	7.49	1.18
Mixed algal flakes	India	64.4	2.34	5.97	15.4	39.2	6.6	0.121	0.271	0.45	2.71
Aphanothece + *Nostoc* bloom	IRRI	71.3	1.62	4.76	12.8	37.8	7.9	0.113	0.320	2.25	6.13
Aphanothece bloom	IRRI	43.8	2.49	4.11	23.1	38.1	9.3	0.181	0.569	2.07	3.95
Nostoc bloom	IRRI	55.9	2.76	5.63	18.9	38.7	6.9	0.159	0.475	1.56	2.10
Nostoc bloom	IRRI	45.6	2.44	4.15	21.4	36.6	8.8	0.081	0.350	3.11	7.77
Aphanothece + *Gloeotrichia* bloom	IRRI	58.8	1.75	3.82	20.4	44.5	11.6	0.074	0.388	3.29	7.20
Nostoc bloom	IRRI	55.2	2.64	5.32	18.9	37.6	7.1	0.142	0.285	1.92	1.04
Aphanothece bloom	IRRI	nd	2.93	nd	nd	nd	nd	nd	nd	nd	nd
Nostoc commune colonies	Mangatarem	49.6	2.72	4.99	21.4	39.3	7.9	0.109	0.122	5.23	1.16
Mean		52.1	2.52	4.81	20.8	39.9	8.5	0.115	0.329	2.86	4.15
CV %		22	19	14	23	7	18	34	40	73	70
Maximum		71.3	3.20	5.97	29.7	45.1	11.6	0.181	0.569	7.49	8.30
Minimum		30.7	1.62	3.82	12.8	36.6	6.6	0.050	0.122	0.45	1.04

Table 10. Analysis of a bloom of mucilaginous strains

	With ash	Ash free
Dry matter	1.31	ns
Ash (%)	58.8	ns
C (%)	20.4	44.5
N (%)	1.75	3.82
C:N	9.93	
P (%)	0.07	0.16
K (%)	0.40	0.85
Mg (%)	3.29	7.18
Ca (%)	7.20	15.7
Na (%)	2.03	4.42
Fe (%)	1.35	2.94
Al (%)	2.20	4.78
Cu (ppm)	30.3	66.1
Mn (ppm)	705	1535
Zn (ppm)	52	114
B (ppm)	641	1397
Chlorophyll *a* (%)	0.15	0.33
Phycocyanin (%)	1.36	2.94

[a] Dry matter is expressed on a fresh weight basis; other data are expressed on a dry matter basis

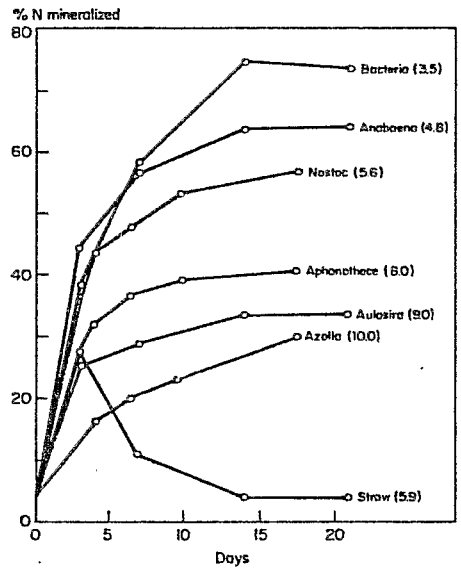

Fig. 7. Nitrogen mineralization rates of different BGA compared with bacteria, *Azolla*, and straw. *Figures* in *parenthesis* are C/N values

particles. Therefore, values presented in Table 10 for major cations and oligoelements do not show the composition of the algae but that of a mixture of algal material and soil particles. Soil where the bloom developed had 84.4% ash, 1.23% C, and 0.13% N in the upper horizon. Assuming a 40% C content in ash-free algal material and 7% ash in algal material free of soil, and using values from Table 1 for ash and C content, the content of soil particles in the harvested material is about 60% of the dry weight.

A similar calculation from ash and N contents from Table 1, assuming 7% ash in algal material free of soil particle, led to a similar value of about 61% of soil particles in the harvested material.

The high boron content of the harvested material is in agreement with the boron toxicity reported at the IRRI farm.

Mineralization study

Results of the mineralization study (Fig. 7) show a clear correlation between the C:N ratio of the material and the percentage of N mineralized at a given time. Depending on the C:N ratio of the strain, between 30% and 65% of BGA nitrogen was mineralized in 3 weeks.

General discussion

Components

Dry matter. Dry matter content in laboratory-grown strains averaged 3.7%. The highest values were recorded for a *Fischerella* strain (13%) and an *Anabaena* strain (8.5%). The lowest values were recorded for mucilaginous strains of *Nostoc* (0.28%) and *Aphanothece* (0.30%). In blooms produced on soil, the average value was close to that of laboratory strains (4.02%). The highest value was recorded for *Aulosira* (6.4%) and the lowest for a mucilaginous *Gloeotrichia* (0.94%).

A few values recorded from field samples showed that species forming mucilaginous colonies of a definite shape (*Aphanothece, Nostoc, Gloeotrichia*) usually have a low dry matter content of about 1%–2%. Roger and Kulasooriya (1980) reported values of 2.2% for *Nostoc* and 0.74% for *Gloeotrichia* spp. Mucilaginous BGA can develop very impressive blooms, but the corresponding dry matter is low. Roger et al. (1985) recorded blooms of *Aphanothece* and *Nostoc* ranging from 7 to 33 t/ha fresh weight, corresponding to 74–132 kg/ha dry weight and 1.5–2.6 kg N/ha only.

Another characteristic of dry matter content is its large variability, as shown by coefficients of variation of 50%–80% (Table 11). The variability is due partly to the nature of the strain. Mucilaginous genera have less dry matter than nonmucilaginous ones. However, larger variability was also observed between species of the same genus. For example, values ranging from 1.0% to 8.5% have been recorded for *Anabaena* and values ranging from 0.28% to 6.3% have been recorded for *Nostoc* (Appendix). In labora-

Table 11. Summarization of the data on the composition of N_2-fixing BGA. Data are expressed as percentage of dry weight except dry matter (% fresh weight) and C:N

		Dry matter	Ash	N	N (ash free)	C	C (ash free)	C:N	P	K	Mg	Ca
Data from	Mean	na	6–7	7.0	7.5[a]	43.0	46.0[a]	5.4	0.96	na	na	na
the literature	*n*	–	2	24	24	5	5	5	9	–	–	–
n variable	Max.	–	–	11.0	11.8	48.0	51.6	7.4	2.10	–	–	–
	Min.	–	–	2.8	3.0	38.0	40.9	4.3	0.18	–	–	–
	CV %	–	–	34	34	12	12	22	74	–	–	–
Laboratory mass	Mean	nd	7.4	7.42	8.02	41.5	45.1	5.6	0.59	0.55	0.55	0.56
cultures at expo-	Max.	–	11.8	8.55	9.24	44.6	49.3	6.3	0.75	0.63	1.09	1.02
nential phase of	Min.	–	5.6	6.22	6.90	39.7	43.5	4.8	0.40	0.46	0.39	0.49
growth. *n* = 7	CV %	–	30	10	9	4	5	9	19	14	44	28
Laboratory flask	Mean	3.85	nd	5.48	5.89[a]	41.6	44.7[a]	8.1	nd	nd	nd	nd
cultures less than	Max.	8.50	–	8.26	8.88	67.3	72.0	13.0	–	–	–	–
4 weeks old	Min.	0.28	–	3.40	3.65	31.9	34.3	4.8	–	–	–	–
n = 22	CV %	54	–	28	28	20	20	31	–	–	–	–
Laboratory flask	Mean	3.56	nd	4.32	4.64	nd	nd	nd	nd	nd	nd	nd
cultures older	Max.	13.64	–	8.02	8.60	–	–	–	–	–	–	–
than 4 weeks	Min.	0.30	–	1.79	1.92	–	–	–	–	–	–	–
n = 24	CV %	83	–	34	34	–	–	–	–	–	–	–
Artificial blooms	Mean	4.02	26.7	4.85	6.26	32.4	42.8	7.1	0.31	0.34	0.64	2.05
produced on soil	Max.	6.41	37.2	6.44	7.43	39.4	45.4	10.0	0.39	0.47	1.50	2.62
four composite	Min.	0.94	15.0	3.01	4.00	28.8	40.0	5.3	0.14	0.19	1.31	1.53
samples	CV %	68	34	32	25	15	5	29	37	35	79	23
Field samples	Mean	nd	52.1	2.52	4.81	20.8	39.9	8.5	0.12	0.32	2.86	4.15
n = 11	Max.	–	71.3	3.20	5.97	29.7	45.1	11.6	0.18	0.57	7.49	8.30
	Min.	–	30.7	1.62	3.82	12.8	36.6	6.6	0.05	0.12	0.45	1.04
	CV %	–	22	19	16	23	7	18	34	40	73	70
Pooled values	Mean	3.28	27.6	5.34	6.03	36.3	43.7	7.71				
	Max.	13.64	71.3	11.00	11.8	67.9	72.0	13.0	2.10	0.63	7.49	8.30
	Min.	0.28	5.6	1.62	1.9	12.8	36.6	4.3	0.05	0.12	0.39	0.49

[a] Extrapolated on the basis of 7% ash content

tory cultures, apparently there was a decrease in dry matter content in older mucilaginous strains and an increase in nonmucilaginous ones. The range of variation observed from pooled data (0.18%–13.64%) shows that the fresh weight of a standing bloom of BGA in a rice field does not give any information about its agronomical significance.

Ash. Laboratory mass cultures had an average ash content of 7.4%, ranging from 5.6% to 11.8%, whereas field samples, consisting mainly of mucilaginous strains, had an average ash content of 52%, ranging from 31% to 71%. Blooms produced in trays, where there was minimum disturbance and where demineralized water was used, had lower ash contents (15%–37%)than those grown in the field, indicating that, as with most aquatic plants, ash content of BGA is related to the quantity of soil particles in suspension in the floodwater (Roger and Watanabe 1984). Ash content may be especially high in sheath-forming strains that may adsorb clay and silt in their mucilage. The wide range of ash content observed in field samples (31%–71%) shows that the dry weight of a standing bloom of BGA in a rice field gives little information about its agronomic significance.

Protein and nitrogen. When the results were expressed in percentage dry weight, a large difference in average N_t content between laboratory cultures (5.2%) and field samples (2.5%) was observed. Because of the difference in ash content of the materials, comparisons were made on ash-free material. Nitrogen content ranged from 1.9% to 11.8% and averaged 6% of ash-free dry weight.

In the literature, different values have been reported for the average protein or N content in BGA: 50% protein or 7.9% N (Fogg et al. 1973), 37%–66% protein or 5.8%–10.4% N (Mishustin and Shil'nikova 1971), and 20%-45% protein or 3.2%–7.1% N (Holm-Hansen 1968). Wolk (1973) stated that so long as N is available in abundant and easily assimilable form and the algae are not producing copious amounts of mucilage, N accounts for about 10% of cell dry weight. Twenty-four N measurements in N_2-fixing BGA recorded from the literature range from 3% to 11% and average 7.02% (Table 1). The

variability of the estimates for the average protein content of BGA is most probably due to the fact that only few data were considered in the calculations. In addition, most of these data refer to laboratory strains, which may have led to biased estimation. Laboratory strains are frequently grown in optimized conditions and, usually, under light intensities much lower than those in natural environments. Within certain limits, an inverse correlation between light intensity and photopigment content is general among photosynthetic organisms; BGA are no exception to this rule (Cohen Bazire and Bryant 1982). Therefore, one can expect a higher pigment content and lower ash content in laboratory-grown material, which leads to a higher relative protein content.

The protein and N contents in BGA exhibit variations related with the nature of strains, the physiological state of the culture (mainly its age), and environment. High protein contents were reported for *Anabaena cylindrica* (43%, Collyer and Fogg 1955) and *Aphanothece halophitica* (76%, Tindal et al. 1977) and some non N_2-fixing species such as *Spirulina* spp. (62%–73%). On the other hand, a value lower than 30% has been reported for *Calothrix* sp. (Williams and Burris 1952). Our results did not allow us to find out if some species or genera exhibited consistently higher N contents than others. The highest average value observed with *Anabaena* strains (Table 4) was probably because most of the cultures analyzed were less than 4 weeks old.

From the ranges of variations among strains (Fig. 1) it appears that intrageneric variability of the N content is large, as shown by the coefficient of variation ranging from 20% to 42% and averaging 30% (Table 4). Data from the literature give little information about intrageneric variability but show that intraspecific variability may be high. For example, protein contents ranging from 35% to 63% were reported for *Anabaena cylindrica* (Table 1).

Large variations have been observed during the growth cycle of some strains. Nitrogen content of *A. cylindrica* increased during the first stages of growth, from about 5% at 2 days to about 10% at 11 days (Cobb and Myers 1964). The highest protein content of an axenic culture of *Apanothece stagnina* (40%–46%) was obtained at the end of the lag phase and the beginning of the exponential phase (3rd–8th day) (De Cano and De Halperin 1978). The general tendency for the protein content of BGA to decrease as the culture ages is clear, and was reported for natural populations of *Aphanizomenon flos-aquae*. Such populations contained 30%–42% protein, with the highest protein contents observed at blooming (Shnyukova et al. 1978). Our results also showed a decrease in N content in aging material. This was observed in laboratory cultures (Fig. 2) and in artificial and natural blooms. In laboratory cultures average N content was 8.02% in cultures at the exponential phase of growth, 5.89% in flask cultures less than 4 weeks old, and 4.64% in cultures more than 4 weeks old. Average N content in ash-free dry matter was 6.76% in soil-based inocula harvested 2 weeks after inoculation, 6.3% in blooms produced on soil which were harvested 4 weeks after submersion, and 4.8% in natural samples which comprised a high proportion of mucilaginous strains harvested after several weeks of growth.

Pigments. Phycocyanin and chlorophyll *a* were measured in flask cultures only. Quantitatively, biliproteins are the most important pigments in BGA. They usually represent 1%–10% of the dry weight of the cell (Chapman 1973) although under certain circumstances it may be more: 18.4% in *Tolypothrix tenuis* grown under fluorescent light (Hattori and Fujita 1959).

Usually phycocyanin is the major biliprotein in BGA. It may account for as much as 40% of the total protein in filaments of *Anabaena cylindrica* (Fay 1969). In the studied samples, phycocyanin content ranged from 0% to 15.5% dry weight. Samples less than 4 weeks old had a higher content (6.59%) than older ones (3.90%). Similarly a decrease was observed for chlorophyll, from 1.11% to 0.75%. Average and extreme values were within the range of values reported in the literature (Table 1).

Phosphorus. The P level in BGA may fluctuate widely depending on whether or not the algae are growing under P-limited conditions. Blue-green algae assimilate more P than they require and store the excess as polyphosphate which can be used in P-deficient conditions (Batterton and Van Baalen 1968). From Table 1 it appears that P content in N_2-fixing BGA reported in the literature varies from 0.1% to 2.2%. Larger values (0.8%–4.2%) have been reported for BGA in general by Mishustin and Shil'nikova (1971), who did not indicate the origin of their data. Data summarized from the literature collected by Healey (1982) suggest that P content in BGA growing in a P-sufficient medium averages 1.38% (highest value 1.56%, lowest 0.75%, 9 data), whereas P content in BGA growing in P-deficient media could be as low as 0.03%. Our strains grown on GO medium as mass cultures were comparatively deficient in P as indicated by contents ranging from 0.4% to 0.75% and averaging 0.59%. An accurate evaluation of P content in BGA grown on soil or field samples is impossible because of the high ash (soil) content of the algal material. Because P content is usually lower

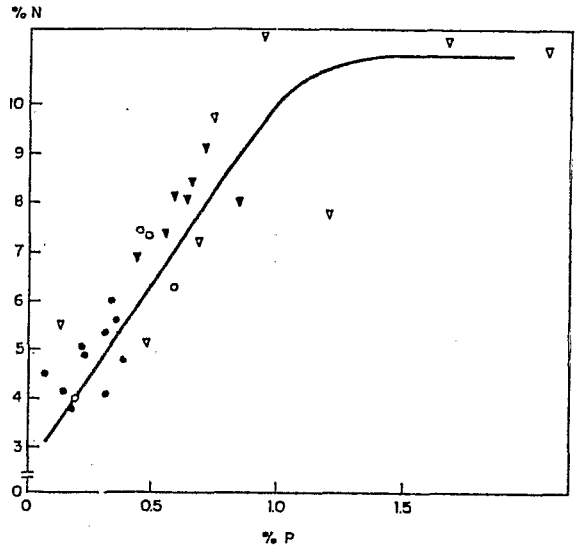

Fig. 8. Correlation between N and P contents (ash-free basis) in N_2-fixing BGA: ▽ Data from the literature; ▼ laboratory mass cultures; o artificial blooms on soil; • field samples

in soil than in the BGA material, noncorrected values are underestimated, whereas values corrected for ash content are overestimated because all the P contained in the mixture of BGA and soil is attributed to the BGA. Even when values corrected for ash are considered, it appears that according to Healey's data, artificial blooms produced on soil (where P was applied basally at a rate of 20 kg/ha) were deficient in P, as indicated by an average content of 0.42% P on an ash-free basis (Healey 1982). A similar observation was made with field samples which exhibited an average P content of 0.25% on an ash-free basis.

Figure 8 shows a highly significant positive correlation between N and P contents expressed on an ash-free basis. The general shape of the curve shows that at P concentrations higher than 1% there was no more increase in N content, indicating a "luxury consumption" in P. The optimal value of 1% was attained in laboratory cultures only. BGA grown on soil and natural samples had concentrations lower than 0.5%, confirming that P availability is a major limiting factor for BGA growth in natural environments (Roger and Kulasooriya 1980).

Carbon. In cultures at the exponential phase of growth, average C content was 45% of ash-free dry matter and exhibited very low variability (CV = 5%). In flask cultures, the average value was similar (45%) but the variability was greater (CV = 20%). In artificial and natural blooms, C content was slightly lower than in laboratory cultures (40%–43%) and variability was low (CV = 5%–7%). Average C content in laboratory cultures (45%, 29 data) was very close to that calculated from data collected from the literature (46%, 9 data). The lower C content in artificial and natural blooms may be due to P deficiency in these materials. Healey and Hendzel (1975) reported C contents of 45%–48% in P-sufficient (1.56% P in the cell) and slightly P-deficient (0.70% P in the cell) *Anabaena variabilis*. In a strongly deficient medium, cellular P was 0.13% and C content 38%. However, more data are needed to draw definite conclusions. A major characteristic of C in N_2-fixing BGA seems to be a low variability compared with other elements. Therefore, an increase in C:N ratio observed when BGA material is aging is mostly due to a decrease in N content.

Carbohydrates. Generally, the percentage of carbohydrates found in N_2-fixing BGA is very variable. The mean from 11 reports (Table 1) is 38%. Values range from 13% to 85%. The carbohydrate content of BGA varies with the nature and age of the organism as well as with some environmental factors (Mehta and Vaidya 1978). Polysaccharides are the most common carbohydrates in BGA. They are a principal constituent of the cell wall and envelopes that form during the differentiation of heterocysts and spores. The cells of certain BGA characteristically form mucilaginous capsules which are polysaccharide in nature. Production of extracellular polysaccharides depends on the age of culture, the growth temperature, and the form of N available (Sangar and Dugan 1972). Extracellular mucilage can account for as much as 44% of the dry weight of cultures (Moore and Tischer 1964; Wolk 1973).

Polysaccharide content, expressed as sugars, was studied only in laboratory cultures grown in flasks. Values recorded for individual samples ranged from 20% to 114% and averaged 53%. The highest value, larger than 100%, clearly demonstrates that there are problems in sugar determination. Similar observations were made by Collyer and Fogg (1955), who pointed out that some high values encountered in the literature may be due to methodological artifacts inherent to the phenol-sulfuric acid method.

Average generic values (Table 4) ranged from 30% (*Anabaena*) to 64% (*Gloeotrichia*). These was no clear-cut difference between mucilaginous colony-forming genera (*Nostoc, Gloeotrichia*) and the other genera. The lower value recorded for *Anabaena* strains is probably because most of the samples were less than 4 weeks old. Our results showed an increase in sugar content in older cultures (Fig. 2). This is in agreement with the observations of Mehta and Vai-

dya (1978), who reported that in *Nostoc* cultures, total polysaccharides, including extracellular ones, yielded 39% of the dry matter in 20-day-old cultures and 48% in 45-day-old cultures.

Major cations. Although in laboratory cultures K, Mg, and Ca exhibited average concentrations of the same order of magnitude (0.5%) (Table 11), very large differences were observed in soil-grown BGA and in natural samples. This was obviously due to the high ash content in these samples, which tremendously increased the Ca and Mg concentrations in the harvested material.

Potassium content is about 1000 ppm in the soils of the area where field samples of BGA were collected. Therefore, average K content of the ash-free algal material collected from the field was about 0.5%. This is similar to K content in laboratory cultures, which indicates that K probably does not limit BGA growth.

Variability of the composition

An analysis of the variability of the composition of laboratory cultures showed that intrageneric variability was slightly larger than intergeneric variability. Multivariate analysis showed that culture age is at least as important as the nature of the strain in explaining the variability of the composition.

Pooled data showed a very large variability of the composition. For example, the ratio between higher and lower values recorded for N content was more than 6 (Table 11). However, pooled data included those from laboratory cultures in which variability is, as a rule, wider than that known from nature (Komareck 1971): coefficients of variation were 31% for N and 20% for C in laboratory cultures, but 16% for N and 7% for C in field samples. However, even when only the data from artificial blooms and natural samples are considered, variability is still high. When average and extreme values obtained for dry matter, ash, and N contents are combined, it appears that N content in 1 tonne of fresh N_2-fixing BGA averages 1.25 kg but may vary from 4 to 0.1 kg. Therefore, the data on N_2-fixing biomass in kilograms fresh weight or dry weight per hectare give little information on its agronomic significance, which depends mainly upon its N content.

Implications for agronomic use of BGA in rice cultivation

Total algal biomass evaluations in rice fields range from a few kg/ha to 58 tonnes/ha fresh weight or 500 kg dry weight. Reported N_2-fixing algal biomasses range within the same limits (Roger and Kulasooriya 1980; Roger et al. 1985; Roger et al. 1986). The highest biomass currently reported on a fresh weight basis in a rice field is 58 tonnes/ha for an *Aphanothece* bloom in which 98.6% water and 54% ash contents limited the N content to 13 kg/ha (Roger et al. 1985). The highest biomass reported on a dry weight basis is 481 kg/ha, corresponding to 53 kg N/ha (Singh 1976). Values reported by Singh indicate an N content of 11% before correction for the ash content. This is very high especially when considering the author's indication that heavy rains frequently disturbed the field, which implies that ash content should have been high.

Assuming a maximum biomass of 500 kg dry weight/ha and using average ash and N values obtained for artificial blooms and field samples (Table 11), it appears that the potential N contribution of an N_2-fixing bloom is 15–25 kg N/ha.

Using average values for dry matter, ash, and N contents we are able to calculate that the average biomass corresponding to 10 kg N is about 8 tonnes fresh weight. This is equivalent to a continuous layer of 0.8 mm algal material over 1 ha of a rice field. In other words, an algal bloom of agronomic significance is visible to the naked eye.

When looking at BGA as a source of nitrogen for rice the C:N ratio is a major factor determining N mineralization. Extreme C:N values were 4.3 and 13 (Table 11). They were observed in laboratory cultures, which confirm that variability is larger in artificial conditions than in nature. When data from blooms produced on soil and natural samples are considered, values range from 5.3 to 11.6 and average 8. This indicates that BGA have a better nitrogen availability than organic fertilizers such as farmyard manure and green manures. Previous studies by Tirol et al. (1982) showed that availability of BGA nitrogen to two successive crops of rice was similar to that of ammonium sulfate.

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Appendix

Analysis of flask cultures of N_2-fixing BGA isolated from rice fields

	Ref.[a] no.	Age[b]	Dry weight	N	C	C:N	Sugars	Chloro-phyll *a*	Phyco-cyanin
Anabaena	3	A	6.94	8.26	51.6	6.25	40.2	1.77	13.1
	2	A	1.80	5.20		12.9	36.6	2.45	6.59
	4	A	2.67	6.98	50.5	7.24	19.9	1.82	13.4
	6	A	1.01	7.86	47.7	6.07	32.2	1.63	15.5
	5	A	8.50	7.97	38.4	4.82	21.8	1.79	14.9
	7	B	3.52	3.68	nd	nd	27.3	0.23	6.33
			4.07	6.66	51.1	7.46	30.0	1.61	11.6
Aphanothece	8	B	0.30	2.67	nd	nd	76.7	0.13	1.31
Aulosira	9	A	5.60	3.84	29.7	7.73	62.5	0.31	3.99
Calothrix	10	A	4.27	4.68	39.1	8.35	62.3	0.44	3.36
	11	A	4.16	6.66	35.6	5.34	41.8	1.77	5.50
	13	A	5.44	5.23	32.1	6.14	36.3	1.12	3.68
	13	B	4.97	6.23	nd	nd	88.1	0.32	5.65
	13	B	5.33	6.03	nd	nd	63.7	0.53	3.68
	14	B	5.33	4.65	nd	nd	41.5	0.34	2.45
	14	B	5.81	4.47	nd	nd	68.8	0.21	2.87
	15	B	6.02	6.74	nd	nd	66.9	0.46	3.16
	15	B	6.03	3.70	nd	nd	37.8	0.45	2.22
			6.26	5.38	35.6	6.61	56.4	0.62	3.62
Fischerella	16	A	4.91	3.40	35.2	10.34	65.5	0.33	1.96
	17	A	3.47	4.99	31.9	6.39	37.0	0.58	3.45
	18	A	4.41	5.26	39.7	7.55	43.5	0.93	3.07
	17	B	13.1	1.78	nd	nd	43.4	0.08	1.14
			6.48	3.85	35.6	8.09	47.3	0.48	2.40
Gloeotrichia	19	A	4.55	4.38	42.4	9.69	38.1	1.13	3.49
	20	A	2.75	5.33	36.6	6.88	49.0	1.09	5.82
	20	B	0.80	2.81	nd	nd	66.0	0.08	1.14
	21	B	0.55	8.02	nd	nd	114.1	0.42	6.58
	21	B	0.80	3.95	nd	nd	53.7	0.48	3.44
	21	B	0.80	4.75	nd	nd	64.5	0.48	3.28
			1.70	4.87	39.5	8.28	64.2	0.61	3.95
Nostoc	23	A	1.11	3.52	45.8	13.0	26.6	0.81	9.25
	24	A	3.95	5.12	46.8	9.14	68.4	0.34	6.08
	25	A	4.44	4.00	45.1	11.3	49.2	0.56	3.79
	26	A	6.33	6.89	36.9	5.36	32.0	1.72	10.4
	28	A	0.28	3.71	38.2	10.2	59.2	0.63	6.72
	27	A	3.34	7.15	40.4	5.65	35.4	1.35	4.91
	23	B	2.59	2.97	nd	nd	69.1	0.24	2.35
	24	B	2.98	4.00	nd	nd	48.7	0.32	5.34
	22	B	1.21	5.60	nd	nd	58.5	0.47	6.76
	22	B	1.36	5.56	nd	nd	65.9	0.44	3.74
	22	B	1.36	6.17	nd	nd	49.6	0.34	5.49
	26	B	1.85	4.49	nd	nd	50.6	0.21	5.68
	26	B	2.95	4.20	nd	nd	45.4	0.65	5.15
	26	B	2.96	4.29	nd	nd	55.1	0.44	3.73
			2.62	4.83	42.2	9.12	51.0	0.61	5.67
Oscillatoria	29	A	3.32	6.42	39.0	6.07	40.0	1.05	2.80
	30	B	4.00	4.37	nd	nd	67.0	0.39	2.77
Scytonema	31	B	6.67	2.53	nd	nd	105.1	0.12	1.29
Tolypothrix	32	A	3.12	3.87	44.5	11.5	50.5	0.86	3.32
	32	B	3.64	4.99	nd	nd	69.7	0.47	8.35
No. of data			46	46	22	22	46	46	46
Average			3.72	4.99	41.6	8.09	53.2	0.71	5.19
Higher value			13.1	8.26	67.3	13.2	114.1	2.45	15.5
Lower value			0.28	1.78	29.7	4.82	19.9	0.08	1.14
CV %			66	31	20	31	37	81	67

[a] From Table 2
[b] A: less than 4 weeks old; B: between 4 and 8 weeks old

References

Batterton J, Van Baalen C (1968) Phosphorus deficiency and phosphate uptake in the blue-green alga *Anacystis nidulans*. Can J Microbiol 14:341–348

Black CA (ed) (1965) Methods of soil analysis. American Society of Agronomy. Inc. Madison, pp 1372

Chapman DJ (1973) Biliproteins and bile pigments. In: Carr ND, Whitton BA (eds) The biology of blue-green algae. University of California Press. Botanical Monographs No 9, pp 162–185

Choi YR, Markakis P (1981) Blue-green algae as a source of protein. Food Chem 7:239–247

Cobb HE Jr, Myers J (1964) Comparative studies of nitrogen fixation and photosynthesis in *Anabaena cylindrica*. Am J Bot 51:753–762

Cohen-Bazire G, Bryant DA (1982) Phycobilisomes: composition and structure. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell Scientific Pub, pp 143–190

Collyer DM, Fogg GE (1955) Studies on fat accumulation by algae. J Exp Bot 6:256–275

De Cano M, De Halperin DR (1978) Correlation between dry weight and protein content in an axenic culture of Aphanothece stagnina as a function of time. Phyton Rev Int Bot Exp 36:71–74

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350

Fay P (1969) Cell differentiation and pigment composition in *Anabaena cylindrica*. Arch Mikrobiol 67:62–70

Fogg GE, Stewart WDP, Fay P, Walsby AE (1973) The blue-green algae. Academic, London, p 459

Fowden L (1954) A comparison of the composition of some algal proteins. Ann Bot n.s. 18:257–266

Harper HJ, Daniel KA (1935) Chemical composition of certain aquatic plants. Bot Gaz 96:186–189

Hattori A, Fujita Y (1959) Crystalline phycobilin chromoproteids obtained from a blue-green alga. *Tolypothrix tenuis*. J Biochem 46:633–644

Healey FP (1982) Phosphate. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria, Blackwell Scientific Pub, pp 105–124

Healey FP, Hendzel LL (1975) Effect of phosphorus deficiency on two algae growing in chemostats. J Phycol 11:303–309

Holm-Hansen O (1968) Ecology, physiology, and biochemistry of blue-green algae. Ann Rev Microbiol 22:47–70

IRRI (1985) Nitrogen fixation and *Azolla* management. In: The International Rice Research Institute, Los Baños, Philippines, Annual report for 1984, pp 273–292

Ke Chan Dang, Nikitina KA (1980) Cell composition of cyanobacteria *Anabaena variabilis* depending on the age of the culture. Vestn Mosk Univ Ser XVI Biol 0:27–30

Kokyrsta PN, Chekoi VN (1972) Ser Biol Khim 2:46–48 (in Russian). Cited by Mehta and Vaidya (1978)

Komarek J (1971) Benützung von Kulturen in der Blaualgentaxonomie. Schweiz Z Hydrol 33:553–565

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Mehta VB, Vaidya BS (1978) Cellular and extracellular polysaccharides of the blue-green alga *Nostoc*. J Exp Bot 29:1423–1430

Mishustin EN, Shil'nikova VK (1971) Nitrogen fixation by blue-green algae. In: Biological fixation of atmospheric nitrogen. University Park, Pennsylvania State University Press, pp 284–312

Moore BF, Tischer RG (1964) Extracellular polysaccharides of algae: effects of life-support systems. Science 145:586–588

Myers J, Kratz WA (1955) Relation between pigment content and photosynthetic characterics in blue-green algae. J Gen Physiol 39:11–22

Rippka R, Deruelles J, Waterbury B, Herdman M, Stanier RY (1979) Genetic assignment, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 111:1–61

Roger PA, Grant I, Reddy PM, Watanabe I (1986) The photosynthetic aquatic biomass in wetland rice fields and its effects on nitrogen dynamics. Paper presented at the INSFFER Workshop on Nitrogen and Rice. Griffith, Australia, April 1985 (in press)

Roger PA, Kulasooriya SA (1980) Blue-green algae and rice. The International Rice Research Institute, Los Baños, Philippines, 112 pp

Roger PA, Santiago-Ardales S, Watanabe I (1985) Unicellular mucilaginous blue-green algae impressive blooms but deceptive biofertilizers. Int Rice Res Newsl 10:27–28

Roger PA, Watanabe I (1984) Algae and aquatic weeds as source of organic matter and plant nutrient for rice. In: The International Rice Research Institute, Los Baños. Organic Matter and Rice, pp 147–168

Sanger VK, Dugan PR (1972) Polysaccharide produced by *Anacystis nidulans*: its ecological implications. Appl Microbiol 24:732–734

Shnyukova YI, Tupyk ND, Mushak PO, Kostlan NV (1978) Biochemical changes in *Aphanizomeanon flos-aquae* during the bloom period of a reserviuor. Ukr Bot Zh 35:592–596

Singh, PK (1976) Algal inoculation and its growth in waterlogged rice fields. Phykos 15:5–10

Tindall DR, Yopp JH, Schmid WE, Miller DM (1977) Protein and amino acid composition of the obligate halophile *Aphanothece halophytica* (Cyanophyta) J Phycol 13:127–133

Tirol AC, Roger PA, Watanabe I (1982) Fate of nitrogen from a blue-green alga in a flooded rice soil. Soil Sci Plant Nutr 28:559–570

Venkataraman GS (1981) Blue-green algae for rice production – a manual for its promotion. FAO Soils Bulletin No 46

Williams AE, Burris RH (1952) Nitrogen fixation by blue-green algae and their nitrogenous composition. Am J Bot 39:340–342

Wolk CP (1973) Physiology and cytological chemistry of blue-green algae. Bact Rev 37:22–101

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