# AN ECOLOGICAL STUDY OF SOME JAPANESE AERIAL AND TERRESTRIAL ALGAE\*

### By

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#### Introduction

Recently, many investigations of the ecology of aerial and terrestrial algae especially on their autecological and physiological peculiarities relating with their corresponding habitats have been done. In these studies, several interesting problems have been focused upon *e. g.* the algal composition of soil communities, the physical function of environmental factors such as moisture, light and temperature, the chemical function of environmental factors such as pH and inorganic salts and the nutrition of algae such as the fixation of atmospheric nitrogen. And the hitherto known these results are summerized and discussed by J. W. G. Lund (1962).

As a remarkable differentiation in the photosynthetic patterns of the so-called "shadeform" and "sun-form" in higher green plants has well been known, while a similar phenomenon has recently been noticed in marine and freshwater plankton under field condition<sup>7)</sup> <sup>(11)</sup> <sup>(12)</sup> <sup>(13)</sup> <sup>(23)</sup> and in fresh water macro-algae<sup>14)</sup>. And the details of this phenomenon especially on the relation to temperature are studied in some cultured planktonic algae by Aruga (1965). However, so far as we are aware, few or no detailed investigation of the photosynthesis of aerial and terrestrial algae has been reported.

On the ecology of Japanese aerial and terrestrial algae, there are several reports on their distribution and the floral researches in some peculiar districts  $^{(1)} ^{(2)} ^{(3)} ^{(4)} ^{(5)}$ , how-ever we have no investigation of the autecology and physiology of these algae.

The present paper is devoted primarily to a study of an autecology of certain aerial and terrestrial algae found commonly in Japan especially on their characteristics of photosynthesis relating to the environmental factors corresponding with their habitats.

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#### **Materials and Methods**

The following materials used in this study were collected from the vicinity of Matsue

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City, Shimane Prefecture, Japan; Hormidium flaccidum (KUETZ). A. BR., Trentepohlia aurea (L.) HARIOT, Trentepohlia sp., Zygogonium sp., Nostoc commune VAUCH. and Nostoc sp. When using the algal materials in experiments on photosynthesis, a homogeneous natural population was used.

The dark and light bottle method was used for testing the photosynthesis, and Beckman's oxygen analyzer model 777 was used for measuring the gas pressure of dissolved oxygen produced by photosynthesis.

A crude chlorophyll solution was extracted with methanol, and the optical density was measured by Shimazu-Bausch & Lomb's spectrophotometer, and the ready method of estimating the chlorophyll concentration from the optical density was calculated by the following formura (modified SCOR-UNESCO, 1964)<sup>22)</sup>.

chlorophyll a (mg/L) = 11.64 log 
$$\left[\frac{I_0}{I}\right]_{663}$$
 - 2.16 log  $\left[\frac{I_0}{I}\right]_{645}$  - 0.10 log  $\left[-\frac{I_0}{I}\right]_{630}$ 

#### **Results and Discussion**

1. A peculiar vacuolar pigment of Zygogonium and its ecological meaning. It is well known that there is a peculiar reddish brown or purple pigment of cell sap, the so-called algal anthocyan or phycoporphyrin in such terrestrial algae as Zygogonium, Pleuroaiscus, Mesotaenium and, in some times, in aquatic species of Zygnema and Mougeotia <sup>18)</sup> <sup>19)</sup>. Already in 1958, R. E. Alston examined this pigment extracted from an aquatic form of Zygogonium ericetorum KUETZ., and concluded that this purple pigment is one of the iron-tannin like substance <sup>6)</sup>. From the Japanese material of a terrestrial form of Zygogonium sp. (the zygote of this alga has not been obtained) and an aquatic form of Mougeotia sp. we obtained a similar water soluble purple pigment. And the certain chemical and physical properties of this pigment (Table 1.) and the absorption spectrum of a crude aqueous extract from these algae matches generally that reported by Alston in 1958 (Fig. 1).

	<i>Zygogor</i> (yellowish)	nium (color in na (green)	tural) (purple)	<i>Mougeotia</i> (purp1e)
Solubility in water	soluble	soluble	soluble	soluble
Color in NH <sub>3</sub>	pale maroon	pale maroon	maroon	maroon-red
Color in HC1	decolorized	decolorized	decolorized	decolorized
Color in U. V. ca. 3600 Å	weak yellow- green	weak yellow- green	weak yellow- green	pale purplish opalescence
Color in FeCl <sub>3</sub>	no change	blue	blue	no change

Table 1. Comparison of properties of the pigment extracted from Zygogonium and Mougeotia.

In the case of a terrestrial form of Zygogonium, however, some differences are recognized. Macroscopically, there are distinctly two type of coloration of Zygogonium found in Matsue viz. purple or some times yellowish brown colored material and green colored material were found, and two of the former type are distinctly contain a vacuolar pigment, however, it was recognized that the latter green colored material also contains a yellowish purple vacuolar pigment. Moreover, the absorption spectrum

20



Fig. 1. Absorption spectra of crude aqueous extracts from Zygogonium and Mougeotia.

of a crude aqueous extract of this yellowish purple vacuolar pigment shows stronger absorption in urtra-violet by comparison with that of purple pigment, and although a broad maximum at about 500-550 m $\mu$  appeared, there was no sharp peak, and shows merely a gentle slope (Fig. 2). And these trends are markedly recognized in the materials growing on the slopes of sandstone region. On the contrary, in the case of the materials found in other habitats such as clayey soil derived from shale, the vacuolar pigment extracted from the both green and purple colored plants are matches generally that reported by Alston.

It was also recognized that the crude aqueous extract from purple material is not a stable substance, and gradually decolorized into such a yellowish purple substance under the natural condition. The details of this substance is not examined in this study.

So far as is known, an ecological meaning of such purple or yellowish to brownish



Fig. 2. Absorption spectra of crude aqueous extracts from variously colored materials of *Zygogonium*.

purple pigment has not been examined. For the purpose of this experiment, an attempt has been made to examine an influence of the purple vacuolar pigment on photosynthetic activity of such algal materials as Zygogonium, Hormidium and Spirogyra. The results of photosynthetic activity in comparison between the bottled materials soaking into the aqueous extract of vacuolar pigment and untreated controls is shown in table 2.

There were some significant differences between the treated materials and their corresponding controls, namely the photosynthetic activities of the treated materials decreased about 60-90 percent of that of corresponding controls. However, an extent of inhibition of photosynthetic activity is varied with such factors as the concentration of aqueous extract, the light intensity and the specific peculiarity of photosynthetic pattern of each material examined.

Materia1s	treated		contro1	efficiency %
Zygogonium (green)	69.2	$\frac{O_2 \text{ mm Hg}}{D. W. 100 \text{mg.}}$	86.9	79.6
	71.3		104.2	68.4
Trentepohlia	8.45		10.06	84.0
Hormidium 44.5			45.9	96.9
Spirogyra	270.3		301.2	89.7
	778.5		1256.7	61.9
Caloglossa	36.4		50.1	72.7

 
 Table 2.
 Effect of vacuolar pigment on photosynthetic activities of freshwater and terrestrial algae.

It is possible that the inhibition of photosynthetic activity is caused by the partial decreasing of the effective light (400 m $\mu$ -550 m $\mu$ ). It seems that the purple or reddish brown vacuolar pigments of terrestrial algae have an effect of protection against injurious strong intens of natural sun light especially of U. V.

2. The chlorophyll content of aerial and terrestrial algae. According to L. Bogard (1962), the chlorophyll content of algae varies and the certain ecological factors such as inorganic salts *e. g.* nitrogen and magnecium and the light intensity have influence on the concentration of algal chlorophylls. Sargent (1940) reported that chlorophyll constituted 6.6 percent of the dry weight of "shade-grown" cells of *Chlorella pyrenoidosa* but only 3.3 percent of the dry weight of "sun-grown" cells.

The chlorophyll content of aerial and terrestrial algae examined in this study mostly ranges 0.04-8.64 percent of the dry weight of the cells. Some examples of the relation between chlorophyll concentration and dry weight are given in figure 3.

As will be seen from figure 3, the chlorophyll constituted about 0.2 percent of the dry weight of the cells of yellowish orange form of *Trentepohlia*, on the contrary, the chlorophyll constituted about 7 percent of the dry weight of the cells of *Hormidium*.

A similar phenomenon has been pointed out by H. H. Strain (1951) and H. W. Milner (1953) viz. the chlorophyll (a+b) equivalent of *Chlorella* sp. showed to 0.01 percent of the dry weight of the cells, and the same organism produced chlorophyll equivalent to 6 percent of the dry weight of the cells, and Strain noted that the decrease in the amount of chlorophyll and the corresponded increase in the ratio of carotenoid to chlorophyll due to deficiency of essential element or of water is frequently regarded in such algae as *Trentepohlia* and *Protosiphon*. This phenomenon is also confirmed with the Japanese *Trentepohlia*.

In the case of Zygogonium, the chlorophyll constituted about 1 percent of the dry weight of the cells of purple colored materials and the chlorophyll amount of the cells without a purple vacuolar pigment is slightly smaller than that of colored materials, however, the details and the ecological meanings of this phenomenon were not confirmed. The investigation of this phenomenon must be the subject of future research.

3. Photosynthetic patterns of aerial and terrestrial algae. It is well known fact that there are distinctive differentiation in photosynthetic patterns relating with the



Fig. 3. Relationship between chlorophyll weight and dry weight of *Hormidium*, *Zygogonium* and *Trentepohlia*.

light intensity, namely "shade-form" and "sun-form" in higher seed plants, and it has also been found in many planktonic and macroscopic algae <sup>7</sup>) <sup>11</sup>) <sup>12</sup>) <sup>13</sup>) <sup>14</sup>) <sup>23</sup>). And usually the habitat segregation relating with light intensity is occured as a results of this phenomenon.

Ecologically, the Japanese Zygogonium shows a habitat segregation relating with the light condition.

The photosynthetic characteristics of shade-form and sun-form of Zygogonium are shown in figure 4.

The light saturation point of the sun-form alga is slightly higher than that of the shade-form alga, besides the shade-form alga shows an inhibition of photosynthetic activity under the strong intensity of light. The similar trends are also recognized in



Fig. 4. Photosynthesis-light curves of two forms *viz*. purple colored material (usually grows in sunny area) and green colored material (usually grows in shaded area) of *Zygogonium*.





both cases of Trentepohlia and Nostoc, and the results are shown in figures 5 and 6.

As already shown in above, there are two patterns of coloration in Japanese Zygogonium, and also in the case of *Trentepohlia* there are two patterns of coloration, viz. green-form and yellowish orange-form. The cells of the latter form usually contains a large quantity of  $\beta$ -carotene, and the chlorophyll concentration is smaller than that of green-form. And the photosynthetic efficiency increase in similar propotion to chlorophyll concentration when it is low intensity of light. The green-form of



Fig. 6. Photosynthesis-light curves of two species of terrestrial Nostoc.

*Trentepohlia* however, which thrive mostly in shaded area shows an inhibition of photosynthetic activity under the strong intensity of light.

It thus appears that the habitat segregation of Zygogonium, Trentepohlia and Nostoc is caused by the differentiation of photosynthetic patterns relating with light intensity in these algae.

4. Osmotic tolerance and the influence of the osmotic change of surrounding media on the photosynthetic activities of aerial and terrestrial algae. The moisture relation of an aerial alga *Trentepohlia* especially on the growth pattern and the details of cellular morphological change was discussed by Fritsch (1922) and Howland (1929). On the soil terrestrial alga *Fritschiella tuberosa* IYENGAR, a physiological differentiation relating with the cellular osmotic pressure corresponded with morphologically differentiated tissues *viz*. projecting and prostrate systems in this alga has been recognized by R. N. Singh (1941). These present data show that most aerial and terrestrial algae well tolerate to desiccated condition, and the cellular osmotic pressure is higher than that of aquatic algae.

The influence of the osmotic change of surrounding media on the photosynthetic activities of some aerial and terrestrial algae has been particularly examined here.

In the case of a terrestrial alga Zygogonium sp., the efficiency of photosynthetic activity reaches a maximum at the concentration of 0.2 mol (ca. 20-25 percent of marine water) of sucrose, sucssesively, it gradually decrease, and at the concentration of 0.8 mol of sucrose, the relative photosynthetic activity falls off 50-65 percent of maximum efficiency. However, in the case of freshwater algae such as *Spirogyra* sp. and *Hydrodictyon reticulatum* (L.) LAGERH., the maximum photosynthetic efficiency is shown under the concentration of less than 0.2 mol and the relative photosynthetic activity precipitately falls off, to be attended with the increasing of the medium concentration, less than 20 percent of maximum efficiency.

On the contrary, an aerial alga *Trentepolia* sp. shows a maximum efficiency of photosynthesis in hypertonic condition. The figure 7 shows a decreasing ratio of

photosynthetic activity of the materials treated with varied concentration of marine water during 24 hours.

From figure 7 it is clear that the terrestrial alga Zygogonium sp. shows a conspicuous tolerance against the high concentration of surrounding media (20-80 percent of marine water), on the contrary, in the case of a freshwater alga Spirogyra sp., the photosynthetic activity is extremely deteriorated.

This series of experiments, then, shows clearly that the aerial alga *Trentepohlia* and the soil terrestrial alga *Zygogonium* have physiological peculiarities as a results of adaptation to aerial condition.



Fig. 7. Osmotic effects on photosynthetic activities of terrestrial and aquatic algae.



Fig. 8. Osmotic tolerance found in the decreasing patterns of photosynthetic activities of terrestrial and aquatic algae treated during 24 hours.

#### Résumé

1) A peculiar purple vacuolar pigment of the terrestrial alga Zygogonium sp. was examined, and the chemical and physical properties of this pigment matched generally that reported by Alston (1958).

2) It seems that the purple or reddish brown vacuolar pigments of terrestrial algae have an effect of protection against injurious strong intens of natural sun light especially of U. V.

3) The chlorophyll content of Zygogonium, Hormidium and Trentepohlia mostly ranges 0.04-8.64 percent of the dry weight. The chlorophyll constituted about 0.2 percent of the dry weight of the cells of yellowish orange form of Trentepohlia, 7 percent of the dry weight of the cells of Hormidium and 1 percent of the dry weight of the cells of Zygogonium.

4) A differentiation of photosynthetic patterns *viz.* shade-type (inhibited under strong intensity of the sun light) and sun-type (non inhibited) has been recognized in aerial alga *Trentepohlia* and in terrestrial algae *Zygogonium* and *Nostoc*.

5) Certain aerial and terrestrial algae such plants as *Trentepohlia* and *Zygogonium* show a conspicuous tolerance against hypertonic condition (0.2-0.8 mol of sucrose solution and corresponded concentration of marine water).

#### Literature Cited

- Akiyama, M. (1961): Aerial and Terrestr. Alg. in San-in Reg. Bull. Shimane Univ. 10: 75-89.
- 2) ...... (1965) : Some Soil Alg. from Jap. *ibid.* 15 : 96-117.
- 3) ..... (1966): Soil Alg. Veget. of  $\gamma$ -Irrad. Field and Nat. Strong Radioact. Distr. in Jap. *ibid.* 16: 126-134.
- 4) ..... and K. Nishigami (1967): Soil Alg. Veget. Found in the Newly Recla. Reg. of the Brackish Lake Nakano-umi Jap. Journ. Ecol. 17: 118-121.
- ..... (1967): On Some Terrest. and Subterr. Alg. of the Ongul Isl., Antarctica. Mem. Fac. Educ. Shimane Univ. 1: 36-56.
- Alston, E. N. (1958): An Invest. of the Purple Vacuol. Pigment of Zygog. erricet. Amer. Jour. Bot. 45: 688-692.
- 7) Aruga, Y. (1965-1, 2; 1966): Ecolog. Stud. of Photosynth. and Matter Product. of Phytoplankton. I, II, III. Bot. Mag. Tokyo 78: 280-288, ibid. 78: 360-365, ibid. 79: 20-27.
- Bogorad, L. (1962): Chlorophylls in Physiology and Biochemistry of Algae pp. 385-408. edit. R. A. Lewin New York
- 9) Fritsch, F. E. (1922): The Moist. Relat. of Terrestr. Alg. I. Ann. Bot. 36: 1-20.
- 10) Howland, J. H. (1929): The Moist. Relat. of Terrestr. Alg. IV. Ann. Bot. 43: 173-202.
- Ichimura, S. and Y. Aruga (1958) : Some Charact. of Photosynthesis of Freshwat. Phytopl. Bot. Mag. Tokyo 71: 261-269.
- 12) ...., Y. Saijyo, and Y. Aruga (1962) : Photosynthetic Charact. of Marine Phytopl. and Their Ecolog. Meaning in the Chlorop. Method. Bot. Mag. Tokyo 75: 212-220.
- 13) ...., S. Nagasawa and T. Tanaka (1968): On the Oxygen and Chlorop. Maxima Found in the Metalimn. of a Mesotroph. Lake. *Bot. Mag. Tokyo* 81: 1-10.
- 14) Kumano, S. (1968): Habitat Sgreg. and Speciation in Some Freshwat. Alg. in Experimental Stud. on the Speciation in Alg. edit. H. Hirose pp. 7-11. Kobe

- Lund, J. W. G. (1962): Soil Algae in *Physiology and Biochem. of Alg.* pp. 759-770 edit.
   R. A. Lewin New York
- Milner, H. W. (1953): The Chemical Composit. of Alg. in Algal Culture from Laborat. to Pilot Plant. pp. 285-302. edit. J. S. Burlew Washington
- Ogino, C. (1962): Tannins and Vacuolar Pigments in Physiology and Biochemistry of Alg. pp. 437-443. edit. R. A. Lewin.
- 18) Randhawa, M. S. (1959) : Zygnemataceae New Dehli
- 19) Smith, G. M. (1950): The Freshwat. Alg. of U. S. New York
- 20) Smith, J. H. C. et. al. (1955): Chlorophylls in Modern Methods of Plant Analysis vol. IVpp. 142-171. edit. Paech, K. and M. V. Tracey Berlin
- Strain H. H. (1951) : The Pigment of Algae in Manual of Phycology pp. 243-262. edit.
   G. M. Smith Waltham
- 22) Tamiya, H. and A. Watanabe (1965): Methods and Technique in Phycology Tokyo
- Yentsch, C. S. (1962): Marine Plankton in *Physiology and Biochemistry of Algae* pp. 777 -797. edit. R. A. Lewin