# ON MYXOPHYCEAE LIVING IN STRONG BRINES

by

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1. **Summary of contents.** The occurrence of blue-green algae in saturated salt solutions has been known for a long time. The importance of the algal mat for the salt-industry (2) was recognized long before the organisms themselves were observed. From the scattered literature it appears that no sufficient distinction has been made between the forms that are able to *withstand* the high concentration of electrolyte and organisms that are able to *grow* and *multiply* in such concentrations. It appears that, while the number of resistant species (survivors, or halotolerant organisms) may be large, the group of the truly halophilic bluegreens is very limited. For various samples of salt and brine, obtained from widely scattered localities, nearly always yielded the same form. Truly halophilic forms are considered those that are able to develop in solutions more concentrated than 3 Molar ($\pm$ 17.55 %) NaCl.

The form which reoccurred in the majority of the salts investigated proved to be extremely variable, so much so that adjacent cells often seemed to belong to different species. Inasmuch as the classification of bluegreen algae is notoriously difficult and as it proved impossible to isolate single cells and culture them the question as to the specific identity of the forms remains doubtful. The occurrence of transition-stages seems to point to a great variability, similar to the variability in the purple bacteria as described by Van Niel (15). The presence of "giant-cells" is especially typical for the strong brines. The first author of this paper is therefore inclined to consider most of the forms observed as belonging to one species: *Aphanocapsa litoralis* Hansgirg. (Aphanothece halophytica Frémy, Dzensia salina Woronichin).

The giant-cells of this species often show a curious mottled or variegated appearance, which is due to the distension of the chromatoplast. It appears that the phycocyanin and the chlorophyll are spatially separated in the chromoplast.

Experiments with "hypertonic" electrolytes showed that the giant cells may possibly contain a larger amount of water.

The unpublished laboratory- and field notes of L. G. M. Baas Becking were used freely in this work. They are indicated in the text by the letters (L. N. B-B.).

**2. The occurrence of the algae in strong brines.** Geitler (10) in his survey of the Myxophyceae, gives forty forms that have been found in brackish or salty water, on salty soil or in brines. The same author mentions, in his later work (11) 53 species of the Chroöcoccaceae only which thrive in saline waters. It is impossible to say in how far these forms are merely tolerant to — or actively growing in this milieu. Walter (20), in his remarkable book "Die Hydratur der Pflanze" remarks that: "das Hydraturminimum von 85 % scheint das absolute Minimum für alle lebenden Organismen im aktiven Zustande zu sein". This would correspond to a solution of NaCl of 3.6 Molar. It appears from the literature that Walter's conclusion is well founded. When seawater evaporates the majority of the organisms disappears at approximately this concentration (18). While Walter does not mention the truly halophilic organisms he indicates the possibility of their existence by stating that organisms which are able to develop in a condition of "hydrature" lower than 85 % show a specific reaction to certain electrolytes. This conclusion is also borne out by the facts (3).

Resistance to high concentrations of electrolyte may be caused by the formation of cysts or of protective impervious or electrically charged envelopes.

As said before, the occurrence of algae in the high concentrations of electrolyte, which has been frequently reported in the literature does not mean that the algae grow and develop in such solutions. The surviving forms interest us, therefore, only to the extent that we may be able to identify those forms with those that appear in our cultures.

Bluegreen algae are very resistant to external influences and it is plausible, therefore, that a large number of them should occur under conditions of extreme "physiological drought".

It appears that those bluegreen algae that form the protective carpet between salt and soil in the solar salt-works are *halotolerant* rather than *halophilic*. (Group a). Evidence of halotolerance may also be derived from the failure to grow algae from old salt samples. It appears that even halophilic algae have a limited halotolerance. (Group b.). Forms that are described in the literature but which did not appear in our cultures may also be passive survivors, although on further experiments they might show up. Several examples of such forms are described under Group c.

*a. The halotolerant bluegreen algae which occur in salt works.* Bluegreen algae are of much importance in solar salt manufacture (2). Von Buschman (6) in his classical treatise says, in his description of the saltworks of Istria (Adriatic): "Am vollkommensten soll der Boden der Kristallisationsbeete dann sein, wenn er durch eine Algenart (microcoleus corium?) einen ziemlich festen wachsähnlichen schwarzen Überzug erhalten hat; es gilt diesfalls der Satz: „Schwarzer Grund, weiszes Salz." (l.c. p. 284).

When the bottom is covered with the algal film the salt may be harvested without contamination by the black sulphide muds which lie immediately beneath.

According to Von Buschman (l.c. p. 592) the Portuguese salt from the ancient saline at Setubal is of superior quality because of the algal cover through which the magnesium salts diffuse more quickly than the NaCl. Great care is taken not to injure the algal carpet during harvest.

Fürer (9) is his description of the salines in Southern France remarks: "Die Festigkeit des Bodens der Salzbeete ist besonders wichtig für die Reinheit des Salzes. Man bedeckt ihn mit 1½ mm dickes Filz ("feutre dol") von Algen (Micrococcus corvium) die aus dem Meerwasser durch mehrmaliges schwaches Eindunsten bis zu 8°B° erhalten werden." Von Buschman (6, passim) mentions similar procedure at the Italian salines of San Felice, Comacchio, Margherita di Savoja and Cagliari. The alga which forms the "carpet" is, in most cases, Microcoleus chtonoplastes Thur. (the name given by Fürer must be a typographical error) which is synonymous with Microcoleus corium as mentioned by Von Buschman. The same form was found in a ditch containing seawater near the saltworks at Moss Landing, California (L. N. B-B.). It was encountered by Woronichin (23) in the "Large Tambukan Lake" in Siberia, which lake contains a varying concentration of (chiefly Glauber-) salts ($\pm$ 3.5—11.5 %), and in the "Little Tambukan Lake" another bittersalt lake with a varying concentration of salts ($\pm$ 0.7—5.8 %).

One of us has found Microcoleus chtonoplastes Thur associated with Lyngbya aestuari (Mert) Liebmann (rarely with Microcoleus tenerrimus. Gom), in materials collected by Professor Seurat, Mrs. Gauthier—Lierre and Mr. Feldmann in the saline waters of Tunis and Algiers. The association presented itself almost always as extensive and compact layers which covered the slopes and bottom of the ponds. Unfortunately the concentration of these waters was not indicated, but is known to be variable.

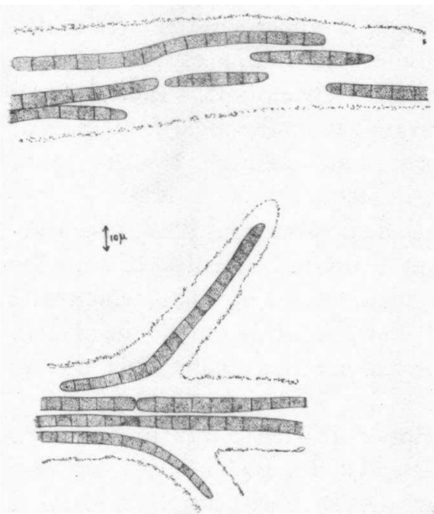


Figure 1.
Microcoleus chthonoplastes Thur.
from San Bartolomeo Cagliari (Sardinia).
1.1 Mol. NaCl; 0.05 Mol. $MgCl_2$;
0.01 Mol. $CaCl_2$ pH 8.

In our cultures Microcoleus appears, however, only in rather low concentrations (1 Mol. = 5.85 %) of NaCl to which small quantities of other salts are added.

0.02 % $KNO_3$ 0.05 Mol. $MgCl_2$
0.02 % $K_2HPO_4$ 0.01 Mol. $CaCl_2$
0.02 % $Na_2SO_4$ pH 8

brought in a "light-box" at a temperature of $\pm$ 22° C.

As infection-material salts from the Government salt works at Gersik-putih (Island of Madura, D.E.I.) and from San Bartolomeo (Sardinia) were used. Both samples were collected within the preceding year. It therefore appears that Microcoleus is unable to develop in 2 Mol. NaCl (11.7 %) and has, therefore, to be considered as a typical halotolerant species. (Figure 1.)

Cavara (7) mentions that, at many salt-works, an Oscillaria belonging to the group Homocystea fulfills the same functions as Microcoleus chthonoplastes Thur. He found that both algae are resistant to any salt-concentration but that the latter form only develops in solutions with an osmotic pressure of 25.2—80.9 atmospheres, corresponding, on a basis of NaCl to solutions of about 0.7—1.9 Molar. (See also Zanco, (24).

Another instance of an algal groundcover was observed in the saline pools near Marina, California. (L. N. B-B.). Here a true green alga, Lochmiopsis sibirica Woronichin and Popova, together with Aphanocapsa marina Hansg. forms a heavy mat. Lochmiopsis is halotolerant, but not halophilic.

*b. The effect of storage in dry salt.* If one endeavors to grow algae from crude salt it appears that samples of salt which have been kept *longer than three years* rarely yield any bluegreens, while the flagellate Dunaliella (1) may be raised from samples as old as seven years.

In 1925 salt was collected from Owen's Lake, California and from Searles' Lake, California. These samples contained living bluegreen algae in 1928, but in 1931 none could be cultured from them, while they still yielded Dunaliella. Salt from the salt desert at Sand Springs, Nevada in 1926 failed to yield bluegreen algae in 1929 while it teemed with them at the time of collection. From the positive cultures of bluegreen algae, described below, it may be said that in no case the infection-material was collected prior to 1928. Salts of uncertain age (French monopoly; Djibouti, Abessynian money-salt; West Indian salts from the collections of the Colonial Institute) remained negative. Samples obtained from the Brazilian Geological Survey and from the Venezuelan salines at the Caracas lagoon (both collected prior to 1928) equally failed to show growth. This seems to indicate that bluegreen algae are not as halotolerant as the polyblepharid flagellates Dunaliella and Asteromonas or as the colourless flagellates which also show a widespread occurrence. This "salt-damage" is also shown in the formation of "giant-cells" which are described later in this paper.

c. *Forms previously described.* It is difficult to say which of the great many forms of algae encountered in salt or brine samples are actually halophilic. We shall mention therefore provisorily a few forms which are certainly halotolerant and may be halophilic. Hase (13) mentions bluegreen algae as the food of the larvae of a curious beetle; Ochthebius quadricollis Mulsant, which is able to live in 27 % brine.

Dr. Flora M. Scott[1]) of the University of California at Los Angeles collected in 1928 at Searles Lake, California, a form resembling a Rivularia with unusually thick filaments (cells $7 \times 15$ μ).

The presence of this alga is indicated by a distinct dull greenish-brown coloration, immediately below the dry white salt crust.

We owe to Woronichin (23) the discription of a number of bluegreen algae from lakes of the Kulundin Steppe which should contain a very high percentage of salt (27°B°). As a mistake has been made in the labelling of the collecting phials great certainty about his data does not exist. The fact remains that

Oscillatoria Tambi Woron.
" brevis Kütz.
" brevis Kütz. var. variabilis Wille and

[1]) In litteris.

Phormidium foveolarum Mont. are able to withstand high concentrations. The uncertainty of these data seems the more probable because Phormidium foveolarum (Mont.) Gom., which is given in this list, is an aerial alga, which causes the formation of cavities in chalk. Maybe Woronichin has taken small torulous hormogones, in various stages of development for Phormidium foveolarum. The alga which Woronichin calls Dzensia salina (21) and also the filamentous Phormidium tenue Gom. are truly halophilic forms and will be treated later. In the neighbourhood of Piatigorsk (22) Woronichin finds in lakes of a salt concentration of 11—21 % (analysis not given; only that chlorine predominated over sulphate) the following species:

Spirulina major Kütz.
Oscillatoria brevis Kütz.
Merismopedia glauca (Ehrb.) Naeg.
Chroococcus turgidus (Kütz.) Naeg.
Phormidium fragile (Menegh.) Gom.

The last species we found in a culture containing 1 Mol. NaCl which had been infected with salt obtained from the salterns of Gersikputih, Pamekasan (Island of Madura, D.E.I.) and of Grissée (Java). The form did not appear in 2 Mol. NaCl, so there is reason to believe that Phormidium fragile is also halotolerant.

This same form Woronichin found in the Large Tambukan Lake, (3.5—11.5 %, mostly $Na_2SO_4$), together with the Spirulina and Chroococcus mentioned above and, moreover, Oscillatoria Tambi Woron., Osc. Kützingiana Naeg., var. crassa Wor., Phormidium ambiguum Gom. and Lyngbya aestuarii (Mert.) Liebmann. We mentioned already the occurrence of Microcoleus chthonoplastes Thur. in this lake. Anabaena torulosa Lagerh., which Woronichin also mentions from this locality, was isolated by us from salts of Teber Lake, Ventura County, Calif., in a solution containing 1 Mol. NaCl. As it failed to develop in 2 Mol. NaCl it is very probable that Anabaena torulosa Lagerh. is also halotolerant but not halophilic. A closely allied form was found (November 1926) in the Soda Lake, Nevada, a crater lake in the Desert, containing at the time about 3.7 % salt at pH 10. (L. N. B-B.). We meet, therefore, in Woronichin's work, with three forms which appear to be truly halotolerant: Anabaena torulosa Lagerh., which also occurs in California and Nevada, Phormidium fragile Gom. which also occurs on Java and Madura and Microcoleus chthonoplastes Thur. which also was found in Java and Sardinia, California, France, Italy and Portugal.

In a very weak culture solution (0.6 Mol. NaCl) infected with sea salt from Dadar (British India, Presidency of Bombay) Aphanocapsa pulchra (Kütz) Rab. appeared. This form was also encountered November 1926 in Pyramid Lake, Nevada, where it covered several miles of the beach. The water of Pyramid Lake contained at that time about 0.1 % salt, pH 9.4 (L. N. B-B.). Aphanocapsa pulchra has to be classed as another halotolerant form.

Evidence for the occurrence of halotolerant, non-halophilic organisms is derived from field observation as well as from cultures. There appeared in our cultures, however, several forms, capable of development only in lower concentrations about which we found, in the literature, no evidence of halotolerance. The following species were found (see page 149).

The absence of these forms in higher concentrations may not be of absolute significance because of the long incubation time even at lower concentrations (several months). An unequal distribution of the material in the salt may be another cause of their absence. No rigid conclusion may therefore be drawn from these data.

| Name of organism. | Highest molarity of NaCl in which grown. | Salt sample from: | Age of Sample |
|---|---|---|---|
| 1. Aphanocapsa marina Hansgirg. ......... | 1 Mol. | Marina, California | 1929 |
| | | Gersik putih, Madura | 1931 |
| 2. Nodularia Harveyana Thur. ............ | 0.8 Mol. | Szovata, Siebenburgen, Rumania | 1928 |
| 3. Nodularia sphaerocarpa. Born & Flah. | 1 Mol. | Marina, California | 1929 |
| 4. Nostoc Linckia Born. | 0.8 Mol. | Szovata, Siebenburgen, Rumania | 1928 |
| 5. Oscillatoria amphibia Ag. ............... | 1 Mol. | San Bartolomeo, Sardinia | 1931 (see Geitler p. 364) (10). |
| 6. Oscillatoria formosa. Bonp. ............. | 1 Mol. | San Bartolomeo, Sardinia | 1931 |
| 7. Phormidium coricum Gom. ............. | 1 Mol. | Gersik putih. (Madura) | 1931 |
| | | San Bartolomeo | 1931 |
| 8. Hydrocoleus lyngbyaceus Kutz., var. rupestris Kutz. fa. uncinata [1]) ............ | 1 Mol. | Gersik putih. | 1931 |
| A transition to the truly halophilic forms seems: | | | |
| 9. Anabaena spec. (sterile).......... | 2.3 Mol. | Setubal, (Portugal) | 1928 |

## 3. The bluegreen algae growing in strong brines.

1. **Spirulina subsalsa Oerst.** This form appeared in concentrations up to 3.2 Mol. NaCl from Sardinian and East Indian salt. (San Bartolomeo, Gersik putih). In almost the same concentration (17 % salt) it was found in 1924 in the salines of Redwood City, California. (L. N. B-B.).

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[1]) fa. uncinata Fremy n. fo. Differt a typo trichomatibus apice plus minusve uncinatis.

2. **Phormidium tenue** (Menegh.) Gom. In 3 Mol. NaCl. from salts of:

*a.* Lake Tekir Ghiol, Rumania (1928)
*b.* Gersikputih, Madura, D.E.I. (1931)
*c.* Pamekasan, Madura, D.E.I. (1931)
*d.* Grissee, Java, D.E.I. (1931)

It was found by Woronichin (22) in a lake on the Kulundinsteppe (27°B°?) and in concentrated alkaline brines of the Sand Springs salt desert, Nevada 1926 and in the concentrated alkaline brines of Searles' Lake, California (1925, 1929). (L. N. B-B.).

3. **Phormidium sp.** still grows in 4 Mol. NaCl, and was isolated from the crust of Sardinian crude salt (San Bartolomeo, 1931).

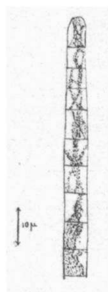

Figure 2. Phormidium Spec. from San Bartolomeo Cagliari (Sardinia) 4 Mol. NaCl; 0.05 Mol. $MgCl_2$; 0.01 Mol. $CaCl_2$. pH 8.

The size of the cells is 6.4 × 6.4—12.8 µ. They have no or inconspicious constrictions. The cells often contain hyaline streaks (see Figure 2).

4. **Dzensia salina** Woron. **or Aphanocapsa litoralis** Hansg., **or Aphanothece halophytica** Frémy sp. nov.

The diagnoses of the forms follow.

*Dzensia salina* Woronichin (21) forms round to oval colonies of 5—10 × 3 mm, sometimes 10 × 8 mm. The cells are 2.5—4 × 3.5 µ and mostly round. In dividing stages they have a diplococcus-like form. In some colonies elongated cells are found (6.8—10 µ rarely 17 µ). The cells are irregularly distributed in a gelatinous matrix. The outside of the colony is covered with a periderm. In young colonies the cells are situated without order, in old colonies the cells form chains surrounded by tubular sheaths, which cross each other in every direction. Typical for this species is the bud-formation of the colony. On the surface of the colony appears a globular elevation of slime, which contains cells. Between the bud and the maternal colony appears a slimy stalk which later breaks. In some cases the bud remains attached to the maternal colony. In this manner double or triple colonies are formed.

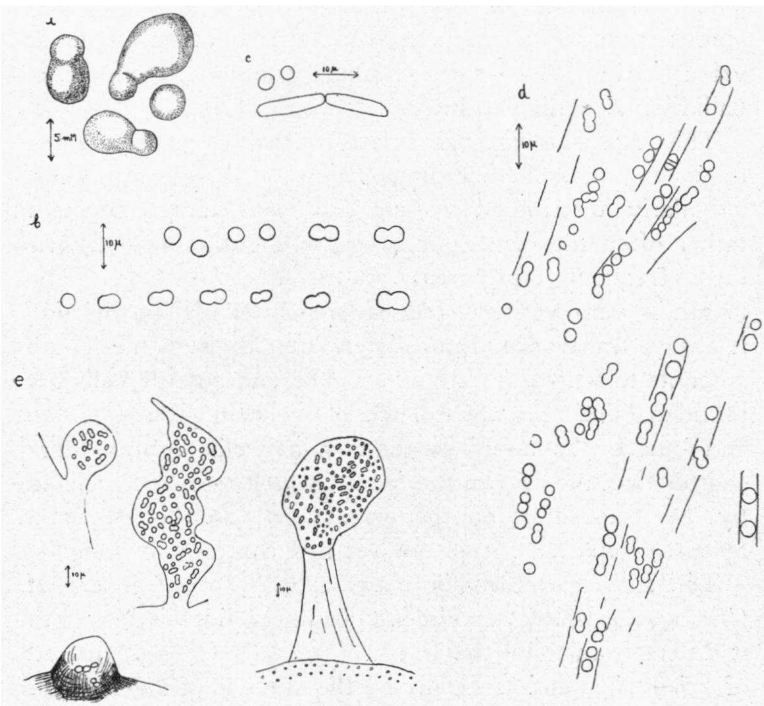


Figure 3.
(After Woronichin) Dzensia salina Woronichin.
a. Colonies. b. Vegetative cells, often in dividing stages.
c. Elongated cells. d. Distribution of the cells in old colonies.
e. and f. Stages of budformation.

Kutschuk Lake, Siberia (16.22 NaCl, 3.15 % $MgSO_4$, 1.227 % $MgCl_2$, 0.109 % $CaSO_4$ and 0.280 % $Na_2CO_3$) often in such masses that the water is of soup-like consistency. (Figure 3).

**Aphanothece halophytica** Frémy n. sp. ad tempus.

*Diagnosis*: Stratum parvum, indefinitum, expansum, tenuissimum, luteolum. Cellulae in pluribus stratis dispositae, rectae, subcylindricae, in medio sat frequenter modice incrassatae 3—4 μ crassae, 10—16 μ (rarius 21 μ) longae ante divisionem, 5—8 μ (rarius 10 μ) post divisionem; apibus rotundatis; tegumento carentes; solitariae aut binae vel interdum 3—4-nae seriatae; contentu homogeneo, pallidissime aerugineo-luteolo.

This alga is found particularly on salt crystals on which it forms a small indefinite yellowish layer. The cells, irrigularly distributed in all directions, are elongated; rather often the centre seems to be slightly swollen. The most frequent dimensions are 3—4 μ × 10—16 μ. The length is very variable and can reach 21 μ. The division is always transversal. Immediately after division the length amounts to 5—8 μ (rarely 10 μ). The ends of the cells are rounded. The cells are isolated or in small groups of two and four. In the latter case the terminal cells are sometimes shorter than the cells in the centre, which may be explained by the supposition that the terminal cells are the result of a recent division and have not yet had the time to elongate.

The alga resembles, in many aspects, other genera of Chroococcaceae. It resembles Gloeothece linearis Näg. but it differs from it by the width of its cells (3—4 μ, instead of 1.5—2.5 μ) and especially by the absence of a gelatinous sheath. Rhabdoderma Schmidt and Lauterb. and particulary Rhabdoderma lineare Schmidt and Lauterb. are similar, but for the width of its cells (3—4 μ, instead of 2 μ) moreover the cells of Rhabdoderma are distributed in one plane. Moreover neither Gloeothece lineare Näg. nor Rhabdoderma lineare Schmidt and Lauterb. are found in saline waters.

From a purely morphological point of view it is not probable that Aphanothece halophytica Frémy belongs to the genus Dzensia for it lacks the tubular sheaths, in which the cells lie in chains and it does not possess a spherical and floating colony. Notwithstanding this we do not have the certainty about the systematic value of our species. For reasons indicated above and which we shall elaborate later, it is possible that Aphanothece halophytica Frémy is a peculiar stationary form of Aphanocapsa litoralis Hansg. The short terminal cells, indicated above, may represent a transition between Aphanocapsa and Aphanothece.

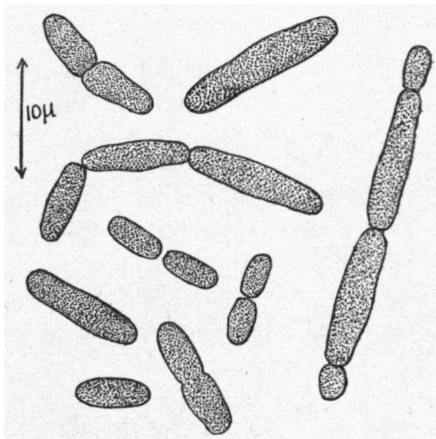


Figure 4.
Aphanothece halophytica Frémy n. sp.

If our alga should be retained as an original species it differs from its congeners by its relatively very long cells.

From saturated brine, Searles' Lake, California. (Figures 4 and 5).

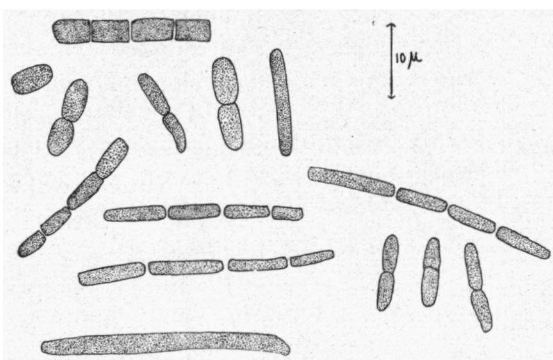


Figure 5.
Aphanothece halophytica Frémy n. sp.
from Searles' Lake, California. Saturated brine.

**Aphanocapsa litoralis** Hansg. (12)

Layer amorphous, mucilaginous, light- to dark brownish, nearly black when dried. Cells spherical or semi-spherical. Diameter 4—6 μ. Contents more or less yellowish. Cells solitary or associated in small numbers and united closely or more or less loosely in the common envelope.

This species, observed frequently by one of the authors, seems very polymorphous. A form with larger cells is indicated by Hansgirg. (see (12), Taf. I., fig. 13).

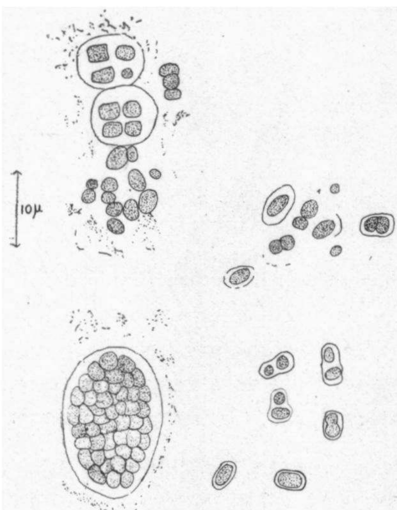


Figure 6.
Aphanocapsa litoralis Hansg. Strain from Lake Tekir Ghiol (Rumania). 3 Mol. NaCl; 0.05 Mol. $MgCl_2$; 0.01 Mol. $CaCl_2$ pH 8.

Forms classed by one of us as belonging to this species were found in samples from Tekir Ghiol (Rumania), San Bartolomeo (Cagliari, Sardinia), Lake Eyre (South Australia), Gersik putih (Madura), Pamekasan (Madura) and Grissee (Java).

*a. Strain from Tekir Ghiol (Rumania).* Cells of clear bluegreen colour surrounded by a common envelope. Diameter of cells 2—4.8 μ spherical. Fairly constant. (Figure 6).

*b. Strain from San Bartolomeo (Cagliari).* Cells not surrounded by a common envelope. Cells 3.2—8 × 4.8—16 μ belonging to three types: (Figure 7).

1. small cells, diameter 4.8 μ;
2. large cells, 8 × 16 μ and
3. elongated cells, 3 × 4.8—16 μ.

The large cells could be classified as Aphanocapsa litoralis var. macrococca (Hansg.). The elongated cells could be named Aphanothece halophytica Frémy.

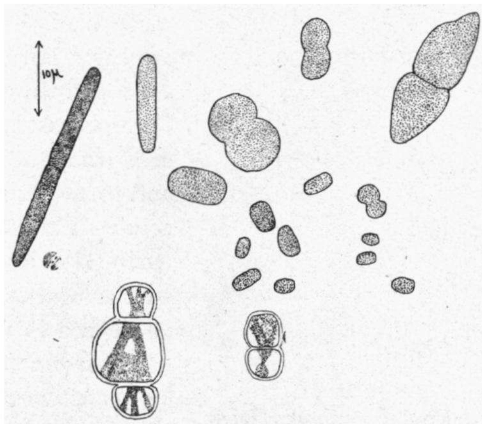


Figure 7. Aphanocapsa litoralis Hansg.
Strain from San Bartolomeo Cagliari (Sardinia).
3.2 Mol. NaCl; 0.05 Mol. $MgCl_2$;
0.01 Mol. $CaCl_2$ pH 8.

c. *Strain from Lake Eyre (South Australia).* (Figures 8 and 9).

Cells clear bluegreen when young, later pale. Young cells 3.2 μ diameter, without visible structure, later

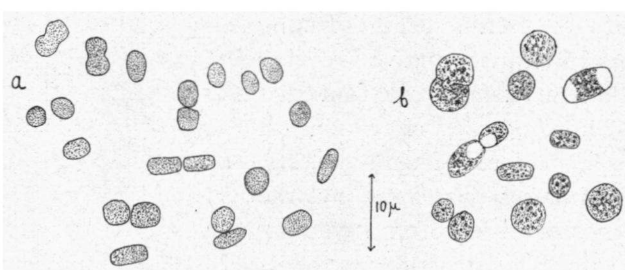


Figure 8. Aphanocapsa litoralis Hansg.
Strain from Lake Eyre (South Australia)
2.4 Mol. NaCl; 0.05 Mol. $MgCl_2$; 0.01 Mol. $CaCl_2$ pH 8.
a. 7 weeks-old culture., b. 9 weeks-old culture.

4.8—6.4 μ, with granular contents. Older cells in a common mucilagenous matrix. Cell division in one plane. In older cultures cells of abnormal size (19 × 25 μ) appear. Part of these cells is hyaline, without chromoplast. The large cells may devide again and form groups of 3—4 cells. Elongated cells (6.4—31 μ) may be found similar to those from Cagliari and Searles' Lake.

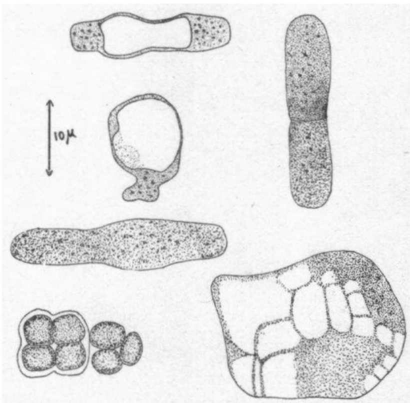


Figure 9. Aphanocapsa litoralis Hansg. Strain from Lake Eyre (South Australia). 2.4 Mol. NaCl; 0.05 Mol. $MgCl_2$.; 0.01 $CaCl_2$ pH 8. Giant cells in a 4 months-old culture.

*d. Strain from Gersik putih (Madura)*. Figures 10 and 11). Size of cells 4.8—32 × 8—13 μ. Large cells, partly hyaline 8 × 41 μ.

*e* and *f. Strains from Grissée and Pamekasan (Madura)*. Are like *d.*

From a purely taxonomic viewpoint it would be well nigh impossible to identify the forms occurring in one culture as belonging to a distinct species of even a genus. Taking a modern treatise, that of Geitler (10) as an example we find that the Chroococceae, to which all these forms most certainly belong, are primarily classified by the presence or absence of a colonial structure. This structure is dependent upon the presence of enough jelly to keep the cells together. This jelly formation is irregular in

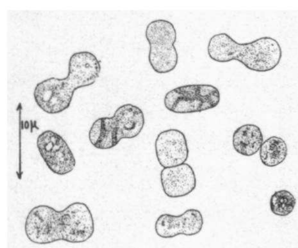


Figure 10. Aphanocapsa litoralis Hansg. Strain from Gersik putih (Madura). 2 Mol. NaCl; 0.05 Mol. $MgCl_2$; 0.01 Mol. $CaCl_2$ pH 8.

the strong brines, and may be absent sometimes. The form of the colonies and the form of the cells is another important diagnostic. Even those characteristics seem to be variable. In certain solutions cell division may be suppressed, and the cells grow to abnormal size. From a single culture from Marina, California (L. N. B-B.) colonies and cells were observed that, according to the taxonomic key should belong to the genera Synechococcus, Aphanothece, Gloeothece, Rhabdoderma, Aphanocapsa and Chroococcus. A good impression may be obtained from the following figures (L. N. B-B.) (Figure 12).

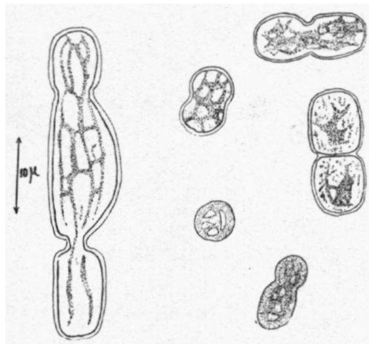


Figure 11.
Aphanocapsa litoralis Hansg.
Strain from Gersik putih (Madura).
3.2 Mol. NaCl.; 0.05 Mol. $MgCl_2$; 0.01 Mol. $CaCl_2$ pH 8.

Comparing these results with the forms mentioned above as Dzensia salina Woron., Aphanothece halophytica Frémy and Aphanocapsa litoralis Hansg., we find an almost complete transition from Aphanocapsa-Dzensia-Aphanothece. It seems, therefore, in this case, just as arbitrary to bring the forms to one genus (whether Dzensia, Aphanothece or Aphanocapsa) as to give different names to the colonies observed. It seems equally profitable to classify Bacteria according to their morphology. Only single-cell cultures would give us the proof. And single cell cultures of these and similar algae have failed. The only conclusion we can reach with any degree of justification would be that there occur in concentrated brines a number of bluegreen algae belonging to the Chroococceae. The shape of the cell, its colour, the ability to form sheaths and common matrices seems to be exceedingly variable.

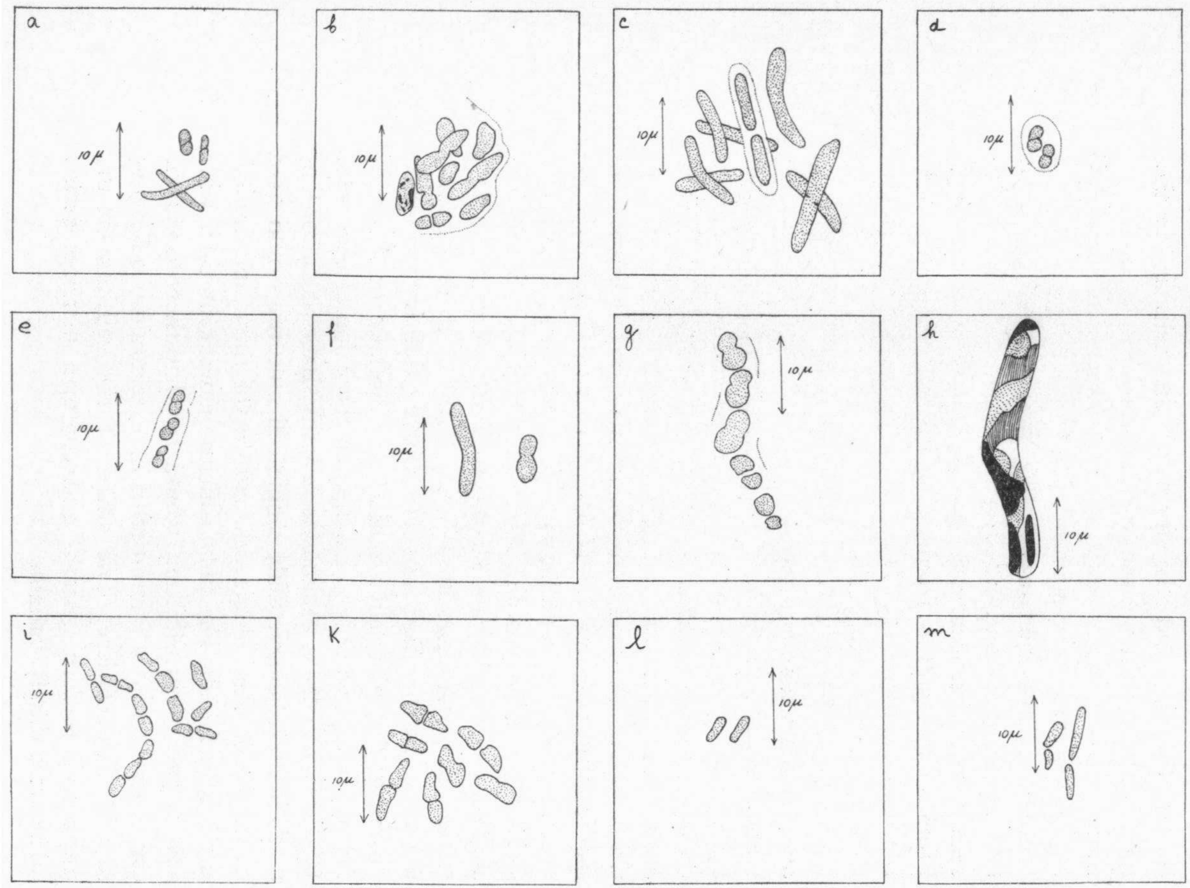


Figure 12. Observed cell forms of Aphanothece halophytica Frémy n. sp. a. Strain from Marina. Saturated brine. b. Strain from Marina. 1 Mol. NaCl; 0.1 Mol. $MgCl_2$; 0.01 Mol. $CaCl_2$ pH 6. c. Strain from Leslie. ± 20 % NaCl pH 8. d. Strain from Marina. 1 Mol NaCl; pH 9. e. Strain from Marina. 1 Mol. NaCl; 0.1 Mol. $MgCl_2$ pH 9. f. Strain from Portugal. 2 Mol NaCl; Yeast Autolysate 5 %. g. Strain from Marina. 1 Mol. NaCl; 0.2 Mol. $MgCl_2$; 0.01 Mol. $CaCl_2$ pH 6. h. Strain from Leslie. 2.5 Mol. NaCl; trace $MgCl_2$ pH 9. i. Strain from Marina. 2 Mol. NaCl; 0.2 Mol. $MgCl_2$; 0.01 Mol. $CaCl_2$ pH. 6. k. Strain from Marina. 1 Mol. NaCl; 0.5 Mol. $MgCl_2$; 0.01 Mol. $CaCl_2$ pH 9. l. Strain from Marina. 1 Mol. NaCl; 0.2 Mol. $MgCl_2$ pH. 6. m. Strain from Marina. 2 Mol. NaCl; 0.2 Mol. $MgCl_2$.

The chemical nature of the culture solution as well as the age of the culture and probably also temperature and illumination are all factors which contribute to this variability. In the literature several Chroococceae are mentioned from salty soil, from salt and from strong brine.

Geitler (11) mentions 53 species of halotolerant Chroococcaceae of which 25 are marine.

Several of these forms may be identical with or closely allied to the forms described by us.

## 4. Cytological and Physiological Notes.

*a. Vacuolisation,* according to Geitler (10) may go parallel with a decrease in mass of the ectoplasm, a disruption and final disappearance of the chromatoplast. In various instances, especially in "giant-cells" this phenomenon was encountered. Parts of the cells became hyaline, through which the coloured ectoplasm would run like threads, or else be pushed towards the ends of the cell. (See figures 9 and 11).

In a 6 months old culture (in 2.5 Mol. NaCl, to which the usual salts and a trace of $MgCl_2$ was added: pH 9) from the Leslie Salt Works, San Mateo, California, there appeared giant cells, about 30—40 $\mu$ long, in which the chromatoplast showed a very curious appearance (L.N.B-B.). At first inspection the cells seemed to be variegated. (See fig. 12 h.). This appearance was apparently caused by the localisation of the pigment: pure green, pure blue and bluegreen patches being visible within the same cell. Camera drawings were made with illumination of varying wave length by means of a small Leitz monochromator. Certain areae never showed dark, while other patches were very distinctly dark between 620—630 m$\mu$ (absorption of phycocyanin) and still others showed up at 670—680 m$\mu$ (Absorption of chlorophyll). The dark bluegreen parts of the chromatoplast appeared dark both from 620—630 mμ and from 670—680 mμ. It seems therefore that on the formation of abnormally large cells the chromatoplast is disrupted and that the chlorophyll and the phycocyanin behave more or less independently. It seems not impossible that each of these two pigments is present in a distinct layer.

*b. "Plasmolysis"* of Myxophyceae cannot be compared with plasmolysis of cells of higher plants, because of the absence of a vacuole in their normal cells. Water may only be withdrawn from the protoplasm of the algal cell. It is evident therefore, that the gas-laws, which seem to hold for plant cells with large vacuoles (Höfler (14)) do not apply here. We rather expect a *swelling* curve, such as Walter (19) found with his experiments on the sporogenous filament of Lemanea, the cells of which contain no vacuoles. Among the various authors who report upon plasmolysis of Myxophyceae Borzi (1886 (4)) never observed actual withdrawal of the protoplasm from the cell-wall. Fischer (8) saw a withdrawal of the protoplasm. The process was reversed after some time. Brand (5) points out that there is a strong adhaesion between protoplasm and wall which is difficult to destroy. He observed plasmolysis in a few cases. Prat (16) comments upon the incompleteness of the plasmolysis, because of the adhaesion. Like Fischer (l.c.) he observed deplasmolysis. Schmid (17) determined the limiting concentrations of several solutions which showed contraction of the filaments of Oscillatoria. This appeared in solutions of saccharose 1 %, $KNO_3$ 0.85 %, NaCl 0.107 % and urea 0.5 %. Deplasmolysis was observed in all cases; saccharose excepted.

For our observations we used a truly halophilic form which is called in this paper Aphanocapsa litoralis Hansgirg. The algal material was washed with the solution to be tested, finally placed in a drop of this liquid. The coverglass was sealed on by means of paraffin-oil to prevent evaporation. The percentage of cells that showed plasmolysis was ascertained after 20—30 minutes.

| Solution. | I. Strain Lake Eyre 2.6 Mol. old 4 months | II. Strain Gersik putih 2 Mol. old 4½ months | III. Strain Gersik putih 2 Mol. old 3 months | IV. Strain Gersik putih 3 Mol. old 3 months |
|---|---|---|---|---|
| 3.8 Mol. NaCl | 19 % | | | |
| 4.0 „ „ | 28 % | 50 % | | |
| 4.5 „ „ | 39 % | 63 % | | |
| 5.0 „ „ | 58 % | 67 % | 63 % | |
| 5.25 (sat.) „ | 80 % | 81 % | 90 % | 97 % |
| 4 Mol. NaCl | | | | |
| 4.5 „ „ | | 33 % | | |
| 5.0 „ „ | | 58 % | | |
| 5.5 (sat.) „ | | 100 % | | |

Neither $MgCl_2$ ($\pm$ 4.8 Mol.) or $CaCl_2$ ($\pm$ 5.6 Mol.) showed any effect, this being probably due to specific action of the kation upon the membrane.

Younger cultures give more complete plasmolysis as older cultures. Strain III for example showed no plasmolysis with 5 Mol. NaCl but with 5.25 Mol. suddenly 97 %. In older cells the percentage of plasmolysis increases with the increasing concentration. The behaviour of the older cultures may be ascribed to the presence of larger, vacuolated cells.

"Deplasmolysis" was observed in all cases. The "type" of plasmolysis was concave which points to a rather high viscosity of the contracting mass.

As in the case of Lemanea the observations on Aphanocapsa could be best accounted for by swelling and shrinking of the protoplasm.

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