

Chinese studies on the edible blue-green alga, *Nostoc flagelliforme*: a review

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Abstract

Nostoc flagelliforme, which is distributed in arid or semiarid steppes of the west and west-northern parts of China, has been used by the Chinese as a food delicacy and for its herbal values for hundreds of years. However, the resource is being over-exploited and is diminishing, while the market demands are increasing with the economic growth. This review deals mainly with the Chinese studies on the ecology, physiology, reproduction, morphology and culture of this species in an attempt to promote research and development of its cultivation technology.

Introduction

Nostoc flagelliforme (Berk. & Curtis) Bornet & Flahault is a terrestrial blue-green alga of great economic value in China. The Chinese have used it as food for about two thousand years, as told in a book called 'Er Ya Zhu' (word interpretation) of the Jin Dynasty (A.D. 265–316). Its herbal values were recognized more than 400 years ago, as recorded with other economic *Nostoc* species in Compendium of Materia Medica (Li Shizhen, 1578). *N. flagelliforme* is called 'Facai' (hair vegetable) in Chinese because of its hair-like appearance. However, the pronunciation of 'Facai' sounds like another Chinese word that means to be fortunate and get rich. Therefore, it symbolizes additionally good luck. *N. flagelliforme* has been consumed in China, especially Guangdong, and among the overseas Chinese on account of its food and herbal values as well as its spiritual image.

In China, *Nostoc flagelliforme* has been collected and traded from old times. The resource is getting less and less as the market demand increases with economic growth. On the other hand, exploitation of land has greatly reduced the area producing *N. flagelliforme*. In Ningxia province, the area was about 3.1×10^6 ha

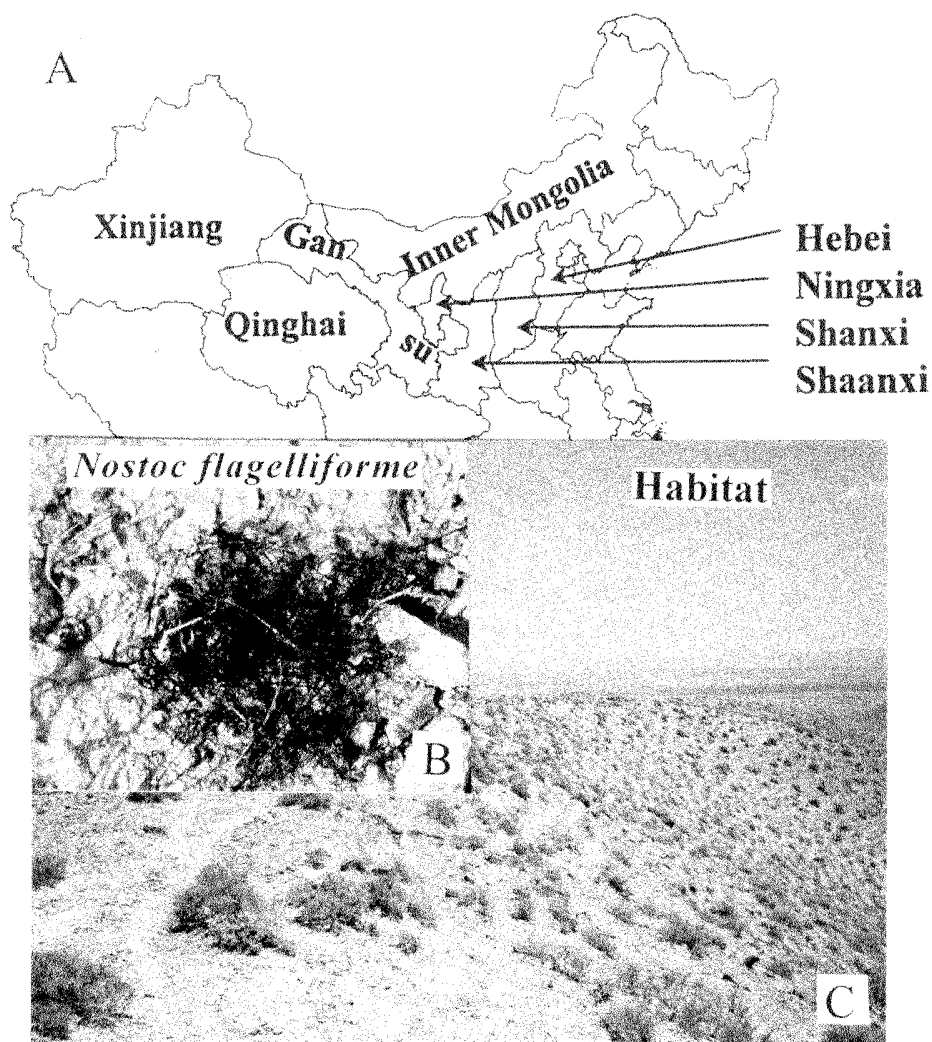
in the 1960s; however, it had decreased to 1.7×10^6 ha by the 1980s (Dai, 1992). To preserve the natural resource and to meet the market demand of *N. flagelliforme*, state-funded technical programs on the biology and cultivation techniques have been carried out since the 1970s. There have been a number of publications on morphology, reproduction, ecology, physiology, biochemistry, resources and culture techniques (Table 1). However, almost all of the research articles have been published in Chinese, and hardly known out of China. The present paper reviews the Chinese studies in an attempt to further the research and development of cultivation technology of this species.

Distribution and geobotanical features

Nostoc flagelliforme is distributed in arid or semiarid areas, including Algeria, China, Czechoslovakia, France, Mexico, Mongolia, Morocco, Russia, Somalia and USA (Li, 1991). In China, it is distributed in the Northern and the west-northern parts, covering eight provinces, Qinghai, Xinjiang, Ningxia, Gansu, Shanxi and Shaanxi, Inner Mongolia and Hebei (Diao, 1996) (Figure 1). *N. flagelliforme* grows on dry desert

Table 1. Main Chinese publications on *N. flagelliforme*

Morphology	Wang & Gu, 1984; Wang & Liang, 1989; Liu & Pan 1997.
Reproduction	Cheng & Cai, 1988; Jiang, 1990; Hua et al., 1994.
Ecology	Zhu, 1982; Sheng et al., 1984; Cui, 1985; Shi, 1986; Chen et al., 1987; Qian et al., 1989; Dai et al., 1989; Zhao, 1996, 1996; Diao, 1996.
Physiology	Wang et al., 1981; Wang et al., 1988; Wang & Zhang, 1988; Mei & Cheng, 1989; 1990; Shi et al., 1992; Zhong et al., 1992.
Biochemistry	Fang et al., 1984; Ni, 1984; Dai, 1985; Ma et al., 1989; Lu et al., 1990; Dai et al., 1991.
Resources	Dai, 1972; He, 1979; Jiang, 1981; Cui, 1985; Yong et al., 1987; Dai, 1987,; 1988; Geng & Jiang, 1991.
Culture	Hu et al., 1987; Wang et al., 1992.

Figure 1. A) Northern and northwest areas of China. B) Mat (6–8 cm in diameter) of *N. flagelliforme*. C) Its habitat in Ningxia province.

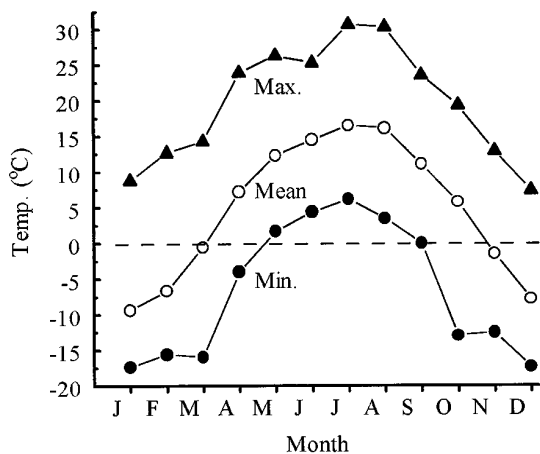


Figure 2. Monthly temperature at Yongden, Gansu province (mean values for 1984–1986, plotted from Qian et al., 1989).

steppes, bare lands or hills in these provinces. It had been described in Japan as a freshwater alga living in streams (e.g. Tanaka, 1991), but this was corrected recently (Aruga, 1992); however, Japanese phycologists were aware of the true organism more than 80 years ago (Okamura, 1913).

Nostoc flagelliforme experiences extreme environmental changes in nature. The habitats of *N. flagelliforme* are located 1000–2800 m above sea level, and are extremely dry (Table 2). The season with rain is from June to August, being responsible for about 70% of the yearly precipitation. Annual evaporation is 10 to 20 times the rainfall. *N. flagelliforme* usually grows in the areas of rainfall between 50–300 mm per year, and co-habitats with *N. commune* in the areas of 250–330 mm rainfall (Shi, 1986). The annual mean temperature is about 2.2–8.6 °C, with the lowest of -17 °C in winter and the highest of 35 °C in summer (Figure 2). In some areas of Gansu province, the air temperature goes as high as 35 °C in summer and as low as -29 °C in winter, a range of 64 °C. Surface temperature on the arid bare lands could rise to about 66 °C in summer, and down to -29 °C in winter. This means that *N. flagelliforme* experiences a yearly temperature difference as big as 95 °C (Qian et al., 1989).

Soil ionic strength and soil texture, which influence cohesiveness and retention of water, affect the abundance of *N. flagelliforme* (Hong, 1987). The soils which have abundant *N. flagelliforme* are the calcic sierozem and light chestnut soil developed from loess mother material during the quaternary period, and is strongly alkaline (pH 8.0–9.5) with plenty of calcic

deposit (Hu et al., 1987; Diao, 1996). Chemical analysis showed that the soil contained less than 2% organic matter, 0.1% total nitrogen or phosphorus but plenty of alkaline oxides (Qian et al., 1989). The niches of *N. flagelliforme* in Qaidamu of Xinjiang Province are around grasses and big stones, being less abundant on bare land in the areas investigated (Hong, 1987).

There are reports on microbes and plants associated with *N. flagelliforme* in these habitats. There were $1.5\text{--}9.8 \times 10^9$ microbes, mostly bacteria, actinomycetes and fungi in 1 g of soil, and these were responsible for 4 to 16 $\mu\text{g CO}_2$ per day (Hu et al., 1987). *N. flagelliforme* shares its habitats with various species of higher plants (Table 3). Vegetation coverage is from 1% to 30%, and there are less than 10 or 20 species of angiosperm. *N. flagelliforme* is most often associated with *Salsola passerina* + *Cleistogenes squarrosa* + *Allium polyrhizum* population in desert steppes of Inner Mongolia (Cui, 1985). In such a community, the vegetation cover was about 30%, of which *N. flagelliforme* accounted for 1% in 18 to 37 mats (1–5 cm in diameter) per square meter.

The standing crop of *N. flagelliforme* was estimated to be in the range of $0.1 \sim 1.3 \text{ g m}^{-2}$ in Ningxia and Inner Mongolia (Shi, 1986; Dai, 1987; Yong et al., 1987; Cui, 1985), about $0.1\text{--}5.0 \text{ g m}^{-2}$ in Gansu province (Qian et al., 1989) and ranged from $0.3\text{--}5.7 \text{ g m}^{-2}$ in Qaidam of Xinjiang Province (Hong, 1987). It usually decreases with increased vegetation coverage in Inner Mongolia on desert or semi-desert steppes (Yong et al., 1987). In non-disturbed areas, mats of *N. flagelliforme* could be as big as 8 cm in diameter, with 18–37 mats m^{-2} , each weighing about 0.6–0.9 g (d. wt). Consequently, the maximum biomass density of *N. flagelliforme* could be as high as $11\text{--}33 \text{ g m}^{-2}$.

Morphology

Nostoc flagelliforme is filamentous, usually not branched, cylindrical or lamellate. False divergent branching, however, does happen occasionally (Dai, 1992). (The term, filament, is used here for the extended macroscopic thallus, and not for the numerous individual trichomes present in the thallus.) *N. flagelliforme* exhibits different shapes according to the habitat, those growing on soil of higher moisture content tending to be flat (Shi et al., 1992). One end of a *N. flagelliforme* filament is usually attached to lumps of soil, stones or plants, seemed to be adhesive. It has been suggested that cell division happens non-uniformly along

Table 2. Environmental features of some habitats of *N. flagelliforme* in China

Province	Elevation (m)	Ann. rainfall (mm)	Rel. humidity (%)	Ann. evaporation (mm)	Annual mean temp. (°C)	Ann. mean sun. exposure (h)	Land type	References
Inner Mongolia	980–1700	100–240	38–55	2997	3.2–8.1	3200	Desert steppe mounds	Young et al. (1987), Cui (1985), Qian et al. (1989)
Qinghai	1700–2800	200–300	43–68	1150–2137	2.2–8.6	2560–3182	Hills, bare lands	Diao (1996)
Gansu	1900	290	45–68	2000–3300	4.4–5.0	3000–3400	Desert steppe, hills	Qian et al. (1989), Hu et al. (1987)

Table 3. Higher plants associated with *N. flagelliforme* (Cui, 1985; Diao, 1996)

Type of habitat	Species
Desert steppe	<i>Stipa grandis</i> , <i>S. krylovii</i> , <i>S. bungeana</i> , <i>S. breviflora</i>
Desert steppe	<i>Stipa govica</i> , <i>S. klemenzii</i> , <i>Ajanian trifida</i>
Sub-desert steppe	<i>Salsola passerina</i> , <i>Reaumuria soongorica</i>
Others	<i>Allium polyrhizum</i> , <i>Androsace filiformis</i> , <i>Arenaria capillaris</i> , <i>Artemisia frigida</i> , <i>A. gmelinii</i> , <i>A. scoparia</i> , <i>A. vestita</i> , <i>Caragana stenophylla</i> , <i>C. microphylla</i> , <i>Cleistogenes squarrosa</i> , <i>Cymbaria dahurica</i> , <i>Eurotia ceratoides</i> , <i>Haplophyllum dauricum</i> , <i>Kalidium gracile</i> , <i>Kochia prostrata</i> , <i>Logochilus ilicifolius</i> , <i>Nitraria sibirica</i> , <i>Peganum harmala</i> , <i>Ptilotrichum elongatum</i> , <i>Reaumuria soongorica</i> , <i>Salsola passerina</i> , <i>Stipa przewalskii</i> , <i>S. tianshanica</i> var. <i>gobica</i> , <i>Thymus mongolicus</i>

the filaments, the ‘top’ (end) of the filament prevails over other parts with faster cell division to provide more space for inner cells of the trichome. This was termed ‘top ascendancy strategy’ (Shi, 1992). It was observed that one end of some filaments was much bigger than the other end and buried in soil about 3–5 mm deep, this being called the Facai’s ‘root’ by peasants; there were bulges in some filaments, called Facai’s seeds. The bulges were proved by growth experiments to be the seeds of angiosperms (Wang & Liang, 1989).

In its natural habitats, *N. flagelliforme* is about 50 cm long, 0.2–1 mm in diameter. Such a filament is a colony of trichomes embedded in a sheath. The vegetative cells are about 4–6 μm long and 4–5 μm wide (Wang & Gu, 1984). The protoplast is often differentiated into a peripheral pigmented region and a central colorless one. Heterocysts occur in the middle or at the ends of the filaments, being bigger than vegetative cells; they appear pale and have a thick cell wall. The sheath of *N. flagelliforme* plays an important role in protecting cells from environmental stresses. During the developmental stage, when the sheath was

not formed, the cells are susceptible to environmental changes. However, after the sheath was formed the cells were resistant to severe environmental changes. It seems that the thicker the sheath the higher the survival probability (Cheng & Cai, 1988). In acidic medium (pH < 6.0), sheath formation was inhibited (Liu & Pan, 1997).

Reproduction

Reproduction of *Nostoc* takes place in four different ways: 1) single cells fragmented from filaments form new colonies; 2) via akinetes; 3) hormogonia disperse and form new colonies; 4) large colonies can bud off small colonies (Dodds et al., 1995). The first three have been reported for *N. flagelliforme* (Jiang, 1990), but not the fourth. Formation of hormogonia is the main way of reproduction. In the part where hormogonium is formed, the filament tapers and appear pale, before being broken due to uneven extension triggered by water absorption (Hua et al., 1994). Akinetes of

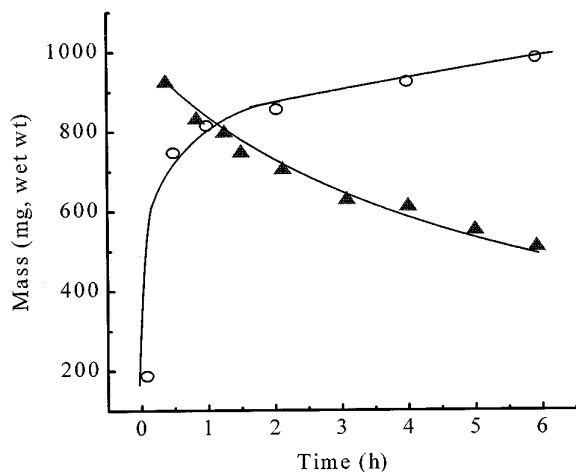


Figure 3. Mass increase with time of *N. flagelliforme* while rewetted (circle), and mass decrease with time while desiccated (triangle) (replotted from Wang & Zhang, 1990).

N. flagelliforme were freed from filaments within the sheath in a state of dormancy, and germinated to form filaments when favorable conditions were met (Jiang, 1990). Occasionally, almost all the vegetative cells in a filament can become akinetes, with cells of various sizes being produced before the filaments disintegrate due to unfavorable conditions (Jiang, 1990). The early stage of development from an akinete in laboratory showed little difference from that of *N. commune* and *N. muscorum* (Cheng & Cai, 1988), being characterized by 1) cell divides to form filament, 2) heterocysts form at ends and subsequently within the filaments, 3) filaments break before spherical colonies are formed.

Ecophysiology

Re-hydration. In its natural habitats with the temperature of 4–23 °C and relative humidity of 37–91%, *N. flagelliforme* usually contains 4–30% water (Dai, 1992). Laboratory experiments showed that *N. flagelliforme* absorbed water fast and lost it slowly (Figure 3). The ability to retain water is an important strategy for *N. flagelliforme* to survive the dry environment. *N. flagelliforme* can absorb water six times its dry weight in one minute, much faster than *N. commune* that takes 3–5 minutes to absorb the equivalent amount. However, it took 5 to 10 h for the rewetted *N. flagelliforme* to lose the water and become dry under windy and dry conditions (Chen et al., 1987). Nevertheless, *N. flagelliforme* loses water faster than other terrestrial

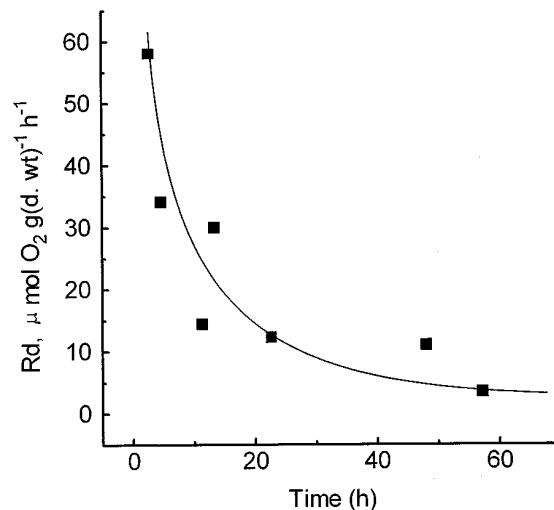


Figure 4. Decline of respiration with time in rewetted *N. flagelliforme* after it had fully recovered. (replotted from Mei & Cheng, 1989).

Nostoc species. Scherer et al. (1984) reported that the ratio of surface to mass (S/M) of *N. flagelliforme* was 82 cm² g⁻¹, while *N. commune* and *Nostoc* sp. were about 37 or 12 cm² g⁻¹, respectively. *N. flagelliforme* from Aldabra Atoll absorbs and loses water much faster than *N. sphaericum*, *N. commune* and *Nostoc* sp. (Whitton et al., 1979). The half-time for rewetting and desiccation was shorter in *N. flagelliforme* than *N. commune* or *Nostoc* sp., inversely correlated with S/M ratios (Scherer et al., 1984). *N. flagelliforme* grows on the surface of bare lands, arid or semiarid areas, where water flows and evaporates off immediately in the hot summer season; therefore, it is beneficial to take up water quickly. Its geometry, however, results in fast desiccation. Fast water uptake and rapid water loss may both be important ecologically and physiologically for *N. flagelliforme* to grow in the dry areas where other terrestrial *Nostoc* species hardly survive. Evaporation of water from the filaments of *N. flagelliforme* helps to dissipate the heat of solar radiation absorbed by the alga. The large surface/mass ratio of *N. flagelliforme* enables it to exchange heat efficiently with the air, which tends to minimize overheating during the summer raining season.

Wind enhances the water-losing process of *N. flagelliforme*. The half-time of desiccation at a wind speed of 2 m s⁻¹ was shortened to one-third and one-fourth that of still air at 20 and 27 °C, respectively (Gao et al., 1998a).

Respiration could not be detected *in situ* during daytime in natural dry *N. flagelliforme* (Mei & Cheng, 1989). However, photosynthetic carbon fixation was active even under extremely dry conditions and strong solar radiation on hot days in July, when land surface temperature was about 44 °C, but was depressed when water was supplied, probably due to enhanced respiration by rewetting (Sheng et al., 1984; Cui, 1985). The dark respiration was detectable at night and reached its maximum at 2100–2200 hours (Sheng et al., 1984; Cui, 1985). In contrast to *in situ* investigations, laboratory experiments showed that rewetting for one day enhanced net photosynthesis of *N. flagelliforme* by 60% compared to that of natural dry samples; however, longer treatment lowered it. Photosynthetic efficiency (F_v/F_m) of fully rewetted and physiologically recovered *N. flagelliforme* started to decline after it lost about 40% of the water (Gao et al., 1998a). Rewetting retrieved the respiration of *N. flagelliforme*, which increased to reach a maximum in 40 min and then decreased to 34% in 8 h and to 5% in 57 h (Figure 4), and declined faster in the dark compared to that exposed to light (Mei & Cheng, 1989). When *N. flagelliforme* was immersed in water for three days, net photosynthesis declined throughout the period (Mei & Cheng, 1989). Daily photosynthetic production of *N. flagelliforme* estimated *in situ* was reduced to zero by watering, while that without being watered was productive [ca. 14.1 mg CO₂ g (d.wt)⁻¹ day⁻¹] (Cui, 1985). This implies that *N. flagelliforme* does not gain any carbon by photosynthesis while immersed after rain; instead, it tends to lose weight due to enhanced respiration, consumption by which exceeds production by photosynthesis. Consequently, less water is required for the alga to be photosynthetically productive than for it to be fully physiologically active.

The recovery time required for maximum photosynthetic and respiratory activities after rewetting is dependent on storage time in dryness. The mats of *N. flagelliforme* after being stored for three months reached maximum photosynthesis in half an hour, and that being stored for 18 months required 3.5 h. In the case of respiration, the mats stored for 3 and 18 months attained a maximum after being rewetted for 5 and 60 min, respectively (Shi et al., 1992). Scherer et al. (1984) reported that rewetted *N. flagelliforme* after stored for two years attained its maximal rates of photosynthesis and respiration in 8–9 h and 2 h, respectively. It seems that the longer the period of dryness, the more time *N. flagelliforme* needs for photosynthetic and respiratory recovery. Photosynthetic recovery

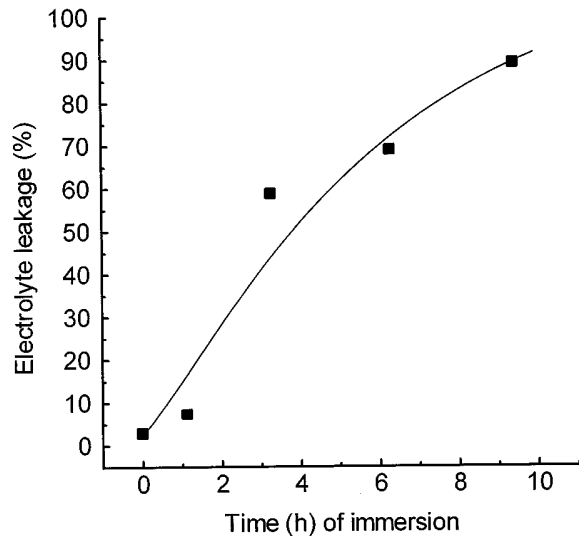


Figure 5. Effects of immersion time (days) on the electrolyte release from *N. flagelliforme* (replotted from Mei & Cheng, 1989).

of *N. flagelliforme* was found to be correlated to the recovery of energy charge (Scherer et al., 1986), and was demonstrated to be a light-dependant process (Gao et al., 1998a). When *N. flagelliforme* was rewetted, its photosynthetic efficiency (F_v/F_m) remained undetectable in the dark for more than 10 h, but was detected immediately when transferred to light. Its recovery was faster at 40 than 4 $\mu\text{mol m}^{-2} \text{s}^{-1}$, indicating a dependency on the light intensity (Gao et al., 1998a). Recovery of nitrogenase activity of *Nostoc* spp. was also delayed due to a long period of dry conditions (Whitton et al., 1979). Physiological activities were found to recover in the order of respiration, photosynthesis and then nitrogen fixation after being rewetted (Scherer et al., 1984). Such a sequence was only obvious for long-stored (> 2 years) samples (Chen et al., 1987).

Immersion or watering is physiologically negative to *N. flagelliforme*. When plant cells suffer from negative environmental changes, the cellular membrane may lose its selective permeability, ions or organic substances being released into the medium, resulting in increased conductivity: increase in conductivity of the medium reflects cellular membrane damage. Rewetting *N. flagelliforme* (24 h) resulted in less than 3% electrolyte leakage; however, immersing (24 h) enhanced the leakage to 9%. Immersing resulted in 60% and 90% electrolyte release, respectively, in 3 and 9 days (Figure 5) (Mei & Cheng, 1989). This indicates that cellular damage occurred when *N. flagelli-*

forme was immersed. *N. flagelliforme* often grows on semi-desert soil or mounds, so usually does not suffer from being submerged after rain. Rain makes *N. flagelliforme* wet, but the water evaporates fast due to high temperature and its geometry. *N. flagelliforme* can become fully dehydrated in 3 to 7 h after precipitation. The strategy for *N. flagelliforme* to survive the arid climate is to lower its physiological activity when becoming dehydrated. In its natural habitats, *N. flagelliforme* absorbs moisture from dew at night to contain about 30% water (Shi, 1986; Hong, 1987). More than half of the photosynthetic efficiency was recognized at this level of water content (Gao et al., 1998a, b). High photosynthetic rates can be expected in the early bright morning before the alga loses water.

Light. Photosynthesis of *N. flagelliforme* was saturated at $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ (conversion made assuming $1 \mu\text{mol m}^{-2} \text{s}^{-1}$ 50 lux) for rewetted air-exposed samples (Mei & Cheng, 1990) and $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ for immersed samples (Shi et al., 1992) under optimum temperatures (25–30 °C). The light compensation point was 40–90 $\mu\text{mol m}^{-2} \text{s}^{-1}$; no photoinhibition was recognized till $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Shi et al., 1992). *In situ* measurement showed that photosynthesis in July was saturated at $967 \mu\text{mol m}^{-2} \text{s}^{-1}$, and the light compensation point was $225 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Cui, 1985). Photosynthesis-saturated photon flux density of *N. flagelliforme* is higher than that reported for some other algae (50–600 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Harris, 1980; Kirk, 1983).

Temperature. The optimum temperature for photosynthesis was from 25–35 °C, that for dark respiration from 35–40 °C (Shi et al., 1992). The optimum temperature for growth has been reported to be in the range of 15–25 °C (Cui, 1983; Wang et al., 1992; Diao, 1996).

N. flagelliforme is heat-tolerant when it is dry. Since *N. flagelliforme* experiences great temperature differences daily and yearly, its heat-resistance is of general concern. When the alga is naturally dry, both dark respiration and net photosynthesis show strong resistance to heat treatment. Dark respiration was not affected by 24 h pretreatment at temperatures from –15 to 85 °C (Mei & Cheng, 1990) (Figure 6). Pretreatment at 105 °C lowered respiration by 47%, and that at 117 °C led to death. Net photosynthesis was not significantly affected by the pretreatment at temperatures of –15 to 45 °C for one day (Figure 7). Pretreatment at temperatures above 45 °C lowered its photosynthetic activity, to only 8% at 95 °C. However, when *N. flagelliforme*

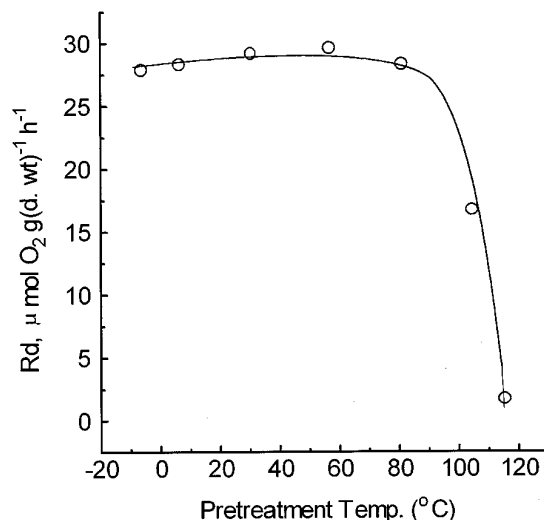


Figure 6. Effects of heat pretreatment on dark respiration of *N. flagelliforme*. Naturally dry *N. flagelliforme* was pretreated at various temperatures for one day before it was rewetted and the dark respiration was measured at 25 °C. (replotted from Mei & Cheng, 1990).

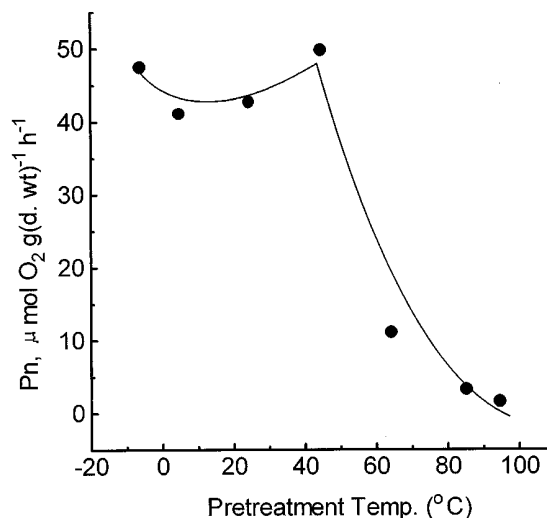


Figure 7. Effects of heat pretreatment on the net photosynthesis of *N. flagelliforme*. Naturally dry *N. flagelliforme* was pretreated at various temperatures for one day before it was rewetted and the net photosynthesis was determined at optimum light and temperature (replotted from Mei & Cheng, 1990).

was wet, pretreatment at 65 °C led to the alga's death (dark respiration reached zero), that at temperatures above 45 °C resulted in zero photosynthetic activity. *N. flagelliforme* can tolerate more heat when it contains less water. Pretreatment at various temperatures

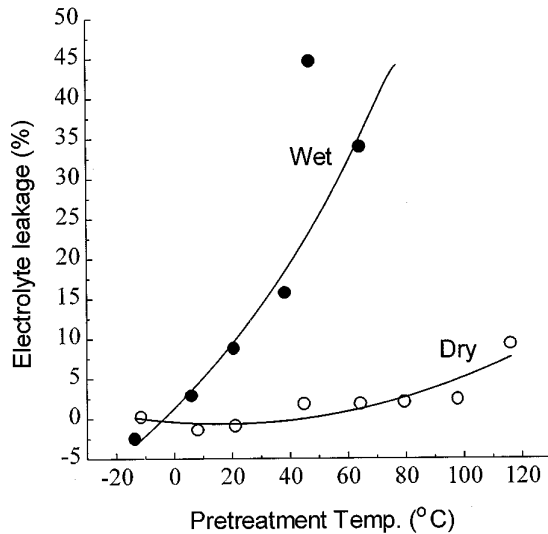


Figure 8. Effects of heat pretreatment on the electrolyte release from *N. flagelliforme*. Comparison was made between naturally dry and rewetted *N. flagelliforme* (replotted from Mei & Cheng, 1990).

showed different effects on the cellular membrane of wet and dry samples. For wet samples, pretreatment at 60 °C gave rise to about 40% electrolyte leakage compared to that for killed cells. However, for dry samples, pretreatment below 100 °C did not cause any leakage; only about 8% leakage was detected when the pretreatment temperature was as high as 115 °C (Figure 8). This indicates that the cellular membrane is prone to be damaged by heat when *N. flagelliforme* is wet. Rapid water loss from *N. flagelliforme* is physiologically necessary for it to cope with high temperature and avoid heat damage after rain in summer, when surface temperature of the habitats sometime rise to 66 °C (Qian et al., 1989). Though rain can make the alga wet and prone to heat damage, it also reduces the surface temperature of the soil. Therefore, rewetting after rain in summer should not bring about heat damage to the alga. In its natural habitats, *N. flagelliforme* may harmonize its dehydration with soil evaporation.

Pretreatment of rewetted *N. flagelliforme* at low temperature (−15 °C) for 24 h enhanced its photosynthesis at room temperature by 30% (Mei & Cheng, 1990). Great daily temperature difference in the habitats of *N. flagelliforme* may benefit its photosynthetic production via reduced respiration at night and enhanced photosynthesis during the daytime.

Salinity. The dry soil on which *N. flagelliforme* grows accumulates salts due to high evaporation and low rain-

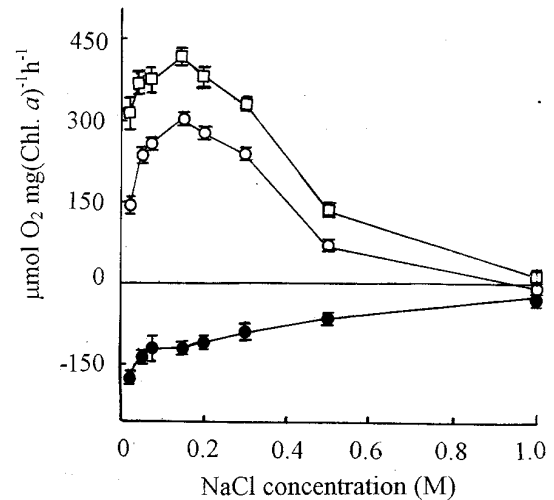


Figure 9. Effects of salinity on gross (□) and net (○) photosynthesis and dark respiration (●) of *N. flagelliforme* (replotted from Shi et al., 1992).

fall. Therefore, physiological responses of *N. flagelliforme* to salinity changes may be expected. Shi et al. (1992) found that *N. flagelliforme* was salinity-tolerant: its photosynthetic activity increased with increased concentration of NaCl, exhibited a maximum at 0.15 m NaCl and dropped to zero in 0.90 m NaCl solution. Respiration decreased accordingly with the concentrated NaCl (Figure 9). *N. flagelliforme* has physiologically acclimated to salts accumulated in soil while growing in a dry arid environment of little rainfall and high evaporation. A description of the mechanism for the decline in respiration with increased salinity is not yet available.

Length. The physiological activity of *N. flagelliforme* seems to be related to its length. Net photosynthesis and dark respiration of *N. flagelliforme* showed maximum values when it was shorter than or equal to 5 mm, both processes decreasing in longer filaments (Shi et al., 1992). The cells embedded in the voluminous gelatinous substance are probably limited in receiving enough substrates for photosynthesis. It is possible that the longer the filaments, the harder it is for the inner cells to obtain water or nutrients. Though longer filaments of *N. flagelliforme* showed reduced photosynthetic rates, it is not enough to imply that longer filaments are less productive because they also show reduced respiration.

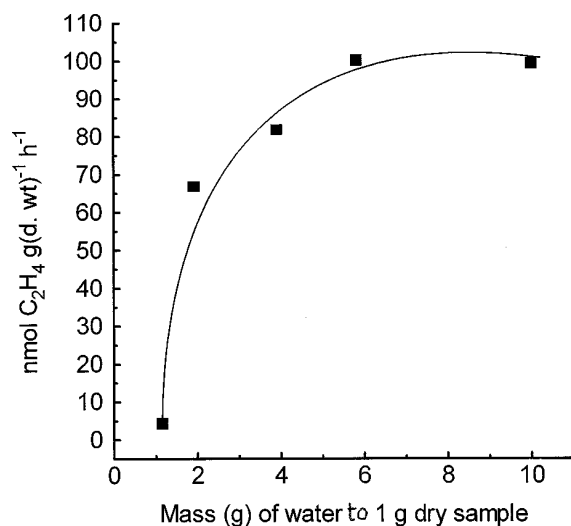


Figure 10. Influence of amount of water absorbed on nitrogenase activity of *N. flagelliforme* (replotted from Wang & Zhang, 1990).

Nitrogenase activity

The activity of nitrogenase in natural dry *N. flagelliforme* was not detectable (Mei & Cheng, 1989). Rewetting *N. flagelliforme* enhanced the activity, but immersing depressed it. The activity of nitrogenase was detectable after *N. flagelliforme* had been wetted for an hour, reached a maximum after 8 h and then decreased with time. Nitrogenase increased with increasing amount of water absorbed, and reached a maximum when the absorbed water was six times the dry weight (Figure 10) (Wang & Zhang, 1988).

The nitrogenase activity of *N. flagelliforme* is extremely sensitive to higher temperatures under wet conditions. Long-term exposure of the wetted filament to high temperature (45 °C) caused rapid decline in nitrogenase activity to zero. Under dry conditions, *N. flagelliforme* is highly resistant to heat exposure. Dry filaments exposed to 55 °C for 5 h daily for 21 days, show no marked change in nitrogenase activity. Repeated drying-wetting cycles induced a gradual increase in nitrogenase activity and enhanced the resistance of the nitrogenase activity to desiccation (Zhong et al., 1992).

High NaCl concentration (0.17–0.43 m) decreases nitrogenase activity quickly (Zhong et al., 1992). Seasonal variation of nitrogenase activity was recognized in *N. flagelliforme* (in China), with the lowest activity in January and the highest in October. The maximum nitrogen fixation rates were in the range of 1 to 3 nmol

N₂ g(d.wt)⁻¹ h⁻¹ (converted from Zhong et al., 1992, assuming ethylene to N₂ ratio of 3:1). The optimal temperature for the nitrogenase activity was 21–28 °C; optimal water content was 10 to 15 times the dry alga, light saturation occurred between 700–900 μmol m⁻² s⁻¹ (Zhong et al., 1992). Nitrogenase activity was only 7% in the dark compared with light, and was slightly enhanced by 1% glucose sprayed on the algal mats. Hydrogen evolution was detected in the light (Wang et al., 1981).

Exploited resource and culture trials

Products of *N. flagelliforme* have been rated to four grades (Dai, 1986). 205 t *N. flagelliforme* were traded in Inner Mongolia, Gansu, Ningxia and Qinghai in 1979 (Shi, 1986), of which 65 t were from Siziwangqi (Inner Mongolia) with the area of approximately 10⁶ ha producing the alga (IMWR, 1988). In Guangdong province, about 100 t *N. flagelliforme* is consumed annually (Geng & Jiang, 1991).

N. flagelliforme is collected throughout the year, most often during the period of October to April from 0400–0800 or the day after rain before it becomes brittle after dew moisture has completely evaporated (Jiang, 1981). In addition to loss by harvesting, pasturing of cattle is rapidly diminishing the resource (IMWR, 1988). The area growing *N. flagelliforme* decreased from 3.1 × 10⁶ ha in the 1960s to 1.7 × 10⁶ ha in the 1980s in Ningxia (Dai, 1987, 1988). The quality of *N. flagelliforme* products is degrading, while its resource is being reduced. One kg of collected *N. flagelliforme* mixture resulted in 900 g good product in the 1970s, but only produced 350 g in the 1980s. In the early 1980s, people were organized to disseminate the segments of *N. flagelliforme* in the hope that homogonia would germinate to increment the biomass. Observation was followed for several years, but no sign of biomass increase was ever seen (Shi, 1986).

It has been believed that *N. flagelliforme* grows (very rapidly) after rain. This is a false impression from its expansion due to water absorption. When *N. flagelliforme* is rewetted, it expands in three dimensions. Filaments 12.5–59.1 mm long elongate in proportion to their length, by 22 ± 4% (*n* = 6) in 15 min, and widen by 71 ± 15% (*n* = 8) in 6 h after rewetting (author, unpublished). Investigation after continuous rain for three days has shown that rain does not enhance growth (Shi, 1986). Water for *N. flagelliforme* to maintain its life and growth comes

from the dew that forms at night. In the northern and west-northern parts of China where *N. flagelliforme* is distributed, the temperature shows a very marked day-night variance. Water vapor becomes dew when temperature drops at night. The relative humidity is below 60% during daytime, and up to 80–90% at night (2200–0800) (Hong, 1987).

Though the photosynthetic activity of *N. flagelliforme* is comparable to that of other blue-green algae (Shi et al., 1992; Scherer et al., 1984), its growth has been reported to be very slow. Cells divided 3 to 4 times in 10 days (25 ° and 500 lux) when *N. flagelliforme* was cultured on solid medium (Cheng & Cai, 1988). The average elongation of *N. flagelliforme*, when cultured in various media in the laboratory, was 10% in half a year (Dai, 1992). The growth of *N. flagelliforme* was enhanced by watering with a dilute solution obtained from the soil of its habitat (Cui, 1983). The maximum elongation rate was as high as 43% in 12 days, and the average rate was about 20% for the same period. However, soil from the alga's environment did not prove to be the best for its growth. Hu et al. (1987) planted the filaments (3 cm long) by interring one end 0.2 cm deep into the soils collected from the habitats of *N. flagelliforme*, Chinese chive and wheat fields, and cultivated outdoors with everyday watering. Soils from the Chinese chive and wheat fields gave rise to better growth than the soil from the alga's habitat, which contained less microbes with weaker respiration. The elongation rate was $105 \pm 24\%$ ($n = 105$) on the wheat field soil over 30 days. Field measurements of 30 filaments (about 18.9 cm long) in the same period showed an average elongation of 12.3%. Clearly, *N. flagelliforme* can grow better under managed conditions. On the other hand, *N. flagelliforme* prefers day-night temperature fluctuations. Growth was better at a temperature regime of 21 °C by day and 3–9 °C at night than that of 29 °C by day and 10–13 °C at night (Cui, 1983). Under laboratory conditions, *N. flagelliforme* grew better in scattered dim light (800 lux) than radiated bright (3000 lux) light (Zhu et al., 1982). The maximum rate was about 30% in 14 days. It has been shown in various culture experiments that *N. flagelliforme* grows faster at the beginning and slower afterwards. Moisture is needed for the growth of *N. flagelliforme*, but it also leads to its disintegration due to enhanced bacterial propagation. Pre-sterilized filaments of *N. flagelliforme* elongated by 40% in 14 days at 30 °C and 1500 lux (12h L:12h D), but untreated samples disintegrated in 7 to 10 days (Wang et al., 1992). Periodic desicca-

tion seems important to prevent *N. flagelliforme* from being disintegrated by bacteria.

Long-term field growth observation showed that *N. flagelliforme* only elongated 6% in a year (Dai, 1992), while a short-term field experiment indicated that it elongated 12% (average of 30 samples) in 29 days (Hu et al., 1987). Daily net photosynthetic production was $14.1 \text{ mg CO}_2 \text{ g (d.wt)}^{-1} \text{ day}^{-1}$ (Cui, 1985) in July, which is converted to $15.4 \text{ mg (d.wt)} \text{ g}^{-1} \text{ day}^{-1}$ (1.5% daily increase by weight) assuming organic carbon content of 25% (Table 4). 1 m filaments of *N. flagelliforme* weighs about 28.4 mg dry (author, unpublished). 1 g (35 m in total length) of *N. flagelliforme* can elongate 27 cm per day, or become 15 mg heavier on the basis of photosynthetic production. Obviously, photosynthetic production does not agree with the growth rate observed *in situ*. Further studies are needed to picture the photosynthesis and growth patterns of *N. flagelliforme* in nature.

Chemical composition

Nostoc flagelliforme contains canthaxanthin in addition to the echinenone, myxoxanthophyll, β -carotenoid, allophycocyanin, phycocyanin and chlorophyll *a* known for other *Nostoc* species (Lu et al., 1990; Fang et al., 1984). It also contains 20–23% protein with 19 amino acids (eight of which are essential for human health), accounting for 39% of the total amino acids by weight (Ni, 1984; Ma et al., 1989; Dai, 1991), about 56–57% carbohydrates (Dai, 1972; IMWR, 1988; Dai, 1988), 1.8% Ca, 3.2% N, 0.1% P (Table 4). The carbon content is about $25.5 \pm 2.9\%$. The C:N:P atomic ratio of typical harvested *N. flagelliforme* has been estimated to be 255:32:1. Other microalgae deprived of P during growth typically have N:P ratio > 30:1 (Atkinson & Smith, 1983). The high N:P ratio of *N. flagelliforme* indicates that its growth may often be P-limited in nature. The alga's C:N ratio is close to that of the Redfield ratio (C:N:P ~ 106:16:1).

The long history of the Chinese use of *N. flagelliforme* indicates that the alga is probably safe to eat. A recent study reported that there were no adverse effects of a population of *N. flagelliforme* on rats in oral acute and subacute toxicity experiments (Takenaka et al., 1998). Nevertheless, more exhaustive studies would be valuable to establish beyond doubt that no adverse effects occur with material obtained from any growth or storage conditions. A hot-water extract of *N. flagelliforme* showed significant macrophage activ-

Table 4. Chemical composition of *N. flagelliforme* in China

Chemical composition	Mean	SD	n	References
Protein (%)	21.3	0.89	3	Ma et al., 1989
Carbohydrate (%)	56			Dai, 1972
Lipid (%)	5.64	0.07	2	Dai, 1985
Water (%)	12.1	0.26	4	Dai et al., 1991
Ash (%)	5.38	0.28	4	Dai et al., 1991
Carbon (%)	25.1	0.61	4	author, unpublished
Nitrogen (%)	3.16	0.22	4	Dai et al., 1991
Phosphorus (%)	0.12	0.02	4	Dai et al., 1991
Calcium (%)	1.83	0.16	4	Dai et al., 1991
Magnesium (%)	0.27	0.06	4	Dai et al., 1991
Iodine (%)	0.25	0.22	2	Geng and Jiang, 1991; Dai et al., 1991
Iron (%)	0.03	0.01	4	Dai et al., 1991
Zinc (ppm)	12.8	4.17	4	Dai et al., 1991
Manganese (ppm)	22.5	6.31	4	Dai et al., 1991
Copper (ppm)	4.95	0.99	4	Dai et al., 1991
Boron (ppm)	14.0	2.70	4	Dai et al., 1991
Cobalt (ppm)	2.78	0.86	4	Dai et al., 1991
Nickel (ppm)	10.6	2.25	4	Dai et al., 1991
vitamin C (ppm)	3.18	0.01	4	Dai et al., 1991

ities, indicating further potential for pharmaceutical use (Takenaka et al., 1997).

Conclusion

This review shows that *N. flagelliforme* is ecologically drought-adapted and physiologically heat-resistant. Rewetting retrieves the physiological activities, but more time is needed for samples stored for longer, and the recovery process is light-dependant. Immersion reduces its photosynthetic production and accelerates its disintegration by bacteria, indicating that drought is not simply an environmental stress, but of physiological and ecological significance. *In situ*, *N. flagelliforme* is productive under conditions where it is dry for much of the time and grows by utilizing the dew formed at night. *N. flagelliforme* can be grown successfully in the laboratory, but further studies are needed to establish its cultivation technology.

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