THE BIOTA IN THE HYPERSALINITY OF MESSOLONGHI SALTERNS

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The Messolonghi salterns were for me a natural laboratory or to put it another way, an exhibition of hidden microscopic life. Life in its extreme is not met only at the hot springs or at the glaciers zone but also in the waters of far more easier accessible places like the hypersaline waters of the salterns.

It is well known, although not proven in an elaborative scientific way, that the good salt production is driven by the solar radiation intensity and is dependent of the hidden biological procedures engaging an array of microscopic organisms (bacteria, cyanobacteria, protozoa, metazoa) in a complicated matrix of relation more or less obscured.

It goes without saying that if we want to put a foothold on the biological procedures that affect the production of salt, first we must study the organisms. To study them we should first learn which organisms are there. Records should be developed with the species, their abundance, their seasonal fluctuations, in parallel with the physicochemical records and the records of salt production. The processing of the collected data may reveal patterns of salt production related to the presence of certain organisms that will lead to more detailed and targeted studies with possible elaboration of manageable plans of actions.

Apart from the managerial point of view, life in hypersalinity has its own fascination. A hydrobiologist can find there a much more comfortable place for the study of microscopic life as the bias of heavy preying upon the tiny creatures is removed, simply because matazoans unable to tolerate high salt are absent, the only exception being the fish species of *Aphanius fasciatus* and the salt shrimp *Artemia* sp. But the short-lived *Aphanius* has certain limits in terms of reproduction and salinity tolerance and *Artemia* is a minute unarmored fragile crustacean. So, with no fish, no shrimps, no mussels, crabs, snails, echinoderms, etc, what is left are the planktonic and benthic life being either scattered or as a part of the slimy biological mats.

On the basic level, that of the primary producers, the richness of species goes inversely with salinity. In the lowest salinity ponds there are many taxa while with increasing salinity the taxa range narrows and finally at the extreme salinities ~>230 ppt exhibits dominance of only few species in great numbers.

In this essay which is written in a simplified friendly scientific manner I'll try to present my findings from samplings made in the hypersaline water of Messolonghi's saltern. The material presented here is selected from a huge depository of microscopic

photos and videos collected so far. It includes cyanobacteria, eukaryotic algae, protozoa, rotifers, copepods and Artemia that were found in the samples. Many of them were vaguely or not at all mentioned in various publications worldwide. So my effort is at the beginning to spark interest for future more detailed studies on them.
I start with the first category (taxon) that of cyanobacteria of the Kingdom Monera.

1. CYANOBACTERIA

The cyanobacteria previously known as blue-green algae, are prokaryotic photosynthetic organisms, microbes in many aspects but with no flagella. Because of their photosynthetic mode of life they are positioned among algae. They lack nucleus, chromosomes, mitochondria and chloroplasts. Instead they have a long looped twisted thread of DNA that contains all of their genetic material in a far smaller genome than that of any eukaryote. They comprise the most productive photosynthetic machinery on earth found practically everywhere. Their ability to withstand intense luminosity and in contrast to other photosynthetic eukaryotes that tend to avoid extremes of sun radiation, they tend to be exposed as much as possible to the sun aided by their flotation apparatus, the gas vacuoles. In their photosynthetic apparatus they contain chlorophyll, carotenoids and phycobiliproteins, that are special pigments absorbing wave lengths beyond that usable to chlorophylls, thus expanding their spectrum of light radiation collection and so harness more efficiently light energy. The color of their phycobiliproteins **phycocyanin** and **phycoerythrin** is cyan and red respectively giving cyanobacteria their well known name "blue-green" algae. The relative proportions of these pigments produces a variable coloration to the algae.
Cyanobacteria are also renown for their ability (at least in some of them) to fix atmospheric nitrogen to usable nitrogen compounds such as ammonia that are nutrient for all the primary producers on earth. Of course they fix nitrogen in order to satisfy their own needs for growth but after their death nitrogen becomes available to other autotrophs and then through the trophic chain to all organisms.
Mostly known as harmful algae (many of them produce toxins) cyanobacteria are well known from fresh waters and only recently their role in the oceans has received much attention. Additionally from a commercial point of view there has been a booming industry of cultivating the valuable cyanobacterium Spirulina as a highly nutritive, antioxidant and unsaturated fatty acids containing healthy food additive. Other uses of cyanobacteria are also under intense experimentation.
Their role in the salterns remain rather obscured. Limited information exists in the literature concerning their biology in such an extreme environment. To put it more plainly, there are vague information even of their correct scientific names for those species that are encountered in hypersalinity. It is difficult to recognise the correct species based on appearance only. First of all they are motionless, they all look quite the same, their color changes astonishingly, they have no distinctive features such as flagella or chloroplasts to ease in identification. The references in the literature

concerning their size, color, shape, etc creates more confusion than conclusions. And not to be forgotten most of the sources concern fresh water cyanobacteria. So, what is out there to use as a base to start off an exploration to the cyanobacterial world of highly saline waters? Sadly the answer is: not many things.

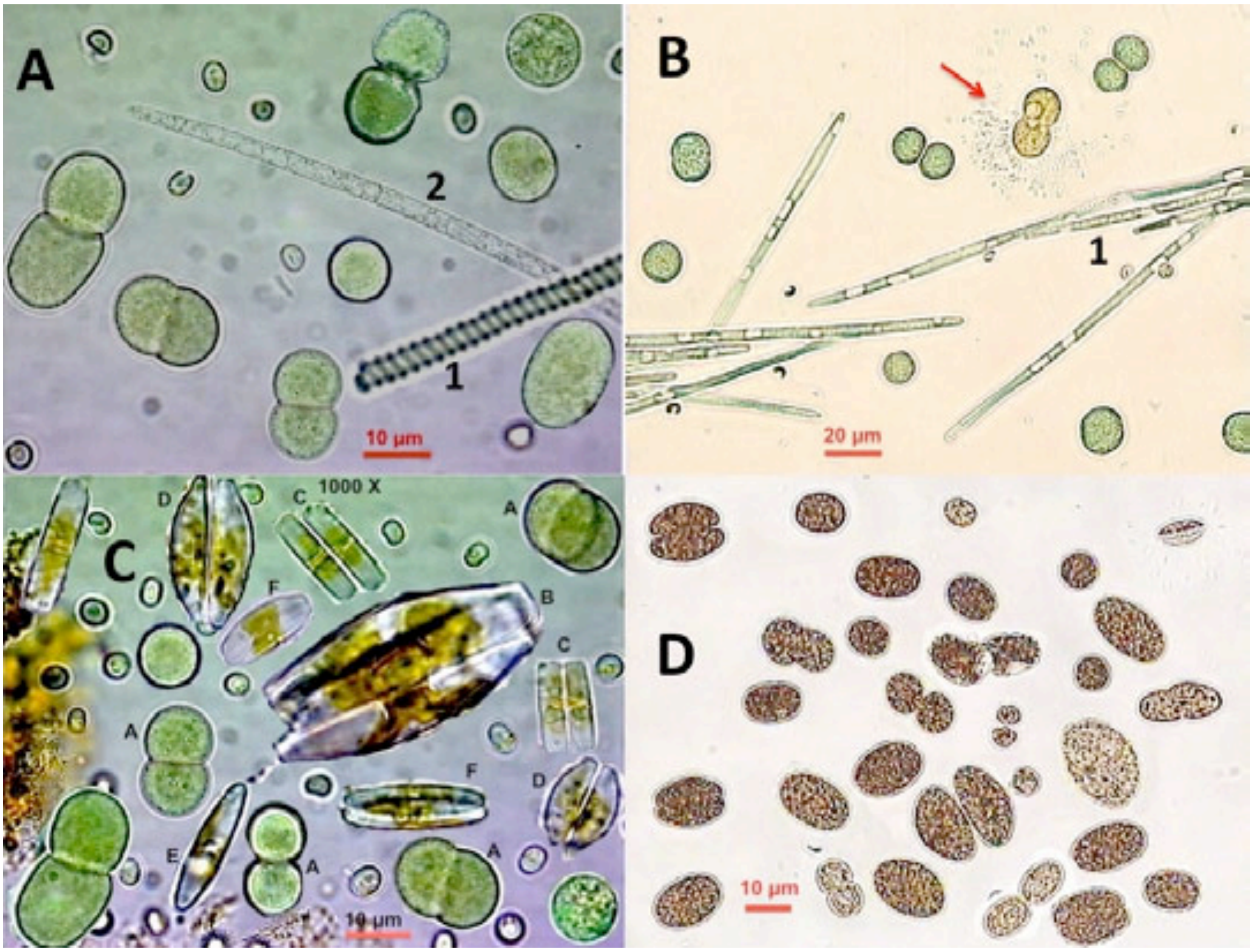

Figure 1. **A**: *Cyanothece* dividing cells (1=*Spirulina*, 2=probably *Cylindrospermopsis*). **B**: Cocciform cyanobacteria of various coloration. Arrow indicates slime, 1=probably *Prochlorothrix*. **C**: An impressive assemblage of various sizes of cyanobacteria and diatoms. **D**: Great variation in shapes and sizes of coccoid (probably *Synechococcus*) cyanobacteria.

That kind of problem threw me in despair after I started recording an astonishing array of various cyanobacteria in various salinities. I searched for information and I found fragments of records for species named as: *Coccochloris, Aphanothece, Synechococcus, Cyanothece* to name but a few. No photos provided and in cases of here and there a photo was presented, its quality made it more confusing than elucidating.

I doubt (I dare to say) for the correct identification of the cyanobacteria in the limited hypersalinity literature. Only after DNA analysis the species identification becomes undisputable. But even so, rarely photographs of the identified species were available. For the layman scientist there is a real confusing situation.

From all around gathered information and before attempting to seek help from experts or to search for funding DNA analyses, I dare to present my collection in order to create a hypersalinity cyanobacteria base to intrigue interest on this topic.

They can be categorized in three groups according to shape and mode of life. 1) **Chroococcales**, round or elongate solitary or colonial cocciform cells. 2) **Oscillatoriales**, multicellular filamentous forms with no nitrogen fixing cells and 3) **Nostocales**, multicellular filamentous forms with nitrogen fixing special cells named "heterocytes".

I start with **Chroococcales**.

In Figure 1 there is an assemblage of cells collected from samples of ~120 ppt salinity water. The green cells (in photo A) are probably of the genus *Cyanothece* and some of them can be seen at various stages of cell division. The protoplasm appears homogeneous and no organelles are visible (not to be forgotten that they are prokaryotes). Next to them are two filamentous cyanobacteria, the spiral one is *Arthrospira* (*Spirulina*) and the other probably *Cylindrospermopsis*. The green-cyan color is due to a combination of chlorophyll and phycocyanin pigments. In the other photos (B, C & D) other cyanobacteria species and diatoms are a good example of the diversity and variation richness in the hypersalinity (more explanation in the Figure's caprion).

Figure 2. Cocciform cyanobacterial cells probably of genus *Synechococcus* exhibiting great variation in coloration and sizes. At the center an amoeba digests some of them.

In Figure 2 from a sample of 100 ppt salinity, the cells are elongated and appear granular for reasons that may be not so clear. It may be due to accumulation of carbohydrates, or air in special compartments named aerotopes that aid in bringing the cells as close as possible to the surface well lit layers in order to intensify photosynthesis. It also may be due to high amount of phycobiliproteins that also contribute to increased photosynthetic rate. From my laboratory cultures I also noticed that the cells become more granular as the culture ages and nutrients are depleted. They are probably of genus *Synechococcus* although their size (~10 μm) is bigger than that found in the literature. The issue of size is really a "mess". Nothing solid can be inferred from the published scientific data as the ranges given for many species are confusing. Besides in the photo the variation of cell sizes is astonishing. So I present my findings and in the future will find out. In the center an amoeba (protozoan) is prominent having engulfed some cells at various stages of digestion, an indication of the biological food web developed in this environment. The green-cyan-brown tint of the cells is due to their blending of pigments with brown to originate from elevated amount of phycoerythrin. The phycocyanin content of the digested cells is seen in Figure 3 as the bluish mass accumulated in the amoeba's protoplasm. Phycocyanin is commercially produced by extraction from cultured cyanobacteria and is highly estimated as antioxidant and natural food colorant.

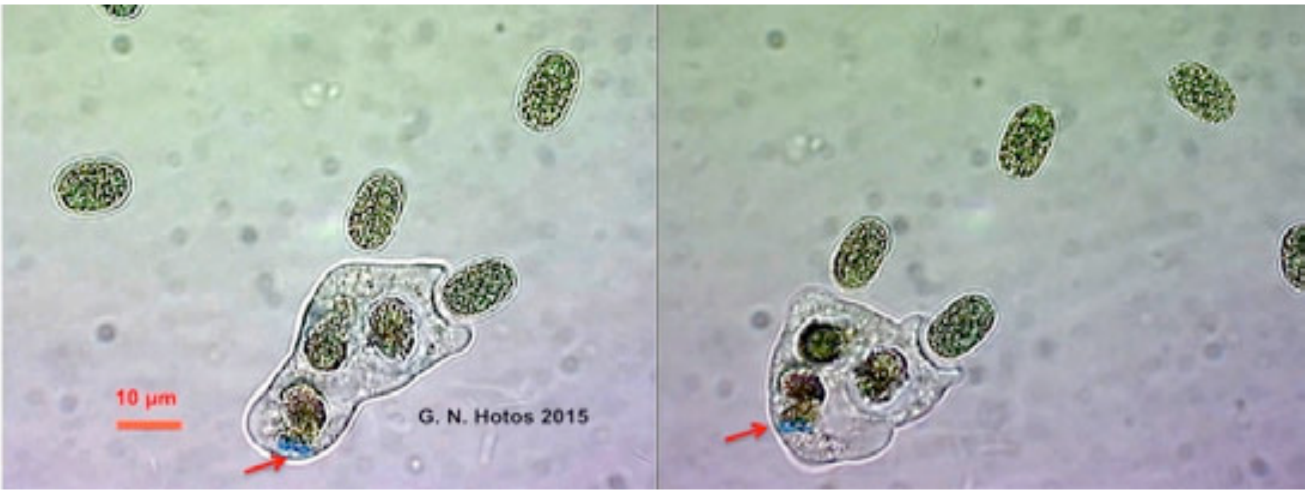

Figure 3. Two frames of cyanobacterial cells digested in amoeba's protoplasm. Arrows indicate mass of phycocyanin (blue colored) from the digested cells.

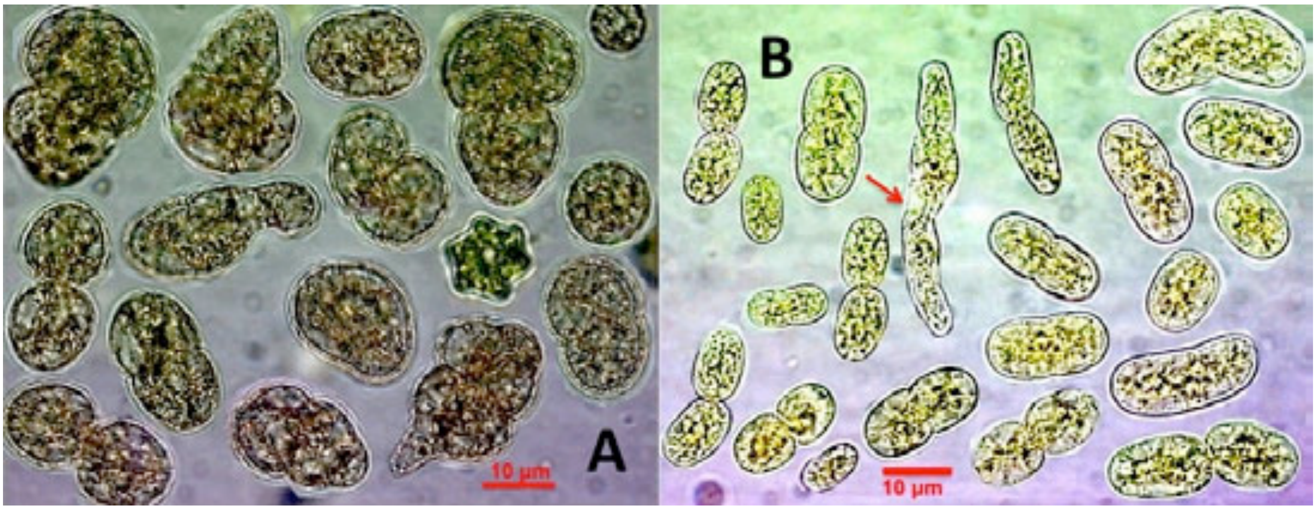

Figure 4. Peculiar involuted expanded cells of cocciform cyanobacteria. The stellate green alga in A is *Asteromonas gracilis* a halotolerant chlorophyte.

In Figure 4 the cocciform cells are deformed to the so called involuted form. In this state they are expanded irregularly, heavily granulated and with asymmetrically shaped daughter cells during cell fission. A prominent feature of the coccoid cyanobacteria is the symmetrical cell division during which they are presented (microscopically) as united half cells remaining so for a long time. It is not clear why involution happens and what follows. It probably occurs in nutrient deficient medium or it is the result of some other unknown physiological state. From my culture observations, this phenomenon is transient and sometimes after some days the involuted cells disappear and the cells regain their typical coccoid form. In Figure 4B the involuted cells are lighter and yellowish in color, very elongated, granular and with great variation in cell sizes. In some of them (arrow) cell division produces a pseudofilament of 4 cells. The specimens of Figures 4 are from laboratory cultures at 120 ppt salinity of specimens from the saltern samples of June 2016.

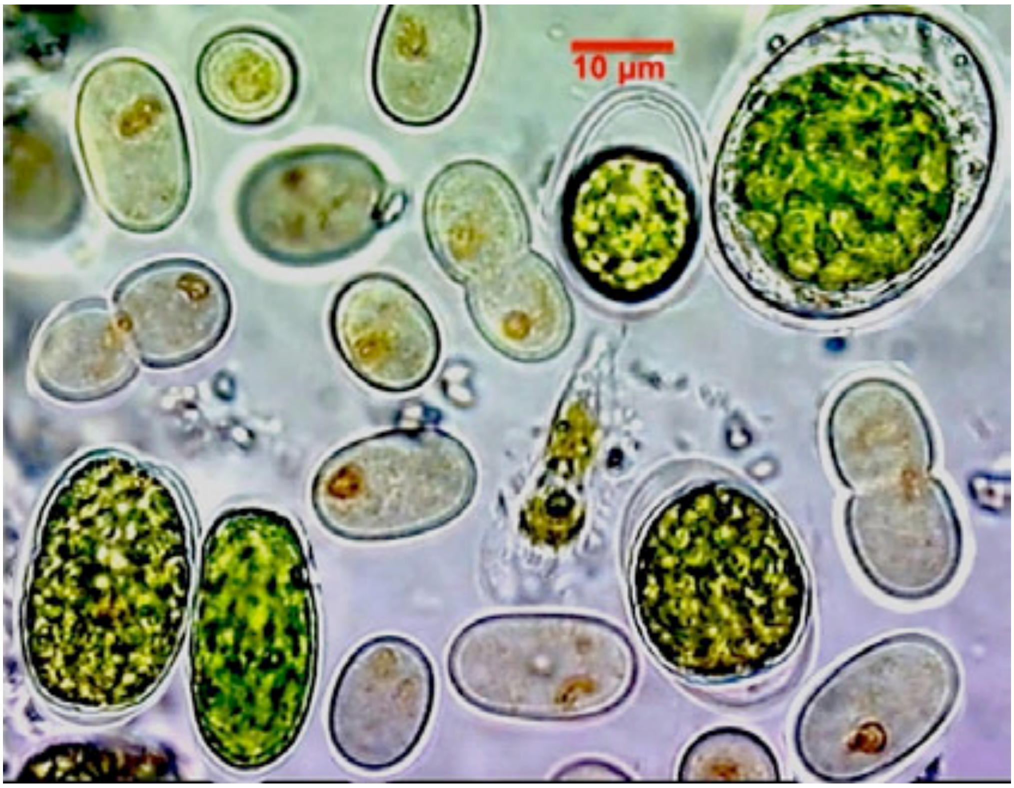

Figure 5. Big non-granular cocciform oval shaped cyanobacteria in high salinity (120 ppt) along with the chlorophyte microalga *Tetraselmis marina* that is in palmelloid stage (immobilized cysts in a capsule).

In Figure 5 from a sample of 120 ppt salinity, there are many cells of cyanobacteria and among them big green palmelloid cells of the chlorophyte *Tetraselmis marina* (well known for its preference of hypersalinity). The cyanobacteria (probably a species of *Synechococcus*) exhibit non-granular light colored protoplasm with a prominent golden structure that is difficult to say if it is carboxysome, cyanophycin body, polyphosphate body or something else.

The variation in shapes, colors and granulation of coccoid cyanobacteria is best seen in Figure 6. This assemblage of unidentified species comes from 110 ppt salinity and there is great variation evident in pictures A, B & C where the protoplasm takes a pale yellowish coloration in contrast to D where granulation is evident (probably genus *Synechococcus*). In B the variation in size of the cells reach an extreme with minute and very big cells and additionally, the division of the big cells creates a peculiar image of "butterfly" like shape. There are also prominent round bodies in the big cells that if not aerotopes then are totally unknown, I met them for the first time (and never again since then). In A the cells are perfect spheres, an unusual image for coccoid cyanobacteria and additionally there are prominent round inclusions probably serving as storage nutrients. There are also many unidentified filamentous cyanobacteria with straight transparent trichomes.

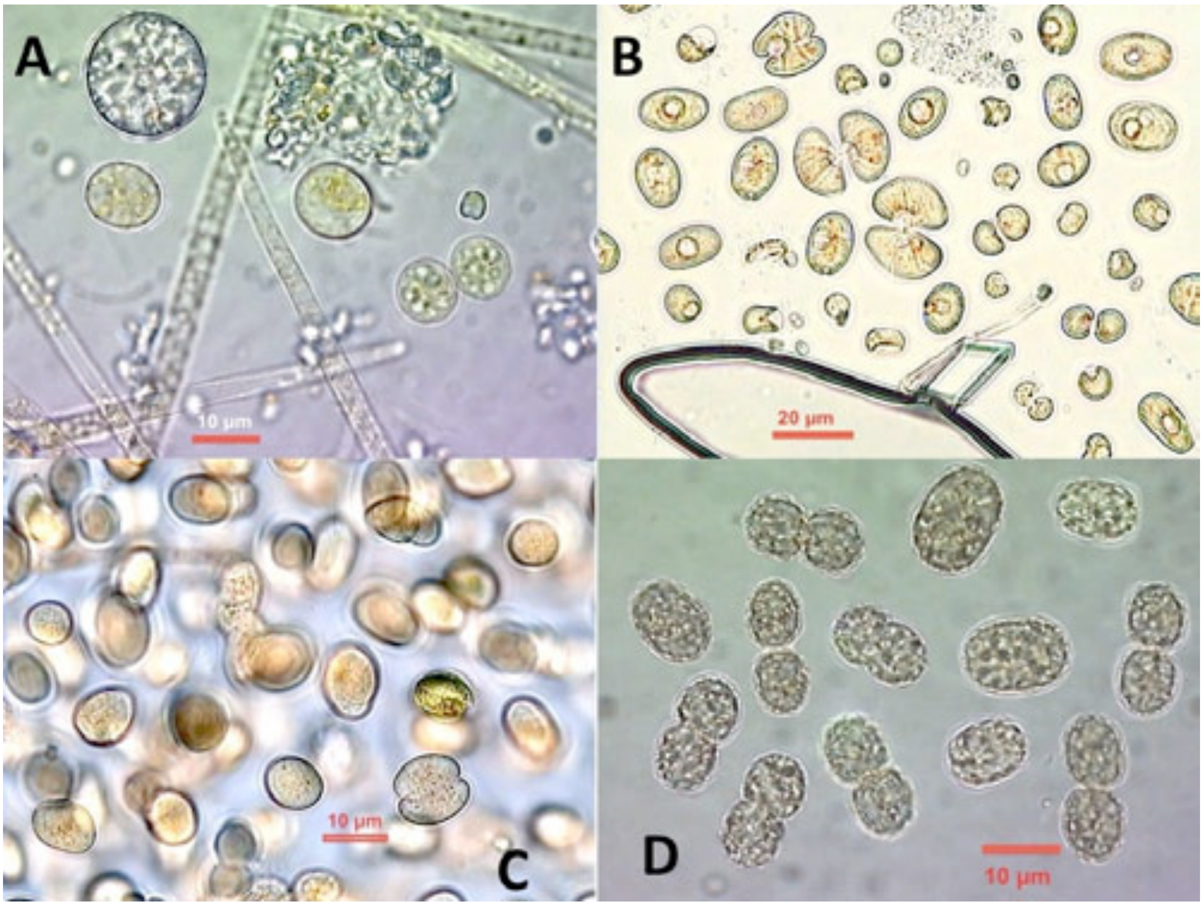

Figure 6. Variation of shapes, color and granulation in cyanobacteria of high salinity.

An irregular deviation of the normal cell division of coccoid cyanobacteria is seen in Figure 7 where apart from the variation in shapes and color of the cells in pictures A, B & C, there are also short chains of cells that deviate from normal. The cells seem to enter the division process and for some unknown reason some of them create instead of pairs, quadrates with the two inner cells smaller and compressed. Nothing was found in the literature about this phenomenon that cannot be attributed to some deterioration of the medium as these samples come from cultures in the laboratory in water of 110 ppt with surplus of nutrients. In C a cell can be seen (marked by arrow) that is surrounded by a thick mass of secreted mucilage. In D the image depicts a typical assemblage from the bottom sample of a hypersaline pond (150 ppt) with coccoid and filamentous cyanobacteria along with pennate diatoms, all mingled in a matrix of bacteria, fungi and mucilage.

So far we have seen solitary coccoid planktonic forms. In Figure 8 depicted are two colonial populations of very small-celled cyanobacteria that form on solid substrates in the ponds. These samples were collected from stone surfaces at 80 ppt salinity and are probably *Microcystis* in photo A and *Synechocystis* in photo B. The cells are very small (less than 5 μm) and actively dividing in B with the colonies heavily condensed.

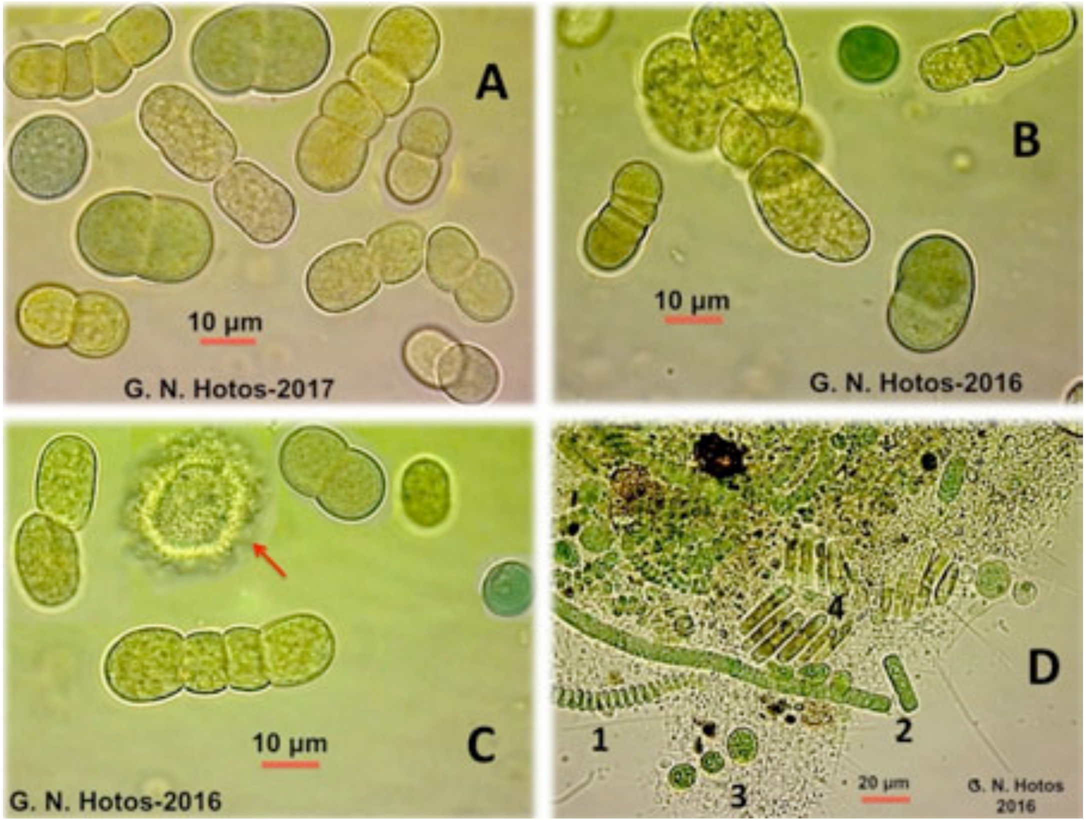

Figure 7. Several species of coccoid cyanobacteria exhibiting multiple uniseriate cell division (A, B, C), heavy mucilage production (arrow in C) and mixed species in biological mat (D).

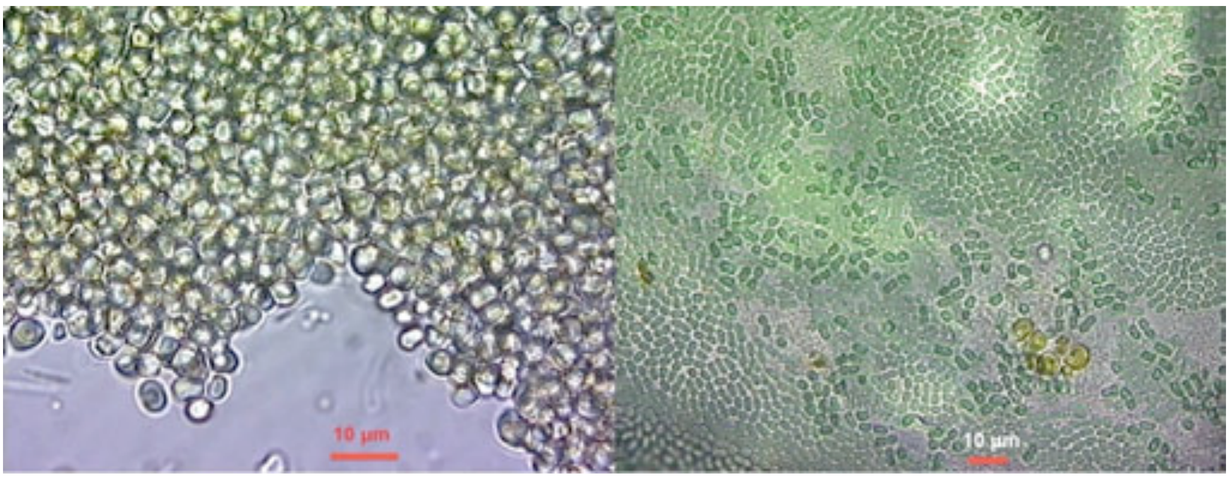

Figure 8. Dense colonies of coccoid cyanobacteria.

Cyanobacteria produce and secrete mucilaginous slime. This is well known and is most evident in the filamentous species where there is a thick outer stroma of slime covering the whole cyanobacterial filament forming a theca. The slimy cover exerts beneficial action on the cyanobacterium as it protects it from UV radiation. While in the filamentous species the slimy theca is uniformally and smoothly enveloping the filament (trichome), in the coccoid species the secreted slime is scattered in a non-organized manner (resembling a "splash") around the cell. It is colorless made from pectin and can be observed by the difference in its refraction index that creates in the water. Not all cells produce slime or at least not all the time. It is assumed that slime is beneficial to the cells as it accumulates nutrients or hosts useful bacteria or aids in floating or deters predators. Its exact role is not fully understood. In terms of salt production a heavy production of slime by a cyanobacterial blooming can have detrimental impact on the production of salt as it negatively interferes with the crystallization and the quality of salt.

In Figure 9 a depiction of the slime accumulation around various forms of coccoid cyanobacteria can be seen. What is interesting in this photo is the much thicker slime mass around the previously mentioned involuted forms with their peculiar shape and division state. As the production of slime takes a lot of energy and material from the cell, it is assumed that these cells are in a high metabolic rate, have enough nutrients and divide actively. It will be a breakthrough in the salt production if we could affect by some manipulation of the cyanobacterial population their slime production (eliminating or depressing its production), thus affecting positively the quality of salt.

Figure 9. Heavy mucilage (slime) depositions around coccoid cyanobacterial cells.

The excretions of cyanobacterial cells are not restricted only to slime but there other chemicals as well that fall into the category of **allelopathic** substances. Allelopathy is a broad term concerning the effect of several substances excreted by algal cells to the neighboring cells of the same or other species. The so called allelopathic interaction is the inhibition of growth of an organism by another. Allelopathy substances are mainly toxins (cyanotoxins in the case of cyanobacteria) and probably some other evaded yet unidentified substances that affect the growth of certain algae (concerning allelopathy between algae). Cyanobacteria endowed with the ability to produce toxins is by no means expected to exert allelopathic action and probably this is one of the factors permitting their blooming and their overwhelming growth in natural waters.
The topic of allelopathy is not well studied and there are still more questions to be answered. In Figure 10 I feel very lucky to have captured and present a really remarkable case of allelopathy of a coccoid cyanobacterium species (probably *Synechococcus*) that creates a clear zone devoid of other species around each of its own cell. The cells of *Synechococcus* (brown, oval and granular) create a broad clear zone around them (indicated by arrows) excluding the cells of the cyanobacterium *Microcystis* (the small colorless round cells). At the same time the seen in the photo big green cells of the chlorophyte *Asteromonas gracilis* do not exhibit such an action. It can not be incidental or artifact, nor a result of slime production (there is no slime in the sample). For me it is a first ever recorded on camera allelopathy of coccoid cyanobacteria from a lab culture in 130 ppt, the same salinity as of the water where I took the sample.

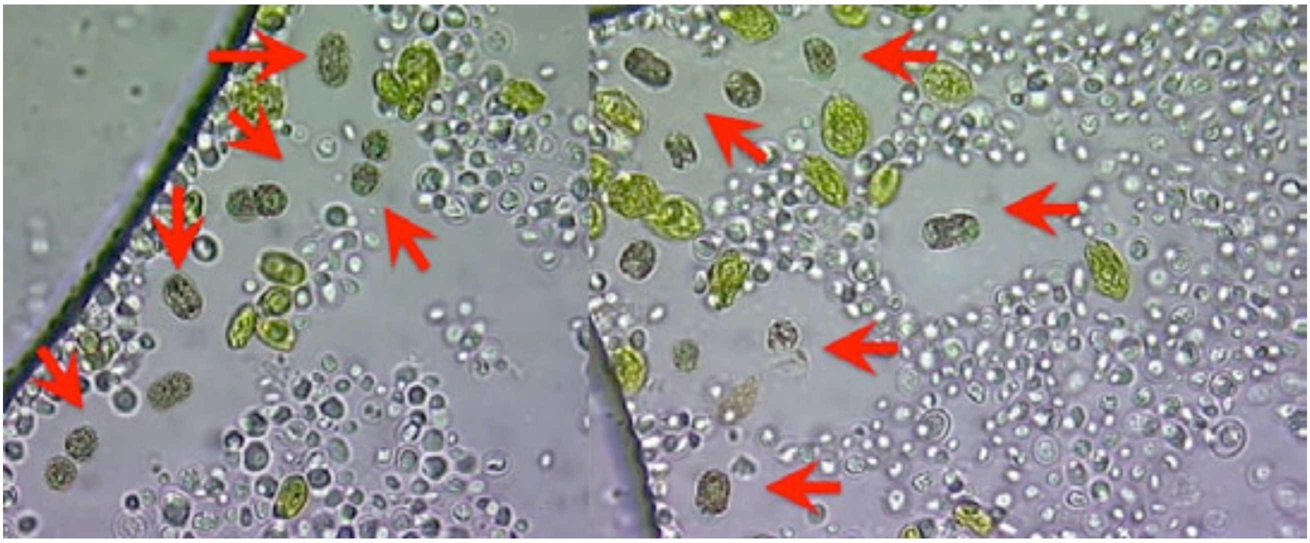

Figure 10. Clear zones devoid of cells around cyanobacterial cells of *Synechococcus* sp. an assumed indication of allelopathy.

(*to be continued with filamentous cyanobacteria*)