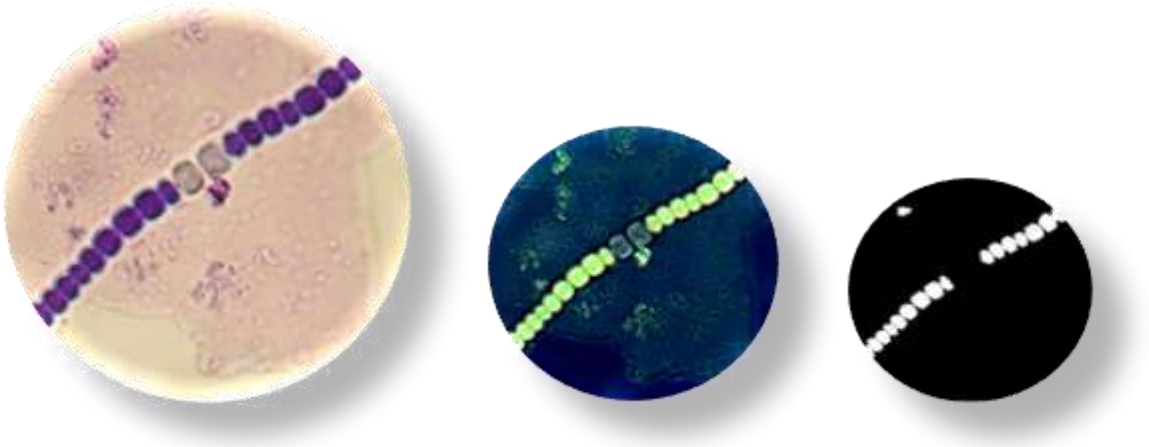
**MINISTRY OF EDUCATION, RESEARCH, YOUTH AND SPORTS**
**UNIVERSITY "OVIDIUS" OF CONSTANȚA**
**FACULTY OF NATURAL SCIENCES AND AGRICULTURAL SCIENCES**
**DOCTORAL SCHOOL - BIOLOGY**

# THE ABSTRACT OF DOCTORAL THESIS

## *"Cyanobacteria in mesothermal sulphurous waters (Obanul Mare – Mangalia)"*



**Scientific Coordinator,**
**Prof. Univ. Dr. IOAN ARDELEAN**

**Doctoral Candidate,**
**BENLIAN (SARCHIZIAN) IRIS**

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**Keywords: cyanobacteria; identification; growth rate; determination of cell's number capable of growth and multiplication; redox properties at population level and individual cell level; cytotoxicity of quantum dots (CdSe / ZnS); digital image analysis.**

# INTRODUCTION

Cyanobacteria have been always in the top of scientific world's attention, the interest from this group of microorganisms is determined because cyanobacteria has a vital role in the evolution of specific biosphere. Thus, I just reminding only metabolic diversity of cyanobacteria in a wide range of environmental factors, including extreme conditions, such as sulfurous mesothermal water, we can affirm that the thesis topic is taking place within the actual international research.

The choice of research topic titled ***"Cyanobacteria in mesothermal sulphurous waters (Obanul Mare - Mangalia)"*** was made in consultation with the existing literature, but insufficient data on cyanobacteria from aquatic sulfurous environments in Romania leded me to approach this new and fascinating topic .

Doctoral research lies in the fact that scientific studies raise only questions and problems, so approach their own research go in was quite difficult, highlighting the originality of methods combining theoretical and practical research, the classical with modern methods, leading to general conclusions of the thesis.

**Aim of the thesis** is to isolate, purify and identify to genus level on the principles of bacterial taxonomy the cyanobacteria strains isolated from natural samples collected from mesothermal sulphurous spring at Obanul Mare - Mangalia, using classical methods improved by adding carbon source before antibiotic , the study of physiological aspects of isolated, such as spectrophotometric methods to determine the growth rate of cyanobacteria cultured aerobically on different media: $BG_0$ and $BG_{11}$ or by calculating cell division rate, determine number of cells capable of growth and multiplication method of Kogure et al. for heterotrophic bacteria (1979), studying the redox properties using spetrophotometric methods at the population level in some strains of cyanobacteria isolated and the automatic analysis of digital images obtained from microscope, marking cyanobacteria with quantum dots (CdSe / ZnS) and study the cytotoxic effect of quantum dots on cyanobacteria.

The doctoral thesis is divided into five chapters, contained in two parts: the current state of knowledge cyanobacteria reported in the latest data of literature (two chapters) and experimental research (including two chapters) and a chapter of general conclusions.

First, the thesis is characterized by the actuality of the topic discussed since its introduction in the sphere of concerns romanian and foreign specialists occurred relatively late, not possible in our country develop advanced techniques in Microbiology at the single cell level, combined with digital image analysis.

In order to accomplish this thesis documentation and I found about 380 titles and suggestive references, of which about 60 were published in the last five years, which allowed me to obtain new experimental results correlated with those obtained internationally. Among them are those who open researches revealed microbiology, general biology, to automatic analysis of digital images, a course of topical international and national, which was made possible through a permanent collaboration with international experts, which allowed development within this thesis.

On my own contributions can say that the original information processing capacity of synthesis and the interpretation of the data , also the transdisciplinarity, which allowed personal opinions strained argument throughout the paper, to emphasize their vision of the phenomena analyzed. Finally, a private contributors is present in the form of proposals submitted at the end of chapters on modern methods used to facilitate their work effort by the researcher to process a large set of precise and reproducible data at a time short.

## CHAPTER 1. CYANOBACTERIA – GENERAL DATA

Chapter 1 sets out the general characteristics of cyanobacteria, the morphological and physiological diversity, as well as the main characteristics of criofile mesophilic and thermophilic cyanobacteria.

Cyanobacteria are the largest and most diverse group of photosinthetic bacteria. Originally, cyanobacteria were considered the following characteristics: large, are bodies oxygenic phototrophs ($H_2O$ used as electron donor with the production of $O_2$), contains PS I and PSII responsible for decomposition by light energy absorbed $H_2O$, contain chlorophyll *a* and *β*-carotene, ficobiliproiens as accessory pigments: photosynthesis is similar to plants.

Cyanobacteria can be found in all aquatic ecosystems, ranging from hydrothermal vents, to arctic areas (Carmichael et al., 1990). Being the oldest oxygen-producing organisms (Schöpf, 2000), cyanobacteria have played a key role in the evolution of the Earth since their first appearance now 2.15 billion years ago (Hoffmann, 1975; Knopf 2006; Ramussen 2008) . Long history of cyanobacteria is responsible for their ability to be better adapted to environmental stress, including rare and abundant nutrients (Paerl, 2006), exposure to UV radiation, high solar radiation and above all at high temperatures ( Paerl et al 1985; Robarts & Zohary 1987; Briand, 2004). These special conditions may favor the dominance of cyanobacteria in many aquatic habitats. Ability to be very tolerant cyanobacteria when subjected to various stress factors suggest that cyanobacteria are likely to benefit from

environmental changes associated with global warming (Paerl and Huisman, 2008, Paerl, 2009).

Recently, Whitton and Potts (2000) have demonstrated morphological diversity of cyanobacteria - filamentous and unicellular forms - which can aggregate into colonies, cells in colonies can be arranged in different ways (radial, flat or irregular). Some have specialized cells for nitrogen fixation (heterocists) cells that can survive under conditions of stress (akinrets) and dispersion (hormogonia).

The chapter concludes with the presentation of cyanobacteria classified as prokaryotes, concerning to Bergey's Manual of illustrated determinative Bacteriology (2001), employing Cyanobacteria group, comprising five orders with 34 genera (Castenholz, 2001).

## CHAPTER 2. THEORETICAL AND PRACTICAL IMPORTANCE OF CYANOBACTERIA

To better understand the structure of cyanobacteria, we studied the importance of theoretical and applied them as cyanobacteria is one of the few groups of organisms can perform photosynthesis and oxygen respiration simultaneously in the same compartment, some species are able to fix nitrogen. This unusual combination of metabolic pathways and metabolic flexibility may be responsible for the development of cyanobacteria and their ability to thrive in extreme conditions. Cyanobacteria are the oldest organisms in terms of evolution: microfosilele found to have 3.5 billion years old were assigned as belonging to cyanobacteria (Schopf, 1993). An important question is the successful combination of cyanobacterial evolution of metabolic pathways.

Characteristic of all species of cyanobacteria is operating photosystem I and II, and the use of water as a source of electrons for photosynthesis. All representatives of cyanobacteria contain chlorophyll and are able to increase photoautrophy, although photohetetrophy and chemoautotrophy and growth are common to many species. Morphology and life cycle of this group is very complex. Combining photosynthesis and respiration in a single compartment is unique. Photosynthesis and respiration require electron transport pathways catalysed by proteins complexela membranes.

The importance of photosynthesis and respiration in cyanobacteria and cyanobacteria use as a model system for the study of nanoparticles on biological prokaryotic or model biological system for the production of nanoparticles has been a very exciting chapter in terms of the information presented. Thus, nanotechnology is being developed in many areas, even in

developing countries have also decided that this new technology could be an investment that can not be ignored, bringing the future economic benefits and social welfare. For new technologies, there is a growing concern about the possible side effects from the use of nanoparticles. Due to increased use of nanotechnology, must be well understood risks associated with exposure to nanoparticles, entry routes and molecular mechanisms of cytotoxicity. Are water-soluble quantum dots with biological applications are usually passivated by different layers of inorganic and / or organic to increase fluorescence yield (Kloepfer et al, 2004). These coatings greatly increase the particle size, making impossible their absorption by microorganisms.

Fluorescent semiconductor quantum dots can be used as layers on/off bacteria and other living cells, they affect the transport of electrons on energy metabolism, both fototrofe bacteria and the heterotrophic bacteria. To explain these results take into account the physicochemical properties of quantum dots in relation to ultrastructural differences of Gram-negative and Gram-positive and cellular localization of the main energy processes, respiration and photosynthesis. In this respect, particular attention increasingly focuses more on the interaction between quantum dots and cyanobacteria for longer periods of time because these prokaryotes oxygen fototrofe have major contributions to the synthesis of organic matter in aquatic environments they inhabit, the consumption of carbon dioxide and molecular oxygen production.

An important issue in all these experiments relate physical relationship between microbial populations and different quantum dots, with special emphasis on quantum dots to position the cell wall and cell membrane. It seems logical to assume that the first site of interaction between the nanoparticles and cells is the cell wall, cell wall however is quite different structure in Gram-negative bacteria (including cyanobacteria) and Gram-positive bacteria.

Physical access of quantum dots to the outer cell membrane (for cell wall) is still an open question, and the ability of nano-sized quantum dots to pass through the cell membrane intact (or previously damaged!) To enter the cytoplasm (Ardelean and al., 2011).

## OBJECTIVES OF THE THESIS

1. Isolation of strains of cyanobacteria in natural samples collected from mesothermal sulphurous spring Obanul Mare- Mangalia;
2. Purification of strains of cyanobacteria using traditional methods;
3. Improving conventional purification methods by adding carbon source before antibiotic;
4. Gender identification at some of the strains of cyanobacteria purified on principiiile bacterial taxonomy;
5. Physiological study of isolated following issues:
a. determination by spectrophotometric methods the growth rate of aerobically grown cyanobacteria (photosynthetic oxygenic) on different culture media - $BG_0$ and $BG_{11}$;
b. Determination of the growth rate of cyanobacteria by frequency of dividing cells;
c. Determination of number of cells capable of growth and multiplication using the method described by Kogure et al (1979);
d. Study of redox properties by spectrophotometric methods at the population level in some strains of cyanobacteria isolated;
e. study the cellular redox properties of some strains of cyanobacteria isolated by automatic analysis of microscopic images;
f. cyanobacteria with quantum dots (CdSe /ZnS);
g. study the cytotoxic effect of quantum dots on cyanobacteria.

## CHAPTER 3. MATERIALS AND METHODS

This chapter of the thesis is focused on experimental description of the study area (being the first time the source is studied cyanobacteria mesothermal from Obanul Mare), how sampling, fixation and preservation of water samples, staining methods used in studying cyanobacteria, cyanobacteria visualization method using light microscopy and comp (epi) fluorescence. Are also precisely described methods for isolation and cultivation of strains of cyanobacteria from mesothermal sulphurous spring at Oban High - Mangalia (genus *Synechocystis sp., Nostoc sp., Anabaena sp., Tychonema sp*., unicellular and filamentous anoxigenic cyanobacteria, filamentous oxygenic and anoxigenic thermotolerant cyanobacteria) , purification of cyanobacteria isolates and obtaining axenic cultures of diazotrophic cyanobacteria using antibiotics tienam, augmentin, nalidixic acid, cephalexin, and lysozyme.

Identification of cyanobacteria strains isolated and the use of digital image analysis to study the morphological and physiological characteristics of the cyanobacteria isolated study studying morphological characters of cyanobacteria using CellC and ImageJ program are described in detail in this chapter.

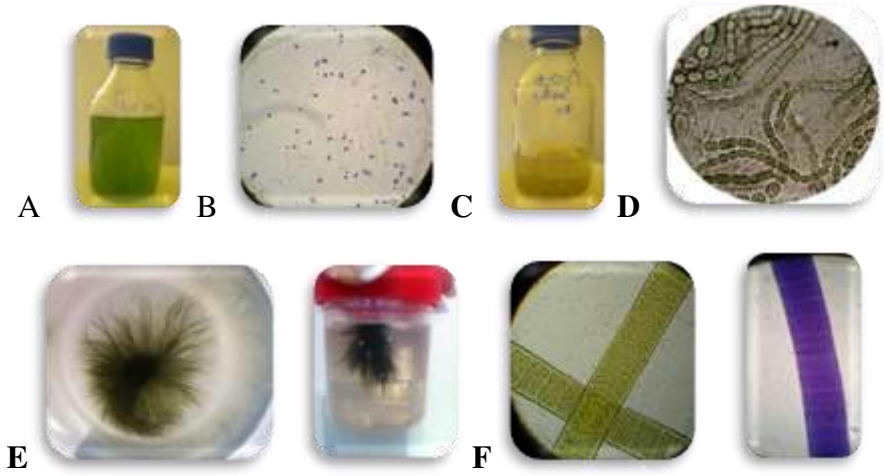



**Figure 1. (Figure 3.7., 3.9., 3.10).** Macroscopic (A, C, E) and microscopic (B, D, F) view of enriched culture of unicellular and filamentous cyanobacteria, E - macroscopic view of enriched culture grown in liquid medium $BG_{11}$ (A) microscopic view of enriched culture grown on $BG_{11}$ liquid medium after staining with crystal violet (original).

There are also presented the methods for determining the growth rate of cyanobacteria in various conditions both in classical but also refer the identical spectrophotometric determination and calculation of frequency of dividing cells in heterocystous cyanobacteria strain *Anabaena sp* and strain *Tychonema* sp., determining the number of cells capable of growth and multiplication described by Kogure et al (1979) in the strain *Anabaena sp*. , in strain *Synechocystis* PCC 6803 and isolated strain of unicellular cyanobacteria *Synechocystis* sp., the last method being applied on natural populations of cyanobacteria from mesothermal spring;studying at the redox properties of some isolated using automated analysis of digital images, spectrophotometric measurement of dehydrogenase activity in some populations of cyanobacteria and quantifying individual proprităților biological redox level (filament of cyanobacteria) from *Anabaena sp*., are all methodologies well used, together with investigating the interaction between quantum dots (CdSe/ZnS) and populations and highlight the fluorescence of quantum dots marked cyanobacteria and study the cytotoxic effect of quantum dots on cyanobacteria.

## CHAPTER 4. RESULTS AND DISCUSSION

Next chapter entitled "Results and discussion" includes original results of experiments conducted for the isolation, cultivation and improvement of methods for purification of strains of cyanobacteria isolates; there was identified following types of cyanobacteria, according to Bergey Manual 2001: *Synechocystis sp. , Synechocystis sp*. - anoxygenic, *Synechococcoccus* sp., *Anabaena* sp., *Oscillatoria* sp., *Nostoc* 1 sp. , *Nostoc* 2 sp., *Tychonema* sp.

Using digital image analysis algorithms obtained by combining mathematical methods from CellC and ImageJ software made it possible for the first time, after consulting the international literature, precise identification in a relatively short time the number of cells analyzed in cyanobacterial filaments from digital images taken in light field microscopy. The two programs allowed me to successfully achieve automatic image files backlit practical steps by steps that are key in obtaining experimental data.

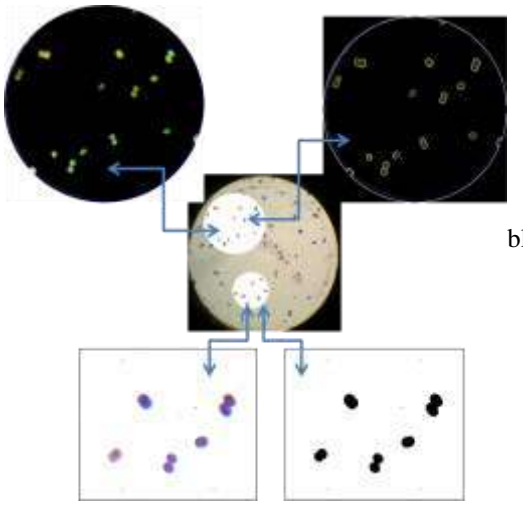


**Figure 2 (Figure 4.3.).** Bright field view of unicellular cyanobacteria of the genus *Synechocystis* sp. by staining with 0.02% crystal violet: A - depth of field microscopic unicellular cyanobacteria in the natural samples collected, B - detection of cell shape and outline of cells; C - image microscopic field with unicellular cyanobacteria, D - detail of various regions of interest , E-shape of cyanobacterial cells viewed using only black and white (Oc.10x, Ob.40x) (10 µmscale bar) (original).

In the Figure 3 there are presented our isolates identified according to Bergey Manual 2001.

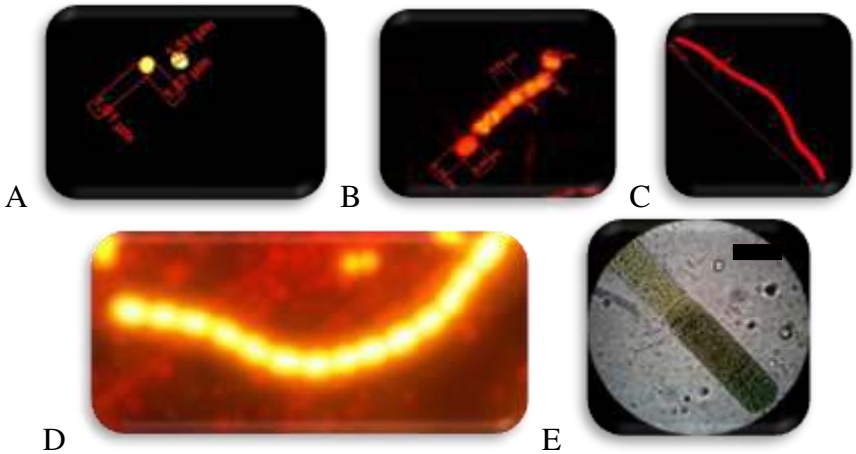



**Figure 3 (Figure 4.27., 4.28, 4.29, 4.30, 4.32).** A - isolate *Synechocystis* sp. oxygenic, B-isolate *Anabaena sp.* ; C - isolate *Oscillatoria* sp., D - isolate *Nostoc sp.* , E-isolate *Tychonema sp.* (original).

The purpose of subchapter for determining the growth rate of cyanobacteria by spectrophotometric method is to determine the rate of growth of cyanobacteria isolates under study aerobic conditions using microplate reader with ultra spectrophotometer, which covers a wide range of wavelengths, from 220 nm to 850 nm, allowing data collection and interpretation in Excell format soon. Measurements were made at D.O. 750 nm, the first time after 3 hours, after 22 hours, after 28 hours, after 124 hours of incubation in continuous light. Cultures of cyanobacteria in the study were distributed to wells microplates and automated reading was performed at 750 nm, achieving concomitant readings by 8 for each crop and each sample for analysis.

**Table 1 (Table 4.3).** Optical densities of the cultures of cyanobacteria grown $BG_0$ (original)

| **Time (hours)** | **D.O.750nm** ***Nostoc sp.*** | **D.O.750nm** ***Oscilatoria sp.*** | **D.O.750nm** ***Nostoc sp.*** | **D.O.750nm** ***Synechocystis sp.*** | **D.O.750nm** ***Anabaena sp.*** | **D.O.750nm** ***Synechocystis sp.*** |
|---|---|---|---|---|---|---|
| 0 | 0,07 | 0,079 | 0,097 | 0,566 | 0,097 | 0,184 |
| 22 | 0,091 | 0,126 | 0,108 | 0,784 | 0,127 | 0,212 |
| 28 | 0,095 | 0,236 | 0,139 | 1,178 | 0,308 | 0,258 |
| 124 | 0,266 | 0,294 | 0,246 | 1,413 | 1,222 | 0,297 |

**Table 2 (Table 4.4).** Optical densities of the cultures of cyanobacteria grown in $BG_{11}$ medium (original).

| **Time (hours)** | **D.O.750nm** ***Nostoc sp.*** | **D.O.750nm** ***Oscilatoria sp.*** | **D.O.750nm** ***Nostoc sp.*** | **D.O.750nm** ***Synechocystis sp.*** | **D.O.750nm** ***Anabaena sp.*** | **D.O.750nm** ***Synechocystis sp.*** |
|---|---|---|---|---|---|---|
| **0** | 0,07 | 0,17 | 0,084 | 0,7 | 0,077 | 0,11 |
| 22 | 0,084 | 0,292 | 0,097 | 0,858 | 0,109 | 0,184 |
| 28 | 0,091 | 0,681 | 0,178 | 1,161 | 0,137 | 0,237 |
| 124 | 0,205 | 0,888 | 0,313 | 1,618 | 0,279 | 0,521 |

The frequency of dividing cell (FCD) is an indirect measure of growth rate in bacteria (Hagström et al., 1979, Campbell & Carpenter, 1986; Carpenter & Campbell, 1988; Nielsen, 2006) and this report extended and applied this technique on populations of filamentous heterocystous cyanobacteria isolated from sulphurous mesothermal spring Obanul Mare - Mangalia (Sarchizian and Ardelean, 2010). This report presents the use of frequency of dividing cells (FDC) method to calculate the growth rate in populations of filamentous (heterocystous and nonheterocystous) cyanobacteria isolated from sulphurous mesothermal spring from Obanul Mare (Mangalia).Septa were counted on heat - fixed preparations of cyanobacteria (300 cells per sample) stained with 0.02% crystal violet, using digital image analysis using two software CellC and ImageJ to clearly observe the shape of the dividing cells form filaments allowing us to obtain accurate data: for heterocyst forming strain

(*Anabaena sp*.) the maximum growth rate on $BG_0$ in light is 0.039 $h^{-1}$ and for non heterocyst forming strain (*Tychonema* sp.) the maximum growth rate on $BG_{11}$ in light is 0.057 $h^{-1}$.

Frequency of dividing cell (FDC) is an indirect measure of the average rate of growth measurement in bacteria (Hagström et al., 1979, Campbell and Carpenter, 1986; Campbell and Carpenter, 1988; Nielsen, 2006),and now is extended and applied on the research conducted in the cyanobacteria populations from mesothermal spring Obanul Mare - Mangalia.

In figure 4 there are presented the images taken at different time during light incubation for our isolate *Anabaena* sp., the arrows indicating the cells in division within the cyanobacterial filaments stained with crystal violet [(initial time (T0), after 12 hours (T1), after 24 hours (T2) and after 60 hours (T5)], and the digital image analysis obtained using ImageJ and CellC software for cell counting and determination of septa.

The calculation of FDC during incubation in light and darkness clearly showed differences between light and dark incubation, which is in strong correlation with differences in growth rate and determined by classical methods, spectrophotometry. The main result in this experiment refers to differences between light and dark incubation, the growth rate is much higher in light compared with the rate of growth in the dark, especially after 24 hours, the results of automated digital image analysis being accord with those obtained by the classical method. Appearance of dividing cells in samples incubated in the dark and corresponding growth rates almost similar in the first 24 hours could be supported by the use of endogenous reserves accumulated in the dark during the light as a source of carbon and energy, agreement with the biological significance of these intracytoplasmic inclusions. The sharp decline in both FDC and the growth rate of long incubation periods in the dark is explained by the the possible reduction of intracellular organic reserves and a change in strategy for cells to survive in hostile conditions, decrease the frequency of cell division is one of the most important responses of bacteria against lack of carbon and energy.

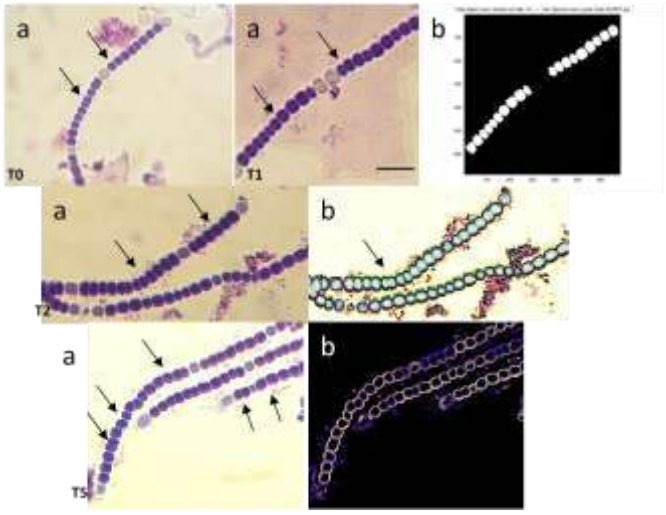
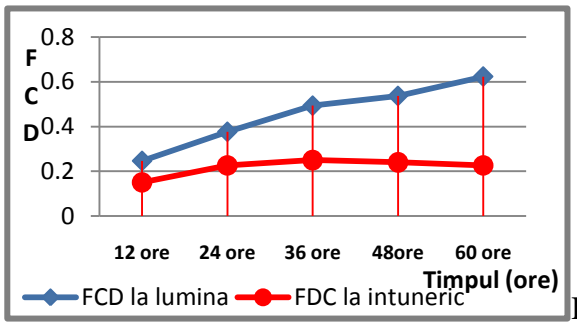



**Figure 4 (Figure 4.40).** Filaments of *Anabaena sp*. cell division during incubation in light after staining with 0.02% crystal violet (T0), after 12 hours (T1), 24 hours (T2) and after 60 hours of incubation (T5), the ) digital image analysis using ImageJ software and CellC for cell counting and determination of division septa, arrows indicate cells in division (Sarchizian and Ardelean, 2012).

The FDC calculated in light and darkness incubation clearly show important differences between light and dark incubations which are in strong correlation (in agreement with the above equation) with the differences in the growth rate. The main result concerns the differences between light and dark incubations, the growing rate being much higher in light as compared with dark conditions, especially after 24 hours. The occurrence of dividing cells in dark incubated samples and the corresponding growth rates almost similar during first 24 hours could be sustained by the use of endogenous reserves accumulated in previous (continuous) light period as a source of carbon and energy in agreement with the biological signification of these reserves. Furthermore, the absence of organic substances in the composition of $BG_0$ medium constrain the cells to have access only to intracellular organic reserves. The sharp decrease both in FDC and in the growth rate at longer incubation times in darkness could be correlated with the diminution of intracellular organic reserves and with a change in the strategy of the cells in order to survive during hostile conditions, the decrease in the frequency of cellular division being one of the most important responses of bacteria against shortage in carbon and energy (e.g. Roszak and Colwell, 1987) .

A 

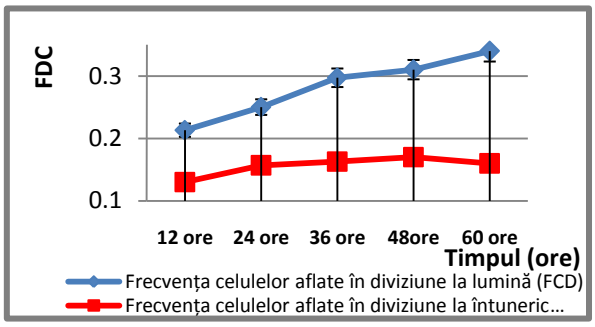
B 


**Figure 5. A (Figure 4.41).** - Comparison of FCD in *Anabaena sp*. incubation under light and dark, **B (Figure 4.44)** - FDC in *Tychonema* sp. incubation under light (blue line) and dark (red line) (original).

The FDC calculated for *Tychonema* sp. incubated in light and darkness clearly show important differences between light and dark incubations which are in strong correlation with the differences in the growth rate .The results obtained in this nonheterocystous strain are in agreement with those obtained in Anabaena sp., the heterocystous strain used in the thesis. The above argumentation based on the diminution of intracellular organic reserves and/or with a change in the strategy of the cells in order to survive during hostile conditions are probably valid also for *Tychonema* sp.

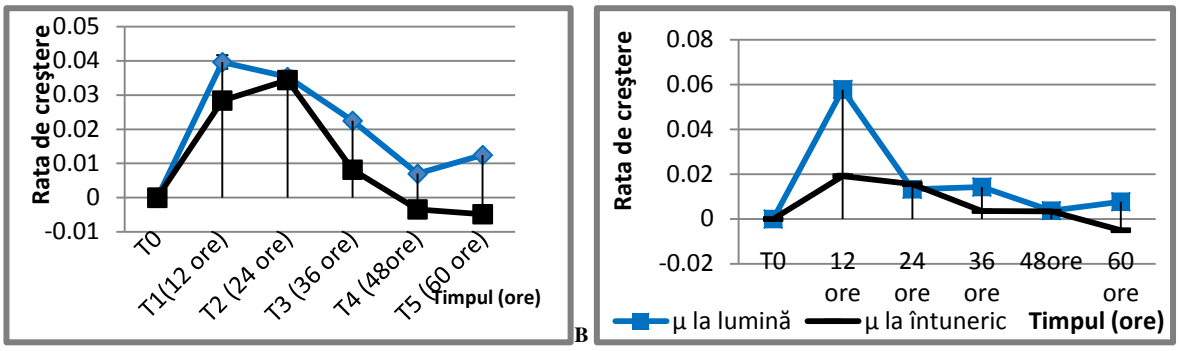
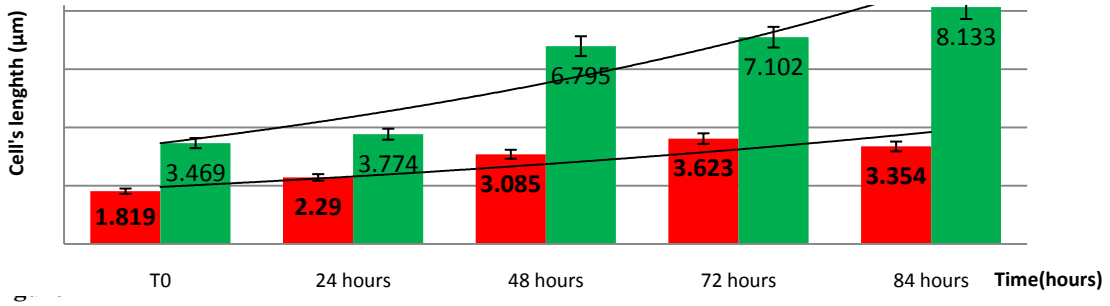



**Figure 6.A - (Figure 4.42)** Growth rate (µ) in culture *Anabaena sp*. incubated in light and dark (light blue line indicates cultivation, growing black line indicates the dark) **B** - **(Figure 4.45.)** - Growth rate (µ) in culture *Tychonema sp. i*ncubated in light and dark (blue line shows the rate of growth to light black line shows the rate of growth in the dark) (original).

Direct viable count method for the quantification of living cells (DVC) was initially developed to distinguish viable heterotrophic bacterial cells (Kogure et al.,1979; Kogure et al.,1984) from cells unable to grow and multiplication in natural samples. This subchapter presents the results concerning the quantification of cyanobacterial cells capable of cellular growth and multiplication using the direct viable count method (DVC). During incubation of cyanobacterial samples in the presence of nalidixic acid as inhibitor of DNA replication , all other metabolic properties remain active. Viable cells may continue to metabolize nutrients and grow but will not be able to divide, thus becoming more elongated after incubation whereas inactive cells do not elongate during the incubation period. Measurements of cells size were performed using two automatic software Image J to measure cell length and CellC software for cell quantification in light microscopy, compared with manual counting and measuring. The results show that the medium cell size increases from 1.81 µm to 3.35 µm in 84 hours of light incubation with nalidixic acid . Up to our knowledge this is the first report on DVC applied to filamentous cyanobacteria. The method was also used on the unicellular cyanobacterium *Synechocystis* PCC 6803 where the results were compared with growth rate calculated taking into account the increase in optical density (Sarchizian and Ardelean,2012).




**(Figure 4.47).** The evolution of the mean and maximum size of cyanobacterial cells during incubation in the presence of nalidixic acid.of *Anabaena sp.* at T0, after 24, 48, 72, 84 hours of (Sarchizian and Ardelean, 2012).

Analyzing the experimental data we found that 64% of the cells are capable of growth and division, 7% of cells belonging to the first class of scale (<1 m) and 57% (89% -32%) of cells in class 1-3 µm can be found at the end of the experiment in class size 3 - 6 µm (67% - 4% = 63%) and 6-9 µm (1%).

**Table 3 (Table 4.9).** Cell size distribution of cyanobacterial filaments during incubation time with nalidixic acid. (original).

| Time (hours) | Cell size distribution (%) | | | |
|---|---|---|---|---|
| | **< 1 µm** | **1-3 µm** | **3-6 µm** | **6-9 µm** |
| **T0** | 7% | 89% | 4% | 0% |
| **12** | 0% | 94% | 6% | 0% |
| **24** | 0% | 90% | 10% | 0% |
| **36** | 0% | 92% | 8% | 0% |
| **48** | 0% | 52% | 46% | 2% |
| **60** | 0% | 80% | 19% | 1% |
| **72** | 0% | 19% | 79% | 2% |
| **84** | 0% | 32% | 67% | 1% |

Direct viable count method applied to *Syneccocystis* PCC 6803. In Figure 8 is presented the cells' shape and size in cultures of *Syneccocystis* PCC 6803 after criystal violet 0,02% staining, obtained automatically with ImageJ software. Digital image analysis allowed us to corectelly identify the sizes of cells. Every digital image was analyed with ImageJ in bright field microscopy and then we utilised the graticula attached to the microscope to measure the size of cells, we observed increase in cell size during the experiment, and their elongation.

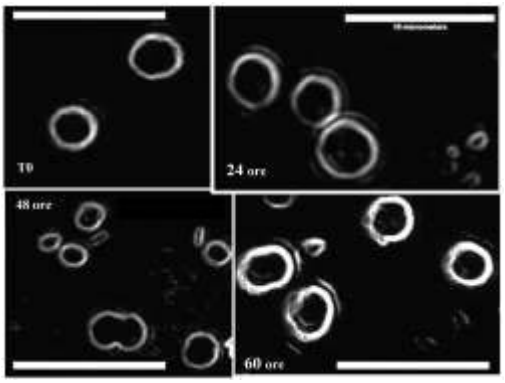



**Figure 8 (Figure 4.48).** Microscopic appearance of cells of *Synechocystis* PCC 6803 during incubation with nalidixic acid after staining with 0.02% crystal violet and cell shape determination using ImageJ software, using the scale of size 10 µm (Sarchizian and Ardelean, 2012).

Through the analysis of cell size by size class (percentage values) we found a significant increase in the number of cells in class 2 - 3 µm after 2 hours of incubation in light in the presence of nalidixic acid, along with enlargement of number of cells class 1 to 2 µm, reaching a value of 47% at the end of 3 hours of incubation.

According to the calculation presented above 71% of the cells are able to grow and multiply; 71% of the cells from the first size class increase their size, thus increasing the

percentage of cells in larges size classes : 26% increases in class 1-2 µm, 41% increase in class 2-3 µm and 4 % increase in class 3-4 µm.

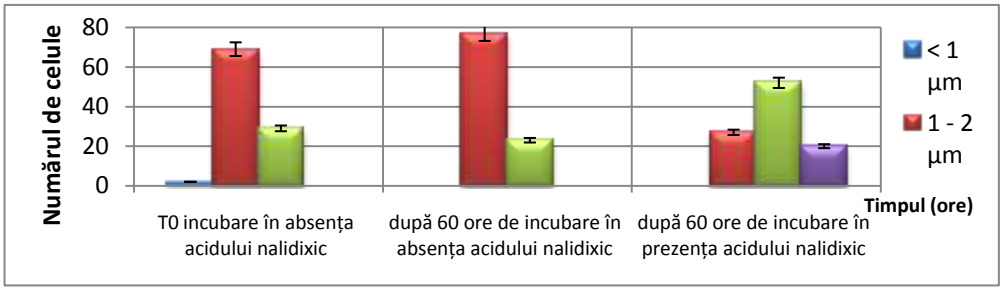



**Figure 9 (Figure 4.49).** Size class distribution of *Synechocystis* PCC 6803 cells incubated in the absence or presence of nalidixic acid at the beginning of the experiment (T0 - 0 hours) and at the end of 60 hours of incubation (Sarchizian and Ardelean, 2012).

As one can see in figure 9, there is a clear difference in size distribution of cells grown in the absence of nalidixic acid or in its presence. Whereas, at different times (T0- 0 hours and T3-60 hours) the distribution is practically the same in populations growing in the absence of nalidixic acid, there is a clear shift towards larger cells in populations grown in the presence of nalidixic acid . The monitoring of cell size distribution in populations without nalidixic acid is needed for accurate quantification of cells capable of growth and division by DVC method (Kogure et al., 1979). In our experiments, practically the same size distribution at different times (e.g. 0 hours and 60 hours) in populations growing in the absence of nalidixic acid is determined by the fact that the work is done on a pure strain during asincron cultivation, the increase in length being undoubtedly continue throughout the cell cycle (Sargent, 1975). The increase in optical density of cultures is practically the same in the absence and in the presence of nalidixic acid.

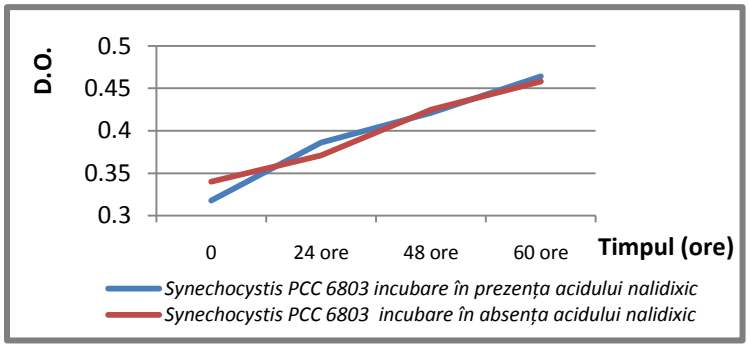



**Figure 10 (Figure 4.50).** Evolution of the optical density in cultures of Synechocystis PCC 6803 increased year presence or absence of nalidixic acid (Sarchizian and Ardelean, 2012).

In the section of quantifying the redox properties of some strains of cyanobacteria experiments followed spectrophotometrically measuring of dehydrogenase activity in some populations of cyanobacteria, analyzing color changes at both the filament and the filament individual cell level. Preliminary experiments performed to quantify the reduction claim MTT or 2.6 diclorphenol indophenol (alone or in the presence of a lipophilic electron carrier -

phenazin metosulfat or 2.6 dichloro benzoquinon) while reducing MTT cellular level, measured in numerical decline in the blue channel.

Dehydrogenase activity spectrophotometric measurement was performed in the presence of DCPIP and DCPIP and FMT separately in the presence and in Table 4.18 are calculated reduction rates in cultures of cyanobacteria.

**Table 4 (Table 4.18).** Reduction rates in cultures of cyanobacteria under study (original).

| **Cyanobactetrial culture** | **Reduction rate DCPIP** (µmoli/min/ D.O.750 nm) | **Reduction rate DCPIP + FMT** (µmoli/min/ D.O.750 nm) |
|---|---|---|
| *Synechocystis sp.* | 3,141 | 6,666 |
| *Synechocystis* PCC 6803 | 1,047 | 3,044 |
| *Anabaena* sp. | 3,455 | 4,14 |

The purpose subchapter showing quantification redox properties at the individual biological (cyanobacteria filament of *Anabaena* sp.) is to investigate the possibility of *Anabaena sp* strain to reduce a artificial acceptor of electrons, with particular emphasis on quantitative determinations within a single cell using automated image analysis for accurate color measurement cells within filaments of cyanobacteria, as up to this time the first report on the use of automated image analysis to measure the reduction of artificial redox carrier within a single cell in cyanobacteria. In this section are presented the quantitative results concerning biotechnological potential of filamentous cyanobacteria strain *Anabaena* sp. the ability to reduce a electron acceptor added artificial extracellular composition of the individual cells of a filament cianobacterian. A particular focus of this chapter is on quantitative determination by automated digital image analysis of cells in each filament capacity to reduce MTT, an artificial electron acceptor. Our results showed a strong decrease (about 4 times in 24 hours) blue signal during MTT reduction by each individual cell analyzed, as a consequence of orange light absorbed by reduced MTT. After consulting international literature, this is the first report on the use of automatic digital image analysis to measure the ability to reduce artificial electron acceptor at a cellular level, the filaments of cyanobacteria. This paper argues for the importance of particular mathematical methods of digital imaging in bright field, a precise methodology for analyzing detailed and objective measurement of color intensity of each individual cell (Sarchizian et al., 2011).

A new way of Microbiology at the cellular level approach is automatic image analysis classical individual bacterial cells obtained using different types of microscopes, to quantify important parameters such as cell enumeration, calculation of cell volume and frequency division cells, in situ classification of bacteria, active bacteria enumeration in terms of breathing and characterization of bacterial growth on a solid medium, biofilm viability and

physiological activity (eg, Yang et al., 2000. Lehmussola et al., 2008,. Chavez de Paz, 2009, Edelstein et al., 2010). Figure 11 presents successively macroscopic appearance of cyanobacteria in suspension culture Anabaena sp. in the presence of MTT at T0 - initial time, T4 - after 4 hours of incubation in light, T6 - after 6 hours incubation in light, T24 - after 24 hours of incubation in light at 28 ° C. Highlighting MTT reduction was visualized using bright-field optical microscopy, which can be observed in filaments of cyanobacteria cells that changes color as a result of MTT reduction.

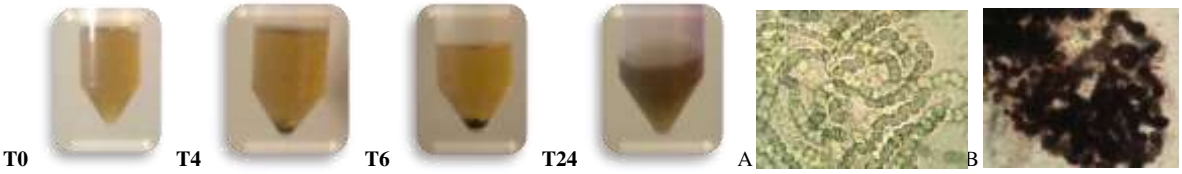



**Figure 11 (Figure 4.55).** Macroscopic appearance of cyanobacterial suspension during incubation in light for studying MTT reduction (T0, initial time, T4, after 4 hours of incubation in the light, T6 - after 6 hours incubation at light T24 - after 24 hours of incubation at light ) **A - (Figure 4.56).** - Digital image of *Anabaena sp* filaments without addition MTT, **B –** the view of culture *Anabaena sp*. after 24 hours of incubation in light in the presence of MTT (Sarchizian, Cîrnu, Ardelean, 2011).

Figure 12 presents the results of automatic analysis of digital color images, while for 10 consecutive cells of a filament of cyanobacteria in the presence of MTT and the average results of pixels in the three RGB channels.

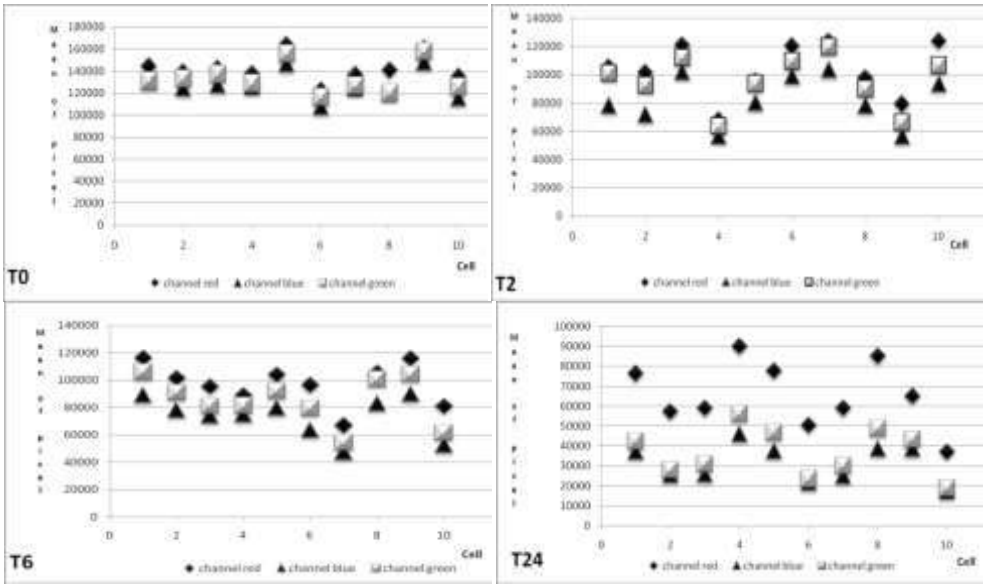



**Figura 12 (Figura 4.57).** Analiza automată de imagine digitală pentru determinarea schimbării de culoare în timp (T0, T2, T6, T24) pentru câte 10 celule consecutive dintr-un filament de *Anabaena* sp. tratată cu MTT şi rezultatele automate ale mediilor pixelilor în trei canale de culoare (Sarchizian, Cîrnu, Ardelean, 2011).

These type of images were further used to measure reduction of MTT occurring at single cell level by automated color image analysis taking into account the change in color due to formation of purple formazan (reduced, insoluble MTT). We observed that all images taken in bright field microscopy can be analyzed with Image J software and can detect any region of interests (ROI's) and after that measure the mean of pixel for each cells from

cyanobacterial filament. This steps used in our study allowed to determine the mean of pixel in channel red, green and blue (RBG) for analyzes cells and also to study the different aspect of MTT reduction in cyanobacterial filaments in time.

In Figure 12 there is a decrease in time, the total intensity of light that passes through every cell of the filament (scale decreases from 20,000 pixels to 1000 pixels at time zero after 24 hours of incubation with MTT), suggesting that analyzed each cell absorbs and / or reflects incident light more therefore less light is available to go through each cell. Since the reduced MTT is colored (purple) and insoluble, both processes (increasing) the absorption of light by colored compounds and light scattering (increasing) the reduced MTT crystals should be considered for light diminished passing through each individual cell of cyanobacteria.

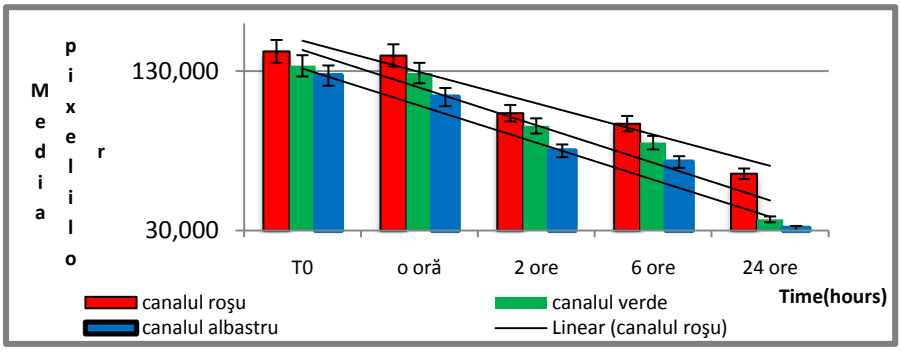
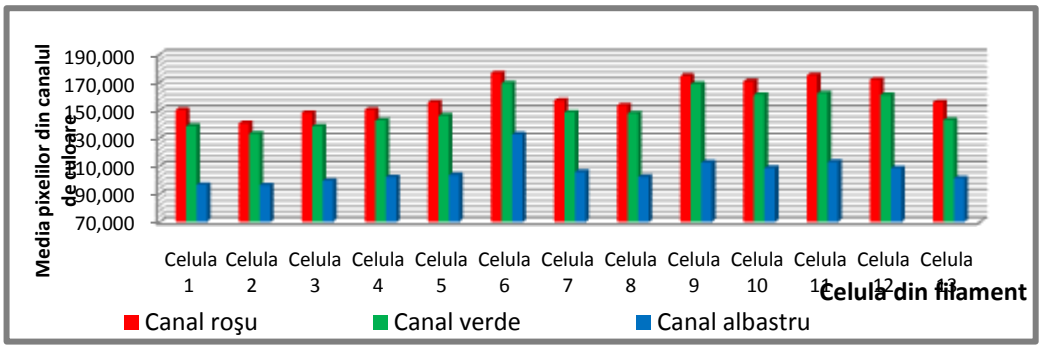



**Figure 13 (Figure 4.58).** Evolution arithmetic mean of pixels in the three color channels: red, green and blue during the incubation period of suspension of cyanobacteria in the presence of MTT (0.5 mg / mL) (Sarchizian, Cîrnu, Ardelean, 2011).

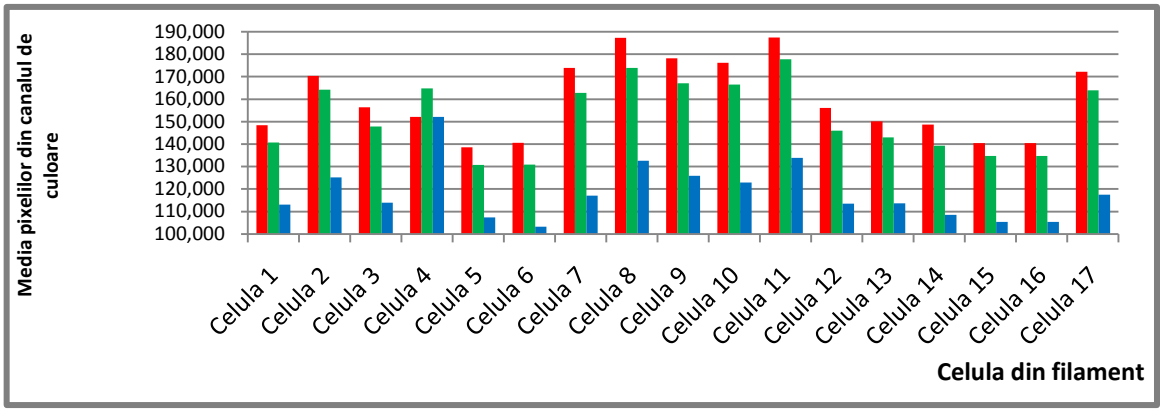
The dramatic decrease in blue channel could logically be attributed to the absorption of the complementary color, orange, by the reduced, purple, MTT; the same for the decrease in red channel as a consequence of absorption of green light by the reduced MTT. When it comes to the decrease in the green channel, its signification is under investigation being probably related to the occurrence of multiple light processes (absorption, reflection, transmission) whose interaction with different (colored) cell components, and processes, is not yet understood (Sarchizian, Cîrnu, Ardelean, 2011).

To quantify the redox properties of individual cell level filament Anabaena sp. results obtained by digital image analysis of cells in filaments cation analyzed for each experimental time to light during cultivation were found under UserGuide regions of interest were analyzed ImageJ and color histograms of each cell. The results of automated digital image analysis of cells in a filament of cyanobacteria at time T0, T1, T2, T3, T4 was considered filaments composed of respectively 13, 17, 24, 19, 16 cells that were defined regions of interest (ROI) as UserGuide ImageJ and were analyzed color histograms of each cell, resulting in the following rezutate, summarized in Figure 14.

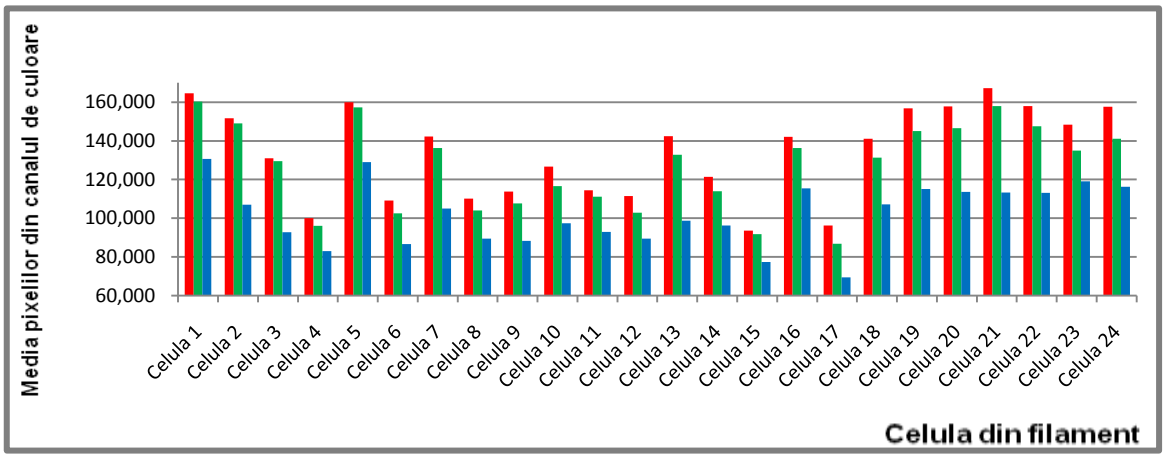
T0


Media pixelilor din canalul de culoare
190,000
170,000
150,000
130,000
110,000
90,000
70,000
Celula 1
Celula 2
Celula 3
Celula 4
Celula 5
Celula 6
Celula 7
Celula 8
Celula 9
Celula 10
Celula 11
Celula 12
Celula 13
Celula din filament
Canal roşu
Canal verde
Canal albastru

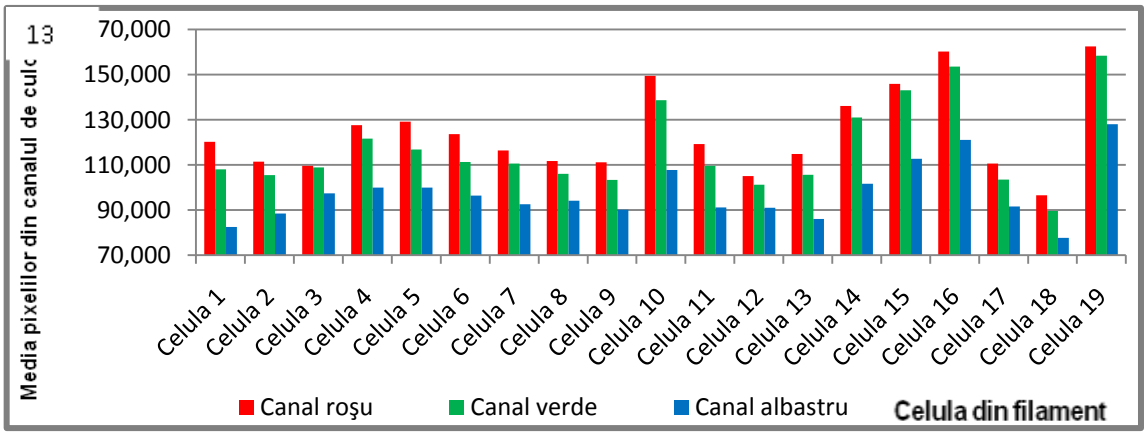
T1


Media pixelilor din canalul de culoare
190,000
180,000
170,000
160,000
150,000
140,000
130,000
120,000
110,000
100,000
Celula 1
Celula 2
Celula 3
Celula 4
Celula 5
Celula 6
Celula 7
Celula 8
Celula 9
Celula 10
Celula 11
Celula 12
Celula 13
Celula 14
Celula 15
Celula 16
Celula 17
Celula din filament

T2


Media pixelilor din canalul de culoare
160,000
140,000
120,000
100,000
80,000
60,000
Celula 1
Celula 2
Celula 3
Celula 4
Celula 5
Celula 6
Celula 7
Celula 8
Celula 9
Celula 10
Celula 11
Celula 12
Celula 13
Celula 14
Celula 15
Celula 16
Celula 17
Celula 18
Celula 19
Celula 20
Celula 21
Celula 22
Celula 23
Celula 24
Celula din filament

T3


13
70,000
150,000
130,000
110,000
90,000
70,000
Celula 1
Celula 2
Celula 3
Celula 4
Celula 5
Celula 6
Celula 7
Celula 8
Celula 9
Celula 10
Celula 11
Celula 12
Celula 13
Celula 14
Celula 15
Celula 16
Celula 17
Celula 18
Celula 19
Canal roşu
Canal verde
Canal albastru
Celula din filament

T4

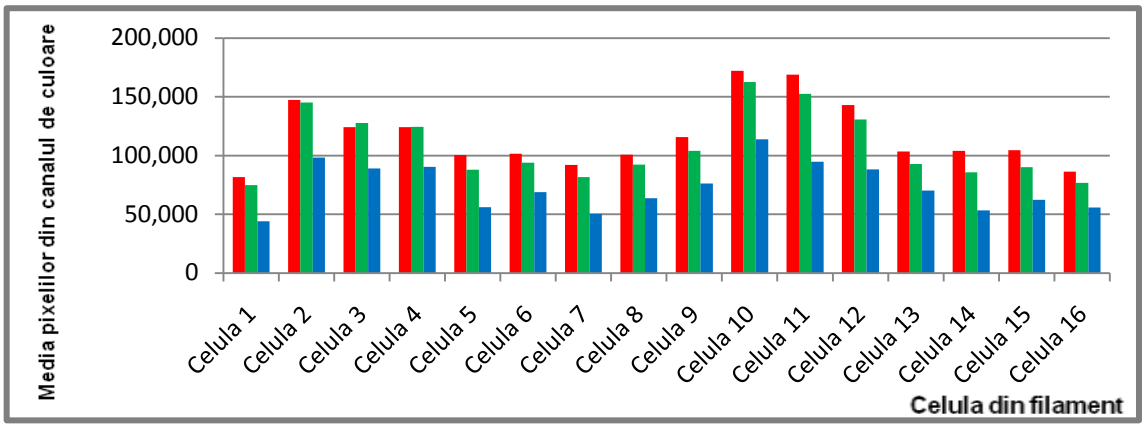



**Figure 14 (Figure 4.67).** Automatic analysis of cyanobacteria cell level of filaments studied (original).

In conclusion, these results show the importance of mathematical methods for image processing and light signals, useful for microbiological research at the cellular level. Our results show a strong decrease in signal blue during MTT reduction by each individual cell analyzed, as a consequence of orange light absorption by reduced MTT. This is the first report on the use of automated digital image analysis to measure the reduction of artificial electron carriers at the cellular level in filamentous cyanobacteria. Also, these results are important for basic research in microbiology at the cellular level, but also for biotechnological research linking redox properties of cyanobacteria by using them as light energy into electricity converters and their use as biosensors.

Using quantum dots to study aims to investigate the cytotoxicity cyanobacteria interaction between quantum dots (CdSe/ZnS) and cyanobacteria in natural samples collected from mesothermal sulphurous spring, as well as enrichment cultures unicellular and filamentous cyanobacteria, while with digital image analysis of cyanobacteria (Armaselu et al., 2011). Digital images taken with epifluorescence microscope showed that unicellular cyanobacteria were stained with quantum dots, and in terms of filamentous cyanobacteria, quantum dots have migrated inward and remained attached to sheath their digital image analysis performed successively digital images obtained from a video attachment cyanobacterial filaments quantum dots tried to explain this change of color of filamentous cyanobacteria labeled with quantum dots by adding additional quantities of quantum dots in enriched cultures of cyanobacteria. Adding quantum dots 0559 the spiral filaments of cyanobacteria have found that adding a second after the quantum dots, the filament of cyanobacteria has deep red color due to the natural fluorescence of chlorophyll color overlay over color quantum dots, and after about 40 seconds filament cyanobacteria broken, noting the toxic effects of cyanobacteria on quantum dots under study.

Each image taken by the video recording was automatically analyzed three color channels RGB and subject to statistical analysis of data with Microsoft Excel. Were analyzed 11 images taken every 10 seconds using Microsoft MovieMaker software. Thus, we found that the green channel pixel value increases with quantum dots attached filament cyanobacteria, increases with decrease in year blue channel.

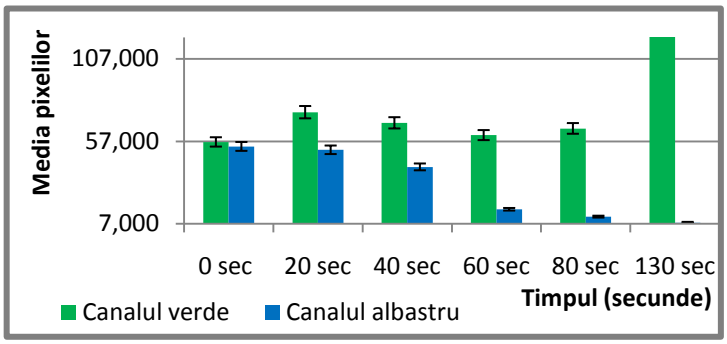



**Figure 15 (Figure 4.77).** Evolution of pixels in the green channel and blue (original).

Cytotoxicity studies were conducted and cyanobacteria isolated from the Black Sea, viewed as the natural fluorescence and bright field, quantum dots in the presence or in the absence thereof 0560. In this case was intended effect on the color of fluorescent quantum dots under study cyanobacteria.

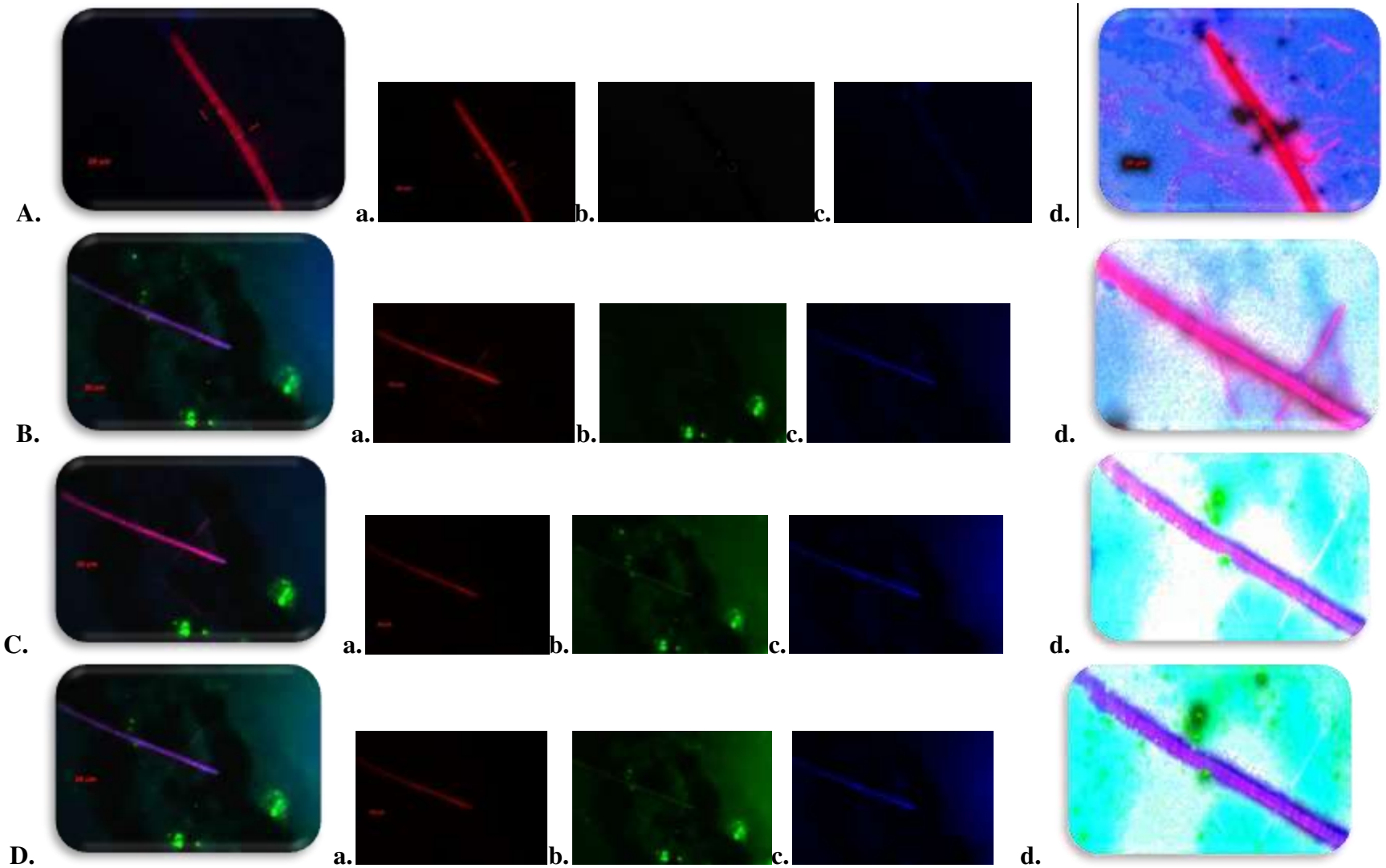



**Figure 16 (Figure 4.78).** Evolution of cyanobacteria filament fluorescence: A-natural fluorescence of cyanobacteria, B-colored cyanobacteria filament after one second from the addition of quantum dots 0560, C-color filament of cyanobacteria after a minute after the addition of quantum dots 0560, D-color cyanobacteria filament after 15 minutes after the addition of quantum dots 0560, to digital image red channel b - channel digital image green digital image channel c-blue d-digital image of the region of interest analyzed after extraction stages image substrate (Armaşelu et al., 2011).

Evolution after addition of fluorescent color, step by step, 0560nm quantum dots is shown in Fig. 16 (Fig. 4.78). Color natural fluorescence of chlorophyll *a* in cyanobacteria filament is red fluorescent color representing adequate cyanobacteria. After the addition of quantum dots in cultured cyanobacteria, we found that they preferentially migrated to the filament of cyanobacteria, remaining attached to them. An explanation of the natural fluorescence color change from red to purple color would be overlap, such as natural fluorescence of chlorophyll a red with green fluorescent quantum dots used in the experiment, and purple is an overlap with the blue red . These findings that have revealed directly by fluorescence microscopy were subsequently explained by digital image analysis performed on digital microphotographs captured during the experiment.In automatic digital analysis using ImageJ software, the software options for determining the intensity of the primary colors red, green and blue color histograms by analyzing each digital image analysis and data processing, we found that the intensity of green color of digital images analyzed increase from T0 to T3 (after 30 minutes of adding quantum dots repeated suspension cyanobacteria), while increasing the intensity of blue color, while red intensity showed a downward curve.

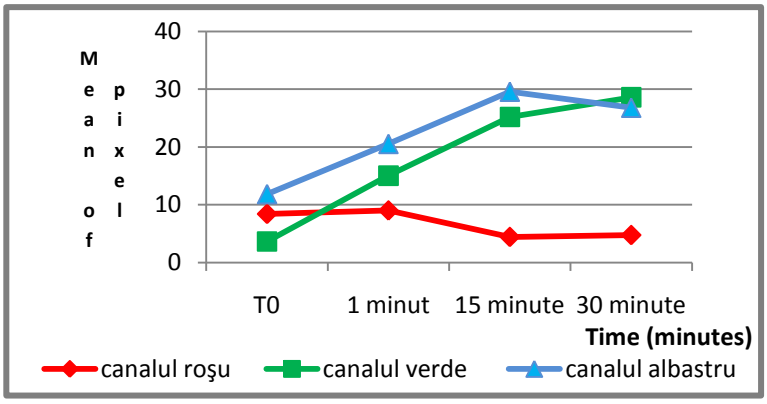



**Figure 17 (Figure 4.79).** Digital analysis of the evolution of fluorescent color in RGB (Armaşelu et al., 2011).

Consulted the literature, we found that this is the first report of automatic digital analysis showing color change fluorescent filaments of cyanobacteria, following the submission, step by step, quantum dots filaments of cyanobacteria (Armaşelu et al., 2011) .

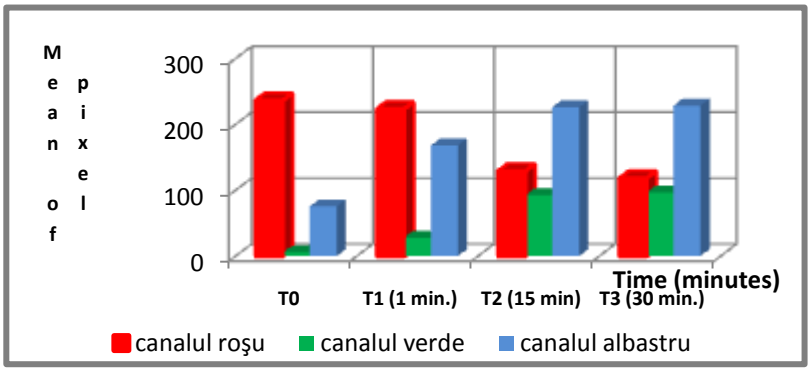



**Figure 18 (Figure 4.80).** Color evolution of cyanobacteria filament (region of interest automatically analyzed) in each color channel: red channel, green channel and the blue channel after addition of quantum dots constant in 0560 extracting suspension of cyanobacteria and substrate each image (original).

To note is that the green channel intensity increases by about 5 times after adding the quantities of quantum dots. After adding a second quantity of quantum dots, the green channel intensity increased further, and after adding multiple quantities of quantum dots, green channel intensity remains almost constant. Regarding the blue channel fluorescence intensity is higher than the red channel or even unprocessed green channel (blue channel was observed in digital images digital analysis early in filamentous cyanobacteria automatic digital).

Cytotoxicity of quantum dots fluorescence at 490nm, 520nm, 560nm and 600nm was investigated in different species of unicellular cyanobacteria, such as *Synechocystis* PCC 6803 culture collection and cultured unicellular cyanobacteria isolated from mesothermal sulphurous spring, denoted *Synechocystis sp* . It is known that quantum dots affects electron transport related to energy metabolism, both fototrofe bacteria and the heterotrophic bacteria.

The Light (Figure 19) and the dark (Figure 20) for *Synechocystis* PCC 6803 collection culture differences were observed in the first 3 hours after adding resazurin when column 9 (quantum dots added before adding resazurinei) shows a slight change color as a result of reducing resazurinei for all quantum dots used in the study (483nm, 522nm, 559nm, 609nm and), these differences being more evident after 7 hours of reaction (results not shown). More differences between the effects of QD incubated together with cyanobacteria either in light or in dark appeared after 24 hours. In light, at 24 hours of incubation in the presence of resazurine in column 1 and 9 the reduction is more advanced then at 7 hours, whereas very low changes in color are visible in other wells, arguing that in light with all types of QD the suppression of metabolic activity by these nanoparticles is very severe.

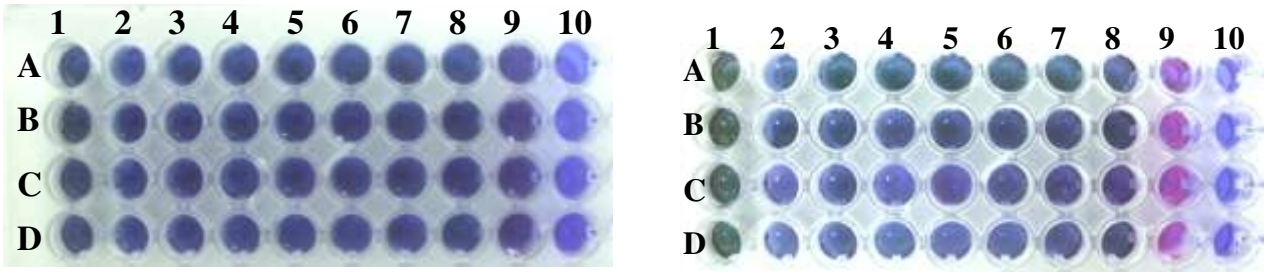
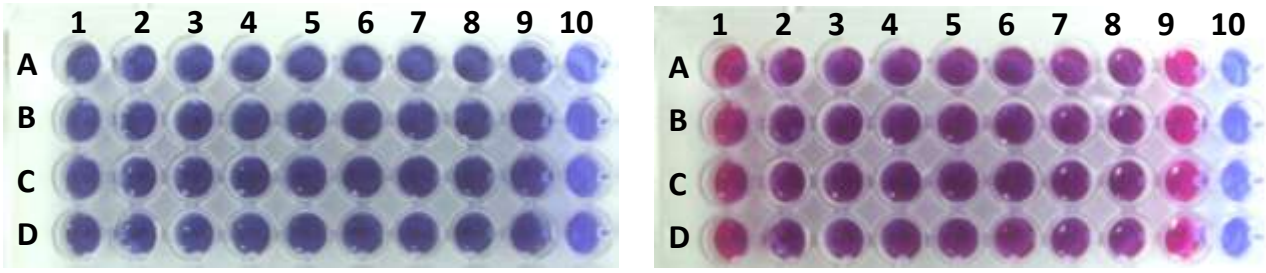



**Figure 19 (Figure 4.81).** Reducing resazurinei (pink-purple) by Synechocystis PCC 6803 incubated with 200 pg dots light cuantice/200µL suspension of cyanobacteria (A-483nm quantum dots, quantum dots B-522nm, 559nm quantum dots C-, D-609nm quantum dots ) at time zero and after 24 hours of incubation in light (Ardelean et al., 2011).

In darkness after 24 hours in column 1 and 9 the reduction is more advanced then at 7 hours; moreover different degrees of reduction are visible in other wells arguing that even in longer incubation times in darkness with all types of QD the ability to reduce resazurin to its

pink, semi reduced form (resorufin) is present in all experimental conditions. This is an important difference as compared with light incubation, suggesting that the cytotoxicity of these QD against *Synechocystis* PCC 6803 is stronger in light than in darkness. Up to our best knowledge this is the first report concerning the higher citotoxicity of QD against cyanobacteria in light than in darkness, results which suggest that the interactions between photosynthetic cells and QD is stronger in light than in darkness.




**Timpul zero: la întuneric** **După 24 de ore: la întuneric**

**Figure 20 (Figure 4.82).** Reducing the Synechocystis PCC 6803 resazurinei incubated in the dark with 200 pg dots cuantice/200µL suspension of cyanobacteria (A-483nm quantum dots, quantum dots B-522nm, 559nm quantum dots C-, D-609nm quantum dots) at time zero and after 24 hours of incubation in the dark (Ardelean et al., 2011).

Given the well-known higher chemical reactivity to light these semiconductor nanoparticles may believe that the higher reactivity could be involved in higher cytotoxicity them to light. Whether there is an interaction of light with quantum dots photosynthetic metabolism of intact cyanobacteria is another interesting question. One can believe that quantum dots located on the cell wall or cell membrane tilacoidele should interact with membranes located in interiorulul cytoplasm by an unknown mechanism and / or because of very small diameters, 4-6 nm of these quantum dots their penetration within the cytoplasm could also be considered (Ardelean et al., 2011).

**Gross dehydrogenase activity.** Preliminary experiments shown that after 21 hours of incubation in darkness or in light with all the 4 types of QD used in these experiments at a concentration of 1 pg QD/1 µL the ability of either *Synechocystis* PCC 6803 or *Synechocystis* sp. to reduce DCPIP alone or in the presence of PMS is completely abolished, showing the cytotoxic effect of these QD in theses experimental conditions. Following these results, new experiments have been designed to measure the cytotoxic effect -if any- at shorter incubation time, namely one or two hours. The incubation of cell suspension with QD were performed in light as well as in darkness in order to further test the interaction in light as compared with the dark incubation, as suggested by microplate assays done with resazurin. Incubation of *Synechocystis* PCC 6803 cultures in darkness together with QD for one or two hours induces interesting effects on the ability of these cells to reduce DCPIP in the presence of PMS.

It noted total inhibition of DCPIP reduction in the presence of FMT by *Synechocystis* sp. incubated for 1-2 hours at light Proving strong cytotoxic effect in light of quantum dots. Interestingly, the incubation light of *Synechocystis* PCC 6803 culture or the culture of Synechocystis sp. quantum dots with one or two hours, completely eliminate the ability of these cells to reduce DCPIP in the presence of FMT again claiming higher cytotoxicity of quantum dots to light than in the dark, in these species of cyanobacteria.

When it comes to the mechanism(s) responsible for the inhibitory effects of QD no original experiments have been done but, in agreement with the literature, one could think that the interactions between cells and QD causes the production of reactive oxygen species (ROS) but other mechanism(s) could also be involved. One important task in all these experiments concerns the physical relationship between QD and different microbial populations, with special emphasis on the position of QD towards cell wall and cell membrane. It seems logically to assume that the first site of interaction between these nanoparticles and cells is at the level of cell wall; however cell wall has rather different structure in Gram- negative bacteria (including cyanobacteria) and Gram-pozitive bacteria. The physical access of QD at the external face of the cell membrane (toward cell wall) is still an open question as well as the ability-if any- of these CdSe/ZnS core-shell quantum dots (with long chain amine capping agent) with dimensions in the range of few nanometers to pass through the intact (or previously damaged!) cell membrane to enter the cytoplasm.

## CONCLUSIONS:

1.The thesis presented the isolation, purification and identification at genus level samples of 8 strains of cyanobacteria from sulphurous mesothermal spring from Oban-Mare (Mangalia).
2. Improved methods for purification of cyanobacteria strains have established the optimal conditions to remove heterotrophic contaminants adding the carbon source before antibiotic; antibiotics tienam, augmentin, cephalexin, nalidixic acid had bactericidal effect on heterotrophic bacteria in cultures this is important fundamanet for making a method of obtaining axenic cultures of cyanobacteria ; augmentin was used for the first time on cyanobacterial cultures in these experiments, having also a strong bactericidal effect on heterotrophic bacterial tested.
3. Determination of the growth rate of cyanobacteria under aerobic conditions in light whatever using atmospheric nitrogen as the only nitrogen source ($BG_0$ culture medium) or nitrate ($BG_{11}$ culture medium) led me to obtain the generation time for cyanobacterial strains (*Nostoc* 1 sp., *Nostoc* 2 sp., *Synechocystis* sp., *Oscillatoria* sp., *Anabaena* sp., *Synechocystis* sp. anoxigenic).
4. Determination of the growth rate by calculating the frequency of dividing cells (FDC) revealed: calculating growth rate in two populations of filamentous cyanobacteria isolated from Obanul Mare (Mangalia), grown in the laboratory to light: the strain of *Anabaena* sp. , the maximum growth rate in $BG_0$ culture medium is 0.039 $hours^{-1}$, and for unformatted strain of heterocysts *Tychonema* sp. maximum growth rate in $BG_{11}$ culture medium is 0.057 $h^{-1}$, based on data from the literature we can say that this is the first report on the use of the method for determining the growth rate using frequency of dividing cells (FDC) applied on filamentous cyanobacteria (heterocysts forming or not) and also, this is the first report on the use of the method for determining the growth rate by calculating FCD coupled with automated image analysis of digital images obtained from bright field microscopy.
5. Using the direct viable count method described by Kogure et al (1979) on filamentous cyanobacteria led me to the following conclusions from experiments: that 64% and 71% of cells are capable of growth and division. The method could be successfully used for direct determination of cells capable of growth and reproduction in natural samples containing filamentous cyanobacteria, including cells within a single filament or to differentiate individual filaments are increasing (containing at least one cell capable of growth and multiplication) to the rest of the filament (which do not contain any such cell), only some cells from filaments analyzed changes its size during the experiment, suggesting that under natural

conditions, only certain cells made cell growth, after the consultations of international scientific literature this method were not applicated on filamentous cyanobacteria, only one reporting unicellular cyanobacteria (Lucilla et al., 1996), also we combined bright field microscopy techniques with digital image analysis in the study of filamentous cyanobacteria treated with nalidixic acid.

6. Studying the redox properties with spectrophotometric methods at the population level in some strains of cyanobacteria isolated led me to obtain different values of overall dehydrogenase activity.

7. During incubation in light of cyanobacteria in the presence of artificial electron acceptor (MTT) changes in color intensity level filament are significant compared to initial time during the channel red, green and blue, decreases as the 91.7%, 89 , 8% and 86.8%, in line with higher speed of reduction of MTT in conditions of light during incubation in the dark in color intensity changes at filametent are very small compared to the initial time (decrease of up to 95-97%) compared with the results obtained during incubation in light, consistent with very low speed to reduce MTT in the dark. Existence of variability for each individual filament of cyanobacteria suggests that metabolic intensity is different for different filaments of cyanobacteria (at individual biological level) individual cell level for all cells in the filament, there is great variability in the values obtained during incubation at light, suggesting that metabolic intensity is different for the individual biological cells, represented by cianobacterian filament, these results represent the first report on the use of automated digital image analysis to measure the reduction of artificial electron at the cellular level in filamentous heterocysts forming cyanobacteria.

8. The use of quantum dots (CdSe/ZnS) in cyanobacteria was found that the intensity of green color of digital images analyzed time increases from initial time to 30 minutes of adding quantum dots repeated suspension of cyanobacteria, as a result of accumulation on the surface of quantum dots on cyanobacterial filaments, while increasing the intensity of blue color, while red intensity showed a declining path as the original time average pixel values in the three color channels, observing to one minute that these values have changed in the 3 color channels. Pixel intensity value of red channel decreased very slowly after the first treatment with quantum dots, but after the second treatment this value drops to half and then becomes stationary. This behavior can be attributed to changes in red fluorescence of chlorophyll a in filamentous cyanobacteria. Green channel can be considered as the next channel variation in the intensity of green fluorescence of quantum dots, experimental methods are successfully applied both on freshwater cyanobacteria and marine cyanobacteria.

9. The study of the cytotoxic effect of quantum dots on cyanobacterial culture in light and in dark cultivation led us to the following conclusions: the cytotoxicity of quantum dots on the culture of *Synechocystis* PCC 6803 is stronger during incubation in the light than in the dark. Interestingly, the incubation in light with quantum dots of *Synechocystis* PCC 6803 culture or our isolated culture *Synechocystis* sp. with one or two hours, completely eliminate the ability of these cells to reduce DCPIP in the presence of PMS once again supporting higher cytotoxicity of quantum dots in light than in the dark, in these species of cyanobacteria. In light cultivation quantum dots inhibit total gross dehydrogenase activity both in culture *Synechocystis* PCC 6803 and *Synechocystis* sp, even after an hour of incubation. Referring to the literature, this is the first report on quantum dots high toxicity against cyanobacteria incubated in light, compared to incubation in the dark. At dark, after 1-2 hours of incubation with quantum dots, is induced in *Synechocystis* PCC 6803 culture a strong increase of dehydrogenase activity (from 260% to 1000%!) while in *Synechocystis* sp. culture there is a decrease of 60-90%.

All results presented in the doctoral thesis conducted us to say that the aims of the study and objectives have been achieved, the results are original, novelty nationally and some even internationally.

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## ANEXX

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- **Sarchizian I.,** Ardelean I.I., 2010. Axenic culture of a diazotrophic filamentous cyanobacterium isolated from mesothermal sulphurous springs (Obanul Mare - Mangalia), Rom. J.Biol- Plant Biol., vol. 55, No.1, p. 47-53.
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**Articles published in proceedings of national conferences:**

- Ghiţă S., **Sarchizian I.,** Ardelean I.I.; Enumerarea şi evidenţierea celulelor bacteriene din medii marine poluate cu hidrocarburi- recomandări metodologice pentru aplicaţii în cercetarea de laborator. Sesiune ştiinţifică „Dezvoltare durabilă în regiunea Mării Negre" Univ. Maritimă Constanţa, ISSN 2069-248X, Ed. Nautica, p: 109-117, (2010).

- **Sarchizian I.,** Ghiţă S., Ardelean I.I.; Aplicaţii ale analizei de imagine digitală pentru măsurarea şi enumerarea bacteriilor heterotrofe şi fotosintetizante utilizând microscopia de epifluorescenţă. Sesiune ştiinţifică „Dezvoltare durabilă în regiunea Mării Negre" Univ. Maritimă Constanţa, ISSN 2069-248X Ed. Nautica, p: 102-108, (2010).

**Participation in international scientific meetings:**

- **Sarchizian, I.,** 2011. Automated Analysis of Unicellular and Filamentous cyanobacteria in bright field and fluorescence microscopy – preliminary results and perspectives , First International School on Biomolecular and Biocellular Computing, Escuela Universitaria de Osuna, Sevilla, Spain, 5-7 Sept.2011 (**prezentare orala**).

**Abstracts published:**

- Ardelean, I.I., Cîrnu, M., Pascu, D., **Sarchizian, I.** , Damian, V., Apostol, I., Iordache, I., Apostol, D., 2012. Metalic Nanoparticle ( Gold, Silver, Aluminium) Citotoxicity against Phototrophic and Heterotrophic Bacteria, NANOCON 2012, 23 – 25. 10. 2012, Brno, Czech Republic.
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- **Sarchizian I.,** Ardelean I.I.; Evidentierea celulelor capabile de crestere si diviziune in populatii naturale de cianobacterii filamentoase din izvorul sulfuros mezotermal de la Obanul Mare (Mangalia); A XXI-a Sesiune de Comunicari stiintifice, Univ. Ovidius Constanta, 25-26 martie 2011.

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**Participation in national scientific meetings:**

- **Sarchizian I.,** Ardelean I.I., Ghiță S. Perspective ale analizei de imagine digitală a cianobacteriilor unicelulare şi filamentoase pentru studierea proprietăților redox la nivel celular, Sesiune de comunicări ştiințifice „Calitatea şi Monitoringul Mediului Înconjurător", Univ. Maritimă Constanța, 31 octombrie 2011 (**prezentare orala**).

- **Sarchizian I.;** Izolarea unei cianobacterii diazotrofe din izvorul sulfuros mezotermal de la Obanul Mare (Mangalia); A XIX-a Sesiune de Comunicari ştiintifice, Univ. Ovidius Constanța, 27-28 martie 2009 (**prezentare orală**).

- **Sarchizian I.,** Ghiță S., Ardelean I.I.; Obținerea culturilor axenice de cianobacterii. Sesiune de comunicări ştiințifice „Calitatea şi Monitoringul Mediului Înconjurător", Univ. Maritimă Constanța, 31 octombrie (2009) (**prezentare orală**).

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- **Sarchizian I.,** Ardelean I.I., Ghiță S.; 2011. Perspective ale analizei de imagine digitală a cianobacteriilor unicelulare şi filamentoase pentru studierea proprietăților redox la nivel celular**,** Simpozionul "Calitatea si monitoringul mediului marin", Ed. Nautica ISSN 2069-248X. (**prezentare orală).**

**Participation in national and international research contracts:**

- Sistem de producere cu laser de nanoparticule pentru biotehnologii (Laser-based manufacturing system for biotech nanoparticles production) (BIO.NANO.LAS) program „*PARTENERIATE IN DOMENIILE PRIORITARE".*

- Contract nr. 159/28/10/2011 proiectul PN-II-ID-PCE-2011-3-0742 "Biodiversitate si distributie cronologica a microorganismelor in straturile de gheata perena din ghetarul Scarisoara (Romania) – program IDEI.

- Am obținut bursă pentru participarea la *First International School on Biomolecular and Biocellular Computing* la Universitatea din Sevilia, Spania, în septembrie 2011.