OVIDIUS UNIVERSITY OF CONSTANȚA
DOCTORAL SCHOOL OF APPLIED SCIENCES
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SUMMARY OF THE PhD THESIS

PHYTOPLANKTON STRUCTURAL AND FUNCTIONAL CHANGES IN MAMAIA BAY OVER THE LAST TWO DECADES

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THESIS INTEGRAL CONTENT

AIM AND OBJECTIVES

A theoretical, descriptive and experimental approach is required for understanding the natural communities' characteristics, such as species richness, abundance, dominant groups and the food web structure. These facts allow the ecosystem status prognosis and give a better picture of environmental changes impact on the biological component. They also represent important knowledge in policy making and management decisions.

The need for a better understanding of the eutrophication impact on coastal ecosystems has been one of the main reasons for exploring the relationships between primary producer communities and nutrient concentrations fluctuations. Changes in nutrient concentrations in the environment can also have effects on the phytoplankton species composition, the amount of food available to zooplankton and the energy transfer to higher trophic levels.

The thesis aims at understanding the factors that control the phytoplankton composition and dynamics in Mamaia Bay in order to estimate the climate change and anthropogenic pressure impact on coastal ecosystems.

The main objectives of the thesis are:

1. Identification of phytoplankton's qualitative and quantitative structure changes in Mamaia Bay in the last two decades.
2. Identifying the multiannual and seasonal evolution of phytoplankton in Mamaia Bay over the last two decades.
3. Identification and analysis of the factors that have triggered the phytoplankton's qualitative and quantitative structure changes in Mamaia Bay in the last two decades.
4. Providing data from a controlled environment (laboratory experiments) related to anthropogenic pressure in terms of temperature rise and nitrogen to phosphorus unbalanced ratios.
5. Highlighting the potential of microalgae (especially *S. costatum*) as a natural alternative to cosmetics and pharmaceutical industries chemicals as well as in biotechnologies.

The personal contributions consist mainly of Mamaia' Bay phytoplankton long-term analysis, providing new data on the evolution of the food chain base, highlighting changes in the phytoplankton multiannual and seasonal structure and the factors that triggered these changes through an theoretical, descriptive and experimental approach using current data processing methods such as PRIMER 7, ANOVA test, *t* test.

Key words: phytoplankton, eutrophication, microalgal cultures, biotechnologies, acidification, Black Sea, experiments, *Skeletonema costatum*.

The doctoral thesis entitled "Phytoplankton structural and functional changes in Mamaia Bay over the last two decades", consists of two parts and is structured in five chapters.

The first part covers the current state of knowledge. The first three chapters present specialized literature information, which reflects the current state of knowledge regarding the phytoplankton evolution and role as the marine ecosystem trophic pyramid basis, the eutrophication and acidification effects on seas and oceans and phytoplankton study methods.

The second part covers the personal contributions. Chapter four presents the last two decades field data comparative study and the experimental studies methodologies. Chapter five highlights the changes that occurred in the last two decades, phytoplankton's qualitative and quantitative structure in Mamaia Bay and the experimental methods application.

PART I. THE STATE OF KNOWLEDGE

The Black Sea is one of the largest inland seas located between Europe and Asia, connected to the Atlantic Ocean by the Mediterranean Sea, the Marmara Sea and the Aegean Sea. In 1933, the Russian oceanographer M. N. Knipovici, attributed to the Black Sea the quality of "*unicum hidrobiologicum*" due to its special physical, chemical and biological peculiarities compared to the other seas and oceans of the world. Peculiarities concern the continental shelf configuration and arrangement, the water column stratification, the lack of vertical circulation, the world highest hydrogen sulphide content in deep waters, the high intake of freshwater with a high level of nutrients and detritus, water masses reduced exchange with the Mediterranean Sea, physical and chemical properties wide spatial and temporal variations, with salinity values reduced by half compared to those found in other seas and oceans and slightly different ionic ratios composition than in the planetary ocean (Knipovici, 1933).

The term phytoplankton comes from the Greek words *phyton* (meaning plant) and *planktos* (meaning wanderer or drifter). Phytoplankton consists of photosynthetic microscopic organisms that grow optimally in all oceans and freshwater bodies upper illuminated layer. Despite its small size, phytoplankton is of enormous importance in the aquatic food chain as a food source for a variety of organisms, fish and crustaceans which in turn provide a food source for larger animals. In addition, phytoplankton provide much of the oxygen we breathe, and fossilized phytoplankton is associated with oil and benthic deposits (Moncheva, 2008).

A better understanding of the phytoplankton natural variability is thus important for predicting the climate change impact on the aquatic ecosystem. A current challenge is to anticipate how changes in phytoplankton's composition will spread to higher trophic levels, as well as how the global warming synergistic effects and other environmental changes will affect the ecosystems. The degree of physical changes and the ability of species to adapt to changing environmental conditions will greatly influence the food web dynamics during future climate warming and will become increasingly variable.

The national phytoplankton study spans over 142 years, the first research on microalgae dating back to the nineteenth century, when Julius Schaarschmidt, professor at the University of Cluj, made the chapter on algae, in the synthesis 'Plantas Romaniae'. Thus, between 1879 and 1958, multiple studies on the phytoplankton qualitative structure have been elaborated by

E. Teodorescu, I. Borcea, E. Vasilescu - Marinescu, S. Negrea. During this period, lists of species were published and less information on the quantitative development of phytoplankton was provided It was not until 1958-1960 that the interest of specialists turned to the phytoplankton quantitative analysis. Many articles were published by H. Skolka, A. Petran, N. Bodeanu, M.-T. Gomoiu, M. Ușurelu, P.-E. Mihnea, Ș. Péterfi and A. Ionescu addressing various issues, starting with the study of the phytoplankton blooms since the '60s, the composition of phytoplankton and its distribution features (1964-1969), studies on the annual and seasonal phytoplankton dynamics, on its quantitative spatio-temporal distribution, the phytoplankton production and the importance of planktonic microphytes as a food source for certain species of molluscs and experimental ecophysiological studies.

A large part of the papers published in the 21st century were written by Bodeanu (2001), Sburlea (2004), Popa (2004), Bologa (2012), Gomoiu (2004) addressing various issues regarding the phytoplankton communities study such as: eutrophication, climate change and their effect, performing simulations using long term data to understand the mechanisms that determine these phenomena and to find out if there is a trend in the events produced. From 1996 until now, Laura Boicenco has carried out the Romanian Black Sea coast phytoplankton qualitative and quantitative study, both from the shallow area and on the continental shelf between Sulina and Vama Veche, to 2000 m depth stations in offshore waters. From 2013 and 2015, respectively, the undersigned and Elena Pantea joined her to carry out the phytoplankton study.

Thus, there is a progress in phytoplankton research, compared to the first papers published in the nineteenth century that were based only on the phytoplankton species inventory. This progress has been made possible by the awareness of the biological and ecological studies importance, especially at European level, and the research project funding for this field, which has led to technological advances for a better understanding of the complex interactions between human activities and the coastal waters.

In the last 20 years, experts from countries bordering the Black Sea such as Oguz, Gilbert, Besiktepe, Dromph, Kopelevich, Moncheva have focused their research on climate change, eutrophication, the toxic potential of some species, non-native species, improved working methods, and the use of remote sensing as a complementary phytoplankton communities research method.

Current estimates suggest that between 4,000 and 5,000 species of marine phytoplankton have been described (Sournia *et al*., 1991). The main pelagic eukaryotes with representatives in both fresh and marine environments are diatoms, haptophytes (coccolithophores) and dinoflagellates that are notable for their relatively recent origins, from the Mesozoic period.

The diatoms, coccolithophores and dinoflagellates appearance in the Mesozoic period fossil record provides a clear illustration of how the microalgae diversification has evolved. Although it is not certain that these groups did not exist before, there is no doubt about their Mesozoic extraordinary development. Another possible trigger would be the end of the Permian period mass extinction, when there was a huge volcanic lava, ash release and dust cover from the area that is now northern Siberia, which led to lower global temperatures. The trend was quickly reversed by atmospheric carbon dioxide accumulation and a severe global warming period (which, together with the marine sediments methane mobilization, raised the ambient temperature by 10-11℃). Life on Earth has suffered a severe setback, being close to total eradication. In less than 0.1 million years, many endangered species disappeared, and the survivor's number has been severely reduced. With the planet cooling over the next approximately 20 million years, both on land and in water, biota has been able to expand into habitats and niches that were otherwise unoccupied (Falkowski, 2002).

Dinoflagellate fossils have been discovered from the early Triassic period, and coccolithophorid fossils from the late Triassic period (around 180 million years ago). Along with diatoms, many new species have appeared in the Jurassic and Cretaceous. In the marine environment, these diatoms, dinoflagellates and haptophytes have assumed a dominant position compared to most other microalgae, except for picocyanobacteria, which continue today.

In marine systems, the primary production stoichiometry is determined by the ratio between the cytoplasm elements (Redfield ratio) which supports the phytoplankton optimal metabolism. The C:N:P ratio is constant in marine phytoplankton (106:16:1), and this primary producers ratio constrains the cycle of all elements. The nitrogen and phosphorus available amount and proportions determine the carbon amount fixed by phytoplankton (Redfield, 1958). Limiting, by any of these elements, constrains any additional carbon accumulation or other nutrients by phytoplankton. In turn, carbon and phytoplankton nutrients determine the nutrients circuit in offshore waters and during the upwelling phenomenon, so that the biotic demand for nitrogen and phosphorus correlates with their availability (Chapin *et al*., 2007).

In more than 200 years since the beginning of the industrial revolution, the carbon dioxide concentration (CO_2) in the atmosphere has increased due to human activities. In particular, the release of CO_2 from fossil fuel use, cement manufacturing and deforestation, since the beginning of the industrial era, has increased the partial pressure of atmospheric carbon dioxide concentration from 280 ppm to current values of 400 ppm (Bates, 2019). During this time, the pH of surface ocean waters decreased by 0.1 units. Although there does not appear to be a significant change, the pH scale is logarithmic, so this change represents an increase in acidity of about 30% (NOAA, 2020).

Although there is a legislative framework for transposing the European marine strategy into Romanian legislation, for updating the national marine water monitoring program according to the Marine Strategy Framework Directive (DCSMM), the Water Framework Directive (WFD), Law 278/2013 on industrial emissions and the Convention on the Protection of the Black Sea against Pollution for Integrated Pollution Prevention and Control, no specific action to assess and reduce acidification is mentioned. Additional Romanian legislation based on Directive 2006/7/EC on the management of bathing water quality, the Nitrates Directive (91/676/EEC) and the Habitats Directive (92/43/EEC) does not address the issue of acidification directly. In 2012, the National Institute for Marine Research and Development ‘Grigore Antipa’ Constanța (NIMRD), published a preliminary report on the Initial Assessment of the current state of the Romanian Black Sea waters according to the requirements of Directive 2008/56/EC of the European Parliament. Currently, it is the only official document that takes this issue seriously, as well as the need to assess the acidification state in territorial waters (Galdies, 2020).

PART II. PERSONAL CONTRIBUTIONS

IV. MATERIAL AND METHODS

4.1. METHODOLOGICAL ASPECTS OF PHYTOPLANKTON QUALITATIVE AND QUANTITATIVE ANALYSIS IN MAMAIA BAY

In order to observe the structural and functional changes of phytoplankton in the last two decades, we processed the data collected from Mamaia Bay. The personal contribution consisted in the processing and interpretation of the entire data set from 2000-2019 and in the samples microscopic analysis starting with 2011, during the license elaboration practice at NIMRD. The first step for the phytoplankton qualitative and quantitative analysis was to combine the sheets in a single excel table and standardize and update the scientific names of each species according to WoRMS. In order to observe the phytoplankton evolution trends in relation to physico-chemical parameters, we performed a phytoplankton qualitative and quantitative comparative analysis for the last two decades, also consulting the available specialized literature.

The physico-chemical data were processed by the NIMRD Measurements and Analyses Laboratory. The personal contribution consisted in combining and arranging the data series from 2000-2019 in a database, calculating the multiannual monthly averages and making and interpreting the graphs.

The data set includes 1797 phytoplankton samples, with a monthly average of 5-6 samples/month in winter, 6-7 samples/month in spring, 7-10 samples/month in summer and 6-8 samples/month during fall. Thus, we considered that, although the data come from a single station, they can provide a closer picture of reality for capturing changes. The variation in the number of samples was due to weather conditions, which are sometimes unfavourable and do not allow safe sampling (especially in winter), and the interest of researchers in case of bloom events.

4.1.1. Sampling area description

The shallow water station in the Mamaia area (2-3 m deep) was chosen to identify the phytoplankton structural and functional changes in the last two decades in the Romanian Black Sea coastal waters because it is the only long term monitoring station (bi-weekly) (**Fig. 21**). There is a network of monitoring stations along the Romanian Black Sea coast that includes coastal, marine and offshore waters, but the sampling frequency is seasonal/annual, the access to these stations being conditioned by the existence of a research vessel, the weather conditions and a budget dedicated to such research. Thus, the station in Mamaia Bay allows a detailed characterization of the coastal area (shallow waters) in terms of phytoplankton evolution, being subject to both the influence of the Danube and the anthropogenic impact of the area (urbanization, tourism and activities in Midia port).

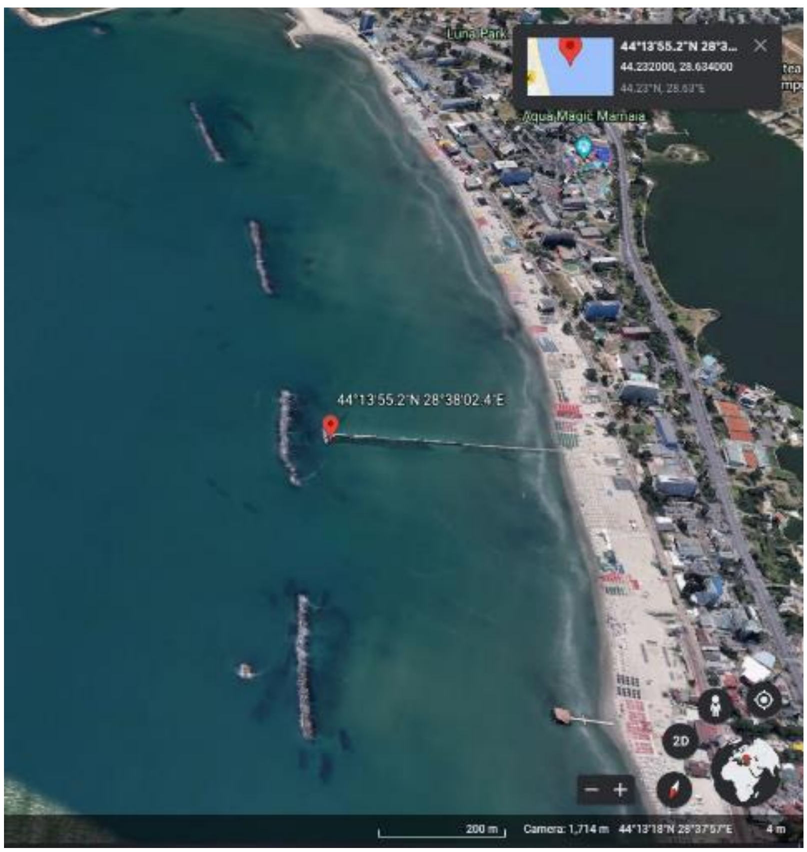

Fig. 21. Station location

4.1.3. Samples processing and data analysis

The phytoplankton identification and counting were performed under an inverted microscope according to the Utermohl method used in laboratories where interest falls mainly on issues of ecology, hydrography and botany (Utermohl, 1958).

For the identification of the species we used both identification keys (Kisselew, 1950, 1951, Proshkina-Lavrenko, 1955, Carmelo R. Thomas, 1993, 1995, Schiller, 1937) and online databases (World Register of Marine Species, Nordic Microalgae, AlgaeBase). All microalgae encountered were identified at the species level, genus or algal group, counting all the cells of each species/genus/group encountered. This information was included in a worksheet, along with the information on the label. Then the worksheets transposed in electronic format (xls) using dBase system software.

We calculated the numerical density (D) and biomass for each species, for each taxonomic class, for each functional and ecological group, for each size class and for the total phytoplankton. The result of the algal cell count in the analysed sample fraction is related to one litre of water, considering the dilution/multiplication factor. PRIMER 7 (Clarke et al., 2014) was used for monthly and annual average phytoplankton calculation and for SIMPER analysis. Graphs and tables were made in Microsoft Excel, and the GraphPad platform available online was used to apply the *t* test. The values of the Danube flow for 2018 were noted from the National Institute of Hydrology and Water Management (http://www.inhga.ro/diagnoza_si_prognoza_dunare) website. The values regarding the global radiation registered at Constanța station were downloaded from the World Radiation Data Center (http://wrdc.mgo.rssi.ru/), the most recent data available being those from 2006.

4.2. EXPERIMENTAL METHODS

4.2.1. Diatoms growth medium

The method for the culture medium was adapted considering the recommendations of ISO 10253:2016 and Andersen, 2005. Thus, the first step was to prepare stock solutions of chelator and micronutrients, macronutrients and vitamins. Then, in a 1000 ml volumetric flask, 200-300 mL of sterilized seawater, 15 mL of the micronutrient stock substance, 0.5 mL of the vitamin stock substance and 1 mL of the macronutrient stock substance were added followed by stirring after the addition of each stock substance. It was brought to the mark with sterilized sea water. If necessary, the pH was adjusted to 8.0 ± 0.2 by adding dilute hydrochloric acid or sodium hydroxide solution. The culture medium was stored in the dark at 4°C and was used for maximum 7 days.

4.2.2. Culture growth conditions

The diatom *Skeletonema costatum* was isolated from Mamaia Bay by filtrations and serial dilutions (Culcea, 2017). The monospecific cultures are grown in an incubator, in 250 mL Erlenmeyer flasks, at 20°C ± 2°C, stirred at 70 rpm. A cycle of 14 hours of light and 10 hours of darkness is provided by fluorescent neon lamps (4500-5000 lx). Subcultures are made every 3-4 days by transferring 1 mL of the exponentially growing stock culture into 100 mL of culture medium.

4.2.3. Testing conditions

S. costatum development at temperatures between 8°C and 20°C ± 2°C was observed by exposing exponentially growing cells to gradually decreasing temperatures. Thereby, the thermostat was set to decrease the temperature every 24 hours, by 1.5°C - 2.0°C until the temperature reached about 8°C. Samples (1 mL) were collected every 24-48 ± 2 hours with a sterile Pasteur pipette. Subcultures were made every 2-4 days from the initial stock culture. The experiment was repeated three times. 36 samples were taken and analysed. The cells average growth rate of the exponential phase was analysed.

S. costatum development was also observed in terms of its tolerance for different nutrient ratios. Thus, exponentially growing cells were exposed to nitrogen limiting conditions (N/P=4.2), phosphorus limiting conditions (N/P=63.3), to optimal conditions according to Redfield, 1958 (N/P=16) and to conditions recommended by ISO 10253:2016 (N/P=18.8). For each nutritional condition we inoculated cells in three replicates, randomly positioned on an orbital stirrer at 80 rotations/minute, at 20°C ± 2°C and 4500-5000 lx. Samples (1 mL) were collected every 24-48 ± 2 hours from each test vessel (12 samples/day). The samples were labelled and fixed with 0.03 ml of 37% formaldehyde. The end of the experiment was 9 days after exposure, corresponding to the beginning of the decline phase. 120 samples were collected and analysed.

4.2.4. Sample and data processing

A haemocytometer was used to estimate the cells density. The average specific growth rate for each test culture was calculated using the formula (ISO 10253:2016): $\frac{\ln N_L - \ln N_0}{t_L - t_0}$, where t_0 is the time of test start, t_L is the time of test termination, N_0 is the nominal initial cell density and N_L is the measured cell density at time t_L

Divisions per day (k) and the population doubling time (T_2) were calculated using the formula $k = r/\ln 2$, $T_2 = \ln 2/r$. The doubling density hours were calculated by multiplying the doubling time by 24 hours (Andersen, 2005). Density and exponential growth rates were plotted on growth curves. The Moving Average analysis tool was used for growth phases better visualization. The *t* test and the ANOVA test were applied to study if there are statistical differences in the development of *S. costatum* under different nutritional conditions.

4.3. METHODOLOGICAL ASPECTS OF TANNIC ACID ANALYSIS FROM *S. COSTATUM* BIOMASS

The personal contribution to this analysis was the biomass production, harvesting and lyophilization of *S. costatum*. The HPLC analysis for the identification and determination of tannic acid was made by Mrs. Associate Professor Dr. Laura Bucur.

The biological material need for tannic acid analysis was obtained by inoculating cells in exponential growth phase into 2 L of culture medium for diatoms (ISO 10253:2016) in a 4 L Erlenmeyer flask. The samples (1 mL) were collected immediately after inoculation and before harvesting (four days after inoculation). Then, the samples were labelled and fixed with 30 µL of 37% formaldehyde. The culture was exposed to continuous lighting (7-8000) lx and stirring (50 rpm). After four days, the biomass was harvested on 0.45 µm Millipore filters using a vacuum filtration system. Wet biomass filters were weighed and lyophilized. The lyophilized biomass was transferred to a paraffin-sealed Petri dish. Identification and determination of tannic acid content was performed by HPLC. The identification and quantification of tannic acid was performed based on the reference substance for which the retention time (RT) was determined at 1.15 min ± 0.05.

V. RESULTS AND DISCUSIONS

5.1. PHYTOPLANKTON QUALITATIVE STRUCTURE IN MAMAIA BAY OVER THE LAST TWO DECADES

Regarding the **multiannual evolution of phytoplankton diversity** during 2000-2019, in the shallow waters of Mamaia Bay, **352 species**, varieties and forms of microalgae belonging to 17 taxonomic classes were identified, the minimum number of 75 species being identified in 2003 and the maximum number of 166 species in 2013 (**Fig. 39**). Although there were fluctuations from one year to another, the general trend of the last 20 years has been **positive** (**R^2=0,63**).

Thus, the **species richness in Mamaia Bay in the last 20 years is higher** considering the number of species **reported until 1969** (320 species) in the shallow waters of the **Romanian Black Sea coast** (from Mangalia, Constanța, Midia, Portița, Sf. Gheorghe, Mila 9, Sulina profiles, down to 31 m depth) (Băcescu *et al.*, 1965, Skolka, 1967, Băcescu *et al.*, 1967, Bodeanu, 1969).

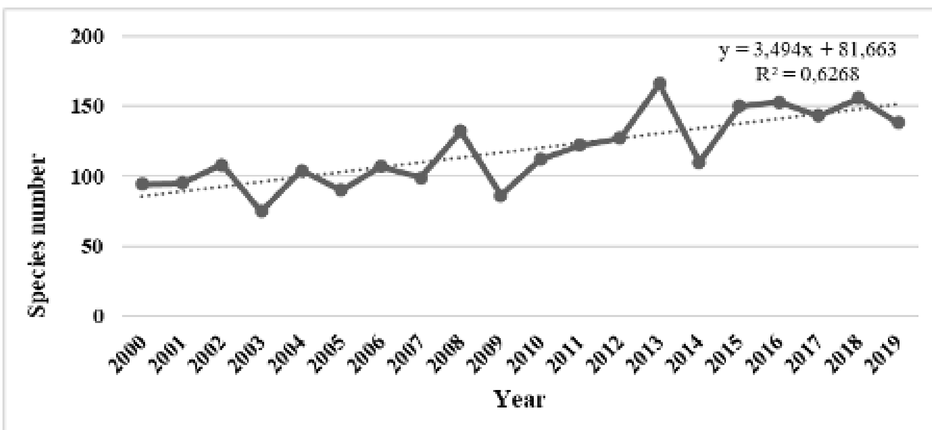

Fig. 39. Multiannul evolution of phytoplankton diversity in the shallow waters of Mamaia Bay

The diatoms (143 species) and dinoflagellates (80 species) dominance was noted during 2000-2019, which together represented 64% of the total number of species, followed by chlorophytes (42 species) and cyanobacteria (35 species), by 12% and 10%, respectively (**Fig. 40**).

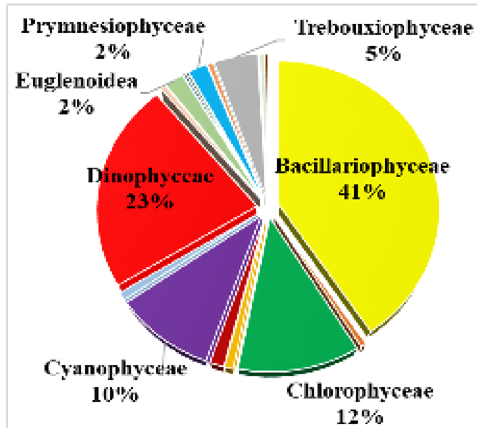

Fig. 40. Phytoplankton taxonomic composition during 2000-2019

280 microalgae were identified during 2010-2019, with 6% more than during 2000-2009. This change is mainly due to the increase with 24% of the dinoflagellates identified in the period 2010-2019 (70 species) compared to the previous period (51 species) (**Fig. 41**).

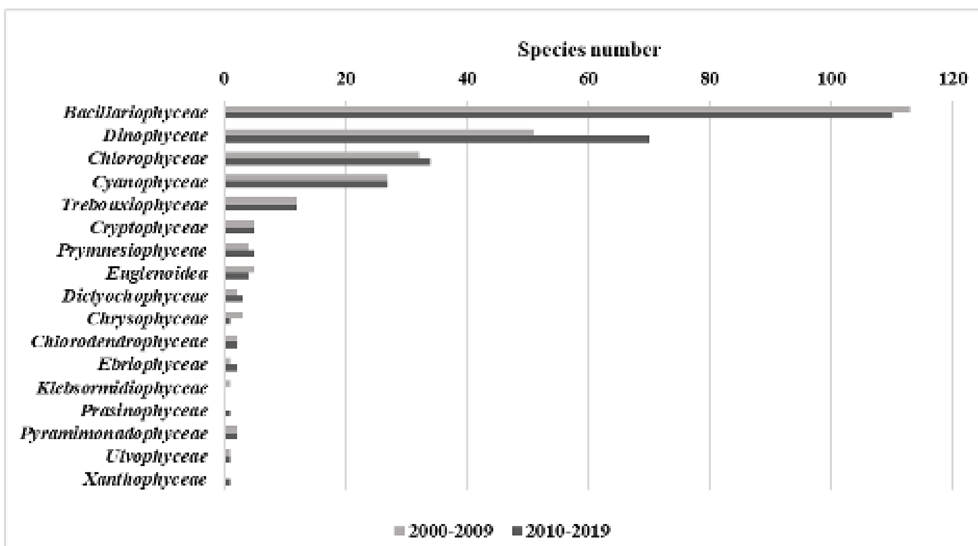

Fig. 41. Phytoplankton diversity by classes in the shallow waters of Mamaia Bay during the last two decades

The ***Chlorophyceae*** class has **increased by 4.8% in the last 10 years** compared to the previous decade. **Beneficial chlorophytes** for the marine ecosystem were present, known for their ability to accumulate heavy metals, to absorb nutrients, but also by the high lipid content (Dao *et al*., 2020; Bogen *et al.*, 2013; Issa *et al*., 1995, Patil *et al*., 1991, Ahmad *et al*., 2020).

One of the main factors influencing the phytoplankton diversity is the Black Sea tributary rivers **freshwater and nutrients intake** which is dispersed by wind and currents along the Romanian coast. Also, **salinity** influences phytoplankton diversity (Larson *et al*., 2013), cell functioning, and growth rate (Kinne, 1971). One of the main causes of seasonal variation in phytoplankton diversity may be the **sampling frequency**. During the summer, the sampling frequency was higher than in the other seasons (with an average of 7-10 samples), which increased the probability of encountering occasional species. Another important factor that could have influenced the positive trend of phytoplankton diversity is the experience gained by involving the study of phytoplankton in numerous national and international **projects** (Nucleus Projects, Emodnet, MISIS, Anemone), **intercalibration** exercises, and new generation microscopes.

Regarding the **functional groups**, the phytoplankton identified in the shallow waters of Mamaia Bay in the last two decades was composed of 245 autotrophic species and 107 heterotrophic species representing 70%, respectively, 30% of the total number of identified species. Considering that the sampling station maximum depth is about 3 m, and the sampling was done only from the surface layer (about 0.5 m), the **lighting conditions support the autotrophic microalgae photosynthetic activity**, being more abundant in this euphotic layer than heterotrophs. In the second decade, the autotrophs remained constant and heterotrophs increased to 90 species (**Fig. 44**). Although, for 2010-2019 autumn, the nutrients concentration was similar to spring, it seems that other parameters such as **temperature** (10-15°C) and a **less intense solar radiation** than spring **limited** the phytoplankton community abundant development, but **favoured the autotrophs diversity**. However, heterotrophic species, represented mainly by dinoflagellates and cryptophytes, prefer the summer' warmer waters and higher salinity.

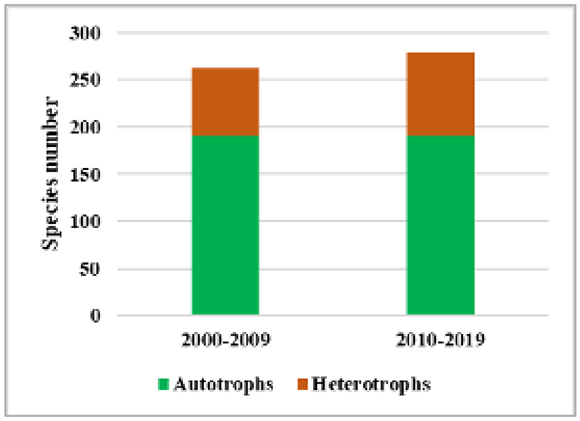

Fig. 44. Phytoplankton structure by functional groups in the shallow waters of Mamaia Bay during the last two decades

Autotrophic phytoplankton was mainly represented by ***Bacillariophyceae*** (35-46%), ***Chlorophyceae*** (11-14%), ***Cyanophyceae*** (9-10%) and ***Trebouxiophyceae*** (3-6%) classes. **Heterotrophic** phytoplankton was mainly represented by **dinoflagellates**, whose contribution varied, in the first decade, between 16% (winter) and 25% (autumn), and in the second decade, between 23% (winter) and 28% (summer) (**Fig. 46**).

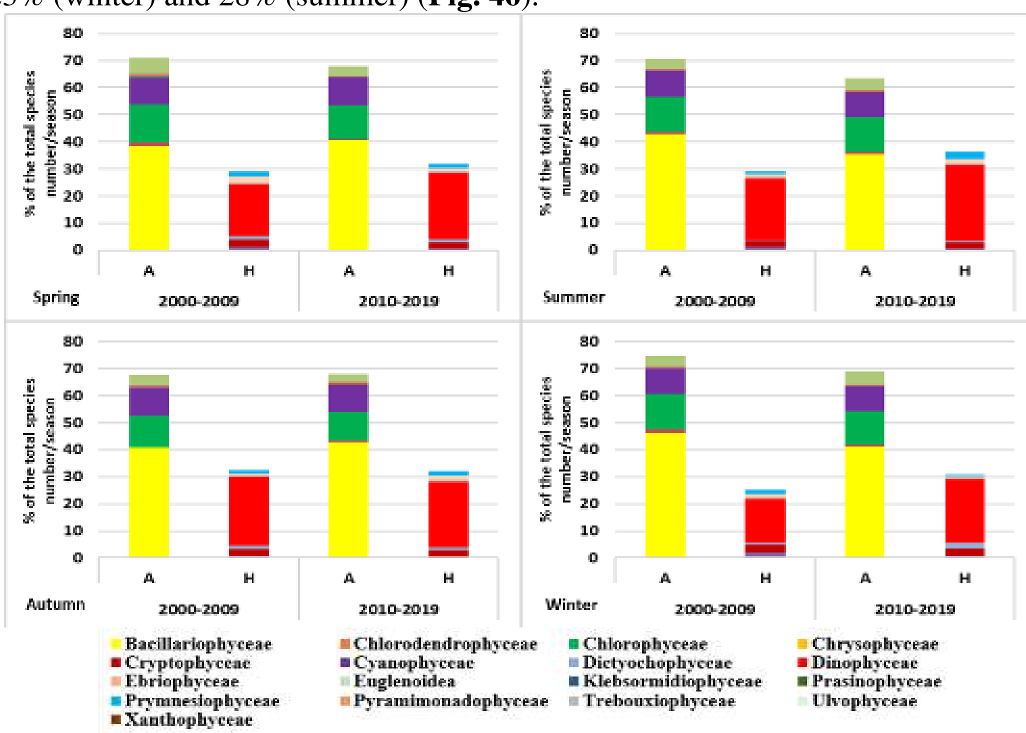

Fig. 46. The seasonal taxonomic composition of the functional groups (A-autotrophic species, H-heterotrophic species) within the phytoplankton community in the shallow waters of Mamaia Bay, during the last two decades

Referring to size structure, the shallow waters phytoplankton from Mamaia Bay in the last two decades comprised **150 nanoplankton** species and **202 microplankton** species (**Fig. 47**). **Microphytoplankton** was mainly represented by ***Bacillariophyceae*** and ***Dinophyceae***, and **nanophytoplankton** by **chlorophytes, diatoms and cyanobacteria**. Microphytoplankton species accounted for over 50% (52-57%) in each season of the two periods analyzed. In the first decade, a larger difference (14%) was observed between the number of nano and microphytoplankton species identified in the autumn season (**Fig. 48**), which corresponded to a **higher concentration of nutrients** (8.95 µM, in September), because nutrient uptake is slower in higher biovolume species, as is the multiplication rate (Sigman and Hain, 2012).

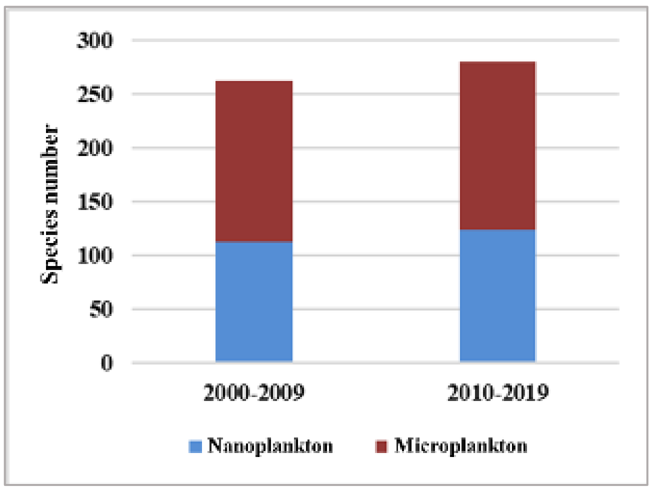

Fig. 47. Phytoplankton size structure in the shallow waters of Mamaia Bay, during the last two decades

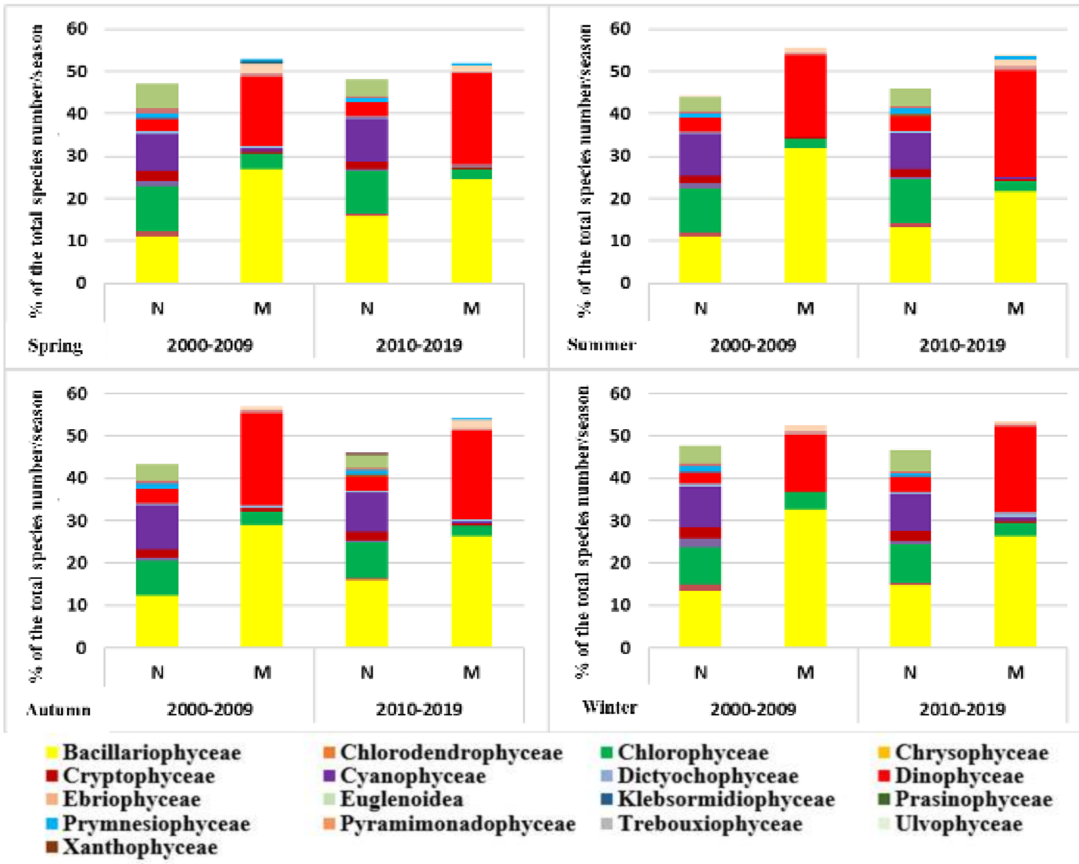

Fig. 48. The seasonal taxonomic composition by size structure (N-nanoplankton, M-microplankton) within the phytoplankton community in the shallow waters of Mamaia Bay, during the last two decades

Regarding the **phytoplankton ecological composition** in the shallow waters of Mamaia Bay in the last two decades, 216 brackish-marine species (MS) and 136 freshwater species (DS) have been identified (**Fig. 49**). The MS contribution was over 50% (54% -66%) in each season of the two analyzed periods. A smaller difference (9%) was observed between the diversity of MS and DS in the first decade spring. In the second decade, the differences between the species number proportion within the two ecological classes are between 31% (winter) and 39% (autumn) (**Fig. 50**). The most important MS classes were ***Bacillariophyceae*** and ***Dinophyceae***, and among the DS classes were ***Chlorophyceae***, ***Bacillariophyceae, Cyanophyceae*** and ***Trebouxiophyceae*** (**Fig. 50**).

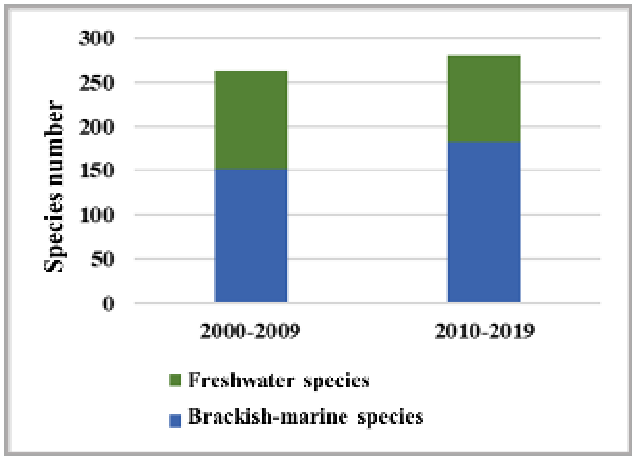

Fig. 49. Phytoplankton ecological composition in the shallow waters of Mamaia Bay, during the last two decades

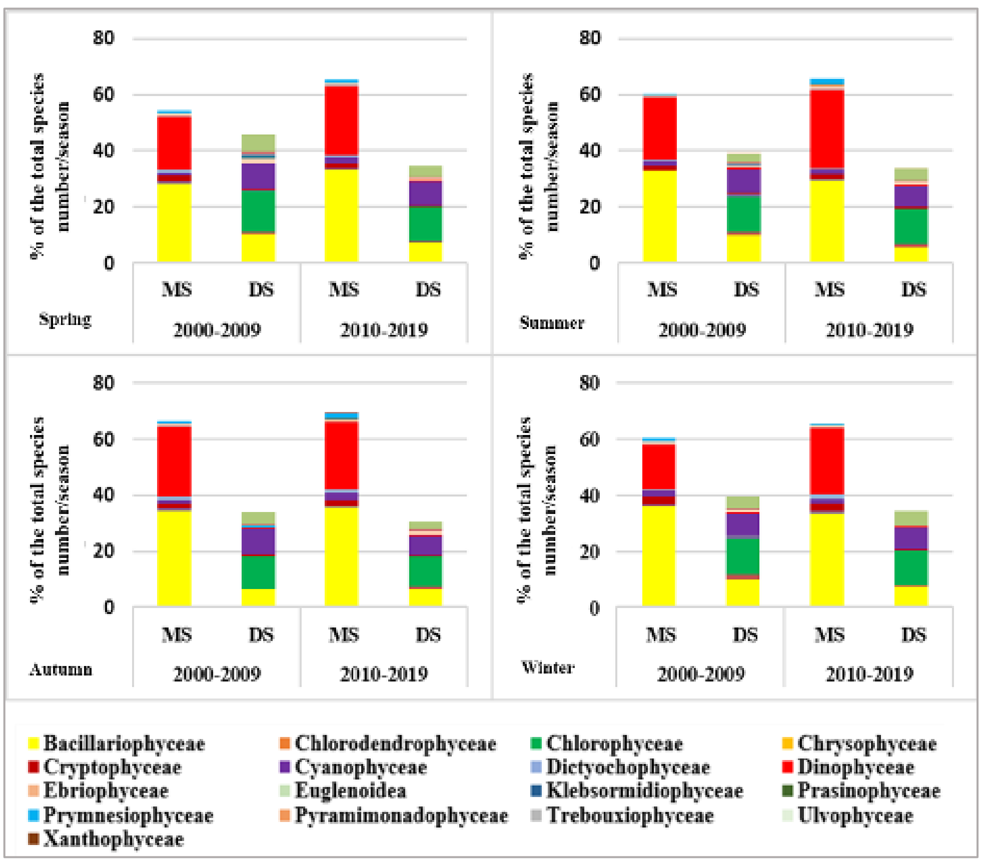

Fig. 50. The seasonal taxonomic composition by ecological groups within the phytoplankton community in the shallow waters of Mamaia Bay, during the last two decades

5.2. PHYTOPLANKTON QUANTITATIVE STRUCTURE IN MAMAIA BAY OVER THE LAST TWO DECADES

In this subchapter, I presented the multiannual and seasonal quantitative variations, the annual and seasonal structure and dynamics by taxonomic classes, by t functional and ecological groups and according to the size structure of the phytoplankton community from the shallow waters of Mamaia Bay. It was highlighted the contribution of each class and the most important species in the phytoplankton seasonal structure of the two periods using the SIMPER analysis (in Primer 7). I presented the statistical significance of the differences between the two analysed periods regarding the functional and ecological groups taxonomic structure and by phytoplankton community size structure.

The phytoplankton decline observed by other authors since 1993 (Boicenco, 2017) has been observed in the last twenty years, the period 2010-2019 being the most obvious (**Fig. 53**). The decline of the trophic pyramid base observed in the last 27 years produces disturbances in the marine food webs and effects on the carbon cycle. In addition to decreasing CO_2 fixation rate, the amount of heat absorbed by phytoplankton also decreases and the microalgae ability to emit sulphate molecules (which helps to form clouds) is reduced.

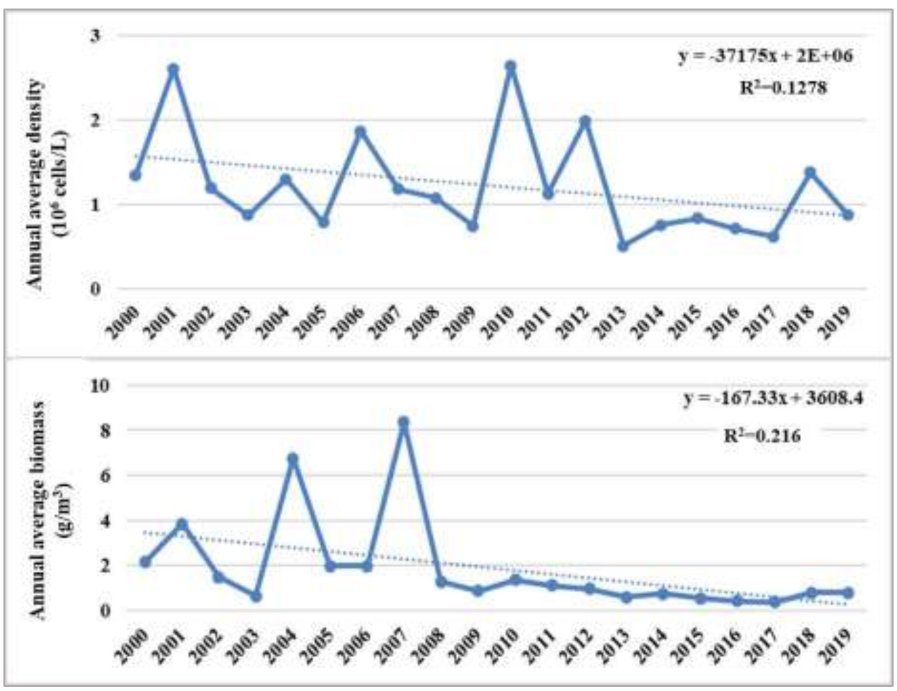

Fig. 53. The multiannual evolution of phytoplankton average density and biomass in the shallow waters of Mamaia Bay

The analysis of the **phytoplankton taxonomic structure** from the shallow waters of Mamaia Bay showed that, in the last two decades, **diatoms** accounted for 65% and 85% of the average density recorded in each period. They were followed by **cyanobacteria** whose contribution was 13% in the first analysed decade, slightly decreasing in the following period (9%). **Dinoflagellates** ranked third with 12% and 3%, respectively.

In the last 10 years, there has been an **increase** in the ***Cryptophyceae*** contribution (3%, with average annual densities between $13.55 \cdot 10^3$ cells/L and $68.83 \cdot 10^3$ cells/L) compared to the period 2000-2009 (1%, with average annual densities of up to $25.89 \cdot 10^3$ cells/L) and a **decrease** in the ***Euglenoidea*** (from 4% to 1%) and ***Chlorophyceae*** (from 2% to 1%) (**Fig. 58**). The contribution of the other classes (*Chlorodendrophyceae, Chrysophyceae, Ebriophyceae, Klebsormidiophyceae, Prasinophyceae, Pyramimonadophyceae, Ulvophyceae* and *Xantophyceae*) was below 1% (0.24% and 0.63%, respectively) in both periods (**Fig. 58**).

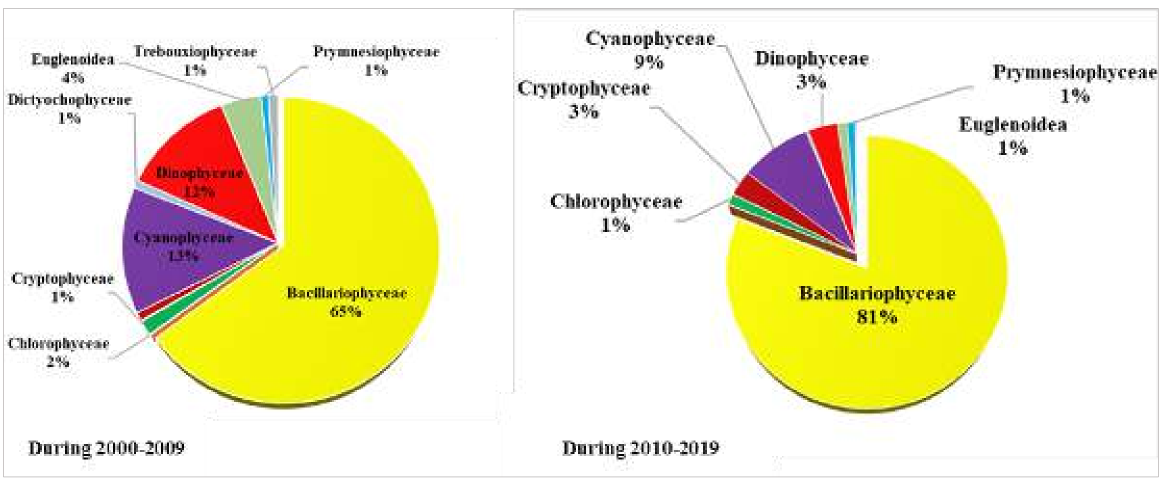

Fig. 58. Phytoplankton taxonomic composition according to the average density recorded in the shallow waters of Mamaia Bay during the last two decades

In the first analysed period, based on the average biomass, the phytoplankton was dominated by **dinoflagellates** which represented **61%,** with annual averages between 0.16 g/m^3 (in 2009) and 6.70 g/m^3 (in 2007), which **decreased in the next period to 35%**, with annual averages between 0.13 g/m^3 (in 2017) and 0.58 g/m^3 (in 2011). **Diatoms** ranked **second** most important group **during 2000-2009**, representing 32% of the total average biomass, recording average annual values between 0.22 g/m^3 (in 2003) and 2.51 g/m^3 (in 2001). However, during **2010-2019**, the phytoplankton structure changed, the **diatoms** being dominant (55% of the total), with average annual values between 0.20 g/m^3 (in 2017) and 1.11 g/m^3 (in 2010) (**Fig. 60**).

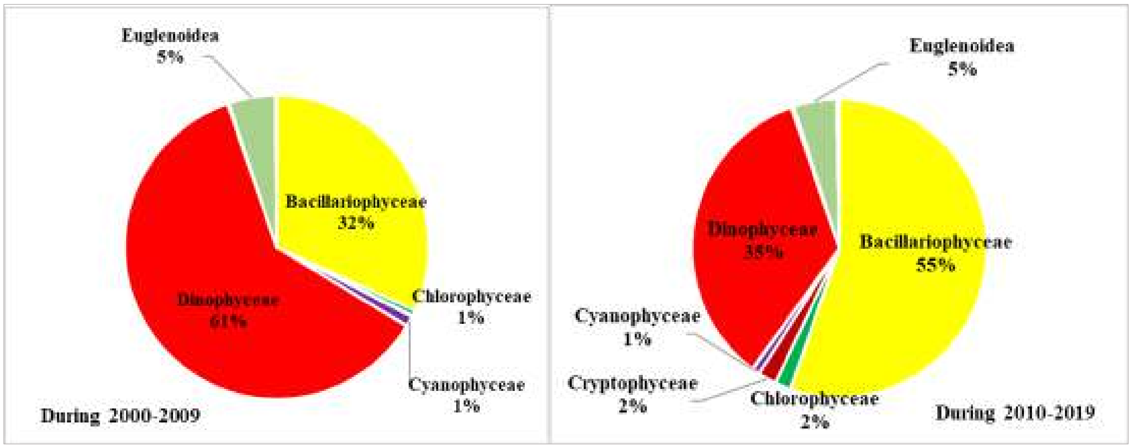

Fig. 60. Phytoplankton taxonomic composition according to the average biomass recorded in the shallow waters of Mamaia Bay during the last two decades

5.2.4. Phytoplankton seasonal structure – SIMPER analysis

The SIMPER analysis in PRIMER 7 (Clarke *et al.*, 2014) was used for the comparative analysis of the phytoplankton seasonal structure in the two periods. The SIMPER analysis is calculated from the Bray-Curtiss dissimilarity matrix, showing the contribution of each class/species in descendant order and their cumulative contribution to the differences for each data set. The SIMPER analysis first classifies the contributions of the species/classes to the average similarity of the samples from each period, then to the average difference between the two periods. The values of the percentage differences between periods vary between 0 and 100, 100 being the maximum difference.

5.2.4.1. The phytoplankton communities' structure during the last two decades spring

Thus, based on the **average monthly density** for each class in the spring season phytoplankton composition, the average difference resulted from SIMPER analysis between the two periods was 46.80%. The main class that determined this difference was ***Bacillaryophyceae***, contributing to the difference between the two periods with 74.6%, followed by ***Dinophyceae*** (with 13.9%) and ***Cyanophyceae*** (3.2%). The **diatoms** average density even more than **doubled** during 2010-2019, compared to 2000-2009 period. This fact was mainly due to the diatom

Skeletonema costatum maximum development during March-April, which coincided with a doubling of the N/P ratio, from 18 (in 2000-2009) to 36. **Cyanobacteria** slightly increased and the **dinoflagellates** average density (the most important being *Gymnodinium aureolum*, *Kryptoperidinium triquetrum, Peridinium* cysts) reduced during 2010-2019 (more than 10 times) compared to the period 2000-2009. Thus, the **limiting phosphorus conditions** (N/P = 36) and a **slight increase in silicates** in the second period, limited the dinoflagellates development and favoured the diatom ***Skeletonema costatum*** bloom.

An even higher difference between the two periods resulted from the SIMPER analysis based on the **average monthly biomass (63.77%).** This situation was mainly due to ***Dinophyceae*** class (78%) which, during 2000-2009, were represented **by higher volume species** (*Gymnodinium aureolum*, *Kryptoperidinium triquetrum, Peridinium* cysts), followed by ***Bacillariophyceae*** (*Skeletonema costatum, Cerataulina pelagica, Cyclotella caspia, Lauderia confervacea*), cumulating 95.3%. In the next period (2010-2019), there was a significant **reduction** of the dinoflagellates' average biomass and a slight increase in the diatoms average biomass compared to the values from 2000-2009, which are closely related to the density variations presented above.

5.2.4.2. The phytoplankton communities' structure during the last two decades summer

Based on the **monthly average density** for each class in the summer season phytoplankton composition, the average difference resulted from SIMPER analysis between the two periods was 45.50%. The main class that determined this difference was ***Bacillaryophyceae***, contributing to the difference between the two periods with 59.69% (the most important species being *Nitzschia tenuirostris*, *Navicula* sp., *Chaetoceros socialis*, *Skeletonema costatum*), followed by ***Cyanophyceae*** with 14.75% (*Microcystis pulverea*), ***Dinophyceae*** with 9.74% (*Scrippsiella acuminata*) and ***Euglenoidea*** with 7.33% (*Eutreptia lanowii*).

The average densities and biomasses recorded during **2010-2019** by each class were two to three times **lower** than the values in the period 2000-2009. These differences may be due to the increase in the summer of the monthly average temperature by 1 - 2°C, slightly higher pH values (up to 0.19 units in June and August), higher phosphate values (with 0.25-0.34 units in June and July), higher silicates (with 1-3 units) and higher dissolved inorganic nitrogen (with 1-

5 units). Also, the N/P ratio was lower in the second period (17-23), but closer to the Redfield ratio (16), considered optimal.

A lower difference between the two periods resulted from the SIMPER analysis based on the **monthly average biomass (37,81%)**. The contributing classes which made up to 94% of the difference between the two periods were ***Bacillariophyceae***, with 48.51% (represented by *Leptocylindrus danicus*, *Cerataulina. bergonii*, *Tabellaria, Navicula* sp., *Nitzschia. tenuirostris*), ***Dinophyceae***, with 29.13% (*Scrippsiella acuminata*, *Akashiwo sanguinea, Peridinium* (chiști), *Prorocentrum micans*, *P. cordatum*, *Protoperidinium granii*) and ***Euglenoidea***, with 16.7% (*Eutreptia lanowii*).

5.2.4.3. The phytoplankton communities' structure during the last two decades autumn

Based on the **monthly average density** for each class in the autumn season phytoplankton's composition, the average difference resulted from SIMPER analysis between the two periods was 47.56%. **Diatoms** contributed over 50% to this difference. *Cerataulina bergonii,* the dominant species in the first period analysed, was replaced by species such as *Nitzschia delicatisima*, *Skeletonema costatum*, *Chaetoceros socialis* and *Leptocylindrus minimus*. Other groups that participated in the differences between the two decades were **cyanobacteria** (with 18.85%) by reducing the average densities recorded ($40\text{-}70 \cdot 10^3$ cells/L to $0.3 \cdot 10^3$ cells/L (*Aphanizomenon flosaquae* and *Microcystis aeruginosa* cases). Among **dinoflagellates**, there was a densities reduction for *Peridinium* (cysts), which also had an impact on biomass (difference of 69.36%), given that the species *Cerataulina pelagica* and *Peridinium* (cysts) are microphytoplanktonic species, with a much larger biovolume than the other dominant diatoms from 2010-2019 period.

These differences could be determined by the **N/P ratio**, which in 2010-2019 October and November reached the value considered optimal according to Redfield, while in 2000-2009 were nitrogen limiting conditions (11-14). One factor that could limit the cyanobacteria development is **salinity**, which increased by about one unit during 2010-2019.

5.2.4.4. The phytoplankton communities' structure during the last two decades winter

Based on the **monthly average density** for each class in the winter season phytoplankton's composition, the average difference resulted from SIMPER analysis between the two periods was 46.62%. **Diatoms** (especially *Skeletonema costatum*) contributed with

68.43% to this difference, followed by cyanobacteria (*Pseudanabaena limnetica*, *Aphanizomenon flosaquae*) with 19.39%. The average diatoms and cyanobacteria densities recorded during 2010-2019 were slightly lower than the 2000-2009 values. These differences may be due to the silicate concentrations reduction by 2-4 units and more severe nitrogen limiting conditions in December and January.

A higher difference between the two periods resulted from the SIMPER analysis based on the **monthly average biomass (69.36%). Dinoflagellates**, especially *Akashiwo sanguinea*, *Peridinium* (cysts) and *Protoperidinium granii* contributed with 50.75% to this difference, followed by **diatoms** (*Skeletonema costatum*, *Thallassiosira nordenskioldii v. aestivalis*) with 25.94% and **euglenoids** (*Eutreptia lanowii* and *Euglena gracilis*) with 11.63%, their values decreasing during the 2010-2019 winter period.

5.2.5. Functional groups density and biomass evolution

The annual average values of autotrophic phytoplankton ranged from $131.28 \cdot 10^3$ cells/L (in 2013) and $2.55 \cdot 10^6$ cells/L (in 2010) and 0.20 g/m^3 (in 2017) and 3.23 g/m^3 (in 2001), registering in five years values of over 10^6 cells/L. The heterotrophic phytoplankton recorded lower values in terms of density, up to $16.04 \cdot 10^6$ cells/L, but much higher in terms of biomass (up to 534 g/m^3), the maximums being recorded in November 2004. Heterotrophs showed higher oscillations during 2000-2007 period, with values between $92.31 \cdot 10^3$ cells/L (in 2003) and $577.44 \cdot 10^3$ cells/L (in 2007) and 0.40 g/m^3 (in 2006) and 9.99 g/m^3 (in 2004). Between 2008-2019, the heterotrophs average annual values decreased below $150 \cdot 10^3$ cells/L and 1 g/m^3 (**Fig. 66**).

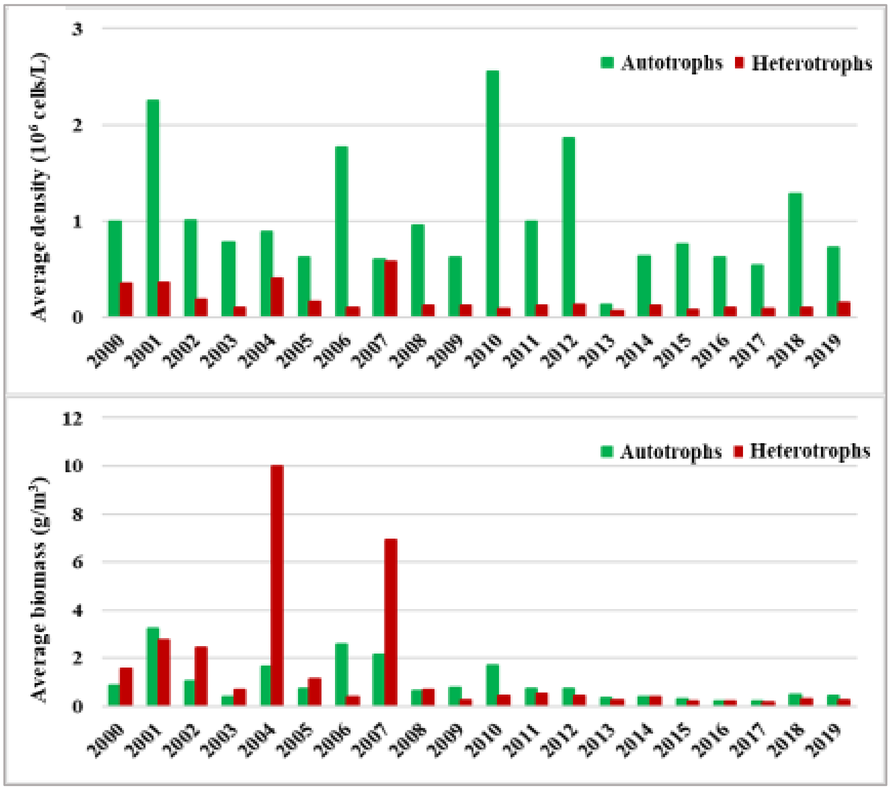

Fig. 66. Multiannual evolution of average density and biomass of phytoplankton functional groups in the shallow waters of Mamaia Bay

The **autotroph's dominance** was also reflected in terms of average monthly **densities recorded in both periods**, a characteristic situation for the studied area, considering the lighting conditions. There has been an **increase in the heterotroph's diversity in the last 10 years** compared to the previous decade, the maximum number being recorded in summer, an increase due to the reduction of phytoplankton quantities compared to 2000-2009 period.

The **autotrophs to heterotrophs ratio** based on the phytoplankton seasonal average **biomass** (**Fig. 69**) showed **major changes for the last 10 years** compared to 2000-2009 period in which dinoflagellates were dominant in each season. Thus, the **autotrophic phytoplankton dominance during 2010-2019 spring, autumn and winter** is an important step towards a more balanced and productive ecosystem due to the higher ratio of prey than predators.

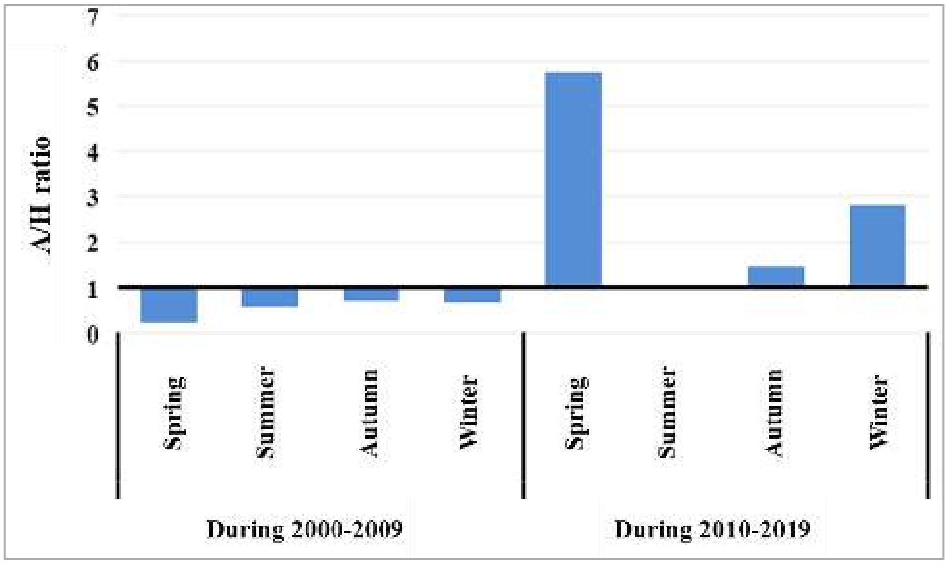

Fig. 69. The autotrophs to heterotrophs ratio based on the phytoplankton seasonal average biomass in the shallow waters of Mamaia Bay during the last two decades

5.2.6. Nano and micro-phytoplankton density and biomass evolution

In the period 2000 to 2019, the **nanophytoplankton average annual values** ranged between $90.36 \cdot 10^3$ cells/L (in 2013) and $2.24 \cdot 10^6$ cells/L (in 2010) and 0.10 g/m^3 (in 2009) and 0.75 g/m^3 (in 2010), recording **values of over 10^6 cells/L in five years**. The **microphytoplankton** average annual density **exceeded 10^6 cells/L only in 2001**, the values being **below $500 \cdot 10^3$ cells/L**, in the period 2010 to 2019. The microphytoplankton average annual biomass was between 0.24 g/m^3 and 114.60 g/m^3, showing higher variations during 2000-2007 period (**Fig. 70**).

Although the **microphytoplankton diversity was higher** (57% of the total), it was observed, in terms of **density,** the **microphytoplankton replacement with nanophytoplankton, being a worldwide problem due to rising temperatures.** However, in terms of **biomass**, although much **lower compared to 2000-2009**, the **microphytoplankton** contribution was **below 50% only in February, March and April**, when the **nanophytoplankton abundance was high enough to compensate for the biovolume difference between the two size classes**. The different results obtained depending on the density and biomass are due to the nanophytoplankton nutrient absorption higher efficiency, leading to a faster multiplication.

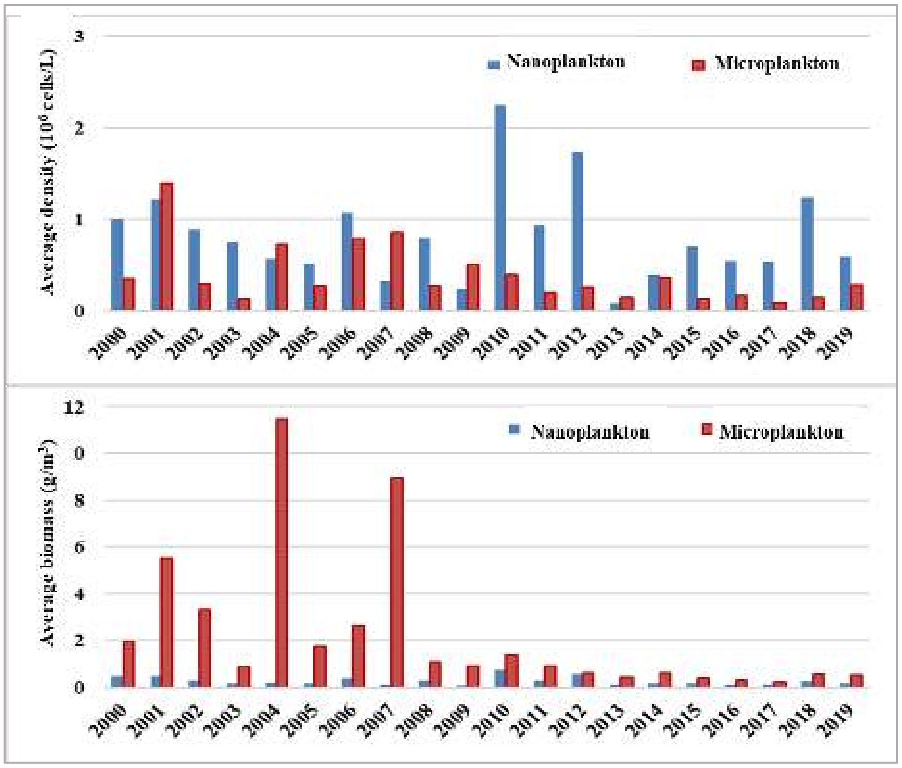

Fig. 70. Multiannual evolution of nano and microphytoplankton average density and biomass in the shallow waters of Mamaia Bay during the last two decades

5.2.7. Ecological groups density and biomass evolution

From 2000 to 2019, the average annual values of marine-brackish microalgae (MS) in the shallow waters of Mamaia Bay, ranged between $137.30 \cdot 10^3$ cells/L (in 2013) and $2.54 \cdot 10^6$ cells/L (in 2001) and 0.35 g/m^3 (in 2017) and 11.63 g/m^3 (in 2004). The MS category recorded values of over 10^6 cells/L in eight years, especially during 2000 to 2012, after which the average annual values fell below this bloom value. Regarding the freshwater species (DS), the annual average values were much lower, between $32.28 \cdot 10^3$ cells/L (in 2011) and $84.52 \cdot 10^3$ cells/L (in 2002) and 0.012 g/m^3 (in 2011) and 0.29 g/m^3 (in 2010) (**Fig. 73**).

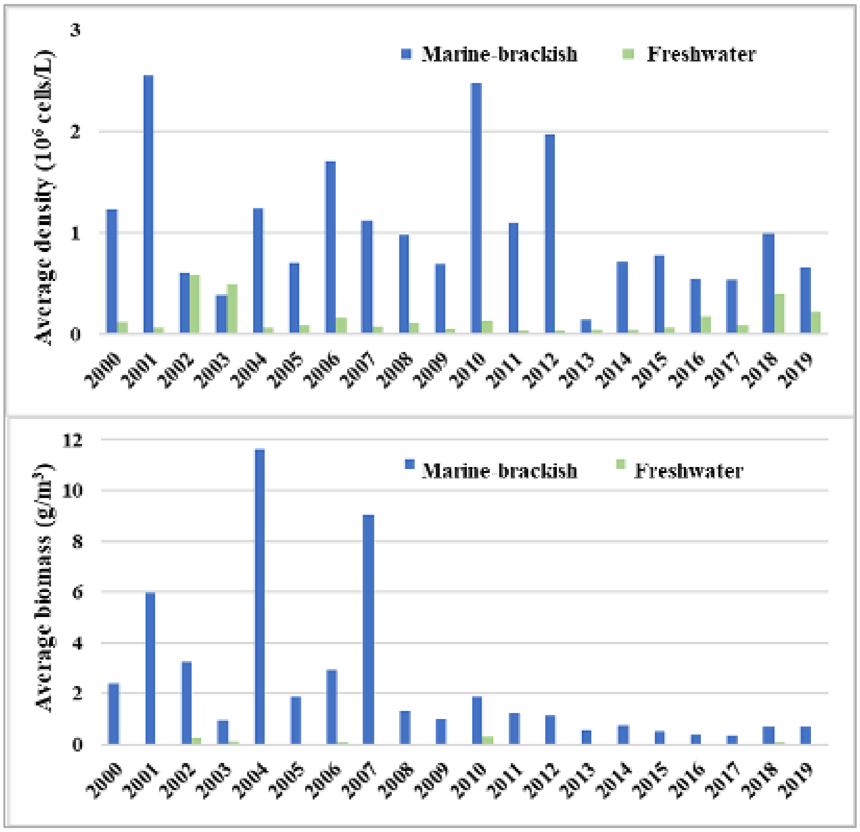

Fig. 73. Multiannual evolution of phytoplankton ecological groups average density and biomass in the shallow waters of Mamaia Bay during the last two decades

Thereby, the phytoplankton community in the shallow waters of Mamaia Bay was mainly represented in the last two decades by **marine-brackish microalgae** adapted to the average salinity conditions in the area and sporadically, there were blooms of some freshwater species, favoured by phosphorus limiting conditions and sometimes lower salinity (11-13 psu).

5.3. EXPERIMENTAL STUDIES

The analysis of phytoplankton from the shallow waters of Mamaia Bay highlighted that the **major changes of the last 20 years community structure were mainly caused by rising temperatures and N/P ratio variations**. For a more precise interpretation of the field data analysis results, we tested these hypotheses in **laboratory-controlled conditions**. The testing species was the diatom, *Skeletonema costatum*, a characteristic and dominant species in the phytoplankton community, which was isolated from the shallow waters of Mamaia Bay, and which can serve as a benchmark for phytoplankton response to current climate change. In this subchapter I presented an experimental approach regarding the **temperature and nutrient variations** (nitrogen and phosphorus) influence on the diatom *Skeletonema costatum* development and, the **exploitation potential** of this species.

Approaching the *Skeletonema costatum* ecophysiological aspects, studied both in the natural environment and in laboratory experiments highlighted that this species, responsible for the most intense and longest annual process of phytoplankton bloom, especially in the last 10 years, has an impressive tolerance for different nutrient ratios (at both limits) and large temperature ranges. In 1987, Dorgham *et al*. considered *Skeletonema costatum* as a eutrophication indicator in the coastal waters of Alexandria, noting that other authors such as Smayda (1965), Purcher-Petrovic and Marasovic (1980), Mihnea (1985) and Revelante and Gilmartin (1985) supported this information. Other known eutrophication indicator species are *Nitzschia closterium, Cerataulina pelagica* și *Prorocentrum micans* (Dorgham *et al*., 1987).

Thereby, I consider that *S. costatum* is an **opportunistic species**, with a high potential to cause **harmful blooms** worldwide. It is important to note that this species blooms have often led to a lower phytoplankton diversity, fish mortality and water column nutrients ratio imbalances. Observing the phytoplankton communities' natural variability in time and space requires an adequate monitoring frequency throughout the Black Sea basin to gain knowledge about the bloom's **triggers** (Moncheva *et al*., 2019).

Although in the natural environment the microalgae rapid multiplication ability can have multiple effects on other trophic levels (Bodeanu, 2002), in biotechnologies, this capacity is explored as a renewable resource and for the by-product's recovery. As a renewable resource, we addressed this issue through a theoretical and experimental study to produce biodiesel from *S. costatum* biomass (Culcea *et al*., 2018). This study was the first attempt to obtain biodiesel from the biomass of a common Black Sea microalgae, isolated from the shallow waters of Mamaia Bay. Thus, the diatom *S. costatum* can be considered a species with potential in biodiesel production due to the rapid development cycle, the growth conditions that are easy to maintain both in the laboratory and in special systems and the high concentration of lipids in dry biomass (12-51%) (Culcea *et al*., 2018).

Microalgae have become some of the most promising and innovative food and pharmaceuticals sources due to bioactive compounds such as vitamins, essential amino acids, polyunsaturated fatty acids, minerals, carotenoids, enzymes and fibers (Matos, 2017). Several pharmacological research studies have shown that oxidative stress and free radicals increased amounts are characteristic of chronic diseases, including cancer, aging and neurodegenerative diseases such as Alzheimer's and Parkinson's and cardiovascular diseases such as atherosclerosis.

Phenolic compounds are secondary metabolites widely distributed in plants with the potential to prevent the occurrence of many degenerative diseases. These compounds are described as radical scavengers, having the ability to prevent various processes of oxidative stress (Jerez-Martel *et al*., 2017). Phenolic compounds have been identified in the biomass of several species of chlorophytes (*Ankistrodesmus* sp., *Spirogyra* sp.), euglenoids (*Euglena cantabrica*) and cyanobacteria (*Nostoc commune*) (Jerez-Martel *et al*., 2017). There are studies that show higher polyphenols concentrations in microalgae (*Chlorella* sp., *Phaeodactylum tricornutum*) grown under stress conditions (low nitrogen concentrations, higher concentrations of iron and copper (Aremu *et al*., 2016, Rico *et al*., 2013).

In the present study, the tannic acid content (a type of polyphenol) determined by the HPLC method in the methanolic extract of *Skeletonema costatum* (**Fig. 85**) was 0.46% (0.46 mg tannic acid/g biomass). Although we obtained a low concentration compared to other available results *(Euglena tuba* - 5.6%, Chaudhuri *et al*., 2014) the tannic acid presence in the methanolic extract justifies further research to highlight *Skeletonema costatum* antioxidant and antimicrobial activity, but also to improve the working method on the biomass harvesting timing, the species exposure to conditions that determine a higher polyphenols concentration, but also to obtain results comparable to other studies. The chromatogram shows the presence of other separate but unidentified organic compounds, which may contribute to the methanolic extract therapeutic potential.

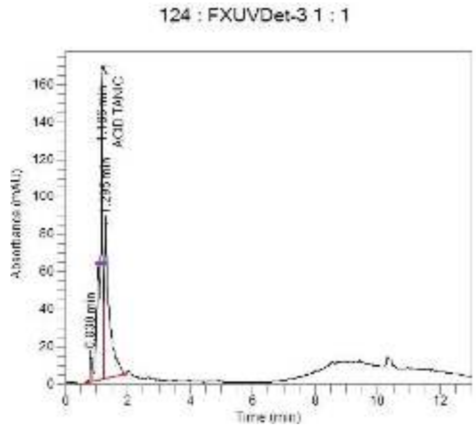

Fig. 85. *Skeletonema costatum* methanolic extract chromatogram
(by Mrs. Associate Professor Dr. Laura Bucur)

According to ISO 10253:2016 *S. costatum* can be used as **biological material** to determine its growth inhibition due to chemicals and mixtures of chemicals. I had the opportunity to participate and improve this type of study within NIMRD "Grigore Antipa" private contracts, an activity which started in 2014. These studies provide data on the potential accidents' prevention during oil exploration and exploitation activities in the Black Sea and for establishing measures for marine ecosystem protection.

VI. CONCLUSIONS

The analysis and interpretation of 1797 phytoplankton samples collected from the shallow waters of Mamaia Bay in the last two decades and the experiments in laboratory-controlled conditions led to the following results:

- 352 species, varieties and forms of microalgae belonging to 17 taxonomic classes have been identified in the shallow waters of the Mamaia Bay during the last two decades. The diatoms were dominant, followed by dinoflagellates, chlorophytes and cyanobacteria, a constant situation for the two decades.
- Although there have been fluctuations from one year to another, the **general trend in phytoplankton diversity** over the last 20 years has been **positive**, which may be due to the lower phytoplankton quantities, the sampling frequency, the more performant microscopes and intercalibration exercises with Black Sea countries experts.
- Seasonally, the **phytoplankton diversity** was **higher during summer in both decades**. This coincided with the highest sampling frequency and the 24% increase in dinoflagellates identified in the last 10 years compared to the previous decade.
- The **phytoplankton quantitative decreasing trend** observed by other authors since 1993 continued in the last twenty years, the period **2010-2019 being the most obvious**.
- Regarding functional groups, the **autotrophic** species were **dominant in diversity and quantities** in both periods, a characteristic situation for the studied area, considering the lighting conditions. There has been an increase in the heterotroph's diversity in the last 10 years compared to the previous decade, the maximum number being recorded in summer, which could relate with the phytoplankton decreased quantities compared to 2000-2009 period.
- The **autotrophs to heterotrophs ratio** based on the phytoplankton seasonal average **biomass** showed **major changes for the last 10 years** compared to 2000-2009 period in which dinoflagellates were dominant in each season. Thus, the **autotrophic phytoplankton dominance during 2010-2019 spring, autumn and winter** is an important step towards a more balanced and productive ecosystem due to the higher ratio of prey than predators.
- Although the **microphytoplankton diversity was higher** (57% of the total), it was observed, in terms of **density,** the **microphytoplankton replacement with nanophytoplankton, being a worldwide problem due to rising temperatures.** However, in terms of **biomass**, although much **lower compared to 2000-2009**, the **microphytoplankton** contribution was **below 50% only in February, March and April**, when the **nanophytoplankton abundance was high enough to compensate for the biovolume difference between the two size classes**. The different results obtained depending on the density and biomass are due to the nanophytoplankton nutrient absorption higher efficiency, leading to a faster multiplication.
- Regarding the ecological groups, the phytoplankton community in the shallow waters of Mamaia Bay was mainly represented in the last two decades by **marine-brackish**

microalgae adapted to the average salinity conditions in the area and sporadically, there were blooms of some freshwater species, favoured by phosphorus limiting conditions and sometimes lower salinity (11-13 psu).

- Changes in the **spring** season of the last 10 years, such as **phosphorus limiting conditions** (N/P=36) and a slight increase in **silicates limited the dinoflagellates development and favoured the diatoms bloom** (especially *S. costatum*).
- The **summer** monthly average temperature increase by 1 - 2°C, slightly higher pH values, higher phosphate values, higher silicates and higher dissolved inorganic nitrogen along with a lower N/P ratio in the last 10 years, but closer to the Redfield ratio, **led to 2-3 times lower average densities and biomasses than those recorded during 2000-2009**.
- The decrease of the phytoplankton community density and biomass was also observed in the last decade **in the autumn** season. This change took place under an **optimal N/P ratio** according to Redfield, while in the period 2000-2009 were nitrogen limiting conditions (11-14).
- The phytoplankton community density and biomass decrease in the **winter** season was due to **lower silicates concentrations** and the **more pronounced nitrogen limiting conditions** in the last 10 years compared to the previous decade.
- The analysis of phytoplankton from the shallow waters of Mamaia Bay highlighted that the **major changes of the last 20 years community structure were mainly caused by rising temperatures and N/P ratio variations**. For a more precise interpretation of the field data analysis results, we tested these hypotheses in **laboratory-controlled conditions**.
- The exposure of *Skeletonema costatum* to **temperature variations** highlighted a high tolerance for 8°C to 20°C and a preference for the 18 - 19°C range compared to lower temperatures of 8°C to 19°C.
- The exposure of *Skeletonema costatum* to **different nitrogen to phosphorus ratios** also showed a **high tolerance of the species**, obtaining the growth curves and high growth rates in all tested conditions. A significant difference was observed in **phosphorus-limiting conditions**, which, in this case as well, **stimulated** a high development of the diatom compared to the nitrogen-limiting or optimal conditions. These results highlight the risk of unbalanced nutrient ratios on *Skeletonema costatum* development, being a blooms key factor.
- Approaching ***Skeletonema costatum*** **ecophysiological response both in the natural environment and in laboratory experiments** I consider that, besides a eutrophication indicator species, *Skeletonema costatum* can also be considered an **opportunistic species**, with a high potential to cause marine ecosystem imbalances.
- The experiment's results demonstrated *Skeletonema costatum* **potential for biotechnological application** (feedstock for **biodiesel** production) and for **cosmetics** and **pharmaceutical** industry (the presence of **tannic acid** in the methanolic extract). These were the first attempts to use the microalgal biomass of a species isolated from the Romanian Black Sea coastal waters, in laboratory-controlled conditions.

In general, we can conclude that the major changes in the phytoplankton's community structure over the last 20 years have been mainly caused by the temperature increase and variations in the N/P ratio. The increase in diversity and the quantitative phytoplankton decrease in the Romanian Black Sea coastal waters in the last 10 years are clear evidence of the efforts made to limit eutrophication since Romania's accession to the European Union (in 2007). Further phytoplankton research, the continuous improvement of methods and acquisition of datasets for evolutionary trends, taxonomic composition and spatial distribution using modern tools are key to the knowledge of the current state of the trophic pyramid base, which is also an important component for the marine ecosystem quality assessment in accordance with the requirements of European Union directives, such as the Marine Strategy Framework Directive (MSFD) and the Water Framework Directive (WFD).

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IX. SCIENTIFIC ACTIVITY DURING DOCTORAL STUDIES

A. PAPERS PUBLISHED IN ISI INDEXED JOURNALS WITH IMPACT FACTOR

1. First attempt to use the biomass of important Black Sea diatom for biodiesel production, **O. Culcea**, S. Nicolaev, D. M. Rosioru, M. M. Fagaras, L. Boicenco, Journal of Environmental Protection and Ecology 19, No 3, ISSN 1311-5065, 2018, **IF = 0,679**.

B. PAPERS PUBLISHED IN ISI INDEXED JOURNALS WITHOUT IMPACT FACTOR

1. Identification of the Romanian Black Sea water types - assessment related to the Marine Strategy Framework Directive implementation, 14th International Multidisciplinary Scientific GeoConference, M.E. Mihailov, S. Nicolaev, L. Buga, S. Jelescu, L. Boicenco, A.D. Spinu, L. Lazar, **O. Vlas,** C. Tabarcea, G. Ganea, SGEM 2014 Conference Proceedings, Book 3, Vol. 2, ISBN 978-619-7105-14-8 / ISSN 1314-2704, 2014.
2. Hyperspectral remote sensing for estimating coastal water quality: case study on coast of Black Sea, Romania, S. G. Ghezehegn, P. Steef, A. Hommersom, De Reus Nils, **O. Culcea**, B. Krommendijk, Proceedings Volume 9239, Remote Sensing for Agriculture, Ecosystems, and Hydrology XVI, 923913, 2014.

C. PAPERS PUBLISHED IN IDB INDEXED JOURNALS

1. Influence of temperature and nutrients on the growth rate of the marine diatom, *Skeletonema costatum* (Greville) Cleve 1873, **O. Vlas**, L. Lazăr, L. Boicenco, E. Pantea, M. Făgăraş, Cercetari marine vol. 50, ISSN 2734-438X ISSN-L 0250-3069, 2020.
2. Performing first integrative evaluation of the ecological status of Romanian Black Sea waters using nested environmental status assessment tool (NEAT), O. Marin, V. Abaza, E. Bișinicu, L. Boicenco, V. Coatu, M. Galaţchi, L. Lazăr, A. Oros, E. Pantea, C. Tabarcea, G. Ţiganov, **O. Vlas**. Cercetari marine vol. 50, ISSN 2734-438X ISSN-L 0250-3069, 2020.
3. Ecological status of Romanian Black Sea waters according to the planktonic communities, L. Boicenco, E. Bișinicu, **O**. **Vlas**, G-E. Harcotă, L. Lazăr, E. Pantea, C. Tabarcea, F. Timofte, "Cercetări Marine ", Issue no. 49, ISSN-L 0250-3069/ISSN 2734-438X, 2019.
4. Black Sea Eutrophication Status - The Integrated Assessment Limitations and Obstacles, L. Lazăr, L. Boicenco, O. Marin, **O. Culcea**, E. Pantea, E. Bișinicu, F. Timofte, V. Abaza, A. Spînu, Cercetări Marine, no. 49, ISSN 2734-438X ISSN-L 0250-3069, 2019.

D. PAPERS PUBLISHED IN National University Research Council (CNCSIS) RECOGNIZED PUBLISHERS:

1. Black Sea eutrophication dynamics from causes to effects (2012-2017), L Lazar, L Boicenco, O Marin, **O. Culcea**, V Abaza, EM Mihailov, Cercetari marine-Recherches marines, Volum nr. 48, ISSN 2734-438X ISSN-L 0250-3069, 2018, editura CD Press.
2. Isolation and Maintenance Methods for *Skeletonema costatum* in Laboratory Cultures, **O. Culcea**, Cercetări Marine, vol. 47, ISSN 2734-438X ISSN-L 0250-3069, 2017.
3. Annual report on the state of the environment in Romania in 2015, L. Alexandrov, E. Bișinicu, L. Boicenco, V. Coatu, **O**. **Culcea**, D. Diaconeasa, C. Dumitrache, A. Filimon, Golumbeanu M., Lazăr L., Malciu V., Marin O., Mateescu R., Maximov V., Micu D., E. Mihailov, M. Nenciu, S. Nicolaev, V. Niță, A. Oros, E. Pantea, V. Pătrașcu, A. Spinu, Stoica E., F. Timofte, T. Zaharia, Cercetări Marine, no. 46 bis, ISSN 2734-438X ISSN-L 0250-3069, 2016.
4. Report on the state of the marine and coastal environment in 2014, L. Alexandrov, E. Bișinicu, L. Boicenco, V. Coatu, **O. Culcea**, D. Diaconeasa, C. Dumitrache, A. Filimon, M. Golumbeanu,

L. Lazăr, V. Malciu, O. Marin, R. Mateescu, V. Maximov, D. Micu, E. Mihailov, M. Nenciu, S. Nicolaev, V. Niță, A. Oros, V. Pătrașcu, A. Spînu, E. Stoica, F. Timofte, D. Țigănuș, T. Zaharia, Cercetări Marine, no. 45, ISSN 2734-438X ISSN-L 0250-3069, 2015.

E. PUBLISHED BOOKS, MONOGRAPHS:

1. BSC, 2019. State of the Environment of the Black Sea (2009-2014/5), Contribuții la capitolul „The State and Dynamics of the Biological Community – Phytoplankton", S. Moncheva, L. Boicenco, A. S. Mikaelyan, A. Zotov, N. Dereziuk, C. Gvarishvili, N. Slabakova, R. Mavrodieva, **O. Vlas**, L. A. Pautova, V. A. Silkin, V. Medinets, F. Sahin, A. M. Feyzioglu. Publications of the Commission on the Protection of the Black Sea Against Pollution (BSC), Istanbul, Turkey, 811 pp., ISBN 978-605-84837-0-5, 2019.
2. State of Environment Report of the Western Black Sea based on Joint MISIS cruise (SoE-WBS), Contribuții la capitolul II. 1. Phytoplankton in MISIS Joint Cruise Scientific Report, S. Moncheva, L. Boicenco, F. Sahin, D. Ediger, **O. Culcea**, R. Mavrodieva, N. Slabakova, V. Doncheva, 2014, Ed. ExPonto, 401 pp, ISBN: 978-606-598-367-0.
3. Report on the MISIS cruise Intercalibration Exercise: Phytoplankton, S. Moncheva, V. Doncheva, L. Boicenco, F. Sahin, N. Slabakova, **O. Culcea**, 2014, 44 pp. ISBN: 978-606-598-359-5.

F. SELECTION OF PAPERS SUBMITTED TO INTERNATIONAL SCIENTIFIC EVENTS AND PUBLISHED AS ABSTRACT

1. **O. Culcea**, S. Nicolaev, D. M. Rosioru, M. M. Fagaras, L. Boicenco, 2018. Biodiesel production using the biomass of the microalgae *Skeletonema costatum* grown in laboratory cultures - Book of abstracts, Water Across Time in Engineering Research, 21 June 2018, Faculty Of Civil Engineering, Ovidius University of Constanta, Editura Ex Ponto, ISBN 978-606-598-663-3, pp. 109.
2. **O. Culcea**, L.Boicenco, L. Lazăr, E. Pantea, 2018. Algal blooms in the Romanian Black Sea waters in the begining of the 21st Century in: Deltas and Wetlands (Book of Abstract), vol. 5, 10 pp, Tulcea, Romania. ISSN 2344-3766.
3. L. Boicenco, **O. Vlas**, L. Lazăr, 2013. Spring Season Phytoplankton Communities in Romanian Black Sea Waters. 4th bi-annual Black Sea scientific conference "Black Sea - challenges towards good environmental status", 2013 Abstracts Book ISBN 978-606-8066-46-2.

G. SCIENTIFIC CONFERENCES PARTICIPATIONS

Abroad:

1. **O. Culcea**, M. Făgăraş, L. Boicenco, E. D. Pantea & L. Lazăr: Key Species That Produced Phytoplankton Blooms in The Romanian Black Sea Waters during 2001-2017. 7th Balkan Botanical Congress – 7BBC, 10-14 September 2018, Novi Sad, Serbia. **Poster**
2. M. Făgăraş & **O. Culcea**: The Danube Delta Biosphere Reserve And Its Importance In The Preservation Of The Coastal Habitats.– 7th Balkan Botanical Congress – 7BBC, 10-14 September 2018, Novi Sad, Serbia. **Poster**.

Local:

1. **O. Culcea**, L.Boicenco, L. Lazăr, E. Pantea. Algal blooms in the Romanian Black Sea waters in the begining of the 21st Century. Symposium "Deltas & Wetlands", 16-20 May 2018, Tulcea, Romania. **Poster**.
2. **O. Culcea**, S. Nicolaev, D. M. Rosioru, M. M. Fagaras, L. Boicenco. Biodiesel production using the biomass of the microalgae *Skeletonema costatum* grown in laboratory cultures. Water Across Time in Engineering Research 21 June 2018, FACULTY OF CIVIL ENGINEERING, Ovidius University of Constanta. **Poster**.
3. L. Boicenco, E. Pantea, L. Lazar and **O. Culcea**. Interannual variations of chlorophyll a concentration in the Romanian Black Sea waters. INTERNATIONAL U.A.B. - B.EN.A.

CONFERENCE ENVIRONMENTAL ENGINEERING AND SUSTAINABLE DEVELOPMENT, MAY 25-27th, 2017, Alba Iulia, România. **Poster**.

4. **O. Culcea** - Isolation and Maintenance Methods for *Skeletonema costatum* in Laboratory Cultures. International Symposium: Protection Of The Black Sea Ecosystem And Sustainable Management Of Maritime Activities PROMARE 2017, 8th Edition, 7-9 September 2017, Constanța, România. **Oral presentation.**
5. L. Boicenco, L. Lazăr, **O. Culcea**, A.-D. Spînu - Phytoplankton quality assessment in the Romanian coastal and transitional areas according to the Water Framework Directive – International Symposium "Protection of the Black Sea Ecosystem and Sustainable Management of Maritime Activities" 7th Edition, PROMARE 2015, Constanta. **Poster**.
6. L. Boicenco, **O. Vlas**, L. Lazăr (2013), Spring Season Phytoplankton Communities in Romanian Black Sea Waters4th BI-ANNUAL BLACK SEA SCIENTIFIC CONFERENCE "BLACK SEA - CHALLENGES TOWARDS GOOD ENVIRONMENTAL STATUS". **Poster**.

H. AWARDS

I. 1st Prize for the best poster presentation at the 4th-Bi-annual Black Sea Scientific Conference, "Black Sea- Challenges towards good environment status", Constanța, Romania, 28-31 October 2013 of the paper "Spring season phytoplankton communities in Romanian Black Sea waters", authors: Laura Boicenco, **Oana Vlas** and Luminita Lazar.

J. 3rd Prize for the best oral presentation at the International Symposium „Protection of the Black Sea ecosystem and sustainable management of maritime activities, 8th Edition, Promare 2017, 7-9 September 2017, Constanța, România of the paper „Isolation and maintenance methods for *Skeletonema costatum* in laboratory cultures", author: **Oana Culcea**.

K. SELECTION OF PARTICIPATIONS IN RELEVANT PROJECTS

1. Updating the integrated monitoring program of the Black Sea marine ecosystem according to the requirements of art. 11 of the Marine Strategy Framework Directive (2008/56 / EC), Contract 28 / 27.12.2013 (11.2013 - 12. 2013) - participation in field activity, collection and analysis of phytoplankton samples, spectrophotometric determination of chlorophyll a, update the scientific names and biovolume of the species.
2. MSFD (Marine Strategy Framework Directive) GUIDING IMPROVEMENT IN THE BLACK SEA INTEGRATED MONITORING SYSTEM (MISIS) – involved in the collection and analysis of phytoplankton samples, updating the list of species and the biovolume of species. Participation in a Black Sea international expedition and in an intercalibration exercise with other phytoplankton specialists. Phytoplankton sampling and analysis, data processing and interpretation and reporting.
3. DG MARE: EMODNet Biology – Operation, development and maintenance of a European Marine Observation and Data Network, contract nr. 2019340/19/04/2017, 2017-2019.
4. Wastewater toxicity study (PROWATOX) - Act adițional 02 din 25.01.2019 la Contract 72/04.07.2018, Beneficiar: Halcrow Romania S.R.L. (JACOBS Ltd.), Client Final: EXXONMOBIL EXPLORATION AND PRODUCTION ROMANIA LIMITED NASSAU (BAHAMAS) SUCURSALA BUCURESTI.
5. PN 45N/2019. 19260202 Ecological, ecophysiological and biotechnological research in Romanian marine ecosystems. Responsible Phase 4: Identifying patterns of microalgae development in the natural environment.
6. PN 45N/2019. 19260202 Ecological, ecophysiological and biotechnological research in Romanian marine ecosystems. Responsible Phase 5: Experimental testing, under laboratory-controlled conditions, of hypotheses regarding the development of the species *Skeletonema costatum*.

L. CONTRIBUTIONS TO SCIENTIFIC RESEARCH

1. Introduction of top methods-methodologies in research by creating a methodology for the use of dispersants in the Black Sea, which could form the basis for a legislative framework elaboration on the use of dispersants in the Black Sea, ROVOCON13-011 / 28.02.2013- member of the project team for conducting experiments to observe the dispersants effect on the species *Skeletonema costatum*, from monospecific cultures grown in the laboratory.
2. Special contributions in applied research: Products and technologies introduced in the economic field of applicability: continuous culture of the microalgae *Skeletonema costatum* as biological material for toxicity tests and experiments, since 2013.