### Phytoplankton quantitative development and species diversity in the Bulgarian Black Sea waters during 2014-2017

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#### Abstract

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The aim of this study is to analyse the characteristics in the development of the phytoplankton communities in the Bulgarian part of Black Sea and in Varna Lake in present days, to trace the occurring changes and to assess their ecological status. During the study period (2014-2017), 196 phytoplankton samples were collected from 52 stations by bathometer type Niskin-5L. The samples were prepared for analysis (fixed and concentrated) and analyzed by routine methods. The analysis of the qualitative and quantitative structure of phytoplankton in the monitored areas revealed the existence of 182 phytoplankton species belonging to 14 taxonomic classes. The share of *Dinophyceae/Bacillariophyceae* group in the phytoplankton composition was 72.53%. A total of 8 species in the coastal and shelf zones and 7 species in the water of Varna Lake were found in bloom-causing concentrations. The highest quantities of phytoplankton were produced during the winter-spring period, with a trend toward increasing the numbers and phytoplankton biomass from 2014 to 2017. The ecological assessment based on results for the phytoplankton determined the ecological status of coastal and shelf water as "*moderate*" and the ecological status of Varna Lake water as "*bad*".

*Keywords:* Black Sea; coast and shelf; Varna Lake; phytoplankton; phytoplankton structure; seasonal dynamics; ecological assessment

#### Introduction

Phytoplankton plays a key role in biogeochemical processes, the global carbon cycle and the transformation of energy along food chains in marine ecosystems. The status of marine phytoplankton affects the commercial fishing, human health (toxic species),  $CO_2$  uptake, and the reaction of the hydroecosystems to the climate change. To anthropogenic loading or climate change, the phytoplankton community responds by changing of the dominant complexes, species diversity, abundance and biomass quantity (Zaitzev, 1992; Moncheva et al., 2013; Petrova, 2014; Gerdzhikov, 2014; Petrova & Gerdzhikov, 2015). The phytoplankton structure and its biomass are the parameters involved in assessments and ecological categorization of marine waters at EU (Directive 2000/60/EC) and national (Ordinance No. H-4, 2012) levels. The Black Sea, defined as the most isolated sea in the world, is no exception of all these processes.

In the 1970s-1980s, the dramatic changes occurring in the Black Sea ecosystem led to a decrease in biodiversity. A natural response to the intensive eutrophication was the high phytoplankton biomasses and abundance, reaching bloomcausing concentrations and hypoxic situations near the bottom, as well as mass fish deaths (Petrova-Karadzhova, 1984; Nesterova, 1987; Velikova et al., 1999; Mee, 1992; Kamburska et al., 2003).

In the last 25 years, the monitoring analyses in different areas of the Black Sea have outlined a steady tendency towards ecosystem recovery (Bodeanu et al., 1998; Moncheva, et al., 2001; Mee et al., 2005; AAQQWBSB, 2005; Petrova, et al., 2006). This process, albeit slowly, occurs in the conditions of modified taxonomic structure of the phytoplankton and dynamics of the nutrients in the marine environment (Borysova et al., 2005; Moncheva et al., 2013; Klisarova et al., 2015).

The aim of this article is to analyse the characteristics of the development of the phytoplankton communities in the Bulgarian part of the Black Sea and in Varna Lake in present days, to trace the occurring changes and to assess their ecological status.

#### **Materials and Methods**

#### Study area

The study was carried out in Bulgarian Black Sea marine area during the period 2014-2017. The monitored water bodies included 52 stations as follows: • For phytoplankton analyses: Varna lake (A-22; I channel; Rowing base); Varna bay (B-1, B-3, B-5, B-7, B-10, B-6; I<sup>st</sup> offshore breakwater and south beach); Cape Galata (G-1, G-3 µ G-5 nautical miles); from station Mussel farm in front of Cape Ilandjik.

• In the case of trawling for assessment of turbot (Scophthalmus maximus) stocks in the shelf zone from Krapets village to Rezovska River: May 2016 - D12-28.03138/42.73305, D14-28.04179/42.56168, D9-28.07711/42.96836, E13-28.13521/42.64471, E17-28.16282/ 42.28221, E19-28.10092/42.15599, E8-28.10843/43.07593, F16-28.18097/42.35305, F18-28.178/42.19571, G6-28.27231/ 43.17465, J5-28.51612/43.27174, J7-28.55314/43.16045, N1-28.85227/43.65155 and May 2017 - C11-27.991158/42.760723, D12-28.026157/42.691906, D14-28.056860/ 42.523510, D16-28.008499/42.411263, D18-28.053497/42.211380, E17-28.151443/ 42.314110, F7-28.236782/43.099716, J6-28.429201/43.177231, L1-28.713987/43.654457, L3-28.730639/43.449490, M4-28.778536/43.368622 (Fig. 1).

#### Sampling, sample preparation and analyses

During the monitoring period, 196 phytoplankton samples were collected from all 52 stations. Phytoplankton samples were collected by bathometers type Niskin-5L at surface – bottom horizons in shallow waters; and from 0 m to 10 m in other areas. The samples were fixed onboard in 2% formalin solution and concentrated by the sedimentary method (Morozova-Vodyanitskaya, 1954). The qualitative and quan-

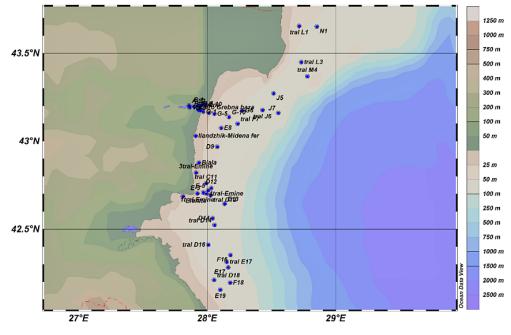


Fig. 1. Map at sampling stations, 2014-2017

titative analyses of the samples were performed with a light microscope OlympusBX41 in counting cells *Sedgewick Rafter* -1 ml and *Palmer-Maloney* -0.05 ml, using standard methods (Moncheva & Parr, 2010).

Measurements of chlorophyll-*a* (Turner Designs Fluorometer), transparency (Secchi disk) and physicochemical parameters of water (multiparametric device - Eutech -PCD650) were performed.

#### **Statistics**

The cell volume was calculated by geometric formulas (Edler, 1979; Olenina et al., 2006). Software Phytomar 2.0 (Institute of Fish Resources – Varna, 2008); Ocean Data View 4.7.4 and Excel 12 (Microsoft Office 2007) were used for calculations and graphs.

#### Ecological status assessment

The ecological status assessment of the water of the investigated marine areas was made according to a Classification system for ecological status of the specific *types of surface water*, category "Coastal marine waters" published in Ordinance No. H-4 (2012).

#### **Results and Discussion**

#### Qualitative composition of phytoplankton

During the 4 years surveyed, growth of a total of 182 phytoplankton species and forms of 14 taxonomic classes was recorded in the Bulgarian marine aquatory. The highest percentage of the taxonomic composition was occupied by class *Dinophyceae* 43.41%, followed by the classes *Bacillariophyceae* 29.12% and *Chlorophyceae* 8.24%. The remaining microalgae classes did not exceed 3.85% (Fig. 2).

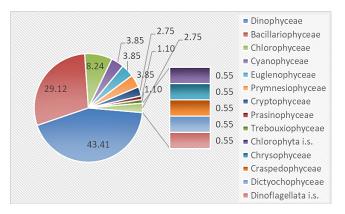


Fig. 2. Taxonomic composition of phytoplankton in the Bulgarian marine areas, 2014-2017

The taxonomic share of the classes of the group "Others" (classes *Chlorophyceae, Cyanophyceae, Euglenophyceae, Prymnesiophyceae, Cryptophyceae, Prasinophyceae, Trebouxiophyceae, Chlorophyta* i.s., *Chrysophyceae, Craspedophyceae, Dictyochophyceae*) decreased from 2014 to 2017, except for the class *Prymnesiophyceae*, whose share increased (Fig. 2 and Fig. 3).

To class *Prymnesiophyceae* were listed the following rare species: *Calciosolenia granii* Schiller, 1925 and *Acanthoica janchenii* Schiller, 1925. The *Dinophyceae/ Bacillariophyceae* group increased its share from 2014 to 2017 (from July to December) as the *Dinophyceae* class (Peridinae) prevailed in the total taxonomic composition (Fig. 3). *Dinophyceae* class was established with a high percentage of participation from April to December, and diatoms dominated in February and March. The share of the classes of the group "*Others*" was the highest in the first half of the year (from February to June), then it did not exceed 30% (Fig. 4).

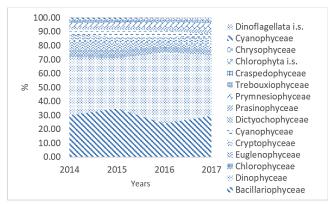


Fig. 3. Annual dynamics in the taxonomic composition of phytoplankton in the Bulgarian marine areas, 2014-2017

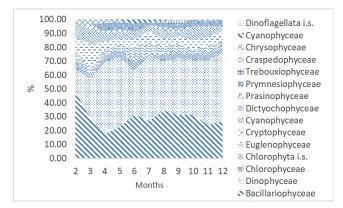


Fig. 4. Monthly dynamics in the taxonomic composition of phytoplankton in the Bulgarian marine areas, 2014-2017

The highest biodiversity of phytoplankton (by number of species) was recorded in spring (May) and late summer (September), (Fig. 5). It correlates with previous studies that established the maximum in biodiversity June - September (Klisarova et al., 2015; Petrova & Gerdzhikov, 2015). The reported slight change might be caused by differences in the intensity and frequency of occurrence of various hydrological impacts on the water column or the periodicity of biogenic supply to the photic water layer (Sommer et al., 1993; Sommer, 1995; Polishchuk, 1999).

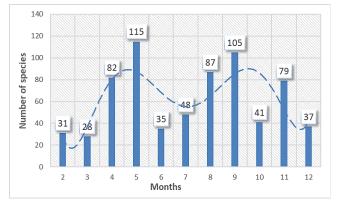
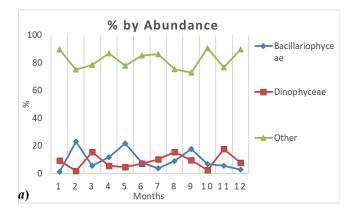


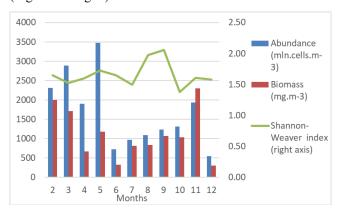
Fig. 5. Monthly dynamics in the number of phytoplankton species in the Bulgarian marine areas, 2014-2017

#### Quantitative development of phytoplankton

In the quantitative structure of the phytoplanktonic communities, microalgae of the group "Others" dominated in number by an average of 82.02%, class *Bacillariophyceae* and class *Dinophyceae* occupied 9.75% and 8.75%, respectively (Fig. 6a). Phytoplankton biomass was dominated by *Dinophyceae* class (50.30%), and *Bacillariophyceae* and "*Others*" were 23.02% and 27.47%, respectively (Fig. 6b).



Compared with our previous studies (Petrova et al., 2014), we found a significant change in the seasonal quantitative structure of the phytoplankton communities. The share of class *Bacillariophyceae* in biomass decreased significantly during the spring and summer seasons, while the group "*Others*" demonstrated high participation in the phytoplankton biomass in winter and spring (Fig. 6). On average, in the period 2014-2017, the highest phytoplankton numbers and biomass were observed in winter and spring (Fig. 7 and Fig. 8).



#### Fig. 7. Average monthly values for abundance (mln. cells.m<sup>-3</sup>), biomass (mg.m<sup>-3</sup>) and Shannon-Weaver index, 2014-2017

The highest values of the Shannon-Weaver Index were recorded in August and September with an assessment of the marine water as water in "*very good*" ecological status on this indicator (Fig. 7).

In summer, comparatively low (below 1000 mln.cells.m<sup>-3</sup> and below 1000 mg.m<sup>-3</sup>) quantitative values of phytoplankton growth were observed (Fig. 7 and Fig. 8), values describ-

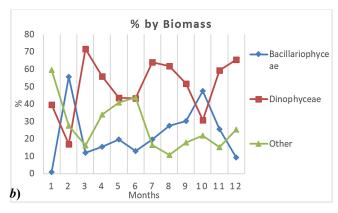
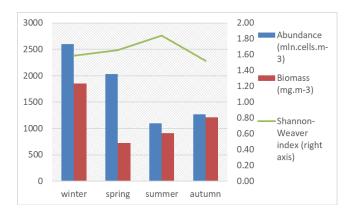


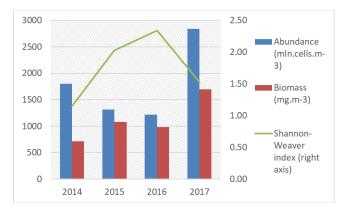
Fig. 6. Phytoplankton quantitative structure, % of the three main taxonomic groups: class *Dinophyceaes*, class *Bacillariophyceae* and the group "*Others*", by months 2014-2017; (*a*) by number, (*b*) by biomass



# Fig. 8. Seasonal averages values for abundance (mln. cells.m<sup>-3</sup>), biomass (mg.m<sup>-3</sup>) and the Shannon-Weaver index, 2014-2017

ing "good" ecological status of our marine ecosystem (Klisarova & Gerdzhikov, 2016; 2017) (Fig. 8).

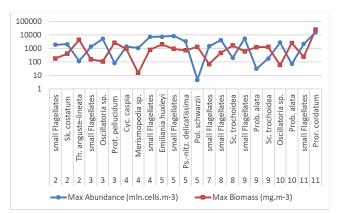
During the study (2014-2017), average values for abundance - 1794 mln.cells.m<sup>-3</sup>, biomass - 1117.76 mg.m<sup>-3</sup> and Shannon-Weaver Index - 1.76, determinates marine water in the ranges of "good" ecological status (Fig. 9). A trend of increasing the average annual quantities of phytoplankton was registered, as in 2017 they were the highest for the past ten years (Fig. 9).



## Fig. 9. Annual average numbers (mln.cells.m<sup>-3</sup>), biomass (mg.m<sup>-3</sup>) and Shannon-Weaver index, 2014-2017

The autumn peak in the seasonal dynamics was recorded in November 2015 when an intense bloom of the peridinae *Prorocentrum cordatum* (biomass 25 g.m<sup>-3</sup>) (Petrova & Gerdzhikov, 2013) and the small Flagellates was observed in the southern part of Varna Bay (Fig. 10). When compared to the so-called "clean period" of the 20<sup>th</sup> century (50-60s), the established summer and autumn values were higher, but significantly lower than those recorded during the eutrophication period (70-80-90s of the 20<sup>th</sup> century) (Klisarova & Gerdzhikov, 2017).

Eight phytoplankton species developed with blooming concentrations >1 mln. cells.m<sup>-3</sup>: *Cyclotella caspia* Grunow, 1878; *Emiliania huxleyi* (Lohmann) Hay & Mohler, 1967; *Merismopedia sp.* Meyen, 1839; *small Flagellates*; *Oscillatoria sp.* Vaucher ex Gomont, 1892; *Prorocentrum cordatum* (Ostenfeld, 1901) Dodge, 1975; *Pseudo-nitzschia delicatissima* (P.T. Cleve, 1897) Heiden, 1928; *Skeletonema costatum* (Greville) Cleve, 1873 (Fig. 10).

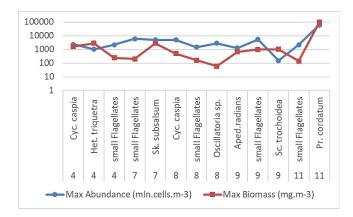


#### Fig. 10. Maximum abundances (mln.cells.m<sup>-3</sup>) and biomass (mg.m<sup>-3</sup>) of blooming and dominant phytoplankton species in Bulgarian coastal waters (excluding Varna Lake), by months, 2014-2017

Six phytoplankton species were developing with high biomass (> 1 g.m<sup>-3</sup>) in the coastal aquatory: *Thalassiosira* anguste-lineata (Schm.) G.Fryxell & Hasle, 1977; Protoperidinium pellucidum Bergh, 1882; Emiliania huxleyi; Polykrikos schwarzii Bütschli, 1873; Scrippsiella trochoidea (Stein, 1883) Balech ex Loeblich III, 1965; Proboscia alata (Bright.) Sundström, 1986; Prorocentrum cordatum. Increased phytoplankton biomass in February and March was caused by the intense development of the diatom *Th.* anguste-lineata and the peridine Prot. pellucidum (Fig.10).

In the Lake of Varna, a total of 7 phytoplankton species in bloom-causing concentrations were recorded: *Apedinella radians* (Lohmann) Campbell, 1973 (Syn. *Ap.spinifera*); *Cyclotella caspia*; *Heterocapsa triquetra* (Ehrenberg, 1840) Stein, 1883; *small Flagellates*; *Oscillatoria sp.*; *Prorocentrum cordatum*; *Skeletonema subsalsum* (Cleve-Euler) Bethge, 1928, (Fig. 11).

Five species developed with high biomass in the lake: *Cyclotella caspia*, *Heterocapsa triquetra*, *Skeletonema subsalsum*, *Scrippsiella trochoidea*, *Prorocentrum cordatum* (Fig. 11).



#### Fig. 11. Maximum abundances (mln.cells.m<sup>-3</sup>) and biomass (mg.m<sup>-3</sup>) of blooming and dominant phytoplankton species in Varna Lake, by months, 2014-2017

The average values of chlorophyll-*a* within the study period were relatively high: on the coast, an average monthly value of 0.98 mg.m<sup>-3</sup> was measured, and in Varna Lake -19.40 mg.m<sup>-3</sup>. According to the classification system of Wasmund et al. (2001), on this indicator our coastal waters were defined as mesotrophic, and the Lake waters - as hypereutrophic.

The average transparency of the seawater was 4.79 m, which determinates the ecological status of water as "*very good*". For Varna Lake the average water transparency was 1.45 m, which characterized the ecological status of water as "*very bad*" (Ordinance No. H-4, 2012).

#### Conclusions

A total of 182 phytoplankton species belonging to 14 taxonomic classes were recorded in Bulgarian Black Sea coastal marine water and in Varna Lake water during the period 2014-2017. The Dinophyceae/Bacillariophyceae group occupied 72.53% of the total taxonomic composition with an increase from 2014 to 2017. The highest biodiversity of phytoplankton was recorded in May and September of the studied period. Eight species in the coastal aquatory and seven species in Varna Lake were recorded in bloom-causing concentrations. Microalgae from the group "Others" (82.02%) dominated in the number of phytoplankton and the Dinophyceae class dominated in the biomass (50.30%). From 2014 to 2017, there was a trend to increase the numbers and biomass of phytoplankton, especially in 2017, when the measured values were the highest for the last decade. The highest quantitative values of the phytoplankton were produced during the winter-spring period of the year, demonstrating a return to the normal annual growth cycle for the temperate latitudes. The ecological assessment of the water in the study areas based on the results obtained for phytoplankton determines the ecological status of coastal water as "*moderate*" and the Varna Lake water as "*bad*".

#### References

- Assessment of the Actual Quantity and Quality of the Waters in the Black Sea Basin District (AAQQWBSB). (2005). Ministry of Environment and Water, Basin Directorate for Black Sea Region – Varna (Bg).
- ttps://www.bsbd.org/UserFiles/File/2005%20%20DOKLAD%20 NA%20BDVARNA% 20ZA%20VODITE (1).pdf (31, July, 2019 date last accessed).
- Bodeanu, N., Moncheva, S., Ruta, G. & Popa, L. (1998). Longterm evolution of the algal blooms in Romanian and Bulgarian Black Sea waters. *Cercetari Marine*, 31, 37-55.
- Borysova, O., Kondakov, A., Paleari, S., Rautalahti-Miettinen, E., Stolberg, F. & Daler, D. (2005). Eutrophication in the Black Sea region: Impact assessment and Causal chain analysis. University of Kalmar, Sweden, 27-27, ISBN: 91-89584-50-3.
- Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Official Journal of the European Communities*, 22.12.2000, L 327/1.
- Edler, L. (1979). Recommendations for marine biological studies in the Baltic Sea phytoplankton and chlorophyll. *Baltic Marine Biologists*, 5-38.
- Gerdzhikov, D. (2014). Ecological assessment of phytoplankton in Bulgarian Black Sea coastal water. Ph.D. thesis, Trakia University, Stara Zagora, Bulgaria (Bg).
- Kamburska, L., Moncheva, S., Konsulov, A., Krastev, A. & Prodanov. K. (2003). The invasion of Beroe ovata in the Black Sea a warming signal for ecosystem concern. In: *Proceedings of Institute of Oceanology* (ed. V. Dachev), 4, Bulgarian Academy of Science - Branch Varna, 111-123.
- Klisarova, D. & Gerdzhikov, D. (2016). Dynamics in chlorophylla concentrations in front of the Bulgarian coast 2011-2016. In: *Proceedings of the Union of Scientists in Bulgaria - Varna, Series "Marine Science"*, 37-42 (Bg).
- Klisarova, D. & Gerdzhikov, D. (2017). Particularities of the phytoplankton dynamics in the Bulgarian coastal aquatory. In: *Proceedings of Institute of Fish Resources (IFR) - Varna*, 28, 63-72 (Bg).
- Klisarova, D., Gerdzhikov, D., Kostadinova, G. & Petkov, G. (2015). Investigation of phytoplankton in the Varna bay (2005-2014). In: Proceedings of the Twelfth International Conference on the Mediterranean Coastal Environment, 06-10 October 2015, E. Özhan (ed.), 299-308, MEDCOAST 2015, Varna, Bulgaria.
- Mee, L. D. (1992). The Black Sea in crisis: A need for concerted international action. *Ambio*, 21(4), 278-286.
- Mee, L. D., Friedrich, J., & Gomoiu, M. T. (2005). Restoring the Black Sea in times of uncertainty. *Oceanography*, 18(2), 101-111.
- Ministry of Environment and Water (2012). Ordinance No.

H-4/14.09.2012 on the characterization of surface water. *State Gazette*, No. 22/05.03.2013 (last change SG No.79/23.09.2014, in force since 23.09.2014) (Bg).

- Moncheva, S., Doncheva, V., & Kamburska, L. (2001). Phytoplankton blooms long-term history off the Bulgarian Black Sea coast in relation to environmental factors: do the 90's manifest a sign of ecosystem health improvement. *Journal of Environmental Protection and Ecology*, 2(4), 997-989.
- Moncheva, S. & Parr, B. (2010). Manual for phytoplankton sampling and analysis in the Black Sea, 68. from http://documents.blacksea – commission.org/Downloads/ Phytoplankton\_20%Manual-Final-1.pdf (26 June, 2016 date last accessed).
- Moncheva, S., Slabakova, V. & Doncheva, V. (2013). Phytoplankton II.2.2.1. In: Initial assessment of the state of the marine environment, according to Article 8 of the RPEMW (Regulation for the protection of the environment in marine waters). Institute of Oceanology, Bulgarian Academy of Science - Branch Varna, 168-180 (Bg).
- Morozova-Vodyanitskaya, N. V. (1954). Phytoplankton of the Black Sea, Part II, In: *Proceedings of the Sevastopol Biological Station*, 8, 11-99 (Ru).
- Nesterova, D.A. (1987). Peculiarities of phytoplankton succession in the North-West part of the Black Sea. *Hydrobiolog. Zhurnal*, 23(1), 16-21 (Ru).
- Olenina, I., Hajdu S., Edler, L., Andersson, A., Wasmund, N., Busch, S., Göbel, J., Gromisz, S., Huseby, S., Huttunen, M., Jaanus, A., Kokkonen, P., Ledaine, I. & Niemkiewicz, E. (2006). Biovolumes and size-classes of phytoplankton in the Baltic Sea, HELCOM Balt. Sea Environmental Proceeding, 106.
- Petrova, D., Velikova, V. & Gerdjikov, D. (2006). Recent State of phytoplankton community in the Varna Bay. *Bulgarian Journal* of Agricultural Science, 12, 247-260.
- Petrova, D. & Gerdzhikov, D. (2013). Development of a traditional blooming phytoplankton species *Prorocentrum cordatum*

Dodge, 1975 along the Bulgarian coast (2008-2010). In: *Proceedings of the Union of Scientists in Bulgaria - Varna, Series "Marine Science"*, 12-17 (Bg).

- Petrova, D. & Gerdzhikov, D. (2015). Phytoplankton taxonomy in the Bulgarian coastal waters (2008-2010). *Bulgarian Journal of Agricultural Science*, 21, 90-99.
- Petrova, D., Kostadinova, G. & Gerdzhikov, D. (2014). Ecological assessment of the phytoplankton community in the Bulgarian Black sea coastal waters. *Agricultural Science and Technol*ogy, 6, 98-103.
- Petrova-Karadzhova, V. J. (1984). Change of planktonic flora in the Bulgarian Black Sea waters under the influence of eutrophication. In: *Proceedings of Institute of Fishery resources -Varna*, XXI, 105-112 (Bg).
- Polishchuk, L. V. (1999). Contribution analysis of disturbancecaused changes in phytoplankton diversity. *Ecology*, 80, 721– 725.
- Sommer, U. (1995). An experimental test of the intermediate disturbance hypothesis using cultures of marine phytoplankton. *Limnology and Oceanography*, 40, 1271-1277.
- Sommer, U., Padisak, J., Reynolds, C. S. & Juhasz-Nagy, P. (1993). Hutchinson's heritage: the diversity-disturbance relationship in phytoplankton. *Hydrobiologia*, 249, 1-7.
- Velikova, V., Moncheva, S. & Petrova, D. (1999). Phytoplankton dynamics and red tides (1987-1997) in the Bulgarian Black Sea. *Journal Water Science and Technology*, 39(8), 27-36.
- Wasmund, N., Andrushaitis, A., Lysiak-Pastuzak, E., Muller-Karulisb, B., Nauscha, G., Neumanna, T., Ojaveerd, H., Oleninae, I., Postela, L. & Witekf, Z. (2001). Trophic Status of the South-Eastern Baltic Sea: a comparison of coastal and open areas. Estuarine. *Coastal and Shelf Science*, 53, 849-864.
- Zaitzev, Yu. P. (1992). Eutrophication in the Black Sea waters. In: Mantoura, R. C., Martin, J. M. & Wollast, R. (eds.) International Workshop on the Black Sea: Focus on the western Black Sea Shelf, New York, USA, 251-279.