pp. 95-100 doi:10.7427/DDI.21.10

10. Phytoplankton assessment in Danube Delta Biosphere Reserve

SPIRIDON Cosmin[1], TÖRÖK Liliana[1], TUDOR Iuliana Mihaela[1], IBRAM Orhan[1], TEODOROF Liliana[1], DESPINA Cristina[1], SECELEANU ODOR Daniela[1], TUDOR Marian[1], GEORGESCU Lucian P. [2]

[1] Danube Delta National Institute for Research and Development, Babadag Street, No. 165, Tulcea, Romania, e-mail: office@ddni.ro

[2]"Dunarea de Jos "University of Galati, European Center of Excellence for the Environment, Faculty of Sciences, 111 Domneasca Street, 800201, Galati, Romania;

Address of author responsible for correspondence: SPIRIDON Cosmin, Danube Delta National Institute for Research and Development, Babadag Street, No. 165, Tulcea, Romania; Email: cosmin.spiridon@ddni.ro

Abstract: The term "plankton" refers to those microscopic aquatic forms having little or no resistance to currents and living free-floating and suspended, in open or pelagic waters. Phytoplankton development has different consequences depending on biomass quality and quantity, the overgrowth result being eutrophication process. The eutrophication intensity can cause both a lower water transparency, by excessive algal growth, to fish death in the area. In this study, it was presented the ecological status and phytoplankton biomass dynamic, in the Danube branches from upstream to downstream. The measurements have been made in 2013, in March, June, September and November, using spectrofluorometer for algal biomass determination and a microscope for qualitative analyses of phytoplankton species. Shannon-Wiener index was calculated to compare phytoplankton species diversity. Also, the biodegradable organic matter loading the ecosystem was determined by computing the Saprobic index. The values obtained do not exceed the eutrophication limits according to the Water Framework Directive, transposed into Romanian legislation by Order 161/2006, with normal concentrations for rheophile ecosystems, as Danube's branches. In this area, water currents and high water turbidity inhibit phytoplankton growth, in contrast to lacustrine ecosystems, where light penetration to depths favors the development of different phytoplankton groups.

Key words: Danube Delta, phytoplankton, rheophile ecosystems

INTRODUCTION

Phytoplankton, with an important role in the monitoring of aquatic ecosystems quality, is represented by the abundance of vegetal microorganisms drifting in the water column. High temperature and sunlight favor the high levels of biogenic elements in surface waters, through a substantial development, respectively the eutrophication of water bodies. [3, 6, 8]

However, the quantity and quality of phytoplankton biomass can be a reference point in assessing the success or failure of some environmental projects that involved changes in water management. Most times, eutrophication of water bodies can have serious consequences for the entire aquatic ecosystem. At the same time, low levels in algal biomass can have serious consequences on the zooplankton populations (lack of food) and also on the entire food chain of aquatic environments. [1, 2]

Generally, in rheophile ecosystems, due to hydrological conditions (depths, water circulation and reduced transparency) phytoplankton abundance is much lower compared to the lacustrine ecosystems. The link between these ecosystems made by the water transfer from rheophile ecosystems to lentic ecosystems, also involves an input of nutrients and organic matter, which have major contributions to the development of phytoplankton populations. [7,13,15]

Thereby, the present study proposes an assessment of phytoplankton biomass and abundance in six representative points, located along the Danube River, from its entry in the Danube Delta (Ceatal Chilia, Ceatal Sfântul Gheorghe, Aval Izmail) to the flow into the Black sea, through 3 arms (Chilia, Sulina and Sfântul Gheorghe). [4]

MATERIALS AND METHODS

Study area

In order to achieve the objectives of this study, it was selected six sampling points, representative for the Danube Delta, as follows: three sampling points, located in the west side of the delta, where the Danube supply the aquatic ecosystems (Ceatal Chilia, Ceatal Sfântul Gheorghe, Aval Izmail) and three sampling points, located in the east side of the Danube Delta (Periprava, Sulina, Sfântul Gheorghe) [4] (figure 1).

The sampling activities were made in 2013 (March, June, September and November).
To establish the ecological status in the selected areas, standardized methods were used for sampling and specific indices were computed (Shannon-Weaver diversity index and Saprobic index). [10,12,14]

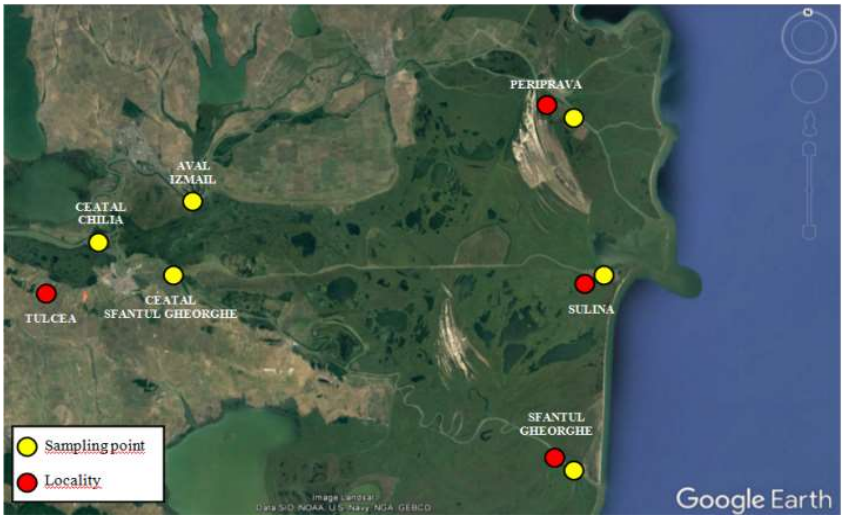

Fig. 1 Map of the sampling points

The samples were collected in 1 liter plastic containers and preserved with 5 mL of Lugol solution. More observations regarding sampling points, as well as site characteristics of area, the weather, water color and other parameters that can indicate water quality, are noted in working notebook. [9]

The phytoplankton samples were collected according to methodology for shallow waters of 2-3m depth, subsurface samples collected at 0.5 m. Qualitative studies have been carried out using a light microscope (Laborlux) at medium (40X) and high magnification (100X). A minimum of 400 cells were enumerated to assure that the count is representative for each sample. [12]

The objectives provide adequate working distance for the counting chamber. Magnification requirements vary with the investigated plankton fraction, the type of microscope, counting chamber and objectives used. [14]

Samples were collected from established depths and, in case of deep waters a larger number of samples were collected from different depths. In case of samples collected from more depths, it is necessary to mix the samples to obtain a single sample from a sampling point. [6]

Before analysis, a concentration of organisms, contained in water samples, must be done. Sedimentation is the selected method for concentration, because it is nonselective and nondestructive, although many of the picoplankton, the smaller nanoplankton and actively swimming flagellates may not settle completely. A volume of 1 L (for general phytoplankton enumeration) is concentrated up to 1 mL for determination to inverted microscope. The concentrated volume varied inversely with the abundance of organisms and is related to sample turbidity.

For microscopic analysis few steps were followed:

a. sedimentation for 7 days
b. siphoning until 100 mL
c. sharing sample in two test tubes, one of 40 mL (for diatoms determination) and 15 mL for quantitative and qualitative analysis of the sample.[12]

Phytoplankton species were identified using a microscope, a list of species was established and it was computed the number of individuals per liter from each sample.

Some phytoplankton species are unicellular, while others are multicellular. To enumerate phytoplankton, it was used a counting chamber, that limits the volume and area for populations densities computing. For cells (organisms), a standard identification of the references point, was done. Dead cells or broken diatom frustules were not counted. Magnification is important in phytoplankton identification and enumeration. [14]

For **"in situ"** phytoplankton biomass determination, it was used bbe FluoroProbe Spectrofluorometer, which can quickly assess the concentration of chlorophyll "a" in water column. The result was obtained by emitting a light beam of different intensities for each group of algae. This device can difference primary groups of algae from mixed populations and can determine organic matter quantity found in decomposition until 100 m depth. [14,16]

Based on relative intensity of fluorescence light for four wavelengths, the following taxonomic groups of algae, were differentiated: green algae to 470 nm LED; blue-green algae to 610 nm LED; diatoms to 525 nm LED; cryptophyceae to 570 nm LED and the measurements accuracy is enhanced by the detection of other fluorescing matter (for example, yellow substances). [12,13,14]

RESULTS AND DISCUTIONS

The results obtained in this study are characteristic for deep reofiles ecosystems, taking into account the diatoms, as dominant specie. Diatoms biomass shows no overgrowth, total biomass being under a half of the maximum allowed limit.

Abundance varied in the range of 424446.5 ind/L and 2044444 ind/L in Aval Izmail sampling point, 437878.6 ind/L and 3070875 ind/L in Periprava sampling point, 265279.1 ind/L and 1622992 ind/L in Ceatal Chilia sampling point, 41195.1 ind/L and 1307916 ind/L in Sulina sampling point, 270360.1 ind/L and 2148897 ind/L in Ceatal Sfantu Gheorghe sampling point and 319112 ind/L and 1915571 ind/L in Sfantu Gheorghe sampling point. **(Table 1)**

Table 1
Abundance of phytoplankton in the studied aquatic ecosystems

	Ceatal Chilia ind/L	**Ceatal Sfantul Gheorghe** ind/L	**Aval Izmail** ind/L	**Periprava** ind/L	**Sulina** ind/L	**Sfantul Gheorghe** ind/L
III.2013	445647	270360	424446	437878	411951	-
VI.2013	1622992	2148897	2044444	821156	1307916	1915571
IX.2013	265279	547759	-	-	451409	392147
XI.2013	-	576559	-	3070875	-	319112

As figure 2 shows, the biomass (represented by chlorophyll "a" concentrations) varied between 4.36 µg/L and 9.75 µg/L in Aval Izmail sampling point, 4.63 µg/L and 8.93 µg/L in Periprava sampling point, 4.46 µg/L and 10.5 µg/L in Ceatal Chilia sampling point, 5.01 µg/L and 6.26 µg/L in Sulina sampling point, between 5.44 µg/L and 8.81 µg/L in Ceatal Sfantu Gheorghe sampling point and between 5.06 µg/L and 8.53 µg/L in Sfantu Gheorghe sampling point.
The chlorophyll "a" values, in the sampling points situated on the Danube branches, frame this water bodies in first quality class (<25 µg/L) according to Romanian legislation [8].

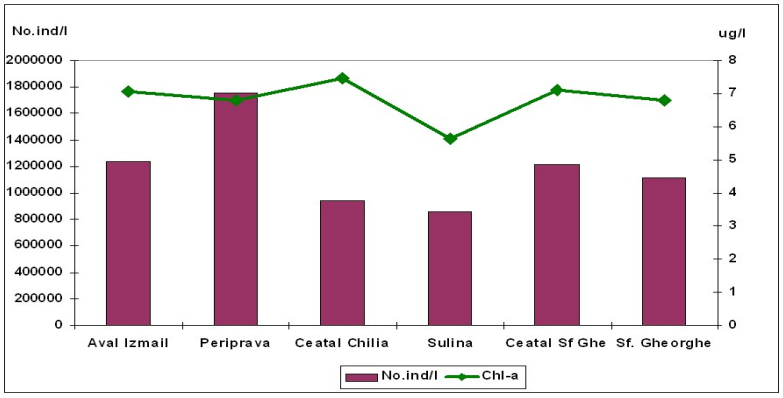

Fig. 2 Abundance and biomass averages in the studied aquatic ecosystem

The species list, identified in the collected samples in 2013, includes 120 species from which 46 Chlorophyceae, 59 Diatoms, 6 Cyanobacteria, 4 Cryptophyta, 3 Euglenophyta and 2 Dinophyta. The presence of the species in all sampling points in 2013 is presented in the **Table 2**. The number of species varied between 13 species (in Periprava in November 2013) and 36 species (in Aval Izmail in June 2013).

The identified species number, representative for reofile ecosystems, is characterized by diatoms abundance.

Table 2
List of species in the studied aquatic ecosystem

Phylum	Ceatal Chilia	Ceatal Sfântul Gheorghe	Aval Izmail	Periprava	Sulina	Sfântul Gheorghe
CHLO	**15**	**21**	**11**	**11**	**13**	**23**
CHRY	**0**	**1**	**0**	**1**	**0**	**1**
CRYP	**3**	**3**	**2**	**1**	**2**	**3**
CYAN	**2**	**0**	**0**	**0**	**1**	**2**
DIAT	**24**	**30**	**29**	**17**	**27**	**23**
DINO	**0**	**0**	**0**	**1**	**0**	**0**
EUGL	**1**	**2**	**1**	**1**	**2**	**2**

The Shannon-Wiener index **(Table 3**), showed a uniformity in the phytoplankton distribution, in the selected area, the values indicating a reduced variation. An exception to this trend is the value obtained for Periprava locality, a sampling point located downstream.
We estimate that uniform values obtained for all six selected sampling points can be attributed to the high water volume and the homogenization degree of Danube waters.

Table 3
Shannon-Wiener index variation in the studied aquatic ecosystem

	Ceatal Chilia	Ceatal Sfântul Gheorghe	Aval Izmail	Periprava	Sulina	Sfântul Gheorghe
III.2013	2.18	2.21	2.28	2.31	2.45	-
VI.2013	1.43	1.53	1.72	1.81	1.96	1.83
IX.2013	2.31	2.38	-	-	2.49	2.36
XI.2013	-	2.47	-	0.37	-	2.47

The saprobic index values framed the surface water in second quality class, except for Periprava sampling point, which saprobic index values frame this water in the third quality class (moderate ecological status) (figure 3).

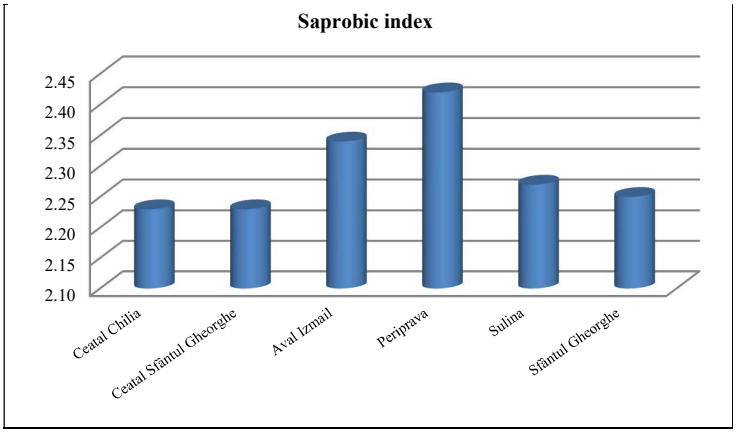

Fig. 3 Saprobic index for the studied aquatic ecosystem

CONCLUSIONS

Phytoplankton, identified as Biological Quality Element under the European Water Framework Directive (2000/60/EC), is suitable to be monitored, to determine anthropogenic influences on aquatic ecosystems. Investigated sections of the Danube River does not have a significant variation of investigated parameters, in 2013, and are classified in good ecological status according to calculated saprobic values.

AKNOLEDGMENTS

This study was supported by the project "Innovative Instruments for Environmental Analysis in North Western Black Sea Basin (Black Sea E- Eye)".

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