SEVERAL CHARACTERISTICS OF PLANKTONIC MICROBIAL COMMUNITIES DECOMPOSING ORGANIC MATTER IN THE AQUATIC ECOSYSTEM OF SFÂNTU GHEORGHE BRANCH, THE DANUBE DELTA

PĂCEŞILĂ Ioan, IONICĂ Doina, DUMITRACHE Cristina Alina

Abstract. Several planktonic physiological groups of microorganisms involved in detrital organic matter decomposition were studied in riverine ecosystem Sfântu Gheorghe branch, the Danube Delta. The total microbial biomass dynamics and bacterial productivity rates were also assessed. The obtained results show a high numeric density of proteolytic microorganisms while the cellulolytic microorganisms were the least abundant. The most physiological groups show maximum density in summer. The annual, seasonal and spatial dynamics of total microbial biomass and bacterial productivity rate did not show clear patterns.

Keywords: planktonic microbial communities, Sfântu Gheorghe branch, the Danube Delta.

Rezumat. Câteva caracteristici ale comunităţilor microbiene planctonice implicate în descompunerea materiei organice detritale din ecosistemul acvatic braţul Sfântu Gheorghe, Delta Dunării. Mai multe grupuri fiziologice de microorganisme planctonice implicate în descompunerea materiei organice detritale au fost studiate în ecosistemele acvatice de pe braţul Sfântu Gheorghe, Delta Dunării. Au fost evaluate, de asemenea, şi dinamica biomasei microbiene totale, precum şi rata producţiei bacteriene. Rezultatele obţinute indică o abundenţă numerică maximă a bacteriilor proteolitice în timp ce grupul microorganismelor celulozolitice este cel mai puţin reprezentat. Aproape toate grupurile de microorganisme studiate au înregistrat densitatea maximă în sezonul de vară. Dinamicile anuale, sezoniere şi spaţiale ale biomasei microbiene totale şi productivităţii bacteriene nu au relevat tipare bine definite.

Cuvinte cheie: comunităţi microbiene planctonice, braţul Sfântu Gheorghe, Delta Dunării.

INTRODUCTION

In aquatic ecosystems heterotrophic microorganisms are the most important users of detrital organic matter resulting from the activity (excrete) or death of aquatic organisms (ZARNEA, 1994; MIHĂESCU, 2000). Through their metabolic activity these microorganisms breakdown most of the detrital organic matter to mineral constituents - recycled by living organisms, and thus it is avoided organic matter accumulation (BOTNARIUC & VĂDINEANU, 1982; SIMON - GRUIŢĂ, 2000; ZARNEA, 1994). Alongside microorganisms at decomposition processes take part others organisms categories, but the role of microorganisms is essential because of their particularly characteristics: ubiquitous presence in the environment, rapid multiplication and growth, high physiological diversity and high metabolic rates (POMEROY et al., 1994; NICOLESCU et al., 2000). The intensity of decomposition processes in an aquatic ecosystem is dependent on physicochemical and biological parameters and, also, on substrate nature. Variations of these parameters are reflected in microbial community structure (BITTON, 2005) and, thus, assessment of microbial physiological groups and biomass can be a method to estimate ecosystem changes.

This paper presents several characteristics of microbial physiological groups involved in the decomposition of detrital organic matter present in river ecosystem Sfântu Gheorghe branch, the Danube Delta.

Biological parameters studied were total microbial biomass, bacterial production and numeric density of bacterial physiological groups of microorganisms.

MATERIAL AND METHODS

Study area. Sfântu Gheorghe is the southernmost of the three main branches through which the Danube flows into the Black Sea. It is the oldest of the branches, has a total length of 108.2 km and carries around 22% of the river flow.

This branch was subject of channelization in the 80's, when six meanders were cut to shorten the navigation route; consequently, different types of sections were formed in the river branch: the free-flowing sector (FS), the meanders section (MS) and the newly built channel (NBC) (GÎŞTESCU & ŞTIUCĂ, 2006). Seven stations, corresponding to these sectors were selected (Fig. 1), as it follows:

- the free-flowing sector (FS): stations S1, S4 and S7;
- the meanders section (MS): stations S2 and S5;
- the newly built channel (NBC): stations S3 and S6.

Sampling. Water samples were collected on water column using a modified Patalas device and kept in sterile bottles until the analyses. Sediment samples were collected from the top layer (the sediment-water interface) with a Corer device and stored in plastic bags. After sampling, both water and sediment samples were introduced in freezing bags and kept at 4^{o}C for transport to the laboratory, where they were processed in short time to avoid major changes in microbial communities' structure.

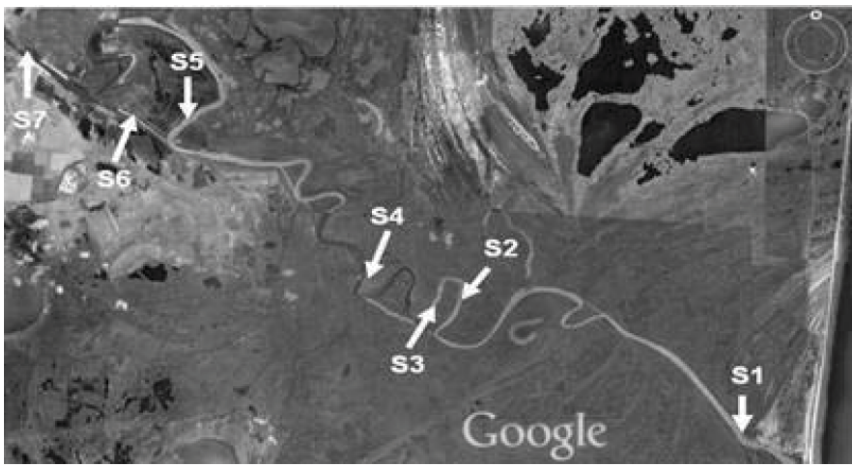


Figure 1. Location of sampling stations along Sf. Gheorghe branch (image from Google Earth).

Total microbial biomass: (stations S1- S7) - lipid phosphate dosage method (SIMON - GRUIŢĂ, 2000). Before processing water samples were filtered through zooplanktonic filter.

Bacterial production) rate: (stations S2 - S7) - was estimated according to the relation: bacterial production (BP) = 0,08 * D (bacterial oxygen consumption) (SIMON - GRUIŢĂ, 2000). Bacterial oxygen consumption was measured through Gaardner - Gran ("black and white bottles") method (NICOLESCU & GIRIP, 1994).

Physiological groups of microorganisms: (stations S5 - S7) - serial dilution method (RODINA, 1972). Samples were inoculated on specific solid and liquid culture media, and incubated at different temperatures for various periods of time depending on the studied physiological type:

- proteolytic microorganisms: Thornton medium, 15 days, 28 ^{o}C;
- amylolitic microorganisms: starch medium, 15 days, 28 ^{o}C;
- denitrifying microorganisms: Rodina medium, 15 days, 28 ^{o}C;
- ammonifying microorganisms: peptone water, 15 days, 28 ^{o}C;
- microorganisms breakdown sulfur proteins: broth media, 15 days, 28 ^{o}C;
- aerobic and anaerobic cellulolytic microorganisms: Stapp medium, 21 days, 37^{o}C (RODINA, 1972).

The following physico-chemical and biological parameters were also determined: pH, temperature, transparency, depth, transparency index, concentrations of oxygen, total organic carbon, nitrite, nitrate and ammonium, dissolved inorganic nitrogen, organic and total phosphorous.

RESULTS AND DISCUSSIONS

Total microbial biomass. Living biomass of microbial communities provides information about active microorganisms existent in an ecosystem (VESTAL & WHITE, 1989). During the study period, planktonic microbial biomass fluctuated within 2.76 - 138 μg CL^{-1} limits. Although seasonal dynamics did not show a regular pattern (Fig. 2a), generally, the highest values were recorded in summer (especially in 2010). A possible explanation of this aspect is represented by phytoplankton activity that usually shows high values in the summer (IONICĂ et al., 2006). Excretion products of phytoplankton and phytoplankton dead bodies represent an important source of carbon and energy for different types of microorganisms (SIMON - GRUIŢĂ, 2000). The annual dynamics shows the highest values in 2008 and minimum in 2009, while spatial dynamics shows the highest values in FS.

Highly significant linear correlations were obtained between microbial biomass dynamics and several physico-chemical parameters: temperature ($r = 0.35$, $n = 63$, $p < 0.01$), transparency ($r = - 0.47$, $n = 63$, $p < 0.001$), transparency index ($r = - 0.37$, $n = 63$, $p < 0.01$) and inorganic dissolved nitrogen ($r = 0.37$, $n = 63$, $p < 0.01$). The negative correlation between the dynamics of microbial biomass and both transparency and transparency index show the importance of substrate availability (particulate and dissolved organic matter) to planktonic microbial biomass production in this ecosystem.

Bacterial production. The assessment of bacterial production rate provides information about the contribution of microbial communities to total ecosystem production (SIMON - GRUIŢĂ, 2000). In the studied ecosystem, bacterial production rate fluctuated within 3 - 130 mg C/L/day limits.

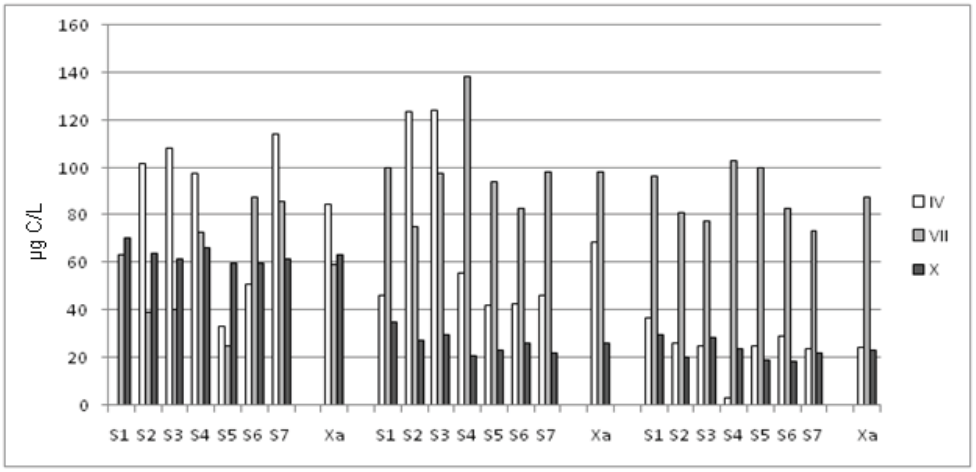
Seasonal dynamics shows the highest values in summer 2008, while in 2009, it did not show a clear pattern (Fig. 2b). The annual average value was higher in 2008 reaching 43.41mg C/L/day. The most important factor controlling BP rate was temperature ($r = 0.4$, $n = 36$, $p < 0.01$).

Physiological groups of microorganisms.

Amylolitic microorganisms are able to synthesize amylase, an enzyme that catalyses the breakdown of starch into sugars (ZARNEA, 1994). It follows from research on the degradation of starch that amylolitic bacteria are a

comparatively numerous group of bacteria (DONDERSKI & KALWASIŃSKA, 2003). In Sfântu Gheorghe branch ecosystem, these microorganisms were rather numerous, reaching values of 4.5 x 10^2 cells/mL. Seasonal dynamics shows the highest density in summer season (Fig. 3a) probably due to higher temperatures and substrate abundance. The importance of substrate availability to amylolitic microorganisms number in this ecosystem is also shown by the significant correlation obtained between their numeric density and total organic carbon concentration ($r = 0.46$, $n = 27$, $p < 0.01$). Annual dynamics shows higher average density in 2008 (Fig. 3c) while spatial dynamics shows higher average density in NBC (Fig. 3b).

a)



b)

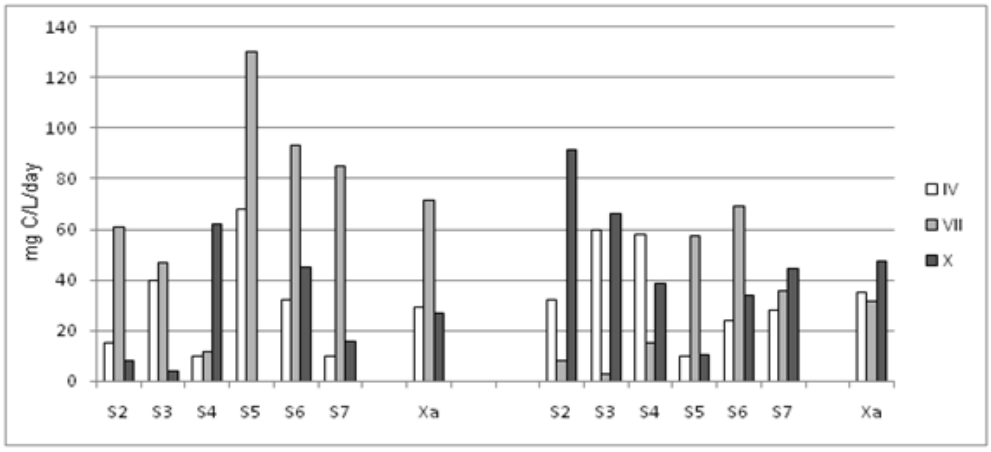


Figure 2. Total microbial biomass dynamics (a) and bacterial production rate (b).

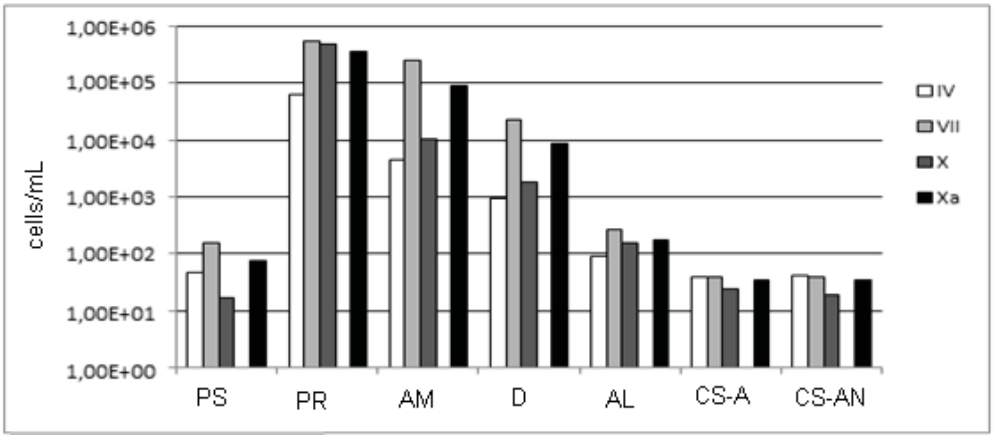
Cellulolytic microorganisms are microorganisms (bacteria, fungi) able to synthesis cellulases, extracellular enzymes that hydrolyse cellulose to cellobiose (ZARNEA & POPESCU, 2012). Cellulose is the structural component of the primary cell wall of green plants and many types of algae (BITTON, 2005) and is also produced by some species of bacteria and protozoans (ZARNEA & POPESCU, 2012). It is the most abundant organic macromolecule in the Earth. Its breakdown processes in aquatic ecosystems and soils are very important in global mineralization processes of organic matter (ZARNEA & POPESCU, 2012). Like starch, cellulose can be used by bacteria as a source of carbon and energy (DONDERSKI & KALWASIŃSKA, 2003). The breakdown processes of cellulose occur in both aerobic and anaerobic conditions (IONICĂ et al., 2006). The microorganisms hydrolysing cellulose represented the least numerous physiological group of organisms isolated from the water column of Sfântu Gheorghe branch. The average densities of both aerobic and anaerobic cellulolytic microorganisms were close in values and less than 1 x 10^2 cells/mL. For both aerobic and anaerobic cellulolytic microorganisms maximum values were recorded in spring (Fig. 3a), the floods period. High values were also recorded in summer, the macrophytes growth period. The annual dynamics shows higher density in 2008 (Fig. 3c) for aerobic microorganisms and 2010 for anaerobic microorganisms.

The highest density was recorded in MS (Fig. 3b) for both types of microorganisms. High significant correlations were also obtained with pH ($r = 0.37$, $n = 27$, $p < 0.05$) for aerobic microorganisms and ammonia ($r = 0.4$, $n = 27$, $p < 0.05$), nitrites $r = 0.4$, $n = 27$, $p < 0.05$), dissolved inorganic nitrogen $r = 0.4$, $n = 27$, $p < 0.05$) and total organic phosphorus

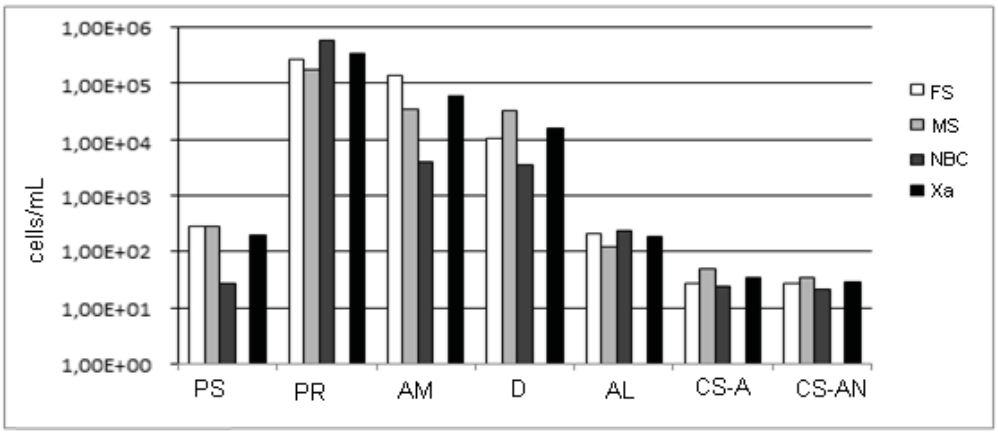
r = 0.62, n = 27, p < 0.001) for anaerobic microorganisms, emphasizing the role of these physico-chemical parameters in controlling the density of cellulolytic physiological group in the water column of studied ecosystem.

Proteolytic microorganisms are able to synthesize extracellular protease, an enzyme that hydrolyses proteins from dead bodies and excretes of aquatic organisms (SIMON-GRUIȚĂ, 2000). This group was the most numerous, with an average density along the study period of 3.78 x 10^5 cells/mL. These bacteria occur in such great number because, in water bodies, proteins, peptides and amino acids are the main components of organic matter. The highest density was recorded in summer season (Fig. 3a), when probably the protein substrate was abundant. The highest annual average density was recorded in 2008 (Fig. 3c). Also, the highest density was recorded in NBC (Fig. 3b). There were no significant correlations between proteolytic bacteria density and physico-chemical parameters, although they certainly influence the dynamics of these microorganisms.

a)



b)



c)

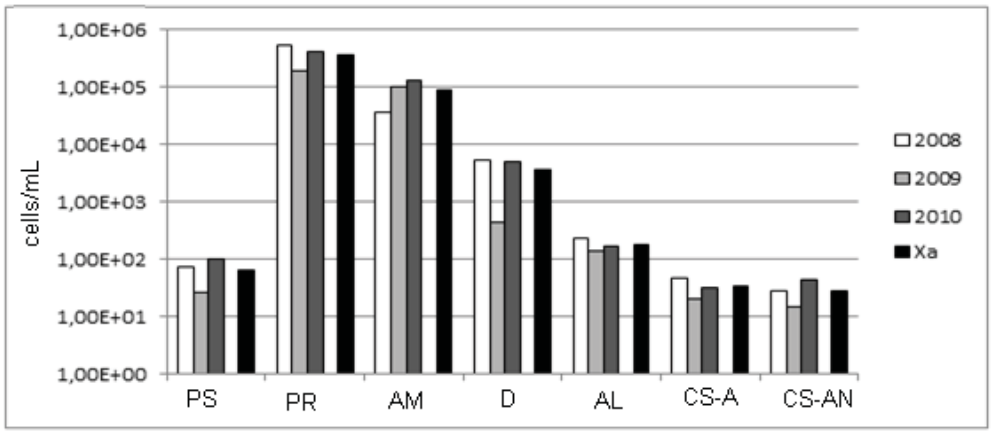


Figure 3. Seasonal (a), spatial (b) and annual (c) dynamics of physiological groups: microorganisms decomposing sulfur proteins (PS), proteolytic microorganisms (PR), ammonifying microorganisms (AM), denitrifying microorganisms (D), amylolitic microorganisms (AL) and aerobic (CS-A) and anaerobic cellulolytic microorganisms (CS-AN).

Ammonifying microorganisms. Ammonification is a very important process through which bacteria convert organic nitrogen (amino acids, urea, uric acid, nucleotides, etc.) within detrital organic matter composition into ammonia (SIMON-GRUIŢĂ, 2000; ZARNEA, 1994). The average density of these microorganisms along the study period was 8.92 x 10^5 cells/mL. Their maximum number was reached in summer and the minimum in spring (Fig. 3a). The annual average density was the highest in 2010 (Fig. 3c), and the spatial dynamics shows most high-density values in FS (Fig. 3b). The number of ammonifying bacteria in this ecosystem was influenced especially by total organic carbon concentration ($r = 0.68$, $n = 27$, $p < 0.01$).

Denitrifying bacteria are microorganisms that through their metabolic activities convert the nitrates from water bodies and soils to molecular nitrogen. This process is very important in nature because it is the major way to the formation of atmospheric nitrogen and a way to balancing nitrogen exchanges between the atmosphere and terrestrial and aquatic environments (ZARNEA, 1994; BOTNARIUC & VĂDINEANU, 1982; SIMON - GRUIŢĂ, 2000). The presence of denitrifying bacteria in the studied ecosystem indicates, on the one hand, the content of easily assimilated organic material and the considerable abundance of nitrates, and on the other hand, the self-cleaning processes taking place in the water column (DONDERSKI & KALWASIŃSKA, 2003). The average density of this physiological group of bacteria was 3.63 x 10^5 cells/mL along the study period. Their maximum number was found in summer and the minimum in spring (Fig. 3a). The annual average density was the highest in 2008 (Fig. 3c) and spatial distribution shows maximum density in MS (Fig. 3b) along the study period. The main physico-chemical parameters that influenced the denitrifying bacteria numeric density were transparency ($r = 0.44$, $n = 27$, $p<0.01$), pH ($r = 0.44$, $n = 27$, $p<0.01$) and oxygen concentration ($r = 0.44$, $n = 27$, $p<0.01$).

Microorganisms decomposing sulfur proteins. Sulfur organic compounds from dead bodies and excretes of aquatic organisms are decomposed by some categories of microorganisms with the formation of H_2S. The resulted H_2S can react with iron and form insoluble sulphides or, in oxidizing conditions, it can be transformed in elementary sulfur (SIMON - GRUIŢĂ, 2000). In the studied period, the numeric density of bacteria decomposing sulfur proteins fluctuated in a wide range, from 1 x 10^0 to 4.5 x 10^2 cells/mL. Like other physiological groups, their maximum number was recorded in summer (Fig. 3a). The annual average density was the highest in 2010 (Fig. 3c) and spatial distribution shows maximum density in FS and MS (Fig. 3b) along the study period. The main physico-chemical parameter that influenced the sulfur proteins decomposing bacteria numeric density was temperature ($r = 0.58$, $n = 27$, $p<0.001$).

CONCLUSIONS

The numeric density of planktonic physiological groups of microorganisms assessed in the water column of the aquatic ecosystem Sfântu Gheorghe branch varied significantly in terms of seasonal, annual and spatial dynamics. The most numerous physiological group of microorganisms was proteolytic bacteria while the least numerous were aerobic and anaerobic cellulolytic microorganisms. Except the cellulolytic microorganisms group whose highest numeric density was recorded in spring, all physiological groups of microorganisms show the highest numeric density in summer. Spatial and annual dynamics of these microbial groups did not show a clear pattern. The dynamics of total microbial biomass and bacterial productivity rates did not show a clear seasonal, annual or spatial pattern. The calculation of linear regression between numeric density of physiological groups, the microbial biomass dynamics and productivity rates and different physico-chemical parameters revealed the significant role of the environmental factors (pH, temperature, transparency of the water column, transparency index, dissolved nutrients and oxygen concentration) in the presence and activity of planktonic bacteria, respectively organic matter decomposition processes, in the investigated ecosystem.

ACKNOWLEDGEMENT

Thanks are to the Romanian Academy for financing the project **"The impact of hydrotechnical changes on ecologic systems located on Sfântu Gheorghe branch**".

REFERENCES

BITTON G. 2005. *Wastewater microbiology*. Edit. Wiley-Liss. New York. 785 pp.

BOTNARIUC N. & VĂDINEANU A. 1982. *Ecologie*. Edit. Didactică şi Pedagogică. Bucureşti. 396 pp.

DONDERSKI W. & KALWASIŃSKA A. 2003. *Occurrence and Physiological Properties of Bacterioplankton of Lake Chełmżyńskie* (*Poland*). Polish Journal of Environmental Studies. Olsztyn. Poland. **12**: 287-295.

GÎŞTESCU P. & ŞTIUCĂ R. 2006. *Delta Dunării-Rezervaţie a biosferei.* Edit. Dobrogea Publishing House. Constanţa. 279 pp.

IONICĂ DORINA. 2006. *Comunităţi microbiene planctonice şi bentonice*. In: Zinevici V., Ionică D., Parpală L., Sandu C., Muşa R., Dobre D. S.: Diversitatea unor comunităţi de microorganisme acvatice în sisteme ecologice din zonele Erenciuc şi Gorgostel (Delta - Dunării). Edit. Ars Docendi. Bucureşti. 302 pp.

NICOLESCU DORINA, IONICĂ DOINA, SIMON-GRUIŢĂ ALEXANDRA, SANDU CRISTINA, NICOLESCU N., ZINEVICI V., TEODORESCU LAURA, DOBRE D. 2000. *Microbial degradation of the organic matter from the Danube Delta lakes. Biotic and abiotic factors controlling the microbial degradation*. Proceedings of the Institute of Biology. Annales Scientifique Session. Bucharest. **2**. 396 pp.

NICOLESCU DORINA & GIRIP ALEXANDRA. 1994. *Dynamique de la degradation aerobie de la matiere organique dans les ecosystemes lacustres du Delta du Danube, expression de la fonctionalite des communautes microbiennes planctoniques dans les conditions des annes 1991-1992.* Revue Roumaine Biology. Biology Vegetal. Roumanian Academy Press. Bucharest. **39**(1): 21-27.

MIHAESCU G. 2000. *Microbiologie generală si virologie*. Edit. Universităţii Bucureşti. 580 pp.

POMEROY L. R., SHELDON J. E., SHELDON W. M. 1994. *Changes in bacterial numbers and leucine assimilation during estimations of microbial respiratory rates in seawater by precision Winkler method.* Applied and Environmental Microbiology. United States. **60**: 328-332.

RODINA A. G. 1972. *Methods in aquatic microbiology*. University Park Press. Baltimore. **23**. 461 pp.

SIMON-GRUIŢĂ ALEXANDRA. 2000. *Rolul bacterioplanctonului în procesele ecologice în ecosistemele acvatice din Delta Dunării*. Teză de doctorat. Universitatea Bucureşti. 350 pp.

VESTAL J. R. & WHITE D. 1989. *Lipid analysis in microbial ecology*. Bioscience. Elsevier. California. **39**: 535-541.

ZARNEA G. 1994. *Tratat de microbiologie generală*. Edit. Academiei Române. Bucureşti. **5**. 1008 pp.

ZARNEA G. & POPESCU O. 2012. *Dicţionar de microbiologie generală şi biologie moleculară.* Edit. Academiei Române. Bucureşti. 1331 pp.

***. Google Earth. *www.google.ro/intl/ro/earth.* (Accessed: March 20, 2013).

Păceşilă Ioan
Institute of Biology Bucharest, Romanian Academy
Splaiul Independenţei, No. 296, Sect. 6, 060031, Bucharest, Romania.
E-mail: pacesilai@yahoo.com

Ionică Doina
Institute of Biology Bucharest, Romanian Academy
Splaiul Independenţei, No. 296, Sect. 6, 060031, Bucharest, Romania.
E-mail: ionica_doina@yahoo.com

Dumitrache Cristina Alina
Institute of Biology Bucharest, Romanian Academy
Splaiul Independenţei, No. 296, Sect. 6, 060031, Bucharest, Romania.
E-mail: coman.cris@yahoo.com

Received: March 31, 2013
Accepted: June 18, 2013