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CHLOROPHYLL-*a* DETERMINATION – A RELIABLE METHOD FOR PHYTOPLANKTON BIOMASS ASSESSMENT

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Phytoplankton biomass assessment is one of the major objectives in an aquatic ecosystem in order to evaluate the amount of energy for the next trophic level and also for trophic state prediction. Spectrometric determination of chlorophyll-*a* content is a simply and quick alternative method for the algal biomass evaluation. Although it is not so precise as the microscopic count, this method becomes soon adopted in the water management. The paper shows a comparison between two methods for algal biomass assessment (microscopic count and determination of chlorophyll-*a* content) in some aquatic ecosystems of Danube Delta in 2000–2002 period.

Key words*:* algal biomass, chlorophyll-*a*, phytoplankton, trophic state

INTRODUCTION

The most accurate method for biomass evaluation is the microscopic count, the mean volume of each phytoplankton species being calculated and multiplied by the number of cells for that species, on different size classes. This method is very laborious and requires also trained persons and special equipment (microscope), as do other methods based on fluorescence and HPLC (Vollenweider 1969, Kling and Bridgeman 1999).

For routine analyses, and especially in monitoring of algal blooms, the spectrophotometric measurement of chlorophyll-*a* content is the most simple and fast. Lots of criteria for classification of lake water quality, based on trophic conditions, use as parameter the chlorophyll-*a* concentration (OECD 1982, Premazzi and Chiaudani 1992).

In spite of the great variability of chlorophyll-*a* content in phytoplankton (may vary with the light conditions, taxonomic composition, nutritional status, etc.), the researchers have shown a strong correlation between the chlorophyll-*a* concentration and biomass of phytoplankton (Raschke 1993, Vörös and Padisák 1991, Vörös *et al.* 2000) or periphyton (Barreto *et al.* 1997).

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The aim of this study was to make a comparison between two methods for algal biomass assessment (microscopic count and determination of chlorophyll-*a* content) in few aquatic ecosystems of the Danube Delta in the 2000–2002 period.

MATERIAL AND METHODS

The Romanian Danube Delta Biosphere Reserve (DDBR) is located in the eastern part of Europe (45° N latitude with 29° E longitude). Half of its surface represents the so-called "Danube Delta" and includes the area between the first bifurcation of the Danube River at Ceatal Chilia and the area bordered by the Black Sea to the east, Chilia branch to the north and Razim-Sinoie lagoon complex to the south (Oosterberg *et al.* 2000: RIZA Report).

Due to its species richness, this reserve occupies the third place in the world, after the Amazon and the Nile Delta. The importance of this area is confirmed by its present status: since 1990, the Danube Delta is included in the World Natural Heritage List and also in the Ramsar Convention List.

The Danube Delta area is divided in 4 main lake complexes: Sontea-Fortuna, Gorgova-Uzlina, Matita-Merhei, Roşu-Puiu. The studied lakes are located in the last complex. Lake Roşu is among the largest lakes of the Danube Delta (with a surface area of 1,365 ha) and it is well known for his camping and also for fishing contests. Potcoava and Tătaru are two smaller backwaters (with a surface area of about 20 ha), located in the northwestern and respectively, in the southeastern part of Lake Roşu (Fig. 1).

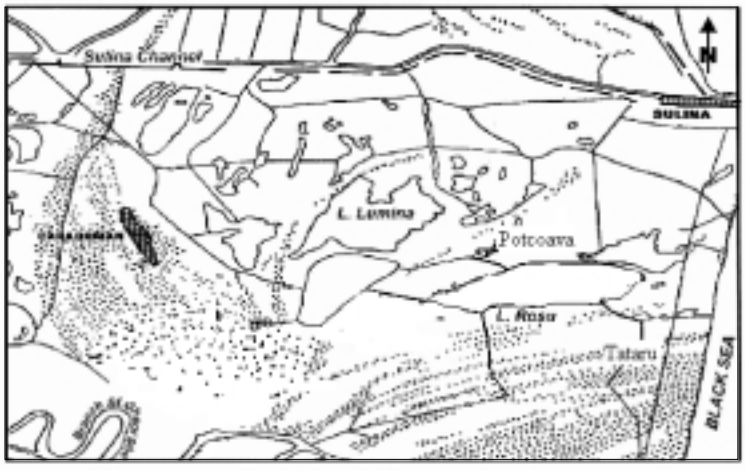

Fig. 1. Sketch map of studied area

The samples were collected in spring, summer and autumn of the years 2000–2002 from 3 stations in Lake Roşu, in 2000 and 2001 in Tătaru backwater and in 2002 in Potcoava backwater (an average sample was collected in both backwaters), on water column, using a Patalas device.

Microscopic count was performed in laboratory, after preservation of samples with 4% formaldehyde. The quantitative analysis of phytoplankton was made by direct Utermöhl technique (Utermöhl 1958). Phytoplankton biomass was calculated by multiplying the mean volume of each species with the number of cells for that species, on different size classes.

Chlorophyll-*a* was measured spectrophotometrically, after extraction in 90% ethyl alcohol, using a standard method (ISO 10260/1992). This method was criticised by Pápista *et al.* (2002), but we found a good correlation between the chlorophyll-*a* concentration (measured by ISO method) and the algal biomass, therefore we used it during our study.

The conversion of results obtained for chlorophyll-*a* from µg chl to mg C was made using the next coefficients: (a) assuming that chlorophyll-*a* constitutes, on the average, 1.5% of the dry weight of algae organic matter, the algal biomass may be estimated by multiplying the chlorophyll-*a* content by a factor of 67 (Raschke 1993); (b) 1 mg of chlorophyll-*a* corresponds to 40–50 mg C, varying with the taxonomic group, cell size, physiological status of the algae (Pourriot and Meybeck 1995).

RESULTS AND DISCUSSIONS

The dynamics of phytoplankton biomass follows the usual pattern for inland aquatic ecosystems in temperate zones: a raising tendency from spring to summer, when the maximum is reached due to the algal blooms, and a decreasing tendency from summer to autumn.

In spring, during all three years of study, the dominant group was Bacillariophyceae (*Fragilaria acus*, *F. ulna*, *Melosira granulata*, *Aulacoseira italica*, *Cyclotella chaetoceras*), decreasing from 80% in Lake Roşu to 63% in Potcoava backwater.

In summer, characteristic for eutrophic lakes, the dominant group was Cyanobacteria (*Microcystis aeruginosa*, *Anabaena spiroides*, *Oscillatoria tenuis*) in all studied lakes: in Lake Roşu with 50% in 2000 and 2002, while in 2001 the percent increased to 70%, in Tătaru backwater were registered 43% in 2000 and 76% in 2001, and in Potcoava backwater were 71% Cyanobacteria in 2002.

In autumn, there are significant differences between Lake Roşu and the other two: Lake Roşu is dominated by Cyanobacteria (*Oscillatoria minima*, *O. tenuis*) with 45% in 2000, 51% in 2001 and 62% in 2002. In Tătaru a high percent

of Chlorophyceae (*Chlorella vulgaris*, *C. ellipsoidea*) was measured in 2000 (over 90%) while in 2001 the dominant group was Cyanobacteria (*Aphanizomenon flos-aquae*, *Oscillatoria limosa*) with 59%. In 2002 Potcoava was dominated by Bacillariophyceae (*Fragilaria acus*, *F. ulna*) (Table 1).

Chlorophyll-*a* values follow the same pattern as the algal biomass values obtained by microscopic count (Table 2).

In spring, before the algal blooms, the mean values in studied ecosystems are ranging between 10–20 µg chl dm^{-3}: 11–16 µg chl dm^{-3} in Lake Roşu, 12 µg chl dm^{-3} in Potcoava and 20 µg chl dm^{-3} in Tătaru. In summer, the differences recorded between the lakes were significantly increased: in the pelagic zone of Lake Roşu (R2) were registered 104 µg chl dm^{-3} in 2000, 125 µg chl dm^{-3} in 2001 and 87 µg chl dm^{-3} in 2002. In Tătaru were recorded 90 µg chl dm^{-3} in 2001 and in Potcoava were 42 µg chl dm^{-3} in 2002. These differences are a consequence of fact that in Lake Roşu the primary production is dominated by phytoplankton, while in the backwaters the macrophytes are predominated. In autumn, the values decreased to 20–45 µg chl dm^{-3} in Lake Roşu, 43 µg chl dm^{-3} in Tătaru and 13 µg chl dm^{-3} in Potcoava.

Researches carried out about physiological adaptation to a sudden light up, show changes in shape and position of chloroplasts, a new configuration of photosynthetic systems and also changes in enzymatic activities involved in e^- transportation and CO_2 fixation (Pourriot and Meybeck 1995).

All the algae (and also the macrophytes), maintained in low light conditions, increase their content of chlorophyll-*a*/biomass unit. The shadow-

Table 1
Dominant algal groups in studied ecosystems (B = Bacillariophyceae, Cy = Cyanobacteria, Chl = Chlorophyceae)

	Lake Roşu and Tătaru backwater				
	R1	R2	R3	Xa lake	Xa backwater
2000					
April	B 81.45%	B 74.45%	B 86.16%	B 80.00%	B 70.60%
July	Cy 57.03%	Cy 44.47%	Cy 58.48%	Cy 53.00%	Cy 43.27%
October	B 42.78%	Cy 53.99%	Cy 43.30%	Cy 45.25%	Chl 93.95%
2001					
April	B 72.75%	B 83.24%	B 80.65%	B 78.88%	B 76.13%
July	Cy 62.27%	Cy 76.96%	Cy 72.48%	Cy 70.57%	Cy 76.03%
October	Cy 46.26%	Cy 62.87%	Cy 43.88%	Cy 50.84%	Cy 59.14%
2002	Lake Roşu and Potcoava backwater				
April	B 80.56%	B 54.63%	B 65.51%	B 66.90%	B 62.67%
July	Cy 53.42%	Cy 62.25%	B 60.24%	Cy 50.96%	Cy 70.67%
October	Cy 64.51%	Cy 51.93%	Cy 68.46%	Cy 61.63%	B 44.11%

Table 2
Chlorophyll-α content and algal biomass in studied ecosystems

	Lake Roşu and Tătaru backwater											
	chl-α (μg dm^{-3})			B (mg C dm^{-3}) (acc. Raschke 1993)			B (mg C dm^{-3})(acc. Pourriot and Meybeck 1995)			B (mg C dm^{-3}) microscopic count		
	IV	VII	X	IV	VII	X	IV	VII	X	IV	VII	X
2000												
R1	–	13.33	14.8	–	0.447	0.496	–	0.599	0.666	0.403	5.960	1.961
R2	–	103.66	19.04	–	3.473	0.638	–	4.665	0.857	0.963	5.050	2.224
R3	–	20.73	27.5	–	0.695	0.921	–	0.933	1.238	0.699	4.285	3.546
Xa Roşu	–	45.91	20.44	–	1.538	0.685	–	2.066	0.920	0.688	5.098	2.577
Xa Tătaru	–	–	0	–	–	0	–	–	0	0.398	0.423	0.132
2001												
R1	16.62	39.88	59.2	0.567	1.336	1.983	0.761	1.795	2.664	0.603	1.684	1.358
R2	19.04	125.3	25.9	0.638	4.198	0.868	0.857	5.639	1.166	0.882	3.866	1.188
R3	12.69	111.06	51.8	0.425	3.720	1.735	0.570	4.998	2.331	1.080	3.856	0.917
Xa Roşu	16.22	92.08	45.63	0.543	3.085	1.529	0.730	4.144	2.053	0.855	3.135	1.154
Xa Tătaru	20.73	89.86	43.07	0.695	3.010	1.443	0.933	4.044	1.938	0.285	2.507	0.652
2002	Lake Roşu and Potcoava backwater											
R1	19.74	32.21	7.40	0.661	1.079	0.248	0.888	1.449	0.333	0.418	2.834	0.715
R2	6.66	87.58	43.19	0.223	2.934	1.447	0.300	3.941	1.944	0.503	3.666	1.150
R3	5.92	55.53	19.25	0.198	1.860	0.645	0.266	2.499	0.866	0.460	4.969	1.799
Xa Roşu	10.78	58.44	23.28	0.362	1.958	0.780	0.485	2.630	1.048	0.460	3.823	1.221
Xa Potcoava	11.84	42.12	13.33	0.397	1.411	0.447	0.533	1.895	0.600	0.493	3.346	0.103

adapted algae contain 2–5 more chlorophyll-*a* than the algae living in bright light. It was demonstrated that adaptations at low lightening conditions may increase the content of auxiliary pigments (phycocyanine on Cyanobacteria or Cryptophyceae, peridinine on Dinoflagellata) or may decrease the ratio carothenoids/chlorophyll-*a* on numerous algae (Pourriot and Meybeck 1995).

Knowing the great variation of chlorophyll-*a* content in phytoplankton, we looked for a correlation between the chl-*a* concentration and algal biomass. We have found a significant linear relationship between them ($r = 0.630$, $p = 0.012$, $n = 15$) confirming the results obtained by other researchers (Raschke 1993) (Fig. 2).

We compared the results obtained by microscopic count (estimating biomass by volumetric method, using the number and specific average volumes and assuming the cell density to 1 g cm^{-3}) with the results obtained by spectrometric determination of chlorophyll-*a* (transformed from µg chl dm^{-3} to mg C dm^{-3}) in order to see the differences between these two methods.

The values of algal biomass obtained by microscopic counting are generally higher than those obtained by chlorophyll-*a* evaluation, but they follow

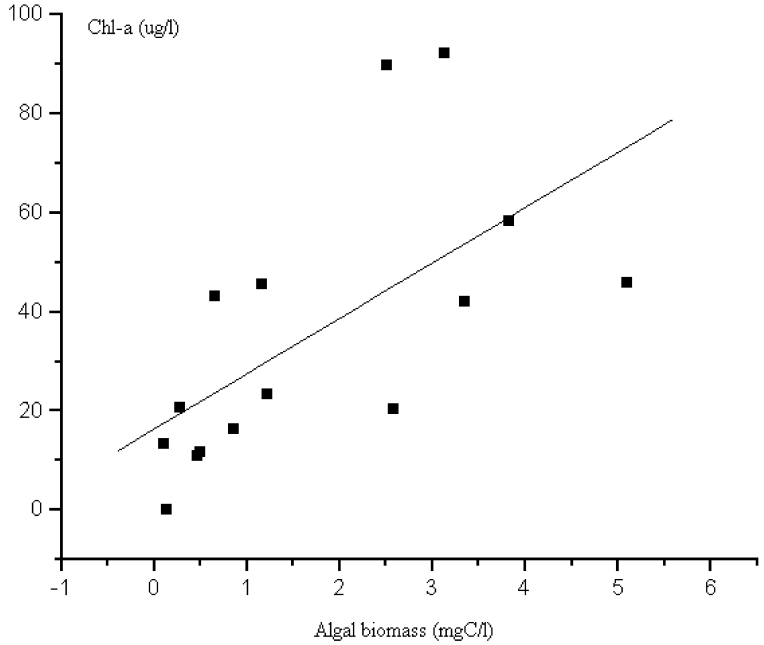

Fig. 2. Correlation between the chlorophyll-*a* concentration and the algal biomass measured and calculated by microscopic count

the same pattern. Figure 3 represents the dynamics of algal biomass (obtained by microscopic counting and by chlorophyll-*a* evaluation) in the studied ecosystems. It may be observed the known tendency, with maximum values in summer, during the algal blooms. In autumn, after the blooms, the values decrease, but they are still higher than in spring. The values recorded in Lake Roşu are higher than in the backwaters due to the predominance of phytoplankton primary producers.

The coefficient used by Pourriot to transform 1 mg chl-*a* in mg C (we chose a mean value of 45 mg C as equivalent of 1 mg chl-*a*) seems to be more

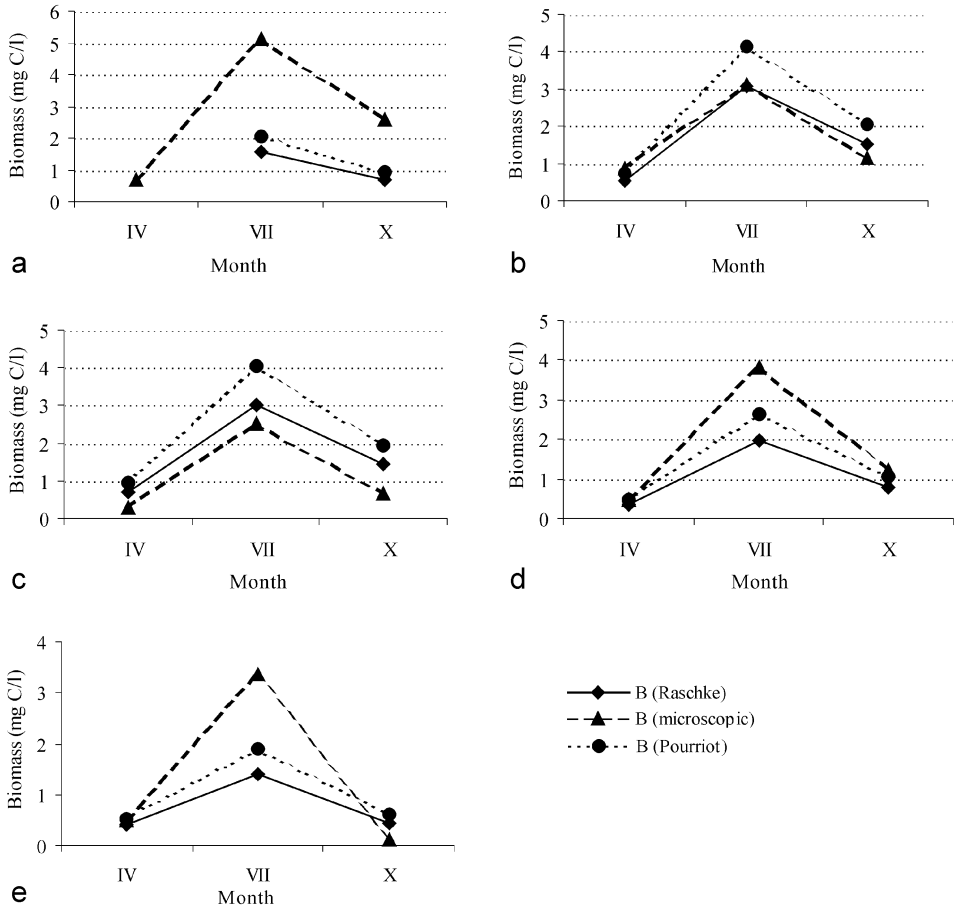

Fig. 3. Dynamics of algal biomass in studied ecosystems (a = Lake Roşu in 2000, b = Lake Roşu in 2001, c = Tătaru backwater in 2001, d = Lake Roşu in 2002, e = Potcoava backwater in 2002)

appropriate for the studied ecosystems because the values obtained in this way are closer to those obtained by microscopic counting (Fig. 3).

CONCLUSIONS

For an appropriate management of algal blooms it is very important to have reliable results in a short time. The spectrometric determination of chlorophyll-*a* content may be used as a faster method for assessing the algal biomass, this representing an important tool for the environmental quality assurance. It seems that the relationship established by Pourriot (1 mg chl-*a* = 40–50 mg C) is more appropriate for algal biomass evaluation in studied lakes. Further studies will be focused on the extension of these analyses in a large number of the Danube Delta lakes, in order to identify a more appropriate conversion relationship between the values obtained by chlorophyll-*a* determination and algal biomass obtained by microscopic counting.

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