TROPHIC STATUS OF DANUBE RIVER AND ITS TRIBUTARIES USING CHLOROPHYLL A AS AN INDICATOR

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Abstract

The study aims to assess the trophic state of Danube River and the main tributaries in the Southern part of Romania through chlorophyll „a" (Chl *a*) dynamics during 2015. The samples were collected with a seasonally frequency. Several environmental variables such as water temperature, pH, salinity, dissolved oxygen, nutrients were considered. In addition, the qualitative analysis of phytoplankton community was performed.
The integrative approach, which included both in-situ and laboratory analysis, highlighted positive correlation between abiotic and biotic parameters.
The chlorophyll „a" dynamics showed a close dependence with the sampling sites characteristics, the point sources of pollution, phytoplankton community and specific changes of chemical parameters.

Keywords*: chlorophyll a, Danube River, freshwater ecosystems, trophic status*

1. Introduction

The development of socio-economic systems is considered to be the most important event at international level and it is corroborated with the industrialization process in Romania, started in the second half of the nineteenth century [1], [2]. This has resulted in the environmental accumulation of a variety of chemical compounds, accumulation concentrated mainly at the river basins level, as it is the case of Danube River [3].
This fact induced significant changes at local and regional ecological systems [4], due to floodplains conversion into agricultural lands and to an extensive use of fertilizers. Moreover, dams were constructed and soils were drained to cut off floodplain areas from the river channel as well as canals were dug to drain water in wet periods or to water the field during dry periods of time. In particular, these large 'land amelioration' works have deprived floodplains of their wetland properties in many parts of the world, particularly in the temperate areas, for example the Rhine [5], Danube and Mississippi basins [6].
Recently, the Millennium Ecosystem Assessment has shown that global food production doubled in the past 40 years, and it has been able to keep up with the increasing human population [7]. However, the assessment also showed that this population increase is taking a toll on the environment causing major biodiversity losses, disruption of biogeochemical cycles, eutrophication and degradation of freshwater resources, as well as loss of regulating ecosystem functions [8].

Although, in the last two decades in the Danube River Basin the nutrients concentration decreased considerably due to development of urban wastewater treatment facilities, the pressures aforementioned (pollution from agriculture, industry and human agglomeration) lead to modification in oxygen budget, composition of aquatic species, and consequently trophic status. Many water quality variables have been described for the assessment of trophic status. At the European level the nutrient status, dissolved oxygen, ammonium, total phosphorus and total nitrogen were recommended as indicators for trophic status assessment [9]. Additionally, phytoplankton composition and chlorophyll *a* as an additional measure of biomass are necessary to assess the trophic level correctly [10].
The paper aims to assess the trophic status of Danube River – Romanian sector and its tributaries considering the relationship between Chlorophyll *a* (Chl *a*) content and the main water quality variables (nutrients, oxygen budget, organic load, salinity, hydrogen ion concentration and water temperature).

2. Materials and Methods

Danube River Basin - Romanian sector and its three main tributaries represented the study area. Sixteen sampling sites were selected along Danube River and its tributaries, as follows: nine on Danube River, two on Jiu River, two on Olt River and three on Arges River (Figure 1). The sampling sites were chosen considering the main points (human agglomerations, industrial and agricultural units, wastewater discharges) and/or nonpoint/diffuse sources of water pollution (pesticides, fertilisers, animal manure, rainfall run-off, etc).

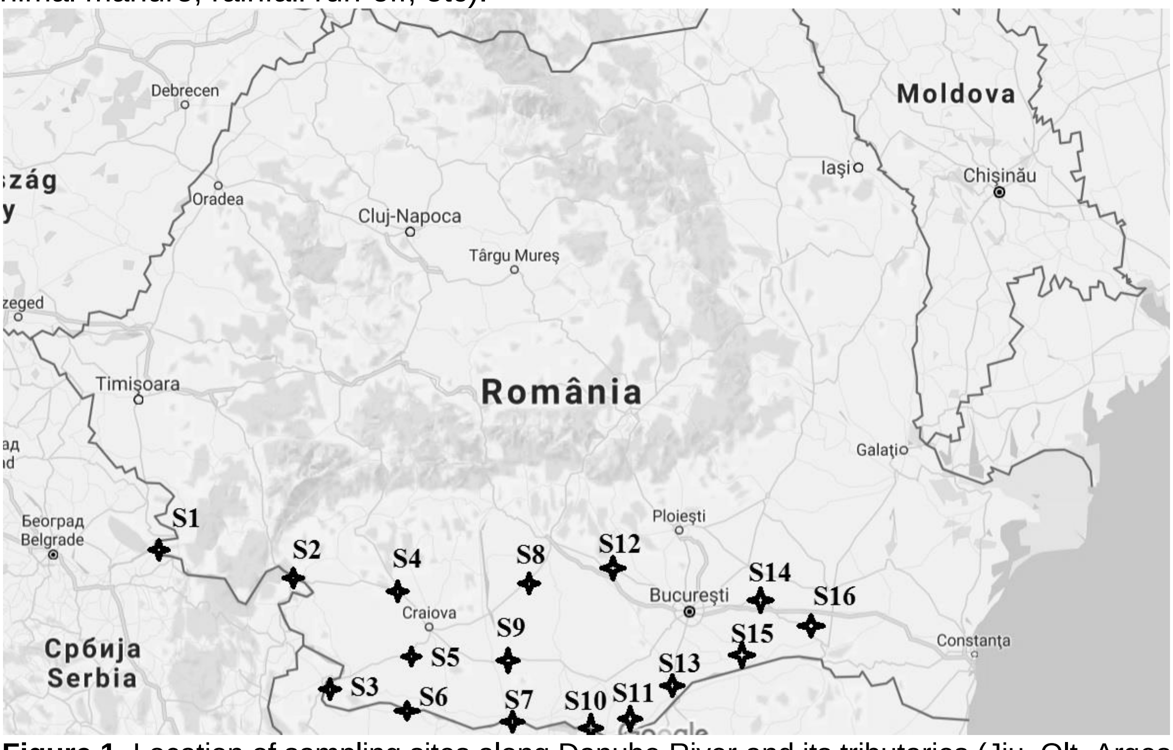


Figure 1. Location of sampling sites along Danube River and its tributaries (Jiu, Olt, Arges Rivers): **S1.** *Bazias* (Danube River); **S2.** *Gura Vaii* (Danube River); **S3.** *Calafat* (Danube River); **S4.** *Filiasi* (Jiu River); **S5.** *Podari* (Jiu River). **S6.** *Rast* (Danube River); **S7.** *Bechet* (Danube River); **S8.** *Slatina Bridge* (Olt River); **S9.** *Izbiceni* (Olt River); **S10.** *Islaz* (Danube River); **S11.** *Turnu Magurele* (Danube River); **S12.** *km 36* (Arges River); **S13.** *Giurgiu* (Danube River); **S14.** *Chirnogi* (Arges River); **S15.** *Oltenita* (Arges River); **S16.** *Calarasi* (Danube River).

The samples were collected with a seasonally frequency (in spring, summer and autumn of 2015).
T°C, pH, DO and conductivity were measured in-situ using 350i Multi-parameter Sentix 41-3, ConOx-3 (WTW, Germany). Nutrients were determined using Romanian standard methods, such as: ISO 7150/1 [11] for $N-NH_4$, ISO 7890/3 [12] for $N-NO_3$, EN 26777 [13] for $N-NO_2$, EN 12260 [14] for TN, EN ISO 6878 [15] for $P-PO_4$ and TP. Moreover, COD and BOD were analyzed according to ISO 6060 [16] and EN 1899 [17] methods.
Chl *a* concentration was determined following ISO 10260 [18] standard method: the water samples were filtered after collection. 0,5 L water sample was vaccum filtered through 47 mm GF/F Whatman filters. Then, Chl *a* was extracted with 90% ethylic alcohol and spectrometrical measured (Specord BU 205, Analytik Jena Germany). After eight hours, the extract absorbance was measured before and after acidification (with HCl 3mol*L^{-1}) at 665 nm and 750 nm. Qualitative analysis of phytoplankton was made using Leica Dmi8 inverted microscope (Leica Microsystems Inc., USA).
Moreover, the total nitrogen to total phosphorus ratio (TN:TP) was used as a measure of the tendency of a water body to support an algal bloom. TN:TP ratio ranging between 25 to 40:1 correspond to un-impacted rivers whereas TN:TP ratio of less than 10:1 correspond to most impacted rivers [19].

3. Results and Discussion

The Chl *a* content as well as the environmental variables: water temperature (T°C), hydrogen ion concentration (pH), dissolved oxygen (DO), organic content (measured as: chemical oxygen demand (COD) and biochemical oxygen demand (BOD)), salinity (conductivity) and nutrients ($N-NH_4$, $N-NO_3$, $N-NO_2$, total nitrogen (TN), $P-PO_3$, total phosphorus (TP)) were strongly related to sampling sites, associated anthropogenic pressures and seasonal variations.

Water quality variables characterization

The water temperature showed in 2015 a seasonal dynamics, the lowest value was measured in spring 5,3°C (Danube River) and the highest water temperature was recorded 24,6°C in summer along Jiu River. In terms of acidification, the pH values measured during 2015 ranged between 6,2 in spring (Danube River) and 8,7 in summer (Olt River).
DO concentration measured along Danube and its tributaries during the three studied seasons of 2015 did not significantly fluctuated. According to US Environmental Protection Agency [20], DO is not a necessary variable to be measured in lotic systems. The organic load (expressed as COD and BOD) was greater at Arges River (12,9 mgO_2*L^{-1} COD and 4,17 mgO_2*L^{-1} BOD) and Olt River (12,4 mgO_2*L^{-1} COD and 3,64 mgO_2*L^{-1} BOD), than Danube River (6,65 mgO_2*L^{-1} COD and 2,26 mgO_2*L^{-1} BOD) (Table 1).
Salinity (measured as conductivity) had seasonal and spatial related patterns. Along Danube River, the conductivity values ranged between 326 µS*cm^{-1} at S13 (Giurgiu) in autumn and 468 µS*cm^{-1} at S10 (Islaz) during summer. The highest conductivity values were measured along Olt River, mainly at S8 (Slatina Bridge), 674 µS*cm^{-1} during spring and 696 µS*cm^{-1} during summer. These values may be explained by the wastewater discharges resulted from industrial activities of SC Oltchim S.A and USG Ciech Chemical Group Govora, dowstream Ramnicu-Valcea county. A negative correlation was observed between Chl a content and conductivity values along Olt River (Table 1).

Table 1. The main environmental variables detected along Danube River and its tributaries during 2015 (*the standard deviation was applied to the average values calculated from the values seasonally detected*

Environmental Variables	***U.M***	***Danube River***	***Jiu River***	***Olt River***	***Arges River***
		Avg ± std	**Avg ± std**	**Avg ± std**	**Avg ± std**
Temperature	°C	15.2 ± 5.53	17.5 ± 3.93	16.8 ± 4.12	14.4 ± 4.65
pH	pH units	7.15 ± 0.51	7.47 ± 0.56	7.87 ± 0.81	7.04 ± 0.56
DO	mgO_2*L^{-1}	6.10 ± 1.14	6.46 ± 0.88	6.56 ± 0.88	5.89 ± 1.66
CCOCr	mgO_2*L^{-1}	6.65 ± 3.16	9.71 ± 7.01	12.4 ± 4.63	12.9 ± 5.84
CBO_5	mgO_2*L^{-1}	2.26 ± 1.06	3.36 ± 2.21	3.64 ± 0.96	4.17 ± 1.78
Conductivity	$\mu S*cm^{-1}$	395 ± 39.8	356 ± 71.1	535 ± 123	409 ± 41.8
$N\text{-}NH_4^+$	$mg*L^{-1}$	0.09 ± 0.07	0.19 ± 0.14	0.18 ±0.153	0.21 ± 0.27
$N\text{-}NO_3^-$	$mg*L^{-1}$	1.64 ± 0.42	2.17 ± 0.56	1.61 ± 0.94	1.68 ±0.41
$N\text{-}NO_2^-$	$mg*L^{-1}$	0.004 ± 0.001	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.001
TN	$mg*L^{-1}$	2.53 ± 0.49	3.48 ± 0.69	2.86 ± 0.39	2.75 ± 0.46
$P\text{-}PO_4^{3-}$	$mg*L^{-1}$	0.05 ± 0.01	0.08 ±0.08	0.04 ± 0.01	0.07 ± 0.03
TP	$mg*L^{-1}$	0.068 ± 0.01	0.12 ± 0.15	0.065 ± 0.02	0.082 ± 0.04
Chlorophyll a	$\mu g* L^{-1}$	**8.24 ± 7.05**	**15.1 ± 24.7**	**4.2 ± 2.51**	**9.21 ± 4.76**

This lotic system also exhibited the most alkaline water, the average value of pH was of 7,87 pH units.

Nutrients concentration varied over a wide range of concentrations showing different peaks at particular lotic ecosystems: $N\text{-}NH_4$ concentrations reached its maximum along Arges River (0,84 $mg*L^{-1}$ at S12 (km 36) in autumn); the greater values of $N\text{-}NO_3$ (2,74 $mg*L^{-1}$) at S4 (Filiasi) and TN (4,62 $mg*L^{-1}$) at S5 (Podari) during summer, $P\text{-}PO_4$ (0,24 $mg*L^{-1}$) and TP (0,42 $mg*L^{-1}$) at S4 (Filiasi) during spring were detected along Jiu River.

Chlorphyll a variation

In terms of spatial distribution, Chl *a* content increased from upstream to downstream Danube River sampling sites; the highest Chl *a* values were recorded at S13 (Giurgiu) (13,9 µg/L) and S16 (Calarasi) (16,8 µg/L).

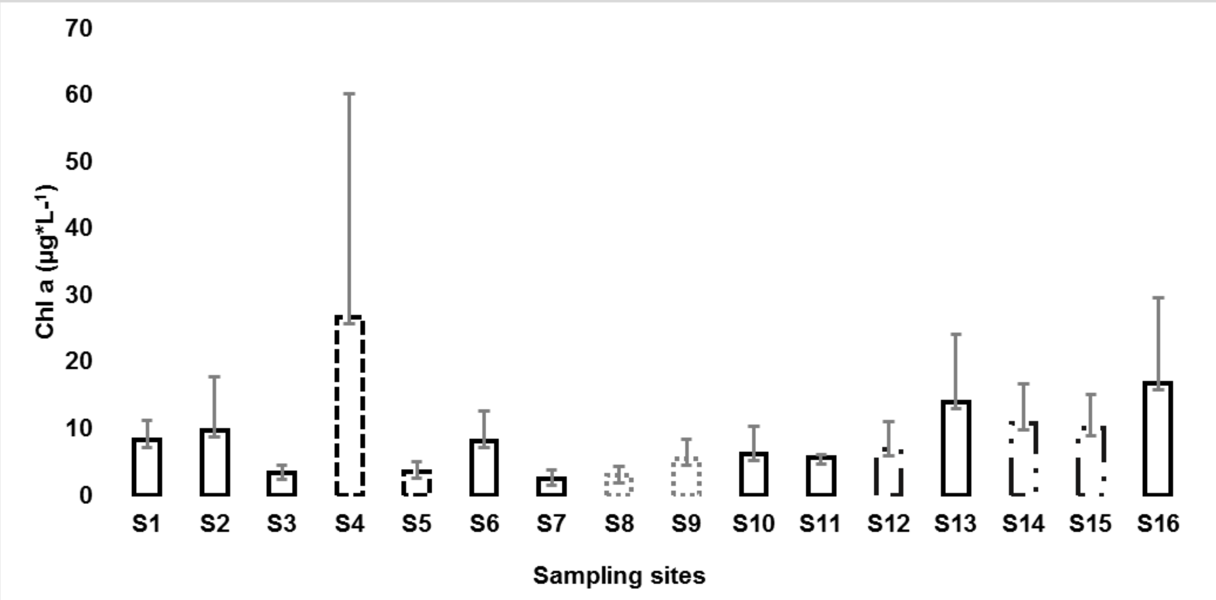


Figure 2. Spatial variation of Chl a during 2015 along Danube River and its tributaries

Legend: Danube River | Jiu River | Olt River | Arges River

Moreover, along Danube`s tributaries, it was observed that the annual concentration of Chl *a* on Jiu River (26,7 µg/L at S4 (Filiasi)) was greater than Chl *a* values obtained on Olt River and Arges River. During 2015, the average Chl *a* concentration ranged from 1,47 µg/L at S8 (Slatina Bridge) to 2,90 µg/L at S9 (Izbiceni) along Olt River. Along Arges River, Chl *a* content varied from 6,88 µg/L at S12 (km 36) to 10,8µg/L at S14 (Chirnogi) (Figure 2).
The seasonal pattern of surface Chl *a* distribution emphasized high variability, which was related to both anthropogenic pressures and meteorological conditions. Chl *a* showed a spring maximum linked mainly with the Danube discharges and nutrients inputs (Figure 3).

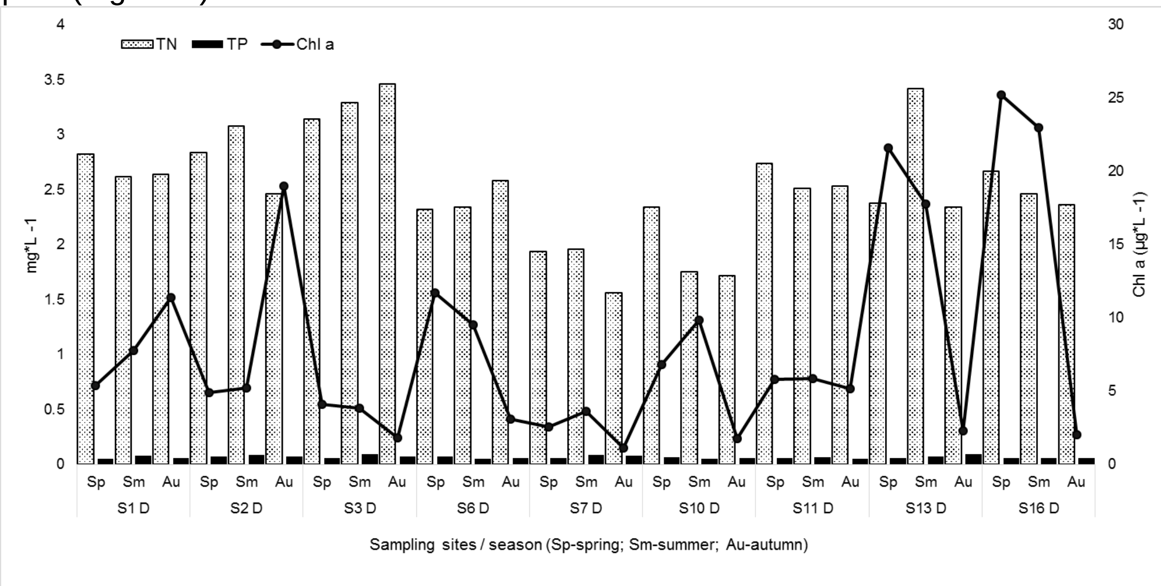
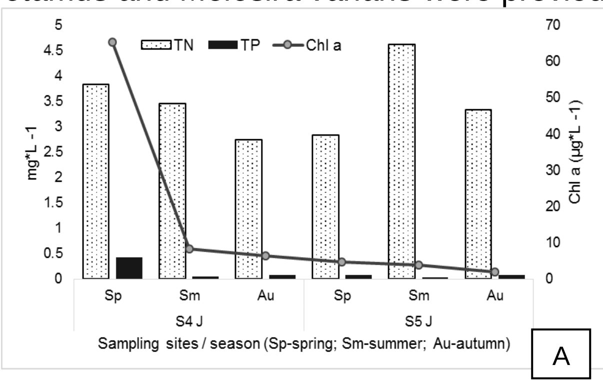
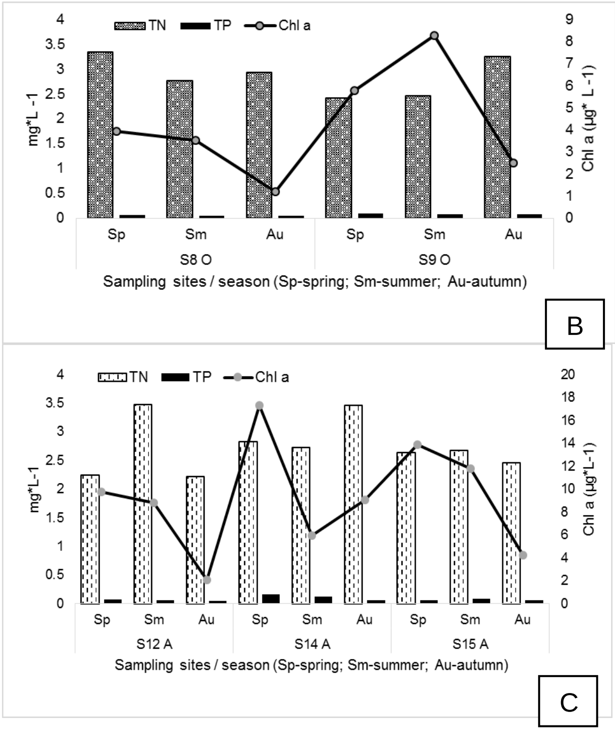


Figure 3. Temporal variation of Chl a during 2015 along Danube River

High Chl *a* content was detected in spring (21,6 µg*L^{-1} at S13 and 25,2 µg*L^{-1} at S16) and summer (17, 7 µg*L^{-1} at S13 and 23 µg*L^{-1} at S16) downstream Danube River sampling sites, S13 (Giurgiu) and S16 (Calarasi), due to Arges water discharges as well as anthropogenic point souces from the aforementioned cites (Giurgiu and Calarasi).
Moreover, the qualitative analysis of phytoplankton community along Danube River sampling sites showed predominantly, the presence of diatoms (Baccilarophycae) and green algae (Chlorophycace). Among diatoms, *Aulacoseira granulata, Skeletonema potamus and Melosira varians* were previously reported along Danube River [10].



Chl *a* dynamics along Jiu River recorded an increase during spring which was correlated with TP concentration. The Chl *a* content detected at S4 (Filiasi) was of 65,4 µg*L^{-1}, decreasing in the next two seasons. The increased Chl *a* value as well as TP concentration may be explaind by the phytoplankton



development during spring and / or wastewater discharges from agriculture units. The phytoplankton community along Jiu River was dominated by the diatoms (*Cymbella ventricosa, Navicula gracilis, Gomphonema acuminatum*) and less frequently Clorophycae (*Ulothrix zonata*) (data not shown).

As for Olt and Arges Rivers, during 2015, the temporal variation of Chl *a* was positive correlated with the TP and P-PO_4 concentration. The highest Chl *a* concentration measured along Arges River at S14 (Chirnogi) during spring was of 17,3 µg*L^{-1}. As in the case of Danube River and Jiu River ecosystems, along Olt River in terms of phytoplankton dominated the diatoms (*Synedra acus, Nitzchia sigmoidea, Gyrosigma acuminatum*) and Chlorophyceae (*Pediastrum duplex*) (data not shown).

Figure 4. Temporal variation of Chl a during 2015 along Danube`s tributaries (Jiu-A, Olt-B, Arges-C)

Trophic status assessment

For the assessment of trophic status, total nitrogen to total phosphorus ratio (TN:TP) was used [19]. Thus, the TN:TP ratio was applied on the values detected along Danube River and its tributaries during 2015 (Table 2).

Table 2. TN:TP ratio calculated along Danube River and its main tributaries (Jiu, Olt and Arges)

TN:TP ratio / lotic systems	***Danube River***	***Jiu River***	***Olt River***	***Arges River***
TN:TP	38:1	54:1	47:1	38:1

Legend:
TN:TP ratio - 25 to 40:1 un-impacted rivers;
TN:TP ratio - < 10:1 most impacted rivers.

The TN:TP values calculated for Danube River and its tributaries emphasized that during the study period, the values were above or in range of the TN:TP ratio corresponding for un-impacted rivers.

Additionally, according with the Romanian Norm 161/2006 [21] for the classification of surface water quality, the average values of Chl *a* measured during 2015 along Danube River and its tributaries framed the studied ecosystems into quality Class I (Table 3).

Table 3. Chlorophyll a content along Danube River and its main tributaries (Jiu, Olt and Arges)

	U.M	***Danube River***	***Jiu River***	***Olt River***	***Arges River***
Chlorophyll a	$\mu g * L^{-1}$	**8.24**	**15.1**	**4.2**	**9.21**
Romanian Norm 161/2006 for Quality Class I	$\mu g * L^{-1}$	**25**			

The dissolved oxygen, ammonium, total phosphorus, total nitrogen alongside chlorophyll a and phytoplankton composition were considered the main water quality indicators for the assessment of trophic status.
In this study, Chl *a* had a positive relationship more with TP and $P\text{-}PO_4$ concentration than with TN, $N\text{-}NH_4$, $N\text{-}NO_3$, $N\text{-}NO_2$, although previous studies found no significant correlation between Chl *a* and P-PO4 and TP, mainly due to phosphorus consumption linked to growth of diatoms [22].
Furthermore, the absorption of nitrate by the phytoplankton community has been widely disscused, in particular during the daylight conditions [23]. The negative relationship between nitrate and Chl *a* corroborated the idea of modulating the nitrate concentration by phytoplankton [24].
Dissolved oxygen variation influenced Chl *a* content along Danube river and its tributaries during high water levels, when allochthonous inputs increased and thus decreasing the oxygen concentration in the water column which in turn could affect aquatic biota [25].
Although, the health of water bodies improved in the last few years, due to wastewater treatment plants construction, Romanian economical transitions (decreasing the industrial production), interruption of agriculture plans, yet, important steps forward must be done to meet the European requirements.

4. Conclusions

The study was done using data collected in 2015 along Danube River – Romanian sector and three of its main tributaries (Jiu, Olt, Arges). It emphasised the spatial and seasonal variation of Chl *a* as well as of various water quality variables used to assess the trophic status.
The higher ranges encountered in the present study, either of Chl *a* or nutrient concentrations, indicated changes in water quality. The environmental variables dynamics suggested that phosphorus and nitrogen were the most important nutrients regulating autotrophic state along the studied lotic systems. It was observed that along Danube River sampling sites as well as its tributaries, Chl *a* dynamics was more related to the increase TP and $P\text{-}PO_4$ than of TN, $N\text{-}NH_4$, $N\text{-}NO_3$, $N\text{-}NO_2$ variation.
The TN:TP ratio calculated for Danube River and its tributaries during 2015 indicated a trophic status linked to un-impacted rivers. Moreover, the average values of Chl *a* measured during 2015 along Danube River and its tributaries framed the studied ecosystems into quality Class I.

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References

[1] Zalasiewicz, J., Williams, M., Steffen, W., Crutzen, P., (2010), The new world of the Anthropocene, *Env. Sci. & Tech*., **44**: 2228–2231.
[2] Stoica, C., Gheorghe, Ş., Păun, I., Stănescu, E., Dinu, C., Petre, J., Lucaciu, I., (2014), Long-term biological changes along Danube Delta systems after industrialization period, *Romaqua*, **1**(91): 14-20.
[3] Keller, E., Barbara, M., Lathja, K., Cristofor, S., (1998), Trace metal concentrations in the sediments and plants of the Danube Delta Romania, *Wetlands*, **18**(1):42-50.
[4] Vadineanu, A, 2004, *Managementul dezvoltarii: o abordare ecosistemică*, Editura Ars Docendi, Bucuresti, 394 (in Romanian).
[5] Nienhuis, N.H., (2008), *Environmental history of the Rhine–Meuse Delta: an ecological story on evolving human–environmental relations coping with climate change and sea-level rise,* Edition no 1, Springer Netherlands, Berlin, 588.
[6] Mitsch, W.J, Gosselink, J.G., (2000), The value of wetlands: importance of scale and landscape setting, *Ecological Economics*, **35**:25–33.
[7] Millennium Ecosystem Assessment, (2005), *Ecosystems and human well-being. Current state and trends*, Hassan, R., Scholes, R., Ash, N.(Eds), Volume 1, Island Press, Washington, DC, 47.
[8] Verhoeven, J.T.A and Setter, T.L., (2010), Agricultural use of wetlands: opportunities and limitations, *Ann. Bot*., **105** (1): 155-163.
[9] Noges, T., (2003), *Final report on the relevant system of indicators and criteria for evaluating the ecological status of a very large non-stratified lake and its river basin in WFD context*, Deliverable D3b, Tartu University, Estonia.
[10] Dokulil, M., Donabaum, U., (2015), *Phytoplankton*, *Joint Danube Survey 3, A comprehensive analysis of Danube water quality,* Liska, I., Wagner, F, Sengi, M., Deutsch, K., Slobodnik, J.(Eds), ICPDR-International Commission for the Protection of the Danube River, Vienna, 369.
[11] ISO 7150-1, (2001), Water quality. Determination of ammonium. Part 1: Manual spectrometric method.
[12] ISO 7890-3, (2000), Water quality. Determination of nitrate. Part 3: Spectrometric method using sulfosalicylic acid.
[13] EN 26777, (2002), Water quality. Determination of nitrite: Molecular absorption spectrometric method.
[14] EN 12260, (2004), Water quality. Determination of nitrogen. Determination of bound nitrogen (TNb) following oxidation to nitrogen oxides.
[15] EN ISO 6878 (2005) Water quality. Determination of phosphorus. Ammonium molybdate spectrometric method (ISO 6878:2004).
[16] ISO 6060, (1996), Water quality. Determination of chemical oxygen demand.
[17] EN 1899, (2003), Water quality. Determination of biochemical oxygen demand after n days (BODn)-Part 1: Dilution and seeding method with allylthiourea addition.
[18] ISO 10260, (1996), Water quality. Measurement of biochemical parameters. Spectrometric determination of the chlorophyll a concentration.
[19] DWAF, (1996), *South African water quality guidelines*, *volume 1: Aquatic ecosystems*, Department of Water Affairs and Forestry, Pretoria, South Africa.
[20] EPA, (1999), Nutrient Criteria. Technical Guidance Manual. Rivers and Streams. Washington DC, https://www.epa.gov/sites/production/files/documents/guidance_rivers.pdf.
[21] Ordin nr. 161, (2006) pentru aprobarea Normativului privind clasificarea calitatii apelor de suprafata in vederea stabilirii starii ecologice a corpurilor de apa (In Romanian), http://www.rowater.ro/dacrisuri/Documente%20Repository/Legislatie/gospodarirea%20apelor/ORD.%20161_16.02.2006.pdf.
[22] Stoica, C., Paun, I., Stanescu, E., Lucaciu, I., Niculescu, D., (2013), Spatial and temporal variation of chlorophyll a along Danube River, *Journal of Environmental Protection and Ecology*, **14** (3): 864-874.

[23] Thomaz, SM., Pagioro, TA., Bini, LM., Roberto, MC., Rocha, RRA., (2004). *Limnology of the Upper Paraná Floodplain habitats: patterns of spatio-temporal variations and influence of the water levels,* In: *The Upper Paraná River and its floodplain: physical aspects, ecology and conservation*, Thomaz, SM., Agostinho, AA., Hahn, NS. (Eds.), Leiden: Backhuys Publishers, 76-102.
[24] Rocha, RRA., Thomaz, SM., Carvalho, P., Goes, LC., (2009), Modelling chlorophyll-a and dissolved oxygen concentration in tropical floodplain lakes (Parana River, Brazil), *Braz.J.Biol.,* **69** (2, Suppl.): 491-500.
[25] Carvalho, P., Bini, LM., Thomaz, SM., Oliveira, LG., Robertson, B., Tavechio, WLG., Darwisch, AJ., (2001) Comparative limnology of South American floodplain lakes and lagoons, *Acta Scientiarum*, **23** (2): 265-273.