

# Spatio-Temporal Characterization of Nutrient Pollution in Lower Danube Area

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*Eutrophication of large river systems, as a result of the increasing content of nutrients, is one of the major issues in water quality facing the authorities in the European countries. It is closely related to the environment, incurring profound effects on biodiversity of aquatic ecosystems, yet not fully understood so far. The present study is based on the characterization of spatio-temporal trend of nutrient pollution of the Danube River during one year, namely, from September 2012 to September 2013 in Calarasi-Braila sector. The studied area was chosen on the basis of some construction works which took place for improving the navigation conditions, which might have a potential negative impact on water quality and on biodiversity. Water quality assessment was carried on in 10 monitoring stations, which revealed significant pressure upstream pollution. The results disclosed the level of eutrophication and the relationship between the parameters analyzed and the influence of the land usage.*

*Keywords: eutrophication, nutrients, surface water, Danube River*

The quality of surface water is an important issue worldwide. Low water quality reflects the negative influence of human activities on the environmental status, which may generate large imbalances in aquatic ecosystems [1-4]. In such cases, sustainable water management has become an important task being achieved by regular assessments of water quality, stipulated by law at national and international level. Such management is accomplished by providing accurate information on the status and trends of the evolution of ecological systems, as well as by adequate evaluation methods of data monitoring [5-7].

As a consequence of the increasing content of nutrients in natural waters, the eutrophication process may occur in large river systems [8-10]. Likewise, the environment is affected and leads to negative consequences on biodiversity of aquatic ecosystems which are not fully known [11-13].

The eutrophication process is generally understood as an enrichment nutrients in water bodies (especially chemical forms of nitrogen and phosphorus) [14], which can enhance the production of algae and higher plants, as well as to increase biomass production at high turbidity, and sometimes entailing an unpleasant odor and taste of the water [15-17]. The development of algal affects water transparency and concentration of dissolved oxygen, such phenomena being accompanied by the disappearance of aquatic fauna and, ultimately, fostering the formation of marshes [15, 16]. The conditions created by modern agriculture, urbanization, and industrialization have led to disruption of nutrient quantity (especially nitrogen and phosphorus forms) and to a considerable increase of their quantity [18], both from diffuse and point sources in surface water and groundwater [15, 16]. Normally, the eutrophication is a natural phenomenon but, more recently, the same term has been used to refer to the presence of extra and unwanted nutrients in water bodies [8, 10]. Therefore, approximately 33 % of the monitoring stations

of the European rivers and lakes and some coastal waters indicate signs of eutrophication [19].

In the aquatic ecosystem, pollutants, including the nutrients, are distributed differentially between biotic and abiotic compartment, this distribution being determined by the water transport and suspended matter, by the chemical and biological transformation processes, and by the distribution processes, such as adsorption and desorption on sediments and on suspended matter, evaporation from the aqueous phase, and bioaccumulation in trophic chain. In the same time, the chemical structure of the pollutant is important because it can directly or indirectly lead to an impaired ecosystem [14]. Unlike other indicators, the presence of nutrients can be influenced by some phenomena, mainly by assessing the diffuse pollution (when by precipitation salts based on nitrogen and phosphorus from the soil surface are entrained), by the denitrification processes, and by the precipitation processes of orthophosphates at alkaline pH, as well [20].

At European level, a great attention has been paid to achieving good qualitative and quantitative status of all water bodies. Water Framework Directive/2000/60/EC (WFD) is the most substantial and complex legislative provision of water management that has been developed by the European Commission, being the most important legal instrument in the area of water and a prerequisite for the successful implementation of the concept of management integrated medium in all European countries.

WFD, provides descriptive definitions for five quality classes that characterize the ecological status of water (high, good, moderate, poor, and bad), classes established on the base of biological quality elements, hydro-morphological, chemical and physico-chemical with specific limits for each EU country [21, 22]. The main goal of WFD is the protection and improvement of the status for all bodies of water to the level of "good state", i.e., to assure a good hydro-chemical and ecological state of water.

Reduction in the presence of nitrates in waters is part of

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WFD, which establishes a comprehensive and transboundary approach regarding the protection of water organized around river basin districts in order to achieve good status of European water bodies by 2015.

Danube River, as one of the most important inland waterway of Europe and a natural support for many species of flora and fauna, some of them being endangered, has been a subject of constant interest both locally and internationally [12, 17]. The states from Danube River Basin, including Romania, is cooperating for implementing the WFD at the Danube Basin level and for achieving a unitary Management Plan under the coordination of the International Commission for the Protection of the Danube River (ICPDR) [21, 23].

The present study intends to characterize the spatio-temporal trend of nutrient pollution in the Danube River during one year, from September 2012 to September 2013 in the Calarasi-Braila stretch, km 375 - km 175, a location where works are in progress (construction of submersible thresholds) to improve navigation conditions. The degree of eutrophication is evaluated for 10 monitored locations, aiming to establish the relationships between physico-chemical parameters and land exploitation impact.

### Experimental part

The Danube River, the second largest river in Europe, with a length of 2857 km and a drainage area of the hydrographic basin of almost 801 500 km<sup>2</sup>, is one of the most important waterways inside Europe. Having 2414 navigable kilometers, the Danube River is classified as European artery E80 by European Agreement on Main Inland Waterways of International Importance, which was adopted by the Interior Transport Committee of the UNECE and ratified by the Romania's Government in 1998 [24, 25].

In Romania, the Danube has a length of 1075 km and owns about 31 % of the total catchment area of the Danube River, rising to approximately 250 000 km<sup>2</sup>. The Danube

River enters Romania loaded with pollutants from upstream and constitutes the most important source of pollution flowing into the Black Sea [26].

Samples were collected from the left bank, right bank and the center of the Danube River in 10 locations indicated in table 1 and preserved according to standard methods for surface water [27]. In this paper, the average values of the indicators were employed.

Table 1 presents the critical points of monitoring with the respective locations, corresponding of the monitoring plan established by this study, their general characteristics, as well as the geographical coordinates of sampling stations [28].

### Sampling

Water samples were collected monthly from 10 locations (table 1) from the some sections of the Lower Danube (fig. 1) between September 2012 (Month 1) - August 2013 (Month 12), and the following nutrients were determined: nitrogen forms, respectively: nitrates (NO<sub>3</sub>-N), nitrites (NO<sub>2</sub>-N), ammonia nitrogen (NH<sub>4</sub>-N), total nitrogen (TN), and phosphorus forms, total phosphorus (TP) and orthophosphates (PO<sub>4</sub>-P), Chlorophyll 'a', as well as the variation of flow rate (Q). It should be noted that in these monitoring stations were not met Danube Commission's recommendations to maintain minimum depths during dry seasons for normal navigation, this being the main reason for which hydrotechnical works for improving the navigation conditions have been started.

During the sampling campaigns, each water sample was collected in a clean polyethylene container, previously decontaminated, and kept at 4 °C during transportation to the laboratory for analysis.

### Reagents and methods

The reagents (Merck) used throughout the analyses were of analytical purity, the solutions were prepared with double-distilled and deionized water, and the

Watercourse	Notation of sampling site	Geographical coordinates		Length (km)	Average flow (m <sup>3</sup> ·s <sup>-1</sup> )
		Longitude	Latitude		
Bala Area	L1	27°34'9.549"	44°11'24.35"	347	4654
	L2			345	3087
	L3			344	1580
	L4			343	3514
Epurasu Island	L5	27°37'2.349"	44°11'59.10"	340	1361
	L6			341	219
	L7			334	1576
Lupu Island	L8	27°54'27.93"	45°4'0.316"	197	2713
	L9			196	1645
	L10			195	4367

Table 1  
SAMPLING SITE LOCATIONS AND THEIR CHARACTERISTICS

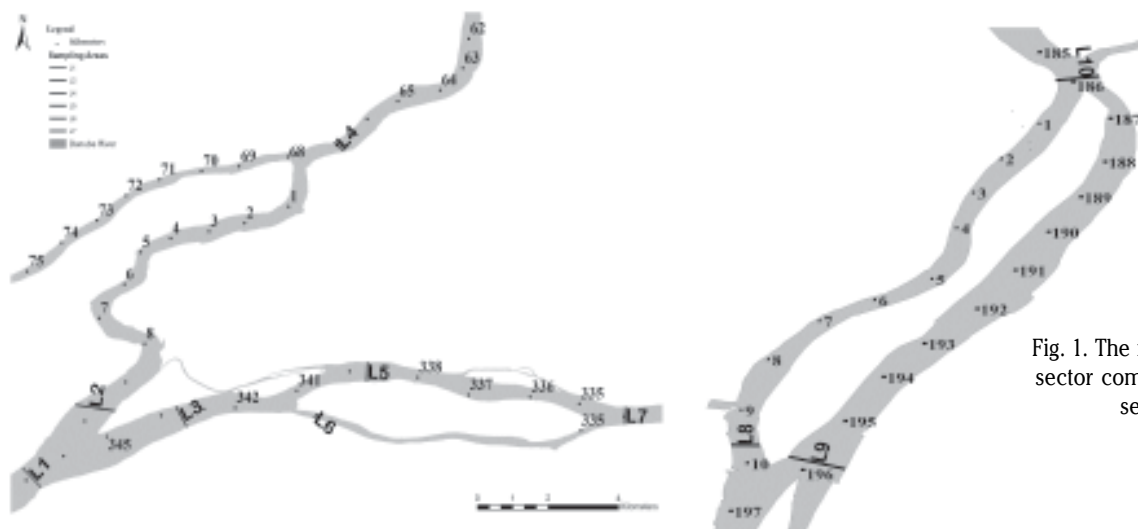


Fig. 1. The map of the Danube sector comprising monitoring sections [7]

determinations were carried out according to the present standard (Standard Operating Procedure) [29-34].

The nitrogen and phosphorus forms were analyzed in dissolved status and results being corroborated with the ones from the annual ICPDR reports [35].

Determination of the nitrate ions was based on the formation of a yellow-colored complex by the reaction of sulfosalicylic acid in an alkaline medium, and the measure of the compound absorbance was done at a wavelength of 415 nm, with LOD 0.005 mg·L<sup>-1</sup> and LOQ 0.014 mg·L<sup>-1</sup>. The reaction of nitrite ions at pH 1.9 with the reagent 4-amino benzene sulfonamide, in the presence of orthophosphoric acid formed a complex of red color, and in this case, the absorbance was measured at 540 nm, with LOD 0.001 mg·L<sup>-1</sup> and LOQ 0.003 mg·L<sup>-1</sup>. The determination of the ammonium is based on measuring the absorbance at a wavelength of 650 nm of a blue compound resulted from the reaction of ammonium ions with ions salicylate and hypochlorite in the presence of sodium nitroprusside, with LOD 0.0011 mg · L<sup>-1</sup> and LOQ 0.0028 mg · L<sup>-1</sup>. The total nitrogen after acidification and distillation using the Kjeldahl method, was determined titrimetric, with LOD 0.016 mg · L<sup>-1</sup> and LOQ 0.042 mg · L<sup>-1</sup>.

Total phosphorus was analyzed spectrophotometrically using unfiltered samples, the concentration being determined after acidification with sulfuric acid and a mineralization with peroxodisulphate of potassium and the orthophosphate was determined spectrophotometrically after acidification with sulfuric acid and with color reagent, forming a blue compound measured at a wavelength of 880 nm. The detection limit LOD was 0.0017 mg · L<sup>-1</sup> and limit of quantification LOQ was 0.0068 mg · L<sup>-1</sup>. Water flow rate was calculated based on the measurements of level.

Determination of Chlorophyll 'a' was based on collecting algae and other suspended matter from a water sample by filtration, extraction of algal pigments from the filter residue into hot ethanol and after acidification on the extract, was measured concentration at wavelength 665 nm, with LOD 0.0026 mg · L<sup>-1</sup> and LOQ 0.0104 mg · L<sup>-1</sup>.

The presented results represent an average of the values obtained by using of a UV-Vis spectrometer Cary 300 Bio on a section.

## Results and discussions

In order to establish the degree of eutrophication (Chlorophyll 'a' concentration), the chemical speciation of nutrients was taken into account [24]. Also, interparametric correlations were performed and the ratio TN/TP of nutrients was calculated, taking into account both the pollutants pressure and impact on environment. It was considered that the assessment of Danube impact monitoring should be conducted on causality and the pressures upstream, knowing that upstream sectors are subject to numerous and varied types of pressure that can alter the hydrological characteristics, physico-chemical, and biological properties of the Danube from entering the country [26].

### Chemical Speciation

In Danube, particles released from the decomposition of macrophyte species, are partially stored on sediments and in suspended matter, and thus significant amounts of inorganic and organic nutrients are discharged in the Nord-Western part of the Black Sea [24, 26]. The amount of organic or inorganic nitrogen in dissolved form or in suspension form constitutes the total nitrogen, the same statement being also in the phosphorus case.

For characterizing the share of all the forms of nitrogen in total nitrogen, and of the orthophosphates (the most

**Table 2**  
NUTRIENTS FORMS PERCENTAGES

Nutrients	Weighting (%)
TIN in TN	74.78
NO <sub>2</sub> -N in TIN	3.73
NO <sub>3</sub> -N in TIN	62.91
NH <sub>4</sub> -N in TIN	33.36
PO <sub>4</sub> -P in TP	39.55

easily assimilated form in the process of photosynthesis) as total phosphorus, there were calculated specific percentages of nutrients which are shown in table 2, on the basis of the average of three measurements.

Table 2 shows that the TIN represents about 75 % of TN, being expected that approximately 25 % (organic nitrogen) to be found in suspended matter, respectively in sediments. Therefore, from the point of view of the eutrophication only 75 % of the TN is bio-available for assimilation and formation of Chlorophyll 'a'. In the TIN level, the major share returns NO<sub>3</sub>-N (about 63 %) and ions NH<sub>4</sub>-N (33 %), in that balance NO<sub>2</sub>-N being situated below the threshold of 4 %. PO<sub>4</sub>-P has a share of about 40 % in TP. It is emphasized that these values were calculated at level of all locations throughout all the period of investigation.

### Interdependences and mutual conditionings between nitrogen content, phosphorus, reports between these (TN/TP) and the assessment of the eutrophication potential

An increase in the water flow rate is reflected by an increase in the concentration of both nitrogen and phosphorus, a conclusion drawn from the increase of pollution diffusion affecting the total balance of nutrients (entrainment from the adjacent banks of fertilizers under conditions of precipitation) [14].

Eutrophication potential, characterized by the ratio TN/TP, has a lower relevance to the investigated sections of the Danube River, an aspect highlighted in the figure 2 a-b, where the temporal variations of the concentrations of Chlorophyll 'a', TN and TP are presented. It was observed that higher values of concentration of Chlorophyll 'a' were recorded during the months May to August, accompanied by increased concentrations of TN, this growth being presented in figure 3.

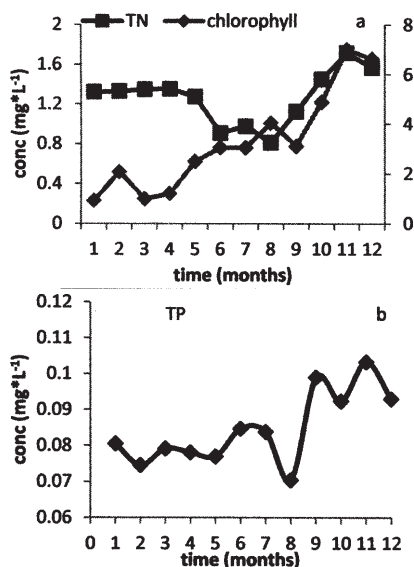


Fig.2. Temporal variation of concentration of Chlorophyll "a" TN (a), of TP (b)

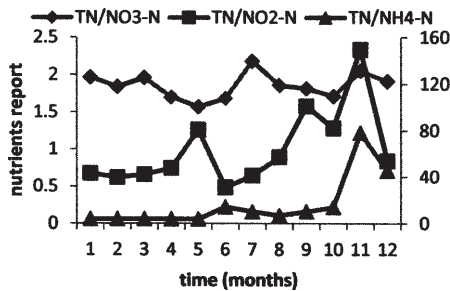


Fig. 3. Temporal variation of nutrients reports

In spatio-temporal terms, the Chlorophyll 'a' presents an increase of concentration in the monitoring area L6, area where the water flow is lower due to construction works in progress (fig. 4). It was found that on the Danube River, loading with nutrients is higher in spring and early summer mainly because of variations in river flow which is increased due to snowmelt.

Variation of Chlorophyll 'a' concentration in function of the water flow shows a high dispersion of points, but it can see a slight increase (fig. 5), and the ratio TN/TP versus the concentration of Chlorophyll 'a' presents seasonal variability, being increased in warm season and decreased in cold season, as shown in figure 6.

Figure 7 shows that the ratio TN/TP indicates an increase with the water flow to a maximum of  $2500 \text{ m}^3 \cdot \text{s}^{-1}$ , after which there is a decrease because at the higher water flow the increases of diffuse pollution figure 8 a-f present the concentrations of TIN (total inorganic nitrogen) and  $\text{NO}_3\text{-N}$  which, in this case, are increasing with the water flow (fig. 8 c), while at  $\text{PO}_4\text{-P}$  are decreasing (fig. 8 e). Also  $\text{NO}_2\text{-N}$  (fig. 8 b) and TN (fig. 8 d) grows with the water discharge, and the  $\text{NH}_4\text{-N}$  (fig. 8 a) and TP (fig. 8 f) presents very low concentrations. The explanation for this differentiation lies in the fact that high rainfall increases the share of diffuse pollution, namely, entrainment of nutrients from topsoil.

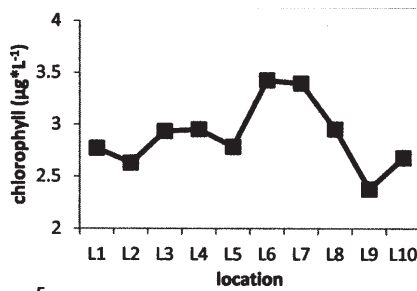


Fig. 4. Variation of Chlorophyll 'a' concentration along monitored locations

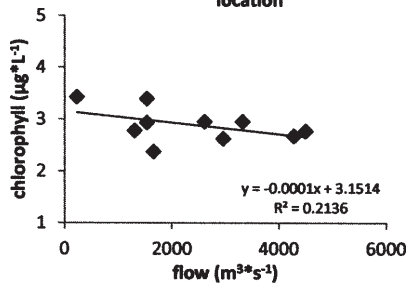


Fig. 5. Spatial variation of Chlorophyll 'a' concentration as a function of the water ischarge

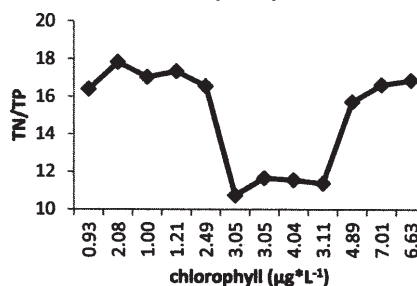


Fig. 6. Variation of the TN/TP ratio as a function of the Chlorophyll 'a' concentration

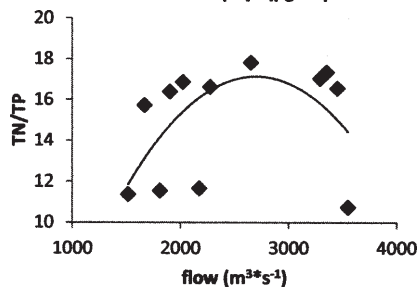
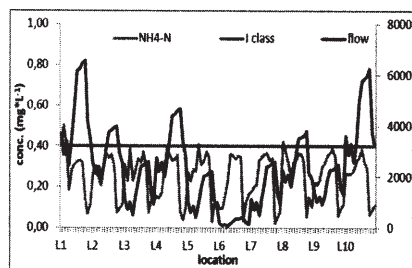
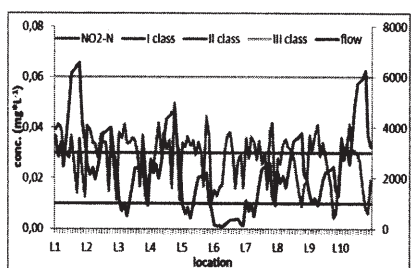


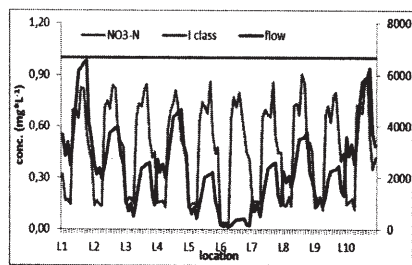
Fig. 7. Dependence of N/P ratio on flow rate



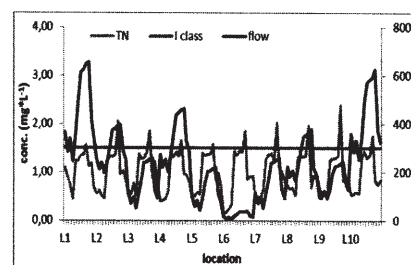
(a)



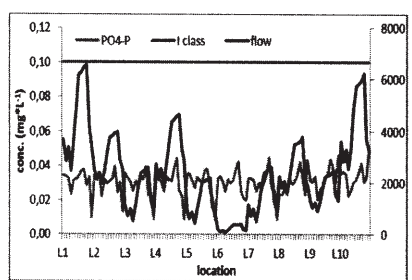
(b)



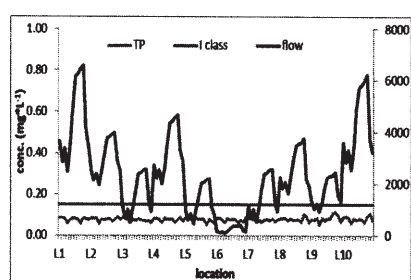
(c)



(d)



(e)



(f)

Fig. 8. a-f Temporal variation of nutrient concentrations connected with flow water and the respective water quality classes

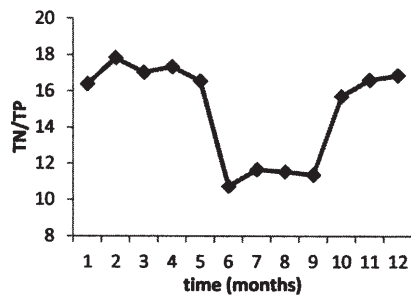


Fig. 9. Variation N/P ratio along the 12 monitoring months

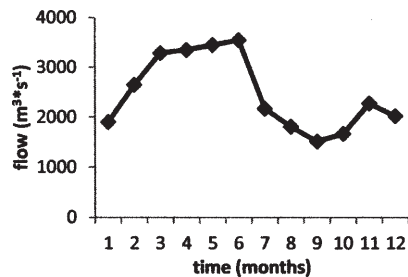


Fig. 11. Monthly average flow across total section of monitoring

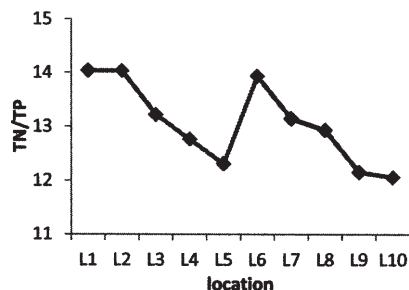


Fig. 10. Variation of N/P ratio along monitored locations

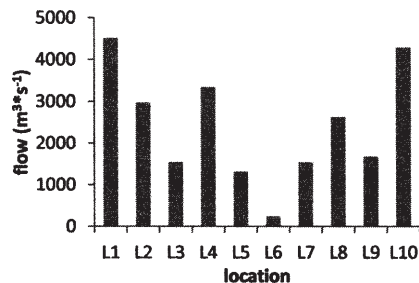


Fig. 12. The monthly water flow on monitored sections

The temporal variation of nutrient concentrations versus flow water rate shows that the monitored nutrient content recorded a relatively low variability, mainly dependent on the sampling time (seasonal variability). Depending on the content of nutrients, the river water was framed in the Class I of water quality, the Class II of water quality only because of the presence of nitrites according to the Order 161/2006 [36, 37].

#### TN/TP

By using the nutrient values obtained through this monitoring, the ratio TN/TP was calculated, which revealed the variation of the eutrophication potential. Values obtained for ratio TN/TP are larger than 10, which indicate the increasing of eutrophication potential. This ratio can be interpreted from two points of view. From the temporal point of view, this report presents decreasing values in cold season and implicitly a decrease of the potential of eutrophication, as seen from figure 9. From the spatial point of view, a decrease from upstream to downstream (comes loaded from upstream) can be observed, except in the location L6, where the flow is small and implicitly, the potential of eutrophication increases (fig. 10).

Danube's nutrients loading is influenced, mainly in spring and early summer, by the high flow variations which depends on snow melt, fact which can be observed in figure 9.

The hydrological regime of the Danube River on the monitored sector presents a seasonal variability (fig. 11), being recorded maximum flows in period January - February, exception making the monitoring location L6, where the river water flow was very small due to the hydrotechnical constructions. A comparison of this phenomenon observed in L6 location with the other 9 locations monitored is shown in figure 12.

#### Conclusions

The results of water analyses showed that the high variability of indicators was due to the actual ecological status of the Danube River as a consequence of the environmental conditions in the period 2012-2013, and not due to the hydrotechnical works that took place during that period of time. It was found that, nutrients loading of Danube River is higher in spring and early summer mainly because of variations in river flow which may be increased due to snowmelt, so is a seasonal variation.

Since both  $\text{PO}_4\text{-P}$ , and soluble forms of N are not influenced, it can be expected that hydrotechnic works specified above will not influence directly the eutrophication potential.

From the water quality point of view, it can be concluded that the actual ecological status of the Danube, in terms of nutrients, can be classified between good and moderate, an appreciation which is in accordance with the range of historical data (ICPDR - TNMN).

The method applied for parametric correlation of the studied indicators is accessible, easy, and provides a global picture of surface water quality, so it can be considered as an useful tool for future environmental assessments.

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