



Eutrophication Induction Via N/P and P/N Ratios Under Controlled Conditions—Effects of Temperature and Water Sources

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Received: 10 November 2019 / Accepted: 20 February 2020 / Published online: 23 March 2020
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Abstract The current research outlines the course of eutrophication processes emerging when some critical physical and chemical factors interact altogether. For this purpose, investigations were carried out, where nitrogen [N as $(\text{NH}_4)_2\text{HPO}_4$ and KNO_3] and phosphorus [P as $(\text{NH}_4)_2\text{HPO}_4$] were added to three different water sources (double distilled water, DDW; tap water, TW; and lacustrine water, LW) and the solutions were incubated at two distinct temperatures (17 and 23 °C). Treatments were kept in 1 dm³ glass jars and the incubation time lasted 7 weeks. The eutrophication process emerged only at 23 °C and was stronger for the lacustrine water (LW). In the case of DDW treatments, this process was observed at N/P = 5.1 and even at 60.0, whereas for the TW, no algal blooming was detected (N/P ratio 17.7–640.0). The lacustrine water (LW) outlined patterns with strong eutrophication at N/P = 4.40, but also at ratios 20.9–71.1. Algal blooming significantly intensified according to LW > TW > DDW but was reversely dependent on the P/N ratios, which followed the range DDW (P/N, 1.6–3.78) > TW (P/N,

0.050–0.100) > LW (P/N, 0.016–0.023). At P = constant (P = 0.10 mg dm⁻³) and the N inputs varying from 0.010 to 2.0 mg dm⁻³, it appeared that the higher the N concentrations, the more intensive the eutrophication process. For N/P ratios, phosphorus regulated for most of the intensity of the process, whereas in the case of P/N, the role of N and P was interchangeable. The main finding of the research is that nitrogen revealed in many cases to be a powerful eutrophication-regulating factor than did phosphorus.

Keywords Eutrophication · N/P · P/N · Water sources · Temperature · Incubation

1 Introduction

In its original use and etymology, ‘eutrophic’ meant ‘good nourishment’, and eutrophication expressed the process by which water bodies grew more productive. About 50 years ago, however, it became clear that this ‘good nourishment’ had considerable environmental impacts in fresh water environments such as lakes and reservoirs (Ferreira et al. 2011). Especially, water bodies, constituting the hydrosphere, are some of the most sensitive ecosystems as they become a sink of biogens (nitrogen, phosphorus) and dissolved organic matter after agricultural lands. Eutrophication is currently defined as the process of enriching water with nutrients, which increases autochthonous organic production and the biomass of algae. In consequence, these changes disturb and deteriorate water quality (Renouf and

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Kenway 2017; Järveläinen et al. 2017). It is estimated that 53% of water bodies in Europe, 54% in Asia, 48% in North America, 41% in South America, and 28% in Africa are threatened by excessive eutrophication (Nyenje et al. 2010).

There are two types of eutrophication: natural and artificial (anthropogenic) (Bartoszek and Czech 2014). The former takes place very slowly after the formation of a water body, when the growth of algae and their role in the environment are minimal. The latter type of eutrophication is caused by intensive land use, misuse of fertilisers and crop protection products, deforestation, growth of urban and rural areas, as well as discharge of untreated sewage to surface waters (Pajewski 2016; Huang et al. 2017; Sender and Jaruga 2017; Serrano et al. 2017; Bhagowati and Ahamad 2018). In the last 30 years, reliable data on the state and eutrophication of waters in Europe have been collected. They enabled researchers preparing various numerical models, which combine all elements (nutrients, physical processes, and biogeochemical cycles), illustrating the state of waters (Ferreira et al. 2011). Next, environmentalists have proposed various methods for assessing this process and changes in water bodies. The methods meet the legal requirements of the Water Framework Directive (WFD-2000/60/EC).

Direct biological response is recognised as one of the basic methods for assessing water eutrophication. This response is manifested by an increased content of chlorophyll A and/or macroalgae, which indicates the first stage of excessive amount of biogens in water (Borja et al. 2011). Secondary eutrophication is represented by low concentration of dissolved oxygen and the presence of harmful blooms (Bricker et al. 2008; Ferreira et al. 2011).

It is assumed that the excessive concentration of nutrients, mostly nitrogen (N) and phosphorus (P), is a key factor accelerating the eutrophication of water ecosystems (Gurkan et al. 2006; Dupas et al. 2015). Most of these elements originate from agricultural and urban areas. Untreated industrial waste discharged into waters is the main cause of increasing eutrophication in developing countries (Beyene et al. 2009).

The Regulation of the Polish Minister of the Environment of 23 December 2002 provides the criteria for identifying waters susceptible to pollution with nitrogen compounds from agricultural sources (Official Journal

No. 241, Pos. 2093). Table 1 shows the threshold values of indicators used for the assessment of eutrophication.

The Regulation defines much more N and P concentrations and the threshold N:P ratio at which eutrophication occurs, but there is no clear information on the P:N ratio. This is an important criterion, especially in countries, where fertilisation with phosphorus compounds is still very common (Zhang et al. 2008; Le et al. 2010; Jiang et al. 2019).

So far, biogens from the agricultural environment have been considered as the most important factors responsible for generating the eutrophication process; however, they should be regarded as largely quantified in scientific literature (Strokal et al. 2016; Huang et al. 2017). The complexity of these processes requires taking into account not only the P:N ratio but also additional key factors, such as the temperature and the type of water in which eutrophication may take place. It is therefore necessary to extend current knowledge with data concerning these factors and their impact on water resources along with potential prediction to global warming.

The main goal of the research was to simulate the eutrophication process under controlled conditions, i.e. with nitrogen and phosphorus, at two temperatures (17 and 23 °C) and using various water sources (double distilled water, tap water, lacustrine water). The specific objectives were to check the influence of several nitrogen and phosphorus concentrations, expressed as N/P and especially P/N on potential algal blooming. The interaction of factors like temperature and waters is intended to enrich current knowledge on the induction of eutrophication, especially for stagnant waters.

2 Materials and Methods

Laboratory tests were carried out in a growth chamber at the Department of Agricultural Chemistry and Environmental Biogeochemistry of Poznan University of Life Sciences (Poznan, Poland) (Fig. 1).

Experimental factors considered for the study are as follows: three sources of water, double distilled water (DDW), tap water (TW), and lacustrine water (LW); two ambient air temperatures, 17 ± 0.5 °C and 23 ± 0.5 °C; and various nitrogen (N) and phosphorus (P) concentrations expressing N:P and P:N ratios.

Table 1 Limit values of basic indicators for eutrophication of waters (eutrophication occurs when these limits are exceeded)

Indicators	Units	Stagnant waters	Flowing waters	Internal sea waters	Coastal sea waters
Total phosphorus	mg P dm ⁻³	> 0.10	> 0.25	> 0.3	> 0.10
Total nitrogen	mg N dm ⁻³	> 1.5	> 5	> 7	> 4
Ammonium nitrogen	mg NH ₄ dm ⁻³	-	> 2.3	> 3.4	> 1.8
Nitrate nitrogen	mg NO ₃ dm ⁻³	-	> 10	> 15	> 8

Regulation of the Polish Minister of the Environment of 23 December 2002 provides the criteria for identifying waters susceptible to pollution with nitrogen compounds from agricultural sources (Official Journal No. 241, Pos. 2093)

2.1 Characteristics of Water Sources and Nitrogen and Phosphorus Concentrations

2.1.1 Double Distilled Water

The water was collected from a water distillation kit Hydrolab HLP5. It is equipped with a UV lamp and a 0.2- μ m microfiltration capsule. The quality of water is dedicated for instrumental (AAS, ICP/MS, IC, HPLC) and biological analyses.

2.1.2 Tap Water

Poznan Agglomeration (about 800,000 inhabitants) is supplied with water from several water purification stations controlled by the Company Aquanet. Most of this water originates from the station located in Mosina (52° 14' 48" N, 16° 50' 42" E). The total volume of water supplied in 2017 reached 46.3 mln m³.

2.1.3 Lacustrine Water

Lake Rusalka (52° 25' 35" N, 16° 52' 40" E) is an artificial shallow water reservoir extending 36.7 ha (Fig. 2). It was created in 1943 and its mean depth is 1.9 m, but maximally 9 m. The shape is elongate, and the shoreline is about 3.3 km long. The close catchment area covers 83.9 ha of which 90% is occupied by forest stands and the remaining 10% by meadows. Due to its location within the Poznan Agglomeration, the lake is highly exposed to touristic pressure at summer time, particularly (Janicka et al. 2017).

2.1.4 Nitrogen and Phosphorus Concentrations in Waters

The knowledge of the preliminary concentrations of nitrogen and phosphorus (Table 2) is fundamental

for further scheduling the laboratory trials. Next, these elements are generally agreed to initiate and control biological development in waters at given concentrations.

2.2 Preparation of Particular N and P Concentrations for Trial Purpose

Stock and working solutions were elaborated by using chemical compounds considered as artificial fertilisers in agrochemical practice. These were diammonium phosphate [(NH₄)₂HPO₄] and potassium nitrate (KNO₃), entirely soluble in water.

The first one contains nitrogen (N) and phosphorus (P), whereas the second one nitrogen and potassium (K). By taking into consideration that (NH₄)₂HPO₄ contains 18% N–NH₄, 20% P–P₂O₅, and KNO₃ 13.7% N–NO₃, two stock solutions were prepared as listed below:

Stock A: 5000 mg (NH₄)₂HPO₄ dm⁻³ contains 900 mg N–NH₄ and 1000 mg P–P₂O₅ dm⁻³;
 Stock B: 767 mg KNO₃ dm⁻³ contains 105 mg N–NO₃ dm⁻³.

2.3 Experimental Sets

The trial consisted of 360 glass jars of 0.50 dm³ (three replicates), grouped into 120 treatments for each source of water: double distilled water, DDW; tap water, TW; and lacustrine water, LW.

Stock A, solution of (NH₄)₂HPO₄, was added in the range 5.0–1000 μ l to particular N:P treatments and the same was done with Stock B (solution of KNO₃) in the range 7100–0 μ l for N/P ratios varying from 150 to 0.75 (Table 3). In the case of the

Fig. 1 Poznan city in the background of the Wielkopolska Region and Poland

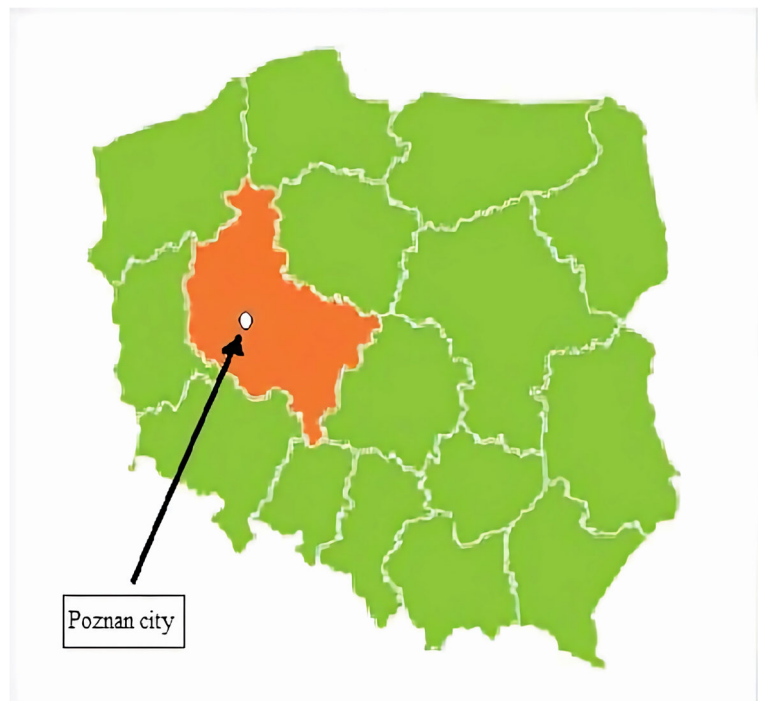


Fig. 2 Lake Rusalka in the background of Poznan Agglomeration

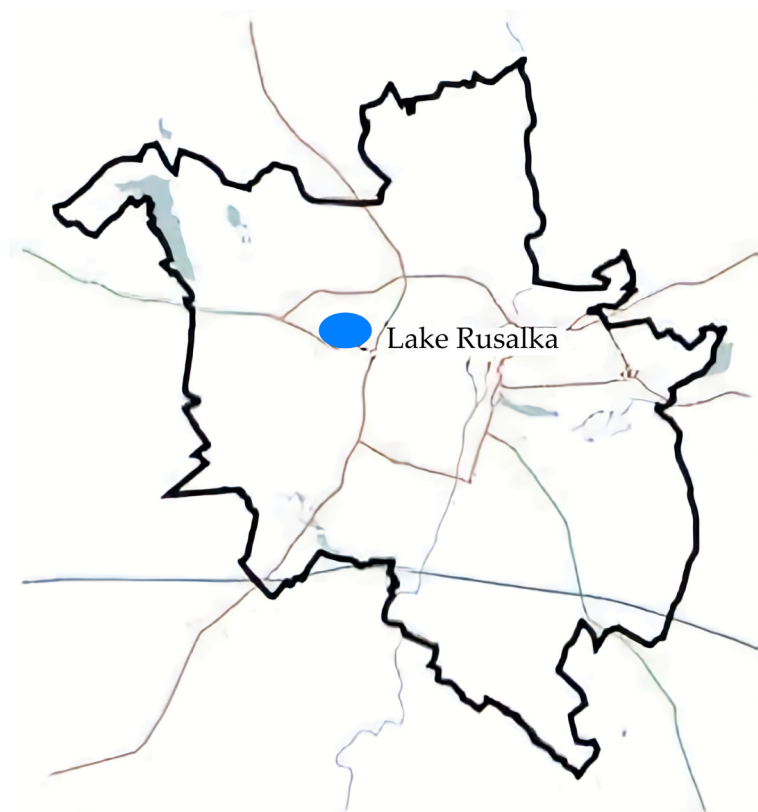


Table 2 Initial total nitrogen (N_{tot}) and phosphorus (P_{tot}) concentrations in waters used for the study

Water source	N_{tot} mg dm^{-3}	P_{tot}
Double distilled water (DDW)	ND ^a	ND
Tap water (TW)	1.70 ^b	0.008 ^c
Lacustrine water (LW)	2.08 ^c	0.100 ^c

^a Not detected; ^b Aquanet (2018), $N\text{-NH}_4$ basically; ^c Authors of the current paper

ratios P/N, stock A was added at a constant volume of 50 μl , but stock B varied within the range 0–9095 μl . This resulted in respective P/N ratios of 10 to 0.05 (Table 4).

All treatments with final volume of 0.50 dm^3 were first weighed, divided into two sets, and kept in rooms at ambient temperatures 17 ± 0.5 °C and 22 ± 0.5 °C. All jars have been weighed at 3-day time intervals and respective volume of double distilled water (DDW) was added for supplementing evaporation. For the treatments kept at 17 ± 0.5 °C, the amounts of water varied from 1.0 to 2.50 cm^3 and in the case of the temperature 22 ± 0.5 °C, much more water was supplemented, i.e., 3.0–5.50 cm^3 . Light cycles were set at 10 h for both temperature rooms and alternated with the dark cycle. The whole trial was run for seven (7) weeks.

Table 3 Concentrations of nitrogen (N) and phosphorus (P) with N/P ratios for the initial treatments (study concept)

N:P ratio	N (mg dm^{-3})	P
150	1.5	0.010
60	1.5	0.025
30	1.5	0.05
15	1.5*	0.10*
6.0	1.5	0.25
3.0	1.5	0.50
2.0	1.5	0.75
1.5	1.5	1.0
1.0	1.5	1.5
0.75	1.5	2.0

*Reference concentrations according to the Regulation of Polish Minister of Environment (December 23, 2002. Book of Laws Nr 241, Pos. 2093) (limit concentrations of N and P for stagnant waters, where eutrophication may emerge (see Table 1))

Table 4 Concentrations of phosphorus (P) and nitrogen (N) with P/N ratios for the initial treatments (study concept)

P:N ratio	P (mg dm^{-3})	N
10.0	0.10	0.010
4.0	0.10	0.025
2.0	0.10	0.05
1.0	0.10	0.10
0.40	0.10	0.25
0.20	0.10	0.50
0.13	0.10	0.75
0.10	0.10	1.0
0.07	0.10*	1.5*
0.05	0.10	2.0

*Reference concentrations according to the Regulation of Polish Minister of Environment (December 23, 2002. Book of Laws Nr 241, Pos. 2093) (limit concentrations of N and P for stagnant waters, where eutrophication may emerge (see Table 1))

2.4 Analytical Procedures

Analyses dealt with the measurement of pH and the determination of both total N and P in waters of the experimental jars at the following terms: before placing jars at given temperatures in growth chambers (pH) and after 7 weeks of incubation (pH, total N, and total P).

A multifunctional device, Elmetron CX701, was used for the potentiometric determination of pH directly in the jars. The electrode was preliminarily calibrated at 20 °C by using pH buffer solutions in the range 4.0 ± 0.02 – 9.0 ± 0.02 (Roti@Calipure, Carl Roth GmbH + Co. KG). For determination of both total nitrogen (N) and phosphorus (P), 25 ml of water aliquots was collected with a 25-ml pipette from the jars and directly filtered. Total N and total P were determined in 10-ml filtrates by the colorimetric flow injection analysis method with the Foss-Tecator FIAstar Analyzer (5000) and FIAstar Sampler (5027) equipment (FIAstar™ 5000 User Manual, 1001 0038 / Rev. 3 2004), respectively.

2.5 Data Elaboration

The Statistica 10.0© Package (StatSoft) was applied for performing the evaluation of data in terms of descriptive statistics. Next, the Excel© sheet facilities were used for elaborating graphs and linear relationships.

3 Results

3.1 Dynamic Changes of pH in the Water Media (Treatments)

Factors involved in the initiation of the eutrophication process are various but in most cases are restrained to the concentrations of phosphorus in waters. For this generally agreed rule, only the ratio of nitrogen (N) to phosphorus (P), i.e., N/P, prevails as a referencing tool. The concept developed in the current trial hypothesised that the phosphorus (P) to nitrogen (N) ratio (P/N), the temperature, and the pH of the solution as well as water sources are imminently important parameters to be taken into consideration.

The pH value of waters used in the study at starting the tests as well as at the end did not change markedly, except for the double distilled (DDW) and tap waters (TW) (Table 5). At the start of the trial, the water pH was slightly acidic (pH = 6.5, DDW) and slightly alkaline (pH = 7.3, TW), but these values rose by approximately 0.60 unit after 7 weeks for both temperatures. No changes were recorded for the lacustrine water, since the initial as well as final pH remained similar and slightly alkaline (range 7.6–7.8).

The mean pH values resumed in Table 6 may be evaluated on the basis of two patterns, i.e., (i) within water sources and temperatures and (ii) among nitrogen and phosphorus ratios, i.e., N/P and P/N.

In the first case, greatest disparities were denoted for the DDW treatments, particularly between those (N/P and P/N) before incubation and kept at 17 and 23 ± 0.5 °C, where the pH difference is approximately 0.50 (for 17 ± 0.5 °C) and about 0.90 (for 23 ± 0.5 °C). Further slight differences occurred also at the tap water (TW) treatments, in similar pattern as for DDW, but the values varied within a narrow range (0.30–0.50). It should be mentioned that no differences were noted between TW and LW, for both incubation temperatures and ratios (N/P and P/N).

The induction of eutrophication and the emergence of green biomass (blooming) were observed strictly for the treatments subjected to the highest temperature, i.e., 23 ± 0.5 °C as compared to 17 ± 0.5 °C, where the waters remained limpid throughout the whole trial. For such reasons, results reported in the paper are only those concerning 23 ± 0.5 °C. Data listed in Table 5 and Table 6 assumed that pH could be one of the additional chemical parameters directly involved in the

eutrophication process. Its changes as induced by N/P and P/N ratios and incubation time are graphically presented by Figs. 3, 4, and 5 (for double distilled water, DDW; tap water, TW; and lacustrine water, LW, respectively).

In the case of the DDW treatments (Fig. 3), the pH value increased progressively with increasing P concentrations in the water (N/P ratio), contrarily to the P/N ratio, where the pH value decreased along with increasing N levels just before the start of the incubation. This finding stresses on the acidifying potential of nitrates in waters, particularly those considered as less burdened, like in the case of the DDW. Interestingly, the pH values of both ratios behave differently after 7 weeks: for N/P, where N = constant, the pH value slightly and gradually decreased with the rise in P concentrations, whereas for P/N, with P = constant, the pH value remained unchanged with increasing N levels in the solutions.

The reference N and P concentrations (Table 3 and Table 4) at which eutrophication could be potentially expected are 1.5 and 0.10 mg dm⁻³, respectively. This results in ratios amounting to 15 (N/P) and 0.07 (P/N), with pH values at the end of the incubation period remaining similar, i.e., around 6.3 (slightly acidic).

Tap water (TW) treatments (Fig. 4) manifested different patterns as compared with the DDW. For those before incubation, a slight shift of pH values was recorded with narrowing N/P ratios, i.e., increasing P solution concentrations. A similar trend, but with slightly dynamic pH rise, took place for the P/N ratio (increase in N concentrations). Data listed in Table 2 show that TW treatments were initially characterised by N and P levels of 1.70 and 0.008 mg dm⁻³, respectively. Ammonium prevailed in the initial N pool and this could be related to the observed trends for treatments before incubation.

The variation of pH after incubation was progressive and significant, practically within the ranges 7.9–8.4 for the N/P and 7.5–8.3 in the case of P/N ratios. The question arises about the driving forces which controlled this hydrosystem. Complex chemical as well as biological interactions may have been taking place throughout the whole incubation period, and they were maintained constantly dynamic by the tender temperature (23 ± 0.5 °C).

Values of pH observed for the lacustrine water (LW) treatments (Fig. 5) before as well as after incubation were higher than those in the case of DDW and TW. One of the possible factors which could initiate such pH

Table 5 The pH of initial waters before and after incubation

Water source	pH before incubation	pH after incubation (7 weeks)	
		17 ± 0.5 °C	23 ± 0.5 °C
Double distilled water (DDW)	6.5	6.9	7.3
Tap water (TW)	7.3	8.1	7.9
Lacustrine water (LW)	7.6	7.8	7.7

rise was probably the joint effect of N (2.08 mg dm⁻³) and P (0.10 mg dm⁻³) and dissolved organic matter (DOM), initially present in the LW, but this was not analysed. This process was clearly illustrated by the course of the lines and particularly the narrowest gap between them.

Such a hydro-ecosystem should be less susceptible to internal alterations activated by external impulses, like inputs of substances with low or high pH or additional N and P compounds. The variations in pH for N/P and P/N treatments before incubation were greater as compared with those after, where the pH fluctuations ranged mostly within 8.00 and 8.25, i.e., not significant.

This should be perceived that water temperature is one of the key factors ruling algal blooming in water media, but the rise of pH above 7.0 and even much more should be attributed to the potentials of these ecosystems

for buffering any sudden changes, particularly chemical. The hypothesis of alkaline pH-induced organic decay should be given due investigations, which is out of the scope of the current tests.

3.2 N/P and P/N Ratios Versus Eutrophication Induction

3.2.1 Double Distilled Water Treatments

Datasets on Tables 3 and 4, respectively, for the ratios N/P and P/N show that N concentration was kept constant for N/P as compared with P/N with P concentration being unchanged. This was intended to trace any alterations that could be more or less responsible for the emergence of blooming considered as eutrophication induction. The resulting N/P and P/N ratios at the end of the incubation period are illustrated by Figs. 6 a and b

Table 6 The pH of test solutions (with incorporated N and P reagents) before and after incubation (7 weeks)

	Double distilled water (DDW)			Tap water (TW)			Lacustrine water (LW)			
	Treatments before incubation	17 ± 0.5 °C	23 ± 0.5 °C	Treatments before incubation	17 ± 0.5 °C	23 ± 0.5 °C	Treatments before incubation	17 ± 0.5 °C	23 ± 0.5 °C	
Descriptive statistics (n = 10) ^a	N/P	N/P			N/P			N/P		
Min.	6.2	6.9	6.8	7.6	7.8	7.9	8.0	7.8	7.7	
Max.	6.9	7.2	7.9	7.8	8.1	8.4	8.1	8.3	8.5	
Mean	6.5	7.1	7.5	7.7	8.0	8.2	8.0	8.1	8.1	
SD ^b	0.24	0.08	0.32	0.08	0.10	0.16	0.05	0.18	0.29	
Descriptive statistics (n = 10)	P/N	P/N			P/N			P/N		
Min.	6.3	7.1	7.4	7.3	7.9	7.5	8.0	7.8	7.5	
Max.	7.4	7.2	7.5	7.6	8.1	8.3	8.2	8.1	8.2	
Mean	6.6	7.1	7.4	7.5	8.0	8.0	8.0	8.0	7.9	
SD ^b	0.33	0.05	0.04	0.09	0.07	0.25	0.07	0.09	0.19	

^aSee Tables 3 and 4; ^bStandard deviation

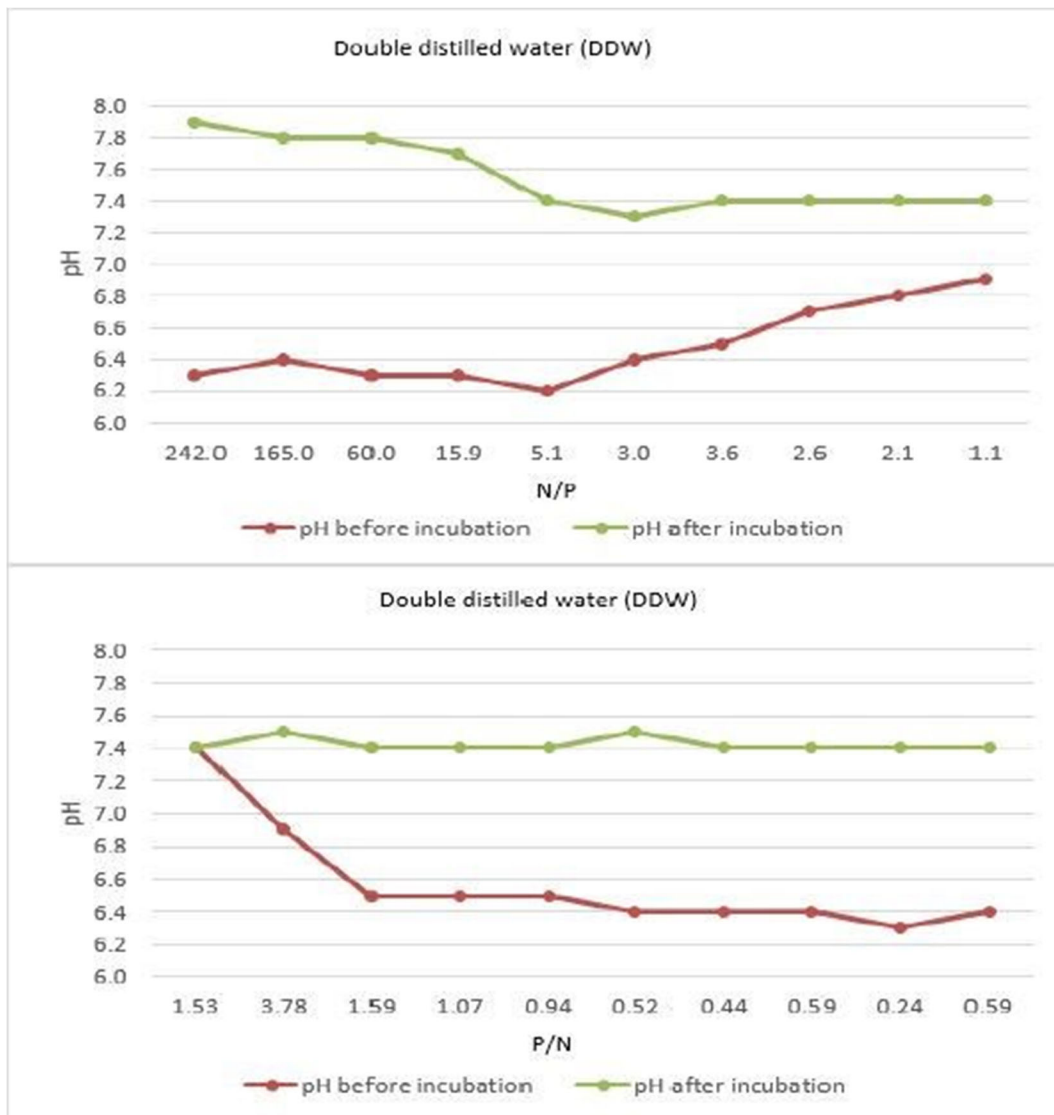


Fig. 3 Changes of double distilled water (DDW) pH as induced by N/P and P/N ratios and incubation time

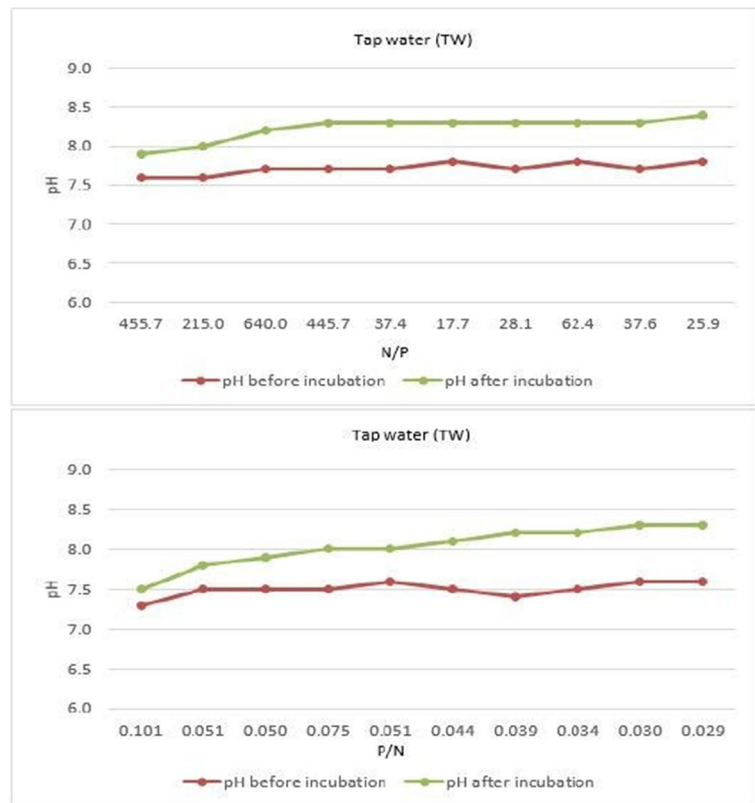
(N/P and P/N, respectively) for the double distilled water (DDW) as water source (Table 2). Next, the response of these ratios as a blooming process was reported on Figures 9 and 10 (N/P) as well as Figures 11 and 12 (P/N).

The ranges for N/P ratios varied within a large scale, that is, between 242.0 and 1.1; hence, they meet all possible criteria that could be favorable or not for the emergence of any blooming activity. The reported N/P ratio, i.e., 15, at which eutrophication may potentially take place was not observed, except two ratios: N/P = 60 and N/P = 5.1. The density as well as the volume of the greenish biomass particles was scarce, even at the

narrowest N/P ratio, assuming that N could have been the limiting factor.

The P/N ratios for water ecosystems are not widely reported in the scientific literature. If most research focuses on establishing optimal N/P ratios for evaluating the productivity of water bodies, practically nothing is reported about P/N-induced aquatic blooming. Hence, data of the current study should be, in some extent, considered as referencing for studies, specifically under controlled conditions. However, they may be a basis for a rough evaluation of stagnant waters, particularly when ambient temperature is around 23 or even higher.

Fig. 4 Changes of tap water (TW) pH as induced by N/P and P/N ratios and incubation time



Figures 11 and 12 reveal that the eutrophication process may potentially emerge at respective concentrations of P and N, but much more at their given ratios, which varied within the range $3.78 < P/N > 0.24$. The green biomass was observed at the ratio 1.6, where N and P concentrations were visibly the lowest. Therefore, if both are low and P was kept constant, then N could be considered as the factor controlling algal blooming.

3.2.2 Tap Water Treatments

Table 2 resumes initial concentrations of N and P of the investigated waters, and hence it appeared that tap water registered N concentration of 1.70 mg dm^{-3} , whereas for P, the level was negligible, i.e., 0.008 mg dm^{-3} . The initial N/P and P/N ratios rose to 212.5 and 0.005, respectively, revealing a lack of adequate conditions for blooming initiation. In both cases, P should be identified as a strictly limiting factor.

The implementation of the concept as listed in Tables 3 and 4 implies that reported N and P concentrations must consider their respective initial water levels (Table 2). Therefore, the highest final N concentration at

the start of the incubation should be 3.20 mg dm^{-3} ($1.70 + 1.5 \text{ mg dm}^{-3}$) for the N/P ratio and 3.70 mg dm^{-3} ($1.70 + 2.0 \text{ mg dm}^{-3}$) in the case of the P/N ratio. These preliminary ratios, resulted from respective N and P concentrations, altered significantly during the 7-week incubation period illustrated by Fig. 7a, b (for N/P and P/N, respectively). No blooming process was detected at the N/P treatments, where the ratios fluctuated within an extremely large range, i.e., $640.0 < N/P > 17.7$. A possible imbalance between N and P could have been responsible for this state.

The trends of N and P changes shown by Fig. 7b resulted in significantly low values of P/N ratios, which varied between 0.101 and 0.0291. It should be pointed out that P was kept constant (Table 4), whereas N concentrations were altered. At the end of the incubation, P concentrations similarly to the N ones rose gradually (but not linearly), implying a possible release of inorganic P. This assumption may have been supported by the intensive blooming process (Figures 13, 14, and 15) as a proof of optimal conditions for the emergence of eutrophication. Finally, it may be formulated that N was the limiting as well as controlling factor and

particularly at its relatively low concentrations in the waters. The intensity of blooming was recorded at the P/N *ca* 0.050 and 0.075 for Figures 13 and 14, respectively, compared with Figure 15 with the value 0.101.

3.2.3 Lacustrine Water Treatments

Natural waters are by essence a mixture of several burden organic as well as inorganic compounds, whose concentrations differ generally with seasons and location. The tested water originated from a peri-urban lake with N and P levels amounting to 2.08 and 0.100 mg dm⁻³ (Table 2), which practically results in N/P and P/N ratios of 20.8 and 0.0048, respectively. This initial ratio reveals some potential susceptibility for the emergence of eutrophication, since being close to N/P = 15, reported as target threshold. Next, the pH measured just at sampling was slightly alkaline (pH = 7.6, Table 5 and Table 6), implying that this ecosystem was strongly buffered, probably due to dissolved organic substances (DOS).

The observed trend in N concentrations (Fig. 8a) at the end of the incubation period showed an irregular

pattern for the whole experimental N inputs (Table 3) as compared with phosphorus concentrations, which exhibited a linear course. The overall N/P ratios varied significantly, i.e., from 110.0 to 4.43, but the potentially productive ones were detected at N/P = 4.40 (Figure 16), N/P = 20.9 (Figure 17), N/P = 51.2 (Figure 18), and N/P = 71.1 (Figure 19).

The first two could be expected to undergo eutrophication, since the value 20.9 (Figure 17) was closer to the threshold (i.e., 15) identified as optimal for blooming. Larger N/P ratios implied practically that phosphorus should be the limiting factor, particularly at N/P = 51.2 (Figure 18) and N/P = 71.1 (Figure 19). This is a proof that nitrogen and phosphorus concentrations in water environments could not be the sole referencing parameter for stating potential ecosystem productivity nor its lack. Additional factors, such as water pH and ambient temperature, may be playing a ground role. The current study outlined 4 of them, i.e., N, P, pH, and temperature.

Data of the treatments as illustrated by Fig. 8b imply that both N and P were simultaneously controlling the observed algal blooming (Figures 20 and 21). In the first case, for P/N in the range 0.016–0.018, nitrogen could

Fig. 5 Changes of lacustrine water (LW) pH as induced by N/P and P/N ratios and incubation time

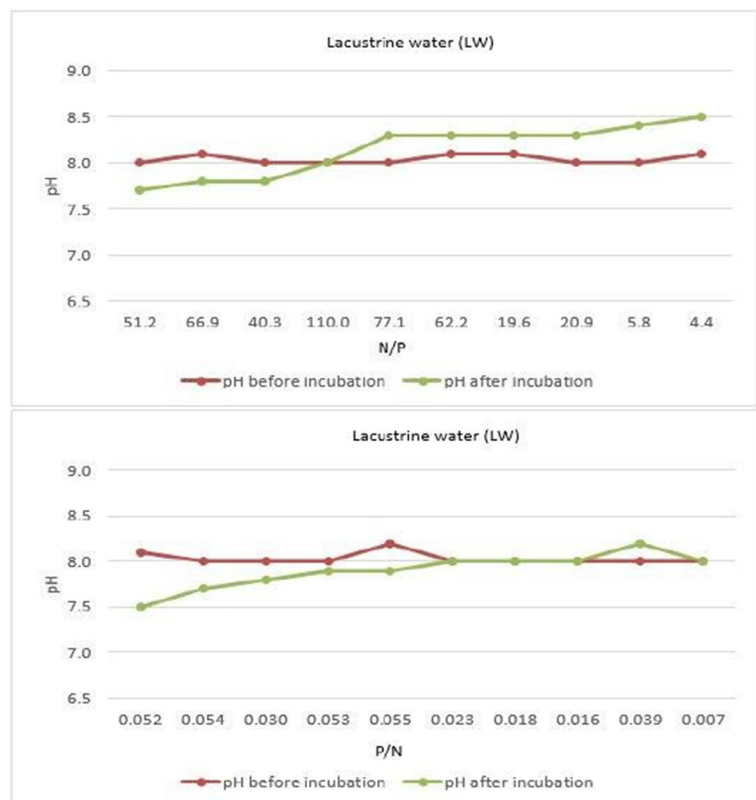
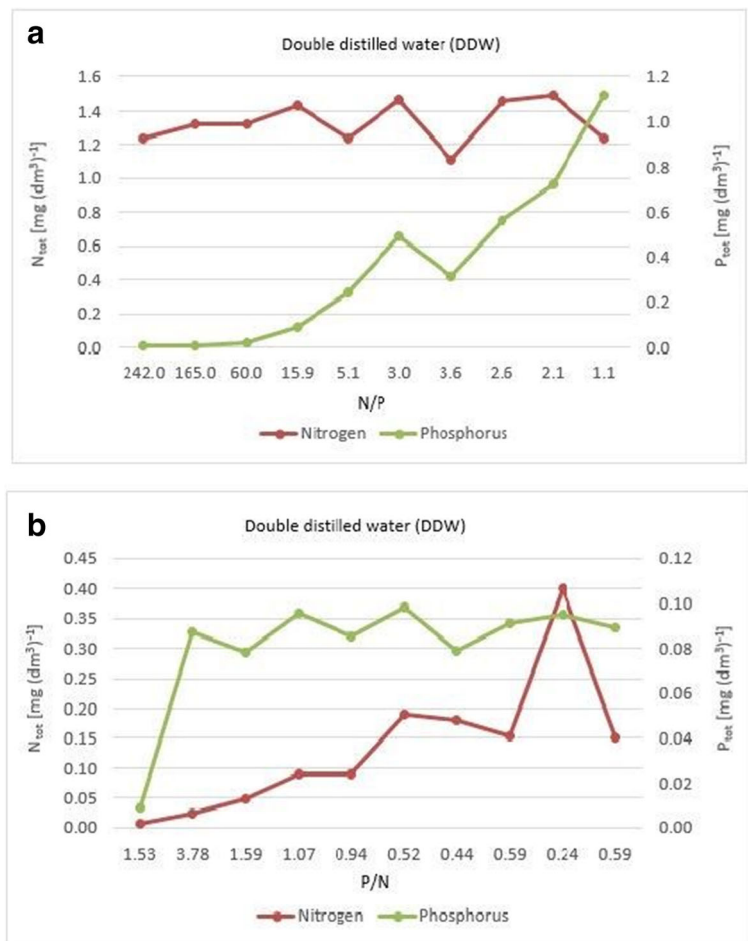


Fig. 6 **a** Variations of N and P concentrations in double distilled water (DDW) and their impacts on N/P ratios at the end of incubation time. **b** Variations of N and P concentrations in double distilled water (DDW) and their impacts on P/N ratios at the end of incubation time



have been responsible for the dynamic emergence of this biomass as compared with the $P/N = 0.023$, where phosphorus initiated the process.

The pattern observed at this level is similar to that occurring in the case of tap water (TW) and double distilled water (DDW): the narrower the P/N ratio, the more intensive the blooming activity.

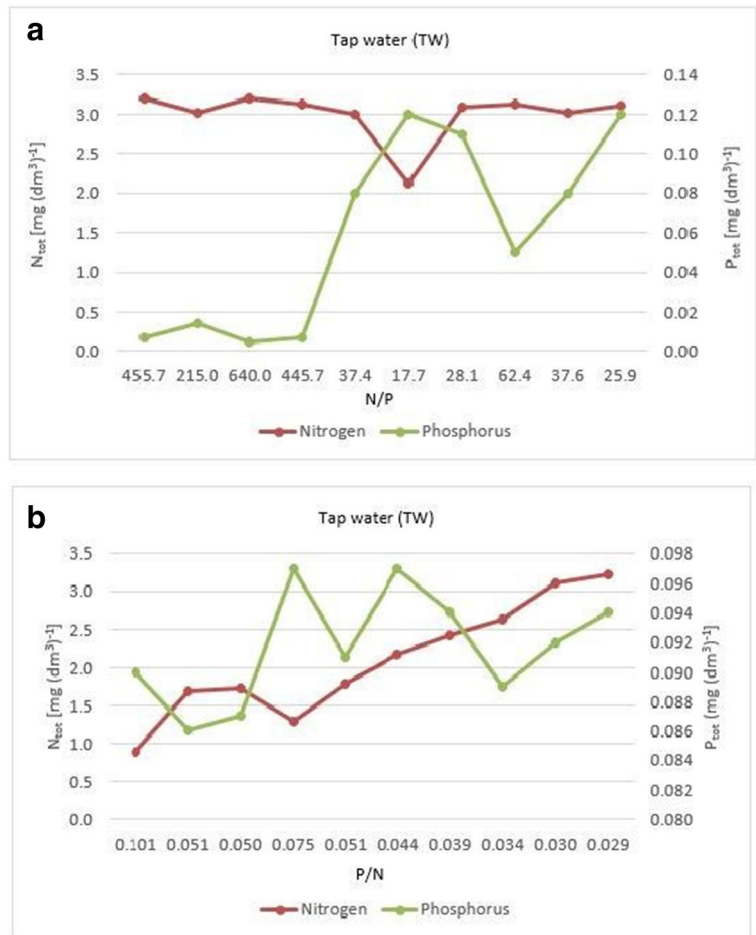
4 Discussion

The quantification of factors, mostly abiotic, being responsible for initiating the process of eutrophication expressed also as algal blooming, has been for several decades a matter of intensive endeavor for biologists, environmentalists, and last but not the least agrochemists too (Daniel et al. 1998; Conley et al. 2009; Ferreira et al. 2011; Qina et al. 2014; Panepinto et al. 2016). The relationship cause to effect of this process focuses much

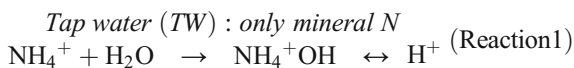
more on parameters such as nitrogen (N), phosphorus (P), N/P ratio, and dissolved organic substances (DOS) (Le et al. 2010; van Roon 2011; Järveläinen et al. 2017; Teurlinx et al. 2019) than to water source, ambient temperature, pH, and decidedly P/N ratio. Scientific data are crucially scarce or lacking even about the effects or role of the latter ones as co-initiators of eutrophication.

From the data, first reported synthetically in Table 2, it appeared that water pH may be explained preliminarily by the concentrations of total nitrogen (N_{tot}), except for the double distilled water (DDW), where no nitrogenous compounds were initially detected and the pH stabilised to *ca* 6.5 (slightly acidic, Table 5—pH before incubation). The observed rule was that the higher the N_{tot} , the higher the pH. This specific feature of the investigated waters may be considered as an important factor regulating chemical as well as biological processes in hydrosystems. The question arises about the pH-controlling value of $N-NH_4$ (for tap water, TW) and N_{tot}

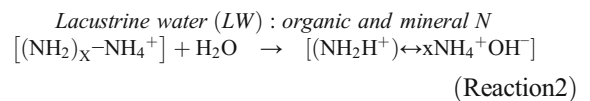
Fig. 7 a Variations of N and P concentrations in tap water (TW) and their impacts on N/P ratios at the end of incubation time. **b** Variations of N and P concentrations in tap water (TW) and their impacts on P/N ratios at the end of incubation time



(lacustrine water, LW), where the latter one is assumed to consist of both N–NH₄ and organic nitrogen (N_{org}). These particular mechanisms manifest their potential efficiency as interactions running at the level: water source–temperature–N/P and P/N ratios–incubation time. The dualistic pattern of these N compounds acting simultaneously for raising and buffering pH may proceed according to the reactions as below (authors conceptual representation):



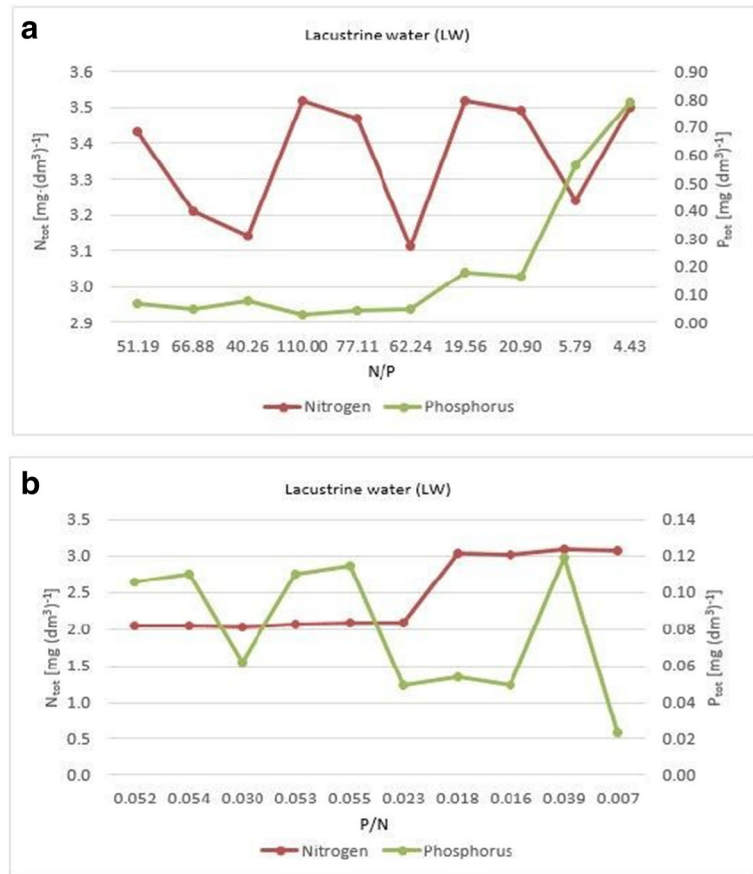
This state is unstable and each additional input of H⁺ shifts the equilibrium to the formation of H₂O leading in turn to the emergence of NH₄⁺. The ammonium/proton interaction efficiently buffers pH in aquatic systems, keeping it neutral to alkaline, even (Table 5).



For simplification, the pool of organic N was represented intentionally by amino acid chains, i.e., (NH₂)_x, due to the heterogenic buildup of this nitrogen forms, particularly in lacustrine waters. Reaction 2 outlines synthetically the dynamic character of buffering mechanisms emerging at the input of protons (H⁺) as well as hydroxyl (OH⁻) ions and the equilibrium kept between the organic and mineral parts of the whole entity.

It is worth mentioning that these mechanisms (Reactions 1 and 2) operated highly and efficiently irrespective of the incubation time (7 weeks), temperatures (i.e., 17 ± 0.5 °C and 23 ± 0.5 °C), and N/P as well as P/N ratios. The resulting pH values (means) are unquestionable proofs of the outlined concept and

Fig. 8 **a** Variations of N and P concentrations in lacustrine water (LW) and their impacts on N/P ratios at the end of incubation time. **b** Variations of P and N concentrations in lacustrine water (LW) and their impacts on P/N ratios at the end of incubation time



initiate a challenging research area on the impact of N versus pH in the blooming intensity within hydrosystems.

Nitrogen and phosphorus in aqueous ecosystems have been targeted as strictly responsible for boosting biological activity which consequently stimulates the emergence of eutrophication (Zeilhofer et al. 2010; Renouf and Kenway 2017; Huang et al. 2017). This process should not proceed straightly as illustrated by Figs. 3, 4, and 5 for DDW, TW, and LW, respectively, but may depend also on N/P as well as P/N ratios. The latter ones influenced pH course, which clearly bears various shapes indicative also of the time course, i.e., $t = 0$ (before incubation) and $t = 7$ weeks of incubation.

The case in Fig. 3 (for double distilled water) deserves additional insight, particularly both ratios. Phosphorus in the case of N/P ratio was added as $(\text{NH}_4)_2\text{HPO}_4$, and any increase of its rate simultaneously raises NH_4 concentrations in the solution. This factor may have been responsible for the dynamic pH increase before the incubation (Reaction 1) and the same pattern

is applied also to the P/N ratio but related to increasing NO_3 concentrations (KNO_3 inputs) over NH_4 .

Curiously, both pH courses (N/P and P/N, DDW) leveled at values around 7.4 or above, after 7 weeks of incubation at 23 ± 0.5 °C. In the case of the P/N treatment, pH remained strictly not altered, irrespective of the ratios. This phenomenon may be attributed to a possible source of alkalinity, which could be generated by a trophic factor. The mean pH difference (ΔpH) (Table 6) between N/P and P/N treatments at 23 ± 0.5 °C and that before incubation amounted to 1.0 and 0.8, respectively. Such a significant pH rise should be considered as a life-controlled process, ‘strategically’ initiated for buffering the water solution against any prompt alteration.

Observations made for the tap water (TW) treatments initially characterised by N and P levels of 1.70 and 0.008 mg dm^{-3} , respectively (Table 2, Fig. 4), as well as the lacustrine water (LW) with natural N (2.08 mg dm^{-3}) and P (0.10 mg dm^{-3}) (Table 2, Fig. 5) revealed an unexpected pH pattern for the particular N/P and P/N

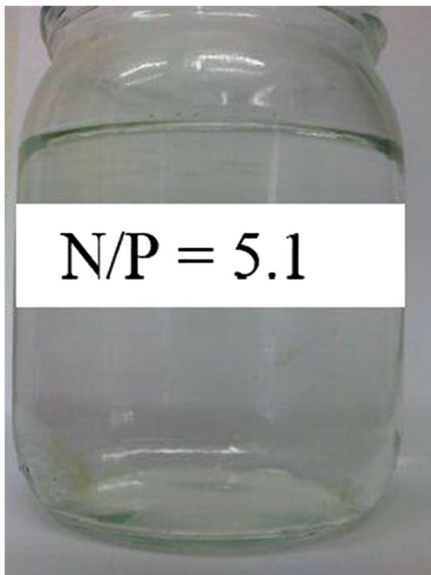


Fig. 9 N/P = 5.1

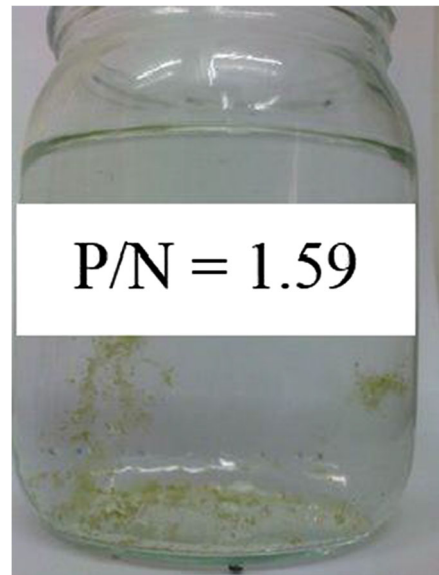


Fig. 11 P/N = 1.59

ratios. For the TW, the difference for the treatments before and after incubation (Table 6) was equally 0.50 for both N/P and P/N ratios, whereas in the case of LW, 0.10 and -0.10 , respectively. This net decrease in the course of pH values at the level of the water sources varied accordingly (mean value basis):

N/P : DDW ($\Delta\text{pH} = 1.00$)
 > TW ($\Delta\text{pH} = 0.50$)
 > LW ($\Delta\text{pH} = 0.10$) (Serie1)

-(N/P, where N concentration = constant (1.50 mg dm^{-3}) and P varied $0.010\text{--}2.0 \text{ mg dm}^{-3}$)



Fig. 10 N/P = 60.0

P/N : DDW ($\Delta\text{pH} = 0.80$)
 > TW ($\Delta\text{pH} = 0.50$)
 > LW ($\Delta\text{pH} = -0.10$) (Serie2)

-(P/N, where P concentration = constant (0.10 mg dm^{-3}) and N varied $0.010\text{--}2.0 \text{ mg dm}^{-3}$).

This specific finding may be resumed by the formulation that incremental P inputs into investigated waters



Fig. 12 P/N = 3.78

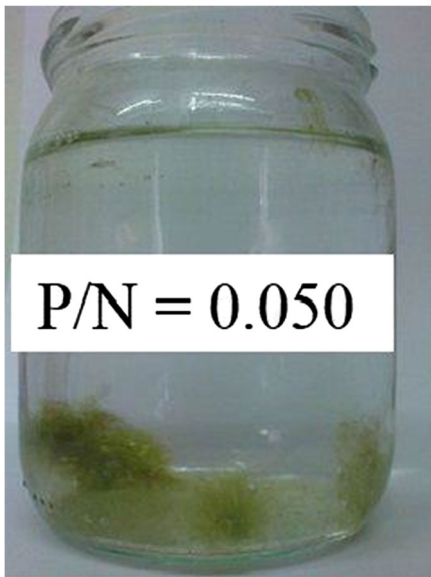


Fig. 13 P/N = 0.050

did not depress their pH as manifested in the case of N inputs, where the process was slight. However, the trends observed for the series 1 and 2 are quite similar, implying that the higher the initial total N (N_{tot}) concentrations in the waters, the much more efficient buffering mechanisms should emerge and consequently less prompt alterations (basically chemical, like pH).

The multiplicity of factors identified as directly responsible for generating the eutrophication is still the hardpan for environmentalists in their endeavor for

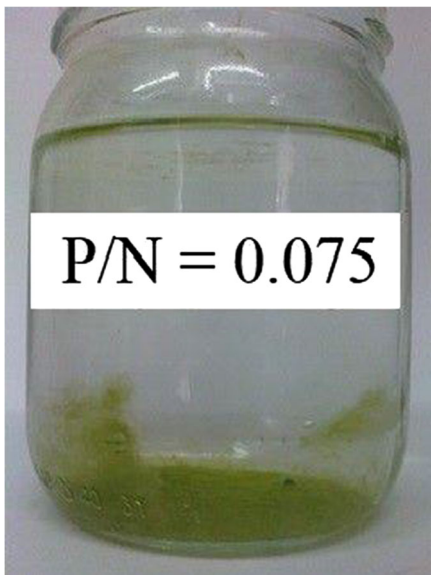


Fig. 14 P/N = 0.075

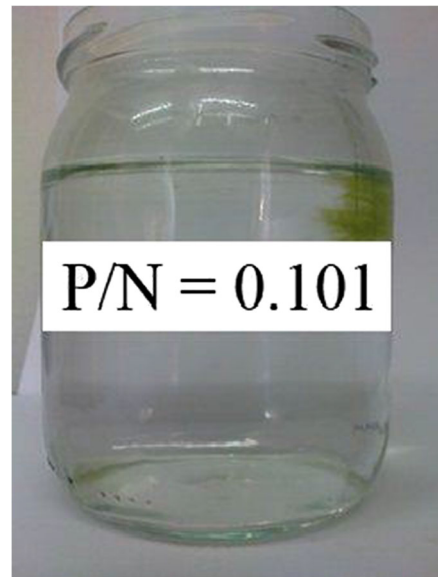


Fig. 15 P/N = 0.101

pointing out key parameters of this process, out of N and P concentrations. The large and unique documentation (illustrations and photos) reported as Figs. 6 a and b, Figs. 7 a and b, and Figs. 8a and b synthesises and sets light at the concept of the current research. Several assumptions may be formulated, but some specifically targeted should help overcoming this complexity: (i) Is really the 'normatively' reported 15/1 (N/P) ratio applicable elsewhere and decisive for algal blooming, irrespective of water source? (ii) Is eutrophication process dependent only on N/P and P/N or also on the temperature? (iii) Is hydrosystem pH a 'transboundary' and key link for N/P, P/N, water temperature, and finally water source?

The Regulation of Polish Minister of Environment (December 23, 2002, 241/2093), (The current study refers to stagnant waters (Table 1) that stipulates



Fig. 16 N/P = 4.43



Fig. 17 N/P = 20.9

eutrophication of waters emerges, when the limit values of basic indicators (i.e., N and P concentrations) are exceeded. This Regulation seems ‘slightly’ precise for the following reasons:

- If limit values of basic indicators are proportionally exceeded, then the N/P ratio is not altered. The Regulation operates.
- If limit values of basic indicators are not proportionally exceeded, then the N/P ratio is accordingly altered. The Regulation operates at the specific levels as below:
 - a. $N/P < 15/1$ (conditions for algal blooming)
 - b. $N/P > 15/1$ (no conditions for algal blooming)

Double distilled water (DDW, Fig. 6a) has manifested some eutrophication process at $N/P = 5.1$ and even 60.0 (Figures 9 and 10, respectively). What worked out for initiating algal blooming at such large N/P ratio? Data for N/P recorded in the case of the tap water (TW) are in line with the $15/1 < N/P$ (no conditions for algal blooming), since the narrowest ratio amounted to 17.7 and the largest up to 640.0 (Fig. 7a). Finally, the



Fig. 18 N/P = 51.2



Fig. 19 N/P = 77.1

lacustrine water (LW) outlined unexpected patterns with intensive eutrophication process (Fig. 8a). Figure 16 ($N/P = 4.40$) fitted the Regulation with $15/1 > N/P$ (conditions for algal blooming) contrarily to the Figures 17, 18, and 19, characterised by N/P values of 20.9, 51.2, and 71.1, respectively. The overview of these findings prompts the formulation that two key factors (out of N and P) have been directly responsible for this process: temperature and pH (particularly in the range 7.4–8.5).

The scientific and environmental literature does neither report about the effect of P/N ratio nor the pH, temperature, and next water source on eutrophication process. Values listed in this study look pioneering in this case and are presented by Figs. 6b, 11 and 12 (DDW), Figs. 7b, 13, 14, and 15 (TW), and Figs. 8b, 20 and 21 (LW). Algal blooming significantly intensified accordingly $DDW < TW < LW$, but was reversely dependent on the P/N ratios, which followed the range $DDW (P/N, 1.6–3.78) > TW (P/N, 0.050–0.100) > LW (P/N, 0.016–0.023)$. At $P = \text{constant}$ ($P = 0.10 \text{ mg dm}^{-3}$) and the N inputs varying from 0.010 to 2.0 mg dm^{-3} , it appeared that the higher the N concentrations, the more intensive the eutrophication process, similar to the N/P case. It means that eutrophication power of N and P is



Fig. 20 P/N = 0.016 – 0.018



Fig. 21 P/N = 0.023

similar in stagnant waters, provided additional hydrosystem key parameters are operating within biological and eco-friendly conditions, i.e., pH within the range 7.4–8.5 and ambient water temperature around 23 °C or even more.

5 Hypotheses–Statements–Conclusions

On the basis of the data outlined in the current research, we hypothesised that the operationally reported N/P ratio (15/1) used for discriminating pros and cons for water bodies for algal blooming should be re-verified. At the era of rainfall water shortage (trends in pH rise in urban water systems) along with temperature rise (global warming), hydrological studies (at various water sources) should involve these two parameters in the evaluation of eutrophication tasks.

Organic nitrogen pools should be releasing as compared to phosphorus, much more dynamically and quantitatively due to temperature rise. This trend may lead to considering the phosphorus to nitrogen ratio, i.e. P/N as a timely algal blooming parameter of due hydro-environmental and indicative value. Data of this study support this statement.

Water pH within the range 7.4–8.5 and ambient water temperature around 23 °C or even more are considered as decidedly responsible for initiating and supporting eutrophication. Lower temperature (i.e., 17 °C), even at similar pH range, did not generate blooming.

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