

N:P ratios to estimate nutrient limitations and trophic state in a shallow freshwater lake

Şiğ bir tatlısu gölünde besin sınırlamaları ve trofik durumu belirlemede N:P oranlarının kullanımı

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Abstract: Understanding nutrient limitation is essential for managing shallow freshwater ecosystems experiencing eutrophication. Kovada Lake, a National Park in Türkiye, suffers from severe nutrient pollution and excessive macrophyte growth, yet its specific limitation status is unclear. This study aimed to diagnose the nutrient limitation and current trophic state of the lake and its inlet canal. Epilimnetic water samples were collected from five stations during the growing season (May–October 2024). Samples were analyzed for Total Nitrogen (TN), Total Phosphorus (TP), Dissolved Inorganic Nitrogen (DIN), Dissolved Inorganic Phosphorus (DIP), and Chlorophyll-a (Chl-a). Molar stoichiometric ratios (TN:TP and DIN:DIP) were then calculated to identify limitation patterns. Results revealed extremely high nutrient concentrations throughout the system. The lake-canal average molar TN:TP ratio was 7.77:1, indicating strong and persistent N limitation. In contrast, the average molar DIN:DIP ratio was 13.97:1, suggesting a N-P co-limitation for immediately bioavailable nutrients. Significant spatial and monthly variations confirmed the system's dynamic nature. The average Chl-a concentration (14.69 µg L⁻¹) confirmed a eutrophic trophic state. Crucially, Chl-a did not correlate with TN or TP but showed significant relations with DIP and DIN:DIP ratio. This study demonstrates that the Kovada Lake system is primarily N-limited. Furthermore, it highlights that in this macrophyte-dominated shallow lake, dissolved nutrient parameters (DIP, DIN:DIP) are more reliable predictors of trophic response than total nutrient ratios. These findings provide a critical diagnostic basis for developing nutrient management strategies.

Keywords: TN:TP ratio, DIN:DIP ratio, Kovada Lake, chlorophyll a, stoichiometry, Redfield ratio

Öz: Şiğ tatlısu ekosistemlerinde ötrofikasyonun yönetimi için besin sınırlamasının anlaşılması büyük önem taşır. Millî Park statüsündeki Kovada Gölü (Isparta, Türkiye), yoğun besin kirliliği ve aşırı makrofit gelişimi ile karakterize edilirken, gölün özgün besin sınırlama durumu belirsizliğini korumaktadır. Bu çalışma, göl ve giriş kanalındaki besin sınırlamasını ve güncel trofik durumu belirlemeyi amaçlamıştır. Epilimnetik su örnekleri, bitki büyüme dönemi boyunca (Mayıs–Ekim 2024) beş istasyondan alınmıştır. Örneklerde Toplam Azot (TN), Toplam Fosfor (TP), Çözünbilir İnorganik Azot (DIN), Çözünbilir İnorganik Fosfor (DIP) ve Klorofil-a (Chl-a) analiz edilmiştir. Besin sınırlamalarını belirlemek için molar stokiyometrik oranlar (TN:TP ve DIN:DIP) hesaplanmıştır. Sonuçlar, göl-kanal sisteminde son derece yüksek besin konsantrasyonları ortaya koymuştur. Göl-kanal ortalama molar TN:TP oranı 7.77:1 olarak bulunmuş ve belirgin, kalıcı bir azot sınırlamasını; buna karşılık, ortalama molar DIN:DIP oranı 13.97:1 biyolojik olarak hemen kullanılabilir besin formları açısından bir azot-fosfor eş-sınırlamasını göstermektedir. Belirgin mekânsal ve aylık değişkenlikler, sistemin dinamik yapısını doğrulamıştır. Ortalama Chl-a konsantrasyonu (14.69 µg L⁻¹), gölün ötrofik trofik durumda olduğunu göstermiştir. Chl-a'nın TN veya TP ile ilişkili olmamasına rağmen, DIP ve DIN:DIP oranı ile anlamlı ilişkiler göstermesi dikkat çekicidir. Bu çalışma, Kovada Gölü sisteminin baskın olarak azot sınırlamalı olduğunu ortaya koymaktadır. Ayrıca, makrofit baskın şiğ göl yapısında, çözünbilir besin parametrelerinin (DIP, DIN:DIP) trofik duurunun belirlenmesinde toplam besin oranlarına göre daha güvenilir göstergeler olduğunu vurgulamaktadır. Bulgular, göldeki besin yönetim stratejilerinin geliştirilmesi için kritik bir tanısal temel sunmaktadır.

Anahtar kelimeler: TN:TP oranı, DIN:DIP oranı, Kovada Gölü, klorofil-a, stokiyometri, Redfield oranı

INTRODUCTION

Concept of limiting nutrients

The ecological health and trophic state of freshwater ecosystems are governed by nutrient dynamics, specifically the proportional availability (stoichiometry) of Nitrogen (N) and Phosphorus (P) (Sterner and Elser, 2002; Conley et al., 2009, Kolzau et al., 2014; Zhang et al., 2023). P is traditionally recognized as the primary limiting nutrient; consequently, controlling P inputs is the central paradigm of eutrophication management (Schindler, 1977; Ekholm, 2008; Søndergaard et al., 2017). While P limitation is often driven by anthropogenic inputs (Schindler, 1977), freshwater systems can also experience N limitation or N-P co-limitation. Shifts in lake N:P

stoichiometry indicates changes in these limitation patterns (Elser et al., 2007).

The Redfield ratio (RR)

The Redfield Ratio (RR) provides a theoretical molar benchmark of 16:1 (N:P) for optimal phytoplankton stoichiometry (Redfield, 1958). While RR widely adopted to assess nutrient dynamics in marine ecosystems (Ptacnik et al., 2010), the 16:1 RR is often an "exception rather than the rule" in freshwaters. Phytoplankton nutrient content varies, necessitating empirical validation with methods like bioassays (Morris and Lewis, 1988; Mischler et al., 2014, Kolzau et al., 2014).

Rationale of N:P ratio

Since N and P cycling mechanisms vary with trophic, the TN to TP ratio can estimate trophic status. The TN:TP ratio is high in oligotrophic and low in eutrophic lakes, declining curvilinearly with increased TP (Downing and McCauley, 1992). The N:P ratio is thus a tool to evaluate nutrient sources (Downing and McCauley, 1992; Ptacnik et al., 2010; Dai et al., 2022). The high ratios (oligotrophic) indicate natural watersheds, while low ratios (eutrophic) often correspond to highly nutrient loadings and even to municipal sewages (Downing and McCauley, 1992). Stoichiometry affects species competition; sediment nutrients are also critical for macrophytes, which in turn can control N:P ratios in shallow waters (Bergström, 2010; Dai et al., 2022).

Trophic state and eutrophication

Excess nutrient inputs lead to eutrophication by supporting excessive primary production (Smith et al., 1999). Understanding nutrient limitation is critical for ecosystem health (Sterner and Elser, 2002). For effective eutrophication control, management must target the specific nutrients responsible for enhanced production (Conley et al., 2009; Savic et al., 2022). The TN:TP ratio model is also used for this trophic state estimation (Miller, 2010).

Parameter selection and effectiveness

While total nutrients (TN, TP) and TN:TP are used for trophic state (Downing and McCauley, 1992; Ekholm, 2008; Bergström, 2010), dissolved forms, Dissolved Inorganic Nitrogen (DIN) and Dissolved Inorganic Phosphorus (DIP), are readily available to primary producers. Limitation may cease if DIP and DIN exceed certain concentrations (Ekholm, 2008). The DIN:TP ratio is sometimes considered a better indicator than TN:TP (Bergström, 2010). The DIN:DIP ratio is a direct measure of nutrient supply and has shown high accuracy (80-90%) in predicting limitation compared with biotests (Morris and Lewis, 1988; Mischler et al., 2014; Søndergaard et al., 2017). Bioassays have used DIN: DIP to define limitation thresholds and seasonal shifts (Mischler et al., 2014; Kolzau et al., 2014). The ratio is also applied in other contexts, such as riverine and atmospheric deposition studies (Ravi et al., 2021; Koçak et al., 2016).

Consequences of imbalanced N:P ratios

Besides determination of nutrient limitation, trophic state and eutrophication tendency, the N:P ratio is used to estimate cyanobacterial bloom risk (Morris and Lewis, 1988; Havens et al., 2003; Ekholm, 2008). N-fixing cyanobacteria, for instance, tend to dominate at low mass TN:TP ratios (Ekholm, 2008). Long-term studies confirm that increased P loads and decreased N:P ratios favor cyanobacterial dominance (Havens et al., 2003). Chronic over-enrichment leads to severe water quality degradation, including low dissolved oxygen, fish kills, and species shifts (Gibson et al., 2000).

Kovada is a eutrophic-hypertrophic shallow freshwater lake. The lake and surrounding forest area were declared and

used as National Park, is experiencing severe eutrophication, water scarcity and shallowing. Numerous limnological studies have been conducted previously to investigate the lake (Lahn, 1948; Atay, 1996; Kazancı et al., 1999; Gülle, 1999; Yüce, 1999; Yeğen et al., 2006; Gürbüz et al., 2009; Zeybek, 2012; Zeybek et al., 2012; Yüce and Ertan, 2014; Çiçek and Yamuç, 2017; Erdoğan and Savaş, 2024; Özdal, 2025; Cevher, 2025). However, a specific diagnosis of its nutrient limitation status is lacking. In this study, we aimed to determine nutrient limitation using TN:TP and DIN:DIP ratios, and to assess the trophic state via DIP-Chlorophyll-a relations in the Kovada Lake-canal system.

MATERIALS AND METHODS

Study area and sampling stations

Kovada Lake (KoL) (Figure 1) is a natural lake of tectonic-karstic origin (Lahn, 1948), located at 37°38' N, 30°53' E in the Antalya Basin (Mediterranean region) of Türkiye. A regulated inlet (Kovada Canal, KoC) carries excess water from Eğirdir Lake to KoL, and a constructed outlet connects KoL to the Aksu River. In regular rainfall years, the water budget is balanced mainly via these inlet and outlet canals. Its surface area varies from 800 to 1100 ha; the depth in the pelagic zone averages 3 m, with a maximum depth of 5 m. The nearest meteorological station's (Eğirdir, 37.87 N-30.85 E, 30 kms in north) 30 years average min-max temperature (°C) /rainfall (mm) data in sampling months were: May 9-20/58, June 13-25/30, July 16-29/7; August 16-29/7, September 12-25/26, October 8-19/62. Main and effective wind directions were N-NE and S with maximum 25-30 km h⁻¹ speed (Meteoblue, 2025). The winds can easily mix the lake water and cause turbidity in windy days.

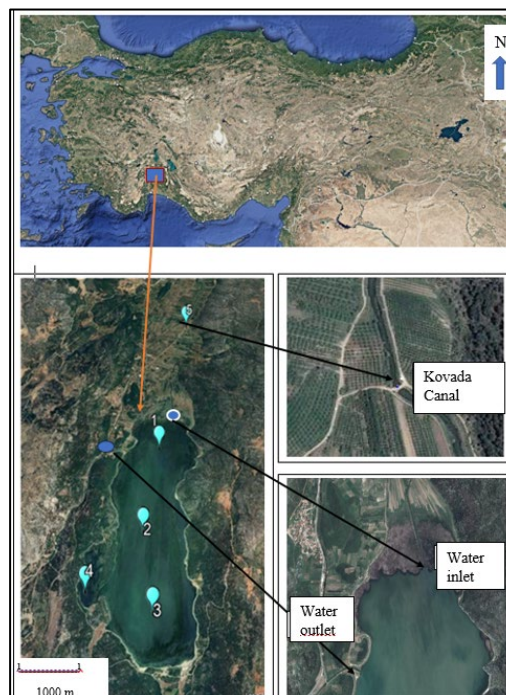


Figure 1. Kovada Lake, Kovada Canal and sampling stations (Cevher, 2025)

KoL and its surrounding area (6,534 ha) were designated as "Kovada Lake National Park" in 1970, and KoL designated as a first-class natural protected area in 1992 (Kazancı et al., 1999; Bahadır, 2014; Cevher 2025). The lake's biota includes 12 fish species (mainly cyprinids) and 1 crayfish (Kazancı et al., 1999; Yeğen et al., 2006); 10 macrophyte species, predominantly *Stuckenia pectinata*, *Myriophyllum spicatum*, and *Potamogeton crispus* (Özdal, 2025); and 32 zooplankton species belonging to 4 families (Gülle, 1999; Erdoğan and Savaş, 2024). In KoL and KoC, 51 phytoplankton taxa were observed, belonging to Bacillariophyta (37), Chlorophyta (8), Charophyta (4), and Cyanobacteria (2) (Çiçek and Yamuç, 2017). In KoC, 43 phytoplankton taxa were reported: Bacillariophyta (23), Chlorophyta (15), Cyanophyta (3), and Euglenophyta (2). The macrophyte community of KoC is composed of *Myriophyllum* sp., *Potamogeton* sp., *Phragmites* sp., *Juncus* sp., and *Lemna* sp. (Kazancı et al., 1999; Yüce and Ertan, 2014).

The lake's littorals and nearby forest provide an important wildlife habitat, ecosystem services, and a recreation area for trekking, climbing, and camping (Bahadır, 2014).

Sampling, analyses and calculations

Five sampling stations (SS), four within the lake and one on the canal, were chosen for water sampling and in situ measurements (Figure 1). Samplings were conducted during the plankton-macrophyte growing season (May–October) in 2024. Epilimnetic water samples taken from 20–30 cm under the lake surface and canal were analyzed for Total Phosphorus (TP), Total Nitrogen (TN), orthophosphate (PO_4^{3-}), nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), and Chlorophyll-a (Chl-a) standard spectrophotometric methods (APHA, 1995). The analyses were performed in the chemistry laboratory of the Eğirdir Fisheries Research Institute.

Calculation molar TN:TP = TN μmol^{-1} / TP μmol^{-1}

TN $\mu\text{mol}/\text{L} = \text{mg}^{-1} \text{TN} \times 1000/14,01$ (atomic weight of N, g/mol)

TP $\mu\text{mol}/\text{L} = \text{mg}^{-1} \text{TP} \times 1000/30,97$ (atomic weight of P, g/mol)

Alternatively, TN:TP mass ratio was calculated as TN (mg^{-1})/TP (mg^{-1}) and multiplied by 2,21 for molar TN:TP (Downing & McCauley, 1992).

Calculation molar DIN:DIP = $[\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+]/[\text{PO}_4^{3-}]$

$[\text{NO}_3^-] = \text{mg}^{-1} \text{NO}_3^- \times 1000/62,00$ (molecular weight of NO_3^-)

$[\text{NO}_2^-] = \text{mg}^{-1} \text{NO}_2^- \times 1000/46,01$ (molecular weight of NO_2^-)

$[\text{NH}_4^+] = \text{mg}^{-1} \text{NH}_4^+ \times 1000/18,04$ (molecular weight of NH_4^+)

$[\text{PO}_4^{3-}] = \text{mg}^{-1} \text{PO}_4^{3-} \times 1000/94,97$ (molecular weight of PO_4^{3-})

Mass DIN:DIP = $(\text{NO}_3^- \text{N} \text{mg}^{-1} + \text{NO}_2^- \text{N} \text{mg}^{-1} + \text{NH}_4^+ \text{N} \text{mg}^{-1})/(\text{PO}_4^{3-} \text{P} \text{mg}^{-1})$

$\text{PO}_4^{3-} \text{P} \text{mg}^{-1} = \text{PO}_4^{3-} \times (\text{Atomic weight of P (30,97)}/\text{Molecular$

weight of PO_4^{3-} (94,97)) = $\text{PO}_4^{3-}(\text{mg}^{-1}) \times 0,326$ and, N compounds are converted to N as; $\text{NO}_2^- \text{N} (\text{mg}^{-1}) = \text{NO}_2^- \times 0,304$; $\text{NO}_3^- \text{N} (\text{mg}^{-1}) = \text{NO}_3^- \times 0,226$; $\text{NH}_4^+ \text{N} (\text{mg}^{-1}) = \text{NH}_4^+ \times 0,776$

Mass data (in μg^{-1}) were converted to molar concentrations (μmol^{-1}) and molar ratios by dividing the mass values of TN by 14 and TP by 31 (Downing and McCauley, 1992). The percentages, DIP:TP% and DIN:TN%, were also calculated.

Data analysis

After Shapiro-Wilk (S-W) normality tests, descriptive statistics, and other data analysis were performed. To compare two characteristic point one-sample and dependent (paired) two-sample t-tests; to compare stations and months ANOVA and Kruskal-Wallis (K-W) test; to monitor the relations Spearman correlations, and to construct a relationship the regression analysis performed by using SPSS v26 (IBM, SPSS 2017), and graphs were created by Excel and SPSS. The significance level was set at $p = 0.05$.

RESULTS

Nutrient concentrations, ratios and percentages

The epilimnion nutrient concentrations, ratios, and percentages for the sampling stations (SSs) in KoL and KoC are presented in Table 1. The averages of TN, TP, and TN:TP varied significantly among the SSs (K-W, $p < 0.05$); TN was highest (3.332 mg^{-1}) in SS-3 and lowest (1.380 mg^{-1}) in SS-4, whereas TP was highest (0.772 mg^{-1}) in SS-4 and lowest (0.176 mg^{-1}) in SS-1. The TN:TP ratio was highest (15.187) in SS-1 and lowest (1.800) in SS-4 (Table 1; Figure 2a, b). The average TN:TP was 7.77 for the KoL-KoC system. The variability of the molar TN:TP ratio was lowest (45.15 %CV) in SS-4. The other nutrients, DIN:DIP ratios, and dissolved nutrient percentages did not vary significantly among the SSs ($p > 0.05$). TN, TP, and TN:TP were similar (K-W, $p > 0.05$) among sampling months. In contrast, NO_3^- , NO_2^- , NH_4^+ , PO_4^{3-} , DIN, DIP, DIN:DIP, %DIP to TP, and %DIN to TN varied significantly ($p < 0.05$) by month, with lower values observed in September–October (Figure 3).

Correlations between the nutrients and ratios

Spearman's rho correlation coefficients were calculated, because not normal distributed (S-W, $p < 0.05$) data and the results are presented in Table 2. The TN:TP ratio was positively related to TN mg^{-1} ($r = 0.670$, $p < 0,01$) and negatively related to TP mg^{-1} ($r = -0.902$, $p < 0,01$). The DIN:DIP ratio was negatively related to DIP mg^{-1} ($r = -0.898$, $p < 0,01$). No significant correlations were found between DIN and TN, NO_2^- , NO_3^- , or NH_4^+ . The correlations between TN and DIN, as well as between TN and DIP, were as expected (Table 2).

Table 1. Mean nutrient concentrations, molar N:P and DIN:DIP rates and nutrient percentages (N=25) of Kovada Lake-canal system's sampling stations of year 2024

Nutrients, Ratios	Sampling Stations	Means	St. Dev.	lower CI 95%	upper CI 95%	Kruskal-Wallis	CV%
TN mg L ⁻¹	I.SS	2.419	0.469	1.836	3.002	p<0.05*	19.40
	II.SS	2.180	0.384	1.703	2.657		17.63
	III.SS	3.332	0.545	2.655	4.009		16.36
	IV.SS	1.380	0.299	1.009	1.751		21.68
	Kovada Canal	1.478	0.287	1.122	1.834		19.39
Average		2.158	0.397	1.665	2.651		18.40
TP mg L ⁻¹	I.SS	0.176	0.078	0.079	0.273	p<0.05*	44.32
	II.SS	0.444	0.228	0.161	0.727		51.33
	III.SS	0.442	0.142	0.265	0.619		32.24
	IV.SS	0.772	0.118	0.626	0.918		15.22
	Kovada Canal	0.298	0.111	0.159	0.436		37.41
Average		0.426	0.135	0.258	0.594		31.77
OP mg L ⁻¹	I.SS	0.187	0.120	0.037	0.336	p>0.05	64.41
	II.SS	0.194	0.131	0.032	0.357		67.37
	III.SS	0.181	0.136	0.012	0.350		75.03
	IV.SS	0.236	0.160	0.037	0.435		68.02
	Kovada Canal	0.209	0.161	0.009	0.409		77.23
Average		0.201	0.142	0.025	0.377		70.40
Nitrite mg L ⁻¹	I.SS	0.038	0.015	0.019	0.057	p>0.05	39.78
	II.SS	0.034	0.022	0.007	0.061		62.90
	III.SS	0.031	0.017	0.010	0.053		55.95
	IV.SS	0.031	0.016	0.011	0.051		51.76
	Kovada Canal	0.037	0.017	0.016	0.058		46.07
Average		0.034	0.017	0.013	0.056		50.84
Nitrate mg L ⁻¹	I.SS	1.300	0.400	0.803	1.797	p>0.05	30.77
	II.SS	1.260	0.385	0.782	1.738		30.53
	III.SS	1.300	0.316	0.907	1.693		24.33
	IV.SS	1.240	0.472	0.654	1.826		38.08
	Kovada Canal	1.220	0.602	0.473	1.967		49.32
Average		1.264	0.435	0.724	1.804		34.41
Ammonium mg L ⁻¹	I.SS	0.616	0.655	-0.197	1.428	p>0.05	106.34
	II.SS	0.297	0.201	0.047	0.546		67.82
	III.SS	0.207	0.113	0.067	0.347		54.52
	IV.SS	0.216	0.139	0.044	0.389		64.13
	Kovada Canal	0.177	0.111	0.040	0.315		62.53
Average		0.303	0.244	0.000	0.605		80.53
DIP mg L ⁻¹	I.SS	0.061	0.039	0.012	0.110	p>0.05	64.41
	II.SS	0.063	0.043	0.010	0.116		67.37
	III.SS	0.059	0.044	0.004	0.114		75.03
	IV.SS	0.077	0.052	0.012	0.142		68.02
	Kovada Canal	0.068	0.053	0.003	0.133		77.23
Average		0.066	0.046	0.008	0.123		70.40
DIN mg L ⁻¹	I.SS	0.783	0.564	0.083	1.484	p>0.05	72.05
	II.SS	0.525	0.180	0.302	0.748		34.19
	III.SS	0.464	0.136	0.294	0.633		29.42
	IV.SS	0.458	0.147	0.276	0.640		32.03
	Kovada Canal	0.425	0.199	0.178	0.671		46.86
Average		0.531	0.245	0.226	0.835		46.18
TN:TP	I.SS	15.187	4.924	9.072	21.301	p<0.05*	32.43
	II.SS	7.369	6.180	-0.304	15.043		83.86
	III.SS	8.294	3.130	4.407	12.180		37.74
	IV.SS	1.800	0.341	1.377	2.223		18.93
	Kovada Canal	6.178	4.510	0.578	11.778		73.00
Average		7.766	3.817	3.026	12.505		49.15
DIN:DIP	I.SS	16.738	9.064	5.484	27.992	p>0.05	54.15
	II.SS	12.842	8.930	1.754	23.930		69.54
	III.SS	14.812	12.464	-0.664	30.288		84.15
	IV.SS	9.996	8.268	-0.270	20.261		82.71
	Kovada Canal	15.472	16.937	-5.557	36.502		109.46
Average		13.972	11.132	0.149	27.795		79.68
Percent DIP/TP	I.SS	37.705	28.054	2.872	72.538	p>0.05	74.40
	II.SS	17.031	9.651	5.047	29.015		56.67
	III.SS	12.508	7.000	3.816	21.199		55.96
	IV.SS	9.591	5.810	2.377	16.804		60.58
	Kovada Canal	29.154	23.436	0.054	58.254		80.39
Average		21.198	14.790	2.833	39.562		69.77
Percent DIN/TN	I.SS	30.863	17.380	9.283	52.443	p>0.05	56.31
	II.SS	24.503	9.592	12.593	36.413		39.15
	III.SS	14.291	4.727	8.421	20.160		33.08
	IV.SS	33.692	10.824	20.252	47.132		32.13
	Kovada Canal	30.873	19.060	7.206	54.540		61.74
Average		26.844	12.317	11.551	42.138		44.48

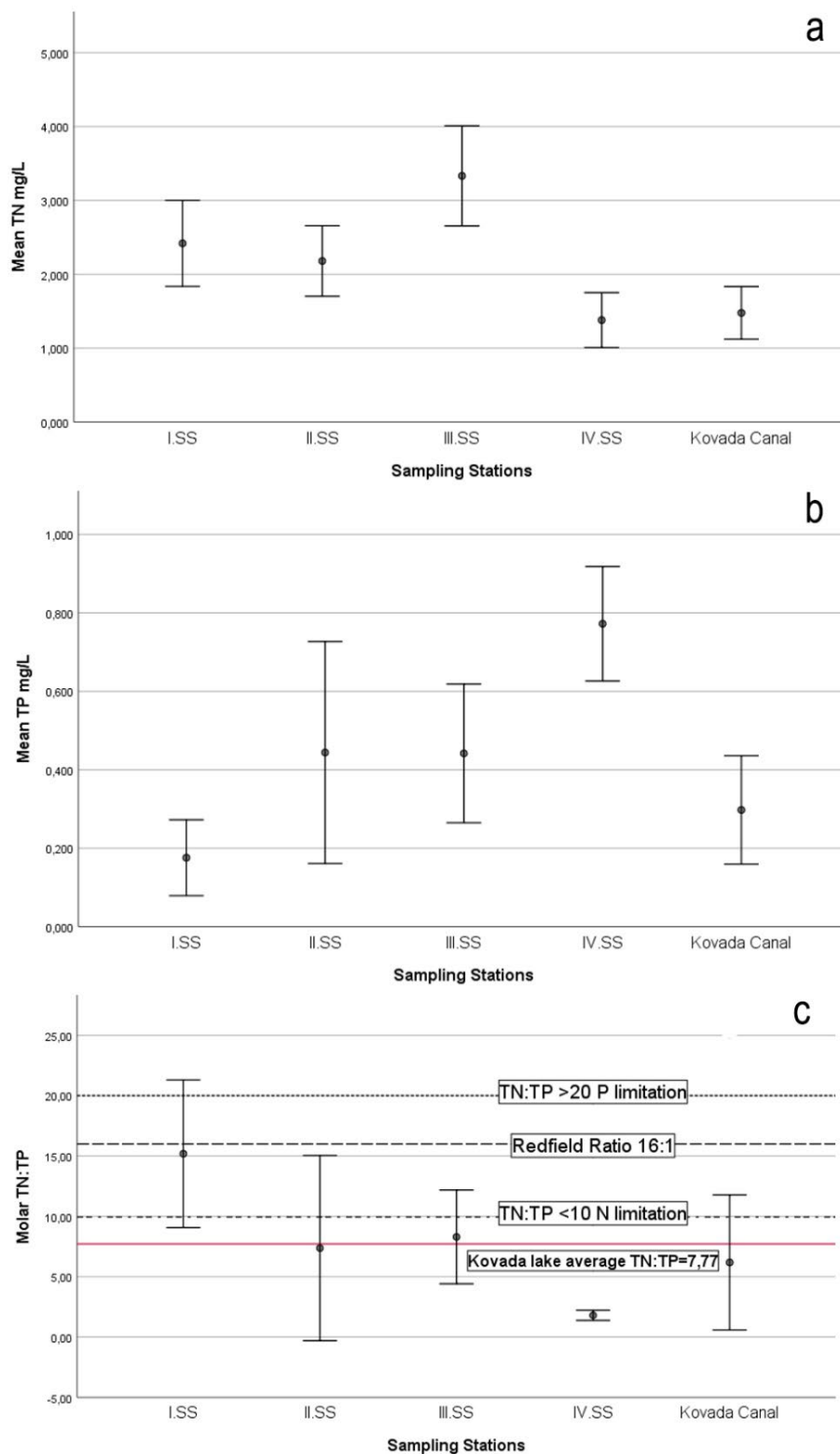


Figure 2. TN(a), TP(b), TN:TP(c) averages and thresholds in sampling stations of Kovada Lake-canal system (bar 95%CI)

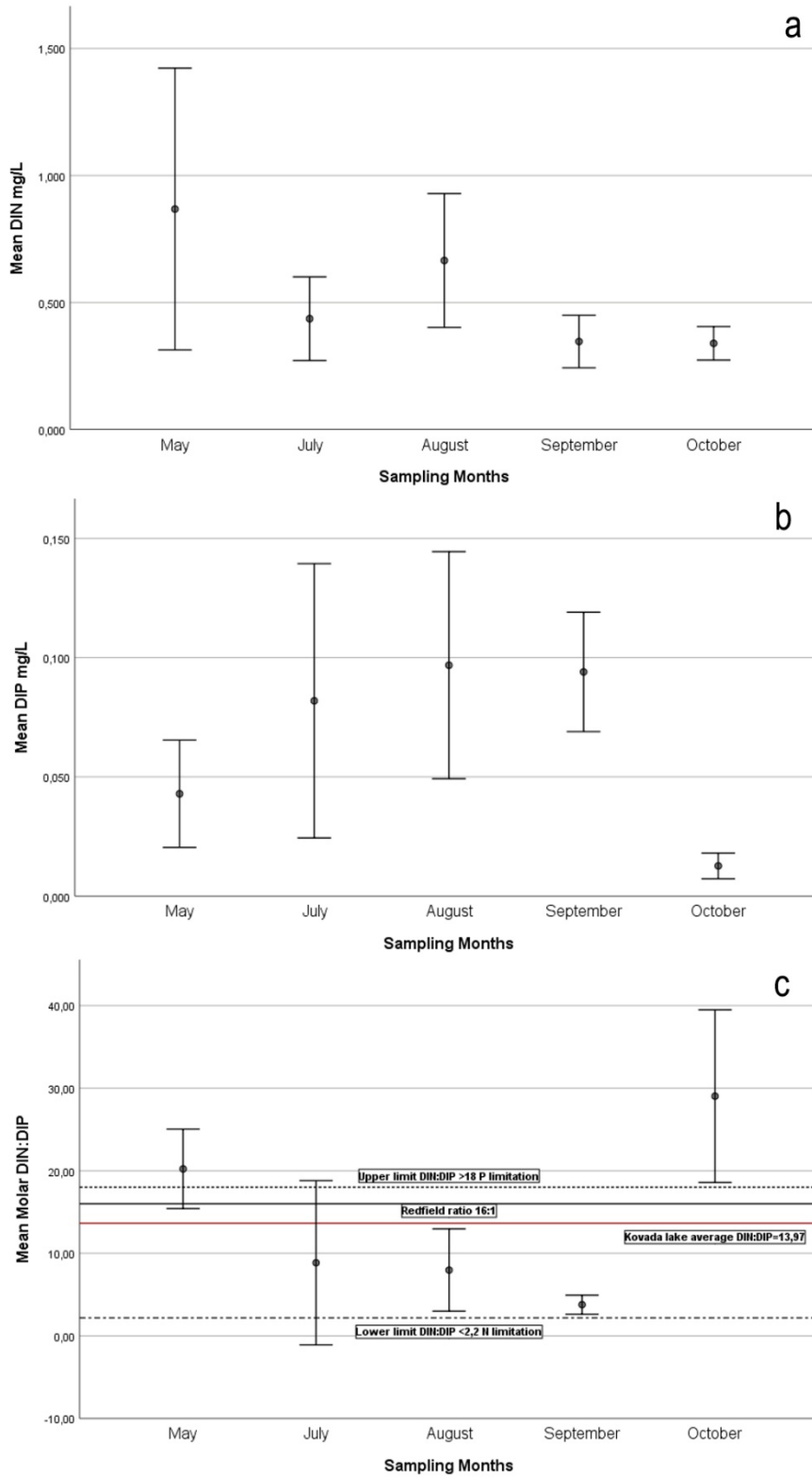


Figure 3. DIN (a), DIP(b) DIN:DIP (c) averages and thresholds of Kovada Lake-canal system in sampling months of year 2024 (bar 95%CI)

Table 2. The Spearman's rho correlation coefficients between the nutrients and ratios in Kovada Lake-canal system in year 2024

Nutrients, Ratios	TN mg L ⁻¹	TP mg L ⁻¹	OP mg L ⁻¹	Nitrite mg L ⁻¹	Nitrate mg L ⁻¹	Ammonium mg L ⁻¹	DIP mg L ⁻¹	DIN mg L ⁻¹	TN:TP	DIN:DIP	Percent DIP/TP	Percent DIN/TN
TN mg L ⁻¹		-0,337	-0,069	-0,006	0,029	0,168	-0,069	0,165	,672**	0,146	0,171	-,507**
TP mg L ⁻¹	-0,337		0,137	-0,276	0,082	-0,155	0,137	-0,022	-,902**	-0,076	-,561**	0,192
OP mg L ⁻¹	-0,069	0,137		,448*	-0,075	0,224	1,000**	0,034	-0,143	-,898**	,689**	0,037
Nitrite mg L ⁻¹	-0,006	-0,276	,448*		,407*	,689**	,448*	,627**	0,189	-0,243	,568**	,511**
Nitrate mg L ⁻¹	0,029	0,082	-0,075	,407*		0,317	-0,075	,721**	-0,031	0,338	-0,052	,574**
Ammonium mg L ⁻¹	0,168	-0,155	0,224	,689**	0,317		0,224	,847**	0,116	-0,020	0,313	,562**
DIP mg L ⁻¹	-0,069	0,137	1,000**	,448*	-0,075	0,224		0,034	-0,143	-,898**	,689**	0,037
DIN mg L ⁻¹	0,165	-0,022	0,034	,627**	,721**	,847**	0,034		0,045	0,259	0,095	,711**
TN/TP	,672**	-,902**	-0,143	0,189	-0,031	0,116	-0,143	0,045		0,118	,495*	-0,384
DIN:DIP	0,146	-0,076	-,898**	-0,243	0,338	-0,020	-,898**	0,259	0,118		-,646**	0,193
Percent DIP/TP	0,171	-,561**	,689**	,568**	-0,052	0,313	,689**	0,095	,495*	-,646**		-0,031
Percent DIN/TN	-,507**	0,192	0,037	,511**	,574**	,562**	0,037	,711**	-0,384	0,193	-0,031	

** Correlation is significant at p= 0.01 * Correlation is significant at p= 0.05

TN, TP to TN:TP relations

No significant relationship was found between the sampling stations' average TN and TP ($p > 0.05$), indicating they are independent of each other. A significant positive power relationship was found between TN and the TN:TP ratio ($TN:TP = 2.1845 * TN^{1.4906}$; $r^2 = 0.4824$; $r = 0.695$; $p < 0.05$). Based on this, four sampling stations (SS-II, SS-III, SS-IV, and KoC) indicated N limitation, while SS-I indicated N-P co-limitation.

Conversely, a strong negative logarithmic relationship was found between TP and the TN:TP ratio ($TN:TP = -7.929 * \ln(TP) + 0.0943$; $r^2 = 0.8085$, $r = -0,8891$; $p < 0.05$) (Figure 4b). Both the TN and TP regression models indicated the same nutrient limitation patterns across the stations (Figure 4). Despite these average conditions, some individual TN:TP measurements showed P limitation ($N:P > 20$).

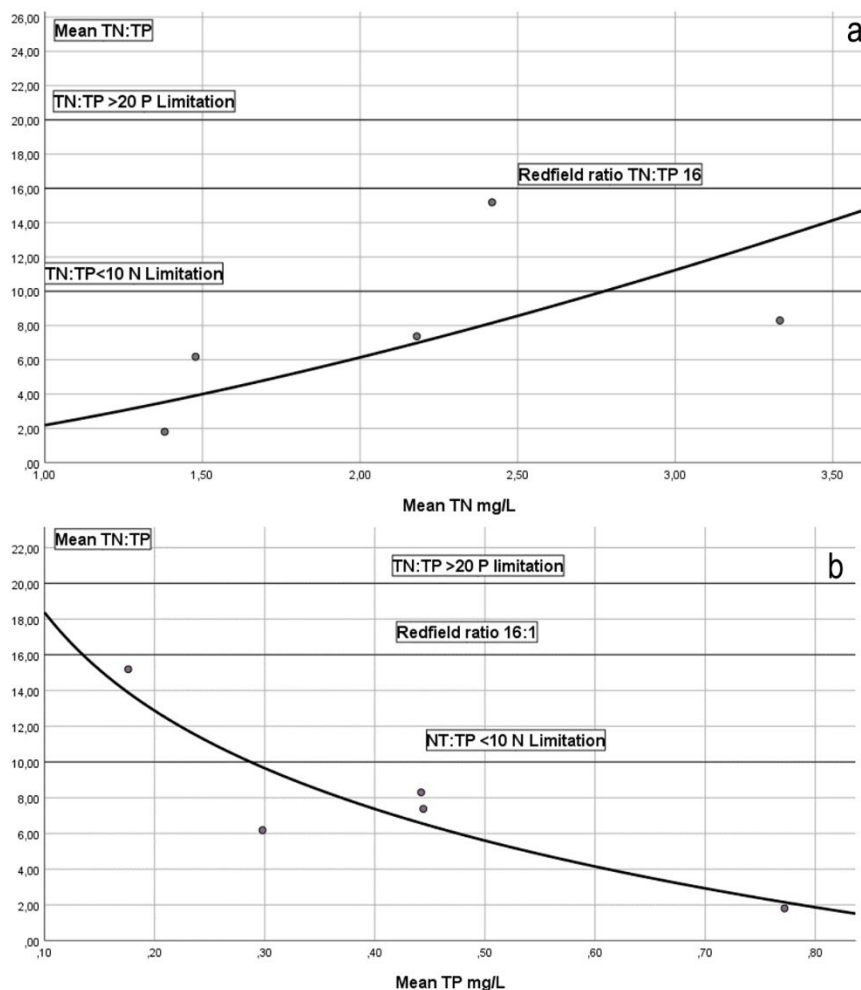


Figure 4. TN to molar TN:TP (a), TP to TN:TP (b) relations in Kovada Lake and canal

The molar TN:TP ratios were not normally distributed (S-W, $p < 0.05$) and varied significantly among sampling stations (K-W, $p < 0.01$). The highest mean ratio was observed in SS-I, near the canal's entrance (TN:TP = 15.187), indicating N-P co-limitation and closely approximating the Redfield Ratio (16:1). The lowest mean ratio was in SS-IV (1.800), indicating clear N limitation. The other sampling stations and the overall lake average (7.74) also showed N limitation (Figure 4a, b). SS-IV was distinct from the other stations, being shallower, more densely vegetated with macrophytes, and having a weaker connection to the main lake body (Figure 1).

DIN, DIP, DIN:DIP relations

No significant relationships were found between the average molar concentrations of DIN and DIP, DIN and DIN:DIP, or DIP and DIN:DIP ($p > 0.05$). Based on individual (non-averaged) data, a negative logarithmic relationship was found between DIP and the DIN:DIP ratio (DIN:DIP = $-18,66 \cdot \ln(\text{DIP}) - 36,921$, $r^2=0,5587$ $r = -0,747$). However, individual DIP and DIN:DIP values were not suitable for evaluating nutrient limitation. This was due to extremely high DIN values skewing

the ratio, which resulted in very high variation (79.68% CV) (Table 1, Figure 5a). Therefore, to evaluate nutrient limitation using this ratio, average station values were used (Figure 5b). No significant difference was found in the average DIN:DIP ratios among the SSs (K-W, $p > 0.05$). All SSs indicated N-P co-limitation. The average DIN:DIP for the entire lake system was 13.972 (SD = 10.866, indicating high variation), with a 95% CI between 9.491 and 18.453. This average falls within the recommended ratios (2.2–18) that indicate N-P co-limitation. Based on these station averages, potential N-P co-limitation (DIN:DIP ratios between 2.2 and 16) was found in four SSs, whereas SS-I (ratio > 16:1) tended toward P limitation. SS-IV had the lowest DIN:DIP ratio, tending toward N limitation, and may be loaded with DIP from an unknown source. The average DIN:DIP ratios were found to be significantly different among the sampling months (K-W, $p < 0.05$). The ratio in October was highest (29.019), differing from the other months and indicating P limitation. This corresponds to the period when the Kovada Canal had the lowest flow (resulting in lower DIN load) and the lake exhibited its lowest DIN concentration (0.339 mg L⁻¹, Table 1).

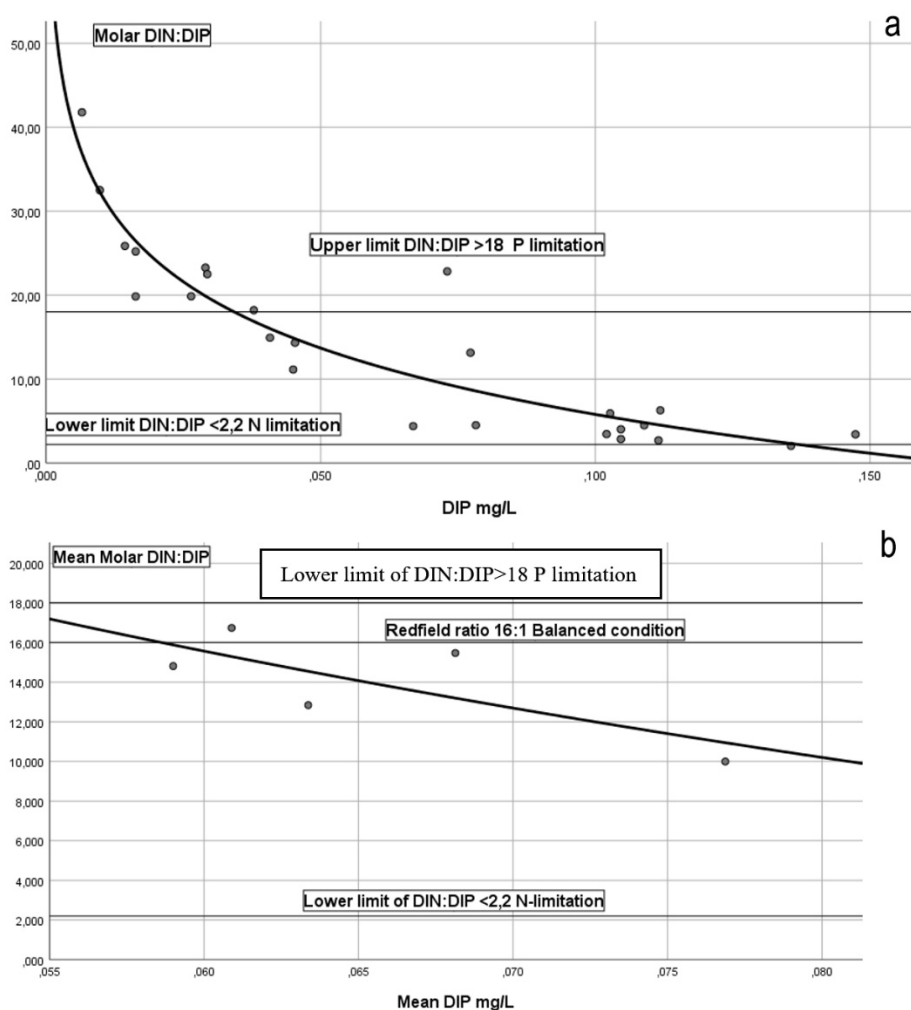


Figure 5. DIP to Molar DIN:DIP (a, individual data), DIP to Molar DIN:DIP (b, means) relations in sampling stations of Kovada Lake-canal system

Dissolved-available nutrient percentages

The percentage of DIN to TN (lowest in SS-III, 14.29%; highest in SS-IV, 33.69%) and DIP to TP (lowest in SS-IV, 9.59%; highest in SS-I, 37.71%) did not vary significantly among sampling stations or months ($p > 0.05$). The lake-wide average percentage of DIN to TN was 25.34% (SD = 13.07%), and the DIP to TP percentage was 19.20% (SD = 18.17%). In KoC, these averages were 30.87% (SD = 19.06%) for DIN:TN and 29.15% (SD = 23.44%) for DIP:TP (Table 1).

TN, TP, DIN, DIP, TN:TP and DIN:DIP ratios and Chlorophyll-a

To assess the effect of nutrients ("cause factors") on primary production ("effect factor"), relationships with Chl-a

were examined. No significant relationships were found between Chl-a and TN, TP, or the TN:TP ratio ($p > 0.05$). However, Chl-a responded strongly to DIP. A significant power relationship was found between individual values (Chl-a = $45.887 * DIP^{0.447}$; $p < 0.05$; $r^2=0.328$, $r = 0.572$) (Figure 6a). A significant exponential relationship was also found between Chl-a and the DIN:DIP ratio (Chl-a = $17.606 * (DIN:DIP)^{-0.028}$; $p < 0.05$; $r^2=0.196$, $r = -0.443$) (Figure 6b). When plotting the average DIN:DIP ratio and average Chl-a for each station, all stations fell within the N-P co-limitation range. The corresponding Chl-a averages varied between 11.16 and 18.31 $\mu\text{g/L}$; no station showed clear P limitation. The KoL-KoC system average Chl-a of 14.69 $\mu\text{g/L}$ indicates a "eutrophic trophic state" according to the OECD (1982) classification (8–25 $\mu\text{g/L}$) (Figure 6c).

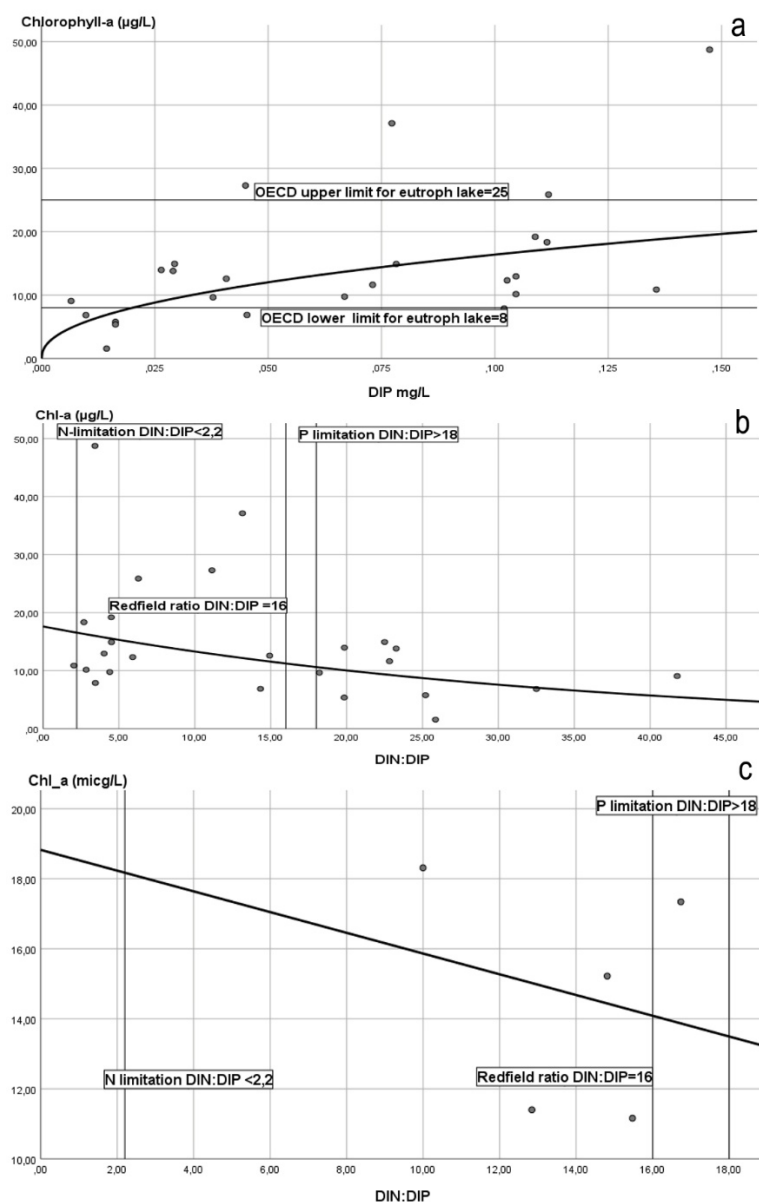


Figure 6. DIP-Chl-a (a, individual), DIN:DIP-Chl-a (b, individual) and DIN:DIP-Chl-a (c, average) relations in Kovada Lake-canal system

DISCUSSION

This study aimed to predict nutrient limitations and trophic state using total and dissolved nutrient concentrations, Chl-a, and the TN:TP and DIN:DIP ratios in Kovada Lake and its inlet canal. The canal carries excess water from Eğirdir Lake (a mesotrophic-eutrophic system) to the lake and the downstream Aksu River (Antalya basin). The canal also serves as the discharge medium for the Eğirdir wastewater treatment facility, drainage from the Boğazova plain (which has intensive horticulture, e.g., fertilizer application for apples, cherries) and wastes from fruit juice factories and storage facilities (Kazancı et al. 1999). The canal is also used for irrigation, which includes the application of plant protection chemicals. Therefore, enormous nutrient, organic, and suspended matter loadings to Kovada Lake are frequent. This results in high temporal (in KoC) and spatial (in KoL SSs) fluctuations in nutrients, Chl-a, N:P and DIN:DIP ratios, and available nutrient percentages (Table 1; Figures 2a, b; 3a, b; 4b; 6a).

Prediction of the limiting nutrient by N:P ratios

N or P limitation of algal growth is a consequence of the TN, TP, and TN:TP ratio in lakes (Guildford and Hecky, 2000). N limitation is common in freshwaters with high P levels from anthropogenic sources or P-rich soils (Ekholm, 2008). In the case of total nutrients, the average molar TN:TP ratio of 7.77 for the KoL-KoC system indicates strong N limitation (Table 1, Figure 2c). This is apparently due to the high TP and DIP loads from the domestic, agricultural, and industrial wastes entering the system (Kazancı et al., 1999). While the Redfield Ratio (RR) of 16:1 (often within a 14:1–18:1 margin) serves as a benchmark (ratios < 16:1 suggest N limitation), the presence of dense aquatic macrophyte, such as in KoL, may lead to variable stoichiometry (Dai et al., 2022).

Nutrient concentrations and ratios in the KoL-KoC system varied among SSs and months (Table 1; Figures 2, 4, 6). The system's average molar TN:TP (7.77:1) is significantly lower than the RR 16:1 (t-test, $p < 0.01$), confirming N limitation and explaining the observed ecosystem dynamics (e.g., eutrophication, excessive macrophyte growth). The trophic state, estimated as hypereutrophic (KoC) and eutrophic (KoL) in 2010 (Zeybek et al., 2012), appears to have progressed toward hypereutrophic in both systems (Cevher, 2025). This is reflected in the excessive macrophyte cover (Özdal, 2025) and the dominance of eutrophic-indicator zooplankton species (Erdoğan and Savaş, 2024). Generally, molar TN:TP ratios < 10 imply N limitation, 10–20 mixed limitation, and > 20 P limitation, though no strict guidelines exist (Kolzau et al., 2014). The observed ratio of 7.77 aligns with thresholds for N limitation proposed by various authors (e.g., <8 or <20) (Guildford and Hecky, 2000; Savic et al., 2022).

Prediction of the limiting nutrient by DIN:DIP ratios

In contrast, the average DIN:DIP ratio (13.97:1) was not significantly different from the RR (t-test, $p = 0.360$). This implies that the dissolved nutrient pool is relatively balanced,

consistent with N-P co-limitation, which supports the observed excessive macrophyte growth. Field observations confirmed that nearly the entire lake surface was covered by macrophyte by September and October. The available dissolved nutrient levels indicate a constant nutrient load occurs (Table 1, Figure 3a, b). In KoL and KoC, both total (TN, TP) and soluble inorganic forms (DIN, DIP) were consistently high, often in excess of autotrophic demand (Table 1). DIN decreased slightly in the growing season while DIP remained constant, suggesting a tendency toward N limitation (Morris and Lewis, 1988). Our estimations are consistent with previous work: Yüce and Ertan (2014) reported annual mean NO_3 (2.0 mg/L) and PO_4 (0.3 mg/L) in KoC, which yields a DIN:DIP ratio of 10.2 (N or N-P co-limitation). Our estimation of molar DIN:DIP in KoC was 15.47, approaching the P limitation level (~16). The DIN:DIP ratios at other KoL sampling points varied from 9.996 (SS-IV) to 16.738 (SS-I), averaging 13.972 for the system (Table 1). As these samples reflect the growing season, the DIN:DIP ratio might shift, possibly toward higher ratios, in the winter-spring months.

Chlorophyll a and DIN:DIP ratios

Chl-a concentrations in lakes are often dependent on TP and DIP, with weaker relationships to TN and DIN (Guildford and Hecky, 2000). In the KoL-KoC system, no relationship was found between Chl-a and TN, TP, or the TN:TP ratio. This suggests that total nutrient concentrations do not accurately reflect the biologically available nutrient pool in this macrophyte-dominated system. In contrast, a significant relationship was estimated between Chl-a and both DIP and the DIN:DIP ratio (Figure 6a,b,c). This indicates that DIP and the DIN:DIP ratio are good estimators of Chl-a (and likely other primary producer biomass) and the trophic status of the lake (OECD, 1982; Søndergaard et al., 2017; Cevher 2025).

CONCLUSIONS

Kovada Lake presents a unique system: it is a shallow, light-penetrating lake fed by nutrient-rich inflows (from Eğirdir Lake and agricultural/domestic drainage), covered by dense macrophytes (Özdal, 2025), and protected from interventions like fishery or macrophyte removal (National Park). The rainfall is very low in late spring, whole summer and early autumn months, of course temperatures are highest of the year, N-NE and S winds (Meteoblue, 2025), parallel to maximum length direction of the lake driven severe water turbidity by sediment release. In the period water abstraction for irrigation is peaked. Evaporation is highest in the period thus actual concentrations of nutrients will be increase. The nutrient limitation in such a system is affected by complex factors, including morphology, hydrology, external loading, sediment dynamics, and macrophyte abundance (Bergström, 2010). While the TN:TP ratio is a primary indicator of nutrient limitation (Guildford and Hecky, 2000), interpreting it in isolation is misleading, especially in complex systems. Our findings, showing high variability (Figure 2a, b), emphasize that ratios must be combined with absolute nutrient concentrations and biological

data. Furthermore, limitation may cease entirely when concentrations are high; if DIP exceeds $\sim 5 \mu\text{g L}^{-1}$ and DIN exceeds $\sim 300\text{--}500 \mu\text{g L}^{-1}$, neither N nor P may be limiting (Ekholm, 2008).

The distinction between nutrient forms is also critical. Molar ratios are essential as they reflect biological uptake (stoichiometry). While TN:TP is robust for long-term ecological and trophic assessment, the DIN:DIP ratio is superior for assessing short-term (growing season) nutrient bioavailability. In shallow, macrophyte-dominated systems like Kovada, macrophytes are key determinants of N:P ratios (Elser et al., 2007; Søndergaard et al., 2017). Their abundance and stoichiometry must be considered (Dai et al., 2022; Özdal, 2025) as they influence biological availability (Ekholm, 2008). In the KoL-KoC system, both total and available nutrient concentrations were found in excess, sufficient to support prolific algal and macrophyte growth. A key consequence of imbalanced (low) N:P ratios is the risk of cyanobacterial blooms. N-fixing cyanobacteria are often favored at mass N:P ratios < 22 (molar ~ 48.6) (Ekholm, 2008). Given that the KoL-KoC system harbors cyanobacteria species (Gürbüz et al., 2009; Yüce and Ertan, 2014; Çiçek and Yamuç, 2017), this risk must be considered, although hydrology and light are also critical factors.

Although many limitation thresholds exist (Guildford and Hecky, 2000; Kolzau et al., 2014), they must be adapted regionally. Before implementing remediation, site-specific thresholds should be confirmed, ideally with bioassays (Ekholm, 2008; Kolzau et al., 2014). Given the extremely high nutrient concentrations (Table 1), management actions such as

macrophyte buffer zones at the canal inlet area, canal meandering, and the implementation of 'good agricultural practices' in the catchment are needed to control pollution. The N:P ratios and DIP-Chl-a relationships evaluated here serve as critical diagnostic tools for the future assessment and management of Kovada Lake.

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AUTHORSHIP CONTRIBUTIONS

Osman Çetinkaya: Conceptualization, Methodology, Data Curation, Visualization, Writing-Original Draft Preparation, Writing-Reviewing and Editing. Cemal Cevher: Investigation, Methodology, Visualization, Original Draft Preparation

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ETHICAL APPROVAL

No specific ethical approval was necessary for this study, as no animals were used, nor were any questionnaires applied.

DECLARATION OF AI USE

AI was used for grammatical and style improvements of English text.

DATA AVAILABILITY

The data that support the results in this study are available from the corresponding author upon reasonable request.

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