Iranian Journal of Fisheries Sciences
http:doi.org/ 10.22092/ijfs.2024.132467 2025, 24(2) 177-191

Research Article

Spatio-temporal dynamics of eutrophication in Anzali international wetland system using a trophic state index

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Keywords

Anzali wetland,
Classification tree model,
Chlorophyll-*a*,
Eutrophication,
Nitrogen,
Phosphorus

Article info

Received: May 2024
Accepted: July 2024
Published: March 2025

Abstract

Eutrophication phenomenon is a global concern in wetland ecosystems. In the present research, the trophic state index (TSI) was initially determined in four main parts of the Anzali wetland using Carlson's TSI. Then, the key variables driving the eutrophication phenomenon were identified in the wetland using principal component analysis (PCA) and classification tree model (CT). Sampling was conducted monthly at 4 different and main parts of the wetland for one year (April 2019-March 2020). Based on the TSI results, all sampling sites are categorized as a hypertrophic state. However, among the sites, the Pirbazar and Sorkhankol had the highest (88.44) and the lowest TSI (76.86), respectively. The decrease in the ratio of total nitrogen (TN) to total phosphorus (TP) in the Pirbazar (5.54), shipping channel (6.58), and Sorkhankol (9.27) indicated the limitation of TN in these sites. On the contrary, the high ratio of TN/TP in the Abkanar (14.85) indicated the limitation of TP in this section. The low average ratio of the TN to TP in the four different parts of the wetland (9.06) revealed the limitation of TN in the entire wetland. Considering the TSI average in the whole wetland (22.83), it can be concluded that all parts of the wetland are classified as a hypertrophic condition. Based on the PCA biplot (the first component), the most influential factors driving the eutrophication in the wetland were TP, water transparency and chlorophyll-*a* (chl-*a*), respectively. According to the CT model's prediction, water temperature, TP, and water transparency contributed more than other factors to the wetland eutrophication process.

Introduction

Eutrophication phenomenon (water enrichment) is one of the main factors of water quality destruction in many countries of the world (Le Moal *et al*., 2019; Li *et al*., 2019; Zhang *et al*., 2021). The trophic state in aquatic ecosystems is mainly related to the primary plant production such as algae or other aquatic plants (Dodds and Cole, 2007). Variations in the nutrient concentration (mainly phosphate and nitrate) may cause significant changes in the algal biomass so-called chlorophyll-*a* (chl-*a*). This can, in turn, cause changes in water transparency (López-Abbate *et al*., 2019; Deyab *et al*., 2021).

Considering that eutrophication is a complex phenomenon, extensive studies are needed to determine the impact of the most important factors causing this phenomenon on aquatic ecosystems. Therefore, in addition to the role of important water quality factors in creating the eutrophication phenomenon, the occurrence of eutrophication in aquatic ecosystems may be significantly influenced by various factors such as structural, water depth and the volume of water ecosystems (Hamilton *et al*., 2001; Taranu and Gregory-Eaves, 2008; Liu *et al*., 2010; Troost *et al*., 2014; Li *et al*., 2019).

Using the trophic state index (TSI) for wetland ecosystems can be a valuable method to quantify the relationship between the amount of nutrients (TP and TN), chl-*a*, and water transparency. One of the most common indicators in the classification of aquatic ecosystems is Carlson's TSI (1977). The most important parameters used in estimating the Carlson's TSI are water transparency, chl-*a*, TN, and TP (Bekteshi and Cupi, 2014; Zarkami *et al*., 2022). Later on, a modified TSI was proposed by Lamparelli (Lamparelli, 2004) and almost the same parameters that were used in Carlson's TSI were employed for the wetlands and lakes. Xu *et al*. (2001) and Du *et al*. (2019) used the physical-chemical and biological factors consisting of TP, TN, and chemical oxygen demand (COD), water transparency and chl-*a* to calculate the TSI in wetlands and lakes. Several studies (e.g., Rahmati *et al*., 2012; Eizadkhasti *et al*., 2014; Vahidi *et al*., 2016) applied Carlson's TSI to study eutrophication in aquatic ecosystems.

The occurrence of eutrophication in wetlands largely depends on the nutrient inputs coming from the watershed. Phosphate and nitrate are the most common nutrients that can cause eutrophication in such fragile ecosystems. Nevertheless, phosphate concentration plays a more prominent role in the enrichment of wetlands (Zarkami *et al*., 2022). The main sources of nitrogen pollution in wetlands are related to agricultural runoffs, while most of the phosphorus pollution comes from municipal, industrial effluents, and phosphorus-based detergents (Beusen *et al*., 2016). Nowadays, anthropogenic activities (e.g., agriculture) are one of the most important threats to the destruction of wetland ecosystems around the world (Baigun *et al*., 2022). Such activities have caused excessive nutrients to enter these ecosystems leading to eutrophication.

In the last few decades, most of Iran's wetlands have faced the problem of eutrophication. Among the most important destructive activities threatening these ecosystems are agricultural activities, applications of chemical fertilizers, herbicides and pesticides, and lack of monitoring of water treatment resulting from industrial activities (Sadeghi *et al*., 2021).

Anzali international wetland (the study areas) is a typical example of such a destructed wetland. In recent years, the depth of this wetland has been reduced due to the increase in the amount of sediments and nutrients (Zarkami *et al*., 2022). This ecosystem has been exposed to extreme fragility due to the expansion of various industries and agricultural activities, the drying up of some parts of the wetland, the increase of the tourism industry in the region and the arrival of invasive species. The pollutants in the Anzali wetland originate from point and non-point sources. The pollutants of the point sources contain the wastewater of the residential areas and industrial factories due to the lack of wastewater treatment while the pollutants of the non-point sources originate from the forests and other agricultural activities like rice fields. Wetland destruction and fluctuations in the sea water level provide a good opportunity to increase the invasive plant species (e.g., *Azolla* and water hyacinth) (JICA, 2019). According to the earlier findings (Abedini *et al*., 2018; Zarkami *et al*., 2022), the density of invasive plant species has been increasing in the Anzali wetland.

The main goal of the present research is to determine the TSI in the main parts of Anzali wetland consisting of Pirbazar, Sorkhankol, shipping channel (Polghazian) and Abkanar using Carlson's TSI (with Lamparelli's modification). Therefore, with the practical application of the given index, it would be possible to identify the intensity of pollution caused by eutrophication in the main parts of the wetland in the different seasons. In the next step, the principal component analysis (PCA) helped determine the most influential variables causing the eutrophication in the wetland. Since the flexibility and transparency of the classification tree model (CT model) is very high, the application of this method can be of great importance in the management of aquatic ecosystems (Zarkami *et al*., 2022). Therefore, this model can determine the key factors contributing to the phenomenon of eutrophication in the Anzali wetland.

Materials and methods

Description of the study area

The Anzali International Wetland is one of the most important wetlands in Iran. It is located in the southwest of the Caspian Sea in Guilan province and Bandar Anzali city. The wetland area is about 195 ha and the geographic coordinate is 37° 22' to 37° 32' north latitude and 49° 14' to 49° 36' east longitude (Zarkami and Saharkhiz, 2020).

In the present research, the samplings were carried out on a monthly basis from four main and most important parts of the Anzali wetland consisting of the shipping channel, Abkanar, Sorkhankol and Pirbazar during one year (April 2019-March 2020) (Fig. 1). One sample was taken at each site and the sample containing water was taken from the water column by a Rotner. The sampling sites covered the main and different parts of Anzali wetland (including the western, eastern, Sorkhankol and central parts). Shipping channel connects the Anzali wetland to the Caspian Sea. Abkanar located in the western part of the wetland is the largest part of the wetland and it has relatively better water quality than other parts of wetland. Sorkhankol wildlife refuge is another important part of the wetland that has a high species richness such as waterfowl and fish species. This wildlife refuge supports important species in this ecosystem. Pirbazar situated in the eastern part of the wetland is shallower than other parts of the wetland. Due to the proximity of Pirbazar to the residential areas and various factories as well as the entrance of polluted rivers, the water quality is poor in this part of the wetland (Zarkami *et al*., 2022).

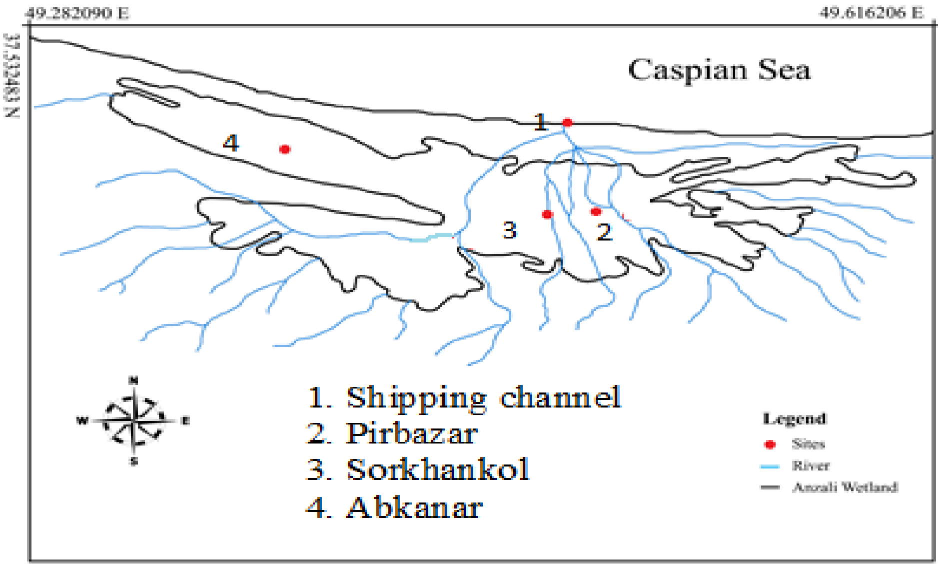

Figure 1: Sampling sites for investigating the TSI in the four main parts of the Anzali wetland (2019-2020).

Sampling methods of the environmental variables

The variables measured at each sampling site consisted of chl-*a*, water turbidity, bicarbonate, ammonium (NH_4^+), pH, water transparency, total nitrogen (TN), total phosphorus (TP), dissolved oxygen (DO), chemical oxygen demand (COD), biological oxygen demand (BOD5), electrical conductivity (EC), and water temperature (Table 2). The geographic coordinates for measuring the variables at each site were determined using a GPS device. For the measurement of the chl-*a*, a specific volume of water was filtered using a 0.45 micron filter paper and then it was extracted on ethanol paper and its absorbance was read at wavelengths of 665-750 nm. Onsite monitoring was used for measuring the water temperature (mercury thermometer), dissolved oxygen (iodometric titration), electrical conductivity and pH (multi-parameter device). For measuring the rest of the parameters, the samples were immediately transferred to the laboratory and analyzed by the standard method (APHA, 2012).

Calculation of Carlson's TSI

In the present research, a multi-parameter evaluation method of TSI was used to determine the eutrophication status in the main parts of the Anzali wetland. In the given method, the range of TSI for the key variables such as Secchi disk (SD), chlorophyll-a (chl-*a*), and total phosphorus (TP) is proposed to assess the TSI in aquatic ecosystems. The equations for the SD, chl-*a*, TP, and TN are assessed from 0 to 100 (more information can be found on the Carlson, 1977, and modified by Lamparelli, 2004). In general, in this classification system, the TSI fluctuates from an extremely low trophic to an extremely high trophic environment. If TSI is ≤47, the conditions may be considered very oligotrophic, and if TSI>67, the conditions are hypertrophic and other TSI ranges are in between (Table 1).

Table 1: Classification of aquatic ecosystems based on Carlson's TSI (Carlson, 1977).

Trophic classification	TSI ranges
Very oligotroph	TSI ≤ 47
Oligotroph	47 < TSI ≤ 52
Mesotroph	52 < TSI ≤ 59
Eutroph	59 < TSI ≤ 63
Super eutroph	63 < TSI ≤ 67
Hypereutroph	TSI > 67

Data analysis method

First, the distribution of data (minimum, maximum, mean and standard deviation) was calculated for each variable in the sampling sites using general descriptive statistics. Then, the Kolmogorov-Smirnov test was used to check the normality of the data. After identifying the non-normal data for each variable (if any), they were converted to the logarithm scale (SPSS software). Then, the normalized data were used for the different tests (e.g., one-way ANOVA and PCA). The Pearson correlation coefficient was used to show the relationship between the recorded variables. One-way analysis of variance test (ANOVA) was used to check the significance of influencing variables in the phenomenon of eutrophication based on different sampling sites/seasons. PCA (PAST software) was employed to identify the most important variables at each component and the relative contribution of the variables to the wetland eutrophication. In the PCA, all factors are recorded in Table 2. 13 factors representing 13 main components) were introduced to the relevant software. The CT model (WEKA software) (Witten *et al*., 2011) was employed to predict the key variables and their impact on eutrophication in the wetland. The model's output was the amount of chl-*a* concentration that was classified into three categorical classes (low, medium, and high) and the model's inputs were the other variables measured in the sampling sites (Table 2).

Table 2: General descriptive statistics for representing the data distribution (minimum, maximum, mean and standard deviation) for the recorded variables in the sampling sites in Anzali wetland (the number of samples taken for a 12-month in the four main parts of the Anzali wetland: 48). TN: total nitrogen; TP: total phosphorus; S1: Abkanar; S2: Pirbazar; S3: Sorkhankol; S4: Shipping channel (wave breaking section).

Variables	Statistics	S1	S2	S3	S4
COD (mg/L)	max	41	24	12	22
	min	17	4	2	7
	mean	31.42	15.42	7.83	16.08
	SD	8.88	7.63	3.21	5.23

Table 2 (continued):

Variables	Statistics	S1	S2	S3	S4
BOD (mg/L)	max	18	11	6	13
	min	8	2	3	2
	mean	12.67	6.42	4.08	7.67
	SD	3.08	3.58	0.99	3.31
Turbidity (FTU)	max	51	47	69	69
	min	4	12	10	8
	mean	18.30	24.75	33.75	19.58
	SD	15.52	10.79	13.38	16.68
HCO_3^- (mg/L)	max	176.90	341.60	268.40	262.30
	min	15.30	170.80	128.10	125.10
	mean	94.57	242.23	196.73	224.44
	SD	50.69	63.77	39.74	43.91
NH_4^+ (mg/L)	max	6.90	1.99	0.19	1.56
	min	2.87	0.21	0.01	0.30
	mean	4.73	0.93	0.11	0.80
	SD	1.12	0.66	0.06	0.39
Chl-*a* (µg/L)	max	109.52	260.48	148.00	233.21
	min	3.26	0.59	1.18	1.48
	mean	27.04	51.38	51.72	57.49
	SD	31.52	77.24	53.97	78.62
pH	max	10.03	8.18	8.79	8.69
	min	7.95	7.11	6.98	6.87
	mean	8.80	7.62	8.01	7.83
	SD	0.68	0.29	0.63	0.52
WT (°C)	max	28.50	29.00	8.79	28.00
	min	10.50	10.80	6.98	9.50
	mean	19.27	19.11	8.01	17.87
	SD	7.74	7.74	0.63	7.01
Transparency (cm)	max	175	100	50	150
	min	30	15	25	40
	mean	103.75	60.00	38.33	76.25
	SD	56.17	27.71	10.07	30.31
TN (mg/L)	max	2.55	3.18	1.77	2.02
	min	0.29	0.54	0.45	0.46
	mean	1.04	1.33	1.02	1.12
	SD	0.71	0.76	0.38	0.45
TP (mg/L)	max	0.16	0.61	0.21	0.38
	min	0.01	0.05	0.02	0.02
	mean	0.07	0.24	0.11	0.17
	SD	0.05	0.18	0.05	0.11
DO (mg/L)	max	12.50	9.50	11.50	11.50
	min	9.00	2.80	4.90	7.00
	mean	11.18	5.83	8.25	8.87
	SD	0.95	1.89	1.99	1.26

Table 2 (continued):

Variables	Statistics	S1	S2	S3	S4
EC (µS/cm)	max	3050	1708	13910	14250
	min	254	442	431	333
	mean	867.08	1175.25	3604.66	6085.25
	SD	967.75	478.45	466.26	4480.74

Results

General descriptive statistics

Table 2 shows the results of descriptive statistics (the minimum, maximum, mean and standard deviation) for all factors that were measured in the sampling sites. As indicated in the table, among the recorded variables, the highest and lowest chl-*a* concentrations were recorded in the Pirbazar (260.48 µg/L) and Abkanar (109.52 µg/L), respectively. The maximum and minimum TN concentrations were also found in the same sites (3.18 and 0.29 mg/L), respectively. During the research period, the concentration of TP (another important nutrient) was also higher in the Pirbazar than other sampling sites (0.24 mg/L), while the lowest amount of this nutrient was observed in the Abkanar (0.01 mg/L). Abkanar had the highest water transparency (1.75 m) while the lowest transparency was observed in the Pirbazar (0.15 m). Therefore, by testing the data pre-processing of these four key and influential factors for calculating TSI, it was determined that the amount of nutrient pollution in the Pirbazar was usually higher than other sites and the lowest nutrient pollution was observed in the Abkanar.

The results of the Kolmogorov-Smirnov test (KS) showed that only DO and pH were normally distributed ($p>0.05$), while the other variables had a non-normal distribution (as stated already, the data of all variables in Table 2 were converted to the normal distribution using logarithm scale, except for the DO and pH).

Correlation coefficient between the recorded variables

The results of Pearson correlation coefficient showed that chl-*a* had a positive and high correlation with several factors such as TP (r= 0.58, $p<0.01$), TN (r=0.50, $p<0.05$), water temperature (r=0.74, $p<0.01$) and electrical conductivity (r= 0.58, $p<0.01$) indicating that an increase at each of the above factors may lead to an increase in the amount of chl-*a* in the Anzali wetland.

Relationship between environmental variables and sampling sites/seasons

Based on the results of one-way analysis of variance (ANOVA), TP, EC, BOD and pH had a significant difference either with the sampling sites or seasons ($p<0.05$ for all variables), while NH_4^+, water transparency, DO, HCO_3^- and COD had a significant difference only with sampling sites ($p<0.05$ for all). TN, WT, water turbidity and chl-*a* had a significant difference only with the sampling seasons ($p<0.05$ for all).

Carlson's TSI

In order to examine the trophic conditions in the main parts of the wetland, the four influencing factors for the eutrophication "chl-*a*, TP, TN and the depth of transparency" were used by Carlson's TSI (with Lamparelli's modification). Then, their averages (based on the ratio of TN/TP) were put in the relevant equations.

First, the ratio of TN/TP was calculated using the total average. In this case, the ratio of TN/TP was obtained 7. Given that the obtained number is <10, it indicates the limitation of nitrogen. Therefore, the following formula is used for the four parts of the Anzali wetland:

TSI (AVG) = 1/3 [TSI (chl) + TSI (SD) + TSI (TN)]

TSI (chl-*a*) = 16.8 + 14.4 * ln (chl-*a*), (mg/m^3)

TSI (SD) = 60.0 – 30.0 * ln (SD), (m)

TSI (TN) = 59.6 + 21.5 * ln (TN), (mg/L)

By putting the averages in these 3 equations and then in the total equation, the number ‘’84’’ was obtained which indicates the hypertrophic condition in the Anzali wetland. Considering the amount of TSI (by calculating the ratio of TN/TP), it can be concluded that there is a limitation of TN in the Pirbazar, the shipping channel, and Sorkhankol, whereas the limitation of TP (14.85) was observed in the Abkanar (Table 3). By calculating the average ratio of TN/TP in four different parts of the wetland (9.06), one can conclude that there is a limitation of TN in all mentioned parts. Considering the average of the TSI in the different sampling sites of the wetland (22.83), it is obvious that all of the four parts of the wetland are classified as a hypertrophic condition. Since the TSI obtained in the four parts of the wetland lies in the range of 60-100, it specifies that the entire wetland is categorized as hypertrophic.

Table 3: Classification of sampling sites in the Anzali wetland based on the TSI in a one-year.

Sampling sites	TN/TP	TSI	TSI level
Shipping channel	6.58	87.50	Hypertrophic
Sorkhankol	9.27	76.86	Hypertrophic
Pirbazar	5.54	88.44	Hypertrophic
Abkanar	14.85	80.09	Hypertrophic
Total four parts	**9.06**	**83.22**	**Hypertrophic**

Principal component analysis (PCA)

According to the PCA results, components 1, 2 and 3 accounted for 32.74, 23.81 and 14.41 % of the variation, respectively (in total, 3 components accounted for 70.96 % of the variation). Based on KMO and Bartlett's results of PCA, the obtained number is 0.71 (p>0.50) which indicates the acceptability of the test. The probability value in the given test (p<0.01) shows that the relationship between the variables causing eutrophication state in the wetland is significant.

Based on the analysis of PCA biplot (Fig. 2), the most influential factors of the first component, in the process of eutrophication consist of TP, water transparency, and chl-*a*, respectively. The variables in the second component were pH, BOD5, and COD, respectively.

The CT model

Among the 12 variables introduced to the CT model (as model’s inputs) and one variable " chl-*a*" (as the model’s output with three categorical classes: low, medium and high), the induced model predicted that the variables ‘’water temperature, TP and water transparency’’ may be more important variables than others for deciding the eutrophication status in the wetland (Fig. 3).

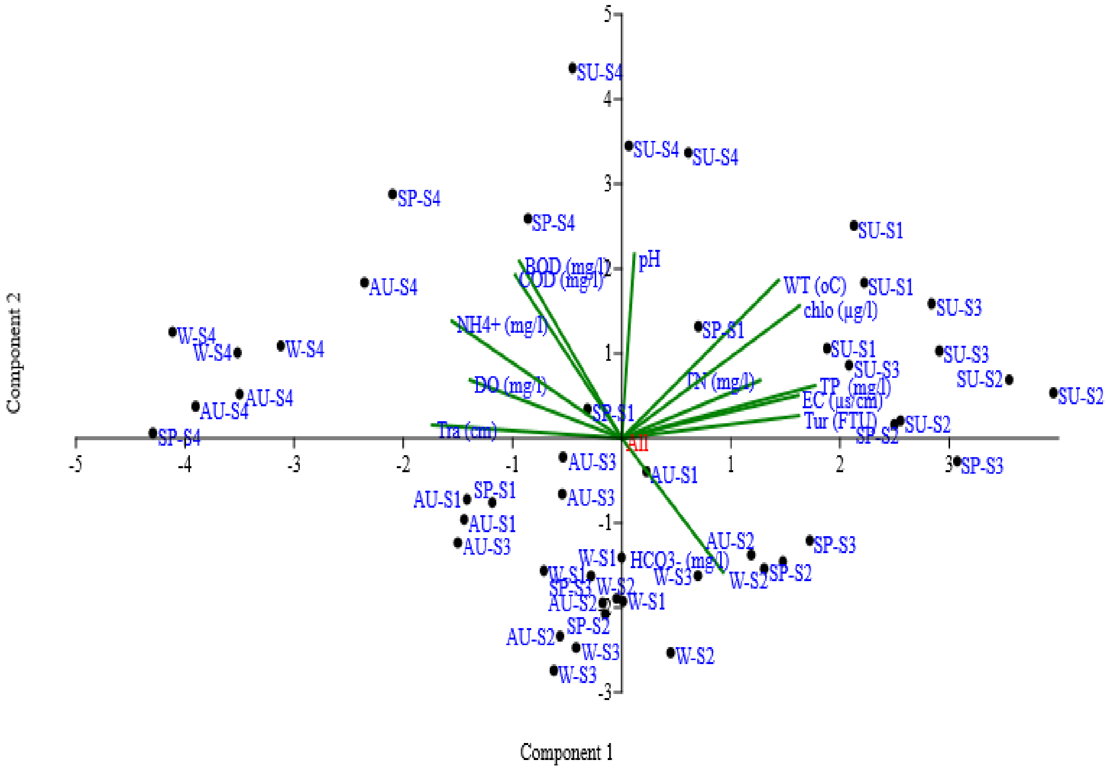

Figure 2: PCA biplot (PCAs 1 and 2) for illustrating the effect of the most important variables on the eutrophication in the Anzali wetland for one year: 2019-2020 (the type of matrix: "correlation"). Tra: transparency, Tur: turbidity, chlo: chlorophyll-a, total phosphorus: TP, total nitrogen: TN, dissolved oxygen: DO, bicarbonate: HCO_3^-, electrical conductivity: EC, ammonium: NH_4^+, acidity: pH, chemical oxygen demand: COD, biological oxygen demand: BOD5, water temperature: WT. S1: shipping channel, S2: Sorkhankol, S3: Pirbazar, S4: Abkanar, SP: spring, AU: autumn, WI: winter, SU: summer. The codes (next to the circles) indicate the sampling sites (S1, S2, S3, and S4) and seasons (AU, W, SP, and SU).

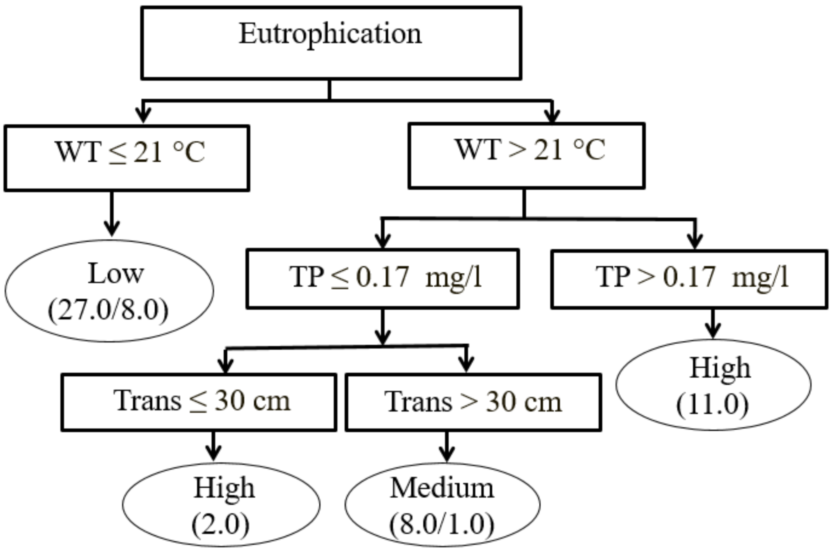

Figure 3: CT model for predicting the eutrophication condition based on the amount of chl-*a* in the main parts of the Anzali wetland. The model output: chl-*a* (with three categorical classes: low, medium, and high) and model inputs: all recorded variables presented in Table 2. WT: water temperature, TP: total phosphorus, Trans: water transparency, PCF: 0.25; ≠ folds cross-validation: 3 folds. The numbers in parentheses represent the number of instances in which the rules of the model are specified as true/false; total instances: 48.

The model prediction was based on the medium pruning confidence factor (PCF): 0.25, the number of folds cross-validation: 3 times, the size of the tree: 7 and the number of leaves: 4. The model's output, which is based on two important criteria for model validation, (i.e. percentage of correctly classified instances or CCI %) and Cohen's Kappa statistics or *k*) showed that the model prediction was reliable so that more than 60 % of the data were correctly classified and Cohen's Kappa (*k*) was also more than 0.40 (CCI=64.58%; Cohen Kappa=0.46).

Based on the model's outcomes, when the water temperature shows an increase particularly in the hot season (>21°C), this condition may stimulate the growth of phytoplankton. This might subsequently increase the amount of chl-*a* and the intensity of eutrophication in the wetland. According to the general rule extracted from the model, when the water temperature gradually decreases (<21°C), the amount of chl-*a* may also show a decrease. The model's decision confirmed that when the water temperature rises particularly in the hot seasons (>21°C) together with an increase in the concentration of TP in the given seasons (>0.17 mg/L), the concentration of chl-*a* may rise. When the concentration of TP decreases (<0.17 mg/L), the increase/decrease of water transparency may play a decisive role in the increase/decrease of chl-*a* in the wetland. If the level of water transparency in the wetland decreases (due to the increase of various factors such as phosphate), the concentration of chl-*a* will also increase. If the level of water transparency in water becomes relatively high (>30 cm), the level of chl-*a* in the wetland will reach an intermediate level.

Discussion

Given that chl-*a*, total phosphorus, total nitrogen and water transparency are the most important factors driving the eutrophication in the wetland ecosystems, the trophic state index (TSI) was initially determined based on these important factors in the four main parts of the Anzali international wetland. In addition to the aforementioned variables, the earlier reports (Liu *et al*., 2010; Zhang *et al*., 2021) stated that other factors may also contribute to the eutrophication phenomenon in wetlands. For that reason, after calculating Carlson's TSI, we applied the PCA and the CT techniques to determine other important drivers (Abedini *et al*., 2018; Zarkami *et al*., 2022) that may influence the eutrophication in the Anzali wetland.

The results of Carlson's TSI showed that all four parts of the Anzali wetland are classified as hypertrophic condition. The obtained results were to a high extent consistent with the previous research (Zarkami *et al*., 2022). However, among the studied areas, the Pirbazar section had the maximum concentration of nutrient pollutants (based on the highest TSI) indicating that the intensity of eutrophication in the Pirbazar was higher than the rest of the areas. Despite this, the Sorkhankol section had relatively the lowest TSI than the other three sampling sites. The outcomes of Carlson's TSI are in line with the earlier reports in which the wetland was classified as a hypertrophic system (Abedini *et al*., 2018; Zarkami *et* *al*., 2022). In another study, the wetland was classified as a mesotrophic system. Therefore, by comparing the outcomes of the present research with the previous studies (Mirzajani *et al*., 2010), one can conclude that the eutrophication situation in the Anzali wetland has reached a critical level and it is expected that this phenomenon still will continue to occur. The main reason to intensify this problem is the increase of different types of effluents (e.g., agricultural and urban activities) in the catchment area of the Anzali wetland and finally to enter the effluents into the wetland (Abedini *et al*., 2018; Zarkami *et al*., 2022).

The results of PCA and CT techniques showed that when the concentration of nutrients (especially TP) shows an increase in the wetland, the amount of chl-*a* also increases. The advantage of the PCA and CT techniques applied in the present research compared to the previous studies is that both methods were able to give an additional information for the management of the Anzali wetland in the near future.

The concentration of nutrients and subsequently the amount of chl-*a* in the Pirbazar and shipping channel (wave breaking) sections were higher than the other two sites. This problem may be attributed to the influx of different types of wastewater (*e.g.*, urban, industrial, and also agricultural effluents) (Babaei *et al*., 2007). According to the results of the correlation analysis as well as the PCA and CT methods, the maximum concentration of chl-*a* was observed in the areas where the water transparency was relatively lower than other parts of the wetland. The aquatic ecosystems that have clear water (less water turbidity) and also low nutrients may not provide good conditions for the production of phytoplankton communities (Liu *et al*., 2010; Troost *et al*., 2014).

The results of the present research showed that the most important factors causing the eutrophication (e.g., total phosphorus) were positively correlated not only with chl-*a* but also with other factors such as water turbidity in the wetland. The outcomes of the PCA and the CT model indicated that total phosphorus is the most important nutrient than total nitrogen for stimulating the algal blooms in the wetland. The obtained results were in accordance with the previous reports (Bekteshi and Cupi, 2014) confirming that the nitrate concentration (unlike phosphate) is not more often used to estimate the TSI in lakes and wetlands (Carlson, 1977; Liu *et al*., 2010; Bekteshi and Cupi, 2014). Nevertheless, the presence of both nutrients (NO_3^- and PO_4^{-3}) can stimulate the photosynthetic activity and they can, in turn, increase the production of algae in the wetland and hence exacerbating the eutrophication problem in this valuable ecosystem.

The outcomes of the applied methods (PCA and CT) confirmed that a large amount of chl-*a* (especially in the summer season) may escalate the water turbidity in the wetland so that the amount of these two factors in the Pirbazar is more striking than other parts of the wetland. The correlation analysis also showed that electrical conductivity, chl-*a*, TP and TN have a close relationship with the level of water turbidity in the wetland demonstrating that the escalation of water turbidity (especially at lower depths) may to a high extent contribute to the water enrichment (Silvino and Barbosa, 2015; Zarkami *et al.*, 2022). Like the outcomes of the present research, another research conducted in the Gomishan wetland (in the southeast of the Caspian Sea) revealed that the main cause of the eutrophication in the wetland is attributed the proliferation of phosphorus originating from various sources such as agriculture and household (Omrani and Dorri, 2017). The correlation analysis specified that water turbidity and water transparency had a positive and negative correlation with chl-*a* in the Anzali wetland, respectively so that this issue also was already confirmed in the earlier reports (Zarkami *et al*., 2022).

The ratio of TN/TP is one of the most important criteria in calculating the trophic state in aquatic ecosystems (Janse, 2005). In general, this ratio is usually low in the highly eutrophic wetlands, in contrast, it is high in the oligotrophic ecosystems. Although all the sampling sites located in the Anzali wetland were classified in the hypertrophic condition, the Pirbazar and shipping channels had the lowest ratio of TN/TP (5.54 and 6.58, respectively). The ratio of TN/TP in the Sorkhankol was at the intermediate level (9.27), while Abkanar had the highest ratio of TN/TP (14.85) due to having higher water depth that other areas. The previous research also confirmed that the eutrophication in the Pirbazar and shipping channel reached a critical condition. In general, the intensity of eutrophication in the Anzali wetland as well as other eutrophic wetlands can vary depending on the type of human activities, weather conditions, depth, size of wetlands, and the amount of pollutants coming from the surface and underground waters. The shallower depth and smaller size of the wetland, the intensity of eutrophication will be more so that reaching the final stages of eutrophication will be sooner. Since, in the last few decades, the depth of the Anzali wetland has become shallower, this problem has intensified the eutrophication problem (Zarkami *et al*., 2022).

Due to the various agricultural and industrial activities in the Anzali wetland catchment and the rivers entering this wetland, numerous types of pollutants come into the wetland causing the wetland more eutrophic. Another source of pollution in the Anzali wetland is the effluents resulting from the wastes deposited in the Saravan forest area. During the rainfall, these effluents enter the surface and underground waters, and then they come into the Anzali wetland through different rivers (Jafari *et al*., 2022) strengthening the trend of eutrophication in the wetland. Considering the increasing trend of eutrophication in the Anzali wetland, it is necessary to take serious actions especially to control the polluting sources. To reach this goal, the wetland restoration projects are successful in terms of eutrophication.

Conclusions

The results of the TSI showed that all four parts of the wetland are in the hypertrophic condition indicating a severe eutrophication in this ecosystem. Considering the average ratio of TN/TP (9.06), it can be concluded that there is a limitation of TN in the entire parts of the wetland. This implicitly indicates that the state of eutrophication is strengthened in all parts of the wetland.

Based on the outcomes of both techniques (PCA and CT model), TP and water transparency are the most important driving factors in the process of eutrophication in the wetland. According to the prediction of the CT model, the situation of eutrophication in the summer season is more remarkable than in other seasons.

Acknowledgments

The authors would like to thank the Guilan Environment Protection Office for giving us the opportunity for field sampling.

Conflicts of interest

It is hereby declared that the authors declare no conflict of interest.

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