PHYTOPLANKTON MONITORING AND GENETIC ANALYSIS OF LAKE BUTRINTI: ADVANCED METHODS AND ENVIRONMENTAL IMPLICATIONS

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ABSTRACT

Lake Butrinti, located near Saranda in southern Albania, is a unique brackish lagoon shaped by tectonic activity and characterized by significant ecological, historical, and economic value. Its distinct nature has sparked scientific interest in understanding the factors that influence water quality and the lagoon's capacity to support diverse life forms. This paper reviews the findings on water quality based on physical (temperature, turbidity, salinity, and pH), chemical (oxygen, OD, COD, BOD, nitrites, nitrates, phosphates, heavy metals, pesticides, and organic matter), and biological indicators (phytoplankton chlorophyll a, Carlson Trophic State Index-TSIC, biomass, and cytotoxicity testing via bio-reporting bacteria). The data reported data were organized to depict annual, seasonal, and spatial dynamics more clearly. Furthermore, the methodologies applied to assess phytoplankton diversity, including microscopy, chemotaxonomy, specific-PCR, CARD-FISH, and sequencing, are reviewed, with a discussion of their respective advantages and limitations. The findings indicate that anthropogenic activities, including past interventions in the hydrological network, fishing practices, commercial aquaculture, and pollution, along with the effects of climate change, have negatively impacted the lagoons on phytoplankton diversity, and their advantages versus limitations were discussed. In conclusion, anthropogenic activities (former interventions in the hydrological network, fishing, commercial aquaculture farming, and water pollution) have negatively impacted the lagoon's physical, chemical, and biological integrity. To prevent significant ecological and socio-economic consequences, such as dystrophic crises, reduced fishing and mussel production, ecological degradation, biodiversity loss, and decreased aesthetic value, there is a pressing need for more comprehensive and systematic research. Such research should employ both conventional and advanced methods to monitor changes, mitigate adverse effects, and safeguard the ecological integrity and economic sustainability of the lake.

Keywords: Albanian lagoons, physic-chemical parameters, algal bloom, analytical methods

1. INTRODUCTION

Lake Butrinti is a unique brackish lagoon shaped by tectonic activity and is characterized by significant ecological, historical, and economic importance. Covering an area of approximately 16.3 km² with an average depth of 14 m (Topi *et al*. 2013c; Guri *et al*. 2014), it receives freshwater from the Pavllo and Bistrica rivers and saltwater from the Ionian Sea through the Vivari Canal. The lake is also connected to Lake Bufi via the Rreza/Bufi Canal, with the surrounding bedrock primarily composed of limestone (Hounslow and Chepstow-Lusty, 2004). Subterranean springs emerge along the eastern lakeshores, and two pumping stations discharge drainage waters from the Vurgu and Vrina plains at Manastiri (northern shore) and Dajlani (southern shore), respectively (Miho *et al*. 2013). The lagoon is a vital ecosystem supporting rare habitats and biodiversity (Miho, 1994; Miho and Witkowski, 2005; Bego and Malltezi, 2010; Bushati, 2013; Miho *et al*. 2013), with the cultivation of *Mytilus galloprovincialis* playing a key role in the local economy (Bego and Malltezi, 2010; Miho *et al*. 2013). The area holds outstanding cultural value and is protected under national and international legislation, including its designation as a UNESCO World Heritage Site (UNESCO, Topi *et al*. 2013b). Moreover, Lake Butrinti forms a core component of the Ramsar transitory-marine area of Çuka Channel-Butrinti-Stillo Cape (13,500 ha; DCM 531/2002; Site No.1290, 28.3.2003).

However, increasing anthropogenic pressures, including the diversion of the Bistrica River in the late 1950s, waste disposal, and growing tourism, have exacerbated environmental stress (Miho 1994; Xhulaj *et al*., 2008). River diversion and reclamation of the Vurgu Plain significantly reduced the surface area of Lake Butrinti and its associated wetlands, decreased freshwater input, and led to increased mineralization of the lagoon. Consequently, Butrinti is classified as a meromictic lake with longstanding water stratification (epilimnion and hypolimnion) and a history of anoxic conditions that facilitate hydrogen sulfide (H_2S) accumulation, limiting benthic life (Pano, 1984; Miho, 1994; Moisiu *et al*. 2016; Bacu and Zaho, 2022).

2. MATERIALS AND METHODS

This review synthesizes findings on water quality based on physical (temperature, turbidity, salinity, and pH), chemical (oxygen, OD, COD, BOD, nitrites, nitrates, phosphates, heavy metals, pesticides, and organic matter), and biological parameters (phytoplankton chlorophyll a, Carlson Trophic State Index – TSIC, biomass, and cytotoxicity testing using bio-reporting bacteria). Data were collected from studies conducted over the past decades, including Pano (1984), Miho (1994), Hounslow and Chepstow-Lusty (2004), Miho and Witkowski (2005), Xhulaj *et al*. (2008), Bego and Malltezi (2010), Miho *et al*. (2013), Bushati (2013), Topi *et al*. (2013 a), Guri *et al*. (2014), Moisiu *et al*. (2016), and Bacu and Zaho (2022), among others. The reviewed data are presented in a structured format to facilitate a clearer depiction of the annual, seasonal, and spatial dynamics of these parameters. Additionally, methodologies previously employed for phytoplankton diversity assessment, including microscopy, chemotaxonomy, specific PCR, CARD-FISH, and sequencing, are examined. Their respective capacities and limitations are critically discussed, drawing on findings from Bacu *et al*. (2022), Bacu and Zaho (2022), Bacu *et al*. (2024), Omeri *et al*. (2024), and other relevant studies.

An overview on physical and chemical characteristics of waters and sediments

Physical characteristics

Butrinti region has a typical Mediterranean climate, which is reflected in both water temperature (Table 1) and precipitation patterns (Dedej and Bino, 2003; Topi *et al.* 2013(c); Velaj, 2015).

Table 1. Epilimnion temperature (˚C) of Lake Butrinti

References	Year of measurement	Winter-autumn	Spring-summer
Osmani Miri and Peja, 2012	2012	9˚C	28˚C

Bushati, 2013	2006-2010	6˚C	28˚C
NEA 2015	2013	19.3˚C	24.2˚C

Rainfall is abundant, with higher levels occurring in autumn and winter and lower levels in spring and summer. The annual average precipitation is approximately 1,500 mm (Miho, 1994; Dedej and Bino, 2003; Bego and Malltezi, 2010; Zotaj, 2010; Topi e*t al*. 2013a; Pano, 2015; Velaj, 2015).

The tidal cycle at Lake Butrinti follows a six-hour periodicity (Pano, 2015). However, hydrometeorological factors, including components of the water balance, wind regime, and wave dynamics, also influence water level oscillations. These factors do not affect not the pattern of fluctuations, but their amplitude.

Owing to its connection with the Ionian Sea, Lake Butrinti is influenced by marine winds, with average speeds of 3.2–3.5 m/s in winter and 2.2–2.3 m/s in summer (Bego and Malltezi, 2010; Topi *et al*. 2013c; Zotaj, 2010).

Water transparency (Table 2) varies in response to rainfall, wind intensity, and plankton growth (Miho, 1994; Bushati, 2013; Miho *et al*. 2013). The highest transparency values are generally recorded during winter, late spring, and early summer (Bushati, 2013).

Table 2. Transparency of the Lake Butrinti based on Secchi disc measurements (m)

References	Depth (m)
Miho, 1994; Bushati, 2013	0.8/1 - 4
Pano, 2015	0.8 - 3.2
Heywood, 2017	2.8 - 3
Çako *et al.* 2013	1.85 - 4.32

The sediment archive of Lake Butrinti provides evidence of variations in the Mediterranean climate and anthropogenic impacts on the lake's trophic status and hydrology. Lake sediments are excellent recorders of environmental change, capturing signals from within the basin and from the surrounding catchment. When coupled with robust chronological constraints, lacustrine sediment archives can effectively reveal climatic, human, and tectonic influences on the environment (Xhulaj *et al*. 2008; Ariztegui *et al*. 2010). Sediments consist primarily of carbonates, organic matter, and clay layers (Morellón *et al*. 2016) and are characterized by finely stratified silt resulting from the seasonal deposition of calcite, which is rich in organic materials and clays. The presence of heavy metals in lake sediments has also been investigated by Topi *et al*. (2012) and Omeri *et al*. (2024).

Organic carbon (Corg) levels in the sediments exhibited an average value of 1.792 ± 0.336% dry weight (d.w.), while total nitrogen (TN) averaged 0.197 ± 0.043% d.w. Their spatial distribution is relatively uniform between the northwestern and southeastern regions of the lagoon (Moisiu *et al.* 2016).

Chemical characteristics

The salinity of Lake Butrinti is influenced by its connection with the Ionian Sea through the Vivari Canal, contact with Lake Bufi, freshwater inflows from the Bistrica and Pavllo rivers, and precipitation and evaporation processes (Miho *et al*. 2013; Pano, 1984; 2015). Reported salinity values vary among authors (Tables 3 and 4); however, a consistent finding is that salinity increases with depth (Pano, 1984; Miho, 1994; Pano, 2015; Moisiu *et al*. 2016). Seasonal variation is also evident, with higher salinity values observed during summer compared than in winter (Pano, 1984; Dedej and Bino, 2003; Miho *et al*. 2013). In the epilimnion, salinity ranges from 14–35‰, reaching maximum levels in late summer and minimum levels in winter. Below a depth of 6 m, salinity remains consistently higher, between 20–35‰. In contrast, salinity in the adjacent seawaters is significantly higher, ranging from 30.4‰ at the surface to 40.9‰ at a depth of 5 m (Miho, 1994; Bushati, 2013; Miho *et al*. 2013).

Table 3. Salinity (‰) in hypolimnion and epilimnion

References	Hypolimnion (under 6 meter)	Epilimnion (surface waters)
Miho, 1994	35.1‰	18‰
Anonymous, 2010; Miho *et al*. 2013	20-35‰	14-35‰
Moisiu *et al.* 2016	35‰	24.3‰

Table 4. Salinity (‰) in summer and winter

References	Summer	Winter
Pano, 1984	26‰	13‰
Dedej and Bino, 2003	33‰	15‰

pH

The waters of Lake Butrinti are slightly alkaline (Table 5). Generally, alkalinity is lower during summer compared to winter, as reported by Dedej and Bino (2003), Miho *et al*. (2013), and Heywood (2017), with some exceptions noted by Pano (1984). Regarding vertical distribution, pH values tend to decrease from the surface to greater depths (Pano, 1984; Miho, 1994; Bushati, 2013).

Table 5. Epilimnion pH values during winter and summer at Lake Butrinti

References	Summer	Winter
Pano 1984; Miho 1994; Bushati 2013	8.7	7.9
Miho *et al*., 2013; Dedej and Bino 2003	6.5	9.5
NEA, 2015	7.18	8.53
Çako *et al*., 2013	7.8	8.08

Oxygen, COD, BOD and TOC

The oxygen levels in Lake Butrinti are influenced by multiple factors, including gas exchange, photosynthesis (Pano, 2015), wind, and temperature (Pano, 1984; Gabeira, 2023). Seasonal trends show that dissolved oxygen (DO) concentrations are generally higher in winter than in summer (Pano, 1984; Miho, 1994; Miho *et al*. 2013), and that DO levels decrease with depth (Table 6). Miho *et al*. (2013) stated that epilimnion waters are consistently well oxygenated, often exhibiting saturation levels above 100%, with maxima recorded in late winter and early spring. However, below depths of 4–5 m, oxygen levels decline to less than 50%, and at 6–8 m, they drop further, oscillating between 0 and 2 mg/L. In contrast, the hypolimnion is characterized by the presence of hydrogen sulfide (H_2S) and the absence of oxygen (Miho, 1994; Dedej and Bino, 2003; Bushati, 2013; Pano, 2015; Velaj, 2015).

Table 6. Oxygen level in different depths of Lake Butrinti

References	Epilimnion	5 m	6-8 m	10 m and below
Miho *et al.* 2013; Bushati 2013	5 mg/l	14 mg/l	0-3 mg/l	0
Dedej and Bino, 2003	8-9 mg/l		0	0

The chemical oxygen demand (COD) in Lake Butrinti ranged from 1.52 to 9.04 mg/L across the surface, mid-depth, and bottom layers, indicating poor water quality according to the Norwegian Water Institute (NIVA) classification (6.5–15 mg/L) (Heywood, 2017). The biological oxygen demand (BOD) ranged from 0.5 to 1.25 mg/L, reflecting moderate water quality. For 2023–2024, the maximum COD values were recorded in September, with minimum values observed in April. BOD peaked in November and reached its lowest value in March (Omeri *et al*. 2024). Organic matter concentrations ranged from 2 to 17 mg/L (Dedej and Bino, 2003), with higher levels in April than in August, suggesting that phytoplankton are the principal contributors to organic matter production during the spring season (Miho *et al*. 2013).

Nutrients (N and P)

Phosphorus is a key limiting nutrient for aquatic organisms; at elevated concentrations, it can be harmful by promoting eutrophication and algal blooms in coastal transitional waters such as Lake Butrinti. Consequently, phosphate levels in natural waters are among the most critical environmental concerns. Many regulations focus on limiting total phosphorus concentrations to prevent harmful algal blooms and maintain water quality, with typical standards ranging from 0.01 - 0.10 mg/L (Boyd 2001). However, data on nutrient content (N and P) in Lake Butrinti are limited and sporadic. Heywood (2017), based on the Official Environment Report of Albania (2014), reports total phosphorus concentrations from 0.03 to 0.35 mg/L during monitoring in 2013 (two seasonal phases). Nitrate ($N–NO_3$) values ranged from 0.1 to 1.6 mg/L, whereas nitrite (N–

NO_2) values ranged from 0.01 to 0.02 mg/L. Despite limited data, the phosphate concentrations often exceed 0.1 mg/L.

Kolitari *et al*. (2013) documented a significant increase in nitrite and phosphate levels between 2004 and 2011, with nitrite peaking in June and phosphate in October. High nutrient concentrations, combined with elevated temperatures, evaporation, limited water exchange, and low flow conditions, particularly in summer, likely contribute to dystrophic crises and periodic mussel die-offs. This situation may worsen due to nutrient leaching from increasing agricultural and urban activities in the catchment areas, including the Vurgu and Vrina plains and the urban centers of Ksamil and Manastiri, where untreated waters are discharged into the lake via the Manastiri and Dajlani pumping stations.

Table 7. Nutrient (N and P) presence at surface waters of Butrinti

	Heywood 2017	Kolitari *et al.*, 2013	Pano 2015
Nitrites	Low	High	Low
Nitrates	Low	-	0
Phosphates	High	High	-

Sulfides and hydrogen sulfide

Miho *et al*. (2013) reported that sulfides and hydrogen sulfide (H_2S) are consistently present in Lake Butrinti waters below 7 m depth. In some cases, particularly during hot summers, sulfides are also detected in surface waters, causing crises among sedentary biota, especially mussels. Sulfate concentrations were relatively high, reaching up to 2.86 mg/L, with a vertical distribution similar to that of salinity, and the lowest concentrations were observed in the surface layers (up to 2.01 mg/L). The presence of H_2S is attributed to sulfur-reducing bacteria that can reduce acidic sulfur salts under anaerobic conditions. Its concentration increases with depth, reaching values exceeding 5.0 mg/L at the lake bottom (Miho, 1994; Dedej and Bino, 2003; Pano, 2015; Velaj, 2015).

Heavy metals

Few studies have addressed the presence of heavy metals in the waters and sediments of Lake Butrinti (Topi *et al*. 2012; 2013a,b). Lead (Pb) concentrations were higher in both the hypolimnion and epilimnion during winter, while cadmium (Cd) is detected at both the surface and depth during autumn. Chromium (Cr) predominantly occurred at depth, with peak values observed in the summer. Copper (Cu) is present throughout the water column, with higher concentrations in the summer. Mercury (Hg) has also been detected. Overall, the annual heavy metal concentrations generally fell within the normal range, with the notable exception of mercury. The average Hg concentration across all seasons (0.79 µg/L) exceeded the EU guideline of 0.05 µg/L (Bushati, 2013; Table 8). More recent studies (Omeri *et al*. 2024) have reported that Cd, Cr, and Pb levels during 2023–2024 also surpassed the EU reference values.

Table 8. Heavy metal concentrations in Lake Butrinti waters compared to EU standards (µg L^{-1}) (Topi *et al*. 2012; 2013a,b; Omeri *et al*. 2024)

Reference	Cd	Cr	Cu	Pb	Hg
Topi *et al.* 2013	0.078	1.41	8.26	1.5	0.79
Topi *et al.* 2012	0.075	11.06	20.15	1.78	0.23
Omeri *et al.* 2024	1.4	41	18	29	0.001
EU Standard	**1**	**20**	**50**	**7.2**	**0.05**

Pesticides

Nuro and Marku (2011) reported the presence of pesticides in the waters of Butrinti, including DDT, Lindane, HCB, Aldrins, and Heptachlors. The concentration of organochlorine pesticides ranged from 7.3 to 30.7 ng/L, whereas the general EU limit for pesticide residues (EQS 2013/39/EU) is approximately 0.01 ng/L (0.00001 µg/L). These values indicate that pesticide levels in Butrinti waters substantially exceed the EU standards. This finding provides further evidence of the adverse impact of agricultural and urban activities in the surrounding areas. Given that such activities have intensified in recent years; it is reasonable to suspect that the associated environmental impact may now be even greater.

Investigation of the toxicity of waters at Butrinti Lake using bio-reporting bacteria

Physicochemical analyses of waters and sediments of Butrinti Lake confirmed the presence of contaminants that may affect the organisms inhabiting the ecosystem (Fig. 1a) and the adjacent marine protected area. While the measurement of individual chemical parameters provides valuable insight into water quality, assessing cytotoxicity using luminescent bio-reporting bacteria allows for the simultaneous evaluation of the combined effects of all pollutants. This was achieved by monitoring the changes luminescence emitted by the bacteria (Fig. 1b). Biosensors based on luminescent bacteria are highly sensitive to toxic substances and are valuable tools for monitoring water safety and chemical quality (Bond and Martin, 2005; Woutersen *et al*. 2010).

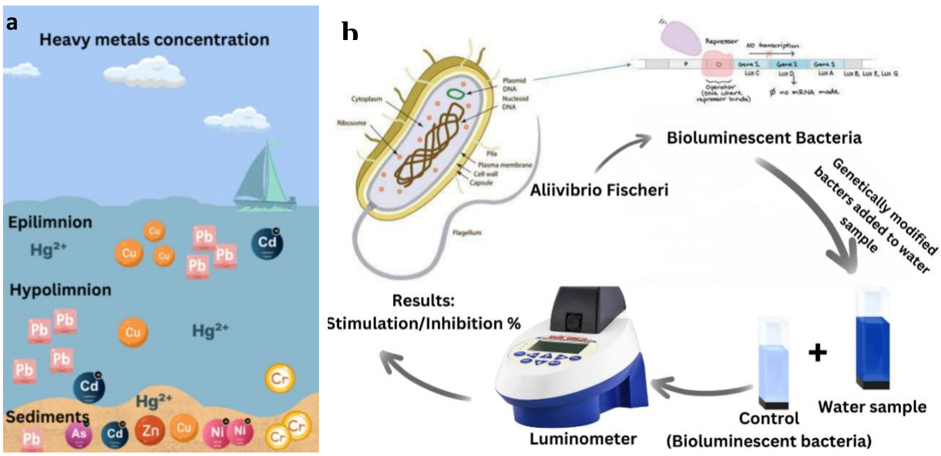


Fig. 1: (*a*) HM distribution in the epi/hypolimnion and sediments of Butrinti Lake based on previously reported data. ***(b)*** Use of bioluminescent bio-reporting bacteria for the detection of cytotoxicity. Figures were prepared using the Canva software.

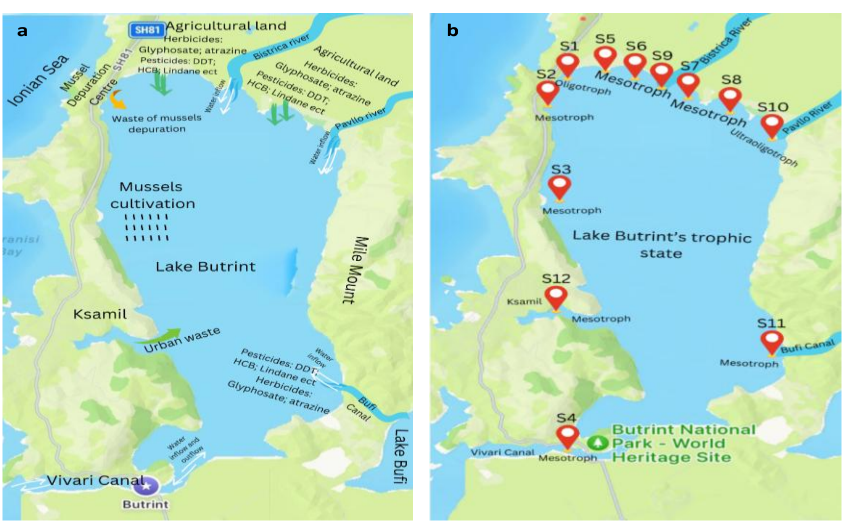
Water samples from 12 stations in Butrinti Lake were analysed for cytotoxicity (Omeri *et al*. 2024) using luminescent *Vibrio fischeri* according to ISO 11348-3:2007. The results revealed the impact of various categories of pollutants, including pesticides, heavy metals, urban waste, and aquaculture by-products, together with the hydrological characteristics of the lake, on bacterial toxicity (Fig. 2). Data on heavy metal concentrations in sediments (Bacu *et al*. 2024; Fig. 2d) and water (Table 8) were used to interpret the cytotoxicity levels at the corresponding stations (Fig. 2a–d). The findings indicate that the hydrological regime plays a significant role in determining water cytotoxicity. Although Cd, Cr, and Pb concentrations in water indicated a "Moderately Polluted" status (according to EU standards; Table 8), and Cr and Ni in sediments range from "Moderately Polluted" (Stations 6–10) to "Heavily Polluted" (Stations 4, 6–11) (according to USEPA, 1991 Guidelines), the Cytotoxicity Induction Factor (IF) was significantly high only at Station 2.

This location corresponds to the Vivari Canal, an area characterized by high fluctuations in inflow and outflow between Butrinti Lake and the Ionian Sea. Moreover, stations situated near agricultural areas exhibited IF values ranging from 1.5 to 2, highlighting the role of agricultural pollutants in the degradation of water quality.

Phytoplankton data

Butrinti Lake is considered one of the most important transitional ecosystems along the Albanian coast, offering substantial ecological and economic benefits, as well as high biodiversity and productivity. The lagoon is extensively used for aquaculture, particularly for farming mussel (*Mytilus galloprovincialis*). Phytoplankton, primarily diatom species, account for the highest primary production in surface water layers (Miho and Witkowski, 2005; Bushati, 2013; Miho *et al*. 2013). Experts recognize phytoplankton as a reliable bioindicator of trophic status (Bushati, 2013; Kolitari *et al*.2013).

The Trophic State Index (TSI) categorizes water bodies based on their biological productivity. Data from various studies (Miho *et al.* 2013; Bushati, 2013; Çako *et al*. 2013; Kolitari *et al*. 2013; Bacu *et al*. 2022; Bacu and Zaho, 2022; Topi *et al*. 2013c; Omeri *et al*. 2024) indicate a shift in the surface waters of Butrinti Lake from oligotrophic toward mesotrophic conditions.



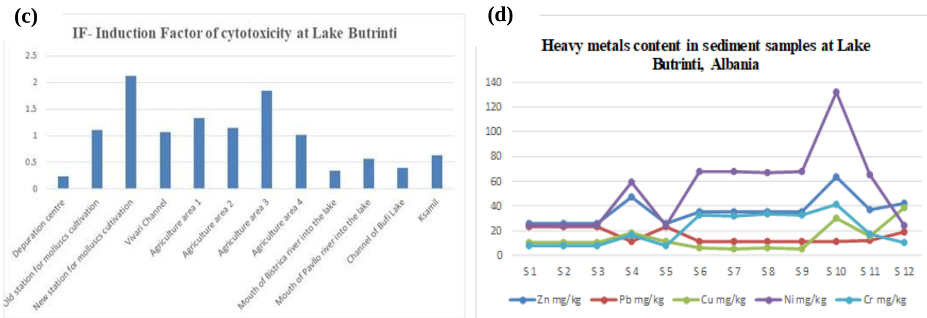


Fig. 2: ***(a)*** Factors affecting the cytotoxicity of water in the Butrinti aquatic ecosystem. ***(b)*** Trophic state of 12 stations in shallow waters at Lake Butrinti (acc to Omeri *et al*. 2024); ***(c)*** Induction Factor of cytotoxicity in waters (acc to Bacu *et al*. 2024); ***(d)*** Heavy metals content in sediment samples at Lake Butrinti (Bacu *et al*. 2024).

Algal blooms

Previous studies have indicated that Lake Butrinti provides favourable conditions for the growth of algal blooms. Consequently, dystrophic crises, including monospecific blooms, may occur and pose significant ecological risks (Miho, 1994; Bacu and Zaho, 2022). The majority of phytoplankton species belong to the *Bacillariophyceae* (diatoms) and *Dinophyceae* (dinoflagellates) (Table 9).

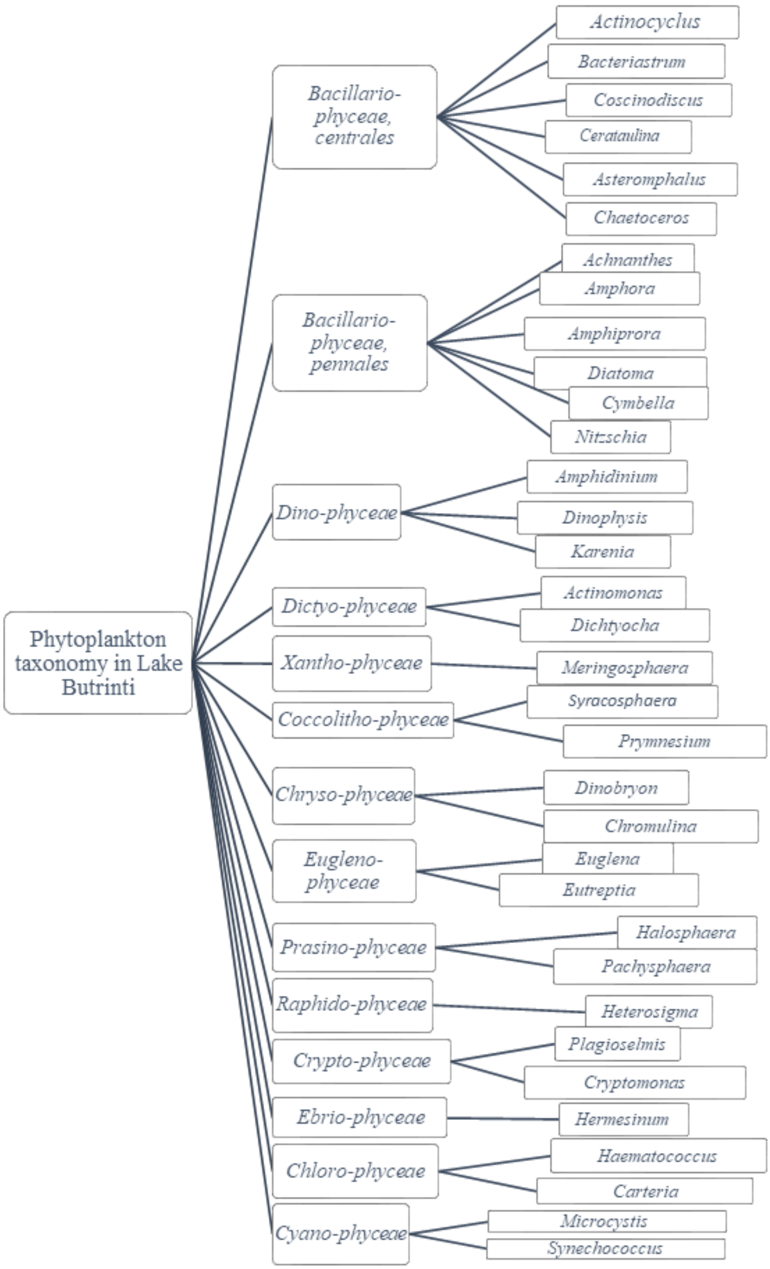


Fig. 3: Phytoplankton diversity in Lake Butrinti up to the genus level. The figure was prepared using previous data from Protić (1907), Miho (1994); Dedej (2005), and Bushati 2013.

Table 9. Algal blooms recorded in Butrinti Lake over the years.

Month	*Year*	*Species*
April	1987	*Pseudo-nitzschia* spp.
May	1987	*Prorocentrus micans*
March	1990	*Chaetoceros* spp., *Bacertiastrum* spp., *Cyclotella caspia, etc.*
March	1991	*Chaetoceros* spp. *(C. wighamii)*
January	2006	*Pseudo-nitzschia delicatissima and P. seriata*
October	2006	*Pseudo-nitzschia delicatissima and P. seriata*

Diversity of microalgae

Studies conducted between 1987 and 1991 reported 90 species of microalgae, of which 60 were diatoms (Miho, 1994; Xhulaj *et al*. 2008), followed by dinoflagellates (Miho, 1994). These species were classified into five classes: *Bacillariophyceae, Dinophyceae, Chrysophyceae, Euglenophyceae, and Cyanophyceae*, with diatoms occupying a dominant position (Miho, 1994; Peja *et al*. 1996; Xhulaj *et al*. 2008). Between 2006 and 2010, Bushati (2013) recorded approximately 460 taxa of microscopic algae across 13 classes, with diatoms and dinoflagellates being the predominant groups. Over 20 taxa have been identified as toxic, including five diatom species and 15 dinoflagellates (Miho *et al*. 2013). Bacu *et al.* (2022) reported the presence of unicellular cyanobacteria, such as *Prochlorococcus* and *Synechococcus*, emphasizing that favourable natural and anthropogenic conditions may promote their blooms, potentially posing ecological risks. Figure 3 presents a dendrogram of the phytoplankton identified to date in Lake Butrinti, classified at the genus level.

Methods used to determine phytoplankton diversity

Several analytical techniques have been employed to investigate phytoplankton diversity in Lake Butrinti, including optical, electron, and fluorescence microscopy, specific PCR assays, flow cytometry, chemotaxonomy, and catalyzed reporter deposition–fluorescence *in situ* hybridization (CARD-FISH) (Fig. 5).

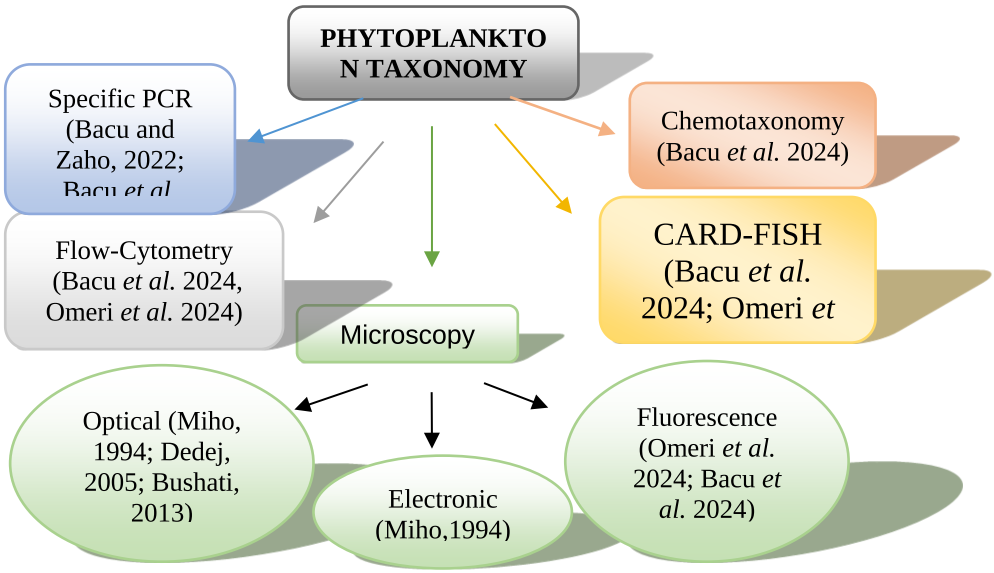


Fig. 4: Techniques used to investigate phytoplankton diversity in Lake Butrinti.

Microscopy

Most phytoplankton taxonomy results for Lake Butrinti have been obtained using optical microscopy (Miho, 1994; Dedej, 2005; Bushati, 2013), with electron microscopy applied only in a few cases (Miho and Witkowski, 2005). These methods rely on the observation of morphological characteristics of phytoplankton samples, often enhanced by staining with dyes such as Lugol's solution and Methylene Blue.

DNA based methods (CARD-FISH, specific PCR, Genome Sequencing)

CARD-FISH (catalyzed reporter deposition–fluorescence *in situ* hybridization) was used to detect ribosomal RNA (rRNA), messenger RNA (mRNA), and functional genes encoded on the chromosome (Fig. 5a). This advanced FISH method is widely applied for the identification of phytoplankton and other environmental microorganisms. This technique involves designing a specific oligonucleotide probe targeting the gene or RNA of interest, which is labelled with horseradish peroxidase (HRP) enzyme. In the presence of tyramide, the signal is amplified via fluorescence, enabling the sensitive detection of the target. CARD-FISH allows for the prediction of the relative abundance of individual microbial lineages based on their rRNA gene phylogeny. It can also be combined with direct enumeration methods, such as microscopy and flow cytometry, to estimate the absolute abundance of microscopic lineages in environmental samples (Piwosz *et al*. 2021).

Hybridized probes are typically visualized using epifluorescence microscopy, although flow cytometry is occasionally used. Although the main steps of the CARD-FISH protocol are generally consistent across studies, minor modifications may be applied, such as additional staining with DAPI (Fazi *et al*. 2007).

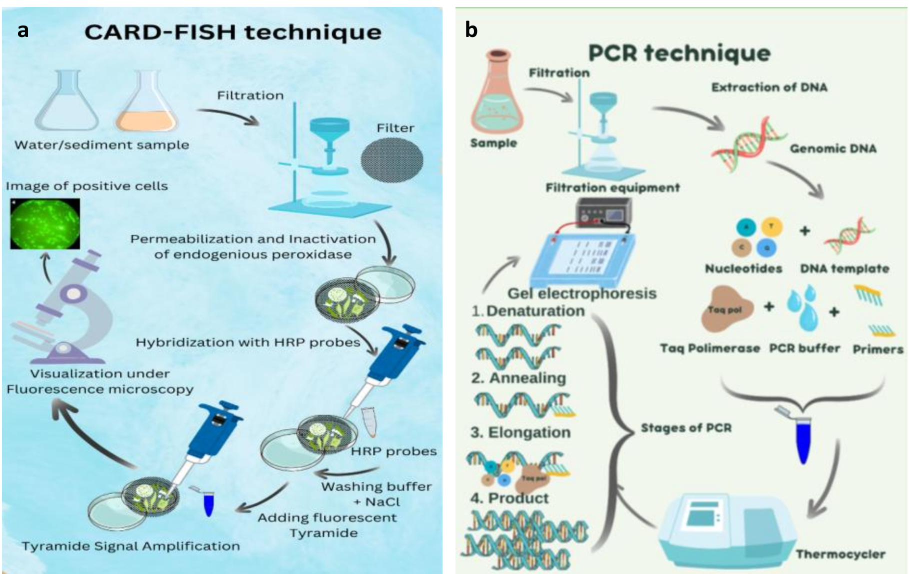


Fig. 5: ***(a)*** CARD-FISH (protocol in brief) used to investigate phytoplankton taxonomy at the class level. ***(b)*** PCR (protocol in brief) was used to determine phytoplankton genetic diversity up to the genotype/subspecies level.

Recently, CARD-FISH has been applied to assess phytoplankton biomass in Lake Butrinti (Bacu *et al*. 2024; Omeri *et al*. 2024). For the hybridization of the filter sections, different HRP-labelled oligonucleotide probes were used to target *Bacteria* (probes EUB338 I–III) and *Archaea* (probe ARCH915). The results indicated that 39.4% of the detected species belonged to *Proteobacteria*, 30% to *Bacteroidetes*, and 10% to *Cyanobacteria*. In the sediment samples, the proportion of *Cyanobacteria* fell below 1%.

Specific Polymerase Chain Reaction

PCR is one of the most commonly used molecular techniques for taxonomic analyses. It exponentially amplifies specific DNA sequences through a three-step procedure that is repeated over numerous cycles (Fig. 4b). The most frequently used genetic markers for phytoplankton taxonomy are 16S rRNA and 18S rRNA, which are universally present in bacteria, often in multiple copies or operons. The conserved function of the 16S rRNA gene over time enables more accurate phylogenetic and evolutionary analyses, and its length (~1,500 bp) is suitable for such computational analyses. The 18S rRNA gene is primarily used for phylogenetic analysis and biodiversity assessment, particularly in closely related centric diatoms (Armbrust and Galindo, 2001). The internal transcribed spacer (ITS) region is valuable for molecular systematics and population genetics; however, the presence of multiple non-identical rRNA operons can complicate comparative studies and restriction enzyme analyses (Boyer *et al*. 2001). Investigations of 16S–23S rDNA ITS diversity in Lake Butrinti (Bacu *et al*. 2022, 2024; Omeri *et al*. 2024) have revealed variations in ITS regions attributable to water pollutants. For pico-cyanobacteria, multiple ITS products were detected: ITS-a showed three sizes (1000, 900, and 300 bp), while ITS-b exhibited two sizes (550 and 350 bp).

Phytoplankton Genome Sequencing

Genome sequencing can identify subspecies and can be applied to whole genomes or specific genomic fragments (Tang *et al*. 2019). Commonly used markers include 16S rRNA (Honda *et al*. 1999; Sánchez-Baracaldo, 2015), 18S rRNA (Wang *et al*. 2014), and rbcL (Salmaso *et al*. 2022). In Lake Butrinti, DNA sequencing has been used to investigate polygenic relationships among species groups (Bacu *et al*. 2025, unpublished data) through meta-rDNA amplicon sequencing of the hypervariable V4 region of 16S rRNA, followed by taxonomic analysis.

Pigment- based method (Chemotaxonomy) for phytoplankton diversity evaluation

Chemotaxonomy uses phytoplankton pigments to investigate taxonomy, often at the class level, via CHEMTAX software (Fig. 6). Pigments are extracted from filtered water samples and serve as markers for broad taxonomic groups (Latasa, 2007; Develi *et al*. 2012; Matek *et al*. 2023; Peltomaa *et al*. 2023). A simplified CHEMTAX approach applied to Lake Butrinti samples revealed changes in phytoplankton community composition associated with climate change (Bacu *et al*. 2024).

Table 10. An example of chemotaxonomy based evaluation of the main taxa at Butrinti Lake considering the simplified CHEMTAX approach

Stations	klb/kla	klc/kla	Taxonomy
B1-0	0.030481377	0.083801827	*Haptophyceae, Bacillariophyceae, Chrysophyceae, Proclorophyceae*
B1-8	3.932308084	0.00687638	*Proclorophyceae, Prasinophyces*
B2-0	0.855646268	0.711292537	*Chrysophyceae, Haptophyceae, Proclorophyceae, Prasinophyceae*
B2-22	1.791247622	0.271359971	*Dinoflagjelate, Haptophyceae, Chrysophyceae, Diatoms, Proclorophyceae, Prasinophyces*
B3-0	1.350116587	2.318815951	*Prasinophyces*
B3-19	0.359337349	0.428915663	*Dinoflagjelate, Prasinophyceae, Chrysophyceae, Proclorophyces, Euglenophyceae, Prasinophyceae*

The method was applied to samples collected from stations B1–B3, with sampling performed at both the surface and maximum depth: B1-0 (Station 1, depth 0 m), B1-8 (Station 1, depth 8 m), B2-0 (Station 2, depth 0 m), B2-22 (Station 2, depth 22 m), B3-0 (Station 3, depth 0 m), and B3-19 (Station 3, depth 19 m), as reported by Bacu *et al*. (2024).

Each method described above has specific advantages and limitations (Table 11), which should be considered in relation to the research objectives, whether focusing on phytoplankton taxonomy, water quality and trophic state assessment, phytoplankton abundance, community composition, genetic diversity, or the evaluation of pollutant effects on cytotoxicity and subspecies-level genetic variation.

Table 11. Summary of main uses and limitations of methods for water quality and phytoplankton diversity evaluation, applied at Lake Butrinti

Methods	**Purpose of use**	**Limitations**	**References**
Physical-chemical parameters of waters and sediments	Determine the quality of waters according to accepted standards.	Cannot be used to investigate phytoplankton community composition	USEPA, 1991
Biomarkers	Determine Trophic State/Quality of waters according to accepted standards.	Cannot be used to interpret the chemical composition of waters	USEPA, 1991
Biosensors (single-cell fiber-optics, luminescent bacteria, etc)	Evaluate the cytotoxicity imposed by different pollutants in water and sediments.	Higher sensitivity is required in complex wastewater environments, long-term stability, and regulatory barriers must be addressed.	Fdez-Sanromán *et al.* 2025
Microscopy (optical, electronic, fluorescence)	Identification to species level, evaluation of abundance.	Time-consuming; The reliability of identification depends on the skills of the identifier. Morphologically similar species cannot be distinguished from each other.	Peltomaa *et al.* 2023
Flow cytometry	Counting, analysis, and identification of phytoplankton species and groups.	Standard flow cytometry (FCM) is restricted to a low taxonomic resolution, making it unsuitable for identifying indicator species. Imaging flow cytometers (IFC) can overcome these limitations.	Dunker *et al* 2019
Chemotaxonomy	Monitoring phytoplankton communities;	Limitations due to overlapping profiles of some biomarkers.	Peltomaa *et al.* 2023

	Taxonomy at the class level identification;		
CARD-FISH	Estimates of relative abundance (percent contribution to total eukaryotic numbers) of individual microbial lineages defined by their rRNA gene phylogeny; It can be separately optimized for each target group (probe), which is not possible for PCR with primers that target many different templates.	Imperfect probe coverage and specificity; poor detection of low abundance or inactive community members, and difficulties in counting aggregated cells, autofluorescence of chloroplasts might also interfere with probe signals; and tyramides sometimes bind unspecifically.	Kubota,2013 Piwosz *et al.* 2021
Amplicon and shotgun Sequencing & Metabarcoding	Species identification based on the similarity or divergence of the molecular sequence of an unknown organism to a vouchered reference sequence in the database.	Intragenomic variation with many copies of individual genes causes overestimation of cell numbers and diversity; An annotated barcode library and choice of barcode and primers are essential.	Alemzadeh. *et al.* 2014 Gelis *et al.* 2024

3. CONCLUSIONS

Lake Butrinti represents a dynamic aquatic ecosystem shaped by the combined influences of freshwater inflow, saline intrusion from the Ionian Sea, and contributions from Lake Bufi. Over time, it has exhibited a trend toward mesotrophic conditions, underscoring the need for continuous monitoring of anthropogenic activities and climate change, the two primary influencing factors of water quality. Strict regulation of fertilizer and pesticide use in agriculture, proper treatment of urban wastewater, controlled diversion of the Bistrica River during summer, and ongoing maintenance of the Butrinti Canal (enlargement and deepening) are essential to mitigate potential ecological and socio-economic impacts, such as dystrophic crises, declines in fishing and mussel production, loss of biodiversity, degradation of ecological quality, and reduced environmental aesthetic value. Furthermore, there is a clear need for more comprehensive and systematic research employing both conventional and advanced methods to monitor ecological changes, mitigate negative effects, and safeguard the integrity and economic value of the lake. Emphasis should be placed on the specific strengths of each analytical approach—physicochemical, biological, DNA-based, and biosensor methods—and their integration into a unified monitoring framework to ensure food safety. Integrating these methodologies into the monitoring program of the Albanian National Agency of Environment would strengthen long-term ecological assessment and provide a robust evidence base to guide environmental policy implementation in Albania.

DECLARATIONS

Data accessibility: There are no databases to be made public**.**

Declaration of AI use: There has been no use of AI when writing the actual paper.

Author's contribution:

Xh.O.: writing-original, editing draft: A.B.: conceptualization, validation, writing-review and editing.

Authors approval

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