AN INTEGRATED APPROACH BASED ON THE USE OF MONITORING AND RESEARCH PROTOCOLS FOR WATER QUALITY ASSESSMENT IN ALBANIA

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ABSTRACT

Water quality is one of the most critical environmental issues in the context of climate change, as are the methodologies applied to evaluate it. In Albania, standardized methods have traditionally been employed for the measurement of physical-chemical indicators, trophic state (ISO 17025), and bacteriological indicators (ISO16649-3:2015). However, the use of any these methodologies in isolation does not provide a comprehensive understanding of the condition of fresh, marine or lagoon ecosystems. The present study discusses an integrated approach to water quality evaluation that combines standardized monitoring protocols with advanced biotechnological research methods. These include CARD-FISH, fluorescence microscopy, and flow cytometry for biomass quantification; factorial analysis of bacterial DNA in relation to environmental parameters; assessment of phytoplankton genetic diversity; and chemotaxonomic analyses. In addition, the application of novel standardized monitoring protocols employing bioreporter bacteria for cytotoxicity testing is presented. The results obtained from the implementation of these methodologies in Albania indicate that an integrated, multidisciplinary approach provides a more comprehensive and reliable framework for the assessment of water quality.

Keywords: single-cell biosensors, CARD-FISH, chemotaxonomy, Flow cytometry, trophic state

1. INTRODUCTION

Monitoring of water quality in rivers, lakes, lagoons, and coastal areas has long been part of the services provided by the Department of Biotechnology, University of Tirana, to the National Agency of Environment of Albania until 2015. Since then, the department's capacities and expertise in this field have been primarily devoted to research activities. However, according to the Albanian Law on Higher Education Institutions, university departments are expected to engage in teaching, research, and third-party services. The latter plays a crucial role for both universities and external stakeholders, including public and private institutions and businesses.

ISO protocols and/or research methodologies have been applied to address the following parameters: i) physical-chemical indicators, ii) pathogenic bacteria using indicator species, iii) trophic state (Carlson Index), iv) cytotoxicity sensing, v) phytoplankton community composition, vi) use of phytoplankton DNA as a biomarker, vii) factorial analysis of DNA / environmental parameters, viii) pico-cyanobacteria PCR-based subspecies diversity exploration, ix) invasive species investigation & Monitoring conditions for algal blooms, and x) CARD-FISH and fluorescence imaging.

The results obtained from the application of these methodologies demonstrate that the use of ISO protocols or research-based methods alone does not provide a complete understanding of the condition of freshwater, marine, or lagoon ecosystems. Therefore, this study proposes an integrated approach that combines both standardized and research-oriented protocols for a more comprehensive evaluation of water quality. This approach enables simultaneous assessment of the chemical composition of water, the presence of pathogenic bacteria of public health importance, trophic state (reflecting the ecological health of aquatic environments), and the impacts of various pollutants (e.g., heavy metals, pesticides, herbicides) on aquatic organisms. Furthermore, it allows the examination of environmental influences on phytoplankton community structure and biomass.

The implementation of this integrated methodology in Albanian waters supports the conclusion that such an approach not only provides an accurate evaluation of the current state of aquatic ecosystems but also enhances understanding of microbial community interactions with environmental and anthropogenic factors—thus contributing to predictive modeling of future ecological conditions.

2. MATERIALS AND METHODS

The data presented in this study were obtained and analyzed using the following methodologies, applied to several major Albanian aquatic ecosystems, including Lake Butrint, Lake Ohrid, and Lake Shkodra. All procedures were conducted within research projects undertaken at the Department of Biotechnology, University of Tirana.

2.1. Physicochemical analysis of water quality

Water quality was assessed using physicochemical indicators. Temperature, pressure, dissolved oxygen, electrical conductivity, salinity, and pH were measured in situ. The remaining parameters were analyzed in the laboratory using standardized protocols, as listed below:

1. Chemical oxygen demand (COD): ISO 15705:2002
2. Biochemical oxygen demand (BOD_5): EPA Method 5210
3. Total suspended solids (TSS): S SHEN 872:2005
4. Total dissolved solids (TDS): APHA/AWWA 2540C/2022
5. Phosphates: APHA/AWWA 4500-P E/2022
6. Nitrates: ISO 7890-3:1988
7. Nitrites: ISO 6777:1984
8. Ammonium: APHA/AWWA 4500-NH_3 F/2022
9. Total nitrogen: ISO 11905-1:1997
10. Total phosphorus: ISO 6878:2004
11. Major and trace elements (K, Na, Ca, Mg, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn): APHA/AWWA 3111B/2022

2.2. Microbiological analysis

The presence of pathogenic bacteria was investigated using indicator species according to ISO 16649-3:2015.

2.2. Trophic state determination

The trophic state of each water body was calculated using the Carlson Trophic State Index (Carlson, 1977).

2.4. Cytotoxicity sensing

Cytotoxicity was evaluated using *Vibrio fischeri* bioluminescent bacteria following ISO 11348-3:2007.

2.5. Phytoplankton community analysis

Phytoplankton community composition was determined through chemotaxonomic methods following Mackey et al. (1996). Factorial analysis was performed to explore correlations between phytoplankton DNA characteristics and environmental parameters.

2.6. Molecular and cytometric analyses

Subspecies diversity of pico-cyanobacteria was explored using the 16S rDNA ITS region. CARD-FISH and fluorescence imaging were employed for phytoplankton biomass evaluation, complemented by flow cytometry to quantify biomass and characterize cell populations.

3. RESULTS and DISCUSSIONS

- **Water quality evaluation based on physicochemical indicators: Case study of Lake Butrint (2023)**

Data on the physicochemical characteristics of Lake Butrint have been extensively published and reviewed due to the lake's environmental, economic, and cultural importance (Bacu *et al.* 2022). In this study, we analyzed a comprehensive set of parameters, including pH, temperature, electrical conductivity, salinity, total suspended solids (TSS), total dissolved solids (TDS), total organic carbon (TOC), chemical oxygen demand (COD), biochemical oxygen demand (BOD), and a range of elements (P, N, Zn, Fe, Al, Cr, As, Cd, Hg, Pb, Cu, and Ni), to assess the water quality of Lake Butrint during 2023. The assessment was conducted in accordance with the Water Framework Directive (75/440/EEC) of the European Union and Council Decision No. 379, dated 25 May 2016, approving the regulation "Drinking Water Quality." In addition, reference was made to the standards for urban wastewater discharges established under Albanian legislation (VKM No. 177, dated 31 March 2005). Based on these criteria, Lake Butrint was classified as being in a mesotrophic to eutrophic state. Seasonal fluctuations in the measured parameters were analyzed, and regression curves with corresponding equations were generated to describe variations over time. Depth profiles of selected physicochemical parameters (Fig. 1) indicate the presence of anoxic conditions at depths of 8 m and below, reflecting reduced oxygen availability and potential stratification effects within the water column.

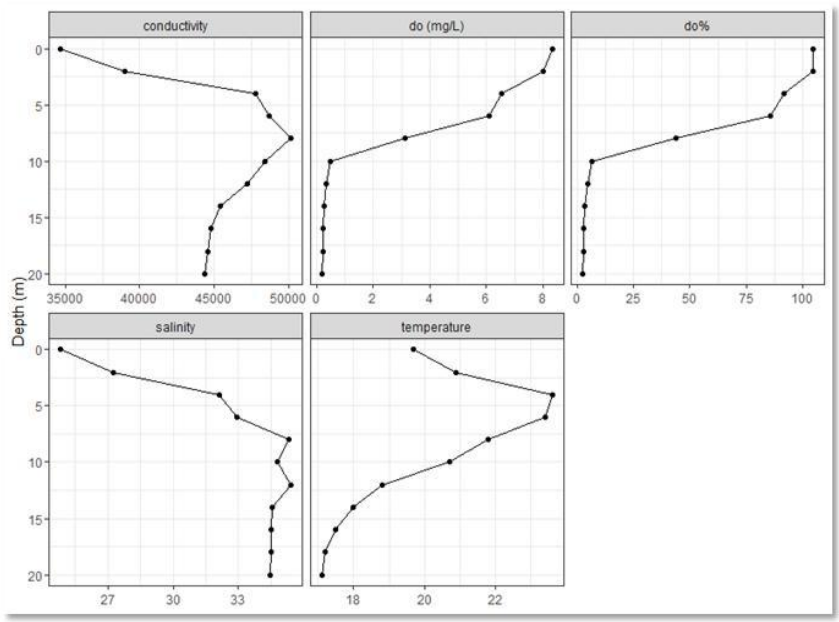

Fig.2. Depth profiles of some Physical-chemical parameters measured at Butrinti Lake.

- **Investigation of pathogenic bacteria using indicator species: Case study of Lake Butrint**

The presence of pathogenic bacteria in Lake Butrint was investigated through the monitoring of *faecal coliforms* and *faecal streptococci* during the period 2021–2022. The results were evaluated in accordance with European Union Directive 75/440/EEC on the quality required of surface waters intended for the abstraction of drinking water, as well as with the WHO/UNEP and EU guidelines for surface water classification based on the 90th and 95th percentile values.

These bacterial indicators were selected due to their reliability in reflecting recent faecal contamination and their wide use as standard parameters in microbiological water quality assessment. The observed concentrations of faecal indicator bacteria varied seasonally, with higher counts typically recorded during warmer months, reflecting increased microbial activity and potential runoff from surrounding anthropogenic sources.

Table 1. *Faecal coliform* presence at Butrinti Lake, 2022

	November 2021	December 2021	January 2022	March 2022	April 2022	May 2022	June 2022	November 2022
Station 1	11000	900	1500	<3	400	700	1500	2300
Station 2	11000	<3	<3	700	400	700	400	4300
Station 3	11000	900	<3	<3	<3	1500	400	15000

In November 2021 and 2022, the microbial load of *Faecal coliforms* at all three monitoring stations exceeded the limit value established by the European Union (2,000 bacteria per 100 mL of water).

Table 2. *Faecal streptococci* presence at Butrinti Lake, 2022

Fecal streptococci	November	December	January	March	April	May	June
Station 1 "Mussel Gathering Ponit"	<3	1100	1500	<3	<3	<3	<3
Station 2 "In the city of Ksamil"	<3	1100	430	<3	<3	<3	<3
Station 3 "Butrinti National Park"	<3	2800	2400	400	<3	<3	<3

Similarly, during December 2021 and January 2022**,** the *Faecal streptococci* load at Station 3 surpassed the EU threshold of 2,000 bacteria per 100 mL**.**

- The presence of *Escherichia coli* in mussels was also monitored over a two-month period (June–July 2022) following the ISO/TS 16649-3:2015 protocol

Table 3. *E. coli* presence in mussels at Butrinti Lake, 2022.

	Dilutions		Station I			Station II			Station III
1-15 June	MPN		**330**			**460**			**<18**
15-30 June	MPN		**<18**			**<18**			**45**
1-15 July	MPN		**<18**			**<18**			**61**
15-31 July	MPN		**<18**			**18**			**<18**

As shown in Table 3, Stations 1 and 2 exhibited a high microbial load of *Escherichia coli* during the first weeks of June 2022, exceeding acceptable microbiological limits for surface waters.

• Cytotoxicity sensing using bio-reporting bacteria- at Vlora, Albania- 2023

Luminescent bioreporter bacteria were employed to assess the cumulative toxic effects of various pollutant categories present in water and sediment samples on the native microbiota. Measurements were performed in November 2023 at eight sampling stations in the Bay of Vlora, Albania. The Luminescence Induction Factors (IF), expressing either stimulation (S) or inhibition (I) of bacterial luminescence, ranged from 0.39–3.82 S to 0.51 I (Fig. 2). Stations 1, 2, 4, 6, and 8 exhibited IF values above 1.5 S, indicating a significant level of toxicity, whereas Stations 5 and 7 showed values between 0.5 and 1.0 S, representing borderline or moderate toxicity levels.

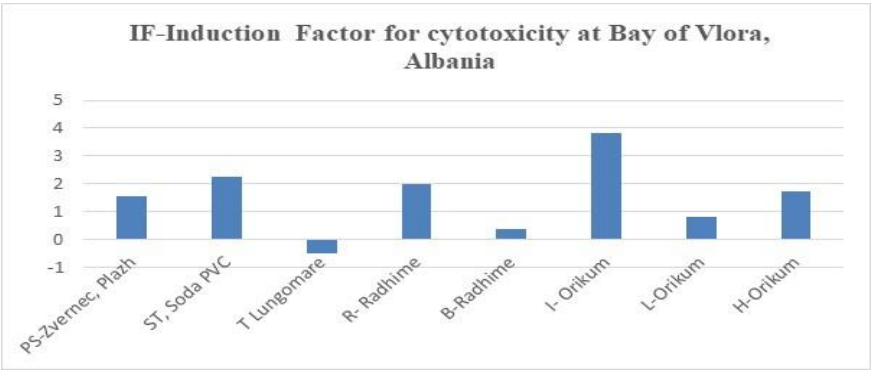

Fig. 2. Variation of cytotoxicity in Vlora Bay based on bioluminescent bio-reporting bacteria (November 2023).

- Trophic State seasonal variation- Case of Butrinti Lake

The Trophic State Index (TSI)**,** as proposed by *Carlson (1977),* utilizes algal biomass to classify the trophic condition of aquatic systems.

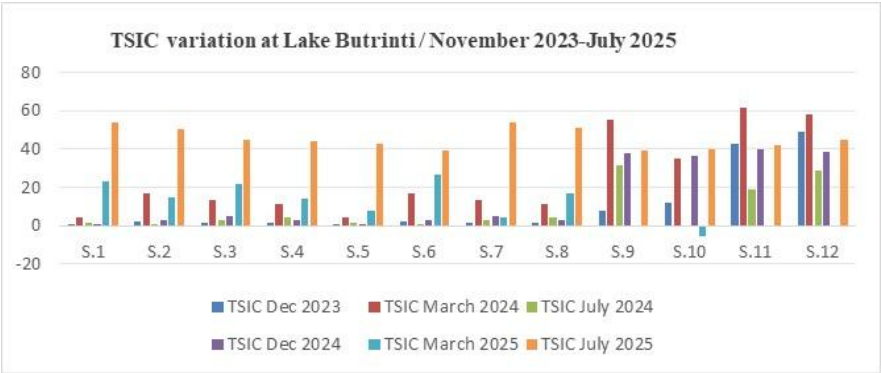

Fig. 3. Trophic State (Carlson Index based on chlorophyll a) at Lake Butrinti shallow waters from November 2023 to July 2025.

Results indicate both spatial and temporal variations in the trophic state of Butrinti Lake, demonstrating the influence of climate change in driving this parameter toward eutrophic conditions during 2025**.**

- Analysis of Phytoplankton Community composition based on photosynthetic pigments-Example from Lake Ohrid, Albania

CHEMTAX software software algorithm (Mackey *et al*. 1996; Wright *et al*. 1996) estimates the relative contribution of major algal groups to total chlorophyll *a* (Chl-*a*) by calculating specific pigment-to-Chl-*a* ratios. This approach enables the quantification of dominant phytoplankton taxa and supports the assessment of seasonal and interannual variations in community composition (Fig. 4).

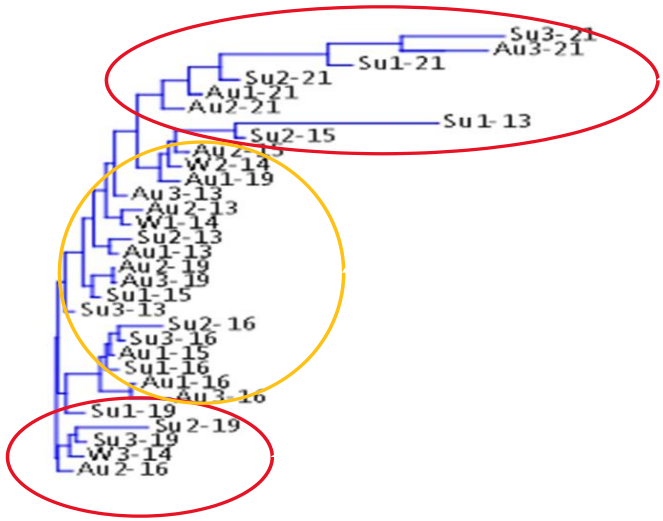

Fig. 4. Dendrogram of similarity among phytoplankton composition (based on pigment ratios chlab/chla, chlc/chla, carotenoids /chla) at three stations (S1-S3) during three seasons (W-winter; Su-Summer; Au-Autumn) at Lake OHRID through the years 2016-2021. Dendrogram was build using Past 4.03. Abbreviation example explanation: Su3-21 stands for Summer-Station3-Year 2021.

The phytoplankton composition observed in 2021 stands distinct from that of all other study years (Fig. 4). The summer–autumn assemblages of 2013, 2014, 2015, and 2019 form a coherent cluster, while 2016 and 2021 each constitute separate groups.

Table 4. An example of Chemotaxonomy based evaluation of the main taxa at Ohrid Lake, based on simplified CHEMTAX approach, applied at samples from 3 stations.

	June 2016 chlb/chla	June 2016 chlc/chla	July 2016 chlb/chla	July 2016 chlc/chla	September 2016 chlb/chla	Sepember 2016 chlc/chla	June 2019 chlb/chla	June 2019 chlc/chla	July 2019 chlb/chla	Jul 2019 chlc/chla	September 2019 chlb/chla	September 2019 chlc/chla
Ohri 1	0.85	1.34	0.97	1.23	0.36	0.75	0.63	0.55	0.86	1.24	0.36	0.75
Ohri 2	1	1.78	0.8	1.05	0.58	0.97	0.88	0.98	0.7	1.02	0.63	0.97
Ohri 3	0.81	1.47	1.18	1.41	0.59	0.97	0.48	0.36	0.82	1.1	0.59	0.97
Ohri1 Taxonomy	*Chlorophyta, Prasinophyta, Euglenophyta*	*Crysophyta, Dinophyta, Cryptophyta, Haptophyta*	*Chlorophyta, Prasinophyta, Euglenophyta*	*Crysophyta, Dinophyta, Cryptophyta, Haptophyta*	*Prasinophyta, Proclorophyta*	*Dinophyta, Cryptophyta*	*Procolorophyta, Chlorophyta, Euglenophyta, Prasinophyta*	*Dynophyta*	*Chlorophyta, Prasinophyta, Euglenophyta*	*Crysophyta, Dinophyta, Cryptophyta, Haptophyta*	*Prasinophyta, Proclorophyta*	*Dinophyta, Cryptophyta*
Ohri 2 Taxonomy	*Proclorophyta, Prasinophyta*	*Crysophyta, Dinophyta, Cryptophyta, Haptophyta*	*Chlorophyta, Prasinophyta, Euglenophyta*	*Crysophyta, Dinophyta, Cryptophyta, Haptophyta*	*Prasinophyta, Proclorophyta*	*Crysophyta, Dinophyta, Cryptophyta*	*Chlorophyta, Prasinophyta, Euglenophyta*	*Dinophyta, Cryptophyta*	*Chlorophyta, Prasinophyta, Proclorophyta, Euglenophyta*	*Crysophyta, Dinophyta, Cryptophyta*	*Procolorophyta, Chlorophyta, Euglenophyta, Prasinophyta*	*Crysophyta, Dinophyta, Cryptophyta*
Ohri 3 Taxonomy	*Chlorophyta, Prasinophyta, Euglenophyta*	*Crysophyta, Dinophyta, Cryptophyta, Haptophyta*	*Proclorophyta, Prasinophyta, Chlorophyta*	*Crysophyta, Dinophyta, Cryptophyta, Haptophyta*	*Prasinophyta, Proclorophyta*	*Crysophyta, Dinophyta, Cryptophyta*	*Proclorophyta, Prasinophyta*	*Cryptophyta, Chrysophyta*	*Chlorophyta, Prasinophyta, Euglenophyta*	*Crysophyta, Dinophyta, Cryptophyta*	*Prasinophyta, Proclorophyta*	*Crysophyta, Dinophyta, Cryptophyta*

This clear differentiation suggests that multivariate analysis of phytoplankton composition can serve as a sensitive indicator for assessing the impact of climate change on aquatic ecosystem dynamics.

- Phytoplankton DNA as a bioindicator of trophic state and exploration of picophytoplankton subspecies diversity

It has been hypothesized that particulate DNA concentrations can provide reliable estimates of living biomass (*Holm-Hansen et al. 1968; Dortch et al. 1983*). Indeed, cellular DNA has been shown to be linearly correlated with cell carbon across various phytoplankton species. Early studies (*Holm-Hansen, 1969*) reported discrepancies between biomass estimates derived from DNA and those obtained from particulate carbon, chlorophyll, or ATP, which were attributed to the presence of substantial fractions of detrital DNA. However, this interpretation was later contested for coastal waters (*Falkowski and Owens, 1982; Dortch et al. 1983*). Subsequent work (*Paul et al., 1985*) demonstrated that particulate DNA correlates closely with particulate organic carbon (POC) and bacterial abundance across diverse aquatic environments. DNA content has thus emerged as a scalable cellular component, co-varying with carbon and nitrogen content in phytoplankton cells. This relationship supports the use of total DNA content as an accurate and independent estimator of cellular carbon biomass in unicellular pelagic phytoplankton (*Veldhuis et al. 2008*). Figures **5** and **6** illustrate applications of phytoplankton DNA as a biomarker for assessing water quality in several Albanian lakes.

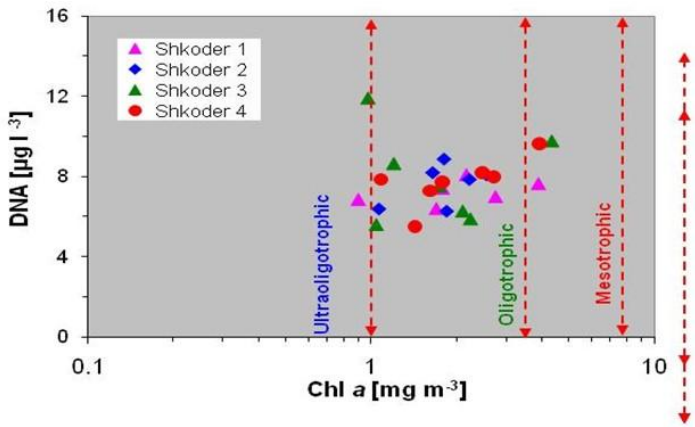

Fig. 5. Evaluation of Trophic State at Lake of Shkodra, Albania based on bioindicators (phytoplankton DNA and chla). Most of the stations have oligotrophic waters.

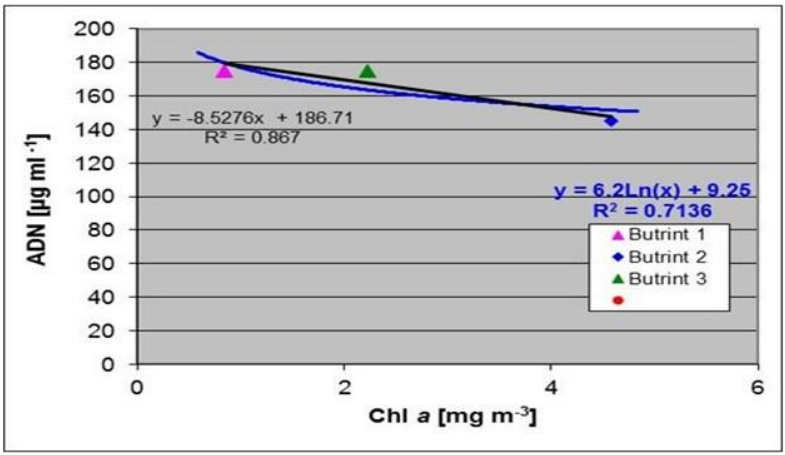

Fig. 6. Correlation between DNA and chla content at Butrinti Lake, Albania. There is a strong correlation R^2=0,867, which proves the possibility to use the phytoplankton DNA as a reliable biomarker for the quality of waters, along with chla.

The quantification of total phytoplankton DNA represents a reliable parameter for assessing phytoplankton biomass, as the cellular DNA content of phytoplankton remains relatively constant under varying environmental conditions. Measurement of this parameter provides an estimate of the number of phytoplankton cells per unit volume of sampled water, independent of their physiological or metabolic activity. Consequently, it reflects the actual biomass of phytoplankton under specific environmental conditions.

In parallel, molecular analyses aimed at exploring subspecies-level diversity were conducted through the amplification of 16S–23S cyanobacterial ribosomal DNA (Fig. 7). This approach has been demonstrated to effectively identify pico-cyanobacteria across diverse aquatic environments worldwide (*Honda et al. 1999; Robertson et al., 2001*).

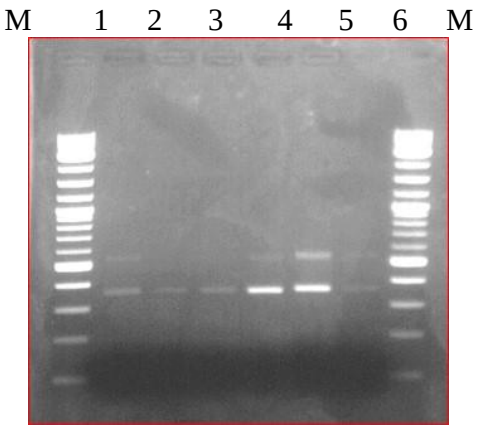

Fig. 7. ITS bf/br 16S-23S ITS rADN amplicon for genus Synechoccocus from Butrinti Lake (Bacu *et al*. 2022a). Primers were designed on the sequence of picophytoplankton 16S-23S rADN according to Lavin *et al*. (2008). Samples of picophytoplankton from different stations have ITS - Internal Transcribed Spacers of two dimensions (350bp and 550bp). From left to right: M-molecular marker 100bp; 1-6 are sampling stations; M-molecular marker 100bp).

- CARD-FISH and Fluorescence Imaging and Flow-Cytometry for phytoplankton biomass evaluation- Case of Lake Butrinti, Albania.

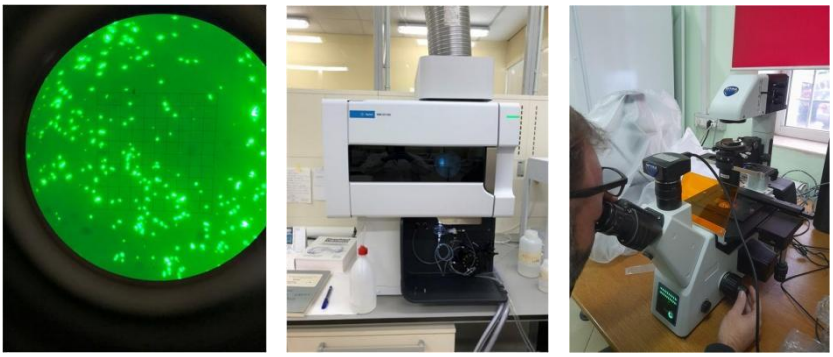

Fig. 8. DAPI staining, Flow-cytometry and fluorescence microscopy were employed to analyze the phytoplankton biomass at Lake Butrinti, Albania in the frame of Bilateral Protocol Italy -Albania 2021-2022. Fram left to right: Image of DAPI staining of filters where phytoplankton was trapped; Flow-cytometer apparatus at Water Research Institute, CNR, Rome, Italy (partner institution); Fluorescence microscope at the Laboratory of Molecular Biotechnology, Department of Biotechnology, University of Tirana, Albania.

A clear dominance of Proteobacteria was observed across all sequenced samples from Lake Butrinti, accounting for approximately 39.4% of the total microbial community. The composition differed notably between water and sediment samples. In water and superficial sediment layers, Bacteroidetes exhibited comparable dominance, contributing up to 30% of the community in some samples. The deep-water microbial community showed greater similarity to that of shallow sediment samples, indicating potential sediment–water microbial exchanges. In surface water samples, Cyanobacteria represented the third most abundant phylum (~10%), whereas their relative abundance dropped below 1% in deeper sediment samples.

4. CONCLUSIONS

Each of the ISO-based methodologies described above provides valuable information on distinct categories of water quality indicators currently applied in Albania. However, a truly integrated assessment framework—combining biotechnological tools such as CARD-FISH**,** fluorescence microscopy, and flow cytometry for biomass evaluation, with factorial analysis of bacterial DNA–environment interactions, phytoplankton genetic diversity**,** and chemotaxonomic profiling—offers a more comprehensive understanding of aquatic ecosystems. Such an approach enables the spatiotemporal analysis of phytoplankton biomass and composition and their correlation with physico-chemical parameters, thus supporting the prediction and interpretation of microbial community dynamics under changing climatic conditions. Furthermore, the incorporation of advanced bioanalytical methodologies (e.g., cell biosensors and chemotaxonomic techniques) facilitates the evaluation of cytotoxic effects on microbiota and associated aquatic food webs caused by pollutants of various origins, including toxic algal blooms and agricultural or industrial discharges. These approaches substantially enhance the resolution and interpretive power of traditional monitoring methods based solely on physico-chemical parameters.

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:

AB - conceptualisation, writing-original, editing draft, evaluation**; XhO**-writing original**; RS-**writing original**; KP-**writing original

Authors consent:

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

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