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PRIMARILY RESULTS OF PHYTOPLANKTON DNA AND VARIATION TO ENVIRONMENTAL FACTORS IN DURRES`S BAY COASTAL WATERS (ALBANIA)

Laura Gjyli[1], Ariola Bacu[2], Jerina Kolitari[3], Silvana Gjyli[4]*

Address(es): MSc. Laura Gjyli,
[1]University "Aleksander Moisiu", Durres, Faculty of Professional Studies, Department of Medicine, Lagjia Nr. 1, Rruga e Currilave, 2000 Durres, Albania, phone number: +355 52 39162.
[2]University of Tirana, Faculty of Natural Sciences, Department of Biotechnology, Bulevardi "ZOG I", 2000 Tirane, Albania.
[3]Agricultural University of Tirana, Aquaculture and Fisheries Laboratory, Lagjia Nr. 4, Rruga Skenderbej, 2000 Durres, Albania .
[4]University of Tirana, Faculty of Natural Sciences, Department of Chemistry, Bulevardi "ZOG I", 2000 Tirane, Albania.

*Corresponding author: lauragjyli@yahoo.com

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ABSTRACT

After isolation of phytoplankton DNA in coastal waters of Durres Bay, Albania, quantification and analysis of quality were investigated with spectrophotometric analysis. Analysis of UV absorption by the nucleotides provides a simple and accurate estimation of the concentration of nucleic acids in a sample. This method is however limited by the quantity of DNA and the purity of the preparation. Also biotic environment factors as Chlorophyll a and abiotic environment factors as temperature, salinity, pH, dissolved oxygen, turbidity, nitrate, phosphate were investigated to assess DNA quantities in different environment conditions. The Chlorophyll a was studied also to access the level of trophy. The sample stations were: Golem Beach (GB), Channel of Plepa (ChP), Hekurudha Beach (HB), Ex-Fuel Quay in Marine Durres Harbour (EFQ), Water Channel of Durres City (WChDC) and Currila Beach (CB). Samples are taken in one meter depth from the water surface. Water samples were collected monthly from April to October 2011. The most abundant stations with phytoplankton DNA are Channel of Plepa and Water Channel of Durres City. This confirms that there are spills of fresh waters, sewage or agricultural water spills, often discharge in coastal waters. Referring Mutliple Regression Analysis and single regression analysis, the association between phytoplankton DNA and environment factors was strong (R^2 = 0.75). Basing in single correlation and statistically significance (*p-value ≤ 0.05),* the enviroment factors that correlated to phytoplankton DNA were pH, salinity and phosphate; *explaining thus* the variation of total phytoplankton in Durres Bay coastal waters.

Keywords: Phytoplankton DNA, Chlorophyll a, environment factors, trophy

INTRODUCTION

Durres Bay is located between Selita Cape (Lagj) in south and Durres Cape in north, that are 20 km far away from each others. The coastline of Durres Bay is 7 km in the east and the highest width is in Golem Beach. In this bay are known two types of coasts: the low coast where there are dominated storage processes, and high coast where are dominated abrasion processes. The first coast covers from Dajlan Bridge to Karpen, whereas the second coast covers the Karpen-Lagj Cape and Currila area **(www.updurres.com.al.).**
Coastal water are a very dynamic environment since they are influenced by both terrestrial inputs, natural and anthropogenic, as well as from inshore – offshore water exchanges, weather conditions and wind – driven water movements. In addition, coastal bathymetry complicates the system response to various inputs. All these physical mechanisms and the fact that nutrient transformations, nutrient uptake and phytoplankton growth proceed at a high rate, suggest that the trophic status of a coastal area should not be considered as an almost static entity **(Giovanardi and Vollenweider, 2004).**
The first aim of this study was to access DNA phytoplankton in marine coastal waters of the Durres Bay. The most commonly used methodologies for quantifying the amount of nucleic acid in a preparation are: (i) gel electrophoresis; and (ii) spectrophotometric analysis. At present, we have used only the spectrophotometric analysis. Analysis of UV absorption by the nucleotides provides a simple and accurate estimation of the concentration of nucleic acids in a sample. The ratio of OD_{260}/OD_{280} should be determined to assess the purity of the sample. This method is however limited by the quantity of DNA and the purity of the preparation. In the estimation of total genomic DNA, the presence of RNA, sheared DNA etc. could interfere with the accurate estimation of total high molecular weight genomic DNA **(Hoisington *et.al.*, 1994).**
The fact that relationships between gross macromolecular composition (e.g., RNA, DNA, and protein content) and growth rate of cyanobacteria can be described in simple mathematical terms, and appear to be independent of the specific environmental factors that determines the growth rate, supports the idea of using gross biochemical composition to estimate in situ the growth rate of natural microbial populations **(Dortch *et.al.*, 1983; Kemp *et.al.*, 1993).**
The second aim of this study was to assess variation of quantity of phytoplankton DNA in water volume in different environment factors like temperature, salinity, pH, dissolved oxygen, turbidity, nitrate, phosphate and Chl a, to estimate the difference in quantity of phytoplankton biomass. The measurement of DNA quantity in water volume indicates values commensurate with real quantity of phytoplankton, in due period, in due environment conditions, so we can assess the difference of quantity in phytoplankton biomass.
An another aim of this study was to classify the sample stations according level of trophy. Chlorophyll a concentration is also an indicator for assessing trophic status **(Kitsiou and Karydis, 2001).** Marine coastal systems are classified in different trophic levels: oligotrophic, mesotrophic, eutrophic and hypertrophic **(modified from OECD, 1982; Håkanson and Jansson, 1983; Wallin *et al.*, 1992; Håkanson *et al.*, 2007).**

MATERIAL AND METHODS

Water Samples Stations

Areas where samples of water were taken in Durres Bay are: Golem Beach (GB), Channel of Plepa (ChP), Hekurudha Beach (HB), Ex-Fuel Quay in Marine Durres Harbour (EFQ), Water Channel of Durres City (WChDC) and Currila Beach (CB). Samples were taken in one meter depth from the water surface. Water samples were collected monthly from April to October 2011.

Samples Collection

Sea water (~ 4 L) was collected for environmental DNA isolation (2 L) and to assess environment factors (2 L) in coastal of Durres Bay. Water samples from six stations were taken monthly between 6–9 a.m. in 2-litre plastic container in one meter depth. The plastic container was filled up, and preserved in an ice chest. Thereafter, the samples were taken to the laboratory for analyses.

Figure 1 Locations of Water Samples in Durres Bay

Filtration of Water for isolation phytoplankton DNA

The water sample for environmental DNA isolation was drawn through 47 mm diameter 0.7 µm pore size, GF/F filter under gentle vacuum. The filter was stored in 4°C in 1 mL of STE (100 mM NaCl, 10mM Tris HCl, 1 mM EDTA (pH 8.0) until DNA extraction **(Pichard *et al.*, 1993; Sambrook *et al.*, 1989)**.

DNA extractions and evaluation

The phytoplankton DNA was extracted according to **Pichard *et al.* (1993)** based on the **Fuhrman *et al.* (1988)** study with some modifications (**Bacu *et.al.*, 2011**). DNA Quantification and Quality Analysis was evaluated with Spectrophotometric Determination based on **Sambrook *et al.* (1989).**

Water quality /Environment Factor Analysis)

Environmental factors studied following APHA *et al.* and APHA are temperature, *p*H, dissolved oxygen ,salinity, turbidity, dissolved oxygen, nitrate, phosphate and Chl a. A blank sample was prepared for confirmation of the results while water temperature was measured *in situ*. Determination of chlorophyll in this way is therefore carried out in a fluorometer which has been previously calibrated with various known chlorophyll a solutions (**Apha, 1985; Apha *et al.*, 1998; Grasshoff *et al.*, 1999; Grasshoff, 1976).**

Statistical analyses

Routine estimation was carried out using MegaStat.xla and MultiBase to test for differences in the values of parameters monthly and sampling sites and to assess correlation between DNA and environmental factors **(MegaStat.xla, 2007, MultiBase, 2012).**

RESULTS AND DISCUSSION

Total phytoplankton DNA in all water samples stations along the coastal Durres Bay

The quantity content of total phytoplankton DNA in Durres Bay was assessed for the first time in April-October 2011. The phytoplankton DNA quantity in studied stations ranged (Table 1, Figure 2) between 132 - 676 µg/mL. The richest month with phytoplankton DNA was August, whereas the poorest month was May.
In April, the richest stations with phytoplankton DNA were (WChDC) and (HB) reaching 189 µg/mL, whereas the poorest station was (EFQ) with 132 µg/mL.
May indicated the poorest month with phytoplankton DNA in all stations, except (EFQ) where there was a little increase of DNA quantity (+30 µg/mL). As (EFQ) is a station that communicates less than other stations with open sea, may be a reason for this exception.
In June, there was an increase of phytoplankton DNA in all stations. The maximum was 211 µg/mL in (GB) and minimum was 192 µg/mL in (EFQ).
July continued with a progression of phytoplankton DNA in all stations with maximum 340 µg/mL in (ChP) and minimum 250 µg/mL in (EFQ).
August indicated the most abundant month with phytoplankton DNA, reaching maximum 626 µg/mL (WChDC) and minimum 300 µg/mL (HB). This confirms there are waste waters of Durres city, especially from Durres's Villa Hill discharged in (WChDC). There is a pump that sucked polluted urban runoff discharged to coastal waters.
In September, a downfall of phytoplankton DNA in all stations was noticed, except (ChP), where there was an increase of phytoplankton DNA compared with August. The maximum was 676 µg/mL in (ChP). This confirms the impact of abundant waste waters spill, usually as sewage from around hills and the hotels near this area. A big channel pours wastewater directly in sea and a bad smell is felt in (ChP).
In October, a little increase of DNA phytoplankton was shown in all stations, expect (CB) and (ChP), where the last has a drastic downfall. The maximum was 400 µg/mL in (HB) and minimum 260 µg/mL in (CB).
The diference in quantities of phytoplankton DNA between stations for the same month were little, except (ChP), (WChDC), and (GB). This confirms the abundant dirty water spill in these stations by anthropogenic influence.
(ChP) and (WChDC) had the highest averages in percentage (Table 1, Figure. 3) with 19.3% and 18.7%. Then there were ranked (GB), (HB), (EFQ), (CB) and in the end 14.7% (EFQ). This confirms that spills of polluted waters and sewage in (ChP) and (WChDC) are reasons for a high level of phytoplankton DNA. The reason of lowest phytoplankton DNA in (EFQ) may be the high level of heavy metals in basin that impede the growth of phytoplankton **(Wilbur Smith Associates, 2002).**
According to coefficient variation (Table 1), the varibility of (ChP) and (WChDC) were higher than other stations. This confirms once more that the anthropogenic influence brings this situation in environment, especially in these stations.

Table 1 The quantity of total phytoplankton DNA (µg/mL), Minimum, Maximum, Mean, Standard Deviation and Coefficient Variation (CV) of (GB), (ChP), (HB), (EFQ), (WChDC) and (CB) according to months

Stations	April	May	June	July	August	September	October	Min	Max	Mean	Standard Deviation	CV
GB	186	138	211	260	470	270	330	138	470	266	109.21	0.41
ChP	168	147	200	340	360	676	300	147	676	313	180.68	0.58
HB	189	158	203	290	300	220	400	158	400	252	83.45	0.33
EFQ	132	162	192	250	350	250	330	132	350	238	82.07	0.34
WChDC	189	137	198	300	626	300	370	137	626	303	163.56	0.54
CB	179	167	200	270	370	300	260	167	370	249	72.90	0.29

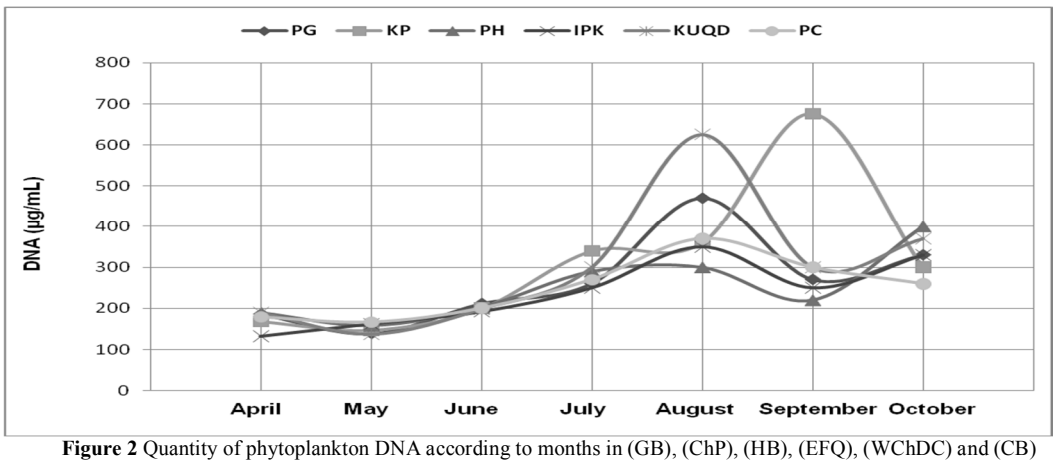

Figure 2 Quantity of phytoplankton DNA according to months in (GB), (ChP), (HB), (EFQ), (WChDC) and (CB)

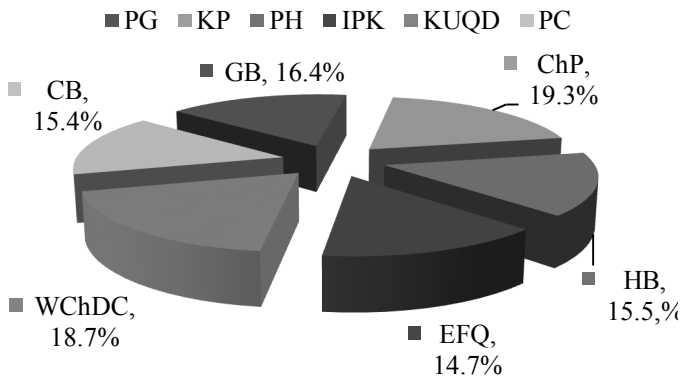

Figure 3 The average of phytoplankton DNA quantities according stations in (GB), (ChP), (HB), (EFQ), (WChDC) and (CB)

In Spring, (Table 2, Figure 4) the quantites of phytoplankton DNA were lower than in Summer and Autumn, with maximum 174 µg/mL in (HB), whereas minimum was 147 µg/mL in (EFQ).
In Summer, there was an increase of phytoplankton DNA quantity in all stacions, with maximun 375 µg/mL in (WChDC) and minimum 264 µg/mL in (HB & EFQ).
In Autumn, the phytoplankton DNA continues to increase only in these stations: (ChP), (HB), (EFQ). The maximum was in (ChP) 488 µg/mL and minimum (CB) 280 µg/mL.
The quantity in percent of phytoplankton DNA (Table 2, Figure 5) was highest in Autumn 42%, whereas in Summer and Spring were respectively 38% and 20%. This confirms that in Autumn, the environmental conditions were the best to produce phytoplankton in coastal waters of Durres Bay.

Table 2 The quantity of phytoplankton DNA (µg/mL), Minimum, Maximum, Mean, Standard Deviation (St. Dev.)and Coefficient Variation (CV) of (GB), (ChP), (HB), (EFQ), (WChDC) and (CB) according to seasons.

Stations	Spring	Summer	Autumn
GB	162	314	300
ChP	158	300	488
HB	174	264	310
EFQ	147	264	290
WChDC	163	375	335
CB	173	280	280
Min	147	264	280
Max	174	375	488
Mean	163	299	334
St. Dev.	9.94	41.77	77.82
CV	0.06	0.14	0.23

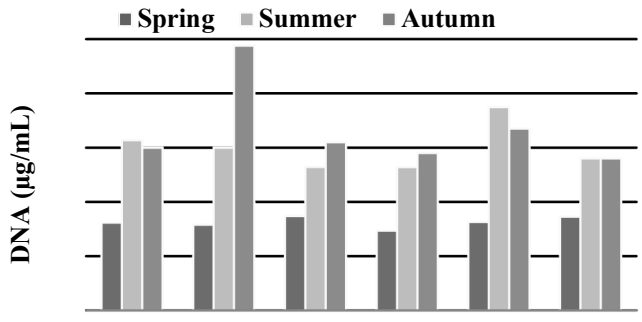

Figure 4 Quantity of total phytoplankton DNA according to seasons in (GB), (ChP), (HB), (EFQ), (WChDC) and (CB).

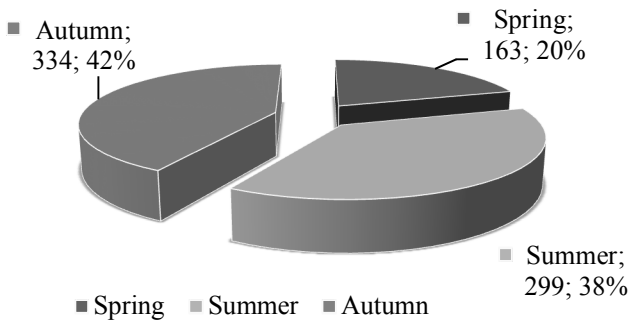

Figure 5 The spreading of total phytoplankton DNA quantities according seasons.

Environment Factors (biotic factors)
Total Chl a variation and level of trophy according concentration of Chl a

The quantity averages of total Chl a (Table 3, Figure 6) showed for a high level of trophy. The total average for all stations was 46.04 µg/L. All the stations were categorized in hypertrophic level (**Håkanson *et al.*, 2007**). Only (ChP) was included in eutrophic level in June, whereas (CB) was included in eutrophic level in May. The station with highest variability was (ChP). Maybe the flows in this area that determine the variation of Chl a.
Water pollution of Durres Bay from flows of sewage of reservoir Shkallnur-Arapaj and discharge in Kavalishenca-Kavaja Rock area, flows no inspect of sewage in Port-Currila area and throw of garbage along coast are negative for environment and human health. The high eutrophication in Durres Bay, is a result of anthropogenic factor. Therefore it is recommended to build sanitation, especially in Golem area, process that has begun, and to build plants to process sewage and polluted fresh waters of city Durres. Also it is necessary to inform community about this environment situation and to protect coastline from pollution and to stop building along coastline.

Table 3 The concentration of Chl a (µg/L), Minimum, Maximum, Mean, Standard Deviation and Coefficient Variation of (GB), (ChP), (HB), (EFQ), (WChDC) and (CB) according to seasons.

Months	GB	ChP	HB	EFQ	WChDC	CB
May	70.66	70.31	70.32	48.49	67.86	14.81
June	60.46	14.11	50.21	87.69	64.42	24.56
July	31.70	49.30	67.30	26.80	39.20	22.70
August	49.10	54.70	31.30	68.70	71.90	55.30
September	47.70	36.90	29.50	53.70	33.50	23.40
October	23.70	21.50	49.70	52.30	41.80	31.70
Average	47.22	41.14	49.72	56.28	53.11	28.75
min	23.70	14.11	29.50	26.80	33.50	14.81
max	70.66	70.31	70.32	87.69	71.90	55.30
Dev. St	17.45	21.15	17.22	20.46	16.76	14.08
CV	0.37	0.51	0.35	0.36	0.32	0.49

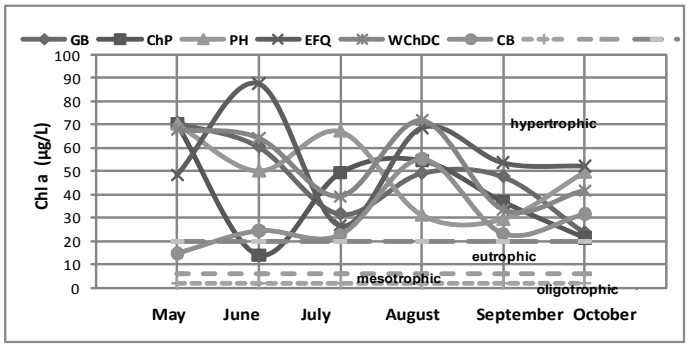

Figure 6 Concentration of Chl a (µg/L) according to months in (GB), (ChP), (HB), (EFQ), (WChDC) and (CB).

Environment Factors (abiotic factors)

The maximum value of temperature was 27°C, (Table 4) in all stations, and minimum 18°C in (CB). The mean temperature of all stations indicated 23.5°C. Referring mean temperature of all stations, water temperatures were mostly high. This happens because temperatures of surface are in influence of air temperatures. Coefficient of variation (CV) tell us for a little variability between temperature values.

The maximum value of pH (8.67) was observed in (ChP) and the minimum value of pH (7.59) was observed in (EFQ). The mean pH was 8.15. Referring standard deviation and CV, the variability of pH values were little. According to **WAC 173-201A (1997, 2011)** these pH values were within pH standard "range", expect the maximum value of pH in ChP. There are spills of fresh waters, sewage or agricultural water discharge in coastal waters from the channel in this area, that brings this situation.

The maximum value of dissolved oxygen (DO) (7.68 mg/L) was observed in (CB). The minimum value of DO (4.1 mg/L) was observed in (EFQ). The mean of DO was 5.87 mg/L. Referring standard deviation and CV, the variability of DO values were very little. DO in different depths decreased with the increased of the depth. According to **WAC 173-201A (1997, 2011)** these DO concetrations in surface water were within standard "range", expect minimum value in (EFQ). There are some reasons that brings the low level of DO. Maybe the little communication with open sea, because there is only one channel for water circulation of Durres's Basin Harbour with open sea. Also maybe the human activity that brings organic and chemical spills or garbage during anchorage of sea-chrafts waiting for operation in Harbour.

Referring percent water saturation of oxygen (CB) was the station with two extreme values (64.1-101%), and the mean saturation in all stations was 77.6%. These values are greater than 60 per cent saturation, a value considered adequate to support aquatic life. Lower saturation values ranging between 40-60 per cent saturation, indicate significant levels of oxygen depletion **(EPA, 2008).**

The maximum value of salinity(37.4 ‰) was observed in (CB). The minimum value of salinity (32.2 ‰) was observed in (ChP). The mean of salinity of all stations was 36.8 ‰ . The minimum of salinity confirms the impact of discharge of wastewater from human activity in (ChP). According salinity of albanian coastal waters these values are within (30-39 ‰) in all studied stations **(Shumka, 2005).** Water salinity mean of all stations results within diapason mean of salinity in Durres Bay (35.8-38.22 ‰) **(OSI, 2002).**

The maximum and minimum values of turbidity were in (ChP), respectively 13.4 and 0.41 NTU. The mean turbidity was 4.41 NTU. This confirms us for high suspended load in the water of (ChP), reducing the amount of light penetrating the water and affecting in color of the water. There are sewage spills and waste discharge that brings this situation in (ChP). Referring standard deviation and CV, the variability of turbidity values were highest.

The maximum of nitrates concetration (5.8 mg/L)was observed in (HB) and the minimum (0.06 mg/L) in (ChP) . The mean nitrate concetration was 1.7 mg/L. The maximum and mean are considered to be elevated when compared to the recommended standard of 0.1 mg/L. Natural levels of nitrate in surface waters seldom exceed 0.1 mg/l as N, but waters influenced by human activity normally contain up to 5 mg/l as N with levels over 5 mg/l as N indicating pollution by animal or human waste or fertilizer runoff **(Chapman, 1992).** The mean of nitrates concetration, was 17 times than recommended standard.

The maximum of phosphates concetration (0.6 mg/L)was observed in (CB)and the minimum (0.019 mg/L) in (ChP). The mean nitrates concetration was 0.17 mg/L. In most natural surface waters, phosphorus ranges from 0.005 to 0.020 mg/L PO4-P **(Chapman, 1992).** The mean of phosphates concetration, was 8 times higher than upper limit of recommended standard. Nitrogen and phosphorus may be released into the environment as a result of many human activities.

Excessive loads of nutrients can cause the eutrophication of coastal waterways. The general pattern of change involves a shift from large macrophytes (including seagrasses) towards fast-growing macroalgae and phytoplankton (including harmful species found in blooms **(Anderson *et al.*, 2002)** that can capture and use light more efficiently (**Nielson and Jernakoff, 1996; Duarte, 1995).**

Table 4 Mean, Maximum, Minimum, Standard Deviations and Variation Coefficients of abiotic factors: in (GB), (ChP), (HB), (EFQ), (WChDC) and (CB)

	T (°C)	pH	O_2 (mg/L:)	O_2 (%)	Salinity (‰)	Turbidity (FTU)	NO_3^- (mg/L)	PO_4^{3-} (mg/L)
Min	18	7.59	4.1	64.1	32.2	0.41	0.06	0.019
Min Sample	CB	EFQ	EFQ	CB	ChP	ChP	ChP	ChP
Max	27	8.67	7.68	101	37.4	13.4	5.8	0.6
Max Sample	All stations	ChP	CB	CB	CB	ChP	HB	CB
Mean	23.5	8.15	5.87	77.6	36.8	4.41	1.70	0.17
Stand. Dev.	3.127	0.310	1.080	8.628	0.832	3.417	1.862	0.197
CV	0.133	0.038	0.184	0.111	0.023	0.774	1.095	1.146

Legend: T (°C) = Temperature, pH = Pehash; **O_2** % = Oxygen Saturation , **O_2** mg/l = Dissolved Oxygen, Salinity (%) = Salinity, Turbidity (NTU)= Turbodity, **NO_3^-** = Nitrate; **PO_4^{3-}** = Phosphate.

The relationship between phytoplankton DNA and environmental factors

According to Multiple Regression Analysis between dependent variable (total phytoplankton DNA) and independent variables (Chl a and abiotic environment factors: temperature, salinity, pH, dissolved oxygen, turbidity, nitrate, phosphates), the coefficient of determination resulted (R^2 = 0.75). This means that 75% of the total variation in phytoplankton DNA (dependent variable) can be explained or accounted for by variation in environment factors (independent variables). Basing in Pearson's correlation and statistically significance (*p-value ≤ 0.05),* the phytoplankton DNA was related with these enviromental factors: pH (r= 0.6, *p-value<0.01*), salinity (r= - 0.5), *p-value=<0.01)* and phosphate (r= 0.4 *p-value=<0.05); explaining thus* the variation of phytoplankton DNA in Durres Bay coastal waters.

CONCLUSION

In Autumn, there was the highest level of phytoplankton DNA. The most abundant stations with phytoplankton DNA were Channel of Plepa and Water Channel of Durres City. This confirms that there are waste water as sewage or agricultural water discharged near coastline, that have a significant effect on waters quality and marine life of Durres Bay**.**

Whereas the station with the least phytoplankton DNA, was Ex-Fuel Quay in Marine Durres Harbour. This shows the inhibition of phytoplankton growth in this area. The inhibition itself may come as a result of chemical wastes such as petroleum, heavy metals.

The correlation between phytoplankton DNA and environment factors was strong. Especially pH, phosphates correlated positively with phytoplankton DNA, wheras salinity correlates negativety , explaining phytoplankton dynamics in coastal environments.

Coastal waters of Durres Bay, based in Chl a content values is characterized by a high trophic state, evaluated as hypertrophic level. Nitrogen and phosphorus concentrations were higher than standard levels. Excessive loads of nutrients can cause the eutrophication of coastal waterways. Therefore it is recommended to build sanitation, especially in Golem area, process that has begun, and to build plants to process sewage and other polluted waters of city Durres.

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