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RESEARCH ARTICLE **(Open Access)**

Dynamics of picophytoplankton and presence of cyanobacteria *Synechoccocus* in coastal waters of Durrës Bay (Albania)

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Abstract:

Cyanobacteria belonging to species *Synechococcus* and *Prochlorococcus* are important components of the picophytoplankton community and present in all oceanic systems. It is reported that coastal aquatic environments are typically more highly productive and dynamic than open oceans, and their dynamics reflects the level of trophy and pollution in the specific areas. Marine water samples were collected monthly from June to October 2012 in one meter depth at sample stations of the Bay of Durrës 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). Environmental factors as total chlorophyll a, temperature, pH, salinity, dissolved oxygen, percent oxygen saturation, turbidity, macronutrient (N and P) were measured to conclude on the level of trophy of the sampled area, and to explain the dynamics of picophytoplankton populations. The existence of *Synechococcus* was proved via the amplification of 16S-23S specific ribosomal DNA. Total phytoplankton and picophytoplankton DNA content were measured via spectrophotometry in order to understand the growth rate of the populations in the sampled area. The cyanobacteria *Synechococcus* investigated in all stations of Durres Bay, including in water bodies of (EFQ) and (CB), where a high level of heavy metals is present, was confirmed. This suggests that *Synechococcus* populations in these areas have developed mechanisms to combat heavy metal exposure. The correlation between phytoplankton DNA content and environmental factors was strong. Stations were categorized in hypertrophic level, which might be as a result of the excessive loads of nutrients in wastewaters discharged in Durrës's Bay.

Keywords: picophytoplankton, PCR, Durres Bay, heavy metals, level of trophy, environmental factor

1. Introduction

Phytoplankton vary more than 100-times in cell size from small picoplankton (0.2-2 μm) and nanoplankton (2-20 μm) to large microplankton (20-200 μm) [13]. Picoplankton, generally defined as plankton in the size range from 0.2 to 2 μm, consist mostly of heterotrophic bacteria and picophytoplankton (cyanobacteria and picoeukaryotes). Picophytoplankton are also major contributors to phytoplankton biomass in oligotrophic oceanic ecosystems. In particular, cyanobacteria belonging to the genera *Synechococcus* and *Prochlorococcus* are important components of the picophytoplankton community in the oceanic waters of the world [11]. Picophytoplankton are a small or major component of the phytoplankton community and present in all oceanic systems. They dominate in the low chlorophyll biomass areas, such as the (sub) tropical regions, but also contribute considerably (up to 20%) in the high chlorophyll biomass areas [19]. *Synechococcus*, which is large (ca. 1 μm in diameter) relative to *Prochlorococcus*, is ubiquitously distributed from the open ocean to the coastal sea [17].

For measuring natural microbial growth rates involves the use of biochemical "indexes" such as RNA, DNA and protein content that are correlated with growth rate. The fact that these relationships can be described in simple mathematical terms, and appear to be independent of the specific environmental factor that determines the growth rate, supports the idea of using gross biochemical composition to estimate the in situ growth rate of natural microbial populations. In particular, ribosome (or rRNA) content is a good candidate as a biochemical index for growth rate in natural microbial populations [4].

The most important mechanisms by which bacteria combat heavy metal exposure and subsequent

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accumulation is through internal metal sequestration. In the prokaryotic cyanobacteria, metal ion sequestration within the cell is performed by the class II metallothioneins [29]. The smt locus of *Synechococcus* PCC 7942 contains a metal-regulated gene, smtA. This operon encodes a class II metallothionein and a divergently transcribed repressor of smtA transcription, smtB [15].

The first aim of this study was to use molecular analysis to show the existence of cyanobacterial genus *Synechococcus.* The second aim of this study was to measure total phytoplankton DNA content to understand growth rate in natural microbial populations as picophytoplankton and cyanobacteria at different coastal water areas of Durres Bay. Also referring previous studies of heavy metals presence in coastal water of Durres Bay, we could understand the impact of heavy metals in growth of *Synechococcus,* and in DNA phytoplankton concentrations. An another aim of this study was to assess variation of phytoplankton DNA quantity 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. Also an another aim was to measure Chlorophyll a concentration, as an indicator for assessing trophic status of waters bodies [12] of Durres Bay**.**

2. Material and Methods

2.1. Water Samples Stations and Samples Collecton

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 June to October 2012. 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 [9].

Figure 1. Locations of Water Samples in Durres Bay

2.3. Filtration of Water for isolation phytoplankton DNA, DNA extractions and evaluation

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 [23]. The phytoplankton DNA was extracted according to Pichard *et al.* [21] and Paul [20], based on the Fuhrman *et al.* [7] study with little modifications. DNA Quantification and Quality Analysis was evaluated with spectrophotometry based on Sambrook *et al* [23].

2.5. Polymerase Chain reaction

Primers used to amplify cyanobacterial rDNA were 16S-1247F and 16S-241R according to Rocap *et al*. [22]. PCR was run in a 20 ml solution. Sigma taq polymerase (0.5 U), $MgCl_2$ (2 mM), buffer (1X), deoxynucleoside triphosphates, and 100 pmol of each primer. Temperature cycle was: 94 °C for 4 min, followed by 40 cycles of 94 °C for 1 min, 52 °C for 1min, and 72 °C for 3 min. Final step was a 10 min stretch at 72 °C. DNA products were checked by a 1 % agarose gel under the UV transiluminator.

2.6. Water quality

Environmental factors studied following APHA [2], Grasshoff *et al.* [9], 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.

2.7. 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 [14, 16].

3. Results and Discussion

The water samples are taken during October-June 2012. The water samples to assess the physico-chemical parameters are taken in: (GB), (ChP), (HB), (EFQ), (WChDC) and (CB Maximum, minimum, mean, standard deviation and variation coefficient of physico-chemical waters of the obtained data are given in Table 1. Also is presented mean all above parameters to compare 2012 level with 2011.

3.1 Physico-chemical water properties

Table 1. Maximum, minimum, mean, standard deviation and variation coefficient of physico-chemical & biological properties in 2012 and mean of obtained data in 2011 for all stations: (GB), (ChP), (HB), (EFQ), (WChDC) and (CB).

		T (°C)	pH	O_2 (mg/L)	O_2 (%)	Salinity (‰)	Turbidity (NTU)	NO_3^- (mg/L)	PO_4^{3-} (mg/L)	Chl a (µg/L)
2012	**Max**	28.9	8.20	9.31	112.3	38.6	68	2.1	0.41	39.57
	Sample	ChP	GB	ChP	EFQ	WChDC&CB	WChDC	EFQ	ChP	WChDC
	Min	23	7.97	5.32	66.7	34.2	0.32	0.3	0.17	6.92
	Sample	WChDC	GB	EFQ	GB&EFQ	ChP	CB	CB	CB	CB
	Mean	25.9	8.09	7.09	83.13	37.59	8.12	0.93	0.25	21.82
	St.Dev.	1.55	0.05	1.19	13.93	0.98	12.13	0.45	0.05	8.99
	CV	0.06	0.01	0.17	0.17	0.03	1.49	0.49	0.20	0.41
2011	**Mean**	23.5	8.15	5.87	77.61	36.77	4.41	1.70	0.17	46.04

Legend: T (°C) = Temperature, pH = Pehash; O_2 (%) = Oxygen Saturation , O_2 mg/l = Dissolved Oxygen, Salinity (%) = Salinity, Turbidity (NTU) = Turbidity, NO_3^- = Nitrate; PO_4^{3-} = Phosphate, Max = Maximum; Min = Minimum; St. Dev. = Standard Deviation; CV = Coefficient of Variation.

Surface water temperatures for all stations ranged between 23 - 28.9 °C respectevily in (WChDC) and (ChP) (Table 1), whereas mean 25.9 °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) tells us for a little variability between temperature values. Comparing mean temperatures of 2012 and 2011 the temperature of 2012 were 2.4 °C higher than in 2011.

The pH values ranged between 7.97 - 8.20 respectivelly both in (GB), whereas average 8.09 (Table 1). According to **WAC 173-201A (1997, 2011)** these pH values were within pH standard "range". CV tells us for a little variability between pH values. Comparing mean temperatures of 2012 and 2011 the pH of 2012 was 0.1 unit lower than in 2011, informing that pH was almost the same situation.

Dissolved Oxygen (DO) values for all stations ranged between 5.32 - 9.31 mg/L respectively in (EFQ) and (ChP), whereas average 7.09 mg/L (Table 1). Variability was low between DO values. According to **WAC 173-201A (1997, 2011)**, DO values were within DO standard "range" classified in extraordinary quality & excellent quality of aquatic life dissolved oxygen criteria in marine water**,** except minimum in (EFQ) classified in good quality. May be little communication to open sea and high temperatures in Marine Durres's Harbour basin, that brings low DO. DO concentration of 2012 was 1.2 mg/L bigger than 2011, informing that oxygenation of these coastal water bodies were improved.

Percent oxygen saturation values for all stations ranged between 66.7- 112.3 % respectively in (GB) & (EFQ) and (EFQ), whereas average 83.13 % (Table 1). Variability was low between percent water saturation of oxygen values. These values were 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 [6]. Percent water saturation of oxygen of 2012 was 5.5 % bigger than 2011, informing that saturation oxygenation of these coastal water bodies were improved.

Salinity values for all stations ranged between 34.2- 38.6 ‰ respectively in (ChP), and (WChDC) & (CB) whereas average 37.59 ‰ (Table 1). Maybe spills of fresh waters, sewage or agricultural water discharge in coastal waters from the channel in this area, that brings this situation in (ChP). Variability was very low between salinity values. According salinity of albanian coastal waters these values are within (30-39 ‰) in all studied stations [24]**.** Water salinity mean of all stations results within diapason mean of salinity in Durres Bay (35.8-38.22 ‰) [18].

Salinity mean of 2012 was 0.8 ‰ bigger than 2011, informing that these coastal water bodies have been more saline.

Turbidity values for all stations ranged between 0.32 – 68 NTU, respectively in (CB) and (WChDC), whereas average 8.12 NTU (Table 1). Variability was extremely high between turbidity values. The highest level in (WChDC) informs for waste waters discharge, including sewage in this area during the water pumps were working. Turbidity mean of 2012 was 1.8 times higher than 2011, informing that these coastal water bodies had an increase of turbidity.

Nitrate concentrations for stations ranged between 0.3 - 2.1 mg/L respectively in (CB) and (EFQ), whereas average 0.93 mg/L (Table 1). Variability was high between nitrate concentrations. Mean values of 2012 & 2011 converted as NO_3-N, (0.38 mg/L, 0.93 mg/L) are considered to be elevated when compared to the recommended standard of 0.1 mg/L NO_3-N [5]. Nitrate concentration of 2012 was 1.8 times smaller than 2011, informing that nitrate level of these coastal water bodies were reduced, improving situation of coastal waters.

Phosphate concentrations for stations ranged between 0.17- 0.41 mg/L respectively in (CB) and (ChP), whereas average 0.25 mg/L (Table 1). In most natural surface waters, phosphorus ranges from 0.005 to 0.020 mg/L PO_4-P [5]. Mean values of 2012 & 2011 converted as PO_4-P, (0.081 mg/L, 0.055 mg/L) are considered to be elevated when compared to the high level of recommended standard. Variability of phosphate concentrations was lower than nitrate concentrations. Nitrogen and phosphorus may be released into the coastal environment as a result of many human activities. Phosphate concentration of 2012 was 1.4 times greater than 2011, informing that phosphates level of these coastal water bodies were increased.

3.2 Biological parameters: Level of trophy according Chl a concentrations

Differences of Chl a concentration (Figure 2, Table 1) among stations during the same month were not high. Chl a concentration for all stations ranged between 6.92 - 39.57 µg/L, respectively in (CB) and (WChDC), whereas average 21.82 µg/L. Variability was high between Chl a concentrations. All the stations are categorized in hypertrophic level [10], except (CB) in eutrophic state. All the values of June, some values of July & October are classified in eutrophic level. Waste waters became the dominant source of water pollution. Wastewater treatment and waste disposal management should be of the highest priority. Hypereutrophic water bodies are very nutrient-rich waters characterized by frequent and severe nuisance algal blooms and low transparency [25]. Chl a concentration of 2012 was 2.1 times lower than 2011 (informing that level of trophy in these coastal water bodies were decreased. Situation of these coastal water bodies were improved. Maybe given its impact on coastal water bodies setting of appropriate filters in (ChP) during beach season of 2012. Also maybe given its impact the sanctions by the local authority to buildings and businesses subjects, that discharge wastewater along the coast of Durres Bay during beach season of 2012.

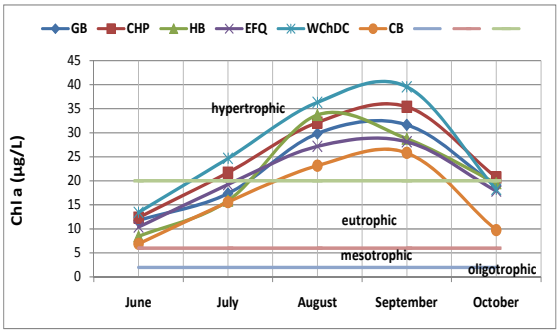

Figure 2. Chl a concentrations according to months in (GB), (ChP), (HB), (EFQ), (WChDC) and (CB).

3.3 Total Phytoplankton DNA quantity, presence of Synechococcus and impact of heavy metals in their growth

August reached the maximum of total phytoplankton DNA concentration (189 µg/mL) (Figure 3) in WChDC, whereas October the minimum of total phytoplankton DNA (30 µg/mL) in CB. Differences of total phytoplankton DNA concentrations among stations during the same month were considerable high between stations. Average of all stations was 99.4 µg/mL. Total phytoplankton DNA concentration of 2012 was 2.7 times lower than 2011, (Table 2) confirming that phytoplankton quantity and also picophytoplankton of these coastal water bodies were decreased.

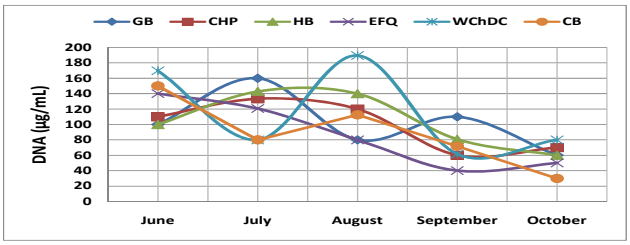

Figure 3. Total phytoplankton DNA concentration in 2012 according to months in (GB), (ChP), (HB), (EFQ), (WChDC) and (CB) stations.

The quantity in percent of phytoplankton DNA (Figure 4) was highest in Summer 66%, whereas in Autumn and Spring was 34%. This confirms that in Summer, the environmental conditions were better than Autumn to produce phytoplankton in coastal waters of Durres Bay.

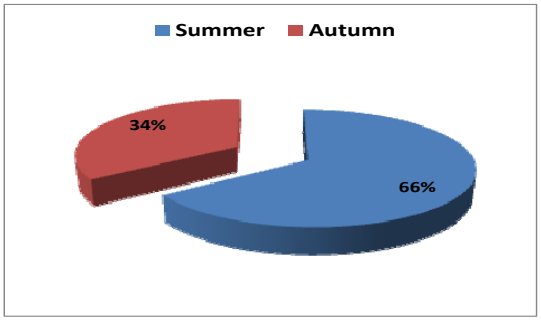

Figure 4. The spreading of total phytoplankton DNA quantities according seasons.

(WChDC) and (HB) had the greatest averages in percentage (Table 2, Figure 5) with 19.5 % and 17.6 %. Then there were ranked (GB) and (ChP). In the end were ranked (CB) and (EFQ) respectively with 14.9 % and in the end 14.4 % (EFQ). The smallest mean was in (EFQ).

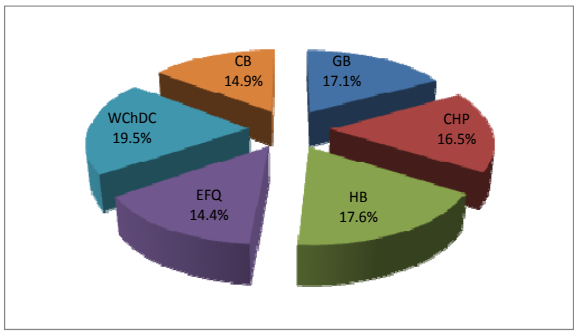

Figure 5. The percent average of phytoplankton DNA quantities according stations in (GB), (ChP), (HB), (EFQ), (WChDC) and (CB) stations.

Comparing data phytoplankton DNA content of 2012 to 2011 (Table 2), we observed: (ChP) is ranked in fourth place in 2012, wheras in 2011 in first place for total phytoplankton DNA. Maybe given its impact on coastal water body setting of appropriate filters in (ChP) during beach season of 2012. Also maybe given its impact, sanctions by the local authority for those buildings and businesses subjects, that discharge polluted water along the coast of Durres Bay, especially in Golem Area.

Table 2. Maximum, minimum, mean, standard deviation and variation coefficient of total phytoplakton DNA quantities in 2011 & 2012 for (GB), (ChP), (HB), (EFQ), (WChDC) and (CB) stations.

		GB	CHP	HB	EFQ	WChDC	CB
2011	**Max**	470	676	400	350	626	370
	Min	138	147	158	132	137	167
	Mean	266.42	313.09	251.53	238.01	302.81	249.37
	St.Dev.	109.21	180.68	83.45	82.07	163.56	72.90
	CV	0.41	0.58	0.33	0.34	0.54	0.29
2012	**Max**	160	133	143	140	189	150
	Min	60	60	60	40	62	30
	Mean	102.00	98.67	104.73	86.12	116.14	88.87
	St.Dev.	37.68	32.02	36.38	43.48	58.82	45.09
	CV	0.37	0.32	0.35	0.50	0.51	0.51

Legend: Max = Maximum; Min = Minimum; St. Dev. = Standard Deviation; CV = Coefficient of Variation.

Spills of polluted waters and sewage in (WChDC) may be the reason for the highest level of phytoplankton DNA in 2012. Comparing 2012 to 2011, (WChDC) was respectively in first place and second place.

The smallest mean of total phytoplankton DNA quantity was again as in 2011 to (CB) & (EFQ). There is something that that impede the growth of phytoplankton in (EFQ). The same situation is showed with inhibition of heterotrophs bacteria growth in Fuel Quay [8]. The inhibition itself may come as a result of chemical wastes such as petroleum, heavy metals. The reason of lowest phytoplankton DNA in (EFQ) may be the high level of heavy metals in basin that impede the growth of phytoplankton. Also in (CB) may be the same reason. Referring the previous studies for heavy metals in surface sediments in Durres's Marine Harbour basin [28] for heavy metals in surface sediments and water in Currila area [1], and comparing to accepted background levels of Mediterranean sediments and Adriatic sediments, we can say: (EFQ) has grater heavy metals than (CB). The levels of heavy metals in (EFQ) & (CB) are greater than background levels in Mediterranean sediments and Adriatic sediments, expect values of Cd and Pb in (CB), that are higher than values reported from different studied carried out in Adriatic Sea, but these values aren't higher than values in Mediterranean Sea.

According to coefficient variation (Table 2), the varibility of (WChDC), (CB) and (EFQ) were higher than other stations. This confirms once more that the anthropogenic influence brings this situation in environment, especially in these stations.

Results indicated that genus of picophytoplankton *Synechococcus* was present all in coastal bodies, into six stations of sampling (Figure 6). The product was about 400-450 bp and was reproduced successfully as in Bacu *et al*. [3]. This confirmed that heavy metals in Durres harbour basin and Currila area permit the growth of *Synechoccocus*. *Synechococcus* has mechanisms to combat heavy metal exposure.

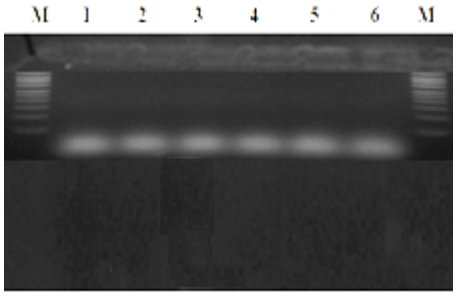

Figure 6. PCR products of ribosomal fragments from cyanobacterial strains of *Synechoccocus* amplified from phytoplankton DNA in all stations.

Legend: M = Marker; 1 = (GB); 2= (ChP), 3 = (HB); 4 = (EFQ); 5 = (WChDC); 6 = (CB).

3.4 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, phosphate) studied for all samples 2011 - 2012, the coefficient of determination resulted (R^2 = 0.74). This means that 74% of the total variation in phytoplankton DNA (dependent variable) can be explained or accounted for by variation in environmental factors (independent variables). Basing in Pearson's correlation and statistically significance (*p-value ≤ 0.05),* the phytoplankton DNA was related with these environmental factors: pH (r = 0.49, p-value ≤ 0.01), Chl a (r = 0.41, p-value ≤ 0.01), correlated positively, whereas salinity (r = - 0.59, p-value ≤ 0.01), dissolved oxygen (r = - 0.49, p-value ≤ 0.01), percent oxygen saturation (r = - 0.33, p-value ≤ 0.01) correlated negatively, explaining thus the variation of phytoplankton DNA in Durres Bay coastal waters. The high level of phytoplankton as excessive algal blooms can also significantly reduce oxygen levels and prevent life from functioning at lower depths creating dead zones beneath the surface.

4. Conclusions

The presence of cyanocacteria *Synechococcus* was investigated in all stations of Durres Bay, based on the PCR amplification of a cyanobacterial ribosomal DNA, including in water bodies of Fuel Quay in Marine Durres Harbour and Currila Area, where there are high level of heavy metals. This confirms that, *Synechococcus* has mechanisms to combat heavy metal exposure. The station with the least phytoplankton DNA, was (EFQ) 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 quantity of phytoplankton DNA, was reduced comparing 2012 to 2011. The correlation between phytoplankton DNA and environment factors was strong. Especially pH and chlorophyll a, salinity, dissolved oxygen and percent oxygen saturation explained much phytoplankton and picophytoplankton dynamics in coastal environments.

Coastal waters of Durres Bay, based in Chl a content values were reduced in 2012 comparing to 2011 improving coastal situation, but still they are characterized by a high trophic state, evaluated as hypertrophic level, except Currila Beach which is in eutrophic level. Excessive loads of nutrients in waste waters as sewage can cause the eutrophication of coastal water bodies Durres's Bay. Therefore it is recommended to build sanitation, especially in Golem area, process that has begun in early 2012, and to build plants to process sewage or other polluted waters of city Durres.

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