Nitrogen and Phosphorus in Coastal Sediments covered by Cyanobacteria Mats

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Abstract

Background. Biocommunities of phototrophic microorganisms (principally cyanobacteria) developing on the surface sediments of shallow, warm and semi-isolated coastal environments, bind or trap small particles on their polysaccharide sheaths creating organosedimentary structures. Those structures are called algal mats and they are commonly flat and laminar in shape. They are also called recent stromatolites due to their remarkable similarity to the fossil ones.

Objectives. Not many geochemical studies exist concerning cyanobacterial mats in Mediterranean coasts, in comparison with the biological ones. Considering the importance of nitrogen and phosphorus in the cyanobacteria mat development, the present study aims to contribute to the knowledge of their chemical behaviour in such environments.

Methods. Sediment cores of about 10 cm length along with water samples were collected during the course of a year from two Greek coastal areas. Sediments were fractionated in three distinct layers according to their structure. A wet oxidation method for the simultaneous determination of total nitrogen and total phosphorus was applied to all sediment samples. The total organic carbon (TOC), the total inorganic phosphorus and the natural pigment content in the sediment samples were also determined. Standard spectrophotometric methods were used for the determination of the concentration of dissolved nutrients. In situ measurements of pH, temperature and salinity were also carried out.

Results and Discussion. The sampling areas were found to be in the mesotrophic level. The group of aerobic filamentous cyanobacteria dominated the sediment biocommunities. The pH of the sediment showed a gradual reduction downwards. The highest content of TOC, total nitrogen and total phosphorus were detected in the surface layer of the sediment samples. The contribution of organic phosphorus to the total phosphorus ranged from 0% and 50% with an average value of 26.6%. A depletion of organic phosphorus in the anoxic middle layer of the sediment was detected. A good positive correlation between total nitrogen and chlorophyll-a, as well as total nitrogen and TOC in the surface sediment layer, was observed. A good negative correlation between total phosphorous in sediments and dissolved phosphates in seawater was also observed.

Conclusions. The high content of total nitrogen recorded in the surface sediments indicate that cyanobacteria accumulate nitrogen acquiring it from the sediment, the marine environment and the air (in the form of N₂). A proportional accumulation in phosphorus also happens, although to a lesser degree. The temporal fluctuation of nitrogen content in the sediment’s surface layer agrees with the temporal fluctuation of primary production. The cyanobacterial mat acts as a natural pump that transfers phosphorus from seawater to the surface of the sediment.

Recommendations and Outlook. A further, more extensive, multidisciplinary study of cyanobacterial mats is needed for the determination of the biogeochemical processes that take place there and for the effective environmental management of the areas where these forms develop.

Keywords: Coastal sediments; cyanobacterial mats; filamentous cyanobacteria; organosedimentary structures; recent stromatolites; total nitrogen; total phosphorus

Introduction

In coastal marine environments with shallow, warm and calm waters, it is sometimes possible to observe the presence of organosedimentary structures, which are produced by sediment trapping, binding and/or precipitation as a result of the growth and metabolic activity of microorganisms, principally cyanobacteria. Cyanobacteria are a group of prokaryotic, oxygenic phototrophic microorganisms and it is well established that they can build thick mats of flat morphology. The gradient of light within the mat forces different species and functional groups of microorganisms (phototrophs, chemotrophs) to particular vertical stratified positions in the mat (Brock 1976, Golubic 1976, 1983, Stal 2000).

Those structures are called microbial or cyanobacteria or algal mats and one of their typical properties is their laminated structure in which different functional groups of microorganisms occur in vertically stratified layers. This type of lamination can be attributed to different growth periods, seasonal events, periodical events (e.g. tides) or episodic or erratic events (e.g. storms). The sediment trapping is usually carried out in shallow waters by the action of filamentous cyanobacteria. These cyanobacteria secrete sticky organic matter known as mucilage. Fine-grained extraneous particles, usually lime mud that is washed across the musculaginous mats, stick to the mat. After they have trapped and bound a sheet of extraneous particles, the cyanobacteria grow another layer of mat through and over the trapped and bound
particles. By repeating such trapping and binding of extraneous particles and such growing of mats over the bound particles, these cyanobacteria produce a deposit characterized by millimetre-thin laminae. According to Friedman and Sanders (1972) and Brock (1976), the favourable environmental conditions for the formation of a cyanobacterial mat are:

- Swallow protected and warm waters.
- Conditions supporting the presence and growth of cyanophytes.
- The growth rate of the mat-building organisms must be faster than consumption by grazing microorganisms.
- Low sedimentation rates to allow stabilized colonization of the surface by the mat-building organisms.
- Destructive forces such as burrowing organisms and mechanical and chemical erosion must be absent or at least not capable of preventing the accretion of organisms.

Calcification is the general process of consolidation and preservation of microbial mats. Laminated rocks dating from the Precambrian or later eras are known as stromatolites (Krumbein 1983). Still living, well-laminated and consolidated stromatolites can be found in Exuma Cays in the Bahamas islands (Reid and Browne 1991) as well as in Sharks’ Bay in Australia. Nowadays, microbial mats built by cyanobacteria show remarkable similarities with fossil stromatolites and are also known as recent stromatolites (Stal 2000). However, the most important difference between recent stromatolites and fossil ones is that the great majority of recent stromatolites do not form consolidated rocks. Such formations of recent stromatolites can be found in some coastal places all over the world as well as in Mediterranean coasts.

The typical structure of a Mediterranean cyanobacterial mat consists of three layers: The upper one, 0.5–1.2 mm thick, includes the cyanobacterial mat; the middle one, up to 5 cm thick, of dark colour, is anoxic and has a characteristic H₂S odour; the third one, 5 to 10 cm, represents the background sediment (Fig. 1).

These structures have also been observed in various Greek coastal areas. The most important of them are located in the peninsula of Attika (Anavissos and Rafina), in the areas of Sitia and Ierapetra (Eastern Crete island), on the island of Lesvos (East Aegean sea) and on the island of Zante (Ionian sea). The areas of Anavissos and Rafina were selected for this study. Anavissos is considered to have ideal conditions for the development of such formations rather than Rafina, where those formations are not so extended (Fig. 2).

The majority of studies concerning cyanobacterial mats takes a biological point of view and mostly refers to tropical environments, so there is a lack of information about their geochemistry, especially in the Mediterranean. The behaviour of various forms of nitrogen and phosphorus is studied in this paper.

Nitrogen and phosphorus are very important elements for the development of cyanobacterial mats. The nitrogen content in cyanobacteria may amount up to 10% of their dry weight and is quantitatively the third most important element essential for fixing amino acids and proteins. Any shortage of it immediately affects the amount of phytobiliproteins and, consequently, the efficiency of light harvesting for photosynthesis. Cyanobacteria acquire nitrogen from various sources including sediment, soil, water-column and air. Nitrogen in marine sediments occurs mainly in organic forms principally as amino acids or proteins. The most important inorganic forms of nitrogen are NH₄⁺ and NO₃⁻ ions, which are mainly dissolved in pore-water (Touchette and Burkholder 2000), and are considered to be the available forms for biological processes.

The content of phosphorus in the cyanobacterial cells is about 0.6% of their dry mass, although an excess of this element can be stored as polyphosphates. It is a critical element for fixing ATP and DNA. The main source of phosphorus for cyanobacteria is orthophosphates found in seawater (Stal 2000). In microbial mats the occurrence of organic forms of phosphorus is low as it is cleaved hydrolytically by phosphatases to phosphoric ions that are immediately taken up by the cyanobacteria. In addition, the content of free orthophosphates in microbial mats is expected to be negligible because, as soon as orthophosphates are liberated, the microbial community takes them up.

1. Materials and Methods
1.1 Sampling

The area of Anavissos (see Fig. 2) is a closed and shallow bay in the south-east of Athens, having a surface of about
20,000 square metres. A line of beach rocks obstructs the bay’s entrance and, as a result, hinders the renewal of its water, which takes place at both ends of the 3 m wide entrance. The maximum tidal height is about 10 cm. Some small fishery and recreation crafts are secured inside the bay. At the shallow bottom of the cove (average depth of about 0.6 metres), a layer of cyanobacteria can be observed (thickness 0.2 mm–2 mm).

The samplings in this area were carried out on a monthly basis from July 2000 to June 2001 at three different sites: Point 1, which is continuously covered by seawater, point 2, which is covered by seawater during the most hours of the day, and point 3, which is mostly not submerged.

The area of Rafina (see Fig. 2) is an open beach to the northeast of Athens. The length of the coast is about 300 metres and is mostly covered by sand. This beach is a 'high energy' one, as waves penetrate the coastline carrying seawater up to 6 metres inland. Human activities are rare during winter, while this beach becomes very crowded by summer visitors. In this area, samplings were carried out seasonally from November 2000 to August 2001 at one selected point (point 4) located in the centre of the beach and 4 metres away from the coastline (see Fig. 1). This point is usually in contact with the waves.

Water samples for nutrient determination were collected in plastic bottles pretreated overnight with 1M HCl solution and rinsed afterwards twice with redistilled water. The samples, after filtration through 0.45 µm nitrocellulose membrane filters were stored at –18°C until the time of analysis.

Sediment cores of about 10 cm were also collected and fractionated in the main layers described above. The three layers were named a, b and c, respectively. Subsamples were either kept alive for culturing purposes or fixed in 3% formaldehyde solution in ambient water for examination under light microscope.

### 1.2 Measurement and determination procedures

The physicochemical parameters of the seawater (pH, Salinity, dissolved Oxygen and Temperature) were measured in situ by the use of a portable salinometer (YSI Model 63). The pH in the sediments was also measured in situ using a pH-electrode, convenient for measurements into the wet sand (ORION KNipHE 7120BN), and a portable pH-meter. Measurements were carried out in depths of 0.5, 1.0, 2.0 and 5.0 cm inside the sediment.

The upper layers of the sediment samples were examined microscopically in order to detect their cyanobacterial load. After freeze-drying, the separated layers were sieved through 0.24 mm sieves and stored under inert atmosphere, in darkness at –18°C. The sediment samples were analysed for their grain-size by a 5100 MicroMeritics sedigraph.

The pigments were determined in all layers of the sediment samples, after their extraction with methanol using an HPLC Waters 600D apparatus equipped with a diode array detector and a C18 column. A linear gradient elution procedure was applied for the pigments’ separation (Hatzianestis and Sklivagou 2000, Wright and Jeffrey 1997). The Total Organic Carbon (TOC) in sediments was determined titrimetrically using the K_2Cr_2O_7 oxidation method (Gaudette et al. 1974).

A wet oxidation method for the simultaneous oxidation of total nitrogen to nitrates and total phosphorus to phosphates was applied to the sediment samples (Ladakis et al. 2003). This is a modification of an existing method applied to seawater samples (Valderama 1981, Junco et al. 1983). A reference material (RM) with certified values in total nitrogen and phosphorus (VKI Loam Soil A) was used to check the effectiveness of the method in every oxidation procedure performed. The recovery of the RM for both elements (P < 0.05) is presented in Table 1.

| Table 1: Recovery of total nitrogen and total phosphorus in VKI Loam Soil
<table>
<thead>
<tr>
<th>Certified value</th>
<th>Value found</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total N (mg/g)</td>
<td>1.57 ± 0.06</td>
<td>1.50 ± 0.04</td>
</tr>
<tr>
<td>Total P (mg/g)</td>
<td>0.785 ± 0.044</td>
<td>0.757 ± 0.040</td>
</tr>
</tbody>
</table>

The inorganic forms of nitrogen (NH_4^+, NO_2^−, NO_3^−) which remained in the sediment after the removal of pore-water by lyophilization, were extracted by shaking for 30min with 0.5M NaCl.

The inorganic phosphorus was extracted using 1M HCl and shaking for 16 hours (Aspila et al. 1976). The free orthophosphates were extracted by 1M NH_4Cl solution at pH 7 and shaking for 30 min (Xu et al. 2001). The organic phosphorus was calculated by subtracting the amount of inorganic phosphorus from the amount of the total phosphorus.

The determination of the produced NH_4^+, NO_2^−, NO_3^− and PO_4^{3−} ions as well as the seawater nutrients was based on the spectrophotometric methods of Grasshoff and Kremling (1977). A continuous flow autoanalysers from Brann + Luebbe, suitable for nutrient determination, was used for this purpose.

### 1.3 Microbiological analysis and culturing methods

The live samples as well as the fixed ones were analyzed microscopically immediately after collection. The cyanobacteria with apparent dominance were identified and separated by micromanipulation. A light microscopic analysis and a photodocumentation of live and preserved samples were carried out using a standard Zeiss microscope. Cultures were started from natural populations in the MN medium suitable for marine cyanobacteria.

A small and thin section of mats (approximately 3 × 3 mm) dominated by cyanobacteria was excised under sterile conditions and used as an inoculum in liquid culture media. The cultures were maintained at room conditions. Each transfer was accompanied by a microscopic analysis. The identification of cyanobacteria was carried out to the systematic approach and terminology of Anagnostidis and Komarek (1988).
2 Results and Discussion

2.1 Seawater characteristics

The Salinity in Anavissos ranged between 34.5 psu and 39.8 psu inside the cove and between 35.5 psu and 38.4 psu outside (typical for East Mediterranean coasts). One low value (18.2 psu) determined during March 2001 inside the cove, indicates an occasional freshwater inflow. Salinity in Rafina ranged between 35.0 psu and 38.3 psu.

The water temperature in Anavissos varied between 14.7°C (in January) and 33.2°C (in July) inside, and between 14.6°C and 26.8°C outside the cove. Rafina’s water temperature varied between 15.0°C (in winter) and 25.8°C (in summer).

The fluctuation of the seawater’s pH in Anavissos was between 7.97 and 8.27 while Rafina’s pH ranged between 7.92 and 8.13. The water in all stations was saturated with O₂.

The annual mean values of dissolved nutrients in the areas of Anavissos and Rafina are presented in Table 2. According to Karidis et al. (1998) criteria for the eutrophication levels in the area of Anavissos, the water of the cove is in the Upper Mesotrophic level while the Lower Mesotrophic level dominates the water outside the cove. On the other hand, the water in the area of Rafina is in the Lower Mesotrophic level. According to Justic et al. (1995) criteria, no specific limiting factor for nutrients was found in the area of Anavissos, since nitrogen is the limiting factor during spring in the area of Rafina. The annual average N/P ratio (in atoms) is 21 (both inside and outside the cove) and 24 for Anavissos and Rafina, respectively. Measurements in the open Saronikos Gulf during the same period have shown that its water was in the Lower Mesotrophic level, while nitrogen was the limiting factor and the N/P ratio was lower than 10 in all cases.

The microscopic analysis of live, preserved and cultured material taken from the surface layer of the samples revealed that cyanobacteria was the dominant group of the system phototrophs. Chlorophytes and diatoms were also present, but to a lesser degree. The composition of the cyanobacterial communities of the surface layer did not differ significantly among the samples, although the cyanobacteria total population in Rafina’s sampling station was found to be quantifiably lower. Six taxa of cyanobacteria (five filamentous, non-heterocystous and one colonial) were identified. The filamentous Microcoleus chthonoplastes was the dominant and most abundant phototroph bearing a thick polysaccharide sheath. Other filamentous cyanobacteria that were detected in considerable amounts were: Spirulina subsalsa, Lyngbya aestuari, Schizothrix sp. and occasionally Oscillatoria sp. and the chroococcoid Chroococcus turgidus. Lyngbya aestuari has trichomes which are surrounded by a thick polysaccharide sheath that is often yellow brown due to the pigment scytonemine. Material from the underlying layers was also cultured. The presence and growth of Microcoleus chthonoplastes was ascertained in cultured material from layer b. This phenomenon was not recorded when specimens from the deeper layer (layer c) were cultured, indicating the absence of such biocommunities there.

Knowing that layer b is anoxic, the presence and growth of Microcoleus chthonoplastes filaments is interpreted by the fact that this particular mat forming cyanobacterium has the ability to grow either aerobically or anaerobically. Our findings agree with those of Golubic (1976), Bauld (1984), Stal et al. (1985) and Stal (2000).

2.2 Biocommunities

In every case, the surface layers contained more clay than the underlying ones. The mean content of sand, silt and clay in the surface layer (a) was 43.5%, 29.1% and 27.4% respectively, whereas in the second layer (b) the corresponding values were 63.2%, 17.9% and 18.9%, respectively. The binding and cementation capabilities of cyanobacteria that affect mainly small particles can explain this fact.

2.3 Grain-size of the sediment samples.

The general trend recorded was a gradient decrease as sediment depth increases until the edge of the second layer, and a slight increase when entering to the third layer. This is due to the anoxic conditions prevailing in layer (b) that produce H₂S (Revsbeck et al. 1983). The Anavissos sediments’ pH varied from 7.80–7.15 in the surface layer and from 6.70–6.40 at a depth of 5 cm. In Rafina (point 4), pH fluctuated between 7.63 at the surface of the sediment and 6.10 at a depth of 5 cm.

Table 2: Concentration of dissolved nutrients (in µg N l⁻¹, µg P l⁻¹)

<table>
<thead>
<tr>
<th></th>
<th>Anavissos cove’s water</th>
<th>Anavissos outer water</th>
<th>Rafina</th>
</tr>
</thead>
<tbody>
<tr>
<td>[NO₃]⁻</td>
<td>Min 8.68</td>
<td>Max 31.8</td>
<td>Mean 17.8</td>
</tr>
<tr>
<td>[NO₂]⁻</td>
<td>Min 0.56</td>
<td>Max 1.82</td>
<td>Mean 0.84</td>
</tr>
<tr>
<td>[NH₄]⁺</td>
<td>Min 1.96</td>
<td>Max 24.5</td>
<td>Mean 10.6</td>
</tr>
<tr>
<td>[PO₄]³⁻</td>
<td>Min 0.93</td>
<td>Max 14.2</td>
<td>Mean 4.34</td>
</tr>
<tr>
<td>Org N</td>
<td>Min 91.6</td>
<td>Max 560</td>
<td>Mean 190</td>
</tr>
<tr>
<td>Org P</td>
<td>Min 1.24</td>
<td>Max 14.2</td>
<td>Mean 2.79</td>
</tr>
</tbody>
</table>
2.5 Nitrogen in sediment

The monthly fluctuation of the total nitrogen content in sampling point 1, as well as the seasonal fluctuation in points 2 and 4, are presented in Fig. 3 and Fig. 4 respectively. The inorganic forms of nitrogen represented only 0.6–1.7% of the total Nitrogen in the surface layer and less than 6% in the underlying ones. The Total nitrogen content in some marine and coastal areas all over the world is presented in Table 3. Unfortunately, information concerning the Mediterranean areas was scarce.

The highest content were measured in the surface layer of the sediments and were found to be 2–5 times greater than the ones prevailing in the coastal areas referred to in Table 3. The content of total nitrogen in layer b was lower than in the surface layer, but still higher than in coastal sediments. The values for layer c were near to the minimum reported levels of Table 3. The lowest values of total nitrogen in the surface layer for all sampling stations of Anavissos were recorded during winter (see Fig. 4a). A significant decrease was observed during January and February that was fol-

![Fig. 3: Monthly variation of total Nitrogen at point 1 (Anavissos)](image)

![Fig. 4: a) Seasonal variation of sediment total Nitrogen at point 2 (Anavissos); b) Seasonal variation of sediment total Nitrogen at point 4 (Rafina)](image)

Table 3: Concentration of total nitrogen in sediments of some coastal areas

<table>
<thead>
<tr>
<th>Region</th>
<th>tot N (mg/g)</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venice lagoon</td>
<td>1.25</td>
<td>Sfriso et al. (1995)</td>
</tr>
<tr>
<td>Mississippi estuaries, Gulf of Mexico</td>
<td>1.6</td>
<td>Ruttenberg and Goni (1997)</td>
</tr>
<tr>
<td>Kertinge Nor, Denmark (area 1)</td>
<td>0.3–1.7</td>
<td>Hansen and Kristensen (1997)</td>
</tr>
<tr>
<td>Kertinge Nor, Denmark (area 2)</td>
<td>0.7–1.3</td>
<td>Hansen and Kristensen (1997)</td>
</tr>
<tr>
<td>Swed Skaggarak coast (with microbial mat)</td>
<td>0.90–2.10</td>
<td>Pihl et al. (1999)</td>
</tr>
<tr>
<td>Swed Skaggarak coast (without microbial mat)</td>
<td>0.30–0.70</td>
<td>Pihl et al. (1999)</td>
</tr>
<tr>
<td>Southern Chilean inlets, Chile (area 1)</td>
<td>0.3–1.7</td>
<td>Silva and Prego (2002)</td>
</tr>
<tr>
<td>Southern Chilean inlets, Chile (area 2)</td>
<td>0.6–2.1</td>
<td>Silva and Prego (2002)</td>
</tr>
<tr>
<td>Southern Chilean inlets, Chile (area 3)</td>
<td>0.4–1.3</td>
<td>Silva and Prego (2002)</td>
</tr>
<tr>
<td>Acheloos river estuaries, Greece</td>
<td>0.29–0.59 (mean 0.38)</td>
<td>Ladakis et al. (2003)</td>
</tr>
<tr>
<td>Alonnissos island, Greece</td>
<td>0.39–0.55 (mean 0.47)</td>
<td>Ladakis et al. (2003)</td>
</tr>
<tr>
<td>Anavissos, Greece (surface layer)</td>
<td>1.31–4.89 (mean 3.08)</td>
<td>Present work</td>
</tr>
<tr>
<td>Anavissos, Greece (layer b)</td>
<td>0.42–1.67 (mean 1.03)</td>
<td>Present work</td>
</tr>
<tr>
<td>Anavissos, Greece (layer c)</td>
<td>0.11–0.97 (mean 0.46)</td>
<td>Present work</td>
</tr>
<tr>
<td>Rafina, Greece (surface layer)</td>
<td>0.84–1.63 (mean 1.23)</td>
<td>Present work</td>
</tr>
<tr>
<td>Rafina, Greece (layer b)</td>
<td>0.28–0.36 (mean 0.32)</td>
<td>Present work</td>
</tr>
<tr>
<td>Rafina, Greece (layer c)</td>
<td>0.15–0.30 (mean 0.24)</td>
<td>Present work</td>
</tr>
</tbody>
</table>
followed by a slight increase of nitrogen content in the underlying layers (see Fig. 3). During this period, the degradation rate exceeds the primary production, so it is possible that part of the produced NH$_4^+$ and NO$_3^-$ enriches the underneath layers via pore-water (Stal 2000). In autumn and spring, the content of total nitrogen was increased (see Fig. 4). This fluctuation agrees with the annual fluctuation of the primary production as there is a good correlation between the content of Chlorophyll-a and total N as well as TOC and total N in the surface sediment of all sampling points ($r$ coefficient ranged between 0.75 and 0.80 for Chlorophyll-a and between 0.87 and 0.93 for TOC; $t$-test for $P < 0.05$ was positive for all $r$-values counted). Two typical correlation plots for point 1 are presented in Fig. 5 and Fig. 6.

One main difference in surface layer’s seasonal fluctuation of the total nitrogen content was detected in point 4, as greatest values were recorded during winter and not during spring as was the case in Anavissos (points 1, 2 and 3). During this period, sea waves frequently have enough energy to drench the sampling point, so the cyanobacterial mat covered with seawater is able to feed on nutrients more efficiently while it is less exposed to the air. On the other hand, the winter temperature in the Mediterranean is not so low as to prevent the growth of cyanobacteria. No remarkable fluctuation of total nitrogen content was found for the underlying layers. Values in both b and c layers were similar and very low (see Fig. 4b).

The vertical distribution of total nitrogen in Rafina was similar to that in Anavissos (high content in the surface layer, which gradually decreases as one moves deeper into the sediment). Nevertheless, the surface layer content is 2–2.5 times lower from the prevailing one in Anavissos (see Fig. 4b and Table 3). This is due to the smaller population of cyanobacteria there, because of low nutrient supply, as this sampling point is enriched with nutrients only when it is reached by sea waves.

The points that remain submerged in seawater continuously or most of the day (points 1 and 2) have a higher fluctuation range of total nitrogen content than the ones (point 3 and 4) exposed to the air most of the day (3.4 versus 2.4 mg/g). The average annual content value, concerning those points, was also slightly higher (Fig. 7).

The growth of the cyanobacterial mat seems to be favoured in the presence of seawater as it is protected from the atmospheric oxygen that destroys the cyanobacterial nitrogenase in that way.

No correlation was detected between any form of dissolved nitrogen in the water column and total nitrogen in the sediment surface. It seems that the nitrogen in the water does not affect the content of the nitrogen in the sediment surface. Pihl et al. (1999), Reise and Siebert (1994) and Lowthion et al. (1985) also report this conclusion.

The content of total nitrogen in layer c was very low and has narrow temporal fluctuation (see Fig. 3 and 4). This fact in combination with the negative results in the culture attempts indicates that this layer is not directly affected by the biocommunities that colonize the sediment surface. A slight influence is possible only through pore-water.

### 2.7 Phosphorus in sediments

Phosphorus consists mostly of inorganic insoluble compounds. The contribution of organic phosphorus to the total in the studied areas ranges from 0% to 50% with an average value of 26.6%. Free orthophosphates represent 5% of the total in the surface layer and less than 2% in the underlying ones. The monthly fluctuation of the organic phosphorus content in sampling point 3, as well as the seasonal one in
points 1 and 2 (Anavissos), are presented in Fig. 8 and Fig. 9, respectively. The decrease of content in Anavissos during January–February that was observed in the case of nitrogen, was also observed in the case of phosphorus.

As observed in Fig. 8 and Fig. 9, the more covered by seawater the sediment is, the higher content of organic phosphorus is determined: Sampling point 1 is the most, while sampling point 3 is the least enriched in organic phosphorus in all cases. The highest content of total phosphorus was determined in the surface layer. This was 2–3 times higher than in the underlying layers. The background layer c had a higher content of organic phosphorus than the middle layer b. This anoxic layer seems to be depleted of both inorganic and organic phosphorus. The depletion of organic phosphorus in this layer is ascribed to the bacterial enzymatic degradation of organic compounds containing phosphorus. In the case of inorganic phosphorus, the production of sulfide is probably responsible for its depletion as it can react with ferric phosphate according to: \[2\text{FePO}_4 + 3\text{H}_2\text{S} \rightarrow 2\text{FeS} + 2\text{H}_3\text{PO}_4 + \text{S}_0\] (Ehrlich 1996). The produced phosphate ions can reach either the surface or the background layer via pore-water. As soon as phosphate ions reach the surface layer they are consumed by cyanobacteria. Phosphate ions reaching layer c can react with \(\text{Ca}^{2+}\) (originated from the dissolution of \(\text{CaCO}_3\) due to the low pH of this layer), thus yielding \(\text{Ca}_3(\text{PO}_4)_2\). According to the prevailing conditions in the sediment as well as the microbial activity, \(\text{Ca}_3(\text{PO}_4)_2\) can be redissolved, releasing phosphate ions to the pore-water (Ehrlich 1996).

The points that remain submerged in seawater continuously or most of the time had a negative correlation between the total P in the sediment and dissolved phosphate concentration (point 1, \(r = -0.72\); point 2, \(r = -0.74\); t-test for P <0.05 was positive for both r values). No correlation was found in point 3, which is mostly exposed to the air. This observation can be explained if we consider that the main source of phosphorus to the mat is the phosphate ions in the seawater and that the mobility of phosphorus increases when the sediment is covered with seawater. We suggest that the cyanobacterial mat acts as a natural pump that transfers phosphorus from the seawater to the sediment surface. Taking into account that this area is small, almost isolated with low inputs of dissolved phosphate, such negative correlation could be detectable (Fig. 10).

The annual fluctuation of total phosphorus content in sampling point 4 (Rafina) is presented in Fig. 11. The content of
total phosphorus in the surface layer was found to be double compared to the two underlying layers. Nevertheless, content was lower than the ones determined in Anavissos samples (Average annual means: Rafina 0.116 mg P/g; Anavissos 0.287 mg P/g).

The sampling point 4 is only occasionally covered with seawater, which is the main source of phosphorus, so that the accumulation of this element to the sediment is low and does not follow the typical seasonal fluctuation. As is the case with nitrogen, the lowest values of total phosphorus' content in this point were recorded during summer, when drenching by sea waves is less frequent.

In general, the values of total phosphorus in the investigated areas are lower than the prevailing ones in coastal areas affected by human activities (urban or industrial), lagoons or river discharges, since a considerable load of organic phosphorus is normally expected in such areas. On the other hand, the values are on the same level as the ones existing in Mediterranean coastal areas (Table 4).

### 2.8 Correlation between total nitrogen and total phosphorus

A fair correlation between total nitrogen and total phosphorus content was detected in the surface layer of the sediments in the Anavissos samples (t-test for P <0.05 was positive for all r values). No correlation could be detected in the case of Rafina as only four pairs of values were available to test. This correlation indicates the importance of nitrogen and phosphorus for the cyanobacterial growth. r values as well as N/P values are presented in Table 5.

### Conclusions and Outlook

In general, the development of the studied organosedimentary forms depends on the appropriate sediment characteristics, the abundance of cyanobacteria capable of binding small grains, the favourable hydrology, the adequate supply of nutrients, the low values of toxic pollutants and the environmental conditions that discourage the occurrence of grazing organisms. The relatively high water temperature and the sunlight abundance that characterize the area of the Mediterranean Sea are conditions that favour the cyanobacterial mat development in certain semi-solated coastal areas, notwithstanding the oligotrophic character of its water. The development of the cyanobacterial mat in Rafina shows, even if the conditions are not optimum (no frequent drenching by seawater, continuous exposure to air and sunlight), that they are still capable of creating and conserving a cyanobacterial mat. The main bio-chemical characteristics of these two sampling stations (Anavissos and Rafina) were found to be similar. The high content recorded for total nitrogen in the surface sediments indicate that cyanobacteria accumulate nitrogen by taking it from the sediment, the sea-water and the air. When the cyanobacteria die, part of the cells' nitrogen remains into the sediment, enriching it. Total nitrogen fluctuation agrees with the primary production one as it is well correlated with TOC and chlorophyl a, respectively. Nitrogen is usually the limiting factor for the filamentous cyanobacteria growth. A proportional accumulation in phosphorus also happens, although to a lesser degree compared with nitrogen. The cyanobacterial mats act as a natural pump that transfers phosphorus.
from the seawater to the surface sediment. The vertical distributions of nitrogen and phosphorus are similar concerning the decrease in layer (b), but the prevailing trends in layer (c) are different. Although nitrogen and phosphorus content in Mediterranean cyanobacterial mats are higher when compared with typical coastal sediments, they remain lower than those of estuarine or heavily polluted sediments.

In general, the chemical behaviour of those two basic elements in the formation and existence of cyanobacterial mats and organosedimentary forms, presents interesting similarities and differences that need to be studied further.

4 References


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