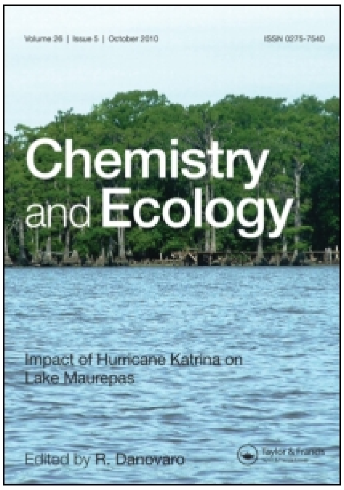

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Biochemical composition and early diagenesis of organic matter in coastal sediments of the NW Adriatic Sea influenced by riverine inputs

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Biochemical composition and early diagenesis of organic matter in coastal sediments of the NW Adriatic Sea influenced by riverine inputs

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River inputs influence trophodynamic and biogeochemical processes of adjacent continental shelves. In order to provide new insights on the influence of continental inputs on the benthic trophic state and early diagenesis of sediment organic matter we collected surface sediments in the NW Adriatic Sea at three stations located at increasing distance from the Po River. Sediment samples were collected in four periods characterized by different river outflows and analysed for chloropigment content (chlorophyll-a and phaeopygments), protein, carbohydrate and lipid concentrations, prokaryote abundance and aminopeptidase activity. Sediments of the NW Adriatic Sea displayed high organic loads, tightly coupled with the outflow dynamics of the Po River. A major flooding event was responsible of an enhanced accumulation of organic material on the sea bottom. The resulting increased nutrient load in the sediment impaired organic matter degradation processes. The results of the present study suggest that the enhanced trophic state of marine coastal sediments subjected to riverine inputs are related not only to the increased nutrient inputs, but that they may be amplified by impaired degradation processes.

Keywords: river inputs; sediment organic matter; trophic state; enzymatic degradation

1. Introduction

Eutrophication is one of the most widely spread phenomena associated with human utilization of coastal oceans [1,2]. Changes in delivery of river-borne nutrients (e.g. such as phosphate, nitrate and silicate), owing to land-use changes and anthropogenic emissions, have greatly contributed to the spreading of this phenomenon in coastal ecosystems worldwide [3,4]. The external nutrient loads entering marine coastal areas increase primary productivity and the export of organic matter to the sediment, with cascade effects on benthic trophodynamic and biogeochemical processes [5]. The magnitude of organic matter inputs to the sea floor, indeed, influences the oxygen concentration at the water-sediment interface as well as the depth of the oxic-anoxic interface within sediments [6]. When the supply rate of organic matter increases, benthic microbial metabolism is stimulated, resulting in increased oxygen consumption rates [7]. Such rates depend not only

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upon the organic C load reaching the sediment [8], but also upon the biochemical composition of the organic compounds [9]. To this regard, recent investigations pointed out that an enrichment in the biopolymeric fraction (as the sum of protein, carbohydrate and lipid) of sediment organic matter might shift the benthic system towards conditions of higher oxygen demand [10,11].

The North-Western Adriatic Sea, due to the runoff of the Po River (which contributes up to 50% of the entire freshwater inputs of the entire Adriatic basin) is one of the most productive regions of the Mediterranean Sea [12]. Adriatic coastal sediments are typically characterised by the accumulation of large amounts of organic matter [13–15] which, locally, might determine hypoxic conditions at the sediment-water interface [16–18].

We investigated sedimentation rates, quantity and biochemical composition of organic matter, extracellular aminopeptidase activities (as a proxy of early diagenesis of organic matter) and prokaryote abundance in coastal sediments of the North-Western Adriatic Sea in four periods (April and October 1999 and April and October 2000) at three stations at increasing distance from the Po river outflow.

The main aim of this study was assessing the spatial and temporal patterns of early diagenesis of organic matter along a putative gradient of trophic state (i.e. organic enrichment) as influenced by the Po river outflow.

2. Methods

2.1. *Study area and sampling*

Sediment sampling was carried out in three different areas of the NW Adriatic Sea using the R/V Daphne (Figure 1). The P. Garibaldi station (ca. 12 m depth) is close to the Po Delta, whereas the Ravenna (ca. 11 m depth) and the Cesenatico stations (ca. 10 m depth) are located at increasing distance from the Po Delta, at about 15 miles distance from each other. At each sampling site, temperature and salinity of surface water column were synoptically measured by a multiparametric CTD-probe (911 Seabird). During each cruise the surface water temperature did not display significant differences between stations and ranged from 10.54 °C (P. Garibaldi; April 1999) to 20.76 °C (Ravenna; October 2000). In both years, salinity decreased with increasing distance from the Po river and ranged from 31.96 (P. Garibaldi) to 37.26 (Cesenatico).

Undisturbed sediment samples were collected using a box-corer on April 1999, October 1999, April and October 2000. These dates were selected on the basis of the regime of the Po river outflow and the typical seasonality of the sampling area. During each cruise, 4 to 7 deployments were carried out at each site. From three independent deployments, 3 different cores were randomly collected from the box-corer. Upon recovery, the top first cm of each core was frozen at −20 °C and stored until analysis for organic matter determinations. Aliquots of the surface sediments were treated on board for the measurement of extracellular enzymatic activities as described below. The values of the monthly averaged Po river discharge rates were obtained from the Magistrato delle Acque del Po (Parma, Italy).

2.2. *Sedimentation rates*

Quantitative estimates of the sediment accumulation rates in the investigated sites were obtained from the same stations previously investigated [19]. Sediments were dated using the short-lived radionuclide ^{210}Pb. In order to determine ^{210}Pb activities of the northern stations, the measurement of its daughter nuclide ^{210}Po was accomplished by alpha spectrometry [20]. The sediment accumulation rate was obtained by the activity-depth profile of excess ^{210}Pb. Excess ^{210}Pb was

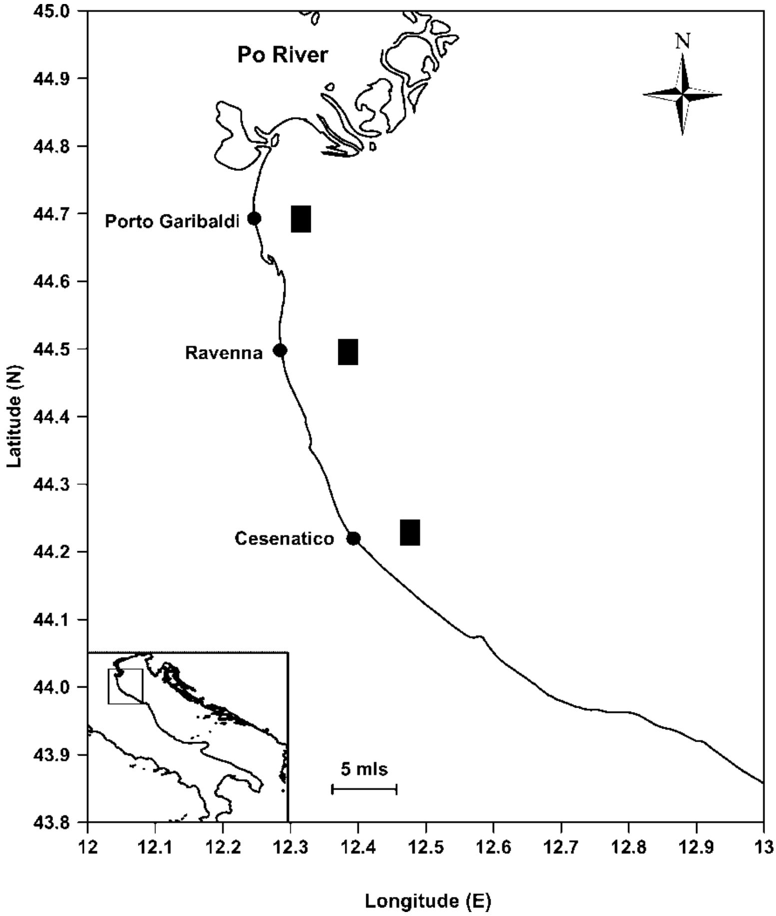

Figure 1. Sampling area and station locations.

calculated from the difference of total and supported ^{210}Pb. The value of supported ^{210}Pb activity was assumed constant along the core and estimated from values of the deepest sediment samples, where ^{210}Pb is in radioactive equilibrium with its parent, ^{214}Pb. In contrast, sediment levels of the southernmost station (Cesenatico) were prepared for ^{210}Pb counting via gamma spectrometry using a gamma-x type germanium detector [21], which allowed also the simultaneous determinations of the ^{214}Pb activities.

2.3. *Sediment biochemistry*

Analyses of chlorophyll-a and phaeopigments in the sediments were carried out fluorometrically after extraction with 90% acetone (24 h in the dark at 4 °C) [22]. After centrifugation (800 rpm, 10 min), the supernatant was used to determine functional chlorophyll-a, and acidified with 0.1 N HCl to estimate the amounts of phaeopigments [23].

Protein analyses were carried out according to Hartree [24], modified by Rice [25] to compensate for phenol interference. Concentrations are expressed as bovine serum albumin (BSA) equivalents. Carbohydrates were analysed spectrophotometrically and expressed as glucose equivalents [26]. Lipids were extracted by direct elution with chloroform and methanol and analysed spectrophotometrically [27]. Lipid concentrations are reported as tripalmitine equivalents. All analyses were carried out on three replicates. For each biochemical analysis, blanks were made using the same sediments previously treated in a muffle furnace (550 °C, 4 h). All data were normalised to sediment dry weight after desiccation (60 °C, 24 h).

Biopolymeric organic carbon (BPC) was defined as the sum of the carbon equivalents of total carbohydrates, proteins and lipids (utilising conversion factors of 0.4, 0.49 and 0.75, respectively) [28].

2.4. *Prokaryotic abundance and extracellular enzymatic activity*

For prokaryote analyses, sediment sub-samples were diluted 100 to 500 times, stained with Acridine Orange (0.01% final concentration) and filtered on black Nuclepore 0.2 μm filters [29]. Filters were analysed under epifluorescence microscopy using a Zeiss Axioplan microscope equipped with a 50-W lamp. Ten to 50 fields were viewed at ×1000 magnification and a minimum of 400 prokaryotic cells were counted. Prokaryotic counts were normalised to sediment dry weight after desiccation (60 °C, 24 h).

Extracellular enzymatic activity of aminopeptidase (L-Leucine-4-methylcoumarinyl-7-amide, Leu-MCA) was determined fluorometrically [30]. This activity was selected in respect to others (e.g. ß-glucosidase) because previous studies carried out along the NW Adriatic Sea demonstrated the dominance of proteins among the main biochemical classes of organic compounds [8]. Extracellular enzymatic activity measurements were carried out on sediment sub-samples ($n = 3$) diluted 1:1 with 0.2 μm filtered seawater by adding 100 μl of Leu-MCA (final concentration 400 μM). Sediment samples were incubated in the dark at *in situ* temperature for 1 h. Additional experiments with increasing fluorogenic substrate concentrations were carried out to assess saturation conditions. After incubation, samples were centrifuged and supernatants were analysed fluorometrically. Data were normalized to dry weight (60 °C, 24 h) and reported as nanomoles of fluorescent dye released per g of sediment per hour.

2.5. *Data analysis*

A Spearman-Rank correlation analysis was performed to test for possible relationships among all of the investigated variables. A two-way analysis of variance (ANOVA) was carried out to test for temporal and spatial differences among sampling periods (time) and areas (space). When significant differences were observed a post-hoc Tukey's test was also performed.

3. Results and Discussion

3.1. *Effects of the Po River outflow on the quantity and quality of sediment organic matter*

In the North-Western Adriatic Sea, the Po River is the main freshwater input and accounts for approximately more than 50% of the total riverine discharge into this shallow and semi-confined basin [12]. These inputs are among the major factors affecting the physico-chemical characteristics, biogeochemical cycles and the trophic state of the adjacent continental shelf [31]. The dispersal of the freshwaters from the Po river discharge follows the physical structure of the mixed water masses and the main cyclonic circulation of the basin.

The Po River discharge displays large interannual variability, while annually two discharge peaks of approximately 2000 $m^3 s^{-1}$ are typically observed. The first peak occurs in spring (May–June) after the snowmelt-derived waters entering the Po River from alpine regions, and the second peak occurs in October–November following the intense autumn rainfall.

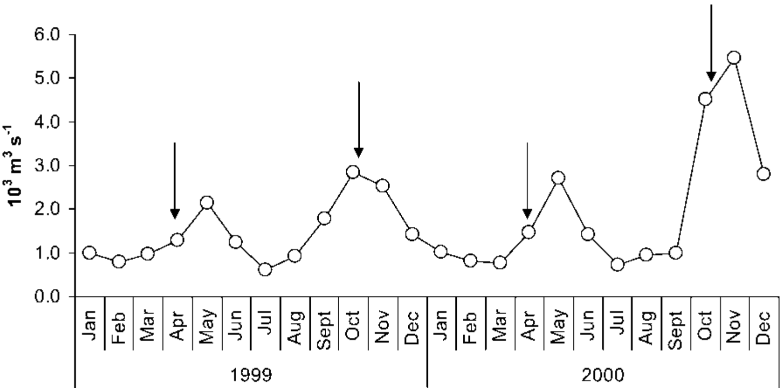

Figure 2. The Po River discharge rates (monthly averaged) in 1999 and 2000. Arrows indicate sampling periods.

During the present study, the Po River discharge widely fluctuated among the two years (Figure 2). In particular, the event occurred in October 2000 represented the largest and prolonged flood ever occurred during the last century [31].

The spreading southward of the Po River outflow and its wide temporal fluctuations were reflected by changes in the quantity and biochemical composition of sediment organic matter.

ANOVA and post-hoc Tukey's tests revealed that the concentrations of chlorophyll-a in the sediment (here used as a proxy of microphytobenthos biomass) [32] increased significantly with increasing distance from the Po River, with values in the Cesenatico station (average of the four sampling periods $1.6 \pm 0.7\,\mu g\,g^{-1}$) double than those in the P. Garibaldi one ($0.8 \pm 0.2\,\mu g\,g^{-1}$; Figure 3a–b). Previous studies reported similar spatial patterns of microphytobenthos biomass in the North-Western Adriatic Sea [16,17] and led us to hypothesise that freshwater inputs

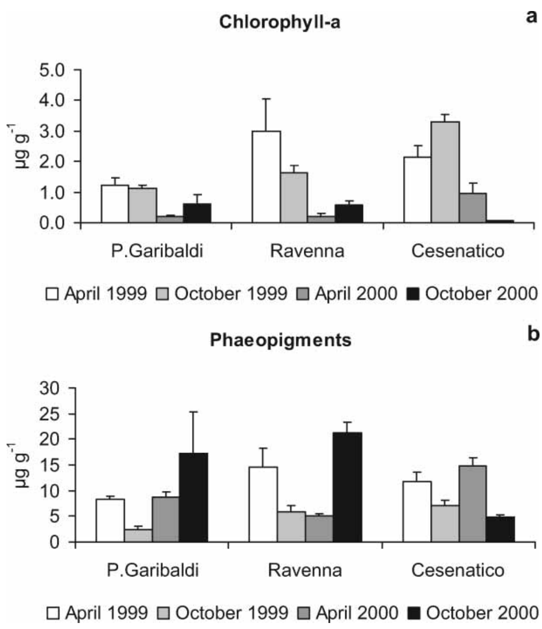

Figure 3. Chlorophyll-a (a) and phaeopigmet (b) concentrations in the N Adriatic Sea coastal sediments in April and October 1999 and April and October 2000. Error bars represent standard deviations of triplicate determinations.

coming from the Po River, carrying relevant amounts of suspended material, might reduce microphytobenthos productivity because of the shading of the surface layers of the water column.

The sediment contents of protein, carbohydrate, lipid and biopolymeric C were high when compared with other Mediterranean locations at the same depth (Figure 4) [32], but similar to data previously collected in the same region [13,29]. According to the threshold levels proposed by Dell'Anno et al. [32] and Pusceddu et al. [33], the sediments of the N Adriatic Sea, with biopolymeric C sediment contents ranging from 1.2 to 4.7 mg C g^{-1}, can be classified as from meso- to eutrophic.

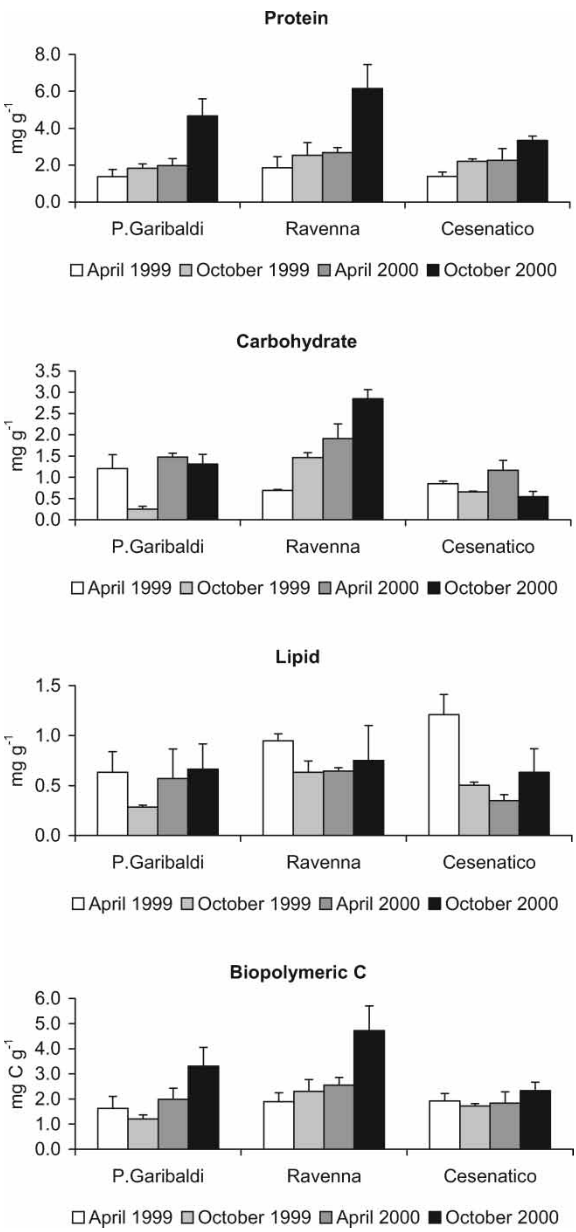

Figure 4. Protein, carbohydrate, lipid and biopolymeric C contents of the N Adriatic Sea coastal sediments in April and October 1999 and April and October 2000. Error bars represent standard deviations of triplicate determinations.

At all sampling periods, the levels of the three classes of biochemical compounds and of biopolymeric C increased significantly from the northernmost station (P. Garibaldi) to the intermediate one (Ravenna), then decreased in the southernmost station (Cesenatico; Figure 4). The Northernmost station, located just at the seaside border of the deltaic system of the Po River, is subject to a relevant hydrodynamic stress letting the material from the river to be continuously spread offshore to the south.

Excess ^{210}Pb activities of the three stations showed particularly disturbed depth profiles, far from the expected exponential shape, which implies that other processes (i.e. physical mixing or bioturbation) were active during sediment deposition and burial. Since $^{210}Pb_{ex}$ profiles were not suitable for sophisticated model calculations, a crude estimate of sediment accumulation was obtained by dividing the thickness of maximum penetration of $^{210}Pb_{ex}$ by 100 yr. This calculation led to mass accumulation rates in the Ravenna station (150 mg cm^{-2} y^{-1}) significantly higher than in the two other stations (120 mg cm^{-2} y^{-1} in both P. Garibaldi and Cesenatico stations).

The organic matter content in the sediments of the investigated area was thus in good agreement with the calculated mass accumulation rates, resulting in the Ravenna station higher than in the two other areas (P. Garibaldi and Cesenatico stations).

Concentrations of biopolymers in the sediment displayed also significant interannual changes, with the highest values observed concurrently with the October 2000 Po River flooding.

Overall, our results indicate that the quantity of organic loads in the sediment of the North-Western Adriatic Sea is tightly coupled with the outflow dynamics of the Po River. This result is consistent with previous investigations that demonstrated the seeding role of the Po River freshwater front occurring seasonally in this region and determining relevant shifts in the benthic trophic state [29].

These shifts were also evident from the analysis of the temporal patterns in the composition of the deposited organic matter. Although in all sampling periods proteins were the dominant biochemical compound (on average 57 ± 2%), followed by lipids (23 ± 3%) and carbohydrates (20 ± 3%), the different fractions displayed clear temporal changes. In particular, in October 1999 and October 2000 the sediments of all of the three stations experienced an increasing contribution of the protein fraction, and a decrease of the carbohydrate and lipid fractions. This resulted in the increase of the values of the protein to carbohydrate ratio (Figure 5). Values of this ratio >1.0 are a peculiar feature of meso-eutrophic sediments [33]. In this regard, the sediments of the North-Western Adriatic Sea were characterised by values of the ratio always above 1 (on average 2.9 ± 0.6) confirming that the sediments of this area are characterised by meso-eutrophic conditions. The contribution of the autotrophic biomass to the BPC pool was generally low (on average less than 10% of the biopolymeric C), indicating that a large fraction of the organic C pools in the sediments was detrital (i.e. non living) or of heterotrophic origin. However, the analysis of the temporal variability of such fraction revealed that, at all stations, the values in 1999

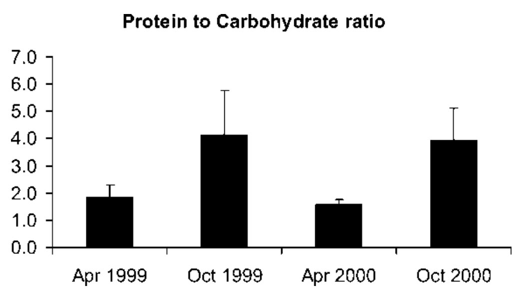

Figure 5. Values of the protein to carbohydrate ratio in the sediments of the N Adriatic Sea coastal sediments in April and October 1999 and April and October 2000. Error bars represent standard deviations of triplicate determinations.

were significantly higher than those in 2000. This result suggests that 2000 was characterised by a more relevant fraction of detrital material in the sediment, likely as a consequence of highly organic inputs entering the system through the Po river discharge.

3.2. *Effects of the Po River outflow on prokaryote abundance and organic matter degradation*

The sediments of the North-Western Adriatic Sea were characterised by values of prokaryote abundance typically encountered in organically enriched coastal sediments [10,29]. In both years, the highest prokaryote abundance occurred in October, when values were about double than in April (Figure 6a). Prokaryote abundance was significantly correlated with the concentrations of biopolymeric C in the sediment (Figure 6b). Although correlation analysis does not allow us to infer cause-effect relationships, this result suggests a tight coupling between the available resources and prokaryote distribution. Temperature may also contribute to the observed temporal patterns of prokaryote abundance, since in October water temperature was ca. 10 °C higher than in April.

The highest temperature in October coupled with higher prokaryotic abundance might also induce higher extracellular enzymatic activities, with consequent faster organic matter degradation rates. Such effect was observed in October 1999, but not in October 2000 (Figure 7). Therefore, temperature alone cannot explain the observed patterns of enzymatic activities.

Substrate availability stimulates extracellular enzymatic activities [34]. However, when concentrations of organic substrates exceed certain threshold levels, enzymatic activities may be inhibited [35]. This might impair the balance between degradation and utilization processes of organic compounds, thus promoting their accumulation in the sediment and letting the system shift towards increased oxygen demand rates.

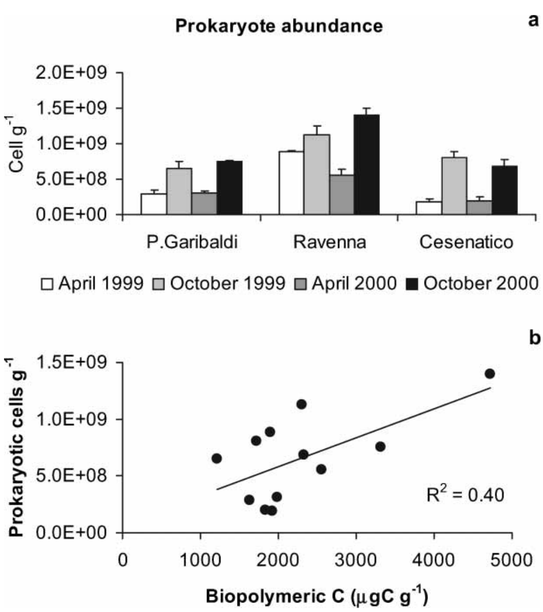

Figure 6. Prokaryote abundance in the N Adriatic Sea coastal sediments in April and October 1999 and April and October 2000 (a) and the relationship between prokaryote abundance and biopolymeric C (b). Error bars represent standard deviations of triplicate determinations.

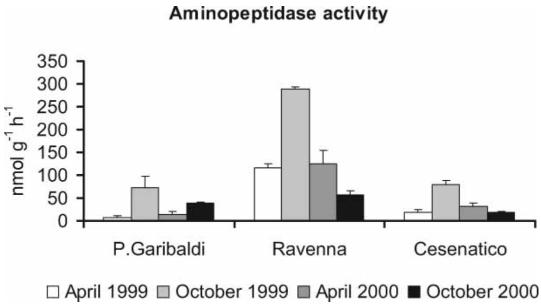

Figure 7. Values of the aminopeptidase activity in the N Adriatic Sea coastal sediments in April and October 1999 and April and October 2000. Error bars represent standard deviations of triplicate determinations.

Here we report that the lowest values of the aminopeptidase activity occurred in October 2000 were coupled with the highest protein content in the sediment. This applies also when the values of aminopeptidase activity are normalised to the total prokaryote abundance. The relationship between aminopeptidase activity per prokaryote cell and the values of the sediment protein concentrations is described by a hamp-shaped curve (Figure 8). This curve allows identifying the level of 3.0 mg g^{-1} of sediment protein concentration as the threshold level at which the degradation process mediated by prokaryotes is progressively suppressed.

The decrease of degradation rates coupled with increased organic matter loads to the sea bottom, as observed in the North-Western Adriatic Sea after the flood event in 2000, might have acted as a positive feedback mechanism enhancing accumulation processes and finally determining enhanced oxygen demand. In extreme conditions, such a mechanism may promote hypoxia or even anoxia at the sediment-water interface.

Large sectors of the world coastal oceans are increasingly experiencing the spreading of eutrophication processes which are traditionally seen as increased nutrient inputs of anthropogenic origin [1], and, more recently as increased organic matter content of sediments [33]. The results of this study suggest that changes in the trophic state of marine coastal sediments may be related not only to the increase of nutrient loads to the sea bottom, but that they may be amplified by changes in organic matter degradation processes.

In a proximate scenery of increased exploitation of the coastal oceans, the synergy of these factors might contribute to the profound impairment of the coastal ecosystems functioning.

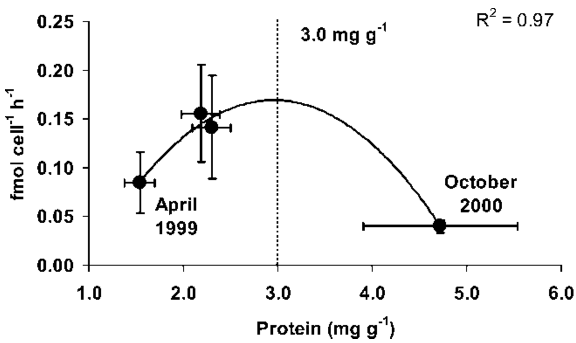

Figure 8. The relationship between aminopeptidase activity per prokaryote cell and protein concentrations in the N Adriatic Sea coastal sediments. Error bars represent standard deviations of triplicate determinations.

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