BIOGEOCHEMICAL CHARACTERISTICS OF SEDIMENTS UNDER THE CANOPY OF INVASIVE ALGA *Caulerpa racemosa* var. *cylindracea* (PELJEŠAC PENINSULA, ADRIATIC SEA)

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ABSTRACT

Sediments under the invasive alga *Caulerpa racemosa* var. *cylindracea* were investigated in the southern Adriatic Sea (Priježba Cove, Pelješac Peninsula, Croatia). To identify the potential impact of C*aulerpa racemosa* var. *cylindracea* on granulometric composition, organic matter, organic carbon, total nitrogen, phosphorus content and redox potential, we investigated sites where the alga has developed the densest canopy in relation to sites unaffected with the alga. Vertical distribution of sediment particles along the cores showed highest variability in surface layers at invaded sites related to control that indicates changes in environmental conditions during the sedimentation. Prevailing coarse grains in surface sediments at invaded sites can be attributed to alga's capacity to trap sediment particles as well as to coastal weathering and transporting process. Organic carbon (C-ORG), total nitrogen (N-TOT) and total phosphorus content (TP) was also elevated in the surface sediment at invaded sites. C-ORG/N-TOT and C-ORG/TP ratios as well as negative redox-potential indicated the origin of the organic matter from marine seagrasses captured by the canopies and its degradation under the anoxic conditions. Statistical analyses emphasized the differences between invaded and control sites in Priježba Cove, and indicated gravel and sand contents, total phosphorus and redox-potential as the key parameters contributing to the presented differences.

KEYWORDS: sediment, grain size, carbon, nitrogen, phosphorus, redox-potential, *Caulerpa racemosa* var. *cylindracea*

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1 INTRODUCTION

The green alga *Caulerpa racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman & Boudouresque (here after *C. racemosa*), as an invasive species [1], was recorded for the first time in the Adriatic Sea in Croatia in 2000 [2, 3]. The competitive success of this species, shortly after its appearance, was assigned to the ability of adaptation to different depths and environmental conditions in the water column as well as to different types of substrate such as tide pools, on pebbles, rocks, dead *Posidonia oceanica* (here after *P. oceanica*) "matte", sand and mud [4]. *C. racemosa* was found in polluted waters as well as inside relatively pristine areas [5, 6]. *C. racemosa* can establish highly abundant colonies in most of the habitats modifying the composition of the benthic communities that result in decrease of total macrophyte cover [7-9] or changing the macroalgal diversity and vegetational characteristics of the epiphytic macroalgal assemblage on *P. oceanica* rhizomes [10]. This invasive alga may form compact multilayered mats up to 15 cm thick that trap sediment material thus possibly contributing to the siltation of the assemblages [2, 11, 12]. The main mechanism of its invasion is overgrowing by dense canopy that result in large particle retention capacity per structure surface area [13].

There are numerous papers that comprise *C. racemosa* taxonomy, morphology, seasonal dynamics, reproduction cycle, ecological impacts of this invasive species on the benthic communities [3, 4, 14, 15]. However, still limited information is available concerning the characteristics of sediments below the *C. racemosa* canopy [12, 16, 17] or the effects of particle trapping and hydrodynamics near the seabed [13].

In this paper we investigated the variability of biogeochemical parameters in the sediments (granulometric composition, organic matter, organic carbon, nitrogen, phosphorus content and redox potential) at the sites where *C. racemosa* has developed the densest canopy in relation to unaffected sites. In order to determinate the potential impact of *C. racemosa* on "natural" ratios between carbon, nitrogen and phosphorus as well as to the origin of organic matter, vertical distribution of all parameters in sediment cores at invaded and non-invaded sites were also studied.

2 MATERIALS AND METHODS

2.1 Sampling area

Field research has taken place in October 2004 at Priježba Cove (Pelješac Peninsula, Adriatic Sea) by RV "Bios" (Fig. 1). The Bay (average depth; 32 m) is exposed to strong hydrodynamics and is not affected by human activities. The sampling date was chosen to coincide with the annual maximum development of *C. racemosa* to assess its highest impact.

Well developed meadow of the seagrass *P. oceanica* was formed at the seabed of the Bay from 5 to 30 m depth. Inside the meadow a few sandy and rocky areas (at 10-20 m depth) were present. *C. racemosa* was observed approximately 1000 m distanced away from the coastline in the investigated area at water column depths of 0-50 m. On the edge of *P. oceanica* meadow *C. racemosa* has formed dense canopy, penetrating inside the meadow to around 1 m. Inside the continuous *P. oceanica* meadow, *C. racemosa* was not frequent. However, on the sandy bottom surrounded by *P. oceanica* meadow, *C. racemosa* has developed a dense patchy colony and completely covered the seagrass rhizomes at the centre of the colony.

Two sites of *P. oceanica* covered with the densest canopy of the *C. racemosa* were chosen: the dead mats of *P. oceanica* (A) and the sandy bottom surrounded by seagrass *P. oceanica* meadow (B). Each "infected" site was compared with a control site not affected by *C. racemosa* distanced away 10–20 m (A-REF, B-REF). Another control bare sandy site with no algae at all (REF), approximately 500 m distanced away, was sampled as well. The depth of the investigated sites was as follows: site A and A-REF 14 m; site B and B-REF 13 m, and REF site 7 m, while temperature and salinity of seawater ranged from 19.5 to 19.9°C and 38.4 to 38.6, respectively.

2.2 Sampling methods and laboratory analyses

Sediment samples for granulometric and chemical analysis were collected by SCUBA-divers using transparent plastic tubes (approximately 20 cm long and 8 cm in diameter) which were vertically inserted into the sediment to preserve an undisturbed core. The sediment cores in duplicates for the determination of redox-potential were capped with rubber caps and immediately emerged on board. Redox-potential (E_H) in sediment cores was measured „in situ" by vertical penetration of Pt electrode with Ag/AgCl reference electrode, with quinhidrone buffer solutions in pH=4 and pH=7 prepared according to Metrohm. For determination of granulometric composition, organic matter (OM), carbonates (CA), organic carbon (C-ORG), total nitrogen (N-TOT), as well as total and inorganic phosphorus contents (TP, IP), each sampled core was divided into slices-subsamples (1 cm thick), frozen (at -40°C) and freeze dried (Christo Alpha 1-5) until the laboratory analysis. The granulometric composition of gravel (>2 mm) and sand (0.063-2 mm) particles was determined by sieving. Obtained values were used for generating of cumulative granulometric curves. Silt (0.004-0.063 mm) and clay (<0.004 mm) particles content were determined from the curves. Granulometric parameters: mean size (Mz), sorting (So), skewness (Sk) and kurtosis (Kg) were calculated according to Folk and Ward (1957) [18]. Sediment type was determined according to Folk's classification [19]. Gravimetrical methods were used to determine OM and CA contents [20, 21].

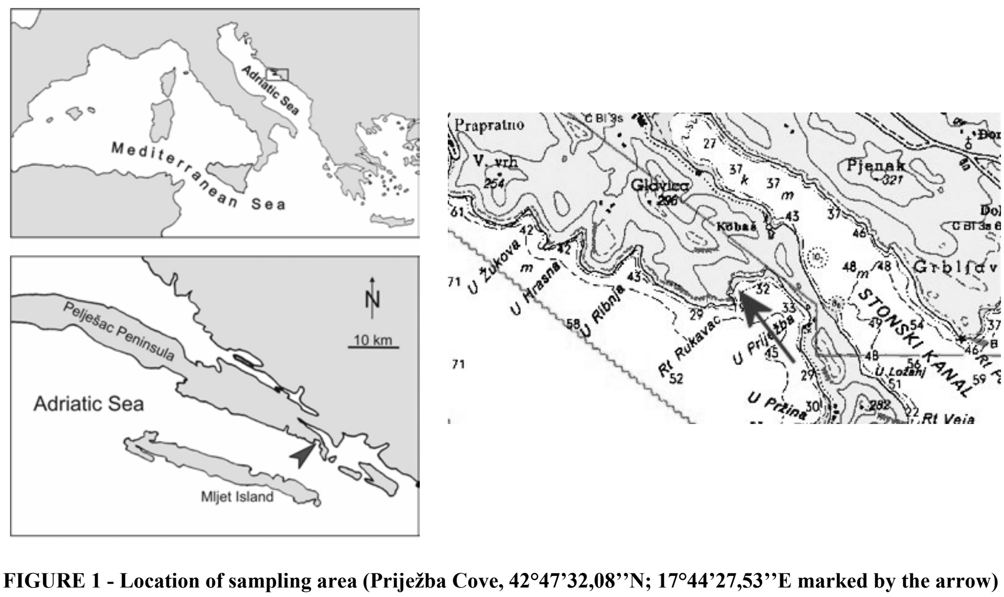

FIGURE 1 - Location of sampling area (Priježba Cove, 42°47'32,08''N; 17°44'27,53''E marked by the arrow)

Organic carbon and total nitrogen content were determined using CHNS-O analyzer (EA 1110, CE instruments). Before the analyses, freeze-dried sediment samples were grounded and prepared according to Ujiié et al. (2001) [22] by acidification of the sediments with HCl to remove carbonates.

The contents of TP and IP in sediments were estimated according to Aspila et al. (1976) [23], while certified reference sediments PACS-2 (Canadian Institute for National Measurement Standards NRC-CNRC), and BCR-684 (European Commission Community Bureau) were used for method evaluation.

2.3 Statistical analysis

Non-parametric Spearman rank order correlations were used to assess the relationships between granulometric, chemical and physical parameters. The significance of differences in those parameters was tested via non-parametric Kruskal-Wallis test (K-W), followed by the post hoc Dunn's Multiple Comparison test to compare individual pairs of sites. The analysis were performed using the statistical package StatSoft Inc. (2000) STATISTICA (http//www.statsoft.com).

Analysis of similarities (ANOSIM), hierarchical cluster analysis (CLA) and multi dimensional scaling (MDS) ordination were used to illustrate relationships between the variables at different sites in the layer of 0-3 cm depth. To reveal similarities between sites, the Euclidian distance was computed on standardised and log (x+1) transformed data matrix for the following parameters: So, gravel, sand, silt, clay, OM, N-TOT, C-ORG, IP, TP and E_H. The log transformation was used to meet the assumption of normality and down-weight the extreme observations. To show the contribution of granulometric and chemical variables to the dissimilarities among the sampling sites, similarity percentages analysis (SIMPER) was performed on the standardised and log-transformed data matrix, with the cut-off for the low contribution fixed at 90%. This analysis identified parameters that were principally responsible for the sample grouping in the ordination analysis. The above mentioned analyses were performed using the statistical package Primer 5 (version 5.2.9) [24].

3 RESULTS AND DISCUSSION

The coarse fractions had the highest percentages in sediments of all sites, in relation to negligible portions of fine sized grains (Table 1). The vertical distribution of all granulometric fractions (gravel, sand, silt and clay) in sediment cores at the investigated sites are in detail presented in Fig. 2.

Gravel content ranged from 5 to 90% at A-REF and B sites, respectively. Average values were from 11 to 22% for all sites except for site A with an average gravel content of 49%. Wider ranges at sites A and B, in comparison to other sites, could be explained by the highest gravel content in the surface sediment layer at site B (0-1 cm), and in deeper sediment at site A (9-10 cm depth) (Fig. 2, Table 1). Kruskal-Wallis test (K-W test) of differences in vertical distribution of gravel was significant between site A and A-REF; A and B sites ($p<0.001$), as well as between A and B-REF but at lower significant level ($p<0.05$).

Sand was the dominant particle size with average values from 50 to 88% for all sites, with the lowest average at site A (Table 1). Wider ranges at A and B sites are consequence of vertical distribution of sand in sediment cores at site A that is inversely to gravel content (higher sand content in surface layer) and at site B where higher portions of sand were in deeper sediment layers (Fig. 2, Table 1). However, those differences were not significant (K-W test $p>0.05$), due to high vertical variability within the station. The only statistically significant difference was found between sites A and A-REF (K-W test $p<0.001$). Similar ranges and averages of sand content at referential sites were result of relatively equal distribution of sand particles along the cores (K-W tests; $p>0.05$).

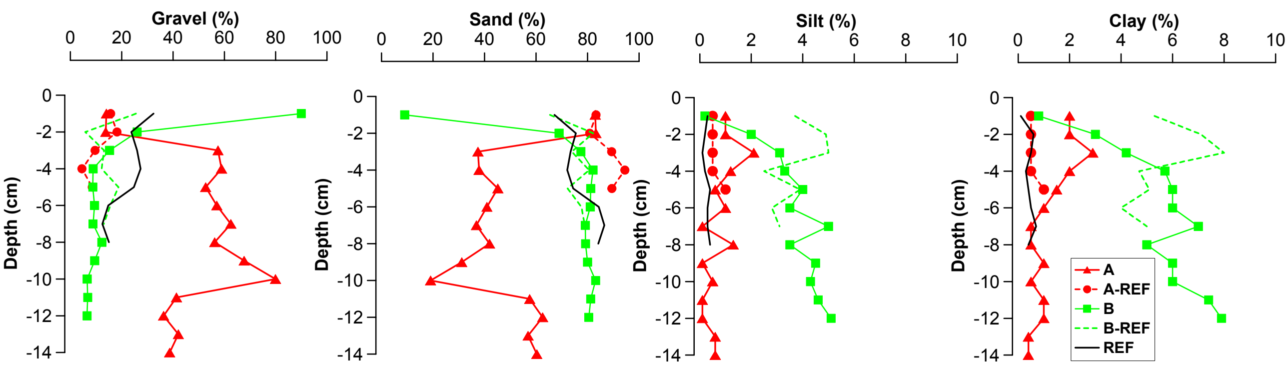

FIGURE 2 - Vertical distribution of granulometric fractions (gravel, sand, silt and clay) in the sediment cores at five investigated stations in Priježba Cove

TABLE 1 - Investigated parameters presented as average value ± standard deviation and ranges in the sediments at five investigated stations in Priježba Cove (granulometric composition, CA, OM, C-ORG, N-TOT, TP, E_H, the ratios of C-ORG/TP and C-ORG/N-TOT determined in sediments)

Station / Parameter	A	A-REF	B	B-REF	REF
Gravel (%)	48.5±18.9 13.8-80.0	11.3±5.5 4.5-18.2	17.4 ±23.5 6.5-90.0	14.9 ±6.1 5.8-25.6	22.1±7.1 12.4-32.4
Sand (%)	49.6±18.6 19.0-83.2	87.5± 5.4 80.8-94.5	73.6 ±20.7 9.0-83.2	75.8± 5.8 65.4-82.2	77.2±7.0 67.2-86.6
Silt (%)	0.74± 0.58 0.1-2.1	0.6± 0.2 0.5-1.0	3.6 ±1.4 0.2-5.1	3.7± 0.9 2.5-5.0	0.3 ±0.1 0.1-0.4
Clay (%)	1.2±0.8 0.4-2.9	0.6±0.2. 0.5-1.0	5.4±2.0 0.8-7.9	5.6±1.4 4.0-8.0	0.4±0.2 0.1-0.7
Mean size (μm)	2362±1278 870-5098	822±186 660-1097	1682±3710 467-13454	451±123 274-675	1081±222 793-1464
Sorting	1.8±0.3 1.3-2.6	1.1±0.1 1.0-1.3	1.7±0.2 1.5-1.9	1.8±0.3 1.4-2.2	1.5±0.3 1.1-1.8
Skewness	-0.05±0.15 -0.32-0.2	0±0.04 -0.06-0.05	0.2±0.24 -0.01-0.86	-0.32±0.1 -0.45-(-0.16)	-0.2±0.1 -0.32-(-0.07)
Kurtosis	1.4±0.4 1-2.6	1.0±0.1 1-1.1	0.9±0.3 0.6-1.5	1.2±0.5 0.9-2.3	1.3±0.2 1-1.4
Carbonates (%)	89±6 81-96	89±2 85-91	88±5 76-96	77±5 71-82	88±13 61-98
Organic matter (%)	4.0±2.5 1.2-9.1	2.0±0.1 1.8-2.1	3.4±1.0 2.7-6.6	13.6±10.5 5.0-32.8	1.6±0.32 1.3-2.3
C-ORG (%)	4.21±1.65 1.36-6.38	0.79±0.35 0.45-1.38	1.26±1.19 0.47-4.87	2.93±1.48 1.74-5.35	0.88±0.18 0.63-1.18
N-TOT (%)	0.49±0.17 0.24-0.77	0.21±0.02 0.18-0.23	0.12±0.10 0.05-0.42	0.23±0.13 0.11-0.48	0.13±0.03 0.08-0.17
TP (%)	0.038±0.005 0.005-0.044	0.023±0.001 0.022-0.024	0.045±0.026 0.031-0.097	0.033±0.007 0.027-0.040	0.022±0.001 0.020-0.022
Eh (mV)	-222±115 -276-85	15±80 -130-93	-72±73 -115-85	40±44 40-83	130±44 73-180
C-ORG/N-TOT	9.5±1.9 5.7-11.9	4.4±1.5 2.9-6.9	12.1±1.9 7.4-16.7	16.5±5.8 7.3-22.5	8.4±1.7 6.1-10.5
C-ORG/TP	355±89 274-449	107±41 81-155	116±22 90-130	327 ±121 217-457	94±11 81-102

Fine grained particles content (silt and clay) ranged from 0.1 to 5.1% for silt and 0.1 to 8.0% for clay (Fig. 2) with highest content in sediments at B and B-REF sites, respectively. The highest difference in vertical distribution among sites was found between B and REF site as well as between B-REF and REF (K-W test $p<0.001$).

Vertical distribution of sediment particles along the cores of investigated sites showed highest variability in surface layers at sites A and B invaded with *C. racemosa* related to the reference sites. This indicates significant changes in environmental conditions during the sedimentation especially between site A and other sites. The main difference was in prevailing of gravel particles that can be explained as a consequence of *C. racemosa*'s ability to trap sediment particles during its high vegetative growth. Previous studies of *C. racemosa* also show higher coarse grained particle content in sediments trapped inside the canopy [2, 12, 16]. However, the granulometric composition of sediments below the canopy is not reported at all. Detailed examinations of particle trapping rates among the all *Caulerpa* species show for *C. racemosa* the largest particle retention capacity per surface area structure [13].

The content of gravel particles in sediments at investigated Priježba Cove area is mostly result of coastal weathering and its transportation process in the deeper area. This could be an explanation for the high level of gravel content in sediment surface layer at site B. On the other hand at site A, *C. racemosa* was surrounded by *P. oceanica* meadow that reduced input of those particles in the sediment.

According to Folk's classification [19], prevalent sediment type at investigated sites was gravelly sand (in 20 subsamples), followed with sandy gravel (12 subsamples) and gravelly muddy sand (11 subsamples), while slightly gravelly sand and gravel were determined only once.

Mean grain size (Mz) ranged from medium sand (270 μm at B-REF station) to pebble (13454 μm in 0-1 cm interval at B station) with average value of very coarse sand (1503.8±2084.2 μm) (Table 1).

Statistically significant correlation was established between Mz, gravel and sand content (Table 2), that is due to prevailing coarse grained particles in sediments at investigated sites.

TABLE 2 - Spearman rank order correlation between granulometric parameters (mean size, Mz; sorting, So; skewness, Sk; kurtosis, Kg), granulometric composition (gravel, sand, silt and clay), carbonate (CA), organic matter (OM), total nitrogen (N-TOT), organic carbon (C-ORG) and total phosphorus (TP) content in sediment samples (N=46) (Correlations printed in bold are significant at p<0.05)

	Mz	So	Sk	Kg	Gravel	Sand	Silt	Clay	OM	CA	N-TOT	C-ORG
So	0.29											
Sk	0.56	-0.02										
Kg	-0.29	0.50	-0.39									
Gravel	0.87	0.48	0.39	0.00								
Sand	-0.85	-0.56	-0.34	-0.02	-0.98							
Silt	-0.31	0.29	-0.31	0.16	-0.33	0.13						
Clay	-0.27	0.29	-0.27	0.10	-0.31	0.12	0.99					
OM	-0.06	0.45	-0.36	0.57	-0.02	-0.13	0.72	0.70				
CA	0.11	-0.17	0.40	-0.31	0.17	-0.02	-0.76	-0.72	-0.87			
N-TOT	0.22	0.01	0.24	0.20	0.36	-0.37	-0.05	-0.03	0.28	-0.15		
C-ORG	0.28	0.25	0.14	0.15	0.37	-0.46	0.31	0.35	0.54	-0.42	0.89	
TP	0.91	0.43	0.66	-0.16	0.82	-0.86	-0.03	0.01	0.15	-0.05	0.41	0.53

Sediment was mainly poorly sorted (sorting range: 1.0-2.6), with exceptions in subsamples at site A (3-4 cm and 8-9 cm) and at B-REF (0-1 cm) where sediment sorting (So) was very poorly (>2) (K-W test: between A and A-REF p<0.001; B and A-REF, B-REF and A-REF p<0.01). On the other hand, the highest values of So were in sediments with prevailing sand particles that was in accordance to significant correlation between So and sand content in sediments (r=-0.56; Table 2). Low So values indicate different origin (terrigenous and biogenic) of settled particles in investigated sediments of Pelješac Peninsula [25]. Recent investigations of the sediments below the seagrass meadows on the west coast of Sardinia also indicated to poor sorted sediment [26]. Curve skewness (Sk) in investigated sediments ranged from -0.45 to 0.86 at B-REF and B station, respectively (Table 1). Negative values indicated the existence of fine grains in investigated sediment sample. Kurtosis (Kg) was in the range from very platykurtic (0.58 at site B) to very leptokurtic (2.56 at site A) with dominating leptokurtic and mesokurtic curves.

Carbonate content (CA) in sediments varied between 61 and 98% at A and REF stations, respectively, without clear vertical pattern (Fig. 3, Table 1). Relatively low CA ranges are obvious for all sites except for REF, where the widest range was result of highest CA variability along the sediment core (Fig. 3). The lowest CA average was at B-REF site and statistically significant differences were found among B-REF site and other sites (KW p<0.05). CA content >60% were also determined in sediments at shallower coastal stations along the east Adriatic coast [21, 27]. Carbonate content in sediments at north-west part of the Prižeba Cove reported here, were due to geological origin of sediment that was partly derived from weathering of Cretaceous and Eocene carbonate rocks [25], and partly from remains of skeletal parts or fragments of organisms mostly abundant in a coarse-grained fraction. Negative correlations between CA and fine grained particles were obtained, while the absence of correlation between CA and coarse sized grains was found (Table 1). That indicates the presence of noncarbonated particles in coarser granulometric fraction and is in accordance with previously reported data for the middle Adriatic [27]. According to investigations of sediment collected inside the *P. oceanica* meadows in different Mediterranean sites biogenic carbonates were associated with sand fraction [28].

The organic matter content (OM) ranged from 1.2 to 32.8% at A and B-REF sites, respectively (Fig. 3, Table 1). Lower averages at A, A-REF, B and REF sites (1.6 - 4.0%)

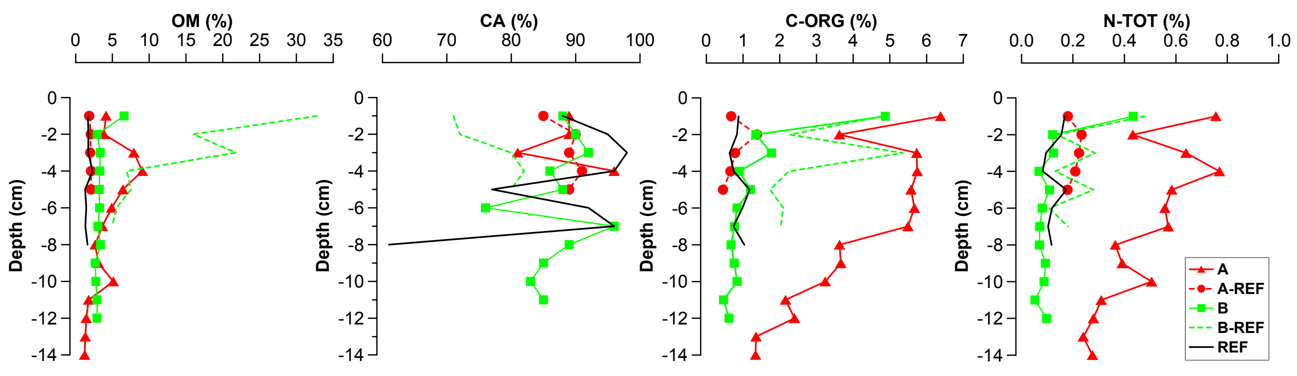

FIGURE 3 - Vertical distribution of carbonate (CA), organic matter (OM), organic carbon (C-ORG) and total nitrogen (N-TOT) content in sediments at five investigated sites in Prižeba Cove

are in the range of OM reported for the middle Adriatic sediments [27]. Highest OM average (13.6±10.5%) and maximum at B-REF site were due to seagrass remains observed in surface sediment layers (≤4 cm). This finding is consistent with results of Como et al. (2007) [29], who also explain sediment enrichment with organic matter and organic carbon by persistence of seagrass leaf litter. Significant correlation was obtained between OM and fine-grained particles such as silt and clay (Table 2). Similar result is reported for fine-grained sediments at the Middle Adriatic [27].

Organic carbon content (C-ORG) in sediments ranged between 0.45 and 6.38% at sites A-REF and A, respectively (Figure 3, Table 1). Elevated C-ORG content at invaded sites and site B-REF was particularly identified in surface sediment layer (≤4 cm), similar to OM (Fig. 3). Significant differences in vertical distribution were found at site A in reference to sites A-REF, B and REF (K-W: $p<0.01$). C-ORG values in sediments at those three stations are in accordance with data reported for the middle Adriatic off-shore sediments (0.3-1.8%) [27, 30, 31]. Wide range of C-ORG, as we found at sites A, B and B-REF, was also determined in the middle and north Adriatic sediments (0.5-10.1%) but in the anthropogenic influenced coastal areas where increased input of organic matter to water column was detected [32]. According to Holmer et al. (2009) [17] similar organic enrichment was found in Mallorca, Spain where C-ORG content in sediments with *Caulerpa* species was up to 11 times higher than at sites unaffacted of the algae.

Total nitrogen content (N-TOT) varied from 0.05 to 0.77% (at invaded sites A and B) with highest average value and variability at same site as for C-ORG content (site A). N-TOT values at five investigated sites in Prიježba Cove reported in this paper were higher than values found in the middle Adriatic sediments (0.02-0.15%) but were more similar to values in the sediments of eutrophicated coastal area [32]. Increased values of C-ORG and N-TOT contents at sites invaded by *C. racemosa* (A, B) and station B-REF could be assigned to high quantity of *Posidonia oceanica* leaves found in the sample.

Total phosphorus content (TP) in sediments ranged between 0.005 and 0.097%, while higher average values were found at A and B related to other sites (Fig. 4, Table 1). Differences in vertical distribution were obtained between site B vs. A-REF and REF sites (K-W test: $p<0.05$). Determined TP contents are generally lower than the average phosphorus content of 0.041±0.013 found in the middle Adriatic sediments [33]. However, our findings are in accordance with TP ranges obtained in sediments beneath the meadows of green macroalgae *Ulva* and *Enteromorpha* in Spain [34]. The highest TP content found in surface layer at site B is within the range previously determined in the Adriatic marine environments of higher trophic status, as well as in the fine-sized sediments of the open sea [33].

Inorganic and organic phosphorus (IP and OP) content in investigated samples was also determined and their concentrations in surface sediment layer (0-1 cm), as well as their portions in total phosphorus are shown in Figure 4. Calculated IP portion in TP in sediments, ranged from 54 to 99% at site B and control sandy REF site, respectively. According to previous investigations in the middle Adriatic Sea area the similar range of IP portion is estimated in sandy sediments [33]. Higher OP portion at sites affected with *Caulerpa racemosa* and at *Posidonia oceanica* meadows can be result of the organic material trapped inside the canopy or from seagrass leaves. The content of TP in *P. oceanica* leaves was estimated at 0.06-0.19% [35], or in some green algae leaves at up to 10 times higher values than was recorded in the sediment [34]. High TP content in the surface layer, at sites A and B-REF and especially at site B in Prijεžba Cove, was also in accordance with elevated C-ORG, N-TOT and OM contents in the same samples (Fig. 3 and 4, Table 1). Additionally, statistical analysis showed significant positive correlation between TP and gravel content ($r=0.82$) that is in discrepancy with previously reported correlations among TP and fine-sized particles in middle Adriatic sediments [33]. The

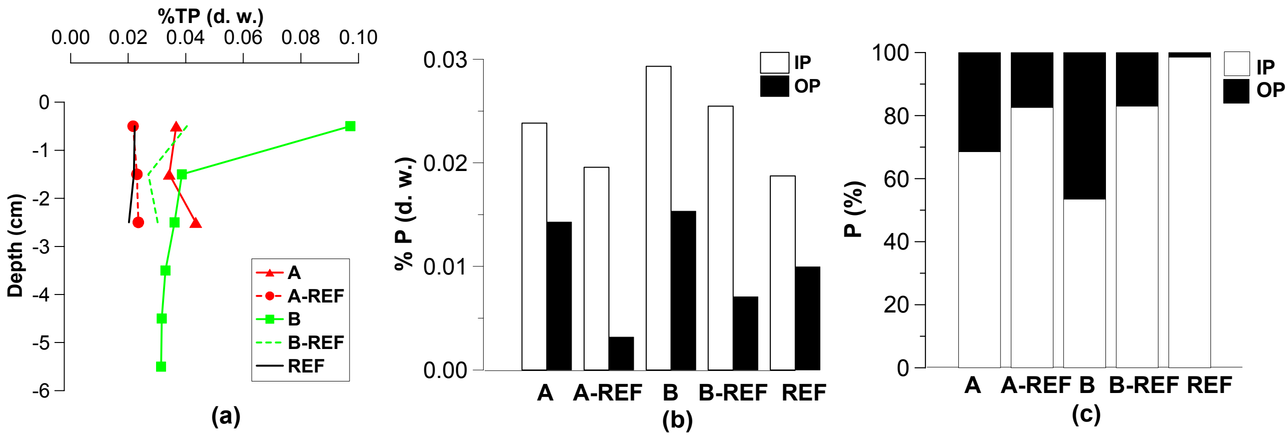

FIGURE 4 - Vertical distribution of total phosphorus content (TP) in the sediment cores (a), concentrations of inorganic and organic phosphorus (IP and OP) in the surface sediment layer of (0-1 cm) (b), and their portions in TP at five investigated sites in Prježba Cove (c)

lack of correlations between TP and fine-sized particles during our investigation could be caused by the coarser material captured in the canopy of *C. racemosa* that dominated in the surface sediment layer at invaded sites.

Redox potential (E_H) in the investigated sediments ranged between -276 and 180 mV with more negative values at infected sites A and B (Fig. 5, Table 1). Minimum E_H in these sediments is among the lowest E_H recorded for the middle Adriatic Sea area, including the sediments under the fish farm cages [32]. Extremely negative potential indicates high concentrations of sulphide ions (S^{2-}) as well as degradation of organic matter in the absence of oxygen [36]. As reported by Holmer et al. (2009) [17], the presence of *Caulerpa* species in *P. oceanica* meadows also coincided with enhanced sulfate reduction rates and increased pools of sulfides in the sediments. The main distinction in E_H vertical profiles among the investigated sites in Prijezba Cove was depth of redox-cline. At sites invaded with *Caulerpa racemosa* (site A and B), redox-cline was established in the surface sediment layer (0.5-1 cm), while at other investigated sites positive E_H was up to 3 cm sediment depth or along the whole sediment core (Fig 5). Statistical analysis showed significant difference in vertical distribution only between site A vs. B-REF and REF site (K-W: $p<0.05$, $p<0.01$, respectively).

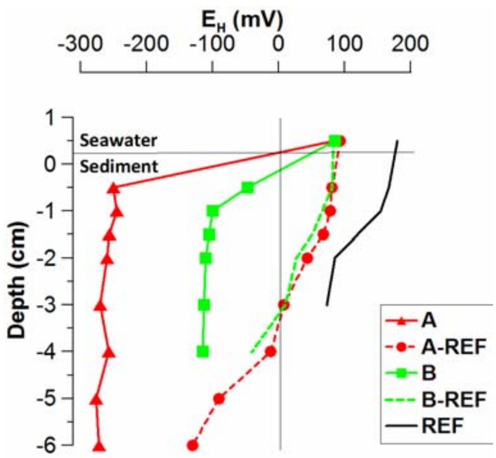

FIGURE 5 - Vertical profiles of redox potential in sediments at five investigated sites in Priježba Cove

Molar ratios of organic carbon, total phosphorus and total nitrogen (C-ORG/TP and C-ORG/N-TOT) were also calculated in order to investigate potential impact of the *Caulerpa racemosa* on the origin of organic matter in the sediments. The atomic ratios of C-ORG/N-TOT in sediments at all investigated stations ranged from 2.9 to 22.5 at A-REF and B sites, respectively (Table 1).

Average ratio was higher at invaded sites A and B and at B-REF, which was similar to C-ORG content distribution (Table 1). Vertical variability of this parameter at invaded site A and referent stations A-REF and REF is shown in Figure 6. According to Stein (1991) [37], Vilares and Carballeira (2003) [34], the value of C-ORG/N-TOT ratio >10 indicates to the origin of organic matter from the marine seagrasses. Higher C-ORG/N-TOT ratios found at invaded sites are probably consequence of seagrasses remains captured by the canopies of *C. racemosa*, or seagrass debris found in sediment core at B-REF.

Organic carbon and total phosphorus ratio (C-ORG/TP) in investigated sediment samples ranged from 81 to 457. High values were recorded at sites A and B-REF (Fig. 5, Table 1). According to Algeo and Ingall (2007) [38], the ratio of C-ORG/P in marine sediments is strongly influenced by benthic redox conditions, since oxygen exhaustion enhances preservation of organic carbon and diffusive loss of remineralized phosphorus. Furthermore, the ratio of C-ORG/TP higher than 200 indicates the remineralization process of phosphorus forms (that implicates anoxic conditions), or elevated input of organic carbon into the sediments [38]. This association between sedimentary C-ORG/TP and redox state of investigated sediments was particularly evident at site A through decreased E_H with depth that indicates the anoxic pathways of degradation of organic material accumulated below the canopy [38, 39]. In contrast to the invaded site A, measured values of E_H at control sites (A-REF and REF) were almost constantly positive (Fig. 5 and 6). On the other hand, the relationship between the above referred parameters at sites B and B-REF were less pronounced, which were most likely influenced by the extremely high values of TP and C-ORG in the surface sediment layer (Fig. 3 and 4).

In order to clearly demonstrate the relationships between the granulometric, chemical and physical variables and reveal the similarities between the investigated sites, analysis of similarities (ANOSIM), hierarchical cluster analysis (CLA) and multi dimensional scaling (MDS) ordination methods were applied. Following parameters were included in those analyses: sorting, gravel, sand, silt, clay, organic matter, total nitrogen, organic carbon, inorganic and total phosphorus and redox potential. However, due to the particular importance of surface sediment in processes of accumulation and degradation of organic matter in sediments [33], we reduced the data matrix to the surface sediment samples of 0-3 cm depth.

Two-way nested ANOSIM test confirmed significant differences between the samples at investigated sites (Global R=0.607; p=0.001). The separation of the three main groups and further distinction of the subgroups was confirmed by the hierarchical cluster dendrogram (Fig. 7). Within the first group, the highest similarity was established between control sites (REF, A-REF and B-REF) including 2-3 cm of sediment core at invaded site B. The second group comprised of the surface sediment (0-1 cm) at site B and surface sediment of 0-2 cm at site A. The sediment layer of 2-3 cm at invaded station A was clustered separately in the third group (Fig. 7).

Similarity percentages analysis (SIMPER) identified gravel and sand contents, total phosphorus and redox po-

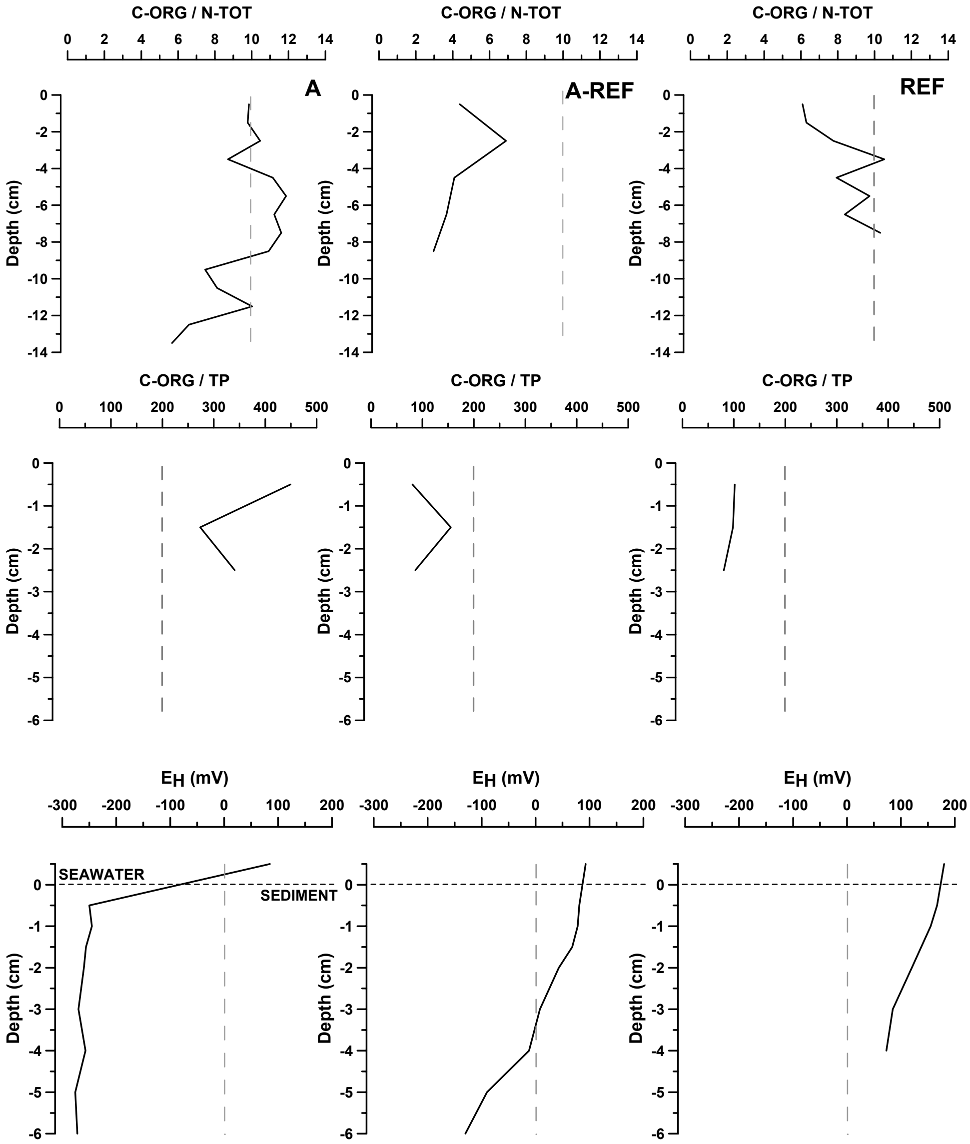

FIGURE 6 - Vertical profiles of the ratios of organic carbon and total nitrogen (C-ORG/N-TOT), organic carbon and total phosphorus (C-ORG/ TP) and redox potential (EH) in sediment core at sites A, A-REF and REF in Priježba Cove

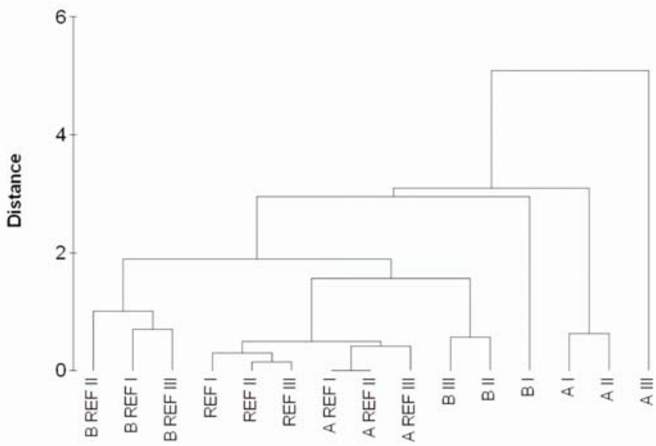

FIGURE 7 - Dendrogram of the sampling layers (denoted by Roman numerals) at five sites in the Priježba Cove, using group average clustering from Euclidian distance similarity on standardised and log transformed data matrix of selected granulometric, physical and chemical variables (I, 0-1 cm; II, 1-2 cm; III, 2-3 cm)

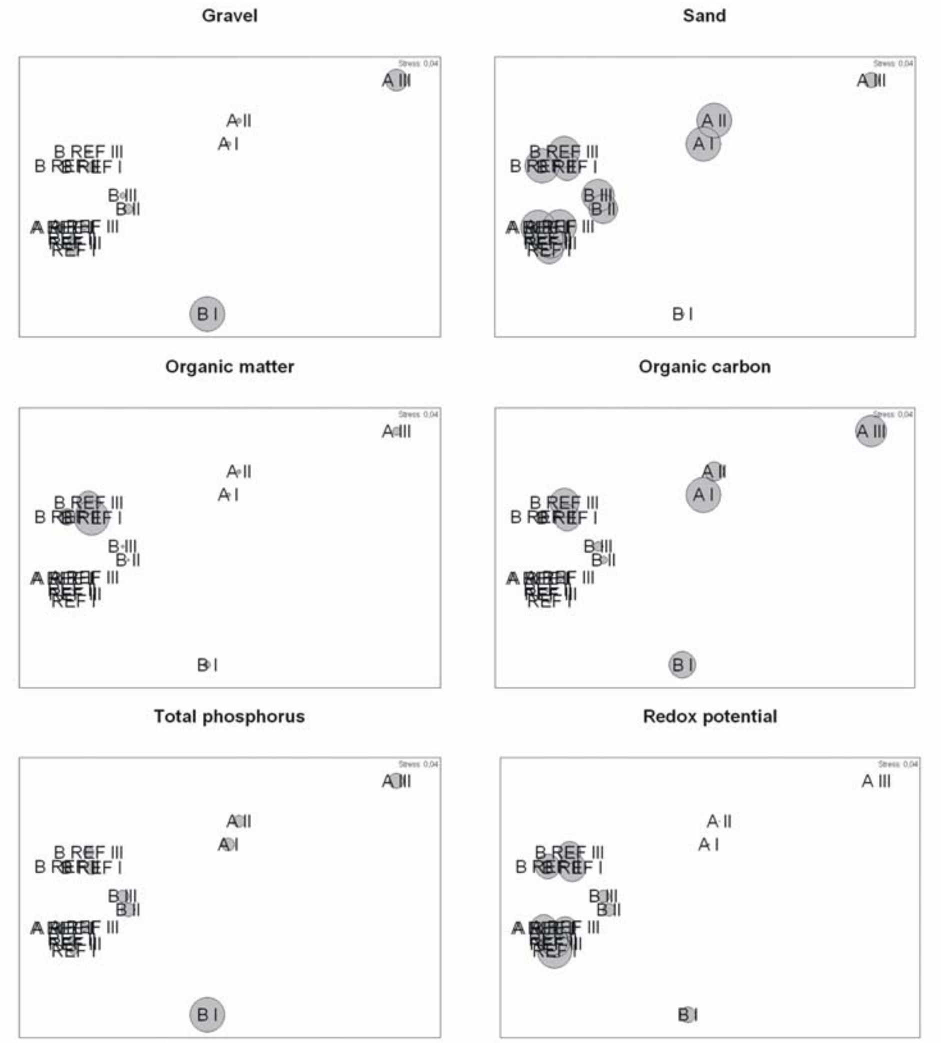

FIGURE 8 - MDS ordination of the sampling layers (denoted by Roman numerals) with selected granulometric, chemical and physical parameters represented as superimposed bubbles increasing in size with increasing content of gravel, sand, OM, C-ORG, TP contents and redox potential values at five sites in Priježba Cove based on standardised and log transformed Euclidian distance similarity matrix (I, 0-1 cm; II, 1-2 cm; III, 2-3 cm)

tential among the key parameters regardless of the sampling site. Average similarity within the sites was always higher at the referent sites in comparison to invaded ones. Cross comparison of the investigated parameters revealed further spatial distinction in the characteristics of sediments. The lowest dissimilarity of 4.79% was found between sites A-REF and REF. The analysis identified gravel and sand content, TP, IP and OM as the top five parameters contributing to the observed differences accounting for 73.71% of the total. On the other hand, the highest dissimilarity of 40.36% was recorded between sites A and REF (Fig. 8). The five most responsible parameters for such separation were E_H, TP, C-ORG, OM and sand content (64.72%). Therefore we have chosen these three sites for detailed analysis of the origin of organic matter as shown in Fig.5.

Similarity among the sampling layers at five investigated sites are illustrated in the MDS ordination diagram derived from the Euclidian distance similarities matrix based on standardized and log (X+1) transformed selected parameters (Fig. 8). Concerning the granulometric composition of sediment, gravel content contributes to dissimilarity between sites due to its increased content at site B (0-1 cm), site A (2-3 cm) related to sediment layers at other investigated sites. Low sand content in the surface sediment layer (0-1 cm) at site B distinguishes this sample compared to others. Clay and organic matter content are higher in sediments of referential sites, while the total phosphorus is more represented at sites invaded with *Caulerpa racemosa*. The C-ORG content strongly contributes to distinction of sediment layers between invaded and control sites, as well as within each investigated site. Redox potential was more negative at site A and in surface layer at site B related to positive values at referential sites.

4 CONCLUSIONS

Variability of biogeochemical parameters along the sediment cores was highest in surface layer at sites invaded with *C. racemosa* related to unaffected sites that indicates changes in environmental conditions during the sedimentation.

Prevailing coarse grains in surface sediments at invaded sites can be attributed to high *Caulerpa*'s retention capacity to trap sediment particles as well as to coastal weathering and transporting process.

Organic carbon, total nitrogen and phosphorus content were elevated in the surface sediment layer at invaded sites related to control. C-ORG/N-TOT and C-ORG/TP ratios as well as negative redox potential at invaded sites indicated the origin of organic matter from marine seagrasses captured by the canopies and its degradation under hypoxic and anoxic conditions.

Statistical analyses emphasized the differences between invaded and control sites and indicated gravel and sand contents, total phosphorus and sediment redox-potential as the key parameters contributing to the presented differences.

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REFERENCES

[1] Boudouresque, C.F. and Verlaque, M. (2002) Assessing scale and impact of ship-transported alien macrophytes in the mediterranean. Ciesm Workshop Monographs 20: 53-61.

[2] Žuljević, A., Antolić, B. and Onofri, V. (2003) First record of Caulerpa racemosa (Caulerpales, Chlorophyta) in the Adriatic Sea, The Journal of the Marine Biological Association of the United Kingdom 83, 711–712.

[3] Nuber, N., Gornik, O., Lauc, G., Bauer, N., Žuljević, A., Papeš, D. and Zoldoš, V. (2007) Genetic evidence for the identity of *Caulerpa racemosa* (Forsskal) J. Agardh (Caulerpales, Chlorophyta) in the Adriatic Sea. European Journal of Phycology 42(1), 113–120.

[4] Klein, J. and Verlaque, M. (2008) The *Caulerpa racemosa* invasion: A critical review. Marine Pollution Bulletin 56, 205–225.

[5] Žuljević, A., Antolić, B., Despalatović, M. and Onofri, V. (2004). The spread of the invasive variety of Caulerpa *racemosa* in the Adriatic Sea. Rapports de la CIESM. 37, 466.

[6] Ruitton, S., Verlaque, M., Boudouresque, C.F. (2005) Seasonal changes of the introduced *Caulerpa racemosa var. cylindracea* (Caulerpales, Chlorophyta) at the northwest limit of its Mediterranean distribution. Aquatic Botany 82, 55–70.

[7] Piazzi, L., Meinesz, A., Verlaque, M., Açali, B., Antolic, B., Argyrou, M., Baltana, D., Ballesteros, E., Calvo, S., Cinelli, F., Cirik, S., Cossu, A., d'Archino, R., Djellouli, A.S., Javel, F., Lanfranco, E., Mifsud, C., Pala, D., Panayotidis, P., Peirano, A., Pergent, G., Petrocelli, A., Ruitton, S. and Žuljević, A. (2005) Invasion of *Caulerpa racemosa* var. *cylindracea* (Caulerpales, Chlorophyta) in the Mediterranean Sea: an assessment of the spread. Cryptogamie, Algologie 26, 189-202.

[8] Antolić, B., Žuljević, A., Despalatović, M., Grubelić and I., Cvitković, I. (2008) Impact of the invasive green alga *Caulerpa racemosa* var. *cylindracea*on the epiphytic macroalgal assemblage of *Posidonia oceanica* seagrass rhizomes in the Adriatic Sea. Nova Hedwigia 86(1-2), 155-167.

[9] Box, A., Martin, D. and Deudero, S. (2010) Changes in seagrass polychaete assemblages after invasion by *Caulerpa racemosa* var. *cylindracea* (Chlorophyta: Caulerpales): community structure, trophic guilds and taxonomic istinctness. Scientia Marina 74(2), 317-329.

[10] Žuljević, A., Thibaut, T., Despalatović, M., Cottalorda, J.M., Nikolić, V., Cvitković, I. and Antolić, B. (2011). Invasive alga *Caulerpa racemosa var. cylindracea* makes a strong impact on the Mediterranean sponge Sarcotragus spinosulus. Biological invasions 13, 2303-2308.

[11] Argyrou, M., Demetropoulos, A. and Hadjichristophorou, M. (1999) Expansion of the macroalga Caulerpa racemosa and changes in softbottom macrofaunal assemblages in Moni Bay, Cyprus. Oceanologica Acta 22, 517–528.

[12] Piazzi, L., Balata, D., Foresi, L., Cristaudo, C. and Cinelli, F. (2007) Sediment as a constituent of Mediterranean benthic communities dominated by *Caulerpa racemosa* var. *cylindracea*. Scientia Marina 71(1), 129–135.

[13] Hendricks, I.E., Bouma, T.J., Morris, E,P. and Duarte, C.M. (2010) Effects of seagrasses and algae of the Caulerpa family on hydrodynamics and particle-trapping rates. Marine Biology 157, 473–481.

[14] Bulleri, F., Balata, D., Bertocci, I., Tamburello, L. and Benedetti-Cecchi, L. (2010) The seaweed *Caulerpa racemosa* on mediterranean rocky reefs: from passenger to driver of ecological change. Ecology 91(8), 2205–2212.

[15] Žuljević, A., Antolić, B., Nikolić, V., Despalatović, M., Cvitković, I. (2012) Absence of successful sexual reproduction of *Caulerpa racemosa* var. *cylindracea* in the Adriatic Sea. Phycologia 51,(3), 283-286.

[16] Piazzi, L., Balata, D., Ceccherelli, G. and Cinelli, F. (2005) Interactive effect of sedimentation and *Caulerpa racemosa* var. *cylindracea* invasion on macroalgal assemblages in the Mediterranean Sea. Estuarine, Coastal and Shelf Research 64(2-3), 467-474.

[17] Holmer, M., Marbà, N., Lamote, M. and Duarte C.M. (2009) Deterioration of Sediment Quality in Seagrass Meadows (Posidonia oceanica) Invaded by Macroalgae (Caulerpa sp.). Estuaries and Coasts 32, 456-466.

[18] Folk, R.L. and Ward, W.C. (1957) Brazos river bar: a study in the significance of grain size parameters. Journal of Sedimentary Petrology 27, 3-26.

[19] Folk, R.L. (1954) The distinction between grain size and mineral composition in sedimentary rock nomenclature. Journal of Geology 62, 344-356.

[20] Loring, D.H. and Rantala, R.T.T. (1992). Manual for geochemical analyses of marine sediments and suspended particulate matter. Earth-Science Reviews 32, 235-283.

[21] Bogner, D., Ujević, I. and Barić, A. (2005) Trace metals (Cd, Pb, Cu, Zn and Cr) distribution in sediment along east coast of the Adriatic Sea (Croatia). Fresenius Environmental Bulletin 14(1), 50-58.

[22] Ujiié, H., Hatakeyama, Y., Gu, X.X., Yamamoto, S., Ishiwatari, R. and Maeda, L. (2001) Upward decrease of organic C/N rations in the Okinawa Trough cores: proxy for tracing the post-glacial retreat of the continental shore line. Palaeogeography, Palaeoclimatology and Palaeoecology 165, 129-140.

[23] Aspila, K.I., Agemian, H. and Chau, A.S.Y. (1976) A semiautomated method for the determination of inorganic, organic and total phosphate in sediments. Analyst 101, 187-197.

[24] Clarke, K.R. and Gorley, R.N. (2001) PRIMER v5: User Manual/Tutorial. PRIMER-E: Plymouth.

[25] Raić, V., Papeš, J., Ahac, A., Korolija, B., Borović, I., Grimani, I. and Marinčić, S. (1980) Osnovna geološka karta SFRJ 1:100000, List Ston K33-48, Institut za geološka istraživanja Zagreb, Savezni geološki zavod, Beograd.

[26] De Falco, G., Baroli, M., Cucco, A. and Simeone, S. (2008) Intrabasinal conditions promoting the development of a biogenic carbonate sedimentary facies associated with the seagrass *Posidonia oceanica*. Continental Shelf Research 28(6), 797-812

[27] Matijević, S., Bogner, D., Morović, M., Tičina, V. and Grbec, B. (2008) Characteristics of the sediment along the eastern Adriatic coast (Croatia). Fresenius Environmental Bulletin 17(10b), 1763-1772

[28] De Falco, G., Ferrari, S., Cancemi, G. and Baroli, M. (2000) Relationships between sediment distribution and *Posidonia oceanica* seagrass. Geo-Marine Letters 20, 50-57.

[29] Como, S., Magni, P., Casu, D., Floris, A., Giordani, G., Natale, S., Fenzi, G.a:, Signa, G. and De Falco, G. (2007) Sediment characteristics and macrofauna distribution along a human-modified inlet in the Gulf of Oristano (Sardinia, Italy). Marine Pollution Bulletin 54(6), 733-744.

[30] Faganeli, J., Pezdič, J., Ogorelec, B., Mišič, M. and Najdek, M. (1994) The origin of sedimentary organic matter in the Adriatic. Continental Shelf Research 14(4), 365-384.

[31] Najdek, M., Travizi, A., Bogner, D. and Blazina, M. (2007) Low impact of marine fish farming on sediment and meiofauna in Limski Channel (northern Adriatic, Croatia). Fresenius Environmental Bulletin 16(7), 784-791.

[32] Matijević, S., Kušpilić, G. and Barić, A. (2006). Impact of a fish farm on physical and chemical properties of sediments and water column in the middle Adriatic Sea. Fresenius Environmental Bulletin 15, 1058-1063.

[33] Matijević, S., Kušpilić, G., Kljaković-Gašpić, Z. and Bogner, D. (2008) Impact of fish farming on the distribution of phosphorus in sediments in the middle Adriatic area. Marine Pollution Bulletin 56, 535-548.

[34] Villares, R. and Carballeira, A. (2003) Seasonal variation in the concentrations of nutrients in two green macroalgae and nutrient levels in sediments in the Rias Baixas (NW Spain). Estuarine, Coastal and Shelf Sciences 58, 887-900

[35] Invers, O., Pérez, M., Romero, J. (1995) Alkaline phosphatase activity as a tool for asses- sing nutritional conditions in theseagrass. Posidonia oceanica (L.) Delilei. Scientia Marina 59, 41–47

[36] Matijević, S., Kušpilić, G. and Kljaković-Gašpić, Z. (2007) The redox potential of sediment from the middle Adriatic region. Acta Adriatica 48(2), 191–204.

[37] Stein, R. (1991). Accumulation of organic carbon in marine sediments. Lecture Notes in Earth Sciences. Heidelberg (Springer) 34, 217 pp.

[38] Algeo, T.J. and Ingall, E. (2007) Sedimentary Corg:P ratios, paleocean ventilation and phanerozic atmospheric pO_2. Palaeogeography, Palaeoclimatology and Palaeoecology 256, 130-155.

[39] Colman, A.S., Holland, H.D. (2000) The global diagenetic flux of phosphorus from marine sediments to the oceans, redox sensitivity and the control of atmospheric oxygen levels. Marine authigenesis: From Global to Microbial, SEPM Special Publ. 66, 53-75.

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