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PRIMARY RESEARCH PAPER

Validation of the Macrophyte Quality Index (MaQI) set up to assess the ecological status of Italian marine transitional environments

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Abstract The paper couples the results obtained by applying the expert and the rapid Macrophyte Quality Indices set up to assess the ecological status of the Italian transitional environments according to the requirements by the Water Framework Directive (2000/60/CE). The indices were validated by comparing the composition of the macrophyte assemblages and the values of some bio-physico-chemical parameters of the water column of 20 stations of the Venice lagoon sampled monthly for one year between 2003 and 2005. In 5 stations out of the 20, the ones which fall within the 5 classes of ecological status suggested by the Water Framework Directive, sedimentation rates, sediment grain-size, and nutrient and pollutant (metals, Polychloro-Dibenzo-Dioxins/Furans, Polycyclic Aromatic Hydrocarbons, Pesticides and Polychlorinated Biphenils) concentrations in surface sediments were also determined. Results showed strong relationships between the trends of these environmental parameters and the composition and structure of macrophyte associations, as well as with the Macrophyte Quality Index assessment. Chlorophyceae showed a trend opposite to Rhodophyceae whose presence was concentrated in oxygenated and transparent environments. Chlorophyceae and the species characterised by low scores prevailed in turbid areas where nutrient and pollutant concentrations were high. Results allowed the identification of the conditions of the "reference sites" (confinement areas and sites with high water renewal) and the integration of the dichotomic key used for the application of the R-MaQI.

Keywords Transitional environments · Ecological indices · Environmental quality · Water Framework Directive · Macrophytes · Mediterranean Sea eco-region

Introduction

The protection and improvement of coastal and transitional waters are among the environmental priorities of the European Community as stated in the Water Framework Directive (WFD: 2000/60/EC) entered into force in December 2000 (Casazza et al., 2003a, b, 2004; Borja, 2005). After the WFD came into force, studies on coastal waters and transitional environments started in Spain and Greece (Borja et al., 2000, 2003, 2004, 2006, 2007; Orfanidis et al., 2001, 2003; Simboura & Zenetos, 2002; Panayotidis et al., 2004; Simboura 2004, Simboura et al., 2005; Arévalo et al., 2007; Ballesteros et al., 2007; Pinedo et al., 2007). Some researchers from those countries proposed to assess the ecological status of estuarine

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environments by studying macrobenthic communities (AZTI Marine Biotic Index-AMBI—Borja et al., 2000, 2003, 2004, 2007; Biotic index-BENTIX—Simboura & Zenetos, 2002, Simboura 2004) or seaweed taxonomic associations (Borja et al., 2004; Ecological Evaluation Index-EEI—Orfanidis et al., 2001, 2003; Panayotidis et al., 2004) because they mainly consist of sessile or quite sedentary organisms with a relatively long life span. Biological communities, which consist of different species, show a different tolerance even to brief environmental stress and can change their structure and taxonomic composition according to the environmental conditions.

Italy started to comply with the assessment of coastal waters in the early 1990s (Giovanardi & Tromellini, 1992; Ignatides et al., 1992; Innamorati & Giovanardi, 1992; Vollenweider et al., 1998) but transitional waters were disregarded for a long time. Those authors proposed the assessment of the trophic status of marine coastal waters by TRIX (Trophic Index), an index based on the elaboration of two groups of environmental variables, i.e. some trophic factors (nutrient and oxygen concentrations) and the concentration of Chl *a*. TRIX can be applied to coastal waters, but it is unsuitable for transitional environments (i.e. lagoons, bays, estuaries) which are affected by high environmental changes due not only to the proximity of the mainland and the shallowness of the bottoms but also to the presence of seagrasses and macroalgae which dominate over the phytoplankton (the only primary producer considered in that index).

The results obtained by some macroalgal taxonomic studies (Sfriso et al., 2002, 2006a, b; Sfriso & La Rocca, 2005) in the Venice lagoon can be considered one of the first attempts to assess the ecological status of transitional environments by ecological quality elements in Italy, because they gave evidence of a high correlation between the Rhodophyceae/Chlorophyceae (R/C) ratio and the ecological status of the environment. Their rationale was based on the fact that, in general, in Mediterranean transitional environments the number of Chlorophyceae taxa prevails in eutrophic and polluted areas whereas the number of Rhodophyceae is more abundant in less polluted areas. However, since some species belonging to *Gracilaria*, *Polysiphonia*, *Porphyra*, *Gracilariopsis*, *Grateloupia*, etc. prevail in "Bad-condition" waters, the authors improved the index by excluding those Rhodophyceae from the calculation. The new index was named "Corrected R/C index" (Sfriso et al., 2006b). The results were successfully used, but as the number of "excluded species" increased with the increase of transitional environments, it became difficult to apply the index.

Concurrently, some national programmes such as "NITIDA" (New trophic state and ecological integrity descriptors of coastal marine and transitional environments), co-funded by the Italian Ministry of Education, University and Research (MIUR) in 2003, started to implement the WFD requirements. NITIDA which included 5 projects carried out by different Universities and Research Centres (Ancona, Bari, Ferrara, Parma and Venezia) implemented the WFD mainly by selecting the biological indicators (macrofauna, macroalgae, seagrasses, phytoplankton, bacteria). The main objective was to assess the ecological status of transitional waters by working in different Italian lagoons (i.e. Lesina, Goro, Venice, Orbetello, etc.). The results obtained by Venice University were employed to set up a new Quality Index, based mainly on macrophytes (MaQI = Macrophyte Quality Index) in 2 versions for an expert (Sfriso et al., 2006a) and for a rapid assessment (Sfriso et al., 2007).

This paper aims at integrating and validating with hydrological and sedimentary parameters the results obtained by applying the expert and the rapid procedures. Particular attention was devoted to the choice of the reference sites and to the relationships between the different ecological conditions, single macrophyte taxa or taxa assemblages and the subdivision of the results into the 5 classes of ecological status suggested by the WFD.

Materials and methods

MaQI structure

The index was set up in 20 stations situated in the Venice lagoon. It was also calibrated in 17 additional sampling sites of the lagoons of Lesina, Orbetello, Marano, Goro and in the Mar Piccolo at Taranto.

It is an environmental assessment determination which takes into account the ecological value of all the macroalgal taxa and marine seagrasses found in the mentioned Italian transitional environments. MaQI is composed of an expert (E-MaQI) procedure (Sfriso et al., 2006a), which is recommended when a new area is studied, and can then be repeated with a 3–6-year frequency or when the ecological conditions of the study area are changing, and of a rapid (R-MaQI) procedure (Sfriso et al., 2007), which is recommended to the ARPAs (Agenzie Regionali per la Protezione dell'Ambiente, i.e. Regional Agencies for the Environment Safeguard) for routine assessment campaigns.

Expert-Macrophyte Quality Index (E-MaQI)

The E-MaQI took into consideration the macroalgal taxa present in many Italian transitional environments and their scores (Table 1).

A large number of macroalgae present in the study sites were collected by SCUBA divers in a surface ranging from 15 to 50 m according to the area morphology. This width surface was judged to be the most suitable to find all the taxa present in the selected areas because samples taken in limited surfaces, as those obtained by using sampling frames, did not allow a complete species collection. In fact, macroalgae are differently distributed in the bottom depending on the kind of substrata (hard or soft), the exposure, light availability and interferences of local disturbances such as currents and anthropic structures. Sampling was carried out monthly in the Venice lagoon, and in May and July–August in the other sites.

All the collected taxa were determined at least at species level. It was very important to determine also the small epiphytes because many of them, especially the Corallinaceae, are characteristic of "Good-High" environments. In fact, during anoxic crises, water pH decreases markedly hampering the deposition of the calcareous crusts of these species which cannot survive.

After determining all the macroalgae, a score (0 = tolerant taxa, 1 = indifferent taxa, 2 = sensitive taxa) was associated to each macroalgal taxon (Table 1) according to Sfriso et al. (2006a, 2007) and the mean score of all the recorded taxa was also calculated.

The WFD requires that the final score must range in an interval between 0 ("Bad" status) and 1 ("High" status), with reference to the best environmental conditions found in the stations defined as "reference sites". Sfriso et al. (2007), by applying the E-MaQI in the studied Italian lagoons, found the highest macroalgal mean score in a high water renewal station of the Venice lagoon (score: 1.03 at st. 5 = Santa Maria del Mare). A little lower value was also found in a confinement station of Lesina lagoon (score 1.00 at st. 3 in the central part of the lagoon). As a consequence, the environmental conditions found in those stations were considered as the "reference conditions" for high water renewal and confined environments, respectively. A mean score equivalent to 1.0 was considered to show the highest environmental quality. The ratio between the mean macroalgal scores resulting from the taxa found in the study areas and the highest value found in the "reference station" represented the Ecological Quality Ratio (EQR = mean score/highest score ratio).

As EQR values were plotted in a continuum from 0 to 1 and for practical reasons that range was subdivided into five equivalent classes ("Bad" conditions: 0–0.20, "Poor" conditions: 0.21–0.40, "Moderate" conditions: 0.41–0.60, "Good" conditions: 0.61–0.80, "High" conditions: 0.81–1.0), it was considered that scores could fall close to the borderline between two adjacent classes and that small changes could create confusion in the classification. Therefore the assessment of the ecological status of each sampling site was proposed by a "class binomial". The first class corresponded to the class where the EQR value fell, according to the mean macroalgal score, and the second one to the immediately upper or lower score-interval. For example, if the EQR ranged between 0.31 and 0.40 the environment classification would be "Poor-Moderate". On the contrary, in the case of EQR between 0.21 and 0.30, the classification would be "Poor-Bad".

Rapid-Macrophyte Quality Index (R-MaQI)

The R-MaQI is a routine ecological index based on the expert index (E-MaQI), the Rhodophyceae/Chlorophyceae ratio (Sfriso et al., 2002, 2006a, b; Sfriso & La Rocca, 2005) and the general environmental conditions found in all the study areas (Sfriso et al., 2007). It takes into consideration the presence/absence, the biomass and species assemblages of some macroalgae and seagrasses and the variability of some physico-chemical parameters such as water

Table 1 Macroalgae recorded in the Italian transitional environments and related scores

		Score
Chlorophyceae		
1	*Blidingia marginata* (J. Agardh) P. J. L. Dangeard *ex* Bliding	0
2	*Blidingia minima* (Nägeli) *ex* Kützing) Kylin	0
3	*Blidingia ramifera* (Bliding) Garbary & Barkhouse	0
4	*Blidingia subsalsa* (Kjellman) Kornmann & Sahling *ex* Scagel	0
5	*Bryopsis corymbosa* J. Agardh	1
6	*Bryopsis cupressina* J. V. Lamouroux	0
7	*Bryopsis cupressina* J. V. Lamouroux var. *adriatica* (J. Agardh) M. J. Wynne	0
8	*Bryopsis duplex* De Notaris	2
9	*Bryopsis feldmannii* Gallardo & Furnari	1
10	*Bryopsis hypnoides* J. V. Lamouroux	1
11	*Bryopsis muscosa* J. V. Lamouroux	1
12	*Bryopsis plumosa* (Hudson) C. Agardh	1
13	*Bryopsis cfr. secunda* J. Agardh	1
14	*Chaetomorpha aerea* (Dillwyn) Kützing	0
15	*Chaetomorpha linum* (O. F. Müller) Kützing	2
16	*Chaetomorpha mediterranea* (Kützing) Kützing	0
17	*Cladophora albida* (Nees) Kützing	1
18	*Cladophora fracta* (O. F. Müller) Kützing	1
19	*Cladophora laetevirens* (Dillwyn) Kützing	0
20	*Cladophora lehmanniana* (Lindenberg) Kützing	1
21	*Cladophora liniformis* Kützing	2
22	*Cladophora glomerata* (Linnaeus) Kützing	1
23	*Cladophora hutchinsiae* (Dillwyn) Kützing	2
24	*Cladophora prolifera* (Roth) Kützing	2
25	*Cladophora ruchingeri* (C. Agardh) Kützing	1
26	*Cladophora rupestris* (Linnaeus) Kützing	1
27	*Cladophora sericea* (Hudson) Kützing	0
28	*Cladophora vadorum* (Areschoug) Kützing	0
29	*Cladophora vagabunda* (Linnaeus) C. Hoek	0
30	*Codium fragile* (Suringar) Hariot subsp. *tomentosoides* (Goor) P. C. Silva	1
31	*Derbesia tenuissima* (Moris & De Notaris) P. & H. Crouan	0
32	*Enteromorpha multiramosa* Bliding	0
33	*Entocladia leptochaete* Huber	0
34	*Entocladia viridis* Reinke	0
35	*Gayralia oxysperma* (Kützing) K. L. Vinogradova *ex* Scagel & al. f. *oxysperma*	1
36	*Lola implexa* (Harvey) A. *et* G. Hamel	2
37	*Monostroma obscurum* (Kützing) J. Agardh	2
38	*Pedobesia simplex* (Meneghini *ex* Kützing) M.J. Wynne *&* Leliaert	0
39	*Rhizoclonium lubricum* Setchell & N. L. Gardner	0
40	*Rhizoclonium tortuosum* (Dillwyn) Kützing	0
41	*Ulothrix flacca* (Dilllwyn) Thuret	0
42	*Ulothrix implexa* (Kützing) Kützing	0
43	*Ulva clathrata* (Roth) G. Agardh	0
44	*Ulva compressa* Linnaeus	0

Table 1 continued

		Score
45	*Ulva curvata* (Kützing) De Toni	0
46	*Ulva fasciata* Delile	0
47	*Ulva flexuosa* Wulfen	0
48	*Ulva flexuosa* Wulfen subsp. *pilifera* (Kützing) Wynne	0
49	*Ulva kylinii* (Bliding) Hayden et al.	0
50	*Ulva intestinalis* Linnaeus	0
51	*Ulva intestinalis* Linnaeus f. *cornucopiae* (Lyngbye) Sfriso *et* Curiel	0
52	*Ulva laetevirens* Areschoug	0
53	*Ulva linza* Linnaeus	0
54	*Ulva prolifera* O. F. Müller	0
55	*Ulva prolifera* O. F. Müller subsp. *gullmariensis* (Bliding) E. Taskin	0
56	*Ulva ralfsii* (Harvey) Le Jolis	0
57	*Ulva rigida* C. Agardh	0
58	*Ulva rotundata* Bliding	0
59	*Ulvella lens* P. & H. Crouan	0
60	*Valonia aegagropila* C. Agardh	2
Rhodophyceae		
61	*Acrochaetium savianum* (Meneghini) Nägeli	1
62	*Acrochaetium microscopicum* (Nägeli *ex* Kützing) Nägeli	1
63	*Acrochaetium virgatulum* (Harvey) Batters	1
64	*Acrosorium ciliolatum* (Harvey) Kylin	1
65	*Agardhiella subulata* (C.Agardh) Kraft *et* Wynne	1
66	*Aglaothamnion caudatum* J. Agardh	2
67	*Aglaothamnion feldmanniae* Halos	1
68	*Aglaothamnion tenuissimum* (Bonnemaison) Feldmann-Mazoyer var. *tenuissimum*	1
69	*Alsidium corallinum* C. Agardh	2
70	*Anotrichium furcellatum* (J. Agardh) Baldock	2
71	*Antithamnion cruciatum* (C. Agardh) Nägeli	1
72	*Antithamnion nipponicum* Yamada *et* Inagaki	1
73	*Antithamnionella spirographidis* (Schiffner) E. M. Wollaston	1
74	*Bangia atroporpurea* (Roth) C. Agardh	1
75	*Callithamnion corymbosum* (J. E. Smith) Lyngbye	1
76	*Callithamnion tetragonum* (Withering) C. Agardh	1
77	*Catenella caespitosa* (Withering) L. M. Irvine in Parke & Dixon	1
78	*Caulacanthus ustulatus* (Turner) Kützing	1
79	*Centroceras clavulatum* (C. Agardh) Montagne	2
80	*Ceramium ciliatum* (J. Ducluzeau) var. *ciliatum*	2
81	*Ceramium ciliatum* (J. Ducluzeau) var. *robustum* (J. Agardh) Feldmann-Mazoyer	2
82	*Ceramium cimbricum* H. E. Petersen	1
83	*Ceramium codii* (H. Richards) Feldmann-Mazoyer	2
84	*Ceramium deslongchampii* Chauvin *ex* Duby	1
85	*Ceramium flaccidum* (Kützing) Ardissone	2
86	*Ceramium circinatum* (Kützing) J. Agardh	2
87	*Ceramium siliquosum* (Kützing) Maggs & Hommersand var. *siliquosum*	1

Table 1 continued

		Score
88	*Ceramium siliquosum* (Kützing) Maggs & Hommersand var. *zostericola* (Feldmann-Mazoyer) G. Furnari	1
89	*Ceramium tenerrimum* (G. Martens) Okamura	2
90	*Ceramium virgatum* Roth	1
91	*Chylocladia verticillata* (Lightfoot) Bliding	2
92	*Chondracanthus acicularis* (Roth) Fredericq	2
93	*Chondracanthus teedei* (Mertens *ex* Roth) Kützing	2
94	*Chondria capillaris* (Hudson) M. J. Winne	1
95	*Chondria coerulescens* (J. Agardh) Falkenberg	2
96	*Chondria dasyphylla* (Woodward) C. Agardh	2
97	*Chondrophycus papillosus* (C. Agardh) Garbary *et* J. Harper	2
98	*Colaconema daviensii* (Dillwyn) Stegenga	1
99	*Corallina elongata* J. Ellis & Solander	2
100	*Corallina officinalis* Linnaeus	2
101	*Cruoria cruoriaeformis* (P. & H. Crouan) Denizot	1
102	*Cryptonemia lomation* (A. Bertoloni) J. Agardh	2
103	*Dasya baillouviana* (S. G. Gmelin) Montagne	1
104	*Dasya punicea* (Zanardini) Meneghini ex Zanardini	2
105	*Heterosiphonia japonica* Yendo	2
106	*Erythrotrichia bertholdii* Batters	1
107	*Erythrotrichia carnea* (Dillwyn) J. Agardh	1
108	*Erythrocladia discigera* (Berthold) F. Schmitz in Engler & Pranti	1
109	*Erythrocladia irregularis* Rosenvinge	1
110	*Erythrotrichia investiens* (Zanardini) Bornet	1
111	*Gastroclonium reflexum* (Chauvin) Kützing	2
112	*Gelidium crinale* (Turner) Lamouroux	2
113	*Gelidium pusillum* (Stackhouse) Le Jolis	0
114	*Gelidium spathulatum* (Kützing) Bornet	0
115	*Gymnogongrus griffithsiae* (Turner) Martius	0
116	*Gracilaria armata* (C. Agardh) Greville	2
117	*Gracilaria bursa -pastoris* (S.G. Gmelin) P. C. Silva	1
118	*Gracilaria* cfr. *compressa* (C. Agardh) Greville	2
119	*Gracilaria dura* (C. Agardh) J. Agardh	1
120	*Gracilaria longa* Gargiulo, De Masi *et* Tripodi	0
121	*Gracilaria gracilis* (Stackhouse) Steentoft, Irvine *et* Farnham	0
122	*Gracilaria* sp.	2
123	*Gracilariopsis longissima* (S. G. Gmelin) Steentoft et al.	0
124	*Grateloupia dichotoma* J. Agardh	2
125	*Grateloupia turuturu* Yamada	0
126	*Grateloupia filicina* (J.V. Lamouroux) C. Agardh	2
127	*Griffithsia shousboei* Montagne	2
128	*Haliptilon squamatum* (Linnaeus) H. W.Johansen, L.M. Irvine *et* A.M. Webster	2
129	*Halymenia floresii* (Clemente y Rubio) C. Agardh	2
130	*Hydrolithon boreale* (Foslie) Y. M. Chamberlain	2
131	*Hydrolithon cruciatum* (Bressan) Chanberlain	2

Table 1 continued

		Score
132	*Hydrolithon farinosum* (J.V. Lamouroux) Penrose *et* Chamberlain var. *farinosum*	2
133	*Hildenbrandia rubra* (Sommerfelt) Meneghini	2
134	*Hypnea musciformis* (Wulfen) J. V. Lamouroux	2
135	*Hypnea spinella* (C. Agardh) Kützing	2
136	*Hypnea sp.*	2
137	*Laurencia obtusa* (Hudson) Lamouroux	2
138	*Lithophyllum pustulatum* (J.V.Lamouroux) Foslie	2
139	*Lomentaria clavellosa* (Thurner) Gaillon	1
140	*Lomentaria clavellosa* (Thurner) Gaillon v. *clavellosa* f. *reducta* Ercegović	1
141	*Lomentaria ercegovicii* Verlaque et al.	2
142	*Lomentaria hakodatensis* Yendo	2
143	*Lomentaria uncinata* Meneghini *ex* Zanardini	1
144	*Nemalion helminthoides* (Velley) Batters	2
145	*Neosiphonia elongella* (Harvey) M.S. Kim *et* I. K. Lee	0
146	*Neosiphonia harveyi* (J. W. Bailey) M. S. Kim et al.	1
147	*Nitophyllum punctatum* (Stackhouse) Greville	2
148	*Osmundea truncata* (Kützing) K. W. Nam et Maggs	2
149	*Phyllophora sicula* (Kützing) Guiry *et* L.M. Irvine	2
150	*Plenosporium borreri* (J.E. Smith) Nägeli	0
151	*Pneophyllum fragile* Kützing	2
152	*Polysiphonia breviarticulata* (C. Agardh) Zanardini	0
153	*Polysiphonia denudata* (Dillwyn) Greville *ex* Harvey	1
154	*Polysiphonia deusta* (Roth) Sprengel	1
155	*Polysiphonia elongata* (Hudson) Sprengel	0
156	*Polysiphonia fibrillosa* (Dillwyn) Sprengel	0
157	*Polysiphonia fucoides* (Hudson) Greville	2
158	*Polysiphonia flocculosa* (C. Agardh) Kützing	2
159	*Polysiphonia furcellata* (C. Agardh) Harvey	0
160	*Polysiphonia morrowii* Harvey	1
161	*Polysiphonia sanguinea* (C. Agardh) Zanardini	0
162	*Porphyra leucosticta* Thuret	1
163	*Porphyra linearis* Greville	1
164	*Pterothamnion plumula* (J. Ellis) Nägeli	2
165	*Pterothamnion crispum* (Ducluzeau) Nägeli	2
166	*Radicilingua reptans* (Kylin) Papenfuss	2
167	*Radicilingua thysanorhizans* (Holmes) Papenfuss	1
168	*Rhodophyllis divaricata* (Stackhouse) Papenfuss	1
169	*Rhodymenia ardissonei* Feldmann	1
170	*Rhodymenia ligulata* Zanardini	1
171	*Rytiphlaea tinctoria* (Clemente) C. Agardh	2
172	*Sahlingia subintegra* (Rosenvinge) Kornmann	1
173	*Spermothamnion repens* (Dillwyn) Rosenvinge	1
174	*Spyridia filamentosa* (Wulfen) Harvey	1
175	*Stylonema alsidii* (Zanardini) K. M. Drew	1
176	*Stylonema cornu-cervi* Reinsch	1

Table 1 continued

		Score
Phaeophyceae		
177	*Asperococcus bullosus* J.V. Lamouroux f. *bullosus*	1
178	*Asperococcus ensiformis* (Delle Chiaje) M. J. Wynne	1
179	*Asperococcus fistolosus* (Hudson) Hooker	1
180	*Cladosiphon zosterae* (J. Agardh) Kylin	2
181	*Colpomenia sinuosa* (Mertens *ex* Roth) Derbès *et* Solier	2
182	*Cystoseira barbata* (Stackhouse) C. Agardh var. *barbata*	1
183	*Cystoseira compressa* (Esper) Gerloff & Nizamuddin	2
184	*Desmarestia viridis* O. F. Müller	0
185	*Dictyopteris polypodioides* (A.P. De Candolle) J.V. Lamouroux	2
186	*Dictyota dichotoma* (Hudson) J.V. Lamoroux var. *dichotoma*	1
187	*Dictyota dichotoma* (Hudson) J.V. Lamoroux var. *intricata* (C. Agardh) Greville	1
188	*Dictyota linearis* (C. Agardh) Greville	1
189	*Corynophlaea umbellata* (C. Agardh) Kützing	1
190	*Ectocarpus fasciculatus* Harvey var. *fasciculatus*	0
191	*Ectocarpus siliculosus* (Dillwyn) Lyngbye var. *arctus* (Kützing) Gallardo	0
192	*Ectocarpus siliculosus* (Dillwyn) Lyngbye var. *crouaniorum* (Thuret) Gallardo	1
193	*Ectocarpus siliculosus* (Dillwy) Lyngbye var. *hiemalis* (P. *et* H. Crouan *ex* Kjellman) Gallardo	1
194	*Ectocarpus siliculosus* (Dillwyn) Lyngbye var. *siliculosus*	0
195	*Feldmannia irregularis* (Kützing) Hamel	1
196	*Fucus virsoides* J. Agardh	2
197	*Hincksia granulosa* (J.E. Smith) P. C. Silva	1
198	*Hincksia mitchelliae* (Harvey) P. C. Silva	0
199	*Hincksia ovata* (Kjellman) P. C. Silva	1
200	*Hincksia sandriana* (Zanardini) P. C. Silva	1
201	*Hincksia secunda* (Kützing) P. C. Silva	1
202	*Kuckuckia spinosa* (Kützing) Kornmann	1
203	*Leptonematella fasciculata* (Reinke) P. C. Silva	0
204	*Myrionema strangulans* Greville	1
205	*Petalonia fascia* (Müller) Kuntze	1
206	*Petalonia zosterifolia* (Reinke) Kuntze	1
207	*Protectocarpus speciosus* (Børgesen) Kornmann	1
208	*Punctaria latifolia* Greville	0
209	*Punctaria tenuissima* (C. Agardh) Greville	2
210	*Pilayella littoralis* (Linnaeus) Kjellman	1
211	*Sargassum muticum* (Yendo) Fensholt	1
212	*Scytosiphon dotyi* M.J. Wynne	0
213	*Scytosiphon lomentaria* (Lyngbye) Link	0
214	*Sorocarpus* sp.	1
215	*Stictyosiphon adriaticus* Kützing	0
216	*Stictyosiphon soriferus* (Reinke) Rosenvinge	2
217	*Taonia pseudociliata* (J. V. Lamouroux) Nizamiuddin & Godeh	2
218	*Undaria pinnatifida* (Harvey) Suringar	0
Chrysophyceae		
219	*Vaucheria dichotoma* (Linnaeus) C. Agardh fo. *marina* Hauck	0

transparency, salinity, oxygen saturation, sediment grain-size and nutrient concentrations. The R-MaQI does not require taxonomic experts; it is friendly and easy to apply. The placement of the environment in a defined ecological class is almost immediate, notwithstanding the presence of a low number of macrophyte taxa or their complete absence.

The index is structured as a dichotomic key where the conditions of soft and hard substrata are considered separately as reported by Sfriso et al. (2007), but it was in part revised (Table 2). According to the environmental conditions found in the Italian lagoons, in soft substrata, the presence/absence of seagrasses allows a rapid distinction between the "Bad-Poor" and "Moderate-Good-High" classes both in high water renewal and confined areas. The following class separation can be obtained by taking into account the seagrass species, the population structure and their association with some macroalgae. In hard substrata and in the "Bad-Poor" classes of soft substrata, class distinction is based on the presence/absence or abundance of some macroalgal taxa such as Ulvaceae and Cladophoraceae considered at the genus or family level. For example, when macroalgae are almost missing or Ulvaceae and Cladophoraceae are sporadically present, waters are very turbid and the environmental conditions are highly instable, so the environment can be immediately assessed in the "Bad" class. In the presence of a low number of macroalgal taxa, which may belong to other families such as Gracilariaceae, but are able to bloom during the year, the environmental conditions are certainly better and the environment can be classified in the "Poor" class, although after blooming, a collapse usually follows.

The "Moderate" class is characterised by the appearance of seagrasses in the soft substrata and by the fact that the number of taxa of Rhodophyceae overcomes the one of Chlorophyceae. The "Good" and "High" classes are discriminated by the dominance of well structured seagrass-populations and by the presence or dominance of macroalgae such as Corallinaceae which grow in high-quality environments and are characterised by low nutrient and pollutant concentrations, high pH and good water oxygenation.

Usually, the presence of a high number of macroalgae is associated to a "Good" or "High" ecological status. However, the presence of few high-score taxa can show as high-quality an environment as the presence of a large number of taxa.

Study areas

Venice lagoon has a total surface of 549 km^2 and exchanges waters with the sea through 3 large mouths (Lido, Malamocco, Chioggia) whose width and depth vary from 400 to 900 metres and 12–20 (up to 50) metres, respectively. The lagoon mean depth is ca. 1 m and ca. 60% of its waters is exchanged with sea waters at every tidal change, although in areas close to the mainland tidal exchanges can last even 15–20 days.

The Venice lagoon is a very polymorphous environment with very different trophic and contamination levels. There are mesotrophic or hyperdystrophic areas which can be scarcely or highly contaminated. In addition, the environment exhibits areas with hyperaline, mesoaline or hypoaline conditions, and also areas affected by river outfalls, urban sewage, industrial effluents, harbour activities, clam-harvesting and areas intensively drained by seawaters. The lagoon is colonised by seagrasses, macroalgae and phytoplankton each prevailing on the other according to the different ecological conditions (Sfriso & Facca, 2007).

Such conditions have made that environment the most suitable field for our purposes. Twenty sampling sites (Fig. 1) were monitored monthly for one year (between 2003 and 2005) by collecting macroalgae and recording some hydrological parameters (i.e. water temperature, chlorinity, oxygen saturation, suspended solids, chlorophyll *a* and phaeopigments, reactive phosphorus, ammonium, nitrites and nitrates). Out of the 20 sampling sites, 5 stations (i.e. sts. 5, 7, 12, 14, 20), which fell in the five different classes of ecological status proposed by the WFD, were also analysed separately. In fact, the availability of the nutrient concentrations (total, inorganic and organic carbon and phosphorus, total nitrogen) and the knowledge of the contamination status (organic and inorganic micropollutants) in surface sediments allow a more complete environment assessment.

The lagoon of Lesina is a shallow coastal pond (depth ca. 0.8 m, width ca. 50 km^2), which communicates with the southern Adriatic Sea through two narrow and shallow inlets: Acquarotta and Schiapparo Canals (width ca. 4–20 m, depth ca. 2–4 m).

Table 2 Dichotomical key for a rapid assessment of the ecological status of the Mediterranean transitional environments by Sfriso et al. (2007), revised

Hard substrata: Absence, or presence of a very low number of seaweeds, mostly Chlorophyceae.
Soft substrata: Absence of seagrasses…………………………………………………………………1

Hard substrata: The Rhodophyceae number is prevailing on the Chlorophyceae one.
Soft substrata: Presence of seagrasses……………………………………………………………3

1) Macrophyte are missing or almost missing. Dominance of some species of Chlorophyceae, especially Ulvaceae and Cladophoraceae. Seasonal growth of some Rhodophyceae or Phaeophyceae, but with negligible biomass.
Waters are very turbid and seasonally changeable but, on average, Secchi disk is <0.5-0.8 m, due both to phytoplankton blooms and sediment re-suspension phaenomena. Presence of anoxic sediments and persistent water anoxia in spring-summer. High variability of environmental parameters such as transparency and salinity.

Ecological status: BAD

1) Seasonal growth of some seaweeds, but some of them can bloom…………………………………2

2) Presence of a low seaweed number. Monospecific seaweed blooms can occur: especially Ulvaceae, Clodophoraceae and Gracilariaceae.
Water turbid, seasonally changeable but for long periods <1 m.
Oxygen saturation up to 300-400%, followed by macroalgal collapse and anoxia.

Ecological status: POOR

2) Presence of many seaweeds but, no one absolutely dominant. Seagrasses begin to be present……………………………………………………………………………………………3

3) Soft substrata:
Presence of poor *Ruppia* spp., *Nanozostera noltii* and/or *Zostera marina* populations.
Hard substrata:
Seaweed biomass composed by many Chlorophyceae and Rhodophyceae, but the number of the latter begins to be higher.
Waters are quite transparent (1-2 m) for most of the year. Anoxia are lacking but hypoxic conditions can occur.

Ecological status: MODERATE

3) Presence of many species with high quality score. High biomasses of laminar Ulvaceae are missing. The Rhodophyceae number is clearly prevailing on the Chlorophyceae one.
Seagrass beds well organised………………………………………………………………4

4) Soft substrata:
Ruppia spp., *Nanozostera noltii* and/or *Zostera marina* beds are well organised. *Cymodocea nodosa* can be present.
Many seaweeds can be associated to seagrass populations. The latter can also show high Chlorophyceae (i.e. *Chaetomorpha linum,* filamentous Ulvaceae), or more rarely Rhodophyceae (*Gracilaria* spp., *Polysiphonia* spp., etc.), biomasses.

Hard substrata:
Seaweed biomass composed by many species with high environmental score (Table 1), which are sensitive to the environment stressors, begin to be present.
Dominance of some genera such as *Ceramium* spp., *Dictyota* spp., *Cystoseira* spp.; *Sargassum muticum*, etc. Presence of calcified seaweeds.
Transparent waters (2-3 m) for most of the year. Environmental parameters such as oxygen and salinity show only long period or seasonal changes.

Ecological status: GOOD

4) Soft substrata:
Seagrass beds very dense and well organised. *Cymodocea nodosa* and *Posidonia oceanica* L., if present, are abundant especially in high renewal waters. *Ruppia* spp. negligible or missing in high renewal waters but can be dominant in confined environments.
Seaweeds are numerous, especially Rhodophyceae, but each taxon, rarely presents abundant biomasses. Many taxa are epiphytic species and many of these forms calcareous crusts on seagrass leaves.
Hard substrata:
Presence of many taxa which are sensitive to eutrophication, pollution, turbidity or other environmental stressors. Calcified species are numerous (*Corallina* spp., *Hydrolithon* spp.; *Lithophyllum* spp. etc.).
Waters are clear (>3 m) for most of the year.
Environmental parameters such as oxygen and salinity show low seasonal changes. Sediments are mostly coarse or sandy and well oxidised.

Ecological status: HIGH

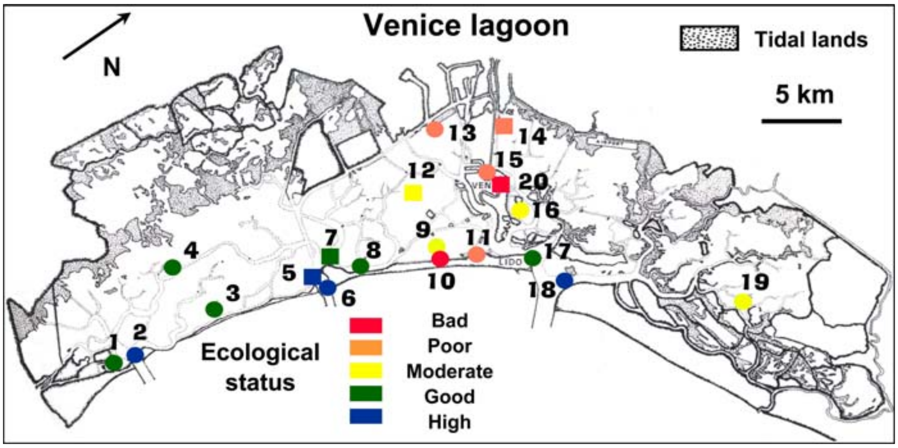


Fig. 1 Venice lagoon and the 20 sampling sites monitored for one year. Squares indicate the 5 areas characterised by a different ecological status where nutrient concentrations and pollutants were also considered

Therefore, water exchange is low and salinity fluctuates in a wide range which depends on the river inputs and the seasonal rainfalls. The lagoon exhibits very homogeneous conditions which are characteristic of a highly confined environment. Except for the area close to Lesina centre, the lagoon shows mesotrophic conditions and low contamination levels. Four sites covering the main differences in that environment were sampled in May and July 2004 (Fig. 2).

The lagoon of Orbetello is a smaller basin of ca. 27 km^2, ca. 1–1.5 m deep, which is divided into two basins (Ponente lagoon: ca. 15 km^2 and Levante lagoon: ca. 12 km^2) by the city of Orbetello and the bridge which connects the city with the Argentario rocky promontory. Ponente lagoon is connected with the sea through two small, shallow and narrow canals: Fibbia canal (3 km long) and Nassa canal (0.5 km long). Levante lagoon is connected with the sea through Ansedonia canal (1.5 km long). As a consequence the water exchange with the sea is very reduced, and Ponente basin is frequently affected by macroalgal blooms and anoxia.

In this lagoon four sampling sites were monitored in August 2005 (Fig. 2). Three of them were selected in Ponente basin: one in the middle of the basin where environmental conditions are quite good, and the others close to Porto Scalo and Nassa oyster farms where environmental conditions are strongly affected by aquaculture and high seaweed production and collapse. Another station was selected in the centre of Levante basin which exhibits environmental conditions similar to the ones in the central part of Ponente lagoon.

Sacca di Goro, which is placed in the southern part of the Po delta, is a large marine embayment (width: ca. 20 km^2 and mean depth ca. 60 cm). It communicates with the northern Adriatic Sea through a large (ca. 1.5 km wide) and shallow (ca. 1 m depth) inlet which extends from Volano Lido to the “Scannone”, a long sandy bank which widens year by year reducing the lagoon mouth. The basin is affected by the outflows from Po di Volano, Po di Gorino and other canals regulated by pumping plants. Sampling was carried out in May and July 2004 in four stations representative of the main environmental differences of the basin (Fig. 2).

Mar Piccolo of Taranto is a marine bay of ca. 20.7 km^2, ca. 8 km long and 3 km wide, subdivided into two smaller basins called the First and the Second inlets, separated by two land promontories. Two canals connect the First inlet with Mar Grande basin allowing a good water renewal. Both basins are rather deep: 12 metres in the First inlet and 8 in the Second inlet. Most freshwater inputs come from ca. 30 submarine springs. Two stations of the lagoon, one in each of the basins, were sampled in late July 2006.

Marano lagoon and Grado lagoon are an unique geographical complex separated by the administrative border between Udine and Gorizia districts. The two lagoons are situated in the Northern Adriatic Sea between the Tagliamento and Isonzo rivers. Their surface is ca. 160 km^2 with a coastal extension of ca. 32 km^2 and a mean width of ca. 5 km. They are separated into two basins of similar surface by Porto Buso inlet. The lagoons’ hydrodynamics and morphology are very similar to Venice showing shallow waters and a high water exchange which

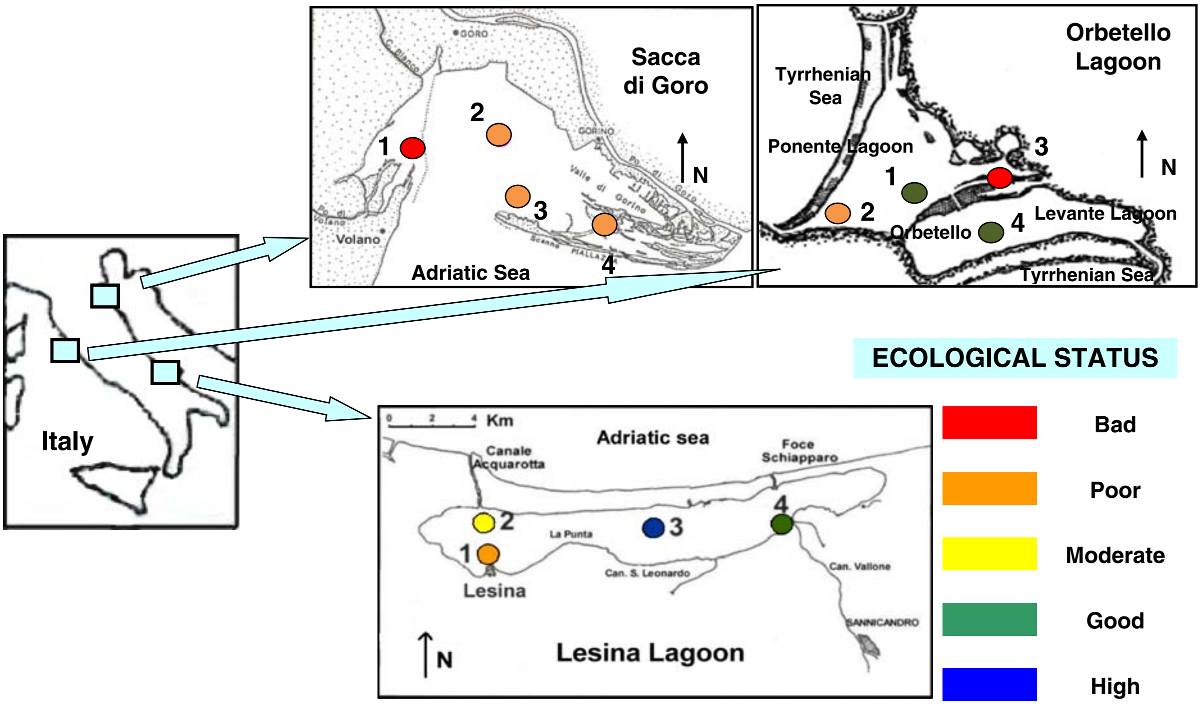


Fig. 2 Classifications of some areas in the lagoons of Lesina, Orbetello and Sacca di Goro

discriminates areas with marine characteristics from confined areas which are close to the tidal lands. The mean water exchange is 8.7×10^3 m^3 s^{-1} during a syzygial tide (Dorigo, 1965) whereas the average freshwater inflow is 108 m^3 s^{-1}, 78 m^3 s^{-1} of which enters Marano lagoon (Marocco, 1995). Marano lagoon is not affected by the anthropic pressures of Venice lagoon, but its sediments are contaminated by high concentrations of Hg and As, of natural and industrial origins. Samplings occurred in April 2007 in four areas placed between Porto Buso inlet and the salt marshes close to the tidal lands.

MaQI validation

The validation of E-MaQI was set up in 20 stations of the Venice lagoon by relating the results obtained by sampling macroalgae and the main hydrological parameters (temperature, chlorinity, oxygen saturation, chlorophyll *a*, phaeopigments, suspended solids and nutrient concentrations). Additionally 5 stations out of the original twenty were chosen to test also the relationship between macroalgal taxa and the concentrations of nutrients (total, inorganic, organic carbon and phosphorus, total nitrogen), and organic (PCBs = Polychlorinated Biphenils, PAHs = Polycyclic Aromatic Hydrocarbons and PCDD/F = polychloro-Dibenzo-Dioxins/Furans) and inorganic (some metals, i.e. Pb, As, Hg, Cu, Zn, Cd, Cr, Ni) pollutants in surface sediments, because they represented the 5 classes of the different ecological status suggested by the WFD.

The physico-chemical parameters, the nutrient concentrations in the water column and surface sediments and the pollutant concentrations in surface sediments used to characterise the selected sampling areas were retrieved from previous projects or from literature (Argese et al., 1997; Sfriso, 2000, 2005, 2007; Secco et al., 2005; Sfriso et al., 2005a, b; Zonta et al., 2006a, b).

Macrophyte sampling procedures and environmental parameters

Seaweeds and seagrasses were recorded by hand during low tides and by SCUBA divers. All the taxa were sorted and examined fresh when possible, or after fixation with 4% formaldehyde, neutralised with hexamethylenetetramine, by means of a stereoscope and a light microscope. When possible, all the macrophytes were determined at species, subspecies, variety and form levels by means of the most recent taxonomic keys and nomenclature revisions (Furnari et al., 1999, 2003; Guiry & Guiry, 2007; Sfriso & Curiel, 2007).

Statistical analyses

The relationship between the bio-physico-chemical parameters, the nutrient concentrations in the water column and the macroalgal taxa found in the 20 stations of the Venice lagoon was investigated by means of the Spearman's correlation coefficients and the cluster analysis. The STATISTICA STAT SOFT vs 7 software package (STATISTICA, 2006) was used to carry out the statistical analyses. The correlation analysis was also applied to the five stations, representing the 5 classes of different ecological status.

Finally all the stations were analysed by applying the canonical correspondence analysis (CCA) in order to show how the environmental variables and pollutants were correlated to the different macroalgal taxa. Data were processed using CANOCO v 4.5 software (CANOCO, 2002; Ter Braak & Smilauer, 1998).

Results

Station assessment

All the 5 classes of ecological status were found in the Venice lagoon (Figs. 1, 3), and in the 20 stations, 219 seaweed and 4 seagrass taxa were recorded. On a yearly basis, the number of taxa ranged from ca. 175 (Table 3) in an area placed near the Malamocco inlet (st. 5: S. Maria del Mare) to 38 in a very polluted canal at Lido Island (st. 10: Ca' Bianca canal) and in a canal in Venice historical centre (st. 20: Misericordia canal). Only stations 10 and 20 showed "Bad" ecological status. Among the remaining group, four stations exhibited "Poor" status (sts. 11, 13, 14, 15), five "Moderate" (sts. 9, 12, 16, 19) or "Good" (sts. 1, 3, 4, 7, 8, 17) status, respectively, and four (sts. 2, 5, 6, 18) "High" status.

At Lesina all the classes, except for "Bad", were recorded (Fig. 2). In May and July 2004 only 30 macroalgal taxa (i.e. 16 Chlorophyceae and 14 Rhodophyceae) and 2 seagrasses (*Ruppia cirrhosa* (Petagna) Grande and *Nanozostera noltii* (Hornemann) Tomlinson *et* Posluzny were found. No Phaeophyceae were recorded in this lagoon. The number of species ranged from 18 at st. 1, close to the Lesina centre, to 10 at st. 2 near the Acquarotta. Station 3, placed in the central part of the basin, showed the best environmental conditions of all the confined areas of the studied lagoons with a mean score of 1.00. This result was very close to the value found in the high renewal reference station placed in Venice lagoon (1.03). Station 3 exhibited a well-structured *N. noltii* population and some macroalgae with a high ecological value such as *Valonia aegagrophyla* C. Agardh and *Chaetomorpha linum* (O. F. Müller) Kützing. Moreover, both *N. noltii* and macroalgae were densely covered by small crustose taxa such as *Litophyllum pustulatum* (J.V. Lamouroux) Foslie, *Hydrolithon boreale* (Foslie) Y. M. Chanberlain and *Hydrolithon farinosum* (J.V. Lamouroux) D. Penrose *et* Y. M. Chamberlain.

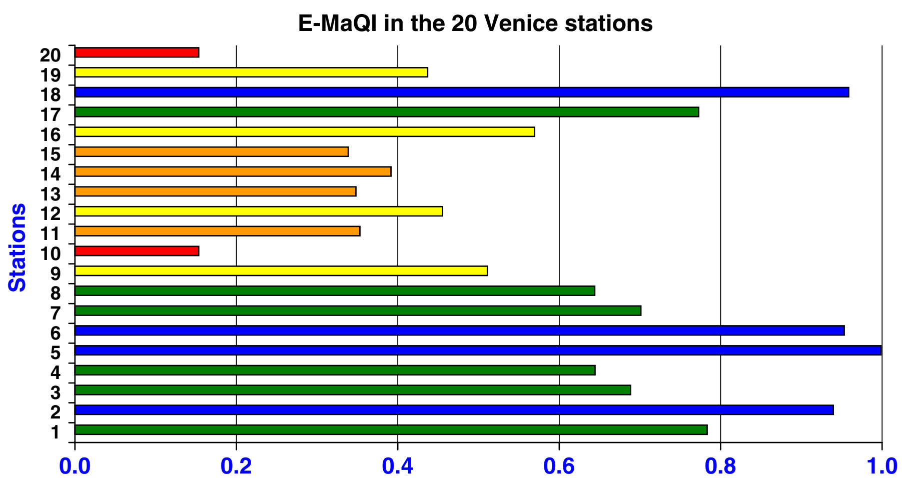


Fig. 3 Ecological status of the 20 areas of the Venice lagoon by applying the E-MaQI

Table 3 Number and percentage of macroalga taxa, R/C ratio and E-MaQI recorded in the 20 Venice stations on annual basis

Stations	Taxa No.	Chlor. No.	Rhod. No.	Phaeo. No.	Chlor. %	Rhod. %	Phaeo. %	R/C	E-MaQI
St. 1 (P. Unione)	150	47	79	24	31	53	16	1.7	0.81
St. 2 (Baia S. Felice)	155	42	87	26	27	56	17	2.1	0.97
St, 3 (Porto Secco)	109	35	57	17	32	52	16	1.6	0.71
St. 4 (Petta di Bò)	112	37	56	19	33	50	17	1.5	0.66
St. 5 (S. Maria del Mare)	175	44	102	29	25	58	17	2.3	1.03
St. 6 (Alberoni dike)	164	45	91	28	27	55	17	2.0	0.98
St. 7 (Alberoni Ottagono)	108	35	57	16	32	53	15	1.6	0.72
St. 8 (Malamocco)	100	34	50	16	34	50	16	1.5	0.66
St. 9 (Lido watershed)	94	37	44	13	39	47	14	1.2	0.53
St. 10 (Ca'Bianca canal)	38	24	10	4	63	26	11	0.4	0.16
St. 11 (Casinò canal)	55	26	21	8	47	38	15	0.8	0.36
St. 12 (Sacca Sessola)	80	32	37	11	40	46	14	1.2	0.47
St. 13 (Trezze)	52	24	22	6	46	42	12	0.9	0.36
St. 14 (San Giuliano)	61	29	23	9	48	38	15	0.8	0.40
St. 15 (Piazzale Roma)	43	19	16	8	44	37	19	0.8	0.35
St. 16 (Celestia)	103	39	43	21	38	42	20	1.1	0.59
St. 17 (San Nicolò)	153	48	77	28	31	50	18	1.6	0.80
St. 18 (Punta Sabbioni)	157	43	85	29	27	54	18	2.0	0.99
St. 19 (Palude Maggiore)	79	30	37	12	38	47	15	1.2	0.45
St. 20 (Misericordia canal)	38	23	9	6	61	24	16	0.4	0.16
Mean	101	35	50	17	38	46	16	1.3	0.61
Std.	45	9	29	9	11	10	2	0.5	0.27
Max	175	48	102	29	63	58	20	2.3	1.03
Min	38	19	9	4	25	24	11	0.4	0.16

Chlor. = Chlorophyceae; Rhod. = Rhodophyceae; Phaeo. = Phaeophyceae; R/C = Rhodophyceae/Chlorophyceae; E-MaQI = Expert-Macrophyte Quality Index; No. = number of species; %Chlor., %Rhod., %Phaeo. = percentages of these classes on the total taxa number

Just like Lesina, the lagoon of Orbetello showed areas with different ecological status, but not one exhibited high quality conditions (Fig. 2). In Orbetello, 21 macroalgae and 2 seagrasses (*Ruppia cirrhosa, Nanozostera noltii*) were recorded and the species richness ranged from 1 (st. 3) to 17 (st. 1).

The lagoon of Goro showed very homogeneous conditions. The four studied areas ranged from "Bad" to "Poor" status. In Goro neither seagrasses nor Phaeophyceae were found and Ulvaceae were the main population. The number of taxa ranged from 5 (st. 1) to 9 (sts. 2 and 4).

By applying E-MaQI in some stations at Marano lagoon (3 stations) and in Mar Piccolo at Taranto (2 stations), EQR values resulted to be lower than the "reference conditions" found in Venice and Lesina. EQR ranged between 0.38 ("Poor" conditions) and 0.87 ("High" conditions) at Marano and between 0.80 ("Good" conditions) and 0.95 ("High" conditions) in Mar Piccolo.

MaQI validation

Table 1 reports the scores assigned to each macroalgal taxon found at Venice, Goro, Lesina and Orbetello integrating the results reported in Sfriso et al. (2006a, 2007) with additional taxa found during successive sampling campaigns and at Marano and Taranto.

Macroalgal parameters (i.e. the total taxa, the number of Rhodophyceae, Chlorophyceae and Phaeophyceae, the percentage of the same classes, the Rhodophyceae/Chlorophyceae ratios and the E-MaQI determination) are shown in Table 3. The number of taxa ranged from 38, in two canals placed in Venice and Lido islands (sts. 10 and 20), to 175 at Santa Maria del Mare (st. 5), the station considered as the "reference area" when comparing environments with high water renewal.

Table 4 reports the mean results of the bio-physico-chemical parameters recorded in the water column of the 20 stations. Values are very different, especially those referring to the oxygen saturation (range: 74–229%), suspended solids (range 14–65.5 mg l^{-1}), chlorophyll *a* (range 0.9–13.2 μg l^{-1}) and nutrient concentrations.

The Spearman's coefficients (Table 5) show very significant correlations between macroalgal parameters and DIN, nitrates, ammonium, RP, chlorinity and interesting correlations with FPM and Phaeo *a*. The correlation was direct with chlorinity but inverse with the nutrient concentrations. Moreover, if we examine data closely, it is possible to observe that Chlorophyceae and Rhodophyceae percentages exhibit inverse correlations, thus confirming that the R/C ratio can also be correctly employed to classify the environment as proposed by Sfriso et al. (2006a, b).

The cluster analysis by using the Euclidean distances helps discriminate the station associations clearly (Fig. 4). There are two main clusters, one with "High" (sts. 2, 5, 6 18) and "Good" (sts. 1, 7) ecological status stations and another which includes all the others. The latter contains one cluster with two "Good" stations (sts. 3, 8), colonised by seagrasses, and another with two sub-clusters, one including "Bad" (sts. 10, 20) and "Poor" (sts. 13, 14) stations and another also divided into two groups grouping "Poor" (sts. 11, 15) and "Moderate-Good" (sts 4, 7, 9, 12, 16, 19) stations.

By examining the values of the parameters recorded only in the five stations (sts. 5, 7, 12, 14, 20, Table 6) we can observe that, except for the Phaeophyceae percentage, macroalgae are significantly correlated with the oxygen saturation, the amount of FPM and SPM, the sediment grain-size, salinity and in the case of the Chlorophyceae and Rhodophyceae percentages with PCDD/F, Pesticides, Pb, As, Cd and Zn concentrations (Table 7).

The canonical correspondence analysis (CCA) was applied to all the 219 taxa reported in Table 1 keeping the analysis in the 20 stations without pollutants (Fig. 5) separate from the one in the 5 stations where pollutants in surface sediments were also available (Fig. 6). The analysis was performed by considering both the taxa and the stations versus the environmental variables.

In the first case (taxa versus variables in the 20 stations) the taxa characterised by score 0, 1 and 2 were considered separately, in order to obtain a clearer separation of responses (Figs. 5, 6). The taxa with score 0 were mainly plotted according to DIN, RP, FPM and Chl *a* vectors (Fig. 5a). The species which shifted from that arrangement were rare or occasional taxa recorded in a small number of stations. The taxa with score 2 were clearly opposite to the trophic variables and associated with high salinity and oxygen levels (Fig. 5c). The taxa with score 1 were scattered over the whole plotted area (Fig. 5b). The inverse analysis (stations versus the environmental variables, Fig. 5d) showed that the stations of low environmental status were plotted according to the trophic vectors.

The same results were obtained by considering the 5 stations of different ecological status (sts. 5, 7, 12, 14, 20, Fig. 6). In that case most of the taxa with score 0 were plotted according to trophic and pollutant vectors whereas taxa with score 2 were plotted on the opposite side characterised by high oxygenation (OD), water transparency (Wtran) and salinity. The taxa with score 1 were scattered over the whole plotted area. The inverse analysis (Fig. 6c) showed that the 5 stations were very differently placed. Sts 20 (Bad) and 14 (Poor) were plotted according to most of the environmental vectors. St. 12 (Moderate) was plotted only according to the Cr and FPM vectors, and sts. 7 (Good) and 5 (High) on the opposite side of most of the environmental variables.

Discussion and conclusions

Reference sites

One of the main difficulties was the choice of the "Reference stations" according to the WFD requirements, because transitional environments exhibit very changeable conditions. Moreover the ecological differences between the considered transitional environments are very high. Venice, Marano and Grado lagoons exhibit high water exchanges, but they also have wide confined areas. In these basins trophic and pollution conditions are very different and salinity

Table 4 Mean bio-physico-chemical parameters recorded in 20 Venice stations (12 monthly samples)

Stations	Temp. (°C)	Chlorinity (g l^{-1})	Oxygen (Sat %)	Chl. *a* (μg l^{-1})	Phaeo. *a* (μg l^{-1})	Chl. *a* Tot (μg l^{-1})	FPM (mg l^{-1})	RP (μM)	NH_4^+ (μM)	NO_2^- (μM)	NO_3^- (μM)	DIN
St. 1 (P. Unione)	16.0	18.4	111	1.17	0.60	1.78	18	0.77	12.54	1.06	6.20	19.8
St. 2 (Baia S. Felice)	15.7	18.4	116	1.35	0.80	2.15	15	0.40	14.47	0.85	7.15	22.5
St, 3 (Porto Secco)	15.5	18.4	229	0.67	0.24	0.90	28	0.66	4.85	0.77	6.10	11.7
St. 4 (Petta di Bò)	16.9	17.0	158	1.14	1.30	2.43	33	0.51	5.19	0.75	7.91	13.8
St. 5 (S. Maria del Mare)	18.3	18.7	200	1.08	0.73	1.81	24	0.69	6.79	0.83	6.68	14.3
St. 6 (Alberoni dike)	16.1	18.1	162	0.56	0.75	1.31	14	0.05	6.51	0.35	15.40	22.3
St. 7 (Alberoni Ottagono)	16.3	18.0	140	1.01	3.25	4.26	52	0.46	4.01	0.87	11.60	16.5
St. 8 (Malamocco)	16.8	18.4	218	2.30	1.57	3.86	29	0.15	5.88	0.45	8.80	15.1
St. 9 (Lido watershed)	17.7	18.1	167	0.87	0.65	1.52	33	0.74	8.02	1.02	9.37	18.4
St. 10 (Ca'Bianca canal)	16.5	18.0	117	0.88	1.40	2.29	33	1.09	35.55	0.68	10.39	46.6
St. 11 (Casinò canal)	16.2	17.3	148	1.69	1.72	3.41	21	1.12	19.33	0.83	14.91	35.1
St. 12 (Sacca Sessola)	16.1	17.3	138	1.59	2.04	3.63	64	0.66	8.81	1.33	13.92	24.1
St. 13 (Trezze)	18.8	16.4	107	6.20	6.98	13.18	52	1.85	17.76	1.95	20.80	40.5
St. 14 (San Giuliano)	17.1	14.4	73	4.20	7.13	11.33	65	1.53	23.23	2.15	22.89	48.3
St. 15 (Piazzale Roma)	15.6	17.5	145	1.59	1.80	3.39	28	0.34	8.98	0.62	18.28	27.9
St. 16 (Celestia)	17.8	17.9	114	2.18	2.05	4.23	39	0.93	9.11	0.87	7.43	17.4
St. 17 (San Nicolò)	17.5	18.7	128	1.95	1.46	3.41	30	0.75	9.82	0.66	7.82	18.3
St. 18 (Punta Sabbioni)	16.1	18.3	112	1.44	0.62	2.06	26	0.36	9.83	1.22	9.24	20.3
St. 19 (Palude Maggiore)	14.8	17.8	169	0.76	0.30	1.06	38	0.92	8.59	1.01	9.19	18.8
St. 20 (Misericordia canal)	16.1	17.5	107	1.80	1.96	3.76	32	1.45	33.80	0.91	17.19	51.9
Mean	16.6	17.7	143.0	1.7	1.9	3.6	33.7	0.8	12.7	1.0	11.6	25.2
Std.	1.02	0.99	39.82	1.33	1.92	3.16	14.57	0.46	9.07	0.44	5.09	12.4
Max	18.8	18.7	229.0	6.2	7.1	13.2	65.5	1.9	35.6	2.1	22.9	51.9
Min	14.8	14.4	73.4	0.6	0.2	0.9	14.0	0.1	4.0	0.4	6.1	11.7

Temp. = temperature; FPM = Filtered Particulate Matter; Chl. *a* = chlorophyll *a*; Phaeo. *a* = phaeophytin *a*; RP = Reactive Phosphorus; DIN = Dissolved Inorganic Nitrogen

Table 5 Spearman's correlation matrix in the 20 Venice stations sampled monthly during one year

	Temp.	Chlorinity	Oxygen	FPM	Chl. *a*	Phaeo. *a*	Chl. *a* Tot.	RP	Ammonium	Nitrites	Nitrates	DIN
No. Taxa	0.07	**0.56**	0.24	**−0.48**	−0.37	**−0.46**	−0.43	**−0.60**	**−0.58**	−0.30	**−0.62**	**−0.70**
No. Chlorophyceae	0.13	**0.52**	0.14	−0.40	−0.35	−0.43	−0.41	**−0.50**	**−0.53**	−0.25	**−0.66**	**−0.67**
No. Rhodophyceae	0.05	**0.56**	0.27	**−0.49**	−0.37	**−0.46**	−0.43	**−0.61**	**−0.59**	−0.30	**−0.60**	**−0.69**
No. Phaeophyceae	0.07	**0.56**	0.18	**−0.50**	−0.35	**−0.46**	−0.43	**−0.60**	**−0.57**	−0.33	**−0.61**	**−0.68**
% Chlorophyceae	0.01	**−0.46**	−0.40	0.34	0.29	0.38	0.36	**0.68**	**0.85**	0.24	**0.56**	**0.86**
% Rhodophyceae	0.01	0.44	0.43	−0.28	−0.27	−0.34	−0.32	**−0.63**	**−0.83**	−0.18	**−0.54**	**−0.84**
% Phaeophyceae	−0.11	0.30	0.06	−0.37	−0.26	−0.36	−0.33	**−0.50**	**−0.48**	−0.37	−0.32	**−0.49**
R/C	0.00	**0.53**	0.39	−0.43	−0.35	−0.43	−0.41	**−0.66**	**−0.71**	−0.28	**−0.58**	**−0.76**
E-MaQI	0.03	**0.51**	0.29	**−0.45**	−0.33	−0.40	−0.38	**−0.65**	**−0.66**	−0.27	**−0.56**	**−0.72**

In bold significant values: $P < 0.05$ for $r > |0.45|$

Temp. = temperature; FPM = Filtered Particulate Matter; Chl. *a* = chlorophyll *a*; Phaeo. *a* = phaeophythin *a*; RP = Reactive Phosphorus; DIN = Dissolved Inorganic Nitrogen; R/C = Rhodophyceae/Chlorophyceae; E-MaQI = Expert-Macrophyte Quality Index

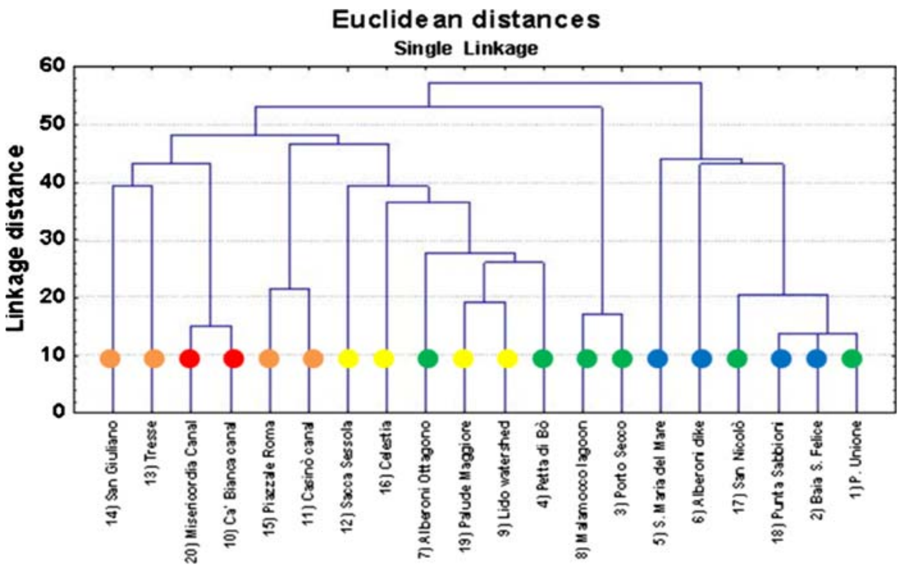
gradients are marked. Vice versa, Orbetello and Lesina lagoons are almost closed environments with negligible water renewal and low pollution differences inside the same basin. Mar Piccolo is a deeper basin which is also considered as a marine embayment, whereas Sacca di Goro is a brackish basin in the Po delta; it is strongly affected by the river Po and shows continual morphological changes. These environments present different habitats which are hardly comparable, especially when we consider high water renewal areas and confinement areas where water renewal is negligible. In the last decades, most of the transitional environments, and particularly their inner areas, have been affected by a high anthropic impact which made the presence of natural and uncontaminated environments very rare. For example, natural or low contaminated environments do not exist at present in Venice or in Marano lagoons, where confinement areas and salt marshes exhibit conditions markedly low than before the 1980s, but they still survive in the lagoon of Lesina. Unfortunately, environmental data to support those changes are rare or do not exist and the comparison is based on the memory of fishermen who know well the environmental changes that occurred in the last decades. As a consequence, whereas relatively high conditions and "reference areas" can still be recorded in Venice and Marano areas characterised by high water exchanges, for confinement areas high environmental conditions must be searched in other basins such as Lesina. Lesina lagoon, with the exception of its western area which is strongly influenced by the wastes from its centre and the effluents of a buffalo farm, shows high and natural conditions and st. 3 in the central part of the lagoon was selected as "reference site" for confinement areas.

The conditions recorded in the two "reference sites" were considered as the "reference conditions" for these extremely different environments and employed to adjust the dichotomic key set up for R-MaQI by Sfriso et al. (2007). The almost equivalent mean macroalgal scores found in these two stations were considered the reference values to normalise the other results recorded in other areas (EQR value determination).

MaQI validation

The presence and abundance of the main macrophyte taxa (i.e. Chlorophyceae, Rhodophyceae and

Fig. 4 Cluster analyses of the 20 Venice areas



Phaeophyceae) and the single species recorded in specific transitional environments are put in relation with the main hydrological and sedimentary parameters, including the organic and inorganic pollutants found in the same areas. Results show that the environment assessment, based on the macrophyte assemblages, appears well supported by the different environmental conditions, the recorded pollutant concentrations (Tables 4, 6) and the statistical elaborations of these data (Tables 5, 7; Figs. 4, 5). In fact, in all the 20 stations of the Venice lagoon, all the considered parameters show increasing or decreasing values according to the change of the macroalgal composition (i.e. number and percentage of taxa), the Rhodophyceae/Chlorophyceae ratio and the mean macroalgal score determined by applying the E-MaQI. Stations with a similar ecological status appear well grouped by the cluster analysis (Fig. 4) showing similar macrophyte assemblages and the single species appear well related with or in opposition to the main variables associated to the ecological status of the considered stations. In fact, the CANOCO analysis (Figs. 5, 6) also confirmed that species associated to high scores grow only in areas where pollutants are low, waters are transparent and no anoxia occur.

Such results are particularly evident by examining Tables 6 and 7 where we can see that the number of taxa, the R/C ratio and the percentage of Rhodophyceae, with the exception of the oxygen concentration and water transparency which are directly correlated to the ecological status, are significantly and inversely correlated to all of the bio-physico-chemical parameters of the water column and to the concentration of nutrients and pollutants in the surface sediments. An exactly opposite behaviour is exhibited by the Chlorophyceae percentage, whereas no significant correlation is displayed by the Phaeophyceae percentage.

In addition, the analysis of the seasonal variation of those parameters per single station (data not reported) put in evidence that the high seasonal variability of salinity, turbidity, oxygen saturation and the nutrient concentrations usually characterise "Bad" or "Poor" environments. In contrast, the areas which exhibit low seasonal variations, also in the presence of low salinity values and relatively high nutrient concentrations, may exhibit "Good" or "High" conditions as it was observed in the lagoon of Lesina.

E-MaQI and R-MaQI coupling

The application of the E-MaQI is rigorous, but time consuming. It can be applied only by experts in macroalgal taxonomy. Its application is suggested when the environment is assessed for the first time; it can then be repeated with a 3–6-year frequency or in case of evident environmental changes. This procedure allows a precise classification of the environment by a class binomial that includes some variance degree. The results are reliable in the presence of a complete list of the taxa which colonise the study areas and in the presence of at least 15 taxa. Samples may

Table 6 Environmental parameters and pollutants in the 5 stations of different Ecological Status in Venice lagoon

Parameters	High (5) SMM	Good (6) Alberoni	Moderate (12) S. Sessola	Poor (14) S. Giuliano	Bad (20) Misericordia	Parameter trend
OD (%)	200	140	138	117	88	↓
FPM (mg DWT l^{-1})	24	52	64	70	75	↑
SPM (g DWT m^{-2} d^{-1})	180	625	2073	1963	2450	↑
Chl. *a* tot (μg l^{-1})	0.52	0.94	1.59	1.33	7.55	↑
RP (μM)	0.69	0.46	0.66	0.91	2.45	↑
DIN (μM)	14.3	16.5	24.1	41.0	122.5	↑
Corg (sed.) (mg DWT g^{-1})	8.3	7.6	7.9	11.2	26.0	↑
Ntot (sed.) (mg DWT g^{-1})	0.89	0.67	0.89	1.47	2	↑
Ptot (sed.) (μg DWT g^{-1})	568	350	360	544	726	↑
Porg (sed.) (μg DWT g^{-1})	70	56	58	132	192	↑
Water Transparency (cm)	100	100	90	88	65	↓
Sed. fraction < 63 μm (%)	33	42	78	89	97	↑
Chlorinity (PSU)	33.7	32.5	31.2	26.5	27.2	↑
PCDD/F (sed.) (pg DWT g^{-1})	47	44	115	750	605	↑
PAHs (sed.) (ng DWT g^{-1})	100	252	582	925	10000	↑
Pesticides (sed.) (ng DWT g^{-1})	0.50	0.39	0.90	5.82	20	↑
PCBs (sed.) (ng DWT g^{-1})	2.00	0.52	1.18	6.51	3992	↑
Pb (sed.) (μg DWT g^{-1})	13	19	26	63	214	↑
As (sed.) (μg DWT g^{-1})	7	9	11	28	37	↑
Hg (sed.) (μg DWT g^{-1})	0.4	0.27	0.53	0.83	4.0	↑
Cu (sed.) (μg DWT g^{-1})	6	13	18	44	296	↑
Zn (sed.) (μg DWT g^{-1})	26	81	162	413	1152	↑
Cd (sed.) (μg DWT g^{-1})	0.5	0.4	1.00	2.4	5.66	↑
Cr (sed.) (μg DWT g^{-1})	20	54	26	37	84.1	↑
Ni (sed.) (μg DWT g^{-1})	13	64	41	36	38	↑
Total taxa	175	108	80	61	38	↓
Chlorophyceae (No.)	44	35	32	29	23	↓
Rhodophyceae (No.)	102	57	37	23	9	↓
Phaeophyceae (No.)	29	16	11	9	6	↓
R/C	2.32	1.63	1.16	0.79	0.39	↓
E-MaQI	1.00	0.70	0.46	0.39	0.15	↓
Chlorophyceae (%)	25.1	32.4	40.0	47.5	60.5	↑
Rhodophyceae (%)	58.3	52.8	46.3	37.7	23.7	↓
Phaeophyceae (%)	16.6	14.8	13.8	14.8	15.8	↔

OD = Dissolved Oxygen; FPM = Filtered Particulate Matter; SPM = Settled Particulate Matter; Chl. *a* tot = total chlorophyll *a*; RP = Reactive Phosphorus; DIN = Dissolved Inorganic Nitrogen; Corg = organic Carbon; Ntot = total Nitrogen; Ptot = total Phosphorus; Porg = organic Phosphorus; sed. = sediment; R/C = Rhodophyceae/Chlorophyceae; E-MaQI = Expert-Macrophyte Quality Index

cover the main seasonal changes but their number should never be lower than 2, one in April–May and the other in September–October. When the number of taxa is lower than 15, the observation of the possible blooming of some nitrophilous species and the measurement of some physico-chemical parameters (i.e. dissolved oxygen, chlorinity, water transparency, percentage of grain-size <63 μm and, when necessary, nutrient concentrations in the water column) help the environment classification, as confirmed by the CANOCO analyses. It is important to underline that the presence of abundant populations with few score 2-

Table 7 Correlation matrix between macroalgal parameters and some pollutants and environmental variables in the 5 stations of different ecological status

	No. Taxa	No. Chlor.	No. Rhod.	No. Phaeo.	R/C	E-MaQI	% Chlor.	% Rhod.	% Phaeo.
OD	**0.98**	**0.99**	**0.98**	**0.97**	**0.97**	**0.97**	**−0.94**	**0.92**	0.36
FPM	**−0.99**	**−0.97**	**−1.00**	**−1.00**	**−0.97**	**−0.97**	**0.89**	**−0.86**	−0.55
SPM	**−0.93**	**−0.92**	**−0.94**	**−0.92**	**−0.95**	**−0.96**	**0.91**	**−0.88**	−0.46
Chl *a* tot	−0.67	−0.77	−0.66	−0.61	−0.74	−0.76	**0.86**	**−0.88**	0.21
RP	−0.62	−0.73	−0.61	−0.54	−0.71	−0.72	0.85	**−0.88**	0.34
DIN	−0.71	−0.81	−0.71	−0.64	−0.78	−0.79	**0.90**	**−0.93**	0.24
Corg	−0.64	−0.75	−0.63	−0.56	−0.72	−0.73	0.86	**−0.89**	0.33
Ntot	−0.69	−0.78	−0.69	−0.61	−0.78	−0.77	**0.90**	**−0.93**	0.29
Ptot	−0.28	−0.39	−0.28	−0.18	−0.41	−0.39	0.60	−0.66	0.68
Porg	−0.67	−0.77	−0.67	−0.60	−0.76	−0.75	**0.89**	**−0.92**	0.32
Water transparency	0.80	**0.87**	0.79	0.74	**0.86**	**0.88**	**−0.95**	**0.96**	−0.07
Sed. fraction < 63 μm	**−0.93**	**−0.92**	**−0.94**	**−0.91**	**−0.96**	**−0.96**	**0.93**	**−0.91**	−0.37
Chlorinity	**0.87**	**0.87**	**0.88**	0.83	**0.91**	**0.87**	**−0.90**	**0.90**	0.17
PCDD/F	−0.77	−0.84	−0.77	−0.70	−0.85	−0.82	**0.94**	**−0.96**	0.20
PAHs	−0.63	−0.74	−0.62	−0.56	−0.70	−0.72	0.83	−0.86	0.29
Pesticides	−0.69	−0.79	−0.69	−0.62	−0.77	−0.77	**0.90**	**−0.93**	0.28
PCBs	−0.59	−0.71	−0.59	−0.52	−0.67	−0.69	0.81	−0.85	0.34
Pb	−0.70	−0.80	−0.70	−0.64	−0.78	−0.78	**0.90**	**−0.93**	0.25
As	−0.82	**−0.87**	−0.82	−0.76	**−0.89**	−0.86	**0.95**	**−0.97**	0.09
Hg	−0.60	−0.71	−0.59	−0.52	−0.68	−0.69	0.83	−0.86	0.38
Cu	−0.65	−0.75	−0.64	−0.58	−0.72	−0.74	0.85	**−0.88**	0.29
Zn	−0.76	−0.84	−0.75	−0.69	−0.83	−0.83	**0.93**	**−0.96**	0.19
Cd	−0.75	−0.83	−0.75	−0.68	−0.83	−0.82	**0.93**	**−0.96**	0.21
Cr	−0.65	−0.75	−0.64	−0.61	−0.67	−0.67	0.74	−0.76	0.15
Ni	−0.43	−0.39	−0.42	−0.49	−0.30	−0.31	0.16	−0.11	−0.61

In bold significant values: $P < 0.05$ for $r > |0.86|$

No. = Number; Chlor. = Chlorophyceae; Rhod. = Rhodophyceae; Phaeo. = Phaeophyceae: E-MaQI = Expert-Macrophyte Quality Index; OD = Dissolved Oxygen; FPM = Filtered Particulate Matter; SPM = Settled Particulate Matter; Chl. *a* tot = total Chlorophyll *a*; RP = Reactive Phosphorus; DIN = Dissolved Inorganic Nitrogen; Corg = organic Carbon; Ntot = total Nitrogen; Ptot = total Phosphorus; Porg = organic Phosphorus; PCDD/F = Polychloro-Dibenzo-Dioxins/Furans; PAHs = Polycyclic Aromatic Hydrocharbons; PCBs = Polychlorinated Biphenyls

taxa or the simultaneous presence of many score 2-taxa (Table 1), but with negligible biomass, is sufficient to classify a “High-status” environment.

The same results can be obtained by applying the R-MaQI which can be used also by non-experts and provides an almost immediate classification. That index was set up as a dicothomic key (Table 2) where the ecological conditions of the study areas, the composition and structure of the seagrass meadows, the R/C ratio and the expert index are compared with the “reference areas”. In the Mediterranean sea, only 7–8 seagrasses are present (Buia et al., 2003), and the density of their populations is an important diagnostic parameter because these plants are very sensitive to eutrophication, pollution and environmental stressors.

R-MaQI was set up by considering only taxa or macrophyte assemblages whose presence/absence and abundance can be considered as indicators of particular environmental conditions (Table 2). We found out that in the studied Italian lagoons, the complete absence of seagrasses is, in general, strictly associated with marked environmental stress factors. In such environments, the lack of seagrasses allows a rapid discrimination between “Bad-Poor” and “Moderate-Good-High” environmental conditions. “Bad” and “Poor” classes can be distinguished by the

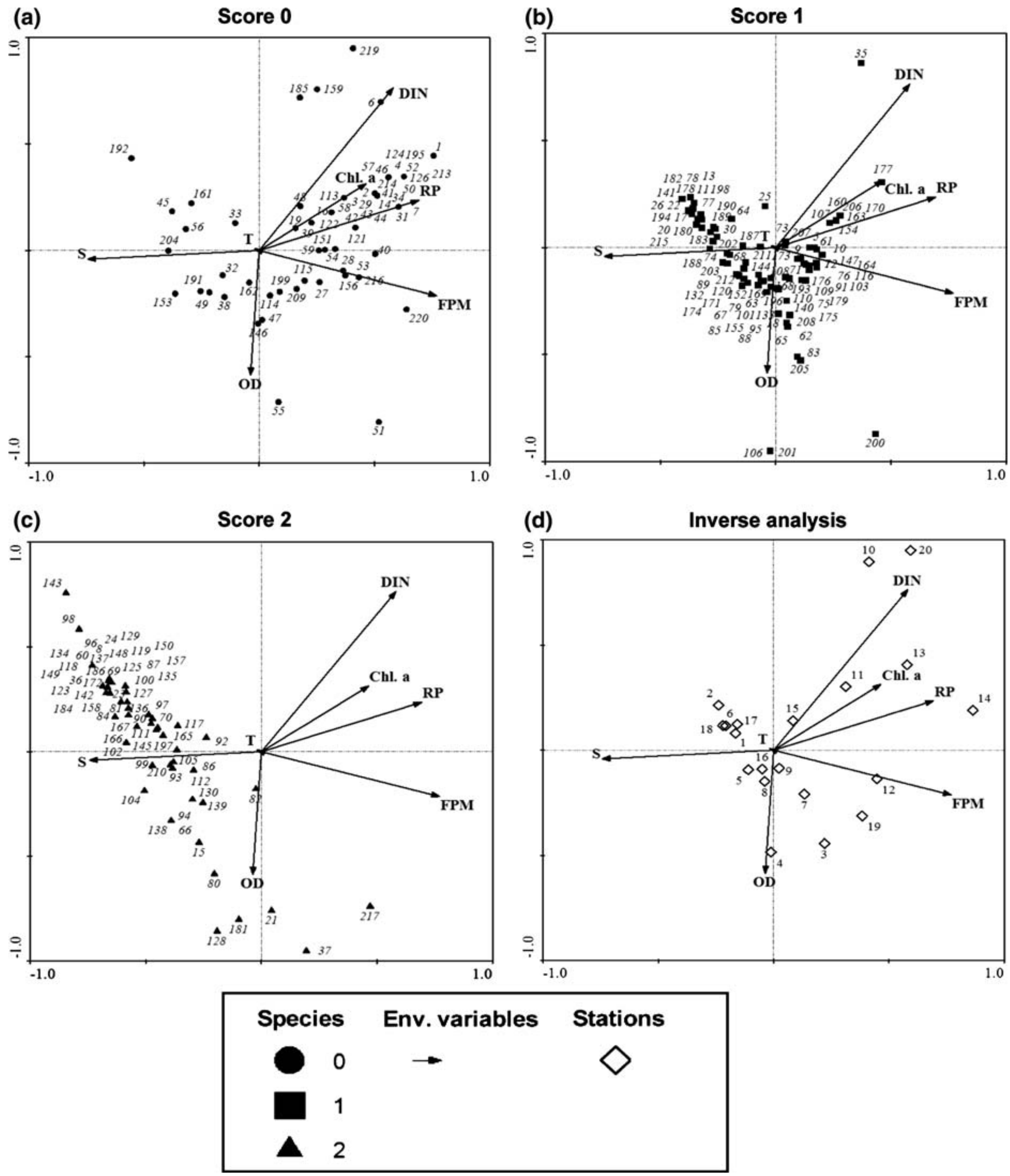


Fig. 5 CANOCO plotters by considering all the 20 Venice areas and some environmental variables and nutrient concentrations

presence or absence of blooming macroalgae, respectively, whereas “Moderate-Good-High” classes take into account both the seagrass population structure and the seaweed associations and are relatively easy to assess in soft bottoms. In the case of hard substrata, the environment classification depends on the prevalence of some macroalgae and on the abundance of some species with a high score (Table 1) such as the

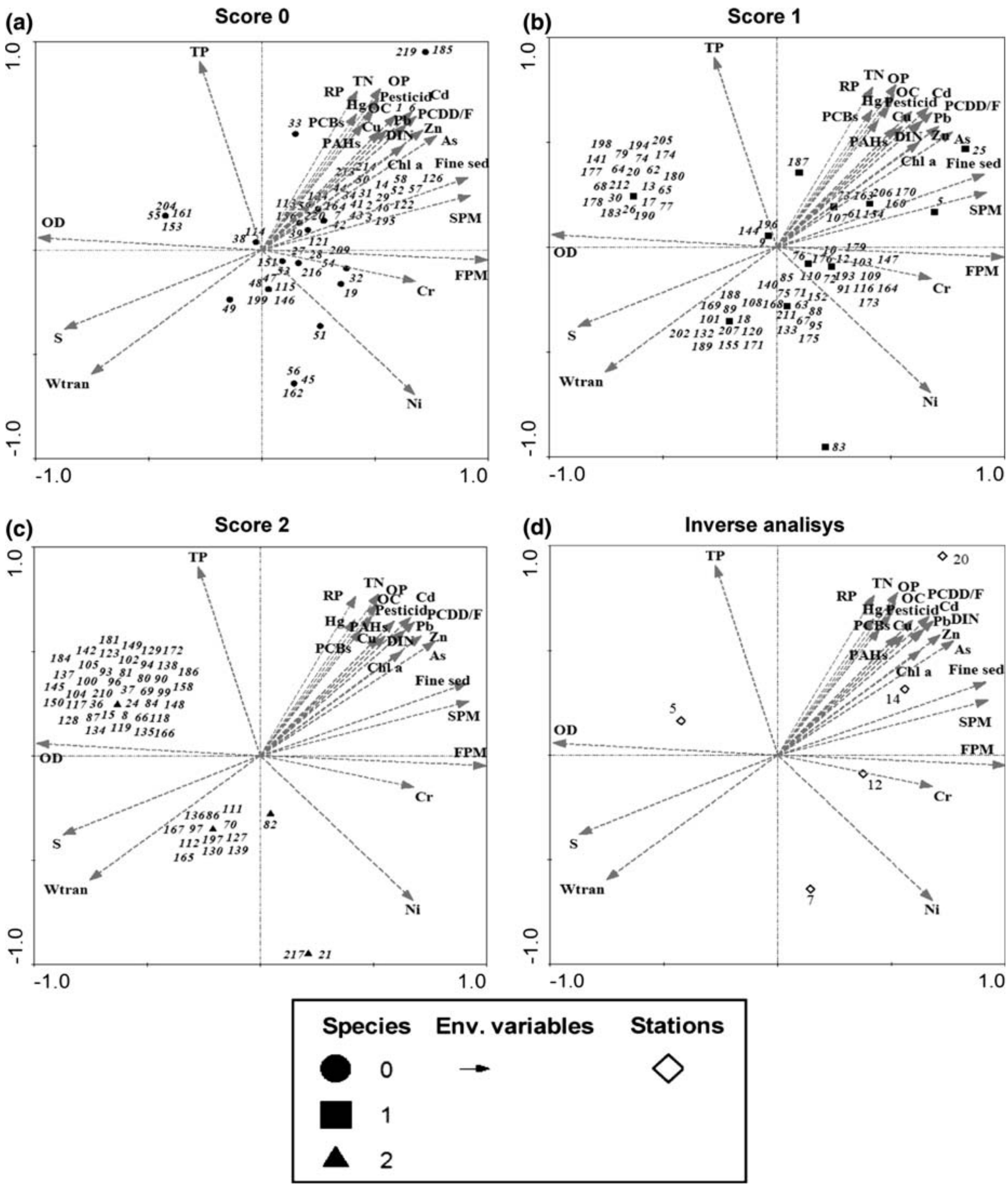


Fig. 6 CANOCO plotters by considering only the 5 stations of different ecological status where nutrient concentrations and pollutants in the surface sediments are also available

Corallinaceae which are very sensitive to eutrophication and pollution. In fact, eutrophic or very polluted environments usually exhibit frequent anoxic crises (Morand & Briand, 1996; Schramm & Nienhuis, 1996), high sedimentation rates (Sfriso et al., 2005a) and pH values lowering down to 7.00

(Sfriso et al., 1987). In those conditions, the presence of calcareous macroalgae is hampered.

Because of its structure, R-MaQI places the studied areas directly into one of the 5 classes of ecological status. As for E-MaQI it uses a classification by a class binomial indicating also the ecological class closer to the main class. This allows to mediate the seasonal changes and reduce doubts due to the presence of ecological conditions which sometimes do not apply to only one class.

The assessment of the Italian Transitional environments by macrophytes and the application of different sampling efforts are also suggested by the Central Institute for the Scientific and Technologic Research Applied to the Sea (ICRAM, 2007) in the "Protocols for sampling and determining the biological and physico-chemical quality elements in the framework of transitional water biomonitoring plans" according to the WFD. ICRAM suggests the application of "Control biomonitoring" every 6 years and "Routine biomonitoring" every year, both by means of two seasonal samplings, in April–May and in September–October.

The sampling of macroalgae is separated from that of seagrasses. However, the protocols include the determination of the biomass coverage, density and taxa determination at genus or species level.

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